UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
61/227,649	07/22/2009		270	C2081-701305		

CONFIRMATION NO. 6229

CORRECTED FILING RECEIPT

37462
LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

Date Mailed: 11/13/2009

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**

Applicant(s)

Stefan Gross, Brookline, MA;
Shengfang Jin, Newton, MA;
Shinsan Su, Cambridge, MA;
Valeria Fantin, Cambridge, MA;
Lenny Dang, Boston, MA;
Katharine Yen, Wellesley, MA;

Power of Attorney:
Catherine McCarty--54301

If Required, Foreign Filing License Granted: 08/11/2009
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/227,649**
Projected Publication Date: None, application is not eligible for pre-grant publication
Non-Publication Request: No
Early Publication Request: No
** **SMALL ENTITY** **
Title

METHODS AND COMPOSITIONS FOR TREATING CANCER

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 (1-866-999-4158).

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).

Attorney Docket No.: C2081-701305

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Stefan Gross et al.
Serial No:	61/227,649
Confirmation No:	6229
Filed:	July 22, 2009
For:	METHODS AND COMPOSITIONS FOR TREATING CANCER

CERTIFICATE OF TRANSMISSION UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being electronically filed in accordance with § 1.6(a)(4), on the 6th day of November, 2009.

/Catherine M. McCarty/
Catherine M McCarty, Reg. No. 54,301

Commissioner for Patents

REQUEST FOR CORRECTED FILING RECEIPT

This is a request for correction of the enclosed Official Filing Receipt Document dated October 23, 2009, in connection with the above-identified application. The inventor name "Stephan Gross" was incorrectly listed. The inventor name should be listed as "**Stefan Gross**".

A copy of the Official Filing Receipt is enclosed with the correction indicated. A supplemental provisional cover sheet is being submitted concurrently to correct the spelling of the inventor's name. It is respectfully requested that the corrected Filing Receipt be issued reflecting the correction of the above noted error and sent to the undersigned at the earliest possible time.

If there is a fee occasioned by this request, please charge the fee to the account of the undersigned, Deposit Account No. 50/2762.

Respectfully submitted,
Gross et al., Applicants

By: /Catherine M. McCarty/
Catherine M. McCarty, Reg. No. 54,301

LANDO & ANASTASI, LLP
One Main Street
Cambridge, Massachusetts 02142
United States of America
Telephone: 617-395-7000
Facsimile: 617-395-7070

Date: November 6, 2009

985000.1

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
61/227,649	07/22/2009		270	C2081-701305		

CONFIRMATION NO. 6229

UPDATED FILING RECEIPT

37462
LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

OC000000038367271

Date Mailed: 10/23/2009

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**

Applicant(s)

~~Stephan~~ Gross, Brookline, MA; → Stefan
Shengfang Jin, Newton, MA;
Shinsan Su, Cambridge, MA;
Valeria Fantin, Cambridge, MA;
Lenny Dang, Boston, MA;
Katharine Yen, Wellesley, MA;

Power of Attorney:
Catherine McCarty--54301

If Required, Foreign Filing License Granted: 08/11/2009
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/227,649**
Projected Publication Date: None, application is not eligible for pre-grant publication
Non-Publication Request: No
Early Publication Request: No
**** SMALL ENTITY ****
Title

METHODS AND COMPOSITIONS FOR TREATING CANCER

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 (1-866-999-4158).

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).

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

PTO/SB/16 (04-07)
Approved for use through 06/30/2010 OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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 i
Stefan		Gross	Brookline	MA	US

Inventor 2 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Shengfang		Jin	Newton	MA	US

Inventor 3 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Shinsan		Su	Cambridge	MA	US

Inventor 4 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Valeria		Fantin	Cambridge	MA	US

Inventor 5 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Lenny		Dang	Boston	MA	US

Inventor 6 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Katharine		Yen	Wellesley	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	METHODS AND COMPOSITIONS FOR TREATING CANCER
Attorney Docket Number (if applicable)	C2081-701305

Correspondence Address

Direct all correspondence to (select one):

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

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

PTO/SB/16 (04-07)
Approved for use through 06/30/2010 OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Customer Number	37462

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 name of the U.S. Government agency and the Government contract number are:

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

PTO/SB/16 (04-07)
Approved for use through 06/30/2010 OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Entity Status

Applicant claims small entity status under 37 CFR 1.27

- (•) Yes, applicant qualifies for small entity status under 37 CFR 1.27
- ( ) 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	/Catherine M. McCarty/			Date (YYYY-MM-DD)	2009-11-06
First Name	Catherine	Last Name	McCarty	Registration Number (If appropriate)	54301

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.

Electronic Acknowledgement Receipt

EFS ID:	6406209
Application Number:	61227649
International Application Number:	
Confirmation Number:	6229
Title of Invention:	METHODS AND COMPOSITIONS FOR TREATING CANCER
First Named Inventor/Applicant Name:	Stephan Gross
Customer Number:	37462
Filer:	Catherine M. McCarty
Filer Authorized By:	
Attorney Docket Number:	C2081-701305
Receipt Date:	06-NOV-2009
Filing Date:	22-JUL-2009
Time Stamp:	14:51:30
Application Type:	Provisional

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Request for Corrected Filing Receipt	C2081_701305_Request_for_Corrected_Filing_Receipt.pdf	26060 b3750bf34398feaafd852af7a90a6089857973a5	no	2

Warnings:	
Information:	

2	Miscellaneous Incoming Letter	C2081_701305_Marked_Up_FR.pdf	167446 e1142892930c933a00d2504a690becf83d0612ec	no	3
Warnings:					
Information:					
3	Provisional Cover Sheet (SB16)	C2081_701305_Supplemental_Coversheet.pdf	1000245 a5850a96320d40927f98455a9588e198abacdee4	no	4
Warnings:					
Information:					
		Total Files Size (in bytes):	1193751		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
61/227,649	07/22/2009		270	C2081-701305		

CONFIRMATION NO. 6229

UPDATED FILING RECEIPT

37462
LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

Date Mailed: 10/23/2009

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**

Applicant(s)

Stephan Gross, Brookline, MA;
Shengfang Jin, Newton, MA;
Shinsan Su, Cambridge, MA;
Valeria Fantin, Cambridge, MA;
Lenny Dang, Boston, MA;
Katharine Yen, Wellesley, MA;

Power of Attorney:
Catherine McCarty--54301

If Required, Foreign Filing License Granted: 08/11/2009
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/227,649**
Projected Publication Date: None, application is not eligible for pre-grant publication
Non-Publication Request: No
Early Publication Request: No
** **SMALL ENTITY** **
Title

METHODS AND COMPOSITIONS FOR TREATING CANCER

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 (1-866-999-4158).

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).

P wsPCT 10.18.09
DUE:
FINAL: 3.18.10
8.18.09 PSP

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
61/227,649	07/22/2009	Stephan Gross	C2081-701305

CONFIRMATION NO. 6229

37462
LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

FORMALITIES LETTER

Date Mailed: 08/18/2009

NOTICE TO FILE MISSING PARTS OF PROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(c)

Filing Date Granted

An application number and filing date have been accorded to this provisional application. The items indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The statutory basic filing fee is missing.
  *Applicant must submit $**220** to complete the basic filing fee for a non-small entity. If appropriate, applicant may make a written assertion of entitlement to small entity status and pay the small entity filing fee (37 CFR 1.27).*

The applicant needs to satisfy supplemental fees problems indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- To avoid abandonment, a surcharge (for late submission of filing fee or cover sheet) as set forth in 37 CFR 1.16(g) of **$50** for a non-small entity, must be submitted with the missing items identified in this notice.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$540** for a non-small entity
- **$220** Statutory basic filing fee.
- **$50** Surcharge.
- The specification and drawings contain more than 100 pages. Applicant owes **$270** for **42** pages in excess of **100** pages for a non-small entity.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/spathammavong/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Attorney Docket No.: C2081-701305

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Stephan Gross et al.
Serial No.:	61/227,649
Filing Date:	July 22, 2009
For:	METHODS AND COMPOSITIONS FOR TREATING CANCER
Examiner:	Not Yet Assigned
Art Unit:	Not Yet Assigned
Confirmation No.:	6229

CERTIFICATE OF TRANSMISSION UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being electronically filed in accordance with §1.6(a)(4) on the 19th day of October 2009.

/Catherine M. McCarty/
Catherine M. McCarty, Reg. No. 54,301

Commissioner for Patents

RESPONSE TO NOTICE TO FILE MISSING PARTS

Sir:

In response to the Notice to File Missing Parts of Application under 37 CFR §1.53(b) mailed August 18, 2009, Applicant submits herewith the following:

1. the basic filing fee; and
2. surcharge

Applicants note that the Applicant is a Small Entity. Therefore, the fee of $270.00 is being paid concurrently herewith on the Electronic Filing System (EFS) by way of Deposit Account authorization. It is understood that this perfects the application and no additional papers or filing fees are required. Please apply any other charges or credits to Deposit Account No. 50-2762, referencing Attorney Docket No. C2081-701305.

Respectfully submitted,

/Catherine M. McCarty/

Catherine McCarty, Reg. No. 54,301
LANDO & ANASTASI, LLP
One Main Street
Cambridge, Massachusetts 02142
United States of America
Telephone: 617-395-7087
Facsimile: 617-395-7070

Date: October 19, 2009
Attorney Docket No.: C2081-701305

980341.1

Electronic Patent Application Fee Transmittal

Application Number:	61227649
Filing Date:	22-Jul-2009
Title of Invention:	METHODS AND COMPOSITIONS FOR TREATING CANCER
First Named Inventor/Applicant Name:	Stephan Gross
Filer:	Catherine M. McCarty/Brenda Kowalczuk
Attorney Docket Number:	C2081-701305

Filed as Small Entity

Provisional Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Provisional Application filing fee	2005	1	110	110
Pages:				
Provis. Appl Size fee per 50 sheets >100	2085	1	135	135
Claims:				
Miscellaneous-Filing:				
Late provisional filing fee/cover sheet	2052	1	25	25
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**270**

Electronic Acknowledgement Receipt	
EFS ID:	6282802
Application Number:	61227649
International Application Number:	
Confirmation Number:	6229
Title of Invention:	METHODS AND COMPOSITIONS FOR TREATING CANCER
First Named Inventor/Applicant Name:	Stephan Gross
Customer Number:	37462
Filer:	Catherine M. McCarty
Filer Authorized By:	
Attorney Docket Number:	C2081-701305
Receipt Date:	19-OCT-2009
Filing Date:	22-JUL-2009
Time Stamp:	12:50:10
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$270
RAM confirmation Number	8019
Deposit Account	502762
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 (National application filing, search, and examination fees) Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)	

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	C2081-701305_Notice_of_NTFMP.PDF	71900 b70a10771e783aa0584292c31e7e8231c1b1b113	no	2
Warnings:					
Information:					
2	Applicant Response to Pre-Exam Formalities Notice	C2081_701305_Transmittal_Response_NTFMP.pdf	25947 6f2b743409833561d43b37213e702f03f4322a0d	no	2
Warnings:					
Information:					
3	Fee Worksheet (PTO-875)	fee-info.pdf	35519 98259001a22ff240e75f04702a9859cbb5f40ab2	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	133366		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

<u>New Applications Under 35 U.S.C. 111</u>
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

<u>National Stage of an International Application under 35 U.S.C. 371</u>
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

<u>New International Application Filed with the USPTO as a Receiving Office</u>
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
61/227,649	07/22/2009		0.00	C2081-701305		

CONFIRMATION NO. 6229

37462
LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

FILING RECEIPT

OC000000037303913

Date Mailed: 08/18/2009

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**

Applicant(s)

Stephan Gross, Brookline, MA;
Shengfang Jin, Newton, MA;
Shinsan Su, Cambridge, MA;
Valeria Fantin, Cambridge, MA;
Lenny Dang, Boston, MA;
Katharine Yen, Wellesley, MA;

Power of Attorney:
Catherine McCarty--54301

If Required, Foreign Filing License Granted: 08/11/2009
The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 61/227,649**
Projected Publication Date: None, application is not eligible for pre-grant publication
Non-Publication Request: No
Early Publication Request: No
Title

METHODS AND COMPOSITIONS FOR TREATING CANCER

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 (1-866-999-4158).

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).

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
61/227,649	07/22/2009	Stephan Gross	C2081-701305

CONFIRMATION NO. 6229

37462
LANDO & ANASTASI, LLP
ONE MAIN STREET, SUITE 1100
CAMBRIDGE, MA 02142

FORMALITIES LETTER

OC000000037303914

Date Mailed: 08/18/2009

NOTICE TO FILE MISSING PARTS OF PROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(c)

Filing Date Granted

An application number and filing date have been accorded to this provisional application. The items indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The statutory basic filing fee is missing.
  *Applicant must submit $**220** to complete the basic filing fee for a non-small entity. If appropriate, applicant may make a written assertion of entitlement to small entity status and pay the small entity filing fee (37 CFR 1.27).*

The applicant needs to satisfy supplemental fees problems indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- To avoid abandonment, a surcharge (for late submission of filing fee or cover sheet) as set forth in 37 CFR 1.16(g) of $**50** for a non-small entity, must be submitted with the missing items identified in this notice.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is $**540** for a non-small entity
- $**220** Statutory basic filing fee.
- $**50** Surcharge.
- The specification and drawings contain more than 100 pages. Applicant owes $**270** for **42** pages in excess of **100** pages for a non-small entity.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/spathammavong/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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

PTO/SB/16 (04-07)
Approved for use through 06/30/2010 OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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 i
Stephan		Gross	Brookline	MA	US

Inventor 2 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Shengfang		Jin	Newton	MA	US

Inventor 3 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Shinsan		Su	Cambridge	MA	US

Inventor 4 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Valeria		Fantin	Cambridge	MA	US

Inventor 5 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Lenny		Dang	Boston	MA	US

Inventor 6 — Remove

Given Name	Middle Name	Family Name	City	State	Country i
Katharine		Yen	Wellesley	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	METHODS AND COMPOSITIONS FOR TREATING CANCER
Attorney Docket Number (if applicable)	C2081-701305

Correspondence Address

Direct all correspondence to (select one):

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

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

PTO/SB/16 (04-07)
Approved for use through 06/30/2010 OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Customer Number	37462

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 name of the U.S. Government agency and the Government contract number are:

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

PTO/SB/16 (04-07)
Approved for use through 06/30/2010 OMB 0651-0032
U.S. Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

Entity Status

Applicant claims small entity status under 37 CFR 1.27

◯ Yes, applicant qualifies for small entity status under 37 CFR 1.27

◉ 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	/Catherine M. McCarty/			Date (YYYY-MM-DD)	2009-07-22
First Name	Catherine	Last Name	McCarty	Registration Number (If appropriate)	54301

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.

METHODS AND COMPOSITIONS FOR TREATING CANCER

The invention relates to the treatment of proliferative disorders such as cancer as well as related methods, compounds and compositions.

BACKGROUND

Isocitrate dehydrogenase, also known as IDH, is an enzyme which participates in the citric acid cycle. It catalyzes the third step of the cycle: the oxidative decarboxylation of isocitrate, producing alpha-ketoglutarate (α-ketoglutarate or α-KG) and CO_2 while converting NAD+ to NADH. This is a two-step process, which involves oxidation of isocitrate (a secondary alcohol) to oxalosuccinate (a ketone), followed by the decarboxylation of the carboxyl group beta to the ketone, forming alpha-ketoglutarate. Another isoform of the enzyme catalyzes the same reaction; however this reaction is unrelated to the citric acid cycle, is carried out in the cytosol as well as the mitochondrion and peroxisome, and uses NADP+ as a cofactor instead of NAD+.

SUMMARY OF THE INVENTION

The inventors have discovered novel methods for treating, identifying, diagnosing and staging a disorder, *e.g.*, a proliferative disorder such as cancer. Described herein are methods, compounds and compositions for the treatment of a proliferative disorder such as cancer (*e.g.*, a cancer of the central nervous system such as a glioma or brain tumor).

Accordingly, in one aspect, the invention features, a method of treating a subject having a disorder characterized by unwanted cell proliferation, *e.g.*, cancer. The method comprises: administering to the subject a therapeutically effective amount of an inhibitor, of a neoactivity of an enzyme (the neoactive enzyme), encoded by a selected or mutant allele of a gene, to thereby treat the subject.

In an embodiment the subject has a cancer characterized by the selected or mutant allele.

In an embodiment the enzyme is in a metabolic pathway.

In one embodiment, the metabolic pathway is selected from a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, nucleotide biosynthetic pathways, or the fatty acid biosynthetic pathway.

In an embodiment the inhibitor reduces the amount of neoactive enzyme or mRNA. In an embodiment the inhibitor interacts directly with, e.g., binds to, the neoactive enzyme or mRNA. In an embodiment, the inhibitor is a small molecule. In an embodiment the inhibitor is a nucleic acid-based inhibitor, such as a double stranded RNA (dsRNA) or antisense RNA that targets neoactive enzyme mRNA.

In an embodiment the method comprises selecting a subject having a cancer characterized by the selected or mutant allele.

In an embodiment the method comprises selecting a subject having a cancer on the basis of the cancer being characterized by the selected or mutant allele.

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g.*, by DNA sequencing, immuno analysis, or assay for enzymatic activity), or receiving such information about the subject, that the cancer is characterized by the selected or mutant allele.

In an embodiment the method comprises administering a second anti-cancer agent or therapy to the subject, *e.g.*, surgical removal or administration of a chemotherapeutic.

In another aspect, the invention features, a method of treating a subject having cancer, wherein the cancer is characterized by a preselected allele, or mutant allele, of an IDH, *e.g.*, IDH1. The method comprises administering to the subject a therapeutically effective amount of an inhibitor of an IDH gene, *e.g.*, IDH1, e.g., a mutant IDH1 gene, for example having a neoactivity.

In an embodiment the cancer is: a cancer of the CNS, *e.g.*, a glioma; prostate cancer, *e.g.*, prostate adenocarcinoma; or a hematological cancer, e.g., B-acute lymphoplastic leukemia (B-ALL).

In an embodiment the inhibitor inhibits one or more of the following: the ability of IDH1 to catalyze the conversion of isocitrate to alpha ketoglutarate; a first degree neoactivity, *e.g.*, the conversion of alpha-ketoglutarate to 2-hydroxyglutarate, e.g., R-2-

hydroxyglutarate; or a second degree neoactivity. In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the inhibitor reduces the amount of an IDH, *e.g.*, IDH1, protein or mRNA. In an embodiment the inhibitor interacts directly with, e.g., binds to, the IDH1 proteinor IDH1 mRNA. In an embodiment, the inhibitor is a small molecule. In an embodiment the inhibitor is a nucleic acid-based inhibitor, such as a double stranded RNA (dsRNA) or antisense RNA that targets an IDH, *e.g.*, IDH1.

In an embodiment the cancer, *e.g.*, a glioma, is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **Fig. 21**). In an embodiment the allele encodes an IDH1 having His at residue 132. In an embodiment the allele encodes an IDH1 having Ser at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A or a G395A mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having cancer, *e.g.*, a glioma, wherein the cancer is characterized by having an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having a cancer, *e.g.*, a glioma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-

hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate, *e.g.*, from MRI measurement, spinal cord fluid analysis, or by analysis of surgical material, *e.g.*, by mass-spectroscopy), or receiving such information about the subject, that the cancer is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132. In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH1, *e.g.*, an IDH1 having an A at nucleotide position 394 or 395, *e.g.*, a mutant allele carrying a C394A mutation or a G395A mutation according to the IDH1 sequence of SEQ ID NO:5.

In an embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH1, *e.g.*, an IDH1 having a C at nucleotide position 394 or a G at nucleotide position 395, according to the IDH1 sequence of SEQ ID NO:5.

In an embodiment, the dsRNA targets an IDH1 having an A at nucleotide position 394 or 395 (*e.g.*, a mutant) and an IDH1 having a C at nucleotide position 394 or a G at nucleotide position 395 (*e.g.*, a wildtype), *e.g.*, by targeting a region of the IDH1 mRNA that is identical between the wildtype and mutant transcripts. In yet another embodiment, the dsRNA targets a particular mutant or polymorphism (such as a single nucleotide polymorphism (SNP)), but not a wildtype allele. In this case, the nucleic acid based inhibitor, *e.g.*, a dsRNA, targets the region of the IDH1 containing the mutation.

As used herein, a "SNP" is a DNA sequence variation occurring when a single nucleotide (A, T, C, or G) in the genome (or other shared sequence) differs between members of a species (or between paired chromosomes in an individual).

In one embodiment, the nucleic acid based inhibitor decreases or inhibits expression of an IDH1 having His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment, the nucleic acid based inhibitor decreases or inhibits expression of an IDH1 enzyme having His at residue

132, *e.g.*, an Arg132His mutation, or an Arg at residue 132, *e.g.*, an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In some embodiments, the nucleic acid based inhibitor, *e.g.*, a dsRNA preferentially or specifically inhibits the product of a mutant IDH1 as compared to the product of a wildtype IDH1. For example, in one embodiment, a dsRNA targets a region of an IDH1 mRNA that carries the mutation (*e.g.*, a C394A or a G395A mutation according to SEQ ID NO:5).

In an embodiment, the nucleic acid based inhibitor is delivered to the brain, *e.g.*, directly to the brain, *e.g.*, by intrathecal or intraventricular delivery. In an embodiment, the nucleic acid-based inhibitor is delivered by infusion using, *e.g.*, a catheter, and optionally, a pump.

In one embodiment, the nucleic acid-based inhibitor is a dsRNA including a sense strand and an antisense strand having a primary sequence presented in **Tables 7, 8, 9, 10, 11, 12, 13** and **14**. In another embodiment, the nucleic acid based inhibitor is an antisense oligonucleotide that includes all or a part of an antisense primary sequence presented in **Tables 7, 8, 9, 10, 11, 12, 13** and **14** or which targets the same or substantially the same region as does a dsRNA from **Tables 7, 8, 9, 10, 11, 12, 13** and **14**.

In some embodiments, the methods described herein can result in reduced side effects relative to other known methods of treating cancer.

In an embodiment the method comprises administering a second anti-cancer agent or therapy to the subject, *e.g.*, surgical removal, administration of an alkylating agent, administration of temoader, or administration of Gleevec.

In an embodiment the cancer, *e.g.*, prostate cancer, *e.g.*, prostate adenocarcinoma, is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His or Cys at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having cancer, e.g., prostate cancer, *e.g.*, prostate adenocarcinoma, wherein the cancer is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having cancer, e.g., prostate cancer, *e.g.*, prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the cancer is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH1, *e.g.*, an IDH1 having an T (or a nucleotide other than C) at nucleotide position 394 or an A (or a nucleotide other than G) at nucleotide position 395, *e.g.*, a mutant allele carrying a C394T mutation or a G395A mutation according to the IDH1 sequence of SEQ ID NO:5.

In an embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH1, *e.g.*, an IDH1 having a C at nucleotide position 394 or a G at nucleotide position 395 (SEQ ID NO:5).

In an embodiment, the dsRNA targets an IDH1 having an C at nucleotide position 394 or a G at 395 (*e.g.*, a wildtype) and an IDH1 having a T at nucleotide position 394 or an A at nucleotide position 395 (*e.g.*, a mutant) according to the sequence of SEQ ID NO:5, *e.g.*, by targeting a region of the IDH1 mRNA that is identical between the wildtype and mutant transcripts. In yet another embodiment, the dsRNA targets a particular mutant or polymorphism (such as a single nucleotide polymorphism (SNP)), but not a wildtype allele. In this case, the nucleic acid based inhibitor, *e.g.*, a dsRNA, targets the region of the IDH1 containing the mutation.

In one embodiment, the nucleic acid based inhibitor decreases or inhibits expression of an IDH1 having His, Ser, Cys, Gly, Val, Pro or Leu, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment, the nucleic acid based inhibitor decreases or inhibits expression of an IDH1 enzyme having His at residue 132, *e.g.*, an Arg132His mutation, or an Cys at residue 132, *e.g.*, an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In some embodiments, the nucleic acid based inhibitor, *e.g.*, a dsRNA preferentially or specifically inhibits the product of a mutant IDH1 as compared to the product of a wildtype IDH1. For example, in one embodiment, a dsRNA targets a region of an IDH1 mRNA that carries the mutation (*e.g.*, a C394T or a G395A mutation according to the sequence of SEQ ID NO:5).

In an embodiment the subject has B-acute lymphoplastic leukemia (B-ALL), and the cancer is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele is an IDH1 having other than an Arg at residue 132 (SEQ ID NO:8). *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having Cys at residue 132. In an embodiment the allele is an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation according to the sequence of SEQ ID NO:5.

In an embodiment the method comprises selecting a subject having cancer, e.g., a B-acute lymphoplastic leukemia (B-ALL) characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 according to the sequence of SEQ ID NO:8.

In an embodiment the method comprises selecting a subject having cancer, *e.g.*, a B-acute lymphoplastic leukemia(B-ALL), on the basis of cancer being characterized by having an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment, the nucleic acid-based inhibitor is a dsRNA that targets IDH1, *e.g.*, an IDH1 having an T at nucleotide position 394, *e.g.*, a mutant allele carrying a C394T mutation according to the IDH1 sequence of SEQ ID NO:5.

In an embodiment, the dsRNA targets an IDH1 having a C at nucleotide position 394 (*e.g.*, a wildtype) and an IDH1 having a T at nucleotide position 394 (*e.g.*, a mutant) according to the sequence of SEQ ID NO:5, *e.g.*, by targeting a region of the IDH1 mRNA that is identical between the wildtype and mutant transcripts. In yet another embodiment, the dsRNA targets a particular mutant or polymorphism (such as a single nucleotide polymorphism (SNP)), but not a wildtype allele. In this case, the nucleic acid based inhibitor, *e.g.*, a dsRNA, targets the region of the IDH1 containing the mutation.

In one embodiment, the nucleic acid based inhibitor decreases or inhibits expression of an IDH1 having His, Ser, Cys, Gly, Val, Pro or Leu, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment, the

nucleic acid based inhibitor decreases or inhibits expression of an IDH1 enzyme having Cys at residue 132, *e.g.*, an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In some embodiments, the nucleic acid based inhibitor, *e.g.*, a dsRNA preferentially or specifically inhibits the product of a mutant IDH1 as compared to the product of a wildtype IDH1. For example, in one embodiment, a dsRNA targets a region of an IDH1 mRNA that carries the mutation (*e.g.*, a C394T mutation (SEQ ID NO:5)).

In another aspect, the invention provides, a method of treating a subject having cancer, *e.g.*, a glioma or brain tumor characterized by a mutant IDH1 or IDH2 gene which encodes a mutant enzyme having a neoactivity. The method comprises:

administering to the subject a therapeutically effective amount of an inhibitor of the neoactivity of the mutant enzyme, wherein the neoactivity is the ability to convert alpha ketoglutarate to 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, to thereby treat the subject. In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

The glioma or brain tumor can be evaluated to determine if it is characterized by a mutant IDH1 or IDH2 using a technique described herein, or other methods known to one skilled in the art.

In an embodiment the subject has cancer, *e.g.*, glioma, and the cancer is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His at residue 132. In an embodiment the allele encodes an IDH1 having Ser at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In one embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide

position 395. In an embodiment the allele is a C394A or a G395A mutation (SEQ ID NO:5).

In one embodiment the method comprises selecting a subject with cancer, *e.g.*, a glioma, wherein the cancer is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having cancer, *e.g.*, a subject having glioma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or a sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the method comprises administering a second anti-cancer agent or therapy to the subject, *e.g.*, surgical removal, administration of an alkylating agent, administration of temoader, or administration of Gleevec.

In an embodiment the subject has cancer, *e.g.*, prostate cancer, *e.g.*, prostate adenocarcinoma, and the cancer is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment, the allele encodes an IDH1 having His or Cys at residue 132. In another embodiment, the allele is an Arg132His mutation, or an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In one embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation (SEQ ID NO:5).

In one embodiment the method comprises selecting a subject having cancer, *e.g.*, prostate cancer, *e.g.*, prostate adenocarcinoma, characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having cancer, *e.g.*, prostate cancer, *e.g.*, prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In one embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject has cancer, *e.g.*, a B-acute lymphoplastic leukemia (B-ALL), and the cancer is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment, the allele encodes an IDH1 having Cys at residue 132. In one embodiment the allele is an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In one embodiment, the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In one embodiment the allele is a C394T mutation.

In one embodiment, the method comprises selecting a subject having a B-acute lymphoplastic leukemia (B-ALL) characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment, the method comprises selecting a subject having a B-acute lymphoplastic leukemia (B-ALL) characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In one embodiment, the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or a sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In another aspect, the invention features, a method of treating a subject, *e.g.*, a subject having a disorder characterized by unwanted cell proliferation, *e.g.*, cancer. The method comprises:

administering to the subject a therapeutically effective amount of a substance, or a compound that induces activity of the substance, or a compound that increases the level of said substance *in vivo*, wherein the subject has a mutation giving rise to a neoactivity and the endogenous level of the substance in cancer cells of the subject is reduced as compared to a cell not having the mutation, *e.g.*, wherein the enzyme is in a metabolic pathway, to thereby treat the subject. In some embodiments, the substance is alpha-ketoglutarate or a prodrug thereof.

In an embodiment the subject has a cancer, e.g., glioma, characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. In another embodiment the subject has prostate cancer characterized by a mutation, or preselected allele, of an IDH

gene, *e.g.*, IDH1. For example, in one embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. For example, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment, the allele encodes an IDH1 having His at residue 132. In another embodiment, the allele encodes an IDH1 having Ser at residue 132. In one embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In one embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A or a G395A mutation (SEQ ID NO:5).

In one embodiment, the method comprises selecting a subject having cancer, for example, glioma or prostate cancer, and the cancer is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In one embodiment the method comprises selecting a subject having cancer, for example, glioma or prostate cancer, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In one embodiment the method comprises administering a second anti-cancer agent or therapy to the subject, *e.g.*, surgical removal, administration of an alkylating agent, administration of temoader, or administration of Gleevec.

In another embodiment the method comprises administering a second anti-cancer agent or therapy to the subject, e.g., surgical removal, radiation therapy, chemotherapy, hormonal therapy, or High-Intensity Focused Ultrasound.

In one embodiment the method further comprises administering an IDH, *e.g.*, IDH1, inhibitor, *e.g.*, an inhibitor described herein.

In another aspect, the invention features, a method of treating a subject having cancer, for example, a glioma or brain tumor characterized by a mutant IDH1 or IDH2 gene which encodes a mutant enzyme having a neoactivity. The method comprises:

administering to the subject a therapeutically effective amount of a alpha ketoglutarate, a prodrug thereof, a compound that increases the level of alpha ketoglutarate in a subject, a compound that activates HIF-1α hydroxylase, or a compound that inhibits HIF-1α, to thereby treat the subject.

In an embodiment the subject has a cancer, such as glioma, and the cancer is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment the allele encodes an IDH1 having His at residue 132. In another embodiment the allele encodes an IDH1 having Ser at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A or a G395A mutation (SEQ ID NO:5).

In one embodiment the method comprises selecting a subject having a cancer such as glioma, wherein the cancer is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having a cancer such as glioma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the method comprises administering a second anti-cancer agent or therapy to the subject, *e.g.*, surgical removal, administration of an alkylating agent, administration of temoader, or administration of Gleevec.

In an embodiment the subject has a cancer such as prostate cancer, *e.g.*, prostate adenocarcinoma, characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His or Cys at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject having a cancer such as prostate cancer, *e.g.*, prostate adenocarcinoma, characterized by an IDH, *e.g.*, IDH1,

allele described herein, *e.g.*, an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having a cancer such as prostate cancer, *e.g.*, prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject has B-acute lymphoplastic leukemia (B-ALL) characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. For example, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having Cys at residue 132. In an embodiment the allele is an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject having B-acute lymphoplastic leukemia (B-ALL), characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having B-acute lymphoplastic leukemia (B-ALL), on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the method further comprises administering an IDH, *e.g.*, IDH1, inhibitor, *e.g.*, an inhibitor described herein.

In one aspect, the invention features a method of selecting a subject for treatment with a compound that is an inhibitor of neoactivity (*e.g.*, a compound that is an inhibitor of neoactivity of an enzyme described herein such as an IDH1 or IDH2 mutant). The method comprises evaluating the subject for the presence of an IDH1 or IDH2 mutant enzyme or gene encoding such enzyme, and selecting a patient for treatment with a compound that inhibits the neoactivity of that enzyme on the basis of that evaluation. For example, if it is determined that the subject has a cancer characterized by a mutant IDH1 or IDH2, selecting the subject for treatment with an inhibitor (*e.g.*, a small molecule or a nucleic acid-based inhibitor) of the neoactivity of that mutant (*e.g.*, conversion of alpha-ketoglutarate to 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject has glioma characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. For example, in one embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132 of SEQ ID NO:8.

For example, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In one embodiment the allele encodes an IDH1 having His at residue 132. In an embodiment the allele encodes an IDH1 having Ser at residue 132. In one embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A or a G395A mutation (SEQ ID NO:5).

In one embodiment the method comprises selecting a subject having glioma, characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having glioma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject has prostate cancer, *e.g.*, prostate adenocarcinoma, characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the subject has an IDH1 allele which encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132,

according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His or Cys at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject has B-acute lymphoplastic leukemia (B-ALL) characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1

having Cys at residue 132. In an embodiment the allele is an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject having B-acute lymphoplastic leukemia (B-ALL) characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject having B-acute lymphoplastic leukemia (B-ALL) , on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In one embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In another aspect, the invention features a method of evaluating a candidate compound, *e.g.*, for use as an anti-proliferative or anti-cancer agent. The method comprises:

optionally supplying the candidate compound;

contacting the candidate compound with an enzyme having the activity of a mutant enzyme having a neoactivity; and

evaluating the ability of the candidate compound to inhibit the activity,

thereby evaluating the compound.

In another aspect, the invention features, a method of evaluating a candidate compound, *e.g.*, for use as an anti-proliferative or anti-cancer agent. The method comprises:

a) optionally supplying the candidate compound (*e.g.*, a candidate nucleic acid-based inhibitor);

b) contacting the candidate compound with an, enzyme, *e.g.*, IDH(1 or 2) enzyme (*e.g.*, a wild-type/non-mutant enzyme or a mutant enzyme);

c) evaluating the ability of the candidate compound to inhibit the forward of non-mutant or wild type enzyme activity, *e.g.*, in the case of IDH, the conversion of isocitrate to α-ketoglutarate (or an intermediate thereof , including the reduced hydroxyl intermediate); and

d) optionally, evaluating the ability of the candidate compound (*e.g.*, a candidate compound which meets a predetermined level of inhibition in the evaluation in c) to modulate a neoactivity of the enzyme (*e.g.*, by the reduction in the formation of a reaction product), or in general an activity of the enzyme other than that evaluated in c, thereby evaluating the compound.

In one embodiment, the method, *e.g.*, step d, comprises evaluating the ability of the candidate compound to modulate, *e.g.*, inhibit, the neoactivty of a mutant IDH (*e.g.*, IDH1 or IDH2). For example, the method could include the evaluation of the presence and/or amount of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate and/or alpha-ketoglutarate. In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen. The evaluation can be *in vitro*, *in vivo* and/or *ex vivo*, for example in a reaction mixture, a biological sample, and/or a subject.

In another aspect, the invention features a method of evaluating, e.g., diagnosing, staging, or providing a prognosis for, a subject, *e.g.*, a subject not having, or not diagnosed as having, 2-hydroxyglutaric aciduria. The method comprises evaluating one or more of the presence, distribution, or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, *e.g.*, in the tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid), or the presence of a selected allele or mutation disclosed herein, in the subject.

In an embodiment the presence, distribution, or level, is indicative of cancer, e.g., indicative of a primary or metastatic lesion.

In an embodiment the presence, distribution, or level, is indicative of a prognosis or outcome, e.g., it is indicative of a less aggressive from of cancer. *E.g.*, in the case of glioma, presence can indicate a less aggressive form of the cancer.

In an embodiment the method includes (*e.g.*, responsive to the evaluation, e.g., the evaluation of the presence, distribution or level) one or more of: transmitting an indication of prognosis or outcome to a party, e.g., the subject; memorializing a value for the evaluation, *e.g.*, the evaluation of the presence, distribution, or level; memorializing an indication of a prognosis or outcome; selecting a course of treatment, *e.g.*, a course of treatment described herein.

In embodiments the method includes evaluating, *e.g.*, by imaging, a preselected organ or portion of the body, *e.g.*, the brain. Suitable imaging methods include *e.g.*, MRI imaging.

In an embodiment the presence, distribution, or level, is compared with a reference level, *e.g.*, the level in a tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid), or reference subject not having abnormal presence, level, or distribution, *e.g.*, a tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) or reference subject not having a mutation in IDH1 having a neoactivity described herein.

In an embodiment, a subject is evaluated by magnetic resonance.

In an embodiment, the evaluation comprises evaluating the presence or elevated amount of a peak corresponding to 2-hydroxyglutarate, *e.g.*, R-2-hydroxyglutarate, as determined by magnetic resonance. For example, a subject can be evaluated for the presence and/or strength of a signal at about 2.5 ppm to determine the presence and/or amount of 2-hydroxyglutarate, *e.g.*, R-2-hydroxyglutarate in the subject.

The presence, level, or distribution can be correlated to and/or predictive of a neoactivity described herein for the mutant enzyme IDH. Similarly, the presence, level, or distribution can be predictive of a response to treatment and thereby used as a noninvasive biomarker for clinical response. *E.g.*, elevated levels can be predictive on better outcome in glioma patients (*e.g.*, longer life expectancy).

In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, *e.g.*, a carcinogen.

In an embodiment the subject is selected from the following: a subject selected at least in part, because said patient has a cancer characterized by a selected IDH, *e.g.*, IDH1 allele, *e.g.*, an IDH mutation; a subject having a selected IDH allele, *e.g.*, a selected IDH1 allele; a subject other than a subject having or diagnosed as having 2-hydroxyglutaric aciduria; or a subject having cancer *e.g.*, glioma, such as an oligodendroglioma, an astrocytoma (*e.g.*, grade II or III), or a secondary glioblastoma; a subject having prostate cancer, *e.g.*, prostate adenocarcinoma; a subject having B-acute lymphoplastic leukemia (B-ALL).

In an embodiment the method comprises determining the sequence of the IDH, *e.g.*, IDH1, gene, *e.g.*, the sequence at position 394 or 395, or determining the identity of amino acid residue 132 (SEQ ID NO:8).

In an embodiment the method further comprises selecting the patient for treatment, *e.g.*, with a compound described herein (*e.g.*, a small molecule or a nucleic acid-based inhibitor). In an embodiment the method further comprises administering a compound described herein (*e.g.*, on the basis of the evaluation of the presence, distribution or level of 2-hydrogyglutarate).

In some embodiments, the method comprises evaluating the possibility of a mutation other than a mutation in (L or D) 2-hydroxyglutarate dehydrogenase, wherein if the 2-hydroxyglutarate, *e.g.*, R-2-hydroxyglutarate level is high is indicative of another mutation. In an embodiment, 2-hydroxyglurarate (*e.g.*, R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (*e.g.*, R-2-hydroxyglutarate) is a toxin, *e.g.*, a carcinogen.

In an embodiment the subject, *e.g.*, a subject having glioma, has a cancer characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an

IDH1 having His at residue 132. In an embodiment the allele encodes an IDH1 having Ser at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having glioma or prostate cancer, wherein the cancer is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having cancer (e.g., glioma or prostate cancer), on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing or immuno analysis or evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, has a cancer characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment

the allele encodes an IDH1 having His or Cys at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, wherein the cancer is cahracterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, on the basis of the cnacer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), has a cnacer characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment

the allele encodes an IDH1 having Cys at residue 132. In an embodiment the allele is an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), on the basis of the patient having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In one aspect, the invention features a method of staging a treatment or prognosing a subject, *e.g.*, a subject having glioma. The method comprises:

evaluating the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, *e.g.*, by imaging, *e.g.*, of the brain, *e.g.*, MRI imaging; and

based on the result of the evaluation, assigning a stage, or selecting a treatment for said patient, *e.g.*, administering a therapeutic agent at a greater (or lesser) dosage, frequency etc., than prior to the evaluation. In some embodiments, the method comprises

increasing the dosage of a drug already being given, or adding another drug to the treatment regime.

In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having glioma, has a cancer, e.g., glioma, characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the subject has an IDH1 allele which encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His at residue 132. In an embodiment the allele encodes an IDH1 having Ser at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having glioma, having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having glioma, on the basis of the patient having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing or immuno analysis or evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate), or receiving such information about the

subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, has a cancer having a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His or Cys at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, wherein the cancer is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, on the basis of the cancer being characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution

or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), has a cancer characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele which encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having Cys at residue 132. In an embodiment the allele is an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), having an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), on the basis of the patient having an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an

IDH1 allele having Cys at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In another aspect, the invention features a method of evaluating or adjusting a treatment regimen. The method comprises treating a subject with a treatment regimen, evaluating the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate in the subject, *e.g.*, by imaging, *e.g.*, of the brain, *e.g.*, MRI imaging; and, based on the result of the evaluation, assigning a stage, or selecting a treatment for said patient, *e.g.*, administering a therapeutic agent at a greater (or lesser) dosage, frequency etc, than prior to the evaluation. In some embodiments, the method comprises increasing the dosage of a drug already being given, or adding another drug to the treatment regime.

In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate)is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having glioma, has a cancer, e.g., glioma, characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His at residue 132. In one embodiment the allele encodes an IDH1 having Ser at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394A or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having glioma, wherein the cancer, e.g., glioma, is characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having glioma, on the basis of the patient having a cancer, e.g., glioma, characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing or immuno analysis or evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, has a cancer. e.g., prostate cancer, wherein the cancer is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His or Cys at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, on the basis of the patient having a cancer

characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), has a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the subject has an IDH1 allele which encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having Cys at residue 132. In an embodiment the allele is an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), on the basis of the patient having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In one aspect, the invention features a method of selecting a patient for treatment with a compound described herein. The method comprises evaluating the subject for the presence and/or amount of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate in the subject, for example, by forming an image such as an MRI image, selecting the patient for treatment with a compound described herein (*e.g.*, a small molecule or a nucleic acid based inhibitor) based on the presence or amount of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate in the subject, and optionally treating the subject with a compound described herein. In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having glioma, has a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the subject has an IDH1 allele which encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any other residue described in Yan *et al.*, N. Eng. J. Med. 360:765-73, at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His at residue 132. In an embodiment the allele encodes an IDH1 having Ser at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Ser mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has an A (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide

position 395. In an embodiment the allele is a C394A or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having glioma, having an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having glioma, on the basis of the patient having an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing or immuno analysis or evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, and the cancer is characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having His or Cys at residue 132. In an embodiment the allele is an Arg132His mutation, or an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394, or an A (or any other nucleotide other than G) at nucleotide position 395. In an embodiment the allele is a C394T or a G395A mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having prostate cancer, *e.g.*, prostate adenocarcinoma, on the basis of the patient having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having His or Ser at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In an embodiment the subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), has a cancer characterized by a mutation, or preselected allele, of an IDH gene, *e.g.*, IDH1. *E.g.*, in an embodiment, the IDH1 allele encodes an IDH1 having other than an Arg at residue 132. *E.g.*, the allele can have His, Ser, Cys, Gly, Val, Pro or Leu, or any residue described in Kang *et al*, 2009, Int. J. Cancer, 125: 353-355 at residue 132, according to the sequence of SEQ ID NO:8 (see also **FIG. 21**). In an embodiment the allele encodes an IDH1 having Cys at residue 132. In an embodiment the allele is an Arg132Cys mutation, according to the sequence of SEQ ID NO:8 (see **FIGs. 2** and **21**).

In an embodiment the IDH1 allele has a T (or any other nucleotide other than C) at nucleotide position 394. In an embodiment the allele is a C394T mutation (SEQ ID NO:5).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), having a cancer characterized by an IDH, *e.g.*,

IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises selecting a subject, *e.g.*, a subject having B-acute lymphoplastic leukemia (B-ALL), on the basis of the patient having a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8).

In an embodiment the method comprises confirming or determining, *e.g.*, by direct examination or evaluation of the subject, or sample *e.g.*, tissue or bodily fluid (*e.g.*, blood (*e.g.*, blood plasma), urine, lymph, or Cerebrospinal fluid) therefrom, (*e.g*, by DNA sequencing, immuno analysis, evaluation of the presence, distribution or level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, or evaluation of the presence, distribution or level of alpha ketoglutarate), or receiving such information about the subject, that the subject has a cancer characterized by an IDH, *e.g.*, IDH1, allele described herein, *e.g.*, an IDH1 allele having Cys at residue 132 (SEQ ID NO:8). In an embodiment, 2-hydroxyglurarate (e.g., R-2-hydroxyglutarate) is a metabolite. In another embodiment, 2-hydroxyglutarate (e.g., R-2-hydroxyglutarate) is a toxin, e.g., a carcinogen.

In another aspect the invention features a pharmaceutical composition of an inhibitor (*e.g.*, a small molecule or a nucleic acid-based inhibitor) described herein.

As used herein, a cancer characterized by a mutation or allele, means a cancer having a substantial number of cells carrying that mutation or allele. In an embodiment at least 10, 25, 50, 75, 90, 95 or 99% of the cells of a cancer, or a representative, average or typical sample of cancer cells, e.g., from a tumor or from blood, carry at least one copy of the mutation or allele. In an embodiment the mutation or allele is present as a heterozygote at the indicated frequencies.

The details of one or more embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts DNA sequence verification of pET41a-IDH1 and alignment against published IDH1 CDS. The sequence of IDH1 (CDS) corresponds to SEQ ID NO:5. The

sequence of pET41a-IDH1 corresponds to SEQ ID NO:6, and the "consensus" sequence corresponds to SEQ ID NO:7.

FIG. 2 depicts DNA sequence verification of R132S and R132H mutants according to the SEQ ID NO:8. The amino acid sequence of IDH1 (SEQ ID NO:8) is provided in FIG. 21.

FIG. 3 depicts separation of wild type IDH1 protein on Ni-Sepharose column.

FIG. 4 depicts protein analysis of wild type IDH1 on SDS gel pre and post Ni column fractionation. T: total protein; I: insoluble fractions; S: soluble fraction; L: sample for loading on Ni-column. The numbers in the figure indicates the fraction numbers. Fractions #17 ~ #27 were collected for further purification.

FIG. 5A depicts separation of wild type IDH1 protein through SEC column S-200.

FIG. 5B depicts protein analysis of wild type IDH1 on SDS gel pre and post S-200 column fractionation. M: molecular weight marker; Ni: nickel column fraction prior to S-200; S200: fraction from SEC column.

FIG. 6 depicts separation of mutant R132S protein on Ni-Sepharose column.

FIG. 7 depicts protein analysis of mutant R132S on SDS gel pre and post Ni column fractionation. M: protein marker (KDa): 116, 66.2, 45, 35, 25, 18.4, 14.4; T: total cell protein; So: soluble fraction; In: insoluble fraction; Ft: flow through. #3-#7 indicate the corresponding eluted fraction numbers.

FIG. 8A depicts separation of mutant R132S protein through SEC column S-200.

FIG. 8B depicts protein analysis of mutant R132S on SDS gel post S-200 column fractionation. M: molecular weight marker; R132S: fraction from SEC column.

FIG. 9 depicts separation of mutant R132H protein on Ni-Sepharose column.

FIG. 10 depicts protein analysis of mutant R132H on SDS gel pre and post Ni column fractionation. M: protein marker (KDa): 116, 66.2, 45, 35, 25, 18.4, 14.4; T: total cell protein; So: soluble fraction; In: insoluble fraction; Ft: flow through; #5-#10 indicate the corresponding eluted fraction numbers; Ni: sample from Ni-Sepharose column, pool #5-#10 together.

FIG. 11A depicts separation of mutant R132H protein through SEC column S-200.

FIG. 11B depicts protein analysis of mutant R132H on SDS gel post S-200 column fractionation. M: molecular weight marker; R132H: fraction from SEC column.

FIG. 12A depicts Michaelis-Menten plot of IDH1 wild-type in the oxidative decarboxylation of ioscitrate to α-ketoglutarate.

FIG. 12B depicts Michaelis-Menten plot of R132H mutant enzyme in the oxidative decarboxylation of ioscitrate to α-ketoglutarate.

FIG. 12C depicts Michaelis-Menten plot of R132S mutant enzyme in the oxidative decarboxylation of ioscitrate to α-ketoglutarate.

FIG. 13A depicts α-KG inhibition of IDH1 wild-type.

FIG. 13B depicts α-KG inhibition of R132H mutant enzyme.

FIG. 13C depicts α-KG inhibition of R132S mutant enzyme.

FIG. 14 depicts IDH1 wt, R132H, and R132S in the conversion α-ketoglutarate to 2-hydroxyglutarate.

FIG. 15A depicts Substrate-Concentration velocity plot for R132H mutant enzyme.

FIG. 15B depicts Substrate-Concentration velocity plot for R132S mutant enzyme.

FIG. 16 depicts IDH1 wt, R132H, and R132S in the conversion α-ketoglutarate to 2-hydroxyglutarate with NADH.

FIG. 17A depicts oxalomalate inhibition to IDH1 wt.

FIG. 17B depicts oxalomalate inhibition to R132H.

FIG. 17C depicts oxalomalate inhibition to R132S.

FIG. 18A depicts LC-MS/MS analysis of the control reaction.

FIG. 18B depicts LC-MS/MS analysis of the reaction containing enzyme.

FIG. 18C depicts LC-MS/MS analysis of the spiked control reaction.

FIG. 19 depicts LC-MS/MS analysis of alpha-hydroxyglutarate.

FIG. 20 depicts LC-MS/MS analysis showing that R132H consumes α-KG to produce 2-hydroxyglutaric acid.

FIG. 21 depicts the amino acid sequence of IDH1 (SEQ ID NO:8) as described in GenBank Accession No. NP_005887.2 (GI No. 28178825) (record dated May 10, 2009).

FIG. 22 depicts the nucleotide sequence of IDH1 (SEQ ID NO:9) as described in GenBank Accession No. NM_005896.2 (GI No. 28178824).

FIG. 23 depicts the progress of forward reactions (isocitrate to α-KG) for the mutant enzyme R132H and R132S.

FIG. 24A depicts LC-MS/MS analysis of derivitized 2-HG racemic mixture.

FIG. 24B depicts LC-MS/MS analysis of derivitized R-2HG standard.

FIG. 24C depicts LC-MS/MS analysis of a coinjection of derivitized 2-HG racemate and R-2-HG standard.

FIG. 24D depicts LC-MS/MS analysis of the deriviatized neoactivity reaction product.

FIG. 24E depicts LC-MS/MS analysis of a coinjection of the neoactivty enzyme reaction product and the R-2-HG standard.

FIG. 24F depicts LC-MS/MS analysis of a coinjection of the neoactivity enzyme reaction product and the 2-HG racemic mixture.

FIG. 25 depicts the inhibitory effect of 2-HG derived from the reduction of α-KG by ICDH1 R132H on the wild-type ICDH1 catalytic oxidative decarboxylation of isocitrate to α-KG.

FIG. 26A depicts levels of 2-HG in CRL-2610 cell lines expressing wildtype or IDH-1 R132H mutant protein.

FIG. 26B depicts levels of 2-HG in HTB-14 cell lines expressing wildtype or IDH-1 R132H mutant protein.

FIG. 27 depicts human IDH1 genomic DNA: intron/2nd exon sequence.

FIG. 28 depicts levels of 2-HG in human brain tumor tissue expressing the IDH-1 R132H mutant protein compared to human brain tumor tissue expressing wild type IDH1.

FIG. 29A depicts the structural analysis of R132H mutant IDH1. On left is shown an overlay structure of R132H mutant IDH1 and WT IDH1 in the 'closed' conformation. On the right is shown an overlay structure of WT IDH1 in the 'open' conformation with mutant IDH1 for comparison.

FIG. 29B depicts the close-up structural comparison of the R132H IDH1 (left) and wild-type (WT) IDH1 (right) active-site containing both αKG and NADPH. In addition to changes at residue 132, the position of the catalytic residues Tyr 139 and Lys 212 are different and αKG is oriented differently relative to NADPH for catalytic hydride transfer in the WT versus R132H mutant enzymes.

FIG. 30A depicts expression of IDH1 in 293T cells transfected with the indicated amount of wild-type (WT) or R132H mutant IDH1. The expression level of IDH1 was confirmed by Western blot as shown. Re-probing of the same blot with antibody against IDH2 is also shown as a control.

FIG. 30B depicts an assay for testing the ability of 293T protein lysates, from cells transfected with the listed amounts of wild-type or R132H mutant IDH1 cDNA, or empty vector to generate NADPH from $NADP^+$ in the presence of 0.1 mM isocitrate

FIG. 30C depicts an assay for testing the ability of the same cell lysates described in FIG. 30B to consume NADPH in the presence of 0.6 mM αKG.

FIG. 31A depicts the enzymatic properties of IDH1 R132H mutants when ecombinant human wild-type (WT) and R132H mutant (R132H) IDH1 enzymes were assessed for oxidative decarboxylation of isocitrate to αKG with $NADP^+$ as cofactor. Different concentrations of enzyme were used to generate the curves.

FIG. 31B depicts the enxymatic properties of IDH R132 mutants when WT and R132H mutant IDH1 enzymes were assessed for reduction of αKG with NADPH as cofactor. Different concentrations of enzyme were used to generate the curves.

FIG. 31C depicts kinetic parameters of oxidative and reductive reactions as measured for WT and R132H IDH1 enzymes are shown. K_m and k_{cat} values for the reductive activity of the WT enzyme were unable to be determined as no measurable enzyme activity was detectable at any substrate concentration.

FIG. 32A depicts the LC-MS/MS analysis identifying 2HG as the reductive reaction product of recombinant human R132H mutant IDH1.

FIG. 32B depicts the diacetyl-L-tartaric anhydride derivatization and LC-MS/MS analysis of the chirality of 2HG produced by R132H mutant IDH1. Normalized LC-MS/MS signal for the reductive reaction (rxn) product alone, an R(-)-2HG standard alone, and the two together (Rxn + R(-)-2HG) are shown as is the signal for a racemic mixture of R(-) and S(+) forms (2HG Racemate) alone or with the reaction products (Rxn + Racemate).

FIG. 33A depicts SDS-PAGE and Western blot analyses of C-terminal affinity-purification tagged IDH1 R132S protein used for crystallization.

FIG. 33B depicts the chromatogram of FPLC analysis of the IDH1 R132S protein sample.

FIG. 34 depicts crystals obtained from a protein solution contained 5 mM NADP, 5 mM isocitrate, 10 mM Ca2+. Precipitant solution contained 100 mM MES (pH 6.0) and 20% PEG 6000 using a hanging drop method of crystallization.

FIG. 35 depicts crystal obtained from a protein solution contained 5 mM NADP, 5 mM α-ketoglutarate, 10 mM Ca2+. Precipitant contained 100 mM MES (pH 6.5) and 12% PEG 20000.

DETAILED DESCRIPTION

The inventors have discovered that certain mutated forms of an enzyme (*e.g.*, IDH1 or IDH2) have a gain of function, referred to herein as a neoactivity, which can be targeted in the treatment of a proliferative disorder such as cancer. For example, in the case of a metabolic pathway enzyme, a gain of function or neoactivity can serve as a target for treatment of cancer. Described herein are methods and compositions for the treatment of a proliferative disorder such as cancer. The methods include, *e.g.*, treating a subject having a glioma or brain tumor characterized by a preselected IDH1 allele, *e.g.*, an allele having A at position 394 (*e.g.*, a C394A mutant) or an A at position 395 (*e.g.*, a G395A mutant) according to the sequence of SEQ ID NO:5, that encodes a an IDH1 having His at position 132 (*e.g.*, an Arg132His mutation) or Ser at position 132 (*e.g.*, an Arg132Ser mutant) and having a neoactivity disclosed herein, by administering to the subject a therapeutically effective amount of an inhibitor of IDH1, e.g., a small molecule or nucleic acid. The nucleic acid based inhibitor is, for example, a dsRNA, *e.g.*, a dsRNA that comprises the primary sequences of the sense strand and antisense strands of **Tables 7-14**. The dsRNA is composed of two separate strands, or a single strand folded to form a hairpin structure (*e.g.*, a short hairpin RNA (shRNA)). In some embodiments, the nucleic acid based inhibitor is an antisense nucleic acid, such as an antisense having a sequence that overlaps, or includes, an antisense sequence provided in **Tables 7-14**.

Neoactivity of an enzyme

Neoactivity, as used herein, means an activity that arises as a result of a mutation, *e.g.*, a point mutation, *e.g.*, a substitution, *e.g.*, in the active site of an enzyme. In an embodiment the neoactivity is substantially absent from wild type or non-mutant enzyme. This is sometimes referred to herein as a first degree neoactivity. An example of a first degree neoactivity is a "gain of function" wherein the mutant enzyme gains a new

catalytic activity. In an embodiment the neoactivity is present in wild type or non-mutant enzyme but at a level which is less than 10, 5, 1, 0.1, 0.01 or 0.001 % of what is seen in the mutant enzyme. This is sometimes referred to herein as a second degree neoactivity. An example of a second degree neoactivity is a "gain of function" wherein the mutant enzyme has an increase, for example, a 5 fold increase in the rate of a catalytic activity possessed by the enzyme when lacking the mutation.

In some embodiments, a non-mutant form the enzyme, *e.g.*, a wild type form, converts substance A (*e.g.*, isocitrate) to substance B (*e.g.*, α-ketoglutarate), and the neoactivity converts substance B (*e.g.*, α-ketoglutarate) to substance C (*e.g.*, 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate). In some embodiments, the enzyme is in a metabolic pathway, *e.g.*, a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.*, IDH1 or IDH2.

In some embodiments, a non-mutant form the enzyme, *e.g.*, a wild type form, converts substance A to substance B, and the neoactivity converts substance B to substance A. In some embodiments, the enzyme is in a metabolic pathway, *e.g.*, a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway.

Isocitrate Dehydrogenases

Isocitrate dehydrogenases (IDHs) catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate (*i.e.*, α-ketoglutarate). These enzymes belong to two distinct subclasses, one of which utilizes NAD(+) as the electron acceptor and the other NADP(+). Five isocitrate dehydrogenases have been reported: three NAD(+)-dependent isocitrate dehydrogenases, which localize to the mitochondrial matrix, and two NADP(+)-dependent isocitrate dehydrogenases, one of which is mitochondrial and the other predominantly cytosolic. Each NADP(+)-dependent isozyme is a homodimer.

IDH1 (isocitrate dehydrogenase 1 (NADP+), cytosolic) is also known as IDH; IDP; IDCD; IDPC or PICD. The protein encoded by this gene is the NADP(+)-dependent isocitrate dehydrogenase found in the cytoplasm and peroxisomes. It contains the PTS-1

peroxisomal targeting signal sequence. The presence of this enzyme in peroxisomes suggests roles in the regeneration of NADPH for intraperoxisomal reductions, such as the conversion of 2, 4-dienoyl-CoAs to 3-enoyl-CoAs, as well as in peroxisomal reactions that consume 2-oxoglutarate, namely the alpha-hydroxylation of phytanic acid. The cytoplasmic enzyme serves a significant role in cytoplasmic NADPH production.

The human IDH1 gene encodes a protein of 414 amino acids. The nucleotide and amino acid sequences for human IDH1 can be found as GenBank entries NM_005896.2 and NP_005887.2 respectively. The nucleotide and amino acid sequences for IDH1 are also described in, *e.g.*, Nekrutenko *et al.*, Mol. Biol. Evol. 15:1674-1684(1998); Geisbrecht *et al.*, J. Biol. Chem. 274:30527-30533(1999); Wiemann *et al.*, Genome Res. 11:422-435(2001); The MGC Project Team, Genome Res. 14:2121-2127(2004); Lubec *et al.*, Submitted (DEC-2008) to UniProtKB; Kullmann *et al.*, Submitted (JUN-1996) to the EMBL/GenBank/DDBJ databases; and Sjoeblom *et al.*, Science 314:268-274(2006).

IDH2 (isocitrate dehydrogenase 2 (NADP+), mitochondrial) is also known as IDH; IDP; IDHM; IDPM; ICD-M; or mNADP-IDH. The protein encoded by this gene is the NADP(+)-dependent isocitrate dehydrogenase found in the mitochondria. It plays a role in intermediary metabolism and energy production. This protein may tightly associate or interact with the pyruvate dehydrogenase complex. Human IDH2 gene encodes a protein of 452 amino acids. The nucleotide and amino acid sequences for IDH2 can be found as GenBank entries NM_002168.2 and NP_002159.2 respectively. The nucleotide and amino acid sequence for human IDH2 are also described in, *e.g.*, Huh *et al.*, Submitted (NOV-1992) to the EMBL/GenBank/DDBJ databases; and The MGC Project Team, Genome Res. 14:2121-2127(2004).

In some embodiments, non-mutant, *e.g.*, wild type, IDH1 catalyzes the oxidative decarboxylation of ioscitrate to α-ketoglutarate thereby reducing NAD^+ ($NADP^+$) to NADP (NADPH), *e.g.*, in the forward reaction:

Isocitrate + NAD^+ ($NADP^+$) → α-KG + CO_2 + NADH (NADPH) + H^+

In some embodiments, the neoactivity of a mutant IDH1 can have the ability to convert α-ketoglutarate to 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate:

α-KG + NADH (NADPH) + H^+ → 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate + NAD^+ ($NADP^+$).

In some embodiments, the neoactivity can be the reduction of pyruvate or malate to the corresponding α-hydroxyl compounds.

In some embodiments, the neoactivity of a mutant IDH1 can arise from a mutant IDH1 having a His, Ser, Cys or Lys, or any other mutations described in Yan H *et al.*, N Engl J Med. 2009 Feb 19;360(8):765-73, at residue 132. In some embodiments, the neoactivity of a mutant IDH2 can arise from a mutant IDH2 having a Gly, Met or Lys, or any other mutations described in Yan H *et al.*, N Engl J Med. 2009 Feb 19;360(8):765-73, at residue 172. Exemplary mutations include the following: R132H, R132C, R132S, R132G, R132L, and R132V.

In some embodiments, a mutant IDH1 and/or IDH2 (*e.g.*, a mutant IDH1 and/or IDH2 having a neoactivity described herein) could lead to a reduced level of α-ketoglurarate. In addition to oxygen, the proline hydroxylases that suppress HIF-1 require α-ketoglurarate as a substrate. Thus, the invention includes a method of treating a subject, *e.g.*, a subject having a disorder characterized by unwanted cell proliferation, *e.g.*, cancer, by administering a therapeutically effective amount of α-ketoglurarate (*e.g.*, high levels as compared to normal metabolic conditions), an α-ketoglurarate prodrug, or a compound that increases the level of α-ketoglurarate to the subject. The cancer can be one described herein, *e.g.*, brain tumor, *e.g.*, glioma or brain tumor, *e.g.*, glioblastoma, *e.g.*, cancer having a mutant gene encoding a mutant gene product having a neoactivity, a mutant gene product having a neoactivity, or a neoactivity, by *e.g.*, inhibiting HIF-1, *e.g.*, HIF-1α.

In some embodiments, the mutant IDH1 and/or IDH2 (*e.g.*, a mutant IDH1 and/or IDH2 having a neoactivity described herein) could lead to an increased level of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate in a subject. The accumulation of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate in a subject, *e.g.*, in the brain of a subject, can be harmful. For example, in some embodiments, elevated levels of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate can lead to and/or be predictive of cancer in a subject such as a cancer of the central nervous system, *e.g.*, brain tumor, *e.g.*, glioma, *e.g.*, glioblastoma multiforme (GBM). Accordingly, in some embodiments, a method described herein includes administering to a subject an inhibitor of the neoactivity

Methods of treating a proliferative disorder

Described herein are methods of treating cancer, *e.g.*, a glioma, *e.g.*, by inhibiting a neoactivity of a mutant enzyme, *e.g.*, an enzyme in a metabolic pathway, *e.g.*, a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.*, IDH1 or IDH2. The cancer can be characterized by the presence of a neoactivity, such as a gain of function in one or more mutant enzymes (*e.g.*, an enzyme in the metabolic pathway, *e.g.*, a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway *e.g.*, IDH1 or IDH2). In some embodiments, the gain of function is the conversion of α-ketoglurarate to 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate.

Described herein are methods of treating cancer by administering a substance, wherein that substance has a reduced level *in vivo* as a result of a neoactivity described herein (*e.g.*, α-ketoglurarate). Also described herein are methods of treating cancer by administering a prodrug of the substance (*e.g.*, α-ketoglurarate), a compound that increases the level of the substance in a subject, or a compound that has similar activity of that substance in a subject (*e.g.*, activation of HIF-1α hydroxylase in a subject or inhibition of HIF-1α).

Compounds for the treatment of cancer

A candidate compound can be evaluated for modulation (*e.g.*, inhibition) of neoactivity, for example, using an assay described herein. A candidate compound can also be evaluated for modulation (*e.g.*, inhibition) of wild type or non-mutant activity. For example, the formation of a product or by product of any activity (*e.g.*, enzymatic activity) can be assayed, thus evaluating a candidate compound. In some embodiments, the activity (*e.g.*, wild type/non-mutant or neoactivity) can be evaluated by measuring one or more readouts from an enzymatic assay. For example, the change in nature and/or amount of substrate and/or product can be measured, *e.g.*, using methods such as fluorescent or radiolabeled substrates. Exemplary substrates and/or products include α-ketoglutarate, CO2, NADP, NADPH, NAD, NADH, and 2-hydroxyglutarate, e.g., R-2-

hydroxyglutarate. In some embodiments, the rate of reaction of the enzyme can also be evaluated as can the nature and/or amount of a product of the enzymatic reaction. In addition to the measurement of potential enzymatic activities, activity (*e.g.*, wild type/non-mutant or neoactivity) can be detected by the quenching of protein fluorescence upon binding of a potential substrate, cofactor, or enzymatic activity modulator to the enzyme.

In one embodiment, assay progress can be monitored by changes in the OD340 or fluorescence of the NAD or NADP cofactor. In another embodiment, the reaction progress can be coupled to a secondary enzyme assay system in continuous mode or endpoint mode for increasing the dynamic range of the assay. For example, an endpoint assay can be performed by adding to the reaction an excess of diaphorase and rezasarin. Diaphorase consumes the remaining NADPH or NADH while producing resorufin from rezasarin. Resorufin is a highly fluorescent product which can be measured by fluorescence at Ex544 Em590. This not only terminates the reaction but also generates an easily detectable signal with greater quantum yield that the fluorescence of the cofactor.

A continuous assay might be implemented through coupling a product of the primary reaction to a secondary enzyme reaction that yields detectable results of greater dynamic range or more convenient detection mode. For example, inclusion in the reaction mix of aldehyde dehydrogenase (ALDH), which is an NADP+ dependent enzyme, and 6-methoxy-2-napthaldehye, a chromogenic substrate for ALDH, will result in the production of the fluorescent product 6-methoxy-2-napthoate (Ex310 Em 360) at a rate dependent on the production of NADP+ by isocitrate dehydrogenase. The inclusion of a coupling enzyme such as aldehyde dehydrogenase has the additional benefit of allowing screening of neoactivity irrespective of whether NADP+ or NAD+ is produced, since this enzyme is capable of utilizing both. Additionally, since the NADPH or NADH cofactor required for the "reverse" assay is regenerated, a coupled enzyme system which cycles the cofactor back to the IDH enzyme has the further advantage of permitting continuous assays to be conducted at cofactor concentrations much below Km for the purpose of enhancing the detection of competitive inhibitors of cofactor binding.

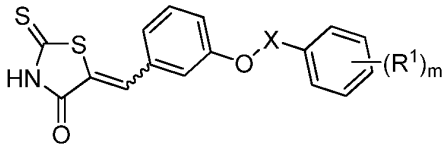
In yet a third embodiment of an activity (*e.g.*, wild type/non-mutant or neoactivity) screen, one or a number of IDH substrates, cofactors, or products can be isotopically

labeled with radioactive or "heavy" elements at defined atoms for the purpose of following specific substrates or atoms of substrates through the chemical reaction. For example, the alpha carbon of a-KG, isocitrate, or 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate may be ^{14}C or ^{13}C. Amount, rate, identity and structure of products formed can be analyzed by means known to those of skill in the art, for example mass spectroscopy or radiometric HPLC.

Compounds that inhibit a neoactivity, *e.g.*, a neoactivity described herein, can include, *e.g.*, small molecule, nucleic acid, protein and antibody.

Exemplary small molecules include, *e.g*, small molecules that bind to enzymes and decrease their activity, *e.g.*, a neoactivity described herein. The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalyzing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically. These inhibitors can modify key amino acid residues needed for enzymatic activity. In contrast, reversible inhibitors bind non-covalently and different types of inhibition are produced depending on whether these inhibitors bind the enzyme, the enzyme-substrate complex, or both. In some embodiments, the small molecule is oxalomalate, oxalofumarate, or oxalosuccinate. In some embodiments, the small molecule is a compound of formula (X), or a compound as listed in **Table 17**.

The compound of formula (X) is provided below:



Formula (X)

wherein X is alkylene (e.g., methylene) or C(O);

R^1 is halo (e.g., fluoro), C_1-C_6 alkyl, hydroxyl, C_1-C_6 alkoxy, cyano, nitro, amino, alkylamino, dialkylamino, amido, -C(O)OH, or C(O)OC_1-C_6alkyl; and

m is 0, 1, 2, or 3.

In some embodiments, the small molecule is a selected inhibitor for the neoactivity (e.g., relative to the wild type activity).

Nucleic acids can be used to inhibit a neoactivity, *e.g.*, a neoactivity described herein, *e.g.*, by decreasing the expression of the enzyme. Exemplary nucleic acids include,

e.g., siRNA, shRNA, aptamer and ribozyme. Art-known methods can be used to select inhibitory molecules, *e.g.*, siRNA molecules, for a particular gene sequence.

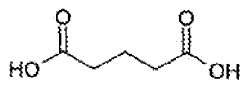
Proteins can also be used to inhibit a neoactivity, *e.g.*, a neoactivity described herein, by directly or indirectly binding to the enzyme and/or substrate, or competing binding to the enzyme and/or substrate. Exemplary proteins include, *e.g.*, soluble receptors, peptides and antibodies. Exemplary antibodies include, *e.g.*, whole antibody or a fragment thereof.

Exemplary candidate compounds, which can be tested for a neoactivity described herein (*e.g.*, a neoactivity associated with mutant IDH1), are described in the following references, each of which are incorporated herein by reference: Bioorganic & Medicinal Chemistry (2008), 16(7), 3580-3586; Free Radical Biology & Medicine (2007), 42(1), 44-51; KR 2005036293 A; Applied and Environmental Microbiology (2005), 71(9), 5465-5475; KR 2002095553 A; U.S. Pat. Appl. US 2004067234 A1; PCT Int. Appl. (2002), WO 2002033063 A1; Journal of Organic Chemistry (1996), 61(14), 4527-4531; Biochimica et Biophysica Acta, Enzymology (1976), 452(2), 302-9; Journal of Biological Chemistry (1975), 250(16), 6351-4; Bollettino - Societa Italiana di Biologia Sperimentale (1972), 48(23), 1031-5; Journal of Biological Chemistry (1969), 244(20), 5709-12.

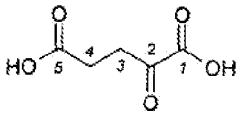
In some embodiments certain compounds (referred to herein as "α-ketoglutarate compounds" or "α-ketogluartates" or "α-ketogluartate esters"), for example, that activate HIFα hydoxylase, can be administered to a subject to treat a cancer described herein (*e.g.*, by inhibiting a neoactivity or by increasing the amount of a substance having a reduced level *in vivo* as a result of the neoactivity). These compounds may be described as α-ketoglutarates bearing (*e.g.*, conjugated to, coupled to) a hydrophobic moiety. Exemplary compounds are described, for example, in WO2006016143, the contents of which is incorporated by reference in its entirety.

For example, these compounds may be described as α-ketoglutarate esters (*i.e.*, esters of α-ketogluartic acid) having a hydrophobic moiety that is, or is part of, an ester group (*i.e.*, -C(=O)OR) formed from one of the acid groups of α-ketogluartic acid.

For reference, the related parent compounds, glutaric acid and α-ketoglutaric acid are shown below.

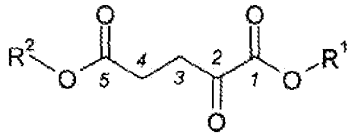


glutaric acid
(pentanedioic acid)



α-ketoglutaric acid
(2-oxo-pentanedioic acid)

Thus, in one embodiment, the compounds have the following formula:



wherein each of R1 and R2 is independently selected from: (i) H; and (ii) a hydrophobic moiety; with the proviso that R1 and R2 are not both H; and pharmaceutically acceptable salts, solvates, amides, esters, ethers, N-oxides, chemically protected forms, and prodrugs thereof.

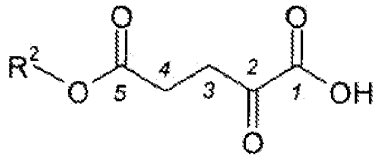
The Groups R1 and R2

In one embodiment, neither R1 nor R2 is H (*i.e.*, diesters).

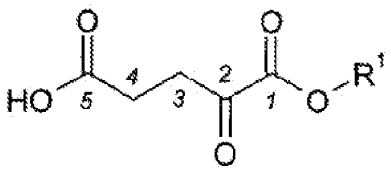
In one embodiment, neither R1 nor R2 is H; and R1 and R2 are different. In one embodiment, neither R1 nor R2 is H; and R1 and R2 are identical.

In one embodiment, exactly one of R1 and R2 is H (*i.e.*, monoesters).

In one embodiment, R1 is H (and R2 is not H):



In one embodiment, R2 is H (and R1 is not H):



The Hydrophobic Moiety/Moieties

As used herein, the term "hydrophobic moiety" includes, but is not limited to, chemical moieties with non-polar atoms or groups that have a tendency to interact with each other rather than with water or other polar atoms or groups. Hydrophobic moieties

are substantially insoluble or only poorly soluble in water. Optionally, the hydrophobic moiety may be selected according to their fusogenic properties or their interactions with components of cellular membranes, such as lectins and lipid head groups. For example, the hydrophobic moiety may comprise a polymer (*e.g.*, a linear or branched polymer); an alkyl, alkenyl, and/or alkynyl group, which may be, for example, linear, branched or cyclic (*e.g.*, C1-C30 alkyl, C2-C30 alkenyl, C2-C30 alkynyl, C3-C30 cycloalkyl, C3-C30 cylcoalkenyl, C3-C30 cycloalkynyl); an aromatic group (*e.g.*, C6- C20 carboaryl, Cs-C20 heteroaryl); or a combination thereof.

Optionally, the hydrophobic moiety may comprise one or more of: a heteroatom, a heterocyclic group, a peptide, a peptoid, a natural product, a synthetic compound, a steroid, and a steroid derivative (*e.g.*, hydrophobic moieties which comprise a steroidal nucleus, *e.g.*, a cholesterol ring system).

It is intended that the hydrophobic moiety be selected so that the α-ketoglutarate compound is capable of performing its intended function, *e.g.*, to cross through lipid membranes into the cytosol/mitochondria.

Examples of hydrophobic moieties include, but are not limited to, those derived from: lipids, fatty acids, phospholipids, sphingolipids, acylglycerols, waxes, sterols, steroids (*e.g.*, cholesterol), terpenes, prostaglandins, thromboxanes, leukotrienes, isoprenoids, retenoids, biotin, and hydrophobic amino acids (*e.g.*, tryptophan, phenylalanine, isoleucine, leucine, valine, methionine, alanine, proline, and tyrosine).

In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently selected from: C1-C30 alkyl; C2-C30 alkenyl; C2-C30 alkynyl; C3-C30 cycloalkyl; C3-C30 cycloalkenyl; C3-C30 cycloalkynyl; C6-C20 carboaryl; C5-C20 heteroaryl; C6-C20 carboaryl-CrC7 alkyl; C5-C20 heteroaryl-d-Cr alkyl; and is unsubstituted or substituted. In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently selected from: C1-C30 alkyl; C2-C30 alkenyl; C2-C30 alkynyl; and is unsubstituted or substituted.

In one embodiment, the bottom of the range (for alkyl, alkenyl, alkynl) is C4. In one embodiment, the bottom of the range is C6. In one embodiment, the bottom of the range is C8. In one embodiment, the bottom of the range is C10. In one embodiment, the bottom of the range is C12.

In one embodiment, the top of the range (for alkyl, alkenyl, alkynl) is C30. In one embodiment, the top of the range is C24. In one embodiment, the top of the range is C22. In one embodiment, the top of the range is C20. In one embodiment, the top of the range is C18. In one embodiment, the top of the range is C16.

In one embodiment, the range (for alkyl, alkenyl, alkynl) is C4-C20. In one embodiment, the range is C6-C18. In one embodiment, the range is C8-C16. In one embodiment, the range is C10-C24. In one embodiment, the range is C12-C22. In one embodiment, the range is C14-C20. In one embodiment, the range is C16-C18.

In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently C1-C30 alkyl and is unsubstituted or substituted.

In one embodiment, the bottom of the range (for alkyl) is C4. In one embodiment, the bottom of the range is C6. In one embodiment, the bottom of the range is C8. In one embodiment, the bottom of the range is C10. In one embodiment, the bottom of the range is C12.

In one embodiment, the top of the range (for alkyl) is C30. In one embodiment, the top of the range is C24. In one embodiment, the top of the range is C22. In one embodiment, the top of the range is C20. In one embodiment, the top of the range is C18. In one embodiment, the top of the range is C16.

In one embodiment, the range (for alkyl) is C4-C20. In one embodiment, the range is C6-C18. In one embodiment, the range is C8-C16. In one embodiment, the range is C10-C24. In one embodiment, the range is C12-C22. In one embodiment, the range is C14-C20. In one embodiment, the range is C16-C18.

In one embodiment, the alkyl group is a linear or branched alkyl group and is unsubstituted or substituted, for example, in one embodiment, the hydrophobic moiety is linear or branched C1-C30 alkyl and is unsubstituted or substituted.

In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently -(CH2)nCH3, wherein n is independently an integer from 0 to 29.

In one embodiment, the bottom of the range for n is 3. In one embodiment, the bottom of the range for n is 5. In one embodiment, the bottom of the range for n is 7. In one embodiment, the bottom of the range for n is 9. In one embodiment, the bottom of the range for n is 11.

In one embodiment, the top of the range for n is 29. In one embodiment, the top of the range for n is 23. In one embodiment, the top of the range for n is 21. In one embodiment, the top of the range for n is 19. In one embodiment, the top of the range for n is 17. In one embodiment, the top of the range for n is 15. In one embodiment, n is independently an integer from 3 to 19. In one embodiment, n is independently an integer from 5 to 17. In one embodiment, n is independently an integer from 7 to 15.

In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently selected from: C6-C20 carboaryl; C5-C20 heteroaryl; C6-C20 carboaryl-C1-C7 alkyl; C5-C20 heteroaryl-C1-C7 alkyl; and is unsubstituted or substituted.

In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently selected from: C6-C12 carboaryl; C5-C12 heteroaryl; C6-C12 carboaryl-C1-C7 alkyl; C5-C12 heteroaryl-C1-C7 alkyl; and is unsubstituted or substituted.

In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently selected from: C6-C10 carboaryl; C5-C10 heteroaryl; C6-C10 carboaryl-C1-C7 alkyl; C5-C10 heteroaryl-C1-C7 alkyl; and is unsubstituted or substituted.

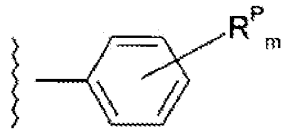
In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently selected from: C6-C20 carboaryl; C6-C20 carboaryl-C1-C7 alkyl; and is unsubstituted or substituted. In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently selected from: C6-C12 carboaryl; C6-C12 carboaryl-C1-C7 alkyl; and is unsubstituted or substituted.

In regard to the phrase "unsubstituted or substituted", any substituents, if present, may be, in one embodiment, as defined below for Rp.

For example, in one embodiment, each carboaryl and heteroaryl group, if present, is unsubstituted or substituted with one or more (*e.g.*, 1 , 2, 3, 4, etc.) substituents independently selected from: halo; cyano; nitro; hydroxy; C1-C7 alkyoxy; C1-C7 alkyl; C1-C7 haloalkyl; and C8-C30 alkyl.

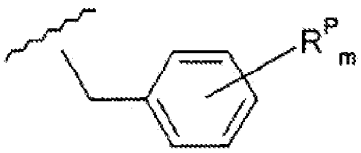
In one embodiment, the above C8-C30 alkyl groups are C10-C24 alkyl. In one embodiment, the above C8-C30 alkyl groups are C12-C22 alkyl. In one embodiment, the above C8-C30 alkyl groups are C14-C20 alkyl. In one embodiment, the above C8-C30 alkyl groups are C16-C18 alkyl.

In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently an optionally substituted phenyl group of formula:



wherein m is independently 0, 1 , 2, 3, 4, or 5, and each Rp, if present, is independently a substituent.

In one embodiment, the hydrophobic moiety, or each hydrophobic moiety, is independently an optionally substituted benzyl group of formula:



wherein m is independently 0, 1 , 2, 3, 4, or 5, and each Rp, if present, is independently a substituent. In one embodiment, m is 0, 1 , 2, or 3. In one embodiment, m is 0, 1, or 2. In one embodiment, m is 0 or 1.

In one embodiment, the substituents, Rp, are independently selected from the following:

(1) carboxylic acid; (2) ester; (3) amido or thioamido; (4) acyl; (5) halo; (6) cyano; (7) nitro; (8) hydroxy; (9) ether; (10) thiol; (11) thioether; (12) acyloxy; (13) carbamate; (14) amino; (15) acylamino or thioacylamino; (16) aminoacylamino or aminothioacylamino; (17) sulfonamino; (18) sulfonyl; (19) sulfonate; (20) sulfonamido; (21) C5-20aryl-C1-7alkyl; (22) C6.20carboaryl and C5.2oheteroaryl; (23) C3-2oheterocyclyl; (24) Ci-7alkyl; C8.30alkyl; C2-7alkenyl; C2-7alkynyl; C3-7cycloalkyl; C3.7cycloalkenyl; C3-7cycloalkynyl.

In one embodiment, the substituents, Rp, are independently selected from the following:

(I) -C(O)OH; (2) -C(=O)OR1, wherein R1 is independently as defined in (21), (22), (23) or (24); (3) -C(=O)NR2R3 or -C(=S)NR2R3, wherein each of R2 and R3 is independently -H; or as defined in (21), (22), (23) or (24); or R2 and R3 taken together with the nitrogen atom to which they are attached form a ring having from 3 to 7 ring

atoms; (4) -C(=O)R4, wherein R4 is independently -H, or as defined in (21), (22), (23) or (24); (5) -F, -Cl, -Br, -I; (6) -CN; (7) -NO2; (8) -OH; (9) -OR5, wherein R5 is independently as defined in (21), (22), (23) or (24); (1O) -SH; (11) -SR6, wherein R6 is independently as defined in (21), (22), (23) or (24); (12) -OC(=O)R7, wherein R7 is independently as defined in (21), (22), (23) or (24); (13) -OC(O)NR8R9, wherein each of R8 and R9 is independently -H; or as defined in (21), (22), (23) or (24); or R8 and R9 taken together with the nitrogen atom to which they are attached form a ring having from 3 to 7 ring atoms; (14) -NR10R11, wherein each of R10 and R11 is independently -H; or as defined in (21), (22), (23) or (24); or R10 and R11 taken together with the nitrogen atom to which they are attached form a ring having from 3 to 7 ring atoms; (15) -NR12C(=O)R13 or -NR12C(=S)R13, wherein R12 is independently -H; or as defined in (21), (22), (23) or (24); and R13 is independently -H, or as defined in (21), (22), (23) or (24); (16) -NR14C(=O)NR15R16 or -NR14C(=S)NR15R16, wherein R14 is independently -H; or as defined in (21), (22), (23) or (24); and each of R15 and R16 is independently -H; or as defined in (21), (22), (23) or (24); or R15 and R16 taken together with the nitrogen atom to which they are attached form a ring having from 3 to 7 ring atoms; (17) -NR17SO2R18, wherein R17 is independently -H; or as defined in (21), (22), (23) or (24); and R18 is independently -H, or as defined in (21), (22), (23) or (24); (18) -SO2R19, wherein R19 is independently as defined in (21), (22), (23) or (24); (19) -OSO2R20 and wherein R20 is independently as defined in (21), (22), (23) or (24); (20) -SO2NR21R22, wherein each of R21 and R22 is independently -H; or as defined in (21), (22), (23) or (24); or R21 and R22 taken together with the nitrogen atom to which they are attached form a ring having from 3 to 7 ring atoms; (21) C5-2oaryl-Ci-7alkyl, for example, wherein C5.20aryl is as defined in (22); unsubstituted or substituted, *e.g.*, with one or more groups as defined in (1) to (24); (22) C6-20carboaryl; C5-20heteroaryl; unsubstituted or substituted, *e.g.*, with one or more groups as defined in (1) to (24); (23) C3.20heterocyclyl; unsubstituted or substituted, *e.g.*, with one or more groups as defined in (1) to (24); (24) C1-7alkyl; C8-3oalkyl; C2-7alkenyl; C2-7alkynyl; C3-7cycloalkyl; C3-7cycloalkenyl; C3.7cycloalkynyl; unsubstituted or substituted, *e.g.*, with one or more groups as defined in (1) to (23), *e.g.*, halo-C1-7alkyl; *e.g.*, amino-C1-7alkyl (*e.g.*, -(CH2)w-amino, w is 1 , 2, 3, or 4); *e.g.*, carboxy-C1-7alkyl (*e.g.*, -(CH2)W-COOH, w is

1 , 2, 3, or 4); *e.g.*, acyl-C1-7alkyl (*e.g.*, -(CH2)W-C(=O)R4, w is 1 , 2, 3, or 4); *e.g.*, hydroxy-C1-7alkyl (*e.g.*, -(CH2)W-OH, w is 1 , 2, 3, or 4); *e.g.*, C1-7alkoxy-C1.7alkyl (*e.g.*, -(CH2)w-O-C1-7alkyl, w is 1 , 2, 3, or 4).

In one embodiment, the substituents, Rp, are independently selected from the following:

(1) -C(=O)OH; (2) -C(=0)0Me, -C(=O)OEt, -C(=O)O(iPr), -C(=O)O(tBu); -C(=O)O(cPr); -C(=O)OCH2CH2OH, -C(=O)OCH2CH2OMe, -C(=O)OCH2CH2OEt; -C(=O)OPh, -C(=O)OCH2Ph; (3) -(C=O)NH2, -(C=O)NMe2, -(C=O)NEt2, -(C=O)N(IPr)2, -(C=O)N(CH2CH2OH)2; -(C=0)-morpholino, -(C=O)NHPh, -(C=O)NHCH2Ph; (4) -C(=0)H, -(C=O)Me, -(C=O)Et, -(C=0)(tBu), -(C=0)-cHex, -(C=O)Ph; -(C=O)CH2Ph; (5) -F, -Cl, -Br, -I; (6) -CN; (7) -NO2; (8) -OH; (9) -OMe, -OEt, -O(iPr), -O(tBu), -OPh, -OCH2Ph; -OCF31 -OCH2CF3; -OCH2CH2OH, -OCH2CH2OMe, -OCH2CH2OEt; -OCH2CH2NH2, -OCH2CH2NMe2, -OCH2CH2N(JPr)2; -OPh-Me, -OPh-OH, -OPh-OMe, -OPh-F, -OPh-CI, -OPh-Br, -OPh-I; (1O) -SH; (11) -SMe, -SEt, -SPh, -SCH2Ph; (12) -OC(=O)Me, -OC(=O)Et, -OC(=O)(iPr), -OC(=O)(tBu); -OC(=O)(cPr); -OC(O)CH2CH2OH, -OC(=O)CH2CH2OMe, -OC(=O)CH2CH2OEt; -OC(=O)Ph, -OC(=O)CH2Ph; (13) -OC(=O)NH2, -0C(=0)NHMe, -0C(=0)NMe2, -OC(=O)NHEt, -OC(=O)NEt2, -OC(=O)NHPh1 -OC(=O)NCH2Ph; (14) -NH2, -NHMe, -NHEt, -NH(iPr), -NMe2, -NEt2, -N(JPr)2, -N(CH2CH2OH)2; -NHPh, -NHCH2Ph; piperidino, piperazino, morpholino; (15) -NH(C=O)Me, -NH(C=O)Et, -NH(C=O)nPr, -NH(C=O)Ph, -NHC(=O)CH2Ph; -NMe(C=O)Me, -NMe(C=O)Et, -NMe(C=O)Ph, -NMeC(=O)CH2Ph; (16) -NH(C=O)NH2, -NH(C=O)NHMe, -NH(C=O)NHEt, -NH(C=O)NPh, -NH(C=O)NHCH2Ph; -NH(C=S)NH2, -NH(C=S)NHMe, -NH(C=S)NHEt, -NH(C=S)NPh, -NH(C=S)NHCH2Ph; (17) -NHSO2Me, -NHSO2Et, -NHSO2Ph1 -NHSO2PhMe, -NHSO2CH2Ph; -NMeSO2Me, -NMeSO2Et1 -NMeSO2Ph1 -NMeSO2PhMe1 -NMeSO2CH2Ph; (18) -SO2Me1 -SO2CF3, -SO2Et, -SO2Ph, -SO2PhMe1 -SO2CH2Ph; (19) -OSO2Me1 -OSO2CF3, -OSO2Et, -OSO2Ph, -OSO2PhMe, -OSO2CH2Ph; (20) -SO2NH2, -SO2NHMe, -SO2NHEt1 -SO2NMe2, -SO2NEt2, -S02-morpholiπo, -SO2NHP h, -SO2NHCH2Ph; (21) -CH2Ph, -CH2Ph-Me, -CH2Ph-OH, -CH2Ph-F, -CH2Ph-CI; (22) -Ph1 -Ph-Me, -Ph-OH, -Ph-OMe, -Ph-NH2, -Ph-F, -Ph-Cl, -Ph-Br, -Ph-I; pyridyl,

pyrazinyl, pyrimidinyl, pyridazinyl; furanyl, thiophenyl, pyrrolyl, imidazolyl, pyrazolyl, oxazolyl, thiazolyl, thiadiazolyl; (23) pyrrolidinyl, imidazolidinyl, pyrazolidinyl, piperidinyl, piperazinyl, azepinyl, tetrahydrofuranyl, tetrahydropyranyl, morpholinyl, azetidinyl; (24) -Me, -Et, -nPr, -iPr, -nBu, -iBu, -sBu, -tBu, -nPe, -nHex; -(CH2)7CH3, -(CHz)9CH3, -(CHz)11CH3, -(CH2)I3CH3, -(CH2)15CH3, -(CH2)17CH3, -(CH2J19CH3; -cPr, -cHex; -CH=CH2, -CH2-CH=CH2; -CF3, -CHF2, -CH2F, -CCI3, -CBr3, -CH2CH2F, -CH2CHF2, and -CH2CF3; -CH2OH, -CH2OMe, -CH2OEt, -CH2NH2, -CH2NMe2; -CH2CH2OH, -CH2CH2OMe, -CH2CH2OEt, -CH2CH2CH2NH2, -CH2CH2NMe2.

In one embodiment, the substituents, Rp, are independently selected from: halo; cyano; nitro; hydroxy; C1-C7 alkyoxy; C1-C7 alkyl; C1-C7 haloalkyl; and C8-C30 alkyl.

In one embodiment, the substituents, Rp, are independently selected from: halo; cyano; nitro; hydroxy; C1-C4 alkyoxy; C1-C4 alkyl; C1-C4 haloalkyl; and C12-C22 alkyl.

In one embodiment, the substituents, Rp, are independently selected from: halo; C1-C4 alkyl; and C1-C4 haloalkyl.

In one embodiment, the substituents, Rp, are independently selected from: fluoro; C1-C4 alkyl; and C1-C4 fluoroalkyl.

In one embodiment, the substituents, Rp, are independently selected from: F, -CH3, -CF3.

As used herein, the term "halo" includes fluoro, chloro, bromo and iodo.

As used herein, the term "alkyl" pertains to monovalent, monodentate, aliphatic (linear or branched) saturated hydrocarbon moieties, for example, methyl, ethyl, n-propyl, i-propyl, etc.

Examples of (unsubstituted) alkyl groups include methyl (C1), ethyl (C2), propyl (C3), butyl (C4), pentyl (C5), hexyl (C6), heptyl (C7), octyl (C8), nonyl (C9), decyl (C10), undecyl (C11), dodecyl (C12), tridecyl (C13), tetradecyl (C14), pentadecyl (C15), and eicodecyl (C20). Examples of (unsubstituted) linear alkyl groups include methyl (C1), ethyl (C2), n-propyl (C3), n-butyl (C4), n-pentyl (amyl) (C5), n-hexyl (C6), and n-heptyl (C7).

Examples of (unsubstituted) branched alkyl groups include iso-propyl (C3), iso-butyl (C4), sec-butyl (C4), tert-butyl (C4), iso-pentyl (C5), and neo-pentyl (C5).

As used herein, the term "alkenyl" pertains to monovalent, monodentate, aliphatic (linear or branched) hydrocarbon moieties having at least one carbon-carbon double bond.

Examples of (unsubstituted) alkenyl groups include ethenyl (vinyl, -CH=CH2), 1-propenyl (-CH=CH-CH3), 2-propenyl (allyl, -CH-CH=CH2), isopropenyl (1-methylvinyl, -C(CH3)=CH2), butenyl (C4), pentenyl (C5), and hexenyl (C6).

As used herein, the term "alkynyl" pertains to monovalent, monodentate, aliphatic (linear or branched) hydrocarbon moieties having at least one carbon-carbon triple bond.

Examples of (unsubstituted) alkynyl groups include ethynyl (ethinyl, -C≡CH) and 2-propynyl (propargyl, -CH2-C=CH).

As used herein, the term "cycloalkyl" pertains to monovalent, monodentate, non-aromatic saturated hydrocarbon moieties having at least one carbon-atom ring (preferably having from 3 to 7 ring carbon atoms).

Examples of cycloalkyl groups include those derived from saturated monocyclic hydrocarbon compounds: cyclopropane (C3), cyclobutane (C4), cyclopentane (C5), cyclohexane (C6), cycloheptane (C7), methylcyclopropane (C4), dimethylcyclopropane (C5), methylcyclobutane (C5), dimethylcyciobutane (C6), methylcyclopentane (C6), dimethylcyclopentane (C7), methylcyclohexane (C7), dimethylcyclohexane (C8), menthane (C10); and saturated polycyclic hydrocarbon compounds: thujane (C10), carane (C10), pinane (C10), bornane (C10), norcarane (C7), norpinane (C7), norbornane (C7), adamantane (C10), decalin (decahydronaphthalene) (C10).

As used herein, the term "cycloalkenyl" pertains to monovalent, monodentate, non-aromatic hydrocarbon moieties having at least one carbon-atom ring (preferably having from 3 to 7 ring carbon atoms) and at least one carbon-carbon double bond. Examples of cycloalkenyl groups include those derived from unsaturated monocyclic hydrocarbon compounds: cyclopropene (C3), cyclobutene (C4), cyclopentene (C5), cyclohexene (C6), methylcyclopropene (C4), dimethylcyclopropene (C5), methylcyclobutene (C5), dimethylcyclobutene (C6), methylcyclopentene (C6), dimethylcyclopentene (C7), methylcyclohexene (C7), dimethylcyclohexene (C8); and

unsaturated polycyclic hydrocarbon compounds: camphene (C10), limonene (Ci0), pinene

As used herein, the term "cycloalkynyl" pertains to monovalent, monodentate, non-aromatic hydrocarbon moieties having at least one carbon-atom ring (preferably having from 3 to 7 ring carbon atoms) and at least one carbon-carbon triple bond.

As used herein, the term "aryl" pertains to monovalent, monodentate, moieties that have an aromatic ring and which has from 3 to 20 ring atoms (unless otherwise specified). Preferably, each ring has from 5 to 7 ring atoms. The ring atoms may be all carbon atoms, as in "carboaryl" groups or the ring atoms may include one or more heteroatoms (*e.g.*, 1 , 2, 3, 4, etc.) (*e.g.*, selected from N1 O, and S), as in "heteroaryl" groups. In this context, the prefixes (*e.g.*, C5-C20, C5-C12, C5-C10, etc.) denote the number of ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms.

Examples of carboaryl groups include those derived from benzene (*i.e.*, phenyl) (C6), naphthalene (Ci0), azulene (C10), anthracene (C14), phenanthrene (C14), naphthacene (C18), and pyrene (C16).

Examples of carboaryl groups which comprise fused rings, at least one of which is an aromatic ring, include groups derived from indane (*e.g.*, 2,3-dihydro-1 H-indene) (C9), indene (C9), isoindene (C9), tetraline (1 ,2,3,4-tetrahydronaphthalene (C10), acenaphthene (C12), fluorene (C13), phenalene (C13), acephenanthrene (C15), and aceanthrene (C16).

Additional examples of carboaryl groups include groups derived from: indene (C9), indane (*e.g.*, 2,3-dihydro-1 H-indene) (C9), tetraline (1 ,2,3,4-tetrahydronaphthalene) (C10), acenaphthene (C12), fluorene (C13), phenalene (C13), acephenanthrene (C15), aceanthrene (C16), cholanthrene (C20).

Examples of monocyclic heteroaryl groups include those derived from: N1: pyrrole (azole) (C5), pyridine (azine) (C6); Ov furan (oxole) (C5); S1: thiophene (thiole) (C5); N1O1: oxazole (C5), isoxazole (C5), isoxazine (C6); N2O1: oxadiazole (furazan) (C5); N3O1: oxatriazole (C5); N1S1: thiazole (C5), isothiazole (C5); N2: imidazole (1 ,3-diazole) (C5), pyrazole (1 ,2-diazole) (C5), pyridazine (1 ,2-diazine) (C6), pyrimidine (1

,3-diazine) (C6) (*e.g.*, cytosine, thymine, uracil), pyrazine (1 ,4-diazine) (C6); N3: triazole (C5), triazine (C6); and, N4: tetrazole (C5).

Examples of polycyclic heteroaryl groups include: Cgheterocyclic groups (with 2 fused rings) derived from benzofuran (O1), isobenzofuran (O1), indole (N1), isoindole (N1), indolizine (N1), indoline (N1), isoindoline (N1), purine (N4) (*e.g.*, adenine, guanine), benzimidazole (N2), indazole (N2), benzoxazole (N1O1), benzisoxazole (N1O1), benzodioxole (O2), benzofurazan (N2O1), benzotriazole (N3), benzothiofuran (S1), benzothiazole (N1S1), benzothiadiazole (N2S); doheterocyclic groups (with 2 fused rings) derived from chromene (O1), isochromene (O1), chroman (O1), isochroman (O1), benzodioxan (O2), quinoline (N1), isoquinoline (N1), quinolizine (N1), benzoxazine (N1O1), benzodiazine (N2), pyridopyridine (N2), quinoxaline (N2), quinazoline (N2), cinnoline (N2), phthalazine (N2), naphthyridine (N2), pteridine (N4); Cnheterocylic groups (with 2 fused rings) derived from benzodiazepine (N2); C13heterocyclic groups (with 3 fused rings) derived from carbazole (N1), dibenzofuran (O1), dibenzothiophene (S1), carboline (N2), perimidine (N2), pyridoindole (N2); and, C14heterocyclic groups (with 3 fused rings) derived from acridine (N1), xanthene (O1), thioxanthene (S1), oxanthrene (O2), phenoxathiin (O1S1), phenazine (N2), phenoxazine (N1O1), phenothiazine (N1S1), thianthrene (S2), phenanthridine (N1), phenanthroline (N2), phenazine (N2).

Heteroaryl groups that have a nitrogen ring atom in the form of an -NH- group may be N-substituted, that is, as -NR-. For example, pyrrole may be N-methyl substituted, to give N-methylpyrrole. Examples of N-substitutents include C1-C7 alkyl; C6-C20 carboaryl; C6- C20 carboaryl-CrC7 alkyl; C1-C7 alkyl-acyl; C6-C20 carboaryl-acyl; C6-C20 carboaryl-CrC7 alkyl-acyl; etc. Heteroaryl groups) which have a nitrogen ring atom in the form of an -N= group may be substituted in the form of an N-oxide, that is, as -N(→O)= (also denoted -N+(→O")=). For example, quinoline may be substituted to give quinoline N-oxide; pyridine to give pyridine N-oxide; benzofurazan to give benzofurazan N-oxide (also known as benzofuroxan).

Molecular Weight

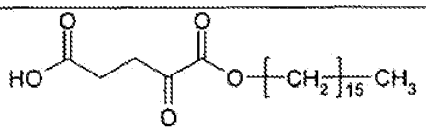
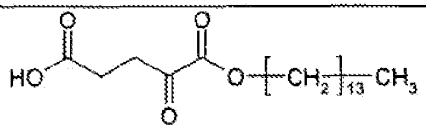
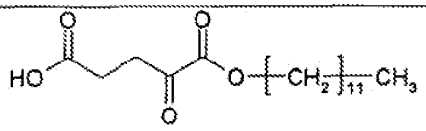
In one embodiment, the compound has a molecular weight of 250 to 1000. In one embodiment, the bottom of range is 275; 300; 325; 350; 375; 400; 425; 450. In one

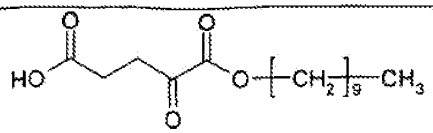
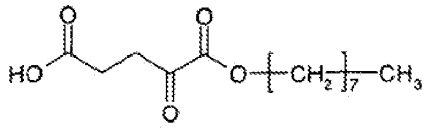
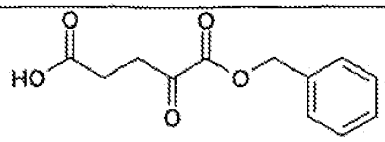
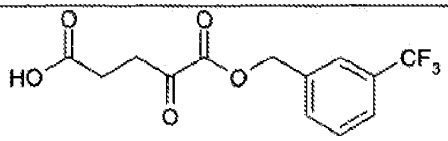
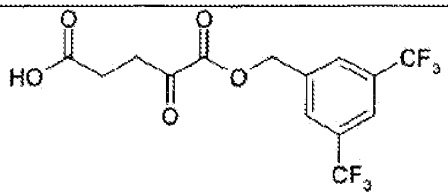
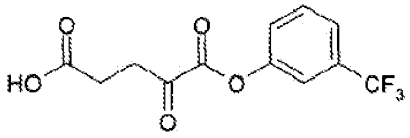
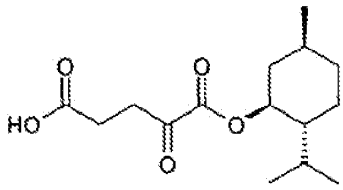
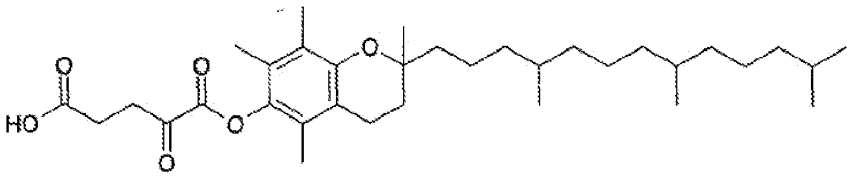
embodiment, the top of range is 900; 800; 700; 600; 500; 400. In one embodiment, the range is 250 to 900. In one embodiment, the range is 250 to 800. In one embodiment, the range is 250 to 700. In one embodiment, the range is 250 to 600. In one embodiment, the range is 250 to 500.

Some Preferred Examples

All plausible and compatible combinations of the embodiments described above are explicitly disclosed herein. Each of these combinations is disclosed herein to the same extent as if each individual combination was specifically and individually recited.

Examples of some preferred compounds include the following:

1	
2	
3	

4	
5	
6	
7	
8	
9	
10	
11	

Additional Compounds - Compounds that Activate HIFα Hydroxylase

In some embodiments, a compound described herein can activate HIFα hydroxylase (for example, HIFα prolyl hydroxylase). Such compounds can be used to treat a cancer described herein (*e.g.*, by inhibition of a neoactivity or by increasing the amount of a substance having a reduced level *in vivo* as a result of the neoactivity). The phrase "a compound that activates HIFα hydroxylase" pertains to a compound that increases the rate or level of HIFα hydroxylase activity whereby the HIFα hydroxylase activity is assessed by the amount of end-product HIF-1α, that is, an increase in the hydroxylation of HIF-1α. Thus, a decrease in HIF-1α protein levels may indicate activation of HIFα hydroxylase.

Suitable methods for determining HIF-1α hydroxylase activation are described herein and/or are well known it the art.

The increase in HIF-1α hydroxylase activity may be a low level increase of about 2 fold to 10 fold; a medium level increase of about 10 fold to 100 fold; or a high level increase of above about 100 fold.

HIF-1α hydroxylases have been described previously and are well known in the art. A preferred HIF-1α hydroxylase is HIFα prolyl hydroxylase. In mammalian cells, three isoforms have been identified, specifically, the prolyl hydroxylase domain (PHD) enzymes (PHD1, PHD2, PHD3), and were shown to hydroxylate HIFα *in vitro*. These enzymes have an absolute requirement for dioxygen as co-substrate. The overall reaction results in insertion of one oxygen atom into the HIFα peptide substrate at the prolyl residue, the other generating succinate from α-ketoglutarate with the release of CO_2.

In one embodiment, the compound acts (or additionally acts) as a substrate or co-factor for a HIF-1α hydroxylase, preferably HIF-1α prolyl hydroxylase.

In one embodiment, the compound that activates HIF-1α hydroxylase is (or additionally is) an α-ketoglutarate compound as described herein.

In one embodiment, the α-ketoglutarate compound described herein is (or additionally is) a compound that activates HIF-1α hydroxylase.

Additional Compounds - Compounds that Increase the Level of α-Ketoαlutarate

In some embodiments, a compound (generally) that increases the level of α-ketoglutarate (*e.g.*, in a cell) can be used in a method described herein. For example, a

compound may increase α-ketoglutarate levels by inhibiting other enzymes such as α-ketoglutarate dehydrogenase and/or branched-chain keto acid dehydrogenase. Blocking these enzymes can have a dual effect of increasing α-ketoglutarate levels and decreasing succinate levels.

Moreover, both enzymes are structural homologs that use lipoic acid as a cofactor. Therefore, a lipoic acid analogue may be another potential inhibitor of these enzymes, and so be a compound that increases the level of α-ketoglutarate

Alternatively, a compound might increase the level of α-ketoglutarate by enhancing glutamate oxaloacetate transaminase (GOT) activity. Glutamate itself will activate GOT activity leading to increased α-ketoglutarate levels.

Moreover, the compound may be selected from upstream metabolites of the TCA cycle including oxaloacetate, citrate, isocitrate, and derivatives thereof.

Additional Compounds - α-Ketoglutarates Generally.

Described herein are α-ketoglutaric acid, α-ketoglutarate salts, and α-ketoglutaric acid derivatives (*e.g.*, esters of α-ketoglutaric acid, generally), and, especially, their use in medicine, for example, in the treatment of a cancer described herein.

In one embodiment, the compound is an α-ketoglutarate bearing (*e.g.*, conjugated to, coupled to) an amino acid moiety (*e.g.*, an α-amino acid moiety) (*e.g.*, an ornithine or arginine moiety).

In one embodiment, the compound is an α-ketoglutarate ester (*i.e.*, an ester of α-ketoglutaric acid) having an amino acid moiety (*e.g.*, an α-amino acid moiety) (*e.g.*, an ornithine or arginine moiety) that is, or is part of, an ester group (*i.e.*, -C(=O)OR) formed from one of the acid groups of α-ketoglutaric acid.

Such compounds are known in the literature (see, *e.g.* Le Boucher *et al.* (1997)) and/or are commercially available and/or may be prepared using conventional synthetic procedures known to the skilled person.

Isomers

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, atropic, stereoisomer, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-

forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

In one embodiment, a compound described herein, e.g., an inhibitor of a neoactivity or 2-HG is an enantiomerically enriched isomer of a stereoisomer described herein. For example, the compound has an enantiomeric excess of at least about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%. Enantiomer, when used herein, refers to either of a pair of chemical compounds whose molecular structures have a mirror-image relationship to each other.

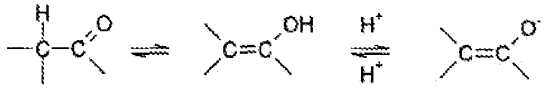
In one embodiment, a preparation of a compound disclosed herein is enriched for an isomer of the compound having a selected stereochemistry, e.g., R or S, corresponding to a selected stereocenter, e.g., the 2-position of 2-hydroxyglutaric acid. For example, the compound has a purity corresponding to a compound having a selected stereochemistry of a selected stereocenter of at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%.

In one embodiment, a composition described herein includes a preparation of a compound disclosed herein that is enriched for a structure or structures having a selected stereochemistry, e.g., R or S, at a selected stereocenter, e.g., the 2-position of 2-hydroxyglutaric acid. Exemplary R/S configurations can be those provided in an example described herein.

An “enriched preparation,” as used herein, is enriched for a selected stereoconfiguration of one, two, three or more selected stereocenters within the subject compound. Exemplary selected stereocenters and exemplary stereoconfigurations thereof can be selected from those provided herein, e.g., in an example described herein. By enriched is meant at least 60%, e.g., of the molecules of compound in the preparation have a selected stereochemistry of a selected stereocenter. In preferred embodiments it is at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%. Enriched refers to the level of a subject molecule(s) and does not connote a process limitation unless specified.

Note that, except as discussed below for tautomeric forms, specifically excluded from the term "isomers," as used herein, are structural (or constitutional) isomers (*i.e.*, isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, -OCH3, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, -CH2OH. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (*e.g.*, C1-7alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hydroxyazo, and nitro/aci-nitro.



Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including 1H, 2H (D), and 3H (T); C may be in any isotopic form, including 12C, 13C, and 14C; O may be in any isotopic form, including 16O and 18O; and the like. Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (*e.g.*, asymmetric synthesis) and separation (*e.g.*, fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods, in a known manner.

Salts

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable

salt. Examples of pharmaceutically acceptable salts are discussed in Berge *et al.*, 1977, "Pharmaceutically Acceptable Salts." J. Pharm. ScL. Vol. 66, pp. 1-19.

For example, if the compound is anionic, or has a functional group which may be anionic (*e.g.*, -COOH may be -COO"), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na+ and K+, alkaline earth cations such as Ca2+ and Mg2+, and other cations such as Al+3. Examples of suitable organic cations include, but are not limited to, ammonium ion (*i.e.*, NH4+) and substituted ammonium ions (*e.g.*, NH3R+, NH2R2+, NHR3+, NR4+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is N(CH3)4+.

If the compound is cationic, or has a functional group that may be cationic (*e.g.*, -NH2 may • be -NH3+), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulfuric, sulfurous, nitric, nitrous, phosphoric, and phosphorous.

Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: 2-acetyoxybenzoic, acetic, ascorbic, aspartic, benzoic, camphorsulfonic, cinnamic, citric, edetic, ethanedisulfonic, ethanesulfonic, fumaric, glucheptonic, gluconic, glutamic, glycolic, hydroxymaleic, hydroxynaphthalene carboxylic, isethionic, lactic, lactobionic, lauric, maleic, malic, methanesulfonic, mucic, oleic, oxalic, palmitic, pamoic, pantothenic, phenylacetic, phenylsulfonic, propionic, pyruvic, salicylic, stearic, succinic, sulfanilic, tartaric, toluenesulfonic, and valeric. Examples of suitable polymeric organic anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

Unless otherwise specified, a reference to a particular compound also includes salt forms thereof.

Solvates

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (*e.g.*, active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

Unless otherwise specified, a reference to a particular compound also includes solvate forms thereof.

Chemically Protected Forms

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form" is used herein in the conventional chemical sense and pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions under specified conditions (*e.g.*, pH, temperature, radiation, solvent, and the like). In practice, well known chemical methods are employed to reversibly render unreactive a functional group, which otherwise would be reactive, under specified conditions. In a chemically protected form, one or more reactive functional groups are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, Protective Groups in Organic Synthesis (T. Green and P. Wuts; 3rd Edition; John Wiley and Sons, 1999). Unless otherwise specified, a reference to a particular compound also includes chemically protected forms thereof.

A wide variety of such "protecting," "blocking," or "masking" methods are widely used and well known in organic synthesis. For example, a compound which has two nonequivalent reactive functional groups, both of which would be reactive under specified conditions, may be derivatized to render one of the functional groups "protected," and therefore unreactive, under the specified conditions; so protected, the compound may be used as a reactant which has effectively only one reactive functional

group. After the desired reaction (involving the other functional group) is complete, the protected group may be "deprotected" to return it to its original functionality.

For example, a hydroxy group may be protected as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH3, -OAc).

For example, an aldehyde or ketone group may be protected as an acetal (R-CH(OR)2) or ketal (R2C(OR)2), respectively, in which the carbonyl group (>C=O) is converted to a diether (>C(OR)2), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

For example, an amine group may be protected, for example, as an amide (-NRCO-R) or a urethane (-NRCO-OR), for example, as: a methyl amide (-NHCO-CH3); a benzyloxy amide (-NHCO-OCH2C6H5, -NH-Cbz); as a t-butoxy amide (-NHCO-OC(CH3)3, -NH-Boc); a 2-biphenyl-2-propoxy amide (-NHCO-OC(CH3)2C6H4C6H5, -NH-Bpoc), as a 9- fluorenylmethoxy amide (-NH-Fmoc), as a 6-nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec); or, in suitable cases (*e.g.*, cyclic amines), as a nitroxide radical (>N-O«).

For example, a carboxylic acid group may be protected as an ester for example, as: an C^alkyl ester (*e.g.*, a methyl ester; a t-butyl ester); a Cvrhaloalkyl ester (*e.g.*, a C1-7trihaloalkyl ester); a triC1-7alkylsilyl-Ci.7alkyl ester; or a C5.2oaryl-C1-7alkyl ester (*e.g.*, a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether (-S-CH2NHC(=O)CH3).

Prodrugs

It may be convenient or desirable to prepare, purify, and/or handle the active compound in the form of a prodrug. The term "prodrug," as used herein, pertains to a compound which, when metabolised (*e.g.*, *in vivo*), yields the desired active compound.

Typically, the prodrug is inactive, or less active than the active compound, but may provide advantageous handling, administration, or metabolic properties.

Unless otherwise specified, a reference to a particular compound also includes prodrugs thereof.

For example, some prodrugs are esters of the active compound (*e.g.*, a physiologically acceptable metabolically labile ester). During metabolism, the ester group (-C(=O)OR) is cleaved to yield the active drug. Such esters may be formed by esterification, for example, of any of the carboxylic acid groups (-C(=O)OH) in the parent compound, with, where appropriate, prior protection of any other reactive groups present in the parent compound, followed by deprotection if required.

Also, some prodrugs are activated enzymatically to yield the active compound, or a compound which, upon further chemical reaction, yields the active compound (for example, as in ADEPT, GDEPT, LIDEPT, etc.). For example, the prodrug may be a sugar derivative or other glycoside conjugate, or may be an amino acid ester derivative.

Chemical Synthesis

Several methods for the chemical synthesis of α-ketoglutarate esters are described herein. These and/or other well known methods may be modified and/or adapted in known ways in order to facilitate the synthesis of additional α-ketoglutarate esters and other compounds described herein, in accordance with standard techniques, from readily available starting materials, and using appropriate reagents and reaction conditions. If necessary and appropriate, the target compounds may be isolated from their reaction mixtures using conventional techniques, for example chromatography such as HPLC or FLASH chromatography.

For example, one general procedure for preparing α-ketoglutarate esters involves the alkylation (*e.g.*, benzylation) of an α-keto acid or its derivative (see, *e.g.*, Takeuchi *et al.*, 1999; Natsugari *et al.*, 1987). Another general procedure involves the esterification of an α-keto acid or its derivative (see, *e.g.*, Hartenstein *et al.*, 1993; Beyerman *et al.*, 1961). Another general procedure involves an ester exchange (see, *e.g.*, Domagala *et al.*, 1980). The synthesis method may be single-step or multi-step.

The synthesis method may employ protective groups, for example, O-protecting groups, such as groups known to be suitable for protecting primary and/or secondary

hydroxyl groups, for example, the O-protecting groups mentioned in "Protective Groups in Organic Chemistry", edited by J.W.F. McOmie, Plenum Press (1973), and "Protective Groups in Organic Synthesis", 3rd edition, T.W. Greene & P.G.M. Wutz, Wiley-lnterscience (1999). Some preferred O-protecting groups include alkylcarbonyl and arylcarbonyl groups (*e.g.*, acyl, *e.g.*, benzoyl), triarylmethyl groups (*e.g.*, triphenylmethyl (trityl) and dimethoxytrityl) and silyl groups (*e.g.*, trialkylsilyl, such as trimethylsilyl).

Nucleic acid based inhibitors

Nucleic acid-based inhibitors for inhibition IDH, *e.g.*, IDH1, can be, *e.g.*, double stranded RNA (dsRNA) that function, *e.g.*, by an RNA interference (RNAi mechanism), an antisense RNA, or a microRNA (miRNA). In an embodiment the nucleic-acid based inhibitor binds to the target mRNA and inhibits the production of protein therefrom, *e.g.*,. by cleavage of the targent mRNA.

Double stranded RNA (dsRNA)

A nucleic acid based inhibitor useful for decreasing IDH1 function is, *e.g.*, a dsRNA, such as a dsRNA that acts by an RNAi mechanism. RNAi refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs). dsRNAs as used herein are understood to include siRNAs. Typically, inhibition of IDH, *e.g.*, IDH1,by dsRNAs does not trigger the interferon response that results from dsRNA-mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

dsRNAs targeting an IDH,*e.g.*, IDH1,enzyme, *e.g.*, a wildtype or mutant IDH1, can be unmodified or chemically modified. The dsRNA can be chemically synthesized, expressed from a vector or enzymatically synthesized. The invention also features various chemically modified synthetic dsRNA molecules capable of modulating IDH1 gene expression or activity in cells by RNA interference (RNAi). The use of chemically modified dsRNA improves various properties of native dsRNA molecules, such as through increased resistance to nuclease degradation *in vivo* and/or through improved cellular uptake.

The dsRNAs targeting nucleic acid can be composed of two separate RNAs, or of one RNA strand, which is folded to form a hairpin structure. Hairpin dsRNAs are typically referred to as shRNAs.

An shRNA that targets IDH, *e.g.*, a mutant or wildtype IDH1 gene can be expressed from a vector, *e.g.*, viral vector, such as a lentiviral or adenoviral vector. In certain embodiments, a suitable dsRNA for inhibiting expression of an IDH1 gene will be identified by screening an siRNA library, such as an adenoviral or lentiviral siRNA library.

In an embodiment, a dsRNA that targets IDH, *e.g.*, IDH1, is about 15 to about 30 base pairs in length (*e.g.*, about 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) basepairs in length. In another embodiment, the dsRNA includes overhanging ends of about 1 to about 3 (*e.g.*, about 1, 2, or 3) nucleotides. By "overhang" is meant that 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. The dsRNA can have an overhang on one or both ends of the dsRNA molecule. In some embodiments, the single-stranded overhang is located at the 3'-terminal end of the antisense strand, or, alternatively, at the 3'-terminal end of the sense strand. In some embodiments, the overhang is a TT or UU dinucleotide overhang, *e.g.*, a TT or UU dinucleotide overhang. For example, in an embodiment, the dsRNA includes a 21-nucleotide antisense strand, a 19 base pair duplex region, and a 3'-terminal dinucleotide. In yet another embodiment, a dsRNA includes a duplex nucleic acid where both ends are blunt, or alternatively, where one of the ends is blunt.

In an embodiment, the dsRNA includes a first and a second strand, each strand is about 18 to about 28 nucleotides in length, *e.g.*, about 19 to about 23 nucleotides in length, the first strand of the dsRNA includes a nucleotide sequence having sufficient complementarity to the IDH, *e.g.*, IDH1, RNA for the dsRNA to direct cleavage of the IDH, *e.g.*, IDH1, mRNA via RNA interference, and the second strand of the dsRNA includes a nucleotide sequence that is complementary to the first strand.

In an embodiment, a dsRNA targeting an IDH, *e.g.*, IDH1, gene can target wildtype and mutant forms of the gene, or can target different allelic isoforms of the same gene. For example, the dsRNA will target a sequence that is identical in two or more of the different isoforms. In an embodiment, the dsRNA targets an IDH1 having G at

position 395 or C at position 394 (*e.g.*, a wildtype IDH1 RNA) and an IDH1 having A at position 395 or A at position 394 (*e.g.*, an IDH1 RNA carrying a G395A and/or a C394A mutation) (**FIG. 2**).

In an embodiment, a dsRNA will preferentially or specifically target a mutant IDH RNA, or a particular IDH polymorphism. For example, in an embodiment, the dsRNA targets an IDH1 RNA carrying an A at position 395, *e.g.*, G395A, and in another embodiment, the dsRNA targets an IDH1 RNA carrying an A at position 394, *e.g.*, C394A mutation.

In an embodiment, a dsRNA targeting an IDH RNA includes one or more chemical modifications. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-deoxyribonucleotides, 2'-O-methyl ribonucleotides, 2'-deoxy-2'-fluoro ribonucleotides, "universal base" nucleotides, "acyclic" nucleotides, 5-C-methyl nucleotides, and terminal glyceryl and/or inverted deoxy abasic residue incorporation. Such chemical modifications have been shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, one or more phosphorothioate substitutions are well-tolerated and have been shown to confer substantial increases in serum stability for modified dsRNA constructs.

In an embodiment, a dsRNA targeting an IDH, *e.g.*, IDH1, RNA includes modified nucleotides while maintaining the ability to mediate RNAi. The modified nucleotides can be used to improve *in vitro* or *in vivo* characteristics such as stability, activity, and/or bioavailability. For example, the dsRNA can include modified nucleotides as a percentage of the total number of nucleotides present in the molecule. As such, the dsRNA can generally include about 5% to about 100% modified nucleotides (*e.g.*, about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% modified nucleotides).

In some embodiments, the dsRNA targeting IDH, *e.g.*, IDH1, is about 21 nucleotides long. In another embodiment, the dsRNA does not contain any ribonucleotides, and in another embodiment, the dsRNA includes one or more ribonucleotides. In an embodiment, each strand of the dsRNA molecule independently includes about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27,

28, 29, or 30) nucleotides, wherein each strand includes about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to the nucleotides of the other strand. In an embodiment, one of the strands of the dsRNA includes a nucleotide sequence that is complementary to a nucleotide sequence or a portion thereof of the IDH1 or IDH2 gene, and the second strand of the dsRNA includes a nucleotide sequence substantially similar to the nucleotide sequence of the IDH1 or IDH2 gene or a portion thereof.

In an embodiment, the dsRNA targeting IDH1 or IDH2 includes an antisense region having a nucleotide sequence that is complementary to a nucleotide sequence of the IDH1 or IDH2 gene or a portion thereof, and a sense region having a nucleotide sequence substantially similar to the nucleotide sequence of the IDH1 or IDH2 gene or a portion thereof. In an embodiment, the antisense region and the sense region independently include about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides, where the antisense region includes about 15 to about 30 (*e.g.*, about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30) nucleotides that are complementary to nucleotides of the sense region.

As used herein, the term "dsRNA" is meant to include nucleic acid molecules that are capable of mediating sequence specific RNAi, such as short interfering RNA (siRNA), short hairpin RNA (shRNA), short interfering oligonucleotide, short interfering nucleic acid, short interfering modified oligonucleotide, chemically modified siRNA, post-transcriptional gene silencing RNA (ptgsRNA), and others. In addition, as used herein, the term "RNAi" is meant to include sequence specific RNA interference, such as post transcriptional gene silencing, translational inhibition, or epigenetics.

Antisense

Suitable nucleic acid based inhibitors include antisense nucleic acids. While not being bound by theory it is believed that antisense inhibition is typically based upon hydrogen bonding-based hybridization of oligonucleotide strands or segments such that at least one strand or segment is cleaved, degraded, or otherwise rendered inoperable.

An antisense agent can bind IDH1 or IDH2 DNA. In embodiments it inhibits replication and transcription. While not being bound by theory it is believed that an

antisense agent can also function to inhibit target RNA translocation, *e.g.*, to a site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more RNA species, and catalytic activity or complex formation involving the RNA.

An antisense agents can have a chemical modification described above as being suitable for dsRNA.

Antisense agents can include, for example, from about 8 to about 80 nucleobases (*i.e.*, from about 8 to about 80 nucleotides), *e.g.*, about 8 to about 50 nucleobases, or about 12 to about 30 nucleobases. Antisense compounds include ribozymes, external guide sequence (EGS) oligonucleotides (oligozymes), and other short catalytic RNAs or catalytic oligonucleotides which hybridize to the target nucleic acid and modulate its expression. Anti-sense compounds can include a stretch of at least eight consecutive nucleobases that are complementary to a sequence in the target gene. An oligonucleotide need not be 100% complementary to its target nucleic acid sequence to be specifically hybridizable. An oligonucleotide is specifically hybridizable when binding of the oligonucleotide to the target interferes with the normal function of the target molecule to cause a loss of utility, and there is a sufficient degree of complementarity to avoid non-specific binding of the oligonucleotide to non-target sequences under conditions in which specific binding is desired, *i.e.*, under physiological conditions in the case of *in vivo* assays or therapeutic treatment or, in the case of *in vitro* assays, under conditions in which the assays are conducted.

Hybridization of antisense oligonucleotides with mRNA (*e.g.*, an mRNA encoding IDH1) can interfere with one or more of the normal functions of mRNA. While not being bound by theory it is believed that athe functions of mRNA to be interfered with include all key functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in by the RNA. Binding of specific protein(s) to the RNA may also be interfered with by antisense oligonucleotide hybridization to the RNA.

Exemplary antisense compounds include DNA or RNA sequences that specifically hybridize to the target nucleic acid, *e.g.*, the mRNA encoding IDH1. The complementary region can extend for between about 8 to about 80 nucleobases. The

compounds can include one or more modified nucleobases. Modified nucleobases may include, *e.g.*, 5-substituted pyrimidines such as 5-iodouracil, 5-iodocytosine, and C5-propynyl pyrimidines such as C5-propynylcytosine and C5-propynyluracil. Other suitable modified nucleobases include N^4-(C_1-C_{12}) alkylaminocytosines and N^4,N^4-(C_1-C_{12}) dialkylaminocytosines. Modified nucleobases may also include 7-substituted-5-aza-7-deazapurines and 7-substituted-7-deazapurines such as, for example, 7-iodo-7-deazapurines, 7-cyano-7-deazapurines, 7-aminocarbonyl-7-deazapurines. Examples of these include 6-amino-7-iodo-7-deazapurines, 6-amino-7-cyano-7-deazapurines, 6-amino-7-aminocarbonyl-7-deazapurines, 2-amino-6-hydroxy-7-iodo-7-deazapurines, 2-amino-6-hydroxy-7-cyano-7-deazapurines, and 2-amino-6-hydroxy-7-aminocarbonyl-7-deazapurines. Furthermore, N^6-(C_1-C_{12}) alkylaminopurines and N^6,N^6-(C_1-C_{12}) dialkylaminopurines, including N^6-methylaminoadenine and N^6,N^6-dimethylaminoadenine, are also suitable modified nucleobases. Similarly, other 6-substituted purines including, for example, 6-thioguanine may constitute appropriate modified nucleobases. Other suitable nucleobases include 2-thiouracil, 8-bromoadenine, 8-bromoguanine, 2-fluoroadenine, and 2-fluoroguanine. Derivatives of any of the aforementioned modified nucleobases are also appropriate. Substituents of any of the preceding compounds may include C_1-C_{30} alkyl, C_2-C_{30} alkenyl, C_2-C_{30} alkynyl, aryl, aralkyl, heteroaryl, halo, amino, amido, nitro, thio, sulfonyl, carboxyl, alkoxy, alkylcarbonyl, alkoxycarbonyl, and the like.

MicroRNA

In some embodiments, the nucleic acid-based inhibitor suitable for targeting IDH, *e.g.*, IDH1, is a microRNA (miRNA). A miRNA is a single stranded RNA that regulates the expression of target mRNAs either by mRNA cleavage, translational repression/inhibition or heterochromatic silencing. The miRNA is 18 to 25 nucleotides, typically 21 to 23 nucleotides in length. In some embodiments, the miRNA includes chemical modifications, such as one or more modifications described herein.

In some embodiments, a nucleic acid based inhibitor targeting IDH has partial complementarity (*i.e.*, less than 100% complementarity) with the target IDH, *e.g.*, IDH1, mRNA. For example, partial complementarity can include various mismatches or non-

base paired nucleotides (*e.g.*, 1, 2, 3, 4, 5 or more mismatches or non-based paired nucleotides, such as nucleotide bulges), which can result in bulges, loops, or overhangs that result between the antisense strand or antisense region of the nucleic acid-based inhibitor and the corresponding target nucleic acid molecule.

The nucleic acid-based inhibitors described herein, *e.g.*, antisense nucleic acid described herein, can be incorporated into a gene construct to be used as a part of a gene therapy protocol to deliver nucleic acids that can be used to express and produce agents within cells. Expression constructs of such components may be administered in any biologically-effective carrier, *e.g.*, any formulation or composition capable of effectively delivering the component gene to cells *in vivo*. Approaches include insertion of the subject gene in viral vectors including recombinant retroviruses, adenovirus, adeno-associated virus, lentivirus, and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly; plasmid DNA can be delivered with the help of, for example, cationic liposomes (lipofectin) or derivatized (*e.g.*, antibody conjugated) polylysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular earners, as well as direct injection of the gene construct or $CaPO_4$ precipitation carried out *in vivo*.

In an embodiment, *in vivo* introduction of nucleic acid into a cell includes use of a viral vector containing nucleic acid, *e.g.*, a cDNA. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, *e.g.*, by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

Retroviral vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous genes *in vivo* particularly into humans. These vectors provide efficient delivery of genes into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F. M. *et al.* (eds.) Greene Publishing Associates (1989), Sections 9.10-9.14 and other standard laboratory manuals. Examples of suitable retroviruses include pLJ, pZIP, pWE, and pEM

which are known to those skilled in the art. Examples of suitable packaging virus lines for preparing both ecotropic and amphotropic retroviral systems include Crip, Cre, 2, and Am. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see, for example, Eglitis *et al.* (1985) Science 230:1395-1398; Danos and Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:6460-6464; Wilson *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano *et al.* (1990) Proc. Natl. Acad. Sci. USA 87:6141-6145; Huber *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury *et al.* (1991) Science 254:1802-1805; van Beusechem *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:7640-7644; Kay *et al.* (1992) Human Gene Therapy 3:641-647; Dai *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu *et al.* (1993) J. Immunol. 150:4104-4115; U.S. Pat. Nos. 4,868,116 and 4,980,286; PCT Pub. Nos. WO 89/07136, WO 89/02468, WO 89/05345, and WO 92/07573).

Another viral gene delivery system utilizes adenovirus-derived vectors. See, for example, Berkner *et al.* (1988) BioTechniques 6:616; Rosenfeld *et al.* (1991) Science 252:431-434; and Rosenfeld *et al.* (1992) Cell 68:143-155. Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 d1324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, Ad7 etc.) are known to those skilled in the art.

Yet another viral vector system useful for delivery of the subject gene is the adeno-associated virus (AAV). See, for example, Flotte *et al.* (1992) Am. J. Respir. Cell. Mol. Biol. 7:349-356; Samulski *et al.* (1989) J. Virol. 63:3822-3828; and McLaughlin *et al.* (1989) J. Virol. 62:1963-1973.

Pharmaceutical compositions

The compositions delineated herein include the compounds delineated herein, as well as additional therapeutic agents if present, in amounts effective for achieving a modulation of disease or disease symptoms, including those described herein.

The term "pharmaceutically acceptable carrier or adjuvant" refers to a carrier or adjuvant that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof and is

nontoxic when administered in doses sufficient to deliver a therapeutic amount of the compound.

Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d-α-tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α-, β-, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-β-cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compounds of the formulae described herein.

The pharmaceutical compositions containing inhibitors of IDH, *e.g.*, IDH1, may be administered directly to the central nervous system, such as into the cerebrospinal fluid or into the brain. Delivery can be, for example, in a bolus or by continuous pump infusion. In certain embodiments, delivery is by intrathecal delivery or by intraventricular injection directly into the brain. A catheter and, optionally, a pump can be used for delivery.

The therapeutics disclosed herein, *e.g.*, nucleic acid based inhibitors, *e.g.* siRNAs can be administered directly to the CNS, *e.g.*, the brain, *e.g.*, using a pump and/or catheter system. In one embodiment, the pump is implanted under the skin. In an embodiment and a catheter attached to a pump is inserted into the CNS, *e.g.*, into the brain or spine. In one embodiment, the pump (such as the IsoMed Drug Pump from Medtronic) delivers dosing, *e.g*, constant dosing, of a nucleic acid based inhibitor. In an embodiment, the pump is programmable to administer variable or constant doses at

predetermined time intervals. For example, the IsoMed Drug pump from Medtronic (or a similar device) can be used to administer a constant supply of the inhibitor, or the SynchroMedII Drug Pump (or a similar device) can be used to administer a variable dosing regime.

Methods and devices described in US patents 7,044,932, 6,620,151, 6,283949, and 6,685,452 can be used in methods described herein.

The pharmaceutical compositions of this invention may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir, preferably by oral administration or administration by injection. The pharmaceutical compositions of this invention may contain any conventional non-toxic pharmaceutically-acceptable carriers, adjuvants or vehicles. In some cases, the pH of the formulation may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents which are commonly used in the

formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions. Other commonly used surfactants such as Tweens or Spans and/or other similar emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

The pharmaceutical compositions of this invention may be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

The pharmaceutical compositions of this invention may also be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

Topical administration of the pharmaceutical compositions of this invention is useful when the desired treatment involves areas or organs readily accessible by topical application. For application topically to the skin, the pharmaceutical composition should be formulated with a suitable ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier with suitable emulsifying agents. Suitable carriers include, but are not limited to, mineral oil,

sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation. Topically-transdermal patches are also included in this invention.

The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

When the compositions of this invention comprise a combination of a compound of the formulae described herein and one or more additional therapeutic or prophylactic agents, both the compound and the additional agent should be present at dosage levels of between about 1 to 100%, and more preferably between about 5 to 95% of the dosage normally administered in a monotherapy regimen. The additional agents may be administered separately, as part of a multiple dose regimen, from the compounds of this invention. Alternatively, those agents may be part of a single dosage form, mixed together with the compounds of this invention in a single composition.

The compounds described herein can, for example, be administered by injection, intravenously, intraarterially, subdermally, intraperitoneally, intramuscularly, or subcutaneously; or orally, buccally, nasally, transmucosally, topically, in an ophthalmic preparation, or by inhalation, with a dosage ranging from about 0.02 to about 100 mg/kg of body weight, alternatively dosages between 1 mg and 1000 mg/dose, every 4 to 120 hours, or according to the requirements of the particular drug. The methods herein contemplate administration of an effective amount of compound or compound composition to achieve the desired or stated effect. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 6 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and

the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Alternatively, such preparations contain from about 20% to about 80% active compound.

Lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the disease, condition or symptoms, the patient's disposition to the disease, condition or symptoms, and the judgment of the treating physician.

Upon improvement of a patient's condition, a maintenance dose of a compound, composition or combination of this invention may be administered, if necessary. Subsequently, the dosage or frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained when the symptoms have been alleviated to the desired level. Patients may, however, require intermittent treatment on a long-term basis upon any recurrence of disease symptoms.

Kits

A compound described herein can be provided in a kit. The kit includes (a) a compound described herein, *e.g.*, a composition that includes a compound described herein, and, optionally (b) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of a compound described herein for the methods described herein.

In one embodiment, the informational material can include information about production of the compound, molecular weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to methods for administering the compound.

In one embodiment, the informational material can include instructions to administer a compound described herein in a suitable manner to perform the methods described herein, *e.g.*, in a suitable dose, dosage form, or mode of administration (*e.g.*, a dose, dosage form, or mode of administration described herein). In another embodiment,

the informational material can include instructions to administer a compound described herein to a suitable subject, *e.g.*, a human, *e.g.*, a human having or at risk for a disorder described herein.

The informational material of the kits is not limited in its form. In many cases, the informational material, *e.g.*, instructions, is provided in printed matter, *e.g.*, a printed text, drawing, and/or photograph, *e.g.*, a label or printed sheet. However, the informational material can also be provided in other formats, such as Braille, computer readable material, video recording, or audio recording. In another embodiment, the informational material of the kit is contact information, *e.g.*, a physical address, email address, website, or telephone number, where a user of the kit can obtain substantive information about a compound described herein and/or its use in the methods described herein. Of course, the informational material can also be provided in any combination of formats.

In addition to a compound described herein, the composition of the kit can include other ingredients, such as a solvent or buffer, a stabilizer, a preservative, a flavoring agent (*e.g.*, a bitter antagonist or a sweetener), a fragrance or other cosmetic ingredient, and/or a second agent for treating a condition or disorder described herein. Alternatively, the other ingredients can be included in the kit, but in different compositions or containers than a compound described herein. In such embodiments, the kit can include instructions for admixing a compound described herein and the other ingredients, or for using a compound described herein together with the other ingredients.

A compound described herein can be provided in any form, *e.g.*, liquid, dried or lyophilized form. It is preferred that a compound described herein be substantially pure and/or sterile. When a compound described herein is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When a compound described herein is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, *e.g.*, sterile water or buffer, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing a compound described herein. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example,

the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained in a plastic sleeve or packet. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (*e.g.*, a pack) of individual containers, each containing one or more unit dosage forms (*e.g.*, a dosage form described herein) of a compound described herein. For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of a compound described herein. The containers of the kits can be air tight, waterproof (*e.g.*, impermeable to changes in moisture or evaporation), and/or light-tight.

The kit optionally includes a device suitable for administration of the composition, *e.g.*, a syringe, inhalant, pipette, forceps, measured spoon, dropper (*e.g.*, eye dropper), swab (*e.g.*, a cotton swab or wooden swab), or any such delivery device. In a preferred embodiment, the device is a medical implant device, *e.g.*, packaged for surgical insertion.

Combination therapies

In some embodiments, a compound or composition described herein, is administered together with an additional cancer treatment. Exemplary cancer treatments include, for example: surgery, chemotherapy, targeted therapies such as antibody therapies, immunotherapy, and hormonal therapy. Examples of each of these treatments are provided below.

Chemotherapy

In some embodiments, a compound or composition described herein, is administered with a chemotherapy. Chemotherapy is the treatment of cancer with drugs that can destroy cancer cells. "Chemotherapy" usually refers to cytotoxic drugs which affect rapidly dividing cells in general, in contrast with targeted therapy. Chemotherapy drugs interfere with cell division in various possible ways, *e.g.*, with the duplication of DNA or the separation of newly formed chromosomes. Most forms of chemotherapy targ*et al*l rapidly dividing cells and are not specific for cancer cells, although some

degree of specificity may come from the inability of many cancer cells to repair DNA damage, while normal cells generally can.

Examples of chemotherapeutic agents used in cancer therapy include, for example, antimetabolites (*e.g.*, folic acid, purine, and pyrimidine derivatives) and alkylating agents (*e.g.*, nitrogen mustards, nitrosoureas, platinum, alkyl sulfonates, hydrazines, triazenes, aziridines, spindle poison, cytotoxic agents, toposimerase inhibitors and others). Exemplary agents include Aclarubicin, Actinomycin, Alitretinon, Altretamine, Aminopterin, Aminolevulinic acid, Amrubicin, Amsacrine, Anagrelide, Arsenic trioxide, Asparaginase, Atrasentan, Belotecan, Bexarotene, endamustine, Bleomycin, Bortezomib, Busulfan, Camptothecin, Capecitabine, Carboplatin, Carboquone, Carmofur, Carmustine, Celecoxib, Chlorambucil, Chlormethine, Cisplatin, Cladribine, Clofarabine, Crisantaspase, Cyclophosphamide, Cytarabine, Dacarbazine, Dactinomycin, Daunorubicin, Decitabine, Demecolcine, Docetaxel, Doxorubicin, Efaproxiral, Elesclomol, Elsamitrucin, Enocitabine, Epirubicin, Estramustine, Etoglucid, Etoposide, Floxuridine, Fludarabine, Fluorouracil (5FU), Fotemustine, Gemcitabine, Gliadel implants, Hydroxycarbamide, Hydroxyurea, Idarubicin, Ifosfamide, Irinotecan, Irofulven, Ixabepilone, Larotaxel, Leucovorin, Liposomal doxorubicin, Liposomal daunorubicin, Lonidamine, Lomustine, Lucanthone, Mannosulfan, Masoprocol, Melphalan, Mercaptopurine, Mesna, Methotrexate, Methyl aminolevulinate, Mitobronitol, Mitoguazone, Mitotane, Mitomycin, Mitoxantrone, Nedaplatin, Nimustine, Oblimersen, Omacetaxine, Ortataxel, Oxaliplatin, Paclitaxel, Pegaspargase, Pemetrexed, Pentostatin, Pirarubicin, Pixantrone, Plicamycin, Porfimer sodium, Prednimustine, Procarbazine, Raltitrexed, Ranimustine, Rubitecan, Sapacitabine, Semustine, Sitimagene ceradenovec, Strataplatin, Streptozocin, Talaporfin, Tegafur-uracil, Temoporfin, Temozolomide, Teniposide, Tesetaxel, Testolactone, Tetranitrate, Thiotepa, Tiazofurine, Tioguanine, Tipifarnib, Topotecan, Trabectedin, Triaziquone, Triethylenemelamine, Triplatin, Tretinoin, Treosulfan, Trofosfamide, Uramustine, Valrubicin, Verteporfin, Vinblastine, Vincristine, Vindesine, Vinflunine, Vinorelbine, Vorinostat, Zorubicin, and other cytostatic or cytotoxic agents described herein.

Because some drugs work better together than alone, two or more drugs are often given at the same time. Often, two or more chemotherapy agents are used as combination

chemotherapy. In some embodiments, the chemotherapy agents (including combination chemotherapy) can be used in combination with a compound described herein, *e.g.*, phenformin.

Targeted therapy

In some embodiments, a compound or composition described herein, is administered with a targeted therapy. Targeted therapy constitutes the use of agents specific for the deregulated proteins of cancer cells. Small molecule targeted therapy drugs are generally inhibitors of enzymatic domains on mutated, overexpressed, or otherwise critical proteins within the cancer cell. Prominent examples are the tyrosine kinase inhibitors such as Axitinib, Bosutinib, Cediranib, desatinib, erlotinib, imatinib, gefitinib, lapatinib, Lestaurtinib, Nilotinib, Semaxanib, Sorafenib, Sunitinib, and Vandetanib, and also cyclin-depdendent kinase inhibitors such as Alvocidib and Seliciclib. Monoclonal antibody therapy is another strategy in which the therapeutic agent is an antibody which specifically binds to a protein on the surface of the cancer cells. Examples include the anti-HER2/neu antibody trastuzumab (HERCEPTIN®) typically used in breast cancer, and the anti-CD20 antibody rituximab and Tositumomab typically used in a variety of B-cell malignancies. Other exemplary antibodies include Cetuximab, Panitumumab, Trastuzumab, Alemtuzumab, Bevacizumab, Edrecolomab, and Gemtuzumab. Exemplary fusion proteins include Aflibercept and Denileukin diftitox. In some embodiments, the targeted therapy can be used in combination with a compound described herein, *e.g.*, a biguanide such as metformin or phenformin, preferably phenformin.

Targeted therapy can also involve small peptides as "homing devices" which can bind to cell surface receptors or affected extracellular matrix surrounding the tumor. Radionuclides which are attached to these peptides (*e.g.*, RGDs) eventually kill the cancer cell if the nuclide decays in the vicinity of the cell. An example of such therapy includes BEXXAR®.

Immunotherapy

In some embodiments, a compound or composition described herein, is administered with an immunotherapy. Cancer immunotherapy refers to a diverse set of therapeutic strategies designed to induce the patient's own immune system to fight the

tumor. Contemporary methods for generating an immune response against tumors include intravesicular BCG immunotherapy for superficial bladder cancer, and use of interferons and other cytokines to induce an immune response in renal cell carcinoma and melanoma patients.

Allogeneic hematopoietic stem cell transplantation can be considered a form of immunotherapy, since the donor's immune cells will often attack the tumor in a graft-versus-tumor effect. In some embodiments, the immunotherapy agents can be used in combination with a compound or composition described herein.

Hormonal therapy

In some embodiments, a compound or composition described herein, is administered with a hormonal therapy. The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an important additional treatment. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial. In some embodiments, the hormonal therapy agents can be used in combination with a compound or a composition described herein.

In some embodiments, a compound or composition described herein, is administered together with an additional cancer treatment (*e.g.*, surgical removal), in treating cancer in nervous system, *e.g.*, cancer in central nervous system, *e.g.*, brain tumor, *e.g.*, glioma, *e.g.*, glioblastoma multiforme (GBM).

Several studies have suggested that more than 25% of glioblastoma patients obtain a significant survival benefit from adjuvant chemotherapy. Meta-analyses have suggested that adjuvant chemotherapy results in a 6-10% increase in 1-year survival rate.

Temozolomide is an orally active alkylating agent that is used for persons newly diagnosed with glioblastoma multiforme. It was approved by the United States Food and Drug Administration (FDA) in March 2005. Studies have shown that the drug was well tolerated and provided a survival benefit. Adjuvant and concomitant temozolomide with radiation was associated with significant improvements in median progression-free survival over radiation alone (6.9 vs 5 mo), overall survival (14.6 vs 12.1 mo), and the likelihood of being alive in 2 years (26% vs 10%).

Nitrosoureas: BCNU (carmustine)-polymer wafers (Gliadel) were approved by the FDA in 2002. Though Gliadel wafers are used by some for initial treatment, they have shown only a modest increase in median survival over placebo (13.8 vs. 11.6 months) in the largest such phase III trial, and are associated with increased rates of CSF leak and increased intracranial pressure secondary to edema and mass effect.

MGMT is a DNA repair enzyme that contributes to temozolomide resistance. Methylation of the MGMT promoter, found in approximately 45% of glioblastoma multiformes, results in an epigenetic silencing of the gene, decreasing the tumor cell's capacity for DNA repair and increasing susceptibility to temozolomide.

When patients with and without MGMT promoter methylation were treated with temozolomide, the groups had median survivals of 21.7 versus 12.7 months, and 2-year survival rates of 46% versus 13.8%, respectively.

Though temozolomide is currently a first-line agent in the treatment of glioblastoma multiforme, unfavorable MGMT methylation status could help select patients appropriate for future therapeutic investigations.

O6-benzylguanine and other inhibitors of MGMT as well as RNA interference-mediated silencing of MGMT offer promising avenues to increase the effectiveness of temozolomide and other alkylating antineoplastics, and such agents are under active study.

Carmustine (BCNU) and cis -platinum (cisplatin) have been the primary chemotherapeutic agents used against malignant gliomas. All agents in use have no greater than a 30-40% response rate, and most fall into the range of 10-20%.

Data from the University of California at San Francisco indicate that, for the treatment of glioblastomas, surgery followed by radiation therapy leads to 1-, 3-, and 5-year survival rates of 44%, 6%, and 0%, respectively. By comparison, surgery followed by radiation and chemotherapy using nitrosourea-based regimens resulted in 1-, 3-, and 5-year survival rates of 46%, 18%, and 18%, respectively.

A major hindrance to the use of chemotherapeutic agents for brain tumors is the fact that the blood-brain barrier (BBB) effectively excludes many agents from the CNS. For this reason, novel methods of intracranial drug delivery are being developed to

deliver higher concentrations of chemotherapeutic agents to the tumor cells while avoiding the adverse systemic effects of these medications.

Pressure-driven infusion of chemotherapeutic agents through an intracranial catheter, also known as convection-enhanced delivery (CED), has the advantage of delivering drugs along a pressure gradient rather than by simple diffusion. CED has shown promising results in animal models with agents including BCNU and topotecan.

Initial attempts investigated the delivery of chemotherapeutic agents via an intraarterial route rather than intravenously. Unfortunately, no survival advantage was observed.

Chemotherapy for recurrent glioblastoma multiforme provides modest, if any, benefit, and several classes of agents are used. Carmustine wafers increased 6-month survival from 36% to 56% over placebo in one randomized study of 222 patients, though there was a significant association between the treatment group and serious intracranial infections.

Genotyping of brain tumors may have applications in stratifying patients for clinical trials of various novel therapies.

The anti-angiogenic agent bevacizumab, when used with irinotecan improved 6-month survival in recurrent glioma patients to 46% compared with 21% in patients treated with temozolomide. This bevacizumab and irinotecan combination for recurrent glioblastoma multiforme has been shown to improve survival over bevacizumab alone. Anti-angiogenic agents also decrease peritumoral edema, potentially reducing the necessary corticosteroid dose.

Some glioblastomas responds to gefitinib or erlotinib (tyrosine kinase inhibitors). The simultaneous presence in glioblastoma cells of mutant EGFR (EGFRviii) and PTEN was associated with responsiveness to tyrosine kinase inhibitors, whereas increased p-akt predicts a decreased effect. Other targets include PDGFR, VEGFR, mTOR, farnesyltransferase, and PI3K.

Other possible therapy modalities include imatinib, gene therapy, peptide and dendritic cell vaccines, synthetic chlorotoxins, and radiolabeled drugs and antibodies.

Patient selection/monitoring

Described herein are methods of treating cancer in a subject and methods of identifying a subject for a treatment described herein. Also described herein are methods of predicting a subject who is at risk of developing cancer (*e.g.*, a cancer associate with a mutation in an enzyme (*e.g.*, an enzyme in the metabolic pathway such as IDH1 and/or IDH2)). The cancer is generally characterized by the presence of a neoactivity, such as a gain of function in one or more mutant enzymes (*e.g.*, an enzyme in the metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.*, IDH1 or IDH2). The subject can be selected on the basis of the subject having a mutant gene having a neoactivity, *e.g.*, a neoactivity described herein. As used herein, "select" means selecting in whole or part on said basis.

In some embodiments, a subject is selected for treatment with a compound described herein based on a determination that the subject has a mutant enzyme described herein (*e.g.*, an enzyme in the metabolic pathway, *e.g.*, a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.*, IDH1 or IDH2). In some embodiments, the mutant enzyme has a neoactivity. The neoactivity of the enzyme can be identified, for example, by evaluating the subject or sample (*e.g.*, tissue or bodily fluid) therefrom, for the presence or amount of a substrate, cofactor and/or product of the enzyme. The presence and/or amount of substrate, cofactor and/or product can correspond to the wild-type/non-mutant activity or can correspond to the neoactivity of the enzyme. Exemplary bodily fluid that can be used to identifty (*e.g.*, evaluate) the neoactivity of the enzyme include amniotic fluid surrounding a fetus, aqueous humour, blood (*e.g.*, blood plasma), Cerebrospinal fluid, cerumen, chyme, Cowper's fluid, female ejaculate, interstitial fluid, lymph, breast milk, mucus (*e.g.*, nasal drainage or phlegm), pleural fluid, pus, saliva, sebum, semen, serum, sweat, tears, urine, vaginal secretion, or vomit.

In some embodiments, a subject can be evaluated for neoactivity of an enzyme using magnetic resonance. For example, where the mutant enzyme is IDH1 and the neoactivity is conversion of α-ketoglutarate to 2-hydroxyglutarate, the subject can be evaluated for the presence of and/or an elevated amount of 2-hydroxyglutarate, e.g., R-2-

hydroxyglutarate relative to the amount of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate present in a subject who does not have a mutation in IDH1 having the above neoactivity. In some embodiments, neoactivity of IDH1 can be determined by the presence or elevated amount of a peak corresponding to 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate as determined by magnetic resonance. For example, a subject can be evaluated for the presence and/or strength of a signal at about 2.5 ppm to determine the presence and/or amount of 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate in the subject. This can be correlated to and/or predictive of a neoactivity described herein for the mutant enzyme IDH. Similarly, the presence, strength and/or absence of a signal at about 2.5 ppm could be predictive of a response to treatment and thereby used as a noninvasive biomarker for clinical response.

Neoactivity of a mutant enzyme such as IDH can also be evaluated using other techniques known to one skilled in the art. For example, the presence or amount of a labeled substrate, cofactor, and/or reaction product can be measured such as a ^{13}C or ^{14}C labeled substrate, cofactor, and/or reaction product. The neoactivity can be evaluated by evaluating the forward reaction of the wild-type/non mutant enzyme (such as the oxidative decarboxylation of ioscitrate to α-ketoglutarate in a mutant IDH1 enzyme) and/or the reaction corresponding to the neoactivity (*e.g.*, the conversion of α-ketoglutarate to 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate in a mutant IDH1 enzyme).

Proliferative disorders

The disclosed methods are useful in treating proliferative disorders, *e.g.* treating solid tumors, soft tissue tumors, and metastases thereof wherein the solid tumor, soft tissue tumor or metastases thereof is a cancer described herein. Exemplary solid tumors include malignancies (*e.g.*, sarcomas, adenocarcinomas, and carcinomas) of the various organ systems, such as those of brain, lung, breast, lymphoid, gastrointestinal (*e.g.*, colon), and genitourinary (*e.g.*, renal, urothelial, or testicular tumors) tracts, pharynx, prostate, and ovary. Exemplary adenocarcinomas include colorectal cancers, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, and cancer of the small intestine. The disclosed methods are also useful in treating non-solid cancers.

The methods described herein can be administered with any cancer, for example those described by the National Cancer Institute. A cancer can be evaluated to determine whether it is using a method described herein. Exemplary cancers described by the National Cancer Institute include: Acute Lymphoblastic Leukemia, Adult; Acute Lymphoblastic Leukemia, Childhood; Acute Myeloid Leukemia, Adult; Adrenocortical Carcinoma; Adrenocortical Carcinoma, Childhood; AIDS-Related Lymphoma; AIDS-Related Malignancies; Anal Cancer; Astrocytoma, Childhood Cerebellar; Astrocytoma, Childhood Cerebral; Bile Duct Cancer, Extrahepatic; Bladder Cancer; Bladder Cancer, Childhood; Bone Cancer, Osteosarcoma/Malignant Fibrous Histiocytoma; Brain Stem Glioma, Childhood; Brain Tumor, Adult; Brain Tumor, Brain Stem Glioma, Childhood; Brain Tumor, Cerebellar Astrocytoma, Childhood; Brain Tumor, Cerebral Astrocytoma/Malignant Glioma, Childhood; Brain Tumor, Ependymoma, Childhood; Brain Tumor, Medulloblastoma, Childhood; Brain Tumor, Supratentorial Primitive Neuroectodermal Tumors, Childhood; Brain Tumor, Visual Pathway and Hypothalamic Glioma, Childhood; Brain Tumor, Childhood (Other); Breast Cancer; Breast Cancer and Pregnancy; Breast Cancer, Childhood; Breast Cancer, Male; Bronchial Adenomas/Carcinoids, Childhood; Carcinoid Tumor, Childhood; Carcinoid Tumor, Gastrointestinal; Carcinoma, Adrenocortical; Carcinoma, Islet Cell; Carcinoma of Unknown Primaiy; Central Nervous System Lymphoma, Primary; Cerebellar Astrocytoma, Childhood; Cerebral Astrocytoma/Malignant Glioma, Childhood; Cervical Cancer; Childhood Cancers; Chronic Lymphocytic Leukemia; Chronic Myelogenous Leukemia; Chronic Myeloproliferative Disorders; Clear Cell Sarcoma of Tendon Sheaths; Colon Cancer; Colorectal Cancer, Childhood; Cutaneous T-CeIl Lymphoma; Endometrial Cancer; Ependymoma, Childhood; Epithelial Cancer, Ovarian; Esophageal Cancer; Esophageal Cancer, Childhood; Ewing's Family of Tumors; Extracranial Germ Cell Tumor, Childhood; Extragonadal Germ Cell Tumor; Extrahepatic Bile Duct Cancer; Eye Cancer, Intraocular Melanoma; Eye Cancer, Retinoblastoma; Gallbladder Cancer; Gastric (Stomach) Cancer; Gastric (Stomach) Cancer, Childhood; Gastrointestinal Carcinoid Tumor; Germ Cell Tumor, Extracranial, Childhood; Germ Cell Tumor, Extragonadal; Germ Cell Tumor, Ovarian; Gestational Trophoblastic Tumor; Glioma, Childhood Brain Stem; Glioma, Childhood Visual Pathway and Hypothalamic; Hairy

Cell Leukemia; Head and Neck Cancer; Hepatocellular (Liver) Cancer, Adult (Primary); Hepatocellular (Liver) Cancer, Childhood (Primary); Hodgkin's Lymphoma, Adult; Hodgkin's Lymphoma, Childhood; Hodgkin's Lymphoma During Pregnancy; Hypopharyngeal Cancer; Hypothalamic and Visual Pathway Glioma, Childhood; Intraocular Melanoma; Islet Cell Carcinoma (Endocrine Pancreas); Kaposi's Sarcoma; Kidney Cancer; Laryngeal Cancer; Laryngeal Cancer, Childhood; Leukemia, Acute Lymphoblastic, Adult; Leukemia, Acute Lymphoblastic, Childhood; Leukemia, Acute Myeloid, Adult; Leukemia, Acute Myeloid, Childhood; Leukemia, Chronic Lymphocytic; Leukemia, Chronic Myelogenous; Leukemia, Hairy Cell; Lip and Oral Cavity Cancer; Liver Cancer, Adult (Primary); Liver Cancer, Childhood (Primary); Lung Cancer, Non-Small Cell; Lung Cancer, Small Cell; Lymphoblastic Leukemia, Adult Acute; Lymphoblastic Leukemia, Childhood Acute; Lymphocytic Leukemia, Chronic; Lymphoma, AIDS- Related; Lymphoma, Central Nervous System (Primary); Lymphoma, Cutaneous T-Cell; Lymphoma, Hodgkin's, Adult; Lymphoma, Hodgkin's, Childhood; Lymphoma, Hodgkin's During Pregnancy; Lymphoma, Non-Hodgkin's, Adult; Lymphoma, Non- Hodgkin's, Childhood; Lymphoma, Non-Hodgkin's During Pregnancy; Lymphoma, Primary Central Nervous System; Macroglobulinemia, Waldenstrom's; Male Breast Cancer; Malignant Mesothelioma, Adult; Malignant Mesothelioma, Childhood; Malignant Thymoma; Medulloblastoma, Childhood; Melanoma; Melanoma, Intraocular; Merkel Cell Carcinoma; Mesothelioma, Malignant; Metastatic Squamous Neck Cancer with Occult Primary; Multiple Endocrine Neoplasia Syndrome, Childhood; Multiple Myeloma/Plasma Cell Neoplasm; Mycosis Fungoides; Myelodysplastic Syndromes; Myelogenous Leukemia, Chronic; Myeloid Leukemia, Childhood Acute; Myeloma, Multiple; Myeloproliferative Disorders, Chronic; Nasal Cavity and Paranasal Sinus Cancer; Nasopharyngeal Cancer; Nasopharyngeal Cancer, Childhood; Neuroblastoma; Non-Hodgkin's Lymphoma, Adult; Non-Hodgkin's Lymphoma, Childhood; Non-Hodgkin's Lymphoma During Pregnancy; Non-Small Cell Lung Cancer; Oral Cancer, Childhood; Oral Cavity and Lip Cancer; Oropharyngeal Cancer; Osteosarcoma/Malignant Fibrous Histiocytoma of Bone; Ovarian Cancer, Childhood; Ovarian Epithelial Cancer; Ovarian Germ Cell Tumor; Ovarian Low Malignant Potential Tumor; Pancreatic Cancer; Pancreatic Cancer, Childhood; Pancreatic Cancer, Islet Cell;

Paranasal Sinus and Nasal Cavity Cancer; Parathyroid Cancer; Penile Cancer; Pheochromocytoma; Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood; Pituitary Tumor; Plasma Cell Neoplasm/Multiple Myeloma; Pleuropulmonary Blastoma; Pregnancy and Breast Cancer; Pregnancy and Hodgkin's Lymphoma; Pregnancy and Non-Hodgkin's Lymphoma; Primary Central Nervous System Lymphoma; Primary Liver Cancer, Adult; Primary Liver Cancer, Childhood; Prostate Cancer; Rectal Cancer; Renal Cell (Kidney) Cancer; Renal Cell Cancer, Childhood; Renal Pelvis and Ureter, Transitional Cell Cancer; Retinoblastoma; Rhabdomyosarcoma, Childhood; Salivary Gland Cancer; Salivary Gland Cancer, Childhood; Sarcoma, Ewing's Family of Tumors; Sarcoma, Kaposi's; Sarcoma (Osteosarcoma)/Malignant Fibrous Histiocytoma of Bone; Sarcoma, Rhabdomyosarcoma, Childhood; Sarcoma, Soft Tissue, Adult; Sarcoma, Soft Tissue, Childhood; Sezary Syndrome; Skin Cancer; Skin Cancer, Childhood; Skin Cancer (Melanoma); Skin Carcinoma, Merkel Cell; Small Cell Lung Cancer; Small Intestine Cancer; Soft Tissue Sarcoma, Adult; Soft Tissue Sarcoma, Childhood; Squamous Neck Cancer with Occult Primary, Metastatic; Stomach (Gastric) Cancer; Stomach (Gastric) Cancer, Childhood; Supratentorial Primitive Neuroectodermal Tumors, Childhood; T- CeIl Lymphoma, Cutaneous; Testicular Cancer; Thymoma, Childhood; Thymoma, Malignant; Thyroid Cancer; Thyroid Cancer, Childhood; Transitional Cell Cancer of the Renal Pelvis and Ureter; Trophoblastic Tumor, Gestational; Unknown Primary Site, Cancer of, Childhood; Unusual Cancers of Childhood; Ureter and Renal Pelvis, Transitional Cell Cancer; Urethral Cancer; Uterine Sarcoma; Vaginal Cancer; Visual Pathway and Hypothalamic Glioma, Childhood; Vulvar Cancer; Waldenstrom's Macro globulinemia; and Wilms' Tumor. Metastases of the aforementioned cancers can also be treated or prevented in accordance with the methods described herein.

The methods described herein are useful in treating cancer in nervous system, *e.g.*, brain tumor, *e.g.*, glioma, *e.g.*, glioblastoma multiforme (GBM), *e.g.*, by inhibiting a neoactivity of a mutant enzyme, *e.g.*, an enzyme in a metabolic pathway, *e.g.*, a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.*, IDH1 or IDH2.

Gliomas, a type of brain tumors, can be classified as grade I to grade IV on the basis of histopathological and clinical criteria established by the World Health Organization (WHO). WHO grade I gliomas are often considered benign. Gliomas of WHO grade II or III are invasive, progress to higher-grade lesions. WHO grade IV tumors (glioblastomas) are the most invasive form. Exemplary brain tumors include, *e.g.*, astrocytic tumor (*e.g.*, pilocytic astrocytoma, subependymal giant-cell astrocytoma, diffuse astrocytoma, pleomorphic xanthoastrocytoma, anaplastic astrocytoma, astrocytoma, giant cell glioblastoma, glioblastoma, secondary glioblastoma, primary adult glioblastoma, and primary pediatric glioblastoma); oligodendroglial tumor (*e.g.*, oligodendroglioma, and anaplastic oligodendroglioma); oligoastrocytic tumor (*e.g.*, oligoastrocytoma, and anaplastic oligoastrocytoma); ependymoma (*e.g.*, myxopapillary ependymoma, and anaplastic ependymoma); medulloblastoma; primitive neuroectodermal tumor, schwannoma, meningioma, meatypical meningioma, anaplastic meningioma; and pituitary adenoma. Exemplary cancers are described in Acta Neuropathol (2008) 116:597–602 and N Engl J Med. 2009 Feb 19;360(8):765-73, the contents of which are each incorporated herein by reference.

Glioblastoma is a common and aggressive type of primary brain tumor in humans. Glioblastomas are also an important brain tumor of the canine, which can be used as a model for developing treatments in humans. Common symptoms of glioblastoma include, *e.g.*, seizure, nausea and vomiting, headache, and hemiparesis, the single most prevalent symptom is a progressive memory, personality, or neurological deficit due to temporal and frontal lobe involvement. The kind of symptoms produced depends on the location of the tumor, more so than on its pathological properties. Glioblastomas can be diagnosed by MRI. More definitive diagnosis of a suspected GBM on CT or MRI might require a stereotactic biopsy or a craniotomy with tumor resection. Glioblastoma tumors can be sporadic or with genetic predisposition. Risk factors can include, *e.g.*, aging; ionizing radiation; viral infection (*e.g.*, SV40 or cytomegalovirus infection); having a low-grade astrocytoma (brain tumor), which might develop into a higher-grade tumor; or having one of the following genetic disorders: Neurofibromatosis, Tuberous sclerosis, Von Hippel-Lindau disease, Li-Fraumeni syndrome, or Turcot syndrome. GBM candidate genes

might include, *e.g.*, IDH1, CDKN2A, TP53, EGFR, PTEN, NF1, CDK4, RB1, PIK3CA and PIK3R1.

Glioblastomas are characterized by the presence of small areas of necrotizing tissue that is surrounded by anaplastic cells (pseudopalisading necrosis), as well as the presence of hyperplastic blood vessels. Glioblastomas can form in either the gray matter or the white matter of the brain; but most GBM arises from the deep white matter and quickly infiltrate the brain, often becoming very large before producing symptoms. The tumor may extend to the meningeal or ventricular wall, leading to the high protein content of cerebrospinal fluid (CSF) (> 100 mg/dL), as well as an occasional pleocytosis of 10 to 100 cells, mostly lymphocytes. Malignant cells carried in the CSF may spread to the spinal cord or cause meningeal gliomatosis. About 50% of GBM occupy more than one lobe of a hemisphere or are bilateral. Tumors of this type usually arise from the cerebrum and may exhibit the classic infiltrate across the corpus callosum, producing a butterfly (bilateral) glioma. The tumor may take on a variety of appearances, depending on the amount of hemorrhage, necrosis, or its age. A CT scan can show a nonhomogeneous mass with a hypodense center and a variable ring of enhancement surrounded by edema. Mass effect from the tumor and edema may compress the ventricles and cause hydrocephalus.

The methods described herein are useful in treating prostate cancer, *e.g.*, by inhibiting a neoactivity of a mutant enzyme, *e.g.*, an enzyme in a metabolic pathway, *e.g.*, a metabolic pathway leading to fatty acid biosynthesis, glycolysis, glutaminolysis, the pentose phosphate shunt, the nucleotide biosynthetic pathway, or the fatty acid biosynthetic pathway, *e.g.*, IDH1 or IDH2.

Prostate cancers, can be classified on the basis of histopathological and clinical criteria, e.g., four-stage TNM system. In the TNM system, clinical T1 (*e.g.*, T1a, T1b and T1c) and T2 (*e.g.*, T2a, T2b and T2c) cancers are found only in the prostate, while T3 (*e.g.*, T3a and T3b) and T4 cancers have spread elsewhere. Several tests can be used to look for evidence of spread. These include computed tomography to evaluate spread within the pelvis, bone scans to look for spread to the bones, and endorectal coil magnetic resonance imaging to closely evaluate the prostatic capsule and the seminal vesicles. The grade of the prostate cancer (how different the tissue is from normal tissue), *e.g.*, G1, G2,

G3 and G4 can be used in conjunction with TNM status to group cases into four overall stages. Types of prostate cancer include, e.g., prostate adenocarcinoma, small cell carcinoma, squamous carcinoma, sarcomas, and transitional cell carcinoma.

Symptoms of prostate cancer include, *e.g.*, elevated PSA (Prostate-Specific Antigen) level, frequent urination, increased urination at night, difficulty starting and maintaining a steady stream of urine, blood in the urine, painful urination, urinary dysfunction, difficulty achieving erection, painful ejaculation, bone pain (*e.g.*, in the vertebrae, pelvis, or ribs), leg weakness, and urinary and fecal incontinence.

The causes or risk factors of prostate cancer include, *e.g.*, age, genetics, race, diet, lifestyle, medications, and other factors. Prostate cancer candidate genes include, *e.g.*, IDH1, IDH2, BRCA1 and BRCA2.

The diagnosis of prostate cancer includes, biopsy (*e.g.*, Gleason score and tumor markers (e.g., PSA)), cystoscopy, and transrectal ultrasonography.

Treatment for prostate cancer include, e.g., active surveillance, surgery (*e.g.*, radical prostatectomy, transurethral resection of the prostate, orchiectomy, and cryosurgegry), radiation therapy including brachytherapy (prostate brachytherapy) and external beam radiation therapy, High-Intensity Focused Ultrasound (HIFU), chemotherapy, cryosurgery, hormonal therapy (*e.g.*, antiandrogens (*e.g.*, flutamide, bicalutamide, nilutamide and cyproterone acetate, ketoconazole, aminoglutethimide), GnRH antagonists (*e.g.*, Abarelix)), or a combination thereof.

All references described herein are expressly incorporated herein by reference.

EXAMPLES

Example 1 IDH1 cloning, mutagenesis, expression and purification

1. Wild type IDH1 was cloned into pET41a, creating His8 tag at C-terminus.

The IDH1 gene coding region (cDNA) was purchased from Invitrogen in pENTR221 vector (www.invitrogen.com, Cat#B-068487_Ultimate_ORF). Oligo nucleotides were designed to PCR out the coding region of IDH1 with NdeI at the 5' end and XhoI at the 3'. (IDH1-f: TAATCATATGTCCAAAAAAATCAGT (SEQ ID NO:1), IDH1-r: TAATCTCGAGTGAAAGTTTGGCCTGAGCTAGTT (SEQ ID NO:2)). The PCR product is cloned into the NdeI/XhoI cleaved pET41a vector. NdeI/XhoI cleavage

of the vector pET41a releases the GST portion of the plasmid, and creating a C-terminal His8 tag (SEQ ID NO:3) without the N-terminal GST fusion. The original stop codon of IDH1 is change to serine, so the junction sequence in final IDH1 protein is: Ser-Leu-Glu-His-His-His-His-His-His-His-His-Stop (SEQ ID NO:4).

The C-terminal His tag strategy instead of N-terminal His tag strategy was chosen, because C-terminal tag might not negatively impact IDH1 protein folding or activity. See, *e.g.*, Xu X *et al*, J Biol Chem. 2004 Aug 6; 279(32):33946-57.

The sequence for pET41a-IDH1 plasmid is confirmed by DNA sequencing. **FIG. 1** shows detailed sequence verification of pET41a-IDH1and alignment against published IDH1 CDS below.

2. IDH1 site directed mutagenesis to create the IDHr132s and IDHr132h mutants.

Site directed mutagenesis was performed to convert R132 to S or H, DNA sequencing confirmed that G395 is mutated to A (creating Arg→His mutation in the IDH1 protein), and C394 is mutated to A (creating Arg→Ser in the IDH1 protein). Detailed method for site directed mutagenesis is described in the user manual for QuikChange® MultiSite-Directed Mutagenesis Kit (Stratagene, cat# 200531). **FIG. 2** shows DNA sequence verification of such mutations. Highlighted nucleotides were successfully changed in the mutagenesis: G395→A mutation allows amino acid Arg132→His; C394→A mutation allows amino acid Arg132→Ser.

3. IDH1 protein expression and purification.

IDHwt, IDHR132S, and IDHR132H proteins were expressed in the *E. coli* strain Rosetta and purified according to the detailed procedure below. Active IDH1 proteins are in dimer form, and SEC column fraction/peak that correspond to the dimer form were collected for enzymology analysis and cross comparison of catalytic activities of these proteins.

A. Cell culturing:

Cells were grown in LB (20 μg/ml Kanamycin) at 37°C with shaking until OD600 reaches 0.6. The temperature was changed to 18°C and protein was induced by adding

IPTG to final concentration of 1 mM. Cells were collected 12-16 hours after IPTG induction.

B. Buffer system:

Lysis buffer: 20mM Tris, pH7.4, 0.1% Triton X-100, 500 mM NaCl, 1 mM PMSF, 5 mM β-mercaptoethanol, 10 % glycerol.

Ni-Column Buffer A: 20 mM Tris, pH7.4, 500mM NaCl, 5 mM β-mercaptoethanol, 10% glycerol.

Ni-column Buffer B: 20 mM Tris, pH7.4, 500 mM NaCl, 5 mM β-mercaptoethanol , 500 mM Imidazole, 10% glycerol

Gel filtration Buffer C: 200 mM NaCl, 50 mM Tris 7.5, 5 mM β-mercaptoethanol, 2 mM $MnSO_4$, 10% glycerol.

C. Protein purification procedure

1. Cell pellet were resuspended in the lysis buffer (1gram cell/5-10 ml buffer).
2. Cells were broken by passing the cell through Microfludizer with at a pressure of 15,000 psi for 3 times.
3. Soluble protein was collected from supernatant after centrifugation at 20,000g (Beckman Avanti J-26XP) for 30 min at 4°C.
4. 5-10 ml of Ni-column was equilibrated by Buffer A until the A280 value reached baseline. The supernatant was loaded onto a 5-ml Ni-Sepharose column (2 ml/min). The column was washed by 10-20 CV of washing buffer (90 % buffer A+10 % buffer B) until A280 reach the baseline (2 ml/min).
5. The protein was eluted by liner gradient of 10-100% buffer B (20 CV) with the flow rate of 2 ml/min and the sample fractions were collected as 2 ml/tube.
6. The samples were analyzed on SDS-PAGE gel.
7. The samples were collected and dialyzed against 200x Gel filtration buffer for 2 times (1 hour and > 4 hours).
8. The samples were concentrated to 10 ml.
9. 200 ml of S-200 Gel-filtration column was equilibrated by buffer C until the A280 value reached baseline. The samples were loaded onto Gel filtration column (0.5 ml/min).

10. The column was washed by 10 CV of buffer C, collect fractions as 2-4 ml/tube.
11. The samples were analyzed on SDS-PAGE gel and protein concentration was determined.

D. Protein purification results

The results for purification of wild type IDH1 are shown in **FIGs. 3, 4, 5A** and **5B**.

The results for purification of mutant IDH1R132S are shown in **FIGs. 6, 7, 8A** and **8B**.

The results for purification of wild type IDH1R132H are shown in **FIGs. 9, 10, 11A** and **11B**.

EXAMPLE 2 ENZYMOLOGY ANALYSIS OF IDH1 WILD TYPE AND MUTANTS

1. Analysis of IDH1 wild-type and mutants R132H and R132S in the oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG).

A. Methods

To determine the catalytic efficiency of enzymes in the oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG) direction, reactions were performed to determine Vmax and Km for isocitrate. In these reactions, the substrate was varied while the cofactor was held constant at 500 uM. All reactions were performed in 150 mM NaCl, 20 mM Tris-Cl, pH 7.5, 10% glycerol, and 0.03% (w/v) BSA). Reaction progress was followed by spectroscopy at 340 nM monitoring the change in oxidation state of the cofactor. Sufficient enzyme was added to give a linear change in absorbance for 10 minutes.

B. ICDH1 R132H and ICDH1 R132S are impaired for conversion of isocitrate to α-KG.

Michaelis-Menten plots for the relationship of isocitrate concentration to reaction velocity are presented in **FIGs. 12A-12C**. Kinetic parameters are summarized in the **Table 1**. All data was fit to the Hill equation by least-squares regression analysis.

Table 1

Enzyme	Vmax (umol/min/mg)	Km (uM)	Hill Constant	Vmax/Km	Relative Catalytic Efficiency

Wt	30.5	56.8	1.8	0.537	100%
R132H	0.605	171.7	0.6	0.0035	0.35%
R132S	95	>1e6	0.479	<9.5e7	<.001%

Both mutant enzymes display a reduced Hill coefficient and an increase in Km for isocitrate, suggesting a loss of co-operativity in substrate binding and/or reduced affinity for substrate. R132H enzyme also displays a reduced Vmax, suggestive of a lower kcat. R132S displays an increase in Vmax, suggesting an increase in kcat, although this comes at the expense of a 20,000 fold increase in Km so that the overall effect on catalytic efficiency is a great decrease as compared to the wild-type enzyme. The relative catalytic efficiency, described as Vmax/Km, is dramatically lower for the mutants as compared to wild-type. The *in vivo* effect of these mutations would be to decrease the flux conversion of isocitrate to α-KG.

C. The ICDH1 R132H and R132S mutants display reduced product inhibition in the oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG).

A well-known regulatory mechanism for control of metabolic enzymes is feedback inhibition, in which the product of the reaction acts as a negative regulator for the generating enzyme. To examine whether the R132S or R132H mutants maintain this regulatory mechanism, the Ki for α-KG in the oxidative decarboxylation of ioscitrate to α-ketoglutarate was determined. Data is presented in **FIGs. 13A-13C** and summarized in **Table 2**. In all cases, α-KG acts as a competitive inhibitor of the isocitrate substrate. However, R132H and R132S display a 20-fold and 13-fold increase in sensitivity to feedback inhibition as compared to the wild-type enzyme.

Table 2

Enzyme	**Ki (uM)**
Wt	612.2
R132H	28.6
R132S	45.3

D. The effect of $MnCl_2$ in oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG).

$MnCl_2$ can be substituted with $MgCl_2$ to examine if there is any difference in oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG).

E. The effect of R132 mutations on the inhibitory effect of oxalomalate on IDH1

The purpose of this example is to examine the susceptibility of IDH1R132S and IDH1R132H in oxidative decarboxylation of isocitrate to α-Ketoglutarate (α-KG) to the known IDH1 inhibitor oxalomalate. Experiments were performed to examine if R132 mutations circumvent the inhibition by oxalomalate.

Final concentrations: Tris 7.5 20 mM, NaCl 150 mM, $MnCl_2$ 2 mM, Glycerol 10%, BSA 0.03%, NADP 0.5 mM, IDH1 wt 1.5 ug/ml, IDH1R132S 30 ug/ml, IDH1R132H 60 ug/ml, DL-isocitrate (5 – 650 uM). The results are summarized in **FIG. 17** and Table 3. The R132S mutation displays approximately a two-fold increase in susceptibility to inhibition by oxalomalate, while the R132H mutation is essentially unaffected. In all three cases, the same fully competitive mode of inhibition with regards to isocitrate was observed.

Table 3

Enzyme	Oxalomalate Ki (uM)
wt	955.4
R132S	510
R132H	950.8

F. Forward reactions (isocitrate to α-KG) of mutant enzyme do not go to completion.

Forward reactions containing ICDH1 R132S or ICDH1 R132H were assembled and reaction progress monitored by an increase in the OD340 of the reduced NADPH cofactor. It was observed (**FIG. 23**), that these reactions proceed in the forward direction for a period of time and then reverse direction and oxidize the cofactor reduced in the early stages of the reaction, essentially to the starting concentration present at the initiation of the experiment. Addition of further isocitrate re-initiated the forward reaction for a period of time, but again did not induce the reaction to proceed to completion. Rather, the system returned to initial concentrations of NADPH. This experiment

suggested that the mutant enzymes were performing a reverse reaction other than the conversion of α-KG to isocitrate.

2. Analysis of IDH1 wild-type and mutants R132H and R132S in the reduction of α-Ketoglutarate (α-KG).

A. Methods

To determine the catalytic efficiency of enzymes in the reduction of α-Ketoglutarate (α-KG), reactions were performed to determine Vmax and Km for α-KG. In these reactions, substrate was varied while the cofactor was held constant at 500 uM. All reactions were performed in 50 mM potassium phosphate buffer, pH 6.5, 10% glycerol, 0.03% (w/v) BSA, 5 mM $MgCl_2$, and 40 mM sodium hydrocarbonate. Reaction progress was followed by spectroscopy at 340 nM monitoring the change in oxidation state of the cofactor. Sufficient enzyme was added to give a linear change in absorbance for 10 minutes.

B. The R132H and R132S mutant enzymes, but not the wild-type enzyme, support the reduction of α-KG.

To test the ability of the mutant and wild-type enzymes to perform the reduction of α-KG, 40 ug/ml of enzyme was incubated under the conditions for the reduction of α-Ketoglutarate (α-KG) as described above. Results are presented in **FIG. 14**. The wild-type enzyme was unable to consume NADPH, while R132S and R132H reduced α-KG and consumed NADPH.

C. The reduction of α-KG by the R132H and R132S mutants occurs *in vitro* at physiologically relevant concentrations of α-KG.

To determine the kinetic parameters of the reduction of α-KG performed by the mutant enzymes, a substrate titration experiment was performed, as presented in **FIGs. 15A-15B**. R132H maintained the Hill-type substrate interaction as seen in the oxidative decarboxylation of isocitrate, but displayed positive substrate co-operative binding. R132S showed a conversion to Michaelis-Menten kinetics with the addition of uncompetitive substrate inhibition, as compared to wild-type enzyme in the oxidative

decarboxylation of isocitrate. The enzymatic parameters of the mutant enzyme are presented in **Table 4**. Since the wild-type enzyme did not consume measurable NADPH in the experiment described above, a full kinetic workup was not performed.

Table 4

Enzyme	Vmax (umol/min/mg)	Km (mM)	Hill Constant	Ki (mM)	Vmax/Km
R132H	1.3	0.965	1.8		1.35
R132S	2.7	0.181	0.479	24.6	14.92

The relative catalytic efficiency of reduction of α-KG is approximately ten-fold higher in the R132S mutant than in the R132H mutant. The biological consequence is that the rate of metabolic flux should be greater in cells expressing R132S as compared to R132H.

D. Analysis of IDH1 wild-type and mutants R132H and R132S in the reduction of alpha-ketoglutarate with NADH.

In order to evaluate the ability of the mutant enzymes to utilize NADH in the reduction of alpha-ketoglutarate, the following experiment was conducted. Final concentrations: NaHCO3 40mM, MgCl2 5mM, Glycerol 10%, K2HPO4 50mM, BSA 0.03%, NADH 0.5mM, IDH1wt 5ug/ml, R132S 30ug/ml, R132H 60ug/ml, alpha-Ketoglutarate 5mM.

The results are shown in **FIG. 16** and **Table 5**. The R132S mutant demonstrated the ability to utilize NADH while the wild type and R132H show no measurable consumption of NADH in the presence of alpha-ketoglutarate.

Table 5: Consumption of NADH by R132S in the presence of alpha-ketoglutarate

	R132S		Mean	SD
Rate (ΔA/sec)	0.001117	0.001088	0.001103	2.05E-05
Umol/min/mg	0.718328	0.699678	**0.709003**	0.013187

Summary

To understand how R132 mutations alter the enzymatic properties of IDH1, wild-type and R132H mutant IDH1 proteins were produced and purified from *E. coli*. When $NADP^+$-dependent oxidative decarboxylation of isocitrate was measured using purified wild-type or R132H mutant IDH1 protein, it was confirmed that R132H mutation impairs the ability of IDH1 to catalyze this reaction (Yan, H. et al. N Engl J Med 360, 765-73 (2009); Zhao, S. et al. Science 324, 261-5 (2009)), as evident by the loss in binding affinity for both isocitrate and $MgCl_2$ along with a 1000-fold decrease in catalytic turnover (**FIGs. 31A** and **31C**). In contrast, when NADPH-dependent reduction of αKG was assessed using either wild-type or R132H mutant IDH1 protein, only R132H mutant could catalyze this reaction at a measurable rate (**FIGs. 31B** and **31C**). Part of this increased rate of αKG reduction results from an increase in binding affinity for both the cofactor NADPH and substrate αKG in the R132H mutant IDH1 (**FIG. 31C**). Taken together, these data demonstrate that while the R132H mutation leads to a loss of enzymatic function for oxidative decarboxylation of isocitrate, this mutation also results in a gain of enzyme function for the NADPH-dependent reduction of αKG.

2: Analysis of mutant IDH1

Expression of R132H mutant IDH1 results in αKG-dependent NADPH consumption

The R132H mutation has been reported to result in loss of function for enzyme activity (Yan, H. et al. N Engl J Med 360, 765-73 (2009); Zhao, S. et al. Science 324, 261-5 (2009)). However, in these studies only the $NADP^+$-dependent oxidative decarboxylation of isocitrate to αKG was assessed. To understand how IDH1 activity is altered in cells by the presence of R132H mutant IDH1, increasing amounts of wild-type and R132H mutant IDH1 were expressed separately or in combination and assessed isocitrate-dependent NADPH production and αKG-dependent NADPH consumption in cell lysates. Consistent with published results, expression of R132H mutant IDH1 resulted in no measurable production of NADPH from isocitrate, and isocitrate-dependent NADPH production increased with increasing amounts of wild-type enzyme (**FIGs. 30A** and **30B**). The ability of the wild-type enzyme to generate NADPH was decreased slightly by co-expression of the R132H mutant IDH1. Opposite results were obtained, however, when NADPH consumption was measured in the presence of αKG.

NADPH consumption by wild-type enzyme was not observed, while R132H mutant IDH1 expression resulted in αKG-dependent NADPH consumption (**FIG. 30C**). If anything, co-expression of wild-type IDH1 with R132H mutant IDH1 facilitated the αKG-dependent consumption of NADPH. These findings demonstrate that in contrast to wild-type IDH1, R132H mutant IDH1 promotes an NADPH-dependent reduction of αKG. Furthermore, as this reduction was not inhibited by co-expression of wild-type IDH1, these data suggest that the novel activity of mutant IDH1 can persist even in the presence of a wild-type IDH1 allele. In fact, it is possible that in the case of a heterodimer of wild-type and mutant IDH1, the αKG and NADPH produced locally by the wild-type subunit could be used as substrates for the mutant subunit explaining the decrease in net NADPH production when wild-type and mutant IDH1 are co-expressed.

The R132H mutant does not result in the conversion of α-KG to isocitrate.

Using standard experimental methods, an API2000 mass spectrometer was configured for optimal detection of α-KG and isocitrate (Table 6). MRM transitions were selected and tuned such that each analyte was monitored by a unique transition. Then, an enzymatic reaction containing 1 mM α-KG, 1 mM NADPH, and ICDH1 R132H were assembled and run to completion as judged by the decrease to baseline of the optical absorbance at 340 nM. A control reaction was performed in parallel from which the enzyme was omitted. Reactions were quenched 1:1 with methanol, extracted, and subjected to analysis by LC-MS/MS.

FIG. 18A presents the control reaction indicating that aKG was not consumed in the absence of enzyme, and no detectable isocitrate was present. **FIG. 18B** presents the reaction containing R132H enzyme, in which the α-KG has been consumed, but no isocitrate was detected. **FIG. 18C** presents a second analysis of the reaction containing enzyme in which isocitrate has been spiked to a final concentration of 1 mM, demonstrating that had α-KG been converted to isocitrate at any appreciable concentration greater than 0.01%, the configured analytical system would have been capable of detecting its presence in the reaction containing enzyme. The conclusion from this experiment is that while α-KG was consumed by R132H, isocitrate was not produced.

This experiment indicates that one neoactivity of the R132H mutant is the reduction of α-KG to a compound other than isocitrate.

Table 6. Instrument settings for MRM detection of compounds

Compound	Q1	Q3	DP	FP	EP	CEP	CE	CXP
α-KG	144.975	100.6	-6	-220	-10	-16	-10	-22
isocitrate	191.235	110.9	-11	-230	-4.5	-14	-16	-24
a-hydroxyglutarate	147.085	128.7	-11	-280	-10	-22	-12	-24

The R132H mutant reduces α-KG to 2-hydroxyglutaric acid.

Using standard experimental methods, an API2000 mass spectrometer was configured for optimal detection 2-hydroxyglutarate (**Table 6** and **FIG. 19**). The reaction products of the control and enzyme-containing reactions from above were investigated for the presence of 2-hydroxyglutaric acid, **FIG. 20**. In the control reaction, no 2-hydroxyglutaric acid was detected, while in reaction containing R132H, 2-hydroxyglutaric acid was detected. This data confirms that one neoactivity of the R132H mutant is the reduction of α-KG to 2-hydroxyglutaric acid.

To determine whether R132H mutant protein directly produced 2HG from αKG, the product of the mutant IDH1 reaction was examined using negative ion mode triple quadrupole electrospray LC-MS. These experiments confirmed that 2HG was the direct product of NADPH-dependent αKG reduction by the purified R132H mutant protein through comparison with a known metabolite standards (**FIG. 32A**). Conversion of αKG to isocitrate was not observed.

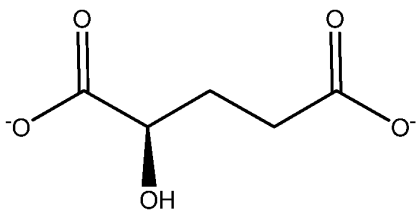
One can determine the enantiomeric specificity of the reaction product through derivitazation with DATAN (diacetyl-L-tartaric acid) and comparing the retention time to that of known R and S standards. This method is described in Struys *et al*. Clin Chem 50:1391-1395(2004). The stereo-specific production of either the R or S enantomer of alpha-hydroxyglutaric acid by ICDH1 R132H may modify the biological activity of other enzymes present in the cell. The racemic production may also occur.

For example, one can measure the inhibitory effect of alpha-hydroxyglutaric acid on the enzymatic activity of enzymes which utilize α-KG as a substrate. In one

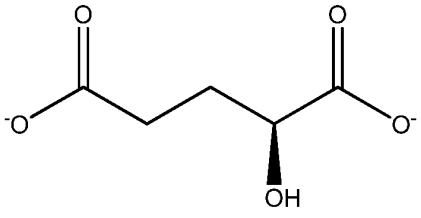
embodiment, alpha-hydroxyglutaric acid may be a substrate- or product- analogue inhibitor of wild-type ICDH1. In another embodiment alpha-hydroxyglutaric acid may be a substrate- or product- analogue inhibitor of HIF1 prolyl hydroxylase. In the former case, inhibition of wild type ICDH1 by the enzymatic product of R132H will reduce the circulating levels of aKG in the cell. In the latter case, inhibition of HIF1 prolyl hydroxylase will result in the stabilization of HIF1 and an induction of the hypoxic response cohort of cellular responses.

ICDH R132H reduces aKG to the R-enantiomer of 2-hydroxyglutarate.

There are two possible enantiomers of the ICDHR132H reductive reaction product, converting alpha-ketoglutarate to 2-hydroxyglutarate, with the chiral center being located at the alpha-carbon position. Exemplary products are depicted below.



R-2-hydroxyglutarate

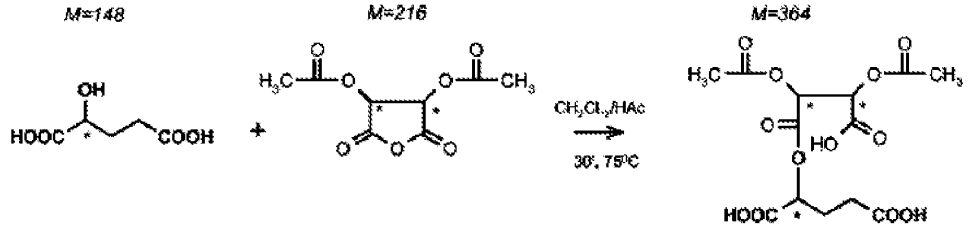


S-2-hydroxyglutarate

These are referred to by those with knowledge in the art as the R (or pro-R) and S (or pro-S) enantiomers, respectively. In order to determine which form or both is produced as a result of the ICDH1 neoactivity described above, the relative amount of each chiral form in the reaction product was determined in the procedure described below.

Reduction of α-KG to 2-HG was performed by ICDHR132H in the presence of NADPH as described above, and the reaction progress was monitored by a change in extinction coefficient of the nucleotide cofactor at 340 nM; once the reaction was judged to be complete, the reaction was extracted with methanol and dried down completely in a stream of nitrogen gas. In parallel, samples of chirally pure R-2-HG and a racemic mixture of R- and S-2-HG (produced by a purely chemical reduction of α-KG to 2-HG) were resuspended in ddH_2O, similarly extracted with methanol, and dried.

The reaction products or chiral standards were then resuspended in a solution of dichloromethane:acetic acid (4:1) containing 50 g/L DATAN and heated to 75°C for 30 minutes to promote the derivitization of 2-HG in the scheme described below:



After cooling to room temperature, the derivitization reactions were dried to completion and resuspended in ddH_2O for analysis on an LC-MS/MS system. Analysis of reaction products and chiral standards was performed on an API2000 LC-MS/MS system using a 2 x 150 mM C18 column with an isocratic flow of 200 µl/min of 90:10 (ammonium formate, pH 3.6:methanol) and monitoring the retention times of the 2-HG-DATAN complex using XIC and the diagnostic MRM transition of 363/147 in the negative ion mode.

It should be noted that retention times in the experiments described below are approximate and accurate to within +/- 1 minute; the highly reproducible peak seen at 4 minutes is an artefact of a column switching valve whose presence has no result on the conclusions drawn from the experiment.

Injection of the racemic mixture gave two peaks of equal area at retention times of 8 and 10 minutes (**FIG. 24A**), while injection of the R-2-HG standard resulted in a major peak of >95% area at 10 minutes and a minor peak <5% area at 8 minutes (**FIG. 24B**); indicating that the R-2-HG standard is approximately 95% R and 5% S. Thus, this method allows us to separate the R and S-2-HG chiral forms and to determine the relative amounts of each in a given sample. Coinjection of the racemic mixture and the R-2-HG standard resulted in two peaks at 8 and 10 minutes, with a larger peak at 10 minutes resulting from the addition of surplus pro-R-form (the standard) to a previously equal

mixture of R- and S-2-HG (**FIG. 24C**). These experiments allow us to assign the 8 minute peak to the S-2-HG form and the 10 minute peak to the R-2-HG form.

Injection of the derivatized neoactivity enzyme reaction product alone yields a single peak at 10 minutes, suggesting that the neoactivity reaction product is chirally pure R-2-HG (**FIG. 24D**). Coinjection of the neoactivity reaction product with the R-2-HG standard results in a major peak of >95% area at 10 minutes (**FIG. 24E**) and a single minor peak of <5% area at 8 minutes (previously observed in injection of the R-2-HG standard alone) confirming the chirality of the neoactivity product as R. Coinjection of a racemic mixture and the neoactivity reaction product (**FIG. 24F**) results in a 60% area peak at 10 minutes and a 40% area peak at 8 minutes; this deviation from the previously symmetrical peak areas observed in the racemate sample being due to the excess presence of R-2-HG form contributed by the addition of the neoactivity reaction product. These experiments allow us to conclude that the ICDH1 neoactivity is a highly specific chiral reduction of α-KG to R-2-HG.

Enzyme properties of other IDH1 mutations

To determine whether the altered enzyme properties resulting from R132H mutation were shared by other R132 mutations found in human gliomas, recombinant R132C, R132L and R132S mutant IDH1 proteins were generated and the enzymatic properties assessed. Similar to R132H mutant protein, R132C, R132L, and R132S mutations all result in a gain-of-function for NADPH-dependent reduction of αKG (data not shown). Thus, in addition to impaired oxidative decarboxylation of isocitrate, one common feature shared among the IDH1 mutations found in human gliomas is the ability to catalyze direct NADPH-dependent reduction of αKG.

Identification of 2-HG production in glioblastoma cell lines containing the IDH-1 R132H mutant protein.

Generation of genetic engineered glioblastoma cell lines expressing wildtype or mutant IDH-1 protein. A carboxy-terminal Myc-DDK-tagged open reading frame (ORF) clone of human isocitrate dehydrogenase 1 (IDH1; Ref. ID: NM_005896) cloned in vector pCMV6 was obtained from commercial vendor Origen Inc. Vector pCMV6

contains both kanamycin and neomycin resistance cassettes for selection in both bacterial and mammalian cell systems. Standard molecular biology mutagenesis techniques were utilized to alter the DNA sequence at base pair 364 of the ORF to introduce base pair change from guanine to adenine resulting in a change in the amino acid code at position 132 from argentine (wt) to histidine (mutant; or R132H). Specific DNA sequence alteration was confirmed by standard methods for DNA sequence analysis. Parental vector pCMV6 (no insert), pCMV6-wt IDH1 or pCMV6-R132H were transfected into immortalized human glioblastoma cell lines ATCC® CRL-2610 (LN-18) or HTB-14 (U-87) in standard growth medium (DMEM; Dulbecco's modified Eagles Medium containing 10 % fetal bovine serum). Approximately 24 hrs after transfection, the cell cultures were transitioned to DMEM containing G418 sodium salt at concentrations of either 750 ug/ml (CRL-2610) or 500 ug/ml (HTB-14) to select those cells in culture that expressed the integrated DNA cassette expressing both the neomycin selectable marker and the ORF for human wild type or R132H. Pooled populations of G418 resistant cells were generated and expression of either wild type IDH1 or R132 IDH1 was confirmed by standard Western blot analysis of cell lysates using commercial antibodies recognizing either human IDH1 antigen or the engineered carboxy-terminal MYC-DDK expression tag. These stable clonal pools were then utilized for metaobolite preparation and analysis.

Procedure for metabolite preparation and analysis. Glioblastoma cell lines (CRL-2610 and HTB-14) expressing wildtype or mutant IDH-1 protein were grown using standard mammalian tissue culture techniques on DMEM media containing 10% FCS, 25 mM glucose, 4 mM glutamine, and G418 antibiotic (CRL-2610 at 750 ug/mL; HTB-14 at 500 ug/mL) to insure ongoing selection to preserve the transfected mutant expression sequences. In preparation for metabolite extraction experiments, cells were passaged into 10 cm round culture dishes at a density of $1x10^6$ cells. Approximately 12 hours prior to metabolite extraction, the culture media was changed (8 mL per plate) to DMEM containing 10% dialyzed FCS (10,000 mwco), 5 mM glucose, 4 mM glutamine, and G-418 antibiotic as before; the dialyzed FCS removes multiple small molecules form the culture media and enables cell culture-specific assessment of metabolite levels. The media was again changed 2 horrs prior to metabolite extraction. Metabolite extraction was accomplished by quickly aspirating the media from the culture dishes in a sterile

hood, immediately placing the dishes in a tray containing dry ice to cool them to -80°C, and as quickly as possible, adding 2.6 mL of 80% MeOH/20% water, pre-chilled to -80°C in a dry-ice/acetone bath. These chilled, methanol extracted cells were then physically separated from the culture dish by scraping with a sterile polyethylene cell lifter (Corning #3008), brought into suspension and transferred to a 15 mL conical vial, then chilled to -20°C. An additional 1.0 mL of 80% MeOH/20% water was applied to the chilled culture dish and the cell lifting procedure repeated, to give a final extraction volume of 3.6 mL. The extracts were centrifuged at 20,000 x g for 30 minutes to sediment the cell debris, and 3.0 mL of the supernatants was transferred to a screw-cap freezer vial and stored at -80°C until ready for analysis.

In preparation for analysis, the extracts were removed from the freezer and dried on a nitrogen blower to remove methanol. The 100% aqueous samples were analyzed by LCMS as follows. The extract (10 µL) was injected onto a reverse-phase HPLC column (Synergi 150mm x 2 mm, Phenomenex Inc.) and eluted using a linear gradient of LCMS-grade methanol (Buffer B) in Aq. 10 mM tributylamine , 15 mM Acetic acid (Buffer A), running from 3% Buffer B to 95% Buffer B over 45 minutes at 200 µL/min. Eluted metabolite ions were detected using a triple-quadrapole mass spectrometer, tuned to detect in negative mode with multiple-reaction-monitoring mode transition set (MRM's) according to the molecular weights and fragmentation patterns for 38 known central metabolites, including 2-hydroxyglutarate (MRM parameters were optimized by prior infusion of known compound standards). Data was processed using Analyst Software (Applied Biosystems, Inc.) and metabolite signal intensities were converted into absolute concentrations using signal build-up curves from injected mixtures of metabolite standards at known concentrations. Final metabolite concentrations were reported as mean of at least three replicates, +/- standard deviation.

Results. Analyses reveal significantly higher levels of 2-HG in cells that express the IDH-1 R132H mutant protein. As shown in **FIG. 26A**, levels of 2-HG in CRL-2610 cell lines expressing the IDH-1 R132H mutant protein are approximately 28-fold higher than identical lines expressing the wild-type protein. Similarly, levels of 2-HG in HTB-14 cell lines expressing the IDH-1 R132H mutant protein are approximately 38-fold higher than identical lines expressing the wild-type protein, as shown in **FIG. 26B**.

Evaluation of 2-hydroxyglutarate (2-HG) production in human glioblastoma tumors containing mutations in isocitrate dehydrogenase 1 (IDH1) at amino acid 132.

Heterozygous somatic mutations at nucleotide position 395 (amino acid codon 132) in the transcript encoding isocitrate dehydrogenase 1(IDH1) can occur in brain tumors.

***Tissue source*:** Human brain tumors were obtained during surgical resection, flash frozen in liquid nitrogen and stored at -80°C. Clinical classification of the tissue as gliomas was performed using standard clinical pathology categorization and grading.

Genomic sequence analysis to identify brain tumor samples containing either wild type isocitrate dehydrogenase (IDH1) or mutations altering amino acid 132. Genomic DNA was isolated from 50-100 mgs of brain tumor tissue using standard methods. A polymerase chain reaction (PCR) procedure was then performed on the isolated genomic DNA to amplify a 295 base pair fragment of the genomic DNA that contains both intron and 2nd exon sequences of human IDH1 (**FIG. 27**). In **FIG. 27**, intron sequence is shown in lower case font; 2nd exon IDH1 DNA sequence is shown in upper case font; forward (5') and reverse (3') primer sequences are shown in underlined font; guanine nucleotide mutated in a subset of human glioma tumors is shown in bold underlined font.

The amplified DNA fragment was then sequenced using standard protocols and sequence alignments were performed to classify the sequences as either wild type or mutant at the guanine nucleotide at base pair 170 of the amplified PCR fragment. Tumors were identified that contained genomic DNA having either two copies of guanine (wild type) or a mixed or monoalellic combination of one IDH1 allele containing guanine and the other an adenine (mutant) sequence at base pair 170 of the amplified product (**Table 15**). The nucleotide change results in a change at amino acid position 132 of human IDH1 protein from arginine (wild type) to histidine (mutant) as has been previously reported.

Table 15. Sequence variance at base pair 170 of the amplified genomic DNA from human glioma samples.

Sample ID	Base 170	IDH1 Amino Acid 132	Genotype
1102	G	arginine	wild type
1822	A	histidine	mutant
496	G	arginine	wild type
1874	A	histidine	mutant
816	A	histidine	mutant
534	G	arginine	wild type
AP-1	A	histidine	mutant
AP-2	A	histidine	mutant

Procedure for metabolite preparation and analysis. Metabolite extraction was accomplished by adding a 10 X volume (m/v ratio) of -80 C methanol:water mix (80%:20%) to the brain tissue (approximately 100mgs) followed by 30 s homogenization at 4 C. These chilled, methanol extracted homogenized tissues were then centrifuged at 14,000 rpm for 30 minutes to sediment the cellular and tissue debris and the cleared tissue supernatants were transferred to a screw-cap freezer vial and stored at -80℃. For analysis, a 2X volume of tributylamine (10 mM) acetic acid (10 mM) pH 5.5 was added to the samples and analyzed by LCMS as follows. Sample extracts were filtered using a Millex-FG 0.20 micron disk and 10 µL were injected onto a reverse-phase HPLC column (Synergi 150mm x 2 mm, Phenomenex Inc.) and eluted using a linear gradient LCMS-grade methanol (50%) with 10 mM tributylamine and 10 mM acetic acid) ramping to 80 % methanol:10 mM tributylamine: 10 mM acetic acid over 6 minutes at 200 µL/min. Eluted metabolite ions were detected using a triple-quadrapole mass spectrometer, tuned to detect in negative mode with multiple-reaction-monitoring mode transition set (MRM's) according to the molecular weights and fragmentation patterns for 8 known central metabolites, including 2-hydroxyglutarate (MRM parameters were optimized by prior infusion of known compound standards). Data was processed using Analyst Software (Applied Biosystems, Inc.) and metabolite signal intensities were obtained by standard peak integration methods.

Results. Analyses revealed dramatically higher levels of 2-HG in cells tumor samples that express the IDH-1 R132H mutant protein. Data is summarized in **Table 16** and **FIG. 28**.

Table 16

Genotype/sample ID	2 HG
wt (1102)	7700
mut (1822)	916000
wt (496)	5440
mut (1874)	1090000
mut (816)	1990000
wt (534)	6030
mut/AP-1	589000
mut/AP-2	200000

To determine if 2HG production is characteristic of tumors harboring mutations in IDH1, metabolites were extracted from human malignant gliomas that were either wild-type or mutant for IDH1. It has been suggested that αKG levels are decreased in cells transfected with mutant IDH1 (Zhao, S. et al. Science 324, 261-5 (2009)). The average αKG level from 12 tumor samples harboring various R132 mutations was slightly less than the average αKG level observed in 10 tumors which are wild-type for IDH1. This difference in αKG was not statistically significant, and a range of αKG levels was observed in both wild-type and mutant tumors. In contrast, increased 2HG levels were found in all tumors that contained an R132 IDH1 mutation. All R132 mutant IDH1 tumors examined had between 5 and 35 μmol of 2HG per gram of tumor, while tumors with wild-type IDH1 had over 100 fold less 2HG. This increase in 2HG in R132 mutant tumors was statistically significant ($p<0.0001$). It was confirmed that (R)-2HG was the isomer present in tumor samples (data not shown). Together these data establish that the novel enzymatic activity associated with R132 mutations in IDH1 results in the production of 2HG in human brain tumors that harbor these mutations.

2HG is known to accumulate in the inherited metabolic disorder 2-hydroxyglutaric aciduria. This disease is caused by deficiency in the enzyme 2-hydroxyglutarate dehydrogenase, which converts 2HG to αKG (Struys, E. A. et al. Am J Hum Genet 76, 358-60 (2005)). Patients with 2-hydroxyglutarate dehydrogenase deficiencies accumulate 2HG in the brain as assessed by MRI and CSF analysis, develop leukoencephalopathy, and have an increased risk of developing brain tumors (Aghili, M.,

Zahedi, F. & Rafiee, J Neurooncol 91, 233-6 (2009); Kolker, S., Mayatepek, E. & Hoffmann, G. F. Neuropediatrics 33, 225-31 (2002); Wajner, M., Latini, A., Wyse, A. T. & Dutra-Filho, C. S. J Inherit Metab Dis 27, 427-48 (2004)). Furthermore, elevated brain levels of 2HG result in increased ROS levels (Kolker, S. et al. Eur J Neurosci 16, 21-8 (2002); Latini, A. et al. Eur J Neurosci 17, 2017-22 (2003)), potentially contributing to an increased risk of cancer. The ability of 2HG to act as an NMDA receptor agonist may contribute to this effect (Kolker, S. et al. Eur J Neurosci 16, 21-8 (2002)). 2HG may also be toxic to cells by competitively inhibiting glutamate and/or αKG utilizing enzymes. These include transaminases which allow utilization of glutamate nitrogen for amino and nucleic acid biosynthesis, and αKG-dependent prolyl hydroxylases such as those which regulate Hif1α levels. Alterations in Hif1α have been reported to result from mutant IDH1 protein expression (Zhao, S. et al. Science 324, 261-5 (2009)). Regardless of mechanism, it appears likely that the gain-of-function ability of cells to produce 2HG as a result of R132 mutations in IDH1 contributes to tumorigenesis. Patients with 2-hydroxyglutarate dehydrogenase deficiency have a high risk of CNS malignancy (Aghili, M., Zahedi, F. & Rafiee, E. J Neurooncol 91, 233-6 (2009)). The ability of mutant IDH1 to directly act on αKG may explain the prevalence of IDH1 mutations in tumors from CNS tissue, which are unique in their high level of glutamate uptake and its ready conversion to αKG in the cytosol (Tsacopoulos, M. J Physiol Paris 96, 283-8 (2002)), thereby providing high levels of substrate for 2HG production. The apparent co-dominance of the activity of mutant IDH1 with that of the wild-type enzyme is consistent with the genetics of the disease, in which only a single copy of the gene is mutated. As discussed above, the wild-type IDH1 could directly provide NADPH and αKG to the mutant enzyme. These data also demonstrate that mutation of R132 to histidine, serine, cysteine, glycine or leucine share a common ability to catalyze the NADPH-dependent conversion of αKG to 2HG. These findings help clarify why mutations at other amino acid residues of IDH1, including other residues essential for catalytic activity, are not found. Finally, these findings have clinical implications in that they suggest that 2HG production will identify patients with IDH1 mutant brain tumors. This will be important for prognosis as patients with IDH1 mutations live longer than patients with gliomas characterized by other mutations (Parsons, D. W. et al. Science 321,

1807-12 (2008)). In addition, patients with lower grade gliomas may benefit by the therapeutic inhibition of 2HG production. Inhibition of 2HG production by mutant IDH1 might slow or halt conversion of lower grade glioma into lethal secondary glioblastoma, changing the course of the disease.

The reaction product of ICDH1 R132H reduction of α-KG inhibits the oxidative decarboxylation of isocitrate by wild-type ICDH1.

A reaction containing the wild-type ICDH1, NADP, and α-KG was assembled (under conditions as described above) to which was added in a titration series either (R)-2-hydroxyglutarate or the reaction product of the ICDH1 R1321H mutant reduction of α-KG to 2-hydroxyglutarate. The reaction product 2-HG was shown to inhibit the oxidative decarboxylation of isocitrate by the wild-type ICDH1, while the (R)-2-hydroxyglutarate did not show any effect on the rate of the reaction. Since there are only two possible chiral products of the ICDH1 R132H mutant reduction of α-KG to 2-HG, and the (R)-2-HG did not show inhibition in this assay, it follows that the product of the mutant reaction is the (S)-2-HG form. This experiment is presented in **FIG. 25**.

To determine the chirality of the 2HG produced, the products of the R132H reaction was derivatized with diacetyl-L-tartaric anhydride, which allowed separating the (S) and (R) enantiomers of 2HG by simple reverse-phase LC and detecting the products by tandem mass spectrometry (Struys, E. A., Jansen, E. E., Verhoeven, N. M. & Jakobs, C. Clin Chem 50, 1391-5 (2004)) (**FIG. 32B**). The peaks corresponding to the (S) and (R) isomers of 2HG were confirmed using racemic and R(-)-2HG standards. The reaction product from R132H co-eluted with R(-)-2HG peak, demonstrating that the R(-) stereoisomer is the product produced from αKG by R132H mutant IDH1.

The observation that the reaction product of the mutant enzyme is capable of inhibiting a metabolic reaction known to occur in cells suggests that this reaction product might also inhibit other reactions which utilize α-KG, isocitrate, or citrate as substrates or produce them as products in vivo or in vitro.

EXAMPLE 3 METABOLOMICS ANALYSIS OF IDH1 WILD TYPE AND MUTANTS

Metabolomics research can provide mechanistic basis for why R132 mutations confer survival advantage for GBM patients carrying such mutations.

1. Metabolomics of GBM tumor cell lines: wild type vs R132 mutants

Cell lines with R132 mutations can be identified and profiled. Experiments can be performed in proximal metabolite pool with a broad scope of metabolites.

2. Oxalomalate treatment of GBM cell lines

Oxalomalate is a competitive inhibitor of IDH1. Change of NADPH (metabolomics) when IDH1 is inhibited by a small molecule can be examined.

3. Metabolomics of primary GBM tumors: wild type vs R132 mutations

Primary tumors with R132 mutations can be identified. Experiments can be performed in proximal metabolite pool with a broad scope of metabolites.

4. Detection of 2-hydroxyglutarate in cells that overexpress IDH1 132 mutants

Overexpression of an IDH1 132 mutant in cells may cause an elevated level of 2-hydroxyglutarate and/or a reduced level of alpha-ketoglutarate. One can perform a metabolomic experiment to demonstrate the consequence of this mutation on the cellular metabolite pool.

EXAMPLE 4 EVALUATION OF IDH1 AS A CANCER TARGET

shRNAmir inducible knockdown can be performed to examine the cellular phenotype and metabolomics profiles. HTS grade IDH1 enzymes are available. The IDH mutations described herein can be used for patient selection.

EXAMPLE 5 siRNAs

Exemplary siRNAs are presented in the following tables. Art-known methods can be used to select other siRNAs. siRNAs can be evaluated, *e.g.*, by determining the ability of an siRNA to silence an IDH, *e.g.*, IDH1, *e.g.*, in an *in vitro* system, *e.g.*, in cultured cells, *e.g.*, HeLa cells or cultured glioma cells. siRNAs known in the art for silencing the target can also be used, see, *e.g.*, *Silencing of cytosolic NADP+ dependent isoccitrate*

dehydrogenase by small interfering RNA enhances the sensitivity of HeLa cells toward stauropine, Lee *et al.*, 2009, Free Radical Research, 43: 165-173.

The siRNAs in **Table 7** (with the exception of entry 1356) were generated using the siRNA selection tool available on the worldwide web at jura.wi.mit.edu/bioc/siRNAext/. (Yuan *et al.* Nucl. Acids. Res. 2004 32:W130-W134.) Other selection tools can be used as well. Entry 1356 was adapted from *Silencing of cytosolic NADP+ dependent isoccitrate dehydrogenase by small interfering RNA enhances the sensitivity of HeLa cells toward stauropine*, Lee *et al.*, 2009, Free Radical Research, 43: 165-173.

The siRNAs in Tables 7, 8, 9, 10, 11, 12, 13 and 14 represent candidates spanning the IDH1 mRNA at nucleotide positions 628 and 629 according to the sequence at GenBank Accession No. NM_005896.2 (SEQ ID NO:9, FIG. 22).

The RNAs in the tables can be modified, *e.g.*, as described herein. Modifications include chemical modifications to enhance properties, *e.g.*, resistance to degradation, or the use of overhangs. For example, either one or both of the sense and antisense strands in the tables can include an additional dinucleotide at the 3' end, *e.g.*, TT, UU, dTdT.

Table 7. siRNAs targeting wildtype IDH1

Position on mRNA (FIG. 22)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
13	GGUUUCUGCAGAGUCUACU		AGUAGACUCUGCAGAAACC	
118	CUCUUCGCCAGCAUAUCAU		AUGAUAUGCUGGCGAAGAG	
140	GGCAGGCGAUAAACUACAU		AUGUAGUUUAUCGCCUGCC	
145	GCGAUAAACUACAUUCAGU		ACUGAAUGUAGUUUAUCGC	
199	GAAAUCUAUUCACUGUCAA		UUGACAGUGAAUAGAUUUC	
257	GUUCUGUGGUAGAGAUGCA		UGCAUCUCUACCACAGAAC	
272	GCAAGGAGAUGAAAUGACA		UGUCAUUUCAUCUCCUUGC	
277	GGAGAUGAAAUGACACGAA		UUCGUGUCAUUUCAUCUCC	
278	GAGAUGAAAUGACACGAAU		AUUCGUGUCAUUUCAUCUC	
280	GAUGAAAUGACACGAAUCA		UGAUUCGUGUCAUUUCAUC	
292	CGAAUCAUUUGGGAAUUGA		UCAAUUCCCAAAUGAUUCG	
302	GGGAAUUGAUUAAAGAGAA		UUCUCUUUAAUCAAUUCCC	
332	CCUACGUGGAAUUGGAUCU		AGAUCCAAUUCCACGUAGG	
333	CUACGUGGAAUUGGAUCUA		UAGAUCCAAUUCCACGUAG	
345	GGAUCUACAUAGCUAUGAU		AUCAUAGCUAUGUAGAUCC	
356	GCUAUGAUUUAGGCAUAGA		UCUAUGCCUAAAUCAUAGC	
408	GGAUGCUGCAGAAGCUAUA		UAUAGCUUCUGCAGCAUCC	
416	CAGAAGCUAUAAAGAAGCA		UGCUUCUUUAUAGCUUCUG	
418	GAAGCUAUAAAGAAGCAUA		UAUGCUUCUUUAUAGCUUC	
432	GCAUAAUGUUGGCGUCAAA		UUUGACGCCAACAUUAUGC	
467	CUGAUGAGAAGAGGGUUGA		UCAACCCUCUUCUCAUCAG	
481	GUUGAGGAGUUCAAGUUGA		UCAACUUGAACUCCUCAAC	
487	GAGUUCAAGUUGAAACAAA		UUUGUUUCAACUUGAACUC	
495	GUUGAAACAAAUGUGGAAA		UUUCCACAUUUGUUUCAAC	
502	CAAAUGUGGAAAUCACCAA		UUGGUGAUUUCCACAUUUG	
517	CCAAAUGGCACCAUACGAA		UUCGUAUGGUGCCAUUUGG	
528	CAUACGAAAUAUUCUGGGU		ACCCAGAAUAUUUCGUAUG	
560	GAGAAGCCAUUAUCUGCAA		UUGCAGAUAAUGGCUUCUC	
614	CUAUCAUCAUAGGUCGUCA		UGACGACCUAUGAUGAUAG	
618	CAUCAUAGGUCGUCAUGCU		AGCAUGACGACCUAUGAUG	
621	CAUAGGUCGUCAUGCUUAU		AUAAGCAUGACGACCUAUG	
691	GAGAUAACCUACACACCAA		UUGGUGUGUAGGUUAUCUC	
735	CCUGGUACAUAACUUUGAA		UUCAAAGUUAUGUACCAGG	
747	CUUUGAAGAAGGUGGUGGU		ACCACCACCUUCUUCAAAG	
775	GGGAUGUAUAAUCAAGAUA		UAUCUUGAUUAUACAUCCC	
811	GCACACAGUUCCUUCCAAA		UUUGGAAGGAACUGUGUGC	
818	GUUCCUUCCAAAUGGCUCU		AGAGCCAUUUGGAAGGAAC	

844	GGUUGGCCUUUGUAUCUGA		UCAGAUACAAAGGCCAACC	
851	CUUUGUAUCUGAGCACCAA		UUGGUGCUCAGAUACAAAG	
882	GAAGAAAUAUGAUGGGCGU		ACGCCCAUCAUAUUUCUUC	
942	GUCCCAGUUUGAAGCUCAA		UUGAGCUUCAAACUGGGAC	
968	GGUAUGAGCAUAGGCUCAU		AUGAGCCUAUGCUCAUACC	
998	GGCCCAAGCUAUGAAAUCA		UGAUUUCAUAGCUUGGGCC	
1001	CCCAAGCUAUGAAAUCAGA		UCUGAUUUCAUAGCUUGGG	
1127	CAGAUGGCAAGACAGUAGA		UCUACUGUCUUGCCAUCUG	
1133	GCAAGACAGUAGAAGCAGA		UCUGCUUCUACUGUCUUGC	
1184	GCAUGUACCAGAAAGGACA		UGUCCUUUCUGGUACAUGC	
1214	CCAAUCCCAUUGCUUCCAU		AUGGAAGCAAUGGGAUUGG	
1257	CCACAGAGCAAAGCUUGAU		AUCAAGCUUUGCUCUGUGG	
1258	CACAGAGCAAAGCUUGAUA		UAUCAAGCUUUGCUCUGUG	
1262	GAGCAAAGCUUGAUAACAA		UUGUUAUCAAGCUUUGCUC	
1285	GAGCUUGCCUUCUUUGCAA		UUGCAAAGAAGGCAAGCUC	
1296	CUUUGCAAAUGCUUUGGAA		UUCCAAAGCAUUUGCAAAG	
1301	CAAAUGCUUUGGAAGAAGU		ACUUCUUCCAAAGCAUUUG	
1307	CUUUGGAAGAAGUCUCUAU		AUAGAGACUUCUUCCAAAG	
1312	GAAGAAGUCUCUAUUGAGA		UCUCAAUAGAGACUUCUUC	
1315	GAAGUCUCUAUUGAGACAA		UUGUCUCAAUAGAGACUUC	
1356	GGACUUGGCUGCUUGCAUU		AAUGCAAGCAGCCAAGUCC	
1359	CUUGGCUGCUUGCAUUAAA		UUUAAUGCAAGCAGCCAAG	
1371	CAUUAAAGGUUUACCCAAU		AUUGGGUAAACCUUUAAUG	
1385	CCAAUGUGCAACGUUCUGA		UCAGAACGUUGCACAUUGG	
1390	GUGCAACGUUCUGACUACU		AGUAGUCAGAACGUUGCAC	
1396	CGUUCUGACUACUUGAAUA		UAUUCAAGUAGUCAGAACG	
1415	CAUUUGAGUUCAUGGAUAA		UUAUCCAUGAACUCAAAUG	
1422	GUUCAUGGAUAAACUUGGA		UCCAAGUUUAUCCAUGAAC	
1425	CAUGGAUAAACUUGGAGAA		UUCUCCAAGUUUAUCCAUG	
1455	CAAACUAGCUCAGGCCAAA		UUUGGCCUGAGCUAGUUUG	
1487	CCUGAGCUAAGAAGGAUAA		UUAUCCUUCUUAGCUCAGG	
1493	CUAAGAAGGAUAAUUGUCU		AGACAAUUAUCCUUCUUAG	
1544	CUGUGUUACACUCAAGGAU		AUCCUUGAGUGUAACACAG	
1546	GUGUUACACUCAAGGAUAA		UUAUCCUUGAGUGUAACAC	
1552	CACUCAAGGAUAAAGGCAA		UUGCCUUUAUCCUUGAGUG	
1581	GUAAUUUGUUUAGAAGCCA		UGGCUUCUAAACAAAUUAC	
1646	GUUAUUGCCACCUUUGUGA		UCACAAAGGUGGCAAUAAC	
1711	CAGCCUAGGAAUUCGGUUA		UAACCGAAUUCCUAGGCUG	
1713	GCCUAGGAAUUCGGUUAGU		ACUAACCGAAUUCCUAGGC	
1714	CCUAGGAAUUCGGUUAGUA		UACUAACCGAAUUCCUAGG	
1718	GGAAUUCGGUUAGUACUCA		UGAGUACUAACCGAAUUCC	
1719	GAAUUCGGUUAGUACUCAU		AUGAGUACUAACCGAAUUC	

1725	GGUUAGUACUCAUUUGUAU		AUACAAAUGAGUACUAACC	
1730	GUACUCAUUUGUAUUCACU		AGUGAAUACAAAUGAGUAC	
1804	GGUAAAUGAUAGCCACAGU		ACUGUGGCUAUCAUUUACC	
1805	GUAAAUGAUAGCCACAGUA		UACUGUGGCUAUCAUUUAC	
1816	CCACAGUAUUGCUCCCUAA		UUAGGGAGCAAUACUGUGG	
1892	GGGAAGUUCUGGUGUCAUA		UAUGACACCAGAACUUCCC	
1897	GUUCUGGUGUCAUAGAUAU		AUAUCUAUGACACCAGAAC	
1934	GCUGUGCAUUAAACUUGCA		UGCAAGUUUAAUGCACAGC	
1937	GUGCAUUAAACUUGCACAU		AUGUGCAAGUUUAAUGCAC	
1939	GCAUUAAACUUGCACAUGA		UCAUGUGCAAGUUUAAUGC	
1953	CAUGACUGGAACGAAGUAU		AUACUUCGUUCCAGUCAUG	
1960	GGAACGAAGUAUGAGUGCA		UGCACUCAUACUUCGUUCC	
1961	GAACGAAGUAUGAGUGCAA		UUGCACUCAUACUUCGUUC	
1972	GAGUGCAACUCAAAUGUGU		ACACAUUUGAGUUGCACUC	
1976	GCAACUCAAAUGUGUUGAA		UUCAACACAUUUGAGUUGC	
1982	CAAAUGUGUUGAAGAUACU		AGUAUCUUCAACACAUUUG	
1987	GUGUUGAAGAUACUGCAGU		ACUGCAGUAUCUUCAACAC	
1989	GUUGAAGAUACUGCAGUCA		UGACUGCAGUAUCUUCAAC	
2020	CCUUGCUGAAUGUUUCCAA		UUGGAAACAUUCAGCAAGG	
2021	CUUGCUGAAUGUUUCCAAU		AUUGGAAACAUUCAGCAAG	
2024	GCUGAAUGUUUCCAAUAGA		UCUAUUGGAAACAUUCAGC	
2035	CCAAUAGACUAAAUACUGU		ACAGUAUUUAGUCUAUUGG	
2067	GAGUUUGGAAUCCGGAAUA		UAUUCCGGAUUCCAAACUC	
2073	GGAAUCCGGAAUAAAUACU		AGUAUUUAUUCCGGAUUCC	
2074	GAAUCCGGAAUAAAUACUA		UAGUAUUUAUUCCGGAUUC	
2080	GGAAUAAAUACUACCUGGA		UCCAGGUAGUAUUUAUUCC	
2133	GGCCUGGCCUGAAUAUUAU		AUAAUAUUCAGGCCAGGCC	
2134	GCCUGAAUAUUAUACUACU		AGUAGUAUAAUAUUCAGGC	
2136	CUGGCCUGAAUAUUAUACU		AGUAUAAUAUUCAGGCCAG	
2166	CAUAUUUCAUCCAAGUGCA		UGCACUUGGAUGAAAUAUG	
2180	GUGCAAUAAUGUAAGCUGA		UCAGCUUACAUUAUUGCAC	
2182	GCAAUAAUGUAAGCUGAAU		AUUCAGCUUACAUUAUUGC	
2272	CACUAUCUUAUCUUCUCCU		AGGAGAAGAUAAGAUAGUG	
2283	CUUCUCCUGAACUGUUGAU		AUCAACAGUUCAGGAGAAG	

Table 8. siRNAs targeting wildtype IDH1

Position on mRNA (FIG. 22)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
611	AACCUAUCAUCAUAGGUCG		CGACCUAUGAUGAUAGGUU	
612	ACCUAUCAUCAUAGGUCGU		ACGACCUAUGAUGAUAGGU	
613	CCUAUCAUCAUAGGUCGUC		GACGACCUAUGAUGAUAGG	
614	CUAUCAUCAUAGGUCGUCA		UGACGACCUAUGAUGAUAG	
615	UAUCAUCAUAGGUCGUCAU		AUGACGACCUAUGAUGAUA	
616	AUCAUCAUAGGUCGUCAUG		CAUGACGACCUAUGAUGAU	
617	UCAUCAUAGGUCGUCAUGC		GCAUGACGACCUAUGAUGA	
618	CAUCAUAGGUCGUCAUGCU		AGCAUGACGACCUAUGAUG	
619	AUCAUAGGUCGUCAUGCUU		AAGCAUGACGACCUAUGAU	
620	UCAUAGGUCGUCAUGCUUA		UAAGCAUGACGACCUAUGA	
621	CAUAGGUCGUCAUGCUUAU		AUAAGCAUGACGACCUAUG	
622	AUAGGUCGUCAUGCUUAUG		CAUAAGCAUGACGACCUAU	
623	UAGGUCGUCAUGCUUAUGG		CCAUAAGCAUGACGACCUA	
624	AGGUCGUCAUGCUUAUGGG		CCCAUAAGCAUGACGACCU	
625	GGUCGUCAUGCUUAUGGGG		CCCCAUAAGCAUGACGACC	
626	GUCGUCAUGCUUAUGGGGA		UCCCAUAAGCAUGACGACC	
627	UCGUCAUGCUUAUGGGGAU		AUCCCAUAAGCAUGACGAC	

Table 9. siRNAs targeting G395A mutant IDH1 (SEQ ID NO:5) (equivalent to G629A of SEQ ID NO:9 (FIG. 22))

Position on mRNA (FIG. 2)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
611	AACCUAUCAUCAUAGGUCA		UGACCUAUGAUGAUAGGUU	
612	ACCUAUCAUCAUAGGUCAU		AUGACCUAUGAUGAUAGGU	
613	CCUAUCAUCAUAGGUCAUC		GAUGACCUAUGAUGAUAGG	
614	CUAUCAUCAUAGGUCAUCA		UGAUGACCUAUGAUGAUAG	
615	UAUCAUCAUAGGUCAUCAU		AUGAUGACCUAUGAUGAUA	
616	AUCAUCAUAGGUCAUCAUG		CAUGAUGACCUAUGAUGAU	
617	UCAUCAUAGGUCAUCAUGC		GCAUGAUGACCUAUGAUGA	
618	CAUCAUAGGUCAUCAUGCU		AGCAUGAUGACCUAUGAUG	
619	AUCAUAGGUCAUCAUGCUU		AAGCAUGAUGACCUAUGAU	

620	UCAUAGGUCAUCAUGCUUA		UAAGCAUGAUGACCUAUGA	
621	CAUAGGUCAUCAUGCUUAU		AUAAGCAUGAUGACCUAUG	
622	AUAGGUCAUCAUGCUUAUG		CAUAAGCAUGAUGACCUAU	
623	UAGGUCAUCAUGCUUAUGG		CCAUAAGCAUGAUGACCUA	
624	AGGUCAUCAUGCUUAUGGG		CCCAUAAGCAUGAUGACCU	
625	GGUCAUCAUGCUUAUGGGG		CCCCAUAAGCAUGAUGACC	
626	GUCAUCAUGCUUAUGGGGA		UCCCCAUAAGCAUGAUGAC	
627	UCAUCAUGCUUAUGGGGAU		AUCCCCAUAAGCAUGAUGA	

Table 10. siRNAs targeting C394A mutant IDH1 (SEQ ID NO:5) (equivalent to C628A of SEQ ID NO:9 (FIG. 22)) (Arg132Ser (SEQ ID NO:8))

Position on mRNA (FIG. 2)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
611	AACCUAUCAUCAUAGGUAG		CUACCUAUGAUGAUAGGUU	
612	ACCUAUCAUCAUAGGUAGU		ACUACCUAUGAUGAUAGGU	
613	CCUAUCAUCAUAGGUAGUC		GACUACCUAUGAUGAUAGG	
614	CUAUCAUCAUAGGUAGUCA		UGACUACCUAUGAUGAUAG	
615	UAUCAUCAUAGGUAGUCAU		AUGACUACCUAUGAUGAUA	
616	AUCAUCAUAGGUAGUCAUG		CAUGACUACCUAUGAUGAU	
617	UCAUCAUAGGUAGUCAUGC		GCAUGACUACCUAUGAUGA	
618	CAUCAUAGGUAGUCAUGCU		AGCAUGACUACCUAUGAUG	
619	AUCAUAGGUAGUCAUGCUU		AAGCAUGACUACCUAUGAU	
620	UCAUAGGUAGUCAUGCUUA		UAAGCAUGACUACCUAUGA	
621	CAUAGGUAGUCAUGCUUAU		AUAAGCAUGACUACCUAUG	
622	AUAGGUAGUCAUGCUUAUG		CAUAAGCAUGACUACCUAU	
623	UAGGUAGUCAUGCUUAUGG		CCAUAAGCAUGACUACCUA	
624	AGGUAGUCAUGCUUAUGGG		CCCAUAAGCAUGACUACCU	
625	GGUAGUCAUGCUUAUGGGG		CCCCAUAAGCAUGACUACC	
626	GUAGUCAUGCUUAUGGGGA		UCCCCAUAAGCAUGACUAC	
627	UAGUCAUGCUUAUGGGGAU		AUCCCCAUAAGCAUGACUA	

Table 11. siRNAs targeting C394U mutant IDH1 (SEQ ID NO:5) (equivalent to C628U of SEQ ID NO:9 (FIG. 22)) (Arg132Cys (SEQ ID NO:8))

Position on mRNA (FIG. 22)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
611	AACCUAUCAUCAUAGGUUG		CAACCUAUGAUGAUAGGUU	
612	ACCUAUCAUCAUAGGUUGU		ACAACCUAUGAUGAUAGGU	
613	CCUAUCAUCAUAGGUUGUC		GACAACCUAUGAUGAUAGG	
614	CUAUCAUCAUAGGUUGUCA		UGACAACCUAUGAUGAUAG	
615	UAUCAUCAUAGGUUGUCAU		AUGACAACCUAUGAUGAUA	
616	AUCAUCAUAGGUUGUCAUG		CAUGACAACCUAUGAUGAU	
617	UCAUCAUAGGUUGUCAUGC		GCAUGACAACCUAUGAUGA	
618	CAUCAUAGGUUGUCAUGCU		AGCAUGACAACCUAUGAUG	
619	AUCAUAGGUUGUCAUGCUU		AAGCAUGACAACCUAUGAU	
620	UCAUAGGUUGUCAUGCUUA		UAAGCAUGACAACCUAUGA	
621	CAUAGGUUGUCAUGCUUAU		AUAAGCAUGACAACCUAUG	
622	AUAGGUUGUCAUGCUUAUG		CAUAAGCAUGACAACCUAU	
623	UAGGUUGUCAUGCUUAUGG		CCAUAAGCAUGACAACCUA	
624	AGGUUGUCAUGCUUAUGGG		CCCAUAAGCAUGACAACCU	
625	GGUUGUCAUGCUUAUGGGG		CCCCAUAAGCAUGACAACC	
626	GUUGUCAUGCUUAUGGGGA		UCCCCAUAAGCAUGACAAC	
627	UUGUCAUGCUUAUGGGGAU		AUCCCCAUAAGCAUGACAA	

Table 12. siRNAs targeting C394G mutant IDH1 (SEQ ID NO:5) (equivalent to C628G of SEQ ID NO:9 (FIG. 22)) (Arg132Gly (SEQ ID NO:8))

Position on mRNA (FIG. 22)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
611	AACCUAUCAUCAUAGGUGG		CCACCUAUGAUGAUAGGUU	
612	ACCUAUCAUCAUAGGUGGU		ACCACCUAUGAUGAUAGGU	
613	CCUAUCAUCAUAGGUGGUC		GACCACCUAUGAUGAUAGG	
614	CUAUCAUCAUAGGUGGUCA		UGACCACCUAUGAUGAUAG	
615	UAUCAUCAUAGGUGGUCAU		AUGACCACCUAUGAUGAUA	
616	AUCAUCAUAGGUGGUCAUG		CAUGACCACCUAUGAUGAU	
617	UCAUCAUAGGUGGUCAUGC		GCAUGACCACCUAUGAUGA	
618	CAUCAUAGGUGGUCAUGCU		AGCAUGACCACCUAUGAUG	
619	AUCAUAGGUGGUCAUGCUU		AAGCAUGACCACCUAUGAU	
620	UCAUAGGUGGUCAUGCUUA		UAAGCAUGACCACCUAUGA	
621	CAUAGGUGGUCAUGCUUAU		AUAAGCAUGACCACCUAUG	

622	AUAGGUGGUCAUGCUUAUG		CAUAAGCAUGACCACCUAU	
623	UAGGUGGUCAUGCUUAUGG		CCAUAAGCAUGACCACCUA	
624	AGGUUGUCAUGCUUAUGGG		CCCAUAAGCAUGACCACCU	
625	GGUUGUCAUGCUUAUGGGG		CCCCAUAAGCAUGACCACC	
626	GUUGUCAUGCUUAUGGGGA		UCCCCAUAAGCAUGACCAC	
627	UUGUCAUGCUUAUGGGGAU		AUCCCCAUAAGCAUGACCA	

Table 13. siRNAs targeting G395C mutant IDH1 (SEQ ID NO:5) (equivalent to G629C of SEQ ID NO:9 (FIG. 22)) (Arg132Pro (SEQ ID NO:8))

Position on mRNA (FIG. 22)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
611	AACCUAUCAUCAUAGGUCG		CGACCUAUGAUGAUAGGUU	
612	ACCUAUCAUCAUAGGUCGU		ACGACCUAUGAUGAUAGGU	
613	CCUAUCAUCAUAGGUCGUC		GACGACCUAUGAUGAUAGG	
614	CUAUCAUCAUAGGUCGUCA		UGACGACCUAUGAUGAUAG	
615	UAUCAUCAUAGGUCGUCAU		AUGACGACCUAUGAUGAUA	
616	AUCAUCAUAGGUCGUCAUG		CAUGACGACCUAUGAUGAU	
617	UCAUCAUAGGUCGUCAUGC		GCAUGACGACCUAUGAUGA	
618	CAUCAUAGGUCGUCAUGCU		AGCAUGACGACCUAUGAUG	
619	AUCAUAGGUCGUCAUGCUU		AAGCAUGACGACCUAUGAU	
620	UCAUAGGUCGUCAUGCUUA		UAAGCAUGACGACCUAUGA	
621	CAUAGGUCGUCAUGCUUAU		AUAAGCAUGACGACCUAUG	
622	AUAGGUCGUCAUGCUUAUG		CAUAAGCAUGACGACCUAU	
623	UAGGUCGUCAUGCUUAUGG		CCAUAAGCAUGACGACCUA	
624	AGGUCGUCAUGCUUAUGGG		CCCAUAAGCAUGACGACCU	
625	GGUCGUCAUGCUUAUGGGG		CCCCAUAAGCAUGACGACC	
626	GUCGUCAUGCUUAUGGGGA		UCCCCAUAAGCAUGACGAC	
627	UCGUCAUGCUUAUGGGGAU		AUCCCCAUAAGCAUGACGA	

Table 14. siRNAs targeting G395U mutant IDH1 (SEQ ID NO:5) (equivalent to G629U of SEQ ID NO:9 (FIG. 22)) (Arg132Leu (SEQ ID NO:8))

Position on mRNA (FIG. 22)	sense (5' to 3')	SEQ ID NO:	antisense (5' to 3')	SEQ ID NO:
611	AACCUAUCAUCAUAGGUCU		AGACCUAUGAUGAUAGGUU	
612	ACCUAUCAUCAUAGGUCUU		AAGACCUAUGAUGAUAGGU	
613	CCUAUCAUCAUAGGUCUUC		GAAGACCUAUGAUGAUAGG	
614	CUAUCAUCAUAGGUCUUCA		UGAAGACCUAUGAUGAUAG	
615	UAUCAUCAUAGGUCUUCAU		AUGAAGACCUAUGAUGAUA	
616	AUCAUCAUAGGUCUUCAUG		CAUGAAGACCUAUGAUGAU	
617	UCAUCAUAGGUCUUCAUGC		GCAUGAAGACCUAUGAUGA	
618	CAUCAUAGGUCUUCAUGCU		AGCAUGAAGACCUAUGAUG	
619	AUCAUAGGUCUUCAUGCUU		AAGCAUGAAGACCUAUGAU	
620	UCAUAGGUCUUCAUGCUUA		UAAGCAUGAAGACCUAUGA	
621	CAUAGGUCUUCAUGCUUAU		AUAAGCAUGAAGACCUAUG	
622	AUAGGUCUUCAUGCUUAUG		CAUAAGCAUGAAGACCUAU	
623	UAGGUCUUCAUGCUUAUGG		CCAUAAGCAUGAAGACCUA	
624	AGGUCUUCAUGCUUAUGGG		CCCAUAAGCAUGAAGACCU	
625	GGUCUUCAUGCUUAUGGGG		CCCCAUAAGCAUGAAGACC	
626	GUCUUCAUGCUUAUGGGGA		UCCCCAUAAGCAUGAAGAC	
627	UCUUCAUGCUUAUGGGGAU		AUCCCCAUAAGCAUGAAGA	

EXAMPLE 6 STRUCTURAL ANALYSIS OF R132H MUTANT IDH1

To define how R132 mutations alter the enzymatic properties of IDH1, the crystal structure of R132H mutant IDH1 bound to αKG, NADPH, and Ca^{2+} was solved at 2.1 Å resolution.

The overall quaternary structure of the homodimeric R132H mutant enzyme adopts the same closed catalytically competent conformation (shown as a monomer in **FIG. 29A**) that has been previously described for the wild-type enzyme (Xu, X. et al. J Biol Chem 279, 33946-57 (2004)). NADPH is positioned as expected for hydride transfer to αKG in an orientation that would produce R(-)-2HG, consistent with our chiral determination of the 2HG product.

Two important features were noted by the change of R132 to histidine: the effect on catalytic conformation equilibrium and the reorganization of the active-site. Locating atop a β-sheet in the relatively rigid small domain, R132 acts as a gate-keeper residue and appears to orchestrate the hinge movement between the open and closed conformations. The guanidinium moiety of R132 swings from the open to the closed conformation with a distance of nearly 8 Å. Substitution of histidine for arginine is likely to change the equilibrium in favor of the closed conformation that forms the catalytic cleft for cofactor and substrate to bind efficiently, which partly explains the high-affinity for NADPH exhibited by the R132H mutant enzyme. This feature may be advantageous for the NADPH-dependent reduction of αKG to R(-)-2HG in an environment where NADPH concentrations are low. Secondly, closer examination of the catalytic pocket of the mutant IDH1 structure in comparison to the wild-type enzyme showed not only the expected loss of key salt-bridge interactions between the guanidinium of R132 and the α/β carboxylates of isocitrate, as well as changes in the network that coordinates the metal ion, but also an unexpected reorganization of the active-site. Mutation to histidine resulted in a significant shift in position of the highly conserved residues Y139 from the A subunit and K212' from the B subunit (**FIG. 29B**), both of which are thought to be critical for catalysis of this enzyme family (Aktas, D. F. & Cook, P. F. Biochemistry 48, 3565-77 (2009)). In particular, the hydroxyl moiety of Y139 now occupies the space of the β-carboxylate of isocitrate. In addition, a significant repositioning of αKG compared to isocitrate where the distal carboxylate of αKG now points upward to make new

contacts with N96 and S94 was observed. Overall, this single R132 mutation results in formation of a distinct active site compared to wild-type IDH1.

EXAMPLE 7 MATERIALS AND METHODS

Summary

R132H, R132C, R132L and R132S mutations were introduced into human IDH1 by standard molecular biology techniques. 293T and the human glioblastoma cell lines U87MG and LN-18 were cultured in DMEM, 10% fetal bovine serum. Cells were transfected and selected using standard techniques. Protein expression levels were determined by Western blot analysis using IDHc antibody (Santa Cruz Biotechnology), IDH1 antibody (proteintech), MYC tag antibody (Cell Signaling Technology), and IDH2 antibody (Abcam). Metabolites were extracted from cultured cells and from tissue samples according to close variants of a previously reported method (Lu, W., Kimball, E. & Rabinowitz, J. D. J Am Soc Mass Spectrom 17, 37-50 (2006)), using 80% aqueous methanol (-80 °C) and either tissue scraping or homogenization to disrupt cells. Enzymatic activity in cell lysates was assessed by following a change in NADPH fluorescence over time in the presence of isocitrate and NADP, or αKG and NADPH. For enzyme assays using recombinant IDH1 enzyme, proteins were produced in *E. coli* and purified using Ni affinity chromatography followed by Sephacryl S-200 size-exclusion chromatography. Enzymatic activity for recombinant IDH1 protein was assessed by following a change in NADPH UV absorbance at 340 nm using a stop-flow spectrophotometer in the presence of isocitrate and NADP or αKG and NADPH. Chirality of 2HG was determined as described previously (Struys, E. A., Jansen, E. E., Verhoeven, N. M. & Jakobs, C. Clin Chem 50, 1391-5 (2004)). For crystallography studies, purified recombinant IDH1 (R132H) at 10 mg/mL in 20 mM Tris pH 7.4, 100 mM NaCl was pre-incubated for 60 min with 10 mM NADPH, 10 mM calcium chloride, and 75 mM αKG. Crystals were obtained at 20°C by vapor diffusion equilibration using 3 μL drops mixed 2:1 (protein:precipitant) against a well-solution of 100 mM MES pH 6.5, 20% PEG 6000. Patient tumor samples were obtained after informed consent as part of a UCLA IRB-approved research protocol. Brain tumor samples were obtained after surgical resection, snap frozen in isopentane cooled by liquid nitrogen and stored at -80 C.

The IDH1 mutation status of each sample was determined using standard molecular biology techniques as described previously (Yan, H. et al. N Engl J Med 360, 765-73 (2009)). Metabolites were extracted and analyzed by LC-MS/MS as described above. Full methods are available in the supplementary material.

Supplementary methods

Cloning, Expression, and Purification of ICDH1 wt and mutants in *E. coli*. The open reading frame (ORF) clone of human isocitrate dehydrogenase 1 (cDNA) (IDH1; ref . ID NM_005896) was purchased from Invitrogen in pENTR221 (Carlsbad, CA) and Origene Inc. in pCMV6 (Rockville, MD). To transfect cells with wild-type or mutant IDH1, standard molecular biology mutagenesis techniques were utilized to alter the DNA sequence at base pair 395 of the ORF in pCMV6 to introduce base pair change from guanine to adenine, which resulted in a change in the amino acid code at position 132 from arginine (wt) to histidine (mutant; or R132H), and confirmed by standard DNA sequencing methods. For 293T cell transfection, wild-type and R132H mutant IDH1 were subcloned into pCMV-Sport6 with or without a carboxy-terminal Myc-DDK-tag. For stable cell line generation, constructs in pCMV6 were used. For expression in E. coli, the coding region was amplified from pENTR221 by PCR using primers designed to add NDEI and XHO1 restrictions sites at the 5' and 3' ends respectively. The resultant fragment was cloned into vector pET41a (EMD Biosciences, Madison, WI) to enable the *E. coli* expression of C-terminus His8-tagged protein. Site directed mutagenesis was performed on the pET41a-ICHD1 plasmid using the QuikChange® MultiSite-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) to change G395 to A, resulting in the Arg to His mutation. R132C, R132L and R132S mutants were introduced into pET41a-ICHD1 in an analogous way.

Wild-type and mutant proteins were expressed in and purified from the E. coli Rosetta™ strain (Invitrogen, Carlsbad, CA) as follows. Cells were grown in LB (20 μg/ml Kanamycin) at 37°C with shaking until OD600 reaches 0.6. The temperature was changed to 18°C and protein expression was induced by adding IPTG to final concentration of 1 mM. After 12-16 hours of IPTG induction, cells were resuspended in Lysis Buffer (20mM Tris, pH7.4, 0.1% Triton X-100, 500 mM NaCl, 1 mM PMSF, 5

mM β-mercaptoethanol, 10 % glycerol) and disrupted by microfluidation. The 20,000g supernatant was loaded on metal chelate affinity resin (MCAC) equilibrated with Nickel Column Buffer A (20 mM Tris, pH7.4, 500mM NaCl, 5 mM β-mercaptoethanol, 10% glycerol) and washed for 20 column volumes. Elution from the column was effected by a 20 column-volume linear gradient of 10% to 100% Nickel Column Buffer B (20 mM Tris, pH7.4, 500 mM NaCl, 5 mM β-mercaptoethanol , 500 mM Imidazole, 10% glycerol) in Nickel Column Buffer A). Fractions containing the protein of interest were identified by SDS-PAGE, pooled, and dialyzed twice against a 200-volume excess of Gel Filtration Buffer (200 mM NaCl, 50 mM Tris 7.5, 5 mM β-mercaptoethanol, 2 mM $MnSO_4$, 10% glycerol), then concentrated to 10 ml using Centricon (Millipore, Billerica, MA) centrifugal concentrators. Purification of active dimers was achieved by applying the concentrated eluent from the MCAC column to a Sephacryl S-200 (GE Life Sciences, Piscataway, NJ) column equilibrated with Gel Filtration Buffer and eluting the column with 20 column volumes of the same buffer. Fractions corresponding to the retention time of the dimeric protein were identified by SDS-PAGE and pooled for storage at -80°C.

Cell lines and Cell Culture. 293T cells were cultured in DMEM (Dulbecco's modified Eagles Medium) with 10% fetal bovine serum and were transfected using pCMV-6-based IDH-1 constructs in six-well plates with Fugene 6 (Roche) or Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions. Parental vector pCMV6 (no insert), pCMV6-wt IDH1 or pCMV6-R132H were transfected into human glioblastoma cell lines (U87MG; LN-18 (ATCC, HTB-14 and CRL-2610; respectively) cultured in DMEM with 10 % fetal bovine serum. Approximately 24 hrs after transfection, the cell cultures were transitioned to medium containing G418 sodium salt at concentrations of either 500 ug/ml (U87MG) or 750 ug/ml (LN-18) to select stable transfectants. Pooled populations of G418 resistant cells were generated and expression of either wild-type IDH1 or R132 IDH1 was confirmed by standard Western blot analysis.

Western blot. For transient transfection experiments in 293 cells, cells were lysed 72 hours after transfection with standard RIPA buffer. Lysates were separated by SDS-PAGE, transferred to nitrocellulose and probed with goat-anti-IDHc antibody (Santa

Cruz Biotechnology sc49996) or rabbit-anti-MYC tag antibody (Cell Signaling Technology #2278) and then detected with HRP-conjugated donkey anti-goat or HRP-conjugated goat-anti-rabbit antibody (Santa Cruz Biotechnology sc2004). IDH1 antibody to confirm expression of both wild-type and R132H IDH1 was obtained from Proteintech. The IDH2 mouse monoclonal antibody used was obtained from Abcam.

Detection of isocitrate, αKG, and 2HG in purified enzyme reactions by LC-MS/MS. Enzyme reactions performed as described in the text were run to completion as judged by measurement of the oxidation state of NADPH at 340 nm. Reactions were extracted with eight volumes of methanol, and centrifuged to remove precipitated protein. The supernatant was dried under a stream of nitrogen and resuspended in H_2O. Analysis was conducted on an API2000 LC-MS/MS (Applied Biosystems, Foster City, CA). Sample separation and analysis was performed on a 150 x 2 mm, 4 uM Synergi Hydro-RP 80 A column, using a gradient of Buffer A (10 mM tributylamine, 15 mM acetic acid, 3% (v/v) methanol, in water) and Buffer B (methanol) using MRM transitions.

Cell lysates based enzyme assays. 293T cell lysates for measuring enzymatic activity were obtained 48 hours after transfection with M-PER lysis buffer supplemented with protease and phosphatase inhibitors. After lysates were sonicated and centrifuged at 12,000g, supernatants were collected and normalized for total protein concentration. To measure IDH oxidative activity, 3 µg of lysate protein was added to 200 µl of an assay solution containing 33 mM Tris-acetate buffer (pH 7.4), 1.3 mM $MgCl_2$, 0.33 mM EDTA, 100 µM β-NADP, and varying concentrations of D-(+)-*threo*-isocitrate. Absorbance at 340 nm, reflecting NADPH production, was measured every 20 seconds for 30 min on a SpectraMax 190 spectrophotometer (Molecular Devices). Data points represent the mean activity of 3 replicates per lysate, averaged among 5 time points centered at every 5 min. To measure IDH reductive activity, 3 µg of lysate protein was added to 200 µl of an assay solution which contained 33 mM Tris-acetate (pH 7.4), 1.3 mM $MgCl_2$, 25 µM β-NADPH, 40 mM $NaHCO_3$, and 0.6 mM αKG. The decrease in 340 nm absorbance over time was measured to assess NADPH consumption, with 3 replicates per lysate.

Recombinant IDH1 Enzyme Assays. All reactions were performed in standard enzyme reaction buffer (150 mM NaCl, 20 mM Tris-Cl, pH 7.5, 10% glycerol, 5 mM $MgCl_2$ and 0.03% (w/v) bovine serum albumin). For determination of kinetic parameters, sufficient enzyme was added to give a linear reaction for 1 to 5 seconds. Reaction progress was monitored by observation of the reduction state of the cofactor at 340 nm in an SFM-400 stopped-flow spectrophotometer (BioLogic, Knoxville, TN). Enzymatic constants were determined using curve fitting algorithms to standard kinetic models with the Sigmaplot software package (Systat Software, San Jose, CA).

Determination of chirality of reaction products from enzyme reactions and tumors. Enzyme reactions were run to completion and extracted with methanol as described above, then derivatized with enantiomerically pure tartaric acid before resolution and analysis by LC-MS/MS. After being thoroughly dried, samples were resuspended in freshly prepared 50 mg/ml (2*R*,3*R*)-(+)-Tartaric acid in dichloromethane:acetic acid (4:1) and incubated for 30 minutes at 75°C. After cooling to room temperature, samples were briefly centrifuged at 14,000g, dried under a stream of nitrogen, and resuspended in H_20. Analysis was conducted on an API200 LC-MS/MS (Applied Biosystems, Foster City, CA), using an isocratic flow of 90:10 (2 mM ammonium formate, pH 3.6:MeOH) on a Luna C18(2) 150 x 2 mm, 5 uM column. Tartaric-acid derivatized 2HG was detected using the 362.9/146.6 MRM transition and the following instrument settings: DP -1, FP -310, EP -4, CE-12, CXP-26. Analysis of the (R)-2HG standard, 2HG racemic mixture, and methanol-extracted tumor biomass (q.v.) was similarly performed.

Crystallography conditions. Crystals were obtained at 20°C by vapor diffusion equilibration using 3 μL drops mixed 2:1 (protein:precipitant) against a well-solution of 100 mM MES pH 6.5, 20% PEG 6000.

Protein characterization. Approximately 90 mg of human cytosolic isocitrate dehydrogenase (HcIDH) was supplied to Xtal BioStructures by Agios. This protein was an engineered mutant form, R132S, with an 11-residue C-terminal affinity-purification tag (sequence SLEHHHHHHHH). The calculated monomeric molecular weight was

48.0 kDa and the theoretical pI was 6.50. The protein, at about 6 mg/mL concentration, was stored in 1-mL aliquots in 50 mM Tris-HCl (pH 7.4), 500 mM NaCl, 5 mM β-mercaptoethanol and 10% glycerol at –80°C. As shown in **FIG. 33A**, SDS-PAGE was performed to test protein purity and an anti-histidine Western blot was done to demonstrate the protein was indeed his-tagged. A sample of the protein was injected into an FPLC size-exclusion column to evaluate the sample purity and to determine the polymeric state in solution. **FIG. 33B** is a chromatogram of this run showing a single peak running at an estimated 87.6 kDa, suggesting IDH exists as a dimer at pH 7.4. Prior to crystallization, the protein was exchanged into 20 mM Tris-HCl (pH 7.4) and 100 mM NaCl using Amicon centrifugal concentrators. At this time, the protein was also concentrated to approximately 15 mg/mL. At this protein concentration and ionic strength, the protein tended to form a detectable level of precipitate. After spinning out the precipitate, the solution was stable at ~10 mg/mL at 4 °C.

Initial attempts at crystallization. The strategy for obtaining diffraction-quality crystals was derived from literature conditions, specifically "Structures of Human Cytosolic NADP-dependent Isocitrate Dehydrogenase Reveal a Novel Self-regulatory Mechanism of Activity," Xu, *et al.* (2005) *J.Biol.Chem.* 279: 33946-56. In this study, two crystal forms of HcIDH wildtype protein were produced. One contained their "binary complex", IDH-NADP, which crystallized from hanging drops in the tetragonal space group $P4_32_12$. The drops were formed from equal parts of protein solution (15 mg/mL IDH, 10 mM NADP) and precipitant consisting of 100 mM MES (pH 6.5) and 12% PEG 20000. The other crystal form contained their "quaternary complex", IDH-NADP/isocitrate/Ca^{2+}, which crystallized in the monoclinic space group $P2_1$ using 100 mM MES (pH 5.9) and 20% PEG 6000 as the precipitant. Here they had added 10 mM DL-isocitrate and 10 mM calcium chloride to the protein solution. First attempts at crystallizing the R132S mutant in this study centered around these two reported conditions with little variation. The following lists the components of the crystallization that could be varied; several different combinations of these components were tried in the screening process.

In the protein solution:

HcIDH(R132S)	always ~10 mg/mL or ~0.2 mM
Tris-HCl (pH 7.4)	always 20 mM
NaCl	always 100 mM
$NADP^+$/NADPH	absent or 5 mM $NADP^+$ (did not try NADPH)
DL-isocitic acid, trisodium salt	absent or 5 mM
calcium chloride	absent or 10 mM
In the precipitant:	100 mM MES (pH 6.5) and 12% PEG 20000 ***OR***
	100 mM MES (pH 6.0) and 20% PEG 6000
Drop size:	always 3 μL
Drop ratios:	2:1, 1:1 or 1:2 (protein:precipitant)

Upon forming the hanging drops, a milky precipitate was always observed. On inspection after 2-4 days at 20 °C most drops showed dense precipitation or phase separation. In some cases, the precipitate subsided and it was from these types of drops small crystals had grown, for example, as shown in **FIG. 34**.

Crystal optimization. Once bonafide crystals were achieved, the next step was to optimize the conditions to obtain larger and more regularly-shaped crystals of IDH-NADP/isocitrate/Ca^{2+} in a timely and consistent manner. The optimal screen focused on varying the pH from 5.7 to 6.2, the MES concentration from 50 to 200 mM and the PEG 6000 concentration from 20 to 25%. Also, bigger drops were set up (5-6 μl) and the drop ratios were again varied. These attempts failed to produce larger, diffraction-quality crystals but did reproduce the results reported above. Either a dense precipitate, oily phase separation or small crystals were observed.

Using α-Ketoglutarate. Concurrent to the optimization of the isocitrate crystals, other screens were performed to obtain crystals of IDH(R132S) complexed with α-ketoglutarate instead. The protein solution was consistently 10 mg/mL IDH in 20 mM Tris-HCl (pH 7.4) and 100 mM NaCl. The following were added in this order: 5 mM NADP, 5 mM α-ketoglutaric acid (free acid, pH balanced with NaOH) and 10 mM calcium chloride. The protein was allowed to incubate with these compounds for at least

an hour before the drops were set up. The precipitant was either 100 mM MES (pH 6.5) and 12% PEG 20000 or 100 mM MES (pH 6.5) and 20% PEG 6000. Again, precipitation or phase separation was primarily seen, but in some drops small crystals did form. At the edge of one of the drops, a single large crystal formed, pictured below. This was the single crystal used in the following structure determination. **FIG. 35** shows crystal obtained from a protein solution contained 5 mM NADP, 5 mM α-ketoglutarate, 10 mM Ca2+. Precipitant contained 100 mM MES (pH 6.5) and 12% PEG 20000.

Cryo conditions. In order to ship the crystal to the X-ray source and protect it during cryo-crystallography, a suitable cryo-protectant was needed. Glycerol is quite widely used and was the first choice. A cryo solution was made, basically as a mixture of the protein buffer and precipitant solution plus glycerol: 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM NADP, 5 mM α-ketoglutaric acid, 10 mM calcium chloride, 100 mM MES (pH 6.5), 12% PEG 20000 and either 12.5% glycerol or 25% glycerol. The crystal was transferred to the cryo solution in two steps. First, 5 μL of the 12.5% glycerol solution was added directly to the drop and incubated for 10 minutes, watching for possible cracking of the crystal. The liquid was removed from the drop and 10 μL of the 25% glycerol solution was added on top of the crystal. Again, this incubated for 10 minutes, harvested into a nylon loop and plunged into liquid nitrogen. The crystal was stored submerged in a liquid nitrogen dewar for transport.

Data collection and processing. The frozen crystal was mounted on a Rigaku RAXIS IV X-ray instrument under a stream of nitrogen gas at temperatures near −170 °C. A 200° dataset was collected with the image plate detector using 1.54 Å wavelength radiation from a rotating copper anode home source, 1° oscillations and 10 minute exposures. The presence of 25% glycerol as a cryoprotectant was sufficient for proper freezing, as no signs of crystal cracking (split spots or superimposed lattices) were observed. A diffuse ring was observed at 3.6 Å resolution, most likely caused by icing. The X-ray diffraction pattern showed clear lattice planes and reasonable spot separation, although the spacing along one reciprocal axis was rather small (b = 275.3). The data was indexed to 2.7 Å resolution into space group $P2_12_12$ with HKL2000 (Otwinowski

and Minor, 1997). Three structures for HcIDH are known, designated the closed form (1T0L), the open form (1T09 subunit A) and semi-open form (1T09 subunit B). Molecular replacement was performed with the CCP4 program PHASER (Bailey, 1994) using only the protein atoms from these three forms. Only the closed form yielded a successful molecular replacement result with 6 protein subunits in the asymmetric unit. The unit cell contains approximately 53.8% solvent.

Model refinement. Using the CCP4 program REFMAC5, rigid-body refinement was performed to fit each of the 6 IDH subunits in the asymmetric unit. This was followed by rigid-body refinement of the three domains in each protein subunit. Restrained refinement utilizing non-crystallographic symmetry averaging of related pairs of subunits yielded an initial structure with R_{cryst} of 33% and R_{free} of 42%. Model building and real-space refinement were performed using the graphics program COOT (Emsley and Cowtan, 2004). A difference map was calculated and this showed strong electron density into which six individual copies of the NADP ligand and calcium ion were manually fit with COOT. Density for the α-ketoglutarate structure was less defined and was fit after the binding-site protein residues were fit using a $2F_o–F_c$ composite omit map. Automated Ramachandran-plot optimization coupled with manual real-space density fitting was applied to improve the overall geometry and fit. A final round of restrained refinement with NCS yielded an R_{cryst} of 30.1% and R_{free} of 35.2%.

a, Å	b, Å	c, Å	α	β	γ	Unit cell volume, Å^3	Z
116.14	275.30	96.28	90°	90°	90°	3.08×10^6	24

Reflections in working set / test set	68,755 / 3,608 (5.0%)
R_{cryst}	30.1%
R_{free}	35.2%

X-ray data and refinement statistics for IDH(R132S)-NADP/α-ketoglurate/Ca^{2+}

Crystal parameters	

Space group	$P2_12_12$
Unit cell dimensions	
a, b, c, Å	116.139, 275.297, 96.283
α, β, γ, °	90.0, 90.0, 90.0
Volume, Å^3	3,078,440
No. protein molecules in asymmetric unit	6
No. protein molecules in unit cell, Z	24
Data collection	
Beam line	
Date of collection	Apr 25, 2009
λ, Å	1.5418
Detector	Rigaku Raxis IV
Data set (phi), °	200
Resolution, Å	25-2.7 (2.8-2.7)
Unique reflections (N, $F > 0$)	73,587
Completeness, %	85.4 (48.4)
$<I> / \sigma I$	9.88 (1.83)

R-merge	0.109 (0.33)
Redundancy	4.3 (1.8)
Mosaicity	0.666
Wilson B factor	57.9
Anisotropy B factor, Å^2	-1.96
Refinement Statistics	
Resolution limit, Å	20.02-2.70
No. of reflections used for *R*-work[a] / *R*-free[b]	68,755 / 3608
Protein atoms	19788
Ligand atoms	348
No. of waters	357
Ions etc.	6
Matthews coeff. Å^3/ Dalton	2.68
Solvent, %	53.8
R-work[a] / *R*-free[b], (%)	30.1 / 35.2
Figure-of-merit[c]	0.80 (0.74)
Average *B* factors	31.0
Coordinates error (Luzzati plot), Å	0.484

R.M.S. deviations	
Bond lengths, Å	0.026
Bond angles, °	2.86

Completeness and *R*-merge are given for all data and for data in the highest resolution shell. Highest shell values are in parentheses.

[a]*R* factor = $\Sigma_{hkl} |F_o - F_c| / \Sigma_{hkl} F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively for all reflections *hkl* used in refinement.

[b]*R*-free is calculated for 5% of the data that were not used in refinement.

[c]Figure of merit = $\sqrt{x^2 + y^2}$, where $x = (\sum_0^{2\pi} P(\alpha)\cos\alpha)/(\sum_0^{2\pi} P(\alpha))$, $y = (\sum_0^{2\pi} P(\alpha)\sin\alpha)/(\sum_0^{2\pi} P(\alpha))$, and the phase probability $P(\alpha) = \exp(A\cos\alpha + B\sin\alpha + C\cos(2\alpha) + D\sin(2\alpha))$, where *A*, *B*, *C*, and *D* are the Hendrickson-Lattman coefficients and α is the phase.

Stereochemistry of IDH(R132S)-NADP/α-ketoglurate/Ca^{2+}

Ramachandran plot statistics	No.of amino acids	% of Residues
Residues in most favored regions [A, B, L]	1824	82.2
Residues in additional allowed regions [a, b, l, p]	341	15.4
Residues in generously allowed regions [-a, -b, -l, -p]	38	1.7
Residues in disallowed regions	17	0.8
Number of non-glycine and non-proline residues	2220	100
Number of end-residues (excl. Gly and Pro)	387	
Number of glycine residues	198	
Number of proline residues	72	

Total number of residues	2877	
Overall <G> -factor[d] score (> -1.0)	-0.65	

Generated by PROCHECK (Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) J Appl Crystallogr 26:283-291.)

[d] G-factors for main-chain and side-chain dihedral angles, and main-chain covalent forces (bond lengths and bond angles). Values should be ideally -0.5 or above -1.0.

Radiation wavelength, Å	1.54
Resolution, Å (outer shell)	20-2.70 (2.80-2.70)
Unique reflections	73,587
Completeness (outer shell)	85.4% (48.4%)
Redundancy (outer shell)	4.3 (1.8)
R_{merge} (outer shell)	10.9% (33%)
<I> / <σ(I)> (outer shell)	9.88 (1.83)

Clinical Specimens, metabolite extraction and analysis. Human brain tumors were obtained during surgical resection, snap frozen in isopentane cooled by liquid nitrogen and stored at -80 C. Clinical classification of the tissue was performed using standard clinical pathology categorization and grading as established by the WHO. Genomic sequence analysis was deployed to identify brain tumor samples containing either wild-type isocitrate dehydrogenase (IDH1) or mutations altering amino acid 132. Genomic DNA was isolated from 50-100 mgs of brain tumor tissue using standard methods. A polymerase chain reaction on the isolated genomic DNA was used to amplify a 295 base pair fragment of the genomic DNA that contains both the intron and 2nd exon sequences of human IDH1 and mutation status assessed by standard molecular biology techniques. Metabolite extraction was accomplished by adding a 10x volume (m/v ratio) of -80 °C methanol:water mix (80%:20%) to the brain tissue (approximately 100mgs) followed by 30 s homogenization at 4 C. These chilled, methanol extracted homogenized tissues were then centrifuged at 14,000 rpm for 30 minutes to sediment the cellular and tissue

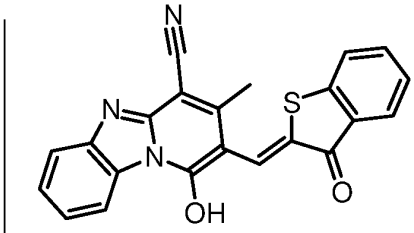
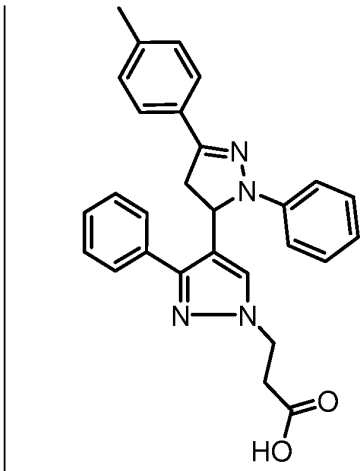
debris and the cleared tissue supernatants were transferred to a screw-cap freezer vial and stored at -80 °C. For analysis, a 2X volume of tributylamine (10 mM) acetic acid (10 mM) pH 5.5 was added to the samples and analyzed by LCMS as follows. Sample extracts were filtered using a Millex-FG 0.20 micron disk and 10 µL were injected onto a reverse-phase HPLC column (Synergi 150mm x 2 mm, Phenomenex Inc.) and eluted using a linear gradient LCMS-grade methanol (50%) with 10 mM tributylamine and 10 mM acetic acid) ramping to 80 % methanol:10 mM tributylamine: 10 mM acetic acid over 6 minutes at 200 µL/min. Eluted metabolite ions were detected using a triple-quadrupole mass spectrometer, tuned to detect in negative mode with multiple-reaction-monitoring mode transition set (MRM's) according to the molecular weights and fragmentation patterns for 8 known central metabolites, including 2-hydroxyglutarate as described above. Data was processed using Analyst Software (Applied Biosystems, Inc.) and metabolite signal intensities were obtained by standard peak integration methods.

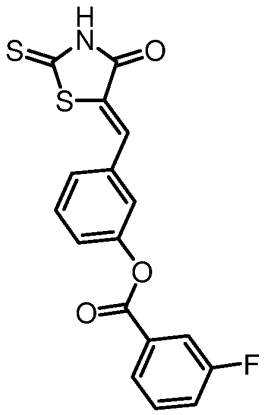
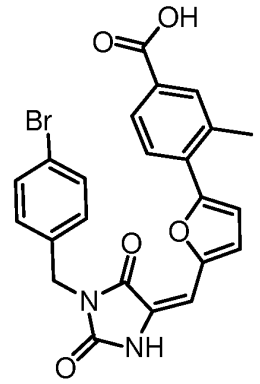
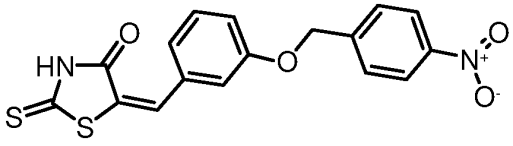
EXAMPLE 9 HIGH THROUGHPUT SCREENING (HTS) FOR IDH1 R132H INHIBITORS

Assays were conducted in a volume of 76 ul assay buffer (150 mM NaCl, 10 mM MgCl2, 20 mM Tris pH 7.5, 0.03% bovine serum albumin) as follows in a standard 384-well plate: To 25 ul of substrate mix (8 uM NADPH, 2 mM aKG), 1 ul of test compound was added in DMSO. The plate was centrifuged briefly, and then 25 ul of enzyme mix was added (0.2 ug/ml ICDH1 R132H) followed by a brief centrifugation and shake at 100 RPM. The reaction was incubated for 50 minutes at room temperature, then 25 ul of detection mix (30 uM resazurin, 36 ug/ml) was added and the mixture further incubated for 5 minutes at room temperature. The conversion of resazurin to resorufin was detected by fluorescent spectroscopy at Ex544 Em590 c/o 590.

Table 17 shows the wild type vs mutant selectivity profile of the top 5 examples of IDH1R132H inhibitors. The IDH1wt assay was performed at 1x Km of NADPH as opposed to IDHR132H at 10x or 100x Km of NADPH. The second example showed no inhibition, even at 100 uM. Also, the first example has IC50=5.74 uM but is shifted significantly when assayed at 100x Km, indicating direct NADPH-competitive inhibitor. The selectivity between wild type vs mutant could be >20-fold.

Table 17

STRUCTURE	LDHa IC50	LDHb IC50	**ICDH IC50 (uM) @ 4 uM (10x Km) NADPH**	ICDH IC50 (uM) @ 40 uM NADPH	IC50 Ratio (40/4)	IDH1wt IC50 @ 1x Km (uM)
 #NAME?	25.43	64.07	5.74	>100	17.42	**16.22**
 #NAME?	5.92	17.40	12.26	41.40	3.38	**NO inhibition**

 #NAME?	8.61	>100	12.79	14.70	1.15	**19.23**
 #NAME?	33.75	>100	14.98	19.17	1.28	**46.83**
 #NAME?	12.76	>100	23.80	33.16	1.39	**69.33**

CLAIMS

1. A method of treating a subject having glioma characterized by a mutant IDH1, which encodes a mutant enzyme having a neoactivity, comprising:

administering to said subject a therapeutically effective amount of an dsRNA inhibitor of the neoactivity of said mutant enzyme,

wherein said mutant comprises Arg132His or Arg132Ser and said neoactivity is the ability to convert alpha ketoglutarate to 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, to thereby treat said subject.

Abstract

A method of treating a subject having glioblastoma characterized by a mutant IDH1 gene which encodes a mutant enzyme having a neoactivity, comprising: administering to said subject a therapeutically effective amount of an siRNA that targets IDH1 of the neoactivity of said mutant enzyme, wherein said neoactivity is the ability to convert alpha ketoglutarate to 2-hydroxyglutarate, e.g., R-2-hydroxyglutarate, to thereby treat said subject.

955677.1

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	METHODS AND COMPOSITIONS FOR TREATING CANCER
First Named Inventor/Applicant Name:	Stephan Gross
Filer:	Catherine M. McCarty/Brenda Kowalczuk
Attorney Docket Number:	C2081-701305

Filed as Large Entity

Provisional Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Provisional application filing	1005	1	220	220
Pages:				
Prov. Appl Size fee per 50 sheets >100	1085	1	270	270
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**490**

Electronic Acknowledgement Receipt

EFS ID:	5749494
Application Number:	61227649
International Application Number:	
Confirmation Number:	6229
Title of Invention:	METHODS AND COMPOSITIONS FOR TREATING CANCER
First Named Inventor/Applicant Name:	Stephan Gross
Customer Number:	37462
Filer:	Catherine M. McCarty
Filer Authorized By:	
Attorney Docket Number:	C2081-701305
Receipt Date:	22-JUL-2009
Filing Date:	
Time Stamp:	17:03:01
Application Type:	Provisional

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Provisional Cover Sheet (SB16)	C2081_701305_Prov_Cover_Sheet.pdf	1000190 414e53c4d24abf988008c0308a7585bf25b1cf4b	no	4

Warnings:	
Information:	

2		C2081_701305_Appln.pdf	953454 5f1e85b3986772ea457acbc326f9cc5b49bd2cab	yes	147
	Multipart Description/PDF files in .zip description				
	Document Description		**Start**	**End**	
	Specification		1	145	
	Claims		146	146	
	Abstract		147	147	
Warnings:					
Information:					
3	Drawings-only black and white line drawings	C2081_701305_Drawings.pdf	3387716 59512d3d99f6cbf830e8ba205edc986fff853e090	no	43
Warnings:					
Information:					
4	Fee Worksheet (PTO-875)	fee-info.pdf	31445 f1ac166cbe457926ebce8de6bd260de7690ca833	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	5372805		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

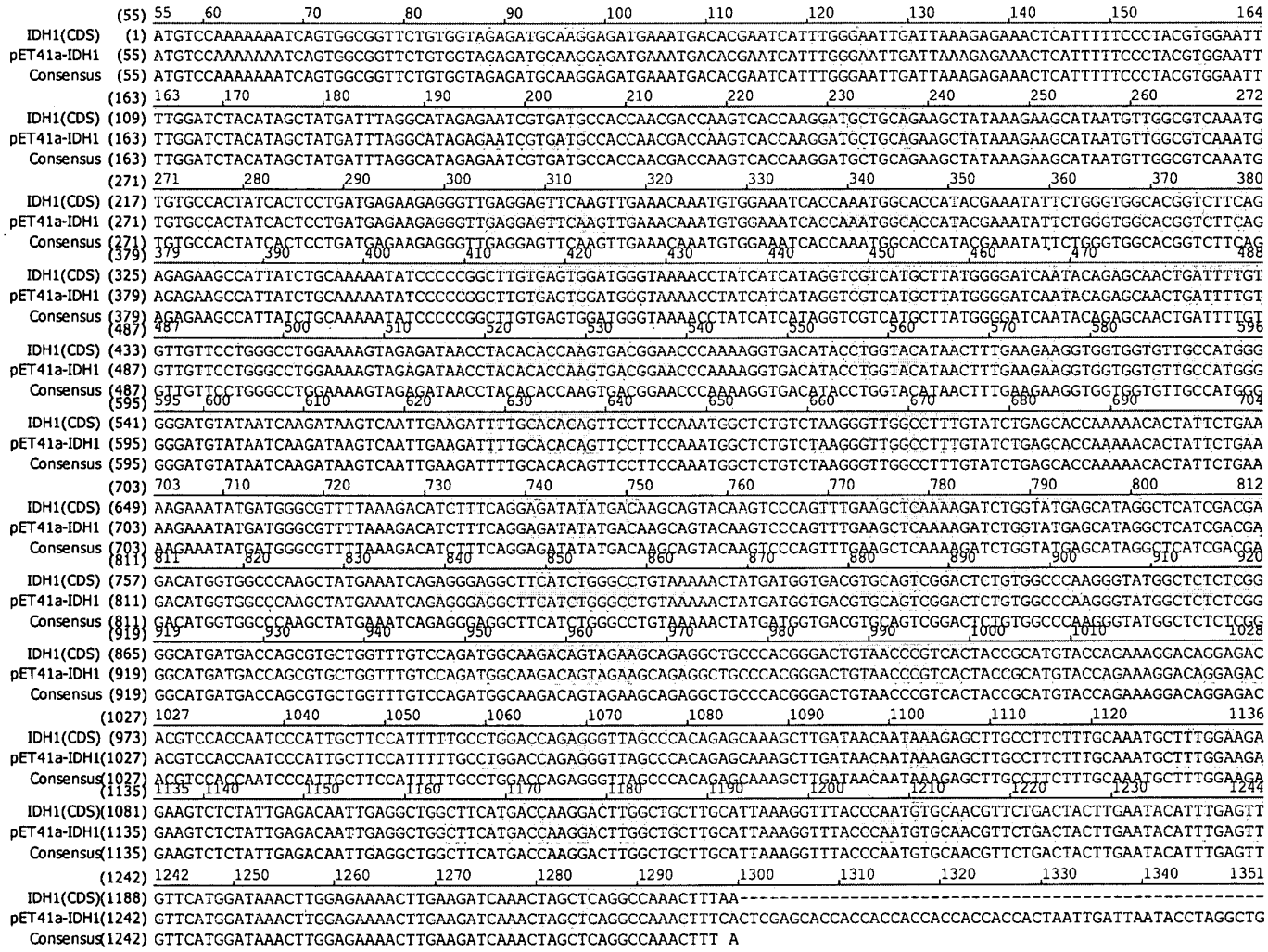

(55) 55 60 70 80 90 100 110 120 130 140 150 164
IDH1(CDS) (1) ATGTCCAAAAAAATCAGTGGCGGTTCTGTGGTAGAGATGCAAGGAGATGAAATGACACGAATCATTTGGGAATTGATTAAAGAGAAACTCATTTTTCCCTACGTGGAATT
pET41a-IDH1 (55) ATGTCCAAAAAAATCAGTGGCGGTTCTGTGGTAGAGATGCAAGGAGATGAAATGACACGAATCATTTGGGAATTGATTAAAGAGAAACTCATTTTTCCCTACGTGGAATT
Consensus (55) ATGTCCAAAAAAATCAGTGGCGGTTCTGTGGTAGAGATGCAAGGAGATGAAATGACACGAATCATTTGGGAATTGATTAAAGAGAAACTCATTTTTCCCTACGTGGAATT
(163) 163 170 180 190 200 210 220 230 240 250 260 272
IDH1(CDS) (109) TTGGATCTACATAGCTATGATTTAGGCATAGAGAATCGTGATGCCACCAACGACCAAGTCACCAAGGATGCTGCAGAAGCTATAAAGAAGCATAATGTTGGCGTCAAATG
pET41a-IDH1 (163) TTGGATCTACATAGCTATGATTTAGGCATAGAGAATCGTGATGCCACCAACGACCAAGTCACCAAGGATGCTGCAGAAGCTATAAAGAAGCATAATGTTGGCGTCAAATG
Consensus (163) TTGGATCTACATAGCTATGATTTAGGCATAGAGAATCGTGATGCCACCAACGACCAAGTCACCAAGGATGCTGCAGAAGCTATAAAGAAGCATAATGTTGGCGTCAAATG
(271) 271 280 290 300 310 320 330 340 350 360 370 380
IDH1(CDS) (217) TGTGCCACTATCACTCCTGATGAGAAGAGGGTTGAGGAGTTCAAGTTGAAACAAATGTGGAAATCACCAAATGGCACCATACGAAATATTCTGGGTGGCACGGTCTTCAG
pET41a-IDH1 (271) TGTGCCACTATCACTCCTGATGAGAAGAGGGTTGAGGAGTTCAAGTTGAAACAAATGTGGAAATCACCAAATGGCACCATACGAAATATTCTGGGTGGCACGGTCTTCAG
Consensus (271) TGTGCCACTATCACTCCTGATGAGAAGAGGGTTGAGGAGTTCAAGTTGAAACAAATGTGGAAATCACCAAATGGCACCATACGAAATATTCTGGGTGGCACGGTCTTCAG
(379) 379 390 400 410 420 430 440 450 460 470 488
IDH1(CDS) (325) AGAGAAGCCATTATCTGCAAAAATATCCCCCGGCTTGTGAGTGGATGGGTAAAACCTATCATCATAGGTCGTCATGCTTATGGGGATCAATACAGAGCAACTGATTTTGT
pET41a-IDH1 (379) AGAGAAGCCATTATCTGCAAAAATATCCCCCGGCTTGTGAGTGGATGGGTAAAACCTATCATCATAGGTCGTCATGCTTATGGGGATCAATACAGAGCAACTGATTTTGT
Consensus (379) AGAGAAGCCATTATCTGCAAAAATATCCCCCGGCTTGTGAGTGGATGGGTAAAACCTATCATCATAGGTCGTCATGCTTATGGGGATCAATACAGAGCAACTGATTTTGT
(487) 487 500 510 520 530 540 550 560 570 580 596
IDH1(CDS) (433) GTTGTTCCTGGGCCTGGAAAAGTAGAGATAACCTACACACCAAGTGACGGAACCCAAAAGGTGACATACCTGGTACATAACTTTGAAGAAGGTGGTGGTGTTGCCATGGG
pET41a-IDH1 (487) GTTGTTCCTGGGCCTGGAAAAGTAGAGATAACCTACACACCAAGTGACGGAACCCAAAAGGTGACATACCTGGTACATAACTTTGAAGAAGGTGGTGGTGTTGCCATGGG
Consensus (487) GTTGTTCCTGGGCCTGGAAAAGTAGAGATAACCTACACACCAAGTGACGGAACCCAAAAGGTGACATACCTGGTACATAACTTTGAAGAAGGTGGTGGTGTTGCCATGGG
(595) 595 600 610 620 630 640 650 660 670 680 690 704
IDH1(CDS) (541) GGGATGTATAATCAAGATAAGTCAATTGAAGATTTTGCACACAGTTCCTTCCAAATGGCTCTGTCTAAGGGTTGGCCTTTGTATCTGAGCACCAAAAACACTATTCTGAA
pET41a-IDH1 (595) GGGATGTATAATCAAGATAAGTCAATTGAAGATTTTGCACACAGTTCCTTCCAAATGGCTCTGTCTAAGGGTTGGCCTTTGTATCTGAGCACCAAAAACACTATTCTGAA
Consensus (595) GGGATGTATAATCAAGATAAGTCAATTGAAGATTTTGCACACAGTTCCTTCCAAATGGCTCTGTCTAAGGGTTGGCCTTTGTATCTGAGCACCAAAAACACTATTCTGAA
(703) 703 710 720 730 740 750 760 770 780 790 800 812
IDH1(CDS) (649) AAGAAATATGATGGGCGTTTTAAAGACATCTTTCAGGAGATATATGACAAGCAGTACAAGTCCCAGTTTGAAGCTCAAAAGATCTGGTATGAGCATAGGCTCATCGACGA
pET41a-IDH1 (703) AAGAAATATGATGGGCGTTTTAAAGACATCTTTCAGGAGATATATGACAAGCAGTACAAGTCCCAGTTTGAAGCTCAAAAGATCTGGTATGAGCATAGGCTCATCGACGA
Consensus (703) AAGAAATATGATGGGCGTTTTAAAGACATCTTTCAGGAGATATATGACAAGCAGTACAAGTCCCAGTTTGAAGCTCAAAAGATCTGGTATGAGCATAGGCTCATCGACGA
(811) 811 820 830 840 850 860 870 880 890 900 910 920
IDH1(CDS) (757) GACATGGTGGCCCAAGCTATGAAATCAGAGGGAGGCTTCATCTGGGCCTGTAAAAACTATGATGGTGACGTGCAGTCGGACTCTGTGGCCCAAGGGTATGGCTCTCTCGG
pET41a-IDH1 (811) GACATGGTGGCCCAAGCTATGAAATCAGAGGGAGGCTTCATCTGGGCCTGTAAAAACTATGATGGTGACGTGCAGTCGGACTCTGTGGCCCAAGGGTATGGCTCTCTCGG
Consensus (811) GACATGGTGGCCCAAGCTATGAAATCAGAGGGAGGCTTCATCTGGGCCTGTAAAAACTATGATGGTGACGTGCAGTCGGACTCTGTGGCCCAAGGGTATGGCTCTCTCGG
(919) 919 930 940 950 960 970 980 990 1000 1010 1028
IDH1(CDS) (865) GGCATGATGACCAGCGTGCTGGTTTGTCCAGATGGCAAGACAGTAGAAGCAGAGGCTGCCCACGGGACTGTAACCCGTCACTACCGCATGTACCAGAAAGGACAGGAGAC
pET41a-IDH1 (919) GGCATGATGACCAGCGTGCTGGTTTGTCCAGATGGCAAGACAGTAGAAGCAGAGGCTGCCCACGGGACTGTAACCCGTCACTACCGCATGTACCAGAAAGGACAGGAGAC
Consensus (919) GGCATGATGACCAGCGTGCTGGTTTGTCCAGATGGCAAGACAGTAGAAGCAGAGGCTGCCCACGGGACTGTAACCCGTCACTACCGCATGTACCAGAAAGGACAGGAGAC
(1027) 1027 1040 1050 1060 1070 1080 1090 1100 1110 1120 1136
IDH1(CDS) (973) ACGTCCACCAATCCCATTGCTTCCATTTTTGCCTGGACCAGAGGGTTAGCCCACAGAGCAAAGCTTGATAACAATAAAGAGCTTGCCTTCTTTGCAAATGCTTTGGAAGA
pET41a-IDH1(1027) ACGTCCACCAATCCCATTGCTTCCATTTTTGCCTGGACCAGAGGGTTAGCCCACAGAGCAAAGCTTGATAACAATAAAGAGCTTGCCTTCTTTGCAAATGCTTTGGAAGA
Consensus(1027) ACGTCCACCAATCCCATTGCTTCCATTTTTGCCTGGACCAGAGGGTTAGCCCACAGAGCAAAGCTTGATAACAATAAAGAGCTTGCCTTCTTTGCAAATGCTTTGGAAGA
(1135) 1135 1140 1150 1160 1170 1180 1190 1200 1210 1220 1230 1244
IDH1(CDS)(1081) GAAGTCTCTATTGAGACAATTGAGGCTGGCTTCATGACCAAGGACTTGGCTGCTTGCATTAAAGGTTTACCCAATGTGCAACGTTCTGACTACTTGAATACATTTGAGTT
pET41a-IDH1(1135) GAAGTCTCTATTGAGACAATTGAGGCTGGCTTCATGACCAAGGACTTGGCTGCTTGCATTAAAGGTTTACCCAATGTGCAACGTTCTGACTACTTGAATACATTTGAGTT
Consensus(1135) GAAGTCTCTATTGAGACAATTGAGGCTGGCTTCATGACCAAGGACTTGGCTGCTTGCATTAAAGGTTTACCCAATGTGCAACGTTCTGACTACTTGAATACATTTGAGTT
(1242) 1242 1250 1260 1270 1280 1290 1300 1310 1320 1330 1340 1351
IDH1(CDS)(1188) GTTCATGGATAAACTTGGAGAAAACTTGAAGATCAAACTAGCTCAGGCCAAACTTTAA----------
pET41a-IDH1(1242) GTTCATGGATAAACTTGGAGAAAACTTGAAGATCAAACTAGCTCAGGCCAAACTTTCACTCGAGCACCACCACCACCACCACCACCACTAATTGATTAATACCTAGGCTG
Consensus(1242) GTTCATGGATAAACTTGGAGAAAACTTGAAGATCAAACTAGCTCAGGCCAAACTTT A

FIG. 1

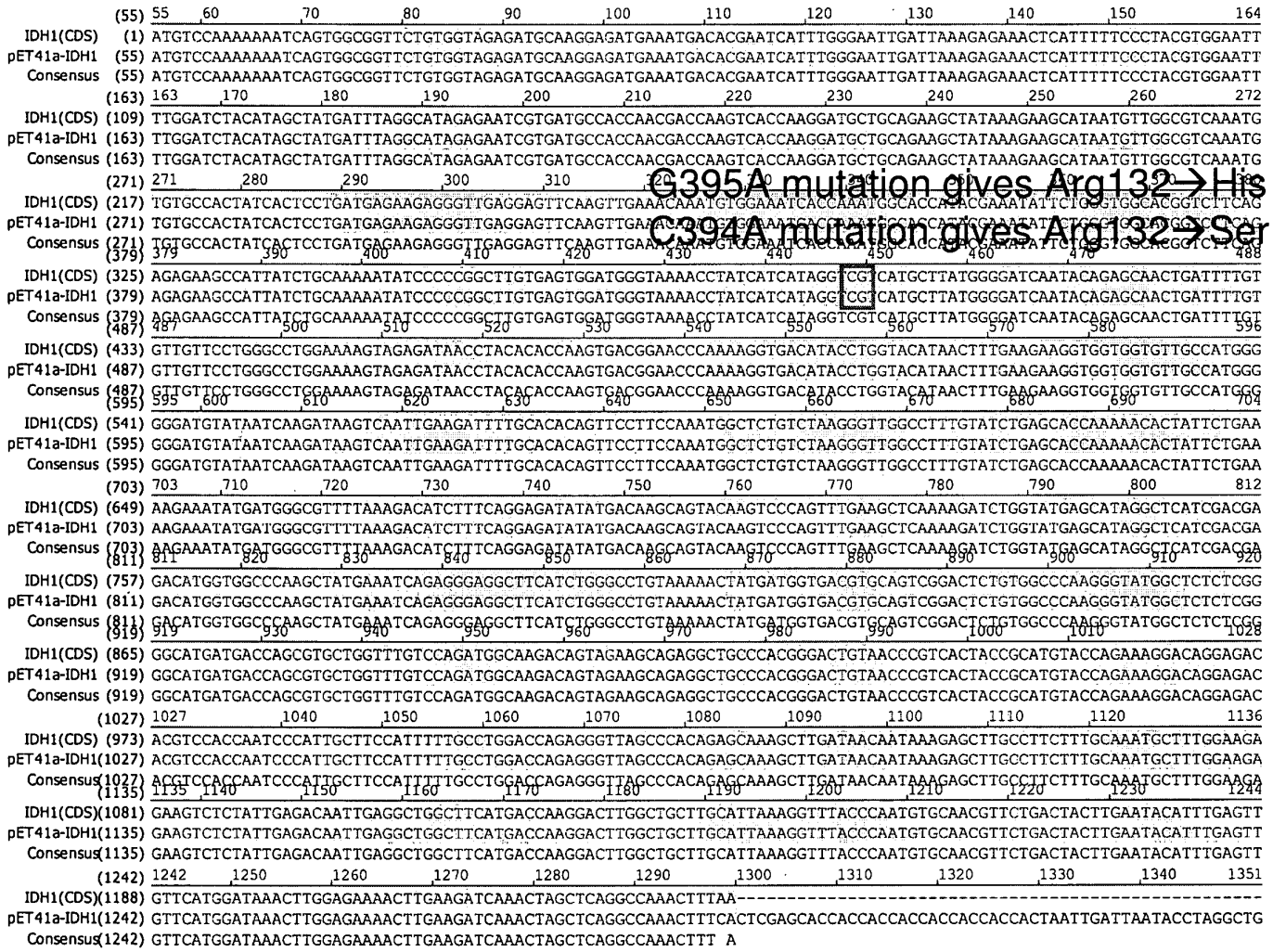


FIG. 2

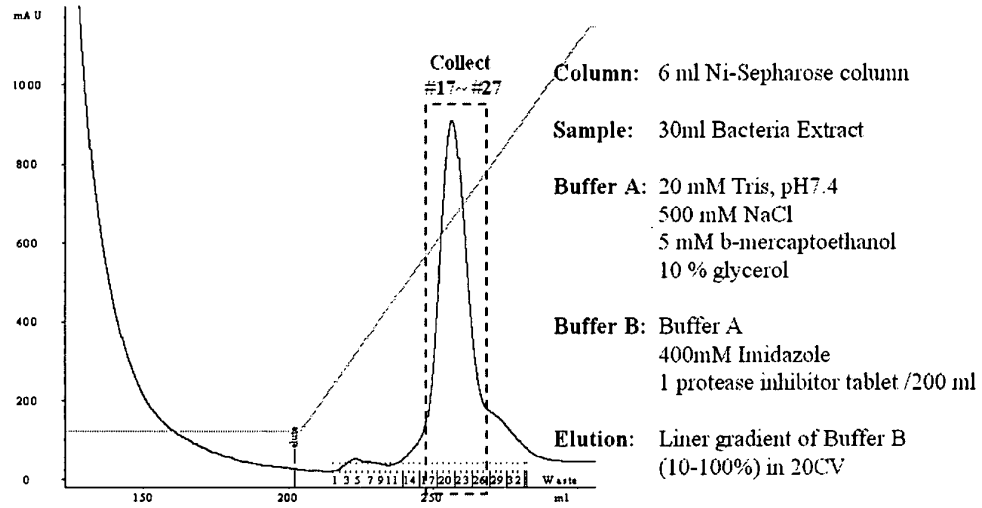

mA U
1000
800
600
400
200
0
150
200
250
ml
Collect
#17~ #27
Column: 6 ml Ni-Sepharose column
Sample: 30ml Bacteria Extract
Buffer A: 20 mM Tris, pH7.4
500 mM NaCl
5 mM b-mercaptoethanol
10 % glycerol
Buffer B: Buffer A
400mM Imidazole
1 protease inhibitor tablet /200 ml
Elution: Liner gradient of Buffer B
(10-100%) in 20CV

FIG. 3

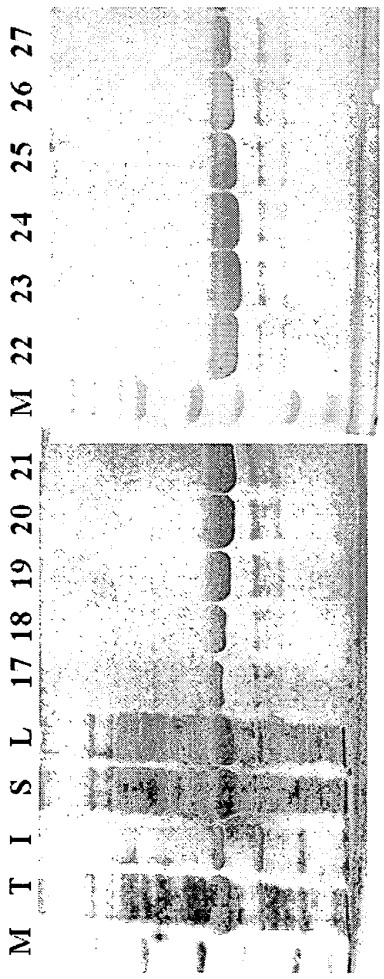
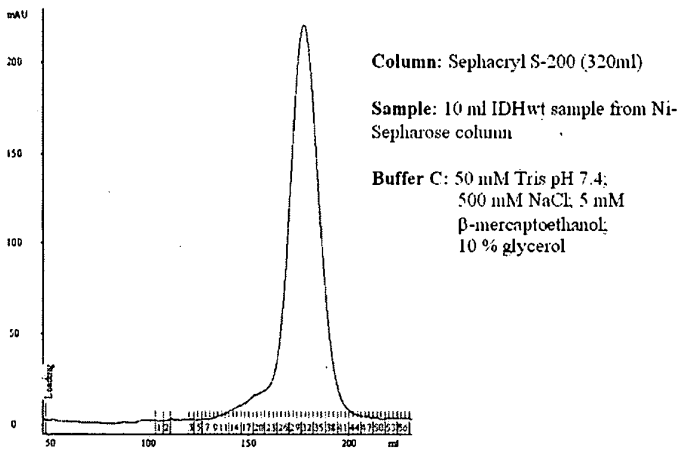


FIG. 4



Column: Sephacryl S-200 (320ml)

Sample: 10 ml IDHwt sample from Ni-Sepharose column

Buffer C: 50 mM Tris pH 7.4; 500 mM NaCl; 5 mM β-mercaptoethanol; 10 % glycerol

FIG. 5A

FIG. 5B

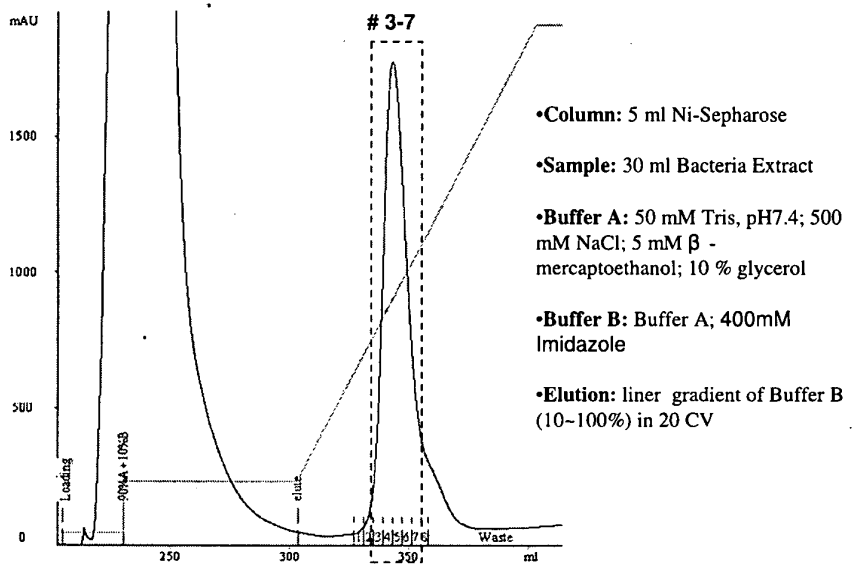


FIG. 6

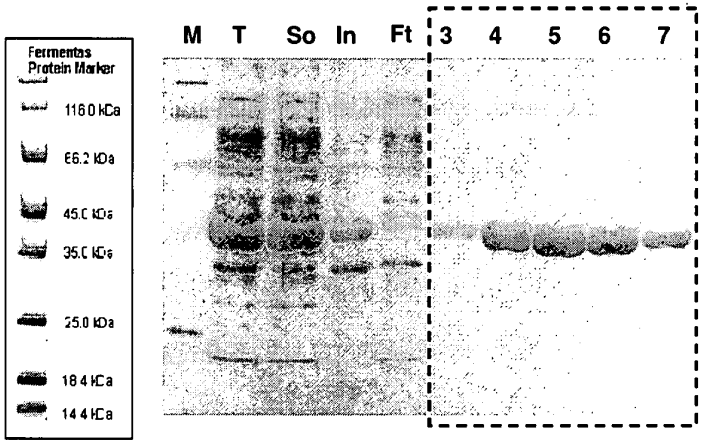


FIG. 7

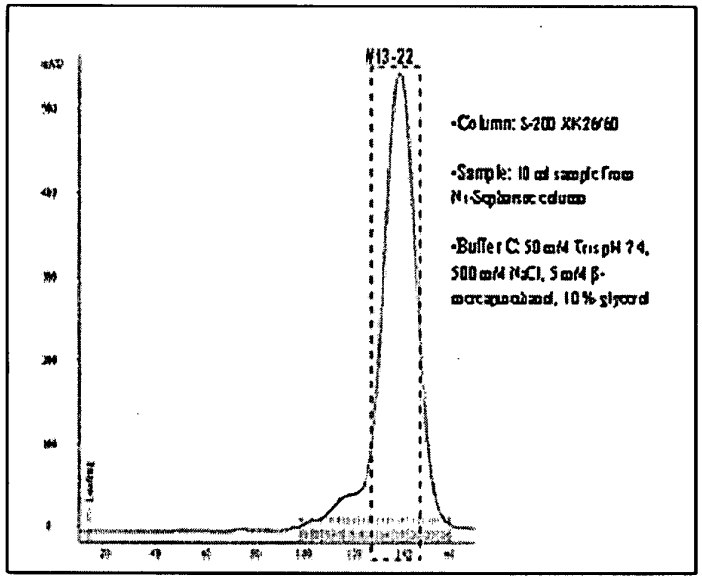


FIG. 8A

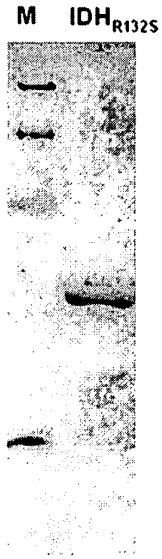


FIG. 8B

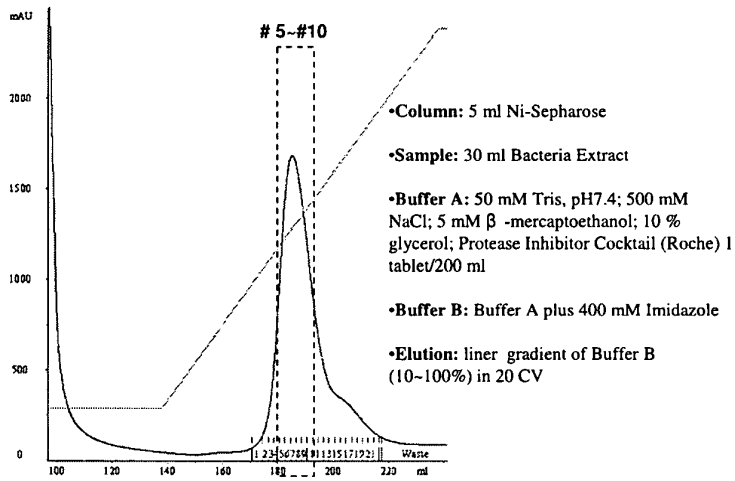


FIG. 9

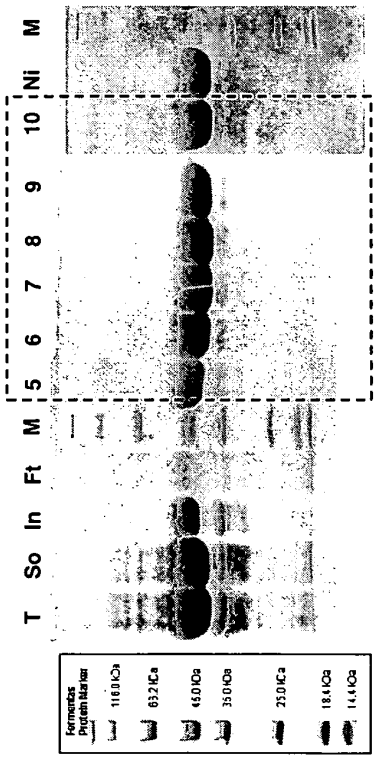

T
So
In
Ft
M
5
6
7
8
9
10
Ni
M
Fermentas Protein Marker
116.0 kDa
85.2 kDa
45.0 kDa
35.0 kDa
25.0 kDa
18.4 kDa
14.4 kDa

FIG. 10

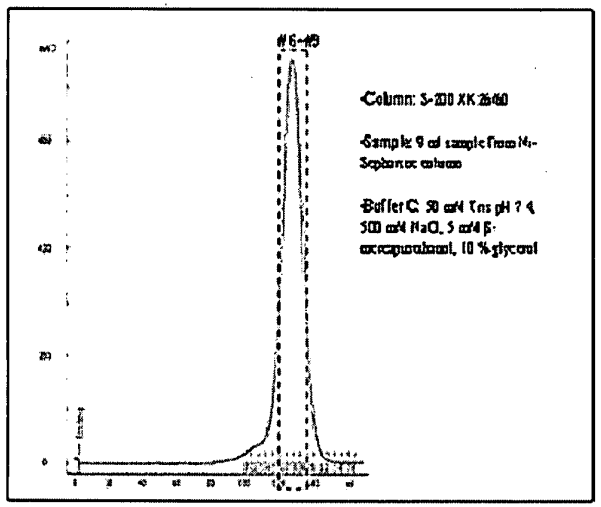


FIG. 11A

FIG. 11B

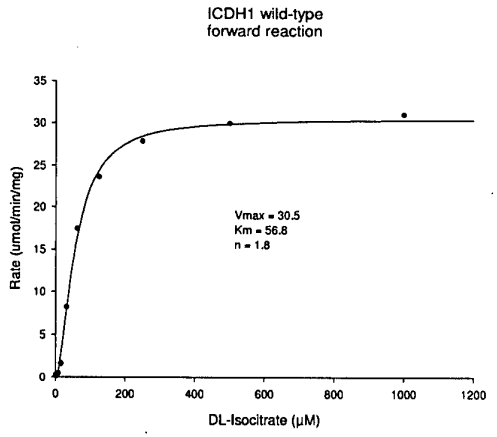


FIG. 12A

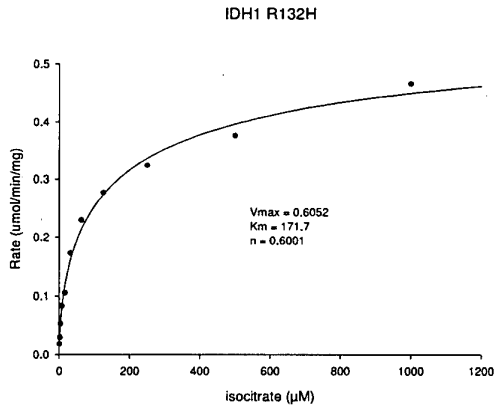


FIG. 12B

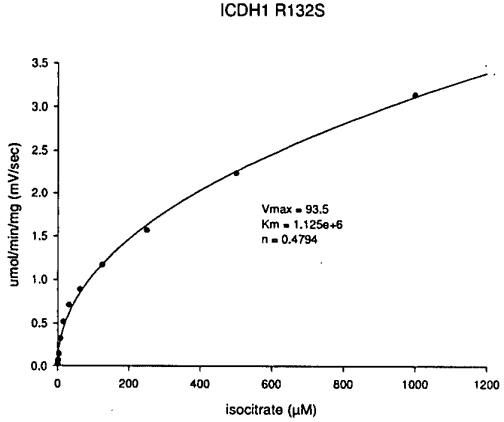


FIG. 12C

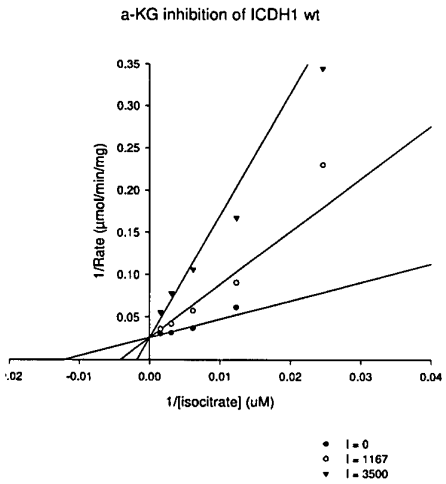


FIG. 13A

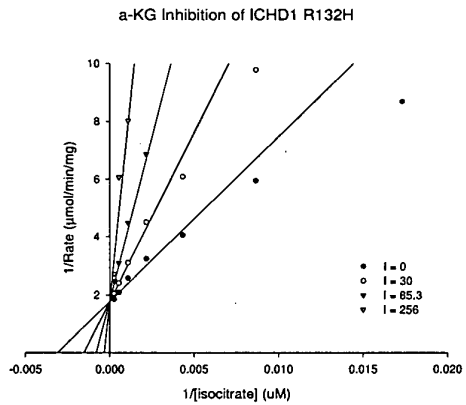


FIG. 13B

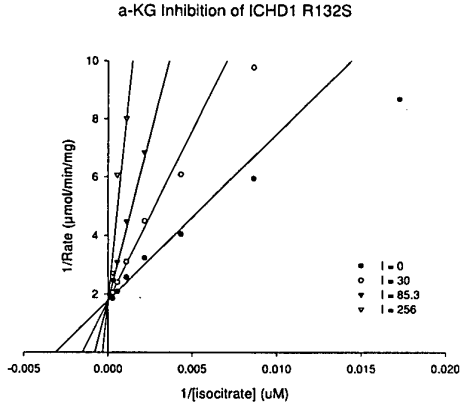


FIG. 13C

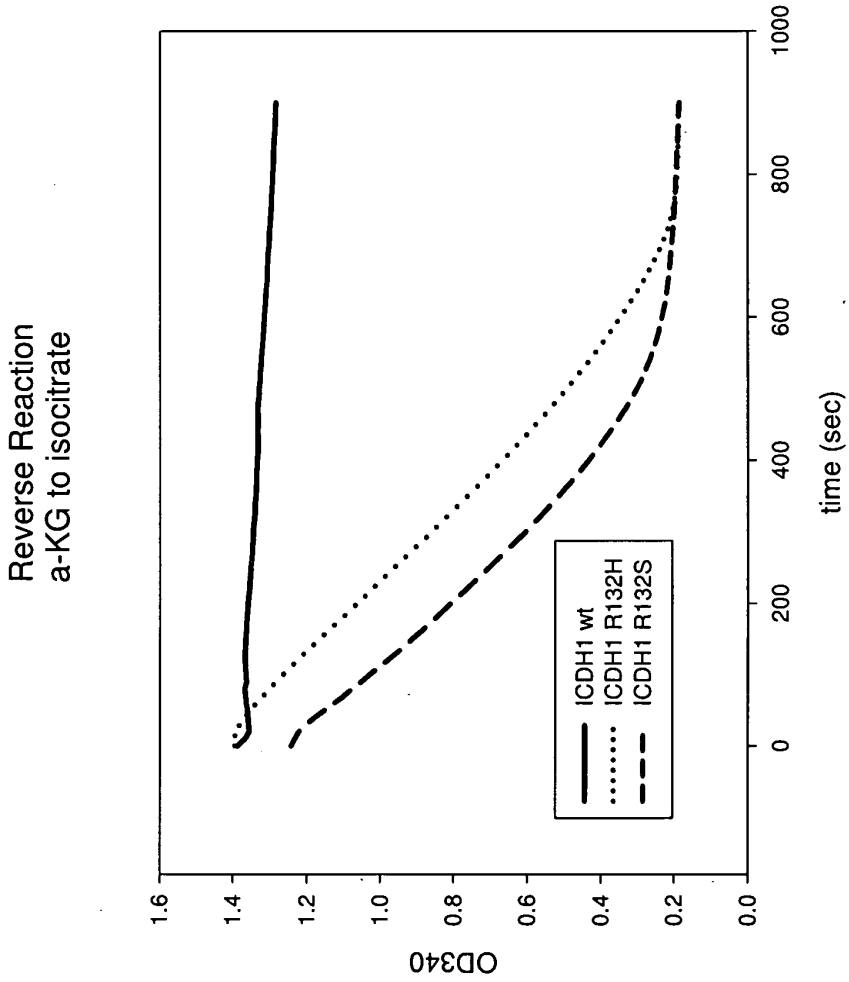

Reverse Reaction
a-KG to isocitrate
OD340
time (sec)
ICDH1 wt
ICDH1 R132H
ICDH1 R132S
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
200
400
600
800
1000

FIG. 14

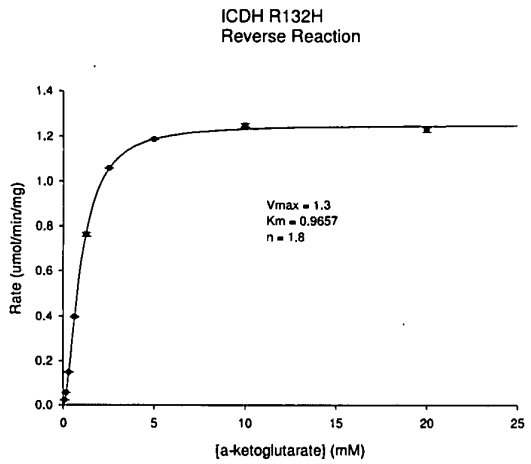


FIG. 15A

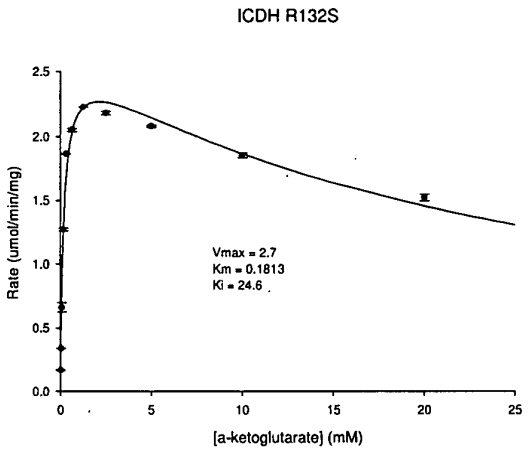


FIG. 15B

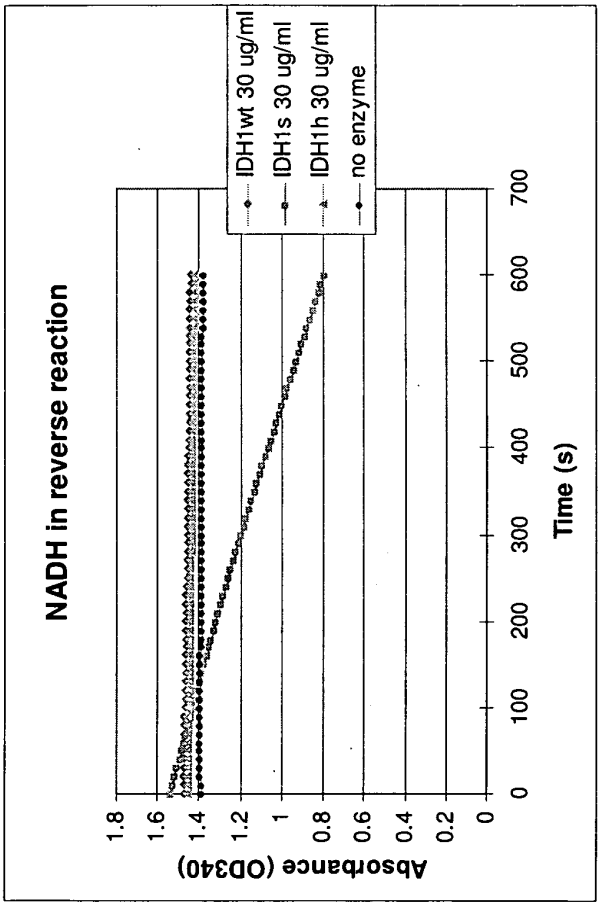

NADH in reverse reaction
IDH1wt 30 ug/ml
IDH1s 30 ug/ml
IDH1h 30 ug/ml
no enzyme
Absorbance (OD340)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
100
200
300
400
500
600
700
Time (s)

FIG. 16

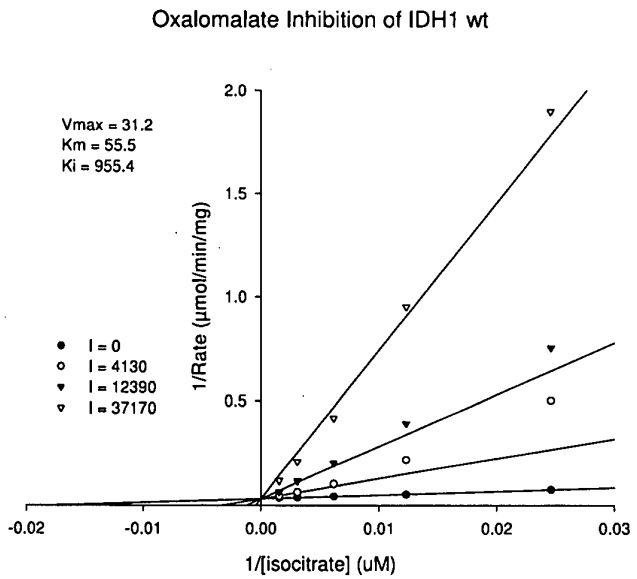


FIG. 17A

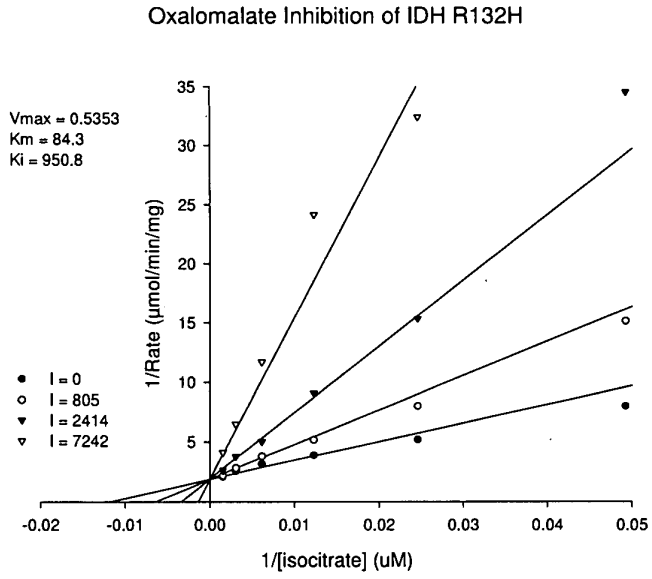


FIG. 17B

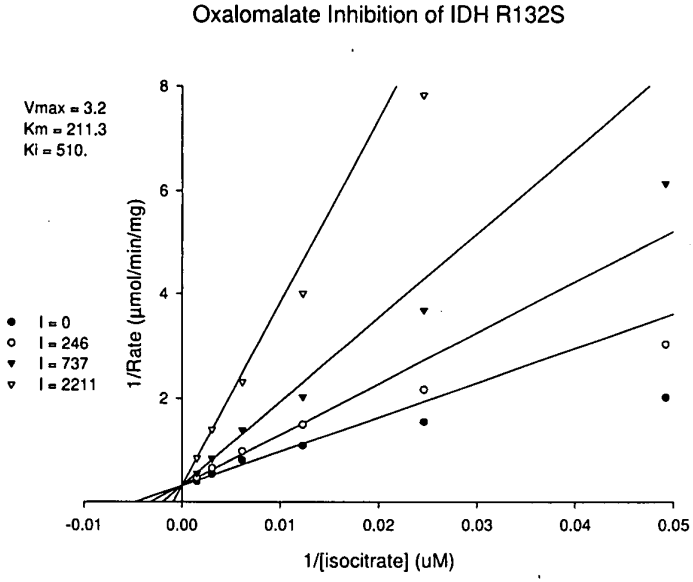


FIG. 17C

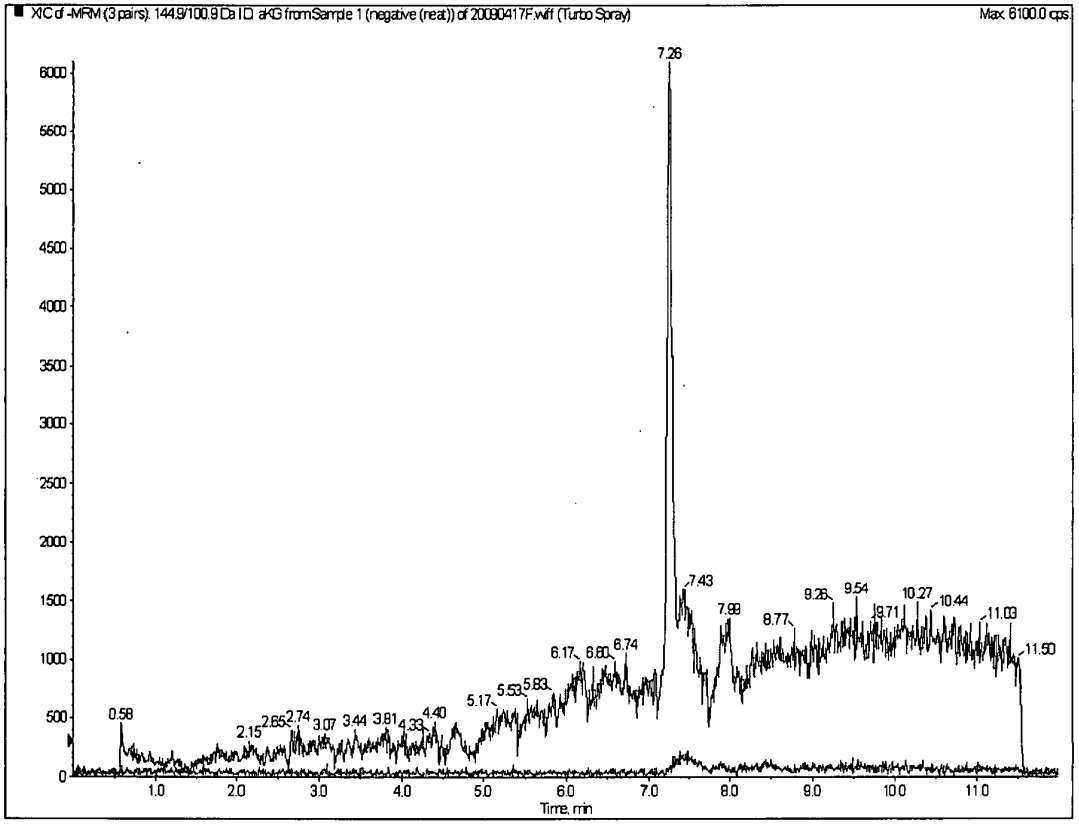


Figure 18A. LC-MS/MS analysis of the control reaction. The presence of a-KG is indicated by the peak at 7.26 minutes (blue) while no isocitrate is observed (red).

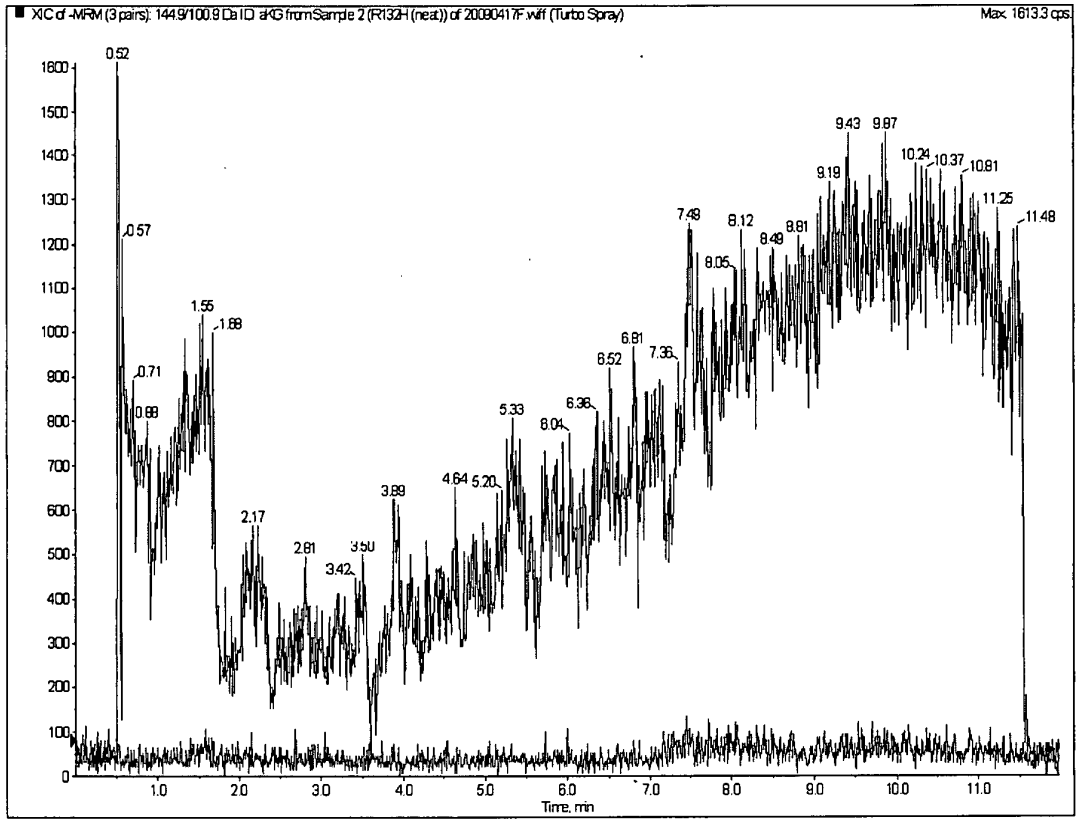


Figure 18B. LC-MS/MS analysis of the reaction containing enzyme. No isocitrate is observed (red), and a-KG has been completely consumed (blue).

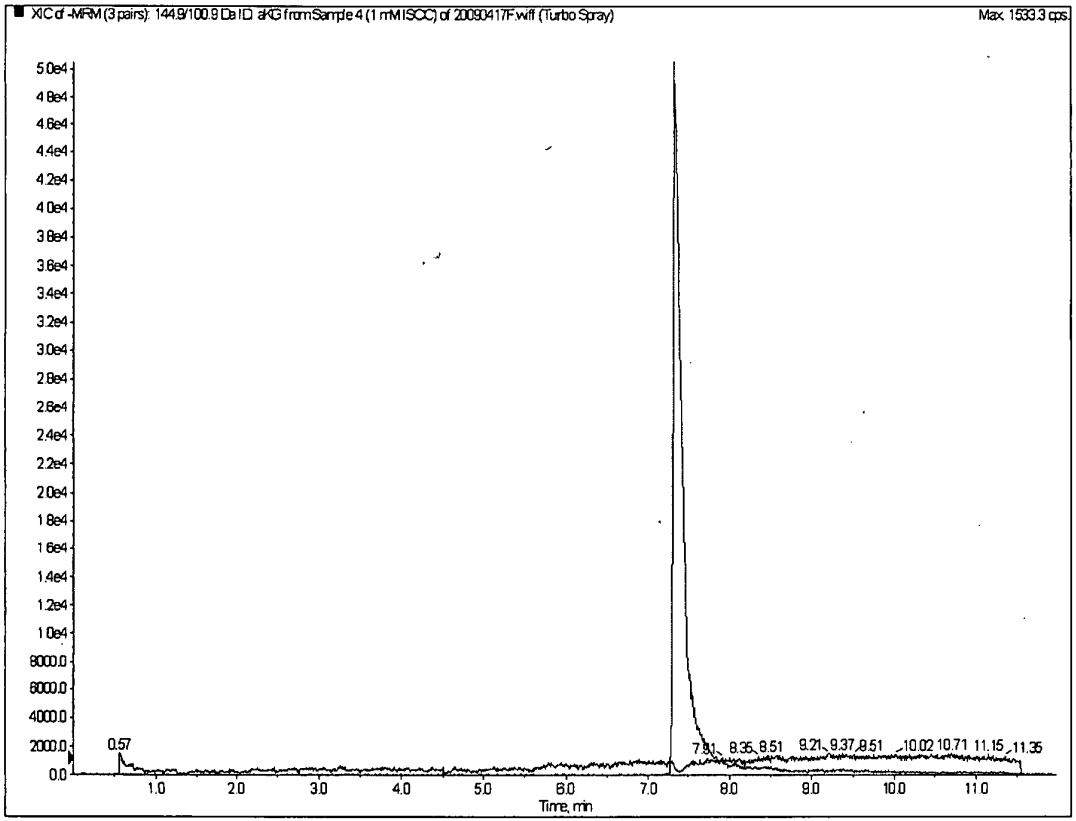


Figure 18C. LC-MS/MS analysis of the spiked control reaction. The LC-MS/MS instrument as configured can readily detect the presumptive final concentration of isocitrate. When the terminated R132H – containing reaction was spiked to a final concentration of 1 mM isocitrate, it was readily observed (red); essentially complete consumption of a-KG was confirmed (blue).

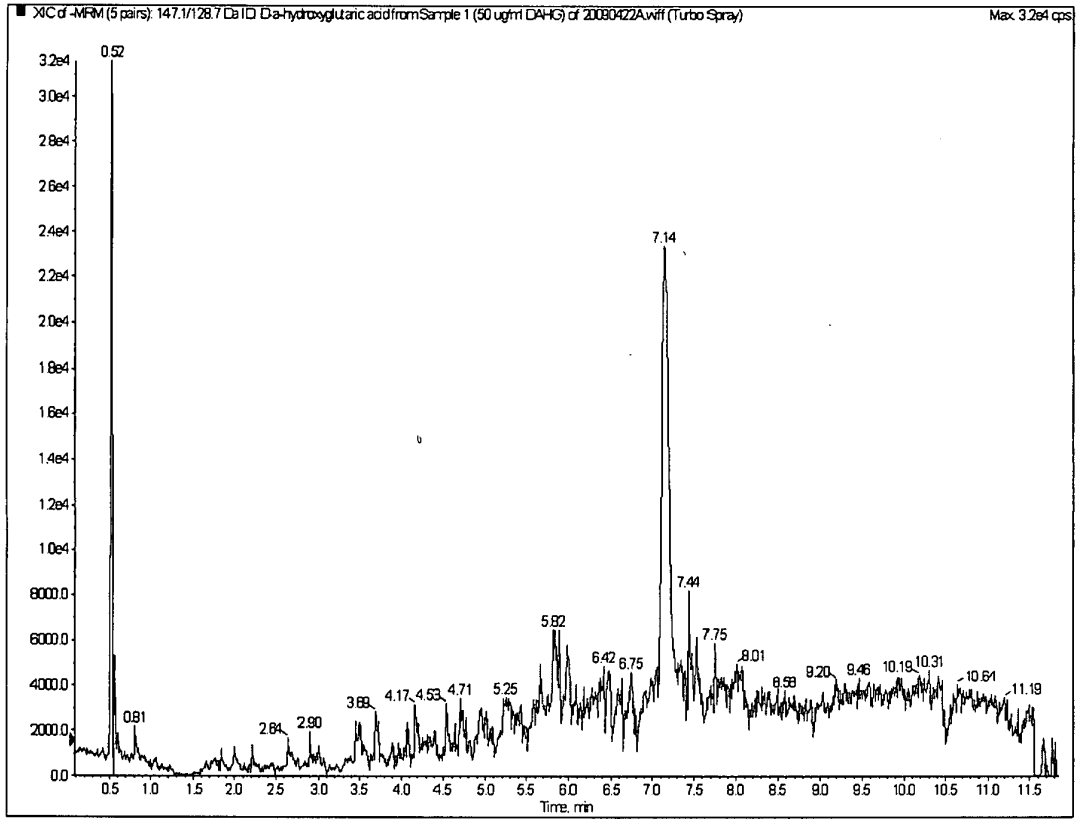


Figure 19. LC-MS/MS analysis of alpha-hydroxyglutarate. The instrument was optimized for the detection of 2-hydroxyglutarate and identified the 147.1/128.7 MRM transition as a peak retained at 7.14 minutes. The peak at 0.52 minutes is an instrument artifact caused by the switching of an inline diversion valve.

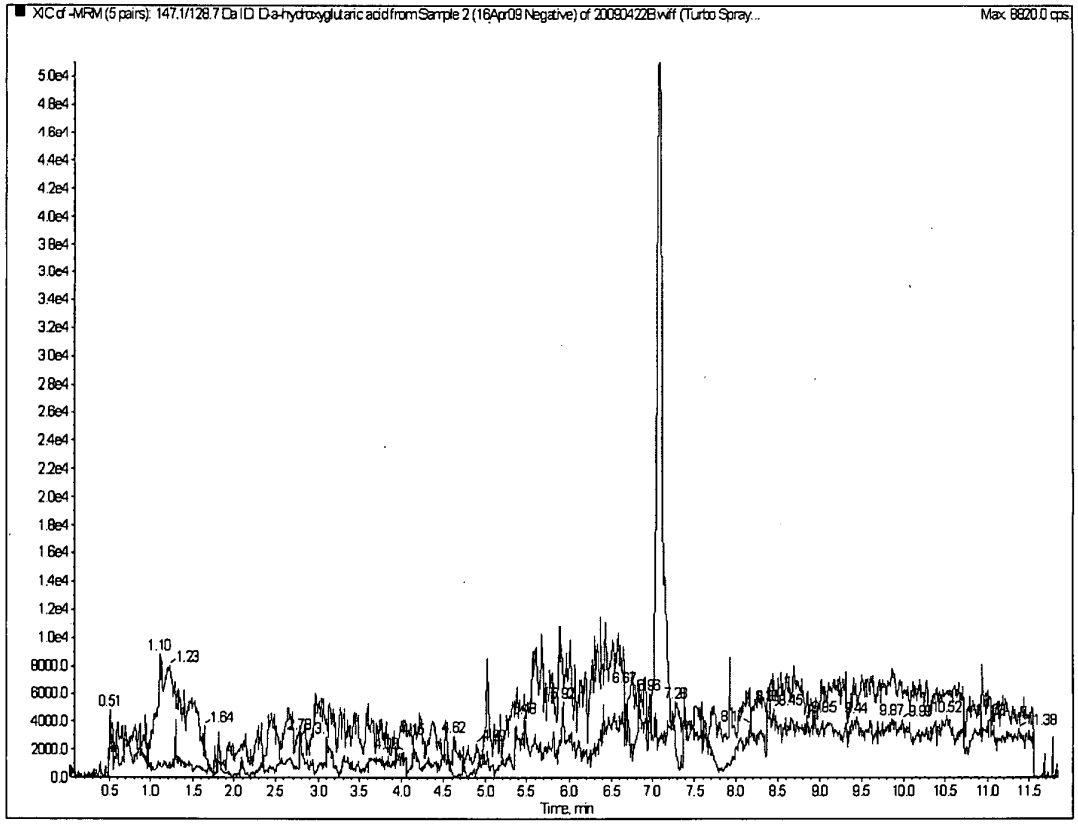
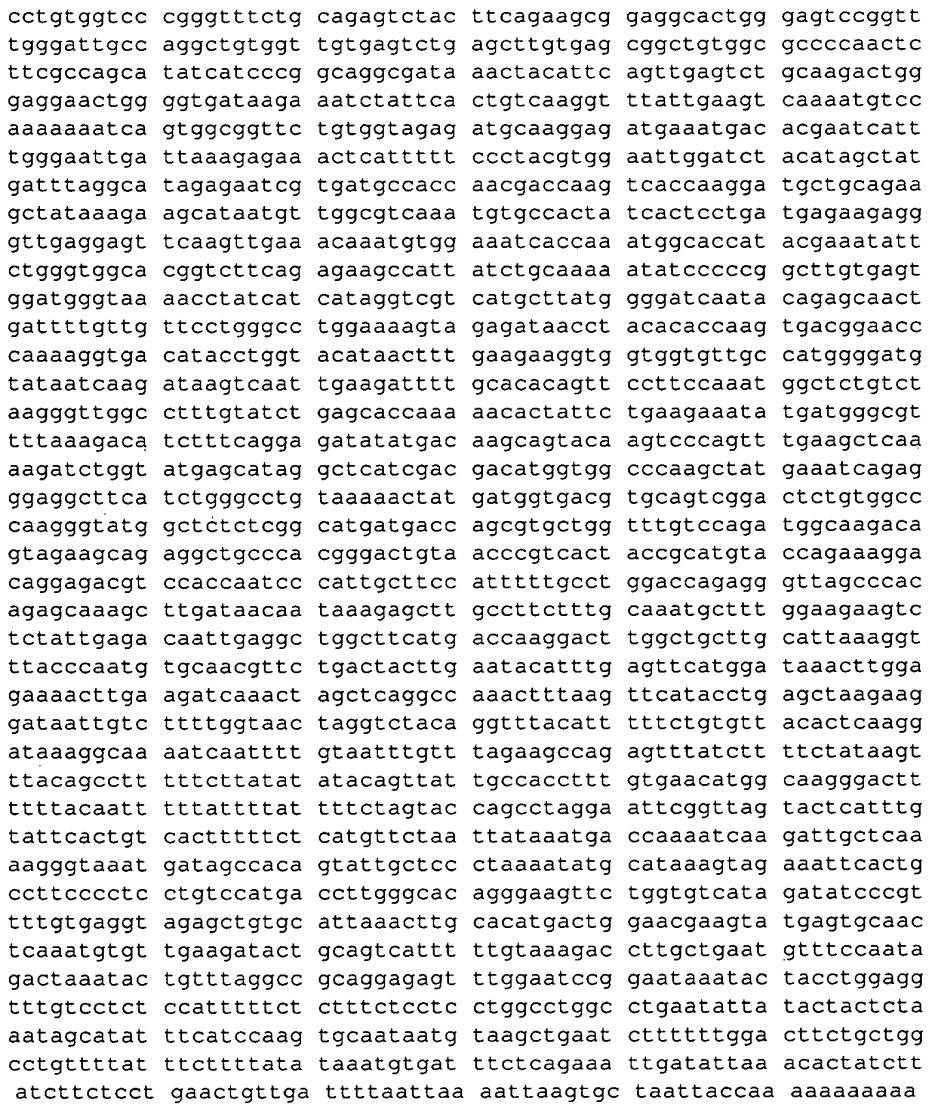


Figure 20 ICDH1 R132H consumes a-KG to produce 2-hydroxyglutaric acid. Using the detection parameters described above, control and ICDH1-containing reactions were subjected to LC-MS/MS analysis to determine the presence of 2-hydroxyglutaric acid. No 2-hydroxyglutaric acid was detected in the control reaction (blue), while in the reaction containing ICDH1 R132H (red), 2-hydroxyglutaric acid was detected, identified both by its unique MRM signature and diagnostic retention time.

```
  1 mskkisggsv vemqgdemtr iiwelikekl ifpyveldlh sydlgienrd atndqvtkda
 61 aeaikkhnvg vkcatitpde krveefklkq mwkspngtir nilggtvfre aiickniprl
121 vsgwvkpiii grhaygdqyr atdfvvpgpg kveitytpsd gtqkvtylvh nfeegggvam
181 gmynqdksie dfahssfqma lskgwplyls tkntilkkyd grfkdifqei ydkqyksqfe
241 aqkiwyehrl iddmvaqamk seggfiwack nydgdvqsds vaqgygslgm mtsvlvcpdg
301 ktveaeeahg tvtrhyrmyq kgqetstnpi asifawtrgl ahrakldnnk elaffanale
361 evsietieag fmtkdlaaci kglpnvqrsd ylntfefmdk lgenlkikla qakl
```

FIG. 21



```
   1 cctgtggtcc cgggtttctg cagagtctac ttcagaagcg gaggcactgg gagtccggtt
  61 tgggattgcc aggctgtggt tgtgagtctg agcttgtgag cggctgtggc gccccaactc
 121 ttcgccagca tatcatcccg gcaggcgata aactacattc agttgagtct gcaagactgg
 181 gaggaactgg ggtgataaga aatctattca ctgtcaaggt ttattgaagt caaaatgtcc
 241 aaaaaaatca gtggcggttc tgtggtagag atgcaaggag atgaaatgac acgaatcatt
 301 tgggaattga ttaaagagaa actcattttt ccctacgtgg aattggatct acatagctat
 361 gatttaggca tagagaatcg tgatgccacc aacgaccaag tcaccaagga tgctgcagaa
 421 gctataaaga agcataatgt tggcgtcaaa tgtgccacta tcactcctga tgagaagagg
 481 gttgaggagt tcaagttgaa acaaatgtgg aaatcaccaa atggcaccat acgaaatatt
 541 ctgggtggca cggtcttcag agaagccatt atctgcaaaa atatcccccg gcttgtgagt
 601 ggatgggtaa aacctatcat cataggtcgt catgcttatg gggatcaata cagagcaact
 661 gattttgttg ttcctgggcc tggaaaagta gagataacct acacaccaag tgacggaacc
 721 caaaaggtga catacctggt acataacttt gaagaaggtg gtggtgttgc catggggatg
 781 tataatcaag ataagtcaat tgaagatttt gcacacagtt ccttccaaat ggctctgtct
 841 aagggttggc ctttgtatct gagcaccaaa aacactattc tgaagaaata tgatgggcgt
 901 tttaaagaca tctttcagga gatatatgac aagcagtaca agtcccagtt tgaagctcaa
 961 aagatctggt atgagcatag gctcatcgac gacatggtgg cccaagctat gaaatcagag
1021 ggaggcttca tctgggcctg taaaaactat gatggtgacg tgcagtcgga ctctgtggcc
1081 caagggtatg gctctctcgg catgatgacc agcgtgctgg tttgtccaga tggcaagaca
1141 gtagaagcag aggctgccca cgggactgta acccgtcact accgcatgta ccagaaagga
1201 caggagacgt ccaccaatcc cattgcttcc atttttgcct ggaccagagg gttagcccac
1261 agagcaaagc ttgataacaa taaagagctt gccttctttg caaatgcttt ggaagaagtc
1321 tctattgaga caattgaggc tggcttcatg accaaggact tggctgcttg cattaaaggt
1381 ttacccaatg tgcaacgttc tgactacttg aatacatttg agttcatgga taaacttgga
1441 gaaaacttga agatcaaact agctcaggcc aaactttaag ttcatacctg agctaagaag
1501 gataattgtc ttttggtaac taggtctaca ggtttacatt tttctgtgtt acactcaagg
1561 ataaaggcaa aatcaatttt gtaatttgtt tagaagccag agtttatctt ttctataagt
1621 ttacagcctt tttcttatat atacagttat tgccaccttt gtgaacatgg caagggactt
1681 ttttacaatt tttatttttat tttctagtac cagcctagga attcggttag tactcatttg
1741 tattcactgt cacttttttct catgttctaa ttataaatga ccaaaatcaa gattgctcaa
1801 aagggtaaat gatagccaca gtattgctcc ctaaaatatg cataaagtag aaattcactg
1861 ccttcccctc ctgtccatga ccttgggcac agggaagttc tggtgtcata gatatcccgt
1921 tttgtgaggt agagctgtgc attaaacttg cacatgactg gaacgaagta tgagtgcaac
1981 tcaaatgtgt tgaagatact gcagtcattt ttgtaaagac cttgctgaat gtttccaata
2041 gactaaatac tgtttaggcc gcaggagagt ttggaatccg gaataaatac tacctggagg
2101 tttgtcctct ccatttttct ctttctcctc ctggcctggc ctgaatatta tactactcta
2161 aatagcatat ttcatccaag tgcaataatg taagctgaat ctttttgga cttctgctgg
2221 cctgttttat ttcttttata taaatgtgat ttctcagaaa ttgatattaa acactatctt
2281 atcttctcct gaactgttga ttttaattaa aattaagtgc taattaccaa aaaaaaaaa
```

FIG. 22

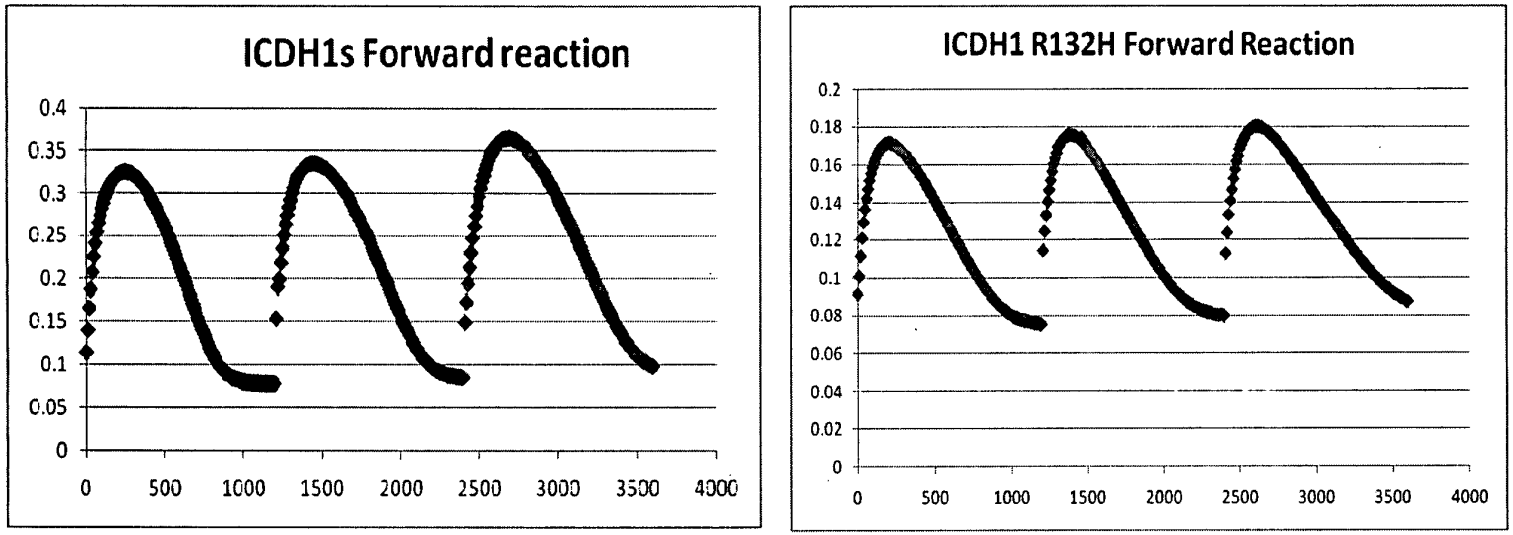


FIG. 23

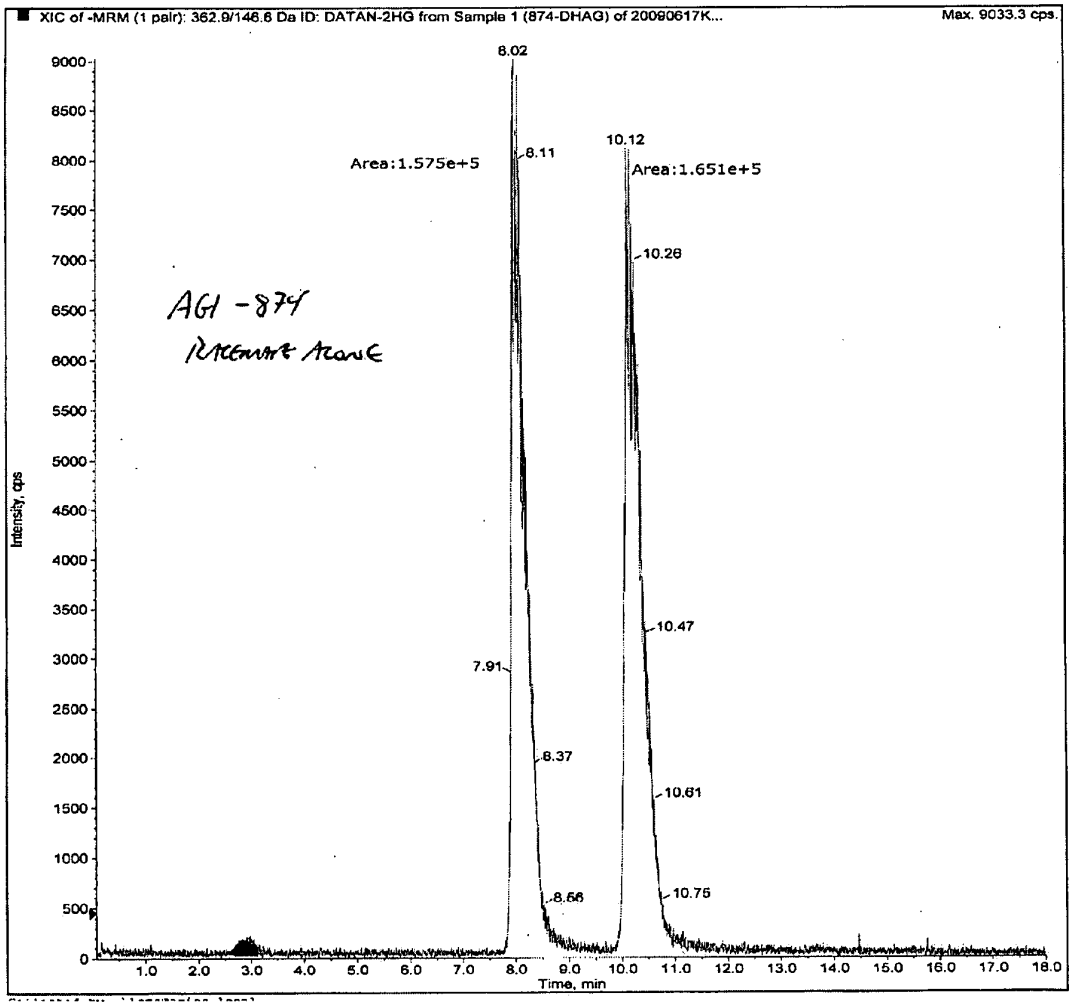

XIC of -MRM (1 pair): 362.9/146.6 Da ID: DATAN-2HG from Sample 1 (874-DHAG) of 20090617K...
Max. 9033.3 cps.
Intensity, cps
Time, min
8.02
8.11
Area:1.575e+5
10.12
Area:1.651e+5
10.28
AGI -874
Racemate Acone
10.47
7.91
8.37
10.61
8.56
10.75

FIG. 24A

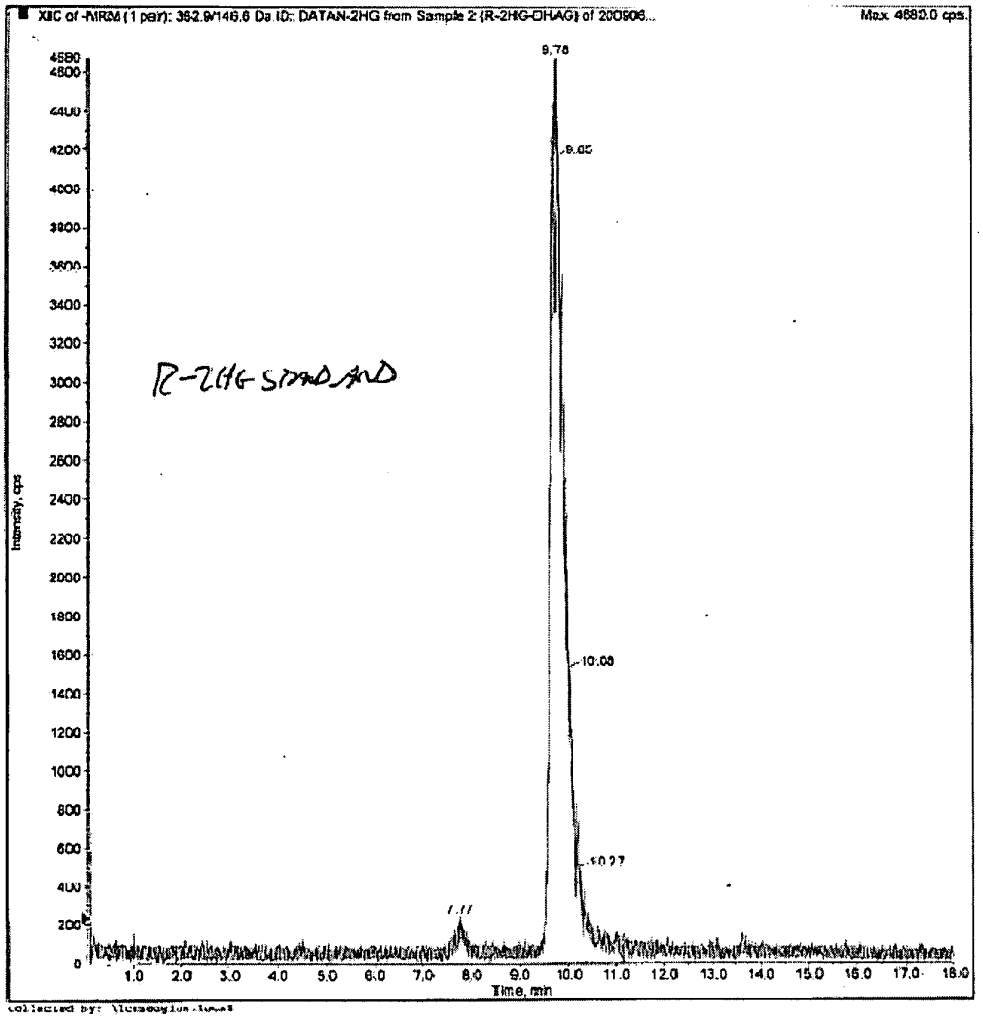

XIC of -MRM (1 pair): 362.9/146.8 Da ID: DATAN-2HG from Sample 2 (R-2HG-DHAG) of 200906...
Max. 4680.0 cps.
9.78
9.05
10.00
10.27
R-2HG STANDARD
Intensity, cps
Time, min

FIG. 24B

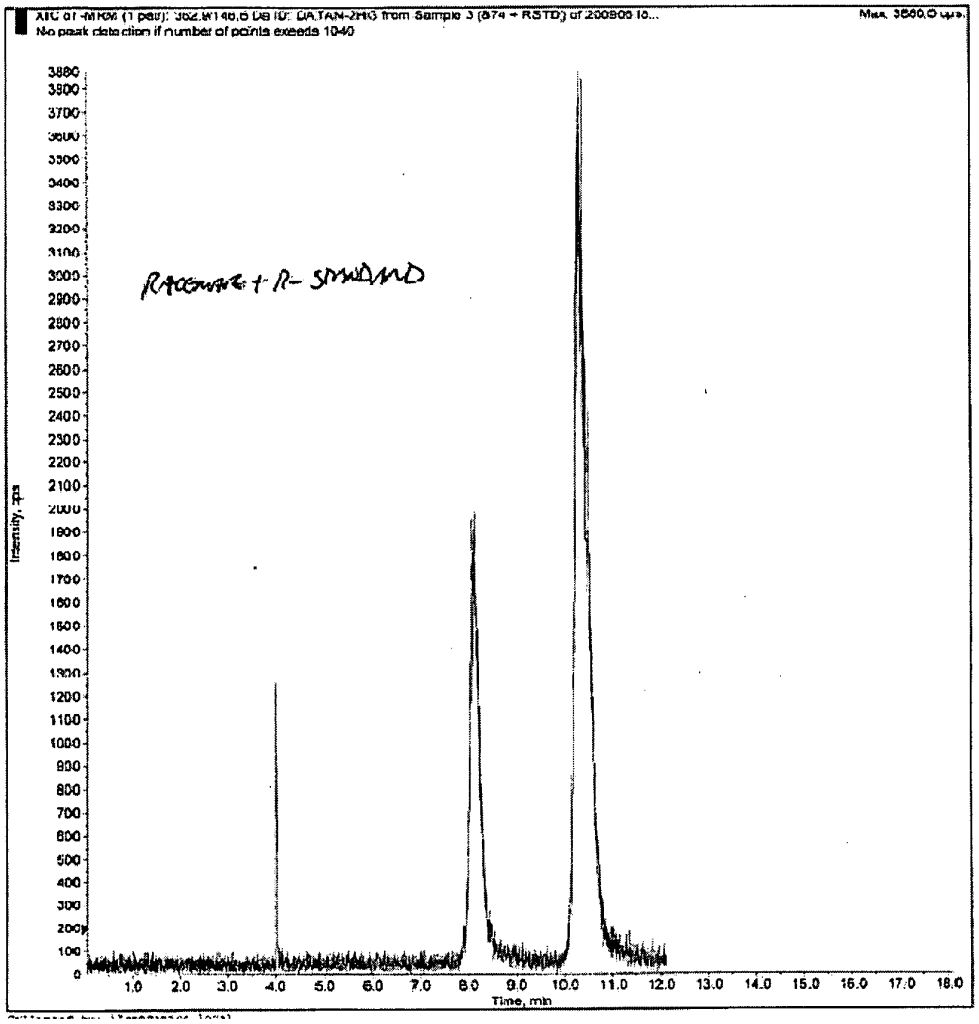

No peak detection if number of points exceeds 1040
RACEMATE + R- STANDARD
Intensity, cps
Time, min

FIG. 24C

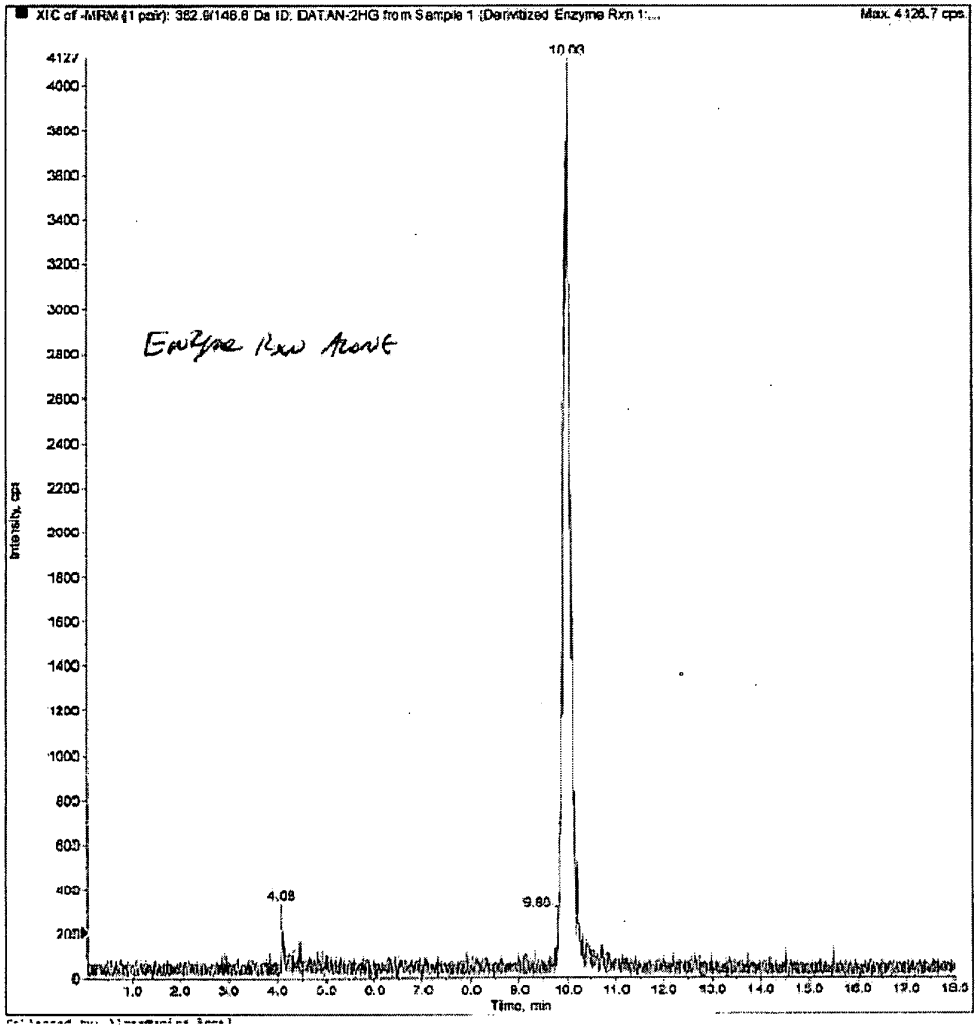

XIC of -MRM (1 pair): 382.9/146.8 Da ID: DATAN-2HG from Sample 1 (Derivatized Enzyme Rxn 1:...
Max. 4126.7 cps.
10.03
Enzyme Rxn Mixture
4.08
9.80
Intensity, cps
Time, min

FIG. 24D

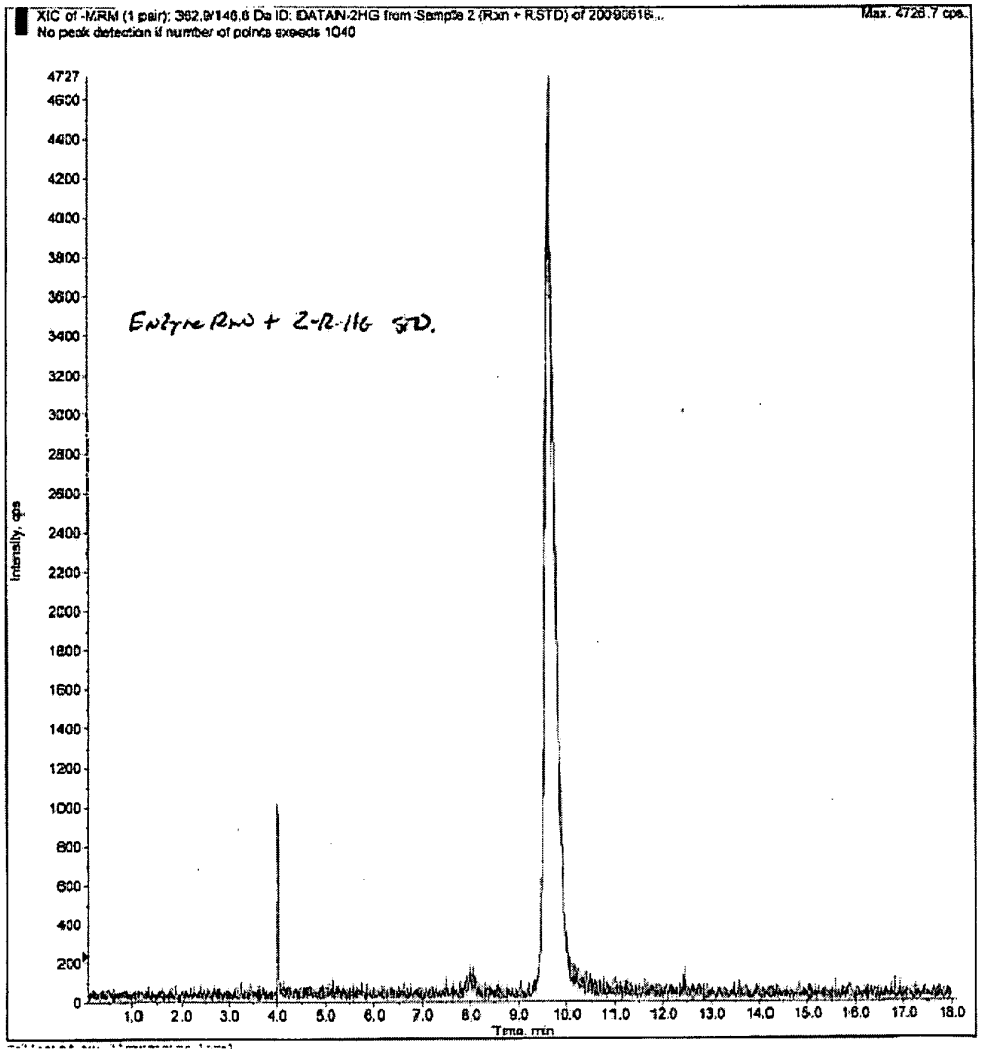

XIC of -MRM (1 pair): 362.9/146.6 Da ID: IDATAN-2HG from Sample 2 (Rxn + R.STD) of 20090618...
Max. 4726.7 cps.
No peak detection if number of points exceeds 1040
ENZYME RXN + 2-R-HG STD.
Intensity, cps
Time, min

FIG. 24E

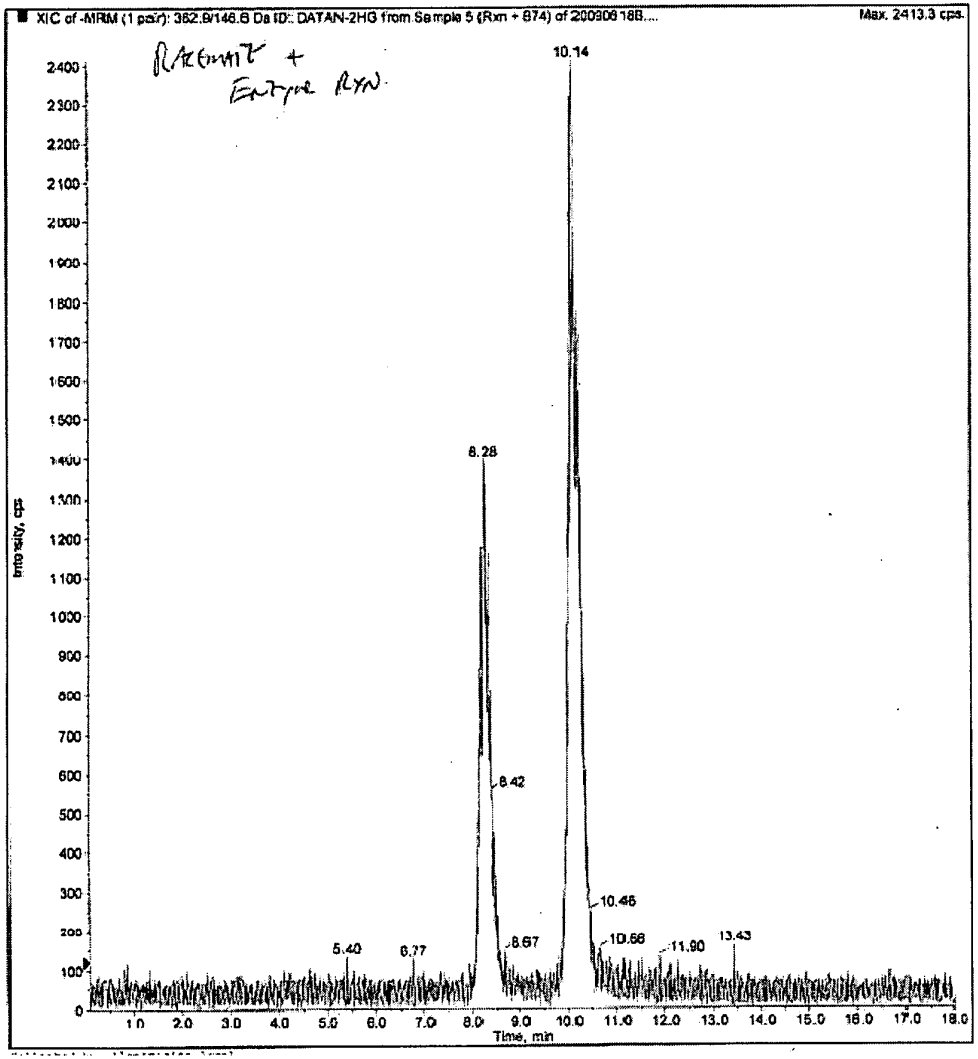


FIG. 24F

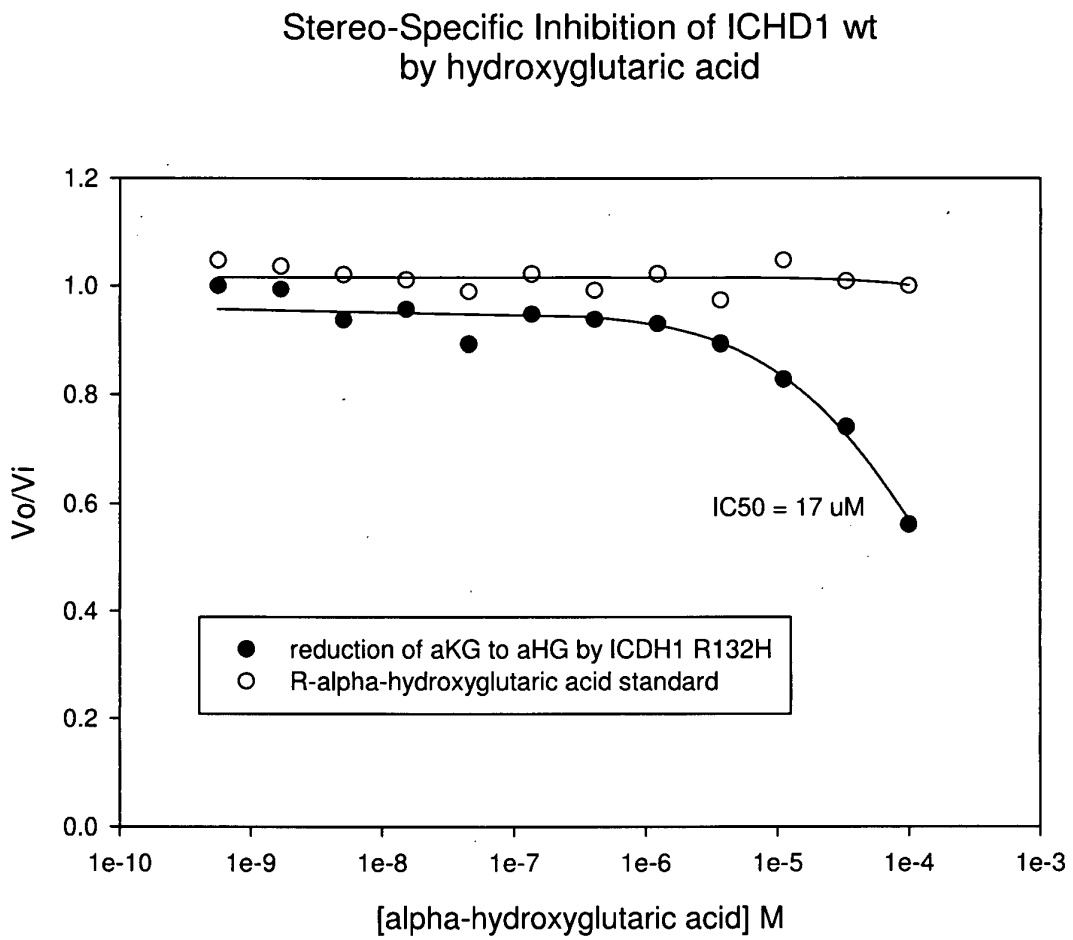

Stereo-Specific Inhibition of ICHD1 wt
by hydroxyglutaric acid
Vo/Vi
1.2
1.0
0.8
0.6
0.4
0.2
0.0
IC50 = 17 uM
reduction of aKG to aHG by ICDH1 R132H
R-alpha-hydroxyglutaric acid standard
1e-10
1e-9
1e-8
1e-7
1e-6
1e-5
1e-4
1e-3
[alpha-hydroxyglutaric acid] M

FIG. 25

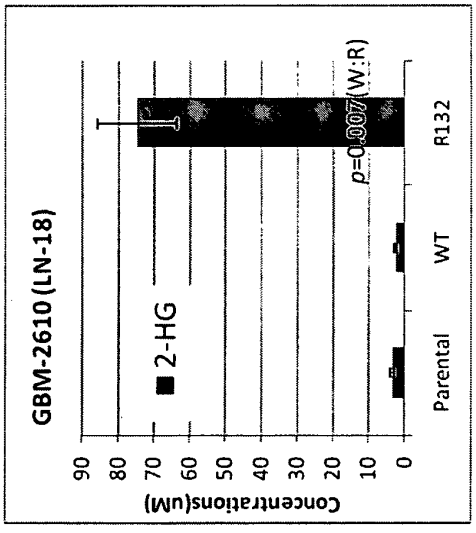


FIG. 26A

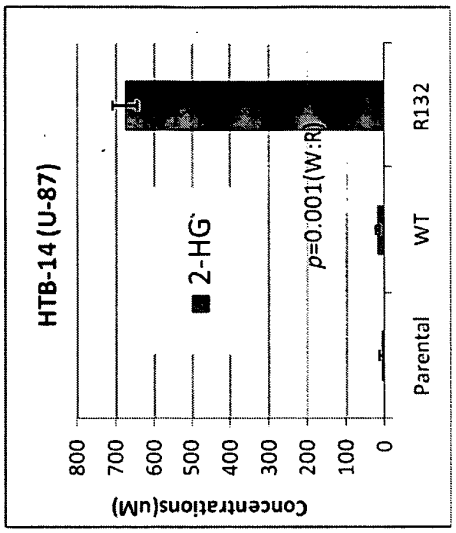


FIG. 26B

gcataatgagctctatatgccatcactgcagttgtaggttataactatccatttgtctgaaaaactttgcttc
taatttttctctttcaagCTATGATTTAGGCATAGAGAATCGTGATGCCACCAAC
GACCAAGTCACCAAGGATGCTGCAGAAGCTATAAAGAAGCATAATGT
TGGCGTCAAATGTGCCACTATCACTCCTGATGAGAAGAGGGTTGAG
GAGTTCAAGTTGAAACAAATGTGGAAATCACCAAATGGCACCATACG
AAATATTCTGGGTGGCJGTCTTCAGAGAAGCCATTATCTGCAAAAATA
TCCCCCGGCTTGTGAGTGGATGGGTAAAACCTATCATCATAGGTCGT
CATGCTTATGGGGATCAAgtaagtcatgttggcaataatgtgattttgcatgbtggcccaga
aatttccaacttgtatgtgttttattcttatcttttggtatctacacccattaagcaaggta

FIG. 27

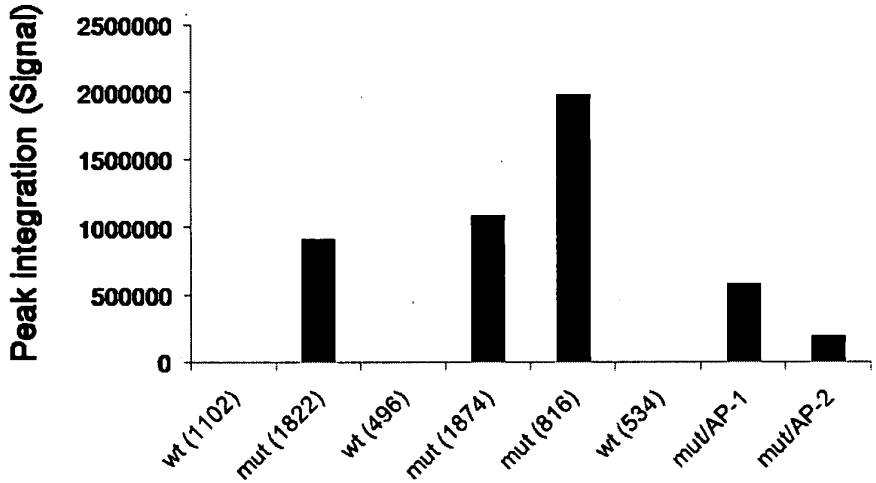


FIG. 28

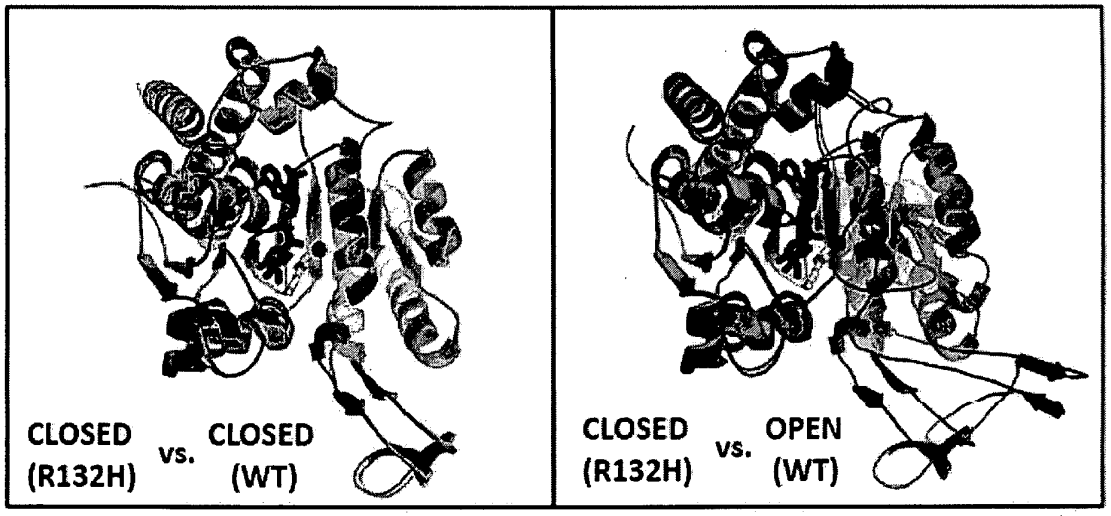


FIG. 29A

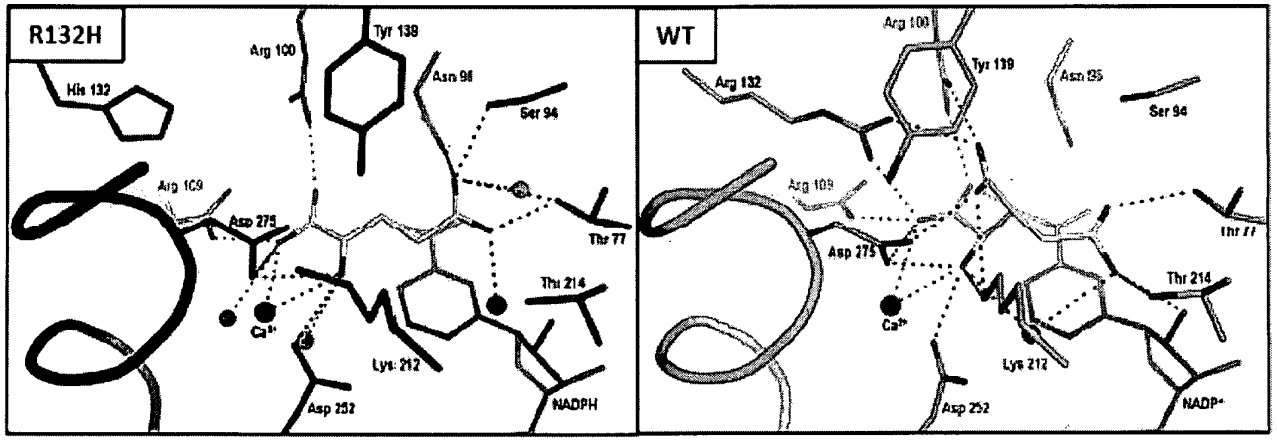


FIG. 29B

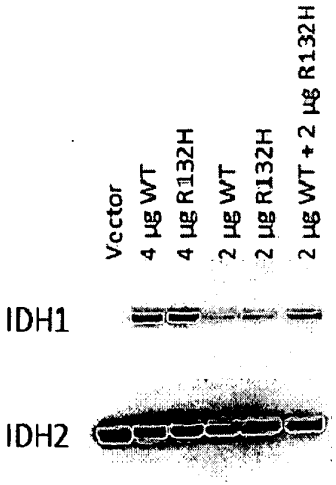


FIG. 30A

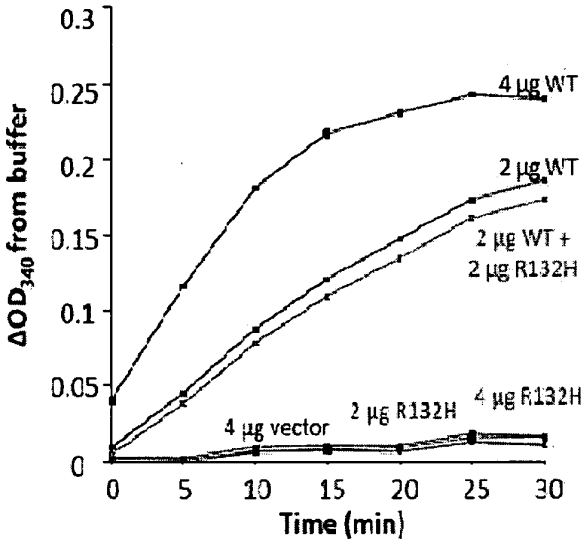


FIG. 30B

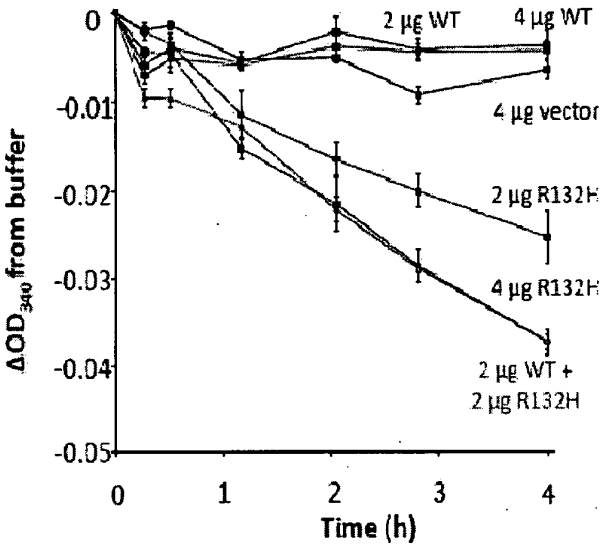


FIG. 30C

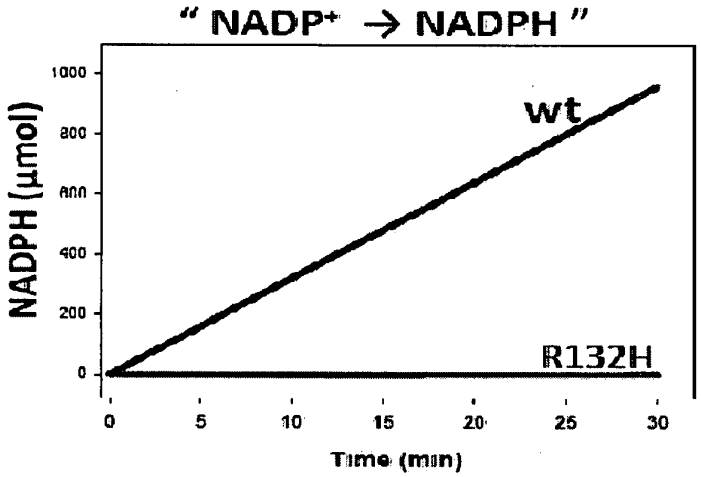


FIG. 31A

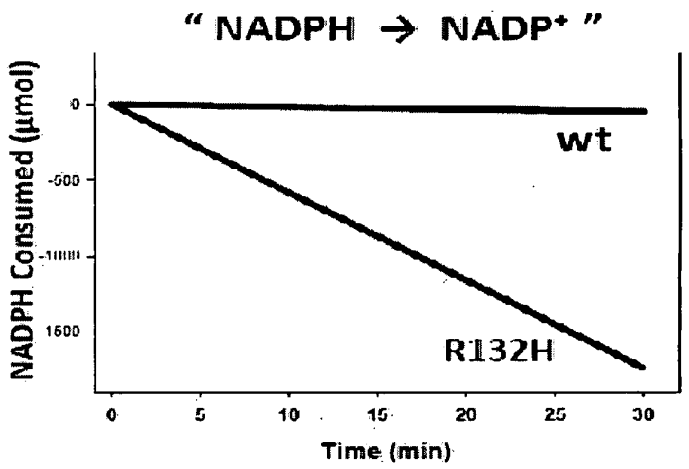


FIG. 31B

Oxidative (→ NADPH)	WT	R132H	Reductive (→ NADP+)	WT	R132H
$K_{M,NADP+}$ (µM)	49	84	$K_{M,NADPH}$ (µM)	n/a*	0.44
$K_{M,isocitrate}$ (µM)	65	370	$K_{M,\alpha KG}$ (µM)	n/a	965
$K_{M,MgCl_2}$ (µM)	29	10085	k_{cat} (s^{-1})	n/a	1.0×10^3
$K_{i,\alpha KG}$ (µM)	1871	24			
k_{cat} (s^{-1})	4.4×10^5	37.5			

*n/a = No measurable enzymatic activity

FIG. 31C

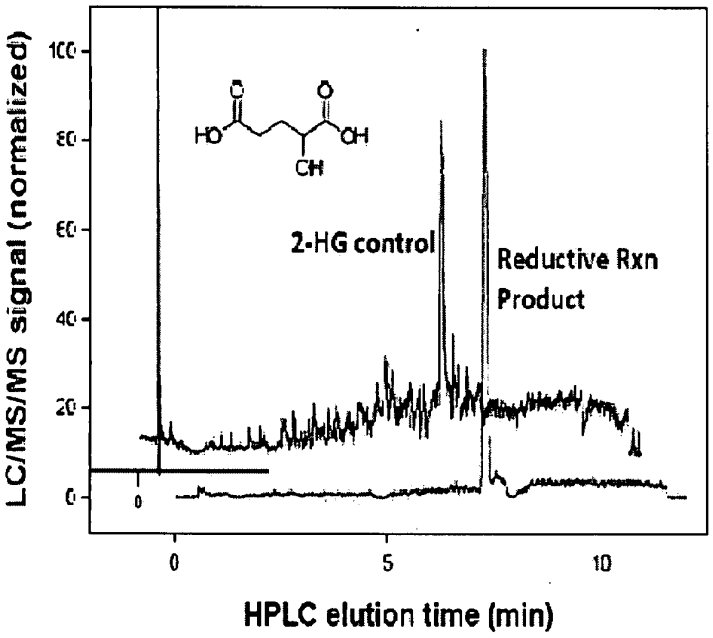


FIG. 32A

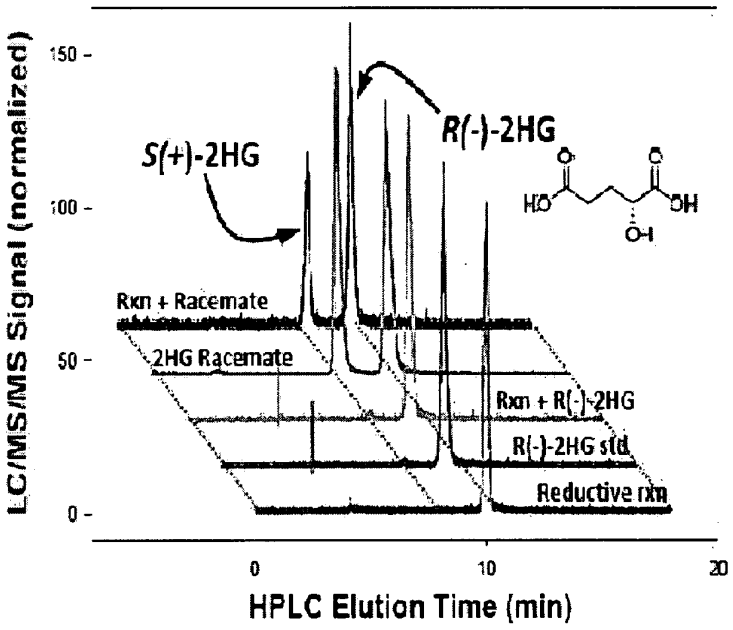


FIG. 32B

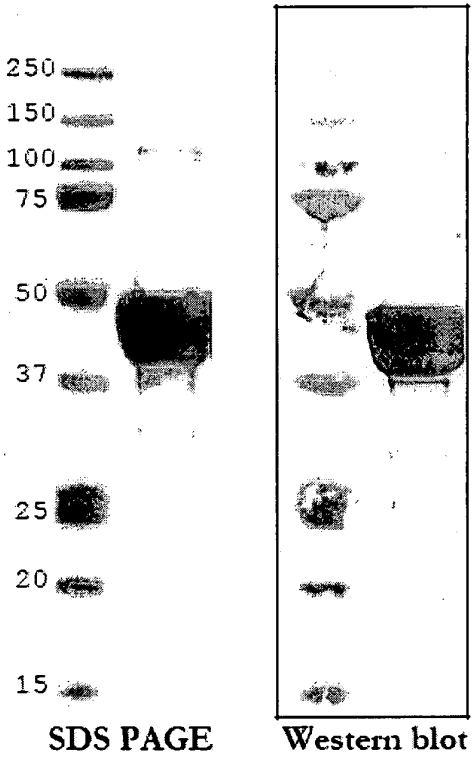


FIG. 33A

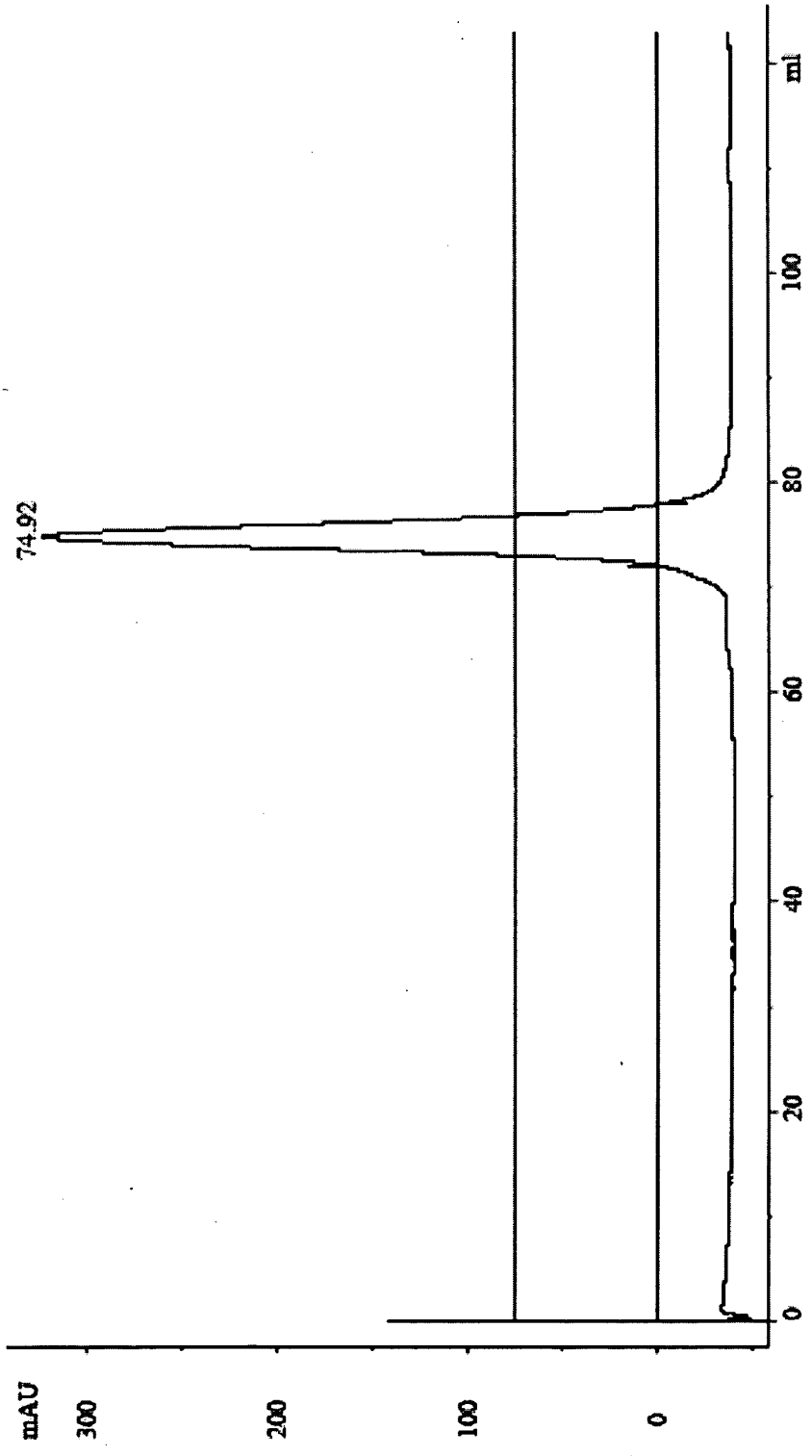


FIG. 33B

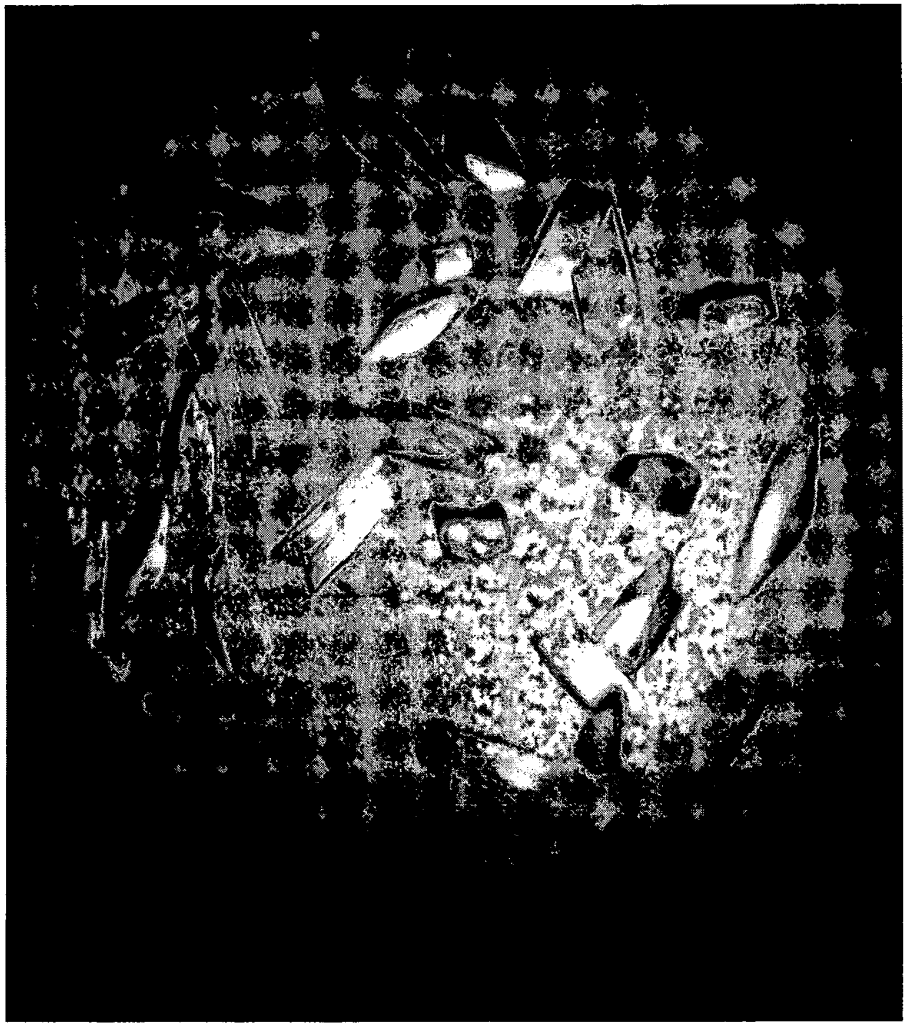


FIG. 34

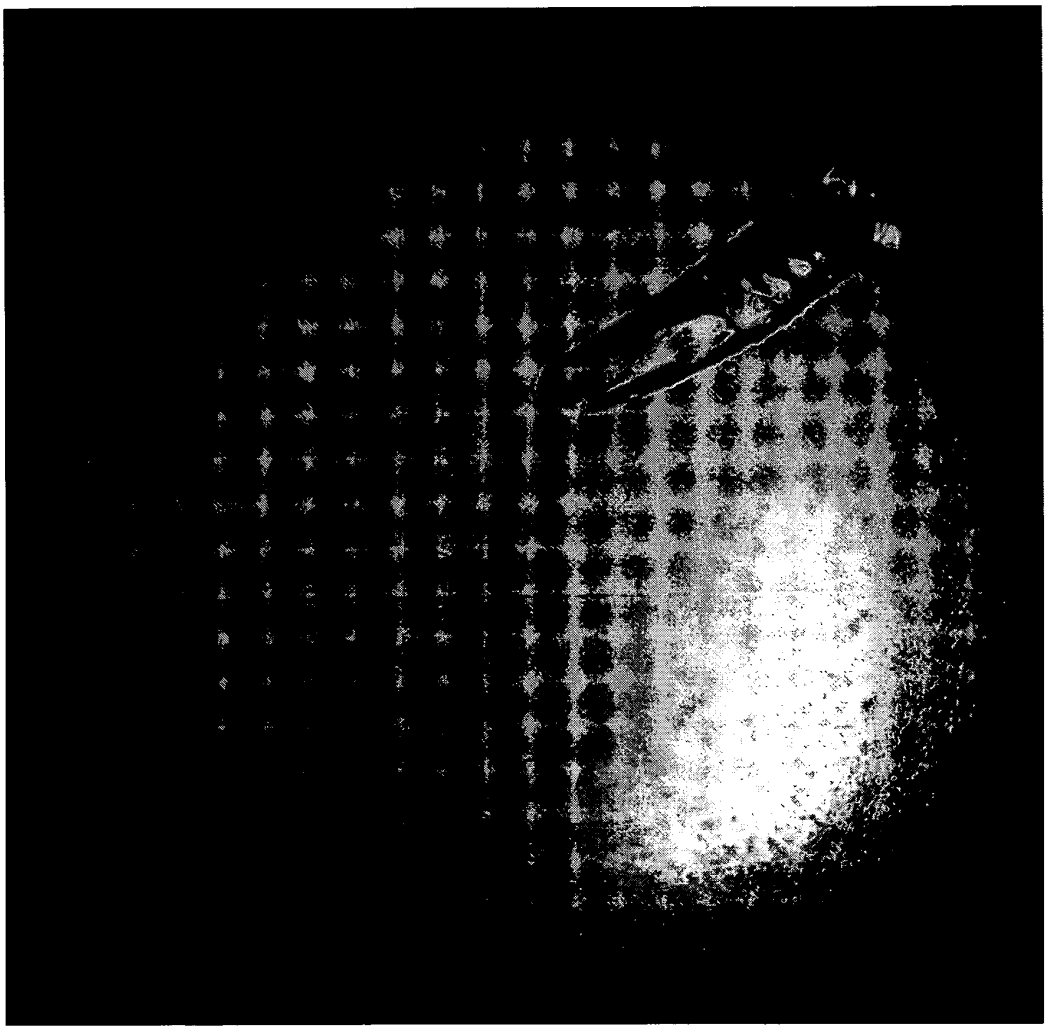


FIG. 35