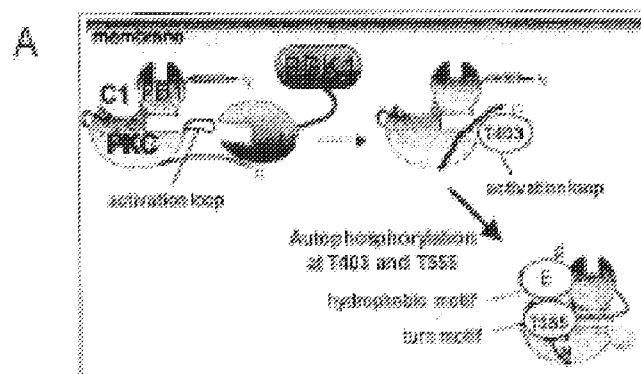




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- (71) Applicant (for all designated States except US): **DANA-FARBER CANCER INSTITUTE, INC.** [US/US]; 450 Brookline Avenue, Boston, MA 02215 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **CHIN, Lynda** [US/US]; 2421 Inwood Drive, Houston, TX 77019 (US). **DRAETTA, Giulio, F.** [IT/US]; 5 Ledgewood Road, Winchester, MA 01890 (US). **MARSZALEK, Joseph, R.** [US/US]; 7106 Spring Run Lane, Katy, TX 77494 (US). **MCGRATH, John, P.** [US/US]; 11 Stearns Rd, Wellesley, MA 02482 (US). **PROTOPOPOVA, Marina** [US/US]; 2425 Dorrington Street, Houston, TX 77030 (US).
- (74) Agents: **SMITH, DeAnn F.** et al.; Foley Hoag LLP, Seaport World Trade Center West, 155 Seaport Blvd., Boston, MA 02210-2600 (US).
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(54) Title: BIOMARKER FOR PKC-IOTA ACTIVITY AND METHODS OF USING SAME

Figure 1



(57) Abstract: The methods of the invention relate to the sUlpfslng detennination that the level of phosphorylation of Thr-412 of human PKC-iota or a corresponding phosphorylatable amino acid of a homolog thereof: serves as a biomarker for PKC-iota enzymatic activity (e.g., kinase activity) useful for preclinical and clinical applications. The present invention, at least in part, is based on the discovery that the level of phosphorylation of the threonine residue at position 412 of human PKC-iota is a reliable biomarker for PKC-iota activity suitable for use in measuring PKC-iota enzymatic activity for preclinical and clinical applications.

BIOMARKER FOR PKC-IOTA ACTIVITY AND METHODS OF USING SAME**Cross-Reference to Related Applications**

This application claims the benefit of priority to U.S. Provisional Application No. 61/494,162 filed on June 7, 2011; the entire contents of which are expressly incorporated herein by reference.

Background of the Invention

The protein kinase C (PKC) family of proteins comprises numerous isozymes found in all eukaryotes (Parker and Murray-Rust (2004) *J. Cell Sci.* 117:131). PKC isozymes can be classified into three subgroups based on structural and functional distinctions. The so-called "conventional PKC" isoforms of group I include the diacylglycerol (DAG)- and Ca^{2+} -responsive isozymes, cPKC- α , cPKC- β I, cPKC- β II, and cPKC- γ (Parker and Murray-Rust (2004) *J. Cell Sci.* 117:131). The so-called "novel PKC" isoforms of group II include nPKC- ϵ , nPKC- δ , nPKC- η and nPKC- θ , which are DAG-sensitive but are Ca^{2+} -insensitive (Parker and Murray-Rust (2004) *J. Cell Sci.* 117:131). The so-called "atypical PKC" isoforms of Group III include aPKC- ι (Selbie *et al.* (1993) *J. Biol. Chem.* 268:24296), aPKC- ζ , aPKC- ζ II (Hirai *et al.* (2003) *Neurosci. Lett.* 348:151), aPKC- μ (protein kinase D isoform) and aPKC- ν (protein kinase D isoform) (Hayashi *et al.* (1999) *Biochim. et Biophys. Acta.* 1450:99). These atypical PKC isoforms are insensitive to both diacylglycerol and calcium and neither bind to nor are activated by phorbol esters. PKCs regulate cellular functions, metabolism and proliferation by phosphorylating proteins in response to transmembrane signals from hormones, growth factors, neuro-transmitters and pharmacological agents.

PKC- ι is of particular interest because it is an oncogene causally linked to growth, invasion, and survival of cancer cells (Fields and Regala (2007) *Pharm. Res.* 55:487; Patel *et al.* (2008) *Cell Prolif.* 31:122). PKC- ι does not contain a Ca^{2+} -binding region and the C-terminal regulatory phosphorylation site within the hydrophobic motif is a non-phosphorylatable phosphomimetic amino acid, glutamic acid (Messerschmidt *et al.* (2005) *JMB* 352:918; Newton *et al.* (2003) *Biochem. J.* 370:351; Diaz-Meco *et al.* (1996) *Molec and Cell Bio.* 16:105). Despite the availability of the crystal structure of human PKC- ι 's catalytic domain and of its numerous phosphorylation sites, a mechanistic understanding of how PKC- ι 's kinase activity is regulated is not well understood

(Messerschmidt *et al.* (2005) *JMB* 352:918; Macek *et al.* (2008) *J. Prot. Res.* 7:2928). This lack of understanding has prevented the identification of biomarkers that reliably report PKC-iota enzymatic activity. Accordingly, there is a great need in the art to identify biomarkers of PKC-iota enzymatic activity in order to measure the inhibition of such activity by effective PKC-iota inhibitors (*e.g.*, small molecule drugs) that may be useful as oncological therapeutics.

Summary of the Invention

The present invention, at least in part, is based on the discovery that the level of phosphorylation of the threonine residue at position 412 of human PKC-iota is a reliable biomarker for PKC-iota activity suitable for use in measuring PKC-iota enzymatic activity for preclinical and clinical applications.

Accordingly, the present invention provides, at least in part, a method of identifying a compound which inhibits kinase activity of human protein kinase C iota (PKC ϵ ; PKC-iota) or a homolog thereof, comprising contacting a sample comprising human PKC-iota or a homolog thereof with the compound and determining the ability of the compound to inhibit Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota, wherein decreased phosphorylation identifies a compound which inhibits kinase activity of human PKC-iota or a homolog thereof. In one embodiment, the inhibition of said Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota is determined by comparing the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the sample relative to a control. In another embodiment, the control is the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the sample relative to said amount in the absence of the compound or at an earlier timepoint after contact of the sample with the compound. In still another embodiment, the inhibition of said Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota is determined by comparing the ratio of the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the sample relative to the total amount of human PKC-iota or homolog thereof to a control. In yet another embodiment, the control is the ratio of the amount of Thr-412 phosphorylated

human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the sample relative to said ratio in the absence of the compound or at an earlier timepoint after contact of the sample with the compound.

The methods described herein can further comprise determining the amount of Thr-
5 564 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota in the sample relative to said amount in the absence of the compound or at an earlier timepoint after contact of the sample with the compound and determining whether the amount changes over time or in response to the compound. Also, the methods can further comprise a step of determining whether the compound directly
10 binds said human PKC-iota or said homolog thereof. In one embodiment, the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples. In another embodiment, the sample comprises cells (*e.g.*, cancer cells such as those selected from the group consisting of any cancer in which PKC-iota is amplified or overexpressed, any cancer having an activating mutation of PKC-iota, and any cancer in which PKC-iota is activated
15 by other kinases). In still another embodiment, the cells are obtained from a subject. In yet another embodiment, the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow.

The amount of Thr-412 phosphorylated human PKC-iota or a corresponding
20 phosphorylatable amino acid in the homolog of human PKC-iota is determined by an immunoassay using a reagent which specifically binds with Thr-412 phosphorylated human PKC-iota or corresponding phosphorylated homolog of human PKC-iota. In one embodiment, the immunoassay is a radioimmunoassay, a Western blot assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a
25 chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay. In another embodiment, the the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human PKC-iota or corresponding homolog of human PKC-iota regardless of phosphorylation state and a detection antibody or fragment thereof which specifically binds
30 with Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylated homolog of human PKC-iota. In still another embodiment, human PKC-iota comprises an amino acid sequence set forth in SEQ ID NOs: 1-2. In yet another embodiment, the compound is a small molecule. In another embodiment, the compound decreases the amount of Thr-412

phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota by at least 50%.

In another aspect, the present invention provides a method for assessing the efficacy of a compound for inhibiting kinase activity of human protein kinase C iota (PKC-iota) or a homolog thereof in a subject, comprising a) detecting in a subject sample at a first point in time, the amount of Thr-412 phosphorylated human PKC-iota or the amount of a human PKC-iota homolog phosphorylated at a corresponding amino acid of human PKC-iota; b) repeating step a) during at one or more subsequent points in time after administration of the compound; and c) comparing the amount of phosphorylated human PKC-iota or homolog thereof detected in step a) with said amount detected in step b), wherein a higher amount of Thr-412 phosphorylated human PKC-iota or the amount of the human PKC-iota homolog phosphorylated at a corresponding amino acid of human PKC-iota in the first point in time relative to at least one subsequent point in time, indicates that the compound inhibits kinase activity of PKC-iota or the homolog thereof. In one embodiment, the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota is determined by an immunoassay using a reagent which specifically binds with Thr-412 phosphorylated human PKC-iota or corresponding phosphorylated homolog of human PKC-iota. In another embodiment, the immunoassay is a radioimmunoassay, a Western blot assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay. In still another embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human PKC-iota or a corresponding homolog of human PKC-iota regardless of phosphorylation state and a detection antibody or fragment thereof which specifically binds with Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylated homolog of human PKC-iota.

In one embodiment, the sample can be selected from the group consisting of *ex vivo* and *in vivo* samples. In another embodiment, the sample comprises cancer cells. In still another embodiment, the cancer cells are cells selected from the group consisting of any cancer in which PKC-iota is amplified or overexpressed, any cancer having an activating mutation of PKC-iota, and any cancer in which PKC-iota is activated by other kinases. In yet another embodiment, the sample is selected from the group consisting of tissue, whole

blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow. In another embodiment, the sample in step a) and/or step b) is a portion of a single sample obtained from the subject or a portion of pooled samples obtained from the subject. In still another embodiment, between the first point in time and the subsequent point in time, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer. In yet another embodiment, human PKC-iota comprises an amino acid sequence set forth in SEQ ID NOs:1-2. In another embodiment, the compound is a small molecule. In still another embodiment, the compound decreases the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota by at least 50%.

In still another aspect, a method is provided for treating a subject afflicted with cancer comprising administering to the subject a compound that inhibits Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota, thereby treating the subject afflicted with the cancer. In one embodiment, the compound is administered in a pharmaceutically acceptable formulation. In another embodiment, the compound is a small molecule. In still another embodiment, the compound directly binds said human PKC-iota or the homolog thereof. In yet another embodiment, the cancer is selected from the group consisting of any cancer in which PKC-iota is amplified or overexpressed, any cancer having an activating mutation of PKC-iota, and any cancer in which PKC-iota is activated by other kinases. In another embodiment, human PKC-iota comprises an amino acid sequence set forth in SEQ ID NOs:1-2. In still another embodiment, the compound is a small molecule. In yet another embodiment, the compound decreases the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota by at least 50%. In another embodiment, the method further comprises administering one or more additional anti-cancer agents.

It will also be understood that certain embodiments of the present invention can be used with more than one method described herein, according to knowledge available to the skilled artisan.

Brief Description of Figures

Figures 1A-1E show that phosphorylation status of Thr-412 is a biomarker of human PKC-iota enzymatic activity. Figure 1A shows a schematic representation of canonical PKC phosphorylation. Figure 1B shows results of Western immunoblotting for phosphorylated and total PKC-iota and phosphorylated and total LLGL2 levels in U20S cells treated with a PKC-iota small molecule inhibitor for 3 hours. Blots were probed for pThr-412-PKC-iota, pThr-564-PKC-iota, total PKC-iota, pSer-653-LLGL2, and total LLGL2 proteins. Figure 1C shows results of Western immunoblotting for phosphorylated and total PKC-iota and LLGL2 levels in A549 cells treated with a PKC-iota small molecule inhibitor for up to 6 hours. Blots were probed for pThr-412-PKC-iota, pThr-564-PKC-iota, total PKC-iota, pSer-653-LLGL2, and total LLGL2 proteins. Figure 1D provides quantitation of the results shown in Figure 1C. Figure 1E shows target engagement in ascites tumor cells after orthotopic xenograft of OVCAR5 cells into mice.

Detailed Description of the Invention

The methods of the invention relate to the surprising determination that the level of phosphorylation of Thr-412 of human PKC-iota or a corresponding phosphorylatable amino acid of a homolog thereof, serves as a biomarker for PKC-iota enzymatic activity (*e.g.*, kinase activity). Specifically, decreased phosphorylation of Thr-412 of human PKC-iota (*e.g.*, by directly or indirectly inhibiting phosphoinositide-dependent kinase-1 (PKD1)-mediated phosphorylation of Thr-412) corresponds with a reduction in PKC-iota substrate phosphorylation (*e.g.*, LLGL2). Such a biomarker is particularly advantageous for preclinical and clinical applications because the biomarker is associated with the PKC-iota oncogene itself rather than being dependent upon downstream indicators of PKC-iota enzymatic activity that may not be expressed in a given cell or tissue of interest.

A. PKC-iota Molecules

As used herein, "PKC-iota" refers to the specific iota isoform of the PKC family of protein kinases and is alternatively known as "PKCI", "PRKCI", and "aPKCI". In addition, "Thr-412" and "Thr-564" of PKC-iota refers to the amino acid numbering of the human PKC-iota polypeptide from the N-terminus. Accordingly, a skilled artisan will readily understand that Thr-412 and Thr-564 of the human PKC-iota polypeptide is conserved across numerous species and that although those specific residues may be referenced herein, the methods of the present invention apply equally well to the corresponding

residues (e.g., phosphorylatable amino acids) of isoforms, homologs, and orthologs in other species corresponding to said Thr-412 and/or Thr-564. In addition, Thr-412 of human PKC-iota (e.g., SEQ ID NO:1) has also been described in the art as Thr-403 and Thr-564 as Thr-555, based on an older nomenclature in which the human PKC-iota polypeptide contained nine fewer amino acids (see, e.g., Newton *et al.* (2003) *Biochem. J.* 370, 351). The PKC-iota inhibitors described herein similarly bind to the human PKC-zeta isoform and modulate its corresponding phosphorylatable residue, Thr-410. Representative PKC-iota homologs, as opposed to other members of the PKC protein kinase family, are provided herein as follows:

10

Human PKC-iota Protein Sequence (SEQ ID NO:1; Gene Acc. NM_002731.4)

```

1 mptqrdsstm shtvaggsg dhshqvrka yyrgdimith fepsisfegl
cnevrdmcsf
61 dneqlftmkw ideegdpctv ssqleleef rlyelnkds llihvfpcvp
15 erpgmpcpge
121 dksiyrrgar rwrklycang htfqakrfnr rahcaictdr iwglgrqgyk
cinckllvhk
181 kchkltvtec grhslpqepv mpmdqssmhs dhaqtvipyn psshesldqv
geekeamnt
20 241 esgkassslg lqdfdllrvi grgsyavll vrlkktdry amkvvkelv
nddedidwvq
301 tekvhfeqas nhpflvglhs cfqtesrlff vieyvnggd1 mfhmqrqrkl
peeharfysa
361 eislalnylh ergiiyrdlk ldnvllldseg hikltdygm keglrpgdtt
25 stfcgtpnyi
421 apeilrgedy gfsvdwwalg vlmfemmagr spfdivgssd npdqntedyl
fqvilekqir
481 iprsisvkaa svlksflnk pkerlgchpq tgfadiqghp ffrnvdwdmm
eqkgvvpf
30 541 pnisgefgl nfdsqftnep vqltpdddi vrkidqsefe gfeyinpllm saaecv

```

Human PKC-iota cDNA Sequence (SEQ ID NO:2; Gene Acc. NM_002740)

```

1 atgccgaccc agaggacag cagcaccatg tccacacgg tcgcaggcgg
cggcagcggg
35 61 gaccattccc accaggtccg ggtgaaagcc tactacgcg gggatatcat
gataacacat
121 tttgaacctt ccattctctt tgagggcctt tgcaatgagg ttcgagacat
gtgttctttt
181 gacaacgaac agctcttcac catgaaatgg atagatgagg aaggagaccc
40 gtgtacagta
241 tcattctcagt tggagttaga agaagccttt agactttatg agctaaacaa
ggattctgaa
301 ctottgatcc atgtgttccc ttgtgtacca gaacgtcctg ggatgccttg
tccaggagaa
45 361 gataaatcca tctaccgtag aggtgcacgc cgctggagaa agctttattg
tgccaatggc
421 cacactttcc aagccaagcg tttcaacagg cgtgctcaat gtgccatctg
cacagaccga
481 atatggggac ttggacgcca aggatataag tgcacaaact gcaaactctt
50 ggttcataag
541 aagtgccata aactcgtcac aattgaatgt gggcggcatt ctttgccaca
ggaaccagtg

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601 atgcccattgg atcagtcattc catgcattct gaccatgcac agacagtaat
 tccatataat
 661 ccttcaagtc atgagagttt ggatcaagtt ggtgaagaaa aagaggcaat
 gaacaccagg
 5 721 gaaagtggca aagcttcattc cagtctaggt cttcaggatt ttgatttgc
 cgggtaata
 781 ggaagaggaa gttatgcaa agtactgttg gttcgattaa aaaaaacaga
 tcgtatttat
 841 gcaatgaaag ttgtgaaaaa agagcttggt aatgatgatg aggatattga
 10 ttgggtacag
 901 acagagaagc atgtgtttga gcaggcatcc aatcatcctt tccttgttgg
 gctgcattct
 961 tgctttcaga cagaaagcag attgttcttt gttatagagt atgtaaatgg
 aggagaccta
 15 1021 atgtttcata tgcagcgaca aagaaaactt cctgaagaac atgccagatt
 ttactctgca
 1081 gaaatcagtc tagcattaaa ttatcttcat gagcgaggga taatttatag
 agatttgaaa
 1141 ctggacaatg tattactgga ctctgaaggc cacattaaac tcactgacta
 20 cggcatgtgt
 1201 aaggaaggat tacggccagg agatacaacc agcactttct gtggtaactcc
 taattacatt
 1261 gctcctgaaa ttttaagagg agaagattat ggtttcagtg ttgactgggtg
 ggctcttgga
 25 1321 gtgctcatgt ttgagatgat ggcaggaagg tctccatttg atattgttgg
 gagctccgat
 1381 aaccctgacc agaacacaga ggattatctc ttccaagtta ttttgaaaaa
 acaaattcgc
 1441 ataccacgtt ctctgtctgt aaaagctgca agtggtctga agagttttct
 30 taataaggac
 1501 cctaaggaa gattgggttg tcatcctcaa acaggatttg ctgatattca
 gggacacccg
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 tccctttaaa
 35 1621 ccaaatattt ctggggaatt tggtttggac aactttgatt ctgagtttac
 taatgaacct
 1681 gtccagctca ctccagatga cgatgacatt gtgaggaaga ttgatcagtc
 tgaatttgaa
 1741 ggttttgagt atatcaatcc tcttttgatg tctgcagaag aatgtgtctg
 40 a

Mouse PKC-iota Protein Sequence (SEQ ID NO:3; Gene Acc.NP_032883.2)

1 mptqrdssst shtvacgggg dhshqvrka pyrgdimith fepsisfegl
 csevrmdcsf
 45 61 dneqpftmkw ideegdpctv ssqleleef rlyelndse llihvfpcvp
 erpgmpcpge
 121 dksiyrrgar rwrklycang htfaqkrfnr rahcaictdr iwglgrqgyk
 cinckllvhk
 181 kohklvtiec grhslppepm mpmdqtmhpd htqtvipynp sshesldqvg
 50 eekeamntre
 241 sgkassslgl qdfdllrvig rgsyakvllv rlkkttdriya mkvkvkelvn
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 301 ekhvfeqasn hpflvglhsc fqtesrlffv ieyvnggdlm fhmqrqrklp
 eeharfysae
 55 361 islalnyihe rgiiyrdkl dnvllldsegh ikltidygmck eglrpgdtts
 tfcgtpnvia
 421 peilrgedyg fsvdwwalgv lmfemmagrs pfdivgssdn pdqntedylf
 qvilekqiri
 481 prslsvkaas vlksflnkdp kerlgchpqt gfadiqghpf frnvdwmmme
 60 qkqvppfkip

541 nisgefglnd fdsqftnepv qltpdddiv rkldqsefeg feyinpilms
aeecv

Mouse PKC-iota cDNA Sequence (SEQ ID NO:4; Gene Acc. NM_008857.3)

5 1 atgcgcaccc agagggacag cagcaccatg toccacacgg tcgcgtgcgg
cggcggcggg
 61 gaccattccc accagggtccg ggtgaaagcc tactaccgcg gggatattat
gataacacac
10 121 tttgagcctt ccattctcctt tgagggaactt tgcagtgcgg ttgcgagatat
gtgttctttt
 181 gacaatgagc agccgttcac catgaaatgg atagatgcgg aaggagaccc
atgtacagtg
 241 tcttctcagt tggagttaga agaggctttc aggtctgtag agctgaacaa
ggattctgaa
15 301 ctcttgattc atgtatttcc atgtgtacca gagcgtcctg gaatgccttg
cccaggggaa
 361 gacaagtcca ttaccggag aggggcacgc cgttgaggaa agctgtattg
tgcaaatggc
20 421 cacacttttc aagccaaacg tttcaatagg cgcgccact gtgccatctg
cacagacaga
 481 atctggggcc tcggacgaca aggatacaag tgcacaaact gcaaacctgt
ggttcataag
 541 aagtgcacac agctgggtcac aattgagtggt gggcggcact ctttgcacc
ggaacccatg
25 601 atgccaatgg accagaccat gcacccagac cacacacaga cagtaattcc
atataatcct
 661 tcaagtcatg agagtttggc ccaagttggt gaagaaaagg aggcaatgaa
caccagggag
30 721 agtgggtaaag cgtcgtccag tctaggtctg caggatttcc atttgcctcg
agttataggg
 781 aggggaagtt acgccaaagt actgttggtt cgtttaaaga aaacagatcg
catttatgca
 841 atgaaagttg tgaagaaaga gctcgtcaat gacgatgcgg atactgattg
ggtgcagaca
35 901 gagaagcatg tgtttgagca ggcgtccaat cacccttttc ttgtcgggct
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 961 ttccagacag aaagcagggtt gttttttgtc atagaatatg taaatggagg
ggacctcatg
40 1021 ttccatattg agcgacagag aaaacttctt gaagagcatg ccagggttta
ctctgcagaa
 1081 atcagtttag cactgaatta tcttcatgag caggggataa tttatagaga
tttgaagttg
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catgtgtaag
45 1201 gaaggattgc ggccgtgaga cacaaccagc actttctgcg gcaactccaa
ttacattgct
 1261 ccagagatct taagaggaga agattatggc ttcagcgttg actggtgggc
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50 1321 cttatgtttg agatgatggc gggaaggctc ccgtttgata tcgttgggag
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gatccgcac
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ctttaaacca
60 1621 aacatttctg gagaatttgg tttggataat ttcgattctc agtttactaa
tgaaccagtc

1681 cagctcactc cagatgatga tgacattgtg aggaagattg atcagttctga
 atttgaagggt
 1741 tttagagtata tcaacccccct cttgatgtct gcagaagagt gtgtctga

5 Rat PKC-iota Protein Sequence (SEQ ID NO:5; Gene Acc. NP_114448.1)

1 mptqrdsstm shtvacgggg dhshqvrkva yyrgdimith fepsisfegl
 csevrmdcsf
 61 dneqpftmkw ideegdpctv ssqleleef rlyelnkds llihvfpcvp
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 10 121 dksiyrrgar rwrklycang htfqakrfnr rahcaictdr iwglgrggyk
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 241 esgkassslg lqdfdllrvi grgsyakvll vrlkktdry amkvvkkelv
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 peeharfysa
 361 eislalnylh ergiiyrdlk ldnvildseg hikltdygm keglrpgdtt
 stfcgtpnyi
 20 421 apeilrgedy gfsvdwalg vlmfemmagr spfdivgssd npdqntedyl
 fqvilekqir
 481 iprsllsvkaa svlksflnkd pkerlgchpq tgfadiqghp ffrnvdwmm
 eqkqvppfk
 541 pnisgefgl nfdsqftnep vqltpdddi vrkidqsefe gfeyinpllm saeecv
 25

Rat PKC-iota cDNA Sequence (SEQ ID NO:6; Gene Acc. NM_032059.1)

1 atgcgaccc agagggacag cagcaccatg totcacacgg tcgcgtgagg
 cggcggcggg
 30 61 gaccattccc accaggtccg ggtgaaagcc tactaccgag gggatattat
 gataacacac
 121 ttcgagcctt ccatctcctt tgaaggactt tgcagtgagg ttcgagatat
 gtgttctttt
 181 gacaatgagc agccattcac catgaaatgg atagatgagg aaggagaccc
 35 gtgcacagtg
 241 tcttctcagt tggagttgga agaggcttct aggtgtatg agttgaacaa
 ggattctgaa
 301 ctcttgatcc acgtgttccc gtgtgtacca gagcgtcctg gaatgccttg
 cccaggggaa
 40 361 gacaagtcca tttaccgcag aggggcgcgc aggtggagaa agctgtattg
 tgcaaatggc
 421 cacacttttc aagccaaacg ctttaacagg cgtgcccact gtgccatctg
 cacagacagg
 481 atctggggac ttggacgaca aggatataaa tgcattcaact gcaaactgct
 45 ggttcataag
 541 aagtgccaca agcttgtcac aattgagtgt gggcggcatt ctttgccacc
 ggaaccatg
 601 atgccaatgg accagtcac catgcacca gaccacacac agacagtaat
 tccatataat
 50 661 ccttcaagtc atgagagttt ggaccaagtt ggtgaagaaa aggaggcaat
 gaacaccagg
 721 gagagtggga aggcgtcatc aagcttaggt ctccaggatt tcgatttgc
 tcgagttata
 781 gggagaggaa gttacgcaa agtactgctg gttogattaa aaaagacaga
 55 tcgcatttat
 841 gcaatgaagg ttgtgaagaa agagctcgtc aatgacgatg aggatattga
 ttgggtacag
 901 acagaaaagc atgtgtttga gcaggcgtcc aatcaccctt tcttgtttg
 tctgcattcc
 961 tgcttccaga cagaaagcag gctgtttttt gtcatagaat atgtgaatgg
 60 aggggatctc

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      1021 atgtttcata tgcagcggca aagaaaactt cctgaagaac atgccagggtt
      ttactcagca
      1081 gaaatcagtc tagcactaaa ttatcttcat gagcgaggga taattttatag
      agattttgaag
5      1141 ttggacaatg tactgctgga ctctgaagga cacatcaaac tcaactgacta
      cggcatgtgt
      1201 aaggaaggat tacggcccg agatacaacc agcaccttct gtgggactcc
      caattacatt
      1261 gctcctgaga tcttaagagg agaagactat ggcttcagcg ttgactgggtg
10      ggctcttgga
      1321 gtactcatgt ttgagatgat ggcggaagg tctccatttg atatcggttg
      gagctctgac
      1381 aatcctgacc aaaacacaga ggattatctg ttccaagtca ttttgagaa
      gcagattcgc
15      1441 ataccgct cctgtctgt gaaagcagca agtgtgtctga agagtttctt
      caacaaggac
      1501 ccaaaggaac gattgggttg tcaccotcaa actggatttg ctgacatoca
      aggacacca
      1561 ttcttccgta atgtggattg ggacatgatg gagcagaagc aagtgggtcc
20      gccctttaa
      1621 ccaaacattt ctggagaatt tggtttgat aactttgact ccagtttac
      caacgaacca
      1681 gtccagctca ctccagatga tgatgacatc gtgaggaaga ttgatcagtc
      tgaatttgaa
25      1741 ggtttcgagt atatcaacc tctcttgatg tctgcagaag agtgtgtctg a

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Cow PKC-iota Protein Sequence (SEQ ID NO:7; Gene Acc. XP_606901.4)

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      1 mptqrdsstm shpiaggig dhshqvrka yyrgdimith fepsisfegl
      cnevrmdcsf
30      61 dneqlftmkw ideegdpctv ssqleleef rlyelnkdse llihvfpcvp
      erpgmpcpge
      121 dksiyrrgar rwrklycang btfqakrfnr rahcaictdr iwglgrqgyk
      cinckllvhk
      181 kchkivtiec grhslppepm mpmdqssmhs dhaqtvipyn psshesldqv
35      geekeamnt
      241 esgkassslg lqdfdlrvi grgsyakvll vrlkkttdriy amkvvkkelv
      nddedidwvq
      301 tekvhfeqas nhpflvglhs cfqtesrlff vieyvnggd1 mfhmqrqrkl
      peeharfysa
40      361 eislalnyih ergiiyrdlk ldnvllldseg hikltdygm ckeglrpgdt
      stfcgtpnyi
      421 apeilrgedy gfsvdwwalg vlmfemmagr spfdivgssd npdqntedyl
      fqvilekqir
      481 iprslsvkaa svlksflnkd pkerlgchpq tgfadiqghp ffrnvdwdmm
45      eqkqvvpfk
      541 pnisgefgld nfdsqftnep vqltpdddi vrkidqsefe gfeyinpllm saeecv

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Cow PKC-iota cDNA Sequence (SEQ ID NO:8; Gene Acc. XM_606901.5)

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50      1 atgccgaccc agagagacag cagcaccatg tctcaccga tcgcaggcgg
      cggcattggg
      61 gaccactctc accagggtccg ggtgaaagcc tactaccgag gggatatcat
      gataacacat
      121 tttgaacott caatatcctt cgagggtctt tgtaatgagg ttcgagacat
55      gtgttctttt
      181 gacaacgaac aacttttcac catgaaatgg atagatgagg aaggagaccc
      gtgtacagta
      241 tcatctcagt tggagttaga agaagccttt aggttttatg agctaaacaa
      ggattctgaa
60      301 ctgttgattc atgtattccc ttgtgtacca gaacgtcctg gaatgccctg
      tccaggggaa

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361 gataaatcca ttacagacg aggtgcacgc cgctggagaa agctttattg
 tgcaaatgga
 421 cacacttttc aagccaagcg ttccaacagg cgtgcgcact gtgccatctg
 cactgaccga
 5 481 atttggggac ttggacgtca aggatataag tgtatcaact gcaagctcct
 gggtcataag
 541 aagtgccaca aacttgctac aattgaatgt gggcggcatt cggtgccacc
 ggaaccaatg
 601 atgcccattg accagtcac catgcactca gaccacgcac agacagtaat
 10 tccatataat
 661 ccttcaagtc atgagagttt ggaccaagtt ggtgaagaaa aggaggcaat
 gaacaccagg
 721 gaaagtggca aagcttcttc cagtttaggt cttcaggact ttgatttgct
 ccgagtaata
 15 781 ggaagaggaa gttatgctaa agtactgttg gtacgattaa aaaaaacaga
 tcgtatttat
 841 gcaatgaaag ttgtgaaaaa agagcttgct aatgatgatg aggatattga
 ctgggtacag
 901 acagagaaac atgtttttga acaggcatcc aatcatcctt ttcttggttg
 20 gctgcattct
 961 tgctttcaga cagaaagcag attgttcttt gttatagagt atgttaattg
 aggagattta
 1021 atgtttcata tgcaacgaca aagaaaactt cctgaagagc atgccagatt
 ttactctgca
 25 1081 gaaatcagtc tagcattaaa ttatcttcat gaacgagggg taatttatag
 agatttgaaa
 1141 ttggacaacg tggtgctgga ctctgaagga cacattaaac tcaactgacta
 tggcatgtgt
 1201 aaggaaggat tacggccagg agatacaact agcactttct gtggtactcc
 30 taattacatc
 1261 gctcctgaaa tottaagagg agaagattat ggtttcagtg tcgactgggtg
 ggctcttgga
 1321 gtactcatgt ttgagatgat ggcaggaagg tctccatttg atattgttg
 gagctcggat
 35 1381 aacctgacg aaaacacaga ggattatctt tttcaagtta ttttgaaaa
 acaaattcgc
 1441 ataccgctt ctttatctgt aaaagctgca agtgcctga agagcttctc
 caacaaggac
 1501 ccaaaggaac gattgggttg tcatccacaa acaggatttg ctgatattca
 40 gggacacca
 1561 ttcttcgaa atgttgattg ggatatgatg gagcaaaagc aggtggtacc
 tccgtttaaa
 1621 ccaaatatct ctggggaatt tggtttgac aactttgatt ctcaagttac
 taatgaacct
 45 1681 gtccagctca ctctgatga tgatgacatt gtgaggaaga tcgatcagtc
 tgaatttgaa
 1741 ggttttgagt acattaatcc tctcttgatg tctgcagaag aatgtgtctg a

Chimpanzee PKC-iota Protein Sequence (SEQ ID NO:9; Gene Acc.
XP_526377.2)
 50 1 mptqrdssm shtvaggsg dshbqvrka yyrgdimith fepsisfegl
 cnevrdmcsf
 61 dneqlftmkw ideegdpctv ssqleleef rlyelndse llihvfpcvp
 erpgmpcpge
 55 121 dksiyrrgar rwrklycang htfaqkrfnr rahcaictdr iwglgrqgyk
 cinckllvhk
 181 kohklvtiec grhslpqpvp mpmdqssmhs dhaqtvipyn psshesldqv
 geekeamnt
 241 esgkassslg lqdfdllrvi grgsyakvll vrlkkttdriy amkvvkkelv
 60 nddedidwvq

301 tekhvfeqas nhpflvgihs cfqtesrlff vieyvnggd1 mfhmqqrkrl
 peeharfysa
 361 eislalnylh ergiiyrdlk ldnvllldseg hikltdygm c keglrpgdtt
 stfcgtpnyi
 5 421 apeilrgedy gfsvdwwalg vlmfemmagr spfdivgssd npdqntedyl
 fgvillekqir
 481 iprsllsvkaa svlksflnkd pkerlgchpq tgfadiqghp ffrnvdwdmm
 eqkqvppfk
 541 pnisgefgld nfdsqftnep vqltpddddd vrkidqsefe gfeyinpllm saeecv
 10

**Chimpanzee PKC-iota cDNA Sequence (SEQ ID NO:10; Gene Acc.
 XM_526377.2)**

1 atgcgcagccc agagggacag cagcaccatg toccacacgg tcgcaggcgg
 cggcagcggg
 15 61 gaccattccc accaggtccg ggtgaaagcc tactaccgcg gggatatcat
 gataaacacat
 121 tttgaacctt ccatctcctt tgagggcctt tgcaatgagg ttcgagacat
 gtgttctttt
 181 gacaacgaac agctcttcac catgaaatgg atagatgagg aaggagaccc
 20 gtgtacagta
 241 tcatctcagt tggagttaga agaagccttt agactttatg agctaaacaa
 ggattctgaa
 301 ctcttgattc atgtgttccc ttgtgtacca gaacgtcctg ggatgccttg
 tccaggagaa
 25 361 gataaatcca totaccgtag aggtgcacgc cgctggagaa agctttattg
 tgccaatggc
 421 cacactttcc aagccaagcg tttcaacagg cgtgctcact gtgccatctg
 cacagaccga
 481 atatggggac ttggacgcc aaggatataag tgcacaaact gcaaactctt
 30 ggttcataag
 541 aagtgccata aactcgtcac aattgaatgt gggcggcatt ctttgcaca
 ggaaccagt
 601 atgcccattg atcagtcac catgcattct gaccatgcac agacagtaat
 tccatataat
 35 661 ccttcaagtc atgagagttt ggatcaagtt ggtgaagaaa aaggagcaat
 gaacaccagg
 721 gaaagtggca aagcttcac cagtctaggt cttcaggatt ttgatttgct
 ccgggtaata
 781 ggaagaggaa gttatgcaa agtactgttg gttcgattaa aaaaaacaga
 40 tcttatttat
 841 gcaatgaaag ttgtgaaaa agagcttggt aatgatgatg aggatattga
 ttgggtgcag
 901 acagagaagc atgtgtttga gcaggcatcc aatcatcctt tcttgtttgg
 gctgcattct
 45 961 tgctttcaga cagaaagcag attgttcttt gttatagagt atgtaaatgg
 aggagacct
 1021 atgtttcata tgcagcgaca aagaaaactt cctgaagaac atgccagatt
 ttactctgca
 1081 gaaatcagtc tagcattaaa ttatcttcat gagcgaggga taatttatag
 50 agatttgaaa
 1141 ctggacaatg tattactgga ctctgaaggc cacattaaac tcaactgacta
 cggcatgtgt
 1201 aaggaaggat taaggccagg agatacaacc agcactttct gtggtactcc
 taattacatt
 55 1261 gctcctgaaa ttttaagagg agaagattat ggtttcagtg ttgactgggtg
 ggctcttgga
 1321 gtgctcatgt ttgagatgat ggcaggaagg tctccatttg atattgttgg
 gagctccgat
 1381 aacctgacc agaacacaga ggattatctc ttccaagtta ttttggaaaa
 60 acaaattcgc

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1441 ataccacggt ctctgtctgt aaaagctgca agtgttctga agagttttot
taataaggac
1501 cctaaggaac gggtgggttg tcatcctcaa acaggatttg ctgatattca
gggacacccg
5 1561 ttcttccgaa atgttgattg ggatatgatg gagcaaaaac aggtggtacc
tccctttaaa
1621 ccaaataatt ctgggggaatt tggtttggac aactttgatt ctgagtttac
taatgaacct
1681 gtccagctca ctccagatga cgatgacatt gtgaggaaga ttgatcagtc
10 tgaatttgaa
1741 ggttttgagt atatcaatcc tcttttgatg tctgcagaag
aatgtgtctg a

Dog PKC-iota Protein Sequence (SEQ ID NO:11; Gene Acc. XM_535855.2)
15 1 mvrpgveapg darmvapalp rnvrlwsgrg grarrggvlg paggaarfrl
prpgaprivr
61 taqvaereav garppppppp papgappaas asalgrgsre mptqrdssam
shaiaggsg
121 dhshqvrka yyrgdimith fepsisfegl cnevrdmcsf dneqlftmkw
20 ideegdpctv
181 ssqleleeaf rlyelndkse llihvfpcvp erpgmpcpge dksiyrrgar
rwrklycang
241 htfaqkrfnr rahcaictdr iwglgrqgyk cinckllvhk kchkltvtec
grhsmpepm
25 301 mpmdqspmps dhvqtvipyn psshesldqv geekeamntr esgkassslg
lqdfdlirvi
361 grgsyakvll vrlkktdriy amkvvkkelv nddedidwvq tekvhfeqas
nhpflvglhs
421 cfqtesrlff vieyvnggd1 mfhmqrqrkl peeharfysa eislalnylh
30 ergiiyrdlk
481 ldnvllldseg hikltdygm ckeglrpgdtt stfcgtpnyi apeilrgedy
gfsvdwwalg
541 vlmfemmagr spfdivgssd npdqntedyl fqvilekqir iprsllsvkaa
svlksfinkd
35 601 pkerlgchpq tgfadiqghp ffrnvdwdmm eqkqvvpfkn pnisgefgl
nfdsqftnep
661 vqltpdddi vrkidqsefe gfeyinpllm saeecv

Dog PKC-iota cDNA Sequence (SEQ ID NO:12; Gene Acc. XM_535855.2)
40 1 atggttcggc ccggcgctga ggctcctgga gatgctcgga tggtcgcgcc
cgccctcccc
61 cggaatgtgc gcctttggag cgggcgaggt gggagggccc ggaggggagg
cgtgctgggc
45 121 ccggcgggag gaggcgcgcg gttcgggctg ccccgcccag gcgcccctcg
ggtcgggcgg
181 accgcgcagg tggccgagcg ggaggccgct ggagcgcgcg cggcccccgc
cccgccgccc
241 ccggcgcgcc gcgcgcccc cgcagcctcg gctccgcgc tggggcgggg
50 gagcagggag
301 atgccgaccc agagggacag cagtgccatg tctcagcga tcgcggggcg
tggcagcggg
361 gaccattccc accaggtccg ggtgaaagcc tactaccgcg gggatatcat
gataacacat
55 421 tttgaacctt caatctcctt tgagggcctt tgcaatgaag ttcgagatat
gtgttctttt
481 gacaacgaac agctttttcac catgaaatgg atagatgagg aaggagaccc
gtgtacagta
541 tcatctcagt tggagttaga agaagccttt aggcctttatg agctgaataa
60 ggattctgaa

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        601 ctcttaattc atgtcttccc ttgtgtacca gaacgtcctg gaatgccttg
tccaggagaa
        661 gacaaatcca totaccgcag aggcgcacgc cgatggagaa agctttattg
tgcaaatggc
5       721 cacacttttc aagccaagcg ttccaacagg cgtgccact gtgccatctg
taccgaccga
        781 atatggggac ttggacgtca gggatataaa tgcatacaact gcaaactcct
ggttcataag
10      841 aagtgccaca aactcgtcac aatcgaatgt gggcggcatt ctatgccacc
ggaaccaatg
        901 atgcctatgg accagtcacc catgccttca gatcatgtac agacagtaat
tccatataat
        961 ccttcaagtc atgagagttt ggaccaagtt ggtgaagaaa aggaggcaat
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15     1021 gaaagtggca aaagcttcac cagtttaggt cttcaggatt ttgatttgc
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        1201 acagagaaac atgttttcga gcaggcatcc aatcatcctt ttcttgtttg
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        1261 tgctttcaga cagaaagcag attgttcttt gtcataagat atgtaaatgg
aggagatcta
25     1321 atgtttcata tgcagcgaca aagaaaactt cctgaagaac atgccagatt
ttactctgca
        1381 gaaatcagcc tagcattaaa ttatcttcat gaacgaggga taatttatag
agatttgaaa
30     1441 ttggacaatg tattgttgga ctcagaagga cacattaagc tcaactgacta
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        1501 aaggaagggc tacggccagg agatacaacc agcactttct gtggaactcc
caattacatt
        1561 gctcctgaaa ttttaagagg agaagattat ggtttcagtg ttgactgggtg
ggcccttgga
35     1621 gtactaatgt ttgagatgat ggcagggaga tctccatttg atattgttgg
aagctctgat
        1681 aaccgcgatc aaaacacaga ggattatctc ttccaagtta ttttgaaaa
acaaatacgc
40     1741 ataccacgtt ctctgtctgt aaaagctgca agtgttctga agagttttct
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        1801 ccaaaggaaac ggctgggttg tcatcctcaa acaggatttg ctgatattca
gggacacca
        1861 ttcttttcgaa atgtcgattg ggatatgatg gagcaaaagc aggtgggtacc
tccctttaaa
45     1921 ccaaataatt ctggggaatt tggtttgac aactttgatt ctcagtttac
taatgaacct
        1981 gtccagctca ctccagatga cgacgacatt gtgaggaaga ttgatcagtc
tgaatttgaa
50     2041 ggttttgagt acatcaatcc ttttttgatg tctgcagaag aatgtgtttg a

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PKC-iota nucleic acid and protein molecules are included that differ due to degeneracy of the genetic code or due to encoding or having “non-essential”, “conservative”, “stereoisomers”, or “unconventional” amino acids that do not appreciably alter the enzymatic (*e.g.*, kinase) and/or Thr-412-regulatory ability of PKC-iota. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Stereoisomers (*e.g.*, D-amino acids)

of the twenty conventional amino acids, unnatural amino acids such as alpha, alpha-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides described herein. There is a known and definite correspondence between the amino acid sequence of a particular protein and the nucleotide sequences that can code for the protein, as defined by the genetic code (shown below). Likewise, there is a known and definite correspondence between the nucleotide sequence of a particular nucleic acid and the amino acid sequence encoded by that nucleic acid, as defined by the genetic code.

10 GENETIC CODE

Alanine (Ala, A)	GCA, GCC, GCG, GCT
Arginine (Arg, R)	AGA, ACG, CGA, CGC, CGG, CGT
Asparagine (Asn, N)	AAC, AAT
Aspartic acid (Asp, D)	GAC, GAT
15 Cysteine (Cys, C)	TGC, TGT
Glutamic acid (Glu, E)	GAA, GAG
Glutamine (Gln, Q)	CAA, CAG
Glycine (Gly, G)	GGA, GGC, GGG, GGT
Histidine (His, H)	CAC, CAT
20 Isoleucine (Ile, I)	ATA, ATC, ATT
Leucine (Leu, L)	CTA, CTC, CTG, CTT, TTA, TTG
Lysine (Lys, K)	AAA, AAG
Methionine (Met, M)	ATG
Phenylalanine (Phe, F)	TTC, TTT
25 Proline (Pro, P)	CCA, CCC, CCG, CCT
Serine (Ser, S)	AGC, AGT, TCA, TCC, TCG, TCT
Threonine (Thr, T)	ACA, ACC, ACG, ACT
Tryptophan (Trp, W)	TGG
Tyrosine (Tyr, Y)	TAC, TAT
30 Valine (Val, V)	GTA, GTC, GTG, GTT
Termination signal (end)	TAA, TAG, TGA

An important and well known feature of the genetic code is its redundancy, whereby, for most of the amino acids used to make proteins, more than one coding nucleotide triplet may be employed (for example, illustrated above). Therefore, a number

of different nucleotide sequences may code for a given amino acid sequence. Such nucleotide sequences are considered functionally equivalent since they result in the production of the same amino acid sequence in all organisms (although certain organisms may translate some sequences more efficiently than they do others). Moreover, 5 occasionally, a methylated variant of a purine or pyrimidine may be found in a given nucleotide sequence. Such methylations do not affect the coding relationship between the trinucleotide codon and the corresponding amino acid. In addition, a skilled artisan will understand how to mutate nucleotides of a specific codon so as to specifically alter an encoded amino acid based on the relevant codon chart. Additional desired nucleic acid 10 and/or amino acid modifications can be engineered using site-directed mutagenesis and PCR-mediated mutagenesis techniques.

The “nucleic acid” can take any of a number of forms (*e.g.*, DNA, mRNA, cDNA) that encode a biomarker described herein. For example, such biomarker nucleic acid molecules include DNA (*e.g.*, genomic DNA and cDNA) comprising the entire or a partial 15 sequence of a PKC-iota gene or the complement or hybridizing fragment of such a sequence. The biomarker nucleic acid molecules also include RNA comprising the entire or a partial sequence of a PKC-iota gene or the complement of such a sequence, wherein all thymidine residues are replaced with uridine residues. A “transcribed polynucleotide” is a polynucleotide (*e.g.*, an RNA, a cDNA, or an analog of one of an RNA or cDNA) which is 20 complementary to or homologous with all or a portion of a mature RNA made by transcription of a biomarker of the present invention, at least in part, and normal post-transcriptional processing (*e.g.*, splicing), if any, of the transcript, and reverse transcription of the transcript.

The terms “homology” or “identity,” as used interchangeably herein, refer to 25 sequence similarity between two polynucleotide sequences or between two polypeptide sequences, with identity being a more strict comparison. The phrases “percent identity or homology” and “% identity or homology” refer to the percentage of sequence similarity found in a comparison of two or more polynucleotide sequences or two or more polypeptide sequences. Two or more sequences can be anywhere from 0-100% similar, or any integer 30 value there between. Identity or similarity can be determined by comparing a position in each sequence that may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same nucleotide base or amino acid, then the molecules are identical at that position. A degree of similarity or identity between

polynucleotide sequences is a function of the number of identical or matching nucleotides at positions shared by the polynucleotide sequences. A degree of identity of polypeptide sequences is a function of the number of identical amino acids at positions shared by the polypeptide sequences. A degree of homology or similarity of polypeptide sequences is a function of the number of amino acids at positions shared by the polypeptide sequences. The term "substantial homology" refers to homology of at least 50%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or more (e.g., about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more). In one embodiment, biomarker nucleic acid molecules encode a protein or portion thereof which includes an amino acid sequence which is sufficiently homologous to an amino acid sequence described herein such that the protein or portion thereof maintains PKC- ϵ enzymatic activity (e.g., kinase activity) and regulation of enzymatic activity by Thr-412 of human PKC- ϵ or a corresponding phosphorylatable amino acid in a homolog thereof.

The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. The alignment can be performed using the Clustal Method. Multiple alignment parameters include GAP Penalty = 10, Gap Length Penalty = 10. For DNA alignments, the pairwise alignment parameters can be Htuple = 2, Gap penalty = 5, Window = 4, and Diagonal saved = 4. For protein alignments, the pairwise alignment parameters can be Ktuple = 1, Gap penalty = 3, Window = 5, and Diagonals Saved = 5. Similarly, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available online), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available online), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0) (available online), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.

Methods for the production of nucleic acids (*e.g.*, PKC-iota) are known in the art and include standard hybridization, PCR, and/or synthetic nucleic acid techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis.

5 A “biomarker protein” is a protein encoded by or corresponding to a biomarker of the present invention, *e.g.*, PKC-iota. The terms “protein” and “polypeptide” are used interchangeably herein. In one embodiment, the protein is at least 50%, 60%, 70%, 80%, 90%, and 95% or more (*e.g.*, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more) homologous to the
10 entire amino acid sequence of a PKC-iota protein described herein. In addition, biologically active portions of PKC-iota proteins described herein are included which have at least 50%, 60%, 70%, 80%, 90%, and 95% or more (*e.g.*, 95.5%, 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9% or more) homology to a fragment of a PKC-iota protein described herein, *e.g.*, a
15 domain or motif, and that is capable of PKC-iota enzymatic activity (*e.g.*, kinase activity) and regulation by Thr-412. Typically, biologically active portions (peptides, *e.g.*, peptides which are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100, 150, 200, 250, 300, 350, 400, 450, or more amino acids in length) comprise a domain or motif, *e.g.*, a PKC-iota kinase domain encompassing Thr-412. Moreover, other biologically active
20 portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein.

Methods for the production of proteins (*e.g.*, PKC-iota) are known in the art and include *e.g.*, the expression of the protein in appropriate cells starting from a cDNA or the production by subsequent addition of amino acids to a starting amino acid (see Current
25 Protocols, John Wiley & Sons, Inc., New York). Furthermore, methods for the production of protein fragments are known in the art and include the cleavage of the protein with appropriate proteases or the generation of nucleic acid fragments encoding the protein fragments and subsequent expression of the fragments in appropriate cells. Methods for the production of mutated proteins, *e.g.*, by exchanging and/or deleting one or more amino
30 acids, are known in the art.

B. Diagnostic Methods

Methods are provided for identifying compounds or agents which inhibit kinase activity of human protein kinase C iota (PKC-iota) or a homolog thereof, comprising: a) contacting a sample comprising human PKC-iota or a homolog thereof with the compound; and b) determining the ability of the compound to inhibit Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota, wherein decreased phosphorylation identifies a compound which inhibits kinase activity of human PKC-iota or a homolog thereof. These methods are also referred to herein as drug screening assays and typically include the step of screening a candidate/test compound or agent for the ability to interact with (*e.g.*, bind to) a PKC-iota protein, to modulate the intra-molecular modification a PKC-iota protein (*e.g.*, phosphorylation), and/or to modulate the interaction of PKC-iota with a target PKC-iota interacting protein. Test compounds or agents which have one or more of these abilities can be used as drugs to treat disorders characterized by aberrant, abnormal, and/or unwanted PKC-iota nucleic acid expression and/or PKC-iota protein activity, such as cancer. Candidate/test compounds include, for example, small organic and inorganic molecules (*e.g.*, molecules obtained from combinatorial and natural product libraries).

The term "sample," "tissue sample," "subject sample," "subject cell or tissue sample" or "specimen" each refer to a collection of similar cells obtained from a tissue of a subject or subject either as *in vitro* (*e.g.*, cultured), *ex vivo*, or *in vivo* (*e.g.*, isolated primary cells) samples. The source of the tissue sample may be solid tissue as from a fresh, frozen and/or preserved organ, tissue sample, biopsy, or aspirate; blood or any blood constituents; bodily fluids such as whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow, amniotic fluid, peritoneal fluid or interstitial fluid; or cells from any time in gestation or development of the subject. The tissue sample may contain compounds that are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics or the like. The sample may further comprise cancer cells, such as ovarian, lung, breast, and multiple myeloma cancer cells or any cancer in which PKC-iota is amplified or overexpressed, has an activating mutation, or is activated by other kinases.

The terms "subject" and "patient" are used interchangeably. As used herein, the terms "subject" and "subjects" refer to an animal, *e.g.*, a mammal including a non-primate (*e.g.*, a cow, pig, horse, donkey, goat, camel, cat, dog, guinea pig, rat, mouse, sheep) and a

primate (*e.g.*, a monkey, such as a cynomolgous monkey, gorilla, chimpanzee and a human).

The term “inhibit” refers to a statistically significant decrease in a metric of interest, such as the reduction of Thr-412-phosphorylated PKC- ι , PKC- ι enzymatic activity (5 *e.g.*, kinase activity), cancer progression, and the like. Such statistically significant decrease can be at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more relative to a control. For example, a test compound administered and analyzed according to the methods described herein can comprise a *bona fide* inhibitor of PKC- ι enzymatic activity (*e.g.*, kinase activity) by decreasing Thr-412-phosphorylated PKC- ι amounts by 10 at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more relative to that of no PKC- ι ligand administration or over a given amount of time. In one embodiment, the term “PKC- ι inhibitor” is a substance, such as a small molecule, which interferes with the phosphorylation of human PKC- ι at Thr-412 or at a corresponding phosphorylation site in a homolog thereof. Exemplary PKC- ι inhibitors are well known in the art and are 15 disclosed, for example, in PCT Publication WO2009/036414 and Pillai *et al.* (2011) *Int. J Biochem Cell Biol.* 43:784-794, such as 1H-imidazole-4-carboxamide, 5-amino-1-[2,3-dihydroxy-4-[(phosphonoxy) methyl] cyclopentyl]-[1R-(1 α , 2 β , 3 β , 4 α)], (ICA-1); each of which is incorporated in its entirety herein by this reference.

The term “altered amount” of a biomarker or “altered level” of a biomarker refers to 20 increased or decreased expression, modification, and/or activity of a biomarker of the present invention, at least in part in a sample as compared to that in a control sample.

The amount of a biomarker in a subject is “significantly” higher or lower than the normal amount of a biomarker, if the amount of the biomarker is greater or less, respectively, than the normal level by an amount greater than the standard error of the assay 25 employed to assess amount, or at least two, three, four, five, ten or more times that amount. Alternatively, the amount of the biomarker in the subject can be considered “significantly” higher or lower than the normal amount if the amount is at least about two, at least about three, at least about four, or at least about five times, higher or lower, respectively, than the normal amount of the biomarker (*e.g.*, in a control sample or the average expression level of 30 the biomarkers of the present invention in several control samples).

“Likely to,” as used herein, refers to an increased probability, that an item, object, thing or person will occur such as at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%,

140%, 150%, 160%, 170%, 180%, 190%, 200%, or more (or any range inclusive). Thus, in one example, a compound that is likely to inhibit PKC-iota enzymatic activity (e.g., kinase activity) has an increased probability of inhibiting Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota.

5 The test compounds of the present invention, at least in part, can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the 'one-bead one-compound' library method; and synthetic library methods using affinity chromatography selection. The
10 biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, K.S. (1997) *Anticancer Drug Des.* 12:145).

 Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt *et al.* (1993) *Proc. Natl. Acad. Sci. U.S.A.* 90:6909; Erb *et al.*
15 (1994) *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.* (1994) *J. Med. Chem.* 37:2678; Cho *et al.* (1993) *Science* 261:1303; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2059; Carrell *et al.* (1994) *Angew. Chem. Int. Ed. Engl.* 33:2061; and in Gallop *et al.* (1994) *J. Med. Chem.* 37:1233.

 Libraries of compounds may be presented in solution (e.g., Houghten (1992)
20 *Biotechniques* 13:412-421), or on beads (Lam (1991) *Nature* 354:82-84), chips (Fodor (1993) *Nature* 364:555-556), bacteria (Ladner USP 5,223,409), spores (Ladner USP '409), plasmids (Cull *et al.* (1992) *Proc Natl Acad Sci USA* 89:1865-1869) or on phage (Scott and Smith (1990) *Science* 249:386-390); (Devlin (1990) *Science* 249:404-406); (Cwirla *et al.* (1990) *Proc. Natl. Acad. Sci.* 87:6378-6382); (Felici (1991) *J. Mol. Biol.* 222:301-310);
25 (Ladner *supra.*).

 In one embodiment, the inhibition of Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota is determined by comparing the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the
30 sample relative to a control. The control can be the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the sample relative to said amount in the absence of the compound or at an earlier timepoint after contact of the sample with the compound. The phosphorylation level

of PKC-iota is generally determined by measuring the amount of phosphorylated PKC-iota protein and, optionally, of unphosphorylated PKC-iota protein, and normalizing the amount of phosphorylated protein to the total protein in the sample being analyzed. The calculated response phosphorylation level in the presence of the test compound and the basal or background phosphorylation levels (*e.g.*, in the absence of the test compound or at a earlier timepoint after test compound administration) are thus not affected by differences in the absolute quantity of the indicator protein at a given time.

The discriminatory time point, or predetermined time after administering the test compound to cells, can be selected to achieve a calibrated statistically significant difference between Thr-412 phosphorylation levels in the sample relative to controls. The difference may be maximal at the predetermined time but that is not required and depends on other parameters of the test. In addition, whereas the calculation of ratios as described herein is beneficial in providing useful comparative numbers, calculation of absolute differences between phosphorylated PKC-iota levels upon administration of test compounds relative to controls, and between test subjects and control subjects, could also be employed and would be effective.

In some embodiments, the methods described above can further comprise determining the amount of Thr-564 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota in the sample relative to said amount in the absence of the compound or at an earlier timepoint after contact of the sample with the compound and determining whether the amount changes over time or in response to the compound. It has been determined herein that Thr-564 phosphorylation does not appreciably change in response to administration of *bona fide* inhibitors of PKC-iota enzymatic activity (*e.g.*, kinase activity).

Phosphorylation is a biochemical reaction in which a phosphate group is added to Ser, Thr or Tyr residues of a protein and is catalyzed by protein kinase enzymes. Phosphorylation normally modifies the functions of target proteins, often causing activation. As part of the cell's homeostatic mechanisms, phosphorylation is only a transient process which is reversed by other enzyme called phosphatases. Therefore, protein phosphorylation levels change over time and can be evaluated in a number of well known manners, including, for example, by immunological approaches. For example, the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota is determined by an immunoassay using a

reagent which specifically binds with Thr-412 phosphorylated human PKC-iota or corresponding phosphorylated homolog of human PKC-iota. Such an immunoassay comprise a number of well known forms, including, without limitation, a radioimmunoassay, a Western blot assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay. General techniques to be used in performing the various immunoassays noted above and other variations of the techniques, such as *in situ* proximity ligation assay (PLA), fluorescence polarization immunoassay (FPIA), fluorescence immunoassay (FIA), enzyme immunoassay (EIA), nephelometric inhibition immunoassay (NIA), enzyme linked immunosorbent assay (ELISA), and radioimmunoassay (RIA), ELISA, etc. alone or in combination or alternatively with NMR, MALDI-TOF, LC-MS/MS, are known to those of ordinary skill in the art.

In one embodiment, the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human PKC-iota or corresponding homolog of human PKC-iota regardless of phosphorylation status and a detection antibody or fragment thereof which specifically binds with Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylated homolog of human PKC-iota. Such an enzyme immunoassay is particularly advantageous because identifying differences in protein levels between several PKC isoforms has traditionally been hampered by the high homology between PKC isoforms and their phosphorylated forms.

Immunological reagents for identifying PKC iota protein, as well as phosphorylated forms of PKC-iota, such as the phosphorylated Thr-412 and Thr-564 forms, are well known in the art (*e.g.*, the anti-PKC-iota mouse monoclonal antibody #610175 from BD Bioscience and the anti-phospho-Thr-564 PKC-iota rabbit polyclonal antibody (available from Invitrogen, Inc.; product #44-968G). Such anti-PKC-iota and/or anti-phospho-PKC-iota antibody reagents (*e.g.*, monoclonal antibody) can be used to isolate and/or determine the amount of the respective proteins such as in a cellular lysate. Such reagents can also be used to monitor protein levels in a cell or tissue, *e.g.*, white blood cells or lymphocytes, as part of a clinical testing procedure, *e.g.*, in order to monitor an optimal dosage of an inhibitory agent. Detection can be facilitated by coupling (*e.g.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent

materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein
5 isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

The screening assays described above can further be adapted to identify
10 candidate/test compounds which modulate (*e.g.*, stimulate or inhibit) the interaction (and most likely PKC- ι activity as well) between a PKC- ι protein and a target molecule with which the PKC- ι protein normally interacts to verify that PKC- ι enzymatic activity has been reduced in accordance with the reduced amounts of phosphorylated Thr-412 PKC- ι levels. Examples of such target molecules or substrates include
15 phosphorylated target proteins in the signaling pathway of PKC- ι (*e.g.*, pSer-653-LLGL2).

In another embodiment, the invention provides assays for screening candidate/test compounds which interact with (*e.g.*, bind to) PKC- ι protein. "Binding compound" shall refer to a binding composition, such as a small molecule, an antibody, a peptide, a peptide
20 or non-peptide ligand, a protein, an oligonucleotide, an oligonucleotide analog, such as a peptide nucleic acid, a lectin, or any other molecular entity that is capable of specifically binding to a target protein or molecule or stable complex formation with an analyte of interest, such as a complex of proteins. "Binding moiety" means any molecule to which molecular tags can be directly or indirectly attached that is capable of specifically binding
25 to an analyte. Binding moieties include, but are not limited to, antibodies, antibody binding compositions, peptides, proteins, nucleic acids and organic molecules having a molecular weight of up to about 1000 daltons and containing atoms selected from the group consisting of hydrogen, fluoride, carbon, oxygen, nitrogen, sulfur and phosphorus. Typically, the assays are cell-based assays. The cell, for example, can be of mammalian origin expressing
30 PKC- ι , *e.g.*, a cancer cell.

In other embodiments, the assays are cell-free assays which include the steps of combining a PKC- ι protein or a biologically active portion thereof, and a candidate/test compound, *e.g.*, under conditions which allow for interaction of (*e.g.*, binding of) the

candidate/test compound to the PKC-iota protein or portion thereof to form a complex, and detecting the formation of a complex, in which the ability of the candidate compound to interact with (*e.g.*, bind to) the PKC-iota polypeptide or fragment thereof is indicated by the presence of the candidate compound in the complex. Formation of complexes between the PKC-iota protein and the candidate compound can be quantitated, for example, using standard immunoassays. Such analyses would identify test compounds as PKC-iota ligands.

To perform the above drug screening assays, it can be desirable to immobilize either PKC-iota or its target molecule to facilitate separation of complexes from uncomplexed forms of one or both of the proteins, as well as to accommodate automation of the assay. Interaction (*e.g.*, binding of) of PKC-iota to a target molecule, in the presence and absence of a candidate compound, can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtitre plates, test tubes, and micro-centrifuge tubes. In one embodiment, a fusion polypeptide can be provided which adds a domain that allows the polypeptide to be bound to a matrix. For example, glutathione-S-transferase/ PKC-iota fusion polypeptides can be adsorbed onto glutathione sepharose beads (Sigma Chemical, St. Louis, Mo.) or glutathione derivatized microtitre plates, which are then combined with the cell lysates (*e.g.*, ³⁵S-labeled) and the candidate compound, and the mixture incubated under conditions conducive to complex formation (*e.g.*, at physiological conditions for salt and pH). Following incubation, the beads are washed to remove any unbound label, and the matrix immobilized and radiolabel determined directly, or in the supernatant after the complexes are dissociated. Alternatively, the complexes can be dissociated from the matrix, separated by SDS-PAGE, and the level of PKC-iota-binding polypeptide found in the bead fraction quantitated from the gel using standard electrophoretic techniques.

Other techniques for immobilizing polypeptides on matrices can also be used in the exemplary drug screening assays of the invention. For example, either PKC-iota or its target molecule can be immobilized utilizing conjugation of biotin and streptavidin. Biotinylated PKC-iota molecules can be prepared from biotin-NHS (N-hydroxy-succinimide) using techniques well known in the art (*e.g.*, biotinylation kit, Pierce Chemicals, Rockford, Ill.), and immobilized in the wells of streptavidin-coated 96 well plates (Pierce Chemical). Alternatively, antibodies reactive with PKC-iota, but which do not interfere with binding of the polypeptide to its target molecule can be derivatized to the

wells of the plate, and PKC-iota trapped in the wells by antibody conjugation. As described above, preparations of a PKC-iota-binding polypeptide and a candidate compound are incubated in the PKC-iota-presenting wells of the plate, and the amount of complex trapped in the well can be quantitated. Methods for detecting such complexes, in addition to those described above for the GST-immobilized complexes, include immunodetection of complexes using antibodies reactive with the PKC-iota target molecule, or which are reactive with PKC-iota polypeptide and compete with the target molecule; as well as enzyme-linked assays which rely on detecting an enzymatic activity associated with the target molecule.

In another aspect, a method for assessing the efficacy of a compound for inhibiting kinase activity of human PKC-iota or a homolog thereof in a subject, comprising a) detecting in a subject sample at a first point in time, the amount of Thr-412 phosphorylated human PKC-iota or the amount of a human PKC-iota homolog phosphorylated at a corresponding amino acid of human PKC-iota; b) repeating step a) during at one or more subsequent points in time after administration of the compound; and c) comparing the amount of phosphorylated human PKC-iota or homolog thereof detected in step a) with said amount detected in step b), wherein a higher amount of Thr-412 phosphorylated human PKC-iota or the amount of the human PKC-iota homolog phosphorylated at a corresponding amino acid of human PKC-iota in the first point in time relative to at least one subsequent point in time, indicates that the compound inhibits kinase activity of PKC-iota or the homolog thereof.

As used herein, "time course" shall refer to the amount of time between an initial event and a subsequent event. For example, with respect to a subject's cancer progression, time course may relate to a subject's disease and may be measured by gauging significant events in the course of the disease, wherein the first event may be diagnosis and the subsequent event may be proliferation, metastasis, etc.

Once binding is confirmed, additional assays, such as kinase assays to determine inhibition of phosphorylation effects, can be performed according to well known methods in the art. For example, assays for determining PKC-iota kinase activity are well known in the art (see, for example, the publications described herein and incorporated by reference in their entirety). Briefly, PKC-iota kinase can be incubated with a suitable substrate in a buffer allowing phosphorylation of PKC-iota. Phosphorylation of the substrate can be detected using a labelled phosphate group, such as the use of the radioactive label ^{32}P

present as the ATP source in the buffer. Alternatively, antibodies specific for the phosphorylated products of PKC- ι catalytic activity can be used to detect activity. As will be apparent to those of ordinary skill in the art, the assays are easily amenable to high through-put technologies using robotics and automated processes. Alternatively, the PKC- ι kinase activity can be assayed using a synthetic substrate. PKC- ι activity can also be assayed by detecting downstream targets of the kinase such as those described herein.

Thr-412 phosphorylated PKC- ι can be analyzed according to any of the methods and using any of the samples described herein (*e.g.*, single subject samples or pooled subject samples). Candidate compounds which produce a statistically significant change in PKC- ι -dependent responses (*e.g.*, inhibition of PKC- ι phosphorylation at Thr-412) can be identified. Such statistically significant changes can be measured according to a number of criteria and/or relative to a number of controls. For example, significant modulation of phosphorylation of Thr-412 can be assessed if the output under analysis is inhibited by 1.1-, 1.2-, 1.3-, 1.4-, 1.5-, 1.6-, 1.7-, 1.8-, 1.9-, 2.0-, 2.1-, 2.2-, 2.3-, 2.4-, 2.5-, 2.6-, 2.7-, 2.8-, 2.9-, 3.0-, 3.1-, 3.2-, 3.3-, 3.4-, 3.5-, 3.6-, 3.7-, 3.8-, 3.9-, 4.0-, 4.1-, 4.2-, 4.3-, 4.4-, 4.5-, 4.6-, 4.7-, 4.8-, 4.9-, 5.0-, 5.5-, 6.0-, 6.5-, 7.0-, 7.5-, 8.0-, 8.5-, 9.0-, 9.5-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-, 20-fold or more different (including any range inclusive), relative to a control. In one embodiment, between the first point in time and the subsequent point in time, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer.

The term "cancer" refer to the presence of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, and certain characteristic morphological features. Cancer cells are often in the form of a tumor, but such cells may exist alone within an animal, or may be a non-tumorigenic cancer cell, such as a leukemia cell. As used herein, the term "cancer" includes premalignant as well as malignant cancers. Cancers include, but are not limited to, B cell cancer, *e.g.*, multiple myeloma, Waldenström's macroglobulinemia, the heavy chain diseases, such as, for example, alpha chain disease, gamma chain disease, and mu chain disease, benign monoclonal gammopathy, and immunocytic amyloidosis, melanomas, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer,

testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like. Also included are any cancers in which the gene encoding PKC-iota (PRKCI) is amplified or overexpressed, has an activating mutation, or the PKC-iota enzyme is hyper-activated by other kinases. In some embodiments, ovarian cancers, including serous cystadenocarcinoma, head and neck cancers, including non-small cell lung cancer (NSCLC), squamous cell carcinoma, pancreatic cancer, colon cancer, prostate cancer, and/or gliomas can be preferred.

“Treat,” “treatment,” and other forms of this word refer to the administration of a PKC-iota ligand to inhibit PKC-iota enzymatic activity (*e.g.*, kinase activity), to cause a cancer to be ameliorated, to extend the expected survival time of the subject and/or time to progression of a cancer or the like.

“Responsiveness,” to “respond” to treatment, and other forms of this verb, as used herein, refer to the reaction of a subject to treatment with a PKC-iota ligand. As an example, a subject responds to treatment with a PKC-iota ligand if PKC-iota enzymatic activity (*e.g.*, kinase activity) in the subject or cell thereof is inhibited by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more relative to that of no PKC-iota ligand administration or over a given amount of time.

C. Treatment Methods

PKC-iota inhibitors described herein can be used to treat cancer. In one embodiment, a method of treating a subject afflicted with cancer is described comprising administering to the subject a compound that inhibits Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota, thereby treating the subject afflicted with the cancer. In another embodiment, such PKC-iota inhibitors can also be used to determine the efficacy, toxicity, or side effects of treatment with such an agent. These methods of treatment generally include the steps of administering atypical PKC-iota modulators in a pharmaceutical composition, as described further below, to a subject in need of such treatment, *e.g.*, a subject with cancer or at risk for developing cancer.

The term “administering” is intended to include routes of administration which allow the agent to perform its intended function of inhibiting PKC-iota enzymatic activity (*e.g.*, kinase activity). Examples of routes of administration which can be used include injection (subcutaneous, intravenous, parenterally, intraperitoneally, intrathecal, etc.), oral,

inhalation, and transdermal. The injection can be bolus injections or can be continuous infusion. Depending on the route of administration, the agent can be coated with or disposed in a selected material to protect it from natural conditions which may detrimentally affect its ability to perform its intended function. The agent may be
5 administered alone, or in conjunction with a pharmaceutically acceptable carrier. The agent also may be administered as a prodrug, which is converted to its active form *in vivo*.

The term "effective amount" of an agent inhibiting PKC- ϵ enzymatic activity is that amount necessary or sufficient to inhibit PKC- ϵ enzymatic activity in the subject or population of subjects as measured, for example, by the levels of Thr-412-phosphorylated
10 PKC- ϵ according to the methods described above. The effective amount can vary depending on such factors as the type of therapeutic agent(s) employed, the size of the subject, or the severity of the disorder.

It will be appreciated that individual dosages may be varied depending upon the requirements of the subject in the judgment of the attending clinician, the severity of the
15 condition being treated and the particular compound being employed. In determining the therapeutically effective amount or dose, a number of additional factors may be considered by the attending clinician, including, but not limited to: the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the desired time course of treatment; the species of mammal; its size, age, and general health; the
20 specific disease involved; the degree of or involvement or the severity of the disease; the response of the individual subject; the particular compound administered; the mode of administration; the bioavailability characteristics of the preparation administered; the dose regimen selected; the kind of concurrent treatment; and other relevant circumstances.

Treatment can be initiated with smaller dosages which are less than the effective
25 dose of the compound. Thereafter, in one embodiment, the dosage should be increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

The effectiveness of any particular agent to treat cancers can be monitored by
30 comparing two or more samples obtained from a subject undergoing cancer treatment. In general, a first sample is obtained from the subject prior to beginning therapy and one or more samples during treatment. In such a use, a baseline of expression of cells from subjects with cancer prior to therapy is determined and then changes in the baseline state of

expression of cells from subjects with cancer is monitored during the course of therapy. Alternatively, two or more successive samples obtained during treatment can be used without the need of a pre-treatment baseline sample. In such a use, the first sample obtained from the subject is used as a baseline for determining whether the expression of cells from subjects with metabolic disorders is increasing or decreasing.

PKC-iota inhibitors can be administered in pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of the inhibitor formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. For example, formulations can be adapted for (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin, buccal, or sublingual surfaces; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; or (5) nasal/aerosol, for example, as an aqueous aerosol, liposomal preparation or solid particles containing the compound, based on well known methods in the pharmaceutical arts.

The phrase "pharmaceutically acceptable" is employed herein to refer to those agents, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil,

cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; 5 (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "pharmaceutically-acceptable salts" refers to the relatively non-toxic, inorganic and organic acid addition salts of the agents that reduce the phosphorylation 10 levels of PKC- ι and/or activity encompassed by the invention. These salts can be prepared in situ during the final isolation and purification of the agents, or by separately reacting a purified agents agent in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, 15 stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like (See, for example, Berge *et al.* (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

In addition, the methods described herein can further comprise treating subjects with 20 PKC- ι inhibitors in addition to administering one or more additional anti-cancer agents and/or use samples from subjects exposed to such anti-cancer agents. Anti-cancer agents are well known to the skilled artisan and include, without limitation, chemotherapy and radiation, as well as immunotherapy, hormone therapy, and gene therapy using nucleic acid molecules and/or proteins that are linked to the initiation, progression, and/or pathology of 25 a tumor or cancer.

Chemotherapy includes the administration of a chemotherapeutic agent. Such a chemotherapeutic agent may be, but is not limited to, those selected from among the following groups of compounds: platinum compounds, cytotoxic antibiotics, antimetabolites, anti-mitotic agents, alkylating agents, arsenic compounds, DNA 30 topoisomerase inhibitors, taxanes, nucleoside analogues, plant alkaloids, and toxins; and synthetic derivatives thereof. Exemplary compounds include, but are not limited to, alkylating agents: cisplatin, treosulfan, and trofosfamide; plant alkaloids: vinblastine, paclitaxel, docetaxol; DNA topoisomerase inhibitors: teniposide, crisnatol, and mitomycin;

anti-folates: methotrexate, mycophenolic acid, and hydroxyurea; pyrimidine analogs: 5-fluorouracil, doxifluridine, and cytosine arabinoside; purine analogs: mercaptopurine and thioguanine; DNA antimetabolites: 2'-deoxy-5-fluorouridine, aphidicolin glycinate, and pyrazoloimidazole; and antimitotic agents: halichondrin, colchicine, and rhizoxin.

- 5 Compositions comprising one or more chemotherapeutic agents (*e.g.*, FLAG, CHOP) may also be used. FLAG comprises fludarabine, cytosine arabinoside (Ara-C) and G-CSF. CHOP comprises cyclophosphamide, vincristine, doxorubicin, and prednisone. In another embodiment, PARP (*e.g.*, PARP-1 and/or PARP-2) inhibitors are used and such inhibitors are well known in the art (*e.g.*, Olaparib, ABT-888, BSI-201, BGP-15 (N-Gene Research
- 10 Laboratories, Inc.); INO-1001 (Inotek Pharmaceuticals Inc.); PJ34 (Soriano *et al.*, 2001; Pacher *et al.*, 2002b); 3-aminobenzamide (Trevigen); 4-amino-1,8-naphthalimide; (Trevigen); 6(5H)-phenanthridinone (Trevigen); benzamide (U.S. Pat. Re. 36,397); and NU1025 (Bowman *et al.*). In still another embodiment, the chemotherapeutic agents are platinum compounds, such as cisplatin, carboplatin, oxaliplatin, nedaplatin, and iproplatin.
- 15 Other antineoplastic platinum coordination compounds are well known in the art, can be modified according to well known methods in the art, and include the compounds disclosed in U.S. Pat. Nos. 4,996,337, 4,946,954, 5,091,521, 5,434,256, 5,527,905, and 5,633,243, all of which are incorporated herein by reference. The foregoing examples of chemotherapeutic agents are illustrative, and are not intended to be limiting.

- 20 Radiation therapy can also comprise an additional anti-cancer agent. The radiation used in radiation therapy can be ionizing radiation. Radiation therapy can also be gamma rays, X-rays, or proton beams. Examples of radiation therapy include, but are not limited to, external-beam radiation therapy, interstitial implantation of radioisotopes (I-125, Pd-103, Ir-192), intravenous administration of radioisotopes such as strontium-89, thoracic
- 25 radiation therapy, intraperitoneal P-32 radiation therapy, and/or total abdominal and pelvic radiation therapy. For a general overview of radiation therapy, see Hellman, Chapter 16: Principles of Cancer Management: Radiation Therapy, 6th edition, 2001, DeVita *et al.*, eds., J. B. Lippencott Company, Philadelphia. The radiation therapy can be administered as external beam radiation or teletherapy wherein the radiation is directed from a remote
- 30 source. The radiation treatment can also be administered as internal therapy or brachytherapy wherein a radioactive source is placed inside the body close to cancer cells or a tumor mass. Also encompassed is the use of photodynamic therapy comprising the administration of photosensitizers, such as hematoporphyrin and its derivatives, Vertoporphin

(BPD-MA), phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A; and 2BA-2-DMHA.

Additional anti-cancer agents include immunotherapy, hormone therapy, and gene therapy. Such therapies include, but are not limited to, the use of antisense polynucleotides, ribozymes, RNA interference molecules, triple helix polynucleotides and the like, where the nucleotide sequence of such compounds are related to the nucleotide sequences of DNA and/or RNA of genes that are linked to the initiation, progression, and/or pathology of a tumor or cancer. For example, oncogenes, growth factor genes, growth factor receptor genes, cell cycle genes, DNA repair genes, and others, may be targeted in such therapies.

Immunotherapy may comprise, for example, use of cancer vaccines and/or sensitized antigen presenting cells. The immunotherapy can involve passive immunity for short-term protection of a host, achieved by the administration of an antibody directed against a cancer antigen or disease antigen (*e.g.*, administration of a monoclonal antibody, optionally linked to a chemotherapeutic agent or toxin, to a tumor antigen).

Immunotherapy can also focus on using the cytotoxic lymphocyte-recognized epitopes of cancer cell lines.

Hormonal therapeutic treatments can comprise, for example, hormonal agonists, hormonal antagonists (*e.g.*, flutamide, bicalutamide, tamoxifen, raloxifene, leuprolide acetate (LUPRON), LH-RH antagonists), inhibitors of hormone biosynthesis and processing, and steroids (*e.g.*, dexamethasone, retinoids, deltoids, betamethasone, cortisol, cortisone, prednisone, dehydrotestosterone, glucocorticoids, mineralocorticoids, estrogen, testosterone, progestins), vitamin A derivatives (*e.g.*, all-trans retinoic acid (ATRA)); vitamin D3 analogs; antigestagens (*e.g.*, mifepristone, onapristone), or antiandrogens (*e.g.*, cyproterone acetate).

In one embodiment, anti-cancer therapy used for cancers whose phenotype is determined by the methods of the invention can comprise one or more types of therapies described herein including, but not limited to, chemotherapeutic agents, immunotherapeutics, anti-angiogenic agents, cytokines, hormones, antibodies, polynucleotides, radiation and photodynamic therapeutic agents. For example, combination therapies can comprise one or more chemotherapeutic agents and radiation, one or more chemotherapeutic agents and immunotherapy, or one or more chemotherapeutic agents, radiation and chemotherapy.

Exemplification

This invention is further illustrated by the following examples, which should not be construed as limiting.

5 **Example 1: Phosphorylation Status of Thr-412 is a Biomarker of Human PKC-iota Enzymatic Activity**

Inhibition of PKC-iota using small molecules has the potential to be an effective oncology therapeutic. In order to measure the inhibition of PKC-iota enzymatic activity by certain agents (e.g., a small molecule inhibitor), a target engagement biomarker is needed
10 that will reliably report PKC-iota enzymatic activity in tumor samples.

The phosphorylation status of autophosphorylation sites generally reflect the level of a protein kinase's activity. PKC's are typically phosphorylated by an upstream kinase (e.g., PDK1 for PKC-iota) in the activation loop (e.g., Thr-412 for human PKC-iota). Phosphorylation of the activation loop typically leads to an autophosphorylation event in
15 the turn motif (e.g., Thr-564 in PKC-iota). A schematic diagram of canonical PKC phosphorylation events is shown in Figure 1A (see Newton *et al.* (2003) *Biochem. J.* 370, 351 for additional details).

Since PKC isoforms are highly homologous and therefore difficult to distinguish, a customized sandwich ELISA technique was developed to accurately measure
20 phosphorylated PKC-iota proteins. Specifically, 96-well assay plates coated with 100 µl of anti-PKCiota mouse monoclonal antibody (available from BD Bioscience; product #610175) diluted 1:500 in PBS were incubated overnight at room temperature in the dark. The assay plates were subsequently washed three times using 200 uL of wash buffer (available from R&D Systems; product #WA126). The assay plates were then blocked with
25 200 µl of 1% BSA in PBS, covered, incubated for one hour at room temperature in the dark, and then washed three times using 200 uL of wash buffer (available from R&D Systems; product #WA126). One hundred microliters of each sample, diluted to 0.01 to 1 mg/ml in dilution buffer (containing 5 mLs 5X sample diluent concentrate (available from R&D Systems; product #DYC001) plus 3.1 mLs 8 M Urea plus 250 µl of 1 M NaF plus
30 16.65 mL water), were added to each well, covered, incubated for 2 hours at room temperature in the dark, and then washed three times using 200 uL of wash buffer (available from R&D Systems; product #WA126). During sample incubation, a stock solution of phospho-specific detection solution was made by diluting 24 µl of anti-PKC zeta/lambda (pT410/412) Phospho (PRKCZ) antibody (available from Epitomics, Inc;

product #2106-1) into 12 mLs of a 1x PBS solution containing 1% BSA. After incubation, 100 uL of detection antibody solution was added to each well containing sample, covered, incubated for another 2 hours at room temperature in the dark, and then washed three times using 200 uL of wash buffer (available from R&D Systems; product #WA126). One
5 hundred microliters of a 1:1000 solution of HRP-conjugated anti-rabbit IgG antibody (available from Cell Signaling; product #7074; 22 uL into 22 mLs of 1x PBS containing 1% BSA) was added to each well containing sample, incubated for 30 minutes at room temperature in the dark, and then washed three times using 200 uL of wash buffer (available from R&D Systems; product #WA126). Finally, luminescence levels are read
10 after 10 minutes of applying 100 uL of HRP chemiluminescent substrate (available from R&D systems; product #DY993).

U2OS cells engineered to overexpress activated PKC-iota protein were treated with small molecules that inhibit PKC-iota enzymatic activity, but that do not inhibit PDPK1 activity. Figure 1B shows that such small molecule PKC-iota inhibitors led to a rapid,
15 robust decrease of phosphorylation at Thr-412 in human PKC-iota, whereas the canonical phosphorylation site at Thr-564 was not affected. In addition, Figure 1B demonstrates that levels of LLGL2 phosphorylated at Ser-653 (*i.e.*, phospho-S653-LLGL2), a validated target substrate of PKC-iota enzymatic activity, was reduced with kinetics similar to that for phospho-Thr-412-PKC-iota, confirming that phosphorylation of Thr-412 is a bona fide
20 biomarker of human PKC-iota enzymatic activity. These results were confirmed using another cell line, human A549 non-small cell lung carcinoma cells (Figures 1C-1D). Studies in mice bearing orthotopic human OVCAR-5 xenograft tumors treated with a small molecule inhibitor of PKC-iota, demonstrated a reduction in phospho-Thr-412-PKC-iota and a corresponding decrease in the levels of the phosphorylated PKCi-iota substrate,
25 LLGL2-pSer-653, in the ascites tumor cells (Figure 1E; ~80% decrease after 6 hours post-150 mg/kg intraperitoneal dosing with an inhibitor of PKC-iota diluted in 2-hydroxypropyl-beta-cyclodextrin (HP-beta-CD)).

Thus, the level of phosphorylation of the threonine at position 412 of human PKC-iota is a target engagement biomarker for PKC-iota enzymatic activity useful for preclinical
30 and clinical applications.

Incorporation by Reference

The contents of all references, patent applications, patents, and published patent applications, as well as the Figures and the Sequence Listing, cited throughout this application are hereby incorporated by reference.

5

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following

10 claims.

What is claimed:

1. A method of identifying a compound which inhibits kinase activity of human protein kinase C iota (PKC-iota) or a homolog thereof, comprising:
 - a) contacting a sample comprising human PKC-iota or a homolog thereof with the
5 compound; and
 - b) determining the ability of the compound to inhibit Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota, wherein decreased phosphorylation identifies a compound which inhibits kinase activity of human PKC-iota or a homolog thereof.
- 10 2. The method of claim 1, wherein the inhibition of said Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota is determined by comparing the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in
15 the sample relative to a control.
3. The method of claim 2, wherein the control is the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the sample relative to said amount in the absence of the
20 compound or at an earlier timepoint after contact of the sample with the compound.
4. The method of claim 1, wherein the inhibition of said Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota is determined by comparing the ratio of the amount of Thr-412 phosphorylated
25 human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the sample relative to the total amount of human PKC-iota or homolog thereof to a control.
5. The method of claim 4, wherein the control is the ratio of the amount of Thr-412
30 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota in the sample relative to said ratio in the absence of the compound or at an earlier timepoint after contact of the sample with the compound.
6. The method of any one of claims 1-5, further comprising determining the amount of
35 Thr-564 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid

in a homolog of human PKC-iota in the sample relative to said amount in the absence of the compound or at an earlier timepoint after contact of the sample with the compound and determining whether the amount changes over time or in response to the compound.

- 5 7. The method of claim 1, further comprising a step of determining whether the compound directly binds said human PKC-iota or said homolog thereof.
8. The method of claim 1, wherein the sample is selected from the group consisting of *in vitro*, *ex vivo*, and *in vivo* samples.
- 10 9. The method of claim 1, wherein the sample comprises cells.
10. The method of claim 9, wherein the cells are cancer cells.
- 15 11. The method of claim 10, wherein the cancer is selected from the group consisting of any cancer in which PKC-iota is amplified or overexpressed, any cancer having an activating mutation of PKC-iota, and any cancer in which PKC-iota is activated by other kinases.
- 20 12. The method of claim 9, wherein the cells are obtained from a subject.
13. The method of claim 1, wherein the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow.
- 25 14. The method of any one of claims 1-5, wherein the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota is determined by an immunoassay using a reagent which specifically binds with Thr-412 phosphorylated human PKC-iota or corresponding
- 30 phosphorylated homolog of human PKC-iota.
15. The method of claim 14, wherein the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an
- 35 immunohistochemical assay, a dot blot assay, or a slot blot assay.

16. The method of claim 15, wherein the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human PKC-iota or corresponding phosphorylated homolog of human PKC-iota and a detection antibody or fragment thereof which specifically binds with Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylated homolog of human PKC-iota.
17. The method of any one of claims 1-5, wherein said human PKC-iota comprises an amino acid sequence set forth in SEQ ID NOs: 1-2.
18. The method of any one of claims 1-5, wherein the compound is a small molecule.
19. The method of any one of claims 1-5, wherein the compound decreases the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota by at least 50%.
20. A method for assessing the efficacy of a compound for inhibiting kinase activity of human protein kinase C iota (PKC-iota) or a homolog thereof in a subject, comprising:
- a) detecting in a subject sample at a first point in time, the amount of Thr-412 phosphorylated human PKC-iota or the amount of a human PKC-iota homolog phosphorylated at a corresponding amino acid of human PKC-iota;
 - b) repeating step a) during at one or more subsequent points in time after administration of the compound; and
 - c) comparing the amount of phosphorylated human PKC-iota or homolog thereof detected in step a) with said amount detected in step b),
- wherein a higher amount of Thr-412 phosphorylated human PKC-iota or the amount of the human PKC-iota homolog phosphorylated at a corresponding amino acid of human PKC-iota in the first point in time relative to at least one subsequent point in time, indicates that the compound inhibits kinase activity of PKC-iota or the homolog thereof.
21. The method of claim 20, wherein the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota is determined by an immunoassay using a reagent which specifically binds with Thr-412 phosphorylated human PKC-iota or corresponding phosphorylated homolog of human PKC-iota.

22. The method of claim 21, wherein the immunoassay is a radioimmunoassay, a Western blot assay, a proximity ligation assay, an immunofluorescence assay, an enzyme immunoassay, an immunoprecipitation assay, a chemiluminescence assay, an immunohistochemical assay, a dot blot assay, or a slot blot assay.
23. The method of claim 22, wherein the enzyme immunoassay is a sandwich enzyme immunoassay using a capture antibody or fragment thereof which specifically binds with human PKC-iota or corresponding homolog of human PKC-iota and a detection antibody or fragment thereof which specifically binds with Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylated homolog of human PKC-iota.
24. The method of claim 20, wherein the sample is selected from the group consisting of *ex vivo* and *in vivo* samples.
25. The method of claim 20, wherein the sample comprises cancer cells.
26. The method of claim 25, wherein the cancer cells are cells selected from the group consisting of any cancer in which PKC-iota is amplified or overexpressed, any cancer having an activating mutation of PKC-iota, and any cancer in which PKC-iota is activated by other kinases.
27. The method of claim 20, wherein the sample is selected from the group consisting of tissue, whole blood, serum, plasma, buccal scrape, saliva, cerebrospinal fluid, urine, stool, and bone marrow.
28. The method of claim 20, wherein the sample in step a) and/or step b) is a portion of a single sample obtained from the subject.
29. The method of claim 20, wherein the sample in step a) and/or step b) is a portion of pooled samples obtained from the subject.
30. The method of claim 20, wherein between the first point in time and the subsequent point in time, the subject has undergone treatment for cancer, has completed treatment for cancer, and/or is in remission from cancer.

31. The method of claim 20, wherein human PKC-iota comprises an amino acid sequence set forth in SEQ ID NOs:1-2.
32. The method of claim 20, wherein the compound is a small molecule.
- 5 33. The method of claim 20, wherein the compound decreases the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota by at least 50%.
- 10 34. A method of treating a subject afflicted with cancer comprising administering to the subject a compound that inhibits Thr-412 phosphorylation of human PKC-iota or a corresponding phosphorylatable amino acid in a homolog of human PKC-iota, thereby treating the subject afflicted with the cancer.
- 15 35. The method of claim 34, wherein the compound is administered in a pharmaceutically acceptable formulation.
36. The method of claim 34, wherein the compound is a small molecule.
- 20 37. The method of claim 34, wherein the compound directly binds said human PKC-iota or the homolog thereof.
38. The method of claim 34, wherein the cancer is selected from the group consisting of any cancer in which PKC-iota is amplified or overexpressed, any cancer having an activating mutation of PKC-iota, and any cancer in which PKC-iota is activated by other kinases.
- 25 39. The method of claim 34, wherein human PKC-iota comprises an amino acid sequence set forth in SEQ ID NOs:1-2.
- 30 40. The method of claim 34, wherein the compound is a small molecule.
41. The method of claim 34, wherein the compound decreases the amount of Thr-412 phosphorylated human PKC-iota or a corresponding phosphorylatable amino acid in the homolog of human PKC-iota by at least 50%.
- 35

42. The method of claim 34, further comprising administering one or more additional anti-cancer agents.

Figure 1

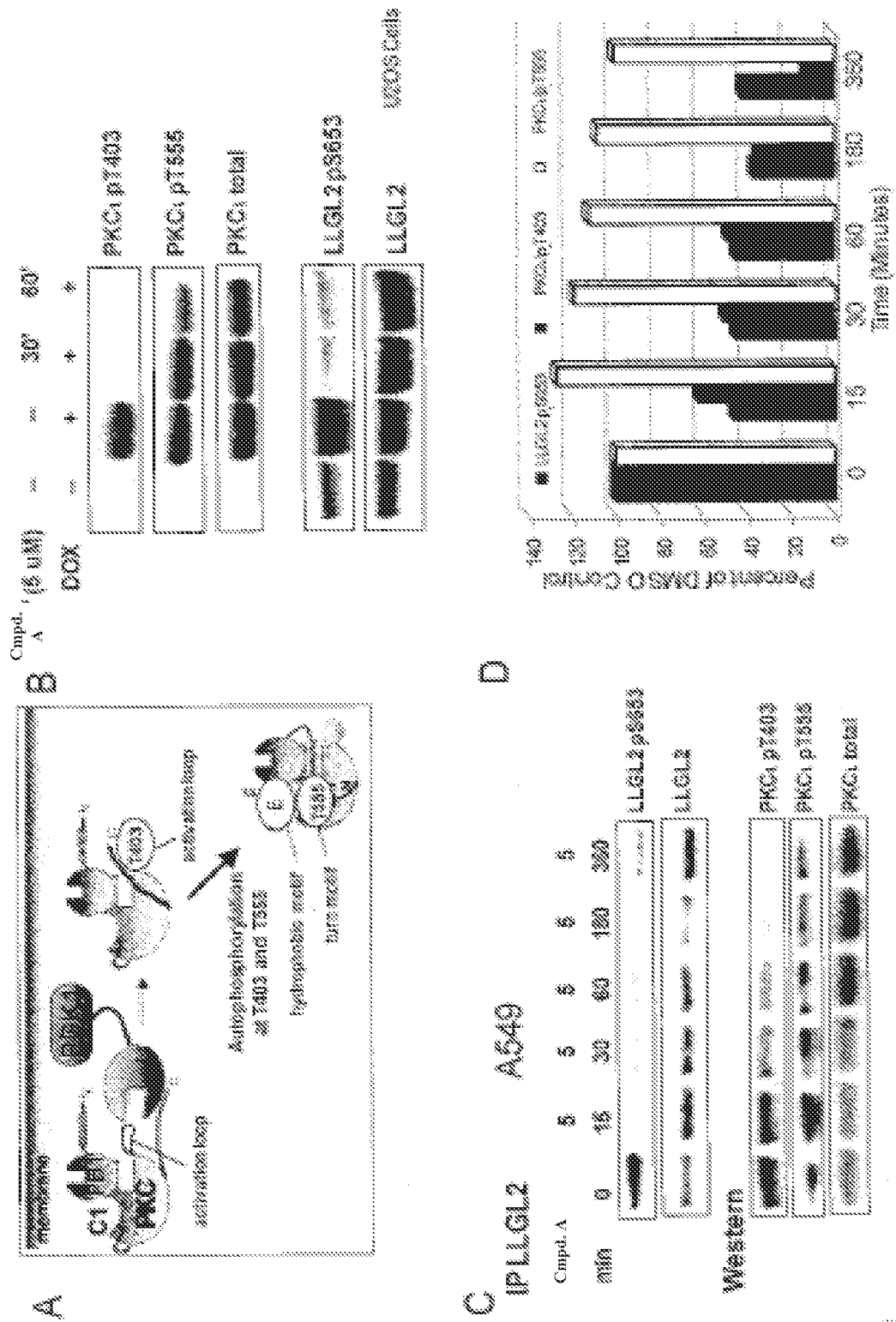
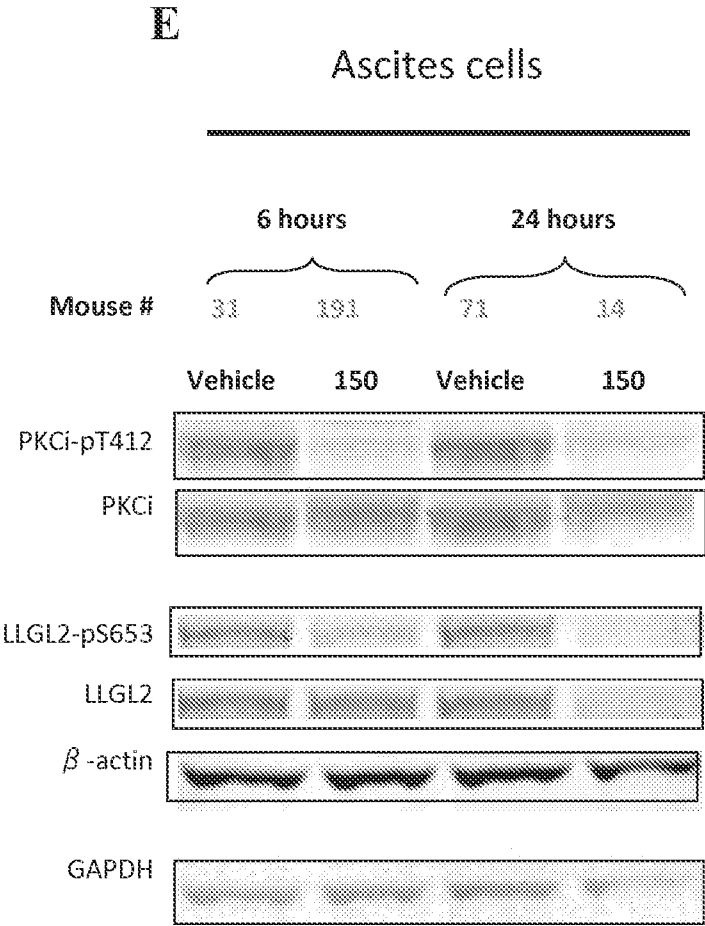


Figure 1 (cont.)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/041026

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - G01N 33/50 (2012.01)

USPC - 435/7.94

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 35/00, 38/00, 48/00; A61P 35/00; G01N33/15, 33/50 (2012.01)

USPC - 435/4, 6.14, 7.1, 7.23, 7.92, 7.94; 514/19.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MicroPatent, Google Patents, Google Scholar, GenCor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0092578 A1 (FIELDS et al) 15 April 2010 (15.04.2010) entire document	1-42
Y	EP 2 077 331 A1 (BAMBA et al) 08 July 2009 (08.07.2009) entire document	1-42
Y	MESSERSCHMIDT et al. 'Crystal Structure of the Catalytic Domain of Human Atypical Protein Kinase C- α Reveals Interaction Mode of Phosphorylation Site in Turn Motif'. J. Mol. Biol. 352:918-931. 30 September 2005. entire document	1-42
Y	US 2001/0001791 A1 (JIROUSEK et al) 24 May 2001 (24.05.2001) entire document	42

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

11 September 2012

Date of mailing of the international search report

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Authorized officer:

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774