

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
26 February 2004 (26.02.2004)

PCT

(10) International Publication Number
WO 2004/016646 A2

- (51) International Patent Classification⁷: **C07K 14/00** (74) Agent: **VOSSIUS & PARTNER**; Siebertstrasse 4, 81675 Munich (DE).
- (21) International Application Number:
PCT/EP2003/008901 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
- (22) International Filing Date: 11 August 2003 (11.08.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
02017791.1 12 August 2002 (12.08.2002) EP (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
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- Published:**
— *without international search report and to be republished upon receipt of that report*
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: PEPTIDE MODULATORS OF TUMOUR SPECIFIC PYRUVATE KINASE SUBTYPE M2 (M2-PK)

(57) Abstract: The present invention relates to (a) peptide(s) capable of specifically interacting with pyruvate kinase subtype M2 (M2-PK), wherein said peptide(s) comprise(s) (an) amino acid sequence(s) as disclosed herein. Furthermore, the invention provides for nucleic acid molecule(s) comprising nucleotide sequence(s) encoding said peptide(s), for vectors comprising said nucleic acid molecule(s). In addition, pharmaceutical and diagnostic compositions are disclosed which comprise the peptide(s) of the invention. The invention also relates to specific uses of the peptide(s) of the invention in preparation of pharmaceutical compositions for the treatment or prevention of, inter alia, proliferative and/or tumorous diseases.



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Peptide modulators of tumour specific pyruvate kinase subtype M2 (M2-PK)

The present invention relates to (a) peptide(s) capable of specifically interacting with pyruvate kinase subtype M2 (M2-PK), wherein said peptide(s) comprise(s) (an) amino acid sequence(s) as disclosed herein. Furthermore, the invention provides for nucleic acid molecule(s) comprising nucleotide sequence(s) encoding said peptide(s), for vectors comprising said nucleic acid molecule(s).

The present invention furthermore relates to compositions comprising the peptide(s) or the nucleic acid molecule(s) of the invention; preferably said, compositions are pharmaceutical or diagnostic compositions.

Additionally, the present invention relates to methods of producing the peptide of the invention, to methods of identifying compounds capable to modulate M2-PK enzyme activity, to methods for identifying (an) inhibitor(s) or activator(s) of the M2-PK enzyme, to methods for identifying (a) compound(s) influencing the activity of the peptide of the invention, as well as, to methods for treating, preventing and/or delaying malignant and/or (benign) cancer diseases and proliferative diseases.

Finally, the present invention discloses uses of the compounds of the invention for the preparation of compositions, in particular, of pharmaceutical and diagnostic compositions for the treatment or the diagnosis of cancer and proliferative diseases

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions etc.) are hereby incorporated by reference.

Living cells possess the potential for proliferation, differentiation and apoptosis and they exhibit a specific metabolic phenotype associated with each of these conditions, characterised by the production of energy and special biosynthetic products. The metabolic phenotype of tumour cells supports the proliferative state associated with the activation of metabolic enzymes that increase glucose carbon utilisation for both

energy production and nucleic acid synthesis (Boros et al., (2002) *Pancreas* 24:26-33). In early studies, Warburg (1930, *The metabolism of tumours*, Constable) demonstrated that the vast majority of tumour cells display a high rate of lactate production from glucose under aerobic conditions. This phenomenon of high glucose catabolism was referred to as aerobic glycolysis and is especially a characteristic of rapidly growing tumours (reviewed in Mathupala et al., (1997) *J Bioenerg Biomembr* 29:339-43). Quantification of regional glucose uptake *in vivo* by positron emission tomography using the glucose analog 2-[(18)F]-fluoro-2-deoxy-D-glucose (DFG), performed in different human cancers, suggest a significant higher glucose uptake in tumours as compared to normal tissue and a positive correlation between glucose uptake and the tumour grade (for recent review, see van der Hiel, (2001) *J Cancer Res Clin Oncol* 127:269-77).

However, cancer cells in solid tumours have special metabolic requirements for their development, which are not comparable with most normal proliferating cells. From a certain tumour size on, an imbalance between supply and consumption of oxygen and nutrients exists because their levels are limited by diffusion, which is frequently caused by abnormalities in the tumour microvasculature (for review, see Carmeliet and Jain, (2000) *Nature* 407:249-57). Those cells grow in gradients of metabolites such as glucose, amino acids and oxygen and it is generally accepted, that the metabolic microenvironment of a tumour can dramatically influence its proliferation and apoptosis rates (for review, see Höckel and Vaupel, (2000) *Int J Oncol* 17:69-79). In accordance, many studies have shown that cancer cells do adapt to these conditions by undergoing a shift from oxidative to highly active glycolytic metabolism, whereas the high energy requirements of the rapidly dividing cells are provided by ATP from glycolysis (Brand and Hermfisse, (1997) *FASEB J* 11:388-95; Nielsen, (2001) *Cancer Res* 61:5318-25). A potential mechanism for establishing this metabolic phenotype of tumours is the hypoxia-induced coordinated upregulation of genes encoding for proteins involved in glucose metabolism, for example Pyruvate kinase type M (reviewed in Maxwell, (2001) *Curr Opin Genet Dev* 11:293-9). This is mediated by HIF-1 α (hypoxia induced factor) (Semenza, (1994) *J Biol Chem* 269:23757-63) and the oncogenic transcription factor c-Myc (Osthus, (2000) *J Biol Chem* 275:21797-800). Since the increase of the glycolytic capacity allows tumour

cells to survive under hypoxic conditions that occur in solid tumours, these cells, however, undergo apoptosis under glucose deprivation (Carmeliet, (1998) *Nature* 394:485-90), because they are dependent on glucose to maintain their ATP level (Eskey, (1993) *Proc Natl Acad Sci U S A* 90:2646-50). Hence, additional parameter must play a role in the adaptation of the cancer cell metabolism to the microenvironment. The metabolic differences also require changes in the metabolic isoenzyme patterns and activities to coordinate energy metabolism and biosynthetic processes in tumour cells (reviewed in Bannasch, (1996) *Prog Liver Dis* 14:161-97).

In human tumours, alternative pathways of energy metabolism exist beside glycolysis, such as the degradation of L-serine- and L-glutamine to lactate, allowing tumour cells to generate energy under hypoglycaemia (reviewed in Newsholme and Board, (1991) *Adv Enzyme Regul* 31:225-46; Boros, (2002) *Pancreas* 24:26-33). To coordinate the relevant pathways, glycolysis, serinolysis and glutaminolysis, tumour cells express the glycolytic key regulatory enzyme pyruvate kinase subtype M2 (M2-PK) (reviewed in Eigenbrodt, (1992) *Crit Rev Oncog* 3:91-115). Three additional tissue-specific expressed pyruvate kinase isoenzymes have been described, the M1-, L-, and R-types (for review, see Yamada and Noguchi, (1999) *Biochem Biophys Res Commun* 256:257-62). Both the M1- and M2-isoenzymes are expressed from the pyruvate kinase M (PKM) gene by alternative splicing (Tani, (1988) *Gene* 73:509-16). M2-PK is the embryogenic form of pyruvate kinase, which replaces the other isoforms in proliferating and cancer cells. M2-PK catalyses the ATP-generating step at the end of the glycolytic pathway, the conversion of phosphoenolpyruvate (PEP) to pyruvate. M2-PK exhibits allosteric properties and occurs in a tetrameric conformation with high affinity to its substrate phosphoenolpyruvate (PEP) as well as in a dimeric conformation with low affinity to PEP (reviewed in Mazurek, (1997) *J Bioenerg Biomembr* 29:315-30). The transition between both conformations regulates the channelling of glucose carbons either to synthetic processes (dimeric form) or to glycolytic energy production (tetrameric form) in the vast majority of all tumour cells. Although the specific activity of M2-PK is lower with respect to the non-allosteric M1-PK isoenzyme in the corresponding host tissue, it appears that the presence of M2-PK is an advantage for the metabolism of a tumour cell. Thus, alterations in the regulation of pyruvate kinase

enzyme activity seem to be more important than the total enzyme activity present. This makes sense, because the shift of M2PK in the low affinity dimeric form leads to the damming up of glycolytic phosphometabolites, which is a prerequisite for the supply with precursor for nucleic acid synthesis. Although tumour cells need a high glycolytic flux for their high ATP demand, a partly inhibited M2-PK fits well with this requirement. It only means that this isoenzyme needs higher levels of substrate to support the same flux (for review, see Eigenbrodt, (1992) Crit Rev Oncog 3:91-115). Consequently, the presence of M2-PK assures at the same time higher steady-state levels of glycolytic phosphometabolites and a high glycolytic flux.

Further evidence for a critical role of this central metabolic regulator in tumorigenesis comes from the identification of M2-PK as a direct target of oncoproteins. Presek and coworkers (1988 FEBS Lett 242:194-8) showed that transformation of Chicken embryo cells by Rous sarcoma virus (RSV) leads to tyrosine phosphorylation of M2-PK, accompanied with a decrease in the M2-PK affinity for PEP. *In vitro*, M2-PK is phosphorylated at tyrosine residues by pp60 v-src, the transforming protein of RSV. In addition, experimental evidence was presented suggesting that the human papillomavirus type 16 E7 oncoprotein directly interacts with M2-PK (Zwerschke, (1999) Proc Natl Acad Sci U S A 96:1291-6). The binding of E7 to M2-PK shifts the equilibrium of M2-PK complexes to the tumor-specific dimeric state, leading to an expansion of the glycolytic phosphometabolites required for biosynthetic processes. Moreover, in a recent study it was shown that M2-PK is an indirect target of the ras oncoprotein (Mazurek, (2001) Oncogene 20:6891-8). Interestingly, expression of oncogenic ras in primary cells leads to tetramerization and a strong upregulation of M2-PK activity. Thus, ras expression strongly induces glucose oxidation leading to the inhibition of nucleic acid synthesis and cell proliferation. Suggesting that tetrameric highly active M2-PK blocks tumour growth.

In summary, the adaptation of cancer cell metabolism to the proliferative state and especially in solid tumours to hypoxia and hypoglycaemia is necessary to maintain the balance between proliferation and apoptosis in tumour cells. This is mediated by the transcriptional upregulation of metabolic enzyme genes, changes in isoenzyme patterns and metabolic enzyme activities and results in perfect coordination of

energy metabolism and biosynthetic processes, especially nucleic acid synthesis. Intermediary metabolic enzymes mediate the growth signalling pathways and promote malignant cell transformation. M2-PK is such an enzyme, which is specifically expressed in cancer cells and plays an central role for the coordination of the tumour metabolism in common cancer.

Therefore, the technical problem of the present invention is to provide for means and methods useful for the treatment and/or the prevention of cancerous and/or proliferative diseases. The solution to said technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a peptide capable of specifically interacting with the (tumor specific) pyruvate kinase subtype M2 (M2-PK), wherein said peptide comprises an amino acid sequence as depicted in table 1 or wherein said peptide is encoded by a nucleic acid molecule as depicted in table 1.

It was surprisingly found that human M2-PK, in the following said M2-PK, binds to short peptide (s) disclosed herein and in particular shown in appended table 1. Said peptides have been found by screening a peptide library (Colas, Nature 380, (1996), 548-550) comprising peptide sequences generated randomly, with a "two hybrid" system in which M2-PK was used as a screening sample. Said peptides comprise preferably 15-25 amino acids and bind to and/or interact with M2-PK protein. It was also found that these peptides are suitable to, e.g., inhibit (pathological) interactions of M2-PK with cellular regulator proteins, e.g. protein kinases, phosphatases, parathymosin alpha and parathymosin alpha like proteins. The applicant has also found that these peptides are suitable to inhibit (pathological) functions of M2-PK, e.g. the shift from the highly active tetrameric into the inactive dimeric form of the enzyme and the resulting damming up of glycolytic phosphometabolites. In addition, a subset of the anti-M2-PK peptides disclosed herein inhibit the shift from the dimeric into the tetrameric conformation. Consequently, the anti-M2-PK peptide-dependent modification of M2-PK leads to deregulation of the M2-PK-function. A constitutive active M2-PK (tetrameric form) would lead to exhaustion of glycolytic phosphometabolites and therefore block DNA synthesis. Concerning the particular

role of M2-PK for tumor cell survival under nutrient deprivation M2-PK is a particularly suitable target for the treatment of solid tumors. Under nutrient deprivation allosteric regulation of M2-PK becomes a bottom line for tumor cell survival. A constitutive active M2-PK (tetrameric form) would lead to exhaustion of glycolytic phosphometabolites and therefore block DNA synthesis. A constitutive inactive M2-PK (dimeric form) would lead to ATP depletion. Both scenarios cause inhibition of cell proliferation and induction of apoptotic cell death (Moley and Mueckler, Apoptosis (2000) 5:99-105). In accordance it was surprisingly found that the peptides disclosed and described herein, the activating as well as the inactivating peptides, are suitable to specifically induce apoptotic cell death in tumor cells. However, most preferably the peptides as disclosed herein specifically interact and functionally inactivate the tumor specific pyruvate kinase. As documented in the appended examples, with the teachings disclosed herein, the person skilled in the art is readily in a position to assess whether an activation or an inhibition of M2-PK is desired. Furthermore, with the examples disclosed herein, the person skilled in the art can deduce whether a peptide of the present invention has an activating or an inhibiting effect in vitro and/or in vivo.

The peptide library comprises randomly generated peptide sequences expressed as "contops" on the surface of bacterial thioredoxin A or human thioredoxin or other human proteins, serving as scaffold proteins. The term "contop" in context of this invention relates to a "constrained peptide epitope". The term contop describes a recombinant protein that consists of a carrier protein (examples: human ubiquitin, human thioredoxin A) and an attached peptide epitope. The carrier protein may be engineered to carry a synthetic acceptor domain which is in a thermodynamically stable conformation and is exposed to solvent. In the "contop", a peptide sequence in the length of 15-25 amino acids may be integrated into the acceptor site, in a constrained conformation. Conformational constraint may be achieved in two ways:

- (i) the overall structure of the carrier protein provides a rigid structure which substantially reduces the number of available conformations of the peptide epitope, the result being an increased affinity for target proteins; or
- (ii) in some cases, for the N-terminal and C-terminal amino acids of the peptide epitope the amino acid cysteine is chosen and the protein is produced under

conditions (oxidizing conditions) where disulfide bonds between the N-terminal and C-terminal cysteine residue are formed.

Disulfide bonding provides a rigid structure which substantially reduces the number of available conformations of the peptide epitope, the result being an increased affinity for target proteins. In order to further increase the affinity(ies) of the screened peptide(s) to M2-PK, a novel method for random mutagenesis of peptide originating from the primary screen was employed (see appended examples). Said novel screening method led to 10^5 new variants of selected contops obtained out of 10^7 individual clones. This two-step screening procedure allows a considerable increase of the total amount of peptides to be screened, and drastically increases, thereby, the binding affinity of the screened peptides. Thus, screening of a total of 10^{12} different peptides could be achieved. In this context, it was surprisingly found that short peptides, particularly the sequences shown in Table 1, bind to the M2-PK. Furthermore, it was found that this peptides inhibit the (pathological) activity of M2-PK.

The term "peptide capable of specifically interacting with M2-PK" as used in accordance with this invention comprises the peptide(s) with the specific amino acid sequence as depicted in the corresponding sequences in Table 1 and as disclosed herein. Peptid-M2-PK interaction was measured in the assays specified in Example 1 and 2. However, the present invention also relates to peptides which are homologous to said depicted amino acid sequences and are capable of specifically interacting with M2-PK. Specific "tests" for inteaction with M2-PK are disclosed herein an exemplified in the appended examples. Said homologous peptides may comprise homologous peptides which are not of natural origin or may be peptides derived from different organisms, like prokaryotic or eukaryotic organisms. Homologes envisaged are also peptides derived from mammalian species. Most preferred are peptides derived from human. However, especially preferred homologous are, in this context, also peptides from mouse, rat cow, sheep, etc. Peptides of the present invention are and may comprise synthetic peptides. As will be discussed herein below peptides of the invention may also be recombinantly produced. Therefore, the present invention also relates to peptides as defined herein, which are recombinantly produced.

In context of the present invention, homology means a sequence identity of at least 60%, in particular an identity of at least 70%, and most preferably an identity to the inventive peptides as disclosed herein of more than 95%.

Additionally, within the scope of the invention are peptides wherein the above mentioned amino acid(s) and/or peptide bonds have been replaced by functional analogous, inter alia by peptidomimetics. Therefore, the present invention also encompasses functional derivatives and/or analogues of the peptides of the invention capable of specifically interacting with M2-PK. Particularly preferred are peptide derivatives and/or analogues that specifically bind to M2-PK, even more preferred are peptide derivatives and analogues capable of interfering, e.g. inhibiting activity of M2-PK. Methods for the preparation of said peptides, (chemical) derivatives and/or analogues are well known to those skilled in the art and comprise, inter alia, recombinant preparation, protein/peptide isolation and purification or proteolytic methods as described, inter alia, in the appended examples. Further methods are known to those of ordinary skill in the art (see, e.g. Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989; Merrifield, J. Am. Chem. Soc. 85 (1963), 2149-2146; Steward, "Solid Phase Peptide Synthesis", WH Freeman Co, San Francisco (1969); Scopes, "Protein Purification, Principles, High Resolution Methods and Applications", VCH Publishers, New York, Weinheim, Cambridge (1989); Wrede, "Concepts in Protein Engineering and Design", Walter de Gruyter, Berlin, New York (1994).)

The term "specifically interacting" with M2-PK means in accordance with this invention that said peptide(s) is (are) capable of, inter alia, binding to M2-PK. Said binding may be direct binding. However, within the scope of this invention, said term defines also (a) peptide(s) which is (are) capable of interacting with M2-PK via "linker" structures, e.g. linker proteins/(poly)peptides. Said term also comprises that said peptide(s) might interact with other/additional proteins. Said interaction of the peptides of the invention with M2-PK and/or further proteins may comprise binding by hydrophobic interaction(s), electrostatic interaction(s) and/or by van-der-Waals forces.

It is particularly preferred that the peptides of the invention are capable of specifically binding to M2-PK, more preferably to a carboxy- (amino acids 500-531) and amino-terminal (amino acids 1-50) fragments of human M2-PK. Even more preferred are peptides which are capable of specifically interacting with the M2-PK specific region of M2-PK comprising amino acids 389-433 of human M2-PK (Tani et al, 1988).

It is even more preferred that the term "specifically interacting M2-PK" comprises the capability of said peptide to interfere with modulation of activity and/or conformationally changes of M2-PK. Most preferred are peptides which are capable of specifically modulating enzyme activity, e.g. inhibition or strong activation of M2-PK by any metabolite or amino acid or cellular or viral protein.

It is preferred that the term "specifically interacting M2-PK" comprises a dissociation constant of the peptide of the invention and M2-PK of less than 10^{-3} M. More preferably of less than 10^{-6} M, more preferably of less than 10^{-7} M and most preferably of less than 10^{-8} M.

In a preferred embodiment the peptide of the invention is a peptide, wherein the amino acid sequence as shown in Table 1 comprises at least one modification.

Beside the above mentioned modifications may be selected from the group consisting of amino acid exchange(s), insertion(s), deletion(s), addition(s), substitution(s), inversion(s) and duplication(s). Said modifications also comprise conservative and/or homologue amino acid exchange(s), for example guidance concerning how to make phenotypically/functionally silent amino acid substitution is given (Bowie (1990), Science 247, 1306-1310). Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln; replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acid substitution. Variants of peptides of this invention include (i) substitutions with

one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example polyethylene glycol) or (iv) fusion of the polypeptide with additional amino acids, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

Thus, the present invention also relates to peptides which are at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably 90%, more preferably at least 95% and most preferably 99% identical homologous to the peptide(s) as shown in Table 1 and which are capable of interacting with M2-PK. However, since the present invention also relates to functional fragments of the peptides of the present invention, i.e. to fragments of said peptides which are capable of specifically interacting with M2-PK, said fragments may comprise peptide fragments which are at least 80% identical to a peptide fragment of the invention which comprises at least 6, preferably at least 9 amino acids. Preferably the peptides as disclosed herein comprise 20 amino acids in length. However, as described herein below, the peptide(s) of the invention may also be comprised in fusion proteins/fusion peptides or in "contops" defined herein. Accordingly, the peptides of the invention may be part of a longer amino acid molecule or may be comprised in (proteinaceous) scaffolds. Specific strategies for obtaining peptides described herein above are known in the art. These methods comprise recombinant and biochemical methods and are, inter alia, disclosed in Sambrook, loc. cit.. Said methods also comprise protein engineering or direct synthesis.

The above mentioned modification may also be due to variations. The term "variant" in this context also comprises allelic variations. Therefore, naturally occurring peptides capable of specifically interacting with M2-PK are called "allelic variants" of the peptides of the invention. These allelic variants can vary either the (poly)nucleotide- and/or peptide level. Naturally as well as non-naturally occurring modifications and/or variants may be produced by mutagenesis techniques.

The present invention also relates to a peptide of the invention which is a fusion (poly)peptide. Said fusion protein may comprise the peptide(s) of the invention as well as (a) functional fragment(s) thereof. Therefore, in addition to the peptides and/or fragment(s) of the present invention, said fusion protein can comprise at least one further domain, said domain being linked by covalent or non-covalent bonds. The linkage can be based on genetic fusion according to the methods known in the art (Sambrook et al., loc. Cit., Ausubel, "Current Protocols in Molecular Biology", Green Publishing Associates and Wiley Interscience, N.Y. (1989)) or can be performed e.g. by chemical cross-linking as described in e.g. WO 94/04686. The additional domain present in the fusion protein comprising the (poly)peptide of the invention may preferably be linked by a flexible linker, advantageously a (poly)peptide linker, wherein said (poly)peptide linker preferably comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of said further domain and the N-terminal end of the peptide, (poly)peptide or antibody vice versa. The above described fusion protein may further comprise a cleavable linker or cleavage site, which, for example, is specifically recognised and cleaved by proteinases or chemical agents.

Additionally, said at least one further domain may be of a predefined specificity or function, such as M2-PK inhibitor function. This can be achieved by chemically linking an inhibitor molecule to said peptide. In this context, it is understood that the peptide(s) and/or fusion proteins of the invention may be further modified by conventional methods known in the art. This allows for the construction of fusion proteins comprising the peptide and/or fragments of the invention and other functional amino acid sequences, e.g., transactivating domains, DNA-binding domains, hormone-binding domains, protein tags (e.g., GST (glutathione-S-transferase), GFP, h-myc peptide, FLAG, HA peptide, Strep), transmembrane domains or fatty acid attachment motifs (CAAX-box) which may be derived from heterologous proteins. The above mentioned modification(s) of the peptide(s), nucleic acid molecules and/or compounds of the invention described herein below may, inter alia, be employed to allow the peptide(s), nucleic acid molecules or compounds of the invention to pass the blood-brain barrier and/or be targeted to specific cellular and/or tissue sites.

It is furthermore envisaged that the peptide of the invention is a chimeric (poly)peptide. Therefore, the fusion protein/(poly)peptide or mosaic (poly)peptide as described herein above may comprise at least two peptide(s) of the invention or at least two fragments and/or epitopes of the peptide(s) of the invention. Said two peptides of the invention or at least two fragments and/or epitopes of the peptide(s) of the invention. Said two peptides of the invention or at least two fragments and/or epitopes of said peptides also comprises the same peptide of the invention or the same fragments or epitopes. Said chimeric (poly)peptide as well as mosaic (poly)peptides may, inter alia, comprise at least two peptides of the invention (or fragment(s)) or epitope(s) thereof which lack amino acids, e.g., amino acids normally intervening between epitopes (or fragments(s)) of the peptides of the invention.

The peptide(s) of the invention as well as the fusion (poly)peptides, chimeric polypeptides and/or mosaic (poly)peptides may be detectably labeled. A variety of techniques are available for labelling biomolecules, are well known to the person skilled in the art and are considered to be within the scope of the present invention. Such techniques are, e.g., described in Tijssen, "Practice and theory of enzyme immuno assays", Burden, RH and von Knippenburg (Eds), Volume 15 (1985), "Basic methods in molecular biology"; Davis LG, Diber MD; Battey Elsevier (1990), Mayer et al., (Eds) "Immunochemical methods in cell and molecular biology" Academic Press, London (1987), or in the series "Methods in Enzymology", Academic Press, Inc.

There are many different labels and methods of labelling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes (like ^{32}P or ^{125}I), colloidal metals, fluorescent compounds/fluorochromes (like fluorescein, rhodamine, Texas Red, etc.), chemiluminescent compounds, and chemi- or bioluminescent compounds (like dioxetanes, luminol or acridiniums).

Furthermore, commonly used labels comprise, inter alia, enzymes (like horse radish peroxidase, β -galactosidase, alkaline phosphatase), biotin or digoxigenin. Labelling

procedures, like covalent coupling of enzyme or biotinyl groups, iodinations, phosphorylations, biotinylations, random priming, nick-translations, tailing (using terminal transferase) are well known in the art.

Detection methods comprise, but are not limited to, autoradiography, fluorescence microscopy, direct and indirect enzymatic reactions, etc.

In a preferred embodiment of the invention the peptide as defined herein and capable of interacting with M2-PK further comprises a signal sequence. Said signal sequence may comprise sequences which are capable of specifically targeting the peptides (as well as fusion (poly)peptides, chimeric (poly)peptides or mosaic (poly)peptides of the invention to specific cellular compartments. Said signal sequences may, inter alia, be useful to target/transport said peptides/(poly)peptides across biological membranes. Signal sequences are well known in the art and comprise ER-sequences, like KDEL-sequences, the leader peptide MQRARPTLWAAALTLLVLLRGPPVARA as well as the so called protein transduction domains (PTDs) like the PTD sequences of the HIV-TAT protein, of the *Drosophila* antennapedia protein, of the Herpes simplex virus translocating protein VP22 and/or an (artificial) poly-arginine sequence (reviewed in Wadia and Dowdy, (2002) *Curr Opin Biotechnol* 13(1):52-6).

For delivery of peptides of the invention or of contops into living cells (either in tissue culture or in cells of living organisms), a protein transduction domain is covalently attached to the peptide or contop. This can be achieved by in frame fusion or by chemical linkage using activated sulfhydryl groups. The PTD is derived from the TAT protein of HIV or other sources described herein above. The biological property of the PTD leads to the adsorption of the contop-PTD fusion protein to the cellular membrane and the subsequent delivery of a substantial part of the fusion protein into the cell where it exerts its influence on M2-PK activity.

In addition, the present invention provides for a peptide of the invention, wherein said peptide comprises at least one non-peptide moiety. Said at least one non-peptide moiety may comprise secondary modifications, like, inter alia, glycosylations or phosphorylations. Furthermore, as mentioned herein above, said moiety comprise

detectable labels, like, e.g., fluorescent substances (FITC, rhodamine, Texas Red, PE, etc.)

Furthermore, said moiety may comprise colloidal metals (like gold particels), biotin, digoxigenin and/or chemi- or bioluminescent compounds.

In yet another embodiment, the present invention relates to a nucleic acid molecule comprising a nucleotide sequence encoding a peptide of the invention which is

- (a) a nucleotide sequence encoding the peptide as set forth in Table 1,
- (b) a nucleotide sequence having the DNA sequence as depicted in Table 1,
- (c) a nucleotide sequence hybridising to a sequence which is complementary to a nucleotide sequence of (a) or (b), or
- (d) a nucleotide sequence being degenerate to the sequence of the nucleotide sequence of (c).

The above mentioned nucleic acid molecule may be a naturally nucleic acid molecule as well as a recombinant nucleic acid molecule. The nucleic acid molecule of the invention may, therefore, be of natural origin, synthetic or semi-synthetic.

With respect to the DNA sequences characterised under (c) above, the term "hybridising" in this context is understood as referring to conventional hybridisation conditions, preferably such as hybridisation in 50%formamide/6xSSC/0.1%SDS and 100µg/ml ssDNA, in which temperatures for hybridisation are above 37°C and temperatures for washing in 0.1 xSSC/0.1 %SDS are above 55°C. Most preferably, the term "hybridising" refers preferably to stringent hybridisation conditions, for example such as described in Sambrook, supra.

It is also immediately evident to the person skilled in the art that regulatory sequences may be added to the nucleic acid molecule of the invention. For example, promoters, transcriptional enhancers and/or sequences which allow for induced expression of the polynucleotide of the invention may be employed. A suitable inducible system is for example tetracycline-regulated gene expression as described, e.g. by Gossen and Bujard (Proc. Natl. Acad. Sci. USA 89 (1992), 5547-

5551) and Gossen et al. (Trends Biotech. 12 (1994), 58-62), or a dexamethasone-inducible gene expression system as described, e.g. by Crook (1989) EMBO J. 8, 513-519.

In a further embodiment, the invention relates to nucleic acid molecules of at least 15 nucleotides in length hybridising specifically with a polynucleotide as described above or with a complementary strand thereof. Specific hybridisation occurs preferably under stringent conditions and implies no or very little cross-hybridisation with nucleotide sequences encoding no or substantially different proteins. Such nucleic acid molecules may be used as probes and/or for the control of gene expression. Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary in length. Preferred are nucleic acid probes of 15 to 60 nucleotides in length. Of course, it may also be appropriate to use nucleic acids of up to 100 and more nucleotides in length. The nucleic acid probes of the invention are useful for various applications. On the one hand, they may be used as PCR primers for amplification of polynucleotides according to the invention. Another application is the use as a hybridisation probe to identify polynucleotides hybridising to the nucleic acid molecules of the invention by homology screening of genomic DNA libraries.

The above described nucleic acid molecules may either be DNA or RNA or a hybrid thereof.

Furthermore, said nucleic acid molecule may contain, for example, thioester bonds and/or nucleotide analogues, commonly used in oligonucleotide anti-sense approaches. Said modifications may be useful for the stabilisation of the nucleic acid molecule against endo- and/or exonucleases in the cell. Said nucleic acid molecules may be transcribed by an appropriate vector containing a chimeric gene which allows for the transcription of said nucleic acid molecule in the cell. Such nucleic acid molecules may further contain ribozyme sequences.

In this respect, it is also to be understood that the polynucleotide of the invention can be used for "gene targeting" and/or "gene replacement", for restoring a mutant gene or for creating a mutant gene via homologous recombination; see for example Mouellic, Proc. Nati. Acad. Sci. USA, 87 (1990), 4712-4716; Joyner, Gene Targeting, A Practical Approach, Oxford University Press.

In a preferred embodiment said nucleic acid molecules are labelled. Methods for the detection of nucleic acids are well known in the art, e.g. Southern and Northern blotting, PCR or primer extension.

The present invention also relates to nucleic acid molecules encoding fusion (poly)peptides, mosaic (poly)peptides or chimeric polypeptides as described herein above.

The nucleic acid molecule(s) of the invention may be a recombinantly produced chimeric nucleic acid molecule comprising any of the aforementioned nucleic acid molecules either alone or in combination. Preferably, the nucleic acid molecule of the invention is part of a vector.

Therefore, the present invention also relates to a vector comprising the nucleic acid molecule of the present invention.

The vector of the present invention may be e.g. a plasmid, cosmid, virus, bacteriophage or another vector used e.g. conventionally in genetic engineering, and may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions.

Furthermore, the vector of the present invention may, in addition to the nucleic acid sequences of the invention, comprise expression control elements, allowing proper expression of the coding regions in suitable hosts. Such control elements are known to the artist and may include a promoter, a splice cassette, translation initiation codon, translation and insertion site for introducing an insert into the vector.

Preferably, the nucleic acid molecule of the invention is operatively linked to said expression control sequences allowing expression in eukaryotic or prokaryotic cells.

Control elements ensuring expression in eukaryotic and prokaryotic cells are well known to those skilled in the art. As mentioned herein above, they usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilisation of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally-associated or heterologous promoter regions. Possible regulatory elements permitting expression in for example mammalian host cells comprise the CMV- HSV thymidine kinase promoter, RSV-promoter (Rous Sarcoma Virus), human elongation factor 1 α -promoter, the glucocorticoid-inducible MMTV-promoter (Moloney Mouse Tumour Virus), metallothionein- or tetracyclin-inducible promoters, or enhancers, like CMV enhancer or SV40-enhancer. For expression in neural cells, it is envisaged that a neurofilament promoter is employed. Said promoters are known in the art and, inter alia, described in Charron (1995), J. Biol. Chem. 270, 25739-25745. For the expression in prokaryotic cells, a multitude of promoters including for example the tac/lac-promoter or the trp promoter has been described. Besides elements which are responsible for the initiation of transcription, such regulatory elements may also comprise transcription termination signals, such as the tk-poly-A site, downstream of the polynucleotide. In this context, suitable expression vectors are known in the art, such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), pSPORT1 (GIBCO BRL), pX (Pagano (1992) Science 255, 1144-1147), yeast two-hybrid vectors, such as pEG202 and pJG4-5 (Gyuris (1993) Cell 75, 791-803), or prokaryotic expression vectors, such as lambda gt11 or pGEX (AmershamPharmacia). Beside the nucleic acid molecules of the present invention, the vector may further comprise nucleic acid sequences encoding for secretion signals. Such sequences are well known to the person skilled in the art. Furthermore, depending on the expression system used leader sequences capable of directing the peptides of the invention to a cellular compartment may be added to the coding sequence of the nucleic acid molecules of the invention and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination

sequences, and, preferably, a leader sequence capable of directing secretion of translated protein, or a protein thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including a C- or N-terminal identification peptide imparting desired characteristics, e.g. stabilisation or simplified purification of expressed recombinant product. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the peptide(s) or fragments thereof of the invention may follow.

Furthermore, the vector of the present invention may also be an expression, a gene transfer or gene targeting vector. Gene therapy, which is based on introducing therapeutic genes into cells by ex-vivo or in-vivo techniques, is one of the most important applications of gene transfer. Suitable vectors, methods or gene-delivering systems for in-vitro or in-vivo gene therapy are described in the literature and are known to the person skilled in the art; see e.g. Giordano, *Nature Medicine* 2 (1996), 534-539; Schaper, *Circ. Res.* 79 (1996), 911-919; Anderson, *Science* 256 (1992), 808-813; Isner, *Lancet* 348 (1996), 370-374; Muhlhauser, *Circ. Res.* 77 (1995), 1077-1086; Onodua, *Blood* 91 (1998), 30-36; Verzeletti, *Hum. Gene Ther.* 9 (1998), 2243-2251; Verma, *Nature* 389 (1997), 239-242; Anderson, *Nature* 392 (Supp. 1998), 2530; Wang, *Gene Therapy* 4 (1997), 393-400; Wang, *Nature Medicine* 2 (1996), 714-716; WO 94/29469; WO 97/00957; US 5,580,859; US 5,589,466; US 4,394,448 or Schaper, *Current Opinion in Biotechnology* 7 (1996), 635-640, and references cited therein. In particular, said vectors and/or gene delivery systems are also described in gene therapy approaches in Hengge (2001), *J. Invest. Dermat.* 116, 979-982; Drug Resist. Updat. 5, (2002), 34-46; Nemunaitis (2002), *Endo. Relat. Cancer* 9, 115-139; Mabjeesh (2001), *Exp. Rev. Anticancer Ther.* 1, 546-554; Tian (2001), *Curr. Gene Ther.* 1, 123-136 or Norris (2001), *Curr. Gene Ther.* 1, 201-226. The nucleic acid molecules and vectors of the invention may be designed for direct introduction or for introduction via liposomes, viral vectors (e.g. adenoviral, retroviral), electroporation, ballistic (e.g. gene gun) or other delivery systems into the cell. Additionally, a baculoviral system can be used as eukaryotic expression system for the nucleic acid molecules of the invention.

As will be discussed herein below, the nucleic acid molecule of the present invention and/or the above described vectors/hosts of the present invention may be particularly useful as pharmaceutical compositions. Said pharmaceutical compositions may be employed in gene therapy approaches.

For gene therapy applications, nucleic acids encoding the peptide(s) of the invention or fragments thereof may be cloned into a gene delivering system, such as a virus and the virus used for infection and conferring disease ameliorating or curing effects in the infected cells or organism.

The present invention also relates to a host cell transfected or transformed with the vector of the invention or a non-human host carrying the vector of the present invention, i.e. to a host cell or host which is genetically modified with a nucleic acid molecule according to the invention or with a vector comprising such a nucleic acid molecule. The term "genetically modified" means that the host cell or host comprises in addition to its natural genome a nucleic acid molecule or vector according to the invention which was introduced into the cell or host or into one of its predecessors/parents. The nucleic acid molecule or vector may be present in the genetically modified host cell or host either as an independent molecule outside the genome, preferably as a molecule which is capable of replication, or it may be stably integrated into the genome of the host cell or host.

The host cell of the present invention may be any prokaryotic or eukaryotic cell. Suitable prokaryotic cells are those generally used for cloning like *E. coli* or *Bacillus subtilis*. Furthermore, eukaryotic cells comprise, for example, fungal or animal cells. Examples for suitable fungal cells are yeast cells, preferably those of the genus *Saccharomyces* and most preferably those of the species *Saccharomyces cerevisiae*. Suitable animal cells are, for instance, insect cells, vertebrate cells, preferably mammalian cells, such as e.g. CHO, MDCK, U2-0-S, MCF-7, SiHa, NIH3T3, MOLT-4, Jurkat, PC-12, PC-3, IMR, NT2N, Sk-n-sh, CaSki, C33A. Further suitable cell lines known in the art are obtainable from cell line depositories, like the American Type Culture Collection (ATCC). In accordance with the present invention, it is furthermore envisaged that primary cells/cell cultures may function as host cells.

Said cells are in particular derived from insects (like insects of the species *Drosophila* or *Blatta*) or mammals (like human, swine, mouse or rat). Said host cells may also comprise cells from and/or derived from cell lines like neuroblastoma cell lines. The above mentioned primary cells are well known in the art and comprise, inter alia, primary astrocytes, (mixed) spinal cultures or hippocampal cultures.

In a more preferred embodiment the host cell which is transformed with the vector of the invention is a cancer cell or a cell (line) derived therefrom.

Hosts may be non-human mammals, most preferably mice, rats, sheep, calves, dogs, monkeys or apes. Said mammals may be indispensable for developing a cure, preferably a cure cancer diseases or proliferative disorders. Furthermore, the hosts of the present invention may be partially useful in producing the (poly)peptides (or fragments thereof) of the invention. It is envisaged that said (poly)peptide (or fragments thereof) be isolated from said host.

Furthermore, the present invention relates to a method of producing a peptide (or (poly)peptide) encoded by the nucleic acid molecule of the invention comprising culturing the host cell of the present invention under suitable conditions that allow the synthesis of said peptide/(poly)peptide and recovering and/or isolating the peptide/(poly)peptide produced from the culture.

In yet another embodiment, the present invention relates to an antibody or a fragment or a derivative thereof, an antiserum or a nucleic acid aptamer specifically recognising the peptide of the invention or the nucleic acid molecule of the invention.

The general methodology for producing antibodies is well-known and has, for monoclonal antibodies, been described in, for example, Köhler and Milstein, *Nature* 256 (1975), 494 and reviewed in J.G.R. Hurrel, ed., "Monoclonal Hybridoma Antibodies: Techniques and Applications", CRC Press Inc., Boca Raton, FL (1982). In accordance with the present invention the term "antibody" relates to monoclonal or polyclonal antibodies. Polyclonal antibodies (antiserum) can be obtained according to conventional protocols. Antibody fragments or derivatives comprise

F(ab')₂, Fab, Fv or scFv fragments; see, for example, Harlow and Lane, "Antibodies, A Laboratory Manual", CSH Press 1988, Cold Spring Harbor, NY. Preferably the antibody of the invention is a monoclonal antibody. Furthermore, in accordance with the present invention, the derivatives of the invention can be produced by peptidomimetics. In the context of the present invention, the term "nucleic acid aptamer" comprises nucleic acids such as RNA, ssDNA (ss = single stranded), modified RNA, modified ssDNA or DNAs which bind a plurality of target sequences having a high specificity and affinity. Nucleic acid aptamers are well known in the art and, inter alia, described in Famulok, Curr. Op. Chem. Biol. 2 (1998), 320-327. The preparation of aptamers is well known in the art and may involve, inter alia, the use of combinatorial RNA libraries to identify binding sites (Gold, Ann. Rev. Biochem. 64 (1995), 763-797). Said other receptors may, for example, be derived from said antibody etc. by peptidomimetics. The specificity of the recognition implies that other known proteins, molecules are not bound. A suitable host for assessing the specificity would imply contacting the above recited compound comprising an epitope of the nucleic acid molecule or the peptide of the invention as well as corresponding compounds e.g. from protein or nucleic acid molecules known in the art, for example in an ELISA format and identifying those antibodies etc. that only bind to the compound of the invention but do not or to no significant extent cross-react with said corresponding compounds.

The present invention additionally relates to a method for identifying a compound capable of interfering with M2-PK-activity comprising the steps of

- (a) testing a collection of compounds for interaction with the peptide of the invention or (a) fragment(s) thereof employing a readout system, and
- (b) identifying compounds that test positive for interaction in step (a).

Said "test for interaction" in step (a) may be carried out by specific immunological and/or biochemical assays which are well known in the art and which comprise, e.g., homogenous and heterogeneous assays as described herein below.

Said interaction assays employing read-out systems are well known in the art and comprise, inter alia, two hybrid screenings (as described, inter alia, in EP-0 963 376, WO 98/25947, WO 00/02911 and the appended examples) GST-pull-down columns, co-precipitation assays from cell extracts as described, inter alia, in Kasus-Jacobi, *Oncogene* 19 (2000), 2052-2059, "interaction-trap" systems (as described, inter alia, in US 6,004,746) expression cloning (e.g. lamda gt10), phage display (as described, inter alia, in US 5,541,109), in vitro binding assays and the like. Further interaction assay methods and corresponding read out systems are, inter alia, described in US 5,525,490, WO 99/51741, WO 00/17221, WO 00/14271 or WO 00/05410.

Similarly, interacting molecules/(poly)peptides may be deduced by cell-based techniques well known in the art. These assays comprise, inter alia, the expression of reporter gene constructs or "knock-in" assays, as described, for, e.g., the identification of drugs/small compounds influencing the gene expression. Said "knock-in" assays may comprise "knock-in" in tissue culture cells, as well as in (transgenic) animals. Examples for successful "knock-ins" are known in the art (see, inter alia, Tanaka, *J. Neurobiol.* 41 (1999), 524-539 or Monroe, *Immunity* 11 (1999), 201-212). Furthermore, biochemical assays may be employed which comprise, but are not limited to, binding of the (poly)peptides of the invention (or (a) fragment(s) thereof) to other molecules/(poly)peptides, peptides or binding of the (poly)peptides of the invention (or (a) fragment(s) thereof) to itself (themselves) (dimerizations, oligomerizations, multimerizations) and assaying said interactions by, inter alia, scintillation proximity assay (SPA) or homogenous time-resolved fluorescence assay (HTRFA).

Further method(s) which may be employed comprises FRET (fluorescence resonance energy transfer; as described, inter alia, in Ng, *Science* 283 (1999), 2085-2089), or fluorescence polarisation assays. These methods are well known in the art and inter alia described in Fernandez, *Curr. Opin. Chem. Biol.* 2 (1998), 547-603.

Said "testing of interaction" may also comprise the measurement of a complex formation. The measurement of a complex formation is well known in the art and comprises, inter alia, heterogeneous and homogeneous assays. Homogeneous

assays comprise assays wherein the binding partners remain in solution and comprise assays, like agglutination assays. Heterogeneous assays comprise assays like, inter alia, immuno assays, for example, ELISAs, RIAs, IRMAs, FIAs, CLIAs or ECLs.

Any measuring or detection step of the method(s) of the present invention may be assisted by computer technology. For example, in accordance with the present invention, said detection and/or measuring step can be automated by various means, including image analysis, spectroscopy or flow cytometry.

Therefore, the detection/measuring step(s) of the method(s) of the invention can be easily performed according to methods known in the art such as described herein. In particular, the detection/measuring step(s) of the method(s) of the invention can be carried out by employing antibodies directed against the (poly)peptides of the invention. Said antibodies may comprise conformation-dependent antibodies. The use of antibodies is particularly preferred in detection methods like ELISA.

The term "readout system" in context with the present invention means any substrate that can be monitored, for example due to enzymatically induced changes. Said "readout system" may also comprise the use of specifically labelled (poly)peptides of the invention. These labels comprise, but are not limited to, radioactive labels, biotin, β -Gal, dioxygenin, fluorescence labels, chemi- or bioluminescent labels or protein labels, like GFP and the like.

The above-described method of identifying compounds capable of interfering with M2-PK-activity may, mutatis mutandis, be employed in drug screening assays. In this context it should be mentioned that the method(s) as described herein may also comprise (high)-throughput screening methods and analysis known in the art. Such high throughput screening methods are maturing rapidly and are reviewed, e.g., in Oldenburg, *Annu. Rev. Med. Chem.* 33 (1998), 301-311 or in Mason, *Pharmainformatics, Trends Supplement* (1999), 34-36.

In yet another embodiment, the present invention relates to the method(s) described herein, which further comprises the step of identifying the nucleic acid molecule(s) encoding the one or more of the above defined compounds, e.g. peptides or polypeptides capable of interfering with M2-PK-activity.

The identification of such nucleic acid molecule(s) is well known in the art and comprises, inter alia, the use of specific and/or degenerate primers. Furthermore, recombinant technologies as described in Ausubel, (1987-2002). Current protocols in molecular biology. Greene Publishing Assoc. and John Wiley & Sons Inc. may be employed.

Compounds identified by the method(s) of the invention may, inter alia, function as potent inhibitors or activators or modulators of M2-PK-activity. Without being bound by theory said inhibitor may, inter alia, function by competing with M2-PK for relevant modulators. As mentioned herein above, said compound may comprise a peptide or a polypeptide, but may also comprise other molecules, like, nucleic acid molecules, nucleic acid aptamers, as well as further candidate agents/compounds, like (small) organic compounds. Small organic compounds have a molecular weight of more than 50 Da yet less than about 2,500 Da, preferably less than about 1,000 Da, more preferably, less than about 500 Da. Candidate agents comprise functional chemical groups necessary for structural interactions with proteins, for example the interaction with M2-PK, and typically include at least an amine, carbonyl, hydroxyl or carbonyl group, preferably at least two of the functional chemical groups, more preferably at least three. The compounds often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the aforementioned functional groups. Candidate agents/compounds are also found among biomolecules including peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogues or combinations thereof, and the like. Candidate agents/compounds to be identified and/or screened by the method of the present invention may be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules. Alternatively, libraries of natural compounds in the form of bacterial,

fungal, plant, and animal extracts are available or readily produced. Additionally, natural and synthetically produced libraries and compounds are readily modified through conventional chemical, physical, and biochemical means. In addition, known pharmacological agents may be subject to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification, etc., to produce structural analogues. In context of the present invention the terms "compound" or "compounds" comprise also mixtures of compounds.

Furthermore, the present invention provides for a method for identifying a compound capable of interfering with M2-PK-activity comprising the steps of

- (a) testing a collection of compounds for interaction with the (poly)peptide identified by the method of described herein above, and
- (b) identifying compounds that test positive for interaction in step (a), and, optionally,
- (c) repeating steps (a) and (b) with the compounds identified one or more times, wherein the newly identified compound replaces the previously identified compound as a bait for the identification of a further interacting compounds.

Embodiments as described herein above apply, *mutatis mutandis*, for the here described method for identifying a compound capable of interfering with M2-PK-activity. Any measuring step or detection step of the method of the present invention as described herein above or below may be computer assisted.

In a further embodiment, the present invention relates for a method for identifying (an) inhibitor(s), activator(s) or modulator(s) of M2-PK-activity comprising the steps of

- (a) contacting the peptide of the invention with a compound to be screened under conditions which permit *in vitro* inactivation of M2-PK, and
- (b) detecting and/or measuring whether M2-PK-activity occurs, wherein the non-occurrence or a decrease M2-PK-activity level is indicative for a putative inhibitor.

As shown in the appended examples or in Zwerschke (1999), PNAS 96, 1291-1296 such an in vitro inhibition of M2-PK may be cell-based, however it is also envisaged that said in vitro inhibition comprises biochemical assays which are devoid of cells.

In addition, the present invention provides for a method for identifying a compound influencing the activity of a peptide of the invention comprising the steps of

- (a) contacting a host carrying an expression vector comprising the nucleic acid molecule of the invention operatively linked to a readout system and/or carrying a peptide of the invention linked to a readout system with a compound or a collection of compounds,
- (b) assaying whether said contacting results in a change of signal intensity provided by said readout system, and, optionally,
- (c) identifying a compound within said collection of compounds that induces a change of signal in step (b), wherein said change in signal correlates with a change in activity of said peptide.

It is particularly preferred said host is a eukaryotic host cell or a prokaryotic host cell. Said eukaryotic host cell may be a mammalian host cell like for example U2-O-S, CasKi, Shia, PC-3, NIH3T3 or MCF-7 or a yeast, said prokaryotic may be E. coli.

The present invention also provides for a method of producing a peptide of the invention or as described in the method of the invention comprising culturing the host as defined in herein above.

The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The peptide(s) of the invention can then be isolated from the growth medium, cellular lysates, cellular membrane fractions or inclusion bodies. Once expressed, the peptide(s) of the present invention can be purified according to standard procedures of the art, including ammonium sulphate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982). Substantially pure proteins of at least

about 60%, at least about 70%, at least about 80% or at least about 90 to 97% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the proteins may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures.

In another embodiment, the present invention relates to a composition comprising the peptide of the invention (and/or the fusion (poly)peptide, mosaic (poly)peptides or the chimeric (poly)peptide of the invention), the nucleic acid molecule of the invention, or the antibody or a fragment or a derivative thereof, an antiserum or a nucleic acid aptamer of the invention. Furthermore, the present invention relates to a composition comprising an inhibitor or a compound as identified or screened by the method of the present invention and disclosed herein above.

The term "composition", as used in accordance with the present invention, comprises at least one peptide (and/or the fusion (poly)peptide, mosaic (poly)peptides or the chimeric (poly)peptide), the nucleic acid molecule, or the antibody or a fragment or a derivative thereof, an antiserum or a nucleic acid aptamer of the invention, or at least one of the inhibitors or compounds as defined herein above and, optionally, further molecules, either alone or in combination, like e.g. molecules which are capable of interacting with the M2-PK and/or are capable of interacting with M2-PK. The composition may be in solid, liquid or gaseous form and may be, inter alia, in a form of (a) powder(s), (a) tablet(s), (a) solution(s) or (an) aerosol(s). In a preferred embodiment, said composition comprises at least two, preferably three, more preferably four, most preferably five peptides (or nucleic acid molecules encoding the peptides as defined herein) of the invention.

It is preferred that said composition is a pharmaceutical composition, optionally comprising a pharmaceutically acceptable carrier and/or diluent.

The here disclosed pharmaceutical composition may be partially useful for the treatment of common cancer disorders, like colon cancer, liver cancer, mamma cancer, prostate cancer.

In addition, inhibitors of the peptides of the present invention, like, inter alia, the herein described antibodies and/or aptamers may be pharmaceutically useful in order to modulate M2-PK-activity by a protein kinase, phosphatase or a interacting protein or metabolite or amino acid thus inhibiting the dimmer-generating, pathological step.

Examples of suitable pharmaceutical carriers, excipients and/or diluents are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, liposomes, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. It is particularly preferred that said administration is carried out by injection and/or delivery, e.g., to a site in a artery or directly into tissue, liver tissue, cervical tissue, skin and other tissues. Preferably, said administration occurs directly at a tumorous side. The compositions of the invention may also be administered directly to the target site, e.g., by biolistic delivery to an external or internal target site, like the liver, lung or brain. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg per dose; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it should also be in the range of 1 ng to 10 mg units per kilogram of body weight per minute, respectively. In context of the present invention, it is preferred that the peptides of the present invention are employed in concentrations of less than 500 µg/ml, more preferred at less than

100 µg/ml, more preferred of less than 10 µg/ml and most preferred of less than 1 µg/ml.

Progress can be monitored by periodic assessment. The compositions of the invention may be administered locally or systemically. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents depending on the intended use of the pharmaceutical composition. Said agents may be drugs acting at cancer diseases, like Tamoxifen, Cisplatin, Doxorubicin, Herceptin or alpha-tocopherol.

The pharmaceutical compositions, as well as the methods of the invention or the uses of the invention described infra can be used for the treatment of all kinds of diseases hitherto unknown or being related to or dependent on pathological M2-PK-activity. They may be particularly useful for the treatment of solid tumours and common cancer diseases or proliferative disorders. They may be desirably employed in humans, although animal treatment is also encompassed by the methods, uses and compositions described herein.

In a preferred embodiment of the invention, the composition of the present invention as disclosed herein above is a diagnostic composition further comprising, optionally,

suitable means for detection. The diagnostic composition comprises at least one of the aforementioned compounds of the invention.

Said diagnostic composition may comprise the compounds of the invention, in particular and preferably the peptide(s) of the present invention, in soluble form/liquid phase but it is also envisaged that said compounds are bound to/attached to and/or linked to a solid support. It is furthermore preferred that the diagnostic composition of the present invention comprises an antibody or a fragment or a derivative thereof or an antiserum or a nucleic acid aptamer or another receptor of the invention specifically recognising the peptide of the present invention. Such a diagnostic composition may be particularly useful in "sandwich assays" or overlay assays.

Solid supports may be used in combination with the diagnostic composition as defined herein or the compounds of the present invention may be directly bound to said solid supports. Such supports are well known in the art and comprise, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, membranes, sheets, duracytes, wells and walls of reaction trays, plastic tubes etc. The compounds of the invention, in particular the peptides of the present invention, may be bound to many different carriers. Examples of well-known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. Appropriate labels and methods for labelling have been identified above and are furthermore mentioned herein below. Suitable methods for fixing/immobilising said compound(s) of the invention are well known and include, but are not limited to ionic, hydrophobic, covalent interactions and the like.

It is furthermore envisaged, that said diagnostic composition comprises a compound(s) of this invention at (micro-)chips. Therefore, said diagnostic composition may, inter alia, comprise the nucleic acid molecules of the invention on

so-called "gene chips" or the peptides (or polypeptides) of the invention on so-called "protein-chips". Said diagnostic compositions may be particularly useful for screening patients for defects underlying, e.g. pathological M2-PK-activity. It is particularly preferred that the diagnostic composition of the invention is employed for the detection and/or quantification of M2-PK.

It is preferred that said compounds of the present invention to be employed in a diagnostic composition are detectably labelled. A variety of techniques are available for labelling biomolecules, are well known to the person skilled in the art and are considered to be within the scope of the present invention. Such techniques are, e.g., described in Tijssen, "Practice and theory of enzyme immuno assays", Burden, RH and von Knippenburg (Eds), Volume 15 (1985), "Basic methods in molecular biology"; Davis LG, Dibner MD; Battey Elsevier (1990), Mayer et al., (Eds) "Immunochemical methods in cell and molecular biology" Academic Press, London (1987), or in the series "Methods in Enzymology", Academic Press, Inc.

There are many different labels and methods of labelling known to those of ordinary skill in the art. Examples of the types of labels, which can be used in the present invention, include enzymes, radioisotopes, colloidal metals, fluorescent compounds, chemiluminescent compounds, and bioluminescent compounds.

Commonly used labels comprise, inter alia, fluorochromes (like fluorescein, rhodamine, Texas Red, etc.), enzymes (like horse radish peroxidase, β -galactosidase, alkaline phosphatase), radioactive isotopes (like ^{32}P or ^{125}I), biotin, digoxigenin, colloidal metals, chemi- or bioluminescent compounds (like dioxetanes, luminol or acridiniums). Labelling procedures, like covalent coupling of enzymes or biotinyl groups, iodinations, phosphorylations, biotinylations, etc. are well known in the art.

Detection methods comprise, but are not limited to, autoradiography, fluorescence microscopy, direct and indirect enzymatic reactions, etc. Commonly used detection assays comprise radioisotopic or non-radioisotopic methods. These comprise, inter alia, Westernblotting, overlay-assays, RIA (Radioimmuno Assay) and IRMA (Immune Radioimmunometric Assay), EIA (Enzyme Immuno Assay), ELISA (Enzyme Linked Immuno Sorbent Assay), FIA (Fluorescent Immuno Assay), and CLIA (Chemiluminescent Immune Assay).

In a further embodiment, the present invention relates to the use of at least one peptide, at least one nucleic acid, antibody or a fragment or a derivative thereof of an antiserum or a nucleic acid-aptamer of the invention or of at least one compound and/or inhibitor as identified or screened by the method of the present invention for the preparation of a composition for the treatment of a malignant and/or benign cancer disease, proliferative disorder including genital and nongenital warts or for the preparation of a composition for the diagnosis of a cancer diseases or proliferative disorder. In particular, the at least one peptide, at least one nucleic acid, antibody or a fragment or a derivative thereof of an antiserum or a nucleic acid-aptamer of the invention or of at least one compound and/or inhibitor as identified or screened by the method of the present invention is to be employed in the treatment and/or the prevention of tumors expressing M2-PK, in particular of tumors expressing M2-PK in aberrant levels or wherein said M2-PK has an aberrant activity. Proliferative diseases includes benign tumours and nongenital and genital warts caused by human papillomaviruses (HPVs) (zur Hausen (1996) Biochim Biophys Acta 1288(2):F55-78).

Furthermore, the present invention relates to the use of at least one peptide of the invention in a diagnostic method for cancer diseases or proliferative disorder comprising the steps of

- (a) contacting said at least one peptide or said at least one nucleic acid molecule with a biological sample comprising or suspected to comprise M2-PK (or (a) fragment(s) thereof) and
- (b) measuring the amount of M2-PK (or (a)fragment(s) thereof) interacting with said peptide.

Said biological sample may, inter alia, be selected from the group consisting of blood, serum, liquor cerebrospinalis, and saliva. However, other biological samples like urine or stool are also envisaged in context of the present invention. As illustrated in the appended examples, it is particularly preferred that said biological sample is a serum sample.

Furthermore, in accordance with this invention, it is preferred that said diagnostic method is an in vitro method. The here described diagnostic method may also comprise further steps. It is, inter alia, envisaged that the above measuring step is carried out in living cells, in particular in living cell cultures. Said measuring step may, e.g. comprise the detection of M2-PK-activity and/or M2-PK Protein. Cells/cell cultures, which may be employed, are well known in the art and comprise cells/cell cultures/ cell lines as described herein above. However, further cells, like epithelial cells may be employed in accordance with the present invention.

The M2-PK (or (a)fragment(s) thereof) to be employed in the methods as described herein above may be a mutated M2-PK (or (a)fragment(s) thereof). It is preferred that since M2-PK is a M2-PK (or (a) fragment(s) thereof as expressed in tumors. Said M2-PK may be a mutated M2-PK or an aberrant isoform.

In an even more preferred embodiment, the present invention relates to the use of a peptide, of a nucleic acid molecule, of an antibody or a fragment or a derivative thereof of an antiserum or of a nucleic acid-aptamer or another receptor of the invention, and/or the use of a composition as defined herein above for the inhibition/activation/modulation of M2-PK.

The present invention also provides for a method for treating, preventing and/or delaying cancer diseases or proliferative disorders comprising the step of administering to a subject suffering from said cancer disease or proliferative disorder and/or to a subject susceptible to said cancer disease or proliferative disorder an effective amount of a peptide of the invention, a nucleic acid molecule of invention and/or a composition as defined herein above.

In particular, it is preferred that said subject is a human. Said cancer disease or proliferative disorder may be selected from the group consisting cancer diseases and cancer diseases or proliferative disorders related to ageing but is by no means limited to said disorders.

In yet another embodiment, the present invention provides for a kit comprising at least one peptide of the invention, an antibody or a fragment or a derivative thereof of an antiserum or a nucleic acid-aptamer or another receptor of the invention and/or a nucleic acid molecule of the invention. Said kit may also comprise a compound or inhibitor as identified or screened by the method of the invention.

Advantageously, the kit of the present invention further comprises, optionally (a) reaction buffer(s), storage solutions and/or remaining reagents or materials required for the conduct of scientific or diagnostic assays or the like. Furthermore, parts of the kit of the invention can be packaged individually in vials or bottles or in combination in containers or multicontainer units.

The kit of the present invention may be advantageously used, inter alia, for carrying out the method of of the Invention and could be employed in a variety of applications referred herein, e.g., as diagnostic kits, as research tools or medical tools. Additionally, the kit of the invention may contain means for detection suitable for scientific medical and/or diagnostic purposes. The manufacture of the kits follows preferably standard procedures, which are known, to the person skilled in the art.

Table 1 shows:

Amino acid sequences and DNA sequences of the M2-PK interacting peptides.

The present invention is additionally described by way of the following illustrative non-limiting examples, that provide a better understanding of the present invention and of its many advantages.

Example 1: Initial peptide screen (in vivo protein-protein interaction assay)

Yeast strain EGY48/pSH1834/pLexA-M2-PK₂₂₋₅₃₁::HIS3 containing the LexA-M2-PK₂₂₋₅₃₁ probe was transformed with the pJM-1 peptide library comprising 6.5×10^8

individual clones (Colas (1996) Nature 380: 548-550). The M2-PK₂₂₋₅₃₁ fragment was derived from the human M2-PK cDNA (Tani et. al, Gene (1988) 20;73(2):509-16).

Transformations were carried out according to Gietz (1992), Nucleic Acids Res. 20: 1425, using 1 µg of the library plasmid DNA per transformation experiment. 3.9×10^6 primary transformants were obtained on glucose Ura⁻, His⁻, Trp⁻ plates. Cells were harvested and suspended in 50 ml of 65% glycerol, 10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂ and stored at -75°C. For the selection, the replating efficiency on 2% galactose/1 % raffinose Ura⁻, His⁻, Trp⁻ plates was determined and after galactose induction for 5 hr in YP galactose medium, about 3.5×10^7 galactose-viable cells were plated on eight 10 cm 2%galactose/1%raffinose Ura⁻, His⁻, Trp⁻ Leu⁻ plates. Leu⁺ colonies were collected on glucose Ura⁻, His⁻, Trp⁻ master plates and retested on glucose Ura⁻, His⁻, Trp⁻ Leu⁻, galactose/raffinose Ura⁻, His⁻, Trp⁻ Leu⁻, glucose X-Gal, Ura⁻, His⁻, Trp⁻ and galactose/raffinose X-Gal, Ura⁻, His⁻, Trp⁻ plates, to identify clones in which both the LEU2 and LacZ reporter genes display galactose-dependent transcription. To eliminate the plasmids pSH1834 and pLexA-M2-PK₂₂₋₅₃₁, positive clones were grown for 2 days in glucose Trp⁻ liquid minimal medium; clones containing only the library plasmid (ura3, his3, leu2, TRP1) were then identified by genetic selection. The plasmids encoding the selected B42 fusion proteins were rescued from the respective yeast strains using E.coli TG1 (Hoffmann (1987), Gene 57: 267-272) and analysed by restriction mapping and DNA sequencing.

Example 2: In vitro interaction analysis

GST pull-down assays were employed to study in vitro interaction (Mannhardt et al., (2000) Mol Cell Biol 20(17):6483-95). GST and GST-M2-PK fusion proteins were purified from bacteria and loaded (each at 10 ng/μl) on glutathione-Sepharose 4B beads. Contops were produced in bacteria and/or transiently transfected U2-O-S cells; extracts from these cells were incubated with the GST proteins, GST proteins were washed and bound proteins analyzed by Western blotting, using anti-Thioredoxin A antibodies (Sigma, Deisenhofen, Germany). Input of the various GST-derived fusion proteins was controlled either by Western blot analysis using a monoclonal antibody against GST (clone GST-2; Sigma Chemical Co., St. Louis, Mo.) or by Coomassie staining.

Example 3: Random mutagenesis / affinity selection of peptides ("intensifier screen")

Plasmids encoding thioredoxin A contop proteins found to interact with M2-PK₂₂₋₅₃₁ were used for random mutagenesis in order to increase their affinity for M2-PK₂₂₋₅₃₁. Random mutagenesis was performed by PCR as described below. The primers SCR 15688 (5'-GACAGTTTTGACACGGATGT-3') and S2 (5'-GCCCTGCTATTCGTCAGCG-3') were used for amplification. PCR-fragments were cloned in the pLexA-yeast vector, and the resulting peptides were assayed in the yeast-based genetic screen for their ability to interact with M2-PK₂₂₋₅₃₁ (see above). PCR amplification was performed using the Vent Polymerase (purchased from Biolabs) using 25 cycles of 94-66-72 °C. To increase the error rate that is required for efficient mutagenesis, in some experiments the concentration of Mg²⁺ was lowered through the inclusion of EDTA at 1 mM. To allow genetic identification of peptide aptamers with improved affinity, after mutagenesis, the DNA was used to transform yeast strain EGY191 (Gyuris et al. (1993), Cell 75, 791-803), which carries a LEU2 reporter gene which is controlled by two LexA binding sites (instead of eight LexA binding sites, present in the LEU2 gene incorporated in yeast strain EGY48,

which was used for the primary screening). Under these conditions, only peptides with very high affinity are revealed as leucine-independent yeast colonies. To reduce the sensitivity of the β -galactosidase assay in a similar way, the reporter gene construct pRB 1840 (Gyuris et al. (1993), Cell 75, 791-803) was used instead of the reporter gene pSH1834, which had been used for the primary screening. pRB 1840 contains only two LexA binding sites and therefore only yields reasonable LacZ expression in yeast colonies expressing peptide aptamers with very high affinity for E7. This procedure allows mutagenesis and subsequent selection according to binding affinity of derivatives of peptides that were identified in the primary screen. Isolated clones were sequenced using a cycle sequencing protocol. Briefly, 0.1 pmol of template DNA was mixed with fluorescent-labelled primer, a thermostable polymerase and the appropriate buffer. Four tubes were prepared with an A-, T-, G-, or C-specific termination mix. DNA mix was added to the tubes, and samples were subjected to PCR with 30 cycles at 95°C - 60°C - 70°C. After cycling, samples were denatured at 95°C and subjected to gel electrophoresis. Analysis was done using an automated DNA-sequencer. Applying this method, the sequence Seq.IDNo.20 in table 1 was determined to be ACGGTTCTTCAGTTGACTGGGGATGCGTGGCGTTTGTCTTTGATGCATAGGTTG AGTCGG Hence, the resulting amino acid sequence Seq.IDNo.19 is deduced as TVLQLTGDAWRLSLMHRLSR.

Example 4: Assessment of M2-PK-activity

The human cancer cell lines MCF-7, CaSKI, U2OS, PC3 and SiHa and the mouse cell line NIH3T3 were purchased from the American Type Culture Collection (Rockville, Md.). Plasmids encoding contops interacting with M2-PK₂₂₋₅₃₁, as identified in the two-hybrid screen, were transfected into the cells using the Effectene transfection reagent (Qiagen, Hilden, Germany). 24 h post-transfection, cells were washed, lysed and the quaternary structure of M2-PK was analysed by gel filtration as described by Zwerschke et al. (1999) PNAS 96, 1291-1296.

Example 5: Assessment of M2-PK in serum of patients

For diagnostic purposes, serum samples of cancer candidate patients and healthy volunteers were subjected to a standard ELISA, whereby instead of detecting antibodies, peptides of the invention or "contops" comprising the same have been employed. Detection was performed with purified high-affinity contops (based on peptides as shown in table 1) which are recombinantly expressed by standard methods in E.coli and/or mammalian cell culture and purified on a Ni-agarose column, at a concentration of 0.001-100 µg/ml. After excessive washing with PBS / 0.05% Tween 20, samples were incubated with a rabbit polyclonal antiserum directed against thioredoxin A (purchased from Santa Cruz, Heidelberg, Germany). Again, after excessive washing with PBS / 0.05% Tween 20, samples were incubated with an HRP-linked anti-rabbit antibody, and signals were detected by measuring HRP-activity.

Example 6: Modulation of M2-PK activity by contops, identification M2-PK-activating contops and M2-PK-inhibiting contops

U2-Os osteosarcoma cells are transfected with a pX-based expression vector coding for a M2-PK binding "contop", comprising a peptide of the invention. Cotransfection with a GFP expression plasmid is used to isolate transfected cells by live cell sorting, using a FACsort. Extracts from GFP-expressing cells will be prepared and subjected to gel filtration, as described (Zwerschke, 1999). To determine the relative amount of hyperactive (tetrameric) M2-PK and inactive (dimeric) M2-PK, pyruvate kinase activity will be performed in the fractions obtained by gel filtration, as described (Zwerschke, 1999). Contops that increase the fraction of tetrameric "M2-PK are referred to a M2-PK-activating contops".

Of the 14 contops tested, three were capable to activate M2-PK.

Example 7: Induction of apoptosis in tumor cells by contops inhibiting M2-PK

Tumor cells derived from other tissues are transfected with a pX-based expression vector coding for a M2-PK binding contop, that was shown to inhibit M2-PK (see Example above). Cotransfection with a GFP expression plasmid is used to visualize transfected cells. The fraction of apoptotic cells in transfected cells is determined by

i) TUNEL staining followed by fluorescence microscopy, ii) nuclear staining with DAPI followed by fluorescence microscopy, iii) propidium iodide staining followed by flow cytometry or iv) annexin V staining followed by flow cytometry.

Contops that inhibit M2-PK can induce apoptosis in U2-Os, PC-3 and MCF-7 tumor cells.

Example 8: Induction of apoptosis in tumor cells by contops activating M2-PK

Tumor cells derived from tissues with prevalent dimeric M2-PK (also referred to as TuM2PK, see above) are transfected with a pX-based expression vector coding for a M2-PK binding contop, that was shown to activate M2-PK (see Example above). Cotransfection with a GFP expression plasmid is used to visualize transfected cells. The fraction of apoptotic cells in transfected cells is determined by i) TUNEL staining followed by fluorescence microscopy, ii) nuclear staining with DAPI followed by fluorescence microscopy, iii) propidium iodide staining followed by flow cytometry or iv) annexin V staining followed by flow cytometry.

Contops that activate M2-PK can induce apoptosis in U2-Os, PC-3 and MCF-7 tumor cells.

Table 1: Peptide and DNA-Sequences of the anti-M2-PK Contops

*SEQ ID NO:1: WDRSPMTCGLLSTLYQIRVS	SEQ ID NO:2: TCGGATCGTACTCTATGACGCTGTGGGCTTCTTAGTACGTTGTATCAGATTAGGGTTTCG
SEQ ID NO:3: HRQFTLLAQLLSTRLANFV	SEQ ID NO:4: CATCGGCAGTTTACTTTTCTTGGCAGCTGTCTCGACTCGTTTGGCGTGGAATTTGTG
* SEQ ID NO:5: SEMMWRYGEGFDYMLQTCAG	SEQ ID NO:6: AGFTCGATGATGTGAGGTATGGGAGGGGTTTGAATATATATGCTTCAGACTTGTGCTGGG
SEQ ID NO:7: RETGLKESRAWSLAYTWLQS	SEQ ID NO:8: AGGAGACGGGTCCTGAAGGAGTCGAGGCGTGGTCTTTGGCGGTAACTTGGCTTCAGTCT
* SEQ ID NO:9: EGMVLPTVWQPANWMCRLSN	SEQ ID NO:10: GAGGGGATGGTTCTGCCCTACGGTTTGGCAGCCTGCTAATTGGATGTGCTCTTAGTAAT
SEQ ID NO:11: GMLSACSSHLTSVSLMWLRA	SEQ ID NO:12: GGGATGCTTAGTGCTTTGTAGTTCTCATCTGACGTCGGTTTCTCTGATGTGGCTTCGTGCG
* SEQ ID NO:13: EGQLRHMGWAWSLASQNFSL	SEQ ID NO:14: GAGGGTCAGCTACGTCATTTGGGGTTGGGCTTGGTCTGGCTAGTCAGAAATTTTCGATT
SEQ ID NO:15: MVSEQYSGTLVSYTHPYTRS	SEQ ID NO:16: ATGGTTTCTGACAGATATTCGGGGACTCTTGTCTTCTTATACGCATCTTATCTCTTCG
* SEQ ID NO:17: NSWTHAWESAWVFAQQRWTI	SEQ ID NO:18: AATTCGTGGACTCATGCTTGGGAGTCGGCGTGGGTGTCTTGTCTCAGCAGCGGTGGACTATT
SEQ ID NO:19: TVLQLTGDAWRLSLMHRLSR	SEQ ID NO:20: ACGGTTCTTCACTTGACITGGGATGCGTGGCGTTTGTCTTGTATGCATAGGTTGAGTCGG
SEQ ID NO:21: GNVPIVNSCTFQQVSVYEAS	SEQ ID NO:22: GGTAAATGTGCTTATGTGAATTCGTGTACTTTTTCAGCAGGTTTCTGTATTATGAGCGCTCG
* SEQ ID NO:23: RADNSWTLAAGLWRRRAFWR	SEQ ID NO:24: CGTCCGGATAATCTTGGACTTTCGGGGCTGGCTGGGTGGCGTAGGGCTTTTTCGGCGG
* SEQ ID NO:25: GTPLAHGVCGLLGMFWSRVS	SEQ ID NO:26: GGGACCGCGTTGGCTCATGGTGTGTGTGGTCTTTTGGGGATGTTTTCGTCGGCGGTTTCT
SEQ ID NO:27: GGTQVCQTLAYRLSLSHCLS	SEQ ID NO:28: GGGGGTACTCAGGTTTGTGACAGCGTTGGCTTATCGTTTGTCTGCTGTCTCATTTGCTTTCT
SEQ ID NO:29: CLINASAVASNYSDLRIWSR	SEQ ID NO:30: TGTCTTGTCTAATGCGTCGGCTGTGTCTAGTAAATATAGTATCTTAGGATTTGGAGTCGG
* SEQ ID NO:31: GWLKYGGGCRGALEPLVCLQ	SEQ ID NO:32: GGGTGGCTGAAGTATGGTGGGGGTGTAGGGGTGCGTTGGAGCCGTTGGTTGTTGTCAG
* SEQ ID NO:33: RGKLHFDASGLSWMMSMLRQS	SEQ ID NO:34: CGGGGTAAAGTTGCATTTTGTATGCGAGTGGTCTTAGTTGGATGTCTATGCTGAGGCAGAT

SEQ ID NO:35: RRGTMHLTGIVSLGLWELS	SEQ ID NO:36: AGCGGGGACTGGGATGCAATTGACGGGGATGTGTCTTTGGGCTCTGTGGGAGTTGTCT
SEQ ID NO:37: KSWHPEGCWSLRQYFFRQS	SEQ ID NO:38: AAGTCTTGGCATCTCTGAGGGGTGTGGTCTCTTAGGCAGTATTTTACTTTTAGGCAGTCT
SEQ ID NO:39: CCLGATSRFLGGSVFHSFA	SEQ ID NO:40: TGTGTGTTGGTGTCTACTAGTCGGGAGTTTTTTGGGTGGTTCGGTTTTTTCATAGTTTTTGGC
SEQ ID NO:41: TDLSYFVGSIDYGRGSLSF	SEQ ID NO:42: ACTGATCTTAGTTATGTGTGTTGGGTGGTCTATGATTAATAGTCTGTGGGAGTCTGAGTTTTT
SEQ ID NO:43: GLRGIALWRWSDTCGGYVSL	SEQ ID NO:44: GGTTTGAGGGGTAATGCTCTGTGGAGGTGGAGTGATACTGTGTGGGGTATGTTCGCTG
SEQ ID NO:45: ALYRPGRGFSMAMLSKIRVS	SEQ ID NO:46: GCGCTGTATCGGCCGGGTAGGGGGTTTTCTATGGCGATGCTGTGCGAAGATTAGGTTAGT
SEQ ID NO:47: NFLCVGWRLSDMLSWVCPRG	SEQ ID NO:48: AATTTTCTTTGTGTGGTGGTGGCGTCTTTCTGATATGCTTTTCTTTGGGTGTCCCGCGGGG
SEQ ID NO:49: CIEEGDTPISFVPMFWSQ	SEQ ID NO:50: TGTATGTAGAGGGGGATACGTCCTCTATTTCTGTTTGTTCGATGTGTTAGTTGGTTCGAG
* SEQ ID NO:51: NKTWHGWDAFESISLAFMA	SEQ ID NO:52: AATAAGACTTGGCATTTGGGGTGGGATTTGGCGGTTTTCTATTTCTTTGGCGTTTTATGGCG
SEQ ID NO:53: SDVFDQWTAWKRVHWRIG	SEQ ID NO:54: AGTGATGTTTTTGATCGTCGTGAGTGGACGGCTGCCGTGGAGCGGGTGCAATGGAGGCTGGG
SEQ ID NO:55: LGSNWRPVFQFSMNRYYVQS	SEQ ID NO:56: CTGGGGTCGAATTGGCGGCCGGTGTTCAGTTTAGTATGAATCGGTATATGTTTCAGTCG
SEQ ID NO:57: RVNIFEIVLERARPGSMSTR	SEQ ID NO:58: CCGGTTAATAATTTTGTAGATTGTTTTTGGAGAGGGCGCTCTGTTCTATGTTCTACGAGG
SEQ ID NO:59: YMLGRQAAMSLRDMFNTRRD	SEQ ID NO:60: TATATGTTGGGTAGGACAGCGGCTATGTCGCTTCGGGATATGTTTAATACTAGGAGGGAT
SEQ ID NO:61: MANIVKAGWGVMCQAQLYHN	SEQ ID NO:62: ATGGCTAATAATTTGTTAAGCTGTTGGGTGTATGTTGTGTCAGGCTCAGCTTTATCATAAT
SEQ ID NO:63: TWTLYTSPAVSLMQVGLWTQ	SEQ ID NO:64: ACTTGGACGCTTTATACCTTCGCCCTGCTGTGTCGTTGATGACAGGTTGGTTTTGTGACTCAG
SEQ ID NO:65: GGHLLPGWDRALFKLQCTFY	SEQ ID NO:66: GGTGGGCATCTTTTGGCGGGGTGGGATAGGGCGTTGTTTAAAGCTTTCAGTGTACGTTTTTAT
* SEQ ID NO:67: DGSYPMFPGMTSCFNGWRWS	SEQ ID NO:68: GATGGGTCTTATCCGATGTTTCTTGGGATGACTTCTGTGTTTTTAATGGGTGGCGTTGGTCG
SEQ ID NO:69: LSWLSLAVLVADLGRWRQS	SEQ ID NO:70: CTGTCTGTGGGTGTCGCTGAGTGCCTGTGTTGTCGATCTGGGATCTGGGTGTTGGCGGACAGT
SEQ ID NO:71: DQRAIYCCKNYLFSPPWRGA	SEQ ID NO:72: GATCAGAGGGCGATTTTATGTTCTAAGAATATTTTGTGTTTCGCCGCCCTGGCGGGGTGCG

Further Literature

- Schneider J, Neu K, Grimm H, Velcovsky HG, Weisse G, Eigenbrodt E. Tumor M2-pyruvate kinase in lung cancer patients: immunohistochemical detection and disease monitoring. *Anticancer Res.* 2002 Jan-Feb;22(1A):311-8.
- Mazurek S, Grimm H, Boschek CB, Vaupel P, Eigenbrodt E. Pyruvate kinase type M2: a crossroad in the tumor metabolome. *Br J Nutr.* 2002 Jan;87 Suppl 1:S23-9. Review.
- Wooll JO, Friesen RH, White MA, Watowich SJ, Fox RO, Lee JC, Czerwinski EW. Structural and functional linkages between subunit interfaces in mammalian pyruvate kinase. *J Mol Biol.* 2001 Sep 21;312(3):525-40.
- Roigas J, Schulze G, Raytarowski S, Jung K, Schnorr D, Loening SA. Tumor M2 pyruvate kinase in plasma of patients with urological tumors. *Tumour Biol.* 2001 Sep-Oct;22(5):282-5.
- Mazurek S, Grimm H, Oehmke M, Weisse G, Teigelkamp S, Eigenbrodt E. Tumor M2-PK and glutaminolytic enzymes in the metabolic shift of tumor cells. *Anticancer Res.* 2000 Nov-Dec;20(6D):5151-4.
- Oremek GM, Sapoutzis N, Kramer W, Bickeboller R, Jonas D. Value of tumor M2 (Tu M2-PK) in patients with renal carcinoma. *Anticancer Res.* 2000 Nov-Dec;20(6D):5095-8.
- Luftner D, Mesterharm J, Akrivakis C, Geppert R, Petrides PE, Wernecke KD, Possinger K. Tumor type M2 pyruvate kinase expression in advanced breast cancer. *Anticancer Res.* 2000 Nov-Dec;20(6D):5077-82.
- Schneider J, Velcovsky HG, Morr H, Katz N, Neu K, Eigenbrodt E. Comparison of the tumor markers tumor M2-PK, CEA, CYFRA 21-1, NSE and SCC in the diagnosis of lung cancer. *Anticancer Res.* 2000 Nov-Dec;20(6D):5053-8.
- Pottek T, Muller M, Blum T, Hartmann M. Tu-M2-PK in the blood of testicular and cubital veins in men with testicular cancer. *Anticancer Res.* 2000 Nov-Dec;20(6D):5029-33.
- Hardt PD, Ngoumou BK, Rupp J, Schnell-Kretschmer H, Kloer HU. Tumor M2-pyruvate kinase: a promising tumor marker in the diagnosis of gastro-intestinal cancer. *Anticancer Res.* 2000 Nov-Dec;20(6D):4965-8.

- Schulze G. The tumor marker tumor M2-PK: an application in the diagnosis of gastrointestinal cancer. *Anticancer Res.* 2000 Nov-Dec;20(6D):4961-4.
- Schneider J, Morr H, Velcovsky HG, Weisse G, Eigenbrodt E. Quantitative detection of tumor M2-pyruvate kinase in plasma of patients with lung cancer in comparison to other lung diseases. *Cancer Detect Prev.* 2000;24(6):531-5.
- Roigas J, Schulze G, Raytarowski S, Jung K, Schnorr D, Loening SA. [Tumor M2 pyruvate kinase in renal cell carcinoma. Studies of plasma in patients] *Urologe A.* 2000 Nov;39(6):554-6. German.
- Olianas MC, Onali P. PD 102807, a novel muscarinic M4 receptor antagonist, discriminates between striatal and cortical muscarinic receptors coupled to cyclic AMP. *Life Sci.* 1999;65(21):2233-40.
- Hugo F, Fischer G, Eigenbrodt E. Quantitative detection of tumor M2-PK in serum and plasma. *Anticancer Res.* 1999 Jul-Aug;19(4A):2753-7.
- Oremek GM, Teigelkamp S, Kramer W, Eigenbrodt E, Usadel KH. The pyruvate kinase isoenzyme tumor M2 (Tu M2-PK) as a tumor marker for renal carcinoma. *Anticancer Res.* 1999 Jul-Aug;19(4A):2599-601.
- Wechsel HW, Petri E, Bichler KH, Feil G. Marker for renal cell carcinoma (RCC): the dimeric form of pyruvate kinase type M2 (Tu M2-PK). *Anticancer Res.* 1999 Jul-Aug;19(4A):2583-90.
- Steinberg P, Klingelhofer A, Schafer A, Wust G, Weisse G, Oesch F, Eigenbrodt E. Expression of pyruvate kinase M2 in preneoplastic hepatic foci of N-nitrosomorpholine-treated rats. *Virchows Arch.* 1999 Mar;434(3):213-20.
- Aisaki K, Kanno H, Oyaizu N, Hara Y, Miwa S, Ikawa Y. Apoptotic changes precede mitochondrial dysfunction in red cell-type pyruvate kinase mutant mouse erythroleukemia cell lines. *Jpn J Cancer Res.* 1999 Feb;90(2):171-9.
- Eigenbrodt E, Basenau D, Holthausen S, Mazurek S, Fischer G. Quantification of tumor type M2 pyruvate kinase (Tu M2-PK) in human carcinomas. *Anticancer Res.* 1997 Jul-Aug;17(4B):3153-6.
- Tian YW, Smith PG, Yeoh GC. The oval-shaped cell as a candidate for a liver stem cell in embryonic, neonatal and precancerous liver: identification based on morphology and immunohistochemical staining for albumin and pyruvate kinase isoenzyme expression. *Histochem Cell Biol.* 1997 Mar;107(3):243-50.

- Tee LB, Kirilak Y, Huang WH, Smith PG, Morgan RH, Yeoh GC. Dual phenotypic expression of hepatocytes and bile ductular markers in developing and preneoplastic rat liver. *Carcinogenesis*. 1996 Feb;17(2):251-9.
- Smith PG, Tee LB, Yeoh GC. Appearance of oval cells in the liver of rats after long-term exposure to ethanol. *Hepatology*. 1996 Jan;23(1):145-54.
- Tee LB, Kirilak Y, Huang WH, Morgan RH, Yeoh GC. Differentiation of oval cells into duct-like cells in preneoplastic liver of rats placed on a choline-deficient diet supplemented with ethionine. *Carcinogenesis*. 1994 Dec;15(12):2747-56.
- Steinberg P, Weisse G, Eigenbrodt E, Oesch F. Expression of L- and M2-pyruvate kinases in proliferating oval cells and cholangiocellular lesions developing in the livers of rats fed a methyl-deficient diet. *Carcinogenesis*. 1994 Jan;15(1):125-7.
- Brinck U, Eigenbrodt E, Oehmke M, Mazurek S, Fischer G. L- and M2-pyruvate kinase expression in renal cell carcinomas and their metastases. *Virchows Arch*. 1994;424(2):177-85.

CLAIMS

1. A peptide capable of specifically interacting with pyruvate kinase subtype M2 (M2-PK), wherein said peptide comprises an amino acid sequence as depicted in table 1 or wherein said peptide is encoded by a nucleic acid sequence as shown in table 1.
2. The peptide of claim 1, wherein the amino acid sequence as shown in table 1 comprises at least one modification.
3. The peptide of claim 2, wherein said modification is selected from the group consisting of insertion, addition, deletion, substitution, inversion and duplication.
4. The peptide according to any one of claims 1 to 3, which is a fusion (poly)peptide.
5. The peptide of any one of claims 1 to 3, which is a chimeric (poly)peptide.
6. The peptide of any one of claims 1 to 5; wherein said peptide further comprises a signal sequence.
7. The peptide of any one of claims 1 to 6; wherein said peptide comprises at least one non-peptide moiety.
8. A nucleic acid molecule comprising a nucleotide sequence encoding a peptide of any one of claims 1 to 6 which is
 - (a) a nucleotide sequence encoding the peptide as set forth in table 1;
 - (b) a nucleotide sequence having the DNA sequence as depicted in any one of the sequences in table 1;
 - (c) a nucleotide sequence hybridising to a sequence which is complementary to a nucleotide sequence of (a) or (b); or

- (d) a nucleotide sequence being degenerate to the sequence of the nucleotide sequence of (c).
9. The nucleic acid molecule of claim 8 which is a recombinant nucleic acid molecule.
 10. The nucleic acid molecule of claim 8 or 9 which is DNA.
 11. The nucleic acid molecule of claim 8 or 9 which is RNA.
 12. A vector comprising a nucleic acid molecule of any one of claims 8 to 11.
 13. The vector of claim 12, which is an expression vector, a gene targeting and/or a gene, transfer vector.
 14. A host transformed with a vector of claim 12 or 13.
 15. The host of claim 14, which is a mammalian, cell, fungal cell, plant cell, insect cell or bacterial cell.
 16. The host of claim 14 which is a non-human transgenic animal.
 17. The host of claim 15, wherein said mammalian or insect cell is a primary cell.
 18. An antibody or a fragment or a derivative thereof, an antiserum or a nucleic acid-aptamer specifically recognising the peptide of any one of claims 1 to 7 or the nucleic acid molecule of any one of claims 8 to 11.
 19. A method for identifying a compound capable of interacting with M2-PK comprising the steps of

- (a) testing a collection of compounds for interaction with the peptide of any one of claims 1 to 7 or (a) fragment(s) thereof employing a readout system; and
 - (b) identifying compounds that test positive for interaction in step (a).
- 20. A method for identifying a compound capable of interacting with M2-PK-activity comprising the steps of
 - (a) testing a collection of compounds for interaction with the compound identified by the method of claim 19; and
 - (b) identifying compounds that test positive for interaction in step (a); and, optionally,
 - (c) repeating steps (a) and (b) with the compounds identified one or more times, wherein the previously identified compound or a bait for the identification of a further interacting compound.
- 21. A method for identifying (an) inhibitor(s) or activator(s) of M2-PK comprising the steps of
 - (a) contacting the peptide of any one of claims 1 to 7 with (a) compound(s) to be screened under conditions which permit in vitro modulation of M2-PK activity; and
 - (b) detecting and/or measuring whether M2-PK-activity is changed, wherein the alteration of M2-PK-activity is indicative for a putative inhibitor
- 22. A method for identifying a compound influencing the activity of a peptide of any one of claims 1 to 7 comprising the steps of
 - (a) contacting a host carrying an expression vector comprising the nucleic acid molecule of any one of claims 8 to 11 operatively linked to a readout system and/or carrying a peptide of any one of claims 1 to 7 linked to a readout system with a compound or a collection of compounds;
 - (b) assaying whether said contacting results in a change of signal intensity provided by said readout system, and, optionally,

- (c) identifying a compound within said collection of compounds that induces

a change of signal in step (b), wherein said change in signal correlates with a change in activity of said peptide.

23. The method of claim 22, wherein said host is a eukaryotic host cell or a prokaryotic host cell.
24. The method of claim 23, wherein said eukaryotic host cell is a mammalian host cell or a yeast cell.
25. The method of claim 24, wherein said prokaryotic host cell is E. coli.
26. A method of producing a (poly)peptide comprising culturing the host as defined in any one of claims 14 to 17 or 22 to 25.
27. A composition comprising the peptide of any one of claims 1 to 7, the nucleic acid molecule of any one of claims 8 to 11, or the antibody or a fragment or a derivative thereof, an antiserum or a nucleic acid-aptamer or another receptor of claim 18, a compound as identified by the method of any one of claims 19, 20 and 22 to 26 and/or an compound as identified by the method of claim 21.
28. The composition of claim 27 which is a pharmaceutical composition, further comprising, optionally, a pharmaceutically acceptable carrier and/or diluent and/or excipient.
29. The composition of claim 19, which is a diagnostic composition, further comprising, optionally, suitable means for detection.
30. Use of at least one peptide as defined in any one of claims 1 to 7, of at least one nucleic acid molecule as defined in any one of claims 8 to 11, of at least one antibody or a fragment or a derivative thereof of an antiserum or a nucleic

acid-aptamer or another receptor of claim 18, of a compound as identified by the method of any one of claims 19, 20, 22 to 26 and/or of an inhibitor as identified by the method of claim 21 for the preparation of a composition for the treatment of a cancer disease or proliferative disorder.

31. Use of at least one peptide as defined in any one of claims 1 to 7, of at least one nucleic acid molecule as defined in any one of claims 8 to 11 or of at least one antibody or a fragment or a derivative thereof of an antiserum or a nucleic acid-aptamer or another receptor of claim 18, of a compound as identified by the method of any one of claims 22 to 26 and/or of an inhibitor as identified by the method of claim 21 for the preparation of a composition for the diagnosis of a cancer disease or proliferative disorder.
32. Use of at least one peptide of any one of claims 1 to 7 or of at least one nucleic acid molecule of any one of claims 8 to 11 in a diagnostic method for a cancer disease or proliferative disorder comprising the steps of
 - (a) contacting said at least one peptide or said at least one nucleic acid molecule with a biological sample comprising or suspected to comprise M2-PK (or (a) fragment(s) thereof) and
 - (b) measuring the amount of M2-PK (or (a) fragment(s) thereof) interacting with said peptide.
33. The use of claim 32, wherein said biological sample is selected from the group consisting of blood, serum, liquor cerebrospinalis and saliva.
34. The use of claim 32 or 33, wherein said diagnostic method is an in vitro method.
35. The use of any one of claims 32 to 34, wherein said M2-PK (or (a) fragment(s) thereof) is a mutated M2-PK (or (a) fragment(s) thereof).

36. Use of a peptide of any one of claims 1 to 7, of a nucleic acid molecule of any one of claims 8 to 11, of an antibody or a fragment or a derivative thereof of an antiserum or a nucleic acid-aptamer or another receptor of claim 18 and/or of a composition as defined in claim 27 or 28 for the inhibition of abnormal metabolism of M2-PK.
37. A method for treating, preventing and/or delaying a cancer disease or proliferative disorder comprising the step of administering to a subject suffering from said a cancer disease or proliferative disorder and/or to a subject susceptible to said a cancer disease or proliferative disorder an effective amount of a peptide as defined in any one of claims 1 to 7, a nucleic acid molecule of any one of claims 8 to 11 and/or a composition as defined in claim 27 or 28.
38. The method of claim 37, wherein said subject is human.
39. The use of any one of claims 30 to 35 or the method of claim 37, wherein said a cancer disease or proliferative disorder is selected from the group consisting Cancer diseases.
40. A kit comprising at least one peptide of any one of claims 1 to 7, a nucleic acid molecule of any one of claims 8 to 11, an antibody or a fragment or a derivative thereof of an antiserum or a nucleic acid-aptamer or another receptor of claim 18, a compound as identified by the method of any one of claims 19, 20, 22 to 25 and/or of an inhibitor as identified by the method of claim 21.

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<120> Peptide modulators of tumor specific pyrovate kinase subtype M2 (M2-PK)

<130> G1988 PCT S3

<150> EP 02 01 7791.1

<151> 2002-08-12

<160> 108

<170> PatentIn version 3.1

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Trp	Asn	Phe	Val
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5/34

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			20

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His Cys Leu Ser
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1 5 10 15Ile Trp Ser Arg
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11/34

Val Cys Leu Gln
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12/34

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13/34

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17/34

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<400> 73

Asn Gly Val Leu Ala Glu Gly Tyr Asn Asp Val Pro Ser Gly Ser Phe
1 5 10 15

24/34

Trp Arg Leu Cys
20

<210> 74

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 74

aatgggggtgc ttgcggaggg ttataatgat gttccgtctg gttcgttttg gcgtctgtgt 60

<210> 75

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 75

His Thr Trp Val Tyr Pro Tyr Thr His Phe Gly Thr Thr Leu Lys Val
1 5 10 15

Arg Asp Ser Gly
20

<210> 76

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 76

catacgtggg tgtatcctta tacgcatttt ggtactacgt tgaagggttcg ggattctggg 60

<210> 77

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 77

Ala Gly Thr Tyr Gln Asp Leu Cys Phe Gln Tyr Lys Leu Phe Ala Ser
1 5 10 15

Trp Phe Arg Ala
20

<210> 78

<211> 60

<212> DNA

<213> artificial sequence

 $\langle 220 \rangle$

<223> sequence encoding contop

<400> 78
gcgggggactt atcaggattt gtgttttcag tataagttgt ttgcgagttg gtttcgggct 60

<210> 79

<211> 20

<212> PRT

<213> artificial sequence

 $\langle 220 \rangle$

<223> contop

<400> 79

Gln Ala Pro Thr Ser Ile Asn Arg Ala Pro His Cys Leu Ser Ser Met
1 5 10 15

Phe Ser Trp Arg
20

<210> 80

<211> 60

<212> DNA

26/34

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 80
caggcgccta ctagtattaa tcgtgcgcct cattgtctgt cttctatggt ttcttggcgg 60

<210> 81

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 81

His Gly Glu Glu Asn Ala Phe Arg Leu Ser Thr Ser Tyr Ile Thr Ser
1 5 10 15

Gln Ala Leu Tyr
20

<210> 82

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 82
catggggagg agaatgcgtt tcgtctttct acgtcgtata ttacttcgca ggcgttgtat 60

<210> 83

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

27/34

<400> 83

Ile Arg Ser Arg Ile Pro Trp His Arg Val Arg Arg Trp Trp Gly Gly
1 5 10 15

Met Glu Arg Ala
20

<210> 84

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 84
attcggagtc ggattccttg gcatcgggtt cgtcgggtggt gggggggtat ggagcgggcg 60

<210> 85

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 85

Glu Gly Trp Ala Thr Ser Cys Gly Ser Leu Leu Ala Gly Leu Gly Arg
1 5 10 15

Ile Trp Cys Ser
20

<210> 86

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 86
gagggttggg cgacttcgtg tgggagtctt ttggcgggtt tgggtcggat ttggtgtagt 60

28/34

<210> 87

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 87

Cys Ala Phe Arg Ser Ile Lys Asn Ile His Ser Leu Met Asn Met Tyr
1 5 10 15

Ile Phe Ser Lys
20

<210> 88

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 88
tgtgcgttta ggtctattaa gaatattcat tcgctgatga atatgtatat ttttagtaag 60

<210> 89

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 89

Cys Arg Trp Met Ser Pro Gly Ser Cys Phe Leu Arg Val Cys Tyr Ser
1 5 10 15

Pro Met Ser Phe
20

29/34

<210> 90

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 90

tgtaggtgga tgcgcgcggg gtcgtgtttt cttcgggtgt gttattctcc tatgtctttt 60

<210> 91

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 91

Ser	Gly	Arg	Pro	Ala	Ala	Phe	Val	Ser	Trp	Ala	Ser	Tyr	Val	Phe	Pro
1				5				10				15			

Phe	His	Ala	Ser
			20

<210> 92

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 92

agtgggaggc cggcggcttt tgtttcttgg gcttcgtatg tttttccttt tcatgcttct 60

<210> 93

<211> 20

<212> PRT

<213> artificial sequence

30/34

<220>

<223> contop

<400> 93

Lys Glu Arg Val Glu Trp Ser Arg Ser His Leu Ile Lys Gly Cys Leu
1 5 10 15

Glu Trp Ser Ser
20

<210> 94

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 94
aaggagcggg tggagtgggc taggtctcat ttgattaagg gttgtctgga gtggagttct 60

<210> 95

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 95

Thr His Glu Ala Ala Leu Lys Gln Ala Gly Thr Leu Gln Leu Gly Leu
1 5 10 15

Leu Tyr Thr Ser
20

<210> 96

<211> 60

<212> DNA

<213> artificial sequence

31/34

<220>

<223> sequence encoding contop

<400> 96

acgcatgagg cggctttgaa gcaggcgggt acgcttcagt tgggtctttt gtatactagt 60

<210> 97

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 97

Val Thr Glu Gly Tyr Met Glu Ser Trp Ser Ala Arg Thr Val Phe Ser
1 5 10 15Val Trp Arg His
20

<210> 98

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 98

gttacggagg ggtatatgga gtcgtggagt gctcgtactg tttttagtgt gtggaggcat 60

<210> 99

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 99

Thr Pro Val Ser Arg Gly Trp Trp Ser Thr Val Glu Pro Cys Tyr Tyr
1 5 10 15

Arg Leu Ser Ile
20

<210> 100

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 100
acgccggtga gtcgggggtg gtggtcgacg gttgagccgt gttattatcg tttgtcgatt 60

<210> 101

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 101

Thr Lys Leu Ser Trp Ser Phe Gly Ala Arg Ala Leu Leu Pro Asn Met
1 5 10 15

Leu Val Gly Gly
20

<210> 102

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 102
acgaagcttt cttggtcgtt tggtgcgagg gctttgttgc ctaatatgct tgtggggggg 60

<210> 103

33/34

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 103

Gly	Leu	Gly	Phe	Arg	Trp	Leu	Ser	Gly	Glu	Asp	Ala	Val	Tyr	Leu	Met
1				5					10					15	

Arg	Phe	Leu	Ser
			20

<210> 104

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400>	104	
ggtcttggtt	ttaggtggct	gagtgggtgag gatgcggtgt atcttatgcg gtttttgtcg
		60

<210> 105

<211> 20

<212> PRT

<213> artificial sequence

<220>

<223> contop

<400> 105

Arg	Gly	Gly	Leu	Phe	Met	Gly	Val	Val	Pro	Thr	Ile	Ser	Gly	Leu	Leu
1				5					10					15	

Thr	Trp	Arg	Arg
			20

<210> 106

<211> 60

<212> DNA

<213> artificial sequence

<220>

<223> sequence encoding contop

<400> 106

aggggtgggt tgtttatggg tgttggtccg actatttctg gtttggtgac ttggcggagg 60

<210> 107

<211> 20

<212> DNA

<213> artificial sequence

<220>

<223> oligonucleotide primer

<400> 107

gacagttttg acacggatgt 20

<210> 108

<211> 19

<212> DNA

<213> artificial sequence

<220>

<223> oligonucleotide primer

<400> 108

gccctgctat tcgtcagcg 19