**Title:** MODULATION OF CALCIUM CHANNEL SPLICE VARIANT IN CANCER THERAPY

**Abstract:** The present invention is directed to calcium channel splice variants, and their modulation in cancer treatment, diagnosis and research. Specifically, the invention relates to a cancer-specific splice variant of the Ca_{1.3} alpha 1 subunit, exhibiting altered activity and a unique response profile to known pharmacological calcium channel inhibitors. Compositions and methods according to embodiments of the invention employ the use of specific inhibitors of this variant, including Azelnidipine and derivatives and salts thereof, in cancer therapy.
MODULATION OF CALCIUM CHANNEL SPLICE VARIANT IN CANCER THERAPY

FIELD OF THE INVENTION

The present invention relates to calcium channel splice variants and their modulators, and to uses thereof in diagnosis, therapy and screening of drug candidates for cancer.

BACKGROUND OF THE INVENTION

Calcium signaling is a common mechanism involved in the majority of cellular functions. Ca^{2+} homeostasis is tightly modulated by multiple channel mechanisms in all excitable and non-excitable cells. Calcium channels include various voltage-dependent channels (Ca_{v}, also referred to as voltage-gated channels) and ligand-gated (receptor-operated) channels.

Among the cellular functions involving Ca^{2+} signaling are many processes that mediate or regulate the development of pathologies, including cardiovascular disorders, hypertension, and several types of cancer. Experiments performed in certain cell culture models suggested that maintenance of intracellular Ca^{2+} pool is required for cell growth, and that blocking Ca^{2+} influx reduces cell growth and invasion. Results from other experimental models suggested that elevated Ca^{2+} levels are positively involved in cancer cell death. For example, it was found that blockade of L-type calcium channel-mediated Ca^{2+} influx suppressed castration-induced apoptotic cell death in prostate epithelial cells. Thus, Ca^{2+} was suggested to play both positive and negative roles in cancer cell expansion (Monteith et al., J Biol. Chem. Vol. 287, no. 38, pp. 31666-31673, 2012; Chen et al., Urologic Oncology: Seminars and Original Investigations, 1-13, 2013).

Pathological deregulation of Ca^{2+} signaling, leading to either increased or decreased intracellular Ca^{2+} levels, has been attributed to differential expression of a variety of Ca^{2+} pumps and channels, notably transient receptor potential (TRP) channels and ORAI-mediated store-operated channels (Monteith et al., 2012, ibid). Stromal interaction molecule (STIM) and ORAI channel subunits (both components of the Calcium release of activated calcium, i.e. CRAC family), as well as Transient receptor potential M7 (TRPM7) channels, were suggested as major players in lung cancer.
Voltage-gated Ca\textsuperscript{2+} channels (Ca\textsubscript{v}) include several families of channels that are activated, i.e. opened, at depolarized membrane potentials. Ca\textsubscript{v} channels are heteromultimers composed of a pore forming a1 subunit, β regulatory subunit, and αl, γ and δ subunits. The topology of the a1 pore subunit is predicted to have four repeated motifs (I-IV), each of which is hexahelical. The S4 transmembrane segments in each motif contain conserved positively charged amino acids that are voltage-sensors and that move outwards upon membrane depolarization, thereby opening the channel. Ca\textsubscript{v} a1 subunits may be classified into three subfamilies having specific functions in different cell types: Ca\textsubscript{v}1 (L-type), Ca\textsubscript{v}2 (N-, P/Q- and R-type), and Ca\textsubscript{v}3 (T-type).

There are four L-type a1 proteins: a1S (Ca\textsubscript{v}1.1), a1C (Ca\textsubscript{v}1.2), a1D (Ca\textsubscript{v}1.3), and a1F (Ca\textsubscript{v}1.4). Although Ca\textsubscript{v} channels are mostly expressed in electrically excitable cells, Ca\textsubscript{v} subunits, including L-type channel subunits, were also found in certain non excitable cells. For example, T cells were found to express the Ca\textsubscript{v}1.1 a1 subunit (Matza et al, 2009, Proc Natl Acad Sci USA 106(24):9785-9790 and Matza et al. (2016, PLoS One 11, e0147379).

Ca\textsubscript{v}1.3 expression was detected in certain hormone-dependent cancer cell lines, including androgen-positive prostate cancer VCaP and LAPC-4 cells, and estrogen-positive Ishikawa endometrial cancer cells (Chen et al, 2013, ibid; Hao et al, FASEB J. 29, 2883-2893, 2015). Certain colon cancer and prostate cancer cells were found to express Ca\textsubscript{v}1.2 or Ca\textsubscript{v}3.2 (Monteith et al, 2012, ibid). In other cancerous cell types, including non-small cell lung cancer (NSCLC) cells, homozygous deletions of the Ca\textsubscript{v} (α2δ12) regulatory subunits have been identified (Carboni et al, 2003, Oncogene 22(4):615-626). However, the role of these channels in tumor development has not been established, and remains mostly unknown.

A range of Ca\textsuperscript{2+} channel blockers were developed as anti-hypertensive agents, and are available for clinical use. These agents are also used to alter heart rate, to prevent cerebral vasospasm, and to reduce chest pain caused by angina pectoris. Dihydropyridine (DHP) Ca\textsuperscript{2+} channel blockers are derived from the molecule dihydropyridine and are often used to reduce systemic vascular resistance and arterial pressure. Non-DHP Ca\textsuperscript{2+} channel blockers include for example Phenylaikylamines such as Verapamil, Benzothiazepines such as Diltiazem, and other types of inhibitors.

Azelnidipine, also known as CS-905, is a DHP Ca\textsuperscript{2+} channel blocker with antihypertensive activity (Oizumi et al., Japan. J. Pharmacol. 51, 57-64, 1989). Azelnidipine has the chemical structure 3-l-Benzhydryl-3-azetidinyl 5-isopropyl 2-amino-6-methyl-4-(m-
nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, and is used for treating ischemic heart disease and cardiac remodeling after myocardial infarction. The drug is marketed in Japan under the brand name CalBlock®, and several generic forms of the drug are available in Japan and China. CalBlock® is available as tablet for oral use, containing 8 or 16 mg of free Azelnidipine. The recommended dose is 8 to 16mg once daily. Azelnidipine formulations are described for example in US 2010/221327.

The proposed involvement of calcium and certain Ca²⁺ channels in cancer-related cellular processes has prompted suggestions for use of Ca²⁺ channel blockers in cancer management. For example, Mibefradil, a T-type calcium blocker, was suggested as a potential inhibitor of cancer metastasis based on its observed effects in HT1080 fibrosarcoma cell cultures. However, other Ca²⁺ channel blockers, including nicardipine, SKF96365, diltiazem, and verapamil, showed no effect at appropriate doses (Huang et al, Cancer Research 64, 2482-2489, 2004). U.S. 6,946,475 discloses compounds useful as cancer cell inhibitors, compositions containing such compounds and methods for inhibiting proliferation of electrically non-excitatable cells. The compounds are described as inhibitors of T-type Ca²⁺ channels.

U.S. 4,690,935 discloses a method for reducing interaction between blood platelets and malignant tumors present in the blood and resulting attachment of the tumors with the platelets in blood vessels in a mammal, which comprises: administering an effective amount of a Ca²⁺ channel blocker compound selected from the group consisting of phenylaikyamines, benzothiapenes, diphenylaikylamines and 1,4-dihydropyridines which is substituted in the 4-position, which reduces the interaction between the platelets and the tumors and resulting attachment of the tumors with the platelets in the blood vessels without treating the tumors, to thereby interfere with the metastatic cascade, and wherein in vitro the Ca²⁺ channel blocker compounds prevent aggregation of tumor cells and platelets.

U.S. 4,988,713 discloses 2-selenomethyl-1,4-dihydropyridines having calcium-antagonistic properties and pharmaceutical compositions containing them. The compounds are suggested to be useful in human therapy as agents able to interact with Ca²⁺ dependent systems and as anti-tumor agents. WO 2016/062275, published after the priority date of the present application, relates to the use of azelnidipine in preparing medicinal composition for treating cancer. WO 2016/062275 reports a cytostatic effect on several cell lines, however the effect was observed at daily doses of 100-200 mg/kg, which are equivalent to doses substantially higher than those approved for clinical use. The publication does not report expression of Ca₁.3
variants or isoforms by tumors or tumor cells. Nor does it suggest the need to determine tumor Ca$_{\text{a},1.3\Delta_{248}}$ expression in a patient to be treated, or any direction or benefit in treating such distinct patient populations.

Other reports have disclosed that certain calcium channel antagonists may in fact act as tumor-promoting agents, and advised that their use may be contraindicated in cancer patients. For example, Guo et al. (PLoS ONE 2014, 9(12): e113649) have reported a direct effect of the DHP L-type calcium blocker, Nifedipine, in promoting the proliferation and migration of breast cancer cells, both in vivo (upon treatment of nude mice with a daily dose of 4.8 mg/kg) and in vitro (at concentrations of 0.01-10 uM). The pro-tumorogenic activities of Nifedipine were observed in the absence of increase in intracellular Ca$^{2+}$ levels, in breast cancer cell lines lacking expression of functional Ca$_{\text{a}}$ channels. The non-DHP L-type calcium blocker, verapamil, had no effect on tumor development. The publication concludes that caution should be taken in prescribing Nifedipine to women, particularly those afflicted with or at a risk of developing breast cancer, as it may promote or aggravate the disease.

In addition, the reported effects of Ca$^{2+}$ channel blockers in clinical practice have raised substantial concerns regarding their involvement in tumor development. Owing to the wide use of Ca$^{2+}$ channel blockers in treating hypertension, multiple epidemiologic surveys were conducted to assess the risk of cancer incidence in blocker users. The results of these studies were inconsistent, and varied considerably depending on the design of the particular study. A large body of evidence implicated Ca$^{2+}$ channel blocker with increased risk for cancer. For example, Ca$^{2+}$ channel blockers were found to be positively associated with breast cancer risk (Li et al, JAMA Intern Med. 2013;173(17):1629-1637). Other studies in prostate cancer patients have reported no excessive risk of prostate cancer incidence, or even found a reverse correlation between the likelihood of prostate cancer risk and the use of Ca$^{2+}$ channel blockers among men without family history (Chen et al, ibid). As the reasons and underlying mechanisms for these strikingly different effects are largely unknown, the ability to predict how a particular subject would react to a particular blocker remains a challenge. This uncertainty would preclude the use of Ca$^{2+}$ channel blockers in cancer therapy, and indeed no such agents are currently indicated for the treatment of cancer.

Variants and isoforms of Ca$_{\text{a}}$ subunits have been described. The Ca$_{\text{a},1.3}$ transcripts undergo post-transcriptional modifications including alternative splicing and A-to-I RNA editing, generating channel isoforms with different electrophysiologic or pharmacologic
properties. For example, Huang et al. (Mol Pharmacol 84:643-653, 2013) describe various Ca_{1.3} isoforms harboring splicing variations, particularly in the IS6 segment and C terminus, and their sensitivities toward different DHP blockers. Huang et al. disclose that alternative splicing could modulate the pharmacological profiles of the channels, as splice variations at the C terminal domains (including the IQ domain and the proximal and/or distal C terminal regulatory domains) resulted in highly variable levels of sensitivity to inhibition by DHPs, while alternative splicing at other domains such as the IS6 segment did not alter DHP sensitivity.

Ca_{1.3} splice variants have also been suggested based on sequence prediction methods and algorithms. For example, accession numbers GI:652817353, XM_005265448.2 and XM_011534096.1 display partial homology to human Ca_{1.3}.

WO 2005/033139 discloses L-type calcium channel nucleic acid sequences and polypeptides, said to be differentially expressed in non-excitable cells and to be useful for a variety of therapeutic and experimental applications. A method for treating or preventing a cancer, an immune system disorder, or an inflammatory condition in a subject, comprising inhibiting expression or activity of the L-type calcium channel polypeptide that is expressed in a non-excitable cell is further disclosed.

There remains an unmet medical need for safe and effective cancer therapies. In addition, the development of diagnostic tools, enabling the identification of patient populations that may benefit from treatment with candidate anti-cancer agents, as well as research tools for screening and development of new therapeutic agents for cancer, are highly desirable.

**SUMMARY OF THE INVENTION**

The present invention is directed to calcium channel splice variants, specifically to a variant of the L-type voltage-gated (Ca_{v}) a1 subunit Ca_{1.3}. The invention relates in some embodiments to the use of the variant, herein disclosed to be expressed specifically in cancer cells, in diagnosis and therapy and in the screening of drug candidates for cancer. The invention further relates to the use of specific modulators including Azelnidipine and derivatives and salts thereof in the treatment of tumors, particularly tumors expressing the Ca_{1.3} variant.

The invention is based, in part, on the surprising discovery of differential expression patterns of Ca_{1.3} variants in normal and malignant cells. Specifically, it is herein disclosed that normal human lung cells express wild-type Ca_{1.3}, characteristic of excitable cells such as
neurons; however lung malignancies, including non-small cell lung carcinoma (NSCLC) cell lines and tumor biopsies, express high levels of a unique splice variant of Ca,1.3. The variant, herein designated Ca,1.3A_{22/48}, was surprisingly found to exhibit distinct structural and functional properties.

The Ca,1.3A_{22/48} transcript comprises deletions of exons 22 and 48, corresponding to part of the intracellular loop connecting motif 1 and motif 2, and to the extracellular loop- S3-S4 linker, respectively. The invention is further based, in part, on the unexpected discovery, that Ca,1.3A22/48 expressing cells exhibit strikingly altered pharmacological activities compared to cells expressing wild-type (also referred to herein as neuronal-type) Ca,1.3. Remarkably, known Ca, antagonists, including Nifedipine and various other dihydropyridines (DHPs) and non-DHPs, failed to inhibit calcium entry to Ca,1.3A_{22/48} expressing cell lines, but rather induced an elevation in intracellular calcium levels. In contradistinction, the DHP Azelnidipine exhibited no stimulatory effect on the cells, and did not induce calcium influx. Rather, Azelnidipine, but not Nifedipine, inhibited proliferation of NSCLC cell lines in vitro; Azelnidipine was also discovered unexpectedly to be a potent and selective anti-cancer agent in vivo, capable of reducing tumor volume and weight significantly in a xenograft murine model of human NSCLC.

Thus, embodiments of the invention are directed to compositions and methods for the treatment and amelioration of cancer, specifically, tumors characterized by Ca,1.3A_{22/48} expression such as lung tumors. Embodiments of the invention employ the use of Ca,1.3A_{22/48} modulators, such as specific Ca,1.3A_{22/48} inhibitors and antagonists. Particularly advantageous embodiments employ the use of Azelnidipine and derivatives and salts thereof.

In one aspect, the invention provides a method of treating a subject having a tumor characterized by Ca,1.3A_{22/48} expression, the method comprising administering to the subject an effective amount of Azelnidipine or a derivative or salt thereof, thereby treating said subject. In a particular embodiment, the method comprises administering an effective amount of Azelnidipine to said subject, thereby treating said subject.

In another embodiment of the methods of the invention, the tumor is a solid tumor, e.g. a lung tumor or a breast tumor. For example, the tumor may be a solid tumor derived from non-excitable cells, including, but not limited to tumors of epithelial or fibroblast origin. In another embodiment the tumor is derived from excitable cells such as neurons. In another embodiment, said tumor is a carcinoma. In a particular embodiment, said tumor is a lung carcinoma. In a further particular embodiment, said tumor is a NSCLC tumor. In another embodiment said tumor
is a breast ductal carcinoma. According to other embodiments of the invention, the method is
used for treating an established tumor in said subject, e.g. by reducing tumor size and/or volume,
wherein each possibility represents a separate embodiment of the invention. Thus, in another
embodiment, treating said subject comprises reducing tumor size and/or volume in said subject.

In another embodiment, Azelnidipine or the derivative or salt thereof is administered
orally. In another embodiment, Azelnidipine or the derivative or salt thereof is administered by
topical, intratumoral of pulmonary administration. In a particular embodiment, said Azelnidipine
or derivative or salt thereof is administered topically. In another embodiment, the tumor is
derived from a tissue or organ lacking Ca$_v$1.3 expression. In yet another embodiment, the tumor
is derived from a tissue or organ expressing neuronal type Ca$_v$1.3. Thus, the compositions and
methods of the invention provide for effective cancer therapy with enhanced safety, as they
enable localized treatment of a tumor with minimized damage to its surrounding tissue.

In another embodiment the method further comprises identifying the subject as being
afflicted with a Ca$_v$1.3A$_{2/48}$ expressing tumor, and administering Azelnidipine or a derivative or
salt thereof to said subject afflicted with the Ca$_v$1.3A$_{2/48}$ expressing tumor. In another
embodiment, the subject is human and the effective amount is 0.05-0.5 mg/kg/day.

In another aspect, there is provided a method to inhibit the proliferation of cells that
exhibit Ca$_v$1.3A$_{2/48}$ expression, comprising contacting the cells with an effective amount of
Azelnidipine, or a derivative or salt thereof. In another embodiment, the cells are tumor cells,
e.g. NSCLC cells. In another embodiment, the subject is human and the effective amount is 5-50
µM.

According to additional embodiments, the invention relates to pharmaceutical
compositions that are particularly useful in the treatment of proliferative diseases, e.g.
proliferative diseases of the lung and other malignancies. Such compositions may optionally be
applied to tumors locally, thereby minimizing side effects associated with the use of calcium
channel blockers, such as hypotension. Thus, in another aspect, there is provided a
pharmaceutical composition comprising as an active ingredient Azelnidipine or a derivative or
salt thereof, formulated for topical, intratumoral or pulmonary administration. In a particular
embodiment, there is provided a pharmaceutical composition comprising as an active ingredient
Azelnidipine or a derivative or salt thereof, formulated for pulmonary administration. In another
embodiment the composition is formulated for administration as an aerosol or mist. In another
embodiment said composition is formulated for use with a nebulizer or inhaler. In another
embodiment, said composition comprises said Azelnidipine or a derivative or salt thereof at an amount effective to inhibit cell proliferation. In a particular embodiment, said composition comprises Azelnidipine at a concentration of 5-50 µM. In another particular embodiment, said composition comprises 1-50 mg Azelnidipine in unit dosage form. In yet another particular embodiment, said composition comprises Azelnidipine as a sole active ingredient.

Other embodiments of the invention are directed to diagnostic methods and assays. In another aspect, the invention provides a method of identifying a subject amenable for treatment by Azelnidipine or a derivative or salt thereof, comprising determining the expression of Ca₅.1.3A22/48 in a biological sample of the subject, wherein expression of Ca₅.1.3A22/48 in the sample indicates that the subject is amenable for treatment by Azelnidipine or a derivative or salt thereof. In another aspect, the invention provides a method of determining whether a subject is afflicted with, or is at risk for developing, a Ca₅.1.3A22/48 expressing tumor, comprising determining the expression of Ca₅.1.3A22/48 in a biological sample of the subject, wherein expression of Ca₅.1.3A22/48 in the sample indicates that the subject is afflicted with, or is at risk for developing, a Ca₅.1.3A22/48 expressing tumor.

In various embodiments of the diagnostic methods of the invention, the sample may include, without limitation, a cell sample, a tissue sample, or a fluid sample. In a particular embodiment, the sample is a tumor sample, e.g. a tumor biopsy. In other embodiment, determining the expression of Ca₅.1.3A22/48 in the sample may employ a variety of immunoassays (e.g. enzyme-linked immunosorbert assay, ELISA) and/or other molecular biology assays (e.g. reverse transcription polymerase chain reaction, RT-PCR) for detecting and determining the expression of a Ca₅.1.3A22/48 polypeptide and/or mRNA transcript. In another embodiment, the methods further comprise the step of administering a therapeutically effective amount of Azelnidipine or a derivative or salt thereof to the subject exhibiting expression of Ca₅.1.3A22/48 in said sample. Thus, in a particular embodiment, the method of identifying a subject amenable for treatment by Azelnidipine or a derivative or salt thereof further comprises the step of administering a therapeutically effective amount of Azelnidipine or a derivative or salt thereof to the subject identified as being amenable for treatment. In another particular embodiment, the method of determining whether a subject is afflicted with, or is at risk for developing, a Ca₅.1.3A22/48 expressing tumor further comprises the step of administering a therapeutically effective amount of Azelnidipine or a derivative or salt thereof, to the subject determined to be afflicted with, or at risk for developing, a Ca₅.1.3A22/48 expressing tumor.
In another aspect there is provided a kit for determining whether a subject is amenable for
treatment by Azelnidipine or a derivative or salt thereof, comprising means for determining the
expression of Ca,1.3A2/48 in a biological sample of the subject. For example, without limitation,
the kit may comprise one or more antibodies, PCR primers or other reagents that may be
employed in various immunoassays and other molecular biology assays known in the art. In
another embodiment, the kit may further comprise instructions for administering Azelnidipine or
a derivative or salt thereof to a subject exhibiting expression of Ca,1.3A2/48 in the sample.

Yet other embodiments of the invention are directed to experimental methods and assays,
useful for the screening, identification and selection of drug candidates or other pharmacological
agents. Thus, in another aspect, there is provided a method for screening of candidate drugs for
cancer therapy, which comprises contacting one or more cells that exhibit Ca,1.3A2/48 calcium
channel expression and do not substantially express other Ca, a subunits with a test substance,
and determining whether the test substance decreases proliferation of the one or more cells,
whereby a test substance that decreases proliferation is identified as a candidate drug for cancer
therapy.

In another aspect there is provided a system for screening of candidate drugs for cancer
therapy. The system comprises one or more cells that exhibit Ca,1.3A22/48 calcium channel
expression and do not substantially express other Ca, a subunits, and means for determining the
effect of a test substance on a biological activity of the one or more cells. In some embodiments,
the biological activity is cell proliferation. In other embodiments, the biological activity is
calcium influx. For example, the system may comprise means for determining whether the test
substance decreases proliferation of the one or more cells, including, but not limited to reagents
and agents for determining DNA synthesis, metabolic markers, proliferation markers or ATP
levels.

In the experimental methods, assays and systems of the invention, said one or more cells
advantageously do not substantially express other Ca, calcium channels (comprising other Ca, a
subunits). Said cells may exhibit ectopic Ca,1.3A2/48 calcium channel expression, or may be
genetically modified to exhibit exogenous Ca,1.3A2/48 calcium channel expression. For example,
without limitation, HEK 293 cells exhibiting ectopic and/or exogenous Ca,1.3A22/48 calcium
channel expression may be used in embodiments of the invention.
Other objects, features and advantages of the present invention will become clear from the following description and drawings.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**Figure 1A.** Expression of Ca\textsubscript{v} subunits in NSCLC cell lines. **Figure 1B.** Schematic representation of the altered Ca\textsubscript{v}1.3-like channel from cancer cells.

**Figure 2.** Expression of Ca\textsubscript{v} subunits in NSCLC biopsies vs. normal control cells.

**Figure 3.** Intracellular free calcium measurement using Fura-2-AM in NSCLC A549 cells after addition of Azelnidipine or Nifedipine (10 µM), and in control HEK 293 cells after addition of Nifedipine.

**Figure 4.** Effect of Azelnidipine (top) and Nifedipine (bottom) on NSCLC A549 proliferation *in vitro.*

**Figure 5.** Effect of Azelnidipine on NSCLC A549 tumor growth *in vivo.* **Figure 5A.** Tumor volume (cm\textsuperscript{3}); **Figure 5B.** Tumor weight (gr); **Figure 5C.** Representative tumors of control and Azelnidipine-treated mice.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is directed to calcium channel splice variants, and provides compositions and methods for cancer treatment, diagnosis and research. Specifically, the invention relates to a cancer-specific splice variant of the Ca\textsubscript{v}1.3 \( \alpha \)I subunit, designated Ca\textsubscript{v}1.3A22/48, and to the use of specific inhibitors and modulators of this splice variant, such as Azelnidipine, for the treatment of tumors characterized by Ca\textsubscript{v}1.3A22/48 expression.

The invention is based, in part, on the discovery of differential expression patterns of Ca\textsubscript{v}1.3 variants in normal and malignant cells. A cancer-specific variant, was unexpectedly identified in non-small cell lung cancer carcinoma (NSCLC) cells. The variant, designated Ca\textsubscript{v}1.3 A\textsubscript{22/48}, lacks exons 22 and 48, corresponding to part of the intracellular loop connecting motif 1 and motif 2, and to the extracellular loop- S3-S4 linker, respectively. The invention is further based, in part, on the unexpected discovery, that Ca\textsubscript{v}1.3 A\textsubscript{22/48} expressing cells exhibit strikingly altered pharmacological activities compared to cells expressing neuronal-type Ca\textsubscript{v}1.3. Remarkably, known Ca\textsubscript{v} antagonists, including the dihydropyridines Nifedipine, Nitrendipine, Nicardipine, Nimodipine and Nisoldipine, and the non-dihydropyridines Diltiazem and...
Verapamil, failed to inhibit calcium entry to Ca\textsubscript{\textit{v},1.3A\textsubscript{22/48}} expressing cell lines, and in fact increased intracellular calcium levels. In contradistinction, Azelnidipine surprisingly exhibited no stimulatory effect on the cells and did not induce calcium influx. \textit{In vitro}, Azelnidipine, but not Nifedipine, inhibited proliferation of NSCLC cell lines. Azelnidipine was also unexpectedly found to be a potent and selective anti-cancer agent \textit{in vivo}, capable of reducing tumor volume and weight significantly in a xenograft mouse model of human NSCLC tumors.

Unexpectedly, the effects of Azelnidipine in eradicating established tumors \textit{in vivo} were substantially more pronounced at a low dose of 2 mg/kg than upon increasing the dose administered to 10 mg/kg. In contradistinction, WO 2016/062275 discloses mere cytostatic effects, exhibited at doses of 100-200 mg/kg. Thus, Azelnidipine is herein demonstrated for the first time to be particularly effective in treating Ca\textsubscript{\textit{v},1.3A\textsubscript{22/48}} expressing tumors, enabling reduction of tumor size and volume, and particularly and advantageously at low and well tolerated doses, thus minimizing the risk of hypotension and other adverse effects. Accordingly, therapeutic modalities according to embodiments of the invention provide for enhanced efficacy and safety in cancer management.

Accordingly, the invention provides compositions, methods, systems and kits for therapeutic, diagnostic and experimental applications, as described herein.

**Ca\textsubscript{\textit{v},1.3} variants**

The term Ca\textsubscript{\textit{v},1.3A\textsubscript{22/48}} as used herein refers to polypeptides, and nucleic acids encoding them, of Ca\textsubscript{\textit{v},1.3} variants, characterized by structural and functional properties as described and exemplified herein. A Ca\textsubscript{\textit{v},1.3A\textsubscript{22/48}} transcript is characterized by deletions of exons 22 and 48 (NCBI ACEview numbering; formerly designated exons 22 and 45), corresponding to part of the intracellular loop connecting motif 1 and motif 2, and to the extracellular loop- S3-S4 linker, respectively. Ca\textsubscript{\textit{v},1.3A\textsubscript{22/48}} polypeptides have been found to mediate induction or enhancement of calcium influx in resting non-excitatoty cells, upon incubation with Nifedipine (e.g. at 10 \textmu M), but not Azelnidipine (e.g. at 10 \textmu M). Ca\textsubscript{\textit{v},1.3A\textsubscript{22/48}} polypeptides have also been found to mediate attenuation or inhibition of cell proliferation upon incubation with Azelnidipine (e.g. at 5-50 \textmu M), but not Nifedipine (e.g. at 1.25-50 \textmu M). Ca\textsubscript{\textit{v},1.3A\textsubscript{22/48}} polypeptides have been further found to mediate Azelnidipine-induced anti-tumor effects in a xenograft murine model (e.g. at daily doses of 2 mg/kg and to a lesser extent 10 mg/kg). A Ca\textsubscript{\textit{v},1.3A\textsubscript{22/48}} transcript may have a nucleic acid sequence comprising SEQ ID NO: 1, as set forth hereinbelow, and may further comprise an additional 5’ sequence:
agatagccaacagtgacaacaaagttcacattgtgactatagagaggaagagagagagagatgaact
ccccctcccgcccttggtgtgacccgagtcgctctcctagctgagttgaatgatgaaaacggtgtcc
catcctgaagggagccgctttctccctcattctagcaagaccaacccgatccgcgtagggctcca
caagctcatcaaccacccacatctcctaccaacctccacctctgtgtctctcatctgactgtcgaccaag
ccccctgagagagcccagggacccccatccgacccacctttccccggaaaacgatctggttctttg
actatcctccacagcccatccttactgtgagatcctgttgaagatgacaacttttgagctttcctc
tcccaccaaggggccctctccgaggaactacttcactctcaatttgctgtgatgtgattgtgggtg
tctctgtgtctattggagttcaactcagccatccgctttgtgaaagattttgcaaggcctttaa
ctctccagctcctcagcttttgagggctgttgctgttgatataagccatcgactcgagaatgcag
ccccctgagagagccagcttcgctttttggctaatcgctggtggatgtggggatgttgacagtcctg
ttcggccatccggaccatcggcaacatcatgatcgtcaccaccccctgcagttcatgtttgcct
tggcactgcgtcccctcagggccatcaacagagcaaaaagcttaaaggacagtcttcagtgactgcg
ttcgctggtggttcgttcctcggagccagtaatcggctcctcggagcttcatcagtttttacatgc
tctgtgcatttctgatcatcaatcgtttgtgctgtcatcatggataatttcgactatctgacccggg
actggtctattttggggcctcaccatttagatgaattcaaaagaatggtcagaatatgaccctgag
ccccctgggggtttgggaagttatgtccacacagggtagcgtgcaagagattagttgccatgaaaag
agatagccaacagtgacaacaaagttcacattgtgactatagagaggaagagagagagagatgaact
ccccctcccgcccttggtgtgacccgagtcgctctcctagctgagttgaatgatgaaaacggtgtcc
catcctgaagggagccgctttctccctcattctagcaagaccaacccgatccgcgtagggctcca
caagctcatcaaccacccacatctcctaccaacctccacctctgtgtctctcatctgactgtcgaccaag
ccccctgagagagcccagggacccccatccgacccacctttccccggaaaacgatctggttctttg
actatcctccacagcccatccttactgtgagatcctgttgaagatgacaacttttgagctttcctc
tcccaccaaggggccctctccgaggaactacttcactctcaatttgctgtgatgtgattgtgggtg
tctctgtgtctattggagttcaactcagccatccgctttgtgaaagattttgcaaggcctttaa
ctctccagctcctcagcttttgagggctgttgctgttgatataagccatcgactcgagaatgcag
ccccctgagagagccagcttcgctttttggctaatcgctggtggatgtggggatgttgacagtcctg
ttcggccatccggaccatcggcaacatcatgatcgtcaccaccccctgcagttcatgtttgcct
tggcactgcgtcccctcagggccatcaacagagcaaaaagcttaaaggacagtcttcagtgactgcg
ttcgctggtggttcgttcctcggagccagtaatcggctcctcggagcttcatcagtttttacatgc
tctgtgcatttctgatcatcaatcgtttgtgctgtcatcatggataatttcgactatctgacccggg
actggtctattttggggcctcaccatttagatgaattcaaaagaatggtcagaatatgaccctgag
ccccctgggggtttgggaagttatgtccacacagggtagcgtgcaagagattagttgccatgaaaag
catgcctctcaacagtgcagggcacagtctgtttaatgcaacctgtttgctttt...
A Ca$_1$-A22 polypeptide may have an amino acid sequence comprising SEQ ID NO: 2, as set forth hereinbelow, and may further comprise an additional N’ sequence:

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STSAPPVGSLSQRKRQQYAKSKKQQNNSNSRPARLFCLSLNNPIRRACISIVEWKFDFIFIL
LAIFANCVALAIYIPFDSENSSTNHNLIKVEYAFILIFVTETFLLKIAYGLLHFNAYVRNGW
NLLDFIVIVGLFSVILEQLTKEGGNHSSGSGDFVKALRAFRLRPLRVLVSQVPSLQVVL
NSIJKAMVPLLHALLVFLVIYIYAIIGLELFQGKMHKTCCFADSDIAEDPAPCAFSNGRQ
CTANGTECRSGWGPNNGITNFDPAFAMLTVFQCTIMEGWTDVLYWVNDIAGIEWPFWVYFVSL
IILSGFPVLNLVLGVSGLFSKEKARKARQDFQKLREKQOLEELKGYLDWTQAEDIDPENE
EEEUGEGKRNTSMPTSETESVENTSVGEGENCCSLGCQLAISKSKLRWRWRWFRRRC
AAVKSSTFYVLWIVLVFVLNLTTLI SSEHYNQPDWLTQIQC1IANKWLALFTCEMLVKKMSLQGA
YFVSLNFROFDCVFVCGGGETILVEIEMSPGLISVFVCRLRFKVTWHTLSNLVASSLLN
SMKSIASLLLFLFIYFSLLGMQFLGKFNDFDEQT38RSTFDNFPQALLTVFQIILTGEDWNA
VMYDGIAYGGPSSGMIVCI YFIILFICGNYILLNVLIAIADVNLADASELSNTAQKEEAEEK
REKKIARESKENKKNPKPVNQANSNEKVTIDDREDEEKDPYPDPCDVPVGEHSEEEEEEDEP
EVPAQRPRIISELNMEKEIAPIGAVFSSFIKTNPIRVGCHKLINEHFTNLILVF IMLSSA
ALIAEDPISRTSNTILYGFDYAFIATFVIELRKLMTTFAGLHKAGFRCNYFNLLDMLWGV
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(SEQ ID NO:1, cDNA complementary to corresponding mRNA transcript).
LAMQHYEQSKMFNDAMDILNMVFTGVFTVEMVLKVIAFKPKGYFSDAWNTFDSLIVIGS IDVA LSEADNSEENSRI SITFFRLFRVMRLVKLSRGEIGRTLLWTFIKSFQALPYVALLIALMLFFI Y AVIGMOMFGKVRMONNNQINRNNNFOQFPQAVLLLFRCATGEAWEIMLACLPGLCDPESVDYN PGEETYCNSFAIVYFISFYMLCFALINIFAVAIMDNFDYILTRDSILGPHHLDEFKRIWSEY DPEAKGRIKHLDVTLRLRIQPLLFGKLCPRVACKRLVAMNPLNSDGTVMFNASLFLALVRT ALKIKTEGNLEQANEELRAVIKIKWKTSMKLLDQWPPAGDDEVTVGKYATFLIQYDFRKFK KRKEQGLVGKYPKNTTIALQAGLRTRDIEIRRAI SCDLDQDEPEETKREEEDEVKRNAGA LLGHNVHNVHSDRDSLQQTNTHPRLHVQRPS IPPASDTEKLPFPAGNSVCHNHNNHNSIGK QVPTSTANLLNANMSKAAHGKRPSSGNLEHVSENGHSSKHDREPQRRSSVVRTRYETYIR SDSGSEQLPTICREDFEIHGFYRDPHCLGEQEOYFSEHEECYEDSSSPWTSWRQNYGYSYRPGRNI DSERFRGYYHHPQGFDDESDPVCDQDSRRSPQRRLPFTAPSHRRRSSFNFECLRRSSQEEFPSS PIFHPRTALPLHMQQQIMAVAGLDSSKAKQKYPSPHSSTRSWATPPATPPYRDWFPCYTLIQE VQSEALDQVNGSLPMLRWSYTDIEPDISYRTFPTASPASLTVFSSFRKNKSDQKRSADSLOTVEAVLIS EGLGARYARDPKFVSATKHEIADACLDITDEMESASTLNNVPRANGDVPLSRHQYDELQD FGPGYSDEEPPGRDEEDLADEMICITTL (SEQ ID NO: 2).

Wild-type Ca,1.3, also referred to herein as neuronal-type Ca,1.3, is identified by accession number NM_000720.3 (nucleic acid sequence: SEQ ID NO: 3; amino acid sequence: SEQ ID NO: 4), incorporated herein by reference. Ca,1.3 variants were postulated, for example in accession numbers XM_011534095.1, XM_0151534096.1 and XM_005265448.2, incorporated herein by reference.

Embodiments of the invention are directed to nucleic acid molecules encoding Ca,1.3 variants. The variants according to embodiments of the invention are characterized by deletions of exons 22 and 48, corresponding to part of the intracellular loop connecting motif 1 and motif 2, and to the extracellular loop-S3-S4 linker, respectively. In some embodiments, the nucleic acid molecule comprises a nucleic acid sequence as set forth in SEQ ID NO: 1, as detailed hereinbelow, and typically comprises an additional 5' sequence. In other embodiments, said nucleic acid molecule (e.g. Ca,1.3A22/48 analog or derivative) further comprises at least one additional modification compared to wild-type Ca,1.3 (accession no. NM_000720.3, SEQ ID NO: 3). For example, the molecule may comprise at least one insertion, deletion, substitution or derivatization. In another embodiment, said nucleic acid molecule further comprises at least one additional modification compared to other Ca,1.3 sequences, as detailed herein. Further embodiments of methods and assays described herein relate to variants in which the additional 5'
sequence corresponds to the respective sequence of wild-type Ca\textsubscript{v}1.3 or a Ca\textsubscript{v}1.3 sequence as described herein.

In another embodiment, said nucleic acid molecule encodes for a Ca\textsubscript{v}1.3 variant polypeptide, which exhibits induction or enhancement of calcium influx into cells expressing a calcium channel complex comprising said Ca\textsubscript{v}1.3 variant polypeptide upon incubation with Nifedipine, but not Azelnidipine (e.g. in resting non-excitatory cells, under conditions as exemplified herein). In another embodiment, said nucleic acid molecule encodes for a Ca\textsubscript{v}1.3 variant polypeptide, which exhibits attenuation or inhibition of cell proliferation in cells expressing a calcium channel complex comprising said Ca\textsubscript{v}1.3 variant upon incubation with Azelnidipine, but not Nifedipine (e.g. under conditions as exemplified herein). In another embodiment, said nucleic acid molecule encodes for a Ca\textsubscript{v}1.3 variant polypeptide, capable of arresting tumor growth or reducing tumor size or volume in a xenograft murine model treated by Azelnidipine (e.g. under conditions as exemplified herein).

In another aspect there is provided an isolated or recombinant polypeptide of a Ca\textsubscript{v}1.3 variant, encoded by said nucleic acid molecule. In another embodiment the polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 2. Typically, the polypeptide comprises an additional N' sequence, which may comprise at least one modification compared to known Ca\textsubscript{v}1.3 polypeptides (e.g. to positions 1-326 of the Ca\textsubscript{v}1.3 polypeptide of SEQ ID NO: 4).

In another aspect there is provided a calcium channel complex comprising the Ca\textsubscript{v}1.3 variant polypeptide as described herein. In another aspect there is provided a host cell genetically modified to express an exogenous Ca\textsubscript{v}1.3 variant polypeptide as described herein.

Azelnidipine, Ca\textsubscript{v}1.3A22/48 inhibitors, and compositions thereof

Azelnidipine has the chemical structure 3-l-Benzhydryl-3-azetidinyl 5-isopropyl 2-amino-6-methyl-4-(m-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. The empirical formula of Azelnidipine is C\textsubscript{33}H\textsubscript{34}N\textsubscript{4}O\textsubscript{6} and its molecular weight is 582.65. The structural formula of Azelnidipine is:
Derivatives and salts of Azelnidipine have been described, for example in EP1961751. The Azelnidipine derivative and salts referred to in connection with the compositions and methods of the invention are those known in the art to retain structural and functional properties of Azelnidipine. The derivatives and salts referred to herein do not induce Ca\(^{2+}\) influx in resting Ca\(_{v}1.3\)A\(_2/48\) expressing cells, and retain the ability to inhibit cell proliferation in Ca\(_{v}1.3\)A\(_2/48\) expressing cells, under conditions in which Azelnidipine inhibits cell proliferation in the absence of induction of Ca\(^{2+}\) influx, as described herein. For example, Azelnidipine inhibits proliferation of A549 and H460 cells as measured 24 or 72 hours post incubation at concentrations of 5-50 µM, as described in Example 3. The derivatives and salts used in embodiments of the invention are pharmacologically equivalent with Azelnidipine.

Other embodiments of the invention employ the use of additional compounds, which are specific Ca\(_{v}1.3\)A\(_2/48\) inhibitors. In some embodiments, the inhibitor is an agent capable of specifically inhibiting the activity of a calcium channel Ca\(_{v}1.3\)A\(_2/48\) subunit, e.g. calcium influx and/or cell proliferation, wherein each possibility represents a separate embodiment of the invention. For example, the inhibitor may be an agent (e.g. Ca\(_{v}1.3\)A22/48-specific antibody or small molecule capable of Ca\(_{v}1.3\)A22/48 binding) that does not induce Ca\(^{2+}\) influx in resting Ca\(_{v}1.3\)A22/48 expressing cells, and inhibits cell proliferation in Ca\(_{v}1.3\)A22/48 expressing cells, under conditions in which Azelnidipine inhibits cell proliferation in the absence of induction of Ca\(^{2+}\) influx, as described herein. Specific Ca\(_{v}1.3\)A\(_2/48\) inhibitors typically do not exert these properties in Ca\(_{v}1.3\)-expressing cells, or in cells lacking Ca\(_{v}1.3\) expression (namely wild-type Ca\(_{v}1.3\)).

Azelnidipine or a derivative or salt thereof and inhibitors as described herein (herein referred to as active ingredients) can be administered according to embodiments of the invention in the form of a pharmaceutical composition, further comprising one or more pharmacologically acceptable carriers, excipients or diluents. The purpose of a pharmaceutical composition is to
facilitate administration of a compound to an organism. The pharmaceutical compositions may be formulated by one having ordinary skill in the art. Suitable pharmaceutical carriers, including, but not limited to fillers, disintegrants, lubricants, glidants, and soluble and insoluble polymers are described in Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field, which is incorporated herein by reference.

The pharmaceutical compositions of the invention are suitable for administration systemically or in a local manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient (e.g. intralesional injection).

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water-based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters such as ethyl oleate, triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the active ingredients, to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., a sterile, pyrogen-free, water-based solution, before use.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries as desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, and sodium carboxymethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone
(PVP). If desired, disintegrating agents, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate, may be added.

Pharmaceutical compositions that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

The pharmaceutical compositions of the invention are also useful for topical and intralesional application. As used herein, the term "topical" means pertaining to a particular surface area and the topical agent applied to a certain area of said surface will affect only the area to which it is applied.

Topical pharmaceutical compositions may comprise, without limitation, non-washable (water-in-oil) creams or washable (oil-in-water) creams, ointments, lotions, gels, suspensions, aqueous or cosolvent solutions, salves, emulsions, coated bandages or other polymer coverings, sprays, aerosols, liposomes and any other pharmaceutically acceptable carrier suitable for administration of the drug topically.

As is well known in the art the physico-chemical characteristics of the carrier may be manipulated by addition of a variety of excipients, including but not limited to thickeners, gelling agents, wetting agents, flocculating agents, suspending agents and the like. These optional excipients will determine the physical characteristics of the resultant formulations such that the application may be more pleasant or convenient. It will be recognized by the skilled artisan that the excipients selected, should preferably enhance and in any case must not interfere with the storage stability of the formulations.

In some embodiments, the pharmaceutical composition is formulated for pulmonary administration. In another embodiment the composition is formulated for administration as an aerosol or mist. In another embodiment said composition is formulated for use with a nebulizer or inhaler.

In another aspect the invention provides a pharmaceutical composition comprising as an active ingredient Azelnidipine or a derivative or salt thereof, formulated for topical, intratumoral
or pulmonary administration, wherein each possibility represents a separate embodiment of the invention. In a particular embodiment, there is provided a pharmaceutical composition comprising as an active ingredient Azelnidipine or a derivative or salt thereof, formulated for pulmonary administration. In another embodiment the composition is formulated for administration as an aerosol or mist. In another embodiment said composition is formulated for use with a nebulizer or inhaler. In another embodiment, said composition comprises said Azelnidipine or a derivative or salt thereof at an amount effective to inhibit cell proliferation. In a particular embodiment, said composition comprises Azelnidipine as a sole active ingredient.

Azelnidipine or a derivative or salt thereof can be administered by inhalation in different ways, such as in pressurized metered-dosage inhalers, in dry powder inhalers, in a liquid solution delivered by nebulizer or small volume liquid inhaler, or in a vaporized formulation suitable for inhalation or nasal aspiration. Pressurized metered dose inhalers (pMDIs) containing Azelnidipine or a derivative or salt thereof, alone or in combination with other therapeutics, with propellants, for example, may be formulated to contain Azelnidipine or a derivative or salt thereof in solution or in dispersion in a propellant, such as HFA 134a or HFA227, alone or in combination with excipients to modify aerosol performance, such as co-solvents (e.g. ethanol, glycerol, polyethylene glycols, propylene glycol), surfactants (e.g. oleic acid) or other excipients such as stabilizers and pH modifiers (e.g. ascorbic acid, sodium edetate, hydrochloric acid). Where Azelnidipine or a derivative or salt thereof is presented as a dispersion in pMDIs then appropriate physical and/or chemical methods may be used to ensure that the aerodynamic particle size upon aerosolization is appropriate for delivery to the respiratory airways, typically less than 10 µm and preferably less than 5 µm.

Dry powder inhalers (DPIs) containing Azelnidipine or a derivative or salt thereof, alone or in combination with other therapeutics, for example, may be formulated to contain Azelnidipine or a derivative or salt thereof as small particles, either alone or in combination with a carrier particle such as lactose or sucrose, to aid aerosolization. Appropriate physical and/or chemical methods may be used to ensure that the aerodynamic particle size upon aerosolization from DPIs is appropriate for delivery to the respiratory airways, typically less than 10 µm and preferably less than 5 µm.

Nebulizers and small volume liquid inhaler preparations of Azelnidipine or a derivative or salt thereof, alone or in combination with other therapeutics, for example, may be formulated to contain Azelnidipine or a derivative or salt thereof in solution or in dispersion in an aqueous
medium, alone or in combination with excipients to modify aerosol performance, such as co-solvents (e.g. ethanol, glycerol, polyethylene glycols, propylene glycol), surfactants (e.g. oleic acid), or other excipients such as stabilizers and pH modifiers (e.g. ascorbic acid, sodium edetate, hydrochloric acid). Where Azelnidipine or a derivative or salt thereof is presented as a dispersion in nebulizers and small volume liquid inhalers then appropriate physical and/or chemical methods may be used to ensure that the aerodynamic particle size upon aerosolization is appropriate for delivery to the respiratory airways, typically less than 10 µm and preferably less than 5 µm.

Vaporized formulations of Azelnidipine or a derivative or salt thereof, alone or in combination with other therapeutics, suitable for inhalation, for example, may be formulated by heating Azelnidipine or a derivative or salt thereof to a high temperature for a short time period, typically less than 1 second, alone or in combination with excipients to modify aerosol performance (e.g. propylene glycol, ethanol). The methods used may ensure that the aerodynamic particle size upon aerosolization is appropriate for delivery to the respiratory airways, typically less than 10 µm and preferably less than 5 µm.

In another embodiment, said composition comprises said Azelnidipine or a derivative or salt thereof at an amount effective to inhibit cell proliferation (i.e. when administered to the subject or applied to the tumor or target cell under the appropriate conditions).

For example, as demonstrated herein, Azelnidipine was found to inhibit cell proliferation at concentrations of 5-50 µM in vitro, and to inhibit tumor development at daily doses of 2-10 mg/kg in a murine model in vivo. Accordingly, exemplary Azelnidipine doses for pharmaceutical compositions of the invention may be 5-50 µM for local (e.g. topical, intratumoral/intraleSIONAL or pulmonary) administration. Exemplary Azelnidipine doses for pharmaceutical compositions for systemic (e.g. oral) administration to human subjects may be 0.8-80 mg, 1-50 mg, 5-25 mg, or 1-6 mg, which may be provided in unit dosage form. In a particular embodiment, said composition comprises Azelnidipine as a sole active ingredient.

**Therapeutic and diagnostic methods**

In another aspect, the invention provides a method of treating a subject having a tumor, comprising administering to the subject an effective amount of Azelnidipine or a derivative or salt thereof, thereby treating the subject. In another embodiment, the tumor is characterized by expression of a Ca,1.3 variant. In another embodiment, the Ca,1.3 variant is characterized by deletion of exon 22. In another embodiment, the Ca,1.3 variant is characterized by deletion of
exon 48. In another embodiment, the Ca\textsubscript{v}1.3 variant is characterized by deletion of exons 22 and 48. In another embodiment, said Ca\textsubscript{v}1.3 variant mediates induction or enhancement of calcium influx in resting non-excitatatory cells, upon incubation with Nifedipine, but not Azelnidipine (under the same conditions, e.g. as described herein), mediates attenuation or inhibition of cell proliferation upon incubation with Azelnidipine, but not Nifedipine, and mediates Azelnidipine-induced anti-tumor effects in a xenograft murine model. In another embodiment said Ca\textsubscript{v}1.3 variant is Ca\textsubscript{v}1.3\textsubscript{A22/48}.

In another aspect, the invention is directed to a pharmaceutical composition comprising an effective amount of Azelnidipine or a derivative or salt thereof, for use in treating a subject having a tumor characterized by Ca\textsubscript{v}1.3\textsubscript{A22/48} expression. In another aspect the invention provides a pharmaceutical composition comprising an effective amount of Azelnidipine or a derivative or salt thereof, for use in inhibiting the proliferation of cells that exhibit Ca\textsubscript{v}1.3\textsubscript{A22/48} expression.

The subject to be treated by the methods and compositions of the invention is a mammalian and preferably a human subject. Unless indicated otherwise, the preferable treatment of human subjects is contemplated.

In another aspect, the invention provides a method of treating a subject having a tumor, preferably a tumor characterized by Ca\textsubscript{v}1.3\textsubscript{A22/48} expression, the method comprising administering to the subject an effective amount of Azelnidipine or a derivative or salt thereof, thereby treating the subject. In a particular embodiment, the method comprises administering an effective amount of Azelnidipine to said subject, thereby treating said subject.

In another aspect, the invention provides a method of treating a tumor characterized by Ca\textsubscript{v}1.3\textsubscript{A22/48} expression in a subject in need thereof, comprising administering to the subject an effective amount of Azelnidipine or a derivative or salt thereof, thereby treating the tumor. In another aspect, there is provided a method to inhibit the proliferation of cells that exhibit Ca\textsubscript{v}1.3\textsubscript{A22/48} expression, comprising contacting the cells with an effective amount of Azelnidipine, or a derivative or salt thereof.

In another embodiment of the methods of the invention, the tumor is a solid tumor, including, but not limited to a lung tumor and a breast tumor. For example, the tumor to be treated by the compositions and methods of the invention may be a solid tumor derived from non excitable cells such as tumors of epithelial or fibroblast origin. In a particular embodiment, said tumor is a carcinoma. In another particular embodiment, said tumor is a lung carcinoma. In a further particular embodiment, said tumor is a NSCLC tumor. In another particular
embodiment said tumor is a breast ductal carcinoma. In another embodiment the tumor is derived from excitable cells such as neurons. In another embodiment the tumor is of hematopoietic origin. Each possibility represents a separate embodiment of the invention. According to other embodiments of the invention, the compositions and methods are used for treating an established tumor in said subject (an existing tumor, detectable by conventional methods used in the art), e.g. by reducing tumor size and/or volume.

In another particular embodiment, the method is used for the treatment of non-hormone dependant tumors (e.g. other than estrogen-dependent or androgen dependant tumors). In a particular embodiment said tumor is other than a prostate tumor, endometrial tumor, colon tumor or a hematopoietic tumor. Each possibility represents a separate embodiment of the invention.

In another embodiment, Azelnidipine or the derivative or salt thereof is administered orally. In another embodiment, Azelnidipine or the derivative or salt thereof is administered by topical, intratumoral of pulmonary administration, wherein each possibility represents a separate embodiment of the invention. In a particular embodiment, said Azelnidipine or derivative or salt thereof is administered topically. In another embodiment, the tumor is derived from (or located in) a tissue or organ lacking Ca$_{\beta}1.3$ expression. In yet another embodiment, the tumor is derived from (or located in) a tissue or organ expressing neuronal type Ca$_{\beta}1.3$. Thus, the compositions and methods of the invention provide for effective cancer therapy with enhanced safety, as they enable localized treatment of a tumor with minimized damage to its surrounding tissue.

In another embodiment, the methods of the invention employ the use of low Azelnidipine doses, thus providing additional safety by minimizing side effects associated with excessive Azelnidipine doses. Thus, according to some embodiments, the methods of the invention comprise administering to the subject (preferably human subject) an effective amount of Azelnidipine selected from the group consisting of 0.01-1 mg/kg/day, preferably 0.05-0.5, 0.1-0.3, 0.01-0.1, or 0.05-0.075 mg/kg/day, or an equivalent amount of a derivative or salt thereof. In certain embodiments, the use of low Azelnidipine doses of 0.05-0.5, 0.1-0.3, 0.01-0.1, or 0.05-0.075 mg/kg/day is contemplated for providing enhanced efficacy and safety.

In another embodiment the method further comprises identifying the subject as being afflicted with a Ca$_{\beta}1.3$A$_{22/48}$ expressing tumor, and administering Azelnidipine or a derivative or salt thereof to said subject afflicted with the Ca$_{\beta}1.3$A$_{22/48}$ expressing tumor. Identifying the subject as being afflicted with a Ca$_{\beta}1.3$A$_{22/48}$ expressing tumor may be performed by various
methods of determining the expression of Ca\textsubscript{v}1.3A\textsubscript{22/48} in a biological sample of said subject, as described herein.

In another aspect, there is provided a method of treating a subject having a tumor characterized by Ca\textsubscript{v}1.3A\textsubscript{22/48} expression, comprising administering to the subject an effective amount of a specific Ca\textsubscript{v}1.3A\textsubscript{22/48} inhibitor, thereby treating said subject. In some embodiments, the inhibitor is an agent capable of specifically inhibiting the activity of a calcium channel Ca\textsubscript{v}1.3A\textsubscript{22/48} subunit, e.g. calcium influx and/or cell proliferation. In another embodiment the inhibitor is Azelnidipine or a derivative or salt thereof. In a particular embodiment, the specific Ca\textsubscript{v}1.3A\textsubscript{22/48} inhibitor is Azelnidipine.

Other embodiments of the invention are directed to diagnostic methods and assays. In another aspect, the invention provides a method of identifying a subject amenable for treatment by Azelnidipine or a derivative or salt thereof, comprising determining the expression of Ca\textsubscript{v}1.3A\textsubscript{22/48} in a biological sample of the subject, wherein expression of Ca\textsubscript{v}1.3A\textsubscript{22/48} in the sample indicates that the subject is amenable for treatment by Azelnidipine or a derivative or salt thereof. In another aspect, the invention provides a method of determining whether a subject is afflicted with, or is at risk for developing, a Ca\textsubscript{v}1.3A\textsubscript{22/48} expressing tumor, comprising determining the expression of Ca\textsubscript{v}1.3A\textsubscript{22/48} in a biological sample of the subject, wherein expression of Ca\textsubscript{v}1.3A\textsubscript{22/48} in the sample indicates that the subject is afflicted with, or is at risk for developing, a Ca\textsubscript{v}1.3A\textsubscript{22/48} expressing tumor, and wherein each possibility represents a separate embodiment of the invention.

As used herein, "determining the expression of Ca\textsubscript{v}1.3A\textsubscript{22/48} in a biological sample" refers to detecting the presence or amount of a Ca\textsubscript{v}1.3A\textsubscript{22/48} polypeptide or transcript in the sample, so as to determine whether a cell, organ or tissue from which the sample is derived (or obtained) is characterized by Ca\textsubscript{v}1.3A\textsubscript{22/48} expression (e.g. a tumor characterized by Ca\textsubscript{v}1.3A\textsubscript{22/48} expression).

A Ca\textsubscript{v}1.3A\textsubscript{22/48} expressing tumor, also referred to herein as a tumor characterized by Ca\textsubscript{v}1.3A\textsubscript{22/48} expression, denotes a tumor characterized by the presence (e.g. cell surface expression) of Ca\textsubscript{v}1.3A\textsubscript{22/48} polypeptide in at least a part of the tumor cells.

A "biological sample of the subject" refers to a sample, typically a cell-containing sample, obtained from the subject. In some embodiments, the sample is obtained from a tissue or organ of interest. For example, biopsies from suspected tumors or lesions may be obtained by conventional methods. In other embodiments, the sample may be a fluid sample, typically a cell-containing fluid sample, e.g. a blood sample, urine sample, saliva sample, or tumor rinse sample,
which may optionally be processed (e.g. by centrifugation) to enrich their relative cell content. In some embodiments, the sample is obtained in a non-invasive manner (e.g. milk samples, urine samples, saliva samples or stool samples). The sample may then be further processed by lysing and purification of protein and/or RNA. Methods for obtaining and processing biological samples are known to those of skill in the art.

In other embodiment, determining the expression of Ca$_v$1.3A$_{22/48}$ in the sample may employ a variety of immunoassays (e.g. enzyme-linked immunosorbent assay, ELISA) and other molecular biology assays (e.g. reverse transcription polymerase chain reaction, RT-PCR) for detecting and determining the expression of a Ca$_v$1.3A$_{22/48}$ polypeptide or transcript.

By means of a non-limiting example, the methods of the invention may involve immunoassays such as Western blot or ELISA using antibodies directed to Ca$_v$1.3A$_{22/48}$ (capable of specifically binding and differentiating between different Ca$_v$1.3 variants) and/or antibodies directed to the intracellular loop connecting motif 1 and motif 2, or to the extracellular loop- S3-S4 linker, or assays based on dipstick technology or antibody array. In some embodiments, the methods of the invention are suitable for automated or semi-automated analysis, and may enable clinical, medium or high-throughput screening of multiple samples. For example, automated ELISA systems such as Biotest’ s Quickstep® ELISA Processor, Maxmat Automated microwell ELISA analyzer (Maxmat S.A., France), or DSX™ Four-Plate System (Dynex Technologies) may conveniently be used. Other assays comprising Ca$_v$1.3A$_{22/48}$ detection by microscopy or cell cytometry (e.g. fluorescence-activated cell sorting, FACS, using suitable antibodies as described above) may be employed. Such techniques are well known to the ordinarily skilled artisan and have been described in many standard immunology manuals and texts.

In addition, various amplification methods, which are sensitive enough to detect to minute amounts of RNA, can also be used to determine whether the tumor or cell expresses Ca$_v$1.3A$_{22/48}$. Such methods include, without limitation, PCR, RT-PCR and in situ PCR (all the above referring also to "nested" PCR, and nested RT-PCR), LCR (ligase chain reaction) and 3SR (self sustained sequence replication). In accordance with a preferred embodiment RT-PCR and nested RT-PCR are used. The amplification products are identified by methods used in the art such as by separation on a gel. Alternatively, if a sufficient quantity of the appropriate cells can be obtained, standard RNA analysis (e.g., Northern analysis, RNase protection, or primer extension) can be performed to determine the level of mRNA expression of the gene of interest.
In another embodiment, the diagnostic methods of the invention further comprise the step of administering a therapeutically effective amount of Azelnidipine or a derivative or salt thereof to the subject exhibiting expression of Ca_{v} 1.3A_{22/48} in said sample (a subject determined to be amenable for treatment by Azelnidipine or a derivative or salt thereof, or to be afflicted with, or is at risk for developing, a Ca_{v} 1.3A_{22/48} expressing tumor, as described herein).

In another aspect there is provided a kit for determining if a subject is amenable for treatment by Azelnidipine or a derivative or salt thereof, comprising means for determining the expression of Ca_{v} 1.3A_{22/48} in a biological sample of a subject. For example, without limitation, the kit may comprise one or more antibodies, PCR primers or other reagents that may be employed in various immunoassays and other molecular biology assays known in the art. Such reagents, e.g. antibodies, primers or probes, may be generated based on the reported sequences of Ca_{v} 1.3 variants, as described herein. For example, without limitation, PCR primers may be directed to Ca_{v} 1.3 exons 22 or 48, and to control sequences from other parts of the Ca_{v} 1.3 transcript. Non-limitative examples for methods and means for determining the expression of Ca_{v} 1.3A_{22/48} in biological samples are provided in the Examples section herein. In another embodiment, the kit may further comprise instructions for administering Azelnidipine or a derivative or salt thereof to a subject exhibiting expression of Ca_{v} 1.3A_{22/48} in the sample.

**Drug screening**

Yet other embodiments of the invention are directed to experimental methods and assays, useful for the screening, identification and selection of drug candidates or other pharmacological agents. Thus, in another aspect, there is provided a method for screening of candidate drugs for cancer therapy, which comprises contacting one or more cells that exhibit Ca_{v} 1.3A_{22/48} calcium channel expression with a test substance, and determining whether the test substance decreases proliferation of the one or more cells, whereby a test substance that decreases proliferation is identified as a candidate drug for cancer therapy.

In another aspect the invention provides a method for identifying an anti-proliferative agent, which comprises contacting one or more cells that exhibit Ca_{v} 1.3A_{22/48} calcium channel expression with a test substance, and determining whether the test substance decreases proliferation of the one or more cells, whereby a test substance that decreases proliferation is identified as an anti-proliferative agent.

In another aspect the invention provides a method for identifying Ca_{v} 1.3A_{22/48} modulators, which comprises contacting one or more cells that exhibit Ca_{v} 1.3A_{22/48} calcium
channel expression with a test substance, determining the effect of said test substance on the biological activity of said $Ca_{\alpha}1.3A_{22/48}$ calcium channel, and selecting those substances which show a significant effect on said biological activity. In one embodiment, the biological activity is calcium influx. In another embodiment the biological activity is cell proliferation. In another embodiment, the effect is an inhibitory effect. In another embodiment, the effect is a stimulatory effect.

In another aspect there is provided a system for screening of candidate drugs for cancer therapy. The system comprises one or more cells that exhibit $Ca_{\alpha}1.3A_{22/48}$ calcium channel expression, and means for determining the effect of a test substance on a biological activity of the one or more cells. In some embodiments, the biological activity is cell proliferation. In other embodiments, the biological activity is calcium influx. For example, the system may comprise means for determining whether the test substance decreases proliferation of the one or more cells, including, but not limited to reagents and agents for determining DNA synthesis, metabolic markers, proliferation markers or ATP levels.

In the experimental methods, assays and systems of the invention, said one or more cells advantageously do not substantially express other $Ca_{\alpha}$ calcium channels (comprising other $Ca_{\alpha}$ subunits). Said cells may exhibit ectopic $Ca_{\alpha}1.3A_{22/48}$ calcium channel expression, or may be genetically modified to exhibit exogenous $Ca_{\alpha}1.3A_{22/48}$ calcium channel expression. For example, without limitation, HEK 293 cells exhibiting ectopic or exogenous $Ca_{\alpha}1.3A_{22/48}$ calcium channel expression may be used in embodiments of the invention.

The following examples are presented in order to more fully illustrate some embodiments of the invention. They should, in no way be construed, however, as limiting the broad scope of the invention.

**EXAMPLES**

**Example 1. Expression of $Ca_{\alpha}1.3$ calcium channels in NSCLC cell lines.**

$Ca_{\alpha}1\beta$ subunit expression was tested in A549 and H460 cells using semi quantitative RT-PCR. High expression of $Ca_{\alpha}1.3$ channels was detected in A549 and H460 cell lines (Fig 1A). Sequence analysis of the 6Kbp cDNA of $Ca_{\alpha}1.3$, revealed that NSCLC A549 and H460 cells, express a variant protein lacking exons 22 and 48 (Fig 1B bottom panel; NCBI ACEview numbering). The cDNA encoding the variant protein, herein designated $Ca_{\alpha}1.3A_{22/48}$, comprises the nucleic acid sequence as set forth in SEQ ID NO: 1, and an additional 5’ section.
In the experiments of Figure 1A, total RNA was extracted from NSCLC A549 and H460 and breast cancer cell line and analyzed for the expression of Ca₉₁₃ subunits. GAPDH and HPRT were used as internal controls. A plasmid containing Ca₉₁₃ subunit was used as positive control for Ca₉₁₃ primers. Results are representative of at least three experiments. In Figure 1B, transmembrane segments are white boxes, except the S4 voltage sensor domain, which is shown in gray.

To confirm these results in primary NSCLC cancer cells, NSCLC tumor biopsies were obtained from two patients, as well as biopsies of their surrounding normal lung tissue. Total RNA was extracted and expression of Ca₉₁₃ was determined using semi quantitative RT-PCR. Interestingly, a Ca₉₁₃-like channel was found to be expressed in both normal and NSCLC tumor tissues (Fig 2 top two rows), however the expression of exons 22 or 48 could not be detected in the tumor biopsies (Fig 2 rows 3 and 4 from top, respectively). These data corroborate the findings in NSCLC cell lines above.

In Figure 2, total RNA was extracted from normal lung or cancerous tissue biopsies and analyzed for the expression of Ca₉₁₃ channel. HPRT was used as internal control. Results are shown for a single patient and are representative of two patients analyzed altogether. The PCR for exons 22 or 48 was designed to include one primer within the designated exon, thus lack of bands indicate lack of exon expression. Results were confirmed by sequencing the bands.

**Example 2. The Ca₉ antagonist Nifedipine increases calcium influx to NSCLC cell lines.**

Since a Ca₉₁₃ variant was found to be highly expressed in NSCLC, known Ca₉ antagonists were tested for calcium entry inhibition. Resting intracellular calcium levels were measured in cancer cell lines using a dual wavelength ratiometric fluorescence imaging system and Fura-2 AM (Fura-2-acetoxymethyl ester, a ratiometric calcium indicator).

Surprisingly, addition of Nifedipine increased intracellular calcium levels in both NSCLC A549 and H460 cells (Fig 3 top shows a typical response using 10µM Nifedipine). This increase in calcium entry after addition of Nifedipine was dose dependent, however significant calcium entry was already detected using as low as 500nM Nifedipine. Higher doses of Nifedipine caused higher increase of calcium entry. Results in Figure 3 show the average of 11 individual cells recorded in a single experiment (Fig 3 top). This experiment was repeated at least five times. 293 cells were used as negative controls since they do not express any
detectable levels of Ca\(_2\) channels and do not respond to Nifedipine (Fig 3 bottom; an average of 10 cells is shown).

In Figure 3, representative experiments are shown as average of 10-15 cells. Ionomycin ionophor stimulation of calcium entry was used as positive control. Calcium was removed from the medium at the end of each experiment via addition of EGTA as negative control. 293 HEK cells, which do not express Ca\(_2\) channels were used as control to show that they do not respond to Nifedipine (10\(\mu\)M). Calculation of the absolute calcium concentration was performed using a standard calcium curve. Results are representative of at least three independent experiments. The experiment was repeated at least 5 times independently. Similar results were obtained with H460 NSCLC and MCF-7 breast cancer cell lines.

The effects of other Dihydropyridines (DHPs) on calcium entry to NSCLC were further tested, including the dihydropyridines Nitrendipine, Nicardipine, Nimodipine and Nisoldipine, and the non-dihydropyridines Diltiazem and Verapamil. The results were in line with those obtained with Nifedipine, as the DHPs did not reduce increased intracellular calcium levels but rather induced calcium entry into the cells.

**Example 3. Azelnidipine is a unique inhibitor of NSCLC growth in vitro and in vivo.**

Surprisingly, unlike Nifedipine or other tested DHPs, Azelnidipine did not stimulate calcium entry to A549 cells (Fig 3 middle panel; average of 10 individual cells) or H460. As positive control Ionomycin was added to show the cells are alive in the culture but are not stimulated by Azelnidipine (Fig 3 middle panel). In fact, Azelnidipine, at 5\(\mu\)M or above, inhibited A549 and H460 proliferation in vitro compared to Nifedipine using XTT proliferation assay (Fig 4). Results are representative of three independent experiments. 293 cells were used as negative control.

In Figure 4, A549 cells were plated with various doses of Nifedipine (bottom) or Azelnidipine (top) at indicated dosage and proliferation was measured after 24 (grey bars) or 72 hours (black bars) by XTT proliferation kit (biological industries, Cat# 20-300-1000). a, b, c, d, p Value= 0.002, 0.0002, 0.0001, 0.00004, respectively. These results are representative of three independent experiments. Similar results were obtained with H460 and MCF-7 cell lines.

The inhibition potential of Azelnidipine was subsequently tested in vivo using a xenograft mouse model, in which NSCLC cell lines are subcutaneously grafted in immunodeficient mice. Following the subcutaneous transfer of NSCLC cells (A549 cell line) and tumor growth, the mice were treated with various doses of Azelnidipine and tumor
development in treated or untreated animals was followed. Figure 5 summarizes the results of three independent experiments performed. Significant inhibition of tumor volume (Fig 5A and C) and tumor weight (Fig 5B) was observed after daily intra-peritoneal administration of 2 or 10 mg/kg Azelnidipine.

In Figure 5, five million A549 cells per animal were injected subcutaneously and tumor formation was followed. Tumor size was measured under the skin using a caliper and volume calculated according the formula W2*L*3.14/6 (shown in Figure 5A). A sizable tumor of 0.3-0.4 cm³ was observed within 4 days after transfer of cells. At this point daily injections (indicated by a black arrow) of Azelnidipine at various doses (of 2 or 10 mg/kg) were initiated (or of vehicle solution as control). 16 animals were used altogether in three independent experiments. The experiments were terminated when tumors reached -1.5 cm³ due to humane reasons. Tumors were then filmed and their weight was measured (shown in Figure 5C and Figure 5B, respectively). Average results are calculated and shown from three independent experiments.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying current knowledge, readily modify and/or adapt for various applications such specific embodiments without undue experimentation and without departing from the generic concept, and, therefore, such adaptations and modifications should and are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments. It is to be understood that the phraseology or terminology employed herein is for the purpose of description and not of limitation. The means, materials, and steps for carrying out various disclosed functions may take a variety of alternative forms without departing from the invention.
CLAIMS

1. A method of treating a subject having a tumor characterized by Ca₅₁.₃₈₂₂/₄₈ expression, comprising administering to the subject an effective amount of Azelnidipine or a derivative or salt thereof, thereby treating said subject.

2. The method of claim 1, which comprises administering an effective amount of Azelnidipine to said subject, thereby treating said subject.

3. The method of claim 1, wherein said tumor is a solid tumor.

4. The method of claim 3, wherein said tumor is a carcinoma.

5. The method of claim 4, wherein said tumor is a lung carcinoma.

6. The method of claim 5, wherein said tumor is a non small cell lung carcinoma (NSCLC).

7. The method of claim 1, wherein treating said subject comprises reducing tumor size and/or volume in said subject.

8. The method of claim 1, wherein Azelnidipine or the derivative or salt thereof is administered orally.

9. The method of claim 1 wherein Azelnidipine or the derivative or salt thereof is administered by topical, intratumoral or pulmonary administration.

10. A method according to claim 1 which comprises: identifying the subject as being afflicted with a Ca₅₁.₃₈₂₂/₄₈ expressing tumor, and administering Azelnidipine or a derivative or salt thereof to said subject afflicted with the Ca₅₁.₃₈₂₂/₄₈ expressing tumor.

11. The method of claim 2, wherein the subject is human and the effective amount is 0.05-0.5 mg/kg/day.

12. A method to inhibit the proliferation of cells that exhibit Ca₅₁.₃₈₂₂/₄₈ expression, comprising contacting the cells with an effective amount of Azelnidipine, or a derivative or salt thereof.

13. The method of claim 12, wherein the cells are tumor cells.

14. The method of claim 13, wherein said cells are NSCLC cells.

15. The method of claim 13, the effective amount is 5-50 µM.
16. A pharmaceutical composition comprising as an active ingredient Azelnidipine or a derivative or salt thereof, formulated for topical, intratumoral or pulmonary administration.

17. The composition of claim 16, formulated for pulmonary administration.

18. The composition of claim 17, formulated for administration as an aerosol or mist.

19. The composition of claim 17, formulated for use with a nebulizer or inhaler.

20. The composition of claim 16, comprising Azelnidipine as a sole active ingredient.

21. The composition of claim 16, comprising Azelnidipine at a concentration of 5-50 µM.

22. The composition of claim 16, comprising 1-50 mg Azelnidipine in unit dosage form.

23. A method of identifying a subject amenable for treatment by Azelnidipine or a derivative or salt thereof, comprising determining the expression of Ca_{1.3A_{22/48}} in a biological sample of the subject, wherein expression of Ca_{1.3A_{22/48}} in the sample indicates that the subject is amenable for treatment by Azelnidipine or a derivative or salt thereof.

24. The method of claim 23, wherein the sample is a cell sample, a tissue sample, or a fluid sample.

25. The method of claim 24, wherein said sample is a tumor sample.

26. The method of claim 23, further comprising the step of administering a therapeutically effective amount of Azelnidipine or a derivative or salt thereof to the subject identified as being amenable for treatment.

27. A method of determining whether a subject is afflicted with, or is at risk for developing, a Ca_{1.3A_{22/48}} expressing tumor, comprising determining the expression of Ca_{1.3A_{22/48}} in a biological sample of the subject, wherein expression of Ca_{1.3A_{22/48}} in the sample indicates that the subject is afflicted with, or is at risk for developing, a Ca_{1.3A_{22/48}} expressing tumor.

28. The method of claim 27, wherein the sample is a cell sample, a tissue sample, or a fluid sample.

29. The method of claim 28, wherein said sample is a tumor sample.
30. The method of claim 27, further comprising the step of administering a therapeutically effective amount of Azelnidipine or a derivative or salt thereof to the subject determined to be afflicted with, or is at risk for developing, a Ca\textsubscript{v}1.3A\textsubscript{22/48} expressing tumor.

31. A method for screening of candidate drugs for cancer therapy, which comprises contacting one or more cells that exhibit Ca\textsubscript{v}1.3A\textsubscript{22/48} calcium channel expression and do not substantially express other Ca\textsubscript{v} subunits with a test substance, and determining whether the test substance decreases proliferation of the one or more cells, whereby a test substance that decreases proliferation is identified as a candidate drug for cancer therapy.

32. A system for screening of candidate drugs for cancer therapy, comprising one or more cells that exhibit Ca\textsubscript{v}1.3A\textsubscript{22/48} calcium channel expression and do not substantially express other Ca\textsubscript{v} subunits, and means for determining whether the test substance decreases proliferation of the one or more cells.

33. A kit for determining if a subject is amenable for treatment by Azelnidipine or a derivative or salt thereof, comprising means for determining the expression of Ca\textsubscript{v}1.3A\textsubscript{22/48} in a biological sample of a subject, and instructions for administering Azelnidipine or a derivative or salt thereof to a subject exhibiting expression of Ca\textsubscript{v}1.3A\textsubscript{22/48} in the sample.
**Figure 1A**

![Image of gel electrophoresis showing bands for Cav 1.1, Cav 1.3, GAPDH, and HPRT in NSCLC cell lines H460, A549, Cav 1.1, and Plasmid control.]

* Bands were sequenced

**Figure 1B**

![Diagram illustrating Exon 22, Motif I, Motif II, Exon 48, Motif III, and Motif IV in Cancer Ca, 1.3.]
Patient #1

<table>
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<th>Normal</th>
<th>NSCLC</th>
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- Ca\textsubscript{v}1.3 N-terminus (1.4 Kb band)
- Ca\textsubscript{v}1.3 C-terminus (1.1 Kb band)
- Ca\textsubscript{v}1.3 expression of Exon 22
- Ca\textsubscript{v}1.3 expression of Exon 48
- HPRT

Figure 2
Figure 3
Figure 4
Figure 5A

Tumor Volume (cm³)

Days post injection of A549 lung cancer cells

- cont
- Azelnidipine 2mg/kg
- Azelnidipine 10mg/kg

Figure 5B

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<th>Treatment</th>
<th>Tumor Weight (g)</th>
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<td>Control</td>
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<td>Azelnidipine 2mg/kg</td>
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<td>Azelnidipine 10mg/kg</td>
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Figure 5C

Mouse #:

1. Control
2. Azelnidipine 2mg/kg

Scale: 5mm
### A. CLASSIFICATION OF SUBJECT MATTER

| IPC (2017.01) | A61K 3/1442700, A61P 35/00 |

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

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

| IPC (2017.01) | A61P, A61K |

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

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C. 

See patent family annex.

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* Special categories of cited documents:
  - "A" document defining the general state of the art which is not considered to be of particular relevance
  - "E" earlier application or patent but published on or after the international filing date
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  - "P" document published prior to the international filing date but later than the priority date claimed

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Date of the actual completion of the international search: 15 Jun 2017

Date of mailing of the international search report: 18 Jun 2017

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Authorized officer
HERMAN Karin

Telephone No. 972-2-5651749

Form PCT/ISA/210 (second sheet) (January 2015)
1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. [X] forming part of the international application as filed:
      [ ] on paper or in the form of an image file.
   b. [ ] furnished together with the international application under PCT Rule liter. 1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:
      [ ] in the form of an Annex C/ST.25 text file (Rule liter. 1(a)).
      [ ] on paper or in the form of an image file (Rule liter. 1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
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B. FIELDS SEARCHED:

* Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
- Databases consulted: BLAST, PATENTSCOPE, THOMSON INNOVATION, Esp@cenet, Google Patents, CAPLUS, BIOSIS, REGISTRY, PubMed, Google Scholar, PatBase
- Search terms used: non-small cell lung cancer carcinoma, NSCLC, Dihydropyridine, CS-905, splice variant of the Cav.3 alpha 1 subunit, Cav.3, Azelnidipine, Calcium Channel Blockers, CalBlock, Cav.3 21/48, exon 22, exon 48

Form PCT/ISA/210 (extra sheet) (January 2015)