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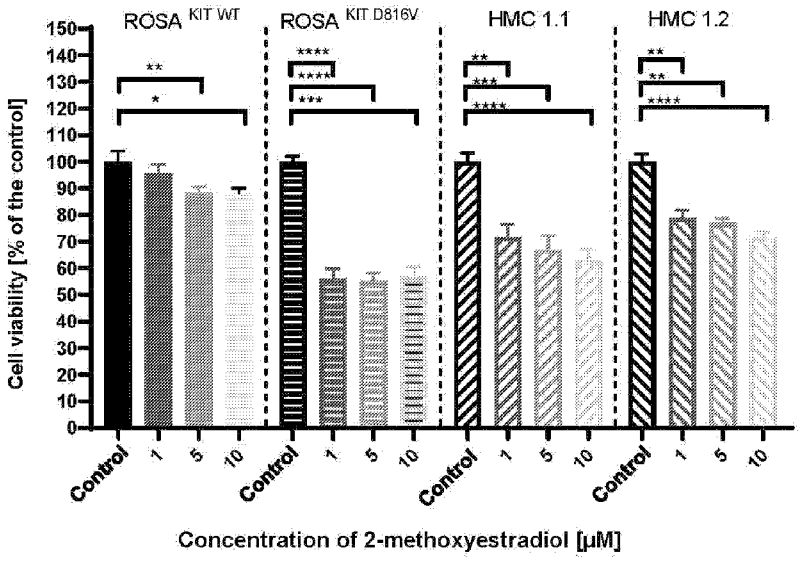
(54) Title: USE OF 2-METHOXYESTRADIOL IN THE TREATMENT OF MASTOCYTOSIS

[Fig. 1]



(57) Abstract: The invention concerns 2-methoxyestradiol to be used in the treatment of mastocytosis, in particular its skin form and/or systemic form.

[Continued on next page]

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Description

Title of Invention : Use of 2-methoxyestradiol in the treatment of mastocytosis

Field of the invention

[0001] The invention concerns use of 2-methoxyestradiol in the treatment of mastocytosis.

State of the art

[0002] Mastocytosis is a heterogenic group of diseases related to pathological accumulation of mastocytes (mast cells, hereinafter referred to as MC or MCs) in various organs, most frequently in bone marrow and skin. The main molecular cause of the disease is somatic mutation pD816V of the c-KIT gene coding the membrane receptor for the stem cell growth factor (stem cell factor, hereinafter referred to as SCF). The mutation makes the MC activation independent of SCF, as well as makes the cells oversensitive to the action of stimulating factors and inhibits their apoptosis process. The pD816V mutation is observed in more than 90% of adult patients suffering from a systemic form of mastocytosis, and in approximately 50% of sick children.

[0003] Neoplastic MCs which infiltrate tissues in the course of this disease demonstrate atypical morphology and changed immunophenotype (presence of the CD4 and CD25 antigens on the surface), where the features constitute important diagnostic criteria. Mastocytosis is characterised by a broad spectrum of clinical forms related to the MC's release of inflammatory reaction mediators, proteolytic enzymes which degrade tissues, growth factors, cytokines, and chemokines [1–3]. The skin symptoms include maculopapular rash with the Darier's symptom typical for the disease, itching, and the formation of blisters. Mastocytosis is also related to the risk of occurrence of anaphylaxis, osteoporosis, and aggressive symptoms related to the impairment of internal organ functions [4–6].

[0004] Described in the patent applications submitted to date were different ways of inhibiting the activity of mast cells (mastocytes) and treating diseases triggered by

their activation, none of them, however, concerns the action of steroid hormone derivatives on those cells.

[0005] Disclosed in international invention application WO2014188423A1 are methods of inhibiting activation of a mast cell and/or treating the induced disease by contacting a neoplastic mast cell with an effective amount of a multivalent compound which binds and activates membrane protein, i.e. the Siglec-7 lectin (Sialic acid-binding Ig-like lectin 7), thus inhibiting activation of the neoplastic mast cell.

[0006] Patent PL201879B has disclosed that hybrid protein binds to (i) mast cells and/or basophils and/or is absorbed thereby and (ii) binds to a protease which splits the proteins of the secretory apparatus of stem cells or basophils. Hybrid protein is composed of IgE or its fragment Fc or an antibody against the IgE receptor of the mast cells and/or basophils, as well as of MCD (inactive but peptide-binding) and proteases. The protein according to the patent application may be used to produce a medicament blocking mast cell degranulation.

[0007] Disclosed in patent EP1410802B1 is a fusion protein which inhibits growth of mastocytes and induces their apoptosis. Fusion protein contains protein transduction domain (PTD) and a variant of the microphthalmia-associated transcription factor MITF. Reduction of the number of mastocytes was evidenced with reduced level of histamine and reduced activity of an MC enzyme, chymase.

[0008] Described in patent EP95909451B1 is a new medicament blocking the activity of mastocytes and being a heterocyclic H1 histamine receptor. The preparation may be potentially used in induced diseases caused by mastocyte degranulation, such as irritable bowel syndrome, abdominal migraine, food allergy, and other diseases related to mastocyte activation.

[0009] A still unexplained problem concerning the pathogenesis of mastocytosis is the clinically observed regression, sometimes full, particularly in puberty, occurring in most sick children. In some of the adult patients, on the other hand, suffering from the chronic form of the disease, it transforms into an aggressive form (agressive systemic mastocytosis - ASM).

[0010] Although the tendency to spontaneous regression of the disease observed in children around puberty might suggest an impact of sex hormones, no research

team has so far demonstrated or proved what hormone might potentially affect the process.

[0011] In the early stages of research on mastocytosis pathogenesis there were no appropriate in vitro cell models available for the disease.

[0012] A solution to the problem came with isolation of two long-lived human MC cell lines: HMC 1.1 and HMC 1.2 from a patient with mast cell leukemia, and experimental introduction of two ROSA cell lines. The ROSA lines were obtained following transformation of the MCs isolated from umbilical blood using lentivirus containing the p.D816V KIT mutation in its genome. The lines enabled an effective search for candidate medicaments to treat and monitor medical conditions, such as allergy, inflammation, autoimmunological disease, or mastocytosis.

[0013] Characteristics of the HMC and ROSA lines

[0014] The difference between the HMC lines comes down to the different KIT gene mutations contained therein. The HMC-1.1 line contains the p.V560G KIT mutation in its cells, while the HMC-1.2 line is characterized by the presence of two KIT gene mutations: p.V560G and p.D816V.

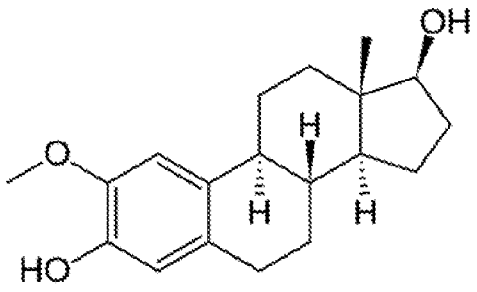
[0015] The ROSA KIT pD816V line contains the p.D816V mutation, while the ROSA KIT WT line contains no mutation of the KIT gene [21–23]. In addition, information on the ROSA line was disclosed in patent EP2773749B, and cell lines were deposited with CNCM (French National Collection of Cultures of Microorganisms) on 02/11/2011 under numbers: CNCM- 1-4551 ROSA-KIT WT; CNCM-1-4552 ROSA KIT D816V; CNCM 1-4553 ROSA KIT Delta 417-419 insY .

[0016] The authors of the invention conducted a number of research studies intended to develop an effective mastocytosis therapy based on hormonal preparations using the cell lines described above. Unexpectedly it turned out that one of the female sex hormones is a promising candidate to be used as a medicament in mastocytosis therapy.

Detailed description of the invention

[0017] The invention concerns 2-methoxyestradiol to be used in the treatment of mastocytosis, especially its skin and/or systemic form.

[0018] 2-methoxyestradiol to be used according to the invention is an agent carrying selective cytotoxic effects on neoplastic mastocytes containing the KIT gene mutation.

[0019] 

[0020] 2-methoxyestradiol (hereainafter referred to as 2-ME) is a physiologically-occurring compound; it is a metabolite of 17β-estradiol (E2) belonging to estrogens, female sex hormones [8]. In addition, it was proved that 2-ME is an effective anticancer agent [9].

[0021] 2-ME, available under the commercial name of Panzem, is in advanced phases of clinical research on the treatment of numerous malignant cancers, including colon cancer, breast cancer, lung cancer, or osteosarcoma [10–13].

[0022] The molecular mechanism of action of 2-ME has not yet been fully understood, however it is known to generate reactive forms of oxygen and nitrogen, thus leading to the nitro-oxidative stress which causes cell apoptosis [8].

[0023] The major anti-proliferation mechanisms include inhibition of the microtubule formation dynamics, inhibition of neoangiogenesis, and regulation of external and internal apoptotic pathways [14].

[0024] It was proved that 2-ME selectively induces neuronal nitric oxide synthase (nNOS) in the 143B osteosarcoma cell line in pharmacological and physiological concentrations. 2-ME increases the presence of nNOS in the nucleus, damaging the DNA by way of the nitro-oxidative stress, which in turn stops the cell cycle and causes apoptosis of the osteosarcoma cells [15–20].

[0025] Induction of nNOS in physiological concentrations implies a hypothesis that 2-ME in human organism is not only an active cell metabolite, but also an independently operating hormone [16].

[0026] In the conducted research it was observed that 2-ME was selectively cytotoxic to neoplastic MCs, specifically to those which contained KIT gene mutations (lines HMC 1.1, HMC 1.2, ROSA KIT D816V), but did not demonstrate any such activity with respect to regular MCs (line ROSA KIT WT).

[0027] It was proved that application of 2-ME in the concentration of 1 μM for 48 hours reduced the viability of the cells of the HMC 1.1 line (containing the p.V560G mutation) down to 71%, whereas at the 2-ME concentration at the level of 5 μM cell viability dropped to 63%, and at the 2-ME concentration of 10 μM to 67%. In the case of the HMC 1.2 cell line (with mutations p.V560G and p. D816V) the number of live cells following the application of 2-ME for 48 hours dropped to the following levels, respectively: 78% for the 2-ME concentration of 1 μM, up to 76% for the concentration of 5 μM, and up to 70% for the concentration of 10 μM.

[0028] In the case of the research conducted on the ROSA line cells, the effects in terms of reduced viability of the mastocyte cells treated with 2-ME proved even more spectacular. The ROSA KIT D816V line (containing mutation p. KIT D816V) proved the most susceptible to 2-ME, since following the treatment of those cells with 2-ME at the concentration of 1 μM their viability dropped as much as to 56%. When higher concentrations were applied, i.e. 5 and 10 μM, the number of live cells dropped to 55% and 51%, respectively. In the case of the ROSA KIT WT line (without KIT gene mutation), the 2-ME concentration of 1 μM inhibited the cell growth by the mere 5%, at the concentration of 5 μM by 12%, and at the concentration of 10 μM by 14%.

[0029] The obtained results of the research are of momentous importance for understanding the pathogenesis of mastocytosis, particularly in children, and explain the phenomenon of disease regression in puberty, they also suggest a potential possibility to develop new therapies of the skin disease and systemic disease using 2-ME.

[0030] In addition, the results of the research indicate that the ROSA KIT D816V and ROSA KIT WT cell lines are very good models for testing effects of active agents on mastocytes.

Figure description

[0031] [Fig.1] presents the effects attained for various 2-ME (2-methoxyestradiol) concentrations applied for 48 hours on the viability of cells of the HMC 1.1, HMC 1.2, ROSA KIT D816V, ROSA KIT WT. lines and control cells.

[0032] An embodiment of the invention is shown below.

Embodiment 1 (variant 1)

[0033] Used in the research were four mastocyte cell lines:

[0034] HMC-1.1 containing the pV560G KIT gene mutation;

[0035] HMC-1.2 containing the pG560V and pD816V KIT gene mutations,

[0036] ROSA KIT D816V containing the pD816V KIT mutation;

[0037] ROSA KIT WT – line containing no KIT mutation [21–23].

[0038] The HMC lines were obtained from the Department of Allergic Diseases. Mayo Clinic. Rochester. MN, USA

[0039] The ROSAKIT lines were obtained from Centre National de la Recherche Scientifique 3 (France), deposited with CNCM (French National Collection of Cultures of Microorganisms) as of 02/11/2011 under numbers: CNCM- 1-4551 ROSA-KIT WT; CNCM-1-4552 ROSA KIT D816V.

[0040] Culture conditions for the HMC line

[0041] The culture was grown in a cell incubator at the temperature of 37 ºC and at 5% CO2 content, in sterile conditions in a chamber with laminar flow of sterile air. Used was Iscove's Modified Dulbecco's Medium (Merck Millipore) supplemented with 1.2 mM of α-thioglycerol, 50 mL of Foetal Bovine Serum (ATCC), and 5 mL of antibiotics (PEN./STREP. 100x, Merck Millipore)

[0042] Culture conditions for the ROSA KIT line

[0043] The culture was grown in a cell incubator at the temperature of 37 ºC and at 5% CO2 content. The laboratory works were conducted in sterile conditions in a chamber with laminar flow of sterile air. Used to grow the culture was Iscove's Modified Dulbecco's Medium (Merck Millipore) added with 50 mL of Foetal Bovine Serum (ATCC), and 5 mL of antibiotics (PEN./STREP. 100x, Merck Millipore), while the medium used for the ROSA KIT WT line was additionally supplemented

with bone marrow stem cell growth factor rhSCF (R&D Systems, Minneapolis, USA) in the final concentration of 80 ng/ml.

[0044] The procedure

[0045] The cells were grown for 48 hours on a 96-well plate at the concentrations 5 ×10^4 cells per well for the HMC 1.1. and HMC 1.2 lines, respectively, and at the concentration of 7.5 ×10^4 cells per well for the ROSA KIT D816V and ROSA KIT WT lines.

[0046] Before the experiments were performed, a working solution of 2-methoxyestradiol was produced by dissolving 5 mg of 2-ME (Sigma-Aldrich, USA) in 500 µL of sterile DMSO (dimethyl sulfoxide), so that its final concentration would be 33 mM. Then, the working 2-ME solution was diluted in 100 µL of DMSO so as to obtain the final solution concentrations of 1, 5, and 10 in 100 µL of DMSO, respectively.

[0047] The control were cells treated with the corresponding amounts of DMSO with no 2-ME added.

[0048] Following 48 hours into incubation, each well was added 50 µL of the XTT solution (Roche Diagnostics GmbH, Mannheim, Germany) prepared in accordance with the manufacturer's instructions. After 4 hours of cell incubation with XTT the level of absorbance was read using the Cytation 3 Imaging Reader (BioTek, USA) at the wavelength of 560 nm.

[0049] Application of 2-ME at the concentration of 1 µM on the HMC 1.1 line for 48 hours reduced proliferation of cells by 29%. In the case of the concentration of 5 µM cell viability dropped to 67%, and in the case of 10 µM to 63%.

[0050] Following the treatment of 2-ME at the concentration of 1 µM the number of live cells in the HMC 1.2. line dropped to 78%, while at the concentration of 5 µM it dropped by 24%, and by 30% at the concentration of 10 µM.

[0051] The ROSA KIT D816V line proved most susceptible to the effects of 2-ME. Following treatment of those cells with 2-ME at the concentration of 1 µM cell viability dropped to 56%. When higher concentrations (5 and 10 µM) were used, the number of live cells dropped to 55% and 51%, respectively.

[0052] In the case of the ROSA KIT WT line, 1 μM of 2-ME inhibited the cell growth only by 5%, with 12% recorded for the concentration of 5 μM, and 14% for the concentration of 10 μM.

[0053] Non Patent Literature

[0054] Nplcit1: Nedoszytko B., Sokołowska-Wojdyło M., Ruckemann-Dziurdzińska K., Roszkiewicz J., Nowicki RJ. Chemokines and cytokines network in the pathogenesis of the inflammatory skin diseases: Atopic dermatitis, psoriasis and skin mastocytosis. Vol. 31, Postepy Dermatologii i Alergologii. Termedia Publishing House Ltd.; 2014. p. 84–91.

[0055] Nplcit2: Broesby-Olsen S., Oropeza AR., Bindslev-Jensen C., Vestergaard H., Møller MB., Siebenhaar F., Kristensen T., Mortz CG. Recognizing mastocytosis in patients with anaphylaxis: Value of KIT D816V mutation analysis of peripheral blood. J Allergy Clin Immunol. 2015;135(1):262–4.

[0056] Nplcit3: Carter MC., Bai Y., Ruiz-Esteves KN., Scott LM., Cantave D., Bolan H., Eisch R., Sun X., Hahn J., Maric I., Metcalfe DD. Detection of KIT D816V in peripheral blood of children with manifestations of cutaneous mastocytosis suggests systemic disease. Br J Haematol. 2018;183(5):775–82.

[0057] Nplcit4: Renke J., Kędzierska-Mieszkowska S., Lange M., Nedoszytko B., Wasilewska E., Liberek A., Renke M., Niedoszytko M., Witkowski J., Skórko-Glonek J., Lipińska B. Mast cells in mastocytosis and allergy – Important player in metabolic and immunological homeostasis. Vol. 64, Advances in Medical Sciences. Medical University of Bialystok; 2019. p. 124–30.

[0058] Nplcit5: Valent P., Akin C., Bonadonna P., Hartmann K., Brockow K., Niedoszytko M., Nedoszytko B., Siebenhaar F., Sperr WR., Oude Elberink JNG., Butterfield JH., Alvarez-Twose I., Sotlar K., Reiter A., Kluin-Nelemans HC., Hermine O., Gotlib J., Broesby-Olsen S., Orfao A., Horny HP., Triggiani M., Arock M., Schwartz LB., Metcalfe DD. Proposed Diagnostic Algorithm for Patients with Suspected Mast Cell Activation Syndrome. Vol. 7, Journal of Allergy and Clinical Immunology: In Practice. American Academy of Allergy, Asthma and Immunology; 2019. p. 1125-1133.e1.

[0059] Nplcit6: Romantowski J., Górska A., Lange M., Nedoszytko B., Gruchała-Niedoszytko M., Niedoszytko M. How to diagnose Mast Cell Activation Syndrome? Practical considerations. Polish Arch Intern Med. 2020;130(4):317–23. 10.20452/pamw.15212

[0060] Nplcit7: Matito A., Azaña JM., Torrelo A., Alvarez-Twose I. Cutaneous Mastocytosis in Adults and Children: New Classification and Prognostic Factors. Vol. 38, Immunology and Allergy Clinics of North America. W.B. Saunders; 2018. p. 351–63.

[0061] Nplcit8: Parada-Bustamante A., Valencia C., Reuquen P., Diaz P., Rincion-Rodriguez R., Orihuela P. Role of 2-methoxyestradiol, an Endogenous Estrogen Metabolite, in Health and Disease. Mini-Reviews Med Chem. 2015;15(5):427–38. 10.2174/1389557515666150226121052

[0062] Nplcit9: Mooberry SL. New insights into 2-methoxyestradiol, a promising antiangiogenic and antitumor agent [Internet]. Vol. 15, Current Opinion in Oncology. 2003. p. 425–30. 10.1097/00001622-200311000-00004

[0063] Nplcit10: Harrison MR., Hahn NM., Pili R., Oh WK., Hammers H., Sweeney C., Kim K., Perlman S., Arnott J., Sidor C., Wilding G., Liu G. A phase II study of 2-methoxyestradiol (2ME2) NanoCrystal® dispersion (NCD) in patients with taxane-refractory, metastatic castrate-resistant prostate cancer (CRPC). Invest New Drugs. 2011;29(6):1465–74. 10.1007/s10637-010-9455-x

[0064] Nplcit11: LaVallee TM., Burke PA., Swartz GM., Hamel E., Agoston GE., Shah J., Suwandi L., Hanson AD., Fogler WE., Sidor CF., Treston AM. Significant antitumor activity in vivo following treatment with the microtubule agent ENMD-1198. Mol Cancer Ther. 2008;7(6):1472–82.

[0065] Nplcit12: Tevaarwerk AJ., Holen KD., Alberti DB., Sidor C., Arnott J., Quon C., Wilding G., Liu G. Phase i trial of 2-methoxyestradiol NanoCrystal dispersion in advanced solid malignancies. Clin Cancer Res. 2009;15(4):1460–5.

[0066] Nplcit13: Kumar BS., Raghuvanshi DS., Hasanain M., Alam S., Sarkar J., Mitra K., Khan F., Negi AS. Recent Advances in chemistry and pharmacology of 2-methoxyestradiol: An anticancer investigational drug. Vol. 110, Steroids. Elsevier Inc.; 2016. p. 9–34.

[0067] Nplcit14: Mueck AO., Seeger H. 2-methoxyestradiol - Biology and mechanism of action. Vol. 75, Steroids. 2010. p. 625–31.

[0068] Nplcit15: Gorska M., Kuban-Jankowska A., Slawek J., Wozniak M. New Insight into 2-Methoxyestradiol- a Possible Physiological Link between Neurodegeneration and Cancer Cell Death. Curr Med Chem. 2016;23(15):1513–27.

[0069] Nplcit16: Gorska M., Kuban-Jankowska A., Zmijewski M., Gammazza AM., Cappello F., Wnuk M., Gorzynik M., Rzeszutek I., Daca A., Lewinska A., Wozniak M. DNA strand breaks induced by nuclear hijacking of neuronal NOS as an anti-cancer effect of 2-methoxyestradiol. Oncotarget. 2015;6(17):15449–63. 10.18632/oncotarget.3913

[0070] Nplcit17: Gorska-Ponikowska M., Kuban-Jankowska A., Daca A., Nussberger S. 2-Methoxyestradiol Reverses the Pro-Carcinogenic Effect of L-Lactate in Osteosarcoma 143B Cells. Cancer Genomics Proteomics. 2017;14(6):483–93.

[0071] Nplcit18: Gorska M., Kuban-Jankowska A., Milczarek R., Wozniak M. Nitro-oxidative Stress Is Involved in Anticancer Activity of 17beta-Estradiol Derivative in Neuroblastoma Cells. Anticancer Res. 2016;36(4):1693–8.

[0072] Nplcit19: Gorska M., Kuban-Jankowska A., Zmijewski M., Gorzynik M., Szkatula M., Wozniak M. Neuronal Nitric Oxide Synthase Induction in the Antitumorigenic and Neurotoxic Effects of 2-Methoxyestradiol. 2014;19(9):13267–81. 10.3390/molecules190913267

[0073] Nplcit20: Gorska-Ponikowska M., Perricone U., Kuban-Jankowska A., Lo Bosco G., Barone G. 2-methoxyestradiol impacts on amino acids-mediated metabolic reprogramming in osteosarcoma cells by its interaction with NMDA receptor. J Cell Physiol. 2017;232(11):3030–49.

[0074] Nplcit21: NILSSON G., BLOM T., KUSCHE - GULLBERG M., KJELLEN L., BUTTERFIELD JH., SUNDSTRÖM C., NILSSON K., HELLMAN L. Phenotypic Characterization of the Human Mast - Cell Line HMC - 1. Scand J Immunol. 1994;39(5):489-98.

[0075] Nplcit22: Sundström M., Vliagoftis H., Karlberg P., Butterfield JH., Nilsson K., Metcalfe DD., Nilsson G. Functional and phenotypic studies of two variants of a

human mast cell line with a distinct set of mutations in the c-kit proto-oncogene. Immunology. 2003;108(1):89–97.

[0076] Nplcit23: Furitsu T., Tsujimura T., Tono T., Ikeda H., Kitayama H., Koshimizu U., Sugahara H., Butterfield JH., Ashman LK., Kanayama Y., Matsuzawa Y., Kitamura Y., Kanakura Y. Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. J Clin Invest. 1993;92(4):1736–44.

Claims

[Claim 1] 2-methoxyestradiol to be used in the treatment of mastocytosis.

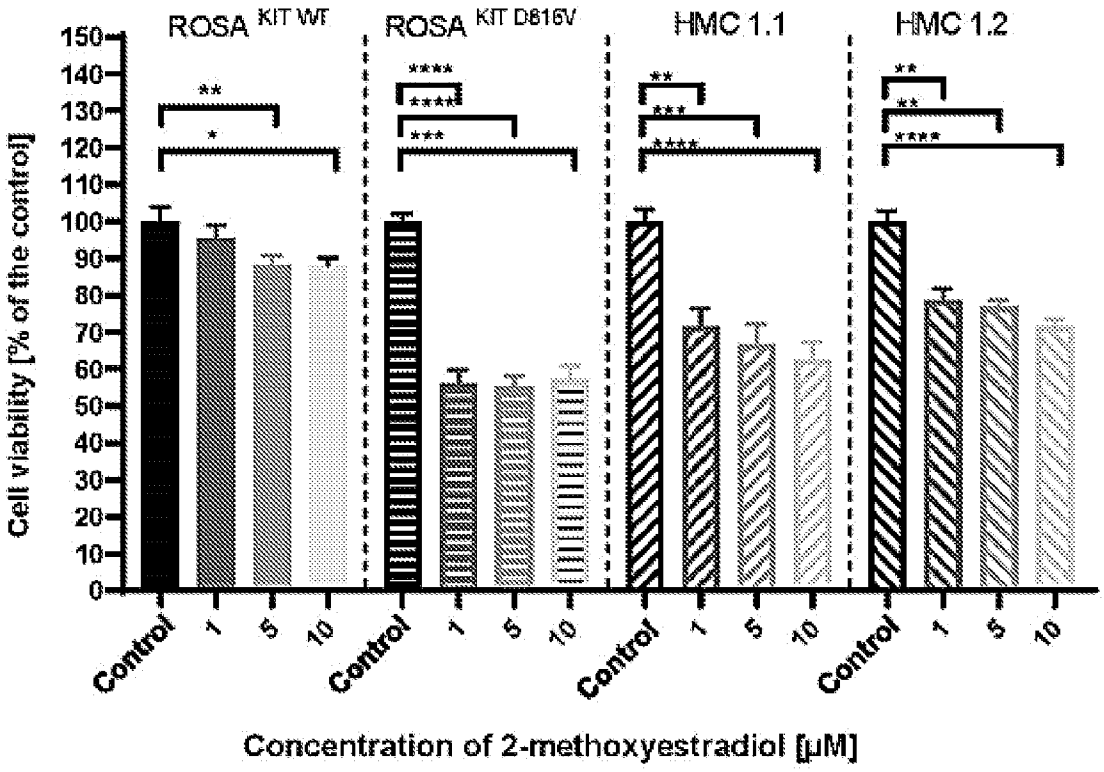
[Claim 2] 2-methoxyestradiol to be used in accordance with Claim 1, where mastocytosis occurs in the skin form and/or systemic form.

[Claim 3] 2-methoxyestradiol to be used in accordance with Claim 1 in the treatment of mastocytosis in children.

[Claim 4] 2-methoxyestradiol to be used in accordance with any of the Claims 1-3 as an agent selectively cytotoxic to neoplastic mastocytes containing a KIT gene mutation.

Drawings

[Fig. 1]



INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2022/050091

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/565 A61P43/00
ADD.

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)
A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2018/050801 A1 (INST NAT SANTE RECH MED [FR]; FOND IMAGINE [FR] ET AL.) 22 March 2018 (2018-03-22) claims 1, 4	1-4
A	US 2021/077503 A1 (BUTTE MANISH J [US] ET AL) 18 March 2021 (2021-03-18) claim 1	1-4
A	WO 2019/152719 A1 (DECIPHERA PHARMACEUTICALS LLC [US]) 8 August 2019 (2019-08-08) claims 25-29	1-4
A	WO 2007/010014 A2 (VALENT PETER [AT]) 25 January 2007 (2007-01-25) claim 1	1-4

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[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 February 2023	27/02/2023

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2022/050091

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PETER VALENT ET AL: "Diagnosis and treatment of systemic mastocytosis: state of the art", BRITISH JOURNAL OF HAEMATOLOGY, JOHN WILEY, HOBOKEN, USA, vol. 122, no. 5, 21 August 2003 (2003-08-21), pages 695-717, XP071161080, ISSN: 0007-1048, DOI: 10.1046/J.1365-2141.2003.04575.X page 707; table 12	1-4
A	SIBONGA J. D. ET AL: "Dose-Response Effects of 2-Methoxyestradiol on Estrogen Target Tissues in the Ovariectomized Rat", ENDOCRINOLOGY, vol. 144, no. 3, 1 March 2003 (2003-03-01) , pages 785-792, XP093022698, US ISSN: 0013-7227, DOI: 10.1210/en.2002-220632 Retrieved from the Internet: URL:http://academic.oup.com/endo/article-pdf/144/3/785/9015451/endo0785.pdf> page 785	1-4
A	WO 97/42958 A1 (AMRAD OPERATIONS PTY LTD [AU]; STEWART ALASTAIR GEORGE [AU]) 20 November 1997 (1997-11-20) claims 1, 8	1-4

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/PL2022/050091

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2018050801 A1	22-03-2018	EP 3512515 A1	24-07-2019
		US 2019247416 A1	15-08-2019
		US 2021308106 A1	07-10-2021
		WO 2018050801 A1	22-03-2018
US 2021077503 A1	18-03-2021	NONE	
WO 2019152719 A1	08-08-2019	AU 2019216351 A1	20-08-2020
		BR 112020015572 A2	02-02-2021
		CA 3089630 A1	08-08-2019
		CN 111886006 A	03-11-2020
		EA 202091763 A1	14-12-2020
		EP 3746060 A1	09-12-2020
		IL 276398 A	30-09-2020
		JP 2021512101 A	13-05-2021
		KR 20200115606 A	07-10-2020
		SG 11202007287X A	28-08-2020
		US 2020352920 A1	12-11-2020
		WO 2019152719 A1	08-08-2019
WO 2007010014 A2	25-01-2007	AT 464911 T	15-05-2010
		AU 2006271652 A1	25-01-2007
		BR PI0613863 A2	15-02-2011
		CA 2615598 A1	25-01-2007
		CN 101267837 A	17-09-2008
		EP 1909837 A2	16-04-2008
		ES 2343542 T3	03-08-2010
		JP 5322639 B2	23-10-2013
		JP 2009501767 A	22-01-2009
		KR 20080039915 A	07-05-2008
		PL 1909837 T3	30-09-2010
		PT 1909837 E	07-07-2010
		US 2009221550 A1	03-09-2009
		WO 2007010014 A2	25-01-2007
WO 9742958 A1	20-11-1997	AT 281170 T	15-11-2004
		CA 2253943 A1	20-11-1997
		CN 1218401 A	02-06-1999
		DE 69731473 T2	27-10-2005
		EP 0923376 A1	23-06-1999
		JP 4228116 B2	25-02-2009
		JP 2000507923 A	27-06-2000
		US 5962445 A	05-10-1999
		US 6200966 B1	13-03-2001
		WO 9742958 A1	20-11-1997