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(54) **Title:** ANTI-HERG1 ANTIBODIES

FIG. 4

Aminoacid sequence (SEQ ID NO:6) of the construct VH-linker-VL relative to the scFv-hERGl

Framework HI CDR HI Framework H2 CDR H2

EVQLQQ[illegible]CKTSGYTFTEYTVHWVKQSHGKSLEWIGGINPNGGTTYNQKFKGKA

Framework H3 CDR H3 Framework 4H Linker

[illegible]GSSGGGGSGGGGS

Framework LI CDR LI Framework L2

GGSALDIVLTQSPLTLSVNIGQPASISCKSSQSLLYTNGKTYLNWLLQRPGQSPKRLIYLVSK

Framework L3 CDR L3 Framework L4

LGSGVI?DGG-FGSSSGTDFTLKISRVEAEDLGVYYCAQGTHFPWTFGGGTKLELKRADAAPTVS

(57) **Abstract:** Here, we describe the detailed structure of an intact murine monoclonal anti-hERGl molecule and the corresponding anti-hERGl scFv antibody production, obtained after the isolation of the mAb anti-hERGl VH and VL. Such scFv has the same 5 specificity of the correspondent whole antibody, and thus it is able to recognize the same anti-hERGl protein, aberrantly expressed in tumours and other diseases

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ANTI-HERG1 ANTIBODIES

FIELD OF THE INVENTION

The present invention relates to the field of antibodies, in particular it relates to anti-hERG1 molecules.

STATE OF THE ART

Over the past two decades, the antibodies' production technology has been significantly improved through antibody engineering; the advent of new technologies in the field of molecular engineering, led to the production of a wide variety of genetically engineered antibodies, such as fragments of type Fab, Fv form of simple chain of scFv, diabodies, triabodies, bispecifics, minibodies, phage antibodies (Holliger & Hudson, 2005). In fact there is a range of applications, in which the Fc-mediated effects are not required and even undesirable, because of their associated toxic effects and their capacity to evoke an immune response able to neutralise the antibody efficacy, when its Fc derived from a non human source.

Among the engineered antibody fragments, the Single Chain Variable Fragment (scFv) is the most popular and one of the smallest recombinant format with an antigen-binding activity function and with the property to be easily manageable for immunological application (Heng & Othman, 2006).

A scFv consists of variable regions of heavy (VH) and light (VL) chains, which are joined together by a flexible peptide linker, without compromising the fidelity of the VH-VL paring and antigen-binding sites. The choice of linker can affect the solubility, expression and correct folding of the scFv. Peptide linkers can vary from 10 to 25 amino acids in length and are typically, composed of hydrophilic amino acids such as glycine (G) and serine (S) (Shen et al., 2008). Hydrophilic sequences prevent intercalation of the peptide within or between the variable domains throughout the protein folding (Argos, 1990). The most common linker used is the (Gly4Ser)3 motif (Huston et al.,1 988), due to its flexibility, neutral charge and solubility (Kortt et al., 2001). The use of scFv in diagnostics and therapy provides several advantages over whole antibodies, especially in solid tumours therapy; in fact the speed of penetration by a fragment versus an intact molecule is the most remarkable advantage. In 1988, it was established that an intact molecule of IgG took fifty-four

hours to penetrate 1 mm into a solid tumour, while a Fab fragment managed the same distance in sixteen hours (Jain, 1987). Moreover, the scFv, as well as all the other antibody fragments format, can be forget into multivalent and multispecific reagents or easily linked to therapeutic tools as radionuclides, toxins or nanoparticles) and engineer to improve their diagnostic and therapeutic efficacy.

hERG1 is a protein which is aberrantly expressed in tumours and other diseases (Arcangeli & Becchetti, 2010). Antibodies against hERG1 have a high potential application in diagnosis and therapy of a large variety of tumours and other diseases which are characterized by an over expression of hERG1 .

In the Italian Patent IT1 367861 is described a hybridoma cell line clone, named A7, able to secrete an anti-hERG1 monoclonal antibody (mAb) specific against the S5-pore extracellular portion of hERG1 .

Aim of the present invention is to provide further antibodies against hERG1 in order to overcome some problems related to the *in vivo* use of murine complete antibodies (immunogenicity and big size of the molecule preventing an efficient permeability).

SUMMARY OF THE INVENTION

Herein is described the detailed structure of an intact murine monoclonal anti-hERG1 molecule and the corresponding anti-hERG1 scFv antibody production, obtained after the isolation of the mAb anti-hERG1 VH and VL. Such scFv has the same specificity of the correspondent whole antibody, and thus it is able to recognize the same hERG1 protein, aberrantly expressed in tumours and other diseases.

Subject-matter of the present invention is therefore a molecule comprising a Heavy chain Variable (VH) domain having SEQ ID NO:3 and a Light chain Variable domain having SEQ ID NO:4, said molecule having specificity against hERG1 S5-pore extracellular portion.

mAb-hERG1 was initially structurally well characterised and then engineered to produce a recombinant single chain variable fragment against hERG1 (scFv-hERG1). The main advantage of scFv over intact whole IgG was its small size; scFv dimensions allow it to penetrate more rapidly. In addition, the lack of constant regions decreased retention by Fc receptors found in most tissues and organs, which further reduced the side effects. These characteristics rendered scFv suitable

for an in vivo administration and an ideal vector for delivery of agents such as radionuclide, enzyme, drugs or toxin. Moreover, it can be easily bound the different tools (es. nanoparticles, Kim et al., 2002) to produce bio-sensors or bio-system for diagnostic and therapeutic purposes. It is worth noting that the structurally characterization of the whole mAb-hERG1 open to the possibility to obtain any format of engineered antibody with a single or a double specificity.

DETAILED DESCRIPTION OF THE INVENTION

Molecule according to the invention can be a murine or fully humanized mAb.

Molecule according to the invention can be a scFv or any other engineered antibody such as Fab, Fv form of simple chain of scFv, diabodies, triabodies, bispecifics, minibodies, phage antibodies.

A molecule according to the invention is useful as diagnostic or therapeutic tool.

Pathologies which can be diagnosed or treated using a molecule according to the invention are all those pathologies characterized by an over expression or mis-expression of hERG1 protein. Among said pathologies can be listed tumours, neurological diseases, endocrine diseases and neuro-endocrine diseases.

A molecule according to the invention, in particular the scFV, can also be used as a pharmaceutical delivery vector: so for example it can be covalently on not bonded to radionuclide, enzyme, drugs or toxin.

Further subject-matter of the present invention are therefore also pharmaceutical compositions comprising a molecule according to the invention.

A molecule according to the invention can be prepared by employing nucleotide sequences SEQ ID NO:1 and SEQ ID NO:2 encoding respectively VH (SEQ ID NO:3) and VL (SEQ ID NO:4).

Particularly preferred according to the invention is a method for preparing a scFv according to the invention, said method comprising the use of nucleotide sequence SEQ ID NO:5 encoding for a scFV having SEQ ID NO:6.

The method according to the invention implies recombinant techniques.

Therefore subject-matter of the present invention are also an expression vector or a plasmid comprising SEQ ID NO:1 and SEQ ID NO:2 as well as genetically modified microorganisms comprising an expression vector according to the invention.

The present invention could be better understood in light of the experimental section below.

BRIEF DESCRIPTION OF THE FIGURES

Fig.1. - Electrophoresis relative to PCR products of variable light (VL) and heavy (VH) chains.

Fig. 2. - VH(SEQ ID NO:1) (A) and VL(SEQ ID NO:2) (B) nucleotide sequences relative to the whole anti-hERG1 mAb. In the bottom panel the corresponding amino acid sequence (SEQ ID NO:3 and SEQ ID NO:4) with the CDR domains underlined is shown.

Fig. 3 - ScFv-hERG1 expression cassette sequence obtained by Automated DNA sequencing service (PRIMM). Underlined nucleotides represent the restriction sites used for the cloning of the CDS for scFv-hERG1 construct; in red bold the peptide linker sequence codifying a glycine-serine stretch (see below).

Fig. 4 - Amino acid sequence of scFv-hERG1 . The highlighted sequences underlined show the scFv-hERG1 CDRs (heavy and light chain) of the recombinant antibody fragments. The framework regions are highlighted in grey and the glycine-serine linker region in bold-italics.

Fig. 5 - Histogram representing the signal obtained in ELISA against S5-Pore peptide testing the aliquot of Not Bound (NB), Wash (W) and Elution (E) obtained from Mut+ and MutS yeast supernatant purification. As expected, aliquot E is the most efficient one, having a higher concentration of scFv-hERG1 .

Fig. 6 - Indirect immunofluorescence on hERG1 positive cell line PANC-1 using the scFv-hERG1 antibody. PANC-1 cells were grown on glass coverslips and fixed with 4% methanol-free formaldehyde (Thermo Scientific) in PBS. As we can see in Figure 6, hERG1 scFv (B) has the same ability to bind hERG1 positive cells than the original mAb anti-hERG1 (A) from which the scFv was obtained.

EXPERIMENTAL SECTION

Initially it was determined the isotype of a mAb-hERG1 clone, named A21 , able to secrete an anti-hERG1 with the same functional characteristics of the one described in IT1 367861 The mAb-hERG1 resulted in a mouse lgG2b and this finding was the starting point to engineer such molecule to obtain different anti-hERG1 antibodies with different structures. To this purpose, we extracted and retro-transcribed the

mRNA of the corresponding hybridoma, and were designed the primers SEQ ID NO:7-10 (listed in Materials and Methods paragraph 1.3 below) to obtain the VH and VL amplificates reported in Fig.1.

The purification of the bands and the relative ligation and transformation in DH5cc bacteria strain, allowed to sequence the two fragments, which resulted from the analysis performed as described in Materials and Methods with the following nucleotide (SEQ ID NO: 1-2) and amino acid (SEQ ID NO:3-4) sequences (Fig.2).

Then it followed the assembling VH and VL chains in the pHenIX vector. To this purpose, it was cloned VH between Sal I (SEQ ID NO:11) and Xho I (SEQ ID NO:12) and VL between ApaL I (SEQ ID NO:13) and Not I (SEQ ID NO:14). The sequence of the new construct obtained by this cloning is SEQ ID NO:5 reported in Fig. 3

Finally, once obtained the scFv-hERG1 construct (SEQ ID N. 15), the expression step was performed in Pichia Pastoris competent cells (as described in Materials and Methods paragraph 2.2 below). Both Mut+ and MutS strains were used and then compared for the scFv-hERG1 production.

Yeast supernatant, containing scFv-hERG1 -6xHis (SEQ ID N: 17), was collected and concentrated with Amicon Ultra-15 10K (Millipore) and purified by chromatography using columns packed with Ni-NTA Agarose resin (Qiagen). Aliquots obtained from purification, marked as Not Bound (NB), Wash (W) and Elution (E), were analyzed through SDS-Page Comassie Staining and therefore by Western Blot, using an anti-6xHis antibody (GeneTex), to verify for the presence of the corresponding scFv-hERG1 (around 30 kDa). Once demonstrated an efficient production of the antibody, the following step was the evaluation of its ability to bind the antigen. To this purpose, it was performed an ELISA using hERG1 S5-Pore peptide coated plates (the same coating which was used for the whole mAb-hERG1 screening). The aliquots Not Bound (NB), Wash (W) and Elution (E), obtained from Mut+ and MutS yeast supernatant purification, were analysed.

MATERIALS AND METHODS

1 *Isolation of mAb anti-hERG1 (mAb-hERG1) heavy (VH) and light (VL) chain variable domains*

1.1 *Determination of mAb-hERG1 isotype*

To this purpose, the Mouse Monoclonal Antibody Isotyping Reagents (Sigma-Aldrich).

1.2 *mRNA extraction and retro-transcription*

To obtain the mAb-hERG1 variable VH and VL chains able to recognise the hERG1 antigen expressed on the cellular membranes, we amplified the hybridoma cell line able to produce and secrete the mAb-hERG1 and total RNA was extracted using TRIzol® Reagent (Ambion), following the manifacturer's instructions. Then, messenger RNA (mRNA) was obtained using Poly(A)Purist® (Ambion).

mRNA was retro-transcribed into the corresponding cDNA encoding the VH and VL sequences using Random Primers (225 ng, final concentration/reaction) and Superscript II Reverse Transcriptase (Invitrogen).

1.3 *PCR amplification*

For the amplification of VH and VL regions, a 5' primer that anneals to the VH and VL framework 1 (FR1) (primer forward) and a primer that anneals to the constant region adjacent to VH and VL domains (primer reverse) were chosen. For VL, a degenerate primer able to recognise the kappa light chain was designed. For VH, a degenerate primer that anneals to the IgG2b heavy chain was designed. The primers used are described in Wang et al., 2000 and are reported below:

NAME	SEQUENCE	SEQ ID NO:
degKappadir	GAYATTGTGMTSACMCARWCTMCA	7
Kapparev	GGATACAGTTGGTGCAGCATC	8
degH2dir	GAGGTCCARCTGCAACARTC	9
IgG2brev	AGGGGCCAGTGGATAGACTGATGG	10

The amplification reaction of VH and VL was performed with the Phusion® High-Fidelity DNA Polymerase (Finnzymes Reagents), following the manifacturer's instructions, as described below.

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Steps	Temperature	Time
1 Initial denaturation	94 °C	2 minutes
2 Denaturation	94 °C	30 seconds
3 Annealing	56 °C (VH); 48°C (VL)	1 minute
4 Extension	72 °C	1 minute
5 Final extension	72 °C	10 minutes

Steps 2,3 and 4 were repeated for 24 times.

1*A VH and VL cloning*

PCR products relative to VH and VL were run on 1% agarose in TAE buffer (Tris, acetic acid and EDTA), then excised from the gel with a scalpel and purified using QIAquick PCR Purification Kit (QIAGEN), according to the manufacturer's instruction. QIAquick Kits contain a silica membrane assembly for binding of DNA in high-salt buffer and elution with low-salt buffer or water. The purification procedure removes impurities from DNA samples.

After purification from agarose gel, PCR products were cloned into pCR™ -Blunt vector (where it is possible to insert blunt PCR products), using a 10:1 molar ratio of insert:vector, according to the following formula:

X ng insert = (10)(Y bp PCR product) (25 ng linearized pCR™ -Blunt)/(3500 bp pCR™ -Blunt)

where X ng is the amount of PCR product of Y base pairs to be ligated. The ligation reaction was incubated at 16°C for one hour.

DH5cc cells were used for the transformation step and the selected colonies were checked for the presence of the insert in the right orientation.

1.5 *Sequencing and analysis of VH and VL*

DNA samples were sequenced by PRIMM s.r.l. and the relative products were analysed using ExPASy translation Tool software and basing on Kabat numbering scheme (www.bioinf.org.uk), in order to find the three scFv Complementary Determining Regions (CDR1 , CDR2 and CDR3).

2 *Anti-hERG1 scFv development*

2.1 *scFv-hERG1 assembly*

To assembly VH and VL, in order to obtain the final scFv construct, phagemid vector pHenIX (Hoogenboom et al., 1991) and specific primers containing restriction enzyme recognition sites were used. Primer's sequences are shown below and restriction sites are reported as underlined. When needed, 2 additional bases (reported in blue) to preserve the sequence frame were included.

NAME	SEQUENCE	SEQ ID NO:
VH for Sal I	5' ACGCGTCGACGAGGTCCAACTGCAACAGTC	11
VH rev Xho I	5' CCGCTCGAGCCAGGGGCCAGTGGATAGACTGATGG	12
VL for ApaL I	5' ACGCGTGCACTGGATATTGTGCTGACACAATCTCCA	13
VL rev Not I	5' ATAAGAATGCGGCCGCGGATACAGTTGGTGCAGCATC	14

After PCR amplication and restriction enzyme cutting, sequences were cloned in the phagemid vector pHenIX using T4 DNA Ligase (New England Biolabs), following the manifacturer's instructions and basing on the formula reported in paragraph 1.4.

2.2 *scFv-hERG1 expression*

For the scFv-hERG1 expression, the yeast host Pichia Pastoris was used. To this purpose, we cloned the scFv-hERG1 construct having SEQ ID 15 into the commercial pPIC9K vector (kindly provided by Prof. E. Gherardi, University of Pavia), which has allowed us to express a 6xHis tagged protein in Pichia Pastoris. The expression cassette was amplified by PCR using primers containing the FspI and AvrII restriction sites at 3' and 5' ends, respectively. The expression cassette was then cutted with with FspI and AvrII and cloned into pPIC9K cutted with Eco53KI and AvrII restriction enzymes (NEB).

Pichia Pastoris competent cells was transformed by electroporation using 0.2 cm cuvette, 2000V, 400 ohm, 25 μF. Yeasts were then seeded on MD plates (Minimal Dextrose Medium: 1.34% YNB, 4x1 0^{-5}% biotin, 2% dextrose) + agar and incubated at 30 °C for 3 days. We decided to express scFv-hERG1 in both Mut+ or MutS strains according to Pichia Expression Kit (Invitrogen) protocol. A protein having

SEQ ID N. 16 was expressed by the yeast. The protein having SEQ ID 16 was cut by the yeast at residue 85 before being secreted in the supernatant thus containing a scFv-hERG1 -6xHis having SEQ ID N. 17 (corresponding to residues 86-365 of SEQ ID N. 16).

2.3 *ELISA assay*

In order to check for the scFv-hERG1 specificity, ELISA assay was performed on yeast supernatant purified aliquots obtained from Mut+ and MutS yeast following a standard protocol and using an anti-6xHis antibody (GeneTex) 1:500 in PBS + 3%BSA as primary antibody, followed by an anti-rabbit IgG-HRP conjugate antibody (Sigma) 1:500 in PBS + 3%BSA. As it can be seen in Figure 6, hERG1 scFv (B) has the same ability to bind hERG1 positive cells than the original mAb anti-hERG1 (A) from which the scFv was obtained.

2.4. Indirect immunofluorescence assay

To compare scFv-hERG1 and the full length antibody (hERG1 mAb) ability to bind hERG1 we performed and indirect immunofluorescence on hERG1 positive cell line PANC-1 . PANC-1 cells were grown on glass coverslips and fixed with 4% methanol-free formaldehyde (Thermo Scientific) in PBS. Coverslips were incubated fifteen minutes in PBS 1% SDS at room temperature for antigen retrieval, treated fifteen minutes with 100 mM glycine at room temperature (to quench residual cross-linking activity of the formaldehyde) and permeabilised four minutes with PBS 0.01 % Triton X-1 00. Unspecific binding sites were blocked thirty minutes at room temperature with PBS 10% FBS. To assay anti-hERG1 mAb binding, coverslips were incubated one hour at room temperature with anti-hERG1 mAb (1 µg/ml in PBS 10% FBS) followed by 45 minutes incubation at room temperature with Alexa-488 labelled anti-mouse antibody (Invitrogen) (1 :500 in PBS 10% FBS). To assay scFv hERG1 binding, coverslips were incubated 2 hours at room temperature with scFv hERG1 (1 :2 in PBS 10% FBS) . To reveal the scFv, coverslips were incubated 1 hour with anti-6xHis antibody (GeneTex) 1:500 in PBS 10% FBS, followed by 45 minutes incubation with Alexa-488 labelled anti-rabbit antibody (Invitrogen) (1 :500 in PBS 10% FBS).

As we can see in Figure 6, hERG1 scFv (B) has the same ability to bind hERG1 positive cells than the full lenght antibody (A).

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5

CLAIMS

I. A molecule comprising a Heavy chain Variable (VH) domain having SEQ ID NO:3 and a Light chain Variable domain having SEQ ID NO:4, said molecule having specificity against hERG1 S5-pore extracellular portion.

2. The molecule according to according to claim 1 which is a monoclonal antibody (mAb).

3. The mAb according to claim 2 which is fully humanised.

4. The molecule according to claim 1 which is an engineered antibody having one, two or more different antigen binding sites.

5. The molecule according to claim 1 which is a Single Chain Variable Fragment (scFV).

6. The scFv according to claim 5 comprising SEQ ID NO:6.

7. The molecule according to any one of claims 1-6 for use as a medicament.

8. The molecule according to any one of claims 1-6 for use in the treatment and/or diagnosis of pathologies characterised by over expression of hERG1 .

9. The molecule for use according to claim 8 wherein the pathologies are selected in the group consisting of tumours, neurological diseases, endocrine diseases and neuro-endocrine diseases.

10. A pharmaceutical composition comprising a molecule according to any one of claims 1-6.

II. A nucleotide sequence comprising SEQ ID NO:1 and SEQ ID NO:2 respectively encoding VH and VL according to claim 1.

12. Nucleotide sequence according to claim 11 and comprising SEQ ID NO:5.

13. An expression vector comprising the nucleotide sequences according to any one of claims 11-12.

14. A genetically-modified micro-organism comprising an expression vector according to claim 13.

15. A method for producing a molecule according to any one of claims 1-6 wherein nucleotide sequences according to any one of claims 11-12 are used.

16. A method for producing the scFv according to claim 6 wherein the nucleotide sequence SEQ ID NO:5, according to claim 12 is used.

FIG. 1

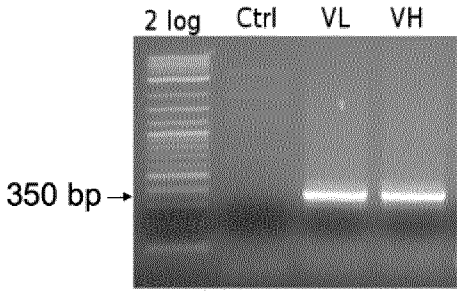


FIG. 2

A

VH: nucleotide (SEQ ID NO:1) and aminoacid (SEQ ID NO:3) sequence
GAGGTCCAACTGCAACAGTCTGGACCTGAACTGGTGAAGCCTGGGGCTTCTGTGAAGATATCCTGC AAGACTTCAGGATACACATTCACTGAATACACCGTTCACTGGGTGAAACAGAGCCATGGAAAGAGCC TTGAATGGATTGGAGGCATTAATCCTAATGGTGGTACTACCTATAATCAGAAGTTCAAGGGCAAGGC CACATTGACTATTGACAAGTCCTCCAGCTCAGCCTTCATGGAGCTCCGCAGCCTGACATCTGAGGAT TCTGCAGTCTATTACTTTGCAACAGGTTGGGGACCTGACTACTGGGGCCAAGGCACCACTCTCACA GTCTCCTCAGCCAAAACAACACCCCCATCAGTCTATCCACTGGCCCCT
Framework 1 CDR H1 Framework 2 CDR H2 EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTVHWVKQSHGKSLEWIGGINPNGGTTYNQKFKGKATLT Framework 3 CDR H3 Framework 4 IDKSSSSAFMELRSLTSEDSAVYYFATGWGPDYWGQGTTLTVSSAKTTPPSVYPLAP

B

VL: nucleotide (SEQ ID NO:2) and amino acid (SEQ ID NO:4) sequence
GATATTGTGCTGACACAATCTCCACTCACTTTGTCGGTTAACATTGGTCAACCAGCCTCTATCTCTTG CAAGTCAAGTCAGAGCCTCTTATATACTAATGGAAAAACCTATTTTAATTGGTTATTACAGAGGCCAG GCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTTCAC TGGCAGTGGATCAGGAACAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTGAAGATTTGGGAGTT TATTACTGCGCGCAAGGTACACATTTTCCGTGGACGTTCGGTGGAGGGACCAAGCTGGAAATCAAA CGGGCTGATGCTGCACCAACTGTATCC
Framework 1 CDR L1 Framework 2 CDR L2 DIVLTQSPLTLSVNIGQPASISCKSSQSLLYTNGKTYFNWLLQRPGQSPKRLIYLVSKLDSGVPDRFTGS Framework 3 CDR L3 Framework 4 GSGTDFTLKISRVEAEDLGVYYCAQGTHFPWTFGGGTKLEIKRADAAPTVS

FIG. 3

Nuclotide sequence (SEQ ID NO:5) of the construct VH-linker-VL relative to the scFv-hERG1

GAGGTCCAACTGCAACAGTCTGGACCTGAACTGGTGAAGCCTGGGGCTTCTGTGAAGATATCCTGCAAGACTTCAGGA
TACACATTCACTGAATACACCGTTCACTGGGTGAAACAGAGCCATGGAAAGAGCCTTGAATGGATTGGAGGCATTAAT
CCTAATGGTGGTACTACCTATAATCAGAAGTTCAAGGGCAAGGCCACATTGACTATTGACAAGTCCTCCAGCTCAGCC
TTCATGGAGCTCCGCAGCCTGACATCTGAGGATTCTGCAGTCTATTACTTTGCAACAGGTTGGGGACCTGACTACTGG
GGCCAAGGCACCACTCTCACAGTCTCCTCAGCCAAAACAACACCCCCATCAGTCTATCCACTGGCCCCTGGCTCGAG
TGGTGGAGGCGGTTCAGGCGGAGGTGGCTCTGGCGGTAGTGCACTGGATATTGTGCTGACACAATCTCCACTC
ACTTTGTCGGTTAACATTGGTCAACCAGCCTCTATCTCTTGCAAGTCAAGTCAGAGCCTCTTATATACTAATGGAAAA
ACCTATTTTAATTGGTTATTACAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACTGGACTCT
GGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGAACAGATTTTACACTGAAAATCAGCAGAGTGGAGGCTGAAGAT
TTGGGAGTTTATTACTGCGCGCAAGGTACACATTTTCCGTGGACGTTCGGTGGAGGGACCAAGCTGGAAATCAAACGG
GCTGATGCTGCACCAACTGTATCC

FIG. 4

Aminoacid sequence (SEQ ID NO:6) of the construct VH-linker-VL relative to the scFv-hERG1

Framework H1 CDR H1 Framework H2 CDR H2

EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTVHWVKQSHGKSLEWIGGINPNGGTTYNQKFKGKA

Framework H3 CDR H3 Framework 4H Linker

TLTIDKSSSSAFMELRSLTSEDSAVYYFATGWGPDYWGQGTTLTVSSAKTTPPSVYPLAP***GSSGGGGSGGGGS***

Framework L1 CDR L1 Framework L2

GGSALDIVLTQSPLTLSVNIGQPASISCKSSQSLLYTNGKTYFNWLLQRPGQSPKRLIYLVSK

Framework L3 CDR L3 Framework L4

LDSGVPDRFTGSGSGTDFTLKISRVEAEDLGVYYCAQGTHFPWTFGGGTKLEIKRADAAPTVS

FIG. 5

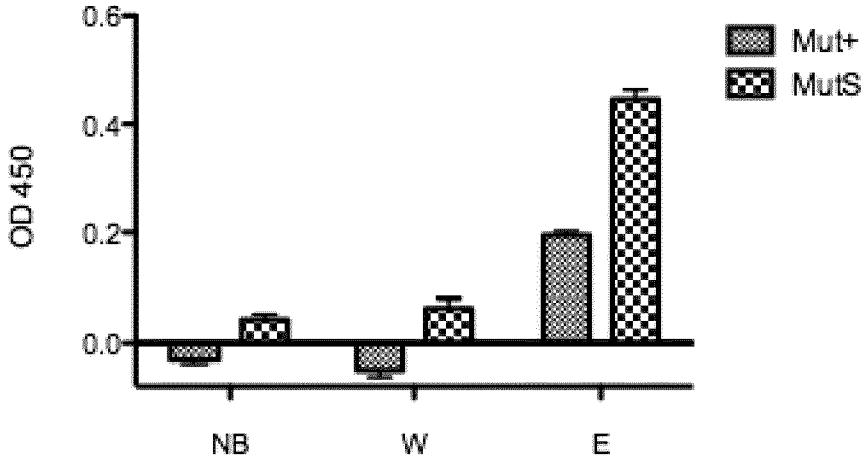
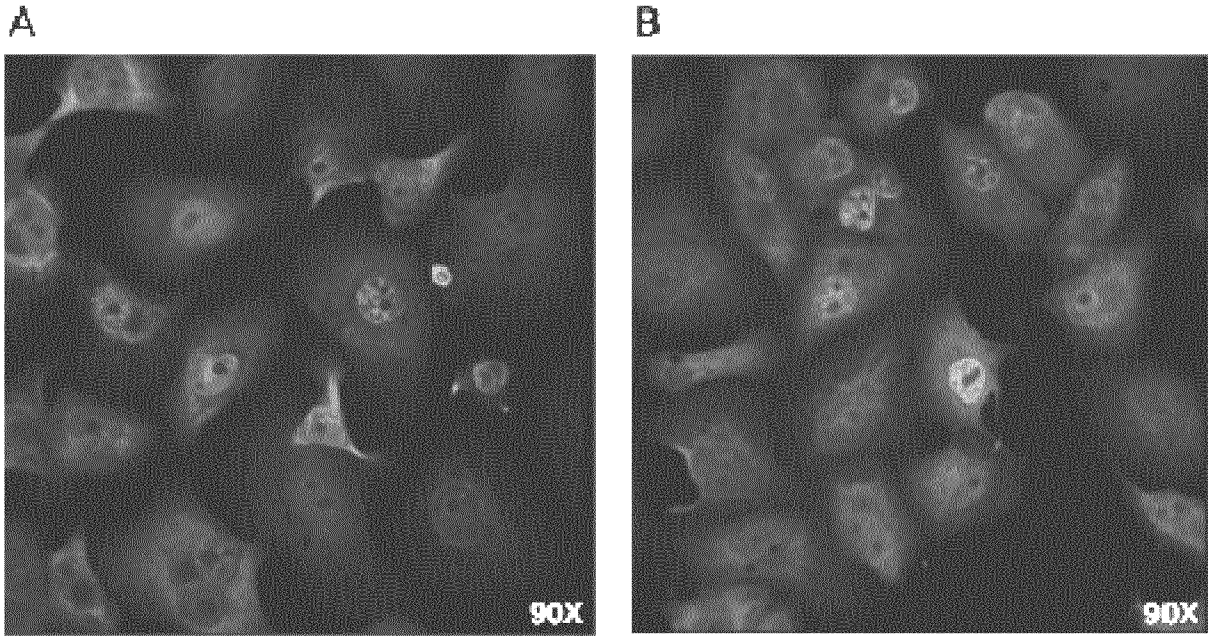


FIG. 6



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/068178

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P35/00
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)
C07K

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 , BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IT FI20 060 008 Al (UNIV FI RENZE) 11 July 2007 (2007-07-11) cited in the application the whole document	1-16
X,P	Epigentek: "HERG1 monoclonal antibody al2\ , 1 January 2015 (2015-01-01) , XP002739553 , Retrieved from the Internet: URL: http://www.epigentek.com/catalog/hergl-monoclonal-antibody-al2-p-3957.html?currency=gb&height=190&width=500&border=l&modal=true&random=1431503689546 [retrieved on 2015-04-15] the whole document	1-16

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

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 November 2015	01/12/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Covone-van Hees , M

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/068178

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