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(54) Title: HLA-G TRANSCRIPTS AND ISOFORMS AND THEIR USES

(57) Abstract: The invention pertains to the field of HLA-G molecules and their therapeutic use. The invention pertains to new HLA-G isoforms, that is to say new RNA transcripts and proteins deriving from the HLA-G gene, pharmaceutical composition comprising thereof, as well as primers specific of these transcripts and antibodies specific of these proteins. The invention further pertains to the diagnostic or therapeutic use of these molecules.

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HLA-G TRANSCRIPTS AND ISOFORMS AND THEIR USES

The invention pertains to the field of HLA-G molecules and their therapeutic use. The invention pertains to new HLA-G isoforms, that is to say new RNA transcripts and proteins deriving from the HLA-G gene, pharmaceutical composition comprising thereof, as well as primers specific of these transcripts and antibodies specific of these proteins. The invention further pertains to the diagnostic or therapeutic use of these molecules.

PRIOR ART

HLA-G is a HLA-class Ib molecule with potent immunomodulatory activities, which is expressed in physiological conditions, where modulation of the immune response is required to avoid allograft recognition (i.e., maternal-fetal interface or transplanted patients). HLA-G was first described to play a crucial role in the maintenance of pregnancy [1] and was found constitutively expressed at the fetal maternal interface in extravillous cytotrophoblasts.

HLA-G has a tolerogenic effect, modulating adaptive and innate immunity by interacting with T or B lymphocytes and NK cells or polymorphonuclear cells. This effect is mediated by the direct binding of both completely soluble and membrane-bound isoforms to inhibitory receptors via the α3 domain. Indeed, B and T lymphocytes, NK cells, and monocytes of the myeloid lineage express the immunoglobulin-like transcript ILT2 (CD85j, ILIRB1) [15]; monocytes, macrophages, and dendritic cells express ILT-4 (CD85d, LILRB2) [16]. The killer cell immunoglobulin-like receptor (KIR2DL4/p49) is specific for HLA-G and is expressed by decidual NK cells. Unlike other inhibitory receptors, it may also mediate activation [17, 18]. In addition, soluble HLA-G triggers the apoptosis of T and NK cells via CD8-like classical class I soluble molecules [19].

HLA-G expression is restricted to some tissues in normal conditions but increases strongly in pathological conditions. Indeed, HLA-G is expressed *de novo* at high levels in several pathological conditions, including solid and hematological tumors. Overexpression of membrane-bound and soluble HLA-G has been detected in different human solid and hematological tumors and might represent a mechanism performed by tumor cells to escape from the control of the immune system, by inhibiting NK and T cells mediated lysis. In particular, high incidence of HLA-G expression has been reported in clear cell renal cell carcinoma (ccRCC) [2, 3], which is among the most common human renal

malignancy [4]. In addition, the role of HLA-G as an immune checkpoint allowing tumor escape has been demonstrated in murine models [5, 6].

On the other hand, the loss of HLA-G mediated control of the immune responses may lead to the onset of autoimmune/inflammatory diseases, caused by an uncontrolled activation of the immune effector cells. Several studies in the last years have demonstrated that HLA-G plays an important role in the control of autoimmune/inflammatory diseases, such as multiple sclerosis (MS), Crohn's disease (CD), psoriasis, pemphigus, celiac disease, systemic lupus erythematosus (SLE), asthma, juvenile idiopathic arthritis, and rheumatoid arthritis (RA) [23].

Seven isoforms of HLA-G have been identified, among which 4 are membrane-bound (HLA-G1, HLA-G2, HLA-G3 and HLA-G4) and 3 are soluble (HLA-G5, HLA-G6 and HLA-G7). All of these HLA-G comprise a peptide signal in their N terminus.

The HLA-G1 protein isoform comprises the three external domains (α1, α2 and α3), the transmembrane region and the cytoplasmic domain. The HLA-G2 protein isoform does not comprise the α2 domain, i.e., the α1 and α3 domains are directly linked, followed by the transmembrane domain and the cytoplasmic domain. The HLA-G3 protein isoform lacks both the α2 and α3 domains, i.e., it comprises the α1 domain directly linked to the transmembrane domain and the cytoplasmic domain. The HLA-G4 protein isoform lacks the α3 domain, i.e., it comprises the α1 domain, the α2 domain, the transmembrane domain and the cytoplasmic domain.

Soluble HLA-G isoforms all lack the transmembrane and cytoplasmic domains. Interestingly, all these soluble HLA-G proteins contain additional amino acids not present in any of the membrane-bound HLA-G, which result from the retention of one intron. More specifically:

- The HLA-G5 protein isoform contains the α1, α2 and α3 domains, as well as an extra C-terminal peptide sequence of 21 amino acid residues encoded by intron 4 (as a result of intron 4 retention after transcript splicing and RNA maturation);
- The HLA-G6 protein isoform corresponds to the HLA-G5 without α2, *i.e.*, HLA-G6 contains α1 and α3 domains, as well as an extra C-terminal peptide sequence of 21 amino acid residues encoded by intron 4 (as a result of intron 4 retention after transcript splicing and RNA maturation);

- The HLA-G7 protein isoform contains only the α1 domain, as well as 2 additional C-terminal amino acid residues encoded by intron 2 (as a result of intron 2 retention after transcript splicing and RNA maturation).

All seven reported HLA-G isoforms result from alternative spicing of one primary transcript, have a similar translation start site and no distinct functional roles have yet been proposed.

Thus far, the numbering of the exons of the HLA-G gene was based on the IMGT/HLA database (also herein called IMGT/HLA nomenclature), and was described as comprising 8 exons, 7 introns and a 3' untranslated end, corresponding respectively to the following domains: exon 1: signal sequence, exon 2: α1 extracellular domain, exon 3: α2, extracellular domain, exon 4: α3 extracellular domain, exon 5: transmembrane domain, exon 6: cytoplasmic domain I, exon 7: cytoplasmic domain II (untranslated), exon 8: cytoplasmic domain III (untranslated) and 3' untranslated region.

However, according to the Ensembl database, the HLA-G gene might possess a supplementary exon at the 5' end that is absent from the IMGT/HLA database. In addition, since the exon 7 corresponds to an untranslated domain, the question remains whether it is relevant to consider it as a exon *per se*.

The presence of this supplementary exon would thus modifies the size of the 5'-untranslated regions (UTR) and the location of the promoter. This may alter the regulation of the gene, by modifying the binding of regulatory proteins and/or miRNA.

Therapeutic approaches based on synthetic HLA-G-derived proteins or antibodies are emerging in mouse models, and these new therapeutic tools may prove useful for the treatment of cancer, infectious diseases, autoimmune/inflammatory diseases, and allogeneic graft rejection. Furthermore, it has been shown that the soluble form of HLA-G1 (also designated HLA-G5) inhibits angiogenesis, and its use as a therapeutic target for preventing pathologic neovascularization has been suggested [28].

In this context, there is thus a need for new therapeutic approaches based on HLA-G molecules.

DESCRIPTION

The inventors have discovered new transcripts of the HLA-G gene, most likely due to alternative splicing.

The inventors have demonstrated the presence of HLA-G transcripts harboring a supplementary sequence at the 5' extremity, corresponding to a region upstream of exon 1 (according to the IMGT/HLA nomenclature). Interestingly, these transcripts, herein called long HLA-G transcripts, also have a 106 bp deletion, compared to the previously known HLA-G transcripts, and possess an ATG that might be used as a translation initiation start point ([29]).

These results confirm the hypothesis that the use of a new nomenclature, based on the Ensembl database, is relevant. The Ensembl nomenclature is therefore used hereafter, unless specifically indicated. In this new nomenclature, illustrated in figure 1, a first exon is located within the supplementary sequence at the 5' extremity, and the previous exon 7 has been suppressed. The exon numbering is therefore modified in consequence: exon 1: corresponds to the newfound sequence; exon 2: signal sequence; exon 3: α1 extracellular domain; exon 4: α2 extracellular domain; exon 5: α3 extracellular domain; exon 6: transmembrane domain; exon 7: cytoplasmic domain I; exon 8: cytoplasmic domain III (untranslated) and 3' untranslated region (compared with IMGT/HLA nomenclature, exon 1 is renumbered exon 2, exon 2 is renumbered exon 3, etc).

The inventors have moreover discovered new HLA-G transcripts which retain either intron 1, 4, 6 or 7, as well as transcripts which retain two introns simultaneously, in particular introns 3 and 4 or introns 3 and 5, which had never been reported before.

The inventors have further shown that the alternative spicing results in the possibility of new translation initiation codons different form the translation initiation codon localized in exon 2, which had so far been considered as the only possible translation initiation codon of HLA-G proteins. In the new transcripts, possible translation initiation codons have been found in exon 1 and in exon 4.

In other terms, the new transcripts encode new HLA-G proteins, which respective structures differ from the HLA-G isoforms which had been disclosed so far.

Within these new transcripts, the inventors have identified several majors features that had not been disclosed in relation with HLA-G proteins before.

A first feature is the presence, in some of the new HLA-G proteins identified, of the five amino-acid residues MKTPR at their N terminal extremity, that is to say upstream of exon 1 (according to the IMGT/HLA nomenclature). This feature results from the initiation of translation in exon 1. For convenience and clarity only, and independently of the actual

length of their amino-acid sequence, the HLA-G proteins having this features are herein called "*long HLA-G*" to highlight the presence of said additional amino-acid residues.

A second feature is the absence of the α1 domain in some of the new HLA-G proteins identified.

A third feature is the absence of the transmembrane domain in some of the new HLA-G proteins identified, said proteins having a peptide sequence distinct from the known soluble HLA-G proteins HLA-G5, HLA-G6 and HLA-G7. For convenience and clarity only, the HLA-G proteins having these features are herein called "*soluble HLA-G*".

A forth feature is the retention, in some of the new HLA-G proteins identified, of at least part of an intron, different from introns 2 or 4, which retention is observed in HLA-G5, HLA-G6 and HLA-G7.It is anticipated that all of the newly identified HLA-G proteins have tolerogenic properties, consistently with the reported function of the already known HLA-G proteins. These proteins may thus be useful in the treatment of autoimmune/inflammatory diseases, such as multiple sclerosis (MS), Crohn's disease (CD), psoriasis, pemphigus, celiac disease, systemic lupus erythematosus (SLE), asthma, juvenile idiopathic arthritis, and rheumatoid arthritis (RA), as well as in the prevention of allogeneic graft rejection.

In addition, based on the knowledge in the field, the newly found HLA-G transcripts and corresponding proteins are expected to play a role in the ability of the cancer cells to evade immune checkpoints, and therefore constitute important therapeutic targets. In this context, the inventors have designed molecules targeting either the new RNA transcripts or the proteins they encode, as well as compositions comprising such molecules, which may be used in the treatment of cancer, in particular in clear cell renal cell carcinoma (ccRCC).

The inventors have demonstrated, as detailed in the experimental part, that the newly identified HLA-G proteins have a surprising angiogenic effect *in vivo*. This effect is in striking contrast with the effects of HLA-G5/soluble HLA-G1 reported in the literature. On the one hand, newly identified HLA-G proteins may thus be useful as therapeutic angiogenesis in the treatment of pathologies where such an angiogenic effect is desired, such as ischemia, which is a symptom found for instance in cardiovascular diseases, peripheral artery diseases and stroke.

Further, the inventors have designed primers and antibodies useful in the detection of these transcripts and of the proteins they encode, which may thus be used in diagnosing cancer.

A first aspect of the invention is thus an isolated HLA-G protein which sequence has at least one of the following features:

- it comprises the five amino-acid residues MKTPR, that is to say SEQ ID NO: 1, in its N terminal part, and/or;
- it is devoid of the α1 domain, that is to say it is devoid of the sequence SEQ ID NO: 3, and/or;
- it is devoid of the transmembrane/cytoplastic domain, that is to say it is devoid of SEQ ID NO: 6, and;
- it comprises amino-acids resulting from retention of at least part of one intron, proviso said intron is not intron 2 or intron 4.

The terms "HLA-G protein" encompass any protein or polypeptide resulting from the expression of the HLA-G gene, preferably the human HLA-G gene of gene ID 3135 (as referred to in the GeneBank database based on genome reference GRCh38.p10). Preferably, a "HLA-G protein" is a protein or polypeptide comprising at least a sequence corresponding to the translation of any of exons 1 to 8 of the human HLA-G gene according to the Ensembl nomenclature. In other terms, a "HLA-G protein" preferably comprises at least one of the following domains: the five amino-acid residues MKTPR in its N terminal part, the peptide signal, the α1 domain, the α2 domain, the α3 domain, the transmembrane domain, and the cytoplasmic domain.

In the context of the invention, the "peptide signal" has the sequence SEQ ID NO: 2.

In the context of the invention, the "α1 domain" has the sequence SEQ ID NO: 3.

In the context of the invention, the "α2 domain" has the sequence SEQ ID NO: 4.

In the context of the invention, the "α3 domain" has the sequence SEQ ID NO: 5.

In the context of the invention, the "transmembrane/cytoplastic domain" has the sequence SEQ ID NO: 6.

The terms "amino-acids resulting from intron retention" should be construed as generally understood in the art. In the context of the invention, the person skilled in the art can easily identify such amino-acids by comparing the sequence of the protein with a reference sequence devoid of any amino-acid resulting from intron retention, such as for instance a reference sequence consisting in the translation of all the exons of the HLA-G gene. A reference sequence appropriate for such comparison is the sequence SEQ ID NO: 7, which corresponds to the peptide sequence of a HLA-G protein having all of the exons and none of the introns of the HLA-G gene, that is to say having the five amino-acid residues

MKTPR in their N terminal part, the peptide signal, the α1, α2 and the α3 domain, the transmembrane/cytoplasmic domain. According to the invention, any amino-acid residue which would constitute an addition in comparison with SEQ ID NO: 7 would result from intron retention.

Preferably, in the context of the invention, a "HLA-G protein which sequence is devoid of transmembrane/cytoplastic domain, that is to say which sequence is devoid of the sequence SEQ ID NO: 6", is a protein which sequence consists of:

- At least part of SEQ ID NO: 1; and/or;
- At least part of SEQ ID NO: 2; and/or;
- At least part of SEQ ID NO: 3; and/or;
- At least part of SEQ ID NO: 4; and/or;
- At least part of SEQ ID NO: 5 and
- proviso said protein does not have the sequence of HLA-G5, HLA-G6 or HLA-G7, that is to say, respectively which does consist of any of the sequences SEQ ID NO: 90, 91 and 92.

The inventors have in particular identified several new HLA-G proteins according to the invention. The isolated HLA-G protein according to the invention is preferably a protein which sequence comprises or consists of a sequence chosen from the group consisting of SEQ ID NO: 7 to SEQ ID NO: 31.

Preferably, the HLA-G protein according to the invention, which sequence comprises the five amino-acid residues MKTPR in their N terminal part is a protein which sequence comprises or consists of a sequence chosen from the group consisting of SEQ ID NO: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16.

Preferably, the HLA-G protein according to the invention which sequence is devoid of α1 domain is a protein which sequence comprises or consists of a sequence chosen from the group consisting of SEQ ID NO: 9, 10, 11, 12, 13, 14, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 29, 30, 31.

Preferably, the HLA-G protein according to the invention which sequence is devoid of transmembrane/cytoplasmic domain is a protein which sequence comprises or consists of a sequence chosen from the group consisting of SEQ ID NO: 8, 10, 12, 14, 16, 18, 20, 21, 23, 25, 27, 28, 30.

The HLA-G protein according to the invention may be a purified protein or a synthetic protein, which may be obtained by conventional techniques known from the person skilled in the art.

Preferably, the invention also encompasses variants of the above proteins, that is to say proteins which sequence has at least 80, 85, 90 or 95% identity with at least one of the above proteins.

In the sense of the present invention, the "percentage identity" or "% identity" between two sequences of nucleic acids or amino acids means the percentage of identical nucleotides or amino acid residues between the two sequences to be compared, obtained after optimal alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly along their length. The comparison of two nucleic acid or amino acid sequences is traditionally carried out by comparing the sequences after having optimally aligned them, said comparison being able to be conducted by segment or by using an "alignment window". Optimal alignment of the sequences for comparison can be carried out, in addition to comparison by hand, by means of the local homology algorithm of Smith and Waterman (1981), by means of the similarity search method of Pearson and Lipman (1988) or by means of computer software using these algorithms (GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI, or by the comparison software BLAST NR or BLAST P).

The percentage identity between two nucleic acid or amino acid sequences is determined by comparing the two optimally-aligned sequences in which the nucleic acid or amino acid sequence to compare can have additions or deletions compared to the reference sequence for optimal alignment between the two sequences. Percentage identity is calculated by determining the number of positions at which the amino acid, nucleotide or residue is identical between the two sequences, preferably between the two complete sequences, dividing the number of identical positions by the total number of positions in the alignment window and multiplying the result by 100 to obtain the percentage identity between the two sequences.

For example, the BLAST program, "BLAST 2 sequences" [27] available on the site http://www.ncbi.nlm.nih.gov/gorf/bl2.html, can be used with the default parameters (notably for the parameters "open gap penalty": 5, and "extension gap penalty": 2; the selected matrix being for example the "BLOSUM 62" matrix proposed by the program);

the percentage identity between the two sequences to compare is calculated directly by the program.

Preferably, the invention further encompasses functional variants of the HLA-G proteins according to the invention, where amino acids residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule.

Preferably, the invention also encompasses modified proteins derived from the above proteins by introduction of any chemical modification into one or more amino acid residues, peptide bonds, N-and/or C-terminal ends of the protein, aimed at increasing the stability, bioavailability or bioactivity of the protein, as long as the modified protein remains functional.

As well known in the art, an alternative to the *in vivo* use of an isolated protein may be the use of a polynucleotide encoding said protein in expressible form or a recombinant vector comprising said polynucleotide. Such vectors are useful for the production of the protein, or for therapeutic use *in vivo*.

Another aspect of the invention relates to an isolated polynucleotide encoding a protein of the invention. The synthetic or recombinant polynucleotide may be DNA, RNA or combination thereof, either single- and/or double-stranded. Preferably the polynucleotide comprises a coding sequence which is optimized for the host in which the protein is expressed.

Another aspect of the invention relates to a recombinant vector comprising said polynucleotide. Preferably, said recombinant vector is an expression vector capable of expressing said polynucleotide when transfected or transformed into a host cell. The polynucleotide is inserted into the expression vector in proper orientation and correct reading frame for expression. Preferably, the polynucleotide is operably linked to at least one transcriptional regulatory sequence and, optionally to at least one translational regulatory sequence. Recombinant vectors include usual vectors used in genetic engineering and gene therapy including for example plasmids and viral vectors.

A further aspect of the invention provides a host cell transformed with said polynucleotide or recombinant vector.

The polynucleotide, vector, and/or cell of the invention may be prepared using well-known recombinant DNA techniques.

It is expected that the proteins of the invention have tolerogenic properties similar to the HLA-G proteins which are already known in the art, in particular since all of the newly found HLA-G proteins herein disclosed harbor a α3 domain.

Another aspect of the invention relates to a pharmaceutical composition, comprising at least one protein, polynucleotide and/or vector of the invention, and, preferably, a pharmaceutically acceptable carrier.

Suitable vehicles or carriers include any pharmaceutically acceptable vehicle such as buffering agents, stabilizing agents, diluents, salts, preservatives, emulsifying agents, sweeteners, etc. The vehicle typically comprises an isotonic aqueous or non-aqueous solution, which may be prepared according to known techniques. Suitable solutions include buffered solutes, such as phosphate buffered solution, chloride solutions, Ringer's solution, and the like.

A further aspect of the invention relates to a protein, polynucleotide, and/or vector of the invention for use as a medicament.

Preferably, the above proteins, polynucleotides and/or vector of the invention are for use in the treatment of autoimmune and/or inflammatory diseases, or in the prevention and/or treatment of allogeneic graft rejection, for use in the prevention and/or treatment of ischemia, preferably ischemia associated with cardiovascular diseases, peripheral artery diseases or stroke, or for use in the prevention and/or treatment of vascular retinopathies.

In the context of the invention, the term vascular retinopathies encompasses angiomatosis of Von Hippel, cavernous retinal hemangioma, coats disease, idiopathic macular telangiectasia (Reese's disease), occlusion of the central artery of the retina, occlusions of arterial branches, occlusion of the cilioretinal artery, occlusion of the ophthalmic artery, retinal vein occlusions.

In the context of medical use, the person skilled in the art may preferably select, among the HLA-G proteins of the invention, those which have the α-3 domain, such as for instance any of the proteins of sequence SEQ ID NO: 7, 8, 9, 10, 13, 14, 17, 18, 19, 20, 21, 22, 23, 26, 27, 31.

Advantageously, the HLA-G protein for use as a medicament, preferably for use in the above treatments, is a protein which sequence comprises or consists of a sequence chosen in the list consisting of SEQ ID NO: 7, 8, 9, 10, 13, 14, 17, 18, 19, 20, 21, 22, 23, 26, 27 and 31.

In the context of the invention, autoimmune/inflammatory diseases preferably refer to multiple sclerosis (MS), Crohn's disease (CD), psoriasis, pemphigus, celiac disease, systemic lupus erythematosus (SLE), asthma, juvenile idiopathic arthritis, and rheumatoid arthritis (RA), yet preferably psoriasis.

The invention also provides a method for the treatment of autoimmune/inflammatory diseases, or for the prevention and treatment of allogeneic graft rejection, for use in the prevention and/or treatment of ischemia, preferably ischemia associated with cardiovascular diseases, peripheral artery diseases or stroke, or for use in the the prevention and/or treatment of vascular retinopathies, comprising: administering to an individual a therapeutically effective amount of at least one protein, polynucleotide and/or vector of the invention, or of the composition as described above.

By "*therapeutically effective amounts*" it is hereby referred to amounts which are, over time, sufficient to at least reduce or prevent disease progression. Typically, said amount can be adjusted by the skilled artisan, depending on the pathological condition, the subject, the duration of treatment, the presence of other active ingredients, etc.

As already said, the newly found HLA-G transcripts and corresponding proteins are important therapeutic targets, in particular for the treatment of cancer. Typically, for therapeutic purposes, RNA transcripts may be targeted by antisens oligonucleotides, while proteins may be targeted by specific antibodies.

For instance, RNA transcripts, which sequence is devoid of the sequence encoding the α1 domain but contains the sequence encoding the signal peptide and the α2 and α3 domains, have a junction between the sequence encoding the signal peptide and the sequence encoding the α2 domain, corresponding to the sequence SEQ ID NO: 32, which is not found in other HLA-G proteins. Similarly RNA transcripts which consist of the sequence encoding the α3 domain and the signal peptide, but are devoid of the α1 and α2 domains, comprise the sequence SEQ ID NO: 33, corresponding to a junction between the sequences encoding the α3 domain and the signal peptide. The RNA transcripts which encode soluble HLA-G comprise the sequence SEQ ID NO: 34.

Another object of the invention is an antisens oligonucleotide having a sequence complementary to at least part of

- SEQ ID NO: 32 ;
- SEQ ID NO: 33, or ;
- SEQ ID NO: 34.

The invention further pertains to a recombinant vector comprising a polynucleotide encoding at least one antisens oligonucleotide of the invention.

The new HLA-G proteins of the invention harbor the specific peptide sequence SEQ ID NO: 35, 36 and 37 encoded by the junction sequences SEQ ID NO: 32, 33 and 34 respectively, which may be used as antigen to produce antibodies specific for such proteins, that is to say anti-HLA-G antibodies specific of certain HLA-G proteins.

Another object of the invention is an antibody specific of at least one of the peptide of sequence SEQ ID NO: 35, 36 or 37.

For the purpose of the present invention, the term "*antibody*" refers to an immunoglobulin that specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of another molecule. The antibody may be monoclonal or polyclonal and may be prepared by techniques that are well known in the art such as immunization of a host and collection of sera (polyclonal), or by preparing continuous hybrid cell lines and collecting the secreted protein (monoclonal), or by cloning and expressing nucleotide sequences or mutagenized versions thereof, coding at least for the amino acid sequences required for specific binding of natural antibodies. Antibodies may include a complete immunoglobulin or fragment thereof, which immunoglobulins include the various classes and isotypes, such as IgA, IgD, IgE, IgG1, IgG2a, IgG2b and IgG3, IgM. Functional antibody fragments may include portions of an antibody capable of retaining binding at similar affinity to full-length antibody (for example, Fab, Fv and F(ab′)2, or Fab′). In addition, aggregates, polymers, and conjugates of immunoglobulins or their fragments may be used where appropriate so long as binding affinity for a particular molecule is substantially maintained.

The terms "*antibody specific of at least one peptide*", should be construed as generally understood in the field, that is to say as indicating that said antibody exhibits a substantial affinity for said at least one peptide, preferably an affinity of about 10^{-5} M (KD) or stronger. The affinity can be determined by various methods well known from the one skilled in the art, which include, but are not limited to, Biacore Analysis, Blitz analysis and Scatchard plot.

Another object of the invention is a pharmaceutical composition comprising an antibody, an antisens oligonucleotide, and/or a vector encoding thereof, according to the invention, and, preferably, a pharmaceutically acceptable carrier.

A further aspect of the invention relates to an antibody, an antisens oligonucleotide, and/or a vector encoding thereof, of the invention for use as a medicament.

Preferably, the above antibody, antisens oligonucleotide and/or vector encoding thereof, of the invention is used in the treatment of cancer.

Non-limitative examples of cancer include esophagus, stomach, colon, pancreas, melanoma, thyroid, lung, breast, kidney, bladder, uterus, ovary and prostate cancer; hepatocellular carcinomas, osteosarcomas, cylindromatose, neuroblastomas, glioblastomas, astrocytomas, colitis associated cancer, multiple myeloma and various types of leukemia and lymphomas such as diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), Hodgkin's lymphoma and MALT lymphoma. In a preferred embodiment said cancer is clear cell renal cell carcinoma (ccRCC).

The invention provides also a method for the treatment of a cancer, comprising: administering to an individual a therapeutically effective amount of the antibody, antisens oligonucleotide and/or vector encoding thereof, of the invention, or of the composition as described above.

As already indicated, the inventors have identified that samples of cancer cells comprise specific HLA-G transcripts, that is to say RNA molecules corresponding to the expression of the human HLA-G gene, These RNA transcripts corresponds to the sequences SEQ ID NO: 38 to 74. The inventors have thus developed an *in vitro* method for the diagnosis of cancer, based on the presence of such RNA transcripts in a biological sample.

The invention further pertains to an *in vitro* method for the diagnosis of a cancer, comprising the detection of at least at least a RNA transcript having a sequence chosen in the list consisting of the sequences SEQ ID NO: 38 to 74 and/or of at least one of the proteins of the invention as disclosed above, in a biological sample of a subject.

The term "*biological sample*" refers to a sample obtained from the subject, including sample of biological tissue or fluid origin. Such samples can be, but are not limited to, body fluid (e.g., blood, blood plasma, serum, or urine), organs, tissues, fractions, and cells isolated from mammals including, humans. Biological samples also may include sections of the biological sample including tissues (e.g., sectional portions of an organ or tissue). Biological samples may also include extracts from a biological sample, for example, an antigen from a biological fluid (e.g., blood or urine). Preferably the subject is a mammal, yet preferably a human.

In the context of the invention, the detection of at least a RNA transcript having a sequence chosen in the list consisting of the sequences SEQ ID NO: 38 to 74, and/or of at least one of the proteins of the invention indicates that the subject has a cancer.

Non-limitative examples of cancer include esophagus, stomach, colon, pancreas, melanoma, thyroid, lung, breast, kidney, bladder, uterus, ovary and prostate cancer; hepatocellular carcinomas, osteosarcomas, cylindromatose, neuroblastomas, glioblastomas, astrocytomas, colitis associated cancer, multiple myeloma and various types of leukemia and lymphomas such as diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), Hodgkin's lymphoma and MALT lymphoma. In a preferred embodiment said cancer is clear cell renal cell carcinoma (ccRCC).

The detection of at least one of the proteins of the invention can be carried out by implementing a suitable immunological method (e.g. ELISA, RIA, immunofluorescence, immunohistochemistry) by means of at least one antibody specific of said protein, as disclosed above.

The detection of the RNA transcripts can be carried out by hybridization, by means of nucleotide probes specific for said RNAs (attached, for example, to a biochip), or by amplification (for example by RT-PCR), by means of nucleotide primers specific for said RNA transcripts.

The inventors have developed nucleotide primers particularly suitable for detecting at least one of the RNA transcripts of the invention:

- the primer int4R, of sequence SEQ ID NO: 75, designed to hybridize with the beginning of intron 4, and which can therefore be used to detect the HLA-G transcripts that have retained this intron;
- the primer Ex1F, of sequence SEQ ID NO: 76, designed to hybridize with a region complementary to exon1, and which can therefore be used to detect the transcripts encoding the long HLA-G proteins ;
- the primer PrPrF, of sequence SEQ ID NO: 77, designed to hybridize with a region located further upstream of the region annotated as the 5'-transcript end of the gene, reported as the promoter region, and which can therefore be used to detect the transcripts encoding the long HLA-G proteins;
- the primer Int3F, of sequence SEQ ID NO: 78, designed to hybridize with a region located in intron 3 and which can be used to detect the HLA-G transcripts that have retained this intron;

- the primer Int5R, of sequence SEQ ID NO: 79, designed to hybridize with a region located in intron 5 and which can be used to detect the HLA-G transcripts that have retained this intron.

Those primers are particularly useful for implementing the diagnosis method of the invention. Thus, in an embodiment, in the method of the invention, the detection of at least a RNA transcript of the invention is performed using at least a primer having a sequence chosen in the list consisting of SEQ ID NO: 75 to 79.

These specific primers may be used in combination with each other or other known primers specific of HLA-G transcripts, such as the primer G526R of sequence SEQ ID NO: 80, which has been disclosed in the art. Further any of the combinations of the primer Int3F and either the primer Int5R or the primer int4R may be used to detect the transcripts having retained two introns (introns 3 and 4, or introns 3 and 5).

In an preferred embodiment, in the method of diagnosis of the invention, the detection of at least a RNA transcript of the invention is performed using at least one of the combinations of:

- the primer Ex1F of sequence SEQ ID NO: 76 or the primer PrPrF of sequence SEQ ID NO: 77, and the primer G526R of sequence SEQ ID NO: 80;
- the primer int4R of sequence SEQ ID NO: 75, and the primer Int3F of sequence SEQ ID NO: 78;
- the primer the primer Int3F of sequence SEQ ID NO: 78 and the primer Int5R of sequence SEQ ID NO: 79;

Another object of the invention is therefore a primer having a sequence chosen in the list consisting of SEQ ID NO: 75 to 79.

The invention further pertains to a kit comprising at least two primers having a sequence chosen in the list consisting of SEQ ID NO: 75 to 80.

Preferably, the kit comprises at least a combination of primers chosen in the list consisting of:

- the primer Ex1F of sequence SEQ ID NO: 76 or the primer PrPrF of sequence SEQ ID NO: 77, and the primer G526R of sequence SEQ ID NO: 80;
- the primer int4R of sequence SEQ ID NO: 75, and the primer Int3F of sequence SEQ ID NO: 78;
- the primer Int3F of sequence SEQ ID NO: 78 and the primer Int5R of sequence SEQ ID NO: 79;

The invention also comprises other provisions that will emerge from the following examples of implementation, which may not be construed as limiting the scope of the invention.

LEGEND OF THE FIGURES

Figure 1 - Schematic representation of the structure of the HLA-G gene. A. IMGT/HLA nomenclature (top) and Ensembl database (bottom). Numbers represent exons and the domains of the HLA-G protein are shown underneath. TM: transmembrane; CT: cytoplasmic tail. B Localization of primers used for the different RT-PCR strategies. Sizes, in bp, for specific amplicons and the translation initiation codons are indicated.

Figure 2 - Differential morphologic and HLA-G staining patterns of eight ccRCC included in this study. A trophoblastic tissue was used as positive control for immunohistochemical study (H&E and immunoperoxidase stains).

Figure 3 - Expression of HLA-G1 in ccRCC patients. RNA were subjected to RT-PCR using the HLA-G1 specific primers G257F and G526R (upper panels) and ACTB primers as controls (lower panels). Lanes 1: adjacent non-tumor region except for tumors of patients 6 and 8. Lanes 2, 3 and 4: different tumor areas. For patients 6 and 8, all regions shown correspond to tumor areas since partial nephrectomies were performed and adjacent tumor regions were not available. M: 100bp size marker.

Figure 4 - Intron retention events found in HLA-G transcripts. Only reads spanning intron-exon junctions have been considered. Reads corresponding exclusively to intron sequences were discarded.

Figure 5 - Molecular validation of main intron retention events. A: Diagrammatic representation of the RT-PCR strategy developed to amplify retained introns. B: Results of the RT-PCR analysis using actin primers as control for the absence of genomic DNA (left) and Int1 and G257R primers to detect the presence of intron 1(right). The band of 523bp reveals the absence of intron 2, which would produce a band of 649bp C: HLA-G transcripts that retain only intron 4 (left panel) or HLA-G transcripts that retain several introns simultaneously (middle and right panels).

Figure 6 - Identification of the 5'-extended transcript HLA-G1. A. Detail of the DNA sequence showing the reduced distance between the two ATGs. The sequence was performed upwards using the G526R primer B. Schematic representation of the 106bp-

deletion; the two ATG are underlined. **Figure 7: A:** Pictures of NSG mice xenografted with RCC7 cells infected with a viral vector encoding the long HLA-G1, taken on day 38 after injection. **B:** Pictures of NSG mice xenografted with RCC7 cells infected with a viral vector encoding the long HLA-G1L, taken on day 38 after injection.

Figure 8: A: Pictures of nude mice xenografted with RCC7 cells expressing either GFP, HLA-G1 or HLA-G1L, on day 25 after intradermal injection. **B**: Pictures of nude mice xenografted with RCC7 cells grown on matrigel and expressing either GFP, HLA-G1 or HLA-G1L, on day 25 after intradermal injection.

Figure 9: Pictures of NSG immunodeficient mice 8 days after injection of a control RCC7 cells (expressing GFP) in the left ear, and of RCC7 cells expressing HLA-G1L in the right ear (A and B).

Figure 10 Pictures of NSG immunodeficient mice 8 days after injection of control RCC7 cells in the left ear, and of RCC7 cells expressing HLA-G1 in the right ear (A and B).

EXAMPLES

A. Detection and analysis of new HLA-G isoforms

1. Materials and Methods

1.1 Tumor and patients

All patients of this study underwent a radical nephrectomy for ccRCC as first therapeutic intervention in the urology department of Saint-Louis Hospital (Paris, France) from November 2014 to April 2015. The median tumor size was of 50 mm (range, 35 to 175). According to the 2010 primary tumor TNM classification, these tumors were classified as pT1a (patient 6), pT1b (patients 1, 3, and 8), and pT3a (patients 2, 4, 5, and 7). Two patients (patients 2 and 4) had visceral metastases at presentation. All these renal tumors were classified as ccRCC by an experienced uropathologist according to the WHO classification of tumors of the kidney [8]. All patients that participated to this study gave their free and informed writing consent. The study was approved by the institutional review boards of Saint-Louis Hospital, Paris.

1.2 Tumor specimen processing

For each tumor and according to the tumor size, we isolated between 3 and 10 samples of 10 × 5 × 5 mm, representing the spatial extent and macroscopic intra-tumor heterogeneity. Half of each sample was snap frozen in liquid nitrogen within 1 h of clamping of the renal artery and the other half was used to perform histological analysis and was documented by photography. Regions that did not contain tumor cells on histopathological examination were also isolated as controls.

1.3 Immunohistochemistry

An immunohistochemical study was performed for each tumor on 4-µm-thick, formalin-fixed and paraffin-embedded tumor tissue sections. The following murine antibodies were used: 4H84, an IgG1 recognizing an epitope located into the alpha1 domain common to all HLA-G isoforms (dilution 1/200, Santa Cruz Biotechnology, Santa Cruz, CA), and two antibodies 5A6G7 and 2A12 recognizing the epitope encoded by the retained intron 5 (Ensembl database) present in soluble HLA-G5 and -G6 isoforms (dilution 1/100, Exbio antibodies, Exbio Co., CR). The staining was performed on automated slide stainers from Roche (BenchMark ULTRA system, Tucson, AZ) using the OptiView DAB IHC Detection Kit (Roche), Cell Conditioning 1 (CC1) short or standard antigen retrieval, an antibody incubation time of 32 min at 37°C, ultraWash procedure, counterstaining with Hematoxylin II for 4 min and bluing reagent for 8 min. Positive and negative controls gave appropriate results for each procedure.

The immunohistochemical analyses were performed by the uropathologist using a BX51 microscope (Olympus France S.A.S, Rungis). Each immunostaining was scored on the basis of membranous and/or cytoplasmic staining by both intensity of staining as negative, weak, moderate, or strong and distribution of staining as negative (0% of tumor area), minimal (0-10% of tumor area), focal (<50% of tumor area), or diffuse (> 50% of tumor area). A trophoblastic tissue was used as the positive control and isotype-specific immunoglobulins were used for negative controls with each run.

1.4 Trophoblast sample preparation

Trophoblastic tissues were obtained from abortions (less than three months of pregnancy). After mechanical dissociation, the samples were preserved in Trizol™ Reagent (LifeTech, ref. 15596-026) at -80°C until RNA extraction using the protocol described below.

1.5 RNA Extraction

Total RNA was isolated from tissue sections manually crushed in Trizol™ Reagent (LifeTechnologie, ref. 15596026). After chloroform separation, the RNA was purified using miRNeasy mini Kit (Qiagen, ref. 217004) according to the manufacturer's instruction, with a DNase treatment extra step (Qiagen, ref. 79254). The RNA purity and concentration was assessed using a Nanodrop spectrophotometer and the Agilent 2100 Bioanalyzer System. RNA Integrity Number (RIN) values were mostly > 8.

1.6 RT-PCR

Reverse transcription of RNA into cDNA was perfomed using GoScript Reverse Transcriptase kit (Promega, ref. A5001) with a thermocycler Eppendorf (MasterCycler, Pro S). The PCR reactions were carried out in a final volume of 10 µL, containing 2 µL of cDNA template, using an ampliTaq polymerase from LifeTech (Ref. N80800166). For amplification, 40 cycles (at 94°C for 30 sec, 55 or 60°C for 30 sec, and 72°C for 30 sec) were conducted. HLA-G and actin (ATCB) primers are described in Table 1. ATCB amplification was performed as control in all the experiments. The PCR amplification product was mixed with 6X loading dye (Promega, ref. G1881) and analyzed on 2% agarose gel stained with 2µL of ethidium bromide at 1 mg/mL for 100 mL of agarose gel. The molecular weight marker used was 1 Kb plus DNA ladder from Invitrogen (Ref. 10787018). Imaging was performed using a ChemiDoc XRS System (Biorad), and interpretation using ImageLab software (Biorad).

Gene	**SEQ ID NO:**	**Sequence (5' to 3')**
PrPr F	77	5'-GTAACATAGTGTGGTACTTTG
Ex1F	76	5'-CCTGGACTCACACGGAAACT
E2 F	81	5'-GGACTCATTCTCCCCAGACG
257 F	82	5'-GGAAGAGGAGACACGGAACA
257 R	83	5'-TGTTCCGTGTCTCCTCTTCC
526 F	84	5'-CCAATGTGGCTGAACAAAGG
526 R	85	5'-CCTTTGTTCAGCCACATTGG
963 R	86	5'-GCAGCTCCAGTGACTACAGC
Int1 F	89	5'-GGCCTCAAGCGTGGCTCTCA
Int3 F	78	5'-CCCAAGGCGCCTTTACCAAA
Int4 R	75	5'-CCACTGCCCCTGGTAC

Int5 R	79	5'-AGCCCTCACCACCGACC
ATCB F	87	5'-TCCTGTGGCATCCACGAAACT
ATCB R	88	5'-GAAGCATTTGCGGTGGACGAT

Table 1. PCR primers for RT-PCR experiments

1.7 RNA sequencing

Indexed complementary DNA libraries were prepared from 1µg of total RNA following the Illumina TRUSEQ protocol. Average size of the AMPure XP beads (Beckman Coulter, Inc.) purified PCR products was 275 bp. The paired-end 150 bp reads sequencing of the transcriptome was performed on equimolar pools of four cDNA libraries on a NextSeq 500 (ILLUMINA).

1.8 High-throughput analysis of HLA-G isoforms

The Ensembl nomenclature will be used throughout the text. Short reads from NGS sequencing were mapped to human Reference Genome NCBI Hg19 using BWA aligner (BWA MEM option) [20]. Low quality mapping reads were filtered out from alignment files and the reads mapping to the HLA-G locus were extracted using samtools (Li et al., 2009). Intron retained detection was performed by selecting reads overlapping an intron and one of the surrounding exons, retention for an intron was assessed only when we detected reads overlapping both 5' and 3' flanking exons. Exon skipping detection was performed by analyzing reads presenting split mapping, searching for discontinuity in the order of mapped exons, eg: a read that is mapped to exon the end of 4 and start of exon 6 but is not mapped to exon 5, presents a skipping of exon5. Each read subset was visually validated with IGV [22]. For the retention of intron n, the percentage of reads pni supporting the event is calculated as the ratio between the reads supporting the events (reads at junction exon n / intron n, internal intronic reads on intron n and reads at junction intron n / exon n+1) and the total number of reads spanning the region where the event occurs (the region starting from the junction between exon n and intron n to the junction between intron n and exon n+1): Let R(i) be the number of reads strictly in region i (the reads are only in region i and do not overlap with other regions) and R(i, j) be the number of reads overlapping both regions i and j. Let S(i) be the number of reads supporting a skipping of exon i (reads overlapping exon n and exon m where m > n+1). The number of reads supporting the retention of intron n is thus IRn = R(exonn, intronn) + R(intronn) +

R(intronn, exonn+1). The total number of reads in the region of the retention of intron n is Tn = IRn + R(exonn, exonn+1) + S(n); pni is thus given by pni = IRn / Tn.
For the skipping of exon n, the percentage of reads pne supporting the event is given by pne = S(n) / Tn. Analysis of potential biases were assessed by using the TopHat2 aligner [24].

2. Results

2.1 Marked subcellular heterogeneity of HLA-G isoforms distribution in ccRCC

In order to consider HLA-G as a potential target for cancer therapy, the expression of HLA-G in tumor cells derived from patients with ccRCC was assessed. To this end, 3 to 10 sections for each tumor were isolated, according to the tumor size. Microscopy analysis performed on hematoxylin and eosin (H&E) stained slides confirmed a morphologic heterogeneity (Fig 2, left panel), classically associated with ccRCC [8]. We further dissected this heterogeneity by immunostaining with specific antibodies directed against HLA-G: 4H84, which recognizes an epitope located into the alpha1 domain common to all seven reported HLA-G isoforms and the antibody 5A6G7 that only recognizes soluble HLA-G5 and HLA-G6 isoforms. This antibody targets the amino acids encoded by the retained intron 5 (previously known as intron 4 according to the IMGT/HLA nomenclature). Trophoblastic cells, which express HLA-G at high levels, were used as positive controls.

Even though all tumors expressed HLA-G in at least one area, this expression was distinct between and inside tumors. Tumors of patients 1 and 2 showed a strong immunostaining with 4H84 antibody in all regions. The staining was membranous and cytoplasmic (Figure 2). Noteworthy, an additional very strong staining of hyaline globules located in the cytoplasm of the tumor cells was also detected. These hyaline globules were well visible on H&E slides and constituted a very uncommon aspect of tumor [9]. On the other hand, using the 5A6G7 antibody, a weak or moderate granular cytoplasmic immunostaining was noticed in the cytoplasm but not in hyaline globules. The expression of HLA-G in tumors from other patients was very different: tumors of patients 6 and 7 presented a diffuse but moderate membrane immunostaining with 4H84 antibody. These two tumors showed no (patient 6) or weak and focal (patient 7) granular intracytoplasmic immunostaining with

5A6G7 which denoted the absence of soluble proteins HLA-G5 and HLA-G6. In two other tumors (patients 4 and 5), the expression of HLA-G evaluated by 4H84 antibody was noted in small microscopic areas of only one tumor region. Of note, the only HLA-G positive area of patient 4's tumor corresponds precisely to intracytoplasmic hyaline globules. No stain was observed in any other region of the tumor.

The immunostaining profiles of tumor cells of patients 3 and 8 were unexpected. No immunostaining was detected with the 4H84 antibody which labels all the reported HLA-G isoforms. The lack of labeling of tumor sections with this antibody normally accounts for the absence of HLA-G expression. However, a diffuse and strong granular intracytoplasmic 5A6G7 immunostaining, and a diffuse, thin and granular intracytoplasmic immunostaining were observed in tumor cells of patients 3 and 8, respectively. This was unpredictable considering our current knowledge on the structure of the seven reported HLA-G isoforms since they all contain the alpha 1 domain recognized by the 4H84 antibody. To try to better understand these differences, we have performed a similar analysis using an antibody that also recognizes the epitope encoded by the retained intron 5 (Ensembl database) present in soluble HLA-G5 and -G6 isoforms named 2A12. The results revealed different and unanticipated immune-staining patterns, notably the labeling of hyaline globules in patients 1 and 2.

Together, the results of the immunohistochemical study clearly demonstrate intra- and inter- heterogeneity of HLA-G expression in ccRCC tumors. However, some immuno-staining patterns were unexpected within the boundaries of our prevailing knowledge on the structure of HLA-G isoforms.

2.2 Survey of HLA-G1 transcripts expressed in ccRCC

To gain a better insight into the HLA-G isoforms that are expressed in ccRCC and clarify the results of the immunohistochemical analysis, a survey of HLA-G isoform diversity was further assessed by RT-PCR. The tumor sections of the eight patients studied above were amplified with the well-known G257F and G526R primers [10] schematically represented in Fig 1B. These primers amplify a region that contains the epitope recognized by the 4H84 antibody. Amplification of actin mRNA was performed for each sample as control. A predicted band of 290 bp, specific for the amplification of HLA-G1 transcripts, was found in all tumor sections for patients 1, 2 and 6 whereas this band was only detected in one or two regions of tumors of other patients (Fig 3). No amplification products were

detected in non-tumoral adjacent tissues. Since the sequence of the different isoforms are highly similar and these RT-PCR conditions do not allow the identification of other isoforms like HLA-G2, -G3, -G6 or -G7 which lack exon 4, the target of primer G526, we undertook a large-scale study by RNAseq in order to provide a comprehensive picture of isoforms expressed in ccRCC.

2.3 RNA-Seq reveals unannotated HLA-G transcripts

RNAseq technology provides the most powerful method to analyze expressed isoforms, offering the opportunity to detect alternative splicing events and unannotated transcripts which are essential for understanding development and disease mechanisms in a species [25].

As a first look, we have undertaken the sequencing of four representative samples at a very high depth of coverage (depth > 300x). Reads were aligned and quantified according to the Ensembl 70 (GRCh37.p8) reference annotation as described in Material and Methods. Alternative spliced isoforms were mainly categorized into two major groups: exon skipping and intron retention, in which a single exon or intron is alternatively spliced or included out of the mature message.

To verify whether the HLA-G expression patterns of ccRCC patients described above constitute a representative subset of general profiles found in ccRCC patients, we have compared our results to those obtained for the "Cancer Genome of the Kidney" (CAGEKID) cohort which includes a hundred ccRCC patients that were treated in four different European countries (Czech Republic, United Kingdom, Romania and Russia). The data that have been generated constitute a high-quality resource that allowed detecting alternative splicing events with high accuracy (Scelo et al., 2014). Moreover, we have deeply assessed whether common factors such as the choice of the aligner for RNAseq data or the reference sequence to study HLA-G might potentially bias our analysis by using two different aligners, BWA MEM and TopHat2. The results confirmed that the data aligned with BWA MEM or TopHat2 produce similar results (supplementary data). Further, the count of reads at the individual level showed a great similarity between the expression profiles of HLA-G transcripts found in our small cohort of ccRCC patients and that of Cagekid. These results are summarized on Tables 2 and 3 and will be discussed more thoroughly in the following sections.

2.4 Undescribed intron retention events in expressed HLA-G transcripts

Intron retention is the rarest type of alternative splicing in mammals and account for only approximately 3% of alternate transcripts [12].So far, only the retention of intron 3 or intron 5 (previously known as intron 2 and intron 4, according to IMGT/HLA nomenclature) was reported in literature for HLA-G transcripts. Transcripts that retain intron 3 encode HLA-G7 [13] and those retaining intron 5 encode HLA-G5 and HLA-G6 [7].

In our RNAseq analysis, introns subsumed by an exon were labeled as retained. The results, represented graphically on Figure 4 and summarized in Table 2, showed that reads representing the retention of introns 3 and 5 were the most abundant. In addition, the data support a number of overall new findings that originate from the retention of four additional introns: 1, 4, 6 and 7. To validate the expression of intron-retained transcripts, we first looked for the presence of transcripts containing the intron 1. To this end, we performed RT-PCR amplifications using a strategy described in Figure 5. First, primer that targets intron 1 (Int1F) was used in combination with G257R, the reverse primer of G257F [13]. Since the presence of introns may be due to contaminating endogenous genomic DNA, all samples were amplified in parallel with actin specific primers located in two different exons. The expected size for the amplification of cDNA derived from mRNA is 320 bp whereas that of genomic DNA is 560 bp. The results show only the amplification of a 320 bp-fragment in all samples, demonstrating the absence of genomic contamination (Figure 5B, left panel). In view of this result, we further amplified tumor samples using primers Int1F and G257R. An amplified band of the expected size (521 bp) was obtained, consistent with the presence of intron 1 in HLA-G transcripts (Figure 5-B, right panel). This event was not reported before in literature since the initiation of transcription of HLA-G was solely assigned to exon 2 [26]. We did not detect a PCR amplification band of 649bp that would correspond to the concomitant retention of intron 2. This is consistent with the results of the RNAseq analysis showing that intron 2 is infrequently retained.

	Patient 1	**Patient 3**	**Patient 4**	**Patient 5**	**B00E4I3**	**B00E4IS**
#reads q30 at HLA-G locus (mean)	4324	1353	238	142	6216	5066
exon1 total reads	6	6	0	0	0	0
exon2 total reads	120	2	0	5	39	15

exon3 total reads	1344	367	11	11	390	384
exon4 total reads	1483	260	37	19	1054	1078
exon5 total reads	1397	319	28	21	2002	1375
exon6 total reads	449	47	0	11	260	187
exon7 total reads	248	10	0	9	2	6
exon8 total reads	1079	676	16	25	1934	1503
retention of intron 1	40	4	1	0	38	12
retention of intron 2	0	3	3	0	4	1
retention of intron 3	2	101	38	0	36	71
retention of intron 4	133	28	2	0	31	84
retention of intron 5	148	28	8	0	179	87
retention of intron 6	37	46	2	7	35	67
retention of intron 7	119	47	0	0	454	96
skipping of exon 4	1	0	0	0	9	7
skipping of exon 5	0	0	0	0	0	0
skipping of exon 6	2	0	0	0	0	0
skipping of exon 7	21	1	0	0	29	31
skipping of exon 4 and 5	2	0	0	0	3	4
skipping of exon 4,5,6 and 7	0	0	0	0	0	0
skipping of exon 4,5 and 7	0	0	0	0	0	0
skipping of exon 5,6 and 7	0	0	0	0	0	0
skipping of exon 6 and 7	2	0	0	0	12	8
raw count of reads start exon2	132	15	0	0	0	0
raw count of reads start exon3	0	5	0	0	1	23
raw count of reads start exon4	0	3	0	0	10	8
raw count of reads start exon5	291	67	6	5	10	3

Table 2. Number of reads for all observed HLA-G splicing events in ccRCC samples
Patients 1, 3, 4 and 5 are representative samples selected for exploring the diversity of HLA-G isoforms. B00E4I3 and B00E4IS are the two samples with the highest HLA-G expression within the CAGEKID (CAncer GEnome of the KIDney) [14].

Further analysis were conducted to validate the retention of intron 4 (Figure 5C). To this end, RT-PCR was performed using primer G257F in combination with a primer that

specifically targets intron 4 (named int4R). Amplification with these primers generated a DNA fragment of 430 bp (Fig 5C, left panel), demonstrating the presence of intron 4 in HLA-G transcripts. The size of the amplified band is also consistent with the presence of a concomitant retention of intron 3. To further assess whether the same transcript might retain several introns simultaneously, we have performed a RT-PCR amplification using primer int3F (whose sequence is complementary to a region of intron 3) in combination with primer int4R. The results reveal a DNA fragment of 380bp, as expected for the retention of introns 3 and 4 in the same transcript (Figure 5C, middle panel). In addition, amplification with Int3F and Int5R primers generated an amplified band of 725pb (Figure 5C, right panel). Of note, the size of this band corresponds to the retention of intron 3 and 5, excluding intron 4. These results clearly demonstrate that tumor samples might express transcripts that retain a single intron and others that retain several different introns which may vary from one transcript to the other. To our knowledge, these events were not previously described.

2.5 Novel HLA-G transcripts with 5'-extended end

The RNAseq data further revealed that some of the reads aligned on either side of exon 1 (Figure 4). Transcripts that originate from this area were not previously reported. In fact, the structure of this region is still a matter of debate since information contained in the Ensembl database suggests that HLA-G transcripts may be initiated at this exon, which is located 5' of the exon 1 defined by IMGT/HLA nomenclature (Fig 1A). The presence or absence of this exon may result in major modifications which include the promoter localization, the length of the 5'-untranslated region and the transcription/translation initiation site. We assess whether HLA-G transcripts may be initiated in this exon or even upstream by RT-PCR amplification. Two specific primers were designed. Primer Ex1F, whose sequence is complementary to a region located in exon1 (Ensembl database) and primer PrPr, whose sequence is complementary to a region located further upstream currently considered as the promoter region (schematically represented in Fig 1B). RT-PCR using these two upstream primers in combination with G526R produced two bands of expected sizes: 690 bp (for Ex1F-G526R) and 725 bp (for PrPr -G526R) respectively (data not shown). To verify the specificity of these fragments, amplified DNA samples were sequenced and nucleotide similarities were searched in public databases using BLAST. The results demonstrated a high degree of similarity with HLA-G except for a deletion of

106 bp fragment. Resulting from this deletion, the distance between the ATG located at the end of exon 1 and the one located in exon 2 was reduced from 118 bp to 12 bp (Fig 6A). As a consequence, the 106-bp deletion brings both ATG in frame. This may now allow the initiation of translation at the ATG located in the first exon and generate a protein that would have a 5'-extended end of five additional amino acids (MKTPR). At present, the only translation initiation start site was attributed to the ATG located in exon 2 (which corresponds to exon 1 defined by IMGT/HLA nomenclature). This transcript was also found in some of the trophoblast samples tested but not all. This indicates that factors regulating it expression are still to be elucidated.

Altogether these results are consistent with the existence of a novel HLA-G transcript, named HLA-G1L, having an extended 5'-end, which might be co-expressed in trophoblasts and ccRCC tumor cells with previously reported HLA-G isoforms.

2.6 Alternatively spliced exons potentially generate novel soluble HLA-G isoforms

Exon skipping is one of the major forms of alternative splicing, which generates multiple mRNA isoforms differing in the precise combinations of their exon sequences. Here, we define an exon skipping event as a pairing between an exon-containing form and an exon-excluding form, occurring at the same exon and with the same flanking introns. The same exon may be involved in multiple exon skipping events.

For HLA-G, only the skipping of exon 4 (HLA-G2), exon 5 (HLA-G4), or both simultaneously (HLA-G3), were reported in literature. In this study, aligned reads with BWA mem reveal the skipping of exons never uncovered before. The main skipping events are reported in Table 2. We also confirmed these results by using TopHat2.

The highest read coverage was consistent with the skipping of exon 7 alone, which contains the stop codon of the protein. However, no major modifications are expected in the encoded protein lacking this exon since a supplementary in-frame stop codon is found at the beginning of exon 8. Most importantly, skipping of exon 7 concomitantly to exon 6, which encodes the transmembrane domain, is highly relevant since their absence may generate isoforms that lack the transmembrane domain and the cytoplasmic tail and therefore would constitute still unreported soluble proteins.

When RT-PCR was performed with primer G963R, whose sequence is complementary to a region of exon 6, no amplification products could be obtained in combination with the forward primers G257F (exon 3) or G256F (exon 4). However, an expected 290bp

amplified fragment was generated when the primer G257F was used in combination with G526R. Together these results are consistent with HLA-G transcripts that possess exons 3 and 4 but lack exon 6. In addition, when these primers were used to analyze samples from patient 1, amplified bands were obtained using the primer combination G526F-G963R whereas no amplification was detected using G257F-G963R, consistent with the expression of transcripts that lack exon 3.

2.7 Alternative spliced HLA-G isoforms lack the alpha-1 domain

Further analysis of RNAseq data reveals that some of the reads might be initiated at exon 4. This was determined by quantifying the raw count of reads within 20 pb upstream of the exon acceptor site. The predicted N-terminal-truncated protein would lack the peptide signal and the alpha1 domain. To assess whether the translation into a protein might start in this region, we have examined the nucleotide sequence of exon 4. This analysis revealed the presence of an in-frame ATG that might serve as a translation initiation codon. Our preliminary results (not shown) reveal that transcripts that lack the alpha-1 domain may lack also the alpha-2 domain and therefore encode only the alpha-3 domain.

Notably, the expression of these isoforms may now provide a hypothesis on the differences of immuno-staining patterns generated following the labeling of some tumor samples with 4H84 and antibodies that have been raised against soluble isoforms, which could not be explained previously within the boundaries of widespread knowledge on the structure of HLA-G isoforms.

Alternative splicing events	%overallSamples	median	%overallSamples CAGEKID	median CAGEKID
retention of intron 1	50	100	25.97	100
retention of intron 2	25	0	41.56	0
retention of intron 3	50	43.9	85.71	8.62
retention of intron 4	50	84.85	75.32	13.89
retention of intron 5	75	82.35	92.21	15.62
retention of intron 6	75	70.66	90.91	17.02
retention of intron 7	50	85.62	90.91	54.17
skipping of exon 4	0	0	38.96	0
skipping of exon 6	0	0	31.17	0
skipping of exon 7	50	7.66	81.82	21.23
skipping of exon 6 and 7	0	0	62.34	2.25

Table 3. Percentage of transcripts for each splicing event observed *Percentage of overall samples is the percentage of samples presenting the event. The last two columns are the same metrics calculated for 77 CAGEKID samples expressing HLA-G.*

B. Pro-tumoral effect of the HLA-G isoforms

1. Materials and Methods

1.1 Production of lentiviruses expressing HLA-G isoforms

The HLA-G1 and HLA-G1L isoforms were introduced into the plasmid pWPXL (10510 bp), between the BamH1 (3499) and NdeI (4334) sites, just 3 'of the EF-1α promoter which directs the expression of two isoforms HLA-G.

For HLA-G1:

The inserted fragment of 3438 bp comprises the HLA-G1 cDNA initiated in the SEQ ID NO. 93 AGTGTGGTACTTT sequence and ending in 3' with the SEQ ID NO. 94 TGGAAGACATGAGAACTTTCCA sequence. This fragment is followed by a "red" variant of the GFP (Aequorea victoria green fluorescent protein jellyfish), named Neptune that has been brought under control of the CMV promoter. Finally, at the 3' end, a molecular barcode was introduced as an integration marker and for *in vivo* monitoring of metastases (Grosselin et al., Stem Cells, 10: 2162-71, 2013).

For HLA-G1L:

The inserted fragment of 3279 bp comprises the HLA-G1L cDNA initiated at the SEQ ID NO. 95 ATATAGTAACATAGTGT sequence and ending in 3 'with the SEQ ID NO. 94 TGGAAGACATGAGAACTTTCCA sequence. This fragment is followed by a "blue" (cyan) variant of GFP, the ECFP which has a bimodal excitation and emission spectrum at 433/445 nm and 475/503 nm leading to a fluorochrome with a gloss and improved photostability. ECFP was put under control of the CMV promoter. Finally, at the 3' end, a molecular barcode was introduced as integration marker and for *in vivo* monitoring of metastases (Grosselin et al., Stem Cells, 10: 2162-71, 2013).

These 2 plasmids were used to produce lentivirus WPXL ΔU3 SIN, envelope VSV-G, OGM group II, class 2 at 1.20E + 08 TU (Transduction Unit) / ml.

2. Results

Each lentivirus contains a different HLA-G isoform. The lentiviruses were transduced in a line of renal cell carcinoma clear (cells RCC7) lineage perfectly characterized, not

expressing HLA-G. For each isoform, two independent transductions were performed to increase the reliability and robustness of our results.

An intradermal injection was performed of each of the RCC7 cell lines transduced, into 5 NSG mice per condition. Non-transduced RCC7 cells are used as a control.

After intradermal injection of the cells, tumor / metastatic growth was evaluated regularly. At the time of sacrifice of the mice, tumors metastases, and different tissues were removed, for immunohistochemical and expression (RNA) analysis. Each isoform is associated with a barcode, making it possible to ensure that the tumors and metastases obtained come from the injected cells.

As can be seen in figure 7, the xenografted mice from the cells from the RCC7 line bearing the long HLA-G1L isoform showed at J 38, a more marked tumor growth, at least partially linked to a more developed intra and peritumoral neovascularization. No intra-tumoral necrotic reworking is observed in these tumors, unlike those resulting from the RCC7 line carrying the HLA-G1 isoform.

Similar experiments were done with nude mice. Figure 8A shows the pictures of nude mice xenografted with RCC7 cells expressing either GFP, HLA-G1 or HLA-G1L, on day 25 after injection. Figure 8B shows the pictures of nude mice xenografted with RCC7 cells grown on matrigel and expressing either GFP, HLA-G1 or HLA-G1L, on day 25 after injection.

C. Pro-angiogenic effect of the HLA-G isoforms

RCC7 cells expressing either GFP, HLA-G1 or HLA-G1L were prepared as disclosed above (point B).

The left ear of NSG mice were injected with control (RCC7 cells expressing GFP), while their right ear were injected with RCC7 cells expressing either HLA-G1 or HLA-G1L. Pictures were taken on day 8. The results are shown in figure 9 (control RCC7 cells vs HLA-G1L) and in figure 10 (control RCC7 cells vs HLA-G1).

The results demonstrate a pro-angiogenic effect of the expression of HLA-G1L, which is not reproduced by the expression of HLA-G1.

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CLAIMS

1. An isolated HLA-G protein which sequence has at least one of the following features:
   - it comprises the five amino-acid residues MKTPR, that is to say SEQ ID NO: 1, in its N terminal part, and/or;
   - it is devoid of the α1 domain, that is to say it is devoid of the sequence SEQ ID NO: 3, and/or;
   - it is devoid of the transmembrane/cytoplastic domain, that is to say it is devoid of SEQ ID NO: 6, proviso said protein does consist of any of the sequences SEQ ID NO: 90, 91 and 92, and/or;
   - it comprises amino-acids resulting from retention of at least part of one intron, proviso said intron is not intron 2 or intron 4.

2. The isolated HLA-G protein according to claim 1, wherein its sequence comprises or consists of a sequence chosen from the group consisting of SEQ ID NO: 7 to SEQ ID NO: 31.

3. The isolated HLA-G protein according to claim 1, wherein its comprises the five amino-acid residues MKTPR in its N terminal part.

4. The isolated HLA-G protein according to claim 1, wherein its sequence comprises or consists of a sequence chosen from the group consisting of SEQ ID NO: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16.

5. The isolated HLA-G protein according to claim 1, wherein its sequence comprises or consists of a sequence chosen from the group consisting of SEQ ID NO: 9, 10, 11, 12, 13, 14, 17, 18, 19, 20, 22, 23, 24, 25, 26, 27, 29, 30, 31.

6. The isolated HLA-G protein according to claim 1, wherein its sequence comprises or consists of a sequence chosen from the group consisting of SEQ ID NO: 8, 10, 12, 14, 16, 18, 20, 21, 23, 25, 27, 28, 30.

7. An isolated polynucleotide encoding a protein as defined in any of claims 1 to 6.

8. A recombinant vector comprising the polynucleotide as defined in claim 7.

9. A protein as defined in any of claims 1 to 6, a polynucleotide as defined in claim 7, and/or vector as defined in claim 8, for use as a medicament, preferably for use in the treatment of autoimmune and/or inflammatory diseases, or in the prevention and/or treatment of allogeneic graft rejection.

10. A protein as defined in any of claims 1 to 6, a polynucleotide as defined in claim 7, and/or vector as defined in claim 8, for use in the prevention and/or treatment of ischemia, preferably ischemia associated with cardiovascular diseases, peripheral artery diseases or stroke.

11. A protein as defined in any of claims 1 to 6, a polynucleotide as defined in claim 7, and/or vector as defined in claim 8, for use in the prevention and/or treatment of vascular retinopathies.

12. An antisens oligonucleotide having a sequence complementary to at least part of
- SEQ ID NO: 32 ;
- SEQ ID NO: 33, or ;
- SEQ ID NO: 34.

13. An antibody specific of at least one of the peptide of sequence SEQ ID NO: 35, 36 or 37.

14. An *in vitro* method for the diagnosis of a cancer, comprising the detection of at least at least a RNA transcript having a sequence chosen in the list consisting of the sequences SEQ ID NO: 38 to 74, and/or of at least one of the proteins as defined in any of claims 1 to 6, in a biological sample of a subject, preferably wherein said detection is performed using at least a primer having a sequence chosen in the list consisting of SEQ ID NO: 75 to 79.

15. A kit comprising at least two primers having a sequence chosen in the list consisting of SEQ ID NO: 78 to 84, preferably comprising at least a combination of primers chosen in the list consisting of:
    - the primer Ex1F of sequence SEQ ID NO: 76 or the primer PrPrF of sequence SEQ ID NO: 77, and the primer G526R of sequence SEQ ID NO: 80;
    - the primer int4R of sequence SEQ ID NO: 75, and the primer Int3F of sequence SEQ ID NO: 78;
    - the primer the primer Int3F of sequence SEQ ID NO: 78 and the primer Int5R of sequence SEQ ID NO: 79;

FIGURES

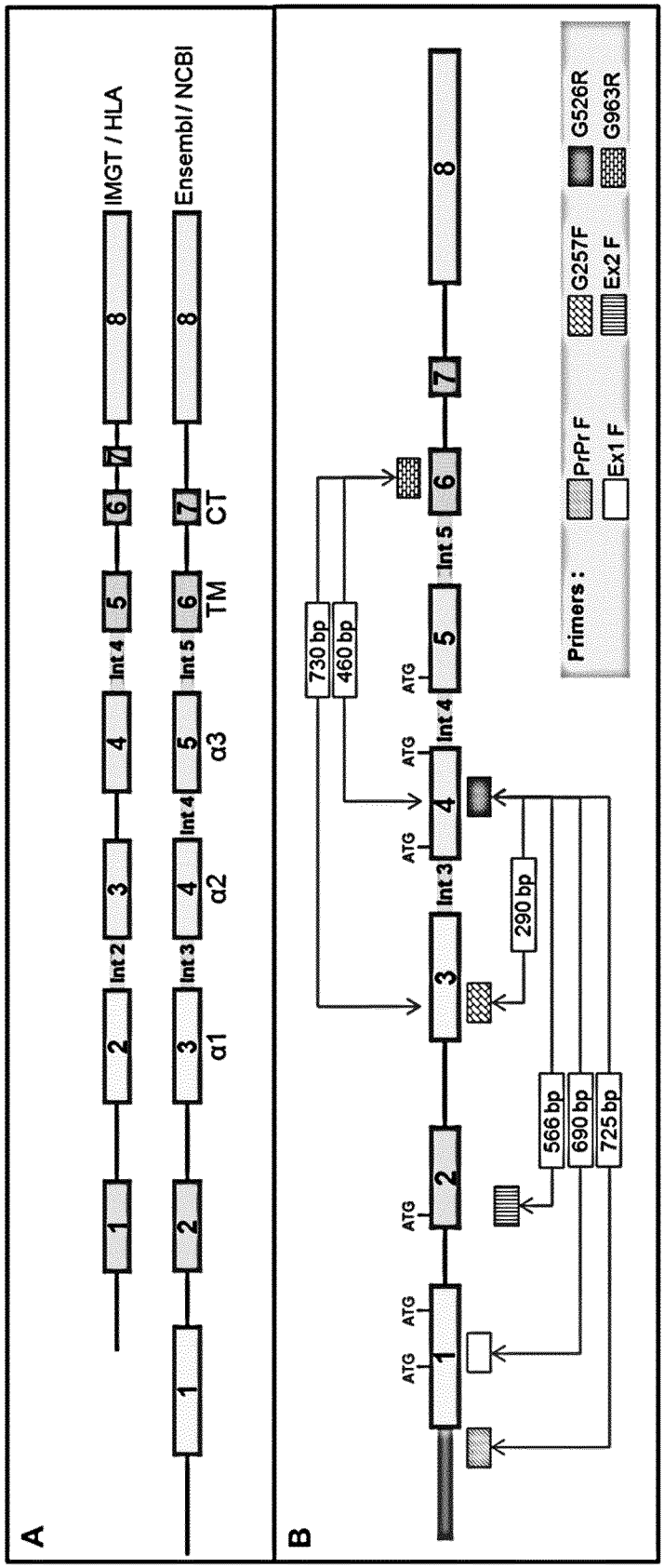
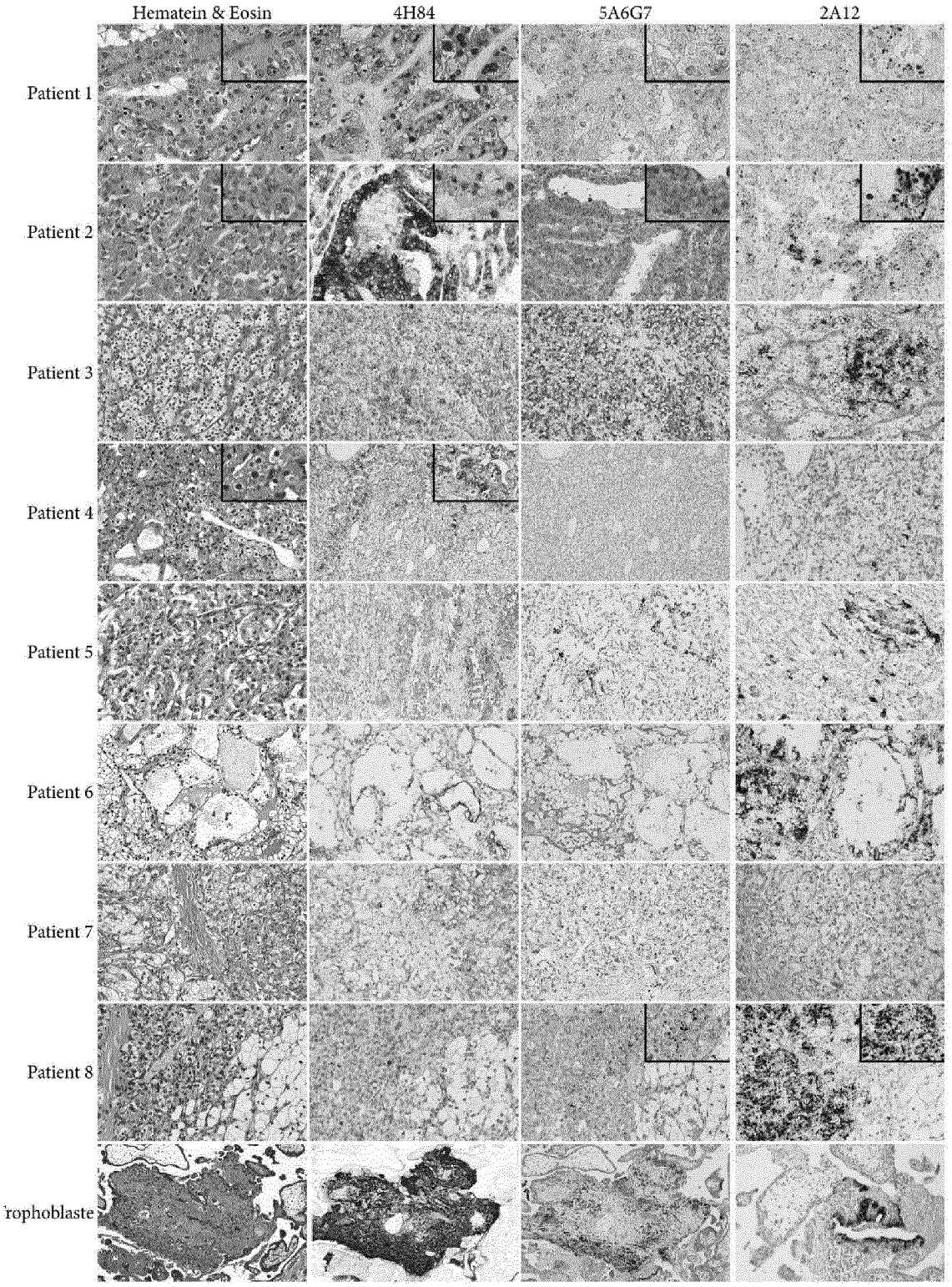


Figure 1


Hematein & Eosin
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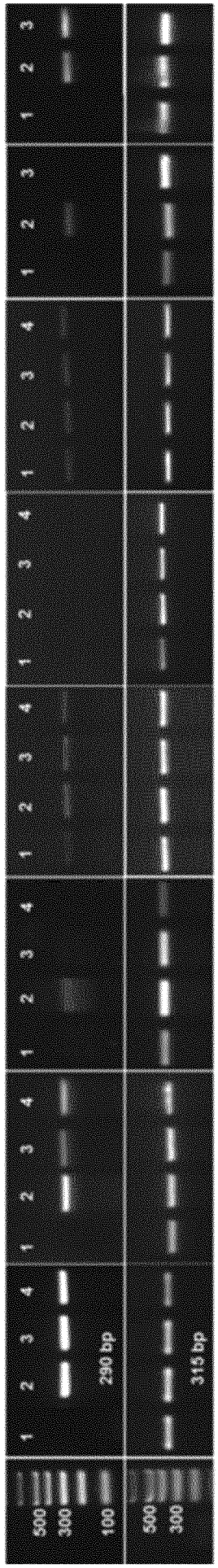
5A6G7
2A12
Patient 1
Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
rophoblaste

Figure 2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Patient 2
Patient 3
Patient 4
Patient 5
Patient 6
Patient 7
Patient 8
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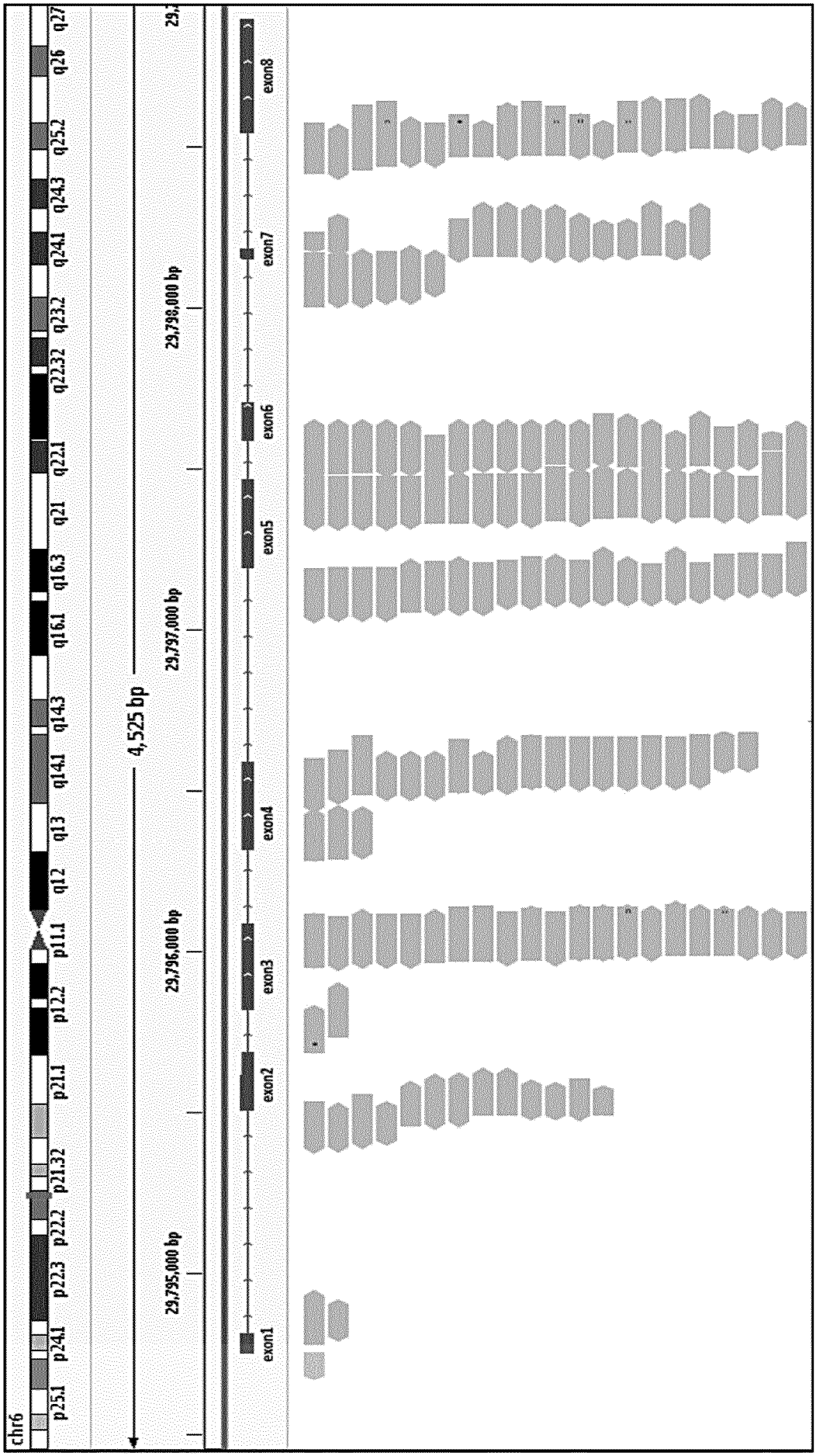
300
100
290 bp
315 bp

Figure 3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p25.1
p24.1
p22.3
p22.2
p21.32
p21.1
p12.2
p11.1
q12
q13
q14.1
q14.3
q16.1
q16.3
q21
q22.1
q22.32
q23.2
q24.1
q24.3
q25.2
q26
q27
4,525 bp
29,795,000 bp
29,796,000 bp
29,797,000 bp
29,798,000 bp
exon1
exon2
exon3
exon4
exon5
exon6
exon7
exon8

Figure 4

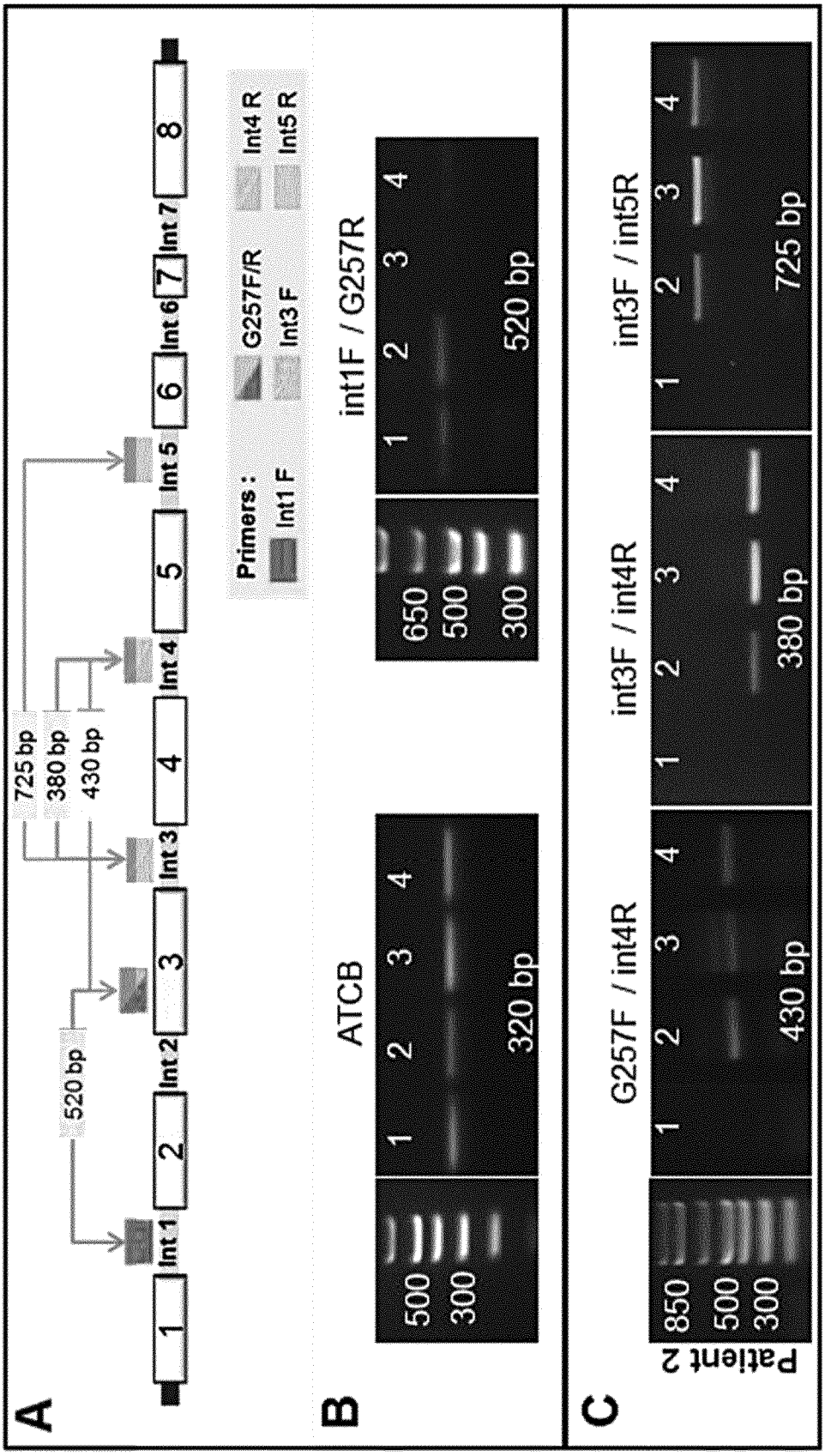
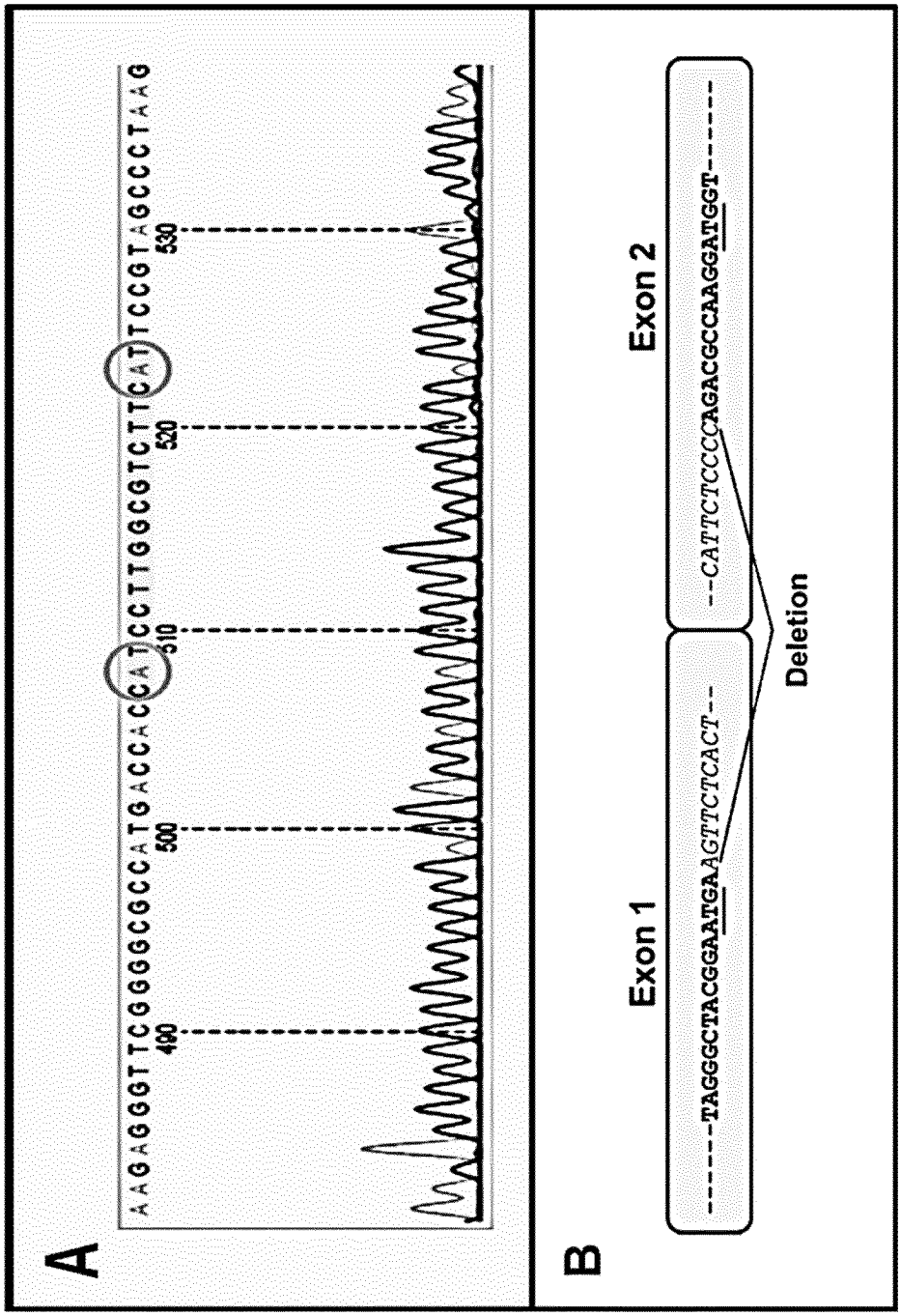

A
1
Int 1
2
Int 2
3
Int 3
4
Int 4
5
Int 5
6
Int 6
7
Int 7
8
520 bp
725 bp
380 bp
430 bp
Primers :
Int1 F
G257F/R
Int3 F
Int4 R
Int5 R
B
ATCB
1 2 3 4
500
300
320 bp
int1F / G257R
1 2 3 4
650
500
300
520 bp
C
G257F / int4R
1 2 3 4
850
500
300
430 bp
int3F / int4R
1 2 3 4
380 bp
int3F / int5R
1 2 3 4
725 bp
Patient 2

Figure 5


A
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490
500
510
520
530
B
Exon 1
Exon 2
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--CATTCTCCCCAGACGCCAAGGATGGT------
Deletion

Figure 6

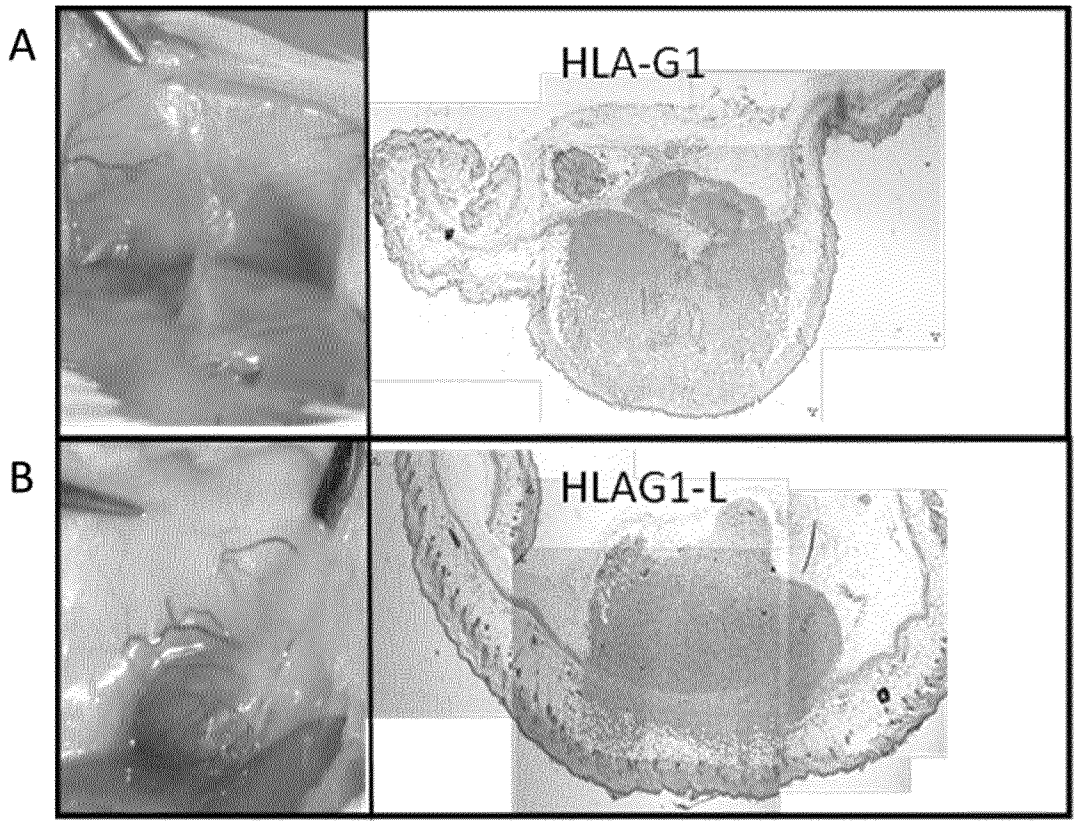
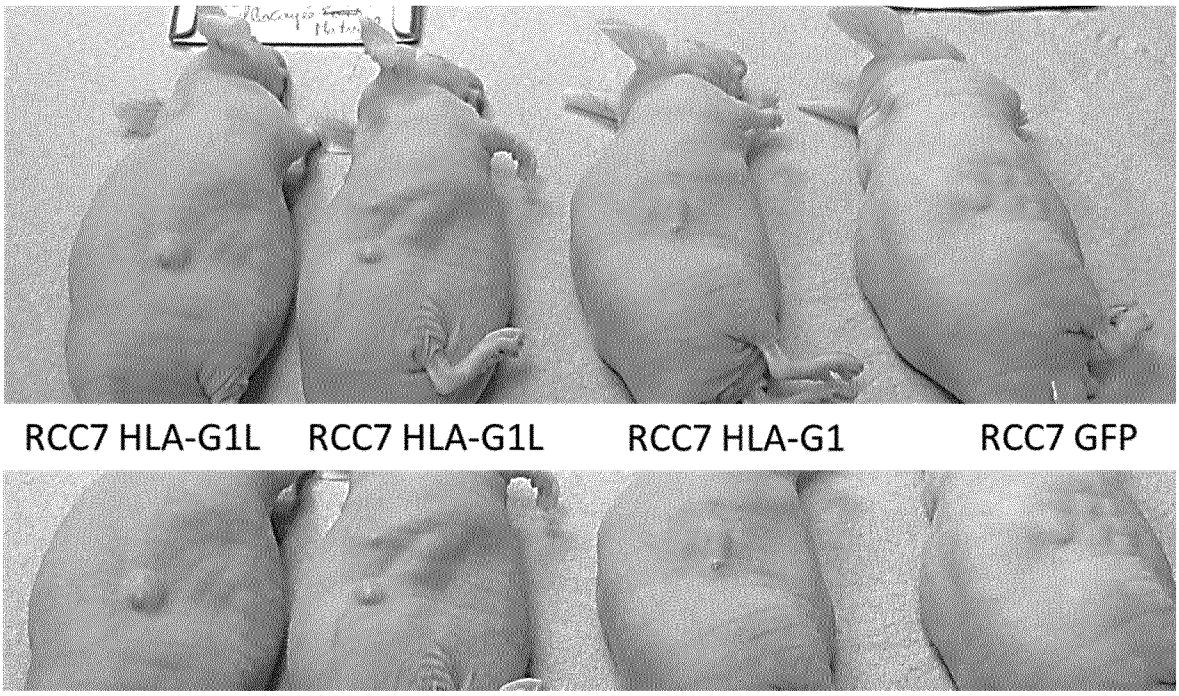


Figure 7

A

RCC7 GFP RCC7 HLA-G1 RCC7 HLA-G1L RCC7 HLA-G1L

B



RCC7 HLA-G1L RCC7 HLA-G1L RCC7 HLA-G1 RCC7 GFP

Figure 8

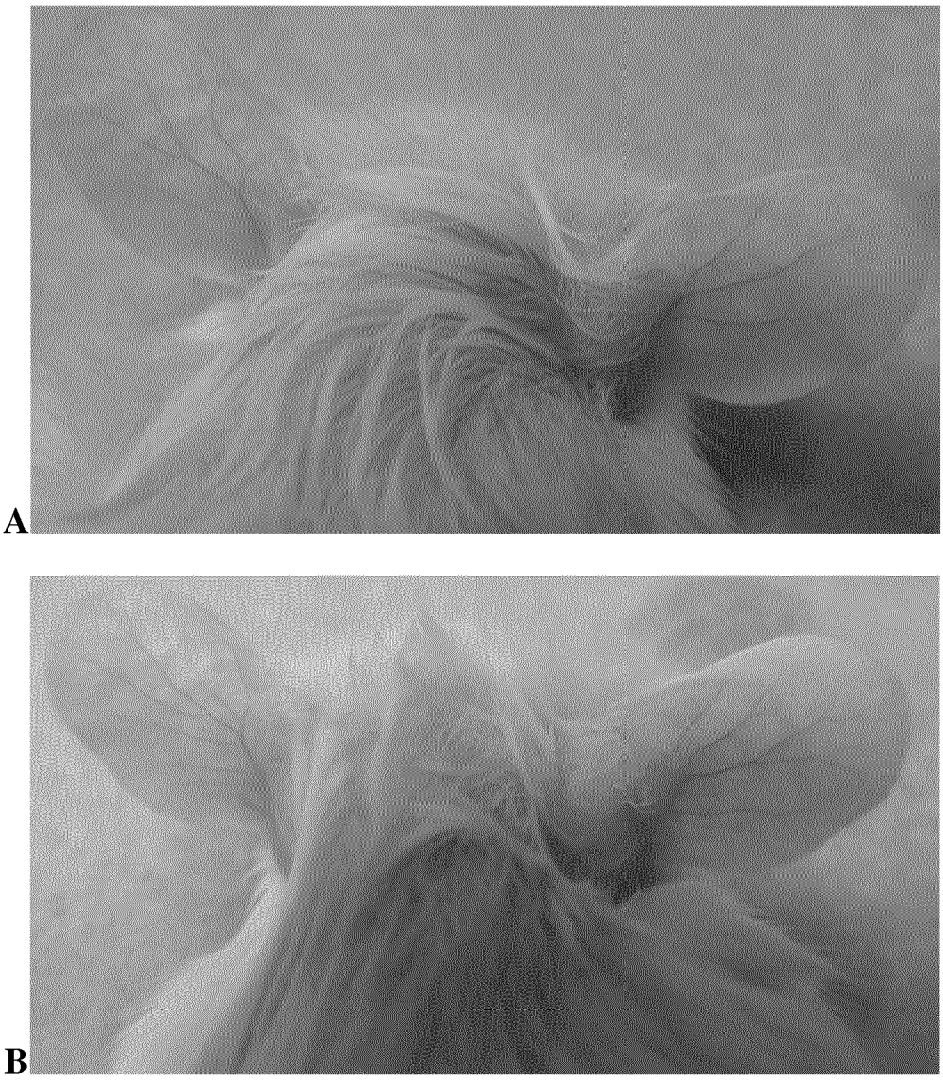


Figure 9

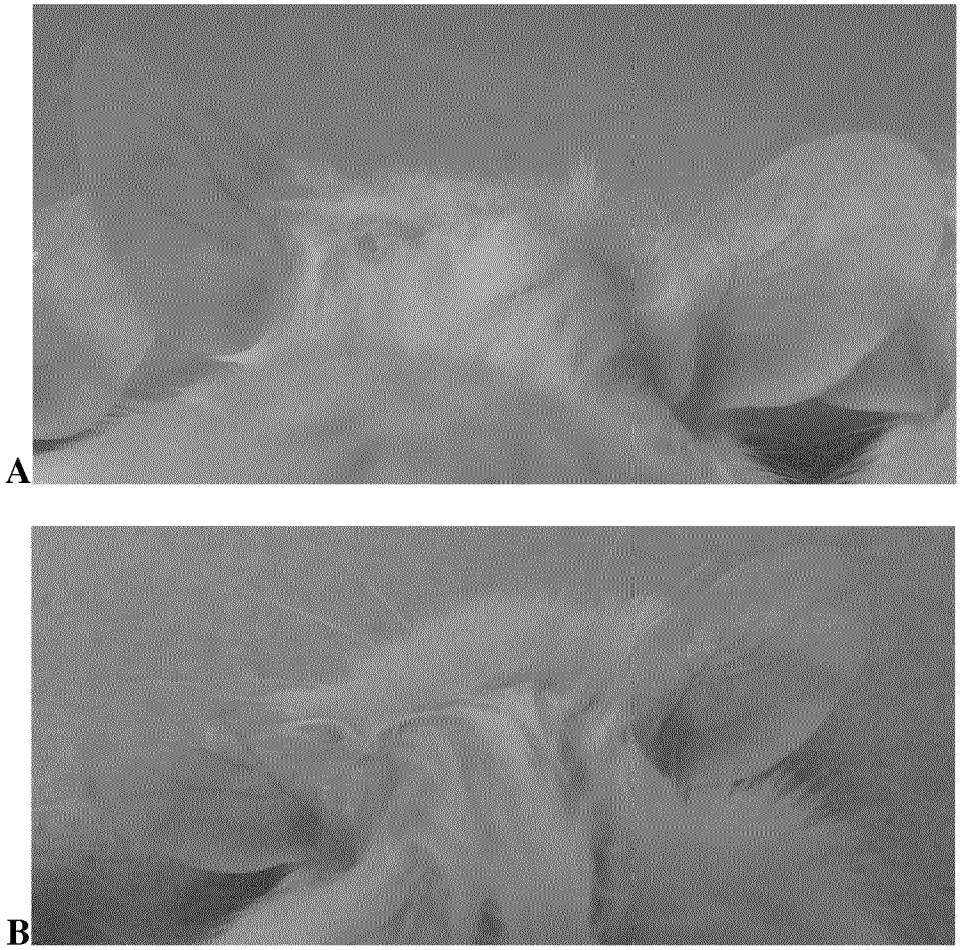


Figure 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/070061

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/74 C07K16/28
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 G01N

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, Sequence Search, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/091078 A2 (AXORDIA LTD [GB]; WALSH JAMES [GB]) 16 August 2007 (2007-08-16) figure 20	1-15
X	US 2004/166519 A1 (CARGILL MICHELE [US] ET AL) 26 August 2004 (2004-08-26) sequences 379, 382, 386,	1-15

-/--

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

[X] See patent family annex.

* 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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2018	04/12/2018

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	Schmitz, Till

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] 23 October 2009 (2009-10-23), "Synthetic construct DNA, clone: pF1KB6430, Homo sapiens HLA-G gene for major histocompatibility complex, class I, G, without stop codon, in Flexi system.", XP002775419, retrieved from EBI accession no. EMBL:AB528641 Database accession no. AB528641 sequence -----	1-15
X	WO 2014/072534 A1 (INTELECTYS [FR]) 15 May 2014 (2014-05-15) sequence 11 -----	1-15
X	DATABASE EMBL [Online] 7 July 1998 (1998-07-07), "Homo sapiens MHC class I antigen HLA-G mRNA (HLA-G*01013 allele), partial cds.", XP002775420, retrieved from EBI accession no. EMBL:AF071017 Database accession no. AF071017 sequence -----	1-15
X	DATABASE EMBL [Online] 26 July 2004 (2004-07-26), "Homo sapiens MHC class I antigen (HLA-G) gene, HLA-G*0103 allele, exons 2, 3 and partial cds.", XP002775421, retrieved from EBI accession no. EMBL:AY672598 Database accession no. AY672598 sequence -----	1-15
A	DATABASE Geneseq [Online] 20 December 2012 (2012-12-20), "Classical HLA capture oligonucleotide, SEQ ID:16141.", XP002775422, retrieved from EBI accession no. GSN:BAF49948 Database accession no. BAF49948 sequence 16141 ----- -/--	12

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DATABASE Geneseq [Online] 20 December 2012 (2012-12-20), "Classical HLA capture oligonucleotide, SEQ ID:13276.", XP002775423, retrieved from EBI accession no. GSN:BAF47083 Database accession no. BAF47083 sequence -----	12
A	CAROSELLA EDGARDO D ET AL: "HLA-G: An Immune Checkpoint Molecule", ADVANCES IN IMMUNOLOGY, ACADEMIC PRESS, US, vol. 127, 1 January 2015 (2015-01-01), pages 33-144, XP009192369, ISSN: 1557-8445 the whole document -----	1-15
X,P	DIANA TRONIK-LE ROUX ET AL: "Novel landscape of HLA-G isoforms expressed in clear cell renal cell carcinoma patients", MOLECULAR ONCOLOGY, vol. 11, no. 11, 13 September 2017 (2017-09-13), pages 1561-1578, XP055422804, AMSTERDAM, NL ISSN: 1574-7891, DOI: 10.1002/1878-0261.12119 the whole document -----	1-15

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2018/070061

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-15(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-15(partially)

   HLA-G protein comprising MKTPR at its N-terminus. Further, polynucleotide, vector, antisense, antibody, method, kit relating thereto, in as far as appropriate.

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2. claims: 1-15(partially)

   As invention 1, but relating to HLA-G protein devoid of alpha 1 domain.

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3. claims: 1-15(partially)

   As invention 1, but relating to HLA-G protein devoid of transmembrane / cytoplasmic domain (SEQ ID NO:6) at the proviso that it does not comprise SEQ ID NOs 90-92.

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4. claims: 1-15(partially)

   As invention 1, but relating to HLA-G proteins comprising amino acids resulting from retention of at least part of one intron, at the proviso that said intron is not intron 2 or intron 4.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2018/070061

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