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(54) Title: piRNA-THERAPEUTICS FOR HUMAN MALIGNANCIES

(57) Abstract: A method of treating malignancy in a subject in need thereof is provided. The method includes administering a therapeutically effective amount of a compound that increases the level of one or more of piR_017723, piR_23656, or piR_016745, or a combination thereof.

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piRNA-THERAPEUTICS FOR HUMAN MALIGNANCIES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/422,319 filed on November 3, 2022, which is incorporated herein by reference in its entirety.

FIELD

[0002] The present disclosure relates to piRNA therapeutics for the treatment of glioblastoma and human malignancies.

SEQUENCE LISTING

[0003] A Sequence Listing is submitted herewith and incorporated by reference herein as an XML file created on October 30, 2023, entitled "0312021-01058_Sequence_Listing.xml" and having a size of 16 KB.

BACKGROUND

[0004] Piwi interacting RNAs (piRNAs) are small non-coding RNA molecules of approximately 24–31 nucleotides in length that often bind to members of the piwi protein family to play regulatory roles. Recently, emerging evidence suggests that in addition to the mammalian germline, piRNAs are also expressed in a tissue-specific manner in a variety of human tissues and modulate key signaling pathways at the transcriptional or post-transcriptional level. In addition, a growing number of studies have shown that piRNA and PIWI proteins, which are abnormally expressed in various cancers, may serve as novel biomarkers and therapeutic targets for tumor diagnostics and treatment. However, the functions of piRNAs in cancer and their underlying mechanisms are not completely understood.

[0005] Glioblastoma multiforme (used interchangeably with glioblastoma) (GBM) is the most malignant and aggressive primary brain tumor. GBM has an ominous prognosis with a survival rate of 14–15 months after diagnosis. Despite worldwide initiatives to optimize therapeutic approaches, GBM is still among the most challenging diseases to treat and the fastest to relapse in clinical oncology. Treatment resistance of GBM and the inevitable tumor recurrence are primarily attributed to the presence of tumor-initiating cells or glioma stem cells (GSCs).

[0006] Because of the foregoing shortcomings, there is an urgent need for an effective GBM treatment along with treatments for other human malignancies.

SUMMARY

[0007] The present disclosure demonstrates that patient derived glioma stem cells (GSCs) do not express DDX4. In some embodiments, DDX4 overexpression induces cytotoxic death of GSCs. In other embodiments, DDX4 expressing GSCs upregulate three specific piRNAs, indicating re-activation of the piRNA biogenesis pathways.

[0008] In one aspect, provided is a method of treating a malignancy in a subject in need thereof, comprising administering a therapeutically effective amount of a compound that increases the expression of at least one piRNA in the subject. The present disclosure provides a diagnostic or prognostic signature for a malignancy such as glioblastoma comprising measured expression levels of piR_017723, piR_23656, or piR_016745, or a combination thereof.

[0009] Also provided is a method of treating a malignancy in a subject in need thereof, comprising administering a therapeutically effective amount of a compound that increases the expression of DDX4 in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0001] The following figures are included to illustrate certain aspects of the present disclosure and should not be viewed as exclusive embodiments. The subject matter disclosed is capable of considerable modifications, alterations, combinations, and equivalents in form and function, as will occur to one having ordinary skill in the art and having the benefit of this disclosure.

[0002] **FIG. 1.** GSC expression profiles for DDX4 and other components of the piRNA biogenesis pathway.

[0003] **FIG. 2.** 2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for assessing DDX4 expression and cell viability.

[0004] **FIG. 3.** piRNA expression in mCherry-DDX4+GSCs.

[0005] **FIGS. 4A-4G.** Volcano plots showing differential expression of endogenous retrotransposons in human glioma stem cells (GSCs) compared to control human neural stem cells. GB4 **(FIG. 4A)**; GB12 **(FIG. 4B)**; GB8 **(FIG. 4C)**; GB11 **(FIG. 4D)**; GB24 **(FIG. 4E)**; GB2 **(FIG. 4F)**; WCR8 **(FIG. 4G)**.

[0006] **FIG. 5A.** Treatment of human GSCs with piRNA mimic. **FIG. 5B.** Cell numbers following treatment with piRNA mimic.

[0007] **FIG. 6.** Treatment of control human astrocytes with piRNA mimics.

DETAILED DESCRIPTION

[0008] Glioblastoma (GBM) is an example of a human malignancy with a universal recurrence rate (at or about 100%). DDX4 is an RNA helicase implicated in mRNA translation and piRNA biogenesis in germ cells. DDX4 upregulates metabolic protein expression related to DNA repair The present disclosure demonstrates that patient derived glioma stem cells (GSCs) do not express DDX4. Further, it was found that DDX4 expressing GSCs upregulate three specific piRNAs, indicating re-activation of the piRNA biogenesis pathways.

[0009] Accordingly, it was demonstrated that treatment of human GSCs with piRNA mimics results in cytotoxic death of the GSCs, whereas treatment of control (i.e., non GSCs) human astrocytes with same piRNA mimics does not affect astrocyte survival, thereby providing a treatment for GBM. Further three piRNAs were upregulated in DDX4-expressing cells and can be used as a diagnostic or prognostic biomarker tool for monitoring glioblastoma treatment.

[0010] Aspects of the present disclosure are directed to methods of treating glioblastoma or a human malignancy in a subject in need thereof. The methods include administering a therapeutically effective amount of a compound that increases the level of one or more of piR_017723, piR_23656, or piR_016745.

[0011] Another aspect of the present disclosure is directed to methods of treating glioblastoma or a human malignancy in a subject in need thereof, in which the methods include administering a therapeutically effective amount of one or more of a nucleic acid that encodes piR_017723 or a mimic thereof, piR_23656 or a mimic thereof, or piR_016745 or a mimic thereof.

[0012] In some embodiments, the one or more of a nucleic acid is incorporated into or encapsulated by nanoparticles, microparticles, micelles, synthetic lipoprotein particles, or carbon nanotubes. In some embodiments, the one or more of a nucleic acid is incorporated into or encapsulated by liposomes. In some embodiments, one or more plasmids or viral vectors comprise the one or more of a nucleic acid.

[0013] Another aspect of the present disclosure is directed to a method of treating glioblastoma or a human malignancy in a subject in need thereof, in which the method includes administering a therapeutically effective amount of one or more of a nucleic acid that encodes piRNA hsa-16745, piRNA hsa-17723, or piRNA hsa-33520.

[0014] In some embodiments, the one or more of a nucleic acid is incorporated into or encapsulated by nanoparticles, microparticles, micelles, synthetic lipoprotein particles, or carbon nanotubes. In some embodiments, the one or more of a nucleic acid is incorporated

into or encapsulated by liposomes. In some embodiments, one or more plasmids or viral vectors comprise the one or more of a nucleic acid.

[0015] Another aspect of the present disclosure is directed to a method of treating glioblastoma or a human malignancy in a subject in need thereof, in which the method includes administering a therapeutically effective amount of DDX4. Another aspect of the present disclosure is directed to a method of treating glioblastoma or a human malignancy in a subject in need thereof, in which the method includes administering a therapeutically effective amount of a compound that results in the overexpression of DDX4.

[0016] A further aspect is directed to methods of treating a malignancy in a subject in need thereof, comprising administering a therapeutically effective amount of a compound that increases the expression of at least one piRNA in the subject. In some embodiments, the malignancy is glioblastoma. In some embodiments, the at least one piRNA is selected from piR_017723, piR_23656, and piR_016745, or a combination thereof.

[0017] In some embodiments, the compound is at least one nucleic acid that encodes a piRNA, or a piRNA mimic, or a combination thereof. In some embodiments, the compound is at least one nucleic acid that encodes for piR_017723, piR_23656, or piR_016745, or a combination thereof. In some embodiments, the compound is at least one nucleic acid that encodes for a piR_017723 mimic, a piR_016745 mimic, or a piR_033520 mimic, or a combination thereof. In some embodiments, the at least one nucleic acid encodes a piRNA mimic selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, or a combination thereof. In some embodiments, the at least one nucleic acid encodes a piRNA mimic having a structure of SEQ ID NO: 1. In some embodiments, the at least one nucleic acid encodes a piRNA mimic having a structure of SEQ ID NO: 2. In some embodiments, the at least one nucleic acid encodes a piRNA mimic having a structure of SEQ ID NO: 3.

[0018] In some embodiments, the piRNA mimic causes cytotoxic cell death in malignant cells but does not cause cytotoxic cell death in non-malignant cells. In some embodiments, the piRNA mimic causes cytotoxic cell death in glioblastoma stem cells (GSCs) but does not cause cytotoxic cell death in astrocytes. In some embodiments, the piRNA mimic is selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, or a combination thereof. In some embodiments, the piRNA mimic has a structure of SEQ ID NO: 1. In some embodiments, piRNA mimic has a structure of SEQ ID NO: 2. In some embodiments, the piRNA mimic has a structure of SEQ ID NO: 3.

[0019] Another aspect provided are methods of treating a malignancy in a subject in need thereof, comprising administering a therapeutically effective amount of a compound that increases the expression of DDX4 in the subject. In some embodiments, the malignancy is

glioblastoma. In some embodiments, the compound is at least one nucleic acid that encodes DDX4. In some embodiments, the at least one nucleic acid causes cytotoxic cell death in malignant cells but does not cause cytotoxic cell death in non-malignant cells. In some embodiments, the at least one nucleic acid causes cytotoxic cell death in glioblastoma stem cells (GSCs) but does not cause cytotoxic cell death in astrocytes.

[0020] In some embodiments, for methods described herein, the at least one nucleic acid is incorporated into or encapsulated by nanoparticles, microparticles, micelles, synthetic lipoprotein particles, or carbon nanotubes. In some embodiments, the at least one nucleic acid is incorporated into or encapsulated by liposomes. In some embodiments, plasmids or viral vectors comprise the at least one nucleic acid.

[0021] In some embodiments, the at least one nucleic acid is administered in an amount sufficient to cause one or more of the following: a decrease in tumor growth, a decrease in tumor cell proliferation, increased tumor cell apoptosis, inhibition of metastatic dissemination of the glioblastoma or a human malignancy improvement in subject survival, reduced ability of tumor cells to form colonies, or reduced ability of tumor cells to migrate. In some embodiments, the at least one nucleic acid is administered before, after, or concurrently with the administration of an antibody therapy. In some embodiments, the at least one nucleic acid is administered before, after, or concurrently with the administration of a radiation therapy.

[0022] Another aspect of the present disclosure is directed to a pharmaceutical composition for treating glioblastoma or a human malignancy. The pharmaceutical composition includes one or more of a nucleic acid or a pharmaceutically acceptable salt thereof that encodes piR_017723 or a mimic thereof, piR_23656 or a mimic thereof, piR_016745 or a mimic thereof, piRNA hsa-16745, piRNA hsa-17723, or piRNA hsa-33520.

[0023] Provided is a pharmaceutical composition comprising at least one nucleic acid or a pharmaceutically acceptable salt thereof that encodes piR_017723 or a mimic thereof, piR_23656 or a mimic thereof, piR_016745 or a mimic thereof, piRNA hsa-16745, piRNA hsa-17723, or piRNA hsa-33520.

[0024] Also provided is a diagnostic or prognostic biomarker for monitoring treatment of glioblastoma comprising the expression levels of at least one of piR_017723, piR_23656, or piR_016745, or a combination thereof, wherein an increase in expression of at least one of piR_017723, piR_23656, or piR_016745, or a combination thereof, is indicative of effective treatment of glioblastoma.

[0025] In some embodiments of the methods described, the at least one nucleic acid, is incorporated into or encapsulated by nanoparticles, microparticles, micelles, synthetic lipoprotein particles, or carbon nanotubes.

[0026] In some embodiments of the methods described, or the at least one nucleic acid, is incorporated into or encapsulated by liposomes.

[0027] In some embodiments of the methods described, one or more plasmids or viral vectors comprise the the at least one nucleic acid.

[0028] In some embodiments of the methods described, the one or more of a nucleic acid is incorporated into or encapsulated by nanoparticles, microparticles, micelles, synthetic lipoprotein particles, or carbon nanotubes.

[0029] In some embodiments of the methods described, the one or more of a nucleic acid is incorporated into or encapsulated by liposomes.

[0030] In some embodiments of the methods described, one or more plasmids or viral vectors comprise the one or more of a nucleic acid.

[0031] In some embodiments, the compound or the at least one nucleic acid is a pharmaceutically acceptable salt of the compound or the at least one nucleic acid.

[0032] In some embodiments, a pharmaceutical composition described herein comprises the compound or pharmaceutically acceptable salt thereof or the at least one nucleic acid or pharmaceutically acceptable thereof.

[0033] In some embodiments, the pharmaceutical composition is administered in an amount sufficient to cause one or more of the following: a decrease in tumor growth, a decrease in tumor cell proliferation, increased tumor cell apoptosis, inhibition of metastatic dissemination of the glioblastoma or a human malignancy improvement in subject survival, reduced ability of tumor cells to form colonies, or reduced ability of tumor cells to migrate.

[0034] In some embodiments, the pharmaceutical composition is administered before, after or concurrently with the administration of an antibody therapy.

[0035] In some embodiments, the pharmaceutical composition is administered before, after or concurrently with the administration of a radiation therapy.

[0036] Many of the active agents utilized for the therapies disclosed herein are nucleic acid-based therapies. Although piRNA, and inhibitors thereof, are typically active as RNA, it will be appreciated that the active agents include one or more modifications to increase activity, reduce degradation, or a combination thereof. Thus, in some embodiments, the piRNA or a functional nucleic acid targeting a piRNA, or any vector or virus including the piRNA or function nucleic acid, include one or more modifications provided the modification does not prevent the nucleic acid's desired activity.

[0037] piRNA mimics include and are not limited to piRNA hsa-16745, piRNA hsa-17723, or piRNA hsa-33520 described herein. Other piRNA mimics are known including and not limited to hsa-piR-33049 as described in the piRNA database (pirnadb.org) and a piR-823 mimic described by Ding et al., *Front Cell Dev Biol.* 2021; 9: 641052.

[0038] The disclosed mimics can be or can include DNA or RNA nucleotides or a combination thereof which typically include a heterocyclic base (nucleic acid base), a sugar moiety attached to the heterocyclic base, and a phosphate moiety which esterifies a hydroxyl function of the sugar moiety. The principal naturally-occurring nucleotides comprise uracil, thymine, cytosine, adenine and guanine as the heterocyclic bases, and ribose or deoxyribose sugar linked by phosphodiester bonds.

[0039] In some embodiments, the nucleic acids include one or more nucleotide analogs that have been chemically modified to improve stability, half-life, or specificity or affinity for a target receptor, relative to a DNA or RNA counterpart. The chemical modifications include chemical modification of nucleobases, sugar moieties, nucleotide linkages, or combinations thereof. As used herein 'modified nucleotide" or "chemically modified nucleotide" defines a nucleotide that has a chemical modification of one or more of the heterocyclic base, sugar moiety or phosphate moiety constituents. In some embodiments, the charge of the modified nucleotide is reduced compared to DNA or RNA oligonucleotides of the same nucleobase sequence. For example, the nucleic acids can have low negative charge, no charge, or positive charge. Typically, nucleoside analogs support bases capable of orthogonal pairing (e.g., hydrogen bonding by Watson-Crick base pairing) to standard nucleoside bases, where the analog backbone presents the bases in a manner to permit such hydrogen bonding in a sequence-specific fashion between the oligonucleotide analog molecule and bases in a standard polynucleotide (e.g., single-stranded RNA or single-stranded DNA). In addition, steric pairing can occur and abasic sites may be present. In some embodiments, the analogs have a substantially uncharged, phosphorus containing backbone.

[0040] The principal naturally-occurring nucleotides include uracil, thymine, cytosine, adenine and guanine as the heterocyclic bases. The nucleic acids can include chemical modifications to their nucleobase constituents.

[0041] Chemical modifications of heterocyclic bases or heterocyclic base analogs may be effective to increase the binding affinity or stability in binding a target sequence. Chemically-modified heterocyclic bases include, but are not limited to, inosine, 5-(1-propynyl) uracil (pU), 5-(1-propynyl) cytosine (pC), 5-methylcytosine, 8-oxo-adenine, pseudocytosine, pseudoisocytosine, 5 and 2-amino-5-(2'-deoxy-.beta.-D-ribofuranosyl)pyridine (2-aminopyridine), and various pyrrolo- and pyrazolopyrimidine derivatives.

[0042] Nucleic acids can also contain nucleotides with modified sugar moieties or sugar moiety analogs. Sugar moiety modifications include, but are not limited to, 2'-O-aminoetoxy, 2'-O-amonioethyl (2'-OAE), 2'-O-methoxy, 2'-O-methyl, 2-guanidoethyl (2'-OGE), 2'-O,4'-C-methylene (LNA), 2'-O-(methoxyethyl) (2'-OME) and 2'-O-(N-(methyl)acetamido) (2'-OMA) 2'-O-aminoethyl sugar moiety substitutions are especially preferred because they are protonated at neutral pH and thus suppress the charge repulsion between the TFO and the target duplex. This modification stabilizes the C3'-endo conformation of the ribose or dexyribose and also forms a bridge with the i-1 phosphate in the purine strand of the duplex.

[0043] In some embodiments, the nucleic acid is a morpholino Oligonucleotide. Morpholino oligonucleotides are typically composed of two more morpholino monomers containing purine or pyrimidine base-pairing moieties effective to bind, by base-specific hydrogen bonding, to a base in a polynucleotide, which are linked together by phosphorus-containing linkages, one to three atoms long, joining the morpholino nitrogen of one monomer to the 5' exocyclic carbon of an adjacent monomer. The purine or pyrimidine base-pairing moiety is typically adenine, cytosine, guanine, uracil or thymine.

[0044] Important properties of the morpholino-based subunits typically include: the ability to be linked in a oligomeric form by stable, uncharged backbone linkages; the ability to support a nucleotide base (e.g. adenine, cytosine, guanine, thymidine, uracil or inosine) such that the polymer formed can hybridize with a complementary-base target nucleic acid, including target RNA, with high T_m, even with oligomers as short as 10-14 bases; the ability of the oligomer to be actively transported into mammalian cells; and the ability of an oligomer:RNA heteroduplex to resist RNAse degradation.

[0045] In some embodiments, oligonucleotides employ morpholino-based subunits bearing base-pairing moieties, joined by uncharged linkages, as described above.

[0046] Nucleic acids connected by an internucleotide bond that refers to a chemical linkage between two nucleoside moieties. Modifications to the phosphate backbone of DNA or RNA may increase the binding affinity or stability oligonucleotides, or reduce the susceptibility of oligonucleotides to nuclease digestion. Cationic modifications, including, but not limited to, diethyl-ethylenediamide (DEED) or dimethyl-aminopropylamine (DMAP) may be especially useful due to decrease electrostatic repulsion between the oligonucleotide and a target. Modifications of the phosphate backbone may also include the substitution of a sulfur atom for one of the non-bridging oxygens in the phosphodiester linkage. This substitution creates a phosphorothioate internucleoside linkage in place of the phosphodiester linkage. Oligonucleotides containing phosphorothioate internucleoside linkages have been shown to be more stable *in vivo*.

[0047] Examples of modified nucleotides with reduced charge include modified internucleotide linkages such as phosphate analogs having achiral and uncharged intersubunit linkages, as discussed above. Some internucleotide linkage analogs include morpholidate, acetal, and polyamide-linked heterocycles.

[0048] In another embodiment, the nucleic acids are composed of locked nucleic acids. Locked nucleic acids (LNA) are modified RNA nucleotides. LNAs form hybrids with DNA which are more stable than DNA/DNA hybrids, a property similar to that of peptide nucleic acid (PNA)/DNA hybrids.

[0049] Therefore, LNA can be used just as PNA molecules would be. LNA binding efficiency can be increased in some embodiments by adding positive charges to it. Commercial nucleic acid synthesizers and standard phosphoramidite chemistry are used to make LNAs.

[0050] In some embodiments, the nucleic acids are composed of peptide nucleic acids. Peptide nucleic acids (PNAs) are synthetic DNA mimics in which the phosphate backbone of the oligonucleotide is replaced in its entirety by repeating N-(2-aminoethyl)-glycine units and phosphodiester bonds are typically replaced by peptide bonds. The various heterocyclic bases are linked to the backbone by methylene carbonyl bonds. PNAs maintain spacing of heterocyclic bases that is similar to conventional DNA oligonucleotides, but are achiral and neutrally charged molecules. Peptide nucleic acids are comprised of peptide nucleic acid monomers.

[0051] Other backbone modifications include peptide and amino acid variations and modifications. Thus, the backbone constituents of oligonucleotides such as PNA may be peptide linkages, or alternatively, they may be non-peptide peptide linkages. Examples include acetyl caps, amino spacers such as 8-amino-3,6-dioxaoctanoic acid (referred to herein as O-linkers), amino acids such as lysine are particularly useful if positive charges are desired in the PNA, and the like. Methods for the chemical assembly of PNAs are well known.

[0052] Nucleic acids optionally include one or more terminal residues or modifications at either or both termini to increase stability, and/or affinity of the oligonucleotide for its target. Commonly used positively charged moieties include the amino acids lysine and arginine, although other positively charged moieties may also be useful. nucleic acids may further be modified to be end capped to prevent degradation using a propylamine group. Procedures for 3' or 5' capping oligonucleotides are well known in the art.

[0053] In some embodiments, the nucleic acid is single stranded or double stranded.

[0054] Compositions and methods of deploying active agents nucleic acid active agents including piRNA and functional nucleic acids can be administered to a subject in need thereof. The piRNA or functional nucleic acid can also be encoded by a vector or virus that is administered to a subject in need thereof. For example, a sequence encoding a piRNA or function nucleic acid can be incorporated into an autonomously replicating plasmid, a virus (e.g., a retrovirus, lentivirus, adenovirus, or herpes virus). Sequence encoding the piRNA or functional nucleic acid can also be integrated into genomic DNA of a subject.

[0055] Nucleic acids can be delivered by a viral vector, for example a commercially available preparation, such as an adenovirus vector. The viral vector delivery can be via a viral system, such as a retroviral vector system which can package a recombinant retroviral genome). The recombinant retrovirus can then be used to infect and thereby deliver to the infected cells nucleic acid encoding the agent. The exact method of introducing the altered nucleic acid into mammalian cells is, of course, not limited to the use of retroviral vectors. Other techniques are widely available for this procedure including the use of adenoviral vectors, adeno-associated viral (AAV) vectors, lentiviral vectors, and pseudotyped retroviral vectors.

[0056] The gliobastoma or human malignancy therapies may further include any well-known therapies to treat cancer, including, but not limited to, surgical removal of the cancer, administration of chemotherapy, administration of radiation, administration of antibody therapies, and administration of anti-cancer drugs.

[0057] The present disclosure also provides pharmaceutical compositions that include effective amounts of the compounds, nucleic acids and the pharmaceutically acceptable salts thereof described above, and a pharmaceutically acceptable carrier. In certain embodiments, the disclosure also provides pharmaceutical compositions and dosage forms comprising any one of the additional therapeutic agents described herein. The carrier(s) are “acceptable” in the sense of being compatible with the other ingredients of the formulation and, in the case of a pharmaceutically acceptable carrier, not deleterious to the recipient thereof in an amount used in the medicament.

[0058] Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of the present disclosure include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-

based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol, and wool fat.

[0059] The compositions or dosage forms may contain any one of the compounds and therapeutic agents described herein in the range of 0.005% to 100% with the balance made up from the suitable pharmaceutically acceptable excipients. The contemplated compositions may contain 0.001%-100% of any one of the compounds and therapeutic agents provided herein, in one embodiment 0.1-95%, in another embodiment 75-85%, in a further embodiment 20-80%, wherein the balance may be made up of any pharmaceutically acceptable excipient described herein, or any combination of these excipients.

Definitions

[0060] As used herein, the term "biological sample" refers to a body fluid or tissue. The body fluid can include, without limitation, whole blood, serum, plasma, peripheral blood, synovial fluid, cerebrospinal fluid, saliva, urine, semen, or other fluid secretion. The term "tissue" can include, without limitation, bone marrow and lymph node, as well as samples of other tissues.

[0061] As used herein, the term “cell” is meant to refer to a cell that is *in vitro*, *ex vivo* or *in vivo*. In some embodiments, an *ex vivo* cell can be part of a tissue sample excised from an organism such as a mammal. In some embodiments, an *in vitro* cell can be a cell in a cell culture. In some embodiments, an *in vivo* cell is a cell living in an organism such as a mammal.

[0062] The term “chemotherapy” refers to the treatment of cancer or a disease or disorder caused by a virus, bacterium, other microorganism, or an inappropriate immune response using specific chemical agents, drugs, or radioactive agents that are selectively toxic and destructive to malignant cells and tissues, viruses, bacteria, or other microorganisms. Chemotherapeutic agents or drugs such as an anti-folate (e.g., methotrexate) or any other agent or drug useful in treating cancer, an inflammatory disease, or an autoimmune disease are preferred. Suitable chemotherapeutic agents and drugs include, but are not limited to, actinomycin D, adriamycin, altretamine, azathioprine, bleomycin, busulphan, capecitabine, carboplatin, carmustine, chlorambucil, cisplatin, cladribine, crisantaspase, cyclophosphamide, cytarabine, dacarbazine, daunorubicin, doxorubicin, epirubicin, etoposide, fludarabine, fluorouracil, gemcitabine, hydroxyurea, idarubicin, ifosfamide, irinotecan, liposomal doxorubicin, lomustine, melphalan, mercaptopurine, methotrexate, mitomycin, mitozantrone, oxaliplatin, paclitaxel, pentostatin, procarbazine, raltitrexed, steroids, streptozocin, taxol, taxotere, temozolomide, thioguanine, thiotepa, tomudex, topotecan, treosulfan, uft (uracil-tegufur), vinblastine, vincristine, vindesine, and vinorelbine.

[0063] As used herein, the phrase "effective amount" or "therapeutically effective amount" refers to the amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal, individual or human that is being sought by a researcher, veterinarian, medical doctor or other clinician.

[0064] The terms "effective amount" or "therapeutically effective amount" refer to an amount, i.e. a dosage, of therapeutic agent administered to a subject (e.g., a mammalian subject, i.e. a human subject), either as a single dose or as part of a series of doses, which is effective to produce a desired therapeutic effect (e.g., effective for influencing, reducing or inhibiting the activity of or preventing activation of a kinase, or effective at bringing about a desired *in vivo* effect in an animal, preferably, a human, such as reduction in intraocular pressure).

[0065] As used herein, the term "individual", "patient", or "subject" used interchangeably, refers to any animal, including mammals, preferably mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, and most preferably humans.

[0066] As used herein, "pharmaceutically acceptable salts" refers to an ionizable therapeutic agent that has been combined with a counter-ion to form a neutral complex. Lists of suitable salts are found, for example, in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418 and Journal of Pharmaceutical Science, 66, 2 (1977).

[0067] The terms "pharmaceutical" and "pharmaceutically acceptable" may refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0068] "Pharmaceutically acceptable carrier" means a carrier that is useful for the preparation of a pharmaceutical composition that is: generally compatible with the other ingredients of the composition, not deleterious to the recipient, and neither biologically nor otherwise undesirable. "A pharmaceutically acceptable carrier" includes both one and more than one carrier. Embodiments include carriers for topical, ocular, parenteral, intravenous, intraperitoneal intramuscular, sublingual, nasal, and oral administration. "Pharmaceutically acceptable carrier" also includes agents for preparation of aqueous dispersions and sterile powders for injection or dispersions.

[0069] As used herein, the term "preventing" or "prevention" of a disease, condition or disorder refers to decreasing the risk of occurrence of the disease, condition or disorder in a subject or group of subjects (e.g., a subject or group of subjects predisposed to or susceptible

to the disease, condition or disorder). In some embodiments, preventing a disease, condition or disorder refers to decreasing the possibility of acquiring the disease, condition or disorder and/or its associated symptoms. In some embodiments, preventing a disease, condition or disorder refers to completely or almost completely stopping the disease, condition or disorder from occurring.

[0070] As used herein, "transformed" and "transfected" encompass the introduction of a nucleic acid (e.g. a vector) into a cell by a number of techniques known in the art.

[0071] As used herein the term "treating" or "treatment" refers to 1) inhibiting the disease; for example, inhibiting a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., arresting further development of the pathology and/or symptomatology), or 2) ameliorating the disease; for example, ameliorating a disease, condition or disorder in an individual who is experiencing or displaying the pathology or symptomatology of the disease, condition or disorder (i.e., reversing the pathology and/or symptomatology).

[0072] The term "treatment" may refer to the application of one or more specific procedures used for the amelioration of a disease. In certain embodiments, the specific procedure is the administration of one or more pharmaceutical agents. "Treatment" of an individual (e.g. a mammal, such as a human) or a cell is any type of intervention used in an attempt to alter the natural course of the individual or cell. Treatment includes, but is not limited to, administration of a therapeutic agent or a pharmaceutical composition, and may be performed either prophylactically or subsequent to the initiation of a pathologic event or contact with an etiologic agent. Treatment includes any desirable effect on the symptoms or pathology of a disease or condition, and may include, for example, minimal changes or improvements in one or more measurable markers of the disease or condition being treated. Also included are "prophylactic" treatments, which can be directed to reducing the rate of progression of the disease or condition being treated, delaying the onset of that disease or condition, or reducing the severity of its onset.

[0073] As used herein, a "vector" is a replicon, such as a plasmid, phage, or cosmid, into which another DNA segment may be inserted so as to bring about the replication of the inserted segment. The vectors described herein can be expression vectors.

Routes of administration and dosage forms

[0074] The pharmaceutical compositions of the present disclosure include those suitable for any acceptable route of administration. Acceptable routes of administration include, but are not limited to, buccal, cutaneous, endocervical, endosinusial, endotracheal, enteral, epidural, interstitial, intra-abdominal, intra-arterial, intrabronchial, intrabursal, intracerebral,

intracisternal, intracoronary, intradermal, intraductal, intraduodenal, intradural, intraepidermal, intraesophageal, intragastric, intragingival, intraileal, intralymphatic, intramedullary, intrameningeal, intramuscular, intranasal, intraovarian, intraperitoneal, intraprostatic, intrapulmonary, intrasinal, intraspinal, intrasynovial, intratesticular, intrathecal, intratubular, intratumoral, intrauterine, intravascular, intravenous, nasal, nasogastric, oral, parenteral, percutaneous, peridural, rectal, respiratory (inhalation), subcutaneous, sublingual, submucosal, topical, transdermal, transmucosal, transtracheal, ureteral, urethral and vaginal. In some embodiments, the at least one nucleic acid is administered in the cerebrospinal fluid (e.g., via injection into spinal fluid).

[0075] Compositions and formulations described herein may conveniently be presented in a unit dosage form, e.g., tablets, sustained release capsules, and in liposomes, and may be prepared by any methods well known in the art of pharmacy. Such preparative methods include the step of bringing into association with the molecule to be administered ingredients such as the carrier that constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers, liposomes or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[0076] In some embodiments, any one of the compounds and therapeutic agents disclosed herein can be administered orally. Compositions of the present disclosure suitable for oral administration may be presented as discrete units such as capsules, sachets, granules or tablets each containing a predetermined amount (e.g., effective amount) of the active ingredient; a powder or granules; a solution or a suspension in an aqueous liquid or a non-aqueous liquid; an oil-in-water liquid emulsion; a water-in-oil liquid emulsion; packed in liposomes; or as a bolus, etc. Soft gelatin capsules can be useful for containing such suspensions, which may beneficially increase the rate of compound absorption. In the case of tablets for oral use, carriers that are commonly used include lactose, sucrose, glucose, mannitol, and silicic acid and starches. Other acceptable excipients may include: a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. For oral administration in a capsule form, useful diluents include

lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added. Compositions suitable for oral administration include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; and pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia.

[0077] Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions or infusion solutions which may contain antioxidants, buffers, bacteriostats, and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, saline (e.g., 0.9% saline solution) or 5% dextrose solution, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets. The injection solutions may be in the form, for example, of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

[0078] Pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can

be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0079] The pharmaceutical compositions of the present disclosure may be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of the present disclosure with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax, and polyethylene glycols.

[0080] The pharmaceutical compositions of the present disclosure may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art..

[0081] Topical compositions of the present disclosure can be prepared and used in the form of an aerosol spray, cream, emulsion, solid, liquid, dispersion, foam, oil, gel, hydrogel, lotion, mousse, ointment, powder, patch, pomade, solution, pump spray, stick, towelette, soap, or other forms commonly employed in the art of topical administration and/or cosmetic and skin care formulation. The topical compositions can be in an emulsion form. Topical administration of the pharmaceutical compositions of the present disclosure is especially useful when the desired treatment involves areas or organs readily accessible by topical application. In some embodiments, the topical composition comprises a combination of any one of the compounds and therapeutic agents disclosed herein, and one or more additional ingredients, carriers, excipients, or diluents including, but not limited to, absorbents, anti-irritants, anti-acne agents, preservatives, antioxidants, coloring agents/pigments, emollients (moisturizers), emulsifiers, film-forming/holding agents, fragrances, leave-on exfoliants, prescription drugs, preservatives, scrub agents, silicones, skin-identical/repairing agents, slip agents, sunscreen actives, surfactants/detergent cleansing agents, penetration enhancers, and thickeners.

[0082] Examples of useful dermatological compositions which can be used to deliver the compounds and therapeutic agents to the skin are known in the art.

[0083] The compounds and therapeutic agents of the present disclosure may be incorporated into compositions for coating an implantable medical device, such as prostheses, artificial valves, vascular grafts, stents, or catheters. Suitable coatings and the general preparation of coated implantable devices are known in the art. The coatings are typically biocompatible polymeric materials such as a hydrogel polymer, polymethyldisiloxane, polycaprolactone, polyethylene glycol, polylactic acid, ethylene vinyl acetate, and mixtures thereof. The coatings may optionally be further covered by a suitable topcoat of fluorosilicone, polysaccharides, polyethylene glycol, phospholipids or combinations thereof to impart controlled release characteristics in the composition. Coatings for invasive devices are to be included within the definition of pharmaceutically acceptable carrier, adjuvant or vehicle, as those terms are used herein.

[0084] According to another embodiment, the present disclosure provides an implantable drug release device impregnated with or containing a compound or a therapeutic agent, or a composition comprising a compound of the present disclosure or a therapeutic agent, such that said compound or therapeutic agent is released from said device and is therapeutically active.

Dosages and regimens

[0085] In the pharmaceutical compositions of the present disclosure, a compound is present in an effective amount (e.g., a therapeutically effective amount).

[0086] Effective doses/amounts may vary, depending on the diseases treated, the severity of the disease, the route of administration, the sex, age and general health condition of the subject, excipient usage, and the possibility of co-usage with other therapeutic treatments such as use of other agents and the judgment of the treating physician.

[0087] In some embodiments, an effective amount of the compounds, nucleic acids and the pharmaceutically acceptable salts thereof described above, can range, for example, from about 0.001 mg/kg to about 500 mg/kg (e.g., from about 0.001 mg/kg to about 200 mg/kg; from about 0.01 mg/kg to about 200 mg/kg; from about 0.01 mg/kg to about 150 mg/kg; from about 0.01 mg/kg to about 100 mg/kg; from about 0.01 mg/kg to about 50 mg/kg; from about 0.01 mg/kg to about 10 mg/kg; from about 0.01 mg/kg to about 5 mg/kg; from about 0.01 mg/kg to about 1 mg/kg; from about 0.01 mg/kg to about 0.5 mg/kg; from about 0.01 mg/kg to about 0.1 mg/kg; from about 0. 1 mg/kg to about 200 mg/kg; from about 0. 1 mg/kg to about 150 mg/kg; from about 0. 1 mg/kg to about 100 mg/kg; from about 0.1 mg/kg to about 50 mg/kg; from about 0. 1 mg/kg to about 10 mg/kg; from about 0.1 mg/kg to about 5 mg/kg; from

about 0.1 mg/kg to about 2 mg/kg; from about 0.1 mg/kg to about 1 mg/kg; or from about 0.1 mg/kg to about 0.5 mg/kg).

[0088] In some embodiments, an effective amount of the compounds, nucleic acids and the pharmaceutically acceptable salts thereof described above is about 0.1 mg/kg, about 0.5 mg/kg, about 1 mg/kg, about 2 mg/kg, or about 5 mg/kg.

[0089] The foregoing dosages can be administered on a daily basis (e.g., as a single dose or as two or more divided doses, e.g., once daily, twice daily, thrice daily) or non-daily basis (e.g., every other day, every two days, every three days, once weekly, twice weekly, once every two weeks, once a month).

Kits

[0090] In some embodiments, provided herein are packaged dosage forms, comprising a container holding a therapeutically effective amount of the compounds, nucleic acids and the pharmaceutically acceptable salts thereof described above, and instructions for using the dosage form in accordance with one or more of the methods provided herein.

[0091] The present dosage forms and associated materials can be finished as a commercial product by the usual steps performed in the present field, for example by appropriate sterilization and packaging steps. For example, the material can be treated by UV/vis irradiation (200–500 nm), for example using photo-initiators with different absorption wavelengths (for example, Irgacure 184, 2959), preferably water-soluble initiators (for example, Irgacure 2959). Such irradiation is usually performed for an irradiation time of 1–60 min, but longer irradiation times may be applied, depending on the specific method. The material according to the present disclosure can be finally sterile-wrapped so as to retain sterility until use and packaged (for example, by the addition of specific product information leaflets) into suitable containers (boxes, etc.).

[0092] According to further embodiments, the described dosage forms can also be provided in kit form combined with other components necessary for administration of the material to the patient. For example, disclosed kits, such as for use in the treatments described herein, can further comprise, for example, administration materials.

[0093] The kits may be designed in various forms based on the specific deficiencies they are designed to treat.

[0094] The dosage forms provided herein may be prepared and placed in a container for storage at ambient or elevated temperature. This is beneficial because transportation of commercially viable dosage forms may benefit from stability at temperatures greater than

those requiring refrigeration or sub-freezing environments during transportation and storage at the site of use.

[0095] When the dosage forms provided herein are stored in a polyolefin plastic container as compared to, for example, a polyvinyl chloride plastic container, discoloration of the dosage form may be reduced. Without wishing to be bound by theory, the container may reduce exposure of the container's contents to electromagnetic radiation, whether visible light (for example, having a wavelength of about 380–780 nm) or ultraviolet (UV) light (for example, having a wavelength of about 190–320 nm (UV B light) or about 320–380 nm (UV A light)). Some containers also include the capacity to reduce adherence or adsorption of the active ingredient to the surface of the container, which could effectively dilute the concentration of active ingredient in the contained solution. Some containers also include the capacity to reduce exposure of the container's contents to infrared light, or a second component with such a capacity. Some containers further include the capacity to reduce the exposure of the container's contents to heat or humidity. The containers that may be used include those made from a polyolefin such as polyethylene, polypropylene, polyethylene terephthalate, polycarbonate, polymethylpentene, polybutene, or a combination thereof, especially polyethylene, polypropylene, or a combination thereof. In some embodiments, the container is a glass container. The container may further be disposed within a second container, for example, a paper container, cardboard container, paperboard container, metallic film container, or foil container, or a combination thereof, to further reduce exposure of the container's contents to UV, visible, or infrared light. Articles of manufacture benefiting from reduced discoloration, decomposition, or both during storage, include dosage forms that include compounds, nucleic acids and the pharmaceutically acceptable salts thereof. The dosage forms provided herein may need storage lasting up to, or longer than, three months; in some cases up to, or longer than one year. The containers may be in any form suitable to contain the contents—for example, a bag, a bottle, or a box.

EXAMPLES

Example 1

[0096] Patient derived glioma stem cells (GSCs) do not express DDX4 but can express other components of the piRNA biogenesis pathway (see e.g., **FIG. 1**). RNA sequences of patient-derived glioma stem cells (GSCs) (GB2, WCR8, GB4, GB8, GB11, GB12, GB24) indicate that DDX4 may not be expressed in GSCs while other components of the piRNA biogenesis pathway may be expressed (e.g. MOV10L1, MOV10, PIWIl2, PIWIL4 and DHX9).

Example 2

[0097] FIG. 2 reveals that expression of DDX4 in GSCs induces cytotoxic death. GSCs are infected with lentivirus expressing DDX4 or control lentivirus expressing EGFP. Five days after infections of GSCs, cellular metabolic activity, which is a marker for cell viability, is quantified using a colorimetric MTT assay. Thus, in some embodiments, expression of DDX4 in GSCs induces significant cytotoxic death ($p<0.000011$).

Example 3

[0098] **FIG. 3** reveals that DDX4 expressing GSCs can upregulate three specific piRNAs, indicating re-activation of the piRNA biogenesis pathways. Small RNA sequencing following expression of DDX4 in GSCs reveals re-activation of three piRNAs.

Example 4

[0099] **FIGS. 4A-4G** show that patient derived GSCs can express specific retrotransposons. The volcano plots show differential expression of endogenous retrotransposons in human glioma stem cells (GSCs) compared to control human neural stem cells.

Example 5

[0100] **FIG. 5A** and **FIG. 5B** indicate that treatment of human GSCs with a piRNA mimic results in cytotoxic death of the GSCs. In some embodiments, transfection of human GSCs with piRNA mimics for 5 days can induce significant reduction of the viable GSC population ($*p<0.05$).

Example 6

[0101] FIG. 6 shows that treatment of control human astrocytes with piRNA mimics does not affect astrocyte survival. Treatment of human astrocytes with piRNA mimics or scramble control piRNA have no effect or substantially no effect on astrocyte survival. Table 1 shows the piRNA mimics tested in this experiment. The oligos are synthesized by Integrated DNA Technologies.

Table 1

piRNA Mimic	ID	Sequence
hsa-piR-17723	SEQ ID NO 1	5'-UGGCAGCUGUGCUGGAUGCCCGCUAUGAGG-3'
hsa-piR-16745	SEQ ID NO 2	5'-UGGAAGUGACUGGUCUGAUGUUGAGGAGA-3'
hsa-piR-33520	SEQ ID NO 3	5-' CGUAGUGUAGUGGUUAUCACGUUCGCC - 3'

[0102] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” As used herein the terms "about" and “approximately” means within 10 to 15%, preferably within 5 to 10%. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0103] The terms “a,” “an,” “the” and similar referents used in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0104] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0105] Certain embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Of course, variations on these

described embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventor expects skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0106] Specific embodiments disclosed herein may be further limited in the claims using consisting of or consisting essentially of language. When used in the claims, whether as filed or added per amendment, the transition term "consisting of" excludes any element, step, or ingredient not specified in the claims. The transition term "consisting essentially of" limits the scope of a claim to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s). Embodiments of the invention so claimed are inherently or expressly described and enabled herein.

[0107] Furthermore, references to patents and printed publications may have been made in this specification. Each of the above-cited references and printed publications are individually incorporated herein by reference in their entirety.

[0108] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed are within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

We claim

1. A method of treating a malignancy in a subject in need thereof, comprising administering a therapeutically effective amount of a compound that increases the expression of at least one piRNA in the subject.

2. The method of claim 1, wherein the malignancy is glioblastoma.

3. The method of claim 1, wherein the at least one piRNA is selected from piR_017723, piR_23656, and piR_016745, or a combination thereof.

4. The method of claim 1, wherein the compound is at least one nucleic acid that encodes a piRNA, or a piRNA mimic, or a combination thereof.

5. The method of claim 4, wherein the compound is at least one nucleic acid that encodes for piR_017723, piR_23656, or piR_016745, or a combination thereof.

6. The method of claim 4, wherein the compound is at least one nucleic acid that encodes for a piR_017723 mimic, a piR_016745 mimic, or a piR_033520 mimic, or a combination thereof.

7. The method of claim 6, wherein the at least one nucleic acid encodes a piRNA mimic selected from SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, or a combination thereof.

8. The method of claim 4, wherein the piRNA mimic causes cytotoxic cell death in malignant cells but does not cause cytotoxic cell death in non-malignant cells.

9. The method of claim 4, wherein the piRNA mimic causes cytotoxic cell death in glioblastoma stem cells (GSCs) but does not cause cytotoxic cell death in astrocytes.

10. The method of claim 4, wherein the at least one nucleic acid is incorporated into or encapsulated by nanoparticles, microparticles, micelles, synthetic lipoprotein particles, or carbon nanotubes.

11. The method of claim 4, wherein the at least one nucleic acid is incorporated into or encapsulated by liposomes.

12. The method of claim 4, wherein plasmids or viral vectors comprise the at least one nucleic acid.

13. A method of treating a malignancy in a subject in need thereof, comprising administering a therapeutically effective amount of a compound that increases the expression of DDX4 in the subject.

14. The method of claim 13, wherein the malignancy is glioblastoma.

15. The method of claim 13, wherein the compound is at least one nucleic acid that encodes DDX4.

16. The method of claim 15, wherein the at least one nucleic acid causes cytotoxic cell death in malignant cells but does not cause cytotoxic cell death in non-malignant cells.

17. The method of claim 15, wherein the at least one nucleic acid causes cytotoxic cell death in glioblastoma stem cells (GSCs) but does not cause cytotoxic cell death in astrocytes.

18. The method of claim 15, wherein the at least one nucleic acid is incorporated into or encapsulated by nanoparticles, microparticles, micelles, synthetic lipoprotein particles, or carbon nanotubes.

19. The method of claim 15, wherein the at least one nucleic acid is incorporated into or encapsulated by liposomes.

20. The method of claim 15, wherein plasmids or viral vectors comprise the at least one nucleic acid.

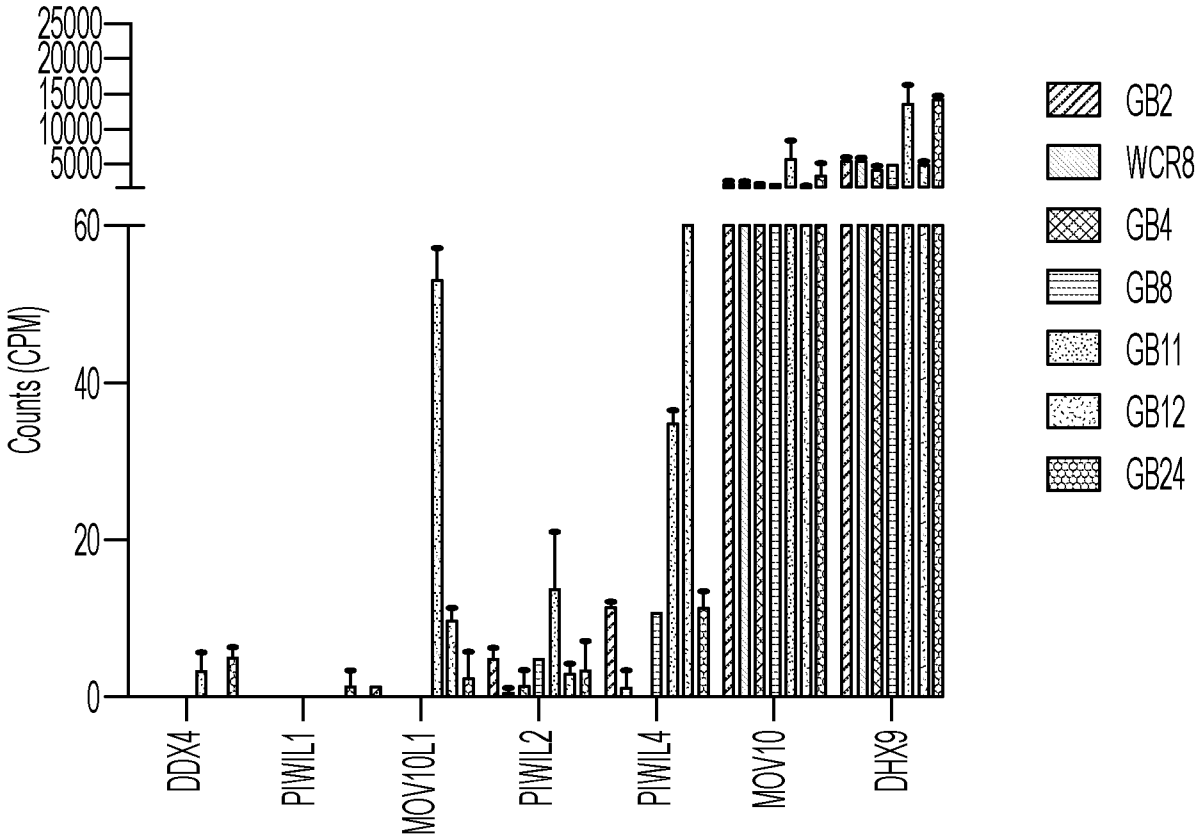
21. The method of any of the preceding claims, wherein the at least one nucleic acid is administered in an amount sufficient to cause one or more of the following: a decrease in tumor growth, a decrease in tumor cell proliferation, increased tumor cell apoptosis, inhibition of metastatic dissemination of the glioblastoma or a human malignancy improvement in subject survival, reduced ability of tumor cells to form colonies, or reduced ability of tumor cells to migrate.

22. The method of claim any of the preceding claims, wherein the at least one nucleic acid is administered before, after, or concurrently with the administration of an antibody therapy.

23. The method of any of the preceding claims, wherein the at least one nucleic acid is administered before, after, or concurrently with the administration of a radiation therapy.

24. A pharmaceutical composition comprising at least one nucleic acid or a pharmaceutically acceptable salt thereof that encodes piR_017723 or a mimic thereof, piR_23656 or a mimic thereof, piR_016745 or a mimic thereof, piRNA hsa-16745, piRNA hsa-17723, or piRNA hsa-33520.

25. A diagnostic or prognostic biomarker for monitoring treatment of glioblastoma comprising the expression levels of at least one of piR_017723, piR_23656, or piR_016745, or a combination thereof, wherein an increase in expression of at least one of piR_017723, piR_23656, or piR_016745, or a combination thereof, is indicative of effective treatment of glioblastoma.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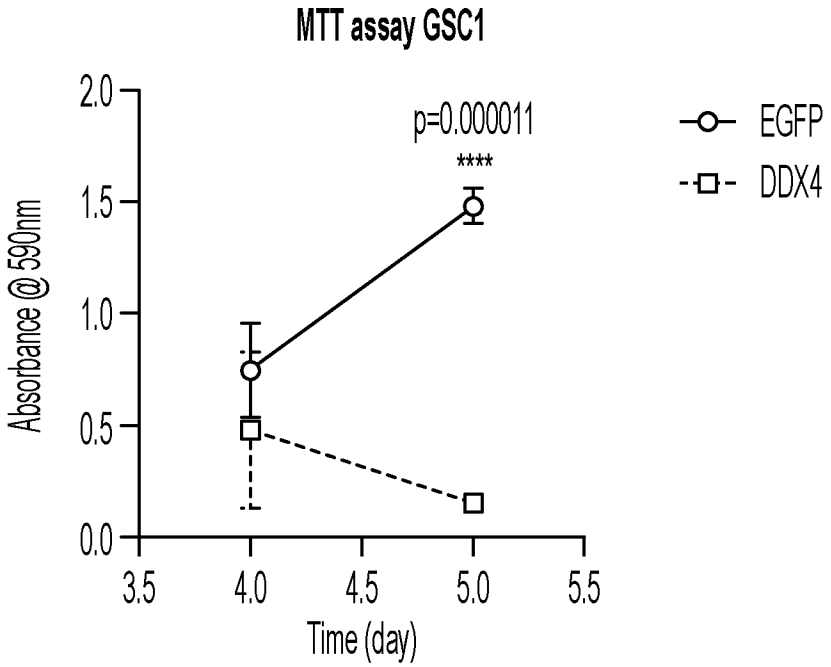
WCR8
GB4
GB8
GB11
GB12
GB24

FIG. 1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****
EGFP
DDX4
Absorbance @ 590nm
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0.0
3.5
4.0
4.5
5.0
5.5
Time (day)

FIG. 2

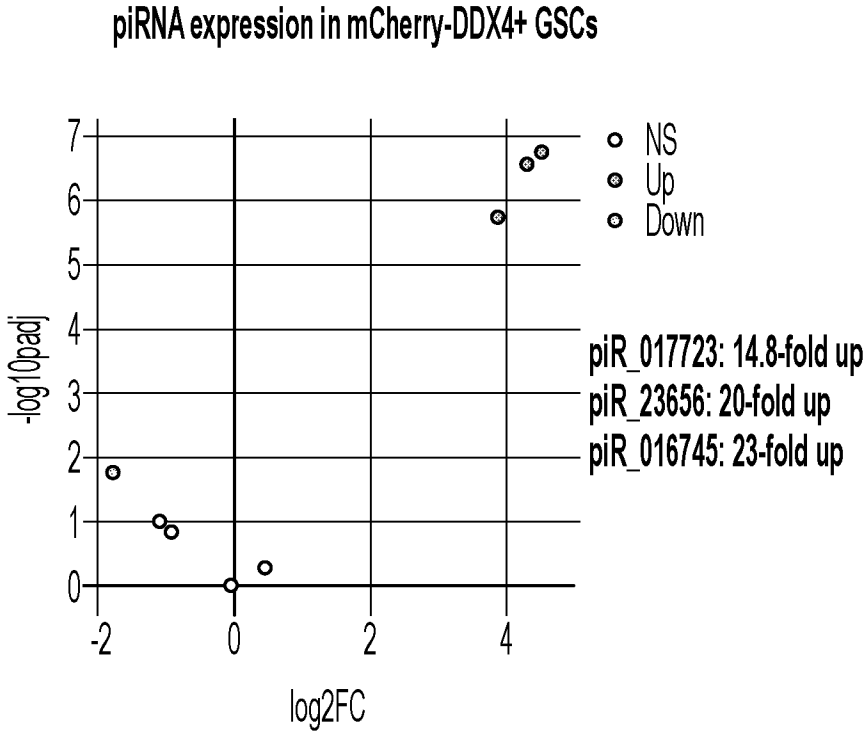
piRNA expression in mCherry-DDX4+ GSCs
-log10padj
log2FC
NS
Up
Down
piR_017723: 14.8-fold up
piR_23656: 20-fold up
piR_016745: 23-fold up

FIG. 3

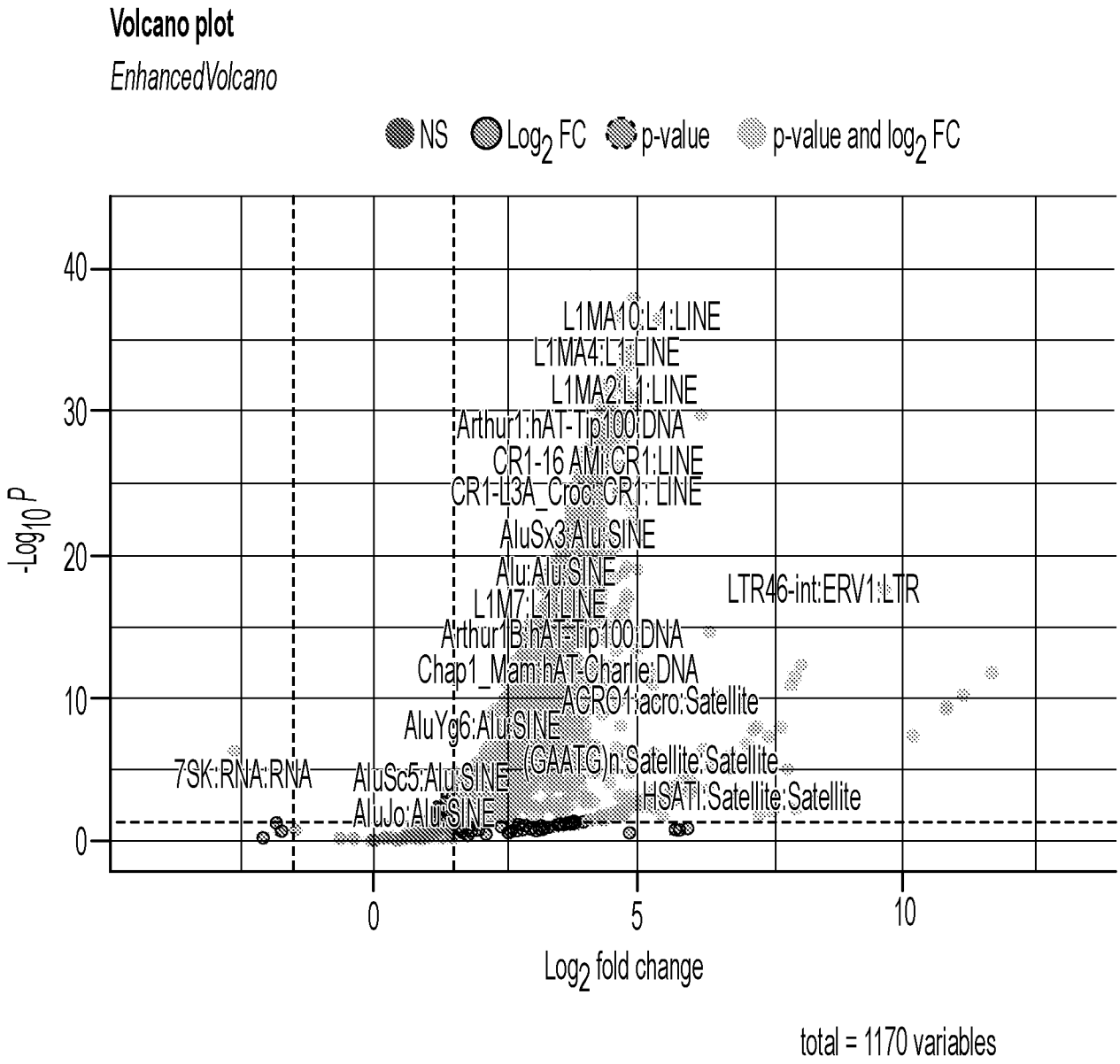
Volcano plot
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
-Log10 P
40
30
20
10
0
L1MA10:L1:LINE
L1MA4:L1:LINE
L1MA2:L1:LINE
Arthur1:hAT-Tip100:DNA
CR1-16_AM:CR1:LINE
CR1-L3A_Croc:CR1:LINE
AluSx3:Alu:SINE
Alu:Alu:SINE
L1M7:L1:LINE
LTR46-int:ERV1:LTR
Arthur1B:hAT-Tip100:DNA
Chap1_Mam:hAT-Charlie:DNA
ACRO1:acro:Satellite
AluYg6:Alu:SINE
(GAATG)n:Satellite:Satellite
7SK:RNA:RNA
AluSc5:Alu:SINE
HSATI:Satellite:Satellite
AluJo:Alu:SINE
0
5
10
Log2 fold change
total = 1170 variables

FIG. 4A

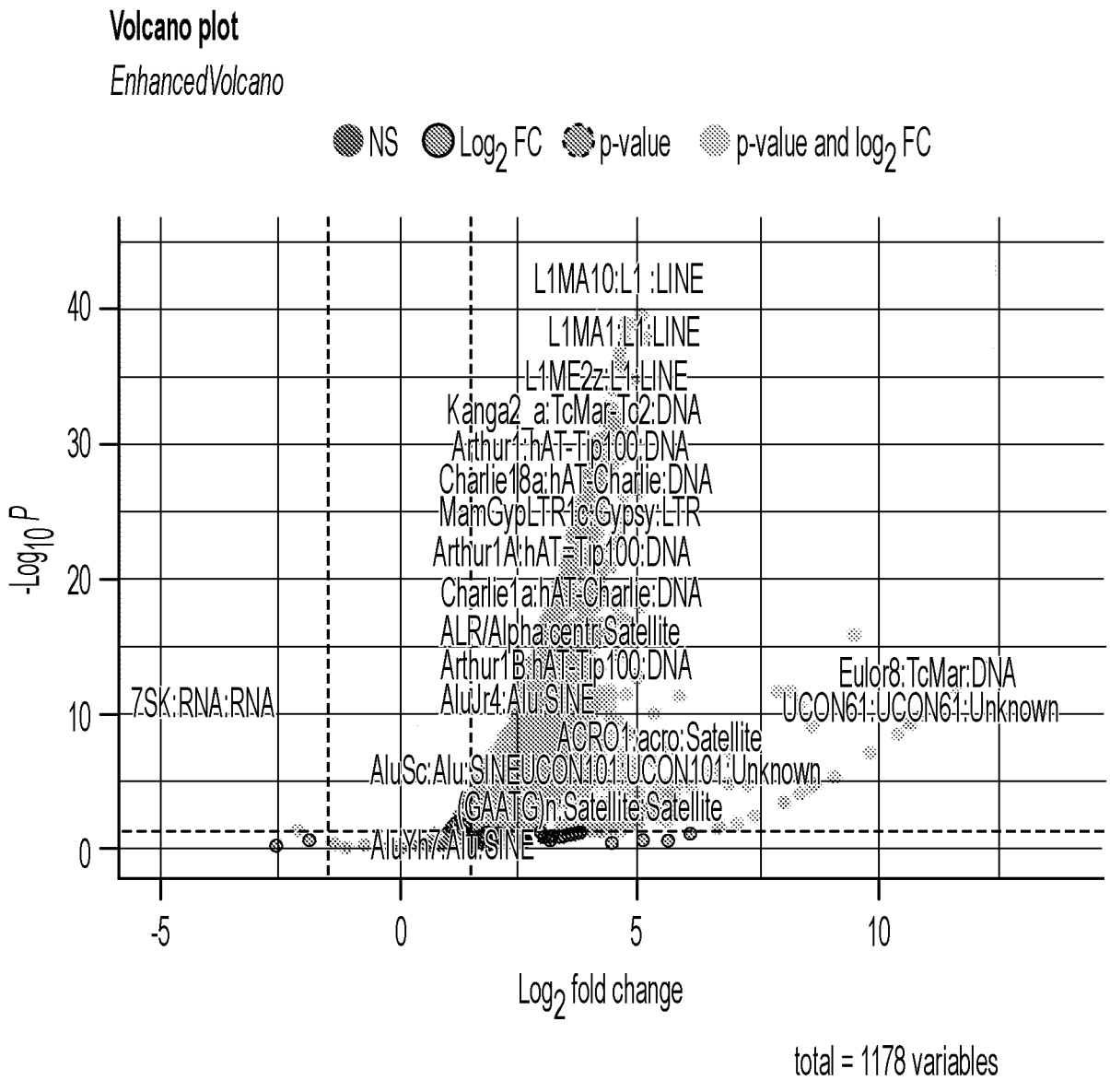
Volcano plot
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
L1MA10:L1:LINE
L1MA1:L1:LINE
L1ME2z:L1:LINE
Kanga2_a:TcMar-Tc2:DNA
Arthur1:hAT-Tip100:DNA
Charlie18a:hAT-Charlie:DNA
MamGypLTR1c:Gypsy:LTR
Arthur1A:hAT-Tip100:DNA
Charlie1a:hAT-Charlie:DNA
ALR/Alpha:centr:Satellite
Arthur1B:hAT-Tip100:DNA
AluJr4:Alu:SINE
7SK:RNA:RNA
Eulor8:TcMar:DNA
UCON61:UCON61:Unknown
ACRO1:acro:Satellite
AluSc:Alu:SINE
UCON101:UCON101:Unknown
(GAATG)n:Satellite:Satellite
AluYh7:Alu:SINE
-Log10 P
40
30
20
10
0
-5
0
5
10
Log2 fold change
total = 1178 variables

FIG. 4B

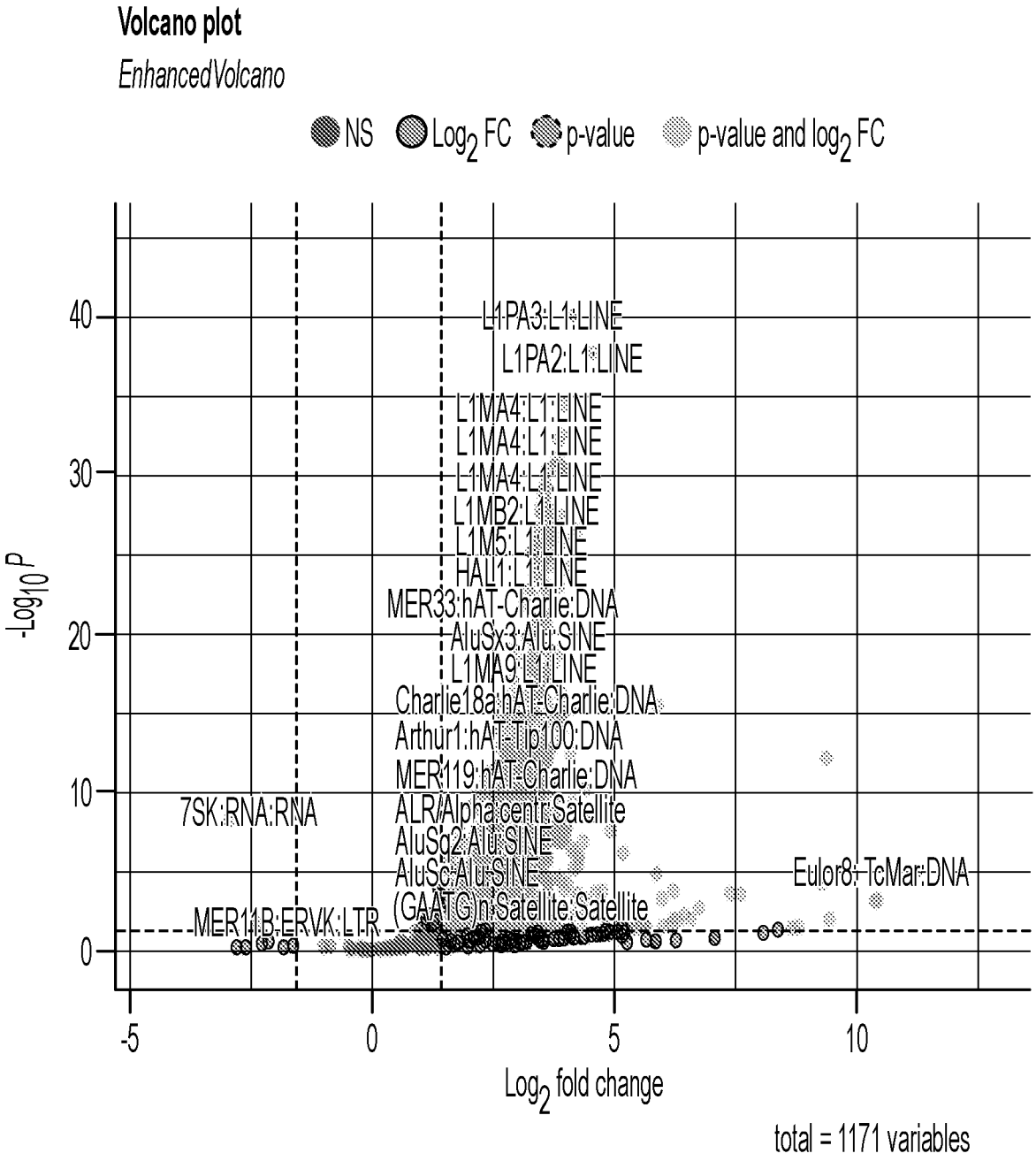
Volcano plot
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
-Log10 P
40
30
20
10
0
-5
0
5
10
Log2 fold change
L1PA3:L1:LINE
L1PA2:L1:LINE
L1MA4:L1:LINE
L1MA4:L1:LINE
L1MA4:L1:LINE
L1MB2:L1:LINE
L1M5:L1:LINE
HAL1:L1:LINE
MER33:hAT-Charlie:DNA
AluSx3:Alu:SINE
L1MA9:L1:LINE
Charlie18a:hAT-Charlie:DNA
Arthur1:hAT-Tip100:DNA
MER119:hAT-Charlie:DNA
7SK:RNA:RNA
ALR/Alpha:centr:Satellite
AluSq2:Alu:SINE
AluSc:Alu:SINE
Eulor8:TcMar:DNA
(GAATG)n:Satellite:Satellite
MER11B:ERVK:LTR
total = 1171 variables

FIG. 4C

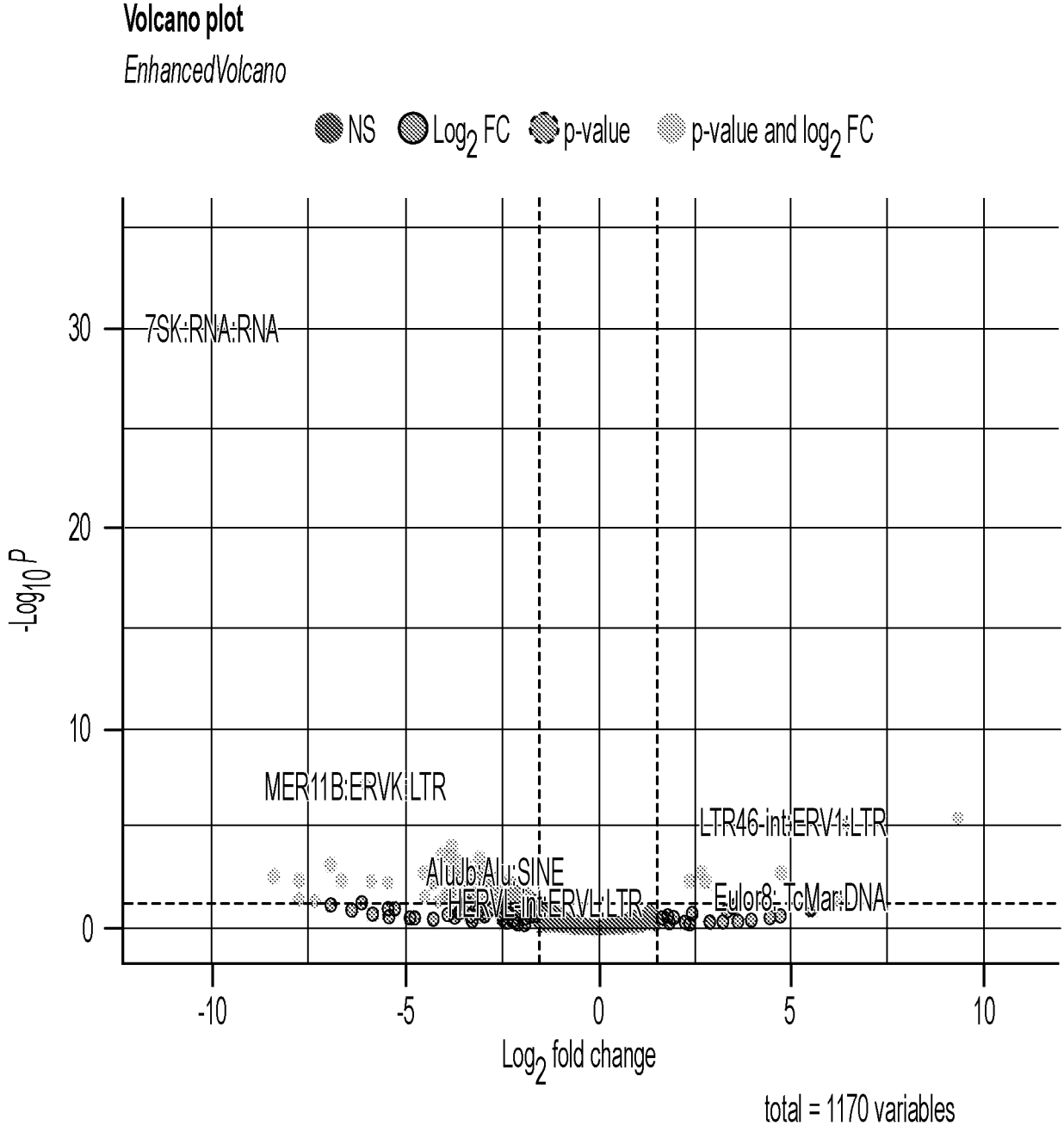
Volcano plot
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
7SK:RNA:RNA
MER11B:ERVK:LTR
LTR46-int:ERV1:LTR
AluJb:Alu:SINE
HERVL-int:ERVL:LTR
Eulor8:TcMar:DNA
-Log10 P
30
20
10
0
-10
-5
0
5
10
Log2 fold change
total = 1170 variables

FIG. 4D

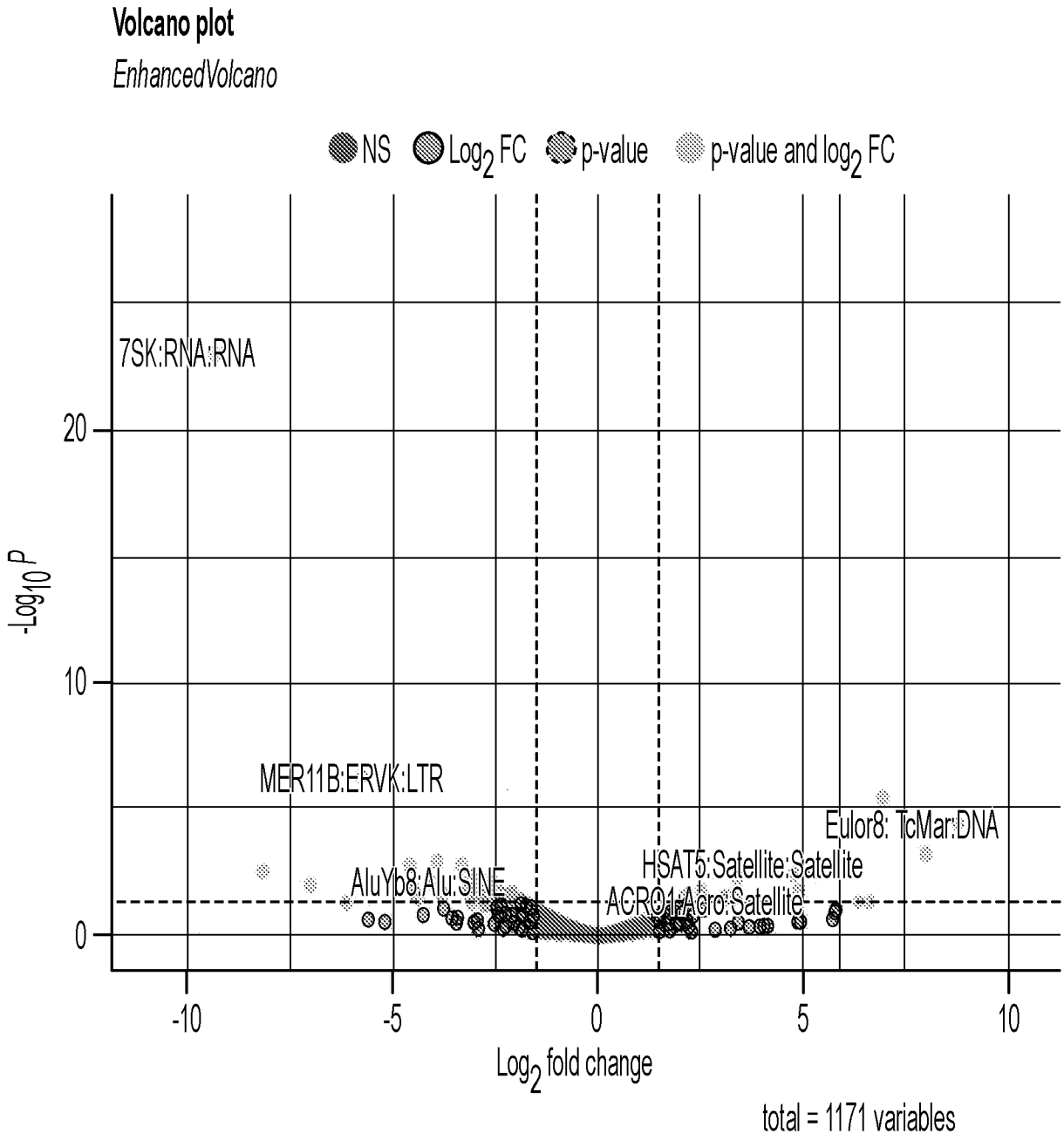
Volcano plot
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
7SK:RNA:RNA
MER11B:ERVK:LTR
AluYb8:Alu:SINE
HSAT5:Satellite:Satellite
ACRO1:Acro:Satellite
Eulor8: TcMar:DNA
20
10
0
-Log10 P
-10
-5
0
5
10
Log2 fold change
total = 1171 variables

FIG. 4E

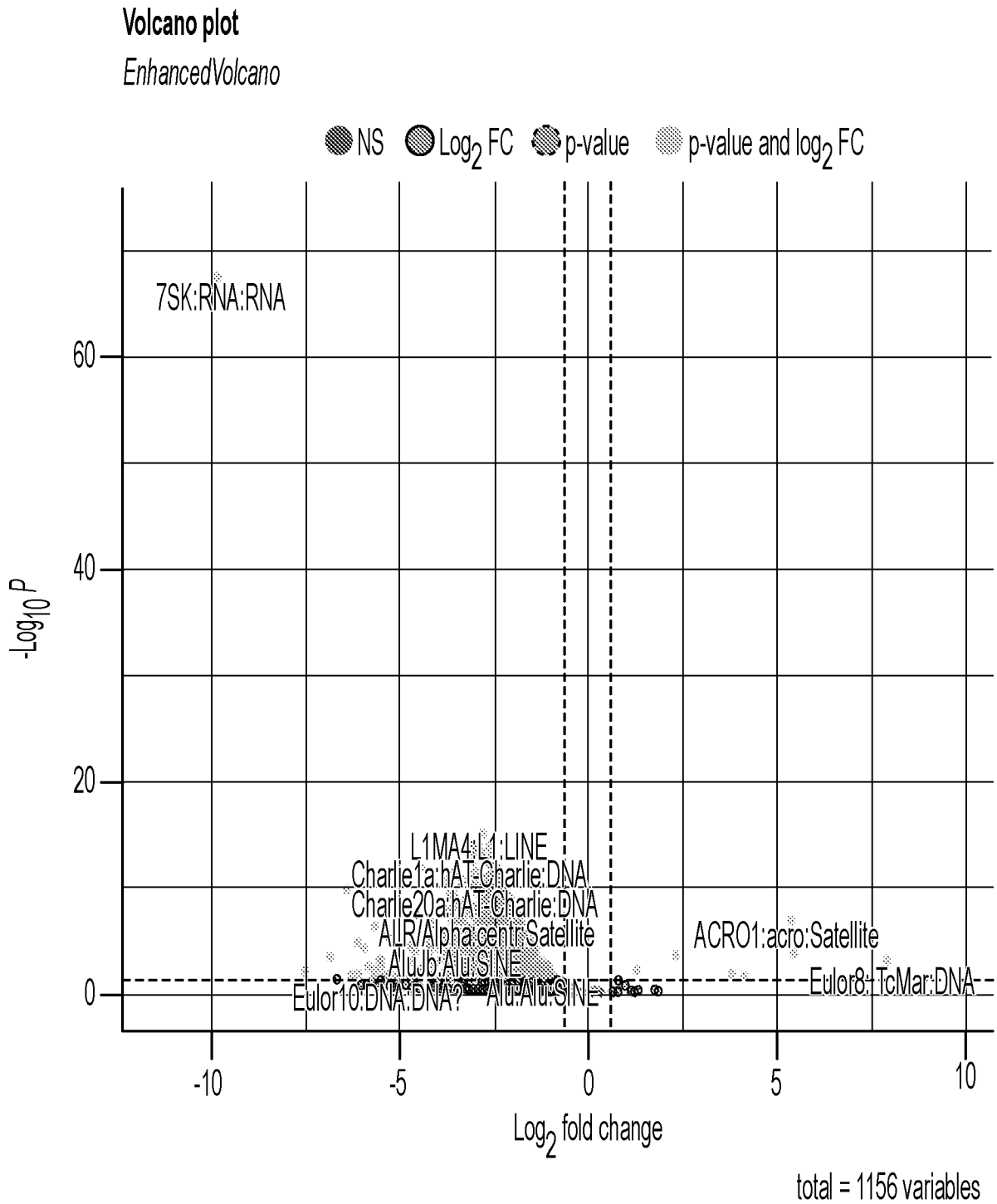
Volcano plot
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
7SK:RNA:RNA
L1MA4:L1:LINE
Charlie1a:hAT-Charlie:DNA
Charlie20a:hAT-Charlie:DNA
ALR/Alpha:centr. Satellite
AluJb:Alu:SINE
Eulor10:DNA:DNA?
Alu:Alu:SINE
ACRO1:acro:Satellite
Eulor8:TcMar:DNA
60
40
20
0
-Log10 P
-10
-5
0
5
10
Log2 fold change
total = 1156 variables

FIG. 4F

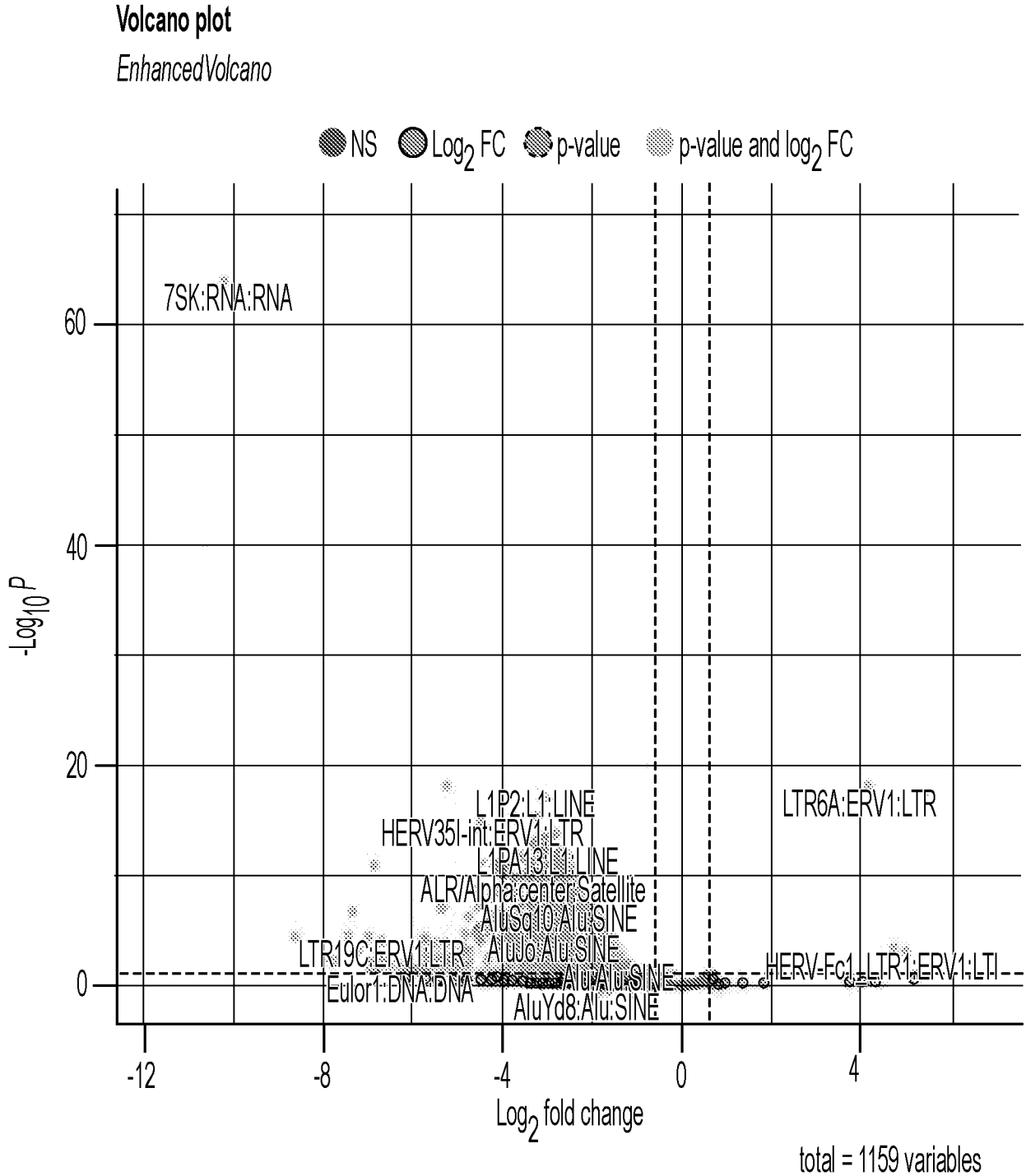
Volcano plot
EnhancedVolcano
NS
Log2 FC
p-value
p-value and log2 FC
7SK:RNA:RNA
L1P2:L1:LINE
HERV35I-int:ERV1:LTR
L1PA13:L1:LINE
ALR/Alpha:center:Satellite
AluSq10:Alu:SINE
LTR19C:ERV1:LTR
AluJo:Alu:SINE
Eulor1:DNA:DNA
AluYd8:Alu:SINE
LTR6A:ERV1:LTR
HERV-Fc1_LTR1:ERV1:LTR
-Log10 P
60
40
20
0
-12
-8
-4
0
4
Log2 fold change
total = 1159 variables

FIG. 4G

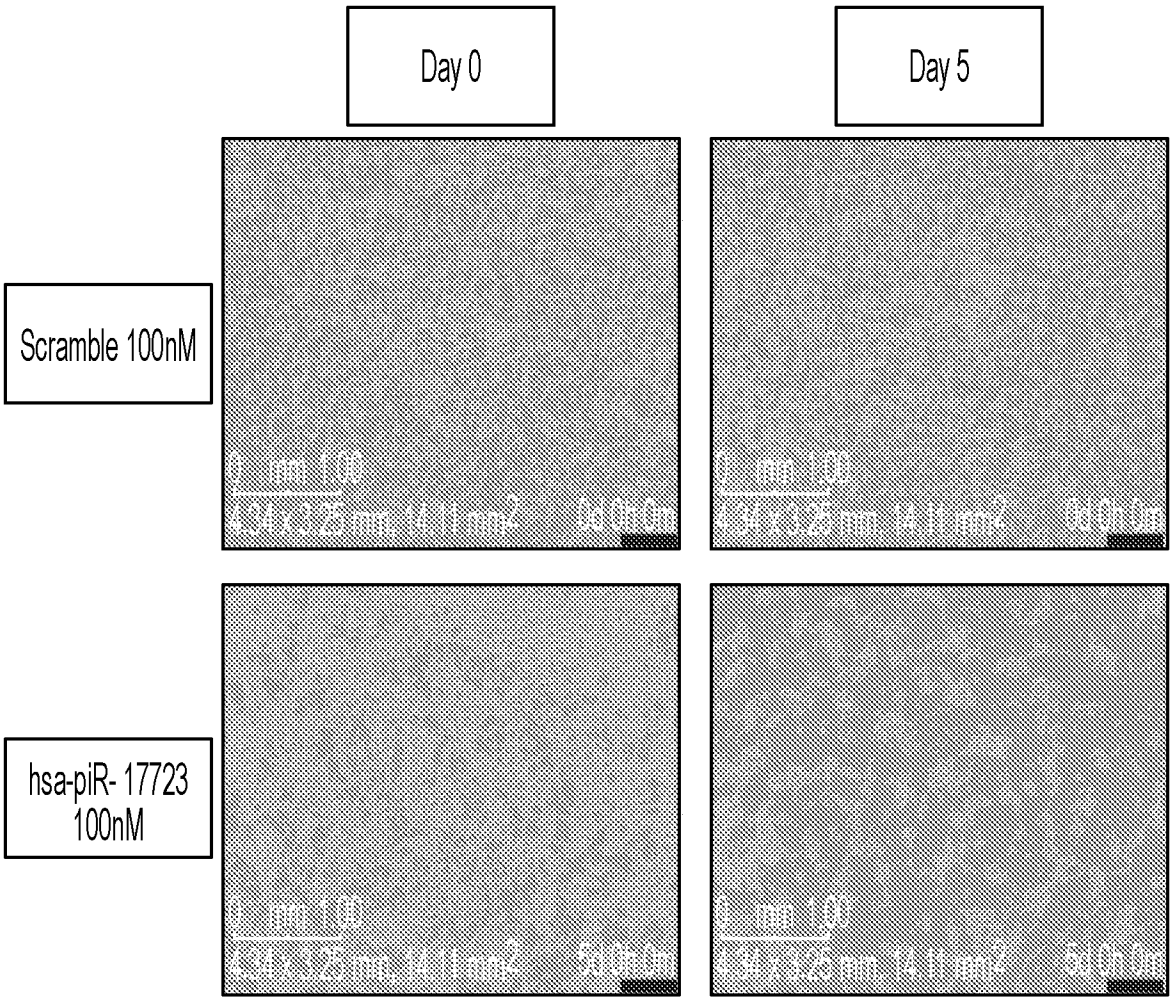

FIG. 5A

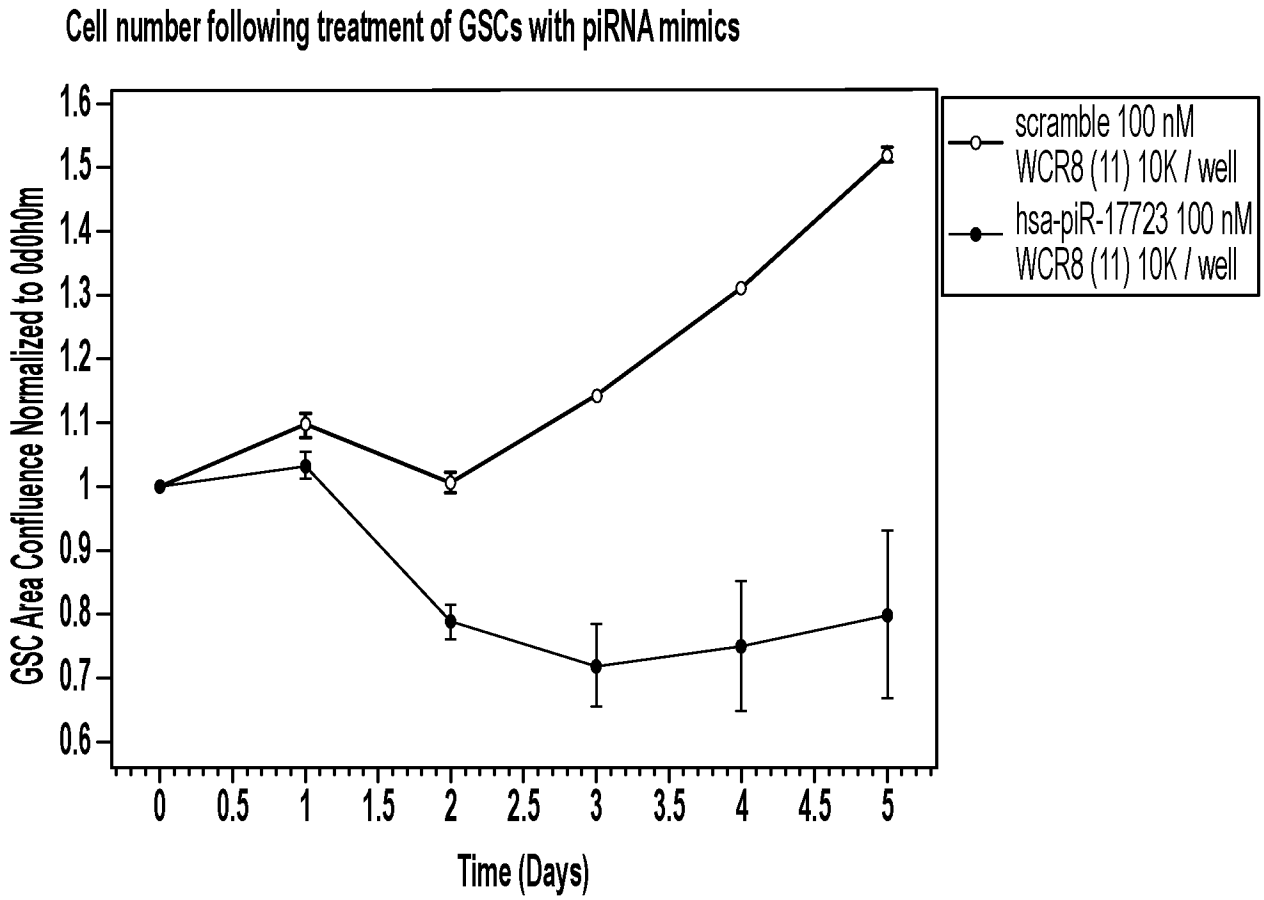
Cell number following treatment of GSCs with piRNA mimics
GSC Area Confluence Normalized to 0d0h0m
1.6
1.5
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Time (Days)
scramble 100 nM WCR8 (11) 10K / well
hsa-piR-17723 100 nM WCR8 (11) 10K / well

FIG. 5B

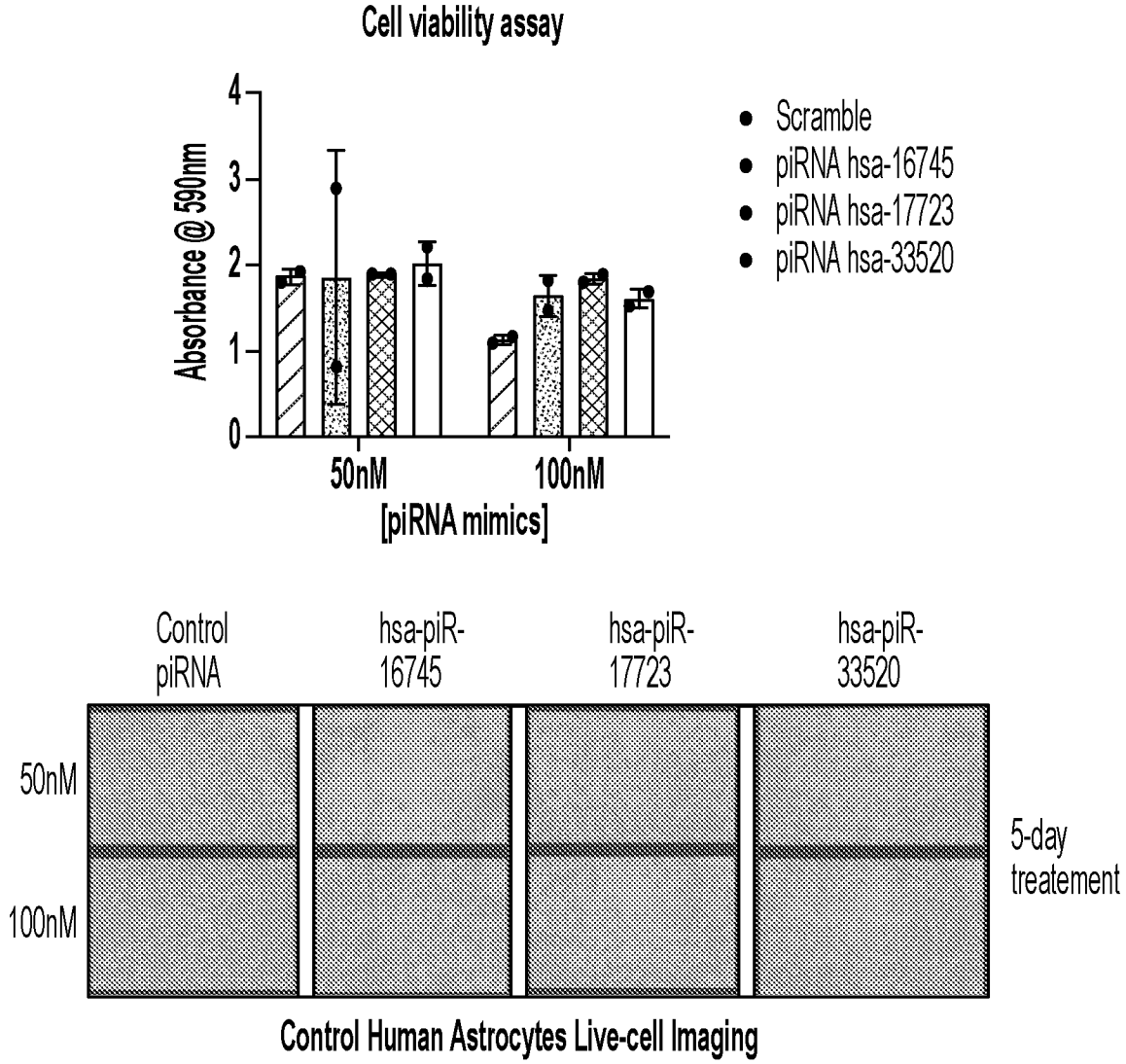
Cell viability assay
Absorbance @ 590nm
0
1
2
3
4
50nM
100nM
[piRNA mimics]
Scramble
piRNA hsa-16745
piRNA hsa-17723
piRNA hsa-33520
Control piRNA
hsa-piR-16745
hsa-piR-17723
hsa-piR-33520
50nM
100nM
5-day treatement
Control Human Astrocytes Live-cell Imaging

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/078611

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 31/7105*** (2024..0); ***A61K 38/43*** (2024..0); *A61K 48/00* (2024..0); ***A61P 35/00*** (2024..0); ***C07K 14/435*** (2024..0); ***C12N 15/113*** (2024..0); ***C12Q 1/6886*** (2024..0)

CPC: ***C12N 15/113; A61P 35/00; A61K 31/7105; A61K 38/43; C07K 14/435;*** *C12N 2310/10; C12N 2320/10; C12Q 1/6886*

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)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	US 10,767,178 B2 (YALE UNIVERSITY) 08 September 2020 (08.09.2020)	
X	entire document	1, 2, 4, 8, 10-12, 21
Y	entire document	3, 5, 9
	US 8,173,616 B2 (ANDERSON et al.) 08 May 2012 (08.05.2012)	
X	entire document	1, 4, 6, 7, 24
	US 2021/0315972 A1 (NORTHWESTERN UNIVERSITY) 14 October 2021 (14.10.2021)	
X	entire document	13, 14
	QU et al., A serum piRNA signature as promising non-invasive diagnostic and prognostic biomarkers for colorectal cancer, Cancer Management and Research, 2019, Vol. 11, Pgs. 3703-3720. entire document	
Y		3, 5

☑ Further documents are listed in the continuation of Box C. ☐ 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
"D" document cited by the applicant in the international application
"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
26 January 2024 (26.01.2024)	**26 February 2024 (26.02.2024)**

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US **Commissioner for Patents** **P.O. Box 1450, Alexandria, VA 22313-1450**	**MATOS** **TAINA**
Facsimile No. **571-273-8300**	Telephone No. **571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/078611

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/078611

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.: **22, 23**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZEPECKI et al., Regulation of human glioma cell migration, tumor growth, and stemness gene expression using a Lck targeted inhibitor, Oncogene, 23 October 2018, Vol. 39, Pgs. 1734–1750. entire document	9
A	US 6,875,854 B1 (CASTRILLON) 05 April 2005 (05.04.2005) entire document	1-21, 24, 25
A	JIA et al., PIWI-interacting RNA sequencing pro#les in maternal plasma-derived exosomes reveal novel non-invasive prenatal biomarkers for the early diagnosis of nonsyndromic cleft lip and palate, EBioMedicine, 24 February 2021, Vol. 39, Pgs. 1-15. entire document	1-21, 24, 25
A	KEE et al., Bone Morphogenetic Proteins Induce Germ Cell Differentiation from Human Embryonic Stem Cells, Stem Cells and Development, 2006, Vol. 15, Pgs. 831-837. entire document	1-21, 24, 25
A	NOYES et al., The germline factor DDX4 contributes to the chemoresistance of small cell lung cancer cells, bioRxiv, 22 April 2022, Pgs. 1-43. entire document	1-21, 24, 25
A	SIEGL, Analysis of PFWI-interacting RNA s in glioblastoma multiforme patients, Masaryk University, Bachelor's thesis, June 2018, Pgs. 64-65 [retrieved on 25 January 2022]. Retrieved from the Internet: <URL: https://theses.cz/id/ak7j2q/?lang=sk>. entire document	1-21, 24, 25