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(54) Title: COMPOUNDS FOR INHIBITING AMANZI

(57) Abstract: The inventions relate to an ASO (antisense oligonucleotide) compound that can inhibit AMANZI transcriptional activity. More specifically, the disclosed ASO comprises a gapmer type of ASO comprising from about 14 to about 25 nucleotide bases; (a) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (b) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (c) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases. Preferably, the gapmer is substantially complementary to a region on AMANZI (SEQ ID NO. 1). More preferably, the gapmer is substantially complementary to a 14-25 base region on AMANZI.



COMPOUNDS FOR INHIBITING AMANZI

PRIOR RELATED APPLICATIONS

[1] This application claims priority to U.S. Provisional Application Serial No. 63/542,512, filed October 4, 2023, the contents of which are herein incorporated in their entirety.

SEQUENCE STATEMENT

[2] The instant application contains a Sequence Listing, which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on October 3, 2024, is named J1742-00203_SL.xml and is 765,657 bytes in size.

FIELD

[3] The inventions relate to compounds and methods for modulating AMANZI transcriptional activity. In particular are provided gapmer type of ASO (antisense oligonucleotide) compounds that can modulate AMANZI transcriptional activity relating to inflammation and activation of the immune system, comprising (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to a 14-25 base region on AMANZI (SEQ ID NO. 1). In particular, the gapmer nucleotide bases can each be linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and the modified nucleotide base modifications can be selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

BACKGROUND

[4] The following includes information that may be useful in understanding the present invention. It is not an admission that any of the information, publications or documents specifically or implicitly referenced herein is prior art, or important, to the inventions described and claimed herein. All publications, patents, related applications, and other written or electronic materials mentioned or identified herein are hereby incorporated herein by reference in their entirety. The information incorporated is as much a part of the application as filed as if all of the text and all other content was repeated in the application and should be treated as part of the text and content of the application as filed.

[5] Long non-coding RNAs (lncRNAs) are non-coding transcripts of more typically than 200 nucleotides (200 nt). lncRNAs can serve as a scaffold for the assembly of chromatin remodeling complexes to robustly influence gene transcription at specific loci. Mattick, J.S., et al. *Nat Rev Mol Cell Biol* **24**, 430–447 (2023)). Long non-coding transcripts are found in many species. DNA (cDNA) sequencing projects such as FANTOM have identified the complexity of these transcripts in humans (Carninci P, et al. (September 2005), *Science*. 309 (5740): 1559–1563).

[6] Inflammation is characterized by a biphasic cycle consisting initially of a proinflammatory phase that is subsequently resolved by anti-inflammatory processes. Interleukin-1 β (IL-1 β) is a master regulator of proinflammation and is encoded within the same topologically associating domain (TAD) as IL-37, which is an anti-inflammatory cytokine that opposes the function of IL-1 β .

SUMMARY

[7] The inventions described and claimed herein have many attributes and embodiments including, but not limited to, those set forth or described or referenced in this Brief Summary. It is not intended to be all-inclusive and the inventions described and claimed herein are not limited to or by the features or embodiments identified in this introduction, which is included for purposes of illustration only and not restriction.

[8] A Master Non coding RNA antagoniZing Inflammation (AMANZI) is a lncRNA encoded within the promoter of IL-1 β . AMANZI is co-transcribed from the opposite strand and opposite direction from IL-1 β and mediates a temporally delayed long-range chromatin looping interaction with the promoter of IL-37, which is otherwise spatially insulated from the active IL-1 β locus. AMANZI is a suppressor of IL-1 β .

[9] The present disclosure provides an ASO (antisense oligonucleotide) compound can lead to over-expression of IL-1 β . The present disclosure provides a gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity. More specifically, the disclosed ASO comprises a Gapmer type of ASO comprising from about 14 to about 25 nucleotide bases; a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases. Preferably, the gapmer is substantially complementary to a 14-25 base region on AMANZI (SEQ ID NO. 1). Preferably, the gapmer nucleotide bases are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from

the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[10] The present disclosure provides a gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, comprising (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary to Region A of AMANZI (SEQ ID NO. 1 bases 10 to 93). Preferably, the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NOs. 2-105 or an 8 mer fragment thereof. Preferably, the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof. Preferably, Region A of AMANZI is SEQ ID NO. 1 base 12 to base 27. More preferably, the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, SEQ ID NO. 45, SEQ ID NO. 46, and combinations thereof. Most preferably the gapmer type of ASO is SEQ ID NO. 42.

[11] The present disclosure provides a gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, comprising (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region B of AMANZI (SEQ ID NO. 1 bases 194 to 253). Preferably, the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NO. 78, SEQ ID NO. 81, SEQ ID NO. 82, SEQ ID NO. 83, SEQ ID NO. 84, and combinations thereof, or an 8 mer fragment thereof. Preferably, the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof. More preferably, the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 78, SEQ ID NO. 81, SEQ ID NO. 82, SEQ ID NO. 83, SEQ ID NO. 84, and combinations thereof. Most preferably the gapmer type of ASO is SEQ ID NO. 84.

[12] The present disclosure a gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region C of AMANZI (SEQ ID NO. 1 bases 519 to 568). Preferably, the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 69, SEQ ID NO. 101, SEQ ID NO. 102, SEQ ID NO. 103, SEQ ID NO. 104, and combinations thereof, or an 8 mer fragment thereof. Preferably, the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof. More preferably, the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 69, SEQ ID NO. 101, SEQ ID NO. 102, SEQ ID NO. 103, SEQ ID NO. 104, and combinations thereof. Most preferably the gapmer type of ASO is SEQ ID NO. 101.

[13] The present disclosure provides a gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary to Region D of AMANZI (SEQ ID NO. 1 bases 377 to 404). Preferably, the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NOs. 228-234, and combinations thereof, or an 8-mer fragment thereof. Preferably, the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[14] The present disclosure provides a gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA

bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region E of AMANZI (SEQ ID NO. 1 bases 574 to 615). Preferably, the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NOs. 206-215, and combinations thereof, or an 8 mer fragment thereof. Preferably, the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[15] The present disclosure provides a pharmaceutical composition comprising a gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity and pharmaceutical excipients, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region D of AMANZI (SEQ ID NO. 1 bases 377 to 404). Preferably, the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID Nos. 228-234, and combinations thereof, or an 8-mer fragment thereof. Preferably, the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[16] The present disclosure provides a pharmaceutical composition comprising a gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity and pharmaceutical excipients, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region E of AMANZI (SEQ ID NO. 1 bases 574 to 615). Preferably, the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NOs. 206-215, and combinations thereof, or an 8 mer fragment thereof. Preferably, the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[17] The gapmer nucleotide bases can each be independently linked by internucleotide bonds throughout the gapmer wherein at least one of said internucleotide bond is a phosphorothiolate (P=S) internucleotide bonds; and wherein nucleotide bases include at least one modified nucleotide base modification selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof. In some aspects, at least one modified internucleoside linkage is a phosphorothioate linkage. In some aspects, all of the internucleotide bonds are phosphorothioate bonds throughout the gapmer.

DETAILED DISCLOSURE

[18] The present disclosure is based on investigations that inhibitors of AMANZI transcriptional activity can lead to over-expression of IL-1 β (beta).

[19] The inventions relate to AMANZI transcriptional modulators and compositions comprising thereof for the modulation of AMANZI transcription. In some embodiments, the AMANZI transcriptional modulators are AMANZI transcriptional inhibitors.

Target AMANZI

[20] (FANTOM CAT no: FTMT20800006495.1) (SEQ ID NO. 1)

[21] Human AMANZI sequence:

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CTGACAATCGTTGTGCAGTTGATGTCCACATTAATACTGGATTTTCCCACGTT
AGAAGAATGTTTAAATTTAGTATATGTGGGACAAAGTGGAAGACACACAGATTT
ATACATGCACATACTTTTCTTCATTCACCTCTTTGTACTTAAGTTTAGGAATCTTC
CCACTTACAGATGGATAAATGGGTACAATGAAGGGCCAATAGCCCTCCCTGTCT
GTATTGAGGGTGTGGGTCTCTACCTTGGGTGCTGTTCTCTGCCTCAGGAGCTCTCT
GTCAATTGCAGGAGCCTCTGAGGAGAAAATTGACCTTTCTTGGCTGGGGCAGAG
AACATACGGTATGCAGGGTTCAGGCTCCTGACGGAGTTGGGGCAACCCTGGAGA
TAAGCTCACACAACCCTGCAAGACCAGGTGCTGTTACCCTAGCCAATCTCATGGA
TGAACCAGATCAATGCCAGATGAGCTCTGCCTAAAATGATTTTTTGGTGAACCTCT
GAAAAGTGGAATATTGTTTCTGTAAGAATATCCATCTGAGACTCTATCTCTTGGT
AATACCAACCAAGAGTTATCAGTTTCTCTTTAACCGAGACACCAGCAAAGTGCCT
GCTCCAGGGTACTGCCAGGGGAGCCCTCCATTTGTAGAATGAATGAGAGTCCA
GGTTATGAACAGTGCCTGGAGTGTAGGAACACCCTCCTTTGCCTCTTTGACAGGT
CTGCATCATAACACTTTTTTTTTTTTTTTGAGACAGAGTCTCACTCTGTGCCCCAG
GCTGGAGTGCAGTGGCACGATCTCGGCCCCCTGCAAGTCCGCCTCCCGGGTCA
CACCATTCTCCTGCCTCAGCCTCCCCAGCAGCTGGGACTACAGGCACCTGCCGCC
ACGCCCCGGCTAATTTTTTTGTATTTTAGTAGAGACAGGGTTTCACCATGTTAGCCA
GGATGGTCTCGATCTCCTGACCTTGTGATCTGCCCCGCTCGGCCTCCCAAAGTGT
TGGGATTACAGGCGTGAGCCACCGTGTCCAGCCTGTAACACTTCTTATAGCACTG
AGTTGAAACCTTGCTCCTCCTGGTTCCTCCAGGAACTGAAATCTTTTTGAGCCA
AGTCTAGCACAGTGCCTGGCATGTACATTCAGGTGGTAGAGTTTGCTGCTTGAAT
GGGTGAATGGGAATTTGACAGCATTTTTATTCAAATTAGTATGTGCCAGGTATCG
TGCTCGCTCTGCATTATCCAAGGGAGTGAGCCTCTGTGCAAGTATTGAGACACG
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AGGGAAATAGGTTCTACTGTGGGAAAAAGAGCATTTTCATGGACTTGCTCTCCAA
GCAGCCTTCTGATTTTTAATTTGGCTCCCAGTATCTTGATATCAGGAGTCAGTCAC
AAGAACTCCATCTTTAGTAAGTTATATTTTCCACAGGAAATCTAAAAGCTGTTCA
ACATGTTAGTTTCCTGTGAATTTGATAAGCCATAATCCATTCCCTAACACTGAGCC
CTCCTGAAATTTGGTGTCTGGTCCTGCAGATAGCTAAAAGCCCTGTCTGGGTGGC
CTAGGGGACTCCTCTGTTTTGCCTCCACAG

Definitions

[22] The following terms have the following meanings:

[23] “2′-substituted nucleoside” means a nucleoside comprising a 2′-substituted sugar moiety. “2′-substituted” in reference to a sugar moiety means a sugar moiety comprising at least one 2′- substituent group other than H or OH.

[24] “2′-deoxynucleoside” means a nucleoside comprising 2′-H furanosyl sugar moiety, as found naturally occurring in deoxyribonucleosides (DNA). A 2′-deoxynucleoside may comprise a modified nucleobase or may comprise an RNA nucleobase (e.g., uracil).

[25] “2′-O-methoxyethyl” (also 2′-MOE and 2′-O(CH₂)₂—OCH₃) refers to an O-methoxy- ethyl modification of the 2′ position of a furanosyl ring. A 2′-O-methoxyethyl modified sugar is a modified sugar.

[26] “2′-O-methoxyethyl nucleotide” means a nucleotide comprising a 2′-O-methoxyethyl modified sugar moiety.

[27] “5-methyl cytosine” means a cytosine modified with a methyl group attached to a 5 position. A 5-methyl cytosine is a modified nucleobase.

[28] “About” means plus or minus 7% of the provided value.

[29] “Active target region” or “target region” means a region to which one or more active antisense compounds is targeted. “Active antisense compounds” means antisense compounds that reduce target gene transcription or resulting protein levels.

[30] “Adjuvant” is defined as any molecule to enhance an antigen-specific adaptive immune response.

[31] “Antisense activity” means any detectable and/or measurable change attributable to the hybridization of an antisense compound to its target nucleic acid. Antisense activity is a decrease in the amount or expression of a target nucleic acid or protein encoded by such target nucleic acid compared to target nucleic acid levels or target protein levels in the absence of the antisense compound.

[32] “Antisense compound” means an oligomeric compound capable of achieving at least one antisense activity.

[33] “alkyl” group refers to a saturated aliphatic hydrocarbon group containing 1-8 (e.g., 1-6 or 1-4) carbon atoms. An alkyl group can be straight or branched. Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, n-heptyl, or 2-ethylhexyl. An alkyl group can be substituted (i.e., optionally substituted) with one or more substituents such as halo; cycloaliphatic [e.g., cycloalkyl or cycloalkenyl]; heterocycloaliphatic [e.g., heterocycloalkyl or heterocycloalkenyl]; aryl; heteroaryl; alkoxy; aroyl; heteroaroyl; acyl [e.g., (aliphatic)carbonyl, (cycloaliphatic)carbonyl, or (heterocycloaliphatic)carbonyl]; nitro; cyano; amido [e.g., (cycloalkylalkyl)carbonylamino, arylcarbonylamino, aralkylcarbonylamino, (heterocycloalkyl)carbonylamino, (heterocycloalkylalkyl)carbonylamino, heteroarylcarbonylamino, heteroaralkylcarbonylamino, alkylaminocarbonyl, cycloalkylaminocarbonyl, heterocycloalkylaminocarbonyl, arylaminocarbonyl, or heteroarylaminocarbonyl]; amino [e.g., aliphaticamino, cycloaliphaticamino, or heterocycloaliphaticamino]; sulfonyl [e.g., aliphatic-S(O)₂-]; sulfinyl; sulfanyl; sulfoxy; urea; thiourea; sulfamoyl; sulfamide; oxo; carboxy; carbamoyl; cycloaliphaticoxy; heterocycloaliphaticoxy; aryloxy; heteroaryloxy; aralkyloxy; heteroarylalkoxy; alkoxycarbonyl; alkylcarbonyloxy; or hydroxy. Without limitation, some examples of substituted alkyls include carboxyalkyl (such as HOOC-alkyl, alkoxycarbonylalkyl, and alkylcarbonyloxyalkyl); cyanoalkyl; hydroxyalkyl; alkoxyalkyl; acylalkyl; aralkyl; (alkoxyaryl)alkyl; (sulfonylamino)alkyl (such as alkyl-S(O)₂-aminoalkyl); aminoalkyl; amidoalkyl; (cycloaliphatic)alkyl; or haloalkyl.

[34] “alkylene” refers to a bifunctional alkyl group.

[35] A “bifunctional” moiety refers to a chemical group that is attached to the main chemical structure in two places, such as a linker moiety. Bifunctional moieties can be attached to the main chemical structure at any two chemically feasible substitutable points. Unless otherwise specified, bifunctional moieties can be in either direction, e.g. the bifunctional moiety “N-O” can be attached in the –N-O- direction or the –O-N- direction.

[36] “Chemically distinct region” refers to a region of an antisense compound that is in some way chemically different than another region of the same antisense compound. For example, a region having 2'-O-methoxyethyl nucleotides is chemically distinct from a region having nucleotides without 2'-O-methoxyethyl modifications.

[37] “Chimeric antisense compound” means an antisense compound that has at least two chemically distinct regions.

[38] “Complementarity” means the capacity for pairing between nucleobases of a first nucleic acid and a second nucleic acid.

[39] “Contiguous nucleobases” means nucleobases immediately adjacent to each other.

[40] “Diluent” means an ingredient in a composition that lacks pharmacological activity, but is pharmaceutically necessary or desirable. For example, the diluent in an injected composition may be a liquid, e.g. saline solution.

[41] “Dose” means a specified quantity of a pharmaceutical agent provided in a single administration, or in a specified time-period. In certain embodiments, a dose may be administered in one, two, or more boluses, tablets, or injections. For example, in certain embodiments where subcutaneous administration is desired, the desired dose requires a volume not easily accommodated by a single injection, therefore, two or more injections may be used to achieve the desired dose. In certain embodiments, the pharmaceutical agent is administered by infusion over an extended period-of-time or continuously. Doses may be stated as the amount of pharmaceutical agent per hour, day, week, or month.

[42] “Chemically Modified” when referring to RNA or DNA bases, has its meaning understood in the art and includes a nucleoside base selected from the group consisting of 2'-substituted nucleoside, '-O-methoxyethyl" (also 2'-MOE and 2'- O(CH₂)₂—OCH₃), 2'-deoxynucleoside, 2'-O-methoxyethyl nucleotide, 5-methyl cytosine, monocyclic nucleosides, Bicyclic nucleoside, 4'-2' bicyclic nucleoside, 4' to 2' bicyclic nucleoside, locked nucleic acid, and Nucleoside mimetic, all as defined herein.

[43] “Fully complementary” or “100% complementary” means each nucleobase of a first nucleic acid has a complementary nucleobase in a second nucleic acid. “Substantially Complementary” in reference to a Gapmer that is “substantially complementary” to the defined region on its target (SEQ ID NO. 1) means that no more than two nucleobases are not capable of hydrogen bonding with one another when the nucleobase sequence of the oligonucleotide and the other nucleic acid are aligned in opposing directions. Complementary nucleobases means nucleobases that are capable of forming hydrogen bonds with one another. For example, for a 10-mer oligonucleotide, a sequence which is substantially complementary can have 8, 9, or 10 bases which are complementary to a second nucleic acid. “Substantially complementary” includes “fully complementary” sequences where there are no bases which are mismatched within the oligonucleotide sequence to a second nucleic acid.

[44] Complementary nucleobase pairs include adenine (A) and thymine (T), adenine (A) and uracil (U), cytosine (C) and guanine (G), 5-methyl cytosine (mC) and guanine (G). Complementary oligonucleotides and/or nucleic acids need not have nucleobase complementarity at each nucleoside. Rather, some mismatches are tolerated. “Fully complementary” or “100%

complementary” in reference to oligonucleotides means that oligonucleotides are complementary to another oligonucleotide or nucleic acid at each nucleoside of the oligonucleotide.

[45] “Contiguous” in the context of an oligonucleotide refers to nucleosides, nucleobases, sugar moieties, or internucleoside linkages that are immediately adjacent to each other. For example, “contiguous nucleobases” means nucleobases that are immediately adjacent to each other in a sequence.

[46] “Gapmer” means a modified oligonucleotide comprising an internal “gap” region having a plurality of DNA nucleosides positioned between external regions having one or more nucleosides, wherein the nucleosides comprising the internal region are chemically distinct from the nucleoside or nucleosides comprising the external regions. The internal region is often referred to as the “gap” and the external regions are often referred to as the “wings.” Unless otherwise indicated, “gapmer” refers to a sugar motif. Unless otherwise indicated, the sugar moieties of the nucleosides of the gap central region of a gapmer are unmodified 2'-deoxyribosyl. Thus, the term “MOE gapmer” indicates a gapmer having a sugar motif of 2'-MOE nucleosides in both wings and a gap of 2'-deoxynucleosides. Unless otherwise indicated, a MOE gapmer may comprise one or more modified internucleoside linkages and/or modified nucleobases and such modifications do not necessarily follow the gapmer pattern of the sugar modifications.

[47] “Hybridization” means the pairing or annealing of complementary oligonucleotides and/or nucleic acids. While not limited to a particular mechanism, the most common mechanism of hybridization involves hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases.

[48] “Immediately adjacent” means there are no intervening elements between the immediately adjacent elements.

[49] “Modified nucleotide base” and “modified nucleoside” is a deoxyribose nucleotide or ribose nucleotide that is modified to have one or more chemical moieties not found in natural nucleic acids. Examples of modified nucleotide bases, and “modified nucleosides” are compounds of Formula Ia, Formula Ib, Formula IIa, or Formula IIb as described herein.

[50] A “Non-bicyclic modified sugar moiety” is a sugar moiety of a modified nucleotide base, wherein the chemical modifications do not involve the transformation of the sugar moiety into a bicyclic or multicyclic ring system.

[51] “Monocyclic nucleosides” are nucleosides comprising modified sugar moieties that are not bicyclic sugar moieties. In certain embodiments, the sugar moiety, or sugar moiety analogue, of a nucleoside may be modified or substituted at any position.

[52] “2'-modified sugar” means a furanosyl sugar modified at the 2' position. Such modifications include substituents as described herein.

[53] “Bicyclic nucleoside” (BNA) are modified nucleoside comprising a bicyclic sugar moiety. Examples of bicyclic nucleosides include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. The synthesis of bicyclic nucleosides have been disclosed in, for example, 7,399,845, WO/2009/006478, WO/2008/150729, US2004-0171570, U.S. Pat. 7,427,672, Chattopadhyaya et al. J. Org. Chem. 2009, 74, 118-134, WO 99/14226, and WO 2008/154401. The synthesis and preparation of the methyleneoxy (4'-CH₂—O-2') BNA monomers adenine, cytosine, guanine, 5-methyl-cytosine, thymine and uracil, along with their oligomerization, and nucleic acid recognition properties have been described (Koshkin et al., Tetrahedron, 1998, 54, 3607-3630). BNAs and their preparation are also described in WO98/39352 and WO 99/14226. Analogs of methyleneoxy (4'-CH₂—O-2') BNA and 2'-thio-BNAs, have also been prepared (Kumar et al., Bioorg. Med. Chem. Lett., 1998, 8, 2219-2222). Preparation of locked nucleoside analogs comprising oligodeoxyribonucleotide duplexes as substrates for nucleic acid polymerases has also been described (WO 99/14226). Furthermore, synthesis of 2'-amino-BNA, a novel conformationally restricted high-affinity oligonucleotide analog has been described in the art (Singh et al., J. Org. Chem., 1998, 63, 10035-10039). In addition, 2'-amino- and 2'-methyldamino-BNA's have been prepared and the thermal stability of their duplexes with complementary RNA and DNA strands has been previously reported. One carbocyclic bicyclic nucleoside having a 4'-(CH₂)₃-2' bridge and the alkenyl analog bridge 4'-CH=CH—CH₂-2' have been described (Freier et al., Nucleic Acids Research, 1997, 25(22), 4429-4443 and Albaek et al., J. Org. Chem., 2006, 71, 7731-7740). The synthesis and preparation of carbocyclic bicyclic nucleosides along with their oligomerization and biochemical studies have also been described (Srivastava et al., J. Am. Chem. Soc., 2007, 129(26), 8362- 8379).

[54] A “4'-2' bicyclic nucleoside” or “4' to 2' bicyclic nucleoside” is a bicyclic nucleoside comprising a furanose ring comprising a bridge connecting two carbon atoms of the furanose ring connects the 2' carbon atom and the 4' carbon atom of the sugar ring.

[55] A “locked nucleic acid” (LNA) is a modified nucleotide base, wherein the chemical modifications are transformation of the sugar moiety into a bicyclic or multicyclic ring system. Two specific examples of locked nucleic acid compounds are β-D-methyleneoxy nucleotides, or “constrained methyl” (cMe) nucleotides; and β-D-ethyleneoxy nucleotides, or “constrained ethyl” (cEt) nucleotides.

[56] “Mismatch” or “non-complementary” means a nucleobase of a first oligonucleotide that is not complementary with the corresponding nucleobase of a second oligonucleotide or target nucleic acid when the first and second oligonucleotide are aligned.

[57] “Motif” means the pattern of unmodified and/or modified sugar moieties, nucleobases, and/or internucleoside linkages, in an oligonucleotide.

[58] “Nucleobase” means an unmodified nucleobase or a modified nucleobase. An “unmodified nucleobase” is adenine (A), thymine (T), cytosine (C), uracil (U), and guanine (G). A “modified nucleobase” is a group of atoms other than unmodified A, T, C, U, or G capable of pairing with at least one unmodified nucleobase. A “5-methyl cytosine” is a modified nucleobase. A universal base is a modified nucleobase that can pair with any one of the five unmodified nucleobases. “Nucleobase sequence” means the order of contiguous nucleobases in a nucleic acid or oligonucleotide independent of any sugar or internucleoside linkage modification.

[59] “Nucleoside” means a compound comprising a nucleobase and a sugar moiety. The nucleobase and sugar moiety are each, independently, unmodified or modified. “Modified nucleoside” means a nucleoside comprising a modified nucleobase and/or a modified sugar moiety. Modified nucleosides include abasic nucleosides, which lack a nucleobase. “Linked nucleosides” are nucleosides that are connected in a contiguous sequence (i.e., no additional nucleosides are presented between those that are linked).

[60] “Nucleoside mimetic” includes those structures used to replace the sugar or the sugar and the base and not necessarily the linkage at one or more positions of an oligomeric compound such as for example nucleoside mimetics having morpholino, cyclohexenyl, cyclohexyl, tetrahydropyranyl, bicyclo or tricyclo sugar mimetics, e.g., non-furanose sugar units. Nucleotide mimetic includes those structures used to replace the nucleoside and the linkage at one or more positions of an oligomeric compound such as for example peptide nucleic acids or morpholinos (morpholinos linked by —N(H)—C(=O)—O— or other non-phosphodiester linkage). Sugar surrogate overlaps with the slightly broader term nucleoside mimetic but is intended to indicate replacement of the sugar unit (furanose ring) only. The tetrahydropyranyl rings provided herein are illustrative of an example of a sugar surrogate wherein the furanose sugar group has been replaced with a tetrahydropyranyl ring system.

[61] “Parenteral administration” means administration through injection (e.g., bolus injection) or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g., intrathecal or intracerebroventricular administration.

[62] “Pharmaceutically acceptable carriers” means physiologically and pharmaceutically acceptable carriers of compounds. Pharmaceutically acceptable carriers retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

[63] “Phosphorothioate linkage” means a linkage between nucleosides where the phosphodiester bond is modified by replacing one of the non-bridging oxygen atoms with a sulfur atom.

[64] “Reducing or inhibiting the amount or activity” refers to a reduction or blockade of the transcriptional expression or activity relative to the transcriptional expression or activity in an untreated or control sample and does not necessarily indicate a total elimination of transcriptional expression or activity.

[65] “Self-complementary” in reference to an oligonucleotide means an oligonucleotide that at least partially hybridizes to itself.

[66] “Side effects” means physiological responses attributable to a treatment other than the desired effects. Side effects include injection site reactions, liver function test abnormalities, renal function abnormalities, liver toxicity, renal toxicity, central nervous system abnormalities, myopathies, and malaise. For example, increased aminotransferase levels in serum may indicate liver toxicity or liver function abnormality. For example, increased bilirubin may indicate liver toxicity or liver function abnormality.

[67] “Single-stranded oligonucleotide” means an oligonucleotide which is not hybridized to a complementary strand.

[68] “Specifically hybridizable” means an antisense compound having a sufficient degree of complementarity between an antisense oligonucleotide and a target nucleic acid to induce a desired effect, while exhibiting minimal or no effects on non-target nucleic acids under conditions in which specific binding is desired, i.e., under physiological conditions in the case of in vivo assays and therapeutic treatments.

[69] “Sugar moiety” means an unmodified sugar moiety or a modified sugar moiety. As used herein, “unmodified sugar moiety” means a 2'-OH(H) ribosyl moiety, as found in RNA (an “unmodified RNA sugar moiety”), or a 2-H(H) deoxyribosyl moiety, as found in DNA (an “unmodified DNA sugar moiety”). Unmodified sugar moieties have one hydrogen at each of the 1', 3', and 4' positions, an oxygen at the 3' position, and two hydrogens at the 5' position. As used herein, “modified sugar moiety” or “modified sugar” means a modified furanosyl sugar moiety or a sugar surrogate.

[70] “Sugar surrogate” means a modified sugar moiety having other than a furanosyl moiety that can link a nucleobase to another group, such as an internucleoside linkage, conjugate group, or terminal group in an oligonucleotide. Modified nucleosides comprising sugar surrogates can be incorporated into one or more positions within an oligonucleotide and such oligonucleotides are capable of hybridizing to complementary oligomeric compounds or target nucleic acids.

[71] “Targeting” or “targeted” means the process of design and selection of an antisense compound that will specifically hybridize to a target nucleic acid and induce a desired effect.

[72] “Target segment” means the sequence of nucleotides of a target nucleic acid to which an antisense compound is targeted. “5' target site” refers to the 5'-most nucleotide of a target segment. “3' target site” refers to the 3'-most nucleotide of a target segment.

[73] “Target nucleic acid” and “target RNA” mean a nucleic acid that an antisense compound is designed to affect, such as AMANZI.

[74] “Target region” means a portion of a target nucleic acid to which an oligomeric compound is designed to hybridize.

[75] “Unmodified nucleotide” means a nucleotide composed of naturally occurring nucleobases, sugar moieties, and internucleoside linkages. An unmodified nucleotide is an RNA nucleotide (i.e. β -D-ribonucleosides) or a DNA nucleotide (i.e. β -D-deoxyribonucleoside).

Antisense ASO Compounds

[76] The present disclosure provides a gapmer antisense oligonucleotide (ASO) compound (“gapmer compound”) that is complementary (for example, from about 91% complementary to about 100% complementary, including 100% complementary over the entire length of the gapmer compound) to a region of AMANZI long non-coding RNA, and that inhibits multiple acute inflammatory gene transcription regulated by the AMANZI long non-coding RNA. In various embodiments of the present disclosure, a gapmer compound comprises a modified oligonucleotide of 12 to 29 linked nucleosides in length. The gapmer compound is substantially complementary (for example, having no more than one nucleotide mismatch over the entire length of the gapmer compound) to a region of AMANZI (SEQ ID NO. 1), and inhibits multiple acute inflammatory gene transcription from being regulated by the AMANZI long non-coding RNA. The gapmer compound comprises: (a) a 5' wing sequence having from about three to about seven wing modified nucleotide bases; (b) a central gap region sequence having from about six to about fifteen 2' deoxynucleotides; and (c) a 3' wing sequence having from about three to about seven wing modified nucleotide bases; wherein the gapmer compound nucleotides are each linked with

phosphorothioate internucleoside linkages, phosphorothiolate internucleoside linkages, or combinations thereof over the entire length of the gapmer compound; and wherein the modified nucleotide base modifications are selected from the group consisting of a 2'-methoxyethyl (MOE) modification, a locked nucleic acid (LNA) modification (for example, a modified sugar containing a cMe or cEt), a 2'-F-ANA modification, a 2'-O-methoxyethyl (2'OMe) modification, or combinations thereof.

[77] The chemical modification of antisense oligonucleotides may enhance their resistance to nucleases and may enhance their ability to enter cells. For example, phosphorothioate oligonucleotides may be used. Other deoxynucleotide analogs include methylphosphonates, phosphoramidates, phosphorodithioates, N3'P5'-phosphoramidates and oligoribonucleotide phosphorothioates and their 2'-O-alkyl analogs and 2'-O-methylribonucleotide methylphosphonates. Alternatively mixed backbone oligonucleotides ("MBOs") may be used. MBOs contain segments of phosphothioate oligodeoxynucleotides and appropriately placed segments of modified oligodeoxy- or oligoribonucleotides. MBOs have segments of phosphorothioate linkages and other segments of other modified oligonucleotides, such as methylphosphonate, which is non-ionic, and very resistant to nucleases or 2'-O-alkyloligoribonucleotides.

[78] In some embodiments, an oligonucleotide sugar moiety is a modified sugar moiety. In some embodiments, the modified sugar moiety can be a sugar moiety which is a conformationally-strained sugar. In some embodiments, the conformationally-strained sugar can be a locked nucleotide (locked nucleic acid, or LNA). In some embodiments, the locked nucleotide can be selected from one of the following types: 2'-O—CH₂-4' (oxy-LNA), 2'-CH₂—CH₂-4' (methylene-LNA), 2'-NH—CH₂-4' (amino-LNA), 2'-N(CH₃)—CH₂-4' (methylamino-LNA), 2'-S—CH₂-4' (thio-LNA), and 2'-Se—CH₂-4' (seleno-LNA). In some embodiments, the conformationally-strained sugar can be a bridged nucleic acid (BNA). Some conformationally-strained sugar can be a locked nucleic acid are shown in Formula III and Formula IV in U.S. Patent NO. 10,465,188, herein incorporated by reference.

[79] Synthesis of antisense oligonucleotides can be performed. See e.g. Stein C.A. and Krieg A.M. (eds), *Applied Antisense Oligonucleotide Technology*, 1998 (Wiley-Liss).

Antisense Compound Motifs

[80] In a gapmer an internal region having a plurality of nucleotides or linked nucleosides is positioned between external regions having a plurality of nucleotides or linked nucleosides that are chemically distinct from the nucleotides or linked nucleosides of the internal

region. In the case of an antisense oligonucleotide having a gapmer motif, the gap segment generally serves as the substrate for endonuclease cleavage, while the wing segments comprise modified nucleosides. The regions of a gapmer (5' wing, gap sequence, and 3' wing) are differentiated by the types of sugar moieties comprising each distinct region. The types of sugar moieties that are used to differentiate the regions of a gapmer may include β -D-ribonucleosides, β -D-deoxyribonucleosides, 2'-modified nucleosides (such 2'-modified nucleosides may include 2'-MOE, and 2'-O—CH₃, among others), and bicyclic sugar modified nucleosides (such bicyclic sugar modified nucleosides may include those having a 4'-(CH₂)_n-O-2' bridge, where n=1 or n=2). Preferably, each distinct region comprises uniform sugar moieties. The wing-gap-wing motif is frequently described as "X—Y—Z", where "X" represents the length of the 5' wing region, "Y" represents the length of the gap region, and "Z" represents the length of the 3' wing region. In general, a gapmer described as "X—Y—Z" has a configuration such that the gap segment is positioned immediately adjacent each of the 5' wing segment and the 3' wing segment. Thus, no intervening nucleotides exist between the 5' wing segment and gap segment, or the gap segment and the 3' wing segment. Often, X and Z are the same chemistry of modified nucleobase, or they are different. Preferably, Y is between 8 and 15 nucleotides. X or Z can be any of 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides. Thus, gapmers include, but are not limited to, forexample 5-10-5, 4-8-4, 4-12-3, 4-12-4, 3-14-3, 2-13-5, 2-16-2, 1-18-1, 3-10-3, 2-10-2, 1-10-1, 2-8-2, 6-8-6 or 5-8-5.

[81] In a preferred embodiment, a gapmer has a gap segment of ten 2'-deoxyribonucleotides positioned immediately adjacent to and between wing segments of five chemically modified nucleosides. In certain embodiments, the chemical modification in the wings comprises a 2'- sugar modification. In another embodiment, the chemical modification comprises a 2'-MOE sugar modification. Preferably, a gap-widened antisense oligonucleotide has a gap segment of eight 2'-deoxyribonucleotides positioned immediately adjacent to and between wing segments of five chemically modified nucleosides. Or the chemical modification comprises a 2'-sugarmodification. Or the chemical modification comprises a 2'-MOE sugar modification.

[82] A gapmer has a gap segment of eight 2'-deoxyribonucleotides positioned immediately adjacent to and between wing segments of five to six chemically modified nucleosides. The chemical modification comprises a 2'-sugar modification, such as a 2'-MOE sugar modification.

Hybridization

[83] Hybridization occurs between a gapmer compound and a target AMANZI nucleic acid (SEQ ID NO. 1). The most common mechanism of hybridization involves hydrogen bonding

(e.g., Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding) between complementary nucleobases of the nucleic acid molecules. Hybridization can occur under varying conditions. Stringent conditions are sequence-dependent and are determined by the nature and composition of the nucleic acid molecules to be hybridized.

Modified Sugar Moieties

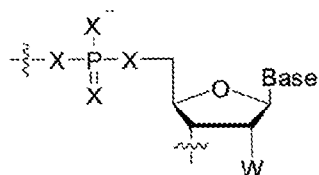
[84] Antisense compounds can optionally contain one or more nucleosides wherein the sugar group has been modified. Such sugar modified nucleosides may impart enhanced nuclease stability, increased binding affinity, or some other beneficial biological property to the antisense compounds. Nucleosides comprise chemically modified ribofuranose ring moieties. Examples of chemically modified ribofuranose rings include, without limitation, addition of substituent groups (including 5' and 2' substituent groups, bridging of non-geminal ring atoms to form bicyclic nucleic acids (BNA), replacement of the ribosyl ring oxygen atom with S, N(R), or C(R₁)(R₂) (R, R₁ and R₂ are each independently H, C₁-C₁₂ alkyl or a protecting group) and combinations thereof. Examples of chemically modified sugars include 2'-F-5'-methyl substituted nucleoside (WO2008/101157 for other disclosed 5',2'-bis substituted nucleosides) or replacement of the ribosyl ring oxygen atom with S with further substitution at the 2'-position (U.S. Patent Application 2005/0130923) or alternatively 5'-substitution of a BNA (WO2007/134181 wherein LNA is substituted with for example a 5'-methyl or a 5'-vinyl group).

[85] Antisense oligonucleotides may be part of compositions which may comprise oligonucleotides to more than one region of AMANZI (SEQ ID NO: 1). Preferably, one AMANZI transcriptional inhibitor gapmer compound is an oligonucleotide having the sequence of all or 1 or 2 base mismatches of that of the sequence of SEQ ID NO: 41-46, 63-64, 66, 67, or 101-104.

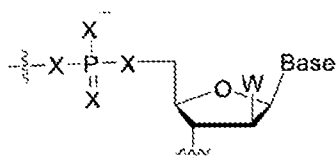
[86] When specific proteins are referred to herein, derivatives, variants, and fragments are contemplated and included. Protein derivatives and variants are well understood to those of skill in the art and can involve insertional, substitutional or deletional amino acid sequence variants known in the art.

Modified Nucleotide Bases

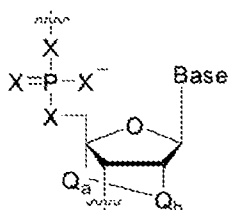
[87] Modified nucleotide bases include Formula Ia Formula Ib, Formula IIa, or Formula IIb:



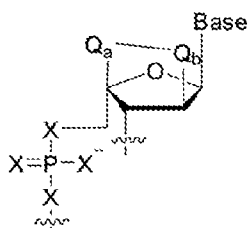
Formula Ia



Formula Ib



Formula IIa



Formula IIb

[88] wherein

each X is independently O or S, wherein 0, 1, or 2 instances of X is S;

each W is independently H, OH, halo, or -O-C₁₋₆ alkyl, wherein the alkyl is optionally substituted with up to three instances of C₁₋₄ alkyl, C₁₋₄ alkoxy, halo, amino, CN, NO₂, or OH; each Q_a is independently a bifunctional C₁₋₆ alkylene, optionally substituted with up to two instances of C₁₋₄ alkyl, C₁₋₄ alkoxy, halo, or OH; and each Q_b is independently a bond or a bifunctional moiety selected from -O-, -S-, -N-O-, -N(R)-, -C(O)-, -C(O)O-, and -C(O)N(R)-, wherein R is an unsubstituted C₁₋₄ alkyl.

[89] In one embodiment, each X is O. In another embodiment, one instance of X is S.

[90] In one embodiment, the gapmer comprises one or more nucleotides of Formula Ia or Formula Ib, wherein W is halo. In a further embodiment, W is fluoro. In another further

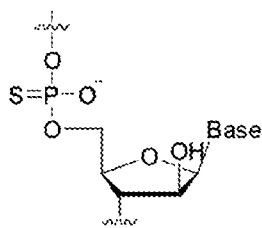
embodiment, the gapmer comprises one or more nucleotides of Formula Ia. In another further embodiment, the gapmer comprises one or more nucleotides of Formula Ib.

[91] In one embodiment, the gapmer comprises one or more nucleotides of Formula Ia or Formula Ib, wherein W is -O-C₁₋₆ alkyl, wherein the alkyl is optionally substituted with up to three instances of C₁₋₄ alkyl, C₁₋₄ alkoxy, halo, amino, or OH. In a further embodiment, W is -O-C₁₋₆ alkyl, wherein the alkyl is optionally substituted with C₁₋₄ alkoxy. In a further embodiment, W is an unsubstituted -O-C₁₋₆ alkyl. In another further embodiment, W is -O-C₁₋₆ alkyl, wherein the alkyl is substituted with C₁₋₄ alkoxy. In a further embodiment, W is selected from methoxy and -O-CH₂CH₂-OCH₃. In one embodiment, the gapmer comprises one or more nucleotides of Formula Ia. In another embodiment, the gapmer comprises one or more nucleotides of Formula Ib.

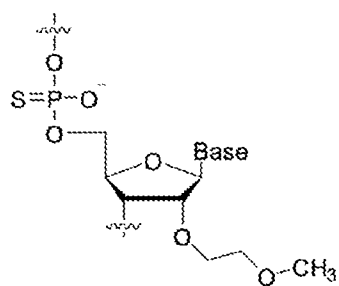
[92] In one embodiment, the gapmer comprises one or more β-D nucleotides of Formula IIa or α-L nucleotides of Formula IIb, wherein Q_a is an unsubstituted bifunctional C₁₋₆ alkylene, and Q_b is a bond or a bifunctional moiety selected from -O-, -S-, -N-O-, and -N(R)-. In a further embodiment, Q_a is selected from -CH₂-, -CH₂-CH₂-, -CH(CH₃)-, -CH₂-CH₂(CH₃)-, and Q_b is a bond or a bifunctional moiety selected from -O-, -S-, -N(R)-O-, and -N(R)-, wherein R is H or C₁₋₆ alkyl.

[93] In one embodiment of Formula IIa or Formula IIb, Q_a is -CH₂- and Q_b is -O-. In another embodiment of Formula IIa or Formula IIb, Q_a is -CH₂-CH₂- and Q_b is -O-. In another embodiment of Formula IIa or Formula IIb, Q_a is -CH₂- and Q_b is -N(R)-O-, wherein R is H or C₁₋₆ alkyl. In another embodiment of Formula IIa or Formula IIb, Q_a is -CH(CH₃)- and Q_b is -O-. In another embodiment of Formula IIa or Formula IIb, Q_a is -CH₂- and Q_b is -S-. In another embodiment of Formula IIa or Formula IIb, Q_a is -CH₂- and Q_b is -N(R)-, wherein R is H or C₁₋₆ alkyl. In another embodiment of Formula IIa or Formula IIb, Q_a is -CH₂-CH(CH₃)- and Q_b is a bond.

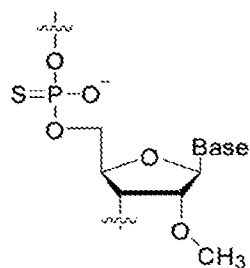
[94] In some embodiments, the gapmer comprises one or more nucleotides selected from the following modified nucleotides:



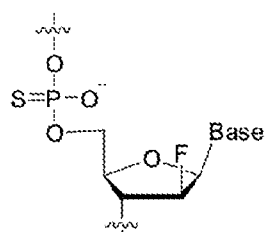
Arabinonucleic acid (ANA)



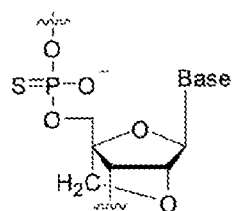
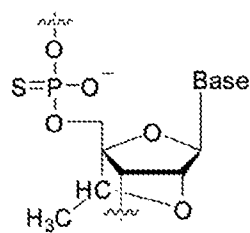
2'-methoxyethyl (MOE)



2'-methoxy (2'-OMe)



2'-fluoroarabinonucleic acid (2'F-ANA)

 β -D-methyleneoxy (cMe) β -D-ethyleneoxy (cEt)

[95] Many other bicyclo and tricyclo sugar surrogate ring systems can be used to modify nucleosides for incorporation into antisense compounds (see, for example, review article: Leumann, *Bioorg. Med. Chem.*, 2002, 10, 841-854).

AMANZI Transcription Modulators

[96] Any transcription regulator that is capable of eliciting a desired inhibition of the protein expression (e.g., transcription) of the AMANZI gene region, and as well as AMANZI transcriptional inhibitor gapmer compounds of this disclosure, may be used in embodiments of the invention as described herein. Any AMANZI transcriptional inhibitor gapmer compound that modulates the AMANZI gene region are also provided in particular embodiments (e.g., those that modulate, block or lessen the transcription of AMANZI).

Administration

[97] The gapmers described herein may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. The compounds and compositions described herein can be delivered in a manner to target a particular tissue, such as the bone marrow or brain. The compounds and compositions described herein are administered parenterally. "Parenteral administration" means administration through injection or infusion. Parenteral administration includes subcutaneous administration, intravenous administration, intramuscular administration, intraarterial administration, intraperitoneal administration, or intracranial administration, e.g. intracerebral administration, intrathecal administration, intraventricular administration, ventricular administration, intracerebroventricular administration, cerebral intraventricular administration or cerebral ventricular administration. Administration can be continuous, or chronic, or short or intermittent.

[98] Parenteral administration is also by infusion. Infusion can be chronic or continuous or short or intermittent, with a pump or by injection. In some embodiments, parenteral administration is subcutaneous.

Formulations

[99] The pharmaceutical compositions of this invention include, for example, compositions comprising an AMANZI transcriptional inhibitor gapmer compound. The

pharmaceutical formulations of this invention may further comprise one or more pharmaceutically acceptable excipients.

[100] The pharmaceutical formulations of this invention may further comprise one or more pharmaceutically acceptable excipients.

[101] The AMANZI transcriptional inhibitor gapmer compounds may be present in the formulation in a substantially isolated form. It will be understood that the product may be mixed with carriers or diluents that will not interfere with the intended purpose of the product and still be regarded as substantially isolated. A product of the invention may also be in a substantially purified form, in which case it will generally comprise about 80%, 85%, or 90%, e.g. at least about 88%, at least about 90, 95 or 98%, or at least about 99% of a oligonucleotide, or dry mass of the preparation.

[102] Pharmaceutically acceptable diluents, carriers and/or excipients include those suitable for veterinary use as well as human pharmaceutical use. By way of example, diluents, carriers and/or excipients include solutions, solvents, dispersion media, delay agents, polymeric and lipidic agents, emulsions and the like. By way of further example, suitable liquid carriers, especially for injectable solutions, include water, aqueous saline solution, aqueous dextrose solution, and the like, and vehicles such as liposomes being also especially suitable for administration of agents.

[103] Suitable carriers and diluents include buffered, aqueous solutions, saline, dextrose, glycerol, isotonic saline solutions, for example phosphate-buffered saline, isotonic water, and the like and combinations thereof. In some embodiments, carriers may include propylene glycol, dimethyl isosorbide, and water, and even more particularly, phosphate buffered saline, isotonic water, deionized water, monofunctional alcohols and symmetrical alcohols. In some embodiments pharmaceutically acceptable carrier or diluent may be or contain a thermosetting poloxamer (which may be a liquid or gel, depending on the temperature), a carboxycellulose (*e.g.* carboxymethylcellulose), a collagen (*e.g.*, a Type I collagen), a collagenous material comprising tropocollagen, a hyaluronan or derived-hyaluronic acid, and/or an oil (*e.g.*, Emu oil). Suitable carriers can be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, and amino acid copolymers.

[104] Compositions may take the form of any standard known dosage form including tablets, pills, capsules, semisolids, powders, sustained release formulation, solutions, suspensions, elixirs, aerosols, liquids for injection, gels, creams, transdermal delivery devices (for example, a transdermal patch), inserts such as ocular inserts, or any other appropriate compositions.

[105] Preferably the AMANZI transcriptional inhibitor gapmer compound is combined with a pharmaceutically acceptable carrier or diluent to produce a pharmaceutical composition.

[106] Pharmaceutically acceptable salts can also be present, *e.g.*, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as citrates, acetates, propionates, malonates, benzoates, and the like.

[107] In addition, if desired substances such as wetting or emulsifying agents, stabilizing or pH buffering agents, or preservatives may also be present. In some embodiments, the pharmaceutical compositions of this invention will comprise suitable ophthalmically acceptable buffers, such as acetate buffers, citrate buffers, phosphate buffers, borate buffers and mixtures thereof. In some embodiments, the buffers useful in the present invention include boric acid, sodium borate, sodium phosphates, including mono, di- and tri-basic phosphates, such as sodium phosphate monobasic monohydrate and sodium phosphate dibasic heptahydrate, and mixtures thereof. In some embodiments, the preservative may be stabilized chlorine dioxide, cationic polymers or quaternary ammonium compounds. In some embodiments the pharmaceutical compositions may also comprise wetting agents, nutrients, viscosity builders, antioxidants, and the like, for example, disodium ethylene diamine tetraacetate, alkali metal hexametaphosphate, citric acid, sodium citrate, sodium metabisulfite, sodium thiosulfate, N-acetylcysteine, butylated hydroxyanisole, butylated hydroxytoluene, polyvinyl alcohol, polyoxamers, polyvinyl pyrrolidone, hydroxypropyl methyl cellulose, hydroxyethylmethyl cellulose, and mixtures thereof and mixtures thereof. In some embodiments, the pharmaceutical formulations of this invention will not include a preservative. In some embodiments, the AMANZI transcriptional inhibitor gapmer composition or formulation comprises sodium phosphate dibasic heptahydrate or potassium phosphate, monobasic or both.

[108] Uptake of nucleic acids by mammalian cells may be enhanced by the use of known transfection techniques including the use of transfection agents. Such techniques may be used with certain AMANZI transcriptional inhibitor gapmer compounds. Examples of useful transfection agents include cationic agents (for example calcium phosphate and DEAE-dextran) and lipofectants (for example lipofectamTM and transfectamTM), and surfactants.

[109] The compositions may be formulated in accordance with standard techniques known in the art, including those as may be found in such standard references as Gennaro AR: Remington: The Science and Practice of Pharmacy, 20th ed., Lippincott, Williams & Wilkins, 2000, for example.

[110] Any container suitable for storing and/or administering a pharmaceutical composition may be used in a combination product of the invention. Suitable containers will be

appreciated by persons skilled in the art. By way of example, such containers include vials and syringes. The containers may be suitably sterilized and hermetically sealed.

[111] Such compositions comprise a pharmaceutically acceptable solvent, such as water or saline, diluent, carrier, or adjuvant. The pharmaceutical compositions may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial (intrathecal or intraventricular, administration).

[112] The compounds may also be admixed, conjugated or otherwise associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor- targeted molecules, or other formulations, for assisting in uptake, distribution and/or absorption.

[113] The term “pharmaceutically acceptable carriers” refers to physiologically and pharmaceutically acceptable carriers of the compounds i.e., carriers that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto. For oligonucleotides, preferred examples of pharmaceutically acceptable carriers and their uses are further described in U.S. Patent 6,287,860, which is incorporated by reference herein. Sodium carriers have been shown to be suitable forms of oligonucleotide drugs.

[114] Formulations include liposomal formulations. The term “liposome” means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

[115] Liposomes also include “sterically stabilized” liposomes which refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Liposomes and their uses are further described in U.S. Patent 6,287,860, which is incorporated by reference herein.

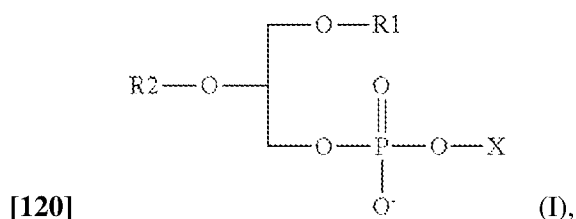
[116] Preferred formulations for topical administration include those in which the oligonucleotides are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearoylphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA).

Lipid Nanoparticles

[117] LNPs are multi-component systems that typically consist of an ionizable amino lipid, a phospholipid, cholesterol, and a polyethylene glycol (PEG)-lipid, with all of the components contributing to efficient delivery of the nucleic acid drug cargo and stability of the particle (Schroeder et al., *J. Intern. Med.* 2010;267:9–21). The cationic lipid electrostatically condenses the negatively charged RNA into nanoparticles and the use of ionizable lipids that are positively charged at acidic pH is thought to enhance endosomal escape. Formulations for delivery, both clinically and non-clinically, are predominantly based on cationic lipids such as DLin-MC3- DMA (MC3). (Kanasty et al. *Nat. Mater.* 2013;12:967–977; and Xue et al. *Curr. Pharm. Des.* 2015;21:3140–3147).

[118] Further LNP's include a nanoemulsion having a perfluorocarbon component (a) consisting of at least one least one perfluorocarbon compound, an emulsifying component (b) such as phospholipids and optionally helper lipids, and an endocytosis enhancing component (c) that comprises at least one compound inducing cellular uptake of the nanoemulsion. A perfluorocarbon compound of component (a) is preferably selected from compounds having the structure $C_mF_{2m+1}X$, $XC_mF_{2m}X$, $XC_nF_{2n}OC_oF_{2o}X$, $N(C_oF_{2o}X)_3$ and $N(C_oF_{2o}+1)_3$, wherein m is an integer from 3 to 10, n and o are integers from 1 to 5, and X is independently from further occurrence selected from Cl, Br and I. Examples of perfluorocarbon compounds are perfluorooctyl bromide and perfluorotributylamine.

[119] Examples of the emulsifying agents include phospholipids, such as the phospholipid compound represented by the formula I:



[121] wherein, R^1 and R^2 are independently selected from H and C_{16-24} acyl residues, which may be saturated or unsaturated and may carry 1 to 3 residues R^3 and wherein one or more of the C-atoms may be substituted by O or NR^4 , and X is selected from H, $-(CH_2)_p-N(R^4)_3^+$, $-(CH_2)_p-CH(N(R^4)_3^+)-COO^-$, $-(CH_2)_p-CH(OH)-CH_2OH$ and $-CH_2(CHOH)_p-CH_2OH$ (wherein p is an integer from 1 to 5); R^3 is independently selected from H, lower alkyl, F, Cl, CN und OH; and R^4 is independently selected from H, CH_3 und CH_2CH_3 , or a pharmacologically acceptable carrier thereof.

[122] Following subcutaneous (s.c.) administration, LNPs and their mRNA cargo are expected to be largely retained at the site of injection, resulting in high local concentrations. Since LNPs are known to be pro-inflammatory, largely attributed to the ionizable lipid present in the LNPs, (Sabnis et al. *Mol. Ther.* 2018;26:1509–1519) then it would be expected that s.c. administration of mRNA formulated in LNPs would be associated with dose-limiting inflammatory responses. Co-administration of dexamethasone with LNP reduces the immune-inflammatory response following i.v. administration (Abrams et al. *Mol. Ther.* 2010;18:171–180). And Chen et al. (*J. Control. Release.* 2018;286:46–54.) showed reduced immune stimulation following systemic administration by incorporating lipophilic dexamethasone prodrugs within LNP-containing nucleic acids.

Dosing

[123] Optimal dosing schedules are calculated from measurements of drug accumulation in the body of the patient. Optimum dosages vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC50s found to be effective in *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μ g to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or at desired intervals. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μ g to 100 g per kg of body weight, once or more daily.

[124] Therapeutically effective amounts include but are not limited to the doses described herein. Described doses and other therapeutically effective amounts are administered in one or more of the therapeutically effective dose regimens described herein.

Articles of Manufacture/Kits

[125] In another embodiment of the invention, an article of manufacture, or “kit”, containing materials useful for inhibiting AMANZI is provided. The kit comprises a container

with a composition comprising one or more modulators, e.g. an AMANZI modulator such as a AMANZI transcriptional inhibitor gapmer compound, for example. Suitable containers include, e.g., bottles, vials, *etc.* The container may be formed from a variety of materials such as glass or plastic. The kit may also comprise a pharmaceutically acceptable carrier. In some embodiments the kit may also include components for administering the compositions, for example, a syringe, needle, microneedle, *etc.*

Manufacture

[126] The oligonucleotides of this invention can be manufactured using solid-phase chemistries for synthesizing oligonucleotides, chemistries known in the art for synthesizing and preparing peptides and peptidomimetics, and chemistries known in the art for synthesizing organic compounds. In one aspect, the formulations of this invention will comprise a salt of the oligonucleotides of this invention, such as the sodium salt of the oligonucleotides of this invention. The kit may also comprise a pharmaceutically acceptable carrier. In one embodiment the formulation may comprise the sodium salt of an gapmer ASO compound as described herein.

[127] In some embodiments, the formulations of this invention are substantially pure. By substantially pure is meant that the formulations comprise less than about 10%, 5%, or 1%, and preferably less than about 0.1%, of any nucleotide or non-nucleotide impurity. In some embodiments the total impurities, including metabolites of the AMANZI transcriptional inhibitor gapmer type of ASO (antisense oligonucleotide) compound, will be not more than 15%. In some embodiments the total impurities, including metabolites of the AMANZI transcriptional inhibitor gapmer type of ASO (antisense oligonucleotide) compound, will be not more than 12%. In some embodiments the total impurities, including metabolites of the AMANZI transcriptional inhibitor gapmer type of ASO (antisense oligonucleotide) compound, will be not more than 11%. In other embodiments the total impurities, including metabolites of the AMANZI transcriptional inhibitor gapmer type of ASO (antisense oligonucleotide) compound, will be not more than 10%.

[128] Sterile compositions comprising the AMANZI transcriptional inhibitor gapmer type of ASO (antisense oligonucleotide) compounds of this invention prepared using aseptic processing by dissolving the AMANZI transcriptional inhibitor gapmer type of ASO (antisense oligonucleotide) compound in the formulation vehicle. In one embodiment, the formulation may also be sterilized by filtration. Excipients used in the manufacture of the formulations of this invention are widely used in pharmaceutical products and released to pharmacopeial standards.

EXAMPLES

EXAMPLE 1

[129] This example provides an *in vitro* screening system of candidate gapmers for inhibiting gene transcription regulated by long non-coding RNA AMANZI. The effect of candidate gapmer compounds were screened for target nucleic acid expression (e.g., messenger RNA) by RT-PCR.

[130] THP-1 human monocytic cell line (derived from an acute leukemia patient) was obtained from InvivoGen. THP1 cells were maintained in complete media, which is composed of RPMI 1640, 1% (2mM) GlutaMAX L-glutamine supplement, 25 mM HEPES, 10% FBS, 100 µg/ml Normocin, Pen-Strep (100 U/ml), Blasticidin (10 µg/ml) and Zeocin (100 µg/ml). Prior to seeding for the screen, the THP-1 monocyte culture was split by 50% to enable the cells to re-enter an exponential growth phase. 250,000 cells were seeded per well in quadruplicate in 96-well plates with 180 µL of complete medium in each well. Each gapmer compound tested was added to the THP-1 cells at a final concentration of 10 µM and mixed gently. Plates were incubated at 37 °C at 5% CO₂ for 24 hours. Then, LPS (10 ng/mL) was added to each well, and plates were incubated at 37 °C at 5% CO₂ for another 24 hours.

[131] Antisense modulation of AMANZI expression on specified genes was assayed by real-time PCR (RT-PCR). RNA analysis was performed on total cellular RNA or poly(A)+ mRNA.

[132] RNA was isolated and prepared using TRIZOL® Reagent (Thermo Fisher Scientific) and Direct-zol RNA Miniprep Kit (Zymo Research) according to the manufacturer's recommended protocols.

[133] Quantitation of target RNA levels was accomplished by quantitative real-time PCR using a CFX Real-time qPCR detection system (Bio Rad). Prior to real-time PCR, the isolated RNA was subjected to a reverse transcriptase (RT) reaction, which produces complementary DNA (cDNA) that is then used as the substrate for the real-time PCR amplification. RT reaction reagents and real-time PCR reagents were obtained from Thermo Fisher Scientific, and protocols for their use are provided by the manufacturer. Gene (or RNA) target quantities obtained by real time PCR were normalized using expression levels of stably expressed housekeeping genes such as HPRT or RPL37A. Total RNA was quantified using a Qubit Fluorometer (Invitrogen/Scientific) and a Qubit RNA HS Assay Kit (Thermo Fisher Scientific Cat. No. Q32852) in accordance with the manufacturer's protocol. The Qubit Fluorometer was calibrated with standards.

[134] A series of assayed gapmer compounds are shown in Table 1. These gapmers were designed to target different regions of the human AMANZI long non-coding RNA (SEQ ID NO. 1). The gapmer compounds in Table 1 are chimeric oligonucleotides (“gapmers”) having different configurations. For instance, gapmers with a configuration of 20 (5-10-5) nucleotides in length were composed of a central “gap” region of ten 2'-deoxynucleotides, which was flanked on both sides (5' and 3' directions) by five-nucleotide “wings”. These wings were composed of 2'-methoxyethyl (2'-MOE) sugar modified nucleosides. The internucleotide (backbone) linkages were phosphorothioate throughout the entire oligonucleotide sequence. Cytidine residues were 5-methylcytidines unless indicated otherwise, in which case they were cytidines residues. Gapmers with a configuration of 16 (3-10-3) nucleotides in length were composed of a central “gap” region comprising ten 2'-deoxynucleotides, which was flanked on both sides (5' and 3' directions) by three-nucleotide “wings”. In some embodiments, the wings were composed of locked nucleic acid (LNA) modified nucleosides employing a cMe locked nucleic acid modification. The internucleotide (backbone) linkages were phosphorothioate throughout the entire oligonucleotide sequence. Cytidine residues were 5-methylcytidines unless indicated otherwise, in which case they were cytidine residues.

[135] Gapmer compounds used are shown in Table 1. Abbreviations of the nucleoside modification in Table 1: M = 2'-methoxyethyl (2'-MOE) modified nucleoside; L = locked nucleic acid (LNA) modified nucleoside (cMe); d = 2'-deoxynucleosides.

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
2	5 - 10 - 5	MMMMMdddddM MMMM	GAACTTGCAGGGG GCCGAGA	789-808
3	5 - 10 - 5	MMMMMdddddM MMMM	CCCTCAATACAGA CAG GGAG	210-229
4	5 - 10 - 5	MMMMMdddddM MMMM	ACTTTTCAGAGTTC ACCAAA	481-500
5	5 - 10 - 5	MMMMMdddddM MMMM	TGGACTCTCATTCA TTCTAC	638-657

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
6	5 - 10 - 5	MMMMMddddddddddM MMMM	CTTGCAGGGGGGCC GAGATCG	786-805
7	5 - 10 - 5	MMMMMddddddddddM MMMM	TTTCAGAGTTCACC AAAAAA	478-497
8	5 - 10 - 5	MMMMMddddddddddM MMMM	AAAAAAAAAAAAAGT GTTATGA	718-737
9	5 - 10 - 5	MMMMMddddddddddM MMMM	CCTGGACTCTCATT CATTCT	640-659
10	5 - 10 - 5	MMMMMddddddddddM MMMM	AGCACGATACCTG GCACATA	1194-1213
11	5 - 10 - 5	MMMMMddddddddddM MMMM	GAGCACGATACCT GGCACAT	1195-1214
12	5 - 10 - 5	MMMMMddddddddddM MMMM	ATCTGGCATTGATC TGGTTC	440-459
13	5 - 10 - 5	MMMMMddddddddddM MMMM	CATCTGGCATTGAT CTGGTT	441-460
14	5 - 10 - 5	MMMMMddddddddddM MMMM	GCCAAATTAAAAA TCAGAAG	1324-1343
15	5 - 10 - 5	MMMMMddddddddddM MMMM	GCGGAACTTGCAG GGGGCCG	792-811
16	5 - 10 - 5	MMMMMddddddddddM MMMM	TAAAAATACAAAA AATTAGC	886-905
17	5 - 10 - 5	MMMMMddddddddddM MMMM	ATACTAATTTGAAT AAAAAT	1177-1196
18	5 - 10 - 5	MMMMMddddddddddM MMMM	TACTAATTTGAATA AAAATG	1176-1195

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
19	5 - 10 - 5	MMMMMddddddddddM MMMM	CAGCTTTTAGATTT CCTGTG	1406-1425
20	5 - 10 - 5	MMMMMddddddddddM MMMM	AGCTTTTAGATTTC CTGTGG	1405-1424
21	5 - 10 - 5	MMMMMddddddddddM MMMM	AAGAAGTGAATGA AGAAAAG	123-142
22	5 - 10 - 5	MMMMMddddddddddM MMMM	TACAAAGAAGTGA ATGAAGA	127-146
23	5 - 10 - 5	MMMMMddddddddddM MMMM	ACAAAGAAGTGAA TGAAGAA	126-145
24	5 - 10 - 5	MMMMMddddddddddM MMMM	GTAAAGAGAAAC TGATAAC	563-582
25	5 - 10 - 5	MMMMMddddddddddM MMMM	AGACCCACACCCT CAATACA	219-238
26	5 - 10 - 5	MMMMMddddddddddM MMMM	ACTAATTGAATA AAAATGC	1175-1194
27	5 - 10 - 5	MMMMMddddddddddM MMMM	GCTTTTAGATTTC TGTGGA	1404-1423
28	5 - 10 - 5	MMMMMddddddddddM MMMM	CAAATTAAAAATC AGAAGGC	1322-1341
29	5 - 10 - 5	MMMMMddddddddddM MMMM	CCAAATTAAAAAT CAGAAGG	1323-1342
30	5 - 10 - 5	MMMMMddddddddddM MMMM	TATCAAATTCACA GGAAACT	1437-1456
31	5 - 10 - 5	MMMMMddddddddddM MMMM	TTATCAAATTCACA GGAAAC	1438-1457

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
32	5 - 10 - 5	MMMMMddddddddddM MMMM	CTAATTTGAATAA AAATGCT	1174-1193
33	5 - 10 - 5	MMMMMddddddddddM MMMM	CCAGGAGGAGCAA GGTTCA	1048-1067
34	5 - 10 - 5	MMMMMddddddddddM MMMM	CTCATCTGGCATTG ATCTGG	443-462
35	5 - 10 - 5	MMMMMddddddddddM MMMM	TGTGACTGACTCCT GATATC	1356-1375
36	5 - 10 - 5	MMMMMddddddddddM MMMM	GCTCCCCTGGGCA GTACCCT	609-628
37	5 - 10 - 5	MMMMMddddddddddM MMMM	CCAAGAGATAGAG TCTCAGA	528-547
38	5 - 10 - 5	MMMMMddddddddddM MMMM	GGAGGAGCAAGGT TTCAACT	1045-1064
39	5 - 10 - 5	MMMMMddddddddddM MMMM	CTTATCAAATTCAC AGGAAA	1439-1458
40	3 - 10 - 3	LLLddddddddddLLL	CATCAACTGCACA ACG	9-24
41	3 - 10 - 3	LLLddddddddddLLL	ACATCAACTGCAC AAC	10-25
42	3 - 10 - 3	LLLddddddddddLLL	GGACATCAACTGC ACA	12-27
43	3 - 10 - 3	LLLddddddddddLLL	TGGACATCAACTG CAC	13-28
44	3 - 10 - 3	LLLddddddddddLLL	TTGTCCCACATATA CT	74-89

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
45	3 - 10 - 3	LLLdddddddddLLL	ACTTTGTCCCACAT AT	77-92
46	3 - 10 - 3	LLLdddddddddLLL	CACTTTGTCCCACA TA	78-93
47	3 - 10 - 3	LLLdddddddddLLL	CTAAACTTAAGTA CAA	142-157
48	3 - 10 - 3	LLLdddddddddLLL	CCTAAACTTAAGT ACA	143-158
49	3 - 10 - 3	LLLdddddddddLLL	TGTACCCATTTATC CA	177-192
50	3 - 10 - 3	LLLdddddddddLLL	ACCGTATGTTCTCT GC	324-339
51	3 - 10 - 3	LLLdddddddddLLL	GGGTTGTGTGAGC TTA	384-399
52	3 - 10 - 3	LLLdddddddddLLL	AATCATTTTAGGCA GA	464-479
53	3 - 10 - 3	LLLdddddddddLLL	AAATCATTTTAGGC AG	465-480
54	3 - 10 - 3	LLLdddddddddLLL	AAAATCATTTTAG GCA	466-481
55	3 - 10 - 3	LLLdddddddddLLL	ACTTTTCAGAGTTC AC	485-500
56	3 - 10 - 3	LLLdddddddddLLL	CACTTTTCAGAGTT CA	486-501
57	3 - 10 - 3	LLLdddddddddLLL	TTCCACTTTTCAGA GT	489-504

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
58	3 - 10 - 3	LLLdddddddddddLLL	ATTCCACTTTTCAGAG	490-505
59	3 - 10 - 3	LLLdddddddddddLLL	AATATTCCACTTTTCA	493-508
60	3 - 10 - 3	LLLdddddddddddLLL	CAATATTCCACTTTTC	494-509
61	3 - 10 - 3	LLLdddddddddddLLL	ACAATATTCCACTTTT	495-510
62	3 - 10 - 3	LLLdddddddddddLLL	TCTCAGATGGATATTC	519-534
63	3 - 10 - 3	LLLdddddddddddLLL	GTCTCAGATGGATATT	520-535
64	3 - 10 - 3	LLLdddddddddddLLL	AGTCTCAGATGGATAT	521-536
65	3 - 10 - 3	LLLdddddddddddLLL	GAGTCTCAGATGGATA	522-537
66	3 - 10 - 3	LLLdddddddddddLLL	AGAGTCTCAGATGAT	523-538
67	3 - 10 - 3	LLLdddddddddddLLL	TAGAGTCTCAGATGGA	524-539
68	3 - 10 - 3	LLLdddddddddddLLL	ATAGAGTCTCAGATGG	525-540
69	3 - 10 - 3	LLLdddddddddddLLL	ACCAAGAGATAGATC	533-548
70	3 - 10 - 3	LLLdddddddddddLLL	TACCAAGAGATAGAGT	534-549

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
71	3 - 10 - 3	LLLdddddddddLLL	TTACCAAGAGATA GAG	535-550
72	3 - 10 - 3	LLLdddddddddLLL	AACTGCACAACGA TTG	5-20
73	3 - 10 - 3	LLLdddddddddLLL	CAACTGCACAACG ATT	6-21
74	3 - 10 - 3	LLLdddddddddLLL	TCAACTGCACAAC GAT	7-22
75	3 - 10 - 3	LLLdddddddddLLL	ATCAACTGCACAA CGA	8-23
76	3 - 10 - 3	LLLdddddddddLLL	GCCCTTCATTGTAC CC	186-201
77	3 - 10 - 3	LLLdddddddddLLL	GCTATTGGCCCTTC AT	193-208
78	3 - 10 - 3	LLLdddddddddLLL	GGCTATTGGCCCTT CA	194-209
79	3 - 10 - 3	LLLdddddddddLLL	AGGGCTATTGGCC CTT	196-211
80	3 - 10 - 3	LLLdddddddddLLL	GAGGGCTATTGGC CCT	197-212
81	3 - 10 - 3	LLLdddddddddLLL	GGAGGGCTATTGG CCC	198-213
82	3 - 10 - 3	LLLdddddddddLLL	ACAGGGAGGGCTA TTG	202-217
83	3 - 10 - 3	LLLdddddddddLLL	AGCACCCAAGGTA GAG	237-252

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
84	3 - 10 - 3	LLLdddddddddLLL	CAGCACCCAAGGT AGA	238-253
85	3 - 10 - 3	LLLdddddddddLLL	GAACCCTGCATAC CGT	335-350
86	3 - 10 - 3	LLLdddddddddLLL	TGAACCCTGCATA CCG	336-351
87	3 - 10 - 3	LLLdddddddddLLL	CCTGAACCCTGCAT AC	338-353
88	3 - 10 - 3	LLLdddddddddLLL	CCCAACTCCGTCA GGA	355-370
89	3 - 10 - 3	LLLdddddddddLLL	AGGGTTGCCCAA CTC	363-378
90	3 - 10 - 3	LLLdddddddddLLL	CCAGGGTTGCCCC AAC	365-380
91	3 - 10 - 3	LLLdddddddddLLL	GATTGGCTAGGGT AAC	416-431
92	3 - 10 - 3	LLLdddddddddLLL	AGATTGGCTAGGG TAA	417-432
93	3 - 10 - 3	LLLdddddddddLLL	GAGATTGGCTAGG GTA	418-433
94	3 - 10 - 3	LLLdddddddddLLL	ATGAGATTGGCTA GGG	420-435
95	3 - 10 - 3	LLLdddddddddLLL	CATGAGATTGGCT AGG	421-436
96	3 - 10 - 3	LLLdddddddddLLL	CCATGAGATTGGC TAG	422-437

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
97	3 - 10 - 3	LLLdddddddddLLL	GGTATTACCAAGA GAT	539-554
98	3 - 10 - 3	LLLdddddddddLLL	TTGGTATTACCAAG AG	541-556
99	3 - 10 - 3	LLLdddddddddLLL	GTTGGTATTACCAA GA	542-557
100	3 - 10 - 3	LLLdddddddddLLL	TGGTTGGTATTACC AA	544-559
101	3 - 10 - 3	LLLdddddddddLLL	CTTGGTTGGTATTA CC	546-561
102	3 - 10 - 3	LLLdddddddddLLL	TAACTCTTGGTTGG TA	551-566
103	3 - 10 - 3	LLLdddddddddLLL	ATAACTCTTGGTTG GT	552-567
104	3 - 10 - 3	LLLdddddddddLLL	GATAACTCTTGGTT GG	553-568
105	3 - 10 - 3	LLLdddddddddLLL	CCAAGAAAGGTCA ATT	303-318
106	3 - 9 - 3	MMMdddddddddMMM	AACACGTCTATAC GC	None
107	3 - 9 - 3	LLLdddddddddLLL	AACACGTCTATAC GC	None

TABLE 1.

[136] The gapmer compounds were analyzed for their effect on IL1B transcription in THP1 cells by quantitative real-time PCR. Similarly, the effect of the gapmer compounds on cytotoxicity and on Toll-like receptor (TLR) signaling activation were analyzed by assaying for the gene transcription of TNFRSF10B and secreted embryonic alkaline phosphatase (SEAP),

respectively. Data are averages from four replicates in which THP1 cells were treated with the gapmer compounds of Table 1.

[137] Table 2 shows the fold change of IL1B, TNFRSF10B, and SEAP gene expression in THP1 cells in the presence of the gapmer compounds in Table 1. Data are normalized by the expression of housekeeping gene RPL37A and represented as fold change relative to negative control (SEQ ID NO. 106 served as a negative control for MOE gapmers, and SEQ ID NO. 107 served as a negative control for LNA gapmers). An expression value < 1.0 means that the transcription of that gene was inhibited, and an expression value >1.0 means that the transcription of that gene is induced. For example, a value of 0.25 means that gene transcription was inhibited by 75%.

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
2	0.832	0.809	0.479
3	0.918	0.913	0.861
4	0.814	0.886	1.039
5	0.732	0.965	0.906
6	0.538	0.978	0.736
7	1.726	1.100	1.442
8	0.709	1.362	0.953
9	1.442	1.140	1.451
10	0.937	0.941	1.231
11	1.230	1.453	1.702
12	1.800	1.531	2.212
13	1.286	1.486	1.725
14	0.671	1.140	0.901

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
15	0.972	1.079	1.041
16	1.330	1.370	1.814
17	0.925	0.853	0.943
18	1.399	1.112	1.219
19	1.519	0.830	1.128
20	1.014	0.779	0.911
21	0.843	0.684	0.707
22	1.191	0.832	1.234
23	0.971	0.804	0.803
24	1.114	0.982	1.273
25	0.793	0.860	0.994
26	0.726	1.001	0.927
27	1.458	1.103	1.311
28	0.967	0.961	0.972
29	1.105	0.951	1.088
30	1.750	1.429	1.777
31	1.130	1.193	1.533
32	1.465	1.353	1.309
33	1.585	1.383	1.191
34	1.294	1.234	1.285

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
35	1.440	1.144	1.550
36	1.081	0.734	0.881
37	1.154	0.979	1.144
38	1.116	0.972	0.830
39	1.470	0.834	1.058
40	1.188	2.530	1.159
41	2.112	3.741	1.630
42	3.289	4.755	2.352
43	2.140	3.236	1.655
44	2.127	4.026	2.033
45	2.059	2.550	1.530
46	2.791	3.779	2.114
47	2.149	3.444	1.371
48	1.945	2.863	1.472
49	1.347	1.493	0.871
50	1.251	0.818	0.918
51	2.408	1.466	1.982
52	0.897	1.503	0.906
53	1.054	0.984	1.094
54	0.938	0.763	0.713

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
55	1.104	0.944	0.763
56	0.920	0.603	0.747
57	1.285	0.625	0.926
58	1.150	0.639	0.922
59	0.817	0.083	0.971
60	0.938	2.096	0.908
61	1.450	1.884	1.014
62	1.524	2.706	1.409
63	2.016	2.490	1.335
64	2.303	3.289	1.329
65	1.844	1.737	1.155
66	2.366	2.899	1.345
67	2.788	2.535	1.218
68	1.897	2.514	1.218
69	2.117	0.990	1.223
70	1.054	0.510	0.956
71	1.073	0.300	1.057
72	0.719	0.947	0.608
73	0.856	0.753	0.784
74	0.645	1.025	0.877

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
75	0.984	0.754	1.204
76	0.322	0.269	0.149
77	1.185	0.899	0.820
78	1.954	1.866	1.195
79	1.775	2.482	1.064
80	1.508	1.548	0.855
81	1.805	1.440	0.832
82	2.189	1.930	1.351
83	2.953	1.528	0.836
84	2.968	1.895	0.922
85	1.607	1.407	0.787
86	1.593	1.412	0.972
87	1.991	1.261	1.404
88	0.868	1.033	0.817
89	1.064	0.793	0.936
90	0.810	0.535	0.843
91	1.095	0.372	0.691
92	1.215	0.584	0.724
93	1.214	0.451	0.952
94	1.151	0.960	1.892

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
95	1.792	0.624	1.253
96	1.778	0.557	2.115
97	1.082	0.632	1.461
98	1.638	1.107	0.694
99	1.152	1.001	0.803
100	1.860	1.741	1.331
101	2.893	1.443	1.380
102	2.016	1.312	1.016
103	2.358	1.430	1.045
104	2.323	1.515	1.283
105	1.410	0.974	1.328

TABLE 2.

[138] Gapmer SEQ ID NOs. 41-47, 51, 63-64, 66-67, 69, 82-84, and 101-104 demonstrated at least two-fold upregulation of human IL1B expression in this assay. Among them, gapmer SEQ ID NO. 42 demonstrated more than three-fold upregulation of human IL1B expression in this assay.

[139] Based on the screening data in Table 1 and 2, three regions (A, B, and C) in human AMANZI sequence (SEQ ID NO. 1) were found effectively targeted by gapmers SEQ ID NOs. 41-46, 63-68, 83-84, and 101-104. Table 3. provides the positions of AMANZI target regions (AMANZI Target Region A, B, and C) in human AMANZI sequence (SEQ ID NO.1) and the average upregulation of IL1B by the gapmers targeted to individual regions.

AMANZI Target Region ID	Target Region in human AMANZI (SEQ ID NO. 1)	SEQ ID NO.	AVERAGE IL-1 β EXPRESSION
A	10-93	41-46	2.420
B	194-253	78, 81-84	2.374
C	519-568	63, 64, 66, 67, 69, 101-104	2.353

TABLE 3.

[140] The gapmers targeted to region A, B, and C in human AMANZI (SEQ ID NO. 1) demonstrated more than 2.3-fold upregulation of IL1B gene expression on average. Gapmers targeting region A are selected from the group consisting of SEQ ID NOs. 41-46. Gapmers targeting region B are selected from the group consisting of SEQ ID NOs. 78, 81-84. Gapmers targeting region C are selected from the group consisting of SEQ ID NOs. 63, 64, 66, 67, 101-104.

[141] Based on screening data in Table 3, a first supplemental group of chimeric phosphorothioate gapmer compounds that target the AMANZI target regions A, B, and C in human AMANZI (SEQ ID NO. 1) were synthesized. Table 4 provides the configuration, chemical modification, and sequence of the chimeric phosphorothioate gapmer compounds of the supplemental chimeric phosphorothioate gapmer compounds. Abbreviations of the nucleoside modification in Table 4: M = 2'-methoxyethyl (2'-MOE) modified nucleoside; L = locked nucleic acid (LNA) modified nucleoside (cMe); d = 2'-deoxynucleosides; 2'Md = 2'OMe modified deoxynucleoside.

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
108	5 - 10 - 5	MMMMMdddddddddMM MMM	GTGGACATCAACT GCACAAC	10-29
109	6 - 8 - 6	MMMMMMdddddddddMM MMM	GTGGACATCAACT GCACAAC	10-29
110	7 - 6 - 7	MMMMMMMMdddddddMM MMMM	GTGGACATCAACT GCACAAC	10-29

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
111	4 - 10 - 4	MMMMdddddddddMMMM	TGGACATCAACTG CACAA	11-28
112	5 - 8 - 5	MMMMMdddddddddMMMM M	TGGACATCAACTG CACAA	11-28
113	4 - 8 - 4	MMMMdddddddddMMMM	GGACATCAACTGC ACA	12-27
114	5 - 6 - 5	MMMMMddddddMMMMM	GGACATCAACTGC ACA	12-27
115	3 - 10 - 4	LLLdddddddddLLLL	TGGACATCAACTG CACA	12-28
116	5 - 8 - 4	LLLLLdddddddddLLLL	TGGACATCAACTG CACA	12-28
42	3 - 10 - 3	LLLdddddddddLLL	GGACATCAACTGC ACA	12-27
117	5 - 7 - 4	LLLLLdddddddddLLLL	GGACATCAACTGC ACA	12-27
118	3 - 10 - 3	LLLdd2'MdddddddddLLL	GGACATCAACTGC ACA	12-27
119	5 - 10 - 5	MMMMMdddddddddMM MMM	CTCTTGGTTGGTAT TA CCAA	544-563
120	6 - 8 - 6	MMMMMMdddddddddMM MMM	CTCTTGGTTGGTAT TACCAA	544-563
121	7 - 6 - 7	MMMMMMMMddddddMMM MMMM	CTCTTGGTTGGTAT TACCAA	544-563
122	4 - 10 - 4	MMMMdddddddddMMMM	CTCTTGGTTGGTAT TACC	546-563

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
123	5 - 8 - 5	MMMMMdddddddddMMMMM	CTCTTGGTTGGTATTACC	546-563
124	4 - 8 - 4	MMMMMdddddddddMMMMM	CTTGGTTGGTATTACC	546-561
125	5 - 6 - 5	MMMMMdddddddddMMMMM	CTTGGTTGGTATTACC	546-561
126	3 - 10 - 4	LLLdddddddddLLLL	CTCTTGGTTGGTATTAC	547-563
127	5 - 8 - 4	LLLLLdddddddddLLLLL	CTCTTGGTTGGTATTAC	547-563
101	3 - 10 - 3	LLLdddddddddLLL	CTTGGTTGGTATTACC	546-561
128	5 - 7 - 4	LLLLLdddddddddLLLLL	CTTGGTTGGTATTACC	546-561
129	3 - 10 - 3	LLLdd2'MdddddddddLLL	CTTGGTTGGTATTACC	546-561
130	5 - 10 - 5	MMMMMdddddddddMMMMMM	ACAGCACCCAAGGTAGAGAC	235-254
131	6 - 8 - 6	MMMMMMdddddddddMMMMMM	ACAGCACCCAAGGTAGAGAC	235-254
132	7 - 6 - 7	MMMMMMMMdddddddMMMMMM	ACAGCACCCAAGGTAGAGAC	235-254
133	4 - 10 - 4	MMMMMdddddddddMMMMM	ACAGCACCCAAGGTAGAG	237-254
134	5 - 8 - 5	MMMMMdddddddddMMMMM	ACAGCACCCAAGGTAGAG	237-254

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
135	4 - 8 - 4	MMMMddddddMMMM	AGCACCCAAGGTA GAG	237-252
136	5 - 6 - 5	MMMMMddddddMMMMM	AGCACCCAAGGTA GAG	237-252
137	3 - 10 - 4	LLLdddddddddLLLL	ACAGCACCCAAGG TAG	239-254
138	5 - 8 - 4	LLLLLdddddddddLLLL	ACAGCACCCAAGG TAG	239-254
83	3 - 10 - 3	LLLdddddddddLLL	AGCACCCAAGGTA GAG	237-252
139	5 - 7 - 4	LLLLLdddddddddLLLL	AGCACCCAAGGTA GAG	237-252
140	3 - 10 - 3	LLLdd2'MdddddddddLLL	AGCACCCAAGGTA GAG	237-252
141	5 - 10 - 5	MMMMMdddddddddMM MMM	TCCACTTTGTCCCA CATATA	76-95
142	6 - 8 - 6	MMMMMMdddddddddMM MMM	TCCACTTTGTCCCA CATATA	76-95
143	7 - 6 - 7	MMMMMMMMddddddMM MMMM	TCCACTTTGTCCCA CATATA	76-95
144	4 - 10 - 4	MMMMdddddddddMMMM	TCCACTTTGTCCCA CATA	78-95
145	5 - 8 - 5	MMMMMdddddddddMM M	TCCACTTTGTCCCA CATA	78-95
146	4 - 8 - 4	MMMMdddddddddMMMM	CACTTTGTCCCACA TA	78-93

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
147	5 - 6 - 5	MMMMMdddddMMMMM	CACTTTGTCCCACA TA	78-93
148	3 - 10 - 4	LLLdddddL	TCCACTTTGTCCCA CAT	79-95
149	5 - 8 - 4	LLLLLdddddL	TCCACTTTGTCCCA CAT	79-95
46	3 - 10 - 3	LLLdddddL	CACTTTGTCCCACA TA	78-93
150	5 - 7 - 4	LLLLLdddddL	CACTTTGTCCCACA TA	78-93
151	3 - 10 - 3	LLLdd2'MdddddL	CACTTTGTCCCACA TA	78-93
152	5 - 10 - 5	MMMMMdddddMMMM MM	GATAGAGTCTCAG ATGGATA	522-541
153	6 - 8 - 6	MMMMMMdddddMMMM MMM	GATAGAGTCTCAG ATGGATA	522-541
154	7 - 6 - 7	MMMMMMMdddddMMMM MMMM	GATAGAGTCTCAG ATGGATA	522-541
155	4 - 10 - 4	MMMdddddMMMM	GATAGAGTCTCAG ATGGA	524-541
156	5 - 8 - 5	MMMMMdddddMMMM M	GATAGAGTCTCAG ATGGA	524-541
157	4 - 8 - 4	MMMdddddMMMM	TAGAGTCTCAGAT GGA	524-539
158	5 - 6 - 5	MMMMMdddddMMMM	TAGAGTCTCAGAT GGA	524-539

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
159	3 - 10 - 4	LLLdddddL	GATAGAGTCTCAG ATGG	525-541
160	5 - 8 - 4	LLLLLdddddL	GATAGAGTCTCAG ATGG	525-541
67	3 - 10 - 3	LLLdddddL	TAGAGTCTCAGAT GGA	524-539
161	5 - 7 - 4	LLLLLdddddL	TAGAGTCTCAGAT GGA	524-539
162	3 - 10 - 3	LLLdd2'MdddddL	TAGAGTCTCAGAT GGA	524-539

TABLE 4.

[142] The first supplemental chimeric phosphorothioate gapmer compounds in Table 4. were analyzed for their effect on IL1B, TNFRSF10B, and SEAP transcription in THP1 cells by quantitative real-time PCR. Table 5 shows the induction of IL1B, TNFRSF10B, and SEAP gene expression by the first supplemental chimeric phosphorothioate gapmers SEQ ID NOs 42, 46, 67, 83, 101 that target AMANZI target regions (region A, B, and C) in human AMANZI sequence (SEQ ID NO.1). Data were normalized by the expression of housekeeping gene RPL37A and represented as fold change relative to negative control (SEQ ID NO. 106 served as a negative control for MOE gapmers, and SEQ ID NO. 107 served as a negative control for LNA gapmers). An expression value < 1.0 means that the transcription of that gene was inhibited, and an expression value >1.0 means that the transcription of that gene is induced. For example, a value of 0.25 means that gene transcription was inhibited by 75%.

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
108	2.145	1.817	1.547
109	1.887	1.511	1.034

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
110	1.630	1.484	1.541
111	2.624	1.497	1.865
112	1.636	1.177	1.229
113	1.621	1.296	1.436
114	2.028	1.331	1.303
115	0.832	1.636	1.258
116	1.602	1.387	1.015
42	1.003	1.372	1.218
117	1.332	1.432	1.054
118	2.185	1.646	1.448
119	0.952	2.415	1.450
120	1.140	0.953	1.809
121	0.620	1.554	1.488
122	1.196	1.188	1.858
123	1.021	0.834	1.224
124	1.128	0.676	1.195
125	2.742	1.330	2.226
126	3.941	1.584	1.722
127	2.327	0.015	1.962

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
101	2.321	0.080	2.031
128	2.206	3.445	1.814
129	3.465	2.001	1.364
130	2.046	1.810	1.401
131	2.150	2.108	1.561
132	1.177	1.254	1.438
133	1.860	1.922	1.583
134	0.609	1.028	1.170
135	1.459	1.846	1.932
136	0.529	0.127	1.215
137	0.818	0.035	1.019
138	0.888	0.170	1.095
83	1.282	0.215	1.132
139	0.811	0.230	0.890
140	0.745	0.140	0.961
141	0.749	1.028	0.514
142	0.939	1.137	0.749
143	1.056	1.222	0.830
144	1.176	1.300	0.894

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
145	1.074	1.127	0.792
146	1.040	1.011	0.762
147	0.776	1.040	0.707
148	2.118	1.263	1.190
149	1.438	0.937	0.852
46	1.305	1.192	0.732
150	2.132	0.979	1.330
151	2.162	0.884	0.953
152	1.217	1.294	1.119
153	1.101	1.030	0.889
154	1.021	0.775	1.120
155	1.137	1.010	0.901
156	1.024	1.231	1.014
157	0.917	0.918	0.805
158	1.140	0.950	0.980
159	1.592	1.250	1.153
160	1.636	1.656	3.616
67	1.402	1.026	1.546
161	1.480	1.326	2.915

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
162	2.559	1.981	1.548

TABLE 5.

[143] Based on screening data in Table 4 and 5, another set of LNA gapmer compounds (second supplemental) that target human AMANZI sequence (SEQ ID NO. 1) were synthesized and tested. Table 6 provides the configuration, nucleoside modification, and sequence of the second supplemental gapmer compounds used in the present examples and embodiments described herein. Abbreviations of the nucleoside modification in Table 6: L = locked nucleic acid (LNA) modified nucleoside (cMe); d = 2'- deoxynucleosides.

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
163	3 - 10 - 3	LLLdddddddddLLL	TCATAACCTGGACTCT	650-665
164	3 - 10 - 3	LLLdddddddddLLL	TCCCCTGGGCAGTACC	611-626
165	3 - 10 - 3	LLLdddddddddLLL	TACAAATGGAGGGCT C	625-640
166	3 - 10 - 3	LLLdddddddddLLL	TCTACAAATGGAGGG C	627-642
167	3 - 10 - 3	LLLdddddddddLLL	ACTCTCATTCTTCTA	639-654
168	3 - 10 - 3	LLLdddddddddLLL	TGGACTCTCATTCTT	642-657
169	3 - 10 - 3	LLLdddddddddLLL	ATAACCTGGACTCTCA	648-663
170	3 - 10 - 3	LLLdddddddddLLL	GTGTTCTTCACTCCA	674-689
171	3 - 10 - 3	LLLdddddddddLLL	GGTGTCTTCACTCC	675-690
172	3 - 10 - 3	LLLdddddddddLLL	AGGGTGTCTTCACT	677-692
173	3 - 10 - 3	LLLdddddddddLLL	CAAAGGAGGGTGTT C	683-698
174	3 - 10 - 3	LLLdddddddddLLL	GCAGACCTGTCAAAG A	701-716

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
175	3 - 10 - 3	LLLdddddddddLLL	GATGCAGACCTGTCAA	704-719
176	3 - 10 - 3	LLLdddddddddLLL	TGATGCAGACCTGTCA	705-720
177	3 - 10 - 3	LLLdddddddddLLL	AGTGTTATGATGCAGA	712-727
178	3 - 10 - 3	LLLdddddddddLLL	GGGGGCCGAGATCGT G	784-799
179	3 - 10 - 3	LLLdddddddddLLL	CTAGGGTAACAGCAC C	410-425
180	3 - 10 - 3	LLLdddddddddLLL	TAGGGTAACAGCACCT	409-424
181	3 - 10 - 3	LLLdddddddddLLL	GCTAGGGTAACAGCA C	411-426
182	3 - 10 - 3	LLLdddddddddLLL	CAGATGGATATTCTTA	516-531
183	3 - 10 - 3	LLLdddddddddLLL	TGGCTAGGGTAACAG C	413-428
184	3 - 10 - 3	LLLdddddddddLLL	TTGGCTAGGGTAACAG	414-429
185	3 - 10 - 3	LLLdddddddddLLL	ATTGGCTAGGGTAACA	415-430
186	3 - 10 - 3	LLLdddddddddLLL	ATTACCAAGAGATAG A	536-551
187	3 - 10 - 3	LLLdddddddddLLL	GATCTGGTTCATCCAT	434-449
188	3 - 10 - 3	LLLdddddddddLLL	TTGATCTGGTTCATCC	436-451
189	3 - 10 - 3	LLLdddddddddLLL	ATTGATCTGGTTCATC	437-452
190	3 - 10 - 3	LLLdddddddddLLL	CTCATCTGGCATTGAT	447-462
191	3 - 10 - 3	LLLdddddddddLLL	GAGTTCACCAAAAAA T	477-492
192	3 - 10 - 3	LLLdddddddddLLL	CTCTTGGTTGGTATTA	548-563
193	3 - 10 - 3	LLLdddddddddLLL	ACTCTTGGTTGGTATT	549-564
194	3 - 10 - 3	LLLdddddddddLLL	AACTGATAACTCTTGG	557-572
195	3 - 10 - 3	LLLdddddddddLLL	AAACTGATAACTCTTG	558-573

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
196	3 - 10 - 3	LLLdddddddddLLL	GAGAAACTGATAACT C	561-576
197	3 - 10 - 3	LLLdddddddddLLL	AGAGAAACTGATAAC T	562-577
198	3 - 10 - 3	LLLdddddddddLLL	CGGTAAAGAGAAAC T	569-584
199	3 - 10 - 3	LLLdddddddddLLL	TCGGTAAAGAGAAA C	570-585
200	3 - 10 - 3	LLLdddddddddLLL	CTCGGTAAAGAGAA A	571-586
201	3 - 10 - 3	LLLdddddddddLLL	TGTCTCGGTAAAGAG	574-589
202	3 - 10 - 3	LLLdddddddddLLL	GTGTCTCGGTAAAGA	575-590
203	3 - 10 - 3	LLLdddddddddLLL	GGTGTCTCGGTAAAG	576-591
204	3 - 10 - 3	LLLdddddddddLLL	TGGTGTCTCGGTAAA	577-592
205	3 - 10 - 3	LLLdddddddddLLL	CTGGTGTCTCGGTAA	578-593
206	3 - 10 - 3	LLLdddddddddLLL	TTGCTGGTGTCTCGGT	581-596
207	3 - 10 - 3	LLLdddddddddLLL	TACCCTGGAGCAGGC A	599-614
208	3 - 10 - 3	LLLdddddddddLLL	GTACCCTGGAGCAGG C	600-615
209	3 - 10 - 3	LLLdddddddddLLL	TCCGTCAGGAGCCTGA	349-364
210	3 - 10 - 3	LLLdddddddddLLL	GTCAGGAGCCTGAAC C	346-361
211	3 - 10 - 3	LLLdddddddddLLL	ACTCCGTCAGGAGCCT	351-366
212	3 - 10 - 3	LLLdddddddddLLL	AACTCCGTCAGGAGCC	352-367
213	3 - 10 - 3	LLLdddddddddLLL	CAACTCCGTCAGGAGC	353-368
214	3 - 10 - 3	LLLdddddddddLLL	CGTCAGGAGCCTGAA C	347-362
215	3 - 10 - 3	LLLdddddddddLLL	CCAACTCCGTCAGGAG	354-369
216	3 - 10 - 3	LLLdddddddddLLL	GCCCCAACTCCGTCAG	357-372

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
217	3 - 10 - 3	LLLdddddddddLLL	TGCCCCAACTCCGTCA	358-373
218	3 - 10 - 3	LLLdddddddddLLL	TTGCCCCAACTCCGTC	359-374
219	3 - 10 - 3	LLLdddddddddLLL	GTTGCCCCAACTCCGT	360-375
220	3 - 10 - 3	LLLdddddddddLLL	GGTTGCCCCAACTCCG	361-376
221	3 - 10 - 3	LLLdddddddddLLL	GGGTTGCCCCAACTCC	362-377
222	3 - 10 - 3	LLLdddddddddLLL	TCCAGGGTTGCCCCAA	366-381
223	3 - 10 - 3	LLLdddddddddLLL	GTGAGCTTATCTCCAG	377-392
224	3 - 10 - 3	LLLdddddddddLLL	GTGTGAGCTTATCTCC	379-394
225	3 - 10 - 3	LLLdddddddddLLL	TGTGTGAGCTTATCTC	380-395
226	3 - 10 - 3	LLLdddddddddLLL	TTGTGTGAGCTTATCT	381-396
227	3 - 10 - 3	LLLdddddddddLLL	GTTGTGTGAGCTTATC	382-397
228	3 - 10 - 3	LLLdddddddddLLL	GGTTGTGTGAGCTTAT	383-398
229	3 - 10 - 3	LLLdddddddddLLL	TTGCAGGGTTGTGTGA	389-404
230	3 - 10 - 3	LLLdddddddddLLL	CTTGCAGGGTTGTGTG	390-405
231	3 - 10 - 3	LLLdddddddddLLL	GGTCTTGCAGGGTTGT	393-408
232	3 - 10 - 3	LLLdddddddddLLL	GCACAACGATTGTCAG	1-16
233	3 - 10 - 3	LLLdddddddddLLL	TGCACAACGATTGTCA	2-17
234	3 - 10 - 3	LLLdddddddddLLL	CTGCACAACGATTGTC	3-18
235	3 - 10 - 3	LLLdddddddddLLL	GTGGGAAGATTCCTAA	154-169
236	3 - 10 - 3	LLLdddddddddLLL	TGTCCCACATATACTA	73-88
237	3 - 10 - 3	LLLdddddddddLLL	TATCCATCTGTAAGTG	167-182

SEQ ID NO.	Configuration	Nucleoside Modification	Sequence of gapmer compound	Complementary position to human AMANZI (SEQ ID NO. 1)
238	3 - 10 - 3	LLLdddddL	AATGTGGACATCAACT	17-32

TABLE 6.

[144] The second supplemental LNA gapmer compounds in Table 6. were similarly analyzed for their effect on IL1B, TNFRSF10B, and SEAP transcription in THP1 cells by quantitative real-time PCR. Table 7 shows the induction of IL1B gene expression by second supplemental LNA gapmers SEQ ID NOs 163-238 that target human AMANZI sequence (SEQ ID NO. 1). Data were normalized by the expression of housekeeping gene RPL37A and represented as fold change relative to negative control (SEQ ID NO. 106 served as a negative control for MOE gapmers, and SEQ ID NO. 107 served as a negative control for LNA gapmers). An expression value < 1.0 means that the transcription of that gene was inhibited, and an expression value >1.0 means that the transcription of that gene is induced.

[145] Gapmer SEQ ID NOs. 173, 201-208, 226, 223-229, and 235 demonstrated at least three- fold upregulation of human IL1B expression in this assay.

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
163	0.615	0.803	0.545
164	1.637	0.392	0.793
165	1.577	1.491	0.922
166	2.310	1.523	1.045
167	1.785	1.110	0.827
168	1.518	1.333	0.780
169	1.525	1.371	0.829
170	1.894	0.922	0.882
171	1.627	0.959	1.092
172	1.439	0.740	1.655

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
173	3.378	1.286	2.818
174	0.859	1.108	0.745
175	0.843	1.108	0.892
176	1.454	1.072	1.067
177	1.249	0.734	1.048
178	2.049	0.189	0.660
179	0.840	0.727	0.703
180	0.715	0.653	0.653
181	0.802	0.371	0.896
182	1.777	0.296	2.569
183	0.766	1.338	0.810
184	1.642	1.412	1.052
185	1.048	1.545	0.954
186	1.411	1.192	0.763
187	2.697	1.424	1.549
188	1.919	1.995	1.326
189	1.512	1.347	0.997
190	1.339	1.150	1.002
191	1.696	0.680	0.900
192	1.741	0.152	1.298
193	0.990	0.033	1.088
194	1.348	0.011	0.498
195	0.885	1.117	0.962
196	0.769	0.898	0.846

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
197	0.862	0.830	0.712
198	0.692	0.962	0.712
199	1.158	1.031	0.921
200	1.210	0.624	1.179
201	3.363	1.592	1.904
202	3.680	1.503	1.719
203	4.634	1.367	1.894
204	4.793	1.049	1.652
205	5.123	2.049	2.301
206	3.417	1.860	1.998
207	7.625	2.269	3.603
208	4.835	1.840	2.442
209	2.294	1.673	1.596
210	2.180	1.408	1.739
211	1.362	1.217	1.287
212	0.936	1.117	1.093
213	1.623	1.090	1.468
214	1.461	0.876	1.419
215	1.006	1.238	1.190
216	3.469	0.417	1.543
217	1.211	0.308	1.254
218	1.203	0.086	1.206
219	1.562	0.059	1.090
220	0.762	0.025	1.223

SEQ ID NO.	IL-1 β EXPRESSION	TNFRSF10B EXPRESSION	SEAP EXPRESSION
221	1.738	1.801	1.357
222	1.917	1.459	1.693
223	3.353	1.501	1.804
224	3.361	1.420	1.835
225	3.973	1.260	1.969
226	4.560	0.900	2.044
227	3.166	0.907	1.802
228	4.370	0.989	1.869
229	3.772	1.239	2.104
230	2.829	0.797	2.054
231	2.773	0.445	1.970
232	1.758	0.944	1.941
233	2.073	1.454	1.496
234	1.792	1.284	1.194
235	3.877	1.131	1.458
236	1.766	0.851	1.155
237	1.834	0.888	1.161
238	1.162	0.683	1.039

TABLE 7.

[146] Based on the screening data in Table 6 and 7, another two regions in human AMANZI sequence (SEQ ID NO. 1) (Regions D and E) were found effectively targeted by gapmers SEQ ID NOs. 201-208 and SEQ ID NOs. 223-229. Table 8. provides the positions of AMANZI target regions (AMANZI Target Regions D and E) and the average upregulation of IL1B by the gapmers targeted to individual regions.

AMANZI Region ID	AMANZI Target Region in human AMANZI (SEQ ID NO. 1)	SEQ ID NO.	AVERAGE IL-1 β EXPRESSION
D	377-404	223-229	3.794
E	574-615	201-208	4.684

TABLE 8.

[147] The gapmers targeted to regions D and E in human AMANZI (SEQ ID NO. 1) demonstrated more than 3.5-fold upregulation of IL1B gene expression on average. Gapmers targeting region D are selected from the group consisting of SEQ ID NOs. 228-234. Gapmers targeting region B are selected from the group consisting of SEQ ID NOs. 206-213.

[148] Table 5 shows results of AMANZI inhibition and corresponding gene activation. A value less than 1, represents inhibition.

[149] THP1 monocytes are heterozygotes for rs16944 (G>A) at the 268th nucleotide of human AMANZI sequence (SEQ ID NO. 1). To understand the allele specific effect on the induction of IL-1 β transcripts in the trained immunity, allele-specific MOE gapmer compounds targeting the region spanning rs16944 were synthesized. Table 6 provides the configuration, nucleoside modification, and sequence of gapmer compounds. Abbreviations of the nucleoside modification in Table 9: M = 2'-methoxyethyl (2'-MOE) modified nucleoside; d = 2'-deoxynucleosides.

SEQ ID NO.	Configuration	Chemistry	Nucleobase Sequence	Complementary position to human AMANZI (SEQ ID NO. 1)	Targeted allele of rs16944
239	5 - 10 - 5	MMMMMddddd ddMMMMM	AGCTCCTGAGG CAGAG AACAA	252-271	A
240	5 - 10 - 5	MMMMMddddd ddMMMMM	AGCTCCCGAGG CAGAG AACAA	252-271	G

TABLE 9.

EXAMPLE 2: AMANZI inhibition in β -glucan-trained monocyte cell line, THP1

[150] Trained immunity was induced in THP1 monocytes as follows; THP1 monocytes were grown with culture medium RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum

(FBS) (Gibco), GlutaMAX (Gibco) and 50 nM 2-Mercaptoethanol (Gibco). THP1 monocytes were then seeded in flat-bottom 96-well plates (Corning, NY, USA) and incubated with the culture medium added with or without 2 µg/ml of β-glucan, together with 10µM of test gapmer SEQ ID NO. 239, 240, or MOE negative control gapmer (SEQ ID NO. 106) for 24 hours at 37 °C. Cells were washed once with 200 µL warm PBS and incubated for 5 days in culture medium. Medium was changed once on day 3. On day 6, cells were restimulated with either 200 µl RPMI or 10 ng/ml of LPS (Serotype 055:B5; Sigma) for another 24h, and the gene expression of IL1B was quantified by RT-qPCR.

[151] Table 10. shows the gene expression of IL1B when THP1 monocytes were trained in the presence of gapmer compounds SEQ ID NO. 239, 240, or MOE negative control gapmer (SEQ ID NO. 106). Data are normalized by the expression of housekeeping gene RPL37A and represented as fold change relative to MOE negative control gapmer (SEQ ID NO. 106).

SEQ ID NO.	IL-1β EXPRESSION
239	5.8
240	0.9
106	1

TABLE 10.

[152] Table 10. shows a differential effect of gapmer compounds SEQ ID NO. 239 and 240 upon the gene expression of IL1B in the trained immunity of THP1 monocytes, in which gapmer compound SEQ ID NO. 239 induced 5.8-fold IL1B transcripts while gapmer compound SEQ ID NO. 240 slightly inhibited IL1B transcription in comparison to negative control gapmer (SEQ ID NO. 107).

Example 3: AMANZI inhibition in LPS stimulated CD14 positive monocytes

[153] Percoll monocytes from healthy donors (n=3) were seeded in serum-free RPMI (Gibco) containing 1% Pen/Strep (Gibco) at 1 x 10⁶ in a 24 well plate (Sarstedt) and incubated at 37 °C at 5% CO₂ for 1 hour. Plated cells were washed with serum-free RPMI and treated with RPMI (ss) + 10% pooled human serum (ss) containing SEQ 24 or SEQ ID 107 gapmer compounds and incubated at 37 °C at 5% CO₂ for overnight. Each treatment was conducted in duplicate. Plated cells were then treated with 10 ng/ml Ultrapure LPS (Invitrogen) and incubated at 37 °C at

5% CO₂ for overnight. Gapmer-treated cells were then centrifuged at 400 rpm for 5 min at room temperature and RNA was isolated using the MagMAX RNA Total RNA Isolation Kit (Thermo Fisher Scientific) in accordance with the manufacturer's recommended protocols. Total RNA was quantified using the NanoDrop® (Thermo Fisher Scientific) manufacturer's recommended protocols. Total RNA underwent a reverse transcriptase reaction (RT) using iScript cDNA synthesis kit (Bio-Rad) according to manufacturer's recommended reagents and protocols to produce complementary DNA used as substrate for quantitative real-time polymerase chain reaction (RT-qPCR). Quantification of target RNA levels was conducted by RT-qPCR with SsoAdvanced Universal SYBR Green according to manufacturer's recommended protocols, using a CFX Real-time PCR detection system (Bio Rad). The target quantities obtained by RT-qPCR were normalized using the expression of a stably expressed housekeeping gene, RPL37A.

[154] Table 11 displays the fold change of IL1B gene expression in LPS-treated monocytes (n=3 donors) in the presence of the gapmer compounds (SEQ ID NO 42). Data were normalized by the expression of housekeeping gene RPL37A and represented as fold change relative to LPS treated monocytes with control gapmer compound (SEQ ID 107). An expression value < 1.0 means that the transcription of that gene was inhibited, and an expression value > 1.0 means that the transcription of that gene was induced. For example, a value of 0.25 means that gene transcription was inhibited by 75%.

SEQ ID NO.	AVERAGE SEQ ID NO: 1 EXPRESSION	IL-1 β EXPRESSION
42	0.47	3.359
107	1	1

TABLE 11.

Example 4: AMANZI inhibition in PBMCs treated with LPS and R848

[155] Peripheral blood mononuclear cells (PBMCs) from healthy donors (n=4), with an average age of 56.3 ± 14.9 years, were purchased cryopreserved from CTL Europe. Cryopreserved PBMCs were thawed in pre-warmed Wash Media (RPMI (Dutch modification) + 20 % FBS + 2 mM GlutaMAX + 1 mM Sodium pyruvate + 1 % Pen/Strep). PBMCs were then washed in pre-warmed Wash Media three times before centrifugation in pre-warmed Wash Media at 500 rpm for 10 min at room temperature. Centrifugation was repeated twice before resuspending cells in pre-warmed Culture Media (RPMI (Dutch modification) + 10 % FBS + 2

mM GlutaMAX + 1 mM Sodium pyruvate + 1 % Pen/Strep). Next, cells were counted and seeded at approximately 1×10^5 cells and were added into each well of a 96-well U-bottom tissue culture plate (CellSTAR) containing a TLR cocktail containing 1 ug/mL Ultrapure LPS (Invitrogen) and 5 ug/mL Resiquimod (Invitrogen) with or without gapmer compounds (SEQ ID 111). Gapmer compounds were added at a low dose (5 μ M) or high dose (30 μ M). Each treatment was conducted in duplicate and plated cells were incubated at 37 °C at 5% CO₂ for 48 hours. Plated cells were then centrifuged at 400 rpm for 5 min at room temperature and RNA was isolated using the MagMAX RNA Total RNA Isolation Kit (Thermo Fisher Scientific) in accordance with the manufacturer's recommended protocols. Total RNA was then quantified using the NanoDrop® (Thermo Fisher Scientific) manufacturer's recommended protocols. Total RNA then underwent a reverse transcriptase reaction (RT) using an iScript cDNA synthesis kit (Bio-Rad), according to the manufacturer's recommended reagents and protocols, to produce complementary DNA. The complementary DNA was used as substrate for quantitative real-time polymerase chain reaction (RT-qPCR) using SsoAdvanced Universal SYBR Green in accordance with manufacturer's recommended protocols. Quantification of target RNA levels was conducted by RT-qPCR using a CFX Real-time PCR detection system (Bio-Rad). The target quantities obtained by RT-qPCR were normalized using the expression of a stably expressed housekeeping gene, RPL37A.

[156] Table 12 displays the fold change of IL1B gene expression in TLR-stimulated PBMCs in the presence of the gapmer compounds (SEQ ID 111). Data were normalized by expression of housekeeping gene RPL37A and represented a fold change relative to PBMCs treated with TLR cocktails without gapmer compounds. An expression value < 1.0 means that the transcription of that gene was inhibited, and an expression value >1.0 means that the transcription of that gene was induced. For example, a value of 0.25 means that gene transcription was inhibited by 75%.

SEQ ID NO.	DONOR ID	GAPMER DOSAGE	IL1B EXPRESSION
111	1	LOW	1.60
111	1	HIGH	0.80
111	2	LOW	1.72

111	2	HIGH	1.13
111	3	LOW	1.15
111	3	HIGH	2.13
111	4	LOW	1.47
111	4	HIGH	3.27

Table 12**Example 5: IL-1 β eRNA inhibition in prostatic adenocarcinoma cell line, PC-3**

[157] PC-3 cells (ATCC) were seeded at 6×10^4 per well in a 24-well plate (Sarstedt) and RPMI 1640 containing GlutaMAX (Gibco), 10% FBS (Gibco) and 1% Pen/Strep (Gibco) and gapmer compound SEQ ID 42 were added. Gapmer compounds were added at 200 nM. Each treatment was conducted in duplicate. Plated cells were incubated at 37 °C at 5% CO₂ for 8 hours. Gapmer-treated cells were then centrifuged at 400 rpm for 5 min at room temperature and RNA was isolated using the MagMAX RNA miRVana Total RNA Isolation Kit (Thermo Fisher Scientific) in accordance with the manufacturer's recommended protocols. Total RNA was quantified using the NanoDrop® (Thermo Fisher Scientific) manufacturer's recommended protocols. Total RNA underwent a reverse transcriptase reaction (RT) using an iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's recommended reagents and protocols to produce complementary DNA to be used as substrate for quantitative real-time polymerase chain reaction (RT-qPCR). Quantification of target RNA levels was conducted by RT-qPCR with SsoAdvanced Universal SYBR Green according to the manufacturer's recommended protocols using a CFX Real-time PCR detection system (Bio-Rad). The target quantities obtained by RT-qPCR were normalized using the expression of a stably expressed housekeeping gene, RPL37A.

[158] Table 13 displays the fold change of IL1B gene expression in PC-3 cells in the presence of the gapmer compounds. Data was normalized by the expression of housekeeping gene RPL37A and represented as fold change relative to untreated PC-3 cells. An expression value < 1.0 means that the transcription of that gene was inhibited, and an expression value > 1.0 means

that the transcription of that gene was induced. For example, a value of 0.25 means that gene transcription was inhibited by 75%.

SEQ ID NO.	IL-1 β EXPRESSION
42	1.51
107	1

Table 13

Example 6: IL-1 β eRNA inhibition in TNF- α treated pancreatic cancer cell line, MIA PaCa2

[159] MIA PaCa-2 cells (ATCC) were seeded in RPMI 1640 + 10% FBS + 1% Pen/Strep at 1×10^6 cells per well in a 24-well plate (Sarstedt) and incubated at 37 °C at 5% CO₂ for 24 hours. Plated cells were treated with media containing 10 ng/ml TNF and incubated at 37 °C at 5% CO₂ for 24 hours. Cells were then treated with media containing SEQ ID 111 or control SEQ ID 106 and incubated at 37 °C at 5% CO₂ for 24 hours. Each treatment was conducted in triplicate. Gapmer-treated cells were then centrifuged at 400 rpm for 5 min at room temperature and RNA was isolated using the MagMAX RNA Total RNA Isolation Kit (Thermo Fisher Scientific) in accordance to manufacturer's protocols. Total RNA was quantified using the NanoDrop® (Thermo Fisher Scientific) manufacturer's recommended protocols. Total RNA underwent a reverse transcriptase reaction (RT) using iScript cDNA synthesis kit (Bio-Rad), according to the manufacturer's recommended reagents and protocols, to produce complementary DNA. Complementary DNA was used as a substrate for quantitative real-time polymerase chain reaction (RT-qPCR). Quantification of target RNA levels was conducted by RT-qPCR with SsoAdvanced Universal SYBR Green according to manufacturer's recommended protocols using a CFX Real-time PCR detection system (Bio-Rad). The target quantities obtained by RT-qPCR were normalized using the expression of a stably expressed housekeeping gene, RPL37A.

[160] Table 14 displays the fold change of IL1B gene expression in TNF α -treated MIA PaCa-2 cells in the presence of the gapmer compounds (SEQ ID NO 111). Data were normalized by the expression of housekeeping gene RPL37A and represented as fold change relative to TNF α treated cells with control gapmer compounds (SEQ ID 106). An expression

value < 1.0 means that the transcription of that gene is inhibited, and an expression value > 1.0 means that the transcription of that gene is induced. For example, a value of 0.25 means that gene transcription was inhibited by 75%.

SEQ ID NO.	IL-1 β EXPRESSION
111	2.05
106	1

Table 14

[161] This disclosure provides for the following embodiments:

[162] A1. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, comprising (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to a 14-25 base region on AMANZI (SEQ ID NO. 1).

[163] A2. The gapmer compound that specifically inhibits AMANZI of embodiment 1, wherein the gapmer nucleotide bases are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[164] A3. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, comprising (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to Region A of AMANZI (SEQ ID NO. 1 bases 10 to 93).

[165] A4. The gapmer type of ASO that can inhibit AMANZI of embodiment 3, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NOs. 41-46 or an 8 mer fragment thereof.

[166] A5. The gapmer type of ASO that can inhibit AMANZI of embodiment 3, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[167] A6. The gapmer type of ASO that can inhibit AMANZI of embodiment 3, wherein Region A of AMANZI is SEQ ID NO. 1 base 10 to base 93.

[168] A7. The gapmer type of ASO that can inhibit AMANZI of embodiment 3, wherein the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 41, SEQ ID NO. 42, SEQ ID NO. 43, SEQ ID NO. 44, SEQ ID NO. 45, SEQ ID NO. 46, and combinations thereof.

[169] A8. The gapmer type of ASO that can inhibit AMANZI of embodiment 7, wherein the gapmer type of ASO is SEQ ID NO. 42.

[170] A9. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to Region B of AMANZI (SEQ ID NO. 1 bases 194 to 253).

[171] A10. The gapmer type of ASO that can inhibit AMANZI of embodiment 9, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NO. 78, SEQ ID NO. 81, SEQ ID NO. 82, SEQ ID NO. 83, SEQ ID NO. 84, and combinations thereof, or an 8 mer fragment thereof.

[172] A11. The gapmer type of ASO that can inhibit AMANZI of embodiment 9, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[173] A12. The gapmer type of ASO that can inhibit AMANZI of embodiment 9, wherein Region B of AMANZI is SEQ ID NO. 1 base 194 to base 253.

[174] A13. The gapmer type of ASO that can inhibit AMANZI of embodiment 9, wherein the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 78, SEQ ID NO. 81, SEQ ID NO. 82, SEQ ID NO. 83, SEQ ID NO. 84, and combinations thereof.

[175] A14. The gapmer type of ASO that can inhibit AMANZI of embodiment 13, wherein the gapmer type of ASO is SEQ ID NO. 84.

[176] A15. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to Region C of AMANZI (SEQ ID NO. 1 bases 519 to 568).

[177] A16. The gapmer type of ASO that can inhibit AMANZI of embodiment 15, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 69, SEQ ID NO. 101, SEQ ID NO. 102, SEQ ID NO. 103, SEQ ID NO. 104, and combinations thereof, or an 8 mer fragment thereof.

[178] A17. The gapmer type of ASO that can inhibit AMANZI of embodiment 15, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[179] A18. The gapmer type of ASO that can inhibit AMANZI of embodiment 15, wherein Region C of AMANZI is SEQ ID NO. 1 base 519 to base 568.

[180] A19. The gapmer type of ASO that can inhibit AMANZI of embodiment 15, wherein the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 69, SEQ ID NO. 101, SEQ ID NO. 102, SEQ ID NO. 103, SEQ ID NO. 104, and combinations thereof.

[181] A20. The gapmer type of ASO that can inhibit AMANZI of embodiment 15, wherein the gapmer type of ASO is SEQ ID NO. 101.

[182] A21. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region D of AMANZI (SEQ ID NO. 1 bases 377 to 404).

[183] A22. The gapmer type of ASO that can inhibit AMANZI of embodiment 21, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NO. 223, SEQ ID NO. 224, SEQ ID NO. 225, SEQ ID NO. 226, SEQ ID NO. 227, SEQ ID NO. 228, SEQ ID NO. 229, and combinations thereof, or an 8 mer fragment thereof.

[184] A23. The gapmer type of ASO that can inhibit AMANZI of embodiment 21, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[185] A24. The gapmer type of ASO that can inhibit AMANZI of embodiment 21, wherein Region D of AMANZI is SEQ ID NO. 1 base 377 to base 404.

[186] A25. The gapmer type of ASO that can inhibit AMANZI of embodiment 21, wherein the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 223, SEQ ID NO. 224, SEQ ID NO. 225, SEQ ID NO. 226, SEQ ID NO. 227, SEQ ID NO. 228, SEQ ID NO. 229, and combinations thereof.

[187] A26. The gapmer type of ASO that can inhibit AMANZI of embodiment 21, wherein the gapmer type of ASO is SEQ ID NO. 226.

[188] A27. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region E of AMANZI (SEQ ID NO. 1 bases 574 to 615).

[189] A28. The gapmer type of ASO that can inhibit AMANZI of embodiment 27, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NO. 201, SEQ ID NO. 202, SEQ ID NO. 203, SEQ ID NO. 204, SEQ ID NO. 205, SEQ ID NO. 206, SEQ ID NO. 207, SEQ ID NO. 208, and combinations thereof, or an 8 mer fragment thereof.

[190] A29. The gapmer type of ASO that can inhibit AMANZI of embodiment 27, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from

the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

[191] A30. The gapmer type of ASO that can inhibit AMANZI of embodiment 27, wherein Region E of AMANZI is SEQ ID NO. 1 base 574 to base 615.

[192] A31. The gapmer type of ASO that can inhibit AMANZI of embodiment 27, wherein the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 201, SEQ ID NO. 202, SEQ ID NO. 203, SEQ ID NO. 204, SEQ ID NO. 205, SEQ ID NO. 206, SEQ ID NO. 207, SEQ ID NO. 208, and combinations thereof.

[193] A32. The gapmer type of ASO that can inhibit AMANZI of embodiment 27, wherein the gapmer type of ASO is SEQ ID NO. 207.

[194] All patents, publications, scientific articles, web sites, and other documents and materials referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced document and material is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such patents, publications, scientific articles, web sites, electronically available information, and other referenced materials or documents. Reference to any applications, patents and publications in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

[195] The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. Thus, for example, in each instance herein, in embodiments or examples of the present invention, any of the terms “comprising”, “consisting essentially of”, and “consisting of” may be replaced with either of the other two terms in the specification. Also, the terms “comprising”, “including”, “containing”, etc. are to be read expansively and without limitation. The methods and processes illustratively described herein

suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. It is also that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants. Furthermore, titles, headings, or the like are provided to enhance the reader's comprehension of this document, and should not be read as limiting the scope of the present invention. Any examples of aspects, embodiments or components of the invention referred to herein are to be considered non-limiting.

[196] The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[197] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[198] Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

CLAIMS

We claim:

1. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, comprising (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to a 14-25 base region on AMANZI (SEQ ID NO. 1).
2. The gapmer compound that specifically inhibits AMANZI of claim 1, wherein the gapmer nucleotide bases are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.
3. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, comprising (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary to Region A of AMANZI (SEQ ID NO. 1 bases 12 to 92).
4. The gapmer type of ASO that can inhibit AMANZI of claim 3, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NOs. 2-105 or an 8 mer fragment thereof.
5. The gapmer type of ASO that can inhibit AMANZI of claim 3, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.
6. The gapmer type of ASO that can inhibit AMANZI of claim 3, wherein Region A of AMANZI is SEQ ID NO. 1 base 12 to base 27.
7. The gapmer type of ASO that can inhibit AMANZI of claim 3, wherein the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 41, SEQ ID NO.

42, SEQ ID NO. 43, SEQ ID NO. 44, SEQ ID NO. 45, SEQ ID NO. 46, and combinations thereof.

8. The gapmer type of ASO that can inhibit AMANZI of claim 7, wherein the gapmer type of ASO is SEQ ID NO. 42.

9. The gapmer type of ASO that can inhibit AMANZI of claim 3, wherein (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region B of AMANZI (SEQ ID NO. 1 bases 519 to 568).

10. The gapmer type of ASO that can inhibit AMANZI of claim 9, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NO. 63, SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 101, SEQ ID NO. 102, SEQ ID NO. 103, SEQ ID NO. 104, and combinations thereof, or an 8 mer fragment thereof.

11. The gapmer type of ASO that can inhibit AMANZI of claim 9, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

12. The gapmer type of ASO that can inhibit AMANZI of claim 9, wherein the gapmer type of ASO is selected from the group consisting of SEQ ID NO. 63, SEQ ID NO. 63, SEQ ID NO. 64, SEQ ID NO. 66, SEQ ID NO. 67, SEQ ID NO. 101, SEQ ID NO. 102, SEQ ID NO. 103, SEQ ID NO. 104, and combinations thereof.

13. The gapmer type of ASO that can inhibit AMANZI of claim 12, wherein the gapmer type of ASO is SEQ ID NO. 101.

14. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region D of AMANZI (SEQ ID NO. 1 bases 377 to 404).

15. The gapmer type of ASO that can inhibit AMANZI of claim 14, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NOs 228-234, and combinations thereof, or an 8 mer fragment thereof.

16. The gapmer type of ASO that can inhibit AMANZI of claim 14, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

17. A gapmer type of ASO (antisense oligonucleotide) that can inhibit AMANZI transcriptional activity, wherein the gapmer comprises (a) from about 14 to about 25 nucleotide bases; (b) a 3' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; (c) a gap region having from at least 8 DNA bases to no more than 19 DNA bases; and (d) a 5' wing region (3' to 5') having from 3 to 7 chemically modified RNA bases; wherein the gapmer is substantially complementary to substantially complementary Region E of AMANZI (SEQ ID NO. 1 bases 574 to 615).

18. The gapmer type of ASO that can inhibit AMANZI of claim 17, wherein the gap region comprises a 10-nucleotide DNA sequence from nucleotide 5 to nucleotide 15 from any of SEQ ID NOs. 206-215, and combinations thereof, or an 8 mer fragment thereof.

19. The gapmer type of ASO that can inhibit AMANZI of claim 17, wherein the gapmer nucleotides are each linked by phosphorothiolate (P=S) internucleotide bonds throughout the gapmer; and wherein the modified nucleotide base modifications are selected from the group consisting of 2'-methoxyethyl (MOE) nucleotides, locked nucleic acid nucleotides (LNA), and combinations thereof.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/059749

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/113

ADD. A61K31/7088

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)

C12N A61K

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RÉBÉ CÉDRIC ET AL: "Interleukin-1[beta] and Cancer", CANCERS, vol. 12, no. 7, 4 July 2020 (2020-07-04), page 1791, XP093235703, CH ISSN: 2072-6694, DOI: 10.3390/cancers12071791 page 2 ----- - / -	1 - 19

☒ 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

"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

20 February 2025

Date of mailing of the international search report

06/03/2025

Name and mailing address of the ISA/

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Authorized officer

Piret, Bernard

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2024/059749

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LU JIAWEI ET AL: "A Potential Suppressive Effect of Natural Antisense IL-1[beta] RNA on Lipopolysaccharide-Induced IL-1[beta] Expression", JOURNAL OF IMMUNOLOGY, vol. 190, no. 12, 15 June 2013 (2013-06-15), pages 6570-6578, XP093235423, ISSN: 0022-1767, DOI: 10.4049/jimmunol.1102487 Retrieved from the Internet: URL:https://watermark.silverchair.com/1102487.pdf?token=AQECAHi208BE49Ooan9kKhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAABb0wggW5BgkqhkiG9w0BBwagggWqMIIFpgIBADCCBZ8GCSqGSib3DQEHATAeBgglghkgBZQMEAS4wEQQMseiI6XWeNcW5XvUqAgEQgIIFcG0uC7yLeEeXGYmqDyGNuQ4vsBInkmTjOUwRpQEGwXiaDeUlgwkyVmaoF-hXjuLTwJ1ncVcgIUJA8lnTErhhHrHoKRxbX> -----	1-19
A	Roux Benoit T. ET AL: "Knockdown of Nuclear-Located Enhancer RNAs and Long ncRNAs Using Locked Nucleic Acid Gapmers : Methods and Protocols" In: "Enhancer RNAs : Methods and Protocols", 24 September 2016 (2016-09-24), Springer New York, New York, NY, XP093232877, ISSN: 1064-3745 ISBN: 978-1-4939-4035-6 vol. 1468, pages 11-18, DOI: 10.1007/978-1-4939-4035-6_2, Retrieved from the Internet: URL:http://link.springer.com/10.1007/978-1-4939-4035-6_2> -----	1-19
X,P	FOK EZIO T. ET AL: "A chromatin-regulated biphasic circuit coordinates IL-1[beta]-mediated inflammation", NATURE GENETICS, vol. 56, no. 1, 13 December 2023 (2023-12-13), pages 85-99, XP093233368, New York ISSN: 1061-4036, DOI: 10.1038/s41588-023-01598-2 Retrieved from the Internet: URL:https://www.nature.com/articles/s41588-023-01598-2> the whole document -----	1-19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2024/059749

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: