SUMMARY

[0006] Provided herein are compositions, methods and kits useful for modulating expression of target genes in the Toll-like receptor pathway. In various embodiments provided are nucleic acid molecule inhibitors of a mammalian gene selected from the group consisting of TLR2, TLR4, MYD88, TICAM1 and TIRAP, having mRNA polynucleotide sequences set forth in SEQ ID NOS: 1-12 which include SEQ ID NO:1 (TLR2 mRNA); SEQ ID NO:2-4 (TLR4 mRNA), SEQ ID NO:5-9 (MYD88 mRNA), SEQ ID NO:10 (TICAM1 mRNA) or SEQ ID NO:11-12 (TIRAP mRNA).

[0007] In particular embodiments provided herein are novel double stranded nucleic acid molecules, in particular double-stranded RNA (dsRNA), that inhibit, down-regulate or reduce expression of a gene selected from the group consisting of TLR2, TLR4, MYD88, TICAM1 and TIRAP, and pharmaceutical compositions comprising one or more such oligonucleotides or a vector capable of expressing the oligonucleotide. Further provided herein are methods for treating inflammation and inflammatory diseases and graft rejection associated with organ transplantation, such as lung transplantation, in which expression of one or more of the TLR2, TLR4, MYD88, TICAM1 and TIRAP genes is associated with the etiology or progression of inflammation and graft rejection associated with organ transplantation.

[0007a] In one aspect, there is provided a double stranded nucleic acid molecule comprising a sense strand and an antisense strand described as the sequence pair set forth as TLR4_14 (SEQ ID NOS: 20624 and 20633, respectively), and having the following structure:

(A2) 5' N1-(N)x - Z 3' (antisense strand)

3' Z'-N2-(N')y –z" 5' (sense strand)

wherein each N2, N and N' is an unmodified or modified ribonucleotide, or an unconventional moiety;

wherein each of (N)x and (N')y is an oligonucleotide in which each consecutive N or N' is joined to the adjacent N or N' by a covalent bond;

wherein each of x and y is independently an integer between 17 and 24;

wherein the sequence of (N')y is complementary to the sequence of (N)x and (N)x is complementary to a consecutive sequence in a target RNA set forth in any one of SEQ ID NO:2-4 (TLR4 mRNA);

wherein N1 is an unmodified or modified ribonucleotide covalently bound to (N)x and mismatched to the target RNA;

wherein z" is a capping moiety covalently attached at the 5' terminus of N2- (N')y;

wherein each of Z and Z' is independently 1-5 consecutive nucleotides, consecutive non-nucleotide moieties or a combination thereof covalently attached at the 3' terminus of the strand in which it is present.

[0008] In some embodiments the double stranded oligonucleotides are chemically modified dsRNA compounds. In some embodiments the dsRNA sense and antisense oligonucleotides are selected from sense oligonucleotides and corresponding antisense oligonucleotides set forth in SEQ ID NOS:13-5846 (targeting TLR2), SEQ ID NOS:5847-12144 (targeting TLR4), SEQ ID NOS:12145-16332 (targeting MYD88), SEQ ID NOS:16333-18242 (targeting TICAM1) and SEQ ID NOS:18243-20606 (targeting TIRAP) .

[0009] Accordingly, in one embodiment provided herein is a nucleic acid molecule having the following double-stranded Structure:

(A1) 5' (N)x – Z 3' (antisense strand)

3' Z'-(N')y –z" 5' (sense strand)

acute aseptic inflammation, neuropathic pain, primary graft failure, ischemia-reperfusion injury, reperfusion injury, reperfusion edema, allograft dysfunction, pulmonary reimplantation response and/or primary graft dysfunction (PGD) in organ transplantation, such as lung transplantation.

[00101a] In one example, there is provided the use of a double stranded nucleic acid molecule as defined herein or a pharmaceutical composition as defined herein in the manufacture of a medicament for the treatment of a subject in need of treatment for a disease or disorder or symptom or condition associated with the expression of a target gene, wherein the nucleic acid molecule or composition is in an amount effective to down regulate gene expression, wherein the gene encodes an RNA having a polynucleotide sequence as set forth in any one of SEQ ID NO:2-4 (TLR4 mRNA).

[00101b] In one example, there is provided the use of a double stranded nucleic acid molecule as defined herein or a pharmaceutical composition as defined herein in the manufacture of a medicament for the treatment of a subject in need of treatment for a posttransplantational disease or injury selected from the group consisting of: primary graft failure, ischemia-reperfusion injury, reperfusion injury, reperfusion edema, allograft dysfunction, pulmonary reimplantation response and primary graft dysfunction (PGD) in organ transplantation.

[00101c] In one example, there is provided the use of a double stranded nucleic acid molecule as defined herein or a pharmaceutical composition as defined herein in preparing a medicament for the treatment of a posttransplantational disease or injury selected from the group consisting of: primary graft failure, ischemia-reperfusion injury, reperfusion injury, reperfusion edema, allograft dysfunction, pulmonary reimplantation response and primary graft dysfunction (PGD) in organ transplantation, wherein when said medicament is being administered, said composition is in an amount effective to down regulate target gene expression, wherein the target gene encodes an RNA having a polynucleotide sequence set forth in any one of SEQ ID NO:2-4 (TLR4 mRNA).

[00101d] In one example, there is provided the use of a double stranded nucleic acid molecule as defined herein or a pharmaceutical composition as defined herein in preparing a medicament for treating or preventing the incidence or severity of a posttransplantational complication selected from the group consisting of primary graft failure, ischemia-reperfusion injury, reperfusion injury, reperfusion edema, allograft dysfunction, pulmonary reimplantation response, primary graft dysfunction (PGD), in a subject in need thereof wherein the complication is associated with expression of a gene encoding an RNA having a polynucleotide sequence set forth in any one of SEQ ID NO:2-4 (TLR4 mRNA).

[00102] In other embodiments the disclosure relates to methods for treating or preventing the incidence or severity of a posttransplantational (e.g. following lung transplantation) complication selected from, without being limited to, primary graft failure, ischemia-reperfusion injury, reperfusion injury, reperfusion edema, allograft dysfunction, pulmonary reimplantation response and/or primary graft dysfunction (PGD), in a subject in need thereof wherein the complication is associated with expression of a gene selected from a gene set forth in Table A1. Such methods involve administering to a mammal in need of such treatment a prophylactically or therapeutically effective amount of one or more double stranded molecules disclosed herein to inhibit or reduce expression or activity of at least one such gene. In other embodiments the disclosure relates to methods for reducing acute or chronic inflammation in a subject in need thereof wherein the inflammation is associated with expression of a gene selected from a gene set forth in Table A1. Such methods involve

CLAIMS

1. A double stranded nucleic acid molecule comprising a sense strand and an antisense strand described as the sequence pair set forth as TLR4_14 (SEQ ID NOS: 20624 and 20633, respectively), and having the following structure:

(A2) 5' N1-(N)x - Z 3' (antisense strand)

3' Z'-N2-(N')y –z" 5' (sense strand)

wherein each N2, N and N' is an unmodified or modified ribonucleotide, or an unconventional moiety;
wherein each of (N)x and (N')y is an oligonucleotide in which each consecutive N or N' is joined to the adjacent N or N' by a covalent bond;
wherein each of x and y is independently an integer between 17 and 24;
wherein the sequence of (N')y is complementary to the sequence of (N)x and (N)x is complementary to a consecutive sequence in a target RNA set forth in any one of SEQ ID NO:2-4 (TLR4 mRNA);
wherein N1 is an unmodified or modified ribonucleotide covalently bound to (N)x and mismatched to the target RNA;
wherein z" is a capping moiety covalently attached at the 5' terminus of N2- (N')y;
wherein each of Z and Z' is independently 1-5 consecutive nucleotides, consecutive non-nucleotide moieties or a combination thereof covalently attached at the 3' terminus of the strand in which it is present.

2. The double stranded nucleic acid molecule of claim 1, wherein at least one of N1, N2, N or N' comprises a modified nucleotide or an unconventional moiety.

3. The double stranded nucleic acid molecule of claim 2, wherein N1-(N)x comprises at least one pyrimidine ribonucleotide and wherein at least one of the pyrimidine ribonucleotides in N1-(N)x comprises a 2' sugar modified pyrimidine ribonucleotide.

4. The double stranded nucleic acid molecule of claim 3, wherein the 2' sugar modified ribonucleotide comprises a 2'-OMe sugar modified ribonucleotide.

5. A pharmaceutical composition comprising the double stranded nucleic acid molecule of any one of claims 2 to 4 in an amount effective to inhibit gene expression, and a pharmaceutically acceptable carrier, wherein the gene encodes an RNA having a polynucleotide sequence set forth in any one of SEQ ID NO:2-4 (TLR4 mRNA).