Only a very limited number of aptamers which could be used to regulate TLR 9 activation in, for example, autoimmune diseases is available at present and their effectiveness and harmlessness for the application in human patients remains to be confirmed. The availability of additional oligonucleotide sequences which inhibit or suppress TLR 9 activation is therefore desired.

Accordingly, it is an object of the present invention to provide new antagonists for the use in therapy of a subject by inhibiting or suppressing the activation of TLR9 in a cell.

Furthermore, it is another object of the present invention to provide a method for inhibiting or suppressing TLR9 activation employing new TLR9 antagonists.

It is also an object of the present invention to provide a pharmaceutical composition and a kit comprising new TLR 9 antagonists.

It is a further object of the present invention to provide a use of novel TLR 9 antagonists for inhibiting or suppressing TLR9 activation.

Summary

This object is solved by the aspects of the present disclosure as specified hereinafter.

In one aspect, the present disclosure refers to use of an aptamer in the manufacture of a medicament for inhibiting or suppressing the activation of TLR9 in a cell in a subject, wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1, wherein the subject is having a disorder selected from an autoimmune disorder, an inflammatory disorder, an autoimmune connective tissue disease (ACTD) and/or a neurodegenerative disorder, preferably the disorder is selected from psoriasis, rheumatoid arthritis, alopecia universalis, acute disseminated encephalomyelitis, Addison's disease, allergy, ankylosing spondylitis, antiphospholipid antibody syndrome, arteriosclerosis, atherosclerosis, autoimmune hemolytic anemia, autoimmune hepatitis, Bullous pemphigoid, Chagas' disease, chronic obstructive pulmonary disease, coeliac disease, cutaneous lupus erythematosus (CLE), dermatomyositis, diabetes, dilated cardiomyopathy (DCM), endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease,

hidradenitis suppurativa, idiopathic thrombocytopenic purpura, inflammatory bowel disease, interstitial cystitis, morphea, multiple sclerosis (MS), myasthenia gravis, myocarditis, narcolepsy, neuromyotonia, pemphigus, pernicious anaemia, polymyositis, primary biliary cirrhosis, rheumatoid arthritis (RA), schizophrenia, Sjogren's syndrome, systemic lupus erythematosus (SLE), systemic sclerosis, temporal arteritis, vasculitis, vitiligo, vulvodynia, Wegener's granulomatosis, traumatic pain, neuropathic pain and acetaminophen toxicity, alternatively wherein the subject is having tumour/cancer, preferably wherein the tumour/cancer is selected from the group consisting of breast cancer, cervical squamous cell carcinoma, gastric carcinoma, glioma, hepatocellular carcinoma, lung cancer, melanoma, prostate cancer, recurrent glioblastoma, recurrent non-Hodgkin lymphoma, colorectal cancer

In another aspect, the present disclosure refers to a method of inhibiting or suppressing the activation of TLR9 in a cell comprising contacting a cell expressing TLR9 with an aptamer, wherein the method is carried out *in vitro/ex vivo* and wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

In yet another aspect, the present disclosure refers to pharmaceutical composition comprising an aptamer and at least one pharmaceutically acceptable excipient, wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

In yet another aspect, the present disclosure refers to kit comprising at least one aptamer and a container, wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

In yet another aspect, the present disclosure refers to the aptamer when used in inhibiting or suppressing TLR9 activation, wherein the aptamer is used *in vitro/ex vivo*, wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

According to the first example of the present disclosure, an aptamer is provided for use in therapy of a subject by inhibiting or suppressing the activation of TLR9 in a cell.

In a preferred example of the first example of the disclosure, the subject is a mammal, preferably the subject is a human.

In another preferred example of the first example of the disclosure, the cell to be contacted and/or the subject to be treated is showing TLR9 overexpression and/or overactivity of TLR9-mediated signaling.

In yet another preferred example of the first example of the disclosure, the cell and/or subject to be treated is tested for TLR9 overexpression and/or overactivity of TLR9-mediated signaling before inhibiting the activation of TLR 9 in the cell and/or subject.

In a preferred example of the first example of the disclosure, the cell is a glial cell, microglial cell, astrocyte, macrophage, B cell and/or dendritic cell, preferably plasmacytoid dendritic cell.

In another preferred example of the first example of the disclosure, the subject is having a disorder selected from an autoimmune disorder, an inflammatory disorder, an autoimmune connective tissue disease (ACTD) and/or a neurodegenerative disorder, preferably the disorder is selected from but not limited to psoriasis, rheumatoid arthritis, alopecia universalis, acute disseminated encephalomyelitis, Addison's disease, allergy, ankylosing spondylitis, antiphospholipid antibody syndrome, arteriosclerosis, atherosclerosis, autoimmune hemolytic anemia, autoimmune hepatitis, Bullous pemphigoid, Chagas' disease, chronic obstructive pulmonary disease, coeliac disease, cutaneous lupus erythematosus (CLE), dermatomyositis, diabetes, dilated cardiomyopathy (DCM), endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hidradenitis suppurativa, idiopathic thrombocytopenic purpura, inflammatory bowel disease, interstitial cystitis, morphea, multiple sclerosis (MS), myasthenia gravis, myocarditis, narcolepsy, neuromyotonia, pemphigus, pernicious anaemia, polymyositis, primary biliary cirrhosis, rheumatoid arthritis (RA), schizophrenia, Sjogren's syndrome, systemic lupus erythematosus (SLE), systemic sclerosis, temporal arteritis, vasculitis, vitiligo, vulvodynia, Wegener's granulomatosis, traumatic pain, neuropathic pain and acetaminophen toxicity.

In a preferred example of the first example of the disclosure, the subject is having tumour/cancer, preferably the tumour/cancer is selected from the group consisting of breast cancer, cervical squamous cell carcinoma, gastric carcinoma, glioma, hepatocellular carcinoma, lung cancer, melanoma, prostate cancer, recurrent glioblastoma, recurrent non-Hodgkin lymphoma, colorectal cancer.

In one preferred example of the first example of the disclosure, the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

According to the second example of the present disclosure, a method of inhibiting or suppressing the activation of TLR9 in a cell is provided comprising contacting a cell expressing TLR9 with an aptamer.

In a preferred example of the second example of the disclosure, the method is carried out *in vitro/ex vivo*.

In another preferred example of the second example of the disclosure, the method additionally comprises a previous step of testing the cell for TLR9 overexpression and/or overactivity of TLR9-mediated signaling.

In a preferred example of the second example of the disclosure, the cell is a mammalian cell, preferably the cell is a human cell.

In one preferred example of the second example of the disclosure, the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

According to the third example of the present disclosure, a pharmaceutical composition is provided comprising an aptamer for use according to the first example of the present disclosure and at least one pharmaceutically acceptable excipient.

According to the fourth example of the present disclosure, a kit is provided comprising at least one aptamer for use according to the first example of the present disclosure and a container.

According to the fifth example of the present disclosure, the use of the aptamer as defined herein for inhibiting or suppressing TLR9 activation is provided.

In a preferred example of the fifth example of the disclosure, the aptamer is used *in vitro/ex vivo*.

Description of Figures

Figure 1 shows the inhibition of TLR 9 activation in ODN 2006 (TLR 9 agonist)-stimulated HEK-Blue™ hTLR9 cells by adding increasing concentrations (in µM) of the aptamer of SEQ ID No. 1 (grey columns). Potential intrinsic effects of increasing concentrations of the aptamer of SEQ ID No. 1 on TLR 9 activation in the absence of stimulation by the TLR 9 agonist ODN 2006 (SEQ ID No. 2) were examined and are shown as black bars. Changes of optical density are caused by TLR 9-activated expression of an alkaline phosphatase.

Figure 2 shows effects of increasing concentrations of the aptamer of SEQ ID No. 1 (in µM) on HEK-Blue™ TNF-α cells stimulated with 100 ng of TNF-α (grey columns) and in the absence of stimulation by TNF-α (black columns). Changes of optical density are caused by TNF-α-activated expression of an alkaline phosphatase.

Claims

1. Use of an aptamer in the manufacture of a medicament for inhibiting or suppressing the activation of TLR9 in a cell in a subject, wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1, wherein the subject is having a disorder selected from an autoimmune disorder, an inflammatory disorder, an autoimmune connective tissue disease (ACTD) and/or a neurodegenerative disorder, preferably the disorder is selected from psoriasis, rheumatoid arthritis, alopecia universalis, acute disseminated encephalomyelitis, Addison's disease, allergy, ankylosing spondylitis, antiphospholipid antibody syndrome, arteriosclerosis, atherosclerosis, autoimmune hemolytic anemia, autoimmune hepatitis, Bullous pemphigoid, Chagas' disease, chronic obstructive pulmonary disease, coeliac disease, cutaneous lupus erythematosus (CLE), dermatomyositis, diabetes, dilated cardiomyopathy (DCM), endometriosis, Goodpasture's syndrome, Graves' disease, Guillain-Barre syndrome, Hashimoto's disease, hidradenitis suppurativa, idiopathic thrombocytopenic purpura, inflammatory bowel disease, interstitial cystitis, morphea, multiple sclerosis (MS), myasthenia gravis, myocarditis, narcolepsy, neuromyotonia, pemphigus, pernicious anaemia, polymyositis, primary biliary cirrhosis, rheumatoid arthritis (RA), schizophrenia, Sjogren's syndrome, systemic lupus erythematosus (SLE), systemic sclerosis, temporal arteritis, vasculitis, vitiligo, vulvodynia, Wegener's granulomatosis, traumatic pain, neuropathic pain and acetaminophen toxicity, alternatively wherein the subject is having tumour/cancer, preferably wherein the tumour/cancer is selected from the group consisting of breast cancer, cervical squamous cell carcinoma, gastric carcinoma, glioma, hepatocellular carcinoma, lung cancer, melanoma, prostate cancer, recurrent glioblastoma, recurrent non-Hodgkin lymphoma, colorectal cancer.

2. The use according to claim 1, wherein the subject is a mammal, preferably wherein the subject is a human.

3. The use according to any of the preceding claims, wherein the subject to be treated is showing TLR9 overexpression and/or overactivity of TLR9-mediated signaling.

4. The use according to any of the preceding claims, wherein the subject to be treated is tested positive for TLR9 overexpression and/or overactivity of TLR9-mediated signaling.

5. The use according to any of claims 3 or 4, wherein TLR9 overexpression and/or overactivity of TLR9-mediated signalling in a subject is given if the TLR9-mediated production of TNFα is increased over a reference value for healthy subjects.

6. The use according to claim 5, wherein the value for the subject to be treated is determined using the method described in the description and is at least 50 pg/ml, preferably at least 75 pg/ml, more preferably at least 100 pg/ml, most preferably at least 125 pg/ml.

7. A method of inhibiting or suppressing the activation of TLR9 in a cell comprising contacting a cell expressing TLR9 with an aptamer, wherein the method is carried out *in vitro/ex vivo* and wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

8. The method of claim 7, wherein the method additionally comprises a previous step of testing the cell for TLR9 overexpression and/or overactivity of TLR9-mediated signaling.

9. The method according to any of claims 7 or 8, wherein the cell is a mammalian cell, preferably wherein the cell is a human cell.

10. Pharmaceutical composition comprising an aptamer and at least one pharmaceutically acceptable excipient, wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

11. Kit comprising at least one aptamer and a container, wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.

12. The aptamer when used in inhibiting or suppressing TLR9 activation, wherein the aptamer is used *in vitro/ex vivo*, wherein the aptamer comprises a nucleic acid sequence of SEQ ID No. 1 (GGT TGG TGT GGT TGG) and/or a nucleic acid sequence being at least 80% identical to SEQ ID No. 1.