(12) UK Patent Application (19) GB (11) 2542425 (13) A

(43) Date of A Publication 22.03.2017

(21) Application No: 1516676.2

(22) Date of Filing: 21.09.2015

(71) Applicant(s):
Mologen AG
(Incorporated in the Federal Republic of Germany)
Fabeckstraße 30, Berlin 14195, Germany

(72) Inventor(s):
Matthias Schroff
Manuel Schmidt
Kerstin Kapp
Alfredo Zurio
Ole Schmeltz Sogaard
Martin Toistrup
Rasmus Offersen

(74) Agent and/or Address for Service:
24IP Law Group Sonnenberg Fortmann
Charlottenstraße 80, 10117, Berlin, Germany

(51) INT CL:
C12N 15/117 (2010.01) ***A61K 39/39*** (2006.01)
A61P 31/18 (2006.01)

(56) Documents Cited:
GB 2523187 A **WO 2012/085282 A1**
WO 2010/039137 A1 **WO 2005/080567 A1**
WO 2001/000232 A2 **US 20100303803 A1**
EU CLINICAL TRIALS REGISTER, EudraCT Number:2014-005634-59, Aarhus University Hospital, trial first available on the database 7 April 2015, "Toll-like receptor 9 enhancement of antiviral immunity in chronic HIV-1 infection: a phase 1b/2a trial (TEACH)" ClinicaTrials.gov website, trial number NCT02443935, "Toll-like Receptor 9 Agonist Treatment in Chronic HIV-1 Infection (TEACH)", published 13 May 2015
Towards an HIV Cure Symposium, 18 &19 July 2015, R Offersen, et al., "A novel TLR-9 agonist (MGN1703) activates NK-cells and enhances NK-cell mediated viral killing of HIV-1 infected CD4+ T cells ex vivo", PE40, page 68.
Critical Reviews in Oncology/Hematology, Vol. 94, B Wittig, et al., epub Dec. 19 2014, "MGN1703, an immunomodulator and toll-like receptor 9 (TLR-9) agonist: From bench to bedside", pages 31-44.
Nucleic Acid Ther., Vol. 25, No. 3, epub 31 Mar. 2015, M Schmidt, et al., "Design and Structural Requirements of the Potent and Safe TLR-9 Agonistic Immunomodulator MGN1703.", pages 130-140.

(58) Field of Search:
INT CL **A61K, A61P, C12N**
Other: **EPODOC, WPI, BIOSIS, MEDLINE, INTERNET, BLASTn**

(54) Title of the Invention: **Means for the treatment of HIV**
Abstract Title: **CG containing sequences of deoxyribonucleic acids for use in treating HIV**

(57) The invention relates to a non-coding sequence of deoxyribonucleic acids comprising at least one sequence motif NNCGNN, wherein N is a nucleotide comprising A, C, T, or G, and C is deoxycytidine, G is deoxyguanosine, A is deoxyadenosine and T is deoxythymidine for the treatment of viral infections. In particular, the non-coding sequence of deoxyribonucleic acids is used in combination with antiretroviral therapy and/or histone de-acetylase inhibitors. The CG DNA molecules may be dumbbell shaped, and may be the construct MGN1703 (also known as dSLIM). The use of the aforementioned CG containing DNA molecules as an adjuvant in therapeutic vaccination for the treatment of HIV is also claimed.

GB 2542425 A

Figure 1A

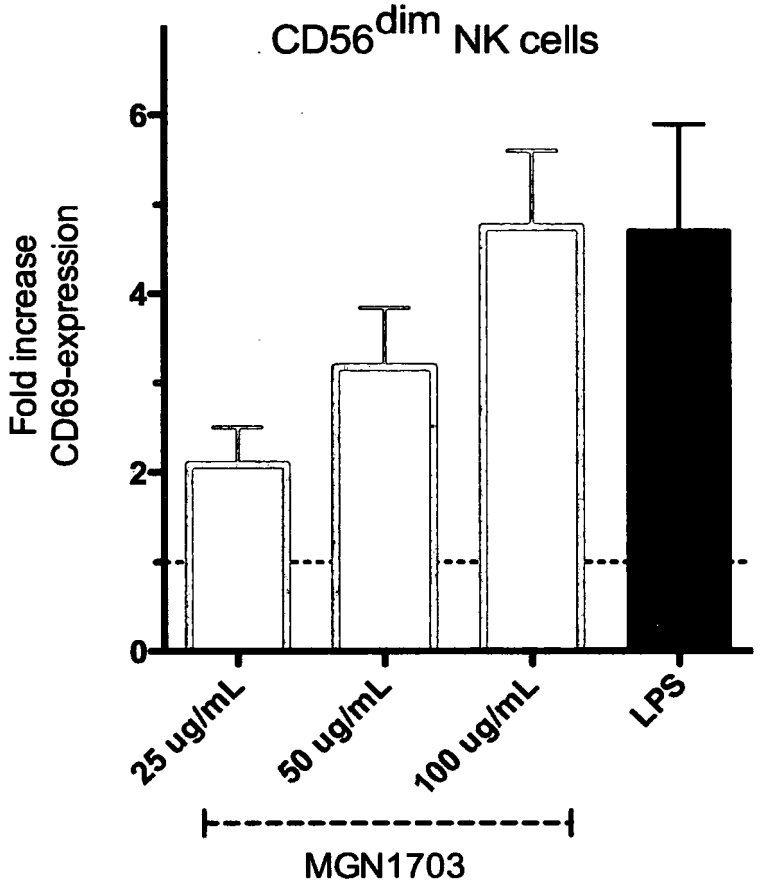


Figure 1B

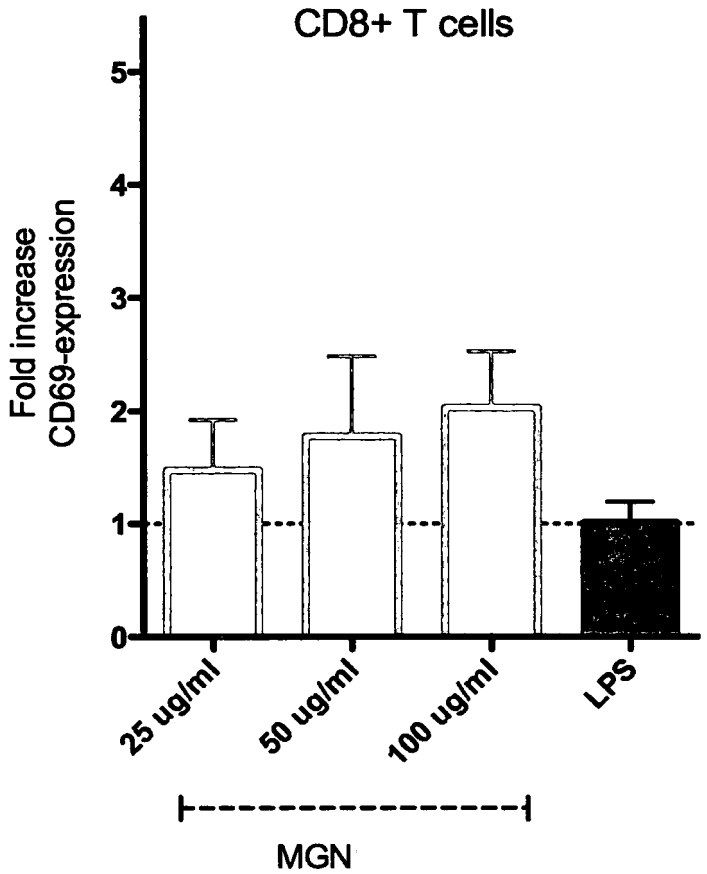


Figure 2

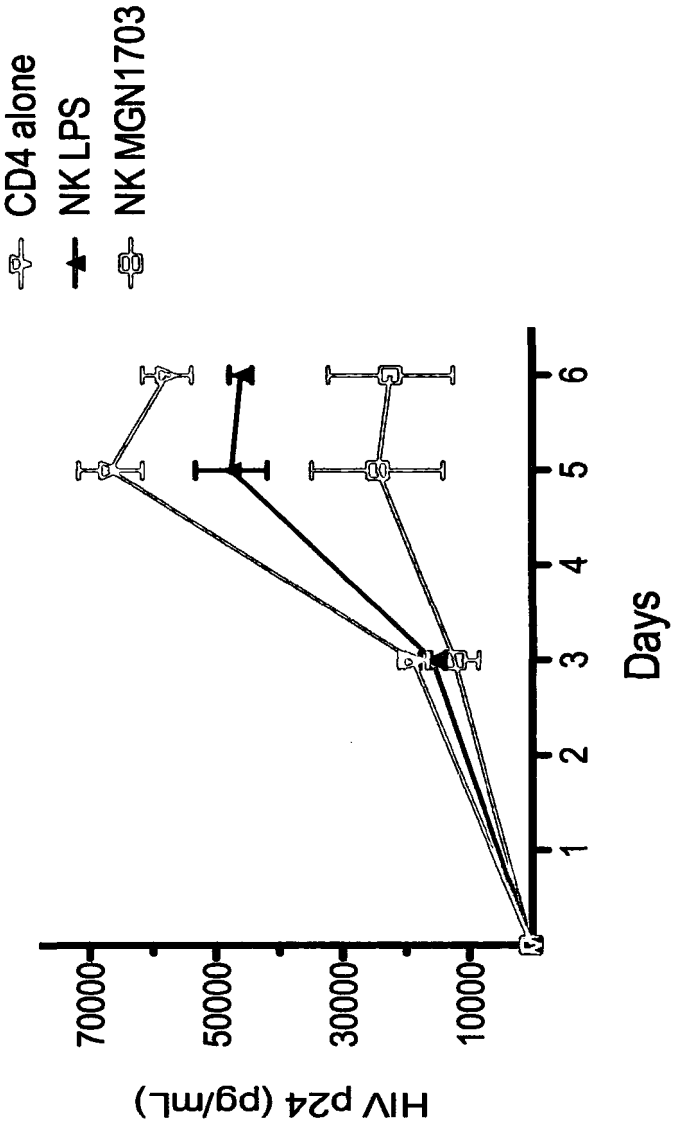

CD4 alone
NK LPS
NK MGN1703
HIV p24 (pg/mL)
70000
50000
30000
10000
1
2
3
4
5
6
Days

Figure 3

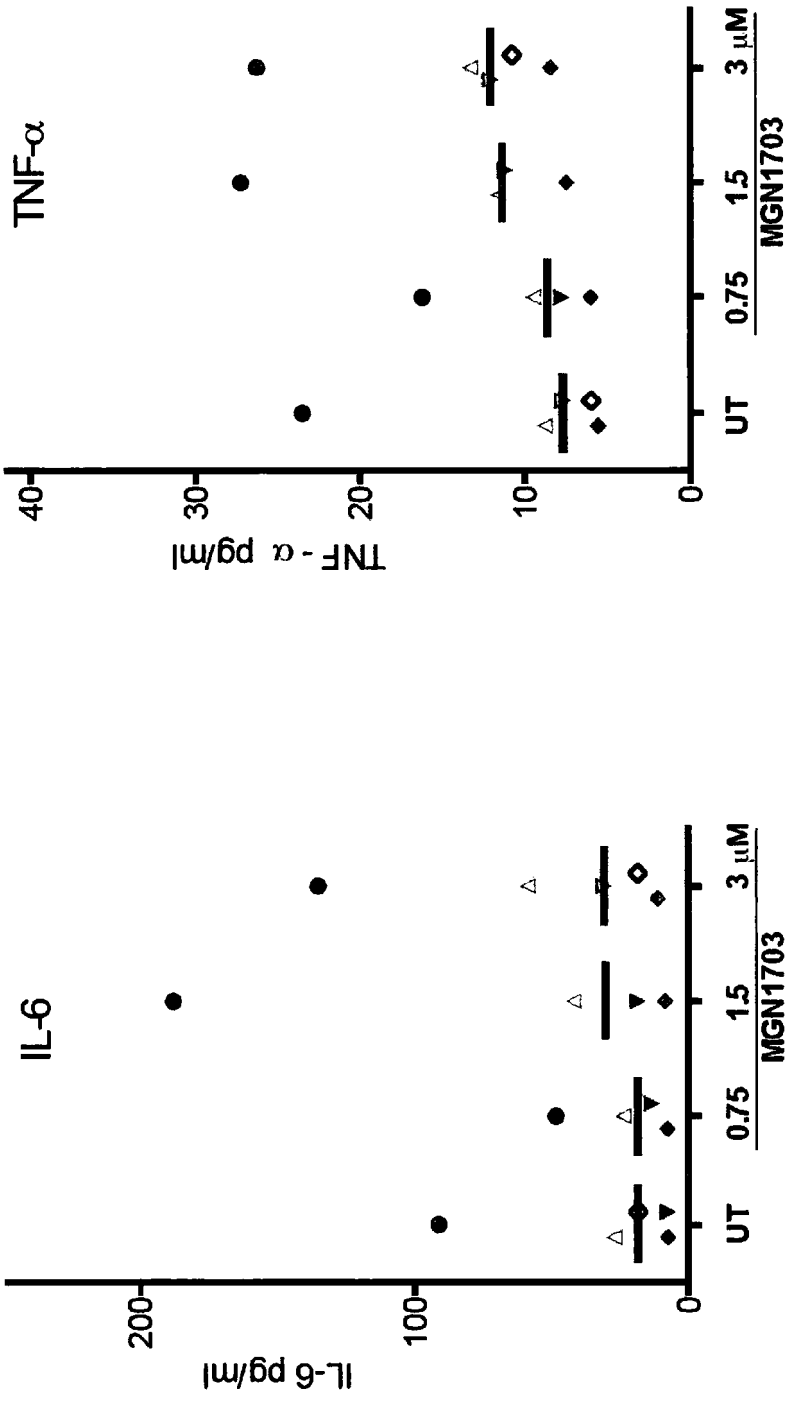

TNF-α
TNF - α pg/ml
0
10
20
30
40
UT
0.75
1.5
3 μM
MGN1703
IL-6
IL-6 pg/ml
0
100
200
UT
0.75
1.5
3 μM
MGN1703

Figure 4A

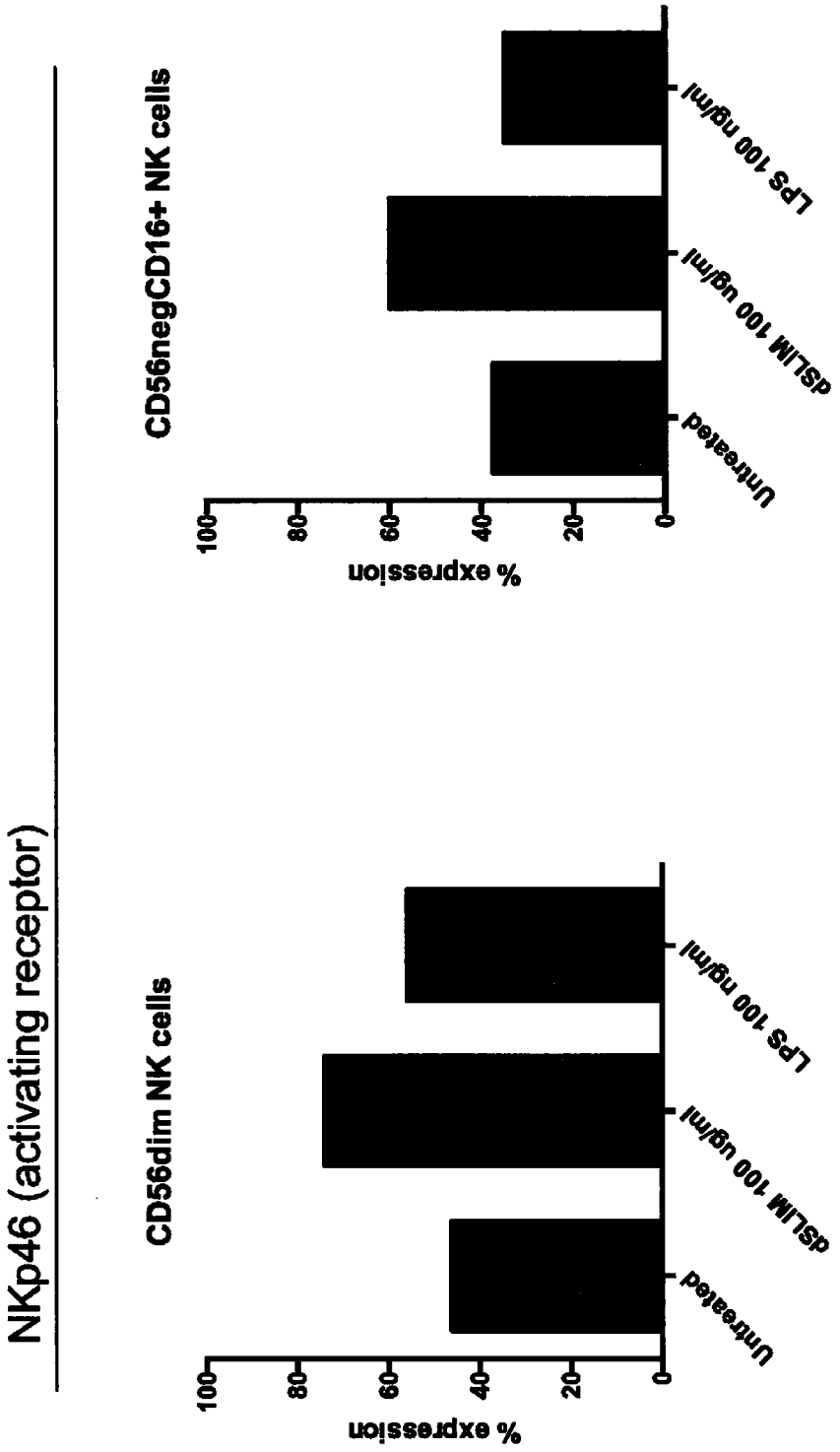

NKp46 (activating receptor)
CD56dim NK cells
% expression
0
20
40
60
80
100
Untreated
dSLIM 100 ug/ml
LPS 100 ng/ml
CD56negCD16+ NK cells
% expression
0
20
40
60
80
100
Untreated
dSLIM 100 ug/ml
LPS 100 ng/ml

Figure 4B

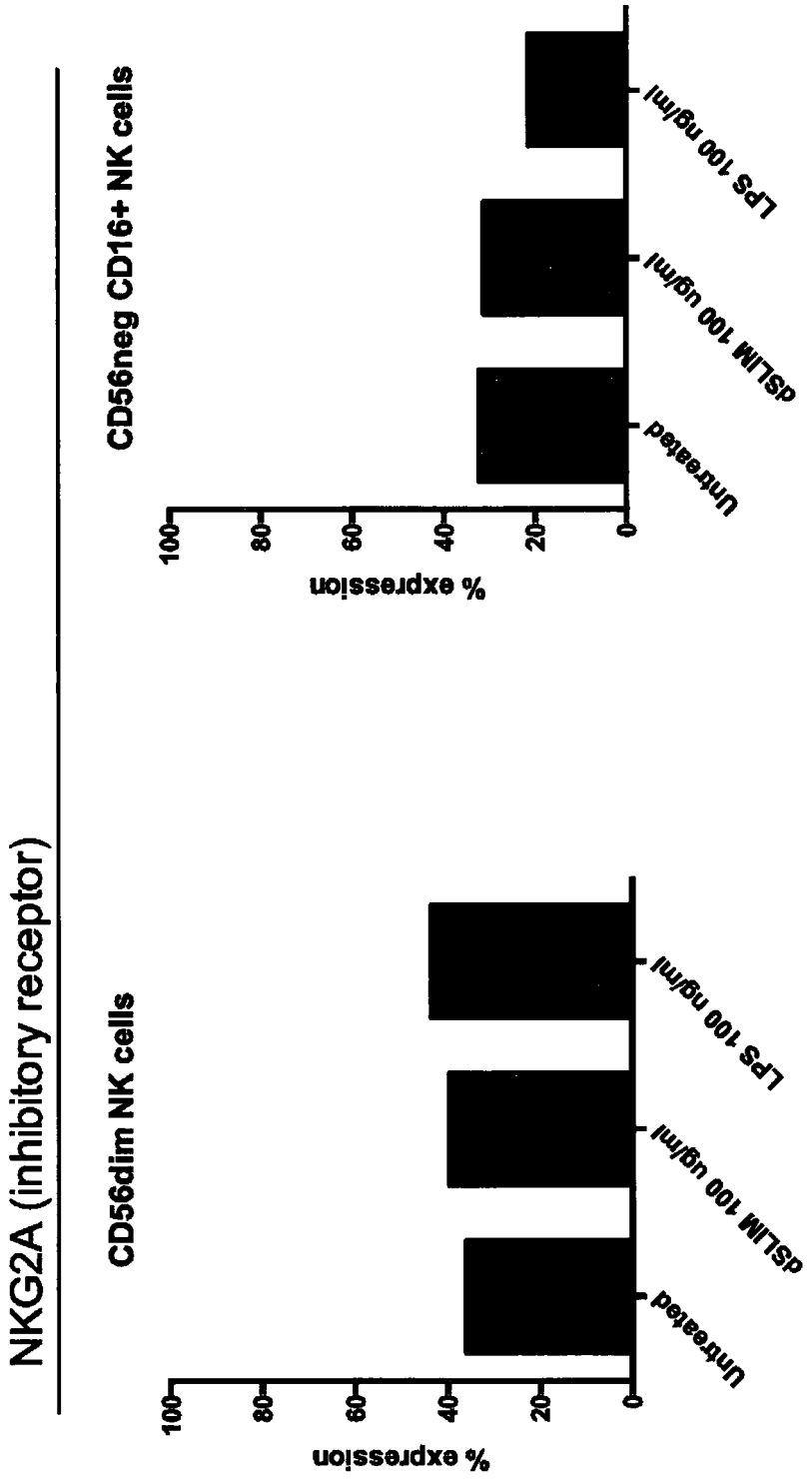

NKG2A (inhibitory receptor)
CD56dim NK cells
% expression
100
80
60
40
20
0
Untreated
dSLIM 100 ug/ml
LPS 100 ng/ml
CD56neg CD16+ NK cells
% expression
100
80
60
40
20
0
Untreated
dSLIM 100 ug/ml
LPS 100 ng/ml

Figure 5A

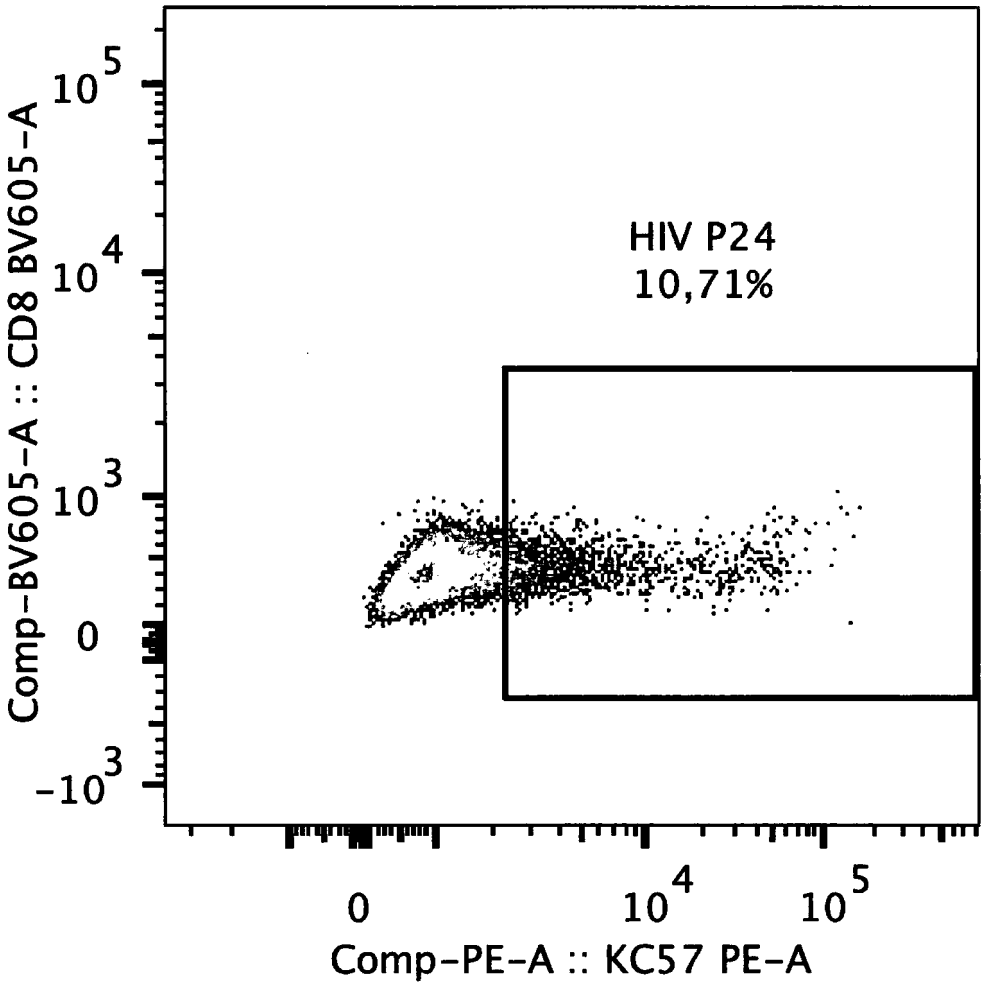

HIV P24
10,71%
Comp-BV605-A :: CD8 BV605-A
Comp-PE-A :: KC57 PE-A
10^5
10^4
10^3
0
-10^3
0
10^4
10^5

Figure 5B

E:T ratio
1:4

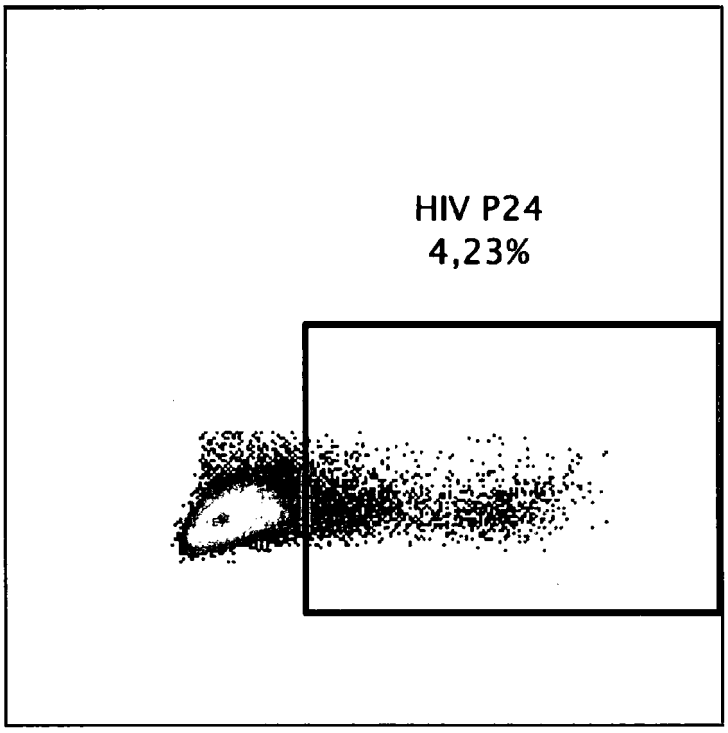
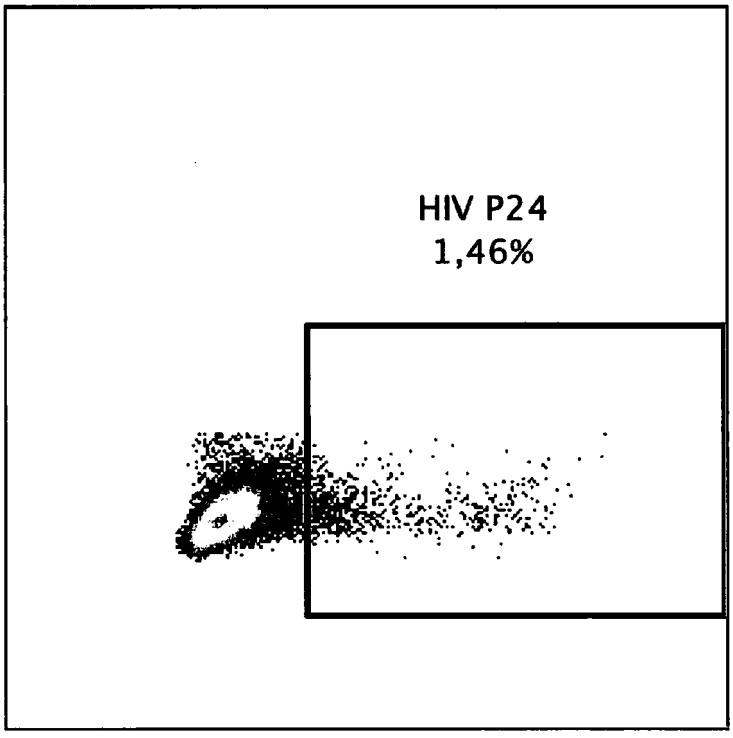


Figure 5C

E:T ratio
1:4



Title: Means for the treatment of HIV

Description

FIELD OF THE INVENTION

[0001] The invention relates to a combination and its use for the treatment of diseases like viral infections.

BRIEF DESCRIPTION OF THE RELATED ART

[0002] The term "immunotherapy" defines the treatment of diseases by stimulating, inducing, enhancing or suppressing an immune response. The strategy of immunotherapies is to fight diseases, such as cancer, infectious diseases, allergy and asthma by activating the immune system.

[0003] Viral infection may result in a state called viral latency, which is characterized by a state of reversible non-productive infection of individual cells. Viral latency provides an important mechanism for viral persistence and escape from immune recognition (Perng and Jones, Interdiscip Perspect Infect Dis, 2010, p 262415).

[0004] It is known that Herpes viruses for instance use genetic programs that allow persistence of their viral genomes with minimal viral gene expression. For retroviruses, stable integration of reverse transcribed viral cDNA into the host cell genome is an important step towards the persistence of viral genomes for the lifespan of the infected cells. There are retroviruses which establish a state of latent infection. The term latency was initially used for HIV-1 in the clinical sense to describe the long asymptomatic period between initial infection and development of AIDS. However, it became clear that HIV-1 replicates actively throughout the progress of infection, even during the asymptomatic period. One of the major mechanism by which HIV-1 escapes from immune response is through rapid evolution of escape mutations that abrogate recognition by neutralizing antibodies and cytolytic T lymphocytes (Bailey et al., Curr Opin Immunol 16, p. 470–476, 2004). Nevertheless, it has become clear

that HIV-1 can establish a state of latent infection at the level of individual T cells. Thus, the removal of the latency reservoir could be a major factor in improving the therapy of HIV.

[0005] Antiretroviral therapy (ART) refers to medications for the treatment of retroviral infections like HIV infections. The drugs do not abolish the virus so that patients will be virus-free after treatment. However, when the drugs – or a selection of them is given in combination they may prevent the growth of the virus. When the virus is slowed down, so is HIV disease. Antiretroviral drugs are referred to as ARV. Combination ARV therapy (cART) is referred to as highly active ART(HAART).

[0006] ARV drugs attack HIV in different ways and they are divided in so-called classes. The first class of anti-HIV drugs which were developed are the "nucleoside reverse transcriptase inhibitors" (also called NRTIs or "nukes".) These drugs block the use of HIV's genetic material to create DNA from RNA. AZT is the most prominent member of this class.

[0007] Another class are the "non-nucleoside reverse transcriptase inhibitors", also called non-nukes or NNRTIs, which also block the transcription of RNA into DNA.

[0008] Members belonging to the class of "protease inhibitors" block the step of cutting raw material for new HIV virus into specific pieces. Finally, there is the class of "entry inhibitors" preventing HIV from entering a cell, and the class of "HIV integrating inhibitors", preventing the integration of HIV genetic material into the host genome. Within the meaning of the present disclosure cART refers to the combinatorial use of at least two member of these classes of ARV.

[0009] Recently it was shown that the state of HIV-1 latency can be disrupted safely in patients with combination antiretroviral therapy (cART) by latency reversing agents (LRA), particularly histone de-acetylase inhibitors (HDACi) (Archin et al., 2012, Nature 487(7408):482-485; Rasmussen et al., 2013, Hum Vaccin Immunother 9(5)). However, it became obvious in the majority of patients that reactivation of

latently infected cells alone does not reduce the HIV-1 reservoir. This seems to be related to insufficient immune mediated killing of reactivated cells. However, the activation of the innate and adaptive immunity bears the risk of activating HIV-infected cells so that the disease will progress. Thus, it is questionable whether the activation of the immune system will help HIV patients.

[0010] The concept of immunotherapy-supported HIV treatment is supported by a recent in vitro study showing that CD8+ T cells from cART-treated aviremic HIV patients are capable of killing reactivated infected autologous CD4+ T cells but only when the CD8+ T cells have been antigen-stimulated prior to co-culture (Shan et al., 2012, Immunity 36(3):491-501).

[0011] Thus, there is a need for compounds which will expose the latent reservoir of HIV infected cells through their activation and targeting of the reactivated cells for immune mediating killing through pharmacological interventions.

[0012] Based on this state of the art, the objective of the instant disclosure is to provide immunostimulating compounds for reactivating latent HIV infected cells and their recognition and destruction by the immune system, and their use as a medicament.

SUMMARY OF THE INVENTION

[0013] With regard to the prior art it is an objective of the instant disclosure to provide a non-coding sequence of deoxyribonucleic acids comprising at least one sequence motif $N^1N^2CGN^3N^4$, wherein N is a nucleotide comprising A, C, T, or G, and C is deoxycytidine, G is deoxyguanosine, A is deoxyadenosine and T is deoxythymidine for the treatment of viral infections.

[0014] It is intended to use the non-coding sequence of deoxyribonucleic acids of the present invention in combination with combination antiretroviral therapy and/or histone de-acetylase inhibitors in viral infections, wherein the viral infection may be latent.

[0015] The non-coding sequence of deoxyribonucleic acids of the instant disclosure is intended to be provided for the treatment of HIV-1, in particular for the treatment of latent HIV-1 infection.

[0016] With the non-coding sequence of deoxyribonucleic acids of the present disclosure, N^1N^2 may be an element taken from the group of GT, GG, GA, AT and AA, N^3N^4 may be an element taken from the group of CT, TG and TT.

[0017] The non-coding sequence of deoxyribonucleic acids can be either linear open-chained on both sides, linear open-chained on one side of a double stranded part with a single stranded hairpin on the respective other side of the double strand or a dumbbell-shaped partially single-stranded covalently closed chain of deoxyribonucleic acids.

[0018] The non-coding sequence of deoxyribonucleic acids may comprise at least three of said sequence motifs $N^1N^2CGN^3N^4$.

[0019] The non-coding sequence of deoxyribonucleic may further be a linear open-chained non-coding sequence of deoxyribonucleic acids comprising at least one nucleotide in L-conformation.

[0020] At least one of the five terminal nucleotides located at the 5'- and/or the 3'-end of a DNA single strand of the linear open-chained non-coding sequence of deoxyribonucleic acids is in L-conformation. It is obvious for a person having ordinary skill in the art that this restriction to the terminal ends means that only within the terminal five nucleotides either on the 5'- and/or the 3'-end nucleotides in l-confirmation are contained.

[0021] It is intended that the non-coding sequence of deoxyribonucleic acids may comprise at least one of the sequence of

a. GTTCCTGGAG ACGTTCTTAG GAACGTTCTC CTTGACGTTG GAGAGAAC (SEQ ID NO:1) or
b. ACCTTCCTTG TACTAACGTT GCCTCAAGGA AGGTTGATCT TCATAACGTT GCCTAGATCA (SEQ ID NO:2), or
c. AACGTTCTTCGGGG CGTT (SEQ ID NO:3), or
d. AGGTGGTAAC CCCTAGGGGT TACCACCTTC ATCGTCGTTT TGTCGTTTTG TCGTTCTT (SEQ ID NO:4).

[0022] The non-coding sequence of deoxyribonucleic acids of the instant disclosure may have a length of 40 to 200 nucleotides or more specifically 48 to 116 nucleotides.

[0023] The above shown sequence of AACGTTCTTCGGGG CGTT may be part of the sequence CCTAGGGGTT ACCACCTTCA TTGGAAAACG TTCTTCGGGG CGTTCTTAGG TGGTAACC CCTAGGGGTT ACCACCTTCA TTGGAAAACG TTCTTCGGGG CGTTCTTAGG TGGTAACC (SEQ ID NO:5).

[0024] It is intended that the sequence motif $N^1N^2CGN^3N^4$ may be part of a single stranded region of the non-coding sequence of deoxyribonucleic acids.

[0025] Another object of the instant disclosure is a method comprising the step of providing a non-coding sequence of deoxyribonucleic acids of the present disclosure simultaneously, alternating or successively with combination antiretroviral therapy and/or histone de-acetylase inhibitors.

[0026] The non-coding sequence of deoxyribonucleic acids may be provided prior to combination antiretroviral therapy and/or histone de-acetylase inhibitors. Alternatively, it is intended that the non-coding sequence of deoxyribonucleic acids may be provided alternatingly with combination antiretroviral therapy and/or histone de-acetylase inhibitors.

[0027] A further object of the instant disclosure is a non-coding sequence of deoxyribonucleic acids with the above disclosed features for use as a medicament.

[0028] The use of a non-coding sequence of deoxyribonucleic acids of the instant disclosure for the manufacture of a pharmaceutical composition comprising the non-coding sequence of deoxyribonucleic acids and possibly additionally ARV and/or histone de-acetylase inhibitors is another object of the instant disclosure.

[0029] The pharmaceutical composition may be a vaccine and it is intended to use the non-coding sequence of deoxyribonucleic acids with the features as disclosed above as an adjuvant in therapeutic or prophylactic vaccination for the treatment of HIV-1.

BRIEF DESCRIPTION OF THE FIGURES

[0030] The disclosure will be further illustrated by examples and figures without being limited to the disclosed embodiments. A non-coding sequence of deoxyribonucleic acids of the present invention is labelled "MGN1703" in Fig. 1-3, and as "dSLIM" in figure 4. It shows:

Fig. 1A/B	MGN1703 upregulates CD69 expression, a marker of cell activation, on peripheral blood immune effector cells (natural killer cells and CD8 T cells)
Fig. 2	MGN1703 stimulated NK cells inhibit the spread of HIV
Fig. 3	Non-coding DNA constructs of the present invention cause negligible changes in pro-inflammatory cytokines IL-6 and TNF α.
Fig. 4A/B	Activating effect of stimulation of NK cells with non-coding immunostimulating sequence of DNA
Fig. 5A/B/C	Treatment of NK cells with non-coding DNA constructs. The non-coding DNA constructs enhance NK cell-mediated killing of HIV-1 producing autologous CD4+ T cells.

DESCRIPTION OF THE INVENTION

[0031] The instant invention provides a compound and method for the treatment of viral infections. In particular, the invention relates to retroviral infection and the treatment of viral latency. The provided non-coding sequences of deoxyribonucleotides shall substantially augment NK cell activity and HIV-specific CD8+ cell cytotoxic activity leading to enhanced killing of HIV-expressing cells.

[0032] Within the meaning of the present disclosure a linear open-chained DNA sequence is designated as oligonucleotide, abbreviated with ODN. Said DNA sequence can be single-stranded or partially or completely double-stranded. The terms oligo, oligonucleotide and oligodeoxynucleotide are used synonymously and do not indicate a limitation of the length of the corresponding DNA sequence. The single components of oligonucleotides are nucleotides.

[0033] An oligo can be manufactured synthetically or be partially or completely of biological origin, wherein a biological origin includes genetically based methods of manufacture of DNA sequences.

[0034] L-DNA or nucleotides in L-conformation refer to nucleotides, which comprises L-deoxyribose as the sugar residue instead of the naturally occurring D-deoxyribose. L-deoxyribose is the enantiomer (mirror-image) of D-deoxyribose. Oligonucleotides partially or completely consisting of nucleotides in L-conformation can be partially or completely single- or double-stranded; however, nucleotides in L-conformation cannot hybridize to nucleotides in D-conformation (Hauser et al., Nucleic Acid Res. 2006 34: 5101-11). L-DNA is equally soluble and selective as D-DNA. Yet, L-DNA is resistant towards enzymatic exoactivity of naturally occurring enzymes, especially exonucleases, so L-DNA is protected against intracellular degradation (Urata et al., Nucleic Acids Res. 1992 20: 3325-32). Therefore, L-DNA is very widely applicable.

[0035] A "stem" according to the present disclosure shall be understood as a DNA double strand formed by base pairing either within the same oligonucleotide (which is then partially self-complementary) or within different oligonucleotides (which are partially or completely complementary). Intramolecular base-pairing designates base-pairing within the same oligonucleotide and base-pairing between different oligonucleotides is termed as intermolecular base-pairing.

[0036] A "loop" within the meaning of the present disclosure shall be understood as an unpaired, single-stranded region either within or at the end of a stem structure. A "hairpin" is a distinct combination of a stem and a loop, which occurs when two self-complementary regions of the same oligonucleotide hybridize to form a stem with an unpaired loop at one end.

[0037] A "solid phase" to which the nucleotides are covalently or non-covalently attached refers to, but is not restricted to, a column, a matrix, beads, glass including modified or functionalized glass, silica or silica-based materials including silicon and modified silicon, plastics (comprising polypropylene, polyethylene, polystyrene and copolymers of styrene and other materials, acrylics, polybutylene, polyurethanes etc.), nylon or nitrocellulose, resins, polysaccharides, carbon as well as inorganic glasses and plastics. Thus, microtiter plates are also within the scope of a solid phase according to the present disclosure.

[0038] Immunomodulation according to the present disclosure refers to immunostimulation and immunosuppression. Immunostimulation means preferentially that effector cells of the immune system are stimulated in order to proliferate, migrate, differentiate or become active in any other form. B cell proliferation for instance can be induced without co-stimulatory signals by immunostimulatory oligonucleotides, which normally require a co-stimulatory signal from helper thymocytes.

[0039] Immunosuppression on the other hand shall be understood as reducing the activation or efficacy of the immune system. Immunosuppression is generally deliberately induced to prevent for instance the rejection of a transplanted organ, to treat

graft-versus-host disease after a bone marrow transplant, or for the treatment of autoimmune diseases such as, for example, rheumatoid arthritis or Crohn's disease.

[0040] An agonist within the meaning of the instant disclosure and in accordance with its common definition represents a chemical or molecule that binds to another molecule, like a receptor or ligand and thus activates the molecule. In contrast to an agonist that activates, an antagonist shall be understood as a chemical or molecule that blocks the interaction of the molecule to which the antagonist binds with a respective agonist. Depending on the context, an antagonist in the understanding of the instant invention may also result in the activation of a process, because the antagonist blocks the interaction of another antagonist with a receptor for instance.

[0041] The term "pharmaceutically applicable or acceptable salts" as used herein includes salts of a compound of the combination, which are prepared with relatively nontoxic (i.e. pharmaceutically acceptable) acids or bases, depending on the particular substituents found on the compounds of the present invention. If, for example, compounds of the present invention contain acidic functionalities, base addition salts may be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Non-limiting examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. If compounds of the present invention contain basic functionalities, acid addition salts may be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Non-limiting examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, phosphoric, partially neutralized phosphoric acids, sulfuric, partially neutralized sulfuric, hydroiodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic. malonic, benzoic, succinic, suberic, fumaric, mandelic, phthalic, benzenesulfonic, p- tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunor-

ic acids and the like. Certain specific compounds of the present invention may contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. Contacting the salt with a base may regenerate the neutral forms of the compounds of the present invention or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention. The compounds of the present invention may possess chiral or asymmetric carbon atoms (optical centers) and/or double bonds. The racemates, diastereomers, geometric isomers and individual optical isomers are encompassed by the present invention. The compounds of the present invention may exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are also encompassed by the present invention. The compounds of the present invention may furthermore exist in multiple crystalline or amorphous forms.

[0042] Deoxyribonucleic acid molecules, consisting of a partially single-stranded, dumbbell-shaped, covalently closed chain of deoxyribonucleoside residues, which contain one or more sequences of the base sequence $N^1N^2CGN^3N^4$, wherein N^1N^2 is an element from the GT, GG, GA, AT or AA group, N^3N^4 is an element from the CT or TT group, as well as C deoxycytosine, G deoxyguanosine, A deoxyadenosine and T deoxythymidine, shall be used in combination with antiretroviral drugs and/ or histone de-acetylase inhibitors.

[0043] The deoxyribonucleic acid molecules relating to the instant disclosure may have a length of up to 200 nucleotides. In particular, sequences with a length between 48 and 116 nucleotide are intended.

[0044] The dumbbell-shaped non-coding sequences of deoxyribonucleic acid molecules may comprise the base sequence $N^1N^2CGN^3N^4$ is their single-stranded regions.

[0045] The immunostimulation may take place *in vitro, ex vivo* or *in vivo*.

[0046] The instant disclosure provides also linear open-chained DNA sequence comprising at least one CpG motif and at least one nucleotide in L-conformation. Due to the partial/complete L-conformation, the DNA sequence is partially or fully resistant to exonucleases. In case that the construct has on one end of a double strand a single stranded-loop, the end is also protected against degradation. Thereby, the ODNs are in total protected against cellular degradation without having the need to use a phosphorothioate backbone, which has been shown to be toxic. In addition, the ODNs only consist of a minimum number of nucleotides, which makes them small and thereby easy to transfect into cells.

[0047] The non-coding sequence of deoxyribonucleic acids comprising at least one sequence motif $N^1N^2CGN^3N^4$ can be single-stranded or partially or completely double-stranded. This includes base-pairing within the same molecule (intramolecular) or within different molecules (intermolecular) or any combination thereof. It is also possible that the construct comprises at least one unpaired, single-stranded region. As a further embodiment, hairpin structures are included. Due to the partial or complete L-conformation, a longer half-life of the construct is ensured as nucleotides in L-conformation are not subject to degradation.

[0048] It is also within the scope of the instant disclosure that at least two molecules, which are single-stranded or partially or completely double-stranded can ligate to each other to form multimeric constructs. These multimeric constructs thus incorporate at least as many CpG motifs as ligation partners, tightly packed within one molecule, and are therefore expected to elicit also a considerable immune response as part of the combination with T-cell regulators. The resulting single-stranded or partially or completely double-stranded multimeric constructs can either be covalently closed comprising nucleotides in L-conformation within the molecule or open multimeric constructs comprising nucleotides in L-conformation at the 5'- and/or the 3'-end for protection against cellular degradation.

[0049] The disclosure further comprises chemical modifications of at least one nucleotide in the non-coding sequence of deoxyribonucleic acids comprising at least

one sequence motif $N^1N^2CGN^3N^4$ with a functional group selected from the group comprising carboxyl, amine, amide, aldimine, ketal, acetal, ester, ether, disulfide, thiol and aldehyde groups. This allows coupling of the DNA construct to a compound selected from the group comprising peptides, proteins, carbohydrates, antibodies, synthetic molecules, polymers, micro projectiles, metal particles or a solid phase by, for example, adsorption, covalent or ionic bonding.

[0050] The modification can be specifically selected for the respective purpose. The construct can thus be used, for example, to shuttle other molecules to the specific cell responding to the CpG motif/s incorporated. In addition, it is possible by such modifications to couple the construct to micro projectiles, which can be used to transfer the construct into the cell. The construct can also be coupled to a solid phase, e. g. a microtiter plate.

[0051] Figure 1 shows that MGN1703 upregulates CD69 expression, a marker of cell activation, on peripheral blood immune effector cells. Total PBMC (n=5 separate donors) were stimulated with MGN1703 or LPS. Following stimulation, cells were analyzed via flow cytometry and CD69 expression on NK cells (left) and CD8+ T cells (right) were characterized. Depicted is mean fold above media control.

[0052] Figure 2 shows that MGN1703 stimulated NK cells inhibit the spread of HIV. CD4-depleted PBMC (one representative donor with SD shown) were stimulated with MGN1703 or LPS. Following stimulation, the cells were incubated with autologous HIV-infected CD4+ cells. HIV capsid protein (Gagp24) was quantitated in culture supernatants over time as a measure of virus spread.

[0053] Figure 3 shows that the non-coding DNA constructs have almost no effect on the pro-inflammatory cytokines IL-6 (Figure 3A) and TNFα (Figure 3B). "UT" denotes untreated controls, "MGN1703" denotes the non-coding DNA constructs applied in concentrations of 0.75µM, 1.5µM, and 3µM from left to right. Thus, the use of a non-coding immunostimulating sequence of deoxyribonucleotides activated viral infected cells without initiating an unintended cytokine based inflammation.

[0054] In order to confirm the activating effect of stimulating NK cells with non-coding immunostimulating sequence of deoxyribonucleotides, the effect on activating or inhibitory receptors of NK cells was investigated. Figures 4A and 4B show that only the expression of activating receptors is increased by the non-coding DNA sequences.

[0055] Figure 5 shows the results of experiments that were conducted to confirm that the viral inhibition shown in figure 2 was a result of HIV-specific killing and not unspecific CD4 T cell death. HIV infected cells were collected on day 6 and stained for intracellular P24 antigen. Figure 5A shows CD4 T-cells which were cultured alone, figure 5B shows CD4 T-cells cultured together with untreated NK cells and figure 5C shows CD4 T-cells cultured together with dSLIM treated NK cells. The square in each figure gates the population of HIV infected cells. Their percentage on the total population is indicated above the square.

[0056] The results in figure 5 confirm that the treatment of NK cells with non-coding DNA constructs according ton the present disclosure is suitable to reduce considerably the number of HIV infected cells.

[0057] In essence, the instant disclosure demonstrates that the treatment of viral infections leading to latency can be supported by the treatment with non-coding DNA constructs according to the present disclosure. Infected cells will be activated or "demasked" so that the immune system will be able to kill them. Thus the combinatorial treatment of HIV infections with cART for instance is supported by applying immunostimulating non-coding DNA.

[0058] Referring to the results described as prior art where cART and HDACi were combined, it seems to be possible that the combinatorial treatment of those two components of HIV treatment with immunostimulating non-coding DNA constructs will be beneficial for the HIV treatment. Corresponding experiments aiming at proving such a beneficial effect are currently performed.

[0059] It has to be noted that the non-coding immunostimulating DNA constructs of the instant disclosure have the advantage of avoiding pro-inflammatory cytokine stimulation. In contrast, non-coding DNA constructs that are stabilized against nuclease degradation by chemical modifications are known to cause pro-inflammatory cytokine release.

[0060] It was surprising that the stimulation of latent retroviral infected cells did not result in an accelerated progression of the HIV infection to AIDS. It was not predictable that the stimulation with constructs according to the above disclosure enhances the success rates of HIC treatment with cART and/or HDACi.

Claims

1. A non-coding sequence of deoxyribonucleic acids comprising at least one sequence motif $N^1N^2CGN^3N^4$, wherein N is a nucleotide comprising A, C, T, or G, and C is deoxycytidine, G is deoxyguanosine, A is deoxyadenosine and T is deoxythymidine for the treatment of viral infections.

2. The non-coding sequence of deoxyribonucleic acids of claim 1 in combination with combination antiretroviral therapy and/or histone de-acetylase inhibitors.

3. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 or 2, wherein the viral infection is latent.

4. The non-coding sequence of deoxyribonucleic acids of any one of claim 1 to 3, for the treatment of HIV-1.

5. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 4, for the treatment of latent HIV-1 infection.

6. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 5, wherein N^1N^2 is an element taken from the group of GT, GG, GA, AT and AA, N^3N^4 is an element taken from the group of CT, TG and TT.

7. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 6, wherein the non-coding sequence of deoxyribonucleic acids is either linear open-chained on both sides, linear open-chained on one side of a double stranded part with a single stranded hairpin on the respective other side of the double strand or a dumbbell-shaped partially single-stranded covalently closed chain of deoxyribonucleic acids.

8. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 7, comprising at least three of said sequence motifs $N^1N^2CGN^3N^4$.

9. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 8, wherein a linear open-chained non-coding sequence of deoxyribonucleic acids comprises at least one nucleotide in L-conformation.

10. The non-coding sequence of deoxyribonucleic acids of claim 9, wherein one of the five terminal nucleotides located at the 5'- and/or the 3'-end of a DNA single strand of the linear open-chained non-coding sequence of deoxyribonucleic acids is in L-conformation.

11. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 10, comprising at least one of the sequence of

    a. GTTCCTGGAG ACGTTCTTAG GAACGTTCTC CTTGACGTTG GAGAGAAC (SEQ ID NO:1) or

    b. ACCTTCCTTG TACTAACGTT GCCTCAAGGA AGGTTGATCT TCATAACGTT GCCTAGATCA (SEQ ID NO:2), or

    c. AACGTTCTTCGGGG CGTT (SEQ ID NO:3), or

    d. AGGTGGTAAC CCCTAGGGGT TACCACCTTC ATCGTCGTTT TGTCGTTTTG TCGTTCTT (SEQ ID NO:4).

12. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 11, wherein the non-coding sequence of deoxyribonucleic acids has a length of 40 to 200 nucleotides.

13. The non-coding sequence of deoxyribonucleic acids of claim 11 or 12, wherein the sequence from claim 10 c) is part of the sequence CCTAGGGGTT ACCACCTTCA TTGGAAAACG TTCTTCGGGG CGTTCTTAGG TGGTAACC CCTAGGGGTT ACCACCTTCA TTGGAAAACG TTCTTCGGGG CGTTCTTAGG TGGTAACC (SEQ ID NO:5).

14. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 13, wherein the non-coding sequence of deoxyribonucleic acids has a length of 48 to 116 nucleotides.

15. The non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 14, where the said sequence motif $N^1N^2CGN^3N^4$ is part of a single stranded region.

16. A method comprising the step of providing a non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 16 simultaneously, alternating or successively with combination antiretroviral therapy and/or histone de-acetylase inhibitors.

17. The method of claim 17, wherein the non-coding sequence of deoxyribonucleic acids is provided prior to combination antiretroviral therapy and/or histone de-acetylase inhibitors.

18. A non-coding sequence of deoxyribonucleic acids of any one of claims 2 to 16 for use as a medicament.

19. A use of a non-coding sequence of deoxyribonucleic acids of any one of claims 2 to 16 for the manufacture of a pharmaceutical composition comprising the non-coding sequence of deoxyribonucleic acids.

20. The use according to claim 20, where the pharmaceutical is a vaccine.

21. The use of a non-coding sequence of deoxyribonucleic acids of any one of claims 1 to 16 as an adjuvant in therapeutic or prophylactic vaccination for the treatment of HIV-1.

Intellectual Property Office

Application No:	GB1516676.2	**Examiner:**	Dr Philip Mountjoy
Claims searched:	1-21	**Date of search:**	7 July 2016

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X	1-21	ClinicaTrials.gov website, trial number NCT02443935, "Toll-like Receptor 9 Agonist Treatment in Chronic HIV-1 Infection (TEACH)", published 13 May 2015 available online at [https://clinicaltrials.gov/archive/NCT02443935/2015_05_13] [accessed 5 July 2016] - See entire document.
X	1-21	EU CLINICAL TRIALS REGISTER, EudraCT Number:2014-005634-59, Aarhus University Hospital, trial first available on the database 7 April 2015, "Toll-like receptor 9 enhancement of antiviral immunity in chronic HIV-1 infection: a phase 1b/2a trial (TEACH)" available online at https://www.clinicaltrialsregister.eu/ctr-search/trial/2014-005634-59/DK [accessed 5 July 2016] - See entire document.
X	1-21	Towards an HIV Cure Symposium, 18 &19 July 2015, R Offersen, et al., "A novel TLR-9 agonist (MGN1703) activates NK-cells and enhances NK-cell mediated viral killing of HIV-1 infected CD4+ T cells ex vivo", PE40, page 68. available online at https://www.iasociety.org/Web/WebContent/File/HIV_Cure_Symposium_2015/Posters/PE40.pdf - See entire document.
X	1, 3-15, and 18-20	Critical Reviews in Oncology/Hematology, Vol. 94, B Wittig, et al., epub Dec. 19 2014, "MGN1703, an immunomodulator and toll-like receptor 9 (TLR-9)agonist: From bench to bedside", pages 31-44. See entire document, particularly the abstract and figure 2 (page 36).
X	1, 3-15, and 18-20	Nucleic Acid Ther., Vol. 25, No. 3, epub 31 Mar. 2015, M Schmidt, et al., "Design and Structural Requirements of the Potent and Safe TLR-9 Agonistic Immunomodulator MGN1703.", pages 130-140. See entire document, particularly the abstract.
X	1, 3-15, and 18-20	GB2523187 A (MOLOGEN) - See entire document, particularly the abstract, page 9, and the claims.
X	1, 3-15, and 18-20	US2010/0303803 A1 (SCHROFF et al.) - See entire document, particularly the abstract, claims, and SEQ ID Nos 9 and 10 (paragraph [0039]).

Intellectual Property Office

X	1, 3-15, and 18-20	WO2005/080567 A1 (MOLOGEN) - See entire document, particularly the abstract and the claims.
X	1-21	WO2010/039137 A1 (IDERA PHARMACEUTICALS) - See entire document, particularly the abstract, claims, and examples 14 and 15 (pages 48-49).
X	1-21	WO01/00232 A2 (SMITHKLINE BEECHAM BIOLOGICALS) - See entire document, particularly the claims.
A	-	WO2012/085282 A1 (MOLOGEN) - See entire document.

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

A61K; A61P; C12N

The following online and other databases have been used in the preparation of this search report

EPODOC, WPI, BIOSIS, MEDLINE, INTERNET, BLASTn

International Classification:

Subclass	Subgroup	Valid From
C12N	0015/117	01/01/2010
A61K	0039/39	01/01/2006
A61P	0031/18	01/01/2006