[0005] The triggering of TLR7 and TLR9 in plasmacytoid dendritic cells precursors (PDC) and B cells by self nucleic acids is key in the pathogenesis of Systemic Lupus Erythematosus (SLE). This leads to the production of type I IFNs from PDC that can be detected by the upregulation of IFN-regulated genes in the blood of patients (IFN-signature) and anti-DNA and anti-RNP antibodies from B cells that form immune complexes (IC) with DNA or RNA from dying cells (Barrat and Coffman, 2008; Marshak-Rothstein, 2006). PDC are the major source of IFN-α induced by nucleic acid-containing ICs. Once activated by self DNA/chromatin or snRNP-containing IC, PDCs migrate from the blood into inflamed tissues including skin and kidney.

[0006] IFN-α and PDC have been proposed to contribute to the pathogenesis of other autoimmune diseases characterized by IFN-α signature as well. Indeed, Type I IFN-producing PDC accumulate in the pancreas, muscle and salivary glands of people affected by diabetes mellitus, dermatomyositis and Sjögren's syndrome respectively, strongly suggesting that dysregulated PDC activation could be a more general feature of autoimmune disease (Barrat and Coffman, 2008; Guiducci et al., 2009; Ueno et al., 2007).

[0007] SLE patients are often treated with strong immunosuppressive regimens, including cytotoxic drugs, antimalarial compounds and glucocorticoids (GC). Glucocorticoids have strong anti-inflammatory effects on both acquired and innate immune functions. They inhibit B and T cell responses and effector functions of monocytes and neutrophils. At the cellular level, GC inhibit NF-kB activity, thought to be the main mechanism by which GC exert their anti-inflammatory effects. In lupus, GC are typically administered orally on a daily basis, as the typical every other day regimens cannot maintain disease control. When doses greater than 40 mg/day are required, patients receive intravenous methylprednisolone (Solumedrol) pulse therapy (e.g., doses up to 30 mg/kg/day or 1 g/day given each day for 3 days). Such treatment can transiently reduce disease activity, but often does not induce remission or prevent end organ damage. The reason why treatment of SLE requires much higher GC doses than many other autoimmune diseases is not clear.

SUMMARY

[0008] The ~~invention~~disclosure relates to inhibitors of TLR9 and/or TLR7 (*e.g.*, immunoregulatory polynucleotides and/or immunoregulatory compounds) and methods of use thereof.

[0008a] In one aspect, there is provided a polynucleotide consisting of the nucleotide sequence selected from the group consisting of SEQ ID NO:73, SEQ ID NO:134, SEQ ID NO:143 and SEQ ID NO:144.

[0008b] In one aspect, there is provided a polynucleotide for use in treating an autoimmune disease in an individual, wherein an elevated inflammatory gene expression pattern as compared to that of control sample(s) from healthy subject(s) is present in a sample from the individual at the onset of treatment, and wherein the polynucleotide consists of the nucleotide sequence selected from the group consisting of SEQ ID NO:73, SEQ ID NO:134, SEQ ID NO:143 and SEQ ID NO:144.

[0008c] In one aspect, there is provided a pharmaceutical composition comprising the polynucleotide as defined above and a pharmaceutically acceptable excipient.

[0009] Provided herein are methods of treating a disease in an individual comprising administering to the individual an effective amount of a TLR7 inhibitor and/or TLR9 inhibitor, wherein treatment is based upon interferon signature and/or differential levels of inflammatory cytokines in a sample. Also provided herein are methods of treating a disease comprising: (a) selecting an individual having an IFN signature and/or differential levels of inflammatory cytokines in a sample; and (b) administering to the selected individual an effective amount of a

GZ'GG motif, wherein I is inosine and Z' is 7-deaza G is a 2'-deoxyribonucleotide (e.g., G is 2'-deoxyguanosine, I is 2'-deoxyinosine and Z' is 7-deaza-2'-deoxyguanosine).

[0014] Also provided herein are methods of regulating an immune response in an individual, comprising administering to the individual a polynucleotide in an amount sufficient to regulate an immune response in the individual, wherein the polynucleotide is selected from the group consisting of SEQ ID NO:64-78, SEQ ID NO:123-135, and SEQ ID NO:141-145. In some embodiments, the polynucleotide comprises one of the group consisting of SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:78, SEQ ID NO:141, SEQ ID NO:143, and SEQ ID NO:144. In some embodiments, the polynucleotide comprises one of the group consisting of SEQ ID NO:73, SEQ ID NO:134, SEQ ID NO:143, and SEQ ID NO:144.

[0015] In certain embodiments, regulating the immune response in the individual treats and/or prevents a disease. In certain embodiments of any of the methods provided herein, an immune response is inhibited. In certain embodiments of any of the methods provided herein, a TLR7 dependent immune response is inhibited. In certain embodiments of any of the methods provided herein, a TLR9 dependent immune response is inhibited. In further embodiments of any of the methods, both TLR7 and TLR9 dependent immune responses are inhibited.

[0016] In certain embodiments of any of the methods provided herein, the disease is an autoimmune disease. In certain embodiments, one or more symptoms of the autoimmune disease is ameliorated. In certain embodiments of any of the methods provided herein, development of the autoimmune disease is prevented or delayed. In certain embodiments, the autoimmune disease is selected from the group consisting of systemic lupus erythematosus (SLE), rheumatoid arthritis, dermatomyositis, and Sjogren's syndrome. In certain embodiments of any of the methods provided herein, the disease is associated with inflammation. In certain embodiments, the inflammation is a sterile inflammation. In certain embodiments, the sterile inflammation is sterile inflammation of the liver.

[0017] The present ~~invention~~disclosure further encompasses a polynucleotide comprising one or more of the group (of 33 sequences) consisting of SEQ ID NOs:64-78, 123-135, and 141-145. The present ~~invention~~disclosure also encompasses a polynucleotide consisting of one of the group (of 33 sequences) consisting of SEQ ID NOs:64-78, 123-135, and 141-145. In some embodiments, the polynucleotide is comprised of all 2'-deoxyribo polynucleotides. In some embodiments one or more nucleotides of the polynucleotide comprises a modification. In some embodiments, the modification comprises a 2'-sugar modification. In a subset of these embodiments, the 2'-sugar modification comprises a 2'-O-methyl sugar modification or a 2'-O-methoxyethyl sugar modification. In certain embodiments, the polynucleotide is comprised of all 2'-deoxyribo

polynucleotides. In certain embodiments, the polynucleotide is a 2'-deoxyribo polynucleotide and a 2'-sugar modification chimeric sequence. In certain embodiments, the polynucleotide is a 2'-deoxyribo polynucleotide and a 2'-O-methyl sugar polynucleotide chimeric sequence. In certain embodiments, the polynucleotide is a 2'-deoxyribo polynucleotide and a 2'-O-methyoxyethyl sugar polynucleotide chimeric sequence. In certain embodiments, the polynucleotide has at least one nucleotide comprising a modified phosphate linkage. In certain embodiments, the polynucleotide comprises only phosphorothioate linkages.

[0018] The ~~invention~~disclosure further relates to kits, preferably for carrying out the methods of the ~~invention~~disclosure. The kits of the ~~invention~~disclosure generally comprise an immunoregulatory polynucleotide and/or an immunoregulatory compound of the ~~invention~~disclosure (generally in a suitable container), and may further include instructions for use of the immunoregulatory polynucleotide and/or immunoregulatory compound in immunoregulation of an individual. In some embodiments, the kit further comprises another therapeutic agent.

[0019] The present disclosure provides methods of treating an autoimmune disease in an individual comprising administering to the individual an effective amount of a TLR7 inhibitor and/or TLR9 inhibitor, wherein an elevated inflammatory gene expression pattern as compared to that of control sample(s) from healthy subject(s) is present in a sample from the individual at the onset of treatment. In some embodiments the TLR7 and/or TLR9 inhibitor is a polynucleotide comprising or consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-78, 80-108, and 110-145, or a polynucleotide comprising or consisting of a nucleotide sequence selected from the group consisting of SEQ ID NOS:64-78, 123-135 and 141-145. In some embodiments the TLR7 and/or TLR9 inhibitor is a polynucleotide comprising the nucleotide sequence of the formula: R_γJGCK$_\alpha$GIGGL$_\beta$-3' (SEQ ID NO:146), wherein each of R, K, and L is a nucleotide, J is U or T, γ is an integer from about 0 to 10, α is an integer from about 1 to about 20, and β is an integer from about 1 to about 20. In some embodiments, each nucleotide of the GIGG of the sequence is a 2'-deoxyribonucleotide (e.g., G is 2'-deoxyguanosine and I is 2'-deoxyinosine). In some embodiments the TLR7 and/or TLR9 inhibitor is a polynucleotide comprising or consisting of a nucleotide sequence selected from the group consisting of SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:78, SEQ ID NO:141, SEQ ID NO:143 and SEQ ID NO:144. In some embodiments the TLR7 and/or TLR9 inhibitor is a polynucleotide comprising or consisting of a nucleotide sequence selected from the group consisting of SEQ ID NO:71, SEQ ID NO:73, SEQ ID NO:78, SEQ ID NO:143 and SEQ ID NO:144. In some embodiments, the TLR7 and/or TLR9 inhibitor is a polynucleotide comprising or consisting of a

CLAIMS

~~1. A polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:73, SEQ ID NO:134, SEQ ID NO:143 and SEQ ID NO:144, wherein the polynucleotide is less than 100 bases or base pairs in length.~~

1.~~2. The polynucleotide of Claim 1, wherein the polynucleotide consists~~ A polynucleotide consisting of the nucleotide sequence selected from the group consisting of SEQ ID NO:73, SEQ ID NO:134, SEQ ID NO:143 and SEQ ID NO:144.

~~3. The polynucleotide of Claim 1, wherein the polynucleotide does not comprise a CG.~~

~~4. The polynucleotide of Claim 1 or Claim 3, wherein the polynucleotide is less than 50 bases or base pairs in length.~~

2.~~5.~~ The polynucleotide of Claim 1~~any one of Claims 1-4~~, wherein one or more nucleotides comprises a modification.

3.~~6.~~ The polynucleotide of Claim 2~~Claim 5~~, wherein the modification comprises at least one phosphorothioate backbone modification.

4.~~7.~~ The polynucleotide of Claim 2 or Claim 3~~Claim 5 or Claim 6~~, wherein the modification comprises a 2'-sugar modification.

5.~~8.~~ The polynucleotide of Claim 3~~Claim 6~~, wherein the polynucleotide consists of the nucleotide sequence of SEQ ID NO:73.

6.~~9.~~ The polynucleotide of Claim 3~~Claim 6~~, wherein the polynucleotide consists of the nucleotide sequence of SEQ ID NO:134.

7.~~10.~~ The polynucleotide of Claim 3~~Claim 6~~, wherein the polynucleotide consists of the nucleotide sequence of SEQ ID NO:143.

8.~~11.~~ The polynucleotide of Claim 3~~Claim 6~~, wherein the polynucleotide consists of the nucleotide sequence of SEQ ID NO:144.

9.~~12.~~ The polynucleotide of any one of Claims 1-8~~Claims 1-11~~, for use in treating an autoimmune disease in an individual.

10.~~13.~~ A polynucleotide for use in treating an autoimmune disease in an individual, wherein an elevated inflammatory gene expression pattern as compared to that of control sample(s) from healthy subject(s) is present in a sample from the individual at the onset of treatment, and wherein the polynucleotide ~~comprises a~~ consists of the nucleotide sequence selected from the group consisting of SEQ ID NO:73, SEQ ID NO:134, SEQ ID NO:143 and SEQ ID NO:144~~, wherein the polynucleotide is less than 100 bases or base pairs in length~~.

11.~~14.~~ The polynucleotide for use according to Claim 9 or Claim 10~~Claim 12 or Claim 13~~, wherein treating the autoimmune disease achieves one or more of the following outcomes:

a.) reduces the inflammatory gene expression pattern in a post-treatment sample from the individual;

b.) reduces a clinical disease activity measure of the autoimmune disease;

c.) reduces a serologic disease measure of the autoimmune disease;

d.) increases plasmacytoid dendritic cells in a post-treatment peripheral blood mononuclear cell sample from the individual; and

e) reduces concurrent corticosteroid use by the individual.

12.~~15.~~ The polynucleotide for use according to any one of Claim 9-11~~ Claims 12-14~~, wherein the individual is a human patient.

13.~~16.~~ The polynucleotide for use according to any one of Claims 9-12~~ Claims 12-15~~, wherein the autoimmune disease is systemic lupus erythematosus (SLE) or cutaneous lupus erythematosus (CLE).

14.~~17.~~ The polynucleotide for use according to any one of Claims 10-13~~ Claims 13-16~~, wherein the elevated inflammatory gene expression pattern comprises an interferon (IFN) signature comprising elevated levels in the sample of one or more biomarkers selected from the group consisting of BATF2, CMPK2, DDX60, EPSTI1, HERC5, HES4, IFI44, IFI44L, IFIT1, IFIT3, IFITM3, ISG15, LAMP3, LOC26010, MX1, OAS1, OAS2, OAS3, OASL, OTOF, RSAD2, RTP4, SERPING1, TRIM6, XAF1, cl02h05 5, Agencourt-7914287 NIH-MCG_71, ISG20, IRF7, and AIM2, as compared to the control sample(s).

15.~~18.~~ The polynucleotide for use according any one of Claim 10-14~~ Claims 13-17~~, wherein the elevated inflammatory gene expression pattern comprises elevated levels in the sample of one or more cytokines selected from the group consisting of IL-1 alpha, IL-1 beta, TNF-alpha, IL-6, IL-17, IFN-alpha, IFN-omega, IFN-lambda1, IFN-lambda2, and IP-10, as compared to the control sample(s).

16.~~19.~~ A pharmaceutical composition comprising the polynucleotide of any one of Claims 1-8~~ Claims 1-11~~ and a pharmaceutically acceptable excipient.

17.~~20.~~ The pharmaceutical composition of Claim 16~~Claim 19~~ comprising a sterile, isotonic solution.