TITLE

[0001] Therapeutic Nuclease Compositions and Methods.

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to US Provisional Application No. 61/480,961, filed April 29, 2011 and US Provisional Application No. 61/617,241, filed March 29, 2012. This application is also related to: International Patent Application No. PCT/US2010/055131, filed November 2, 2010; U.S. Provisional Application No. 61/257,458, filed November 2, 2009; and U.S. Provisional Application No. 61/370,752, filed August 4, 2010. The entire disclosures of the foregoing applications are hereby incorporated by reference in their entirety for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with support from the National Institutes of Health (Grants AI44257, NS065933, and AR048796), the Alliance for Lupus Research, and the Washington State Life Science Discovery Fund (2087750). The government has certain rights in the invention.

BACKGROUND

[0003] Excessive release of (ribo)nucleoprotein particles from dead and dying cells can cause lupus pathology by two mechanisms: (i) Deposition or *in situ* formation of chromatin / anti-chromatin complexes causes nephritis and leads to loss of renal function; and (ii) nucleoproteins activate innate immunity through toll-like receptor (TLR) 7, 8, and 9 as well as TLR-independent pathway(s). Release of nucleoproteins can serve as a potent antigen for autoantibodies in SLE, providing amplification of B cell and DC activation through co-engagement of antigen receptors and TLRs. Thus, there exists a need for a means to remove inciting antigens and/or attenuate immune stimulation, immune amplification, and immune complex mediated disease in subjects in need thereof.

SUMMARY

[0003a] In one aspect, provided herein is a hybrid nuclease molecule comprising a human RNase and a mutant human IgG1 Fc domain, wherein the human RNase is operatively coupled to the Fc domain and wherein the Fc domain includes a P238S mutation and a P331S mutation and has reduced cytotoxicity relative to a hybrid nuclease molecule having an unmodified Fc domain, and wherein the hybrid nuclease molecule comprises: an amino acid

sequence set forth in SEQ ID NO: 96, 92, 62, or 78; or an amino acid sequence set forth in SEQ ID NO: 98 or 94.

[0004] Disclosed herein is a hybrid nuclease molecule comprising a first nuclease domain and a modified Fc domain, wherein the first nuclease domain is operatively coupled to the Fc domain. The Fc domain is modified such that the molecule has reduced cytotoxicity relative to a hybrid nuclease molecule having an unmodified Fc domain. In some examples, the hybrid nuclease molecule has an Fc domain which has been modified to decrease binding to

Fcγ receptors, complement proteins, or both. In some embodiments, the hybrid nuclease molecule has reduced cytotoxity at least 1, 2, 3, 4, or 5-fold compared to a control molecule e.g., a hybrid nuclease molecule without a modified Fc domain.

[0005] In some examples, the hybrid nuclease molecule further includes a first linker domain, and the first nuclease domain is operatively coupled to the modified Fc domain by the first linker domain.

[0006] In some examples, a hybrid nuclease molecule includes a modified Fc domain which is a mutant, IgG1 Fc domain. In some examples, a mutant Fc domain comprises one or more mutations in the hinge, CH2, and/or CH3 domains. In some examples, the Fc domain comprises an amino acid sequence having one or more of the mutations P238S, P331S, SCC, SSS (residues 220, 226, and 229), G236R, L328R, L234A, and L235A. In some examples, a mutant Fc domain includes a P238S mutation. In some examples, a mutant Fc domain includes a P331S mutation. In some examples, a mutant Fc domain includes a P238S mutation and a P331S mutation. In some examples, a mutant Fc domain comprises P238S and/or P331S, and may include mutations in one or more of the three hinge cysteines. In some examples, a mutant Fc domain comprises P238S and/or P331S, and/or one or more mutations in the three hinge cysteines. In some examples, a mutant Fc domain comprises P238S and/or P331S, and/or mutations in one of the three hinge cysteines (located at residue 220 by EU numbering) to SCC (wherein CCC refers to the three cysteines present in the wild type hinge domain). In some examples, a mutant Fc domain comprises P238S and/or P331S, and/or mutations in the three hinge cysteines (located at residues 220, 226 and 229 by EU numbering) to SSS. In some examples, a mutant Fc domain comprises P238S and P331S and mutations in the three hinge cysteines. In some examples, a mutant Fc domain comprises P238S and P331S and SCC. In some examples, a mutant Fc domain comprises P238S and P331S and SSS. In some examples, a mutant Fc domain includes P238S and SCC. In some examples, a mutant Fc domain includes P238S and SSS. In some examples, a mutant Fc domain includes P331S and SCC. In some examples, a mutant Fc domain includes P331S and SSS. In some examples, a mutant Fc domain includes mutations in one or more of the three hinge cysteines. In some examples, a mutant Fc domain includes mutations in the three hinge cysteines. In some examples, a mutant Fc domain includes a mutation in the three hinge cysteines to SCC. In some examples, a mutant Fc domain includes mutations in the three hinge cysteines to SSS. In some examples, a mutant Fc domain includes SCC. In some examples, a mutant Fc domain includes SSS.

[0007] In some examples, a nucleic acid encoding a mutant Fc domain is as shown in SEQ ID NO:59. In some examples, a mutant Fc domain is as shown in SEQ ID NO:60. In some examples, a nucleic acid encoding a mutant Fc domain is as shown in SEQ ID NO:71. In some examples, a mutant Fc domain is as shown in SEQ ID NO:72. In some examples, a nucleic acid encoding a mutant Fc domain is as shown in SEQ ID NO:73. In some examples, a mutant Fc domain is as shown in SEQ ID NO:74. In some examples, a nucleic acid encoding a mutant Fc domain is shown in SEQ ID NO:75. In some examples, a mutant Fc domain is as shown in SEQ ID NO:76. In some examples, a nucleic acid encoding a mutant Fc domain is as shown in SEQ ID NO:87. In some examples, a mutant Fc domain is as shown in SEQ ID NO:88. In some examples, a nucleic acid encoding a mutant Fc domain is as shown in SEQ ID NO:89. In some examples, a mutant Fc domain is as shown in SEQ ID NO:90.

[0008] In some examples, a hybrid nuclease molecule comprises a wild-type, human RNase1 domain linked to a mutant, human IgG1 Fc domain comprising SCC, P238S, and P331S, or to a mutant, human IgG1 Fc domain comprising SSS, P238S, and P331S. In some examples, a nucleic acid encoding a hybrid nuclease molecule is as shown in SEQ ID NO: 61, 77, or 91. In some examples, a hybrid nuclease molecule is as shown in SEQ ID NO:209, 62, 78, 92, or 94.

[0009] In some examples, a hybrid nuclease molecule comprises a wild-type, human RNase1 domain linked via a $(Gly_4Ser)_4$ linker domain to a mutant, human IgG1 Fc domain comprising SCC, P238S, and P331S, or to a mutant, human IgG1 Fc domain comprising SSS, P238S, and P331S. In some examples, a nucleic acid encoding a hybrid nuclease molecule is as shown in SEQ ID NO: 63, or 79. In some examples, a hybrid nuclease molecule is as shown in SEQ ID NO: 64, or 79.

[0010] In some examples, a hybrid nuclease molecule comprises a human DNase1 G105R A114F domain linked via a $(Gly_4Ser)_4$ linker domain to a mutant, human IgG1 Fc domain comprising SCC, P238S, and P331S linked via a NLG linker domain to a wild-type, human RNase1 domain. In some examples, a hybrid nuclease molecule comprises a human DNase1 G105R A114F domain linked via a $(Gly_4Ser)_4$ linker domain to a mutant, human IgG1 Fc domain comprising SSS, P238S, and P331S, linked via a NLG linker domain to a wild-type, human RNase1 domain. In some examples, a nucleic acid encoding a hybrid nuclease molecule is as shown in SEQ ID NO: 65, or 81. In some examples, a hybrid nuclease molecule is as shown in SEQ ID NO: 66, or 82.

In other examples, a hybrid nuclease molecule comprises an amino acid sequence set forth in SEQ ID NO: 62, 64, 78, 80, 92, or 96, or a hybrid nuclease molecule comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 62, 64, 78, 80, 92, or 96. In some ascpects, a hybrid nuclease molecule comprises an amino acid sequence set forth in SEQ ID NO: 96. In other examples, a hybrid nuclease molecule comprises an amino acid sequence set forth in SEQ ID NO: 66, 68, 70, 82, 84, 86, 94, or 98, or a hybrid nuclease molecule comprising an amino acid sequence at least 90% identical to the amino acid sequence set forth in SEQ ID NO: 66, 68, 70, 82, 84, 86, 94, or 98. In other examples, a hybrid nuclease molecule comprises an amino acid sequence set forth in SEQ ID NO: 98.

[0011] In some examples, a hybrid nuclease molecule comprises a wild-type, human RNase1 domain linked via a $(Gly_4Ser)_4$ linker domain to a mutant, human IgG1 Fc domain comprising SCC, P238S, and P331S linked via a NLG linker domain to a human DNase1 G105R A114F domain. In some examples, a hybrid nuclease molecule comprises a wild-type, human RNase1 domain linked via a $(Gly_4Ser)_4$ linker domain to a mutant, human IgG1 Fc domain comprising SSS, P238S, and P331S, linked via a NLG linker domain to a human DNase1 G105R A114F domain. In some examples, a nucleic acid encoding a hybrid nuclease molecule is as shown in SEQ ID NO: 67, or 83. In some examples, a hybrid nuclease molecule is shown in SEQ ID NO: 68, or 84.

[0012] In some examples, a hybrid nuclease molecule comprises a wild-type, human RNase1 domain linked to a mutant, human IgG1 Fc domain comprising SCC, P238S, and P331S linked via a NLG linker domain to a human DNase1 G105R A114F domain. In some examples, a hybrid nuclease molecule comprises a wild-type, human RNase1 domain linked to a mutant, human IgG1 Fc domain comprising SSS, P238S, and P331S linked via a NLG linker domain to a human DNase1 G105R A114F domain. In some examples, a nucleic acid encoding a hybrid nuclease molecule is shown in SEQ ID NO: 69, 85, or 93. In some examples, a hybrid nuclease molecule is shown in SEQ ID NO: 70, 86, 94, or 98.

[0013] In some examples, cytotoxicity induced by a hybrid nuclease molecule is reduced when compared to a control molecule. In some examples, cytotoxicity induced by a hybrid nuclease molecule is reduced about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96,

97, 98, 99, or 100% when compared to a control molecule. In some examples, the hybrid nuclease molecule has about 3-5 fold, or at least about 3 fold reduced cytotoxicity as compared to a hybrid nuclease molecule having an unmodified Fc domain (e.g., a wild type Fc domain).

[0014] In some examples, the activity of a hybrid nuclease molecule having a DNase is not less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or >30-fold less than the activity of a control DNase molecule. In some examples, the activity of a hybrid nuclease molecule having a DNase is about equal to the activity of a control DNase molecule. In some examples, the activity of a hybrid nuclease molecule having an RNase is not less than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or >30-fold less than the activity of a control RNase molecule. In some examples, the activity of a hybrid nuclease molecule having an RNase is about equal to the activity of a control RNase molecule.

[0015] In some examples, a hybrid nuclease molecule is a polypeptide, wherein the amino acid sequence of the first nuclease domain comprises a human, wild-type RNase amino acid sequence, wherein the first linker domain is (Gly4Ser)n, where n is 0, 1, 2, 3, 4 or 5, wherein the amino acid sequence of the Fc domain comprises a human, mutant IgG1 Fc domain amino acid sequence, and wherein the first linker domain is coupled to the C-terminus of the first nuclease domain and the N-terminus of the Fc domain. In some examples, a hybrid nuclease molecule is a polypeptide comprising or consisting of a sequence shown in Table 1.

[0016] In some examples, a hybrid nuclease molecule comprises wild-type, human DNase1 linked to a mutant, human IgG1 Fc domain. In some examples, a hybrid nuclease molecule comprises human DNase1 G105R A114F linked to a mutant, human IgG1 Fc domain by a $(Gly_4Ser)_n$ linker domain where n=0, 1, 2, 3, 4, or 5. In some examples, a hybrid nuclease molecule comprises wild-type, human RNase1 linked to a mutant, human IgG1 Fc domain linked to wild-type, human DNase1. In some examples, a hybrid nuclease molecule comprises wild-type, human RNase1 linked to a mutant, human IgG1 Fc domain linked to human DNase1 G105R A114F. In some examples, a hybrid nuclease molecule is a polypeptide, wherein the amino acid sequence of the first nuclease domain comprises a RNase amino acid sequence, wherein the first linker domain is between 5 and 32 amino acids in length, wherein the amino acid sequence of the Fc domain comprises a human, Fc domain amino acid sequence, and wherein the first linker domain is coupled to the

C-terminus of the first nuclease domain and the N-terminus of the Fc domain. In some examples, the linker domain includes $(Gly_4Ser)_5$ and restriction sites BglII, AgeI, and XhoI. In some examples, a hybrid nuclease molecule is a polypeptide, wherein the amino acid sequence of the first nuclease domain comprises a human RNase amino acid sequence, wherein the first linker domain is a NLG peptide between 5 and 32 amino acids in length, wherein the amino acid sequence of the Fc domain comprises a human, mutant Fc domain amino acid sequence, and wherein the first linker domain is coupled to the C-terminus of the first nuclease domain and the N-terminus of the Fc domain.

[0017] In some examples, the Fc domain does not substantially bind to an Fc receptor on a human cell. In some examples, the Fc domain is modified to decrease binding to Fcγ receptors, complement proteins, or both. In some examples Fc receptor binding is reduced about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% when compared to a control molecule. In some examples, the hybrid nuclease molecule has decrease Fc receptor binding by 3-5 fold, or at least about 3 fold as compared to a hybrid nuclease molecule having an unmodified Fc domain (e.g., a wild type Fc domain).

[0018] In some examples, the serum half-life of the molecule is significantly longer than the serum half-life of the first nuclease domain alone. In some examples, the nuclease activity of the first nuclease domain of the molecule is the same or greater than the nuclease domain alone. In some examples, administration of the molecule to a mouse increases the survival rate of the mouse as measured by a mouse Lupus model assay. In some examples, the hybrid nuclease molecule degrades circulating RNA, DNA or both. In other examples, the hybrid nuclease molecule degrades RNA, DNA or both in immune complexes. In some examples, hybrid nuclease molecule inhibits interferon-α production. In some examples interferon-α production is reduced about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% when compared to a control molecule.

[0019] In some examples, a hybrid nuclease molecule includes a leader sequence. In some examples, the leader sequence is human VK3LP peptide from the human kappa

light chain family, and the leader sequence is coupled to the N-terminus of the first nuclease domain. In examples, the VK3LP has the sequence set forth in SEQ ID NO: 100.

[0020] In some examples, the molecule is a polypeptide. In some examples, the molecule is a polynucleotide.

[0021] In some examples, the first nuclease domain comprises an RNase. In some examples, the RNase is a human RNase. In some examples, the RNase is a polypeptide comprising an amino acid sequence at least 90% identical to an RNase amino acid sequence set forth in Table 1. In some examples, the RNase is a human RNase A family member. In some examples, the RNase is a human pancreatic RNase1.

[0022] In some examples, the first nuclease domain comprises a DNase. In some examples, the DNase is a human DNase. In some examples, the DNase is a polypeptide comprising an amino acid sequence at least 90% identical to a DNase amino acid sequence set forth in Table 1. In some examples, the DNase is selected from the group consisting of human DNase I, TREX1, and human DNase 1L3.

[0023] In some examples, the Fc domain is a human Fc domain. In some examples, the Fc domain is a mutant Fc domain. In some examples, the Fc domain is a mutant Fc domain comprising SSS, P238S, and/or P331S. In some examples, the Fc domain is a human IgG1 Fc domain. In some examples, the Fc domain is a polypeptide comprising an amino acid sequence at least 90% identical to an Fc domain amino acid sequence set forth in Table 1.

[0024] In some examples, the first linker domain has a length of about 1 to about 50 amino acids. In some examples, the first linker domain has a length of about 5 to about 31 amino acids. In some examples, the first linker domain has a length of about 15 to about 25 amino acids. In some examples, the first linker domain has a length of about 20 to about 32 amino acids. In some examples, the first linker domain has a length of about 20 amino acids. In some examples, the first linker domain has a length of about 25 amino acids. In some examples, the first linker domain has a length of about 18 amino acids. In some examples, the first linker domain comprises a gly/ser peptide. In some examples, the gly/ser peptide is of the formula $(Gly_4Ser)_n$, wherein n is a positive integer selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10. In some examples, the gly/ser peptide includes $(Gly_4Ser)_3$. In some examples, the gly/ser peptide includes $(Gly_4Ser)_4$. In some examples, the gly/ser peptide includes $(Gly_4Ser)_5$. In some examples, the first linker domain includes at least one restriction site. In some

examples, the first linker domain includes about 12 or greater nucleotides including at least one restriction site. In some examples, the first linker domain includes two or more restriction sites. In some examples, the first linker domain includes a plurality of restriction sites. In some examples, the first linker domain comprises an NLG peptide. NLG peptides contain an N-linked glycosylation consensus sequence. In examples, the NLG peptide has a sequence as set forth in SEQ ID NO: 99. In some examples, the first linker domain comprises an N-linked glycosylation site.

[0025] In some examples, the first nuclease domain is linked to the N-terminus of the Fc domain. In some examples, the first nuclease domain is linked to the C-terminus of the Fc domain.

[0026] In some examples, the hybrid nuclease molecule further includes a second nuclease domain. In some examples, the first and second nuclease domains are distinct nuclease domains. In some examples, the first and second nuclease domains are the same nuclease domains. In some examples, the second nuclease domain is linked to the C-terminus of the Fc domain. In some examples, the second nuclease domain is linked to the N-terminus of the Fc domain. In some examples, the second nuclease domain is linked to the C-terminus of the first nuclease domain. In some examples, the second nuclease domain is linked to the N-terminus of the first nuclease domain.

[0026a] In one aspect, provided herein is a dimer comprising a hybrid nuclease molecule as described herein, optionally a homodimer.

[0027] Also disclosed herein is a dimeric polypeptide comprising a first polypeptide and a second polypeptide, wherein the first polypeptide comprises a first nuclease domain, and an Fc domain, wherein the first nuclease domain is operatively coupled to the Fc domain. In some examples, the second polypeptide is a second hybrid nuclease molecule comprising a second nuclease domain, and a second Fc domain, wherein the second nuclease domain is operatively coupled to the second Fc domain.

[0027a] In one aspect, provided herein is a composition comprising the hybrid nuclease molecule as described herein or the dimer as described herein, and a pharmaceutically acceptable carrier.

[0028] Also disclosed herein is a pharmaceutical composition comprising at least one hybrid nuclease molecule and/or at least one dimeric polypeptide as described herein, and a pharmaceutically acceptable excipient.

[0029] Also disclosed herein is a nucleic acid molecule encoding a hybrid nuclease molecule disclosed herein. Also disclosed herein is a recombinant expression vector comprising a nucleic acid molecule disclosed herein. Also disclosed herein is a host cell transformed with a recombinant expression vector disclosed herein.

[0030] Also disclosed herein is a method of making a hybrid nuclease disclosed herein, comprising: providing a host cell comprising a nucleic acid sequence that encodes the hybrid

nuclease molecule; and maintaining the host cell under conditions in which the hybrid nuclease molecule is expressed.

[0030a] In one aspect, provided herein is a hybrid nuclease molecule as described herein, the dimer as described herein, or the composition as described herein for use in therapy.

[0030b] In one aspect, provided herein is use of the hybrid nuclease molecule as described herein, the dimer as described herein, or the composition as described herein in the manufacture of a medicament for treating or preventing a condition associated with an abnormal immune response, optionally wherein the condition is an autoimmune disease, preferably wherein the autoimmune disease is selected from the group consisting of insulin-dependent diabetes mellitus, multiple sclerosis, experimental autoimmune encephalomyelitis, rheumatoid arthritis, experimental autoimmune arthritis, myasthenia gravis, thyroiditis, an experimental form of uveoretinitis, Hashimoto's thyroiditis, primary myxoedema, thyrotoxicosis, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, premature menopause, male infertility, juvenile diabetes, Goodpasture's syndrome, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, phacogenic uveitis, autoimmune haemolytic anaemia, idiopathic leucopenia, primary biliary cirrhosis, active chronic hepatitis Hbs-ve, cryptogenic cirrhosis, ulcerative colitis, Sjogren's syndrome, scleroderma, Wegener's granulomatosis, polymyositis, dermatomyositis, discoid LE, systemic lupus erythematosus (SLE), and connective tissue disease.

[0031] Also disclosed herein is a method for treating or preventing a condition associated with an abnormal immune response, comprising administering to a patient in need thereof an effective amount of an isolated hybrid nuclease molecule disclosed herein. In some examples, the condition is an autoimmune disease. In some examples, the autoimmune disease is selected from the group consisting of insulin-dependent diabetes mellitus, multiple sclerosis, experimental autoimmune encephalomyelitis, rheumatoid arthritis, experimental autoimmune arthritis, myasthenia gravis, thyroiditis, an experimental form of uveoretinitis, Hashimoto's thyroiditis, primary myxoedema, thyrotoxicosis, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, premature menopause, male infertility, juvenile diabetes, Goodpasture's syndrome, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, phacogenic uveitis, autoimmune haemolytic anaemia, idiopathic leucopenia, primary biliary cirrhosis, active chronic hepatitis Hbs-ve, cryptogenic cirrhosis, ulcerative colitis, Sjogren's syndrome, scleroderma, Wegener's granulomatosis, polymyositis, dermatomyositis, discoid LE, systemic lupus erythematosus (SLE), and connective tissue disease. In some examples, the autoimmune disease is SLE.

[0032] Also disclosed herein is a method of treating SLE comprising administering to a subject a nuclease-containing composition in an amount effective to degrade immune complexes

containing RNA, DNA or both RNA and DNA. In some examples, the composition includes a pharmaceutically acceptable carrier and a hybrid nuclease molecule as described herein. In other examples, the composition includes a hybrid nuclease molecule comprising an amino acid sequence set forth in SEQ ID NO: 62, 64, 66, 68, 70, 78, 80, 82, 84, 86, 92, 94, 96, or 98.

[0032a] In another aspect, provided herein is a nuclease-containing composition effective to degrade immune complexes containing RNA, DNA or both RNA and DNA, wherein the composition comprises a pharmaceutically acceptable carrier and a hybrid nuclease molecule comprising an amino acid sequence set forth in SEQ ID NO: 96, 98, 92, 94, 62, or 78 for use in therapy.

[0032b] In another aspect, provided herein is a use of a nuclease-containing composition effective to degrade immune complexes containing RNA, DNA or both RNA and DNA, wherein the composition comprises a pharmaceutically acceptable carrier and a hybrid nuclease molecule comprising an amino acid sequence set forth in SEQ ID NO: 96, 98, 92, 94, 62, or 78 in the manufacture of a medicament for treating SLE.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0033] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0034] FIG. 1 shows a prototype structure for creating different embodiments of hybrid nuclease molecules.

[0035] FIG. 2 shows the concentration of RSLV-124 recovered from mouse serum following a single intravenous injection.

CLAIMS

1. A hybrid nuclease molecule comprising a human RNase and a mutant human IgG1 Fc domain, wherein the human RNase is operatively coupled to the Fc domain and wherein the Fc domain includes a P238S mutation and a P331S mutation and has reduced cytotoxicity relative to a hybrid nuclease molecule having an unmodified Fc domain, and wherein the hybrid nuclease molecule comprises:

an amino acid sequence set forth in SEQ ID NO: 96, 92, 62, or 78; or

an amino acid sequence set forth in SEQ ID NO: 98 or 94.

2. The hybrid nuclease molecule of claim 1, comprising an amino acid sequence set forth in SEQ ID NO: 96.

3. The hybrid nuclease molecule of claim 1, comprising an amino acid sequence set forth in SEQ ID NO: 98.

4. A dimer comprising a hybrid nuclease molecule of any of claims 1-3, optionally a homodimer.

5. A composition comprising the hybrid nuclease molecule of any one of claims 1-3 or the dimer of claim 4, and a pharmaceutically acceptable carrier.

6. A nucleic acid molecule encoding the hybrid nuclease molecule according to any one of claims 1-3.

7. A recombinant expression vector comprising a nucleic acid molecule according to claim 6.

8. A host cell transformed with the recombinant expression vector according to claim 7.

9. A method of making the hybrid nuclease molecule of any of claims 1-3, comprising: providing a host cell comprising a nucleic acid sequence that encodes the hybrid nuclease molecule; and maintaining the host cell under conditions in which the hybrid nuclease molecule is expressed.

10. The hybrid nuclease molecule of any one of claims 1-3, the dimer of claim 4, or the composition of claim 5 for use in therapy.

11. Use of the hybrid nuclease molecule of any one of claims 1-3, the dimer of claim 4, or the composition of claim 5 in the manufacture of a medicament for treating or preventing a condition associated with an abnormal immune response, optionally wherein the condition is an autoimmune disease, preferably wherein the autoimmune disease is selected from the group consisting of insulin-dependent diabetes mellitus, multiple sclerosis, experimental autoimmune encephalomyelitis, rheumatoid arthritis, experimental autoimmune arthritis, myasthenia gravis, thyroiditis, an experimental form of uveoretinitis, Hashimoto's thyroiditis, primary myxoedema, thyrotoxicosis, pernicious anaemia, autoimmune atrophic gastritis, Addison's disease, premature menopause, male infertility, juvenile diabetes, Goodpasture's syndrome, pemphigus vulgaris, pemphigoid, sympathetic ophthalmia, phacogenic uveitis, autoimmune haemolytic anaemia, idiopathic leucopenia, primary biliary cirrhosis, active chronic hepatitis Hbs-ve, cryptogenic cirrhosis, ulcerative colitis, Sjogren's syndrome, scleroderma, Wegener's granulomatosis, polymyositis, dermatomyositis, discoid LE, systemic lupus erythematosus (SLE), and connective tissue disease.

12. The use according to claim 11, wherein the autoimmune disease is SLE.

13. A nuclease-containing composition effective to degrade immune complexes containing RNA, DNA or both RNA and DNA, wherein the composition comprises a pharmaceutically acceptable carrier and a hybrid nuclease molecule comprising an amino acid sequence set forth in SEQ ID NO: 96, 98, 92, 94, 62, or 78 for use in therapy.

14. Use of a nuclease-containing composition effective to degrade immune complexes containing RNA, DNA or both RNA and DNA, wherein the composition comprises a pharmaceutically acceptable carrier and a hybrid nuclease molecule comprising an amino acid sequence set forth in SEQ ID NO: 96, 98, 92, 94, 62, or 78 in the manufacture of a medicament for treating SLE.