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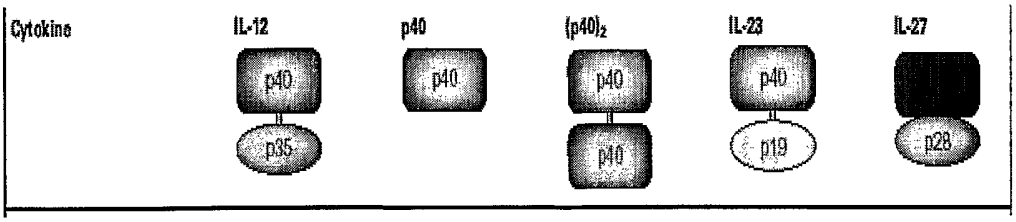
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(54) Title: APTAMERS TO THE HUMAN IL-12 CYTOKINE FAMILY AND THEIR USE AS AUTOIMMUNE DISEASE THERAPEUTICS



(57) Abstract: The present invention provides materials and methods to treat immune disease in which cytokines are involved in pathogenesis. The materials and methods of the present invention are useful in the treatment of autoimmune diseases. The materials and methods of the present invention are directed to nucleic acid ligands capable of binding to human IL-23 and/or human IL-12 cytokines and thus modulate their biological activity and are useful as therapeutic agents in immune, auto-immune and cancer therapeutics.

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Aptamers to the Human IL-12 Cytokine Family and Their Use as Autoimmune Disease Therapeutics

FIELD OF INVENTION

[0001] The invention relates generally to the field of nucleic acids and more particularly to aptamers capable of binding to members of the human interleukin-12 (IL-12) cytokine family, more specifically to human interleukin-12 (IL-12), human interleukin-23 (IL-23), or both IL-12 and IL-23, and to other related cytokines (*e.g.*, IL-27 and p40 dimer). Such aptamers are useful as therapeutics in and diagnostics of autoimmune related diseases and/or other diseases or disorders in which the IL-12 family of cytokines, specifically IL-23 and IL-12, have been implicated. The invention further relates to materials and methods for the administration of aptamers capable of binding to IL-23 and/or IL-12.

BACKGROUND OF THE INVENTION

[0002] Aptamers are nucleic acid molecules having specific binding affinity to molecules through interactions other than classic Watson-Crick base pairing.

[0003] Aptamers, like peptides generated by phage display or monoclonal antibodies ("mAbs"), are capable of specifically binding to selected targets and modulating the target's activity, *e.g.*, through binding aptamers may block their target's ability to function. Created by an *in vitro* selection process from pools of random sequence oligonucleotides, aptamers have been generated for over 100 proteins including growth factors, transcription factors, enzymes, immunoglobulins, and receptors. A typical aptamer is 10-15 kDa in size (30-45 nucleotides), binds its target with sub-nanomolar affinity, and discriminates against closely related targets (*e.g.*, aptamers will typically not bind other proteins from the same gene family). A series of structural studies have shown that aptamers are capable of using the same types of binding interactions (*e.g.*, hydrogen bonding, electrostatic complementarities, hydrophobic contacts, steric exclusion) that drive affinity and specificity in antibody-antigen complexes.

[0004] Aptamers have a number of desirable characteristics for use as therapeutics and diagnostics including high specificity and affinity, biological efficacy, and excellent

pharmacokinetic properties. In addition, they offer specific competitive advantages over antibodies and other protein biologics, for example:

[0005] 1) Speed and control. Aptamers are produced by an entirely *in vitro* process, allowing for the rapid generation of initial leads, including therapeutic leads. *In vitro* selection allows the specificity and affinity of the aptamer to be tightly controlled and allows the generation of leads, including leads against both toxic and non-immunogenic targets.

[0006] 2) Toxicity and Immunogenicity. Aptamers as a class have demonstrated little or no toxicity or immunogenicity. In chronic dosing of rats or woodchucks with high levels of aptamer (10 mg/kg daily for 90 days), no toxicity is observed by any clinical, cellular, or biochemical measure. Whereas the efficacy of many monoclonal antibodies can be severely limited by immune response to antibodies themselves, it is extremely difficult to elicit antibodies to aptamers most likely because aptamers cannot be presented by T-cells via the MHC and the immune response is generally trained not to recognize nucleic acid fragments.

[0007] 3) Administration. Whereas most currently approved antibody therapeutics are administered by intravenous infusion (typically over 2-4 hours), aptamers can be administered by subcutaneous injection (aptamer bioavailability via subcutaneous administration is >80% in monkey studies (Tucker *et al.*, J. Chromatography B. 732: 203-212, 1999)). This difference is primarily due to the comparatively low solubility and thus large volumes necessary for most therapeutic mAbs. With good solubility (>150 mg/mL) and comparatively low molecular weight (aptamer: 10-50 kDa; antibody: 150 kDa), a weekly dose of aptamer may be delivered by injection in a volume of less than 0.5 mL. In addition, the small size of aptamers allows them to penetrate into areas of conformational constrictions that do not allow for antibodies or antibody fragments to penetrate, presenting yet another advantage of aptamer-based therapeutics or prophylaxis.

[0008] 4) Scalability and cost. Therapeutic aptamers are chemically synthesized and consequently can be readily scaled as needed to meet production demand. Whereas difficulties in scaling production are currently limiting the availability of some biologics and the capital cost of a large-scale protein production plant is enormous, a single large-scale oligonucleotide synthesizer can produce upwards of 100 kg/year and requires a relatively modest initial investment. The current cost of goods for aptamer synthesis at the kilogram scale is estimated at $500/g, comparable to that for highly optimized antibodies. Continuing

improvements in process development are expected to lower the cost of goods to < $100/g in five years.

[0009] 5) Stability. Therapeutic aptamers are chemically robust. They are intrinsically adapted to regain activity following exposure to factors such as heat and denaturants and can be stored for extended periods (>1 yr) at room temperature as lyophilized powders.

CYTOKINES AND THE IMMUNE RESPONSE

[0010] The immune response in mammals is based on a series of complex cellular interactions called the "immune network." In addition to the network-like cellular interactions of lymphocytes, macrophages, granulocytes, and other cells, soluble proteins known as lymphokines, cytokines, or monokines play a critical role in controlling these cellular interactions. Cytokine expression by cells of the immune system plays an important role in the regulation of the immune response. Most cytokines are pleiotropic and have multiple biological activities including antigen-presentation; activation, proliferation, and differentiation of CD4+ cell subsets; antibody response by B cells; and manifestations of hypersensitivity. Cytokines are implicated in a wide range of degenerative or abnormal conditions which directly or indirectly involve the immune system and/or hematopoietic cells. An important family of cytokines is the IL-12 family which includes, *e.g.,* IL-12, IL-23, IL-27, and p40 monomers and p40 dimers.

[0011] IL-23 is a covalently linked heterodimeric molecule composed of the p19 and p40 subunits, each encoded by separate genes. IL-12 is also a covalently linked heterodimeric molecule and consists of the p35 and p40 subunits. Thus, IL-23 and IL-12 both have the p40 subunit in common (Figure 1). Human and mouse p19 share ~70% amino acid sequence identity and are closely related to p35 (the subunit unique to IL-12). Transfection assays reveal that like p35, p19 protein is poorly secreted when expressed alone and requires the co-expression of its heterodimerizing partner p40 for higher expression. Together, p40 and p19 form a disulfide-linked heterodimer. The p19 component is produced in large amounts by activated macrophages, dendritic cells ("DCs"), endothelial cells, and T cells. Th1 cells express larger amounts of p19 mRNA than do Th2 cells; however, among these cell types only activated macrophages and DCs constitutively express p40, the other component of IL-23. The expression of p19 is increased by bacterial products that signal through the Toll-like receptor-2, which suggests that p19, and thus IL-23, may function in the immune response to certain bacterial infections.

[0012] One of the shared actions of IL-12 and IL-23 is their proliferative effect on T-cells (Brombacher *et al.*, Trends in Immun. (2003)). However, clear differences exist in the T-cell subsets on which these cytokines act. In the mouse, IL-12 induces proliferation of naïve murine T cells but not memory T cells, whereas the proliferative effect of IL-23 is confined to memory T cells. In humans, IL-12 promotes proliferation of both naïve and memory human T-cells; however, the proliferative effect of IL-23 is still restricted to memory T cells. Also, the action of IL-23 on IFN-γ production is directed primarily toward memory T cells in humans. Although IL-12 can induce IFN-γ production in naïve T-cells and, to a greater extent, memory T-cells, IL-23 has very little effect on IFN-γ production in naïve T-cells. A moderate increase in IFN-γ production is observed in memory T-cells stimulated by IL-23, but this effect is somewhat smaller than that resulting from stimulation with IL-12.

[0013] Thus, IL-23 has biological activity that is distinct from IL-12, however both are believed to play a role in autoimmune and inflammatory diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis, systemic lupus erythamatosus, and irritable bowel diseases (including Crohn's disease and ulcerative colitis), in addition to diseases such as bone resoprtion in osteoporosis, Type I Diabetes, and cancer.

IL-23 AND/OR IL-12 SPECIFIC APTAMERS AS AUTOIMMUNE DISEASE THERAPEUTICS

[0014] While not intending to be bound by theory, it is believed that IL-12 and IL-23 are involved in multiple sclerosis ("MS") pathogenesis. For example, p40 levels are up-regulated in the cerebral spinal fluid of MS patients (Fassbender *et al.*, (1998) Neurology 51:753). In addition, an anti-p40 mAb has been shown to localize to lesions in the brain (Brok *et al.*, JI (2002)169:6554). Furthermore, lower baseline levels of p40 mRNA have been shown to predict clinical responsiveness to IFN-β treatment (Van-Boxel-Dezaire *et al.*, 1999). Thus, a knock-down of both IL-12 and IL-23 via p40 might ameliorate the symptoms of MS. In fact, anti-p40 antibodies have been shown to significantly suppress the development and severity of Experimental Autoimmune Encephalomyelitis ("EAE") in mice (Constantinescu *et al.*, JI (1998) 161:5097) and in marmosets (Brok *et al.*, JI (2002)169:6554).

[0015] Despite the evidence showing that knocking out both IL-23 and IL-12 suppresses the development and symptoms of MS, there is strong evidence that IL-23 is the more

important of the two in MS/EAE pathogenesis in mice, as shown by the effects of IL-12 and IL-23 knock-outs on the EAE mouse model. (Cua *et al.*, (2003) Nature 421:744). For example, EAE can occur in p35 knockout mice, but not p19 or p40 knock-out mice (Cua *et al.*, (2003). Expression of IL-23 but not IL-12 in the CNS rescues EAE in p19/p40 knock-out mice, although over-expression of IL-12 exacerbates EAE, so IL-12 seems to play some role in general TH1 cell development and activation (Cua *et al.*). In humans, over-expression of p40 mRNA but not p35 mRNA has been observed in the Central Nervous System (CNS) of MS patients.

[0016] In addition to playing a general role in activating Th1 cells, IL-12 may be more important for fighting infection than IL-23. In mice, a p19 knock-out induces classic Th1 cell response (high IFN-gamma, low IL-4), whereas the response in p35 and p40 knock-out mice is restricted to Th2 cells (low IFN-gamma, high IL-4) (Cua *et al.*). Additionally, p19 knock-out immune cells produce strong pro-inflammatory cytokines, whereas p40 knock-out immune cells cannot. Lastly, p40, IL-12Rβ1 and IL-12Rβ2 knock-out mice are susceptible to a variety of infections (Adorini, from Contemporary Immunology (2003) pg. 253). Thus inhibiting IL-23 specifically through aptamer therapeutics may effectively fight IL-23 mediated disease while leaving the patient more able to fight infection.

[0017] Both IL-23 and/or IL-12 have been implicated in rheumatoid arthritis as a promoter of end-stage joint inflammation. While not intending to be bound by theory, it is believed that IL-23 affects the function of memory T-cells and inflammatory macrophages through engagement of the IL-23 receptor (IL-23R) on these cells. Studies indicate the IL-23 subunits p19 and/or p40 play a role in murine collagen-induced arthritis ("CIA"), the mouse model for rheumatoid arthritis. Anti-p40 antibodies have been shown to ameliorate the symptoms in murine CIA and prevent development and progression alone and when combined with anti-tumor necrosis factor (anti-TNF) treatment (Malfait *et al.*, Clin. Exp. Immunol. (1998) 111:377, Matthys *et al.*, Eur. J. Immunol. (1998) 28:2143, and Butler *et al.*, Eur. J. Immunol. (1999) 29:2205). Furthermore, p19 and p40 knockout mice have been shown to be completely resistant to the development of CIA while CIA development and severity is exacerbated in p35 knock-out mice (McIntyre *et al.*, Eur. J. Immunol. (1996) 26:2933, and Murphy *et al.*, J. Exp. Med. (2003) 198:1951). Thus, the aptamers and methods of the present invention that bind to and inhibit IL-23 are useful as therapeutic agents for rheumatoid arthritis.

[0018] Both IL-23 and/or IL-12 are also believed to play a dominant role in the recruitment of inflammatory cells in Th-1 mediated diseases such as psoriasis vulgaris, and irritable bowel disease, including but not limited to Crohn's disease and ulcerative colitis. For example, elevated levels of p19 and p40 mRNA were detected by quantitative RT-PCR in skin lesions of patients with psoriasis vulgaris, whereas p35 mRNA was not (Lee *et al.*, J Exp Med (2004) 199(1):125-30). In 2, 4, 6, trinitrobenzene sulfonic acid ("TNBS") colitis, an experimental model of inflammatory bowel disease in mice, treatment with an anti-IL-12 monoclonal antibody proved efficacious in completely ameliorating/preventing mucosal inflammation (Neurath *et al.*, J Exp Med (1995) 182:1281-1290). In another study which evaluated several different IL-12 antagonists in the TNBS colitis model, an anti-IL-12 p40 antibody proved to be the most effective in preventing mucosal inflammation, thus implicating both IL-12 and IL-23 (Schmidt *et al.*, Pathobiology (2002-03); 70:177-183). Thus, the aptamers of the present invention that bind to and inhibit IL-12 and/or IL-23 are useful as therapeutic agents for psoriasis and inflammatory bowel diseases.

[0019] It is also believed that IL-12 and/or IL-23 play a role in systemic lupus erythamatosus ("SLE"). For example, serum obtained from SLE patients were found to contain significantly higher amounts of p40 as a monomer than serum levels of p40 as a heterodimer *e.g.*, IL-12 (p35/p40) and IL-23 (p19/p40), indicating that deficient IL-23 and/or IL-12 production may play a role in the pathogenesis of SLE. Thus, aptamers of the invention which enhance the biological function of IL-23 and/or IL-12 are useful as therapeutics in the treatment of systemic lupus erythamatosus (Lauwerys *et al.*, Lupus (2002) 11(6):384-7).

IL-23 AND/OR IL-12 SPECIFIC APTAMERS AS ONCOLOGICAL THERAPEUTICS

[0020] The anti-tumor activity of IL-12 has been well characterized, and recent studies have shown that IL-23 also possesses anti-tumor and anti-metastatic activity. For example, colon carcinoma cells retrovirally transduced with IL-23 significantly reduced the growth of colon tumors established by the cell line in immunocompetent mice as compared to a control cell line, indicating that the expression of IL-23 in tumors produces an anti-tumor effect. (Wang *et al.*, Int. J. Cancer: 105, 820-824 (2003). Likewise, a lung carcinoma cell line retrovirally engineered to release single chain IL-23 ("scIL-23") significantly suppressed lung metastases in BALB/c mice, resulting in almost complete tumor rejection (Lo *et al.*, J. Immunol 2003, 171:600-607). Thus, aptamers that bind to IL-23 and/or IL-12 and enhance

their biological function are useful as oncological therapeutics for the treatment of colon cancer, lung cancer, specifically lung metastases, and other oncological diseases for which IL-23 and/or IL-12 have an anti-tumor effect.

[0021] There is currently no known therapeutic agent that specifically targets human IL-23. Available agents that target IL-23 include an anti-human IL-23 p19 polyclonal antibody available through R&D Systems (Minneapolis, MN) for research use only, an anti-human p40 monoclonal antibody which targets both IL-12 and IL-23, since both cytokines have the p40 subunit in common, and anti-mouse IL-23 p19 polyclonal and monoclonal antibodies, which target mouse IL-23, not human IL-23 (Pirhonen, *et al.*, (2002), J Immunology 169:5673-5678). As previously explained, an agent that inhibits the activity of both IL-23 and IL-12 may leave patients more vulnerable to infections, and generally can pose more complications in terms of developing a therapeutic agent than an agent that inhibits only IL-23. Since there is evidence that IL-23 plays a more important role than IL-12 for autoimmune inflammation in the brain and joints, a therapeutic specific for only IL-23 may be more advantageous than an agent which targets both cytokines, such as the anti-p40 human mAb.

[0022] Given the advantages of specificity, small size, and affinity of aptamers as therapeutic agents, it would be beneficial to have materials and methods for aptamer therapeutics to treat diseases in which human cytokines, specifically IL-23 and IL-12, play a role in pathogenesis. The present invention provides materials and methods to meet these and other needs.

SUMMARY OF THE INVENTION

[0023] The present invention provides materials and methods for the treatment of autoimmune and inflammatory disease and other related diseases/disorders in which IL-23 and/or IL-12 are involved in pathogenesis.

[0024] In one embodiment, the materials of the present invention provide aptamers that specifically bind to IL-23. In one embodiment, IL-23 to which the aptamers of the invention bind is human IL-23 while in another embodiment IL-23 is a variant of human IL-23. In one embodiment the variant of IL-23 performs a biological function that is essentially the same as a function of human IL-23 and has substantially the same structure and substantially the same ability to bind said aptamer as that of human IL-23.

[0025] In one embodiment, human IL-23 or a variant thereof comprises an amino acid sequence which is at least 70% identical, preferably at least 80% identical, more preferably at least 90% identical to a sequence comprising SEQ ID NOs 4 and/or 5. In another embodiment, human IL-23 or a variant thereof has an amino acid sequence comprising SEQ ID NOs 4 and 5.

[0026] In one embodiment, the aptamer of the invention has a dissociation constant for human IL-23 or a variant thereof of about 100 nM or less, preferably 50 nM or less, more preferably 10 nM or less, even more preferably 1 nM or less.

[0027] In one embodiment, the aptamer of the present invention modulates a function of human IL-23 or a variant thereof. In one embodiment, the aptamer of the present invention stimulates a function of human IL-23. In another embodiment, the aptamer of the present invention inhibits a function of human IL-23 or a variant thereof. In yet another embodiment, the aptamer of the present invention inhibits a function of human IL-23 or a variant thereof *in vivo*. In yet another embodiment, the aptamer of the present invention prevents IL-23 from binding to the IL-23 receptor. In some embodiments, the function of human IL-23 or a variant thereof which is modulated by the aptamer of the invention is to mediate a disease associated with human IL-23 such as: autoimmune disease (including but not limited to multiple sclerosis, rheumatoid arthritis, psoriasis, systemic lupus erythamatosus, and irritable bowel disease (*e.g.*, Crohn's Disease and ulcerative colitis)), inflammatory disease, cancer (including but not limited to colon cancer, lung cancer, and lung metastases), bone resorption in osteoporosis, and Type I Diabetes.

[0028] In one embodiment, the aptamer of the invention has substantially the same ability to bind human IL-23 as that of an aptamer comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-130, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 203-314. In another embodiment the aptamer of the invention has substantially the same structure and substantially the same ability to bind IL-23 as that of an aptamer comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-130, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 203-314.

[0029] In one embodiment, the present invention provides an aptamer that binds to human IL-23 comprising a nucleic acid sequence at least 80% identical, more preferably at least 90% identical to any one of the sequences selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-130, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 203-314. In another embodiment, the present invention provides an aptamer comprising 4 contiguous nucleotides, preferably 8 contiguous nucleotides, more preferably 20 contiguous nucleotides that are identical to a sequence of 4, 8, or 20 contiguous nucleotides in the unique sequence region of any one of the sequences selected from the group of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-130, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 203-314. In yet another embodiment the present invention provides an aptamer capable of binding human IL-23 or a variant thereof comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-130, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 203-314. In another embodiment, the present invention provides an aptamer having the sequence set forth in SEQ ID NO 177, preferably SEQ ID NO 224, more preferably SEQ ID NO 309, more preferably SEQ ID NO 310, and more preferably SEQ ID NO 311.

[0030] In one embodiment, the present invention provides aptamers that specifically bind to mouse IL-23. In another embodiment, the present invention provides aptamers that bind to a variant of mouse IL-23 that performs a biological function that is essentially the same as a function of mouse IL-23 and has substantially the same structure and substantially the same ability to bind said aptamer as that of mouse IL-23.

[0031] In one embodiment, mouse IL-23 or a variant thereof to which the aptamer of the invention binds comprises an amino acid sequence which is at least 80%, preferably at least 90% identical to a sequence comprising SEQ ID NOs 315 and/or 316. In another embodiment mouse IL-23 or a variant thereof has an amino acid sequence comprising SEQ ID NOs 315 and 316.

[0032] In one embodiment, the aptamer of the invention has a dissociation constant for mouse IL-23 or a variant thereof of about 100 nM or less, preferably 50 nM or less, more preferably 10 nM or less.

[0033] In one embodiment, the aptamer of the invention modulates a function of mouse IL-23 or a variant thereof. In one embodiment, the aptamer of the invention stimulates a function of mouse IL-23. In another embodiment, the aptamer of the invention inhibits a function of mouse IL-23 or a variant thereof. In yet another embodiment, the aptamer of the invention inhibits a function of mouse IL-23 or a variant thereof *in vivo*. In yet another embodiment, the aptamer of the invention prevents the binding of mouse IL-23 to the mouse IL-23 receptor. In some embodiments, the function of mouse IL-23 which is modulated by the aptamer of the present invention is to mediate a disease model associated with mouse IL-23 such as experimental autoimmune encephalomyelitis, murine collagen-induced arthritis, and TNBS colitis.

[0034] In one embodiment, the aptamer of the invention has substantially the same ability to bind mouse IL-23 as that of an aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs 124-134 and SEQ ID NOs 199-202. In another embodiment, the aptamer of the invention has substantially the same structure and substantially the same ability to bind mouse IL-23 as that of an aptamer comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs 124-134 and SEQ ID NOs 199-202.

[0035] In one embodiment, the present invention provides aptamers that bind to mouse IL-23 comprising a nucleic acid sequence at least 80% identical, preferably at least 90% identical to any one of the sequences selected from the group consisting of SEQ ID NOs 124-134, and SEQ ID NOs 199-202. In another embodiment, the present invention provides aptamers comprising 4 contiguous, preferably 8 contiguous, more preferably 20 contiguous nucleotides that are identical to a sequence of 4, 8 or 20 contiguous nucleotides in the unique sequence region of any one of the sequences selected from the group consisting of: SEQ ID NOs 124-134 and SEQ ID NOs 199-202. In another embodiment, the present invention provides an aptamer capable of binding mouse IL-23 or a variant thereof comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs 124-134 and SEQ ID NOs 199-202.

[0036] In one embodiment, the materials of the present invention provide aptamers that specifically bind to IL-12. In one embodiment, IL-12 to which the aptamers of the invention bind is human IL-12 while in another embodiment IL-12 is a variant of human IL-12. In one embodiment the variant of IL-12 performs a biological function that is essentially the same as a function of human IL-12 and has substantially the same structure and substantially the same ability to bind said aptamer as that of human IL-12.

[0037] In one embodiment, human IL-12 or a variant thereof comprises an amino acid sequence which is at least 80% identical, preferably at least 90% identical to a sequence comprising SEQ ID NOs 4 and/or 6. In another embodiment, human IL-12 or a variant thereof has an amino acid sequence comprising SEQ ID NOs 4 and 6.

[0038] In one embodiment, the aptamer of the present invention modulates a function of human IL-12 or a variant thereof. In one embodiment, the aptamer of the present invention stimulates a function of human IL-23. In another embodiment, the aptamer of the present invention inhibits a function of human IL-12 or a variant thereof. In yet another embodiment, the aptamer of the present invention inhibits a function of human IL-12 or a variant thereof *in vivo*. In yet another embodiment, the aptamer of the present invention prevents IL-12 from binding to the IL-12 receptor. In one embodiment, the function of human IL-12 or a variant thereof which is modulated by the aptamer of the invention is to mediate a disease associated with human IL-12 such as: autoimmune disease (including but not limited to multiple sclerosis, rheumatoid arthritis, psoriasis, systemic lupus erythamatosus, and irritable bowel disease (*e.g.*, Crohn's Disease and ulcerative colitis)), inflammatory disease, cancer (including but not limited to colon cancer, lung cancer, and lung metastases), bone resorption in osteoporosis, and Type I Diabetes.

[0039] In one embodiment, the present invention provides aptamers which are either ribonucleic or deoxyribonucleic acid. In a further embodiment, these ribonucleic or deoxyribonucleic acid aptamers are single stranded. In another embodiment, the present invention provides aptamers comprising at least one chemical modification. In one embodiment, the modification is selected from the group consisting of: a chemical substitution at a sugar position; a chemical substitution at a phosphate position; and a chemical substitution at a base position, of the nucleic acid; incorporation of a modified nucleotide; 3' capping; conjugation to a high molecular weight, non-immunogenic compound; conjugation to a lipophilic compound; and phosphate backbone modification. In

one embodiment, the non-immunogenic, high molecular weight compound conjugated to the aptamer of the invention is polyalkylene glycol, preferably polyethylene glycol. In one embodiment, the backbone modification comprises incorporation of one or more phosphorothioates into the phosphate backbone. In another embodiment, the aptamer of the invention comprises the incorporation of fewer than 10, fewer than 6, or fewer than 3 phosphorothioates in the phosphate backbone.

[0040] In one embodiment, the materials of the present invention provide a pharmaceutical composition comprising a therapeutically effective amount of an aptamer comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-130, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 203-314, or a salt thereof, and a pharmaceutically acceptable carrier or diluent. In another embodiment, the materials of the present invention provide a pharmaceutical composition comprising a therapeutically effective amount of an aptamer comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO 14, SEQ ID NOs 17-19, SEQ ID NO 21, SEQ ID NOs 27-32, SEQ ID NOs 34-40, SEQ ID NO 42, SEQ ID NO 49, SEQ ID NOs 60-61, SEQ ID NOs 91-92, SEQ ID NO 94, and SEQ ID NOs 103-118, or a salt thereof, and a pharmaceutically acceptable carrier or diluent. In a preferred embodiment, the materials of the present invention provide a pharmaceutical composition comprising a therapeutically effective amount of an aptamer comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO 177, SEQ ID NO 224, and SEQ ID NOs 309-312.

[0041] In one embodiment, the present invention provides a method of treating, preventing or ameliorating a disease mediated by IL-23, comprising administering the composition comprising a therapeutically effective amount of an aptamer comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-130, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 203-314, to a vertebrate. In another embodiment, the present invention provides a method of treating, preventing or ameliorating a disease mediated by IL-23 and/or IL-12, comprising administering the composition comprising a therapeutically effective amount of an aptamer comprising a nucleic acid sequence selected from the group

consisting of: SEQ ID NO 14, SEQ ID NOs 17-19, SEQ ID NO 21, SEQ ID NOs 27-32, SEQ ID NOs 34-40, SEQ ID NO 42, SEQ ID NO 49, SEQ ID NOs 60-61, SEQ ID NOs 91-92, SEQ ID NO 94, and SEQ ID NOs 103-118, to a vertebrate. In a preferred embodiment the composition comprising a therapeutically effective amount of an aptamer administered to a vertebrate comprises a nucleic acid sequence selected from the group consisting of: SEQ ID NO 177, SEQ ID NO 224, and SEQ ID NOs 309-312. In one embodiment the vertebrate to which the pharmaceutical composition is administered is a mammal. In a preferred embodiment, the mammal is a human.

[0042] In one embodiment, the disease treated, prevented or ameliorated by the methods of the present invention is selected from the group consisting of: autoimmune disease (including but not limited to multiple sclerosis, rheumatoid arthritis, psoriasis, systemic lupus erythamatosus, and irritable bowel disease (*e.g.*, Crohn's Disease and ulcerative colitis)), inflammatory disease, cancer (including but not limited to colon cancer, lung cancer, and lung metastases), bone resorption in osteoporosis, and Type I Diabetes.

[0043] In one embodiment, the present invention provides a diagnostic method comprising contacting an aptamer with a nucleic acid sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314 with a composition suspected of comprising IL-23 and/or IL-12 or a variant thereof, and detecting the presence or absence of IL-23 and/or IL-12 or a variant thereof.

[0044] In one embodiment, the present invention provides an aptamer with a nucleic acid sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314 for use as an *in vitro* diagnostic. In another embodiment, the present invention provides an aptamer with a nucleic acid sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314 for use as an *in vivo* diagnostic. In yet another embodiment, the present invention provides an aptamer with a nucleic acid sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ

ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314 for use in the treatment, prevention or amelioration of disease *in vivo*.

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] Figure 1 is a schematic representation of the Interleukin-12 family of cytokines.

[0046] Figure 2 is a schematic representation of the *in vitro* aptamer selection (SELEX™) process from pools of random sequence oligonucleotides.

[0047] Figure 3 is a schematic of the *in vitro* selection scheme for selecting aptamers specific to IL-23 by including IL-12 in the negative selection step thereby eliminating sequences that recognize p40, the common subunit in both IL-12 and IL-23.

[0048] Figure 4 is an illustration of a 40 kDa branched PEG.

[0049] Figure 5 is an illustration of a 40 kDa branched PEG attached to the 5'end of an aptamer.

[0050] Figure 6 is an illustration depicting various PEGylation strategies representing standard mono-PEGylation, multiple PEGylation, and dimerization via PEGylation.

[0051] Figure 7 is a graph showing binding of rRmY and rGmH pools to IL-23 after various rounds of selection.

[0052] Figure 8A is a representative schematic of the sequence and predicted secondary structure configuration of a Type 1 IL-23 aptamers; Figure 8B is a representative schematic of the sequences and predicted secondary structure configuration of several Type 2 IL-23 aptamers.

[0053] Figure 9A is a schematic of the minimized aptamer sequences and predicted secondary structure configurations for Type 1 IL-23 aptamers; Figure 9B is a schematic of the minimized aptamer sequences and predicted secondary structure configurations for Type 2 IL-23 aptamers.

[0054] Figure 10 depicts the predicted G-Quartet structure for dRmY minimer ARC979 (SEQ ID NO 177).

[0055] Figure 11 is a graph showing an increase of NMM fluorescence in ARC979 (SEQ ID NO 177), confirming that ARC979 adopts a G-quartet structure.

[0056] Figure 12 is a graph of the ARC979 (SEQ ID NO 177) competition binding curve analyzed based on total [aptamer] bound using 50 nM IL-23.

[0057] Figure 13 is a graph of the ARC979 (SEQ ID NO 177) competition binding curve analyzed based on [aptamer] bound using 250 nM IL-12.

[0058] Figure 14 is a graph of the direct binding curves for ARC979 (SEQ ID NO 177) under two different binding reaction conditions (1X PBS (without Ca^{++} or Mg^{++}) or 1X Dulbeccos PBS (with Ca^{++} and Mg^{++}).

[0059] Figure 15 is a graph of the direct binding curves for ARC979 (SEQ ID NO 177) phosphorothioate derivatives depicting that single phosphorothioate substitutions yield increased proportion binding to IL-23.

[0060] Figure 16 is a graph of the competition binding curves for ARC979 (SEQ ID NO 177) phosphorothioate derivatives depicting that single phosphorothioate substitutions compete for IL-23 at a higher affinity that ARC979.

[0061] Figure 17 is a graph of the direct binding curves for the ARC979 optimized derivatives ARC1624 (SEQ ID NO 310) and ARC1625 (SEQ ID NO 311), compared to the parent ARC979 (SEQ ID NO 177) aptamer (ARC895 is a negative control).

[0062] Figure 18 is a graph depicting the plasma stability of ARC979 (SEQ ID NO 177) compared to optimized ARC979 derivative constructs.

[0063] Figure 19 is a schematic representation of the TransAM™ assay used to measure STAT3 activity in lysates of PHA blast cells exposed to aptamers of the invention.

[0064] Figure 20 is a flow diagram of the protocol used for the detection of IL-23 induced STAT3 phosphorylation in PHA blasts exposed to aptamers of the invention.

[0065] Figure 21 is a representative graph showing the inhibitory effect of parental IL-23 aptamers of rRfY composition compared to their respective optimized clones on IL-23 induced STAT3 phosphorylation in PHA Blasts using the TransAM™ Assay.

[0066] Figure 22 is a graph of the percent inhibition of IL-23 induced STAT3 phosphorylation by IL-23 aptamers of dRmY composition in the TransAM™ assay (ARC793 (SEQ ID NO 163) is a non-binding aptamer).

[0067] Figure 23 is a graph of the percent inhibition of IL-23 induced STAT3 phosphorylation by parental IL-23 aptamers of dRmY composition (ARC621 (SEQ ID NO 108), ARC627 (SEQ ID NO 110)) compared to their respective optimized clones (ARC979 (SEQ ID NO 177), ARC980 (SEQ ID NO 178), ARC982 (SEQ ID NO 180)) in the TransAM™ assay.

[0068] Figure 24 is a percent inhibition graph of IL-23 induced STAT 3 phosphorylation by ARC979 (SEQ ID NO 177) and two optimized derivative clones of ARC979 (ARC1624 (SEQ ID NO 310) and ARC1625 (SEQ ID NO311)) in the Pathscan® assay.

[0069] Figure 25 is a graph comparing human and mouse IL-23 induced STAT3 activation in human PHA Blasts, measured by the TransAM™ assay.

DETAILED DESCRIPTION OF THE INVENTION

[0070] The details of one or more embodiments of the invention are set forth in the accompanying description below. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the case of conflict, the present Specification will control.

THE SELEX™ METHOD

[0071] A suitable method for generating an aptamer is with the process entitled "Systematic Evolution of Ligands by Exponential Enrichment" ("SELEX™") generally depicted in Figure 2. The SELEX™ process is a method for the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules and is described in, *e.g.*, U.S. patent application Ser. No. 07/536,428, filed Jun. 11, 1990, now abandoned, U.S. Pat. No. 5,475,096 entitled "Nucleic Acid Ligands", and U.S. Pat. No. 5,270,163 (see also WO 91/19813) entitled "Nucleic Acid Ligands". Each SELEX™-identified nucleic acid ligand, *i.e.*, each aptamer, is a specific ligand of a given target compound or molecule. The SELEX™ process is based on the unique insight that nucleic acids have sufficient capacity for forming

a variety of two- and three-dimensional structures and sufficient chemical versatility available within their monomers to act as ligands (*i.e.*, form specific binding pairs) with virtually any chemical compound, whether monomeric or polymeric. Molecules of any size or composition can serve as targets.

[0072] SELEX™ relies as a starting point upon a large library or pool of single stranded oligonucleotides comprising randomized sequences. The oligonucleotides can be modified or unmodified DNA, RNA, or DNA/RNA hybrids. In some examples, the pool comprises 100% random or partially random oligonucleotides. In other examples, the pool comprises random or partially random oligonucleotides containing at least one fixed sequence and/or conserved sequence incorporated within randomized sequence. In other examples, the pool comprises random or partially random oligonucleotides containing at least one fixed sequence and/or conserved sequence at its 5' and/or 3' end which may comprise a sequence shared by all the molecules of the oligonucleotide pool. Fixed sequences are sequences common to oligonucleotides in the pool which are incorporated for a preselected purpose such as, CpG motifs described further below, hybridization sites for PCR primers, promoter sequences for RNA polymerases (*e.g.*, T3, T4, T7, and SP6), restriction sites, or homopolymeric sequences, such as poly A or poly T tracts, catalytic cores, sites for selective binding to affinity columns, and other sequences to facilitate cloning and/or sequencing of an oligonucleotide of interest. Conserved sequences are sequences, other than the previously described fixed sequences, shared by a number of aptamers that bind to the same target.

[0073] The oligonucleotides of the pool preferably include a randomized sequence portion as well as fixed sequences necessary for efficient amplification. Typically the oligonucleotides of the starting pool contain fixed 5' and 3' terminal sequences which flank an internal region of 30–50 random nucleotides. The randomized nucleotides can be produced in a number of ways including chemical synthesis and size selection from randomly cleaved cellular nucleic acids. Sequence variation in test nucleic acids can also be introduced or increased by mutagenesis before or during the selection/amplification iterations.

[0074] The random sequence portion of the oligonucleotide can be of any length and can comprise ribonucleotides and/or deoxyribonucleotides and can include modified or non-natural nucleotides or nucleotide analogs. See, *e.g.*, U.S. Patent No. 5,958,691; U.S. Patent No. 5,660,985; U.S. Patent No. 5,958,691; U.S. Patent No. 5,698,687; U.S. Patent No. 5,817,635; U.S. Patent No. 5,672,695, and PCT Publication WO 92/07065. Random

oligonucleotides can be synthesized from phosphodiester-linked nucleotides using solid phase oligonucleotide synthesis techniques well known in the art. See, *e.g.*, Froehler *et al.*, Nucl. Acid Res. 14:5399-5467 (1986) and Froehler *et al.*, Tet. Lett. 27:5575-5578 (1986). Random oligonucleotides can also be synthesized using solution phase methods such as triester synthesis methods. See, *e.g.*, Sood *et al.*, Nucl. Acid Res. 4:2557 (1977) and Hirose *et al.*, Tet. Lett., 28:2449 (1978). Typical syntheses carried out on automated DNA synthesis equipment yield 10^{14}-10^{16} individual molecules, a number sufficient for most SELEX™ experiments. Sufficiently large regions of random sequence in the sequence design increases the likelihood that each synthesized molecule is likely to represent a unique sequence.

[0075] The starting library of oligonucleotides may be generated by automated chemical synthesis on a DNA synthesizer. To synthesize randomized sequences, mixtures of all four nucleotides are added at each nucleotide addition step during the synthesis process, allowing for random incorporation of nucleotides. As stated above, in one embodiment, random oligonucleotides comprise entirely random sequences; however, in other embodiments, random oligonucleotides can comprise stretches of nonrandom or partially random sequences. Partially random sequences can be created by adding the four nucleotides in different molar ratios at each addition step.

[0076] The starting library of oligonucleotides may be either RNA or DNA. In those instances where an RNA library is to be used as the starting library it is typically generated by transcribing a DNA library *in vitro* using T7 RNA polymerase or modified T7 RNA polymerases and purified. The RNA or DNA library is then mixed with the target under conditions favorable for binding and subjected to step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of binding affinity and selectivity. More specifically, starting with a mixture containing the starting pool of nucleic acids, the SELEX™ method includes steps of: (a) contacting the mixture with the target under conditions favorable for binding; (b) partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules; (c) dissociating the nucleic acid-target complexes; (d) amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids; and (e) reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity nucleic acid ligands to the target molecule. In those instances where RNA aptamers are being

selected, the SELEX™ method further comprises the steps of: (i) reverse transcribing the nucleic acids dissociated from the nucleic acid-target complexes before amplification in step (d); and (ii) transcribing the amplified nucleic acids from step (d) before restarting the process.

[0077] Within a nucleic acid mixture containing a large number of possible sequences and structures, there is a wide range of binding affinities for a given target. A nucleic acid mixture comprising, for example, a 20 nucleotide randomized segment can have 4^{20} candidate possibilities. Those which have the higher affinity constants for the target are most likely to bind to the target. After partitioning, dissociation and amplification, a second nucleic acid mixture is generated, enriched for the higher binding affinity candidates. Additional rounds of selection progressively favor the best ligands until the resulting nucleic acid mixture is predominantly composed of only one or a few sequences. These can then be cloned, sequenced and individually tested for binding affinity as pure ligands or aptamers.

[0078] Cycles of selection and amplification are repeated until a desired goal is achieved. In the most general case, selection/amplification is continued until no significant improvement in binding strength is achieved on repetition of the cycle. The method is typically used to sample approximately 10^{14} different nucleic acid species but may be used to sample as many as about 10^{18} different nucleic acid species. Generally, nucleic acid aptamer molecules are selected in a 5 to 20 cycle procedure. In one embodiment, heterogeneity is introduced only in the initial selection stages and does not occur throughout the replicating process.

[0079] In one embodiment of SELEX™, the selection process is so efficient at isolating those nucleic acid ligands that bind most strongly to the selected target, that only one cycle of selection and amplification is required. Such an efficient selection may occur, for example, in a chromatographic-type process wherein the ability of nucleic acids to associate with targets bound on a column operates in such a manner that the column is sufficiently able to allow separation and isolation of the highest affinity nucleic acid ligands.

[0080] In many cases, it is not necessarily desirable to perform the iterative steps of SELEX™ until a single nucleic acid ligand is identified. The target-specific nucleic acid ligand solution may include a family of nucleic acid structures or motifs that have a number of conserved sequences and a number of sequences which can be substituted or added without significantly affecting the affinity of the nucleic acid ligands to the target. By

terminating the SELEX™ process prior to completion, it is possible to determine the sequence of a number of members of the nucleic acid ligand solution family.

[0081] A variety of nucleic acid primary, secondary and tertiary structures are known to exist. The structures or motifs that have been shown most commonly to be involved in non-Watson-Crick type interactions are referred to as hairpin loops, symmetric and asymmetric bulges, pseudoknots and myriad combinations of the same. Almost all known cases of such motifs suggest that they can be formed in a nucleic acid sequence of no more than 30 nucleotides. For this reason, it is often preferred that SELEX™ procedures with contiguous randomized segments be initiated with nucleic acid sequences containing a randomized segment of between about 20 to about 50 nucleotides and in some embodiments, about 30 to about 40 nucleotides. In one example, the 5'-fixed:random:3'-fixed sequence comprises a random sequence of about 30 to about 50 nucleotides.

[0082] The core SELEX™ method has been modified to achieve a number of specific objectives. For example, U.S. Patent No. 5,707,796 describes the use of SELEX™ in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. U.S. Patent No. 5,763,177 describes SELEX™ based methods for selecting nucleic acid ligands containing photo reactive groups capable of binding and/or photo-crosslinking to and/or photo-inactivating a target molecule. U.S. Patent No. 5,567,588 and U.S. Patent No. 5,861,254 describe SELEX™ based methods which achieve highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. U.S. Patent No. 5,496,938 describes methods for obtaining improved nucleic acid ligands after the SELEX™ process has been performed. U.S. Patent No. 5,705,337 describes methods for covalently linking a ligand to its target.

[0083] SELEX™ can also be used to obtain nucleic acid ligands that bind to more than one site on the target molecule, and to obtain nucleic acid ligands that include non-nucleic acid species that bind to specific sites on the target. SELEX™ provides means for isolating and identifying nucleic acid ligands which bind to any envisionable target, including large and small biomolecules such as nucleic acid-binding proteins and proteins not known to bind nucleic acids as part of their biological function as well as cofactors and other small molecules. For example, U.S. Patent No. 5,580,737 discloses nucleic acid sequences identified through SELEX™ which are capable of binding with high affinity to caffeine and the closely related analog, theophylline.

[0084] Counter-SELEX™ is a method for improving the specificity of nucleic acid ligands to a target molecule by eliminating nucleic acid ligand sequences with cross-reactivity to one or more non-target molecules. Counter- SELEX™ is comprised of the steps of: (a) preparing a candidate mixture of nucleic acids; (b) contacting the candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; (c) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; (d) dissociating the increased affinity nucleic acids from the target; (e) contacting the increased affinity nucleic acids with one or more non-target molecules such that nucleic acid ligands with specific affinity for the non-target molecule(s) are removed; and (f) amplifying the nucleic acids with specific affinity only to the target molecule to yield a mixture of nucleic acids enriched for nucleic acid sequences with a relatively higher affinity and specificity for binding to the target molecule. As described above for SELEX™, cycles of selection and amplification are repeated as necessary until a desired goal is achieved.

[0085] One potential problem encountered in the use of nucleic acids as therapeutics and vaccines is that oligonucleotides in their phosphodiester form may be quickly degraded in body fluids by intracellular and extracellular enzymes such as endonucleases and exonucleases before the desired effect is manifest. The SELEX™ method thus encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX™-identified nucleic acid ligands containing modified nucleotides are described, *e.g.*, in U.S. Patent No. 5,660,985, which describes oligonucleotides containing nucleotide derivatives chemically modified at the 2' position of ribose, 5 position of pyrimidines, and 8 position of purines, U.S. Patent No. 5,756,703 which describes oligonucleotides containing various 2'-modified pyrimidines, and U.S. Patent No. 5,580,737 which describes highly specific nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH_2), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe) substituents.

[0086] Modifications of the nucleic acid ligands contemplated in this invention include, but are not limited to, those which provide other chemical groups that incorporate additional charge, polarizability, hydrophobicity, hydrogen bonding, electrostatic interaction, and

fluxionality to the nucleic acid ligand bases or to the nucleic acid ligand as a whole. Modifications to generate oligonucleotide populations which are resistant to nucleases can also include one or more substitute internucleotide linkages, altered sugars, altered bases, or combinations thereof. Such modifications include, but are not limited to, 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil; backbone modifications, phosphorothioate or alkyl phosphate modifications, methylations, and unusual base-pairing combinations such as the isobases isocytidine and isoguanosine. Modifications can also include 3' and 5' modifications such as capping.

[0087] In one embodiment, oligonucleotides are provided in which the P(O)O group is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), $P(O)NR_2$ ("amidate"), P(O)R, P(O)OR', CO or CH_2 ("formacetal") or 3'-amine (-NH-CH_2-CH_2-), wherein each R or R' is independently H or substituted or unsubstituted alkyl. Linkage groups can be attached to adjacent nucleotides through an -O-, -N-, or -S- linkage. Not all linkages in the oligonucleotide are required to be identical. As used herein, the term phosphorothioate encompasses one or more non-bridging oxygen atoms in a phosphodiester bond replaced by one or more sulfur atom.

[0088] In further embodiments, the oligonucleotides comprise modified sugar groups, for example, one or more of the hydroxyl groups is replaced with halogen, aliphatic groups, or functionalized as ethers or amines. In one embodiment, the 2'-position of the furanose residue is substituted by any of an O-methyl, O-alkyl, O-allyl, S-alkyl, S-allyl, or halo group. Methods of synthesis of 2'-modified sugars are described, *e.g.*, in Sproat, *et al.*, Nucl. Acid Res. 19:733-738 (1991); Cotten, *et al.*, Nucl. Acid Res. 19:2629-2635 (1991); and Hobbs, *et al.*, Biochemistry 12:5138-5145 (1973). Other modifications are known to one of ordinary skill in the art. Such modifications may be pre-SELEX™ process modifications or post-SELEX™ process modifications (modification of previously identified unmodified ligands) or may be made by incorporation into the SELEX™ process.

[0089] Pre- SELEX™ process modifications or those made by incorporation into the SELEX™ process yield nucleic acid ligands with both specificity for their SELEX™ target and improved stability, *e.g.*, *in vivo* stability. Post-SELEX™ process modifications made to nucleic acid ligands may result in improved stability, *e.g.*, *in vivo* stability without adversely affecting the binding capacity of the nucleic acid ligand.

[0090] The SELEX™ method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in U.S. Patent No. 5,637,459 and U.S. Patent No. 5,683,867. The SELEX™ method further encompasses combining selected nucleic acid ligands with lipophilic or non-immunogenic high molecular weight compounds in a diagnostic or therapeutic complex, as described, *e.g.*, in U.S. Patent No. 6,011,020, U.S. Patent No. 6,051,698, and PCT Publication No. WO 98/18480. These patents and applications teach the combination of a broad array of shapes and other properties, with the efficient amplification and replication properties of oligonucleotides, and with the desirable properties of other molecules.

[0091] The identification of nucleic acid ligands to small, flexible peptides via the SELEX™ method has also been explored. Small peptides have flexible structures and usually exist in solution in an equilibrium of multiple conformers, and thus it was initially thought that binding affinities may be limited by the conformational entropy lost upon binding a flexible peptide. However, the feasibility of identifying nucleic acid ligands to small peptides in solution was demonstrated in U.S. Patent No. 5,648,214. In this patent, high affinity RNA nucleic acid ligands to substance P, an 11 amino acid peptide, were identified.

[0092] The aptamers with specificity and binding affinity to the target(s) of the present invention are typically selected by the SELEX™ process as described herein. As part of the SELEX™ process, the sequences selected to bind to the target are then optionally minimized to determine the minimal sequence having the desired binding affinity. The selected sequences and/or the minimized sequences are optionally optimized by performing random or directed mutagenesis of the sequence to increase binding affinity or alternatively to determine which positions in the sequence are essential for binding activity. Additionally, selections can be performed with sequences incorporating modified nucleotides to stabilize the aptamer molecules against degradation *in vivo*.

2' MODIFIED SELEX™

[0093] In order for an aptamer to be suitable for use as a therapeutic, it is preferably inexpensive to synthesize, safe and stable *in vivo*. Wild-type RNA and DNA aptamers are typically not stable *in vivo* because of their susceptibility to degradation by nucleases. Resistance to nuclease degradation can be greatly increased by the incorporation of modifying groups at the 2'-position.

[0094] Fluoro and amino groups have been successfully incorporated into oligonucleotide pools from which aptamers have been subsequently selected. However, these modifications greatly increase the cost of synthesis of the resultant aptamer, and may introduce safety concerns in some cases because of the possibility that the modified nucleotides could be recycled into host DNA by degradation of the modified oligonucleotides and subsequent use of the nucleotides as substrates for DNA synthesis.

[0095] Aptamers that contain 2'-O-methyl ("2'-OMe") nucleotides, as provided herein, overcome many of these drawbacks. Oligonucleotides containing 2'-OMe nucleotides are nuclease-resistant and inexpensive to synthesize. Although 2'-OMe nucleotides are ubiquitous in biological systems, natural polymerases do not accept 2'-OMe NTPs as substrates under physiological conditions, thus there are no safety concerns over the recycling of 2'-OMe nucleotides into host DNA. The SELEX™ method used to generate 2'-modified aptamers is described, *e.g.*, in U.S. Provisional Patent Application Serial No. 60/430,761, filed December 3, 2002, U.S. Provisional Patent Application Serial No. 60/487,474, filed July 15, 2003, U.S. Provisional Patent Application Serial No. 60/517,039, filed November 4, 2003, U.S. Patent Application No. 10/729,581, filed December 3, 2003, and U.S. Patent Application No. 10/873,856, filed June 21, 2004, entitled "Method for *in vitro* Selection of 2'-O-methyl Substituted Nucleic Acids", each of which is herein incorporated by reference in its entirety.

[0096] The present invention includes aptamers that bind to and modulate the function of IL-23 and/or IL-12 which contain modified nucleotides (*e.g.*, nucleotides which have a modification at the 2' position) to make the oligonucleotide more stable than the unmodified oligonucleotide to enzymatic and chemical degradation as well as thermal and physical degradation. Although there are several examples of 2'-OMe containing aptamers in the literature (see, *e.g.*, Green *et al.*, Current Biology 2, 683-695, 1995) these were generated by the *in vitro* selection of libraries of modified transcripts in which the C and U residues were 2'-fluoro (2'-F) substituted and the A and G residues were 2'-OH. Once functional sequences were identified then each A and G residue was tested for tolerance to 2'-OMe substitution, and the aptamer was re-synthesized having all A and G residues which tolerated 2'-OMe substitution as 2'-OMe residues. Most of the A and G residues of aptamers generated in this two-step fashion tolerate substitution with 2'-OMe residues, although, on average, approximately 20% do not. Consequently, aptamers generated using this method

tend to contain from two to four 2'-OH residues, and stability and cost of synthesis are compromised as a result. By incorporating modified nucleotides into the transcription reaction which generate stabilized oligonucleotides used in oligonucleotide pools from which aptamers are selected and enriched by SELEX™ (and/or any of its variations and improvements, including those described herein), the methods of the present invention eliminate the need for stabilizing the selected aptamer oligonucleotides *(e.g.*, by resynthesizing the aptamer oligonucleotides with modified nucleotides).

[0097] In one embodiment, the present invention provides aptamers comprising combinations of 2'-OH, 2'-F, 2'-deoxy, and 2'-OMe modifications of the ATP, GTP, CTP, TTP, and UTP nucleotides. In another embodiment, the present invention provides aptamers comprising combinations of 2'-OH, 2'-F, 2'-deoxy, 2'-OMe, 2'-NH_2, and 2'-methoxyethyl modifications of the ATP, GTP, CTP, TTP, and UTP nucleotides. In another embodiment, the present invention provides aptamers comprising 5^6 combinations of 2'-OH, 2'-F, 2'-deoxy, 2'-OMe, 2'-NH_2, and 2'-methoxyethyl modifications of the ATP, GTP, CTP, TTP, and UTP nucleotides.

[0098] 2' modified aptamers of the invention are created using modified polymerases, *e.g.*, a modified T7 polymerase, having a rate of incorporation of modified nucleotides having bulky substituents at the furanose 2' position that is higher than that of wild-type polymerases. For example, a single mutant T7 polymerase (Y639F) in which the tyrosine residue at position 639 has been changed to phenylalanine readily utilizes 2'deoxy, 2'amino-, and 2'fluoro- nucleotide triphosphates (NTPs) as substrates and has been widely used to synthesize modified RNAs for a variety of applications. However, this mutant T7 polymerase reportedly can not readily utilize (*i.e.*, incorporate) NTPs with bulky 2'-substituents such as 2'-OMe or 2'-azido (2'-N_3) substituents. For incorporation of bulky 2' substituents, a double T7 polymerase mutant (Y639F/H784A) having the histidine at position 784 changed to an alanine residue in addition to the Y639F mutation has been described and has been used in limited circumstances to incorporate modified pyrimidine NTPs. See Padilla, R. and Sousa, R., Nucleic Acids Res., 2002, 30(24): 138. A single mutant T7 polymerase (H784A) having the histidine at position 784 changed to an alanine residue has also been described. Padilla *et al.*, Nucleic Acids Research, 2002, 30: 138. In both the Y639F/H784A double mutant and H784A single mutant T7 polymerases, the change to a

smaller amino acid residue such as alanine allows for the incorporation of bulkier nucleotide substrates, *e.g.*, 2'-OMe substituted nucleotides.

[0099] Generally, it has been found that under the conditions disclosed herein, the Y693F single mutant can be used for the incorporation of all 2'-OMe substituted NTPs except GTP and the Y639F/H784A double mutant can be used for the incorporation of all 2'-OMe substituted NTPs including GTP. It is expected that the H784A single mutant possesses properties similar to the Y639F and the Y639F/H784A mutants when used under the conditions disclosed herein.

[00100] 2'-modified oligonucleotides may be synthesized entirely of modified nucleotides, or with a subset of modified nucleotides. The modifications can be the same or different. All nucleotides may be modified, and all may contain the same modification. All nucleotides may be modified, but contain different modifications, *e.g.*, all nucleotides containing the same base may have one type of modification, while nucleotides containing other bases may have different types of modification. All purine nucleotides may have one type of modification (or are unmodified), while all pyrimidine nucleotides have another, different type of modification (or are unmodified). In this way, transcripts, or pools of transcripts are generated using any combination of modifications, including for example, ribonucleotides (2'-OH), deoxyribonucleotides (2'-deoxy), 2'-F, and 2'-OMe nucleotides. A transcription mixture containing 2'-OMe C and U and 2'-OH A and G is referred to as an "rRmY" mixture and aptamers selected therefrom are referred to as "rRmY" aptamers. A transcription mixture containing deoxy A and G and 2'-OMe U and C is referred to as a "dRmY" mixture and aptamers selected therefrom are referred to as "dRmY" aptamers. A transcription mixture containing 2'-OMe A, C, and U, and 2'-OH G is referred to as a "rGmH" mixture and aptamers selected therefrom are referred to as "rGmH" aptamers. A transcription mixture alternately containing 2'-OMe A, C, U and G and 2'-OMe A, U and C and 2'-F G is referred to as an "alternating mixture" and aptamers selected therefrom are referred to as "alternating mixture" aptamers. A transcription mixture containing 2'-OMe A, U, C, and G, where up to 10% of the G's are ribonucleotides is referred to as a "r/mGmH" mixture and aptamers selected therefrom are referred to as "r/mGmH" aptamers. A transcription mixture containing 2'-OMe A, U, and C, and 2'-F G is referred to as a "fGmH" mixture and aptamers selected therefrom are referred to as "fGmH" aptamers. A transcription mixture containing 2'-OMe A, U, and C, and deoxy G is referred to as a "dGmH" mixture and aptamers selected

therefrom are referred to as "dGmH" aptamers. A transcription mixture containing deoxy A, and 2'-OMe C, G and U is referred to as a "dAmB" mixture and aptamers selected therefrom are referred to as "dAmB" aptamers, and a transcription mixture containing all 2'-OH nucleotides is referred to as a "rN" mixture and aptamers selected therefrom are referred to as "rN" or "rRrY" aptamers. A "mRmY" aptamer is one containing all 2'-O-methyl nucleotides and is usually derived from a r/mGmH oligonucleotide by post-SELEX™ replacement, when possible, of any 2'-OH Gs with 2'-OMe Gs.

[00101] A preferred embodiment includes any combination of 2'-OH, 2'-deoxy and 2'-OMe nucleotides. A more preferred embodiment includes any combination of 2'-deoxy and 2'-OMe nucleotides. An even more preferred embodiment is with any combination of 2'-deoxy and 2'-OMe nucleotides in which the pyrimidines are 2'-OMe (such as dRmY, mRmY or dGmH).

[00102] Incorporation of modified nucleotides into the aptamers of the invention is accomplished before (pre-) the selection process (*e.g.*, a pre-SELEX™ process modification). Optionally, aptamers of the invention in which modified nucleotides have been incorporated by pre-SELEX™ process modification can be further modified by post-SELEX™ process modification (*i.e.,* a post-SELEX™ process modification after a pre-SELEX™ modification). Pre-SELEX™ process modifications yield modified nucleic acid ligands with specificity for the SELEX™ target and also improved *in vivo* stability. Post-SELEX™ process modifications, *i.e.*, modification (*e.g.*, truncation, deletion, substitution or additional nucleotide modifications of previously identified ligands having nucleotides incorporated by pre-SELEX™ process modification) can result in a further improvement of *in vivo* stability without adversely affecting the binding capacity of the nucleic acid ligand having nucleotides incorporated by pre-SELEX™ process modification.

[00103] To generate pools of 2'-modified (*e.g.*, 2'-OMe) RNA transcripts in conditions under which a polymerase accepts 2'-modified NTPs the preferred polymerase is the Y693F/H784A double mutant or the Y693F single mutant. Other polymerases, particularly those that exhibit a high tolerance for bulky 2'-substituents, may also be used in the present invention. Such polymerases can be screened for this capability by assaying their ability to incorporate modified nucleotides under the transcription conditions disclosed herein.

[00104] A number of factors have been determined to be important for the transcription conditions useful in the methods disclosed herein. For example, increases in the yields of

modified transcript are observed when a leader sequence is incorporated into the 5' end of a fixed sequence at the 5' end of the DNA transcription template, such that at least about the first 6 residues of the resultant transcript are all purines.

[00105] Another important factor in obtaining transcripts incorporating modified nucleotides is the presence or concentration of 2'-OH GTP. Transcription can be divided into two phases: the first phase is initiation, during which an NTP is added to the 3'-hydroxyl end of GTP (or another substituted guanosine) to yield a dinucleotide which is then extended by about 10-12 nucleotides; the second phase is elongation, during which transcription proceeds beyond the addition of the first about 10-12 nucleotides. It has been found that small amounts of 2'-OH GTP added to a transcription mixture containing an excess of 2'-OMe GTP are sufficient to enable the polymerase to initiate transcription using 2'-OH GTP, but once transcription enters the elongation phase the reduced discrimination between 2'-OMe and 2'-OH GTP, and the excess of 2'-OMe GTP over 2'-OH GTP allows the incorporation of principally the 2'-OMe GTP.

[00106] Another important factor in the incorporation of 2'-OMe substituted nucleotides into transcripts is the use of both divalent magnesium and manganese in the transcription mixture. Different combinations of concentrations of magnesium chloride and manganese chloride have been found to affect yields of 2'-O-methylated transcripts, the optimum concentration of the magnesium and manganese chloride being dependent on the concentration in the transcription reaction mixture of NTPs which complex divalent metal ions. To obtain the greatest yields of maximally 2' substituted O-methylated transcripts (*i.e.*, all A, C, and U and about 90% of G nucleotides), concentrations of approximately 5 mM magnesium chloride and 1.5 mM manganese chloride are preferred when each NTP is present at a concentration of 0.5 mM. When the concentration of each NTP is 1.0 mM, concentrations of approximately 6.5 mM magnesium chloride and 2.0 mM manganese chloride are preferred. When the concentration of each NTP is 2.0 mM, concentrations of approximately 9.6 mM magnesium chloride and 2.9 mM manganese chloride are preferred. In any case, departures from these concentrations of up to two-fold still give significant amounts of modified transcripts.

[00107] Priming transcription with GMP or guanosine is also important. This effect results from the specificity of the polymerase for the initiating nucleotide. As a result, the 5'-terminal nucleotide of any transcript generated in this fashion is likely to be 2'-OH G. The

preferred concentration of GMP (or guanosine) is 0.5 mM and even more preferably 1 mM. It has also been found that including PEG, preferably PEG-8000, in the transcription reaction is useful to maximize incorporation of modified nucleotides.

[00108] For maximum incorporation of 2'-OMe ATP (100%), UTP (100%), CTP (100%) and GTP (~90%) ("r/mGmH") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), $MgCl_2$ 5 mM (6.5 mM where the concentration of each 2'-OMe NTP is 1.0 mM), $MnCl_2$ 1.5 mM (2.0 mM where the concentration of each 2'-OMe NTP is 1.0 mM), 2'-OMe NTP (each) 500 μM (more preferably, 1.0 mM), 2'-OH GTP 30 μM, 2'-OH GMP 500 μM, pH 7.5, Y639F/H784A T7 RNA Polymerase 15 units/mL, inorganic pyrophosphatase 5 units/mL, and an all-purine leader sequence of at least 8 nucleotides long. As used herein, one unit of the Y639F/H784A mutant T7 RNA polymerase (or any other mutant T7 RNA polymerase specified herein) is defined as the amount of enzyme required to incorporate 1 nmole of 2'-OMe NTPs into transcripts under the r/mGmH conditions. As used herein, one unit of inorganic pyrophosphatase is defined as the amount of enzyme that will liberate 1.0 mole of inorganic orthophosphate per minute at pH 7.2 and 25 °C.

[00109] For maximum incorporation (100%) of 2'-OMe ATP, UTP and CTP ("rGmH") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), $MgCl_2$ 5 mM (9.6 mM where the concentration of each 2'-OMe NTP is 2.0 mM), $MnCl_2$ 1.5 mM (2.9 mM where the concentration of each 2'-OMe NTP is 2.0 mM), 2'-OMe NTP (each) 500 μM (more preferably, 2.0 mM), pH 7.5, Y639F T7 RNA Polymerase 15 units/mL, inorganic pyrophosphatase 5 units/mL, and an all-purine leader sequence of at least 8 nucleotides long.

[00110] For maximum incorporation (100%) of 2'-OMe UTP and CTP ("rRmY") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), $MgCl_2$ 5 mM (9.6 mM where the concentration of each 2'-OMe NTP is 2.0 mM), $MnCl_2$ 1.5 mM (2.9 mM where the concentration of each 2'-OMe NTP is 2.0 mM), 2'-OMe NTP (each) 500μM (more preferably, 2.0 mM), pH 7.5, Y639F/H784A T7 RNA Polymerase 15 units/mL, inorganic pyrophosphatase 5 units/mL, and an all-purine leader sequence of at least 8 nucleotides long.

[00111] For maximum incorporation (100%) of deoxy ATP and GTP and 2'-OMe UTP and CTP ("dRmY") into transcripts the following conditions are preferred: HEPES buffer

200 mM, DTT 40 mM, spermine 2 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), $MgCl_2$ 9.6 mM, $MnCl_2$ 2.9 mM, 2'-OMe NTP (each) 2.0 mM, pH 7.5, Y639F T7 RNA Polymerase 15 units/mL, inorganic pyrophosphatase 5 units/mL, and an all-purine leader sequence of at least 8 nucleotides long.

[00112] For maximum incorporation (100%) of 2'-OMe ATP, UTP and CTP and 2'-F GTP ("fGmH") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), $MgCl_2$ 9.6 mM, $MnCl_2$ 2.9 mM, 2'-OMe NTP (each) 2.0 mM, pH 7.5, Y639F T7 RNA Polymerase 15 units/mL, inorganic pyrophosphatase 5 units/mL, and an all-purine leader sequence of at least 8 nucleotides long.

[00113] For maximum incorporation (100%) of deoxy ATP and 2'-OMe UTP, GTP and CTP ("dAmB") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v), $MgCl_2$ 9.6 mM, $MnCl_2$ 2.9 mM, 2'-OMe NTP (each) 2.0 mM, pH 7.5, Y639F T7 RNA Polymerase 15 units/mL, inorganic pyrophosphatase 5 units/mL, and an all-purine leader sequence of at least 8 nucleotides long.

[00114] For each of the above (a) transcription is preferably performed at a temperature of from about 20 °C to about 50 °C, preferably from about 30 °C to 45 °C, and more preferably at about 37 °C for a period of at least two hours and (b) 50-300 nM of a double stranded DNA transcription template is used (200 nM template is used in round 1 to increase diversity (300 nM template is used in dRmY transcriptions)), and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described herein, is used). The preferred DNA transcription templates are described below (where ARC254 and ARC256 transcribe under all 2'-OMe conditions and ARC255 transcribes under rRmY conditions).

SEQ ID NO 1 (ARC254)
5'-CATCGATGCTAGTCGTAACGATCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCGAGAACGTTCTCTCCTCTCCCTATAGTGAGTCGTATTA-3'

SEQ ID NO 2 (ARC255)
5'-CATGCATCGCGACTGACTAGCCGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTAGAACGTTCTCTCCTCTCCCTATAGTGAGTCGTATTA-3'

SEQ ID NO 3 (ARC256)
5'-CATCGATCGATCGATCGACAGCGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTAGAACGTTCTCTCCTCTCCCTATAGTGAGTCGTATTA-3'

[00115] Under rN transcription conditions of the present invention, the transcription reaction mixture comprises 2′-OH adenosine triphosphates (ATP), 2′-OH guanosine triphosphates (GTP), 2′-OH cytidine triphosphates (CTP), and 2′-OH uridine triphosphates (UTP). The modified oligonucleotides produced using the rN transcription mixtures of the present invention comprise substantially all 2′-OH adenosine, 2′-OH guanosine, 2′-OH cytidine, and 2′-OH uridine. In a preferred embodiment of rN transcription, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2′-OH adenosine, at least 80% of all guanosine nucleotides are 2′-OH guanosine, at least 80% of all cytidine nucleotides are 2′-OH cytidine, and at least 80% of all uridine nucleotides are 2′-OH uridine. In a more preferred embodiment of rN transcription, the resulting modified oligonucleotides of the present invention comprise a sequence where at least 90% of all adenosine nucleotides are 2′-OH adenosine, at least 90% of all guanosine nucleotides are 2′-OH guanosine, at least 90% of all cytidine nucleotides are 2′-OH cytidine, and at least 90% of all uridine nucleotides are 2′-OH uridine. In a most preferred embodiment of rN transcription, the modified oligonucleotides of the present invention comprise a sequence where 100% of all adenosine nucleotides are 2′-OH adenosine, 100% of all guanosine nucleotides are 2′-OH guanosine, 100% of all cytidine nucleotides are 2′-OH cytidine, and 100% of all uridine nucleotides are 2′-OH uridine.

[00116] Under rRmY transcription conditions of the present invention, the transcription reaction mixture comprises 2′-OH adenosine triphosphates, 2′-OH guanosine triphosphates, 2′-O-methyl cytidine triphosphates, and 2′-O-methyl uridine triphosphates. The modified oligonucleotides produced using the rRmY transcription mixtures of the present invention comprise substantially all 2′-OH adenosine, 2′-OH guanosine, 2′-O-methyl cytidine and 2′-O-methyl uridine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2′-OH adenosine, at least 80% of all guanosine nucleotides are 2′-OH guanosine, at least 80% of all cytidine nucleotides are 2′-O-methyl cytidine and at least 80% of all uridine nucleotides are 2′-O-methyl uridine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2′-OH adenosine, at least 90% of all guanosine nucleotides are 2′-OH guanosine, at least 90% of all cytidine nucleotides are 2′-O-methyl cytidine and at least 90% of all uridine nucleotides are 2′-O-

methyl uridine In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2′-OH adenosine, 100% of all guanosine nucleotides are 2′-OH guanosine, 100% of all cytidine nucleotides are 2′-O-methyl cytidine and 100% of all uridine nucleotides are 2′-O-methyl uridine.

[00117] Under dRmY transcription conditions of the present invention, the transcription reaction mixture comprises 2′-deoxy adenosine triphosphates, 2'-deoxy guanosine triphosphates, 2′-O-methyl cytidine triphosphates, and 2'-O-methyl uridine triphosphates. The modified oligonucleotides produced using the dRmY transcription conditions of the present invention comprise substantially all 2'-deoxy adenosine, 2'-deoxy guanosine, 2′-O-methyl cytidine, and 2'-O-methyl uridine. In a preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where at least 80% of all adenosine nucleotides are 2'-deoxy adenosine, at least 80% of all guanosine nucleotides are 2'-deoxy guanosine, at least 80% of all cytidine nucleotides are 2′-O-methyl cytidine, and at least 80% of all uridine nucleotides are 2'-O-methyl uridine. In a more preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where at least 90% of all adenosine nucleotides are 2'-deoxy adenosine, at least 90 % of all guanosine nucleotides are 2'-deoxy guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 90% of all uridine nucleotides are 2′-O-methyl uridine. In a most preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where 100% of all adenosine nucleotides are 2'-deoxy adenosine, 100% of all guanosine nucleotides are 2'-deoxy guanosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, and 100% of all uridine nucleotides are 2′-O-methyl uridine.

[00118] Under rGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2′-OH guanosine triphosphates, 2′-O-methyl cytidine triphosphates, 2′-O-methyl uridine triphosphates, and 2′-O-methyl adenosine triphosphates. The modified oligonucleotides produced using the rGmH transcription mixtures of the present invention comprise substantially all 2′-OH guanosine, 2′-O-methyl cytidine, 2′-O-methyl uridine, and 2′-O-methyl adenosine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all guanosine nucleotides are 2′-OH guanosine, at least 80% of all cytidine nucleotides are 2′-O-methyl

cytidine, at least 80% of all uridine nucleotides are 2′-O-methyl uridine, and at least 80% of all adenosine nucleotides are 2′-O-methyl adenosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all guanosine nucleotides are 2′-OH guanosine, at least 90% of all cytidine nucleotides are 2′-O-methyl cytidine, at least 90% of all uridine nucleotides are 2′-O-methyl uridine, and at least 90% of all adenosine nucleotides are 2′-O-methyl adenosine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all guanosine nucleotides are 2′-OH guanosine, 100% of all cytidine nucleotides are 2′-O-methyl cytidine, 100% of all uridine nucleotides are 2′-O-methyl uridine, and 100% of all adenosine nucleotides are 2′-O-methyl adenosine.

[00119] Under r/mGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2′-O-methyl adenosine triphosphate, 2′-O-methyl cytidine triphosphate, 2′-O-methyl guanosine triphosphate, 2′-O-methyl uridine triphosphate and 2'-OH guanosine triphosphate. The resulting modified oligonucleotides produced using the r/mGmH transcription mixtures of the present invention comprise substantially all 2′-O-methyl adenosine, 2′-O-methyl cytidine, 2′-O-methyl guanosine, and 2′-O-methyl uridine, wherein the population of guanosine nucleotides has a maximum of about 10% 2'-OH guanosine. In a preferred embodiment, the resulting r/mGmH modified oligonucleotides of the present invention comprise a sequence where at least 80% of all adenosine nucleotides are 2′-O-methyl adenosine, at least 80% of all cytidine nucleotides are 2′-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2′-O-methyl guanosine, at least 80% of all uridine nucleotides are 2′-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are 2'-OH guanosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2′-O-methyl adenosine, at least 90% of all cytidine nucleotides are 2′-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2′-O-methyl guanosine, at least 90% of all uridine nucleotides are 2′-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are 2'-OH guanosine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2′-O-methyl adenosine, 100% of all cytidine nucleotides are 2′-O-methyl cytidine, 90% of all guanosine nucleotides are 2′-O-methyl guanosine, and 100% of all uridine nucleotides are 2′-

O-methyl uridine, and no more than about 10% of all guanosine nucleotides are 2'-OH guanosine.

[00120] Under fGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2′-O-methyl adenosine triphosphates, 2′-O-methyl uridine triphosphates, 2′-O-methyl cytidine triphosphates, and 2′-F guanosine triphosphates. The modified oligonucleotides produced using the fGmH transcription conditions of the present invention comprise substantially all 2′-O-methyl adenosine, 2′-O-methyl uridine, 2′-O-methyl cytidine, and 2′-F guanosine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2′-O-methyl adenosine, at least 80% of all uridine nucleotides are 2′-O-methyl uridine, at least 80% of all cytidine nucleotides are 2′-O-methyl cytidine, and at least 80% of all guanosine nucleotides are 2′-F guanosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2′-O-methyl adenosine, at least 90% of all uridine nucleotides are 2′-O-methyl uridine, at least 90% of all cytidine nucleotides are 2′-O-methyl cytidine, and at least 90% of all guanosine nucleotides are 2′-F guanosine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2′-O-methyl adenosine, 100% of all uridine nucleotides are 2′-O-methyl uridine, 100% of all cytidine nucleotides are 2′-O-methyl cytidine, and 100% of all guanosine nucleotides are 2′-F guanosine.

[00121] Under dAmB transcription conditions of the present invention, the transcription reaction mixture comprises 2′-deoxy adenosine triphosphates, 2′-O-methyl cytidine triphosphates, 2′-O-methyl guanosine triphosphates, and 2′-O-methyl uridine triphosphates. The modified oligonucleotides produced using the dAmB transcription mixtures of the present invention comprise substantially all 2′-deoxy adenosine, 2′-O-methyl cytidine, 2′-O-methyl guanosine, and 2′-O-methyl uridine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2′-deoxy adenosine, at least 80% of all cytidine nucleotides are 2′-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2′-O-methyl guanosine, and at least 80% of all uridine nucleotides are 2′-O-methyl uridine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine

nucleotides are 2′-deoxy adenosine, at least 90% of all cytidine nucleotides are 2′-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2′-O-methyl guanosine, and at least 90% of all uridine nucleotides are 2′-O-methyl uridine. In a most preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where 100% of all adenosine nucleotides are 2′-deoxy adenosine, 100% of all cytidine nucleotides are 2′-O-methyl cytidine, 100% of all guanosine nucleotides are 2′-O-methyl guanosine, and 100% of all uridine nucleotides are 2′-O-methyl uridine.

[00122] In each case, the transcription products can then be used as the library in the SELEX™ process to identify aptamers and/or to determine a conserved motif of sequences that have binding specificity to a given target. The resulting sequences are already partially stabilized, eliminating this step from the process to arrive at an optimized aptamer sequence and giving a more highly stabilized aptamer as a result. Another advantage of the 2'-OMe SELEX™ process is that the resulting sequences are likely to have fewer 2'-OH nucleotides required in the sequence, possibly none. To the extent 2'OH nucleotides remain they can be removed by performing post-SELEX™ modifications.

[00123] As described below, lower but still useful yields of transcripts fully incorporating 2' substituted nucleotides can be obtained under conditions other than the optimized conditions described above. For example, variations to the above transcription conditions include:

[00124] The HEPES buffer concentration can range from 0 to 1 M. The present invention also contemplates the use of other buffering agents having a pKa between 5 and 10 including, for example, Tris-hydroxymethyl-aminomethane.

[00125] The DTT concentration can range from 0 to 400 mM. The methods of the present invention also provide for the use of other reducing agents including, for example, mercaptoethanol.

[00126] The spermidine and/or spermine concentration can range from 0 to 20 mM.

[00127] The PEG-8000 concentration can range from 0 to 50 % (w/v). The methods of the present invention also provide for the use of other hydrophilic polymer including, for example, other molecular weight PEG or other polyalkylene glycols.

[00128] The Triton X-100 concentration can range from 0 to 0.1% (w/v). The methods of the present invention also provide for the use of other non-ionic detergents including, for example, other detergents, including other Triton-X detergents.

[00129] The $MgCl_2$ concentration can range from 0.5 mM to 50 mM. The $MnCl_2$ concentration can range from 0.15 mM to 15 mM. Both $MgCl_2$ and $MnCl_2$ must be present within the ranges described and in a preferred embodiment are present in about a 10 to about 3 ratio of $MgCl_2$:$MnCl_2$, preferably, the ratio is about 3-5:1, more preferably, the ratio is about 3-4:1.

[00130] The 2'-OMe NTP concentration (each NTP) can range from 5 μM to 5 mM.

[00131] The 2'-OH GTP concentration can range from 0 μM to 300 μM.

[00132] The 2'-OH GMP concentration can range from 0 to 5 mM.

[00133] The pH can range from pH 6 to pH 9. The methods of the present invention can be practiced within the pH range of activity of most polymerases that incorporate modified nucleotides. In addition, the methods of the present invention provide for the optional use of chelating agents in the transcription reaction condition including, for example, EDTA, EGTA, and DTT.

IL-23 AND/OR IL-12 APTAMER SELECTION STRATEGIES.

[00134] The present invention provides aptamers that bind to human IL-23 and/or IL-12 and in some embodiments, inhibit binding to their receptor and/or otherwise modulate their function. Human IL-23 and IL-12 are both heterodimers that have one subunit in common and one unique. The subunit in common is the p40 subunit which contains the following amino acid sequence (Accession # AF180563) (SEQ ID NO 4):

MCHQQLVISWFSLVFLASPLVAIWELKKDVYVVELDWYPDAPGE

MVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSL
L

LLHKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSS
R

GSSDPQGVTCGAATLSAERVRGDNKEYEYSVECQEDSACPAAEESLPIEVMVDAVH
KL

KYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLTFCVQ

VQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPCS.

[0001] The p19 subunit is unique to IL-23 and contains the following amino acid sequence (Accession # BC067511) (SEQ ID NO 5):

MLGSRAVMLLLLLPWTAQGRAVPGGSSPAWTQCQQLSQKLCTLA

WSAHPLVGHMDLREEGDEETTNDVPHIQCGDGCDPQGLRDNSQFCLQRIHQGLIFYE
K

LLGSDIFTGEPSLLPDSPVGQLHASLLGLSQLLQPEGHHWETQQIPSLSPSQPWQRLL

LRFKILRSLQAFVAVAARVFAHGAATLSP.

[00135] The p35 subunit is unique to IL-12 and contains the following amino acid sequence (Accession # AF180562) (SEQ ID NO 6):

MWPPGSASQPPPSPAAATGLHPAARPVSLQCRLSMCPARSLLLV

ATLVLLDHLSLARNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTLEFYPCTSE
E

IDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTSFMMALCLSS

IYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSS
LE

EPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS.

[00136] The present invention also provides aptamers that bind to mouse IL-23 and/or IL-12 and in some embodiments, inhibit binding to their receptor and/or otherwise modulate their function. Like human, mouse IL-23 and IL-12 are both heterodimers that share the mouse p40 subunit, while the mouse p19 subunit is specific to mouse IL-23 and the mouse p35 subunit is unique to mouse IL-12. The mouse p40 subunit contains the following amino acid sequence (Accession # P43432) (SEQ ID NO 315):

MCPQKLTISWFAIVLLVSPLMAMWELEKDVYVVEVDWTPDAPGETVNLTCDTPEED

DITWTSDQRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWS

TEILKNFKNKTFLKCEAPNYSGRFTCSWLVQRNMDLKFNIKSSSSSPDSRAVTCGMA

SLSAEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIR

DIIKPDPPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETE

EGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDRYYNSSCSKWACVPCRVRS

[00137] The mouse p19 subunit contains the following amino acid sequence (Accession # NP112542) (SEQ ID NO 316):

MLDCRAVIMLWLLPWVTQGLAVPRSSSPDWAQCQQLSRNLCMLAWNAHAP

AGHMNLLREEEDEETKNNVPRIQCEDGCDPQGLKDNSQFCLQRIRQGLAF

YKHLLDSDIF KGEPALLPDSPMEQLHTSLLGLSQLLQPEDHPRETQQMPS

LSSSQQWQRPLLRSKILRSLQAFLAIAARVFAHGAATLTE PLVPTA.

[00138] The mouse p35 subunit contains the following amino acid sequence (Accession # P43431) (SEQ ID NO 317):

MCQSRYLLFLATLALLNHLSLARVIPVSGPARCLSQSRNLLKTTDDMVKTAREKLKH

YSCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPPQKTSLM

MTLCL

GSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKP

PVGEADPYRVKMKLCILLHAFST RVVTINRVMG YLSSA

[00139] Several SELEX™ strategies can be employed to generate aptamers with a variety of specificities for IL-23 and IL-12. One scheme produces aptamers specific for IL-23 over IL-12 by including IL-12 in a negative selection step. This eliminates sequences that recognize the common subunit, p40 (SEQ ID NO 4), and selects for aptamers specific to IL-23, or the p19 subunit (SEQ ID NO 5) as shown in Figure 3. One scheme produces aptamers specific for IL-12 over IL-23 by including IL-23 in the negative selection step. This eliminates sequences that recognize the common subunit, p40 (SEQ ID NO 4) and selects for aptamers specific for IL-12, or the p35 subunit (SEQ ID NO 6). A separate selection in which IL-23 and IL-12 are alternated every other round elicits aptamers that recognize the common subunit, p40 (SEQ ID NO 4), and therefore recognizes both proteins. Once sequences with the desired binding specificity are found, minimization of those sequences can be undertaken

to systematically reduce the size of the sequences with concomitant improvement in binding characteristics.

[00140] The selected aptamers having the highest affinity and specific binding as demonstrated by biological assays as described in the examples below are suitable therapeutics for treating conditions in which IL-23 and/or IL-12 is involved in pathogenesis.

IL-23/IL-12 SPECIFIC BINDING APTAMERS

[00141] The materials of the present invention comprise a series of nucleic acid aptamers of ~25-90 nucleotides in length which bind specifically to cytokines of the human IL-12 cytokine family which includes IL-12, IL-23, and IL-27; p19, p35, and p40 subunit monomers; and p40 subunit dimers; and which functionally modulate, *e.g.*, block, the activity of IL-23 and/or IL-12 in *in vivo* and/or in cell-based assays.

[00142] Aptamers specifically capable of binding and modulating IL-23 and/or IL-12 are set forth herein. These aptamers provide a low-toxicity, safe, and effective modality of treating and/or preventing autoimmune and inflammatory related diseases or disorders. In one embodiment, the aptamers of the invention are used to treat and/or prevent inflammatory and autoimmune diseases, including but not limited to, multiple sclerosis, rheumatoid arthritis, psoriasis vulgaris, and irritable bowel disease, including without limitation Crohn's disease, and ulcerative colitis, each of which are known to be caused by or otherwise associated with the IL-23 and/or IL-12 cytokine. In another embodiment, the aptamers of the invention are used to treat and/or prevent Type I Diabetes, which is known to be caused by or otherwise associated with the IL-23 and/or IL-12 cytokine. In another embodiment, the aptamers of the invention are used to treat and/or prevent other indications for which activation of cytokine receptor binding is desirable including, for example, systemic lupus erythamatosus, colon cancer, lung cancer, and bone resorption in osteoporosis.

[00143] Examples of IL-23 and/or IL-12 specific binding aptamers for use as therapeutics and/or diagnostics include the following sequences listed below.

[00144] Unless noted otherwise, ARC489 (SEQ ID NO 91), ARC491 (SEQ ID NO 94), ARC621 (SEQ ID NO 108), ARC627 (SEQ ID NO 110), ARC527 (SEQ ID NO 159), ARC792 (SEQ ID NO 162), ARC794 (SEQ ID NO 164), ARC795 (SEQ ID NO 165), ARC979 (SEQ ID NO 177), ARC1386 (SEQ ID NO 224), and ARC1623-ARC1625 (SEQ

ID NOs 309-311) represent the sequences of the aptamers that bind to IL-23 and/or IL-12 that were selected under SELEX™ conditions in which the purines (A and G) are deoxy, and the pyrimidines (C and U) are 2'-OMe.

[00145] The unique sequence region of ARC489 (SEQ ID NO 91) and ARC491 (SEQ ID NO 94) begins at nucleotide 23, immediately following the sequence GGGAGAGGAGAGAACGUUCUAC (SEQ ID NO 69), and runs until it meets the 3'fixed nucleic acid sequence GCUGUCGAUCGAUCGAUCGAUG (SEQ ID NO 90).

[00146] The unique sequence region of ARC621 (SEQ ID NO 108) and ARC627 (SEQ ID NO 110) begins at nucleotide 23, immediately following the sequence GGGAGAGGAGAGAACGUUCUAC (SEQ ID NO 101), and runs until it meets the 3'fixed nucleic acid sequence GUCGAUCGAUCGAUCAUCGAUG (SEQ ID NO 102).

SEQ ID NO 91 (ARC489)
GGGAGAGGAGAGAACGUUCUACAGCGCCGGUGGGCGGGCAUUGGGUGGAUGCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 94 (ARC491)
GGGAGAGGAGAGAACGUUCUACAGCGCCGGUGGGUGGGCAUAGGGUGGAUGCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 108 (ARC621)
GGGAGAGGAGAGAACGUUCUACAGGCGGUUACGGGGGAUGCGGGUGGGACAGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 110 (ARC627)
GGGAGAGGAGAGAACGUUCUACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 159 (ARC527)
ACAGCGCCGGUGGGCGGGCAUUGGGUGGAUGCGCUGU

SEQ ID NO 162 (ARC792)
GGCAAGUAAUUGGGGAGUGCGGGCGGGG

SEQ ID NO 164 (ARC794)
GGCGGUACGGGGAGUGUGGGUUGGGGCCGG

SEQ ID NO 165 (ARC795)
CGAUAUAGGCGGUACGGGGGGAGUGGGCUGGGGUCG

SEQ ID NO 177 (ARC979)
ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU

[00147] ARC1623 (SEQ ID NO 309), ARC1624 (SEQ ID NO 310) and ARC1625 (SEQ ID NO 311) represent optimized sequences based on ARC979 (SEQ ID NO 177), where "d"

stands for deoxy, "m" stands for 2'-O-methyl, "s" indicates a phosphorothioate internucleotide linkage, and "3T" stands for a 3'-inverted deoxy thymidine.

SEQ ID NO 309 (ARC1623)

dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGmGmG-s-dG-s-dA-s-dGmU-s-dGmCmGmGdGmCdGdGmGmGmUdGmU-3T

SEQ ID NO 310 (ARC1624)

dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGmGmGdGdAdGmUdGmCmGmG-s-dGmC-s-dG-s-dGmGmGmUdGmU-3T

SEQ ID NO 311 (ARC1625)

dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGmGmGdGdAdGmUdGmCmGmGdGmCdGdGmGmGmU-s-dGmU-3T

[00148] SEQ ID NOS 139-140, SEQ ID NOS 144-145, SEQ ID NO 147, and SEQ ID NOS 151-152, represent the sequences of the aptamers that bind to IL-23 and/or IL-12 that were selected under SELEX™ conditions in which the purines (A and G) are 2'-OH (ribo) and the pyrimidines (C and U) are 2'-Fluoro.

SEQ ID NO 139 (A10.min5)
GGAGCAUACACAAGAAGUUUUUUGUGCUCUGAGUACUCAGCGUCCGUAAGGGAUAUGCUCC

SEQ ID NO 140 (A10.min6)
GGAGUACGCCGAAAGGCGCUCUGAGUACUCAGCGUCCGUAAGGGAUACUCC

SEQ ID NO 144 (B10.min4)
GGAGCAUACACAAGAAGUGCUUCAUGCGGCAAACUGCAUGACGUCGAAUAGAUAUGCUCC

SEQ ID NO 145 (B10.min5)
GGAGUACACAAGAAGUGCUUCCGAAAGGACGUCGAAUAGAUACUCC

SEQ ID NO 147 (F11.min2)
GGACAUACACAAGAUGUGCUUGAGUUAAAUCUCAUCGUCCCCGUUUGGGGAUAUGUC

SEQ ID NO 151
GGGUACGCCGAAAGGCGCUUCCGAAAGGACGUCCGUAAGGGAUACCC

SEQ ID NO 152
GGAGUACGCCGAAAGGCGCUUCCGAAAGGACGUCCGUAAGGGAUACUCC

[00149] Other aptamers that bind IL-23 and/or IL-12 are described below in Examples 1-3.

[00150] These aptamers may include modifications as described herein including *e.g.*, conjugation to lipophilic or high molecular weight compounds (*e.g.*, PEG), incorporation of a

CpG motif, incorporation of a capping moiety, incorporation of modified nucleotides, and incorporation of phosphorothioate in the phosphate backbone.

[00151] In one embodiment, an isolated, non-naturally occurring aptamer that binds to IL-23 and/or IL-12 is provided. In some embodiments, the isolated, non-naturally occurring aptamer has a dissociation constant ("K_D") for IL-23 and/or IL-12 of less than 100 μM, less than 1 μM, less than 500 nM, less than 100 nM, less than 50 nM , less than 1 nM, less than 500 pM, less than 100 pM, and less than 50 pM. In some embodiments of the invention, the dissociation constant is determined by dot blot titration as described in Example 1 below.

[00152] In another embodiment, the aptamer of the invention modulates a function of IL-23 and/or IL-12. In another embodiment, the aptamer of the invention inhibits an IL-23 and/or IL-12 function while in another embodiment the aptamer stimulates a function of the target. In another embodiment of the invention, the aptamer binds and/or modulates a function of an IL-23 or IL-12 variant. An IL-23 or IL-12 variant as used herein encompasses variants that perform essentially the same function as an IL-23 or IL-12 function, preferably comprises substantially the same structure and in some embodiments comprises at least 70% sequence identity, preferably at least 80% sequence identity, more preferably at least 90% sequence identity, and more preferably at least 95% sequence identity to the amino acid sequence of IL-23 or IL-12. In some embodiments of the invention, the sequence identity of target variants is determined using BLAST as described below.

[00153] The terms "sequence identity" in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J Mol. Biol. 48:

443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally, Ausubel *et al.*, infra).

[00154] One example of an algorithm that is suitable for determining percent sequence identity is the algorithm used in the basic local alignment search tool (hereinafter "BLAST"), see, *e.g.* Altschul *et al.*, J Mol. Biol. 215: 403-410 (1990) and Altschul *et al.*, Nucleic Acids Res., 15: 3389-3402 (1997). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (hereinafter "NCBI"). The default parameters used in determining sequence identity using the software available from NCBI, *e.g.*, BLASTN (for nucleotide sequences) and BLASTP (for amino acid sequences) are described in McGinnis *et al.*, Nucleic Acids Res., 32: W20-W25 (2004).

[00155] In one embodiment of the invention, the aptamer has substantially the same ability to bind to IL-23 as that of an aptamer comprising any one of SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314. In another embodiment of the invention, the aptamer has substantially the same structure and ability to bind to IL-23 as that of an aptamer comprising any one of SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

[00156] In one embodiment of the invention, the aptamer has substantially the same ability to bind to IL-23 and/or IL-12 as that of an aptamer comprising any one of SEQ ID NO 14, SEQ ID NOs 17-19, SEQ ID NO 21, SEQ ID NOs 27-32, SEQ ID NOs 34-40, SEQ ID NO 42, SEQ ID NO 49, SEQ ID NOs 60-61, SEQ ID NOs 91-92, SEQ ID NO 94, and SEQ ID NOs 103-118. In another embodiment of the invention, the aptamer has substantially the same structure and ability to bind to IL-23 and/or IL-12 as that of an aptamer comprising any one of SEQ ID NO 14, SEQ ID NOs 17-19, SEQ ID NO 21, SEQ ID NOs 27-32, SEQ ID NOs 34-40, SEQ ID NO 42, SEQ ID NO 49, SEQ ID NOs 60-61, SEQ ID NOs 91-92, SEQ ID NO 94, and SEQ ID NOs 103-118.

[00157] In another embodiment, the aptamers of the invention are used as an active ingredient in pharmaceutical compositions. In another embodiment, the aptamers or compositions comprising the aptamers of the invention are used to treat inflammatory and autoimmune diseases (including but not limited to, multiple sclerosis, rheumatoid arthritis, psoriasis vulgaris, systemic lupus erythamatosus, and irritable bowel disease, including without limitation Crohn's disease, and ulcerative colitis), Type I Diabetes, colon cancer, lung cancer, and bone resorption in osteoporosis.

[00158] In some embodiments aptamer therapeutics of the present invention have great affinity and specificity to their targets while reducing the deleterious side effects from non-naturally occurring nucleotide substitutions if the aptamer therapeutics break down in the body of patients or subjects. In some embodiments, the therapeutic compositions containing the aptamer therapeutics of the present invention are free of or have a reduced amount of fluorinated nucleotides.

[00159] The aptamers of the present invention can be synthesized using any oligonucleotide synthesis techniques known in the art including solid phase oligonucleotide synthesis techniques (see, *e.g.*, Froehler *et al.*, Nucl. Acid Res. 14:5399-5467 (1986) and Froehler *et al.*, Tet. Lett. 27:5575-5578 (1986)) and solution phase methods well known in the art such as triester synthesis methods (see, *e.g.*, Sood *et al.*, Nucl. Acid Res. 4:2557 (1977) and Hirose *et al.*, Tet. Lett., 28:2449 (1978)).

APTAMERS HAVING IMMUNOSTIMULATORY MOTIFS

[00160] The present invention provides aptamers that bind to IL-23 and/or IL-12 and modulate their biological function. More specifically, the present invention provides aptamers that increase the binding of IL-23 and/or IL-12 to the IL-23 and/or IL-12 receptor thereby enhancing the biological function of IL-23 and/or IL-12. The agonistic effect of such aptamers can be further enhanced by selecting for aptamers which bind to the IL-23 and/or IL-12 and contain immunostimulatory motifs, or by treating with aptamers which bind to IL-23 and/or IL-12 in conjunction with aptamers to a target known to bind immunostimulatory sequences.

[00161] Recognition of bacterial DNA by the vertebrate immune system is based on the recognition of unmethylated CG dinucleotides in particular sequence contexts ("CpG motifs"). One receptor that recognizes such a motif is Toll-like receptor 9 ("TLR 9"), a

member of a family of Toll-like receptors (~10 members) that participate in the innate immune response by recognizing distinct microbial components. TLR 9 binds unmethylated oligodeoxynucleotide ("ODN") CpG sequences in a sequence-specific manner. The recognition of CpG motifs triggers defense mechanisms leading to innate and ultimately acquired immune responses. For example, activation of TLR 9 in mice induces activation of antigen presenting cells, up regulation of MHC class I and II molecules and expression of important co-stimulatory molecules and cytokines including IL-12 and IL-23. This activation both directly and indirectly enhances B and T cell responses, including robust up regulation of the TH1 cytokine IFN-gamma. Collectively, the response to CpG sequences leads to: protection against infectious diseases, improved immune response to vaccines, an effective response against asthma, and improved antibody-dependent cell-mediated cytotoxicity. Thus, CpG ODNs can provide protection against infectious diseases, function as immuno-adjuvants or cancer therapeutics (monotherapy or in combination with a mAb or other therapies), and can decrease asthma and allergic response.

[00162] Aptamers of the present invention comprising one or more CpG or other immunostimulatory sequences can be identified or generated by a variety of strategies using, *e.g.*, the SELEX™ process described herein. The incorporated immunostimulatory sequences can be DNA, RNA and/or a combination DNA/RNA. In general the strategies can be divided into two groups. In group one, the strategies are directed to identifying or generating aptamers comprising both a CpG motif or other immunostimulatory sequence as well as a binding site for a target, where the target (hereinafter "non-CpG target") is a target other than one known to recognize CpG motifs or other immunostimulatory sequences and known to stimulates an immune response upon binding to a CpG motif. In some embodiments of the invention the non-CpG target is an IL-23 and/or IL12 target. The first strategy of this group comprises performing SELEX™ to obtain an aptamer to a specific non-CpG target, preferably a target, *e.g.*, IL-23 and/or IL-12, where a repressed immune response is relevant to disease development, using an oligonucleotide pool wherein a CpG motif has been incorporated into each member of the pool as, or as part of, a fixed region, *e.g.*, in some embodiments the randomized region of the pool members comprises a fixed region having a CpG motif incorporated therein, and identifying an aptamer comprising a CpG motif. The second strategy of this group comprises performing SELEX™ to obtain an aptamer to a specific non-CpG target preferably a target, *e.g.*, IL-23 and/or IL-12, where a repressed immune response is relevant to disease development, and following selection appending a CpG motif to the 5'

and/or 3' end or engineering a CpG motif into a region, preferably a non-essential region, of the aptamer. The third strategy of this group comprises performing SELEX™ to obtain an aptamer to a specific non-CpG target, preferably a target, *e.g.*, IL-23 and/or IL-12, where a repressed immune response is relevant to disease development, wherein during synthesis of the pool the molar ratio of the various nucleotides is biased in one or more nucleotide addition steps so that the randomized region of each member of the pool is enriched in CpG motifs, and identifying an aptamer comprising a CpG motif. The fourth strategy of this group comprises performing SELEX™ to obtain an aptamer to a specific non-CpG target, preferably a target, *e.g.*, IL-23 and/or IL-12, where a repressed immune response is relevant to disease development, and identifying an aptamer comprising a CpG motif. The fifth strategy of this group comprises performing SELEX™ to obtain an aptamer to a specific non-CpG target, preferably a target, *e.g.*, IL-23 and/or IL-12, where a repressed immune response is relevant to disease development, and identifying an aptamer which, upon binding, stimulates an immune response but which does not comprise a CpG motif.

[00163] In group two, the strategies are directed to identifying or generating aptamers comprising a CpG motif and/or other sequences that are bound by the receptors for the CpG motifs (*e.g.*, TLR9 or the other toll-like receptors) and upon binding stimulate an immune response. The first strategy of this group comprises performing SELEX™ to obtain an aptamer to a target known to bind to CpG motifs or other immunostimulatory sequences and upon binding stimulate an immune response using an oligonucleotide pool wherein a CpG motif has been incorporated into each member of the pool as, or as part of, a fixed region, *e.g.*, in some embodiments the randomized region of the pool members comprise a fixed region having a CpG motif incorporated therein, and identifying an aptamer comprising a CpG motif. The second strategy of this group comprises performing SELEX™ to obtain an aptamer to a target known to bind to CpG motifs or other immunostimulatory sequences and upon binding stimulate an immune response and then appending a CpG motif to the 5' and/or 3' end or engineering a CpG motif into a region, preferably a non-essential region, of the aptamer. The third strategy of this group comprises performing SELEX™ to obtain an aptamer to a target known to bind to CpG motifs or other immunostimulatory sequences and upon binding stimulate an immune response wherein during synthesis of the pool, the molar ratio of the various nucleotides is biased in one or more nucleotide addition steps so that the randomized region of each member of the pool is enriched in CpG motifs, and identifying an aptamer comprising a CpG motif. The fourth strategy of this group comprises performing

SELEX™ to obtain an aptamer to a target known to bind to CpG motifs or other immunostimulatory sequences and upon binding stimulate an immune response and identifying an aptamer comprising a CpG motif. The fifth strategy of this group comprises performing SELEX™ to obtain an aptamer to a target known to bind to CpG motifs or other immunostimulatory sequences, and identifying an aptamer which upon binding, stimulate an immune response but which does not comprise a CpG motif.

[00164] A variety of different classes of CpG motifs have been identified, each resulting upon recognition in a different cascade of events, release of cytokines and other molecules, and activation of certain cell types. *See, e.g.*, CpG Motifs in Bacterial DNA and Their Immune Effects, Annu. Rev. Immunol. 2002, 20:709-760, incorporated herein by reference. Additional immunostimulatory motifs are disclosed in the following U.S. Patents, each of which is incorporated herein by reference: U.S. Patent No. 6,207,646; U.S. Patent No. 6,239,116; U.S. Patent No. 6,429,199; U.S. Patent No. 6,214,806; U.S. Patent No. 6,653,292; U.S. Patent No. 6,426,434; U.S. Patent No. 6,514,948 and U.S. Patent No. 6,498,148. Any of these CpG or other immunostimulatory motifs can be incorporated into an aptamer. The choice of aptamers is dependent on the disease or disorder to be treated. Preferred immunostimulatory motifs are as follows (shown 5' to 3' left to right) wherein "r" designates a purine, "y" designates a pyrimidine, and "X" designates any nucleotide: AACGTTCGAG (SEQ ID NO 7); AACGTT; ACGT, rCGy; rrCGyy, XCGX, XXCGXX, and $X_1X_2CGY_1Y_2$ wherein X_1 is G or A, X_2 is not C, Y_1 is not G and Y_2 is preferably T.

[00165] In those instances where a CpG motif is incorporated into an aptamer that binds to a specific target other than a target known to bind to CpG motifs and upon binding stimulate an immune response (a "non-CpG target"), the CpG is preferably located in a non-essential region of the aptamer. Non-essential regions of aptamers can be identified by site-directed mutagenesis, deletion analyses and/or substitution analyses. However, any location that does not significantly interfere with the ability of the aptamer to bind to the non-CpG target may be used. In addition to being embedded within the aptamer sequence, the CpG motif may be appended to either or both of the 5' and 3' ends or otherwise attached to the aptamer. Any location or means of attachment may be used so long as the ability of the aptamer to bind to the non-CpG target is not significantly interfered with.

[00166] As used herein, "stimulation of an immune response" can mean either (1) the induction of a specific response (*e.g.*, induction of a Th1 response) or of the production of

certain molecules or (2) the inhibition or suppression of a specific response (*e.g.*, inhibition or suppression of the Th2 response) or of certain molecules.

PHARMACEUTICAL COMPOSITIONS

[00167] The invention also includes pharmaceutical compositions containing aptamer molecules that bind to IL-23 and/or IL-12. In some embodiments, the compositions are suitable for internal use and include an effective amount of a pharmacologically active compound of the invention, alone or in combination, with one or more pharmaceutically acceptable carriers. The compounds are especially useful in that they have very low, if any toxicity.

[00168] Compositions of the invention can be used to treat or prevent a pathology, such as a disease or disorder, or alleviate the symptoms of such disease or disorder in a patient. For example, compositions of the present invention can be used to treat or prevent a pathology associated with IL-23 and/or IL-12 cytokines, including inflammatory and autoimmune related diseases, Type I Diabetes, bone resorption in osteoporosis, and cancer.

[00169] Compositions of the invention are useful for administration to a subject suffering from, or predisposed to, a disease or disorder which is related to or derived from a target to which the aptamers of the invention specifically bind. Compositions of the invention can be used in a method for treating a patient or subject having a pathology. The method involves administering to the patient or subject an aptamer or a composition comprising aptamers that bind to IL-23 and/or IL-12 involved with the pathology, so that binding of the aptamer to the IL-23 and/or IL-12 alters the biological function of the target, thereby treating the pathology.

[00170] The patient or subject having a pathology, *i.e.*, the patient or subject treated by the methods of this invention, can be a vertebrate, more particularly a mammal, or more particularly a human.

[00171] In practice, the aptamers or their pharmaceutically acceptable salts, are administered in amounts which will be sufficient to exert their desired biological activity, *e.g.*, inhibiting the binding of the IL-23 and/or IL-12 to its receptor.

[00172] One aspect of the invention comprises an aptamer composition of the invention in combination with other treatments for inflammatory and autoimmune diseases, cancer, and other related disorders. The aptamer composition of the invention may contain, for example, more than one aptamer. In some examples, an aptamer composition of the invention,

containing one or more compounds of the invention, is administered in combination with another useful composition such as an anti-inflammatory agent, an immunosuppressant, an antiviral agent, or the like. Furthermore, the compounds of the invention may be administered in combination with a cytotoxic, cytostatic, or chemotherapeutic agent such as an alkylating agent, anti-metabolite, mitotic inhibitor or cytotoxic antibiotic, as described above. In general, the currently available dosage forms of the known therapeutic agents for use in such combinations will be suitable.

[00173] "Combination therapy" (or "co-therapy") includes the administration of an aptamer composition of the invention and at least a second agent as part of a specific treatment regimen intended to provide the beneficial effect from the co-action of these therapeutic agents. The beneficial effect of the combination includes, but is not limited to, pharmacokinetic or pharmacodynamic co-action resulting from the combination of therapeutic agents. Administration of these therapeutic agents in combination typically is carried out over a defined time period (usually minutes, hours, days or weeks depending upon the combination selected).

[00174] "Combination therapy" may, but generally is not, intended to encompass the administration of two or more of these therapeutic agents as part of separate monotherapy regimens that incidentally and arbitrarily result in the combinations of the present invention. "Combination therapy" is intended to embrace administration of these therapeutic agents in a sequential manner, that is, wherein each therapeutic agent is administered at a different time, as well as administration of these therapeutic agents, or at least two of the therapeutic agents, in a substantially simultaneous manner. Substantially simultaneous administration can be accomplished, for example, by administering to the subject a single capsule having a fixed ratio of each therapeutic agent or in multiple, single capsules for each of the therapeutic agents.

[00175] Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, topical routes, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination selected may be administered by injection while the other therapeutic agents of the combination may be administered topically.

[00176] Alternatively, for example, all therapeutic agents may be administered topically or all therapeutic agents may be administered by injection. The sequence in which the therapeutic agents are administered is not narrowly critical unless noted otherwise. "Combination therapy" also can embrace the administration of the therapeutic agents as described above in further combination with other biologically active ingredients. Where the combination therapy further comprises a non-drug treatment, the non-drug treatment may be conducted at any suitable time so long as a beneficial effect from the co-action of the combination of the therapeutic agents and non-drug treatment is achieved. For example, in appropriate cases, the beneficial effect is still achieved when the non-drug treatment is temporally removed from the administration of the therapeutic agents, perhaps by days or even weeks.

[00177] Therapeutic or pharmacological compositions of the present invention will generally comprise an effective amount of the active component(s) of the therapy, dissolved or dispersed in a pharmaceutically acceptable medium. Pharmaceutically acceptable media or carriers include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Supplementary active ingredients can also be incorporated into the therapeutic compositions of the present invention.

[00178] The preparation of pharmaceutical or pharmacological compositions will be known to those of skill in the art in light of the present disclosure. Typically, such compositions may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection; as tablets or other solids for oral administration; as time release capsules; or in any other form currently used, including eye drops, creams, lotions, salves, inhalants and the like. The use of sterile formulations, such as saline-based washes, by surgeons, physicians or health care workers to treat a particular area in the operating field may also be particularly useful. Compositions may also be delivered via microdevice, microparticle or sponge.

[00179] Upon formulation, therapeutics will be administered in a manner compatible with the dosage formulation, and in such amount as is pharmacologically effective. The formulations are easily administered in a variety of dosage forms, such as the type of

injectable solutions described above, but drug release capsules and the like can also be employed.

[00180] In this context, the quantity of active ingredient and volume of composition to be administered depends on the host animal to be treated. Precise amounts of active compound required for administration depend on the judgment of the practitioner and are peculiar to each individual.

[00181] A minimal volume of a composition required to disperse the active compounds is typically utilized. Suitable regimes for administration are also variable, but would be typified by initially administering the compound and monitoring the results and then giving further controlled doses at further intervals.

[00182] For instance, for oral administration in the form of a tablet or capsule (*e.g.*, a gelatin capsule), the active drug component can be combined with an oral, non-toxic, pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents, and coloring agents can also be incorporated into the mixture. Suitable binders include starch, magnesium aluminum silicate, starch paste, gelatin, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, polyethylene glycol, waxes, and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol, and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum starches, agar, alginic acid or its sodium salt, or effervescent mixtures, and the like. Diluents, include, *e.g.*, lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine.

[00183] The compounds of the invention can also be administered in such oral dosage forms as timed release and sustained release tablets or capsules, pills, powders, granules, elixirs, tinctures, suspensions, syrups and emulsions. Suppositories are advantageously prepared from fatty emulsions or suspensions.

[00184] The pharmaceutical compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for

regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances. The compositions are prepared according to conventional mixing, granulating, or coating methods, and typically contain about 0.1% to 75%, preferably about 1% to 50%, of the active ingredient.

[00185] Liquid, particularly injectable compositions can, for example, be prepared by dissolving, dispersing, etc. The active compound is dissolved in or mixed with a pharmaceutically pure solvent such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form the injectable solution or suspension. Additionally, solid forms suitable for dissolving in liquid prior to injection can be formulated.

[00186] The compounds of the present invention can be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions.

[00187] Parenteral injectable administration is generally used for subcutaneous, intramuscular or intravenous injections and infusions. Additionally, one approach for parenteral administration employs the implantation of a slow-release or sustained-released systems, which assures that a constant level of dosage is maintained, according to U.S. Pat. No. 3,710,795, incorporated herein by reference.

[00188] Furthermore, preferred compounds for the present invention can be administered in intranasal form via topical use of suitable intranasal vehicles, inhalants, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Other preferred topical preparations include creams, ointments, lotions, aerosol sprays and gels, wherein the concentration of active ingredient would typically range from 0.01% to 15%, w/w or w/v.

[00189] For solid compositions, excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. The active compound defined above, may be also formulated as suppositories, using for example, polyalkylene glycols, for example, propylene

glycol, as the carrier. In some embodiments, suppositories are advantageously prepared from fatty emulsions or suspensions.

[00190] The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, containing cholesterol, stearylamine or phosphatidylcholines. In some embodiments, a film of lipid components is hydrated with an aqueous solution of drug to a form lipid layer encapsulating the drug, as described in U.S. Pat. No. 5,262,564. For example, the aptamer molecules described herein can be provided as a complex with a lipophilic compound or non-immunogenic, high molecular weight compound constructed using methods known in the art. An example of nucleic-acid associated complexes is provided in U.S. Patent No. 6,011,020.

[00191] The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropyl-methacrylamide-phenol, polyhydroxyethylaspanamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

[00192] If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and other substances such as for example, sodium acetate, and triethanolamine oleate.

[00193] The dosage regimen utilizing the aptamers is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the patient; and the particular aptamer or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

[00194] Oral dosages of the present invention, when used for the indicated effects, will range between about 0.05 to 7500 mg/day orally. The compositions are preferably provided in the form of scored tablets containing 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mg of active ingredient. Infused dosages, intranasal dosages and transdermal dosages will range between 0.05 to 7500 mg/day. Subcutaneous, intravenous and intraperitoneal dosages will range between 0.05 to 3800 mg/day.

[00195] Effective plasma levels of the compounds of the present invention range from 0.002 mg/mL to 50 mg/mL.

[00196] Compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily.

MODULATION OF PHARMACOKINETICS AND BIODISTRIBUTION OF APTAMER THERAPEUTICS

[00197] It is important that the pharmacokinetic properties for all oligonucleotide-based therapeutics, including aptamers, be tailored to match the desired pharmaceutical application. While aptamers directed against extracellular targets do not suffer from difficulties associated with intracellular delivery (as is the case with antisense and RNAi-based therapeutics), such aptamers must still be able to be distributed to target organs and tissues, and remain in the body (unmodified) for a period of time consistent with the desired dosing regimen.

[00198] Thus, the present invention provides materials and methods to affect the pharmacokinetics of aptamer compositions, and, in particular, the ability to tune aptamer pharmacokinetics. The tunability of (*i.e.*, the ability to modulate) aptamer pharmacokinetics is achieved through conjugation of modifying moieties (*e.g.*, PEG polymers) to the aptamer and/or the incorporation of modified nucleotides (*e.g.*, 2'-fluoro or 2'-O-methyl) to alter the chemical composition of the nucleic acid. The ability to tune aptamer pharmacokinetics is used in the improvement of existing therapeutic applications, or alternatively, in the development of new therapeutic applications. For example, in some therapeutic applications, *e.g.*, in anti-neoplastic or acute care settings where rapid drug clearance or turn-off may be desired, it is desirable to decrease the residence times of aptamers in the circulation. Alternatively, in other therapeutic applications, *e.g.*, maintenance therapies where systemic

circulation of a therapeutic is desired, it may be desirable to increase the residence times of aptamers in circulation.

[00199] In addition, the tunability of aptamer pharmacokinetics is used to modify the biodistribution of an aptamer therapeutic in a subject. For example, in some therapeutic applications, it may be desirable to alter the biodistribution of an aptamer therapeutic in an effort to target a particular type of tissue or a specific organ (or set of organs). In these applications, the aptamer therapeutic preferentially accumulates in a specific tissue or organ(s). In other therapeutic applications, it may be desirable to target tissues displaying a cellular marker or a symptom associated with a given disease, cellular injury or other abnormal pathology, such that the aptamer therapeutic preferentially accumulates in the affected tissue. For example, as described in copending provisional application United States Serial No. 60/550790, filed on March 5, 2004, and entitled "Controlled Modulation of the Pharmacokinetics and Biodistribution of Aptamer Therapeutics", and in the non-provisional application United States Serial No. 10/---,---, filed on March 7, 2005, also entitled "Controlled Modulation of the Pharmacokinetics and Biodistribution of Aptamer Therapeutics", PEGylation of an aptamer therapeutic (*e.g.*, PEGylation with a 20 kDa PEG polymer) is used to target inflamed tissues, such that the PEGylated aptamer therapeutic preferentially accumulates in inflamed tissue.

[00200] To determine the pharmacokinetic and biodistribution profiles of aptamer therapeutics (*e.g.*, aptamer conjugates or aptamers having altered chemistries, such as modified nucleotides) a variety of parameters are monitored. Such parameters include, for example, the half-life ($t_{1/2}$), the plasma clearance (Cl), the volume of distribution (Vss), the area under the concentration-time curve (AUC), maximum observed serum or plasma concentration (C_{max}), and the mean residence time (MRT) of an aptamer composition. As used herein, the term "AUC" refers to the area under the plot of the plasma concentration of an aptamer therapeutic versus the time after aptamer administration. The AUC value is used to estimate the bioavailability (*i.e.*, the percentage of administered aptamer therapeutic in the circulation after aptamer administration) and/or total clearance (Cl) (*i.e.*, the rate at which the aptamer therapeutic is removed from circulation) of a given aptamer therapeutic. The volume of distribution relates the plasma concentration of an aptamer therapeutic to the amount of aptamer present in the body. The larger the Vss, the more an aptamer is found outside of the plasma (*i.e.*, the more extravasation).

[00201] The present invention provides materials and methods to modulate, in a controlled manner, the pharmacokinetics and biodistribution of stabilized aptamer compositions *in vivo* by conjugating an aptamer to a modulating moiety such as a small molecule, peptide, or polymer terminal group, or by incorporating modified nucleotides into an aptamer. As described herein, conjugation of a modifying moiety and/or altering nucleotide(s) chemical composition alters fundamental aspects of aptamer residence time in circulation and distribution to tissues.

[00202] In addition to clearance by nucleases, oligonucleotide therapeutics are subject to elimination *via* renal filtration. As such, a nuclease-resistant oligonucleotide administered intravenously typically exhibits an *in vivo* half-life of <10 min, unless filtration can be blocked. This can be accomplished by either facilitating rapid distribution out of the blood stream into tissues or by increasing the apparent molecular weight of the oligonucleotide above the effective size cut-off for the glomerulus. Conjugation of small therapeutics to a PEG polymer (PEGylation), described below, can dramatically lengthen residence times of aptamers in circulation, thereby decreasing dosing frequency and enhancing effectiveness against vascular targets.

[00203] Aptamers can be conjugated to a variety of modifying moieties, such as high molecular weight polymers, *e.g.*, PEG; peptides, *e.g.*, Tat (a 13-amino acid fragment of the HIV Tat protein (Vives, *et al.*, (1997), J. Biol. Chem. 272(25): 16010-7)), Ant (a 16-amino acid sequence derived from the third helix of the Drosophila antennapedia homeotic protein (Pietersz, *et al.*, (2001), Vaccine 19(11-12): 1397-405)) and Arg_7 (a short, positively charged cell-permeating peptides composed of polyarginine (Arg_7) (Rothbard, *et al.*, (2000), Nat. Med. 6(11): 1253-7; Rothbard, J *et al.*, (2002), J. Med. Chem. 45(17): 3612-8)); and small molecules, *e.g.*, lipophilic compounds such as cholesterol. Among the various conjugates described herein, *in vivo* properties of aptamers are altered most profoundly by complexation with PEG groups. For example, complexation of a mixed 2'F and 2'-OMe modified aptamer therapeutic with a 20 kDa PEG polymer hinders renal filtration and promotes aptamer distribution to both healthy and inflamed tissues. Furthermore, the 20 kDa PEG polymer-aptamer conjugate proves nearly as effective as a 40 kDa PEG polymer in preventing renal filtration of aptamers. While one effect of PEGylation is on aptamer clearance, the prolonged systemic exposure afforded by presence of the 20 kDa moiety also facilitates distribution of aptamer to tissues, particularly those of highly perfused organs and those at the site of

inflammation. The aptamer-20 kDa PEG polymer conjugate directs aptamer distribution to the site of inflammation, such that the PEGylated aptamer preferentially accumulates in inflamed tissue. In some instances, the 20 kDa PEGylated aptamer conjugate is able to access the interior of cells, such as, for example, kidney cells.

[00204] Modified nucleotides can also be used to modulate the plasma clearance of aptamers. For example, an unconjugated aptamer which incorporates both 2'-F and 2'-OMe stabilizing chemistries, which is typical of current generation aptamers as it exhibits a high degree of nuclease stability *in vitro* and *in vivo,* displays rapid loss from plasma (*i.e.*, rapid plasma clearance) and a rapid distribution into tissues, primarily into the kidney, when compared to unmodified aptamer.

PEG-DERIVATIZED NUCLEIC ACIDS

[00205] As described above, derivatization of nucleic acids with high molecular weight non-immunogenic polymers has the potential to alter the pharmacokinetic and pharmacodynamic properties of nucleic acids making them more effective therapeutic agents. Favorable changes in activity can include increased resistance to degradation by nucleases, decreased filtration through the kidneys, decreased exposure to the immune system, and altered distribution of the therapeutic through the body.

[00206] The aptamer compositions of the invention may be derivatized with polyalkylene glycol ("PAG") moieties. Examples of PAG-derivatized nucleic acids are found in United States Patent Application Ser. No. 10/718,833, filed on November 21, 2003, which is herein incorporated by reference in its entirety. Typical polymers used in the invention include polyethylene glycol ("PEG"), also known as polyethylene oxide ("PEO") and polypropylene glycol (including poly isopropylene glycol). Additionally, random or block copolymers of different alkylene oxides (*e.g.*, ethylene oxide and propylene oxide) can be used in many applications. In its most common form, a polyalkylene glycol, such as PEG, is a linear polymer terminated at each end with hydroxyl groups: $HO\text{-}CH_2CH_2O\text{-}(CH_2CH_2O)_n\text{-}CH_2CH_2\text{-}OH$. This polymer, alpha-, omega-dihydroxylpolyethylene glycol, can also be represented as HO-PEG-OH, where it is understood that the —PEG- symbol represents the following structural unit: $\text{-}CH_2CH_2O\text{-}(CH_2CH_2O)_n\text{-}CH_2CH_2\text{-}$ where n typically ranges from about 4 to about 10,000.

[00207] As shown, the PEG molecule is di-functional and is sometimes referred to as "PEG diol." The terminal portions of the PEG molecule are relatively non-reactive hydroxyl moieties, the –OH groups, that can be activated, or converted to functional moieties, for attachment of the PEG to other compounds at reactive sites on the compound. Such activated PEG diols are referred to herein as bi-activated PEGs. For example, the terminal moieties of PEG diol have been functionalized as active carbonate ester for selective reaction with amino moieties by substitution of the relatively non-reactive hydroxyl moieties, -OH, with succinimidyl active ester moieties from N-hydroxy succinimide.

[00208] In many applications, it is desirable to cap the PEG molecule on one end with an essentially non-reactive moiety so that the PEG molecule is mono-functional (or mono-activated). In the case of protein therapeutics which generally display multiple reaction sites for activated PEGs, bi-functional activated PEGs lead to extensive cross-linking, yielding poorly functional aggregates. To generate mono-activated PEGs, one hydroxyl moiety on the terminus of the PEG diol molecule typically is substituted with non-reactive methoxy end moiety, $-OCH_3$. The other, un-capped terminus of the PEG molecule typically is converted to a reactive end moiety that can be activated for attachment at a reactive site on a surface or a molecule such as a protein.

[00209] PAGs are polymers which typically have the properties of solubility in water and in many organic solvents, lack of toxicity, and lack of immunogenicity. One use of PAGs is to covalently attach the polymer to insoluble molecules to make the resulting PAG-molecule "conjugate" soluble. For example, it has been shown that the water-insoluble drug paclitaxel, when coupled to PEG, becomes water-soluble. Greenwald, *et al., J. Org. Chem.*, 60:331-336 (1995). PAG conjugates are often used not only to enhance solubility and stability but also to prolong the blood circulation half-life of molecules.

[00210] Polyalkylated compounds of the invention are typically between 5 and 80 kDa in size however any size can be used, the choice dependent on the aptamer and application. Other PAG compounds of the invention are between 10 and 80 kDa in size. Still other PAG compounds of the invention are between 10 and 60 kDa in size. For example, a PAG polymer may be at least 10, 20, 30, 40, 50, 60, or 80 kDa in size. Such polymers can be linear or branched. In some embodiments the polymers are PEG. In some embodiment the polymers are branched PEG. In still other embodiments the polymers are 40kDa branched

PEG as depicted in Figure 4. In some embodiments the 40 kDa branched PEG is attached to the 5' end of the aptamer as depicted in Figure 5.

[00211] In contrast to biologically-expressed protein therapeutics, nucleic acid therapeutics are typically chemically synthesized from activated monomer nucleotides. PEG-nucleic acid conjugates may be prepared by incorporating the PEG using the same iterative monomer synthesis. For example, PEGs activated by conversion to a phosphoramidite form can be incorporated into solid-phase oligonucleotide synthesis. Alternatively, oligonucleotide synthesis can be completed with site-specific incorporation of a reactive PEG attachment site. Most commonly this has been accomplished by addition of a free primary amine at the 5'-terminus (incorporated using a modifier phosphoramidite in the last coupling step of solid phase synthesis). Using this approach, a reactive PEG (*e.g.*, one which is activated so that it will react and form a bond with an amine) is combined with the purified oligonucleotide and the coupling reaction is carried out in solution.

[00212] The ability of PEG conjugation to alter the biodistribution of a therapeutic is related to a number of factors including the apparent size (*e.g.*, as measured in terms of hydrodynamic radius) of the conjugate. Larger conjugates (>10 kDa) are known to more effectively block filtration via the kidney and to consequently increase the serum half-life of small macromolecules (*e.g.*, peptides, antisense oligonucleotides). The ability of PEG conjugates to block filtration has been shown to increase with PEG size up to approximately 50 kDa (further increases have minimal beneficial effect as half life becomes defined by macrophage-mediated metabolism rather than elimination via the kidneys).

[00213] Production of high molecular weight PEGs (>10 kDa) can be difficult, inefficient, and expensive. As a route towards the synthesis of high molecular weight PEG-nucleic acid conjugates, previous work has been focused towards the generation of higher molecular weight activated PEGs. One method for generating such molecules involves the formation of a branched activated PEG in which two or more PEGs are attached to a central core carrying the activated group. The terminal portions of these higher molecular weight PEG molecules, *i.e.*, the relatively non-reactive hydroxyl (–OH) moieties, can be activated, or converted to functional moieties, for attachment of one or more of the PEGs to other compounds at reactive sites on the compound. Branched activated PEGs will have more than two termini, and in cases where two or more termini have been activated, such activated higher molecular weight PEG molecules are referred to herein as, multi-activated PEGs. In some cases, not all

termini in a branch PEG molecule are activated. In cases where any two termini of a branch PEG molecule are activated, such PEG molecules are referred to as bi-activated PEGs. In some cases where only one terminus in a branch PEG molecule is activated, such PEG molecules are referred to as mono-activated. As an example of this approach, activated PEG prepared by the attachment of two monomethoxy PEGs to a lysine core which is subsequently activated for reaction has been described (Harris *et al.*, Nature, vol.2: 214-221, 2003).

[00214] The present invention provides another cost effective route to the synthesis of high molecular weight PEG-nucleic acid (preferably, aptamer) conjugates including multiply PEGylated nucleic acids. The present invention also encompasses PEG-linked multimeric oligonucleotides, *e.g.*, dimerized aptamers. The present invention also relates to high molecular weight compositions where a PEG stabilizing moiety is a linker which separates different portions of an aptamer, *e.g.*, the PEG is conjugated within a single aptamer sequence, such that the linear arrangement of the high molecular weight aptamer composition is, *e.g.*, nucleic acid – PEG – nucleic acid (– PEG — nucleic acid)$_n$ where n is greater than or equal to 1.

[00215] High molecular weight compositions of the invention include those having a molecular weight of at least 10 kDa. Compositions typically have a molecular weight between 10 and 80 kDa in size. High molecular weight compositions of the invention are at least 10, 20, 30, 40, 50, 60, or 80 kDa in size.

[00216] A stabilizing moiety is a molecule, or portion of a molecule, which improves pharmacokinetic and pharmacodynamic properties of the high molecular weight aptamer compositions of the invention. In some cases, a stabilizing moiety is a molecule or portion of a molecule which brings two or more aptamers, or aptamer domains, into proximity, or provides decreased overall rotational freedom of the high molecular weight aptamer compositions of the invention. A stabilizing moiety can be a polyalkylene glycol, such a polyethylene glycol, which can be linear or branched, a homopolymer or a heteropolymer. Other stabilizing moieties include polymers such as peptide nucleic acids (PNA). Oligonucleotides can also be stabilizing moieties; such oligonucleotides can include modified nucleotides, and/or modified linkages, such as phosphorothioates. A stabilizing moiety can be an integral part of an aptamer composition, *i.e.*, it is covalently bonded to the aptamer.

[00217] Compositions of the invention include high molecular weight aptamer compositions in which two or more nucleic acid moieties are covalently conjugated to at least one polyalkylene glycol moiety. The polyalkylene glycol moieties serve as stabilizing moieties. In compositions where a polyalkylene glycol moiety is covalently bound at either end to an aptamer, such that the polyalkylene glycol joins the nucleic acid moieties together in one molecule, the polyalkylene glycol is said to be a linking moiety. In such compositions, the primary structure of the covalent molecule includes the linear arrangement nucleic acid-PAG-nucleic acid. One example is a composition having the primary structure nucleic acid-PEG-nucleic acid. Another example is a linear arrangement of: nucleic acid – PEG – nucleic acid – PEG — nucleic acid.

[00218] To produce the nucleic acid—PEG—nucleic acid conjugate, the nucleic acid is originally synthesized such that it bears a single reactive site (*e.g.*, it is mono-activated). In a preferred embodiment, this reactive site is an amino group introduced at the 5'-terminus by addition of a modifier phosphoramidite as the last step in solid phase synthesis of the oligonucleotide. Following deprotection and purification of the modified oligonucleotide, it is reconstituted at high concentration in a solution that minimizes spontaneous hydrolysis of the activated PEG. In a preferred embodiment, the concentration of oligonucleotide is 1 mM and the reconstituted solution contains 200 mM $NaHCO_3$-buffer, pH 8.3. Synthesis of the conjugate is initiated by slow, step-wise addition of highly purified bi-functional PEG. In a preferred embodiment, the PEG diol is activated at both ends (bi-activated) by derivatization with succinimidyl propionate. Following reaction, the PEG-nucleic acid conjugate is purified by gel electrophoresis or liquid chromatography to separate fully-, partially-, and un-conjugated species. Multiple PAG molecules concatenated (*e.g.*, as random or block copolymers) or smaller PAG chains can be linked to achieve various lengths (or molecular weights). Non-PAG linkers can be used between PAG chains of varying lengths.

[00219] The 2'-O-methyl, 2'-fluoro and other modified nucleotide modifications stabilize the aptamer against nucleases and increase its half life *in vivo*. The 3'-3'-dT cap also increases exonuclease resistance. See, *e.g.*, U.S. Patents 5,674,685; 5,668,264; 6,207,816; and 6,229,002, each of which is incorporated by reference herein in its entirety.

PAG-DERIVATIZATION OF A REACTIVE NUCLEIC ACID

[00220] High molecular weight PAG-nucleic acid-PAG conjugates can be prepared by reaction of a mono-functional activated PEG with a nucleic acid containing more than one

reactive site. In one embodiment, the nucleic acid is bi-reactive, or bi-activated, and contains two reactive sites: a 5'-amino group and a 3'-amino group introduced into the oligonucleotide through conventional phosphoramidite synthesis, for example: 3'-5'-di-PEGylation as illustrated in Figure 6. In alternative embodiments, reactive sites can be introduced at internal positions, using for example, the 5-position of pyrimidines, the 8-position of purines, or the 2'-position of ribose as sites for attachment of primary amines. In such embodiments, the nucleic acid can have several activated or reactive sites and is said to be multiply activated. Following synthesis and purification, the modified oligonucleotide is combined with the mono-activated PEG under conditions that promote selective reaction with the oligonucleotide reactive sites while minimizing spontaneous hydrolysis. In the preferred embodiment, monomethoxy-PEG is activated with succinimidyl propionate and the coupled reaction is carried out at pH 8.3. To drive synthesis of the bi-substituted PEG, stoichiometric excess PEG is provided relative to the oligonucleotide. Following reaction, the PEG-nucleic acid conjugate is purified by gel electrophoresis or liquid chromatography to separate fully, partially, and un-conjugated species.

[00221] The linking domains can also have one or more polyalkylene glycol moieties attached thereto. Such PAGs can be of varying lengths and may be used in appropriate combinations to achieve the desired molecular weight of the composition.

[00222] The effect of a particular linker can be influenced by both its chemical composition and length. A linker that is too long, too short, or forms unfavorable steric and/or ionic interactions with the IL-23 and/or IL-12 will preclude the formation of complex between the aptamer and IL-23 and/or IL-12. A linker, which is longer than necessary to span the distance between nucleic acids, may reduce binding stability by diminishing the effective concentration of the ligand. Thus, it is often necessary to optimize linker compositions and lengths in order to maximize the affinity of an aptamer to a target.

[00223] All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced

in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

EXAMPLES

EXAMPLE 1: APTAMER SELECTION AND SEQUENCES

IL-23 Aptamer Selection

[00224] Several SELEX™ strategies were employed to generate ligands with a variety of specificities for IL-23 and IL-12. One scheme, designed to produce aptamers specific for IL-23 vs. IL-12, included IL-12 in a negative selection step to eliminate aptamers that recognize the common subunit and select for aptamers specific to IL-23. A separate SELEX™ scheme in which IL-23 and IL-12 were alternated every other round elicited aptamers that recognized the common subunit and therefore recognized both proteins. In Examples 1A and 1E, selections were done with 2'-OH purine and 2'-F pyrimidine (rRfY) containing pools. Clones from these selections were optimized based on their binding affinity and efficacy in blocking IL-23 activity in a cell based assay. In addition, selections with 2'-OMe nucleotide containing pools, *i.e.*, rRmY (2'-OH A and G, and 2'-OMe C and U), rGmH (2'-OH G and 2'-OMe C, U, A), and dRmY (deoxy A and G, and 2'-OMe C and U) are described in Examples 1B, 1C, and 1D below.

EXAMPLE 1A: Selections against human IL-23 with 2'-Fluoro pyrimidines containing pools (rRfY)

[00225] Three selections were performed to identify aptamers to human ("h")-IL-23 using a pool consisting of 2'-OH purine (ribo-purines) and 2'-F pyrimidine nucleotides (rRfY conditions). The first selection (h-IL-23) was a direct selection against h-IL-23, which is comprised of p19 and p40 domains. The second selection (X-IL-23) utilized h-IL-23 and h-IL-12 in alternating rounds to drive selection of aptamers to the common subunit between the two proteins, p40. In the third selection (PN-IL-23), h-IL-12 was included in the negative selection step to drive enrichment of aptamers binding to the subdomain unique to h-IL-23, p19. As described below, the starting material for this third selection, *i.e.*, the PN-IL-23

selection was a portion of the pool from the h-IL-23 selection, separated from the remainder of the h-IL-23 pool after two rounds of selection against h-IL-23 protein. All three selection strategies yielded aptamers to h-IL-23. Several aptamers are highly specific for h-IL-23, several show cross reactivity between h-IL-23 and h-IL-12, and one is more specific for h-IL-12 vs. h-IL-23.

[00226] Round 1 of the h-IL-23 and the PN-IL-23 selection began with incubation of $2x10^{14}$ molecules of 2'F pyrimidine modified ARC 212 pool (SEQ ID NO 8) (5'gggaaaagcgaaucauacacaaga-N40-gcuccgccagagaccaaccgagaa3'), including a spike of α^{32}P ATP body labeled pool, with 100 pmoles of IL-23 protein (R&D, Minneapolis, MN) in a final volume of 100 µL for 1hr at room temperature. The series of N's in the template (SEQ ID NO 8) can be any combination of nucleotides and gives rise to the unique sequence region of the resulting aptamers.

[00227] After Round 2, the pool was divided into two equal portions, one portion was used for subsequent rounds (*i.e.,* Rounds 3-12) of the h-IL-23 selection and the other portion was used for the subsequent rounds (*i.e.*, Rounds 3-11) of the PN-IL-23 selection. Round 1 of the X-IL-23 selection was conducted similarly, except the pool RNA was incubated with 50 pmoles of h-IL-23 and 50 pmoles of h-IL-12.

[00228] All selections were performed in 1X SHMCK buffer, pH 7.4 (20 mM Hepes pH 7.4, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$). RNA:h-IL-23 complexes and free RNA molecules were separated using 0.45 µm nitrocellulose spin columns from Schleicher & Schuell (Keene, NH). The columns were pre-washed with 1 mL 1X SHMCK, and then the RNA:protein containing solutions were added to the columns and spun in a centrifuge at 1500 g for 2 minutes. Buffer washes were performed to remove nonspecific binders from the filters (Round 1, 2 x 500 µL 1X SHMCK; in later rounds, more stringent washes of increased number and volume to enrich for specific binders), then the RNA:protein complexes attached to the filters were eluted with 2 x 200 µL washes (2 x 100 µL washes in later rounds) of elution buffer (7 M urea, 100 mM sodium acetate, 3 mM EDTA, pre-heated to 95°C). The eluted RNA was phenol:chloroform extracted, then precipitated (40 µg glycogen, 1 volume isopropanol). The RNA was reverse transcribed with the Thermoscript™ RT-PCR system (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, using the 3' primer 5'ttctcggttggtctctggcggagc 3' (SEQ ID NO 10), followed by amplification by PCR (20 mM Tris pH 8.4, 50 mM KCl, 2 mM $MgCl_2$, 0.5 µM of 5' primer

5'taatacgactcactatagggaaaagcgaatcatacacaaga 3' (SEQ ID NO 9), 0.5 μM of 3' primer (SEQ ID NO 10), 0.5 mM each dNTP, 0.05 units/μL Taq polymerase (New England Biolabs, Beverly, MA)). PCR reactions were done under the following cycling conditions: a) 94°C for 30 seconds; b) 55°C for 30 seconds; c) 72°C for 30 seconds. The cycles were repeated until sufficient PCR product was generated. The minimum number of cycles required to generate sufficient PCR product is reported in Tables 1-3 below as the "PCR Threshold".

[00229] The PCR templates were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA). Templates were transcribed using α^{32}P ATP body labeling overnight at 37°C (4% PEG-8000, 40 mM Tris pH 8.0, 12 mM $MgCl_2$, 1 mM spermidine, 0.002 % Triton X-100, 3 mM 2'OH purines, 3 mM 2'F pyrimidines, 25 mM DTT, 0.0025 units/μL inorganic pyrophosphatase, 2 μg/mL T7 Y639F single mutant RNA polymerase, 5 μCi α^{32}P ATP). The reactions were desalted using Bio Spin columns (Bio-Rad, Hercules, CA) according to the manufacturer's instructions.

[00230] Subsequent rounds of all three selections were repeated using the same method as for Round 1, except for the changes indicated in Tables 1-3. Prior to incubation with protein target, the pool RNA was passed through a 0.45 micron nitrocellulose filter column to remove filter binding sequences, then the filtrate was carried on into the positive selection step. In alternating rounds the pool RNA was gel purified. Transcription reactions were quenched with 50 mM EDTA and ethanol precipitated then purified on a 1.5 mm denaturing polyacrylamide gel (8 M urea, 10% acrylamide; 19:1 acrylamide:bisacrylamide). Pool RNA was removed from the gel by electroelution in an Elutrap® apparatus (Schleicher and Schuell, Keene, NH) at 225V for 1 hour in 1X TBE (90 mM Tris, 90 mM boric acid, 0.2 mM EDTA). The eluted material was precipitated by the addition of 300 mM sodium acetate and 2.5 volumes of ethanol.

[00231] The RNA remained in excess of the protein throughout the selections (~1-2 μM RNA). The protein concentration was 1 μM for the first 2 rounds, and then was dropped to varying lower concentrations based on the particular selection. Competitor tRNA was added to the binding reactions at 0.1 mg/mL starting at Round 3 or 4, depending on the selection. A total of 11-12 rounds were completed, with binding assays performed at select rounds. Tables 1-3 below contains the selection details used for the rRfY selections using the h-IL-23, X-IL-23, and PN-IL-23 selection strategies; including pool RNA concentration, protein concentration, and tRNA concentration used for each round. Elution values (ratio of CPM

values of protein-bound RNA versus total RNA flowing through the filter column) along with dot blot binding assays were used to monitor selection progress.

[00232] **Table 1.** Conditions used for h-IL-23 Selection

Round #	RNA pool conc (μM)	protein type	protein conc (μM)	tRNA conc (mg/mL)	neg	%elution	PCR Threshold
1	3.3	IL-23	1	0	none	4.38	10
2	~1	IL-23	1	0	NC	0.85	10
3	0.8	IL-23	0.75	0	NC	10.9	8
4	~1	IL-23	0.5	0.1	NC	0.53	8
5	1	IL-23	0.1	0.1	NC	1.72	11
6	~1	IL-23	0.1	0.1	NC	0.11	12
7	1	IL-23	0.1	0.1	NC	1.15	8
8	~0.5	IL-23	0.05	0.1	NC	0.12	11
9	0.5	IL-23	0.05	0.1	NC	3.54	8
10	~0.5	IL-23	0.05	0.1	NC	0.18	12
11	0.5	IL-23	0.025	0.1	NC	1.09	12
12	~0.5	IL-23	0.025	0.1	NC	0.07	12

[00233] **Table 2.** Conditions used for X-IL-23 Selection

Round #	RNA pool conc (μM)	protein type	protein conc (μM)	tRNA conc (mg/mL)	neg	%elution	PCR Threshold
1	3.3	IL-23/ IL-12	0.5 each	0	none	3.15	10
2	~1	IL-23/ IL-12	0.5 each	0	NC	0.56	10
3	0.8	IL-12	0.75	0	NC	0.58	13
4	~1	IL-23	0.75	0.1	NC	0.37	8
5	1	IL-12	0.5	0.1	NC	0.38	11
6	~1	IL-23	0.1	0.1	NC	0.08	12
7	1	IL-12	0.1	0.1	NC	0.50	9
8	~0.5	IL-23	0.05	0.1	NC	0.10	11

9	0.5	IL-12	0.05	0.1	NC	0.83	11
10	~0.5	IL-23	0.05	0.1	NC	0.17	8
11	0.5	IL-12	0.025	0.1	NC	0.91	12
12	~0.5	IL-23	0.025	0.1	NC	0.05	12

[00234] **Table 3.** Conditions used for PN-IL-23

Round #	RNA pool conc (μM)	protein type	protein conc (μM)	tRNA conc (mg/mL)	neg	neg IL-12 conc (μM)	%elution	PCR Threshold
1	3.3	IL-23	1	0	none	0	4.38	10
2	~1	IL-23	1	0	NC	0	0.85	10
3	0.8	IL-23	0.75	0.1	NC/IL-12	0.75	1.15	10
4	~1	IL-23	0.75	0.1	NC/IL-12	0.75	0.59	10
5	0.7	IL-23	0.5	0.1	NC/IL-12	0.5	4.19	10
6	~1	IL-23	0.1	0.1	NC/IL-12	0.5	0.05	14
7	1	IL-23	0.1	0.1	NC/IL-12	0.5	0.38	10
8	~1	IL-23	0.1	0.1	NC/IL-12	0.3	0.18	15
9	1	IL-23	0.1	0.1	NC/IL-12	0.5	2.81	8
10	~1	IL-23	0.05	0.1	NC/IL-12	0.5	0.21	10
11	~1	IL-23	0.05	0.1	NC/IL-12	0.5	1.35	12

[00235] Monitoring Progress of rRfY Selection. Dot blot binding assays were performed throughout the selections to monitor the protein binding affinity of the pools. Trace ^{32}P-labeled RNA was combined with a dilution series of h-IL-23 and incubated at room temperature for 30 minutes in 1X SHMCK (20 mM Hepes, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.4) plus 0.1 mg/mL tRNA for a final volume of 20 μL. The binding reactions were analyzed by nitrocellulose filtration using a Minifold I dot-blot, 96-well vacuum filtration manifold (Schleicher & Schuell, Keene, NH). A three-layer filtration medium was used, consisting (from top to bottom) of Protran nitrocellulose (Schleicher & Schuell), Hybond-P nylon (Amersham Biosciences) and GB002 gel blot paper (Schleicher & Schuell). RNA that is bound to protein is captured on the nitrocellulose filter, whereas the non-protein bound RNA is captured on the nylon filter. The gel blot paper was included

simply as a supporting medium for the other filters. Following filtration, the filter layers were separated, dried and exposed on a phosphor screen (Amersham Biosciences, Piscataway, NJ) and quantified using a Storm 860 Phosphorimager® blot imaging system (Amersham Biosciences).

[00236] When a significant positive ratio of binding of RNA in the presence of h-IL-23 versus in the absence of h-IL-23 was seen, the pools were cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. For the h-IL-23 and X-IL-23 selections, the Round 8 pool templates were cloned, and 32 individual clones from each selection were assayed in a 1-point dot blot screen (+/- 75 nM h-IL-23, as well as a separate screen at +/- 75 nM h-IL-12). For the PN-IL-23 selection, the Round 10 pool was cloned and sequenced, and 8 unique clones were assayed for protein binding in a 1-point dot blot screen (+/- 200 nM h-IL-23 and a separate screen at +/- 200nM h-IL-12). Subsequently, the Round 10 PN-IL-23 pool was re-cloned for further sequences, as well as the R12 PN-IL-23 pool, and the clones were assayed for protein binding in a 1 point do blot screen (+/- 100 nM h-IL-23 or +/- 200 nM h-IL-12). For K_D determination, the clone transcripts were 5'end labeled with γ^{32}P ATP. K_D values were determined using a dilution series of h-IL-23 (R&D Systems, Minneapolis, MN) in the dot blot assay for all unique sequences with good +/- h-IL-23 binding ratios in the initial screens, and fitting an equation describing a 1:1 RNA:protein complex to the resulting data (fraction aptamer bound = amplitude*([IL-23]/(K_D + [IL-23])) (KaleidaGraph v. 3.51, Synergy Software). Results of protein binding characterization are tabulated in Table 4. Clones with high affinity to h-IL-23 were prepped and screened for functionality in cell-based assays, described in Example 3 below.

[00237] **Table 4.** rRfY Clone binding activity (all measurements were made in the presence of 0.1 mg/mL tRNA)

Round 8 h-IL-23					**1-pt Screen Data**	
SEQ ID NO	**Clone Name**	**K_DIL-23 (nM)**	**K_D IL-12 (nM)**	**K_D IL-12/K_D IL-23**	**+/-IL-23 75 nM**	**+/-IL-12 75 nM**
15	AMX86-B5	195.5	N.B.		5.79	1.01
27	AMX86-C5	80.3	399.8	4.98	6.23	2.65
13	AMX86-D5	27.4	N.B.		7.17	1.52
16	AMX86-D6	25	N.B.		9.82	1.43
24	AMX86-E6	51.3	N.B.		9.02	1.13

22	AMX86-F6	69.1	N.B.		10.17	1.36
18	AMX86-A7	57.7	667.9	11.58	3.99	1.59
14	AMX86-B7	111	934.1	8.42	7.81	1.46
20	AMX86-C7	140.3	N.B.		4.65	0.77
19	AMX86-E7	210.2	267.5	1.27	6.79	1.23
21	AMX86-F7	147	106.4	0.72	13.07	2.49
25	AMX86-H7	89.8	N.B.		10.85	1.26
26	AMX86-C8	107.1	N.B.		5.28	1.17
23	AMX86-D8	294.2	N.B.		6.87	1.08
17	AMX86-G8	133.7	2493.1	18.65	7.26	2.05

Round 8 X-IL-23					**1-pt Screen Data**	
SEQ ID NO	**Clone Name**	**IL-23 K_D (nM)**	**IL-12 K_D (nM)**	**K_D IL-12/K_D IL-23**	**+/-IL-23 75 nM**	**+/-IL-12 75 nM**
41	AMX86-A9	190.5	N.B.		3.55	0.68
35	AMX86-B9	23.7	847.6	35.76	12.88	1.96
32	AMX86-C9	97.9	672.8	6.87	6.07	1.86
33	AMX86-G9	109.4	N.B.		10.03	1.04
39	AMX86-H9	104.6	331.5	3.17	10.35	3.66
34	AMX86-A10	460.9	289.4	0.63	6.64	1.40
28	AMX86-B10	77.8	1038.3	13.35	4.73	2.12
42	AMX86-E10	218.1	904.6	4.15	2.44	1.37
36	AMX86-G10	73.7	356.1	4.83	9.88	2.41
37	AMX86-A11	157.2	182.4	1.16	7.05	3.23
29	AMX86-B11	179.9	5950	33.07	9.23	1.69
30	AMX86-D11	198.9	113.9	0.57	10.26	2.59
38	AMX86-F11	255.64	540.6	2.11	7.33	2.87
40	AMX86-H11	366.9	214.9	0.59	7.56	3.02
31	AMX86-F12	423.7	2910.3	6.87	11.88	2.51

PN-IL-23 Clones					**1-pt Screen Data**		
SEQ ID NO	**Clone Name**	**PN-IL-23**	**IL-23 K_D(nM)**	**IL-12 K_D**	**+/-IL-23 200 nM**	**+/-IL-23 100 nM**	**+/-IL-12 200 nM**

		Round		(nM)			
43	AMX 84-A10	R10	22.3	N.B.	39.6		2.9
44	AMX 84-B10	R10	21.8	N.B.	22.7		1.3
45	AMX 84-A11	R10	17.8	N.B.	32.7		1.8
46	AMX 84-F11	R10	16.6	N.B.	22.5		0.8
47	AMX 84-E12	R10	27.8	N.B.	15.8		0.8
48	AMX 84-C10	R10	94.3	N.B.	17.7		2.2
49	AMX 84-C11	R10	15.5	286.1	23.4		2.7
50	AMX 84-G11	R10	290.7	N.B.	22.3		1.7
51	ARX33-plate1-H01	R12	77.8	N.B.		20.3	1.7
52	AMX 91-F11	R10	201.7	N.B.		11.4	2.2
53	AMX 91-G1	R10	82.3	N.B.		52.2	1.7
54	AMX 91-E3	R10	205.3	N.B.		34.4	2.9
55	AMX 91-H3	R10	265.7	N.B.		18.5	2.3
56	AMX 91-B5	R10	148.5	N.B.		11.2	0.9
57	AMX 91-A6	R10	60.3	N.B.		6.3	1.1
58	AMX 91-G7	R12	63.6	N.B.		38.1	1.9
59	AMX 91-H7	R12	71.0	N.B.		44.7	1.4
60	AMX 91-B8	R12	17.6	409.1		34.0	7.9
61	AMX 91-H8	R12	16.6	243.2		25.2	4.1
62	AMX 91-G9	R12	33.0	N.B.		31.7	1.1
63	AMX 91-D9	R12	44.6	N.B.		25.1	2.1
64	AMX 91-G11	R12	104.4	N.B.		12.5	1.7
65	AMX 91-C12	R12	30.7	N.B.		22.9	1.9
66	AMX 91-H12	R12	60.8	N.B.		48.6	1.2

N.B. = no significant binding observed

[00238] The nucleic acid sequences of the rRfY aptamers characterized in Table 5 are given below. The unique sequence of each aptamer below begins at nucleotide 25, immediately following the sequence GGGAAAAGCGAAUCAUACACAAGA (SEQ ID NO 11) and runs until it meets the 3'fixed nucleic acid sequence GCUCCGCCAGAGACCAACCGAGAA (SEQ ID NO 12).

[00239] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and represent the sequences that bind to IL-23 and/or IL-12 selected under rRfY SELEX™ conditions wherein the purines (A and G) are 2'-OH and the pyrimidines (U and C) are 2'-fluoro. Each of the sequences listed in Table 5 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00240] **Table 5.** rRfY Clone sequences from h-IL-23 Selection (Round 8), X-IL-23 Selection (round 8), PN-IL-23 Selection (Round10/12).

h-IL-23 Selection (Round 8)

SEQ ID NO 13 (AMX(86)-D5)
GGGAAAAGCGAAUCAUACACAAGAGAGGUAUGUGGUUUUGCGGAGCAACUCGUGUCAGCGGUCAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 14 (AMX(86)-B7)
GGGAAAAGCGAAUCAUACACAAGAAUGAAUUCCGUCCACGGGCGCCCGAUGAUGUCAGUUUUCGGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 15 (AMX(86)-B5)
GGGAAAAGCGAAUCAUACACAAGAUUAGUGCGUGUGUUGAAAGGGCUCAUAAUGUCAGUAUCGAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 16 (AMX(86)-D6)
GGGAAAAGCGAAUCAUACACAAGAUUAGGCGUCGUGACAAUAACUGGUCCACGAGCAUGUCAGUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 17 (AMX(86)-G8)
GGGAAAAGCGAAUCAUACACAAGAUGGAAGGCGAUCGUAGCAGUAACCCAAUGAUUGGGACCUAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 18 (AMX(86)-A7)
GGGAAAAGCGAAUCAUACACAAGAUCUCUUUGGCCGACGCAACAAUGCUCUUUUCCGACCUUGCGCUCCGCCAGAGACCA
ACCGAGAA

SEQ ID NO 19 (AMX(86)-E7)
GGGAAAAGCGAAUCCUACCCAAGAUGUUGUUGGCGUUGAUCGUAUGAUUNAUGGAGNGUGUCNGUGCUCCGCCAGAGAC
CAACCGAGAA

SEQ ID NO 20 (AMX(86)-C7)
GGGAAAAGCGAAUCAUACACAAGAUGCGCUAUGUUUGGCUGGGAAUUGUAGCAUUGCUCAAGUGGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 21 (AMX(86)-F7)
GGGAAAAGCGAAUCAUACACAAGAUGUUGAACCUCUUGUGCGUCCCGAUGUUUNGCAAUGUGGAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 22 (AMX(86)-F6)
GGGAAAAGCGAAUCAUACACAAGAAUGUAUACAAUGCCCUAUCGUCAGUUAGGCAUGUGUGGAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 23 (AMX(86)-D8)
GGGAAAAGCGAAUCAUACACAAGACAGAGGCAAUGAGAGCCUGGCGAUGUCAGUCGCAUCUUGCUGCUCCGCCAGAGAC
CAACCGAGAA

SEQ ID NO 24 (AMX(86)-E6)
GGGAAAAGCGAAUCAUACACAAGAUCGCAAAAGGAGUUUGUCUCUGCUCUCGGAGUGUGUCAGUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 25 (AMX(86)-H7)
GGGAAAAGCGAAUCAUACACAAGAGAUGACUACACGCCAGUGUGCGCUUUUUGCGGAGUUAGCGGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 26 (AMX(86)-C8)
GGGAAAAGCGAAUCAUACACAAGAGUCGUGAUGAUUUGGGUUAUGUCAGUUCCCUGUAUGGUUUCGCUCCGCCAGAGAC
CAACCGAGAA

SEQ ID NO 27 (AMX(86)-C5)
GGGAAAAGCGAAUCAUACACAAGAGUUUUAUGUGGGUCCCGAUGAUUAACUUUAUUGGCGCAUUGCUCCGCCAGAGACC
AACCGAGAA

X-IL-23 Selection (Round 8)

SEQ ID NO 28 (AMX(86)-B10)
GGGAAAAGCGAAUCAUACACAAGAGAACGAGUAUAUUUGCGCUGGCGGAGAAGUCUCUCGAAGGGAGCUCCGCCAGAGA
CCAACCGAGAA

SEQ ID NO 29 (AMX(86)-B11)
GGGAAAAGCGAAUCAUACACAAGAGUAUCAUUCGGCUGGUGGGAGAAAUCUCUGUAGAUAUAGAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 30 (AMX(86)-D11)
GGGAAAAGCGAAUCAUACACAAGAUAGCGUCUAUGAUGGCGGAGAAGCAAGUGUAGCAUAACAGGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 31 (AMX(86)-F12)
GGGAAAAGCGAAUCAUACACAAGAGUGUUGAAUGAGCGCUGGUGGACAGAUCUUUGGUUACAGAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 32 (AMX(86)-C9)
GGGAAAAGCGAAUCAUACACAAGACUCAUGGAUAUGGCCUAGCAGCCGUGGAAGCGGUCAUUCUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 33 (AMX(86)-G9)
GGGAAAAGCGAAUCAUACACAAGAUCCCAGCGGUACGUGAGUCUGUUAAAGGCCACCUAAUGUCGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 34 (AMX(86)-A10)
GGGAAAAGCGAAUCAUACACAAGAGUAAUGUGGGUCCCGAUGAUUCGCUGUGCGGCGUUUGUAGCUCCGCCAGAGACCA
ACCGAGAA

SEQ ID NO 35 (AMX(86)-B9)
GGGAAAAGCGAAUCAUACACAAGAGGUUGAGUACGACGGAGUCNUGGCUAACACGGAAACUAGAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 36 (AMX(86)-G10)
GGGAAAAGCGAAUCAUACACAAGAGUCAUGGCUUACAAUUGAAACAAGAGCUCGCGUGACACAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 37 (AMX(86)-A11)
GGGAAAAGCGAAUCAUACACAAGAACGGCUAGGCAUCAAUGGCCAGCAAAAAUAGUCGUGUAAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 38 (AMX(86)-F11)
GGGAAAAGCGAAUCAUACACAAGACCAUCGGACGAGGCGGGUCACCUUUUACGCUUUCGAGCUGGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 39 (AMX(86)-H9)
GGGAAAAGCGAAUCAUACACAAGAUGGUUCCCACGUGAAAGUGGCUAGCGAGUACCCCACUUAUGCUCCGCCAGAGACC
AACCAAGGG

SEQ ID NO 40 (AMX(86)-H11)
GGGAAAAGCGAAUCAUACACAAGAGCGCUUUAGCGGGUAUAGCACUUUUCAUCUAAUGAANCCGUAGCUCCGCCAGAGA
CCAACCGAGAA

SEQ ID NO 41 (AMX(86)-A9)
GGGAAAAGCGAAUCAUACACAAGAUCUACGAUUGUUCAGGUUUUUUGUACUCAACUAAAGGCGAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 42 (AMX(86)-E10)
GGGAAAAGCGAAUCAUACACAAGAUUGUCUCGGAUUGGUCACUCCCAUUUUUGUUCGCUUAACGGCUCCGCCAGAGACC
AACCGAGAA

PN-IL-23 Selection (Round 10 and 12)

SEQ ID NO 43 (AMX(84)-A10)
GGGAAAAGCGAAUCAUACACAAGAAGUUUUUUGUGCUCUGAGUACUCAGCGUCCGUAAGGGAUAUGCUCCGCCAGAGAC
CAACCGAGAA

SEQ ID NO 44 (AMX(84)-B10)
GGGAAAAGCGAAUCAUACACAAGAAGUGCUUCAUGCGGCAAACUGCAUGACGUCGAAUAGAUAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 45 (AMX(84)-A11)
GGGAAAAGCGAAUCAUACACAAGAGAGGUAUGUGGUUUUGCGGAGCAACUCGUGUCAGCGGUCAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 46 (AMX(84)-F11)
GGGAAAAGCGAAUCAUACACAAGAUGUGCUUGAGUUAAAUCUCAUCGUCCCCGUUUGGGGAUAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 47 (AMX(84)-E12)
GGGAAAAGCGAAUCAUACACAAGAAGUUUUUGUGCUCUGAGUACUCAGCGUCCGUAAGGGAUAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 48 (AMX(84)-C10)
GGGAAAAGCGAAUCAUACACAAGAGAUGUAUUCAGGCGGUCCGCAUUGAUGUCAGUUAUGCGUAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 49 (AMX(84)-C11)
GGGAAAAGCGAAUCAUACACAAGAAUGGUCGGAAUCUCUGGCGCCACGCUGAGUAUAGACGGAAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 50 (AMX(84)-G11)
GGGAAAAGCGAAUCAUACACAAGAGUGCUUCGUAUGUUGAAUACGACGUUCGCAGGACGAAUAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 51 (ARX33-plate1-H01)
AGGGAAAAGGAAUCAUACACAAGAUGUAUCAUCCGGUCGUACAAAAGCGCCACGGAACCAUUCGCUCCGCCAGANACCA
ACCGAGAA

SEQ ID NO 52 (AMX(91)-F11)
GGGAAAAGCGAAUCAUACACAAGACGCGUCAGGUCCACGCUGAAAUUUAUUUUCGGCAGUGUAAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 53 (AMX(91)-G1)
GGGAAAAGCGAAUCAUACACAAGAUAUGUGCCUGGGAUGGACGACAUCCCCUGUCUAAGGAUAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 54 (AMX(91)-E3)
GGGAAAAGCGAAUCAUACACAAGAUUACUCCGUUAGUGUCAGUUGACGGAGGGAGCGUACUAUUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 55 (AMX(91)-H3)
GGGAAAAGCGAAUCAUACACAAGACAUUGUGCUUUAUCACGUGGGUGAUAACGACGAAAGUUAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 56 (AMX(91)-B5)
GGGAAAAGCGAAUCAUACACAAGACAGUGUAUGAGGAAGAUUACUUCCAUUCCUGAGCGGUUUUCGCUCCGCCAGAGAC
CAACCGAGAA

SEQ ID NO 57 (AMX(91)-A6)
GGGAAAAGCGAAUCAUACACAAGAUUGGCAAUGUGACCUUCAACCCUUUUCCCGAUGAACAGUGGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 58 (AMX(91)-G7)
GGGAAAAGCGAAUCAUACACAAGACAUGACUGCAUGCUUCGGGAGUAUCUCGGUCCCGACGUUCGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 59 (AMX(91)-H7)
GGGAAAAGCGAAUCAUACACAAGACUUAUCGCCUCAAGGGGGGUAAUAAACCCAGCGUGUGCAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 60 (AMX(91)-B8)
GGGAAAAGCGAAUCAUACACAAGAAUCCUGGCUUCGCAUAGUGUAUGGGUAGUACGACAGCGCGUGCUCCGCCAGAGAC
CAACCGAGAA

SEQ ID NO 61 (AMX(91)-H8)
GGGAAAAGCGAAUCAUACACAAGAACGCAUAGUCGGAUUUACCGAUCAUUCUGUGCCUUCGUGACGCUCCGCCAGAGAC
CAACCGAGAA

SEQ ID NO 62 (AMX(91)-G9)

GGGAAAAGCGAAUCAUACACAAGAAUUGUGCUUACAACUUUCGUUGUACCGACGUGUCAGUUAUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 63 (AMX(91)-D9)
GGGAAAAGCGAAUCAUACACAAGAGUGUAUUACCCCCAACCCAGGGGGACCAUUCGCGUAACAAGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 64 (AMX(91)-G11)
GGGAAAAGCGAAUCAUACACAAGACUUAACAGUGCGGGGCGCAGUGUAUAGAUCCGCAAUGUGUGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 65 (AMX(91)-C12)
GGGAAAAGCGAAUCAUACACAAGACGAUAGUAUGACCUUUUGAAAGGCUUCCCGAGCGGUGUUCGCUCCGCCAGAGACC
AACCGAGAA

SEQ ID NO 66 (AMX(91)-H12)
GGGAAAAGCGAAUCAUACACAAGACGUGUGCUUUAUGUAAACCAUAACGUUCCAUAAGGAAUAUGCUCCGCCAGAGACC
AACCGAGAA

[00241] Those sequences having binding activity to the IL-23 target proteins as determined by the dot blot binding assay described above, and that were functional in cell based assays (described below in Example 3), were minimized (described below in Example 2).

EXAMPLE 1B: IL-23 Selections against human IL-23 with ribo/2'O-Me nucleotide containing pools

[00242] Two selections were performed to identify aptamers containing ribo/2'O-Methyl nucleotides. One selection used 2'O-Methyl A, C, and U and 2'OH G (rGmH), and the other selection used 2'-OMe C, U and 2'-OH G, A (rRmY). Both selections were direct selections against h-IL-23 which had been immobilized on a hydrophobic plate. No steps were taken to bias selection of aptamers specific for the p19 or p40 subdomains. Both selections yielded pools significantly enriched for h-IL-23 binding versus naïve, unselected pool. Individual clone sequences are reported herein, and h-IL-23 binding data is provided for selected individual clones.

[00243] Pool Preparation. A DNA template with the sequence 5'–GGGAGAGGAGAGAACGTTCTACN$_{30}$CGCTGTCGATCGATCGATCGATG–3' (ARC256) (SEQ ID NO 3) was synthesized using an ABI EXPEDITE™ DNA synthesizer, and deprotected by standard methods. The series of N's in the DNA template (SEQ ID NO 3) can be any combination of nucleotides and gives rise to the unique sequence region of the resulting aptamers.

[00244] The template was amplified with the 5' primer 5'-TAATACGACTCACTATAGGGAGAGGAGAGAACGTTCTAC-3' (SEQ ID NO 67) and 3' primer 5'-CATCGATCGATCGATCGACAGC-3' (SEQ ID NO 68) and then used as a template for *in vitro* transcription with Y639F single mutant T7 RNA polymerase. Transcriptions were done at 37° C overnight using 200 mM Hepes, 40 mM DTT, 2 mM spermidine, .01% Triton X-100, 10% PEG-8000, 5 mM $MgCl_2$, 1.5 mM $MnCl_2$, 500 μM NTPs, 500 μM GMP, 0.01 units/μL inorganic pyrophosphatase, and 2 μg/mL Y639F single mutant T7 polymerase. Two different compositions were transcribed, rGmH, and rRmY.

[00245] Selection. Each round of selection was initiated by immobilizing 20 pmoles of h-IL-23 to the surface of Nunc Maxisorp hydrophobic plates for 2 hours at room temperature in 100 μL of 1X Dulbecco's PBS (DPBS ($+Ca^{2+}$, Mg^{2+})). The supernatant was then removed and the wells were washed 4 times with 120 μL wash buffer (1X DPBS, 0.2% BSA, and 0.05% Tween-20). Pool RNA was heated to 90°C for 3 minutes and cooled to room temperature for 10 minutes to refold. In Round 1, a positive selection step was conducted. Briefly, 1 x 10^{14} molecules (0.2 nmoles) of pool RNA were incubated in 100 μL binding buffer (1X DPBS and 0.05% Tween-20) in the wells with immobilized protein target for 1 hour. The supernatant was then removed and the wells were washed 4 times with 120 μL wash buffer. In subsequent rounds a negative selection step was included. The pool RNA was also incubated for 30 minutes at room temperature in empty wells to remove any plastic binding sequences from the pool before the positive selection step. The number of washes was increased after Round 4 to increase stringency. In all cases, the pool RNA bound to immobilized h-IL-23 was reverse transcribed directly in the selection plate by the addition of RT mix (3' primer, (SEQ ID NO 68), and Thermoscript™ RT, (Invitrogen, Carlsbad, CA) followed by incubation at 65°C for 1 hour.

[00246] The resulting cDNA was used as a template for PCR using Taq polymerase (New England Biolabs, Beverly, MA). "Hot start" PCR conditions coupled with a 60°C annealing temperature were used to minimize primer-dimer formation. Amplified pool template DNA was desalted with a Centrisep column (Princeton Separations, Adelphia, NJ) according to the manufacturer's recommended conditions, and used to transcribe the pool RNA for the next round of selection. The transcribed pool was gel purified on a 10 % polyacrylamide gel every round. Table 6 shows the RNA concentration used per round of selection.

[00247] **Table 6.** RNA pool concentrations per round of selection.

Round	rRmY (pmoles pool used)	rGmH (pmoles pool used)
1	200	200
2	110	40
3	65	100
4	50	170
5	80	100
6	100	110
7	50	70
8	120	60
9	120	80
10	130	
11	110	

[00248] The selection progress was monitored using the dot blot sandwich filter binding assay as described in Example 1A. The 5'- ^{32}P-labeled pool RNA was refolded at 90°C for 3 minutes and cooled to room temperature for 10 minutes. Next, pool RNA (trace concentration) was incubated with h-IL-23 DPBS plus 0.1 mg/mL tRNA for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell). The percentage of pool RNA bound to the nitrocellulose was calculated and monitored approximately every 3 rounds with a single point screen (+/- 250 nM h-IL-23). Pool K_D measurements were measured using a titration of h-IL-23 protein (R&D, Minneapolis, MN) and the dot blot apparatus as described above.

[00249] The rRmY h-IL-23 selection was enriched for h-IL-23 binding vs. the naïve pool after 4 rounds of selection (data not shown). The selection stringency was increased and the selection was continued for 8 more rounds. At Round 9 the pool K_D was approximately 500 nM or higher. The rGmH selection was enriched over the naïve pool binding at Round 10. The pool K_D was also approximately 500 nM or higher. Figure 7 is a binding curve of rRmY and rGmH pool selection binding to h-IL-23. The pools were cloned using TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and individual sequences were generated and tested

for binding. A single point binding screen was initially performed on all crude rRmY clone transcriptions using a 1:200 dilution, +/- 200 nM IL-23, plus 0.1 mg/mL competitor tRNA. A 10 point screen was then performed on 24 of the rRmY clones which showed the best binding in the single point screen. The 10 point screen was performed using zero to 480 nM IL-23 in 3 fold serial dilutions. Binding curves were generated (KaleidaGraph v. 3.51, Synergy Software) and K_Ds were estimated by fitting the data to the equation: fraction RNA bound = amplitude*[h-IL-23]/K_D + [h-IL-23]). Table 7 below shows the sequence data for the rRmY selected aptamers that displayed binding affinity for h-IL-23. There was one group of 6 duplicate sequences and 4 pairs of 2 duplicate sequences out of the rRmY clones generated. Table 8 shows the binding characteristics of the rRmY clones thus tested. Clones were also tested from 48 crude rGmH clone transcriptions at a 1:200 dilution and 0.1 mg/mL tRNA was used as competitor. The average binding over background was only about 14%, whereas the average of the rRmY clones in the same assay was about 30%, with 10 clones higher than 40%. The sequences and binding characterization of the rGmH clones tested are not shown.

[00250] The nucleic acid sequences of the rRmY aptamers characterized in Table 7 are given below. The unique sequence of each aptamer in Table 7 begins at nucleotide 23, immediately following the sequence GGGAGAGGAGAGAACGUUCUAC (SEQ ID NO 69), and runs until it meets the 3'fixed nucleic acid sequence GCUGUCGAUCGAUCGAUCGAUG (SEQ ID NO 70).

[00251] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and represent the sequences of the aptamers that bind to IL-23 and/or IL-12 selected under rRmY SELEX™ conditions wherein the purines (A and G) are 2'-OH and the pyrimidines (U and C) are 2'-OMe. Each of the sequences listed in Table 7 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[0002] Table 7 – rRmY (Round 10) Sequences

SEQ ID NO 71
GGGAGAGGAGAGAACGUUCUACAAAUGAGAGCAGGCCGAAGAGGAGUCGCUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 72
GGGAGAGGAGAGAACGUUCUACAAAUGAGAGCAGGCCGAAAAGGAGUCGCUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 73
GGGAGAGGAGAGAACGUUCUACAAAUGAGAGCAGGCCGAAAAGGAGUCGCUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 74

GGGAGAGGAGAGAACGUUCUACGGUAAAGCAGGCUGACUGAAAGGUUGAAGUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 75
GGGAGAGGAGAGAACGUUCUACAGGUUAAGAGCAGGCUCAGGAAUGGAAGUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 76
GGGAGAGGAGAGAACGUUCUACAACAAAGCAGGCUCAUAGUAAUAUGGAAGUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 77
GGGAGAGGAGAGAACGUUCUACAACAAAGCAGGCUCAUAGUAAUAUGGAAGUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 78
GGGAGAGGAGAGAACGUUCUACAAAAGAGAGCAGGCCGAAAAGGAGUCGCUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 79
GGGAGAGGAGAGAACGUUCUACAAAAGGCAGGCUCAGGGGAUCACUGGAAGUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 80
GGGAGAGGAGAGAACGUUCUACAAGAUAUAAUUAAGGAUAAGUGCAAAGGAGACGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 81
GGGAGAGGAGAGAACGUUCUACGAAUGAGAGCAGGCCGAAAAGGAGUCGCUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 82
GGGAGAGGAGAGAACGUUCUACGAGAGGCAAGAGAGAGUCGCAUAAAAAAAGACGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 83
GGGAGAGGAGAGAACGUUCUACGCAGGCUGUCGUAGACAAACGAUGAAGUCGCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 84
GGGAGAGGAGAGAACGUUCUACGGAAAAAGAUAUGAAAGAAAGGAUUAAGAGACGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 85
GGGAGAGGAGAGAACGUUCUACGGAAGGNAACAANAGCACUGUUUGUGCAGGCGCUGUCGAUCNAUCNAUCNAUG

SEQ ID NO 86
GGGAGAGGAGAGAACGUUCUACUAAUGCAGGCUCAGUUACUACUGGAAGUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 87
AGGAGAGGAGAGAACGUUCUACUAGAAGCAGGCUCGAAUACAAUUCGGAAGUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 88
GGGAGAGGAGAGAACGUUCUACAUAAGCAGGCUCCGAUAGUAUUCGGGAAGUCGCUGUCGAUCGAUCGAUCGAU

[00252] **Table 8** – rRmY IL-23 Clone Binding Data.

SEQ	IL-23 K_D

ID No.	(nM)
72	211.4
83	8.2
86	219.3
80	3786.3
75	479.4
74	257.0
81	303.2
77	258.9
73	101.4
88	101.2
84	602.5
78	123.7
76	77.2
87	122.3
71	124.0
85	239.9
82	198.6
79	806.7

**Assays performed in 1X DPBS (+Ca^{2+}, Mg^{2+}), 30 min RT incubation
**R&D IL-23 (carrier free protein)

EXAMPLE 1C: Selections against human IL-23 with deoxy/2'O-Methyl nucleotide containing pools

[00253] An alternative selection was performed to obtain stabilized aptamers specific for IL-23 using deoxy purines (A and G) and 2'-O-Me pyrimidines (C and U) using the h-IL-23 strategy.

[00254] Pool Preparation. A DNA template with the sequence 5'–GGGAGAGGAGAGAACGTTCTACN_{30}CGCTGTCGATCGATCGATCGATG–3' (ARC256, SEQ ID NO 3) was synthesized using an ABI EXPEDITE™ DNA synthesizer, and deprotected by standard methods. The series of N's in the DNA template (SEQ ID NO 3) can be any combination of nucleotides and gives rise to the unique sequence region of the resulting aptamers. The templates were amplified with the 5' primer 5'-

TAATACGACTCACTATAGGGAGAGGAGAGAACGTTCTAC-3' (SEQ ID NO 67) and 3' primer 5'-CATCGATCGATCGATCGACAGC-3' (SEQ ID NO 89) and then used as a template for *in vitro* transcription with Y639F single mutant T7 RNA polymerase. Transcriptions were done at 37° C overnight using 200 mM Hepes, 40 mM DTT, 2 mM spermidine, 0.01% Triton X-100, 10% PEG-8000, 9.6 mM $MgCl_2$, 2.9 mM $MnCl_2$, 2 mM NTPs, 2 mM GMP, 2 mM spermine, 0.01 units/μL inorganic pyrophosphatase, and 2 μg/mL Y639F single mutant T7 polymerase.

[00255] Selection: Each round of selection was initiated by immobilizing 20 pmoles of h-IL-23 to the surface of Nunc Maxisorp hydrophobic plates for 1 hour at room temperature in 100 μL of 1X PBS. The supernatant was then removed and the wells were washed 5 times with 120 μL wash buffer (1X PBS, 0.1 mg/mL tRNA and 0.1 mg/mL salmon sperm DNA ("ssDNA")). In Round 1, a positive selection step was conducted: 100 pmoles of pool RNA (6×10^{13} unique molecules) were incubated in 100 μL binding buffer (1X PBS, 0.1 mg/mL tRNA and 0.1 mg/mL ssDNA) in the wells with immobilized protein target for 1 hour. The supernatant was then removed and the wells were washed 5 times with 120 μL wash buffer. In subsequent rounds a negative selection step was included. The pool RNA was also incubated for 1 hour at room temperature in empty wells to remove any plastic binding sequences from the pool before the positive selection step. Starting at Round 3, a second negative selection step was introduced. The target-immobilized wells were blocked for 1 hour at room temperature in 100 μL blocking buffer (1X PBS, 0.1 mg/mL tRNA, 0.1 mg/mL ssDNA and 0.1 mg/mL BSA) before the positive selection step. In all cases, the pool RNA bound to immobilized h-IL-23 was reverse transcribed directly in the selection plate after by the addition of RT mix (3' primer, (SEQ ID NO 89)), and Thermoscript™ RT (Invitrogen, Carlsbad, CA), followed by incubation at 65°C for 1 hour. The resulting cDNA was used as a template for PCR (Taq polymerase, New England Biolabs, Beverly, MA). "Hot start" PCR conditions coupled with a 68°C annealing temperature were used to minimize primer-dimer formation. Amplified pool template DNA was desalted with a Micro Bio-Spin column (Bio-Rad, Hercules, CA) according to the manufacturer's recommended conditions and used to program transcription of the pool RNA for the next round of selection. The transcribed pool was gel purified on a 10 % polyacrylamide gel every round.

[00256] Protein Binding Analysis. The selection progress was monitored using the sandwich filter binding assay previously described in Example 1A. The 5'- ^{32}P-labeled pool

RNA (trace concentration) was incubated with h-IL-23, 1X PBS plus 0.1 mg/mL tRNA, 0.1 mg/mL ssDNA and 0.1 mg/mL BSA for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell, Keene, NH). The percentage of pool RNA bound to the nitrocellulose was calculated after Rounds 6, 7 and 8 with a seven point screen with h-IL-23 (0.25 nM, 0.5 nM, 1 nM, 4 nM, 16 nM, 64 nM and 128 nM). Pool K_D measurements were calculated as previously described.

[00257] The dRmY IL-23 selection was enriched for h-IL-23 binding vs. the naïve pool after 6 rounds of selection. At Round 8 the pool K_D was approximately 54 nM or higher. The Round 6, 7 and 8 pools were cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and individual sequences were generated. Table 9 lists the sequences of the dRmY clones generated from Round 6, 7 and 8 pools. Protein binding analysis was performed for each clone. Binding assays were performed in 1X PBS +0.1 mg/mL tRNA, 0.1 mg/mL salmon sperm DNA, 0.1 mg/mL BSA, for a 30 minute incubation at room temperature. Table 10 includes the binding characterization for these individual sequences.

[00258] The nucleic acid sequences of the dRmY aptamers characterized in Table 9 are given below. The unique sequence of each aptamer below begins at nucleotide 23, immediately following the sequence GGGAGAGGAGAGAACGUUCUAC (SEQ ID NO 69), and runs until it meets the 3'fixed nucleic acid sequence GCUGUCGAUCGAUCGAUCGAUG (SEQ ID NO 90).

[00259] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and represent the sequences of the aptamers that bind to IL-23 and/or IL-12 selected under dRmY SELEX™ conditions wherein the purines (A and G) are deoxy and the pyrimidines (U and C) are 2'-OMe. Each of the sequences listed in Table 9 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00260] **Table 9.** dRmY IL-23 clone sequences

SEQ ID NO 91 (ARC 489)
GGGAGAGGAGAGAACGUUCUACAGCGCCGGUGGGCGGGCAUUGGGUGGAUGCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 92 (ARC 490)
GGGAGAGGAGAGAACGUUCUACAGCCUUUUGGGUAAGGGGAGGGGUGCCGGUCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 93
GGGAGAGGAGAGAACGUUCUACGUAACGGGGUGGGAGGGGCGAACAACUUGACGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 94 (ARC 491)
GGGAGAGGAGAGAACGUUCUACAGCGCCGGUGGGUGGGCAUAGGGUGGAUGCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 95
GGGAGAGGAGAGAACGUUCUACGGGCUACGGGGAUGGAGGGUGGGUCCCAGACGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 96
GGGAGAGGAGAGAACGUUCUACACGGGGUGGGAGGGGCGAGUCGCAUGGAUGCGCUGUCGAUCGAUCGAUCGAUG

SEQ ID NO 97 (ARC492)
GGGAGAGGAGAGAACGUUCUACUCAAUGACCGCGCGAGGCUCUGGGAGAG GGCGCUGUCGAUCGAUCGAUCGAUG

[00261] **Table 10** - dRmY IL-23 aptamer binding data

SEQ ID No.	IL-23 K_D (nM)	IL-12 K_D (nM)
91	4.0	17.2
92	26.0	37.1
93	186.2	Not tested
94	17.1	93.0
95	432.6	Not tested
96	209.7	Not tested
97	NB	NB

**Assays performed in 1X PBS + 0.1mg/mL tRNA, 0.1mg/mL ssDNA, 0.1mg/mL BSA, 30 min RT incubation
**R&D IL-23 (carrier free protein)
N.B.= no binding detectable

EXAMPLE 1D: Additional Selections against human IL-23 with deoxy/2'O-Methyl nucleotide containing pools

[00262] Introduction: Three selections strategies were used to identify aptamers to h-IL-23 using a pool containing deoxy/2'O-Methyl nucleotides. These selections used 2'O-Me C, and U and deoxy A and G. The first selection strategy (dRmY h-IL-23) was a direct selection against h-IL-23. In the second selection strategy (dRmY h-IL-23/IL-12neg), h-IL-12 was included in the negative selection step to drive enrichment of aptamers binding to p19, the subdomain unique to h-IL-23. In the third selection strategy (dRmY h-IL-23-S), increased stringency was used in the positive selection by including long washes to drive the selection to select for higher affinity aptamers. All three selection strategies yielded aptamers to h-IL-

23. Several aptamers are specific for h-IL-23, and several show cross reactivity between h-IL-23 and h-IL-12.

[00263] dRmY Selection: Round 1 of the dRmY h-IL-23 selection began with $3x10^{14}$ molecules of a 2'O-Me C, and U and deoxy A and G modified RNA pool with the sequence 5'-GGGAGAGGAGAGAACGUUCUAC-N30-GGUCGAUCGAUCGAUCAUCGAUG –3' (ARC520) (SEQ ID NO 98), which was synthesized using an ABI EXPEDITE™ DNA synthesizer, and deprotected by standard methods. The series of N's in the template (SEQ ID NO 98) can be any combination of nucleotides and gives rise to the unique sequence region of the resulting aptamers.

[00264] Each round of selection was initiated by immobilizing 20 pmoles of h-IL-23 to the surface of Nunc Maxisorp hydrophobic plates for 1 hour at room temperature in 100 µL of 1X PBS. The supernatant was then removed and the wells were washed 5 times with 120 µL wash buffer (1X PBS, 0.1 mg/mL tRNA and 0.1 mg/mL salmon sperm DNA ("ssDNA")). In Round 1, 500 pmoles of pool RNA ($3x10^{14}$ molecules) were incubated in 100 µL binding buffer (1X PBS, 0.1 mg/mL tRNA and 0.1 mg/mL ssDNA) in the well with immobilized protein target for 1 hour. The supernatant was then removed and the well was washed 5 times with 120 µL wash buffer. In subsequent rounds a negative selection step was included in which pool RNA was also incubated for 1 hour at room temperature in an empty well to remove any plastic binding sequences from the pool before the positive selection step.

[00265] Starting at Round 3, a second negative selection step was introduced. The pool was subjected to a 1 hour incubation in target-immobilized wells that were blocked for 1 hour at room temperature with 100 µL blocking buffer (1X PBS, 0.1 mg/mL tRNA, 0.1 mg/mL ssDNA and 0.1mg/mL BSA) before the positive selection step (Table 11A). At Round 3, the dRmY h-IL-23 pool was split into the dRmY h-IL-23/IL-12neg selection by subjecting the pool to an additional 1 hour negative incubation step at room temperature in a well that had been blocked for 1 hour at room temperature with 20 pmoles of h-IL-12 and washed 5 times with 120 µL wash buffer, which occurred prior to the positive h-IL-23 positive incubation. The pool was split into additional h-IL-12 blocked wells in later rounds to increase the stringency (See Table 11B).

[00266] An additional method to increase discrimination between h-IL-23 and h-IL-12 binding was to add h-IL-12 to the positive selection along with the pool at a low

concentration, in which the specific h-IL-23 binders would bind to the immobilized h-IL-23, and the h-IL-12 binders would be washed away after the 1 hour incubation. The dRmY h-IL-23-S selection was split from the dRmY h-IL-23 pool at Round 6 with the addition of "stringent washes" in the positive selection, in which after the 1 hour incubation with h-IL-23, the pool was removed, then 100 µL of 1X PBS, 0.1 mg/mL tRNA, and 0.1 mg/mL ssDNA was added and incubated for 30 minutes (Table 11C). This stringent wash procedure was removed and repeated, with the intentions of selecting for molecules with high affinities.

[00267] In all cases, the pool RNA bound to immobilized h-IL-23 was reverse transcribed directly in the selection plate by the addition of RT mix (3' primer, 5'-CATCGATGATCGATCGATCGAC-3' (SEQ ID NO 100)), and Thermoscript™ RT, (Invitrogen, Carlsbad, CA) followed by incubation at 65°C for 1 hour. The resulting cDNA was used as a template for PCR (20 mM Tris pH 8.4, 50 mM KCl, 2 mM $MgCl_2$, 0.5 µM of 5' primer 5'-TAATACGACTCACTATAGGGAGAGGAGAGAACGTTCTAC-3' (SEQ ID NO 99), 0.5 µM of 3' primer (SEQ ID NO 100), 0.5 mM each dNTP, 0.05 units/µL Taq polymerase (New England Biolabs, Beverly, MA)). PCR reactions were done under the following cycling conditions: a): 94°C for 30 seconds; b) 55°C for 30 seconds; c) 72°C for 30 seconds. The cycles were repeated until sufficient PCR product was generated. The minimum number of cycles required to generate sufficient PCR product is reported in Tables 11A-11C as the "PCR Threshold".

[00268] The PCR templates were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA) and used to program transcription of the pool RNA for the next round of selection. Templates were transcribed overnight at 37°C using 200 mM Hepes, 40 mM DTT, 2 mM spermidine, 0.01% Triton X-100, 10% PEG-8000, 9.6 mM $MgCl_2$, 2.9 mM $MnCl_2$, 2 mM NTPs, 2 mM GMP, 2 mM spermine, 0.01 units/µL inorganic pyrophosphatase, and 2 µg/mL Y639F single mutant T7 polymerase. Transcription reactions were quenched with 50 mM EDTA and ethanol precipitated, then purified on a 1.5 mm denaturing polyacrylamide gel (8 M urea, 10% acrylamide; 19:1 acrylamide:bisacrylamide). Pool RNA was removed from the gel by passive elution at 37°C in 300 mM NaOAc, 20 mM EDTA, followed by ethanol precipitation. The selection conditions for each round are provided in the following tables.

[00269] **Table 11A**: dRmY hIL-23 selection conditions

IL-23

Round #	RNA pool conc (μM)	IL-23 conc (μM)	untreated well neg	BSA-blocked well neg	PCR Threshold
1	5	0.2	none	none	18
2	0.6	0.2	1hr	none	17
3	0.75	0.2	1hr	1hr	17
4	1	0.2	1hr	1hr	17
5	0.75	0.2	1hr	1hr	17
6	1	0.2	1hr	1hr	15
7	1	0.2	1hr	1hr	15
8	1	0.2	1hr	1hr	16

[00270] **Table 11B**: dRmY IL-23/IL-12neg selection conditions

IL-23/12neg

Round #	RNA pool conc (μM)	IL-23 conc (μM)	untreated well neg	BSA-blocked well neg	IL-12 neg conc (μM)	# IL-12 wells	IL-12 pos conc (μM)	PCR Threshold
1	5	0.2	none	none	0	0	0	18
2	0.6	0.2	1hr	none	0	0	0	17
3	0.75	0.2	1hr	1hr	0.2	1	0	17
4	1	0.2	1hr	1hr	0.2	1	0	17
5	0.75	0.2	1hr	1hr	0.2	2	0	17
6	1	0.2	1hr	1hr	0.2	2	0	15
7	1	0.2	1hr	1hr	0.2	3	0.02	15
8	1	0.2	1hr	1hr	0.2	3	0.05	15

[00271] **Table 11C**: dRmY hIL-23-S selection conditions

IL-23S

Round #	RNA pool conc (μM)	IL-23 conc (μM)	untreated well neg	BSA-blocked well neg	# 30min positive washes	PCR Threshold

1	5	0.2	none	none	0	18
2	0.6	0.2	1hr	none	0	17
3	0.75	0.2	1hr	1hr	0	17
4	1	0.2	1hr	1hr	0	17
5	0.75	0.2	1hr	1hr	0	17
6	1	0.2	1hr	1hr	2	15
7	1	0.2	1hr	1hr	2	16
8	1	0.2	1hr	1hr	2	16

[00272] Protein Binding Analysis: Dot blot binding assays were performed throughout the selections to monitor the protein binding affinity of the pools as previously described in Example 1A. When a significant positive ratio of binding of RNA in the presence of h-IL-23 versus in the absence of h-IL-23 was seen, the pools were cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Similar sequences were seen in all three selections from the pools having gone through six rounds, and 45 unique clones amongst the three selections were chosen for screening. The 45 clones were synthesized on an ABI EXPEDITE™ DNA synthesizer, then deprotected by standard methods. The 45 individual clones were gel purified on a 10% PAGE gel, and the RNA was passively eluted in 300 mM NaOAc and 20 mM EDTA, followed by ethanol precipitation.

[00273] The clones were 5'end labeled with γ-^{32}P ATP, and were assayed for both IL-23 and IL-12 binding in a 3-point dot blot screen (0 nM, 20 nM, and 100 nM h-IL-23; 0 nM, 20 nM, and 100 nM h-IL-12) (data not shown). Clones showing significant binding in the 20 nM and 100 nM protein conditions for both IL-23 and IL-12 were further assayed for K_D determination using a protein titration from 0 nM to 480 nM (3 fold dilutions) in the dot blot assay previously described. K_D values were determined by fitting an equation describing a 1:1 RNA:protein complex to the resulting data (fraction aptamer bound = amplitude*([IL-23]/(K_D + [IL-23])) + background binding) (KaleidaGraph v. 3.51, Synergy Software). Results of protein binding characterization for the higher affinity clones are tabulated in Table 13, and corresponding clone sequences are listed in Table 12.

[00274] The nucleic acid sequences of the dRmY aptamers characterized in Table 12 are given below. The unique sequence of each aptamer below begins at nucleotide 23, immediately following the sequence GGGAGAGGAGAGAACGUUCUAC (SEQ ID NO

101), and runs until it meets the 3'fixed nucleic acid sequence GUCGAUCGAUCGAUCAUCGAUG (SEQ ID NO 102).

[00275] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and represent the sequences of the aptamers that bind to IL-23 and/or IL-12 selected under dRmY SELEX™ conditions wherein the purines (A and G) are deoxy and the pyrimidines (C and U) are 2'-OMe. Each of the sequences listed in Table 12 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00276] **Table 12**: dRmY clone sequences

SEQ ID NO 103 (ARC611)
GGGAGAGGAGAGAACGUUCUACAGGCAAGGCAAUUGGGGAGUGUGGGUGGGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 104 (ARC612)
GGGAGAGGAGAGAACGUUCUACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 105 (ARC614)
GGGAGAGGAGAGAACGUUCUACAAGGCGGUACGGGGAGUGUGGGUUGGGGCCGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 106 (ARC616)
GGGAGAGGAGAGAACGUUCUACGAUAUAGGCGGUACGGGGGGAGUGGGCUGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 107 (ARC620)
GGGAGAGGAGAGAACGUUCUACAGGAAAGGCGCUUGCGGGGGGUGAGGGAGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 108 (ARC621)
GGGAGAGGAGAGAACGUUCUACAGGCGGUUACGGGGGAUGCGGGUGGGACAGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 109 (ARC626)
GGGAGAGGAGAGAACGUUCUACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 110 (ARC627)
GGGAGAGGAGAGAACGUUCUACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 111 (ARC628)
GGGAGAGGAGAGAACGUUCUACAGGCAAGGCAAUUGGGGAGCGUGGGUGGGGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 112 (ARC632)
GGGAGAGGAGAGAACGUUCUACAAUUGCAGGUGGUGCCGGGGGUUGGGGGCGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 113 (ARC635)
GGGAGAGGAGAGAACGUUCUACAGGCUCAAAAGAGGGGGAUGUGGGAGGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 114 (ARC642)
GGGAGAGGAGAGAACGUUCUACAGGCGCAGCCAGCGGGGAGUGAGGGUGGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 115 (ARC643)
GGGAGAGGAGAGAACGUUCUACAGGCCGAUGAGGGGGAGCAGUGGGUGGGGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 116 ARC644)
GGGAGAGGAGAGAACGUUCUACUAGUGAGGCGGUAACGGGGGGUGAGGGUGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 117 (ARC645)
GGGAGAGGAGAGAACGUUCUACAGGUAGGCAAGAUAUUGGGGGAAGCGGGUGGGGUCGAUCGAUCGAUCAUCGAUG

SEQ ID NO 118 (ARC 646)
GGGAGAGGAGAGAACGUUCUACACAUGGCUCGAAAGAGGGGCGUGAGGGUGGGGUCGAUCGAUCGAUCAUCGAUG

[00277] **Table 13**: Summary of dRmY clone binding

SEQ ID NO	ARC #	Selection	K_D hIL-23 (nM)	K_D hIL-12 (nM)
103	ARC611	R7 hIL-23/12neg	21.3	123.1
104	ARC612	R7 hIL-23/12neg	5.8	41.7
105	ARC614	R7 hIL-23/12neg	3.1	54.4
106	ARC616	R7 hIL-23/12neg	13.1	52.1
107	ARC620	R7 hIL-23/12neg	44.8	178.7
108	ARC621	R7 hIL-23/12neg	28.8	111.9
109	ARC626	R7 hIL-23S	10.1	69.8
110	ARC627	R7 hIL-23S	7	79.5
111	ARC628	R7 hIL-23S	57.8	146.5
112	ARC632	R7 hIL-23S	19.1	63.9
113	ARC635	R7 hIL-23S	171.5	430.9
114	ARC642	R7 hIL-23	37.2	188.3
115	ARC643	R7 hIL-23S	71.6	309.4
116	ARC644	R7 hIL-23	34.5	192.9
117	ARC645	R7 hIL-23	33.5	137.3
118	ARC646	R7 hIL-23	207.9	382.6

*30 min RT incubation for K_D determination in dot blot assay
*1X PBS +0.1mg/mL tRNA, salmon sperm DNA, BSA reaction buffer

Human IL-23 Aptamer Selections Summary

[00278] The different selection conditions and strategies for IL-23 SELEX™ yielded several aptamers, stabilized and/or minimized, having different binding characteristics. The rRfY selected aptamers have affinities approximately in the 15 nM to 460 nM range, and prior to any post-SELEX™ optimization, have cellular potentcy with IC_{50}s approximately in the 50 nM-to 5 μM range. These can be further minimized with appropriate gains in binding characteristics and are expected to show increased potency in cell based assays. These aptamers also show the greatest distinction between IL-23, having a greater than hundred fold discrimination of IL-23 to IL-12.

[00279] The aptamers obtained under the rRmY selection conditions have affinities ranging from approximately 8 nM to 3 μM. However, their cellular potency is lower than the rRfY aptamers' potency. As for the rGmH constructs a single point screen was done, but not carried any further because their extent of binding over background was not as good as the rRmY clones. 48 crude rGmH clone transcriptions were used at a 1:200 dilution and 0.1 mg/mL tRNA was used as competitor. The average binding over background was only about 14%, whereas the rRmY clone's average in the same assay was about 30%, with 10 clones higher than 40 %.

[00280] The dRmY selected aptamers have high affinities in the range of ~3 nM to ~200 nM, and prior to any post-SELEX™ optimization, show a remarkable cellular potency with IC_{50}s in the range of ~50 nM to ~500 nM (described in Example 3 below). Some of these aptamers also have a distinction of approximately 4 fold for IL-23 to IL-12, which may be improved upon by further optimization.

EXAMPLE 1E: Selections against mouse ("m")-IL-23 with 2'-F pyrimidine containing pools (rRfY)

[00281] Introduction: Two selections strategies were used to identify aptamers to mIL-23 using a pool consisting of 2'-OH purine and 2'-F pyrimidine nucleotides (rRfY composition). The first selection strategy (mIL-23) was a direct selection against mIL-23. The second selection strategy (mIL-23S) was a more stringent selection, in which the initial rounds had lower concentrations of RNA and protein in an attempt to drive the selection towards higher affinity binders. Both selection strategies yielded aptamers to mIL-23.

[00282] Selection: Two selections (mIL-23 and mIL-23S) began with incubation of $2x10^{14}$ molecules of 2'F pyrimidine modified pool with the sequence 5' GGAGCGCACUCAGCCAC-N40-UUUCGACCUCUCUGCUAGC 3' (ARC275) (SEQ ID NO 119), including a spike of γ^{32}P ATP 5' end labeled pool, with mouse IL-23 (isolated in-house). The series of N's in the template (SEQ ID NO 119) can be any combination of nucleotides and gives rise to the unique sequence region of the resulting aptamers.

[00283] In Round 1 of the mIL-23 selection, pool RNA was incubated with 50 pmoles of protein in a final volume of 100 µL for 1 hr at room temperature. In Round 1 of the mIL-23S selection, pool RNA was incubated with 65 pmoles of mIL-23 in a final volume of 1300 µL for 1 hr at room temperature. Selections were performed in 1X PBS buffer. RNA:mIL-23 complexes and free RNA molecules were separated using 0.45 µm nitrocellulose spin columns from Schleicher & Schuell (Keene, NH). The columns were pre-washed with 1 mL 1X PBS, and then the RNA:protein containing solutions were added to the columns and spun in a centrifuge at 2000 rpm for 1 minute. Buffer washes were performed to remove nonspecific binders from the filters (Round 1, 2 x 500 µL 1X PBS; in later rounds, more stringent washes of increased number and volume to enrich for specific binders), then the RNA:protein complexes attached to the filters were eluted with 2 x 200 µL washes (2 x 100 µL washes in later rounds) of elution buffer (7 M urea, 100 mM sodium acetate, 3 mM EDTA, pre-heated to 90°C). The eluted RNA was precipitated (40 µg glycogen, 1 volume isopropanol). The RNA was reverse transcribed with the Thermoscript™ RT-PCR system (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, using the 3' primer 5'GCTAGCAGAGAGGTCGAAA 3' (SEQ ID NO 121), followed by PCR amplification (20 mM Tris pH 8.4, 50 mM KCl, 2 mM $MgCl_2$, 0.5µM of 5' primer 5'TAATACGACTCACTATAGGAGCGCACTCAGCCAC 3' (SEQ ID NO 120), 0.5 µM of 3' primer (SEQ ID 121), 0.5 mM each dNTP, 0.05 units/µL Taq polymerase (New England Biolabs, Beverly, MA)). PCR reactions were done under the following cycling conditions: a) 94°C for 30 seconds; b) 60°C for 30 seconds; c) 72°C for 30 seconds. The cycles were repeated until sufficient PCR product was generated. The minimum number of cycles required to generate sufficient PCR product is reported in Table14 as the "PCR Threshold".

[00284] The PCR templates were purified using the QIAquick PCR purification kit (Qiagen, Valencia, CA). Templates were transcribed using α^{32}P GTP body labeling overnight at 37°C (4% PEG-8000, 40 mM Tris pH 8.0, 12 mM $MgCl_2$, 1 mM spermidine,

0.002 % Triton X-100, 3 mM 2'OH purines, 3 mM 2'F pyrimidines, 25 mM DTT, 0.25 units/100 µL inorganic pyrophosphatase, 2 µg/mL T7 Y639F single mutant RNA polymerase, 5uCi α^{32}P GTP).

[00285] Subsequent rounds were repeated using the same method as for Round 1, but with the addition of a negative selection step. Prior to incubation with protein target, the pool RNA was passed through a 0.45 micron nitrocellulose filter column to remove filter binding sequences, then the filtrate was carried on into the positive selection step. In alternating rounds the pool RNA was gel purified. Transcription reactions were quenched with 50 mM EDTA and ethanol precipitated then purified on a 1.5 mm denaturing polyacrylamide gels (8 M urea, 10% acrylamide; 19:1 acrylamide:bisacrylamide). Pool RNA was removed from the gel by passive elution in 300 mM NaOAc, 20 mM EDTA, followed by ethanol precipitation with the addition of 300 mM sodium acetate and 2.5 volumes of ethanol.

[00286] The RNA remained in excess of the protein throughout the selections (~1 µM RNA). The protein concentration was dropped to varying lower concentrations based on the particular selection. Competitor tRNA was added to the binding reactions at 0.1 mg/mL starting at Round 2 or 3, depending on the selection. A total of 7 rounds were completed, with binding assays performed at select rounds. Table 14 contains the selection details including pool RNA concentration, protein concentration, and tRNA concentration used for each round. Elution values (ratio of CPM values of protein-bound RNA versus total RNA flowing through the filter column) along with binding assays were used to monitor selection progress.

[00287] **Table 14**: rRfY mIL-23 Selection conditions:

1. rRfY mIL-23

Round #	RNA pool conc (µM)	protein conc (nM)	neg	tRNA (mg/mL)	%elution	PCR Threshold
1	3.3	500	none	0	2.64	8
2	1	500	filter	0.1	4.24	8
3	~1	200	filter	0.1	0.73	10
4	1	200	filter	0.1	3.71	8
5	~1	100	filter	0.1	0.41	10

6	1	100	filter	0.1	9.27	8
7	~1	100	filter	0.1	0.87	9

2. rRfY mIL-23S (stringent)

Round #	RNA pool conc (μM)	protein conc (nM)	neg	tRNA (mg/mL)	%elution	PCR Threshold
1	0.25	50	none	0	2.79	8
2	0.1	50	filter	0	4.14	8
3	~1	50	filter	0.1	0.16	11
4	1	50	filter	0.1	2.57	8
5	~1	25	filter	0.1	0.42	10
6	0.8	25	filter	0.1	10.29	8
7	~1	25	filter	0.1	0.13	10

[00288] rRfY mIL-23 Protein Binding Analysis: Dot blot binding assays were performed throughout the selections to monitor the protein binding affinity of the pools as previously described. When a significant level of binding of RNA in the presence of mIL-23 was observed, the pools were cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. For both mIL-23 selections, the Round 7 pool templates were cloned, and 16 individual clones from each selection were assayed using an 8-point mIL-23 titration. Seven of the 32 total clones screened had specific binding curves and are listed below in Table 16. Table 15 lists the corresponding sequences. All others displayed nonspecific binding curves similar to the unselected naïve pool. Clones with high affinity to mIL-23 were subsequently screened for protein binding against mouse IL-12, human IL-23 and human IL-12 in the same manner.

[00289] The nucleic acid sequences of the rRfY aptamers characterized in Table 15 are given below. The unique sequence of each aptamer below begins at nucleotide 18, immediately following the sequence GGAGCGCACUCAGCCAC (SEQ ID NO 122), and runs until it meets the 3'fixed nucleic acid sequence UUUCGACCUCUCUGCUAGC (SEQ ID NO 123).

[00290] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and represent the sequences that bind to mouse IL-23 selected under rRfY SELEX™ conditions wherein the purines (A and G) are 2'-OH and the pyrimidines (C and U) are 2'-fluoro. Each of the sequences listed in Table 15 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00291] **Table 15**: mIL-23 rRfY Clone Sequences

SEQ ID NO 124 (ARC1628)
GGAGCGCACUCAGCCACAGGUGGCUUAAUACUGUAAAGACGUGCGCGCAGAGGGAUUUUCGACCUCUCUGCUAGC

SEQ ID NO 125 (ARC1629)
GGAGCGCACUCAGCCACCGUAAUUCACAAGGUCCCUGAGUGCAGGGUUGUAUGUUUGUUUCGACCUCUCUGCUAGC

SEQ ID NO 126 (ARC1630)
GGAGCGCACUCAGCCACUCUACUCGAUAUAGUUUAUCGAGCCGGUGGUAGAUUAUGAUUUCGACCUCUCUGCUAGC

SEQ ID NO 127 (ARC1631)
GGAGCGCACUCAGCCACGCCUACAAUUCACUGUGAUAUAUCGAAUUAUAGCCCUGGUUUCGACCUCUCUGCUAGC

SEQ ID NO 128 (ARC1632)
GGAGCGCACUCAGCCACCGGCUUAAUAUCCAAUAGGAACGUUCGCUCUGAGCAGGCGUUUCGACCUCUCUGCUAGC

SEQ ID NO 129 (ARC1633)
GGAGCGCACUCAGCCACAGCUCGGUGGCUUAAUAUCUAUGUGAACGUGCGCAACAGCUUUCGACCUCUCUGCUAGC

SEQ ID NO 130 (ARC1634)
GGAGCGCACUCAGCCACCUUGGGCUUAAUACCUAUCGGAUGUGCGCCUAGCACGGAAUUUCGACCUCUCUGCUAGC

[00292] **Table 16:** mIL-23 rRfY Clone binding activity

SEQ ID NO	Clone Name	Selection	K_D mIL-23 (nM)	K_D mIL-12 (nM)	K_D hIL-23 (nM)	K_D hIL-12 (nM)
124	ARC1628	R7 mIL-23	2	6	52	161
125	ARC1629	R7 mIL-23	34	103	31	75
126	ARC1630	R7 mIL-23S	14	18	65	239
127	ARC1631	R7 mIL-23S	33	72	39	69
128	ARC1632	R7 mIL-23S	13	16	91	186
129	ARC1633	R7 mIL-23S	17	44	79	195
130	ARC1634	R7 mIL-23S	3	29	39	63

*30min RT incubation for K_D determination
*1X PBS +0.1mg/mL BSA reaction buffer

EXAMPLE 1F: Selections for mouse IL-23 aptamers with specificity against mouse IL-12

[00293] Introduction. One selection was performed to identify aptamers to mouse-IL-23 (mIL-23) with specificity against mouse IL-12 (mIL-12). This selection was split off from the rRfY selection mIL-23S described in the above section starting at Round 3. This selection yielded aptamers to mIL-23 that had ~3-5-fold specificity over mIL-12.

mIL-23S/mIL-12 neg rRfY Selection. To obtain mouse IL-23 aptamers with specificity against mouse IL-12, mouse IL-12 was included in a negative selection, similar to the protein in negative (PN-IL-23) selection described above in Example 1A. The resultant RNA from Round 2 of the mIL-23S selection described in Example 1E above was used to start the R3PN mIL-23/12neg selection, in which mIL-12 was included in the negative step of selection. Nine rounds of selection were performed, with binding assays performed at select rounds. Table 17 summarizes the selection conditions including pool RNA concentration, protein concentration, and tRNA concentration used for each round. Elution values (ratio of CPM values of protein-bound RNA versus total RNA flowing through the filter column) along with binding assays were used to monitor selection progress.

[00294] **Table 17**: rRfY mIL-23S/mIL-12 neg Filter Selection Summary

Round #	RNA pool conc (μM)	protein conc (nM)	neg	tRNA (mg/mL)	neg mIL12 conc (nM)	%elution	PCR cycle #
1	0.25	50	none	0	0	2.79	8
2	0.1	50	filter	0	0	4.14	8
3	~1	500	filter/IL12	0.1	250	1.33	10
4	1	500	filter/IL12	0.1	500	1.68	8
5	1	250	filter/IL12	0.1	250	0.89	9
6	1	200	filter/IL12	0.1	200	1.47	8
7	1	150	filter/IL12	0.1	150	1.39	8
8	1	150	filter/IL12	0.1	150	3.73	8
9	1	150	filter/IL12	0.1	150	2.98	8

Selection buffer: 1X PBS
*1hr positive incubation

[00295] rRfY mIL-23S/mIL-12 neg Protein Binding Analysis. The dot blot binding assays previously described were performed throughout the selection to monitor the protein binding affinity of the pool. Trace ^{32}P-labeled RNA was combined with mIL-23 or mIL-12 and incubated at room temperature for 30 min in 1X PBS plus 0.1mg/mL BSA for a final volume of 30 μL. The reaction was added to a dot blot apparatus (Schleicher and Schuell Minifold-1 Dot Blot, Acrylic). Binding curves were generated as described in previous sections. When a significant level of binding of RNA in the presence of mIL-23 was observed, the pool was cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The Round 9 pool template was cloned, and 10 individual clones from the selection were assayed in an 8-point dot blot titration against mIL-23. Clones that bound significantly to mIL-23 were then screened for binding to mIL-12. Table 18 summarizes protein binding characterization of the binding clones. Four of the 10 total clones screened bound specifically to mIL-23 and mIL-12 at varying affinities. All other clones displayed nonspecific binding curves similar to the unselected naïve pool. The sequences for the four binding clones are listed in Table 19 below.

[00296] **Table 18**: rRfY mIL-23S/mIL-12 neg Clone binding activity

SEQ ID NO	Clone Name	K_D mIL-23 (nM)	K_D mIL-12 (nM)
131	AMX369.F1	63	165
132	AMX369.H1	23	194
133	AMX369.B2	49	252
134	AMX369.G3	106	261

*30min RT incubation for K_D determination
*1X PBS +0.1mg/mL BSA reaction buffer

[00297] The nucleic acid sequences of the rRfY aptamers characterized in Table 19 are given below. The unique sequence of each aptamer below begins at nucleotide 18, immediately following the sequence GGAGCGCACUCAGCCAC (SEQ ID NO 122), and runs until it meets the 3'fixed nucleic acid sequence UUUCGACCUCUCUGCUAGC (SEQ ID NO 123).

[00298] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and represent the sequences that bind to mouse IL-23 selected under rRfY SELEX™ conditions wherein the purines (A and G) are 2'-OH and the pyrimidines (U and C)

are 2'-fluoro. Each of the sequences listed in Table 19 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00299] **Table 19**: rRfY mIL-23S/mIL-12 neg Sequence Information

SEQ ID NO 131 (AMX(369)_F1)

GGAGCGCACUCAGCCACGGUUUACUUCCGUGGCAAUAUUGACCUCNCUCUAGACAGGUUUCGACCUCUCUGCUAGC

SEQ ID NO 132 (AMX(369)_H1) (ARC 1914)

GGAGCGCACUCAGCCACCUGGGAAAAUCUGGGUCCCUGAGUUCUAACAGCAGAGAUUUUUCGACCUCUCUGCUAGC

SEQ ID NO 133 (AMX(369)_B2)

GGAGCGCACUCNGCCACUUCGGAAUAUCGUUGUCUUCUGGGUGAGCAUGCGUUGAGGUUUCNACCUCUCUGCUAGC

SEQ ID NO 134 (AMX(369)_G3)

GGAGCGCACUCAGCCACUGGGGAACAUCUCAUGUCUCUGACCGCUCUUGCAGUAGAAUUUNGACCUCUCUGCUAGC

EXAMPLE 2: COMPOSITION AND SEQUENCE OPTIMIZATION AND SEQUENCES

EXAMPLE 2A: Minimization

[00300] Following a successful selection and following the determination of sequences of aptamers, in addition to determination of functionality *in vitro*, the sequences were minimized to obtain a shorter oligonucleotide sequence that retained binding specificity to its intended target but had improved binding characteristics, such as improved K_D and/or IC_{50}s.

Example 2A.1: Minimization of rRfY Clones:

[00301] The binding parent clones from the rRfY selection described in Example 1A fell into two principal families of aptamers, referred to as Type 1 and Type 2. Figure 8A and 8B show examples of the sequences and predicted secondary structure configurations of Type 1 and Type 2 aptamers. Figure 9A and 9B show the minimized aptamer sequences and predicted secondary structure configurations for Types 1 and 2.

[00302] On the basis of the IL-23 binding analysis described in Example 1 above and the cell based assay data described in Example 3 below, several Type 1 clones from the rRfY PN-IL-23 selection including AMX84-A10 (SEQ ID NO 43), AMX84-B10 (SEQ ID NO 44), and AMX84-F11 (SEQ ID NO 46) were chosen for further characterization. Minimized DNA construct oligonucleotides were transcribed, gel purified, and tested in dot blot assays for binding to h-IL-23.

[00303] The minimized clones A10min5 (SEQ ID NO 139), A10min6 (SEQ ID NO 140) were based on AMX84-A10 (SEQ ID NO 43), the minimzed clones B10min4 (SEQ ID NO 144), and B10min5 (SEQ ID NO 145) were based on AMX84-B10 (SEQ ID NO 44), and the

minimized clone F11min2 (SEQ ID NO 147), was based on AMX84-F11 (SEQ ID NO 46) (Figure 9A). The clones were 5'end labeled with γ-^{32}P ATP, and were assayed in dot blot assays for K_D determination using the same method as for the parent clones. All had significant protein binding (summarized in Table 21), and each was more potent than the respective parent clones from which they are derived when tested in cell based assays as discussed in Example 3 below.

[00304] Additionally, minimized constructs exemplifying Type1 and Type 2 aptamers were made and tested based on the concensus sequence of Type 1 and Type 2 aptamer sequence families. Type1.4 (SEQ ID NO 151) , and Type1.5 (SEQ ID NO 152) are two examples of such minimized constructs based on the Type 1 family sequence, which displayed high IL-23 binding affinity and the most potent activity in the cell based assay described in Example 3, as compared to the other Type 1 minimers described above.

[00305] The resulting rRfY minimers' sequences are listed in Table 20 below. Table 21 shows the minimer binding data for the minimers listed in Table 20.

[00306] For the minimized rRfY aptamers described in Table 20 below, the purines (A and G) are 2'-OH purines and the pyrimidines (C and U) are 2'-fluoro pyrimidines. Unless noted otherwise, the individual sequences are represented in the 5' to 3' orientation. Each of the sequences listed in Table 20 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00307] Table 20 – PN-IL-23 2' F (rRfY) Minimer Aptamer sequences.

SEQ ID NO 135 (A10.min1)
GGAGAUCAUACACAAGAAGUUUUUUGUGCUCUGAGUACUCAGCGUCCGUAAGGGAUCUCC

SEQ ID NO 136 (A10.min2)
GGAGUCUGAGUACUCAGCGUCCGUAAGGGAUAUGCUCCGCCAGACUCC

SEQ ID NO 137 (A10.min3)
GGAGUUACUCAGCGUCCGUAAGGGAUAUGCUCCGACUCC

SEQ ID NO 138 (A10.min4)
GGAGUCUGAGUACUCAGCGUCCCGAGAGGGGAUAUGCUCCGCCAGACUCC

SEQ ID NO 139 (A10.min5)
GGAGCAUACACAAGAAGUUUUUUGUGCUCUGAGUACUCAGCGUCCGUAAGGGAUAUGCUCC

SEQ ID NO 140 (A10.min6)
GGAGUACGCCGAAAGGCGCUCUGAGUACUCAGCGUCCGUAAGGGAUACUCC

SEQ ID NO 141 (B10.min1)
GGAGCGAAUCAUACACAAGAAGUGCUUCAUGCGGCAAACUGCAUGACGUCGAAUAGAUAUGCUCC

SEQ ID NO 142 (B10.min2)
GGAUCAUACACAAGAAGUGCUUCAUGCGGCAAACUGCAUGACGUCGAAUAGAUCC

SEQ ID NO 143 (B10.min3)
GGAUCAUACACAAGAAGUGCUUCACGAAAGUGACGUCGAAUAGAUCC

SEQ ID NO 144 (B10.min4)
GGAGCAUACACAAGAAGUGCUUCAUGCGGCAAACUGCAUGACGUCGAAUAGAUAUGCUCC

SEQ ID NO 145 (B10.MIN5)
GGAGUACACAAGAAGUGCUUCCGAAAGGACGUCGAAUAGAUACUCC

SEQ ID NO 146 (F11.min1)
GGUUAAAUCUCAUCGUCCCCGUUUGGGGAU

SEQ ID NO 147 (F11.min2)
GGACAUACACAAGAUGUGCUUGAGUUAAAUCUCAUCGUCCCCGUUUGGGGAUAUGUC

SEQ ID NO 148 (Type 1.1)
GGCAUACACGAGAGUGCUGUCGAAAGACUCGGCCGAGAGGCUAUGCC

SEQ ID NO 149 (Type1.2)
GGCAUACGCGAGAGCGCUGGCGAAAGCCUCGGCCGAGAGGCUAUGCC

SEQ ID NO 150 (Type1.3)
GGAUACCCGAGAGGGCUGGCGAAAGCCUCGGCGAGAGCUAUCC

SEQ ID NO 151 (Type1.4)
GGGUACGCCGAAAGGCGCUUCCGAAAGGACGUCCGUAAGGGAUACCC

SEQ ID NO 152 (Type1.5)
GGAGUACGCCGAAAGGCGCUUCCGAAAGGACGUCCGUAAGGGAUACUCC

SEQ ID NO 153 (Type 2.1)
GGAAUCAUACCGAGAGGUAUUACCCCGAAAGGGGACCAUUCC

SEQ ID NO 154 (D9.1)
GGAAUCAUACACAAGAGUGUAUUACCCCCAACCCAGGGGGACCAUUCC

SEQ ID NO 155 (C11.1)
GGAAGAAUGGUCGGAAUCUCUGGCGCCACGCUGAGUAUAGACGGAAGCUCCGCCAGA

SEQ ID NO 156 (C11.2)
GGAGGCGCCACGCUGAGUAUAGACGGAAGCUCCGCCUCC

SEQ ID NO 157 (C10.1)
GGACACAAGAGAUGUAUUCAGGCGGUCCGCAUUGAUGUCAGUUAUGCGUAGCUCCGCC

SEQ ID NO 158 (C10.2)
GGCGGUCCGCAUUGAUGUCAGUUAUGCGUAGCUCCGCC

[00308] **Table 21** – PN-IL-23 rRfY Minimer Binding data

SEQ ID No.	Clone Description	+/-IL-23 20 nM	+/-IL-23 100 nM	IL-23 K_D (nM)
135	A10min1	2.2	3.1	
136	A10min2	4.4	6.0	
137	A10min3	0.8	1.6	
138	A10min4	0.9	0.7	
146	F11min1	0.8	0.6	
147	F11min2	7.8	16.9	65
141	B10min1	7.5	33.9	
142	B10min2	1.3	1.6	
143	B10min3	0.6	0.8	
139	A10min5	12.8	40.9	57.8
140	A10min6	13.6	41.7	48.3
144	B10min4	39.4	122.1	36.4
145	B10min5	20.7	89.2	276.9
148	IL-23 Type 1.1	1.4	0.9	
149	IL-23 Type 1.2	0.8	0.7	
150	IL-23 Type 1.3	0.8	0.6	
153	IL-23 Type 2.1	1.7	5.2	
154	D9.1	1.2	3.9	
155	C11.1	1.0	3.5	
156	C11.2	1.1	2.3	
157	C10.1	1.4	4.4	
158	C10.2	1.4	1.5	
151	IL-23 Type 1.4	2.3	11.7	185.3
152	IL-23 Type 1.5	5.2	26.9	31.4

**Assays performed +0.1mg/mL tRNA, 30min RT incubation
**R&D IL-23 (carrier free protein)

Example 2A.2: Minimization of dRmY Selection 1:

[00309] Following the dRmY selection process for aptamers binding to IL-23 (described in Example 1C above) and determination of the oligonucleotide sequences, the sequences were systematically minimized to obtain shorter oligonucleotide sequences that retain the binding characteristics. On the basis of the IL-23 binding analysis described in Example 1A above and the cell based assay data described in Example 3 below, ARC489 (SEQ ID NO 91) (74mer) was chosen for further characterization. 3 minimized constructs based on clone ARC489 (SEQ ID NO 91) were designed and generated. The clones were 5'end labeled with γ–^{32}P ATP, and were assayed in dot blot assays for K_D determination using the same method as for the parent clones in 1X PBS +0.1 mg/mL tRNA, 0.1 mg/mL salmon sperm DNA, 0.1 mg/mL BSA, for a 30 minute incubation at room temperature. Table 22 shows the sequences for the minimized dRmY aptamers. Table 23 includes the binding data for the dRmY minimized aptamers. Only one minimized clone, ARC527 (SEQ ID NO 159), showed binding to IL-23. This clone was tested in the TransAM™ STAT3 activation assay described in Example 3 below, and showed a decrease in assay activity compared to its respective parent, ARC489 (SEQ ID NO 91).

[00310] For the minimized dRmY aptamers described in Table 22 below, the purines (A and G) are deoxy-purines and the pyrimidines (U and C) are 2'-OMe pyrimidines. Unless noted otherwise, the individual sequences are represented in the 5' to 3' orientation. Each of the sequences listed in Table 22 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00311] **Table 22:** Sequences of dRmY Minimized

SEQ ID NO 159 (ARC527)
ACAGCGCCGGUGGGCGGGCAUUGGGUGGAUGCGCUGU

SEQ ID NO 160 (ARC528)
GCGCCGGUGGGCGGGCACCGGGUGGAUGCGCC

SEQ ID NO 161 (ARC529) ACAGCGCCGGUGUUUUCAUUGGGUGGAUGCGCUGU

[00312] **Table 23**: Binding characterization of dRmY selection 1 minimers

SEQ ID NO	Clone Name	K_D (nM)

SEQ ID 159	ARC 527	12.6
SEQ ID 160	ARC 528	NB
SEQ ID 161	ARC 529	NB

**R&D IL-23 (carrier free protein)
N.B.= no binding detectable

Example 2A.3: Minimization of dRmY Selection 2:

[00313] Following the dRmY selection process for aptamers binding to IL-23 (described in Example 1D above) and determination of the oligonucleotide sequences, the sequences were systematically minimized to obtain shorter oligonucleotide sequences that retain the binding characteristics

[00314] Based on sequence analysis and visual inspection of the parent dRmY aptamer sequences described in Example 1D, it was hypothesized that the active conformation of dRmY h-IL-23 binding clones and their minimized constructs fold into a G-quartet structure (Figure 10). Analysis of the functional binding sequences revealed a pattern of G doubles consistent with a G quartet formation (Table 24). The sequences within the G quartet family fell into 2 subclasses, those with 3 base pairs in the 1st stem and those with 2. It has been reported that in much the same way that ethidium bromide fluorescence is increased upon binding to duplex RNA and DNA, that N-methylmesoporphyrin IX (NMM) fluorescence is increased upon binding to G-quartet structures (Arthanari *et al.*, Nucleic Acids Research, 26(16): 3724 (1996); Marathais *et al.*, Nucleic Acids Research, 28(9): 1969 (2000); Joyce *et al.*, Applied Spectroscopy, 58(7): 831 (2004)). Thus as shown in Figure 11, NMM fluorescence was used to confirm that ARC979 (SEQ ID NO 177) does in fact adopt a G-quartet structure. According to the literature protocols, 100 microliter reactions containing ~1 micromolar NMM and ~ 2 micromolar aptamer in Dulbecco's PBS containing magnesium and calcium were analyzed using a SpectraMax Gemini XS fluorescence plate reader. Fluorescence emission spectra were collected from 550 to 750 nm with and excitation wavelength of 405 nm. The G-quartet structure of the anti-thrombin DNA aptamer ARC183 (Macaya *et al.*, Proc. Natl. Acad. Sci., 90: 3745 (1993)) was used as a positive control in this experiment. ARC1346 is an aptamer of a similar size and nucleotide composition as ARC979 (SEQ ID NO 177) that is not predicted to have a G-quartet structure and was used as a negative control in the experiment. As can be seen in Figure 11, ARC183 and ARC979 (SEQ

ID NO 177) show a significant increase in NMM fluorescence relative to NMM alone while the negative control, ARC1346 does not.

[00315] Minimized constructs were synthesized on an ABI EXPEDITE™ DNA synthesizer, then deprotected by standard methods. The minimized clones were gel purified on a 10% PAGE gel, and the RNA was passively eluted in 300 mM NaOAc and 20 mM EDTA, followed by ethanol precipitation.

[00316] The clones were 5'end labeled with γ–^{32}P ATP, and were assayed in dot blot assays for K_D determination using the direct binding assay in which the aptamer was radio-labeled and held at a trace concentration (< 90 pM) while the concentration of IL-23 was varied, in 1X PBS with 0.1 mg/mL BSA, for a 30 minute incubation at room temperature. The fraction aptamer bound vs. [IL-23] was used to calculate the K_D by fitting the following equation to the data:

Fraction aptamer bound = amplitude*([IL-23]/(K_D + [IL-23])) + background binding.

[00317] Several of the minimized constructs from the dRmY Selection 2 were also assayed in a competition format in which cold aptamer was titrated and competed away trace ^{32}P ATP labeled aptamer In the competition assay, the [IL-23] was held constant, the [trace labeled aptamer] was held constant, and the [unlabeled aptamer] was varied. The K_D was calculated by fitting the following equation to the data:

Fraction aptamer bound = amplitude*([aptamer]/(K_D + [aptamer])) + background binding.

[00318] Minimers based upon the G quartet were functional binders, whereas minimers based on a folding algorithm that predicts stem loops (RNAstructure; D.H. Mathews, *et al.*, "Expanded Sequence Dependence of Thermodynamic Parameters Improves Prediction of RNA Secondary Structure". Journal of Molecular Biology, 288, 911-940, (1999)) and that did not contain the pattern of G doubles were non functional (ARC793 (SEQ ID NO 163)).

[00319] Table 25 below summarizes the minimized sequences and the parent clone from which they were derived, and Table 26 summarizes the binding characterization from direct binding assays (+/- tRNA) and competition binding assays for the minimized constructs tested.

[00320] **Table 24:** Alignment of functional clones. (only the regions within the G quartet are represented)

```
AMX(185)_C2 = arc 626   GG-CAA-G-TAA--TTG-GG-GAGTG-C--GG-GCGG-GG 28
AMX(185)_G3 = arc 627   GG-CAA-G-TAA--TTG-GG-GAGTG-C--GG-GCGG-GG 28
AMX(184)_H9 = arc 612   GG-CAA-G-TAA--TTG-GG-GAGTG-C--GG-GCGG-GG 28
AMX(184)_G9 = arc 611   GG-CAA-GGCAA--TTG-GG-GAGTG-T--GG-GTGG-GG 29
AMX(184)_G6 = arc 645   GG-CAA-GAT-A--TTG-GG-GGAAG-C--GG-GTGG-GG 28
AMX(185)_B2 = arc 628   GG-CAA-GGCAA--TTG-GG-GAGCG-T--GG-GTGG-GG 29
AMX(184)_A9 = arc 621   GG-CG--G-TTA---CG-GG-GGATG-C--GG-GTG--GG 25
AMX(184)_C4 = arc 644   GG-CG--G-TAA---CG-GG-GGGTG-A--GG-GTGG-GG 26
AMX(184)_F10 = arc 616  GG-CG--G-T-A---CG-GG-GGGAG-T--GG-GCTG-GG 25
AMX(184)_B11 = arc 614  GG-CG--G-T-A---CG-GG-GAGTG-T--GG-GTTG-GG 25

AMX(185)_A6 = arc 643   GG-CC--GATGA---GG-GG-GAGCAGT--GG-GTGG-GG 28
AMX(184)_A8 = arc 620   GG-CGC---TT---GCG-GG-GGGTG-A--GG-GAGG-GG 26
AMX(184)_H3 = arc 646   GG-CTC-GA-AA--GAG-GG-GCGTG-A--GG-GTGG-GG 28
AMX(185)_G5 = arc 635   GG-CTC-AA-AA--GAG-GG-GGATG-T--GG-GAGG-GG 28
AMX(184)_A4 = arc 642   GG-CGC-AGCCA--GCG-GG-GAGTG-A--GG-GTGG-GG 29
AMX(185)_D1 = arc 632   GG-TGG---T-G--CCG-GG-GGTTG----GG-GGCG-GG 25
```

[00321] The SEQ ID NOS for the clones listed in Table 24 are found in Table 12.

[00322] For the minimized dRmY aptamers described in Table 25 below, the purines (A and G) are deoxy-purines and the pyrimidines (C and U) are 2'-OMe pyrimidines. Unless noted otherwise, the individual sequences are represented in the 5' to 3' orientation. Each of the sequences listed in Table 25 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00323] **Table 25**: dRmY minimer sequences

SEQ ID NO	Parent Clone	Minimer	Minimized Sequence
162	ARC627	ARC792	GGCAAGUAAUUGGGGAGUGCGGGCGGGG
163	ARC614	ARC793	CUACAAGGCGGUACGGGGAGUGUGG
164	ARC614	ARC794	GGCGGUACGGGGAGUGUGGGUUGGGGCCGG
165	ARC616	ARC795	CGAUAUAGGCGGUACGGGGGGAGUGGGCUGGGGUCG
166	ARC626	ARC796	UAAUUGGGGAGUGCGGGCGGGGGGUCGAUCG
167	ARC626	ARC797	GGUGGGGAGUGCGGGCGGGGGGUCGCC
168	ARC627	ARC889	ACAGGCAAGGUAAUUGGGGAGUGCGGGCGGGGUGU
169	ARC627	ARC890	CCAGGCAAGGUAAUUGGGGAGUGCGGGCGGGGUGG
170	ARC627	ARC891	GGCAAGGUAAUUGGGAAGUGUGGGCGGGG
171	ARC627	ARC892	GGCAAGGUAAUUGGGUAGUGAGGGCGGGG
172	ARC627	ARC893	GGCAAGGUAAUUGGGGAGUGCGGGCUGGG

173	ARC627	ARC894	GGCAAGGUAAUUGGGAAGUGUGGGCUGGG
174	ARC627	ARC895	GGCAAGGUAAUUGGGUAGUGAGGGCUGGG
175	ARC627	ARC896	ACAGGCAAGGUAAUUGGGUAGUGAGGGCUGGGUGU
176	ARC627	ARC897	GAUGUUGGCAAGUAAUUGGGGAGUGCGGGCGGGGUUCAUC-3T
177	ARC627	ARC979	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
178	ARC627	ARC980	CCAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGG
179	ARC621	ARC981	GGCGGUUACGGGGGAUGCGGGUGGG
180	ARC621	ARC982	GGCGGUUACGGGGGAUGCGGGUGGGACAGG
181	ARC627	ARC1117	GGCAAGUAAUUGGGGAGUGCGGGCGG
182	ARC627	ARC1118	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGUGU
183	ARC614	ARC1119	GGCGGUACGGGGAGUGUGGGUUGGGGCC
184	ARC614	ARC1120	GGCGGUACGGGGAGUGUGGGCUGGGGCC
185	ARC614	ARC1121	GGUACGGGGAGUGUGGGUUGGG
186	ARC614	ARC1122	GGUACGGGGAGUGUGGGCUGGG
187	ARC614	ARC1123	GGCGGUACGGGGAGUGUGGGUUGGGCC
188	ARC614	ARC1124	GGCGGUACGGGGAGUGUGGGCUGGGCC
189	ARC614	ARC1125	GGUACGGGGAGUGUGGGUUGG
190	ARC614	ARC1126	GGUACGGGGAGUGUGGGCUGG
191	ARC616	ARC1127	GGCGGUACGGGGGGAGUGGGCUGGGGUC
192	ARC616	ARC1128	GGCGGUACGGGGGGAGUGGGCUGGGUC
193	ARC616	ARC1129	GGCGGUACGGGGAGAGUGGGCUGGGGUC
194	ARC616	ARC1130	GGUACGGGGGGAGUGGGCUGGG
195	ARC616	ARC1131	GGUACGGGGGGAGUGGGCUGG
196	ARC616	ARC1132	GGUACGGGGAGAGUGGGCUGGG
197	ARC616	ARC1170	GGCGGUACGGGGGGAGUGGGCUGGG
198	ARC614	ARC1171	GGCGGUACGGGGAGUGUGGGUUGGG

[00324] **Table 26**: protein binding characterization of dRmY minimers

SEQ ID NO	Minimer ARC#	K_D (+tRNA) nM	K_D (-tRNA) nM	K_D (competition) nM
162	ARC792	117		11

164	ARC794	69		14
165	ARC795	40		4
166	ARC796	106		
167	ARC797	50		
168	ARC889	115		
169	ARC890	114		
170	ARC891	177		
171	ARC892	255		
172	ARC893	2857		
173	ARC894	no binding		
174	ARC895	no binding		
175	ARC896	no binding		
176	ARC897	93		
177	ARC979	93	90	9
178	ARC980	139		
179	ARC981	no binding		
180	ARC982	no binding		
181	ARC1117	<parent clone		
182	ARC1118	<parent clone		
183	ARC1119	<parent clone		
184	ARC1120	<parent clone		
185	ARC1121	<parent clone		
186	ARC1122	<parent clone		
187	ARC1123	<parent clone		
188	ARC1124	<parent clone		
189	ARC1125	<parent clone		
190	ARC1126	<parent		

		clone		
191	ARC1127	<parent clone		
192	ARC1128	<parent clone		
193	ARC1129	<parent clone		
194	ARC1130	<parent clone		
195	ARC1131	<parent clone		
196	ARC1132	<parent clone		
197	ARC1170	no binding		
198	ARC1171	no binding		

[00325] The competitive binding data was re-analyzed in a saturation binding experiment where the concentration of ligand (aptamer) was varied and the concentration of receptor (IL-23) was held constant and the [bound aptamer] was plotted versus the [total input aptamer]. ARC979 (SEQ ID NO 177) was used in this analysis.

[00326] The [ARC979] bound saturated at ~ 1.7 nM (Figure 12), which suggested that the concentration of IL-23 that was competent to bind aptamer was 1 nM, or 2 % (1/50) of the input IL-23. The calculated K_D value was 8 nM, which agreed well with the value obtained by fitting the data represented in competition mode (8.7 nM).

[00327] When IL-12 competition binding data was subjected to the same analysis (Figure 13), the fraction active IL-12 was higher (10%), and the specificity of ARC979 for IL-23 vs. IL-12 (33-fold) was greater than what was predicted by the direct binding measurements (2 – 5 fold).

[00328] Subsequently, the direct binding assay was repeated for ARC979 using the binding reaction conditions described previously (1X PBS with 0.1 mg/mL BSA for 30 minute incubation at room temperature) and using different binding reaction conditions (1X Dulbecco's PBS (with Mg^{++} and Ca^{++}) with 0.1 mg/ mL BSA for 30 minutes at room temperature). In both, newly chemically synthesized aptamers were purified using denaturing polyacrylamide gel electrophoresis, 5'end labeled with γ–^{32}P ATP and were tested for direct

binding to full human IL-23. An 8 point protein titration was used in the dot blot binding assay (either {100 nM, 30 nM, 10 nM, 3 nM, 1 nM, 300 pM, 100 pM, 0 pM} or {10 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 10 pM, 0 pM}). K_D values were calculated by fitting the equation y= (max/(1+K/protein))+yint using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). The buffer conditions appeared to affect the binding affinity somewhat. Under the 1X PBS condition, the K_D value for ARC979 was calculated to be ~ 10 nM, whereas under the 1X Dulbecco's PBS condition, the K_D value for ARC979 was calculated to be ~1 nM. (see Figure 14). These K_D values were verified in subsequent assays (data not shown), and are consistent with the IC_{50} value of ~ 6 nM that ARC979 yields in the PHA Blast assay described below in Example 3D.

Example 2A.4: Mouse IL-23 rRfY Minimization

[00329] Based on visual inspection of the parent clone sequences of the mouse IL-23 rRfY aptamers described in Example 1E, and predicted RNA structures using an RNA folding program (RNAstructure), minimized constructs were designed for each of the seven binding mIL-23 clones. PCR templates for the minimized construct oligos were ordered from Integrated DNA Technologies (Coraville, IA). Constructs were PCR amplified, transcribed, gel purified, and tested for binding to mIL-23 using the dot blot binding assay previously described. Trace ^{32}P-labeled RNA was combined with mIL-23 and incubated at room temperature for 30 min in 1X PBS plus 0.1 mg/mL BSA for a final volume of 30 μL. The reaction was added to a dot blot apparatus (Schleicher and Schuell Minifold-1 Dot Blot, Acrylic). Binding curves were generated as described in previous sections. Table 32 lists the sequences of the mIL-23 binding minimized constructs. Table 33 summarizes the protein binding characterization for each rRfY minimized construct that had significant binding to mIL-23.

[00330] Unless noted otherwise, individual sequences listed below are represented in the 5' to 3' orientation and represent the sequences that bind to mouse IL-23 selected under rRfY SELEX™ conditions wherein the purines (A and G) are 2'-OH and the pyrimidines (U and C) are 2'-fluoro. Each of the sequences listed in Table 32 may be derivatized with polyalkylene glycol ("PAG") moieties and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00331] **Table 32** minimized mouse rRfY clone sequences

SEQ ID NO 199 (ARC 1739)

GGGCACUCAGCCACAGGUGGCUUAAUACUGUAAAGACGUGCCC

SEQ ID NO 200 (ARC 1918)

GGAGCGCACUCAGCCACCGGCUUAAUAUCCAAUAGGAACGUUCGCUCU

SEQ ID NO 201

GGGCACUCAGCCACAGCUCGGUGGCUUAAUAUCUAUGUGAACGUGCCC

SEQ ID NO 202

GGGCACUCAGCCACCUUGGGCUUAAUACCUAUCGGAUGUGCCC

[00332] **Table 33:** mIL-23 rRfY Clone K_D Summary

Minimized Clone SEQ ID NO	Parent Clone Name	Parent Clone SEQ ID NO	K_D mIL-23 (nM)
199	ARC1628	124	1
200	ARC1632	128	1
201	ARC1633	129	25
202	ARC1634	130	19

*30min RT incubation for K_D determination
*1X PBS +0.1mg/mL BSA reaction buffer

EXAMPLE 2B: Optimization through Medicinal Chemistry

[00333] Aptamer Medicinal Chemistry is an aptamer improvement technique in which sets of variant aptamers are chemically synthesized. These sets of variants typically differ from the parent aptamer by the introduction of a single substituent, and differ from each other by the location of this substituent. These variants are then compared to each other and to the parent. Improvements in characteristics may be profound enough that the inclusion of a single substituent may be all that is necessary to achieve a particular therapeutic criterion.

[00334] Alternatively the information gleaned from the set of single variants may be used to design further sets of variants in which more than one substituent is introduced simultaneously. In one design strategy, all of the single substituent variants are ranked, the top 4 are chosen and all possible double (6), triple (4) and quadruple (1) combinations of these 4 single substituent variants are synthesized and assayed. In a second design strategy, the best single substituent variant is considered to be the new parent and all possible double

substituent variants that include this highest-ranked single substituent variant are synthesized and assayed. Other strategies may be used, and these strategies may be applied repeatedly such that the number of substituents is gradually increased while continuing to identify further-improved variants.

[00335] Aptamer Medicinal Chemistry is most valuable as a method to explore the local, rather than the global, introduction of substituents. Because aptamers are discovered within libraries that are generated by transcription, any substituents that are introduced during the SELEX™ process must be introduced globally. For example, if it is desired to introduce phosphorothioate linkages between nucleotides then they can only be introduced at every A (or every G, C, T, U etc.) (globally substituted). Aptamers which require phosphorothioates at some As (or some G, C, T, U etc.) (locally substituted) but cannot tolerate it at other As cannot be readily discovered by this process.

[00336] The kinds of substituent that can be utilized by the Aptamer Medicinal Chemistry process are only limited by the ability to generate them as solid-phase synthesis reagents and introduce them into an oligomer synthesis scheme. The process is certainly not limited to nucleotides alone. Aptamer Medicinal Chemistry schemes may include substituents that introduce steric bulk, hydrophobicity, hydrophilicity, lipophilicity, lipophobicity, positive charge, negative charge, neutral charge, zwitterions, polarizability, nuclease-resistance, conformational rigidity, conformational flexibility, protein-binding characteristics, mass etc. Aptamer Medicinal Chemistry schemes may include base-modifications, sugar-modifications or phosphodiester linkage-modifications.

[00337] When considering the kinds of substituents that are likely to be beneficial within the context of a therapeutic aptamer, it may be desirable to introduce substitutions that fall into one or more of the following categories:

(1) Substituents already present in the body, *e.g.*, 2'-deoxy, 2'-ribo, 2'-O-methyl purines or pyrimidines or 5-methyl cytosine.
(2) Substituents already part of an approved therapeutic, *e.g.*, phosphorothioate-linked oligonucleotides.
(3) Substituents that hydrolyze or degrade to one of the above two categories, *e.g.*, methylphosphonate-linked oligonucleotides.

Example 2B.1: Optimization of ARC979 by Phosphorothioate substitution.

[00338] ARC979 (SEQ ID NO 177) is a 34 nucleotide aptamer to IL-23 of dRmY composition. 21 phosphorothioate derivatives of ARC979 were designed and synthesized in which single phosphorothioate substitutions were made at each phosphate linkage (ARC1149 to ARC1169) (SEQ ID NO 203 to SEQ ID NO 223) (see Table 27). These molecules were gel purified and assayed for IL-23 binding using the dot blot assay as described above and compared to each other and to the parent molecule, ARC979. An 8 point IL-23 titration (0 nM to 300 nM, 3 fold serial dilutions) was used in the binding assay. Calculated K_Ds are summarized in Table 28.

[00339] The inclusion of phosphorothioate linkages in ARC979 was well tolerated when compared to ARC979. Many of these constructs have an increased proportion binding to IL-23 and additionally have improved (*i.e.*, lower) K_D values (Figure 15). A similar increase in affinity is seen in competition assays (Figure 16), which further supports that the phosphorothioate derivatives of ARC979 compete for IL-23 at a higher affinity than ARC979.

[00340] Unless noted otherwise, each of the sequences listed in Table 27 below are in the 5'-3' direction, may be derivatized with polyalkylene glycol ("PAG") moieties, and may or may not contain capping (*e.g.*, a 3'-inverted dT).

[00341] Table 27: Sequences of ARC979 phosphorothioate derivatives: Single Phosphorothioate substitutions

SEQ ID NO	ARC#	Phosphorothiote linker between bases (x,y)		Sequence
203	ARC1149	1	2	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
204	ARC1150	2	3	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
205	ARC1151	6	7	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
206	ARC1152	7	8	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
207	ARC1153	8	9	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
208	ARC1154	9	10	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
209	ARC1155	10	11	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
210	ARC1156	11	12	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU

211	ARC1157	12	13	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
212	ARC1158	13	14	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
213	ARC1159	14	15	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
214	ARC1160	18	19	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
215	ARC1161	19	20	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
216	ARC1162	20	21	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
217	ARC1163	21	22	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
218	ARC1164	22	23	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
219	ARC1165	26	27	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
220	ARC1166	27	28	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
221	ARC1167	28	29	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
222	ARC1168	32	33	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU
223	ARC1169	33	34	ACAGGCAAGUAAUUGGGGAGUGCGGGCGGGGUGU

[00342] **Table 28:** K_D summary for ARC979 phopsphorothioate derivatives

SEQ ID NO	ARC#	K_D (+tRNA) nM	K_D (-tRNA) nM	K_D (competition) nM
177	ARC979	93	90	9
203	ARC1149	not tested		
204	ARC1150	not tested		
205	ARC1151		142	
206	ARC1152		232	
207	ARC1153		174	
208	ARC1154		412	
209	ARC1155		168	
210	ARC1156		369	
211	ARC1157		69	
212	ARC1158		192	

213	ARC1159		77	
214	ARC1160		38	5
215	ARC1161		55	6
216	ARC1162		47	6
217	ARC1163		49	8
218	ARC1164		79	
219	ARC1165		55	
220	ARC1166		132	
221	ARC1167		107	
222	ARC1168		82	
223	ARC1169		74	

Example 2B.2: Optimization: 2'-OMe, phosphorothioate and Inosine substitutions

[00343] Systematic modifications were made to ARC979 (SEQ ID NO 177) to increase overall stability and plasma nuclease resistance. The most stable and potent variant of ARC979 was identified through a systematic synthetic approach involving 4 phases of aptamer synthesis, purification and assay for binding activity. The first step in the process was the synthesis and assay for binding activity of ARC1386 (SEQ ID NO 224) (ARC979 with a 3'-inverted-dT). Once ARC1386 (SEQ ID NO 224) was shown to bind to IL-23 with an affinity similar to that of the parent molecule ARC979 (SEQ ID NO 177), all subsequent derivatives of ARC979 were synthesized with a stabilizing 3'-inverted-dT.

[00344] The dot blot binding assay previously described was used to characterize the relative potency of the majority of the aptamers synthesized. For K_D determination, chemically synthesized aptamers were purified using denaturing polyacrylamide gel electrophoresis, 5'end labeled with γ–^{32}P ATP and were tested for direct binding to full human IL-23. An 8 point protein titration was used in the dot blot binding assay (either {100 nM, 30 nM, 10 nM, 3 nM, 1 nM, 300 pM, 100 pM, 0 pM} or {10 nM, 3 nM, 1 nM, 300 pM, 100 pM, 30 pM, 10 pM, 0 pM}) in Dulbecco's PBS (with Mg^{++} and Ca^{++}) with 0.1 mg/ mL BSA. K_D values were calculated by fitting the equation y= (max/(1+K/protein))+yint using KaleidaGraph (KaleidaGraph v. 3.51, Synergy Software). Sequences of the ARC979 derivatives synthesized, purified and assayed for binding to IL-23 as well as the results of the protein binding characterization are tabulated below in Tables 29 and 30. As can be seen in

Table 30, and as previously described in Example 2A.3 above, ARC1386 (SEQ ID NO 224) (which is ARC979 (SEQ ID NO 177) with a 3' inverted dT) has a K_D of 1 nM under these conditions.

[00345] In phase 1 of the optimization process, comprised of ARC1427-ARC1471 (SEQ ID NOs 225-269), each individual purine residue in ARC1386 (SEQ ID NO 224) was replaced by the corresponding 2'-O methyl containing residue. Additionally in phase 1, a series of individual and composite phosphorothioate substitutions were tested based on results generated previously which had suggested that in addition to conferring nuclease stability, phosphorothioate substitutions enhanced the binding affinity of derivatives of ARC979. Finally at the end of phase 1, a series of aptamers were tested that explored further the role of stem 1 in the functional context of ARC979/ARC1386. As seen from the binding data in Table 30, many positions readily tolerated substitution of a deoxy residue for a 2'-O methyl residue. Addition of any particular phosphorothioate did not appear to confer a significant enhancement in the affinity of the aptamers. Interestingly, as can be seen by comparison of ARC1465-1471 (SEQ ID NOs 263-269), stem 1 was important for maintenance of high affinity binding, however its role appeared to be a structural clamp since introduction of PEG spacers between the aptamer core and the 2 strands that comprise stem 1 did not appear to significantly impact the binding properties of the aptamers.

[00346] Based upon the structure activity relationship (SAR) results of the from phase 1 of the optimization process, a second series of aptamers were designed, synthesized, purified and tested for binding to IL-23. In phase 2 optimization, comprised of ARC1539-ARC1545 (SEQ ID NOs 270-276), the data from phase 1 was used to generate more highly modified composite molecules using exclusively 2'-O methyl substitutions. For these and all subsequent molecules, the goal was to identify molecules that retained an affinity (K_D) of ~ 2 nM or better as well as an extent of binding at 100 nM (or 10 nM in phases 3 and 4) IL-23 of at least 50%. The best of these in terms of simple binding affinity was ARC1544 (SEQ ID NO 275).

[00347] In phase 3 of optimization, comprised of ARC1591-ARC1626 (SEQ ID NOs 277-312), the stability of the G-quartet structure of ARC979 (SEQ ID NO 177) was probed by assaying for IL-23 binding during systematic replacement of (deoxy guanosine) dG with deoxy inosine (dI). Since deoxy inosine lacks the exocyclic amine found in deoxy guanosine, a single amino to N7 hydrogen bond is removed from a potential G-quartet for each dG to dI

substitution. As seen from the data, only significant substitutions lead to substantial decreases in affinity for IL-23 suggesting that the aptamer structure is robust. Additionally, the addition of phosphorothioate containing residues into the ARC1544 (SEQ ID NO 275) context was evaluated (comprising ARC1620 to ARC1626 (SEQ ID NOs 306-312). As can be seen in Table 30 the affinities of ARC1620-1626 (SEQ ID NOs 306-312) were in fact improved relative to ARC979 (SEQ ID NO 177). Figure 17 depicts the binding curves for select ARC979 derivatives (ARC1624 and ARC1625) from the phase 3 optimization efforts, showing the remarkably improved binding affinities conferred by the inclusion of select phosphorothioate containing residues, compared to the parent molecule ARC979.

[00348] Phase 4 of optimization, comprised of ARC1755-1756 (SEQ ID NOs 313-314), involved only 2 sequences in an attempt to introduce more deoxy to 2'-O methyl substitutions and retain affinity. As can be seen with ARC1755 and 1756, these experiments were successful.

[00349] Unless noted otherwise, each of the sequences listed in Table 29 are in the 5' to 3' direction and may be derivatized with polyalkylene glycol ("PAG") moieties.

[00350] **Table 29**: Sequence information Phase 1-4 ARC979 optimization

SEQ ID NO	ARC #	Description	Sequence (5' -> 3'), (3T = inv dT), (T=dT), (s=phosphorothioate), (mN = 2'-O Methyl containing residue) (dI = deoxy inosine containing residue)
224	ARC1386	ARC 979 with 3'-inv dT	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
225	ARC1427	ARC979 opt phase 1	mAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
226	ARC1428	ARC979 opt phase 1	dAmCmAdGdGmCdAdAdGmUdAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
227	ARC1429	ARC979 opt phase 1	dAmCdAmGdGmCdAdAdGmUdAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
228	ARC1430	ARC979 opt phase 1	dAmCdAdGmGmCdAdAdGmUdAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
229	ARC1431	ARC979 opt phase 1	dAmCdAdGdGmCmAdAdGmUdAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
230	ARC1432	ARC979 opt phase 1	dAmCdAdGdGmCdAmAdGmUdAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T

231	ARC1433	ARC979 opt phase 1	dAmCdAdGdGmCdAdAmGmUdAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
232	ARC1434	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUmAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
233	ARC1435	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAmAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
234	ARC1436	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUmGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
235	ARC1437	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGmGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
236	ARC1438	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGmGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
237	ARC1439	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGm GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
238	ARC1440	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG mAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
239	ARC1441	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAmGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
240	ARC1442	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUmGmCdGdGdGmCdGdGdGdGmUdGmU-3T
241	ARC1443	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCmGdGdGmCdGdGdGdGmUdGmU-3T
242	ARC1444	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGmGdGmCdGdGdGdGmUdGmU-3T
243	ARC1445	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGmGmCdGdGdGdGmUdGmU-3T
244	ARC1446	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCmGdGdGdGmUdGmU-3T
245	ARC1447	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGmGdGdGmUdGmU-3T
246	ARC1448	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGmGdGmUdGmU-3T
247	ARC1449	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdGmGmUdGmU-3T

248	ARC1450	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdGdGmUmGmU-3T
249	ARC1451	ARC979 opt phase 1	mAmCmAdGdGmCdAdAdGmUdAdAmUmUdGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUmGmU-3T
250	ARC1452	ARC979 opt phase 1	dAmCdAdGdGmCmAmAdGmUdAdAmUmUmGdGdGd GdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
251	ARC1453	ARC979 opt phase 1	dAmCdA-s-dGdGmCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmU dGmCdGdGdGmCdGdGdGdGmUdGmU-3T
252	ARC1454	ARC979 opt phase 1	dAmCdAdG-s-dGmCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdG mCdGdGdGmCdGdGdGdGmUdGmU-3T
253	ARC1455	ARC979 opt phase 1	dAmCdAdGdG-s-mCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdGm CdGdGdGmCdGdGdGdGmUdGmU-3T
254	ARC1456	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdG-s-dGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdGmUdG mU-3T
255	ARC1457	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdG-s-dGdGdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU -3T
256	ARC1458	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdG-s-dGdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
257	ARC1459	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmC-s-dGdGdGmCdGdGdGdGmUdGmU-3T
258	ARC1460	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdG-s-dGdGmCdGdGdGdGmUdGmU-3T
259	ARC1461	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdG-s-dGmCdGdGdGdGmUdGmU-3T
260	ARC1462	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdG-s-dGdGmUdGmU-3T
261	ARC1463	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdG-s-dGmUdGmU-3T
262	ARC1464	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdGdG-s-mUdGmU-3T
263	ARC1465	ARC979 opt phase 1	dAmCdAdGdGmCdAdAdGmUdA-s-dAmUmUdGdGdGdGdA-s-dG-s-mU-s-dG-s-

			mCdGdGdG-s-mCdGdGdGdGmUdGmU-3T
264	ARC1466	ARC979 opt phase 1	dAmCdAPEGdGdGmCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdGPEGmUdGmU-3T
265	ARC1467	ARC979 opt phase 1	mCmGmCdAPEGdGdGmCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdGPEGmUdGmCmG-3T
266	ARC1468	ARC979 opt phase 1	dGdGmCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdG-3T
267	ARC1469	ARC979 opt phase 1	dGdGmCmAmAdGmUdAdAmUmUmGdGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdG-3T
268	ARC1470	ARC979 opt phase 1	dGdGmCdAdAdGmUdA-s-dAmUmUdGdGdGdGdA-s-dG-s-mU-s-dG-s-mCdGdGdG-s-mCdGdGdGdG-3T
269	ARC1471	ARC979 opt phase 1	dGdGmCmAmAdGmUdA-s-dAmUmUmGdGdGdGdA-s-dG-s-mU-s-dG-s-mCdGdGdG-s-mCdGdGdGdG-3T
270	ARC1539	ARC979 opt phase 2	mAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdGmUmGmU-3T
271	ARC1540	ARC979 opt phase 2	dAmCdAdGdGmCdAmAmGmUmAdAmUmUdGdGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
272	ARC1541	ARC979 opt phase 2	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGmGmAmGmUmGmCdGdGdGmCdGdGdGdGmUdGmU-3T
273	ARC1542	ARC979 opt phase 2	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdGmCdGdGmGmCmGmGdGdGmUdGmU-3T
274	ARC1543	ARC979 opt phase 2	mAmCdAdGdGmCdAmAmGmUmAdAmUmUdGdGdGmGmAmGmUmGmCdGdGmGmCmGmGdGdGmUmGmU-3T
275	ARC1544	ARC979 opt phase 2	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGmGmGdGdAdGmUdGmCmGmGdGmCdGdGmGmGmUdGmU-3T
276	ARC1545	ARC979 opt phase 2	mAmCdAdGdGmCdAmAmGmUmAdAmUmUdGmGmGmGmAmGmUmGmCmGmGmGmCmGmGmGmGmUmGmU-3T
277	ARC1591	ARC979 opt phase 3	dAmCdAdIdGmCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdGdGmUdGmU-3T
278	ARC1592	ARC979 opt phase 3	dAmCdAdGdImCdAdAdGmUdAdAmUmUdGdGdGdGdAdGmUdGmCdGdGdGmCdGdGdGdGdGmUdGmU-3T

279	ARC1593	ARC979 opt phase 3	dAmCdAdIdImCdAdAdGmUdAdAmUmUdGdGdGdGd AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
280	ARC1594	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdImUdAdAmUmUdGdGdGdGd AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
281	ARC1595	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdIdGdGdGd AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
282	ARC1596	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdIdGdGd AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
283	ARC1597	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdIdGd AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
284	ARC1598	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdId AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
285	ARC1599	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdIdIdGdGd AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
286	ARC1600	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdIdIdGd AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
287	ARC1601	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdIdId AdGmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
288	ARC1602	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdIdIdIdIdAd GmUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
289	ARC1603	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdImUdGmCdGdGdGmCdGdGdGdGmUdGmU-3T
290	ARC1604	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdImCdGdGdGmCdGdGdGdGmUdGmU-3T
291	ARC1605	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdIdGdGmCdGdGdGdGmUdGmU-3T
292	ARC1606	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdIdGmCdGdGdGdGmUdGmU-3T
293	ARC1607	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdImCdGdGdGdGmUdGmU-3T
294	ARC1608	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdIdIdGmCdGdGdGdGmUdGmU-3T
295	ARC1609	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdIdImCdGdGdGdGmUdGmU-3T

296	ARC1610	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdIdIdImCdGdGdGdGmUdGmU-3T
297	ARC1611	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdIdGdGdGmUdGmU-3T
298	ARC1612	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdIdGdGmUdGmU-3T
299	ARC1613	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdIdGmUdGmU-3T
300	ARC1614	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdGdImUdGmU-3T
301	ARC1615	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdIdIdGdGmUdGmU-3T
302	ARC1616	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdIdIdGmUdGmU-3T
303	ARC1617	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdIdImUdGmU-3T
304	ARC1618	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdIdIdIdImUdGmU-3T
305	ARC1619	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGdGdGdG dAdGmUdGmCdGdGdGmCdGdGdGdGmUdImU-3T
306	ARC1620	ARC979 opt phase 3	dAmC-s-dAdGdGmCdAdAdGmUdAdAmUmUdGmGmGdGdAd GmUdGmCmGmGdGmCdGdGmGmGmUdGmU-3T
307	ARC1621	ARC979 opt phase 3	dAmCdA-s-dG-s-dGmCdAdAdGmUdAdAmUmUdGmGmGdGdAdGmUd GmCmGmGdGmCdGdGmGmGmUdGmU-3T
308	ARC1622	ARC979 opt phase 3	dAmCdAdGdGmC-s-dA-s-dA-s-dGmU-s-dA-s-dAmUmU-s-dGmGmGdGdAdGmUdGmCmGmGdGmCdGdGmGmGmUdGmU-3T
309	ARC1623	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGmGmG-s-dG-s-dA-s-dGmU-s-dGmCmGmGdGmCdGdGmGmGmUdGmU-3T
310	ARC1624	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGmGmGd GdAdGmUdGmCmGmG-s-dGmC-s-dG-s-dGmGmGmUdGmU-3T
311	ARC1625	ARC979 opt phase 3	dAmCdAdGdGmCdAdAdGmUdAdAmUmUdGmGmGd GdAdGmUdGmCmGmGdGmCdGdGmGmGmU-s-

			dGmU-3T
312	ARC1626	ARC979 opt phase 3	dAmC-s-dA-s-dG-s-dGmC-s-dA-s-dA-s-dGmU-s-dA-s-dAmUmU-s-dGmGmG-s-dG-s-dA-s-dGmU-s-dGmCmGmG-s-dGmC-s-dG-s-dGmGmGmU-s-dGmU-3T
313	ARC1755	ARC979 opt phase 4	mAmC-s-dAdGdGmC-s-dAmAmGmUmA-s-dAmUmU-s-dGmGmGmGmAmGmUmGmCmGmGmGmCmGmGmGmGmUmGmU-3T
314	ARC1756	ARC979 opt phase 4	mAmC-s-dAdGdGmC-s-dAmAmGmUmA-s-dAmUmU-s-dGmGmG-s-dG-s-dA-s-dGmU-s-dGmCmGmGmGmCmGmGmGmGmUmGmU-3T

[00351] **Table 30**: Binding Characterization

SEQ ID NO	ARC #	Description	K_D (nM)	% binding at 100 nM (through ARC1619) or at 10 nM (ARC1620 - 1756)
224	ARC1386	ARC 979 with 3'-inv dT	1	69.9
225	ARC1427	ARC979 opt phase 1	3.0	49.4
226	ARC1428	ARC979 opt phase 1	1.8	57.8
227	ARC1429	ARC979 opt phase 1	29.5	48.4
228	ARC1430	ARC979 opt phase 1	14.2	51.6
229	ARC1431	ARC979 opt phase 1	10.0	56.3
230	ARC1432	ARC979 opt phase 1	3.8	57.9
231	ARC1433	ARC979 opt phase 1	2.8	55.2

232	ARC1434	ARC979 opt phase 1	3.0	52.9
233	ARC1435	ARC979 opt phase 1	9.8	51.2
234	ARC1436	ARC979 opt phase 1	15.1	46.9
235	ARC1437	ARC979 opt phase 1	3.9	43.1
236	ARC1438	ARC979 opt phase 1	6.0	36.7
237	ARC1439	ARC979 opt phase 1	4.8	43.5
238	ARC1440	ARC979 opt phase 1	6.7	54.9
239	ARC1441	ARC979 opt phase 1	2.7	49.8
240	ARC1442	ARC979 opt phase 1	2.8	60.5
241	ARC1443	ARC979 opt phase 1	2.0	52.8
242	ARC1444	ARC979 opt phase 1	4.4	58.1
243	ARC1445	ARC979 opt phase 1	2.8	56.3
244	ARC1446	ARC979 opt phase 1	2.1	55.0
245	ARC1447	ARC979 opt phase 1	2.5	56.5
246	ARC1448	ARC979 opt phase 1	2.3	59.5
247	ARC1449	ARC979 opt phase 1	2.6	48.4
248	ARC1450	ARC979 opt phase 1	2.6	46.5
249	ARC1451	ARC979 opt phase 1	10.2	46.1
250	ARC1452	ARC979 opt phase 1	18.9	56.9
251	ARC1453	ARC979 opt	4.4	65.0

		phase 1		
252	ARC1454	ARC979 opt phase 1	2.7	61.6
253	ARC1455	ARC979 opt phase 1	1.6	56.6
254	ARC1456	ARC979 opt phase 1	3.2	55.5
255	ARC1457	ARC979 opt phase 1	3.0	56.1
256	ARC1458	ARC979 opt phase 1	2.9	49.6
257	ARC1459	ARC979 opt phase 1	4.0	50.7
258	ARC1460	ARC979 opt phase 1	5.8	46.1
259	ARC1461	ARC979 opt phase 1	3.7	47.3
260	ARC1462	ARC979 opt phase 1	1.7	53.4
261	ARC1463	ARC979 opt phase 1	3.6	53.5
262	ARC1464	ARC979 opt phase 1	2.4	54.6
263	ARC1465	ARC979 opt phase 1	1.3	57.0
264	ARC1466	ARC979 opt phase 1	1.9	38.7
265	ARC1467	ARC979 opt phase 1	1.7	57.0
266	ARC1468	ARC979 opt phase 1	10.0	49.8
267	ARC1469	ARC979 opt phase 1	49.8	59.8
268	ARC1470	ARC979 opt phase 1	8.6	61.0
269	ARC1471	ARC979 opt phase 1	23.5	62.9
270	ARC1539	ARC979 opt phase 2	6.6	43.8

271	ARC1540	ARC979 opt phase 2	7.5	50.3
272	ARC1541	ARC979 opt phase 2	3.9	57.0
273	ARC1542	ARC979 opt phase 2	1.2	57.6
274	ARC1543	ARC979 opt phase 2	5.9	40.9
275	ARC1544	ARC979 opt phase 2	0.9	58.6
276	ARC1545	ARC979 opt phase 2	0.4 & 62.0 (the binding curve was strongly biphasic)	17.4 & 20.9
277	ARC1591	ARC979 opt phase 3		54.8
278	ARC1592	ARC979 opt phase 3	8.1	54.4
279	ARC1593	ARC979 opt phase 3	13.8	51.0
280	ARC1594	ARC979 opt phase 3	4.2	60.1
281	ARC1595	ARC979 opt phase 3	5.4	53.9
282	ARC1596	ARC979 opt phase 3	11.1	59.0
283	ARC1597	ARC979 opt phase 3	11.2	61.3
284	ARC1598	ARC979 opt phase 3	4.7	61.0
285	ARC1599	ARC979 opt phase 3	7.2	57.7
286	ARC1600	ARC979 opt phase 3	15.6	61.3
287	ARC1601	ARC979 opt phase 3	4.4	58.6
288	ARC1602	ARC979 opt phase 3	40.8	64.4

289	ARC1603	ARC979 opt phase 3	1.6	64.2
290	ARC1604	ARC979 opt phase 3	2.1	50.2
291	ARC1605	ARC979 opt phase 3	7.5	56.8
292	ARC1606	ARC979 opt phase 3	5.0	60.3
293	ARC1607	ARC979 opt phase 3	3.3	61.5
294	ARC1608	ARC979 opt phase 3	9.7	61.1
295	ARC1609	ARC979 opt phase 3	4.7	60.5
296	ARC1610	ARC979 opt phase 3	5.2	60.4
297	ARC1611	ARC979 opt phase 3	1.7	62.1
298	ARC1612	ARC979 opt phase 3	1.9	60.9
299	ARC1613	ARC979 opt phase 3	2.3	58.4
300	ARC1614	ARC979 opt phase 3	1.7	60.5
301	ARC1615	ARC979 opt phase 3	5.8	55.2
302	ARC1616	ARC979 opt phase 3	6.1	59.5
303	ARC1617	ARC979 opt phase 3	4.1	61.9
304	ARC1618	ARC979 opt phase 3	34.0	67.0
305	ARC1619	ARC979 opt phase 3	2.8	52.1
306	ARC1620	ARC979 opt phase 3	0.4	68.0
307	ARC1621	ARC979 opt phase 3	0.5	64.6
308	ARC1622	ARC979 opt	0.3	66.0

		phase 3		
309	ARC1623	ARC979 opt phase 3	0.2	68.7
310	ARC1624	ARC979 opt phase 3	0.4	68.0
311	ARC1625	ARC979 opt phase 3	0.4	75.0
312	ARC1626	ARC979 opt phase 3	0.1	79.2
313	ARC1755	ARC979 opt phase 4	0.8	31
314	ARC1756	ARC979 opt phase 4	0.5	56

*30min RT incubation for K_D determination
*1X Dulbecco's PBS (with Ca^{++} and Mg^{++}) +0.1mg/mL BSA reaction buffer

EXAMPLE 2C: Plasma stability of anti-IL-23 aptamers

[00352] A subset of the aptamers identified during the optimization process was assayed for nuclease stability in human plasma. Plasma nuclease degradation was measured using denaturing polyacrylamide gel electrophoresis as described below. Briefly, for plasma stability determination, chemically synthesized aptamers were purified using denaturing polyacrylamide gel electrophoresis, 5'end labeled with γ-^{32}P ATP and then gel purified again. Trace ^{32}P labeled aptamer was incubated in the presence of 100 nM unlabeled aptamer in 95% human plasma in a 200 microliter binding reaction. The reaction for the time zero point was made separately with the same components except that the plasma was replaced with PBS to ensure that the amount of radioactivity loaded on gels was consistent across the experiment. Reactions were incubated at 37 °C in a thermocycler for the 1, 3, 10, 30 and 100 hours. At each time point, 20 microliters of the reaction was removed, combined with 200 microliters of formamide loading dye and flash frozen in liquid nitrogen and stored at -20 °C. After the last time point was taken, frozen samples were thawed and 20 microliters was removed from each time point. SDS was then added to the small samples to a final concentration of 0.1%. The samples were then incubated at 90 °C for 10 – 15 minutes and loaded directly onto a 15% denaturing PAGE gel and run at 12 W for 35 minutes. Radioactivity on the gels was quantified using a Storm 860 Phosphorimager system

(Amersham Biosciences, Piscataway, NJ). The percentage of full length aptamer at each time point was determined by quantifying the full length aptamer band and dividing by the total counts in the lane. The fraction of full length aptamer at each time-point was then normalized to the percentage full length aptamer of the 0 hour time-point. The fraction of full length aptamer as a function of time was fit to the equation:

m1*e^(-m2*m0)

where m1 is the maximum % full length aptamer (m1=100); and m2 is the rate of degradation.

The half-life of the aptamer ($T_{1/2}$) is equal to the (ln 2) / m2.

[00353] Sample data is shown in Figure 18 and the results for the aptamers tested are summarized in Table 31.

[00354] **Table 31**: plasma stability

SEQ ID NO	ARC #	Description	~T1/2 in human plasma (hrs)
177	ARC979		14
224	ARC1386	ARC 979 with 3'-inv dT	33
307	ARC1621	ARC979 opt phase 3	59
308	ARC1622	ARC979 opt phase 3	54
309	ARC1623	ARC979 opt phase 3	45
310	ARC1624	ARC979 opt phase 3	35
311	ARC1625	ARC979 opt phase 3	31
312	ARC1626	ARC979 opt phase 3	113
313	ARC1755	ARC979 opt phase 4	83
314	ARC1756	ARC979 opt phase 4	96

EXAMPLE 3: FUNCTIONAL CELL ASSAYS

Cell-based assay and minimization of active rRfY IL-23 aptamers

[00355] IL-23 plays a role in JAK/STAT signal transduction and phosphorylates STAT 1, 3, 4, and 5. To test whether IL-23 aptamers showed cell-based activity, signal transduction was assayed in the lysates of peripheral blood mononuclear cells (PBMCs) grown in media containing PHA (Phytohemagglutinin), or PHA Blasts. More specifically, the cell-based assay determined whether IL-23 aptamers could inhibit IL-23 induced STAT-3 phosphorylation in PHA Blasts.

[00356] In essence, lysates of IL-23 treated cells will contain more activated STAT3 than quiescent or aptamer blocked cells. Inhibition of IL-23-induced STAT3 phosphorylation was measured by two methods: by western blot, using an anti-phospho-STAT3 Antibody (Tyr705) (Cell Signaling, Beverly, MA); and by TransAM™ Assay (Active Motif, Carlsbad, CA). The TransAM™ assay kit provides a 96 well plate on which an oligonucleotide containing the STAT consensus binding site (5'TTCCCGGAA-3') is immobilized. An anti-STAT3 antibody that recognizes an epitope on STAT3 that is only accessible when STAT3 is activated is used in conjunction with an HRP-conjugated secondary antibody to give a colorimetric readout that can be quantified by spectrophotometry. (See Figure 19).

[00357] In summary, the cell-based assay was conducted by isolating the peripheral blood mononuclear cells (PBMCs) from whole blood using a Histopaque gradient (Sigma, St. Louis, MO). The PBMCs were cultured for 3 to 5 days at 37°C/5% CO_2 in Peripheral Blood Medium (Sigma) which contains PHA, supplemented with IL-2 (100 units/mL) (R&D Systems, Minneapolis, MN), to generate PHA Blasts. To test IL-23 aptamers, the PHA Blasts were washed twice with 1X PBS, then serum starved for four hours in RPMI, 0.20 % FBS. After serum starvation, approximately 2 million cells were aliquotted into appropriately labeled eppendorf tubes. hIL-23 at a final constant concentration of 3 ng/mL (R&D Systems, Minneapolis, MN) was combined with a dilution series of various IL-23 aptamers as described in Example 1, and the cytokine/aptamer mixture was added to the aliquotted cells in a final volume of 100 µl and incubated at 37°C for 10-12 minutes. The incubation reaction was stopped by adding 1 mL of ice-cold PBS with 1.5 mM Na_3VO_4. Cell lysates were made

using the lysis buffer provided by the TransAM™ STAT 3 assay following the manufacturer's instructions. Figure 20 depicts a flow summary of the protocol used for the cell based assay.

[00358] Parent aptamer and minimized IL-23 aptamers from the various selections with 2'-F pyrimidines-containing pools (rRfY), ribo/2'O-Me containing pools (rRmY), deoxy/2'O-Me containing pools (dRmY), and optimized dRmY aptamers were tested using the TransAM™ method.

Example 3A: Cell Based Assay Results for parent and minimzed clones from rRfY selections

[00359] Full length clones from the rRfY selection described in Example 1A, and select minimized rRfY clones that were described in Example 2A.1, were tested using the TransAM™ STAT3 activation assay. Table 34 summarizes the cell based assay data for IL-23 full length aptamers generated from the rRfY selections described in Example 1A. Table 35 summarizes the activity data of the rRfY minimized clones, described in Example 2A.1, each compared to the activity of their respective parent (full length) clone. The minimized rRfY clones F11min2 (SEQ ID NO 147), A10min5 (SEQ ID NO 139), A10min6 (SEQ ID NO 140), B10min4 (SEQ ID NO 144), B10min5 (SEQ ID NO 145), Type1.4 (SEQ ID NO 151) and Type1.5 (SEQ ID NO 152) each outperformed their respective parent clones (see Figure 21), in addition to all of the full length rRfY clones when tested in the TransAM™ STAT3 activation assay.

[00360] **Table 34**: Cell Based Assay Results: Summary of rRfY Clones Tested

SEQ ID NO	Clone Name	selection	Western Blot	TransAM	TransAM IC_{50}
27	AMX86-C5	R8 h-IL-23	Yes	Yes	3 μM
13	AMX86-D5	R8 h-IL-23	Yes	Yes	> 5 μM
16	AMX86-D6	R8 h-IL-23	Yes	Yes	> 5 μM
24	AMX86-E6	R8 h-IL-23	Yes	No	

22	AMX86-F6	R8 h-IL-23	Yes	No	
18	AMX86-A7	R8 h-IL-23	Yes	No	
25	AMX86-H7	R8 h-IL-23	Yes	No	
35	AMX86-B9	R8 X-IL-23	Yes	No	
32	AMX86-C9	R8 X-IL-23	Yes	No	
33	AMX86-G9	R8 X-IL-23	Yes	No	
39	AMX86-H9	R8 X-IL-23	Yes	Yes	250 nM
28	AMX86-B10	R8 X-IL-23	Yes	Yes	800 nM
36	AMX86-G10	R8 X-IL-23	Yes	Yes	~2 μM
37	AMX86-A11	R8 X-IL-23	Yes	No	
30	AMX86-D11	R8 X-IL-23	Yes	No	
43	AMX84-A10	R10 PN-IL-23	Yes	Yes	400 nM
44	AMX84-B10	R10 PN-IL-23	Yes	Yes	> 1 μM
45	AMX84-A11	R10 PN-IL-23	Yes	Yes	> 5 μM
46	AMX84-F11	R10 PN-IL-23	Yes	Yes	250 nM
47	AMX84-E12	R10 PN-IL-23	Yes	Yes	> 1 μM
48	AMX84-C10	R10 PN-IL-23	No	Yes	250 nM
49	AMX84-C11	R10 PN-IL-23	No	Yes	800 nM
50	AMX84-G11	R10 PN-IL-23	No	Yes	250 nM
51	ARX83-plate1-	R12 PN-IL23	No	Yes	> 5 μM

	H1				
52	AMX91-F11	R10 PN-IL-23	No	Yes	5 μM
53	AMX91-G1	R10 PN-IL-23	No	Yes	2 μM
54	AMX91-E3	R10 PN-IL-23	No	Yes	> 5 μM
55	AMX91-H3	R10 PN-IL-23	No	Yes	50 nM
64	AMX91-G11	R12 PN-IL23	No	Yes	3 μM
65	AMX91-C12	R12 PN-IL23	No	Yes	50 nM
66	AMX91-H12	R12 PN-IL23	No	Yes	350 nM
56	AMX91-B5	R10 PN-IL-23	No	Yes	1 μM
57	AMX91-A6	R10 PN-IL-23	No	Yes	3 μM
58	AMX91-G7	R12 PN-IL23	No	Yes	150 nM
59	AMX91-H7	R12 PN-IL23	No	Yes	50 nM
60	AMX91-B8	R12 PN-IL23	No	Yes	450 nM
61	AMX91-H8	R12 PN-IL23	No	Yes	3 μM
62	AMX91-G9	R12 PN-IL23	No	Yes	50 nM
63	AMX91-D9	R12 PN-IL23	No	Yes	150 nM

[00361] **Table 35**: IL-23 2'F rRfY Minimized aptamer binding compared to parent aptamers.

SEQ ID NO	Clone Name	Selection	W.Blot	TransAM	IC_{50} minimer	IC_{50} Full Length
147	F11min2	R10 PN-IL-23	No	Yes	25 nM	250 Nm

139	A10min5	R10 PN-IL-23	No	Yes	300 nM	1 μM
140	A10min6	R10 PN-IL-23	No	Yes	250 nM	1 μM
144	B10min4	R10 PN-IL-23	No	Yes	500 nM	700 nM
145	B10min5	R10 PN-IL-23	No	Yes	80 nM	700 nM
151	Type1.4	N/A	No	Yes	80 nM	N/A
152	Type1.5	N/A	No	Yes	80 nM	N/A

Example 3B: Cell Based Assay Results for parent and minimzed clones from first dRmY selections

[00362] Parent clones from the dRmY selection described in Example 1C, and minimized dRmY clones from this selection (described in Example 2A.2), were tested for activity using the TransAM™ STAT3 activation assay. The three full length dRmY clones described in Example 1C which showed the highest binding affinity for IL-23, ARC489 (SEQ ID NO 91), ARC490 (SEQ ID NO 92), ARC491 (SEQ ID NO 94) were tested. ARC 492 (SEQ ID NO 97) which exhibited no binding to IL-23 was used as a negative control. ARC489 (SEQ ID NO 91), and ARC491 (SEQ ID NO 94) showed comparable cell based activity in the TransAM™ STAT3 activation assay and preliminary data indicate IC_{50}'s in the 50 nM-500 nM range (data not shown).

[00363] The only minimized clone from the dRmY minimization efforts described in Example 2A.2 which showed binding to IL-23, ARC527 (SEQ ID NO 159), was tested in the TransAM™ STAT3 activation assay and showed a decrease in assay activity compared to its respective full length ARC489 (SEQ ID NO 91) (data not shown).

Example 3C: Cell Based Assay Results for parent and minimized clones from second dRmY selections

[00364] Parent clones from the dRmY selection described in Example 1D, and minimized clones from this selection (described in Example 2A.3) that displayed high affinity to hIL-23 were screened for functionality in the TransAM™ assay using an 8-point IL-23 titration from

0 to 3 μM in 3 fold dilutions in combination with a constant IL-23 concentration of 3 ng/mL. IC_{50}s for the full length clones were calculated from the dose response curves. Figure 22 is an example of the dose response curves for the dRmY clones from the selection described in Example 1D that displayed potent cell based activity in the TransAM™ assay (ARC611 (SEQ ID NO 103), ARC614 (SEQ ID NO 105), ARC621 (SEQ ID NO 108), and ARC627 (SEQ ID NO 110)).

[00365] Minimized dRmY clones (described in Example 2A.3) were screened for functionality and compared to their respective parent clone in the in the TransAM™ assay. IC_{50}s were calculated from the dose response curves. Figure 23 is an example of the dose response curves for some the more potent minimized dRmY clones, ARC979 (SEQ ID NO 177), ARC980 (SEQ ID NO 178), ARC982 (SEQ ID NO 180), compared to the parent full length clones, ARC621 (SEQ ID NO 108) and ARC627 (SEQ ID NO 110). ARC979 (SEQ ID NO 177) consistently performed the best in the TransAM™ assay, with an IC_{50} of 40 nM +/- 10 nM when averaged over the course of three experiments. ARC792 (SEQ ID NO 162), ARC794 (SEQ ID NO 164), ARC795 (SEQ ID NO 165) also displayed potent activity in the TransAM™ assay.

Example 3D: Cell Based Assay Results for Optimized ARC979 Derivatives

[00366] Several of the optimized ARC979 derivatives described in Example 2B.2 that displayed high affinity to hIL-23 were screened for their ability to inhibit IL-23 induced STAT 3 activation using the PHA Blast assay previously described. Inhibition of IL-23-induced STAT3 phosphorylation was measured using the Pathscan® Phospho-STAT3 (Tyr705) Sandwich ELISA Kit (Cell Signaling Technology, Beverly, MA).

[00367] Similar to the TransAM™ Assay method previously described, the Pathscan® Phospho-STAT3 (Tyr705) Sandwich ELISA Kit detects endogenous levels of Phospho-STAT3 (Tyr705) protein by using a STAT3 rabbit monoclonal antibody which has been coated onto the wells of a 96-well plate. After incubation with cell lysates, both nonphospho- and phospho-STAT3 proteins are captured by the coated antibody. A phospho-STAT3 mouse monoclonal antibody is added to detect the captured phospho-STAT3 protein, and an HRP-linked anti-mouse antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color, and the magnitude of optical density for this developed color is proportional to the quantity of phospho-STAT3 protein.

[00368] PHA Blasts were isolated and prepared as described above and treated with hIL-23 at a final constant concentration of 6 ng/mL (R&D Systems, Minneapolis, MN) to induce STAT3 activation, instead of using 3 ng/mL as previously described with the TransAM™ assay. Several clones from the selection described in 2C, were screened by using a 6-point IL-23 titration from 0 to 700nM in 3 fold dilutions in combination with a constant IL-23 concentration of 6 ng/mL of IL-23 (R&D Systems, Minneapolis, MN) to induce STAT3 activation, instead of using 3 ng/mL as previously described with the TransAM™ assay. Lysates of treated cells were prepared using the buffers provided by the Pathscan kit, and the assay was run according to the manufacturer's instructions. IC_{50}s for the full length clones were calculated from the dose response curves.

[00369] ARC979, which displayed an IC_{50} of 40 +/-10 nM using the TransAM™ method, consistently displayed an IC_{50} of 6 +/- 1 nM using the Pathscan® method. As previously mentioned this IC_{50} value is consistent with the K_D value for ARC979 of 1 nM which was repeatedly verified under the direct binding assay conditions described in Example 2B.2. As can be seen from the Table 36, several of the optimized derivatives of ARC979 remarkably displayed even higher potentcy than ARC979 when directly compared using the Pathscan® Method, particularly ARC1624 and ARC1625, which gave IC_{50} values of 2 nM and 4 nM respectively.

[00370] Figure 24 is an example of the dose response curves for several of the optimized clones that displayed both high affinity for IL-23 and potent cell based activity in the Pathscan® assay. Table 36 summarizes the IC_{50}'s derived from the dose response curves for the optimized aptamers tested.

[00371] **Table 36**: IC_{50}s for Optimized ARC979 derivatives in the Pathscan® Assay

SEQ ID NO	Clone	Pathscan® IC_{50} (nM)
177	979	6 +/- 1
275	1544	____
308	1622	9

309	1623	5
310	1624	2
311	1625	4
312	1626	12
313	1755	68
314	1756	19

Example 3E: Cell based assay results for parent and minimized clones from the mouse IL-23 selections

[00372] Using the PHA Blast assay and the TransAM™ method described above, mouse IL-23 was shown to activate STAT3 in human PHA blasts (See Figure 25). Therefore, the ability of the parent clones from the mouse IL-23 selection described in Example 1E, and minimized clones from this selection (described in Example 2A.4) that displayed affinity to mIL-23 to block mouse IL-23 induced STAT3 activation in human PHA blast cells was measured using the TransAM™ assay. The protocol used was identical to that previously described except mouse IL-23 was used to induce STAT 3 activation in PHA Blasts at a concentration of 30 ng/mL, instead of using human IL-23 at a concentration of 3 ng/mL. The results for the parent clones are listed in Table 37 and the results for the minimized clones are listed in Table 38 below.

[00373] **Table 37**: Parent mIL-23-rRfY Clone Activity in the TransAM™ Assay

SEQ ID NO	Clone Name	Selection	IC_{50} (nM)
124	ARC1628	R7 mIL-23	37
125	ARC1629	R7 mIL-23	Not Tested
126	ARC1630	R7 mIL-23S	16.6*
127	ARC1631	R7 mIL-23S	Not Tested
128	ARC1632	R7 mIL-23S	18
129	ARC1633	R7 mIL-23S	31
130	ARC1634	R7 mIL-23S	9

*Multiple experiment average.

[00374] Table 38: Mouse IL-23 rRfY Minimized Clone Activity in the TransAM™ Assay

Minimized Clone SEQ ID NO	Parent Clone	IC_{50} mIL-23 (nM)
199	ARC1628	18 nM
200	ARC1632	inactive
201	ARC1633	7
202	ARC1634	26

The invention having now been described by way of written description and example, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the description and examples above are for purposes of illustration and not limitation of the following claims.

What is claimed is:

1) An aptamer that specifically binds to IL-23.

2) The aptamer of claim 1, wherein IL-23 is a variant of IL-23 that performs a biological function that is essentially the same as a function of IL-23.

3) The aptamer of claim 2 wherein the variant of IL-23 has substantially the same structure and substantially the same ability to bind said aptamer as that of IL-23.

4) The aptamer of claim 3, wherein IL-23 or a variant thereof comprises an amino acid sequence that is at least 70% identical to a sequence comprising SEQ ID NOs 4 and 5.

5) The aptamer of claim 3, wherein IL-23 or a variant thereof comprises an amino acid sequence that is at least 80% identical to a sequence comprising SEQ ID NOs 4 and 5.

6) The aptamer of claim 3, wherein IL-23 or a variant thereof comprises an amino acid sequence that is at least 90% identical to a sequence comprising SEQ ID NOs 4 and 5.

7) The aptamer of claim 3, wherein IL-23 or a variant thereof has an amino acid sequence comprising SEQ ID NOs 4 and 5.

8) The aptamer of claim 7, wherein the aptamer is ribonucleic acid.

9) The aptamer of claim 8, wherein the aptamer is single stranded ribonucleic acid.

10) The aptamer of claim 7, wherein the aptamer is deoxyribonucleic acid.

11) The aptamer of claim 10, wherein the aptamer is single stranded deoxyribonucleic acid.

12) The aptamer of claim 7, wherein the aptamer has a dissociation constant for IL-23 or a variant thereof of about 100 nM or less.

13) The aptamer of claim 12, wherein the aptamer has a dissociation constant for IL-23 or a variant thereof of about 50 nM or less.

14) The aptamer of claim 13, wherein the aptamer has a dissociation constant for human IL-23 or a variant thereof of about 10 nM or less.

15) The aptamer of claim 14, wherein the aptamer has a dissociation constant for human IL-23 or a variant thereof of about 1 nM or less.

16) An aptamer of claim 7, wherein the aptamer comprises at least one chemical modification.

17) The aptamer of claim 16, wherein the modification is selected from the group consisting: of a chemical substitution at a sugar position; a chemical substitution at a phosphate position; and a chemical substitution at a base position, of the nucleic acid.

18) The aptamer of claim 16, wherein the modification is selected from the group consisting of: incorporation of a modified nucleotide; 3' capping; conjugation to a high molecular weight, non-immunogenic compound; conjugation to a lipophilic compound; and phosphate backbone modification.

19) The aptamer of claim 18, wherein the non-immunogenic, high molecular weight compound is polyalkylene glycol.

20) The aptamer of claim 19, wherein the polyalkylene glycol is polyethylene glycol.

21) The aptamer of claim 18, wherein the backbone modification comprises incorporation of one or more phosphorothioates into the phosphate backbone.

22) The aptamer of claim 21, wherein the aptamer comprises the incorporation of fewer than 10 phosphorothioates in the phosphate backbone.

23) The aptamer of claim 22, wherein the aptamer comprises the incorporation of fewer than 6 phosphorothioates in the phosphate backbone.

24) The aptamer of claim 23, wherein the aptamer comprises the incorporation of fewer than 3 phosphorothioates in the phosphate backbone.

25) The aptamer of claim 7, wherein the aptamer modulates a function of IL-23 or a variant thereof.

26) The aptamer of claim 25, wherein the aptamer inhibits a function of IL-23 or a variant thereof.

27) The aptamer of claim 26, wherein the aptamer inhibits a function of IL-23 or a variant thereof *in vivo*.

28) The aptamer of claim 26, wherein the aptamer prevents binding of IL-23 to the IL-23 receptor.

29) The aptamer of claim 1, wherein the aptamer has substantially the same ability to bind IL-23 as that of an aptamer comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

30) The aptamer of claim 1, wherein the aptamer has substantially the same structure and substantially the same ability to bind IL-23 as that of an aptamer comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

31) The aptamer of claim 1, wherein the aptamer comprises a nucleic acid sequence at least 80% identical to any one of the sequences selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

32) The aptamer of claim 31, wherein the aptamer nucleic acid sequence is at least 90% identical to any one of the sequences selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

33) The aptamer of claim 1, wherein the aptamer comprises 20 contiguous nucleotides that are identical to a sequence of 20 contiguous nucleotides in the unique sequence region of any one of the sequences selected from the group of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

34) The aptamer of claim 33, wherein the aptamer comprises 8 contiguous nucleotides that are identical to a sequence of 8 contiguous nucleotides in the unique sequence region of any one of the sequences selected from the group of: SEQ ID NOs 13-66,

SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

35) The aptamer of claim 34, wherein the aptamer comprises 4 contiguous nucleotides that are identical to a sequence of 4 contiguous nucleotides in the unique sequence region of any one of the sequences selected from the group of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

36) An aptamer capable of binding IL-23 or a variant thereof comprising a nucleotide sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118, SEQ ID NOs 124-134, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 199-314.

37) The aptamer of claim 36, further comprising at least one chemical modification.

38) The aptamer of claim 37, wherein the modification is selected from the group consisting: of a chemical substitution at a sugar position; a chemical substitution at a phosphate position; and a chemical substitution at a base position, of the nucleic acid.

39) The aptamer of claim 37, wherein the modification is selected from the group consisting of: incorporation of a modified nucleotide; 3' capping; conjugation to a high molecular weight, non-immunogenic compound; conjugation to a lipophilic compound; and phosphate backbone modification.

40) The aptamer of claim 39, wherein the high molecular weight, non-immunogenic compound is polyalkylene glycol.

41) The aptamer of claim 40, wherein the polyalkylene glycol is polyethylene glycol.

42) The aptamer of claim 39, wherein the backbone modification comprises incorporation of one or more phosphorothioates into the phosphate backbone.

43) The aptamer of claim 42, wherein the aptamer comprises the incorporation of fewer than 10 phosphorothioates in the phosphate backbone.

44) The aptamer of claim 43, wherein the aptamer comprises the incorporation of fewer than 6 phosphorothioates in the phosphate backbone.

45) The aptamer of claim 44, wherein the aptamer comprises the incorporation of fewer than 3 phosphorothioates in the phosphate backbone.

46) The aptamer of claim 1, further capable of binding to IL-12.

47) The aptamer of claim 46, wherein IL-12 is a variant of IL-12 that performs a biological function that is essentially the same as a function of IL-12.

48) The aptamer of claim 47 wherein the variant of IL-12 has substantially the same structure and substantially the same ability to bind said aptamer as that of IL-12.

49) The aptamer of claim 48, wherein IL-12 or a variant thereof comprises an amino acid sequence that is at least 80% identical to a sequence comprising SEQ ID NOs 4 and 6.

50) The aptamer of claim 48, wherein IL-12 or a variant thereof comprises an amino acid sequence that is at least 90% identical to a sequence comprising SEQ ID NOs 4 and 6.

51) The aptamer of claim 48, wherein IL-12 or a variant thereof has an amino acid sequence comprising SEQ ID NOs 4 and 6.

52) The aptamer of claim 51, wherein the aptamer modulates a function of IL-12 or a variant thereof.

53) The aptamer of claim 52, wherein the aptamer inhibits a function of IL-12 or a variant thereof.

54) The aptamer of claim 53, wherein the aptamer inhibits a function of IL-12 or a variant thereof *in vivo*.

55) The aptamer of claim 53, wherein the aptamer prevents binding of IL-12 to the IL-12 receptor.

56) The aptamer of claim 4, wherein IL-23 or a variant thereof is mouse IL-23.

57) The aptamer of claim 56, wherein mouse IL-23 has an amino acid sequence comprising SEQ ID NOs 315 and 316.

58) A pharmaceutical composition comprising a therapeutically effective amount of an aptamer comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NOs 13-66, SEQ ID NOs 71-88, SEQ ID NOs 91-96, SEQ ID NOs 103-118,

SEQ ID NOs 124-130, SEQ ID NOs 135-159, SEQ ID NO 162, and SEQ ID NOs 164-172, SEQ ID NOs 176-178, SEQ ID NOs 181-196, and SEQ ID NOs 203-314, or a salt thereof, and a pharmaceutically acceptable carrier or diluent.

59) A method of treating, preventing or ameliorating a disease mediated by IL-23, comprising administering the composition of claim 58 to a vertebrate.

60) The method of claim 59, wherein the vertebrate is a mammal.

61) The method of claim 60, wherein the mammal is a human.

62) The method of claim 59, wherein said disease is selected from the group consisting of: autoimmune disease, inflammatory disease, cancer, bone resorption in osteoporosis, and Type I Diabetes.

63) The method of claim 62, wherein the autoimmune disease is selected from the group consisting of: multiple sclerosis, rheumatoid arthritis, psoriasis, systemic lupus erythamatosus, and irritable bowel disease.

64) The method of claim 62, wherein the cancer is selected from the group consisting of: colon cancer, lung cancer, and lung metastases.

65) The method of claim 63, wherein the irritable disease is selected from the group consisting of Crohn's Disease and ulcerative colitis.

66) A pharmaceutical composition comprising a therapeutically effective amount of an aptamer comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO 14, SEQ ID NOs 17-19, SEQ ID NO 21, SEQ ID NOs 27-32, SEQ ID NOs 34-40, SEQ ID NO 42, SEQ ID NO 49, SEQ ID NOs 60-61, SEQ ID NOs 91-92, SEQ ID NO 94, and SEQ ID NOs 103-118, or a salt thereof, and a pharmaceutically acceptable carrier or diluent.

67) A method of treating, preventing or ameliorating a disease mediated by IL-12, comprising administering the composition of claim 66 to a human.

68) The method of claim 67, wherein said disease is selected from the group consisting of: autoimmune disease, inflammatory disease, cancer, bone resorption in osteoporosis, and Type I Diabetes.

69) The method of claim 68, wherein the autoimmune disease is selected from the group consisting of: multiple sclerosis, rheumatoid arthritis, psoriasis, systemic lupus erythamatosus, and irritable bowel disease.

70) The method of claim 68, wherein the cancer is selected from the group consisting of: colon cancer, lung cancer, and lung metastases.

71) The method of claim 69, wherein the irritable disease is selected from the group consisting of Crohn's Disease and ulcerative colitis.

72) A diagnostic method comprising contacting an aptamer of claim 36 with a composition suspected of comprising IL-23 or a variant thereof and detecting the presence or absence of IL-23 or a variant thereof.

73) An aptamer according to claim 36 for use as an *in vitro* diagnostic

74) An aptamer according to claim 36 for use as an *in vivo* diagnostic.

75) An aptamer according to claim 36 for use in the treatment, prevention or amelioration of disease *in vivo*.

Figure 1

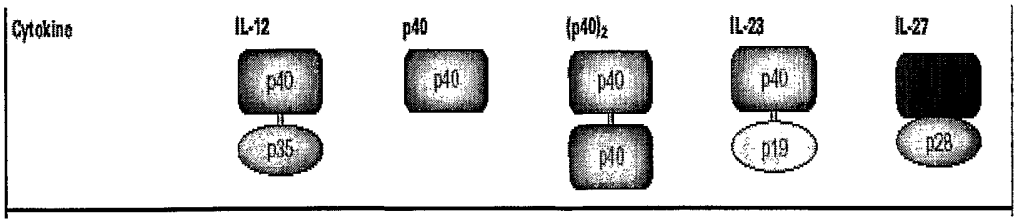


Figure 2

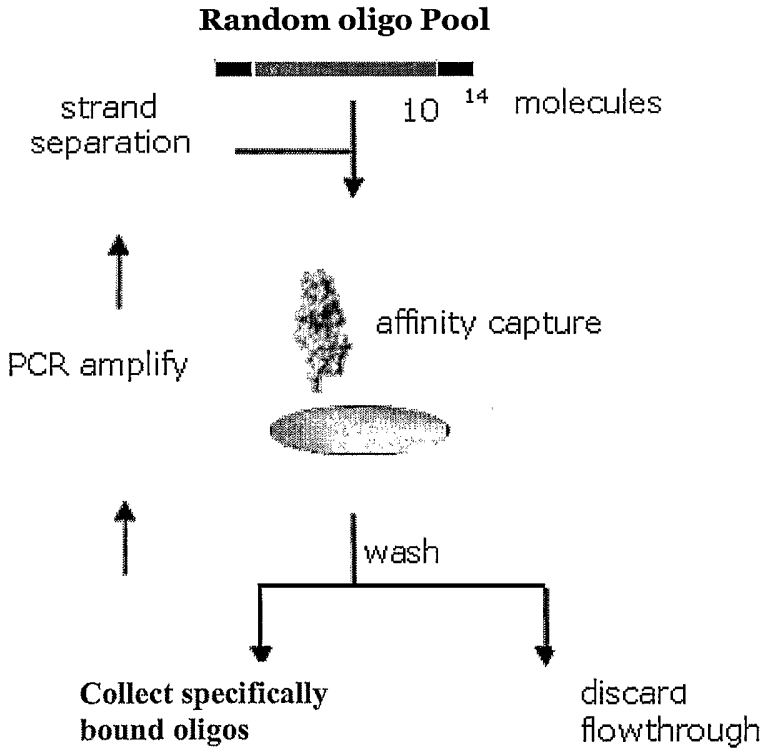


Figure 3

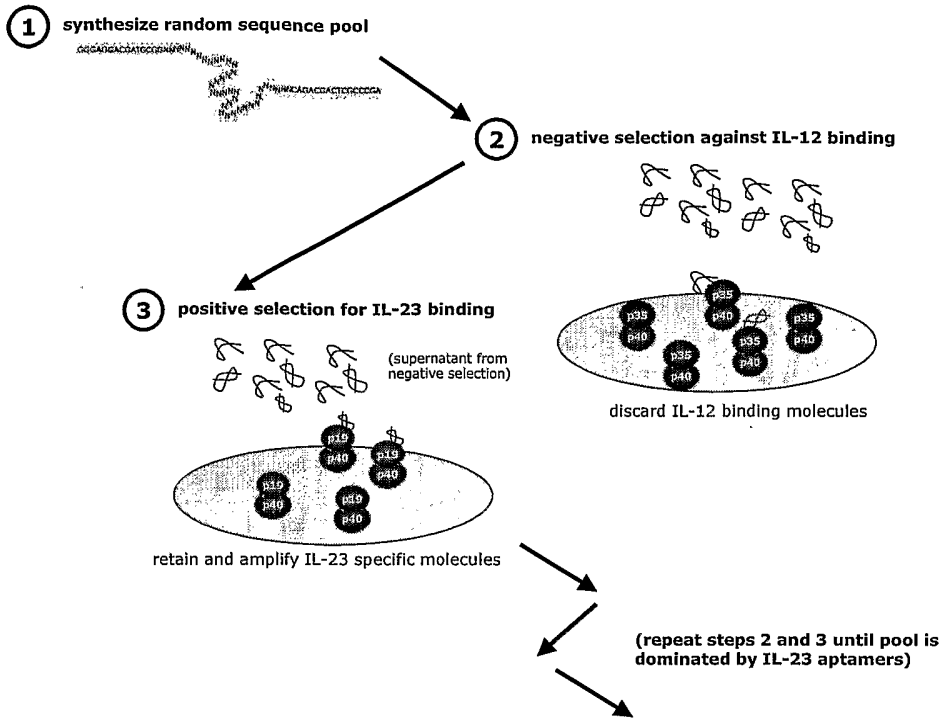

1 synthesize random sequence pool
2 negative selection against IL-12 binding
3 positive selection for IL-23 binding
(supernatant from negative selection)
discard IL-12 binding molecules
retain and amplify IL-23 specific molecules
(repeat steps 2 and 3 until pool is dominated by IL-23 aptamers)

Figure 4

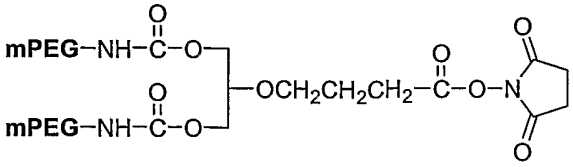


Figure 5

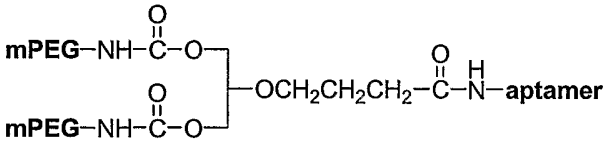


Figure 6

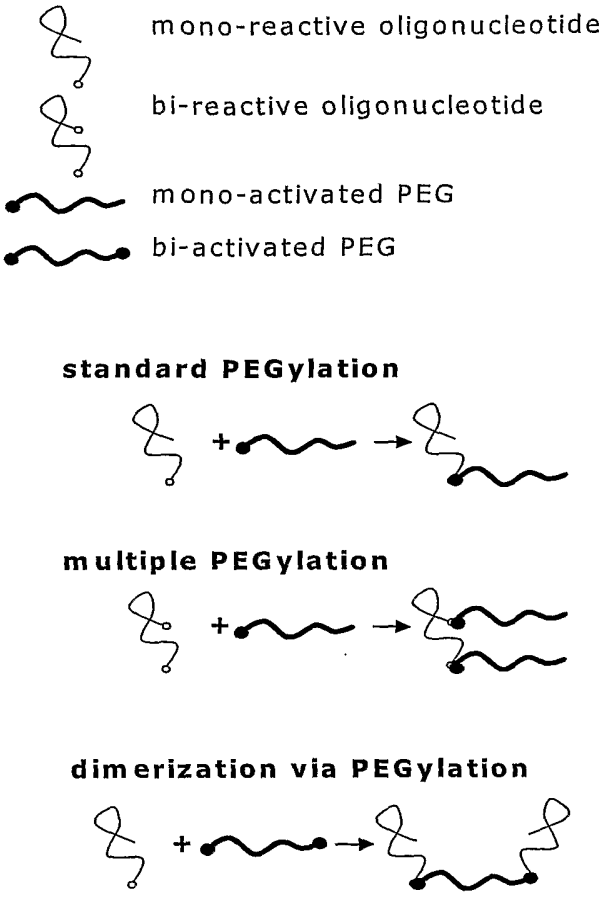

mono-reactive oligonucleotide
bi-reactive oligonucleotide
mono-activated PEG
bi-activated PEG
standard PEGylation
multiple PEGylation
dimerization via PEGylation

Figure 7

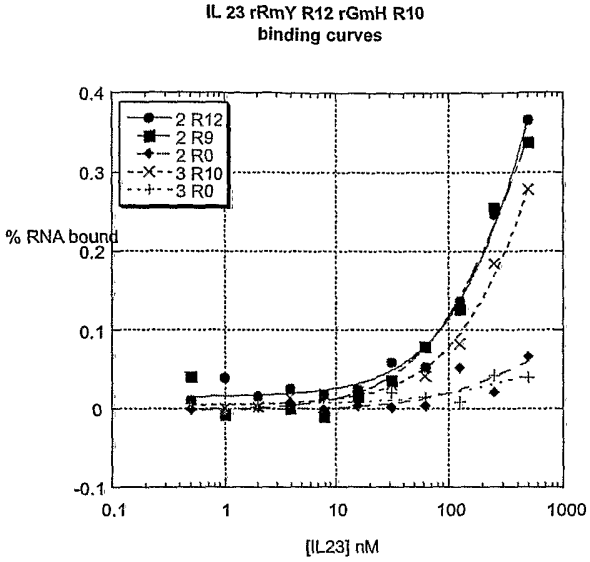
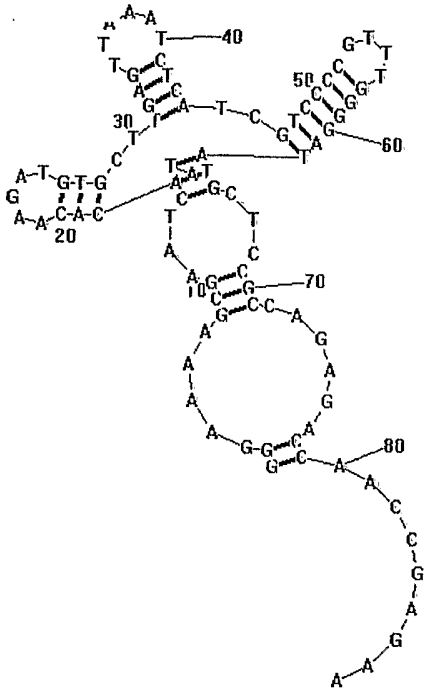

IL 23 rRmY R12 rGmH R10
binding curves
2 R12
2 R9
2 R0
3 R10
3 R0
% RNA bound
0.4
0.3
0.2
0.1
0
-0.1
0.1
1
10
100
1000
[IL23] nM

Figure 8

A)



B)

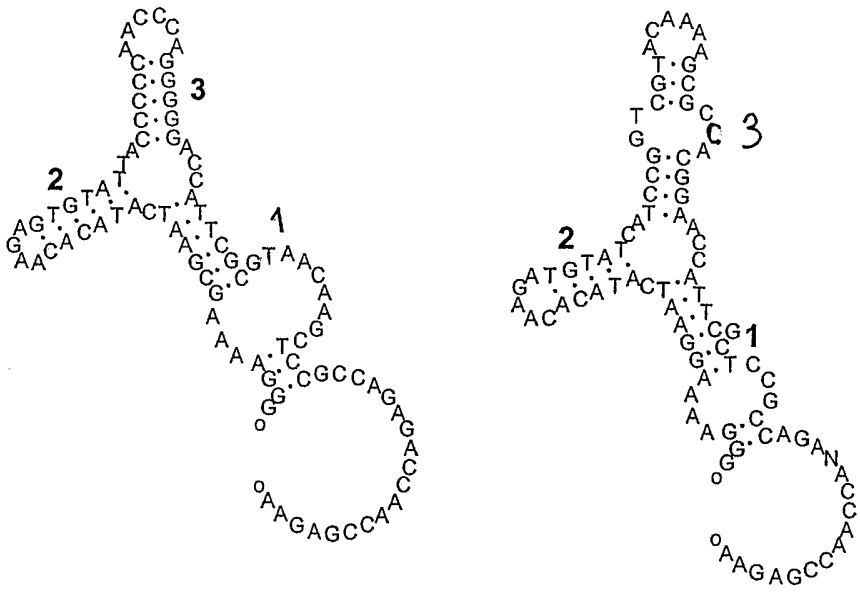
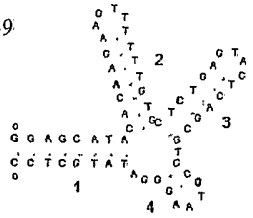


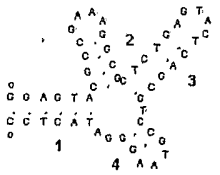
Figure 9

A)

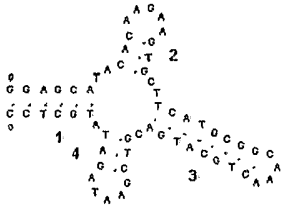
SEQ ID NO 139



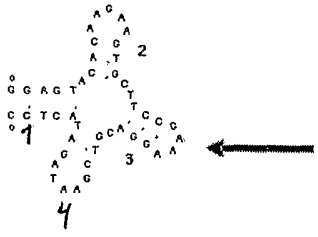
SEQ ID NO 140



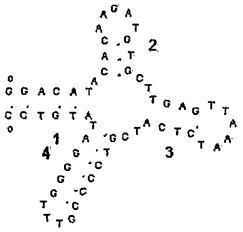
SEQ ID NO 144



SEQ ID NO 145

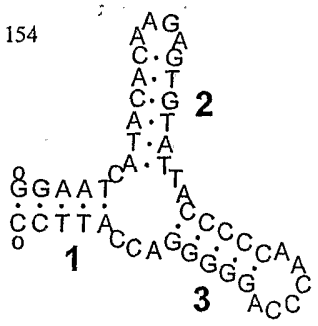


SEQ ID NO 147



B)

SEQ ID NO 154



SEQ ID NO 153

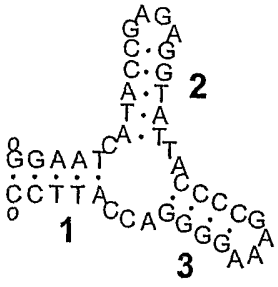
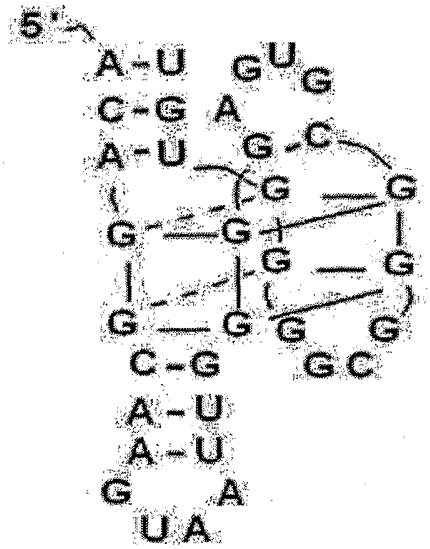


Figure 10



SEQ ID NO 177

Figure 11

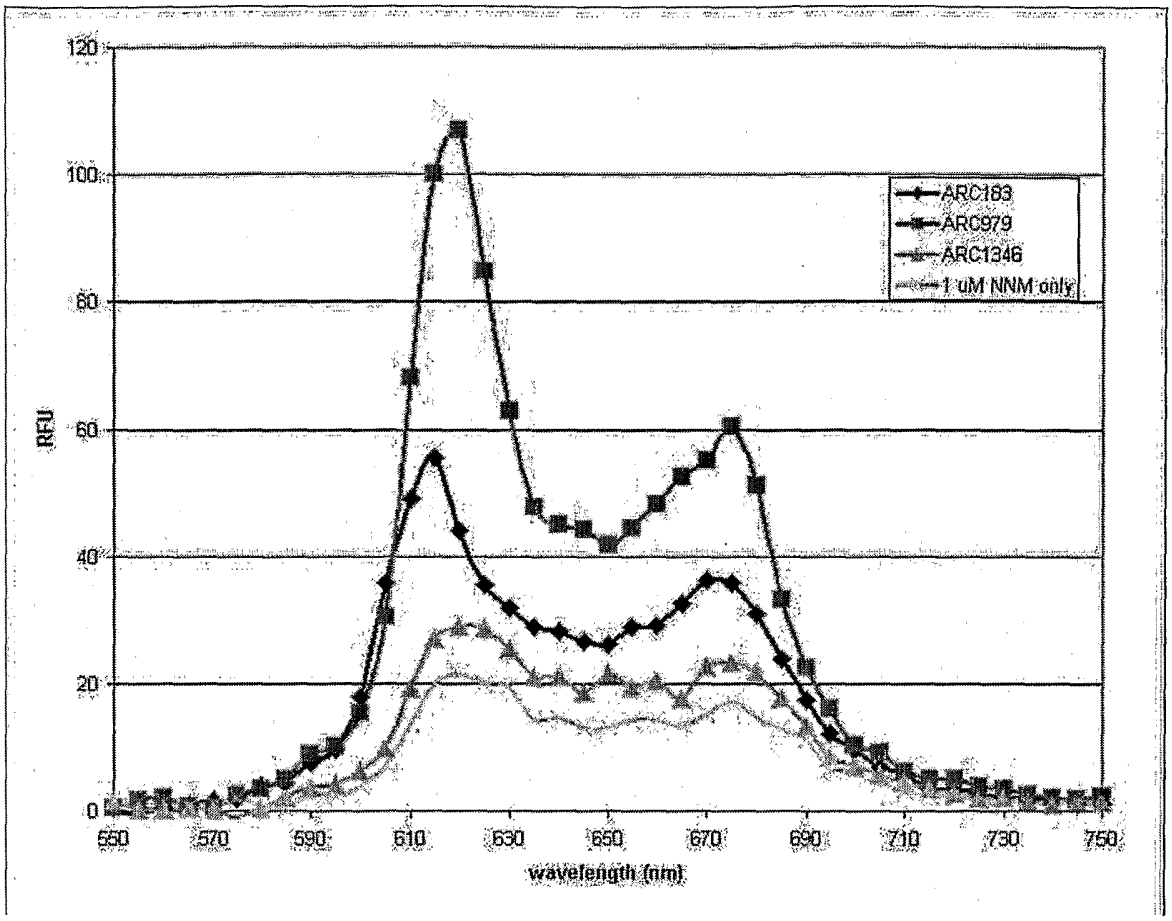

120
100
80
60
40
20
0
RFU
550
570
590
610
630
650
670
690
710
730
750
wavelength (nm)
ARC183
ARC979
ARC1346
1 uM NNM only

Figure 12

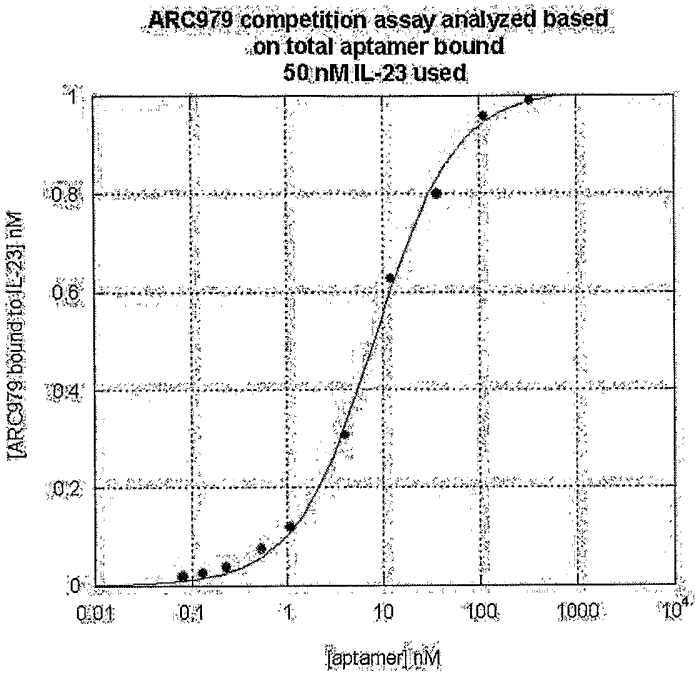

ARC979 competition assay analyzed based on total aptamer bound
50 nM IL-23 used
[ARC979 bound to IL-23] nM
1
0.8
0.6
0.4
0.2
0
0.01
0.1
1
10
100
1000
10^4
[aptamer] nM

Figure 13

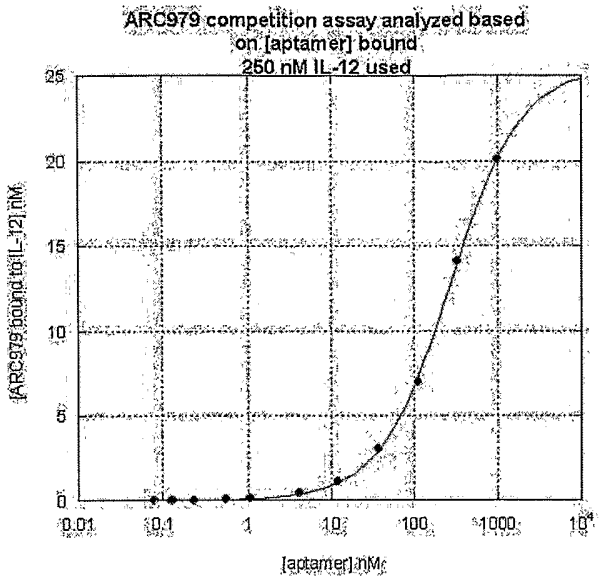

ARC979 competition assay analyzed based on [aptamer] bound
250 nM IL-12 used
[ARC979 bound to IL-12] nM
25
20
15
10
5
0
0.01
0.1
1
10
100
1000
10^4
[aptamer] nM

Figure 14

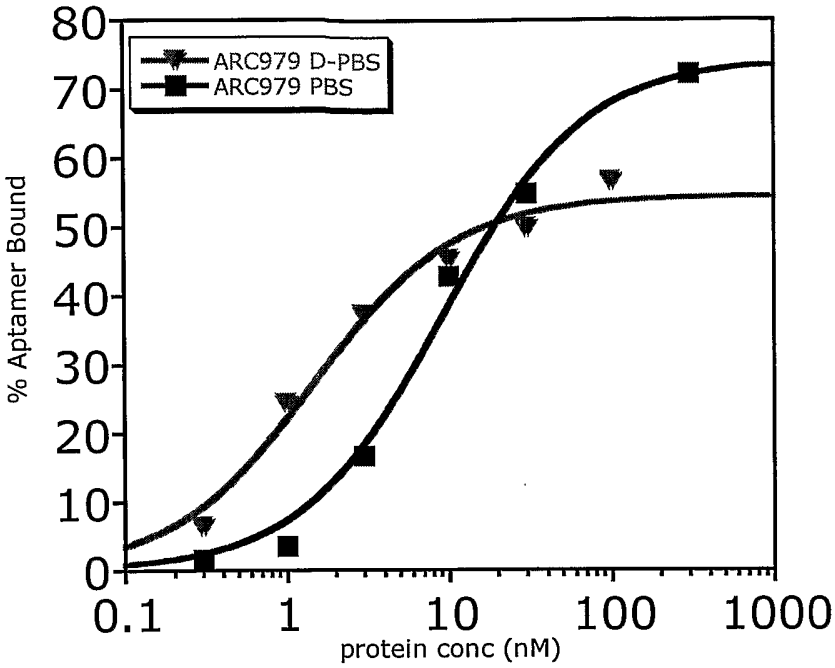

ARC979 D-PBS
ARC979 PBS
% Aptamer Bound
protein conc (nM)
0
10
20
30
40
50
60
70
80
0.1
1
10
100
1000

Figure 15

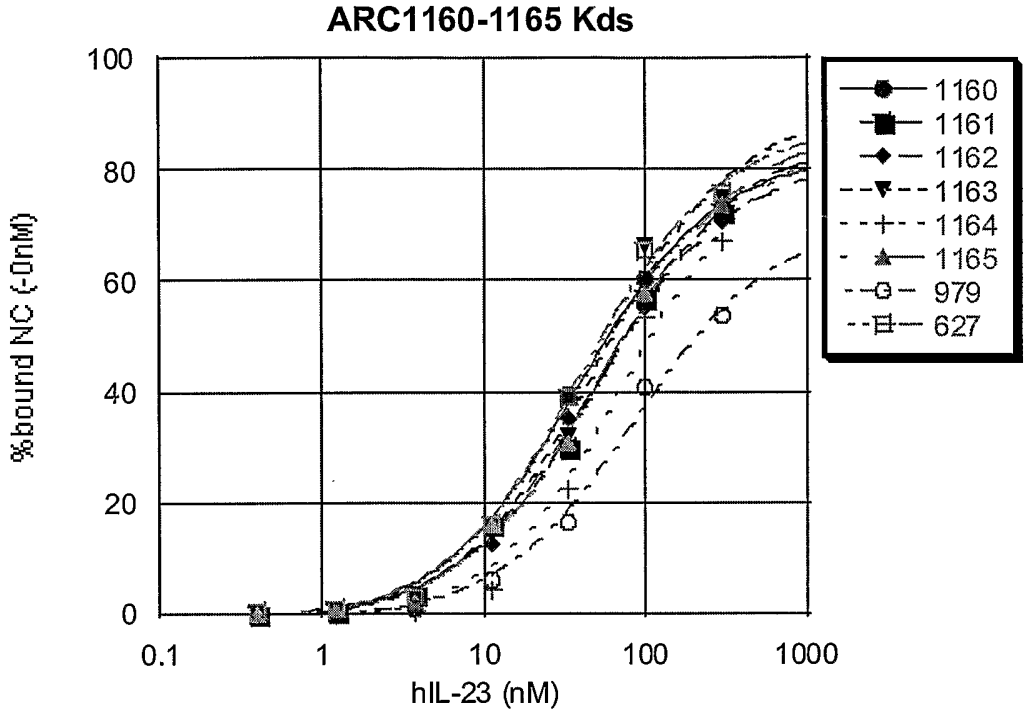

ARC1160-1165 Kds
%bound NC (-0nM)
100
80
60
40
20
0
0.1
1
10
100
1000
hIL-23 (nM)
1160
1161
1162
1163
1164
1165
979
627

Figure 16

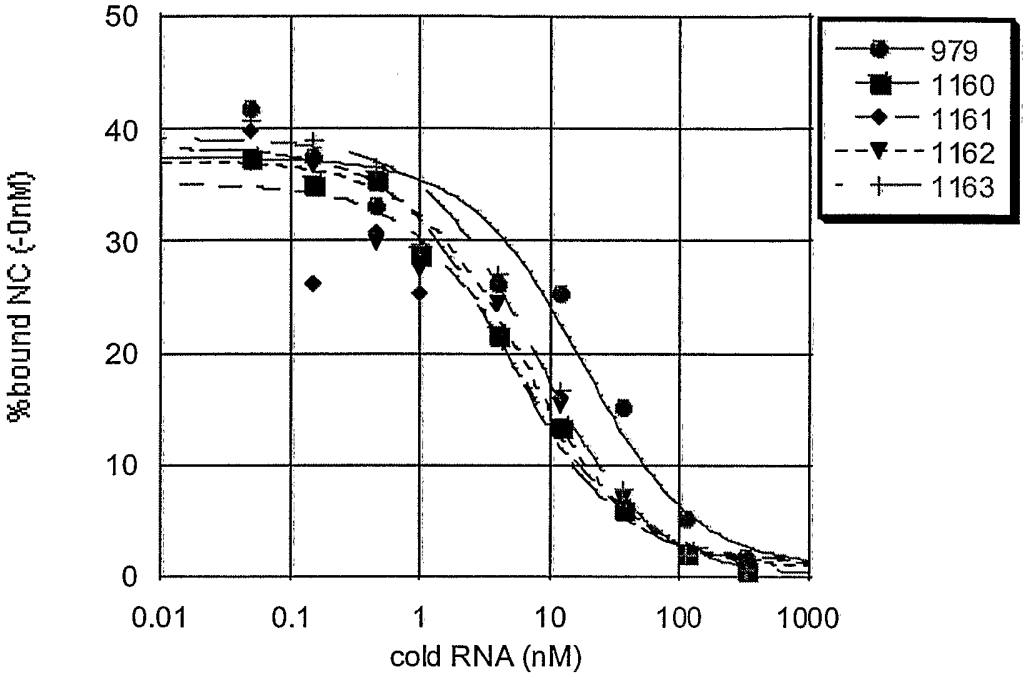

50
40
30
20
10
0
%bound NC (-0nM)
0.01
0.1
1
10
100
1000
cold RNA (nM)
979
1160
1161
1162
1163

Figure 17

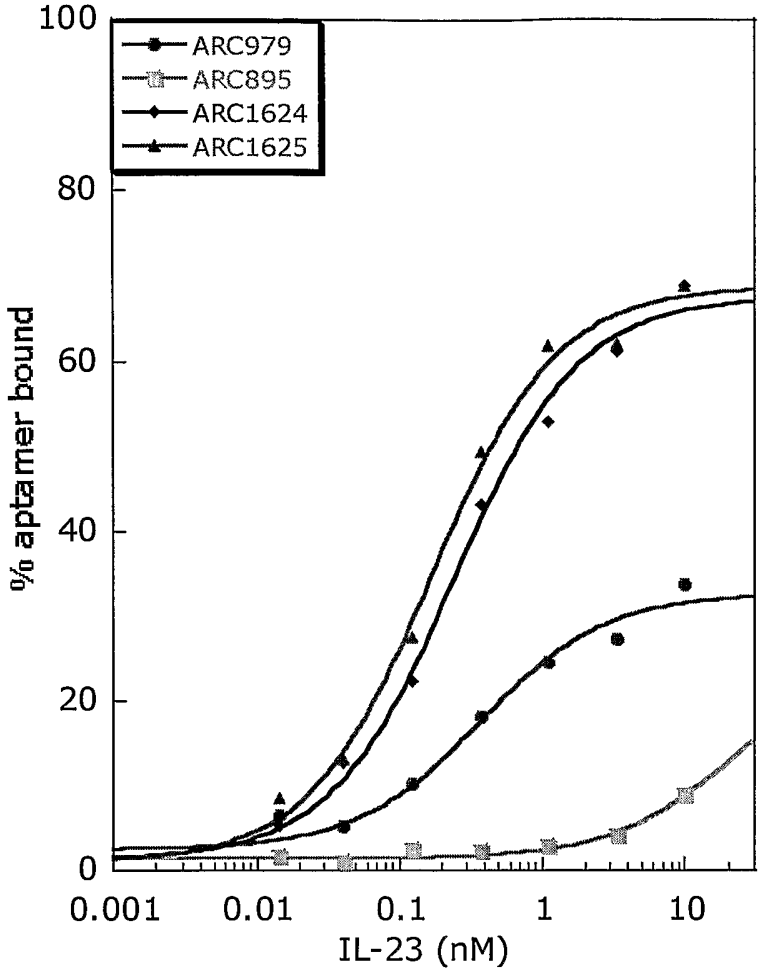

ARC979
ARC895
ARC1624
ARC1625
% aptamer bound
100
80
60
40
20
0
0.001
0.01
0.1
1
10
IL-23 (nM)

Figure 18

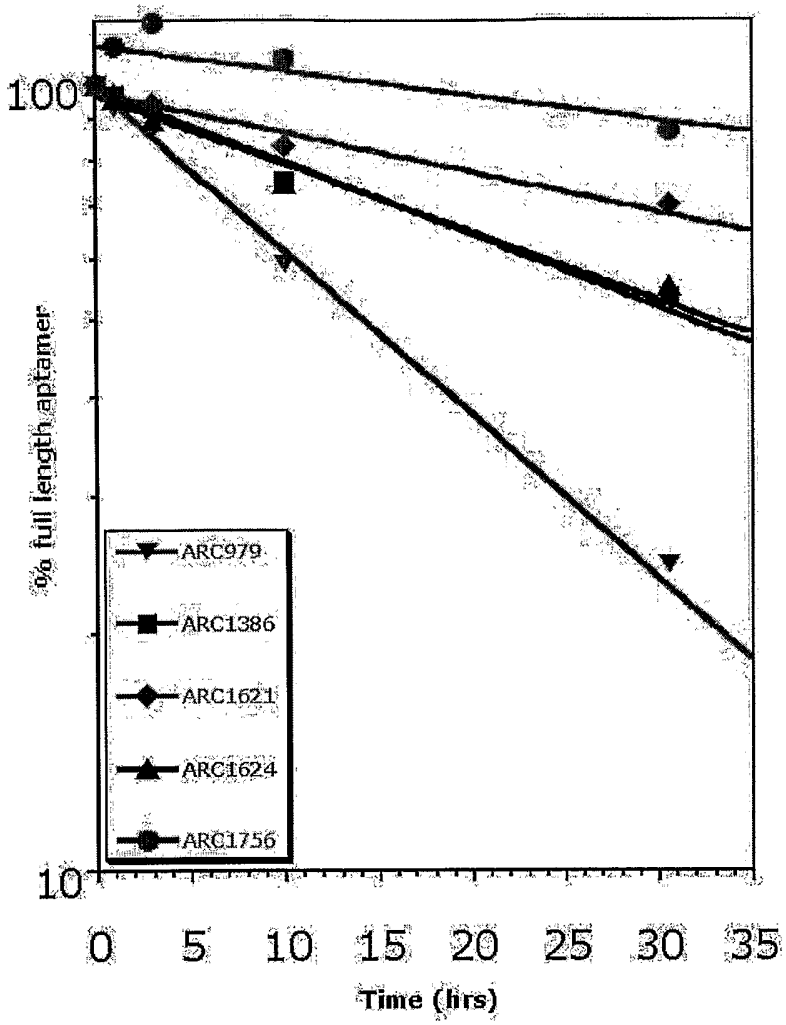

100
10
% full length aptamer
ARC979
ARC1386
ARC1621
ARC1624
ARC1756
0
5
10
15
20
25
30
35
Time (hrs)

Figure 19

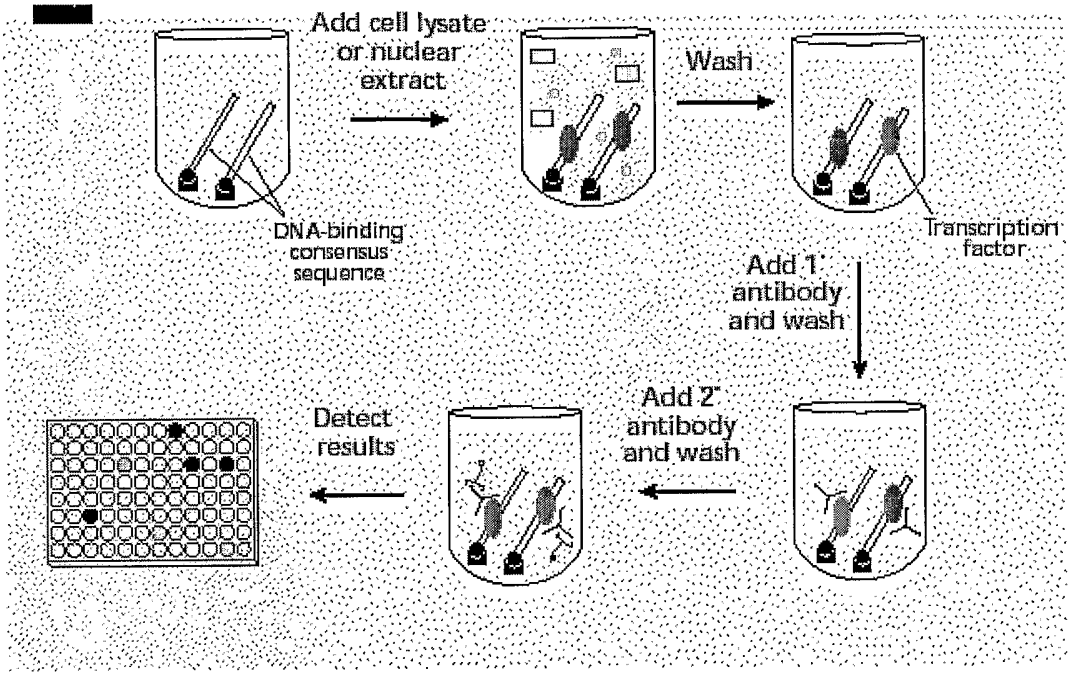

Add cell lysate or nuclear extract
Wash
DNA-binding consensus sequence
Transcription factor
Add 1° antibody and wash
Add 2° antibody and wash
Detect results

Figure 20

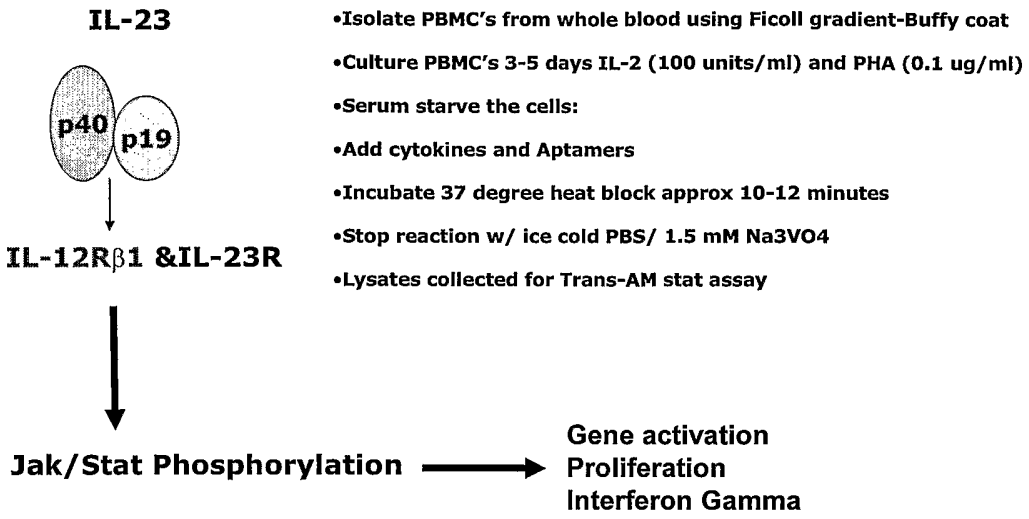

IL-23
p40
p19
IL-12Rβ1 &IL-23R
•Isolate PBMC's from whole blood using Ficoll gradient-Buffy coat
•Culture PBMC's 3-5 days IL-2 (100 units/ml) and PHA (0.1 ug/ml)
•Serum starve the cells:
•Add cytokines and Aptamers
•Incubate 37 degree heat block approx 10-12 minutes
•Stop reaction w/ ice cold PBS/ 1.5 mM Na3VO4
•Lysates collected for Trans-AM stat assay
Jak/Stat Phosphorylation
Gene activation
Proliferation
Interferon Gamma

Figure 21

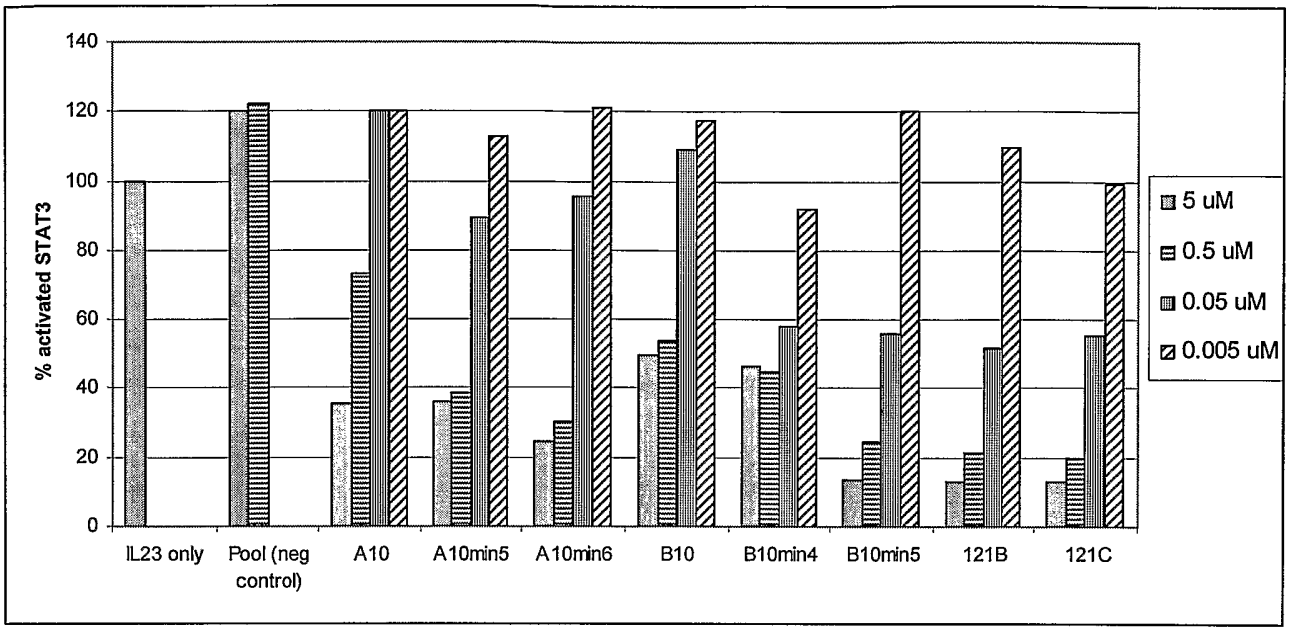

% activated STAT3
140
120
100
80
60
40
20
0
IL23 only
Pool (neg control)
A10
A10min5
A10min6
B10
B10min4
B10min5
121B
121C
5 uM
0.5 uM
0.05 uM
0.005 uM

Figure 22

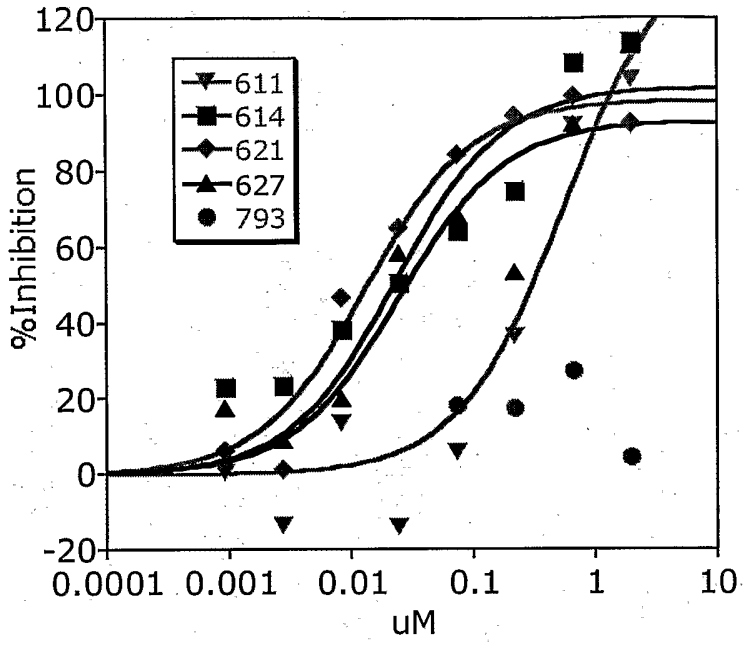

120
100
80
60
40
20
0
-20
%Inhibition
611
614
621
627
793
0.0001
0.001
0.01
0.1
1
10
uM

Figure 23

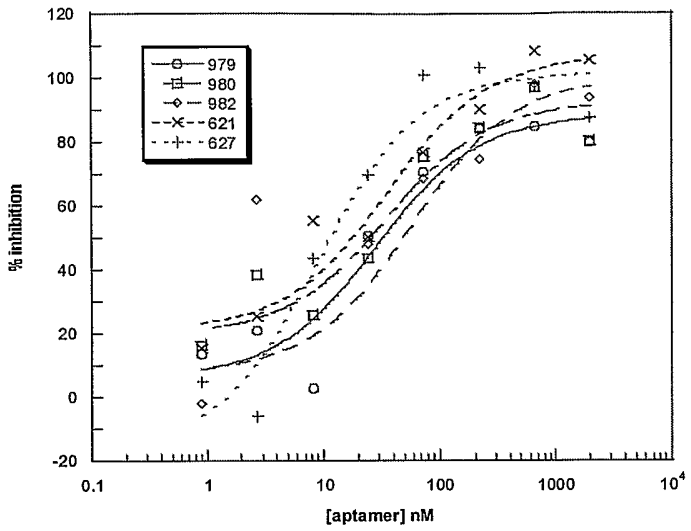

120
100
80
60
40
20
0
-20
% inhibition
0.1
1
10
100
1000
10^4
[aptamer] nM
979
980
982
621
627

Figure 24

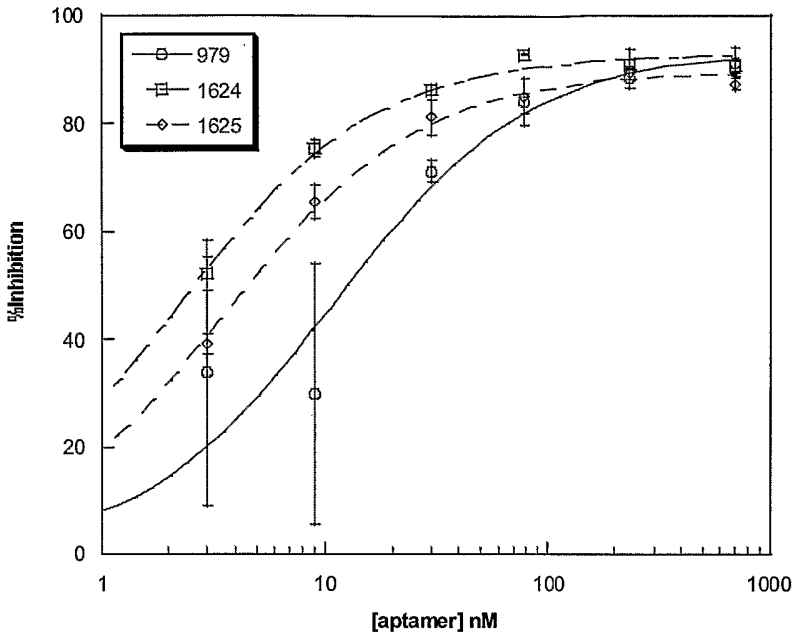
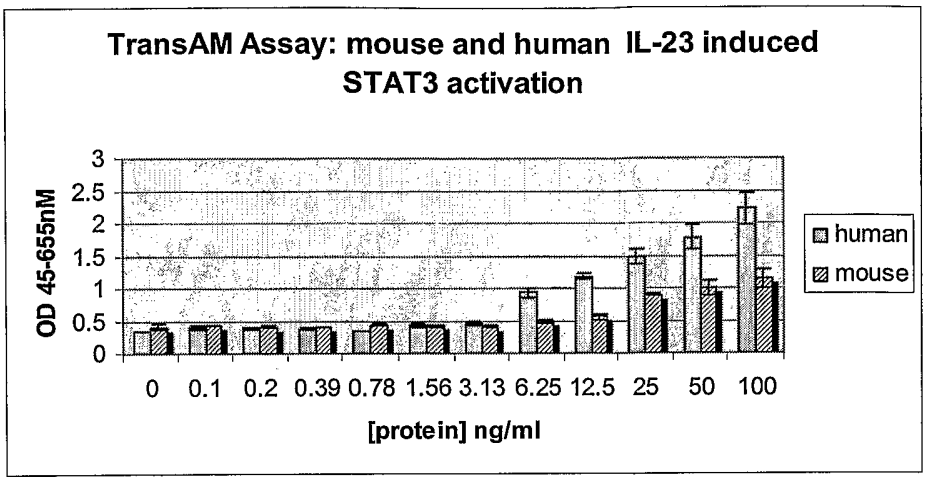

100
80
60
40
20
0
%Inhibition
979
1624
1625
1
10
100
1000
[aptamer] nM

Figure 25


TransAM Assay: mouse and human IL-23 induced STAT3 activation
OD 45-655nM
3
2.5
2
1.5
1
0.5
0
0 0.1 0.2 0.39 0.78 1.56 3.13 6.25 12.5 25 50 100
[protein] ng/ml
human
mouse

SEQUENCE LISTING

<110> Archemix Corp., et al.

<120> Aptamers to the Human IL-12 Cytokine Family and Their Use as Autoimmune Disease Therapeutics

<130> 23239-578-061

<150> 60/550,962
<151> 2004-03-05

<150> 60/608,046
<151> 2004-09-07

<160> 317

<170> PatentIn version 3.3

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cgttctctcc tctccctata gtgagtcgta tta 93

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gttctctcct ctccctatag tgagtcgtat ta 92

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gttctctcct ctccctatag tgagtcgtat ta 92

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<211> 328
<212> PRT
<213> homo sapiens

<400> 4

Met Cys His Gln Gln Leu Val Ile Ser Trp Phe Ser Leu Val Phe Leu
1 5 10 15

Ala Ser Pro Leu Val Ala Ile Trp Glu Leu Lys Lys Asp Val Tyr Val
20 25 30

Val Glu Leu Asp Trp Tyr Pro Asp Ala Pro Gly Glu Met Val Val Leu
35 40 45

Thr Cys Asp Thr Pro Glu Glu Asp Gly Ile Thr Trp Thr Leu Asp Gln
50 55 60

Ser Ser Glu Val Leu Gly Ser Gly Lys Thr Leu Thr Ile Gln Val Lys
65 70 75 80

Glu Phe Gly Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Val
85 90 95

Leu Ser His Ser Leu Leu Leu Leu His Lys Lys Glu Asp Gly Ile Trp
100 105 110

```
Ser Thr Asp Ile Leu Lys Asp Gln Lys Glu Pro Lys Asn Lys Thr Phe
        115                 120                 125

Leu Arg Cys Glu Ala Lys Asn Tyr Ser Gly Arg Phe Thr Cys Trp Trp
    130                 135                 140

Leu Thr Thr Ile Ser Thr Asp Leu Thr Phe Ser Val Lys Ser Ser Arg
145                 150                 155                 160

Gly Ser Ser Asp Pro Gln Gly Val Thr Cys Gly Ala Ala Thr Leu Ser
                165                 170                 175

Ala Glu Arg Val Arg Gly Asp Asn Lys Glu Tyr Glu Tyr Ser Val Glu
            180                 185                 190

Cys Gln Glu Asp Ser Ala Cys Pro Ala Ala Glu Glu Ser Leu Pro Ile
        195                 200                 205

Glu Val Met Val Asp Ala Val His Lys Leu Lys Tyr Glu Asn Tyr Thr
    210                 215                 220

Ser Ser Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn
225                 230                 235                 240

Leu Gln Leu Lys Pro Leu Lys Asn Ser Arg Gln Val Glu Val Ser Trp
                245                 250                 255

Glu Tyr Pro Asp Thr Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Thr
            260                 265                 270

Phe Cys Val Gln Val Gln Gly Lys Ser Lys Arg Glu Lys Lys Asp Arg
        275                 280                 285

Val Phe Thr Asp Lys Thr Ser Ala Thr Val Ile Cys Arg Lys Asn Ala
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Ser Ile Ser Val Arg Ala Gln Asp Arg Tyr Tyr Ser Ser Ser Trp Ser
305                 310                 315                 320

Glu Trp Ala Ser Val Pro Cys Ser
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Ala Gln Gly Arg Ala Val Pro Gly Gly Ser Ser Pro Ala Trp Thr Gln
            20                  25                  30

Cys Gln Gln Leu Ser Gln Lys Leu Cys Thr Leu Ala Trp Ser Ala His
        35                  40                  45

Pro Leu Val Gly His Met Asp Leu Arg Glu Glu Gly Asp Glu Glu Thr
    50                  55                  60

Thr Asn Asp Val Pro His Ile Gln Cys Gly Asp Gly Cys Asp Pro Gln
65                  70                  75                  80

Gly Leu Arg Asp Asn Ser Gln Phe Cys Leu Gln Arg Ile His Gln Gly
                85                  90                  95

Leu Ile Phe Tyr Glu Lys Leu Leu Gly Ser Asp Ile Phe Thr Gly Glu
            100                 105                 110

Pro Ser Leu Leu Pro Asp Ser Pro Val Gly Gln Leu His Ala Ser Leu
        115                 120                 125

Leu Gly Leu Ser Gln Leu Leu Gln Pro Glu Gly His His Trp Glu Thr
    130                 135                 140

Gln Gln Ile Pro Ser Leu Ser Pro Ser Gln Pro Trp Gln Arg Leu Leu
145                 150                 155                 160

Leu Arg Phe Lys Ile Leu Arg Ser Leu Gln Ala Phe Val Ala Val Ala
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Ala Arg Val Phe Ala His Gly Ala Ala Thr Leu Ser Pro
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<212> PRT

<213> homo sapiens

<400> 6

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1               5                  10                  15

Ala Thr Gly Leu His Pro Ala Ala Arg Pro Val Ser Leu Gln Cys Arg
            20                  25                  30

Leu Ser Met Cys Pro Ala Arg Ser Leu Leu Leu Val Ala Thr Leu Val
        35                  40                  45

Leu Leu Asp His Leu Ser Leu Ala Arg Asn Leu Pro Val Ala Thr Pro
    50                  55                  60

Asp Pro Gly Met Phe Pro Cys Leu His His Ser Gln Asn Leu Leu Arg
65                  70                  75                  80

Ala Val Ser Asn Met Leu Gln Lys Ala Arg Gln Thr Leu Glu Phe Tyr
                85                  90                  95

Pro Cys Thr Ser Glu Glu Ile Asp His Glu Asp Ile Thr Lys Asp Lys
            100                 105                 110

Thr Ser Thr Val Glu Ala Cys Leu Pro Leu Glu Leu Thr Lys Asn Glu
        115                 120                 125

Ser Cys Leu Asn Ser Arg Glu Thr Ser Phe Ile Thr Asn Gly Ser Cys
    130                 135                 140

Leu Ala Ser Arg Lys Thr Ser Phe Met Met Ala Leu Cys Leu Ser Ser
145                 150                 155                 160

Ile Tyr Glu Asp Leu Lys Met Tyr Gln Val Glu Phe Lys Thr Met Asn
                165                 170                 175

Ala Lys Leu Leu Met Asp Pro Lys Arg Gln Ile Phe Leu Asp Gln Asn
            180                 185                 190

Met Leu Ala Val Ile Asp Glu Leu Met Gln Ala Leu Asn Phe Asn Ser
        195                 200                 205

Glu Thr Val Pro Gln Lys Ser Ser Leu Glu Glu Pro Asp Phe Tyr Lys
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210 215 220

Thr Lys Ile Lys Leu Cys Ile Leu Leu His Ala Phe Arg Ile Arg Ala
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Val Thr Ile Asp Arg Val Met Ser Tyr Leu Asn Ala Ser
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<210> 8
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nnnngcuccg ccagagacca accgagaa 88

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<212> DNA
<213> artificial

<220>
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<210> 11
<211> 24
<212> RNA
<213> artificial

<220>
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<400> 11
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<210> 12
<211> 24
<212> RNA
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<220>
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<400> 12
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<210> 13
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gucagcuccg ccagagacca accgagaa 88

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<400> 14
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uucggcuccg ccagagacca accgagaa 88

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<212> RNA
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 15
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ucgagcuccg ccagagacca accgagaa 88

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<220>
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<220>
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cagugcuccg ccagagacca accgagaa 88

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ccuagcuccg ccagagacca accgagaa 88

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uugcgcuccg ccagagacca accgagaa 88

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<223> n is a, u, c, or g

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<223> n is a, u, c, or g

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ucngugcucc gccagagacc aaccgagaa 89

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aguggcuccg ccagagacca accgagaa 88

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<212> RNA
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<220>
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<220>
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<220>
<221> misc_feature
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<223> n is a, u, c, or g

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uggagcuccg ccagagacca accgagaa 88

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ggaugcuccg ccagagacca accgagaa 88

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uugcugcucc gccagagacc aaccgagaa 89

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<211> 88
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<213> artificial

<220>
<223> synthetic aptamer

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cagugcuccg ccagagacca accgagaa 88

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aagggagcuc cgccagagac caaccgagaa 90

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uagagcuccg ccagagacca accgagaa 88

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cagagcuccg ccagagacca accgagaa 88

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uucugcuccg ccagagacca accgagaa 88

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ugucgcuccg ccagagacca accgagaa 88

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guagcuccgc cagagaccaa ccgagaa 87

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<220>
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uagagcuccg ccagagacca accgagaa 88

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acaugcuccg ccagagacca accgagaa 88

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uaaugcuccg ccagagacca accgagaa 88

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gcuggcuccg ccagagacca accgagaa 88

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nccguagcuc cgccagagac caaccgagaa 90

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gcgagcuccg ccagagacca accgagaa 88

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<220>
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aacggcuccg ccagagacca accgagaa 88

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gauaugcucc gccagagacc aaccgagaa 89

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<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(88)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 44
gggaaaagcg aaucauacac aagaagugcu ucaugcggca aacugcauga cgucgaauag 60

auaugcuccg ccagagacca accgagaa 88

<210> 45
<211> 88
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(88)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 45
gggaaaagcg aaucauacac aagagaggua ugugguuuug cggagcaacu cgugucagcg 60

gucagcuccg ccagagacca accgagaa 88

<210> 46
<211> 88
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(88)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 46
gggaaaagcg aaucauacac aagaugugcu ugaguuaaau cucaucgucc ccguuugggg 60

auaugcuccg ccagagacca accgagaa 88

<210> 47
<211> 88
<212> RNA
<213> artificial

<220>
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<220>
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<222> (1)..(88)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 47
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auaugcuccg ccagagacca accgagaa 88

<210> 48
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<220>
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<220>
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<400> 48
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cguagcuccg ccagagacca accgagaa 88

<210> 49
<211> 88
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

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ggaagcuccg ccagagacca accgagaa 88

<210> 50
<211> 88
<212> RNA
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<220>
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 50
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auaugcuccg ccagagacca accgagaa 88

<210> 51
<211> 87
<212> RNA
<213> artificial

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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<220>
<221> misc_feature
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<223> n at position 75 is a, u, c, or g

<400> 51
agggaaaagg aaucauacac aagauguauc auccggucgu acaaaagcgc cacggaacca 60

uucgcuccgc caganaccaa ccgagaa 87

<210> 52
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<400> 52
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guaagcuccg ccagagacca accgagaa 88

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<220>
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 53
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auaugcuccg ccagagacca accgagaa 88

<210> 54
<211> 88
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 54
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uauugcuccg ccagagacca accgagaa 88

<210> 55
<211> 88
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<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 55
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uuaugcuccg ccagagacca accgagaa 88

<210> 56
<211> 89
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<400> 56
gggaaaagcg aaucauacac aagacagugu augaggaaga uuacuuccau uccugagcgg 60

uuuucgcucc gccagagacc aaccgagaa 89

<210> 57
<211> 88
<212> RNA
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<220>
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 57
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aguggcuccg ccagagacca accgagaa 88

<210> 58
<211> 88
<212> RNA
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<220>

<223> synthetic aptamer

<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 58
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guucgcuccg ccagagacca accgagaa 88

<210> 59
<211> 88
<212> RNA
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<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 59
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gcaugcuccg ccagagacca accgagaa 88

<210> 60
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<212> RNA
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 60
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cgcgugcucc gccagagacc aaccgagaa 89

<210> 61
<211> 89
<212> RNA
<213> artificial

<220>
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 61
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gugacgcucc gccagagacc aaccgagaa 89

<210> 62
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<212> RNA
<213> artificial

<220>
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<400> 62
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uuaugcuccg ccagagacca accgagaa 88

<210> 63
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

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acaagcuccg ccagagacca accgagaa 88

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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 64
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gugugcuccg ccagagacca accgagaa 88

<210> 65
<211> 88
<212> RNA
<213> artificial

<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

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guucgcuccg ccagagacca accgagaa 88

<210> 66
<211> 88
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(88)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 66
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auaugcuccg ccagagacca accgagaa 88

<210> 67
<211> 39
<212> DNA
<213> artificial

<220>
<223> synthetic primer

<400> 67
taatacgact cactataggg agaggagaga acgttctac 39

<210> 68
<211> 22
<212> DNA
<213> artificial

<220>
<223> synthetic primer

<400> 68
catcgatcga tcgatcgaca gc 22

<210> 69
<211> 22
<212> RNA
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<220>
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<400> 69
gggagaggag agaacguucu ac 22

<210> 70
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<220>
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<400> 70

gcugucgauc gaucgaucga ug 22

<210> 71
<211> 74
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(74)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

<400> 71
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ucgaucgauc gaug 74

<210> 72
<211> 74
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

<400> 72
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ucgaucgauc gaug 74

<210> 73
<211> 74
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

<400> 73
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ucgaucgauc gaug 74

<210> 74
<211> 75
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(75)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

<400> 74
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aucgaucgau cgaug 75

<210> 75
<211> 74
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

<400> 75
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ucgaucgauc gaug 74

<210> 76
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<220>

<223> synthetic aptamer

<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

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aucgaucgau cgaug 75

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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

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aucgaucgau cgaug 75

<210> 78
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<220>
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ucgaucgauc gaug 74

<210> 79
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<212> RNA
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<220>
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aucgaucgau cgaug 75

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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

<400> 80
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gaucgaucga ucgaug 76

<210> 81
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<212> RNA
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<220>
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<220>
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ucgaucgauc gaug 74

<210> 82
<211> 75
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

<400> 82
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aucgaucgau cgaug 75

<210> 83
<211> 75
<212> RNA
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<220>
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<220>
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<400> 83
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aucgaucgau cgaug 75

<210> 84
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<212> RNA
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<220>
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gaucgaucga ucgaug 76

<210> 85
<211> 75
<212> RNA
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<220>
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<220>
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cgaucgaucg aug 73

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<400> 87
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aucgaucgau cgaug 75

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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-O-methyl

<400> 88
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aucgaucgau cgaug 75

<210> 89
<211> 22
<212> DNA
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<220>
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<400> 89
catcgatcga tcgatcgaca gc 22

<210> 90
<211> 22
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<220>
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<400> 90
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<210> 91
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<220>
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<400> 91
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ucgaucgauc gaug 74

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<220>
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<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 92
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aucgaucgau cgaug 75

<210> 93
<211> 75
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aucgaucgau cgaug 75

<210> 94
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ucgaucgauc gaug 74

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<400> 95
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<210> 96
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aucgaucgau cgaug 75

<210> 98
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<220>
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<221> misc_feature
<222> (23)..(52)
<223> n is a, u, c, or g

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gaucgaucau cgaug 75

<210> 99
<211> 39
<212> DNA
<213> artificial

<220>
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<400> 99
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<210> 100
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<220>
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<400> 100
catcgatgat cgatcgatcg ac 22

<210> 101
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<220>
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<210> 102
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<400> 102
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<210> 103
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<222> (1)..(75)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 103
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gaucgaucau cgaug 75

<210> 104
<211> 75
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(75)

<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 104
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gaucgaucau cgaug 75

<210> 105
<211> 75
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(75)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 105
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gaucgaucau cgaug 75

<210> 106
<211> 75
<212> DNA
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<220>
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<220>
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<222> (1)..(75)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 106
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gaucgaucau cgaug 75

<210> 107
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<220>
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<400> 107
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gaucgaucau cgaug 75

<210> 108
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<212> DNA
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<220>
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aucgaucauc gaug 74

<210> 109
<211> 74
<212> DNA
<213> artificial

<220>
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<220>
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aucgaucauc gaug 74

<210> 110
<211> 74
<212> DNA
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<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 110
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aucgaucauc gaug 74

<210> 111
<211> 76
<212> DNA
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<220>
<223> synthetic aptamer

<220>
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<400> 111
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cgaucgauca ucgaug 76

<210> 112
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<212> DNA
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gaucgaucau cgaug 75

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<212> DNA
<213> artificial

<220>
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<220>
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ucgaucaucg aug 73

<210> 114
<211> 74
<212> DNA
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<220>
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<220>
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aucgaucauc gaug 74

<210> 115
<211> 74
<212> DNA
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<220>
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<220>
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<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 115
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aucgaucauc gaug 74

<210> 116
<211> 75
<212> DNA
<213> artificial

<220>
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<220>
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<400> 116
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gaucgaucau cgaug 75

<210> 117
<211> 76
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<220>
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<220>
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<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 117
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cgaucgauca ucgaug 76

<210> 118
<211> 75
<212> DNA

<213> artificial

<220>
<223> synthetic aptamer

<220>
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<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 118
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gaucgaucau cgaug 75

<210> 119
<211> 76
<212> RNA
<213> artificial

<220>
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines are 2'-fluoro

<220>
<221> misc_feature
<222> (18)..(57)
<223> n is a, u, c, or g

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cgaccucucu gcuagc 76

<210> 120
<211> 34
<212> DNA
<213> artificial

<220>
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<400> 120
taatacgact cactatagga gcgcactcag ccac 34

<210> 121
<211> 19

<212> DNA
<213> artificial

<220>
<223> synthetic primer

<400> 121
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<210> 122
<211> 17
<212> RNA
<213> artificial

<220>
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<400> 122
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<210> 123
<211> 19
<212> RNA
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<220>
<223> synthetic fixed region

<400> 123
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<210> 124
<211> 75
<212> RNA
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<220>
<223> synthetic aptamer

<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 124
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gaccucucug cuagc 75

<210> 125
<211> 76

<212> RNA
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<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 125
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cgaccucucu gcuagc 76

<210> 126
<211> 76
<212> RNA
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<220>
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 126
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cgaccucucu gcuagc 76

<210> 127
<211> 75
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 127
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gaccucucug cuagc 75

<210> 128
<211> 76
<212> RNA
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 128
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cgaccucucu gcuagc 76

<210> 129
<211> 76
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

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cgaccucucu gcuagc 76

<210> 130
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 130
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cgaccucucu gcuagc 76

<210> 131
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<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<220>
<221> misc_feature
<222> (46)..(46)
<223> n is a, u, c, or g

<400> 131
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cgaccucucu gcuagc 76

<210> 132
<211> 76
<212> RNA
<213> artificial

<220>
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 132
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cgaccucucu gcuagc 76

<210> 133
<211> 76
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<220>
<221> misc_feature
<222> (12)..(12)
<223> n is a,u, c or g

<220>
<221> misc_feature
<222> (62)..(62)
<223> n is a,u, c or g

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cnaccucucu gcuagc 76

<210> 134
<211> 76
<212> RNA
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<220>
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<221> modified_base
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<220>
<221> misc_feature
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ngaccucucu gcuagc 76

<210> 135
<211> 60
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 135
ggagaucaua cacaagaagu uuuuugugcu cugaguacuc agcguccgua agggaucucc 60

<210> 136
<211> 48
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 136
ggagucugag uacucagcgu ccguaaggga uaugcuccgc cagacucc 48

<210> 137
<211> 39
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 137
ggaguuacuc agcguccgua agggauaugc uccgacucc 39

<210> 138
<211> 50
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 138
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<210> 139
<211> 61
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 139
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c 61

<210> 140
<211> 51
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 140
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<210> 141
<211> 65
<212> RNA
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<220>
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<220>
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<400> 141
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gcucc 65

<210> 142
<211> 55
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 142
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<210> 143
<211> 47
<212> RNA
<213> artificial

<220>
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<220>
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 143
ggaucauaca caagaagugc uucacgaaag ugacgucgaa uagaucc 47

<210> 144
<211> 60
<212> RNA
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<220>
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<220>
<221> modified_base
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<400> 144
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<210> 145
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<212> RNA
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<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 145
ggaguacaca agaagugcuu ccgaaaggac gucgaauaga uacucc 46

<210> 146
<211> 30
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 146
gguuaaaucu caucgucccc guuuggggau 30

<210> 147
<211> 57
<212> RNA
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<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 147
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<210> 148
<211> 47
<212> RNA
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<220>
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<220>
<221> modified_base
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<400> 148
ggcauacacg agagugcugu cgaaagacuc ggccgagagg cuaugcc 47

<210> 149
<211> 47
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<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 149
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<210> 150
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<220>
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<210> 151
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<220>
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<400> 151
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<210> 152
<211> 49
<212> RNA
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<220>
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<220>
<221> modified_base
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<400> 152
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<210> 153
<211> 42
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 153
ggaaucauac cgagagguau uaccccgaaa ggggaccauu cc 42

<210> 154
<211> 48
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 154
ggaaucauac acaagagugu auuaccccca acccaggggg accauucc 48

<210> 155
<211> 57
<212> RNA
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<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 155
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<210> 156
<211> 39
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<400> 156
ggaggcgcca cgcugaguau agacggaagc uccgccucc 39

<210> 157
<211> 58
<212> RNA
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<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 157
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<210> 158
<211> 38
<212> RNA
<213> artificial

<220>
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<220>
<221> modified_base
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<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 158
ggcgguccgc auugauguca guuaugcgua gcuccgcc 38

<210> 159
<211> 37
<212> DNA
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<220>
<223> synthetic aptamer

<220>
<221> modified_base
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<400> 159
acagcgccgg ugggcgggca uuggguggau gcgcugu 37

<210> 160
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<212> DNA
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<220>
<223> synthetic aptamer

<220>
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<400> 160
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<210> 161
<211> 35
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<220>
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<220>
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<400> 161
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<212> DNA
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<220>
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<220>
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<210> 163
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<212> DNA
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<400> 163
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<210> 164
<211> 30
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(30)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 164
ggcgguacgg ggaguguggg uuggggccgg 30

<210> 165
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(36)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 165
cgauauaggc gguacggggg gagugggcug gggucg 36

<210> 166
<211> 31
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(31)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 166
uaauuggggga gugcgggcgg ggggucgauc g 31

<210> 167
<211> 27
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(27)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 167
ggugggggagu gcgggcgggg ggucgcc 27

<210> 168
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 168
acaggcaagg uaauugggga gugcgggcgg ggugu 35

<210> 169
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 169
ccaggcaagg uaauugggga gugcgggcgg ggugg 35

<210> 170
<211> 29
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(29)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 170
ggcaagguaa uugggaagug ugggcgggg 29

<210> 171
<211> 29
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(29)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 171
ggcaagguaa uuggguagug agggcgggg 29

<210> 172
<211> 29
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(29)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 172
ggcaagguaa uuggggagug cgggcuggg 29

<210> 173
<211> 29
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(29)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 173
ggcaagguaa uugggaagug ugggcuggg 29

<210> 174
<211> 29
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(29)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 174
ggcaagguaa uuggguagug agggcuggg 29

<210> 175
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 175
acaggcaagg uaauugggua gugagggcug ggugu 35

<210> 176
<211> 41
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(40)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (41)..(41)
<223> n at position 41 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 176
gauguuggca aguaauuggg gagugcgggc gggguucauc n 41

<210> 177
<211> 34
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 177
acaggcaagu aauuggggag ugcgggcggg gugu 34

<210> 178
<211> 34
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 178
ccaggcaagu aauuggggag ugcgggcggg gugg 34

<210> 179
<211> 25
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(25)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 179
ggcgguuacg ggggaugcgg guggg 25

<210> 180
<211> 30
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(30)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 180
ggcgguuacg ggggaugcgg gugggacagg 30

<210> 181
<211> 26
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(26)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 181
ggcaaguaau uggggagugc gggcgg 26

<210> 182
<211> 32
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(32)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 182
acaggcaagu aauuggggag ugcgggcggu gu 32

<210> 183
<211> 28
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(28)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 183
ggcgguacgg ggaguguggg uuggggcc 28

<210> 184
<211> 28
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(28)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 184
ggcgguacgg ggaguguggg cuggggcc 28

<210> 185
<211> 22
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(22)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 185
gguacgggga guguggguug gg 22

<210> 186
<211> 22
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(22)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 186
gguacgggga gugugggcug gg 22

<210> 187
<211> 27
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(27)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 187
ggcgguacgg ggaguguggg uugggcc 27

<210> 188
<211> 27
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(27)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 188
ggcgguacgg ggaguguggg cugggcc 27

<210> 189
<211> 21
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(21)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 189
gguacgggga gugugggguug g 21

<210> 190
<211> 21
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(21)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 190
gguacgggga gugugggcug g 21

<210> 191
<211> 28
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(28)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 191
ggcgguacgg ggggaguggg cuggggguc 28

<210> 192
<211> 27
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(27)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 192
ggcgguacgg ggggaguggg cuggguc 27

<210> 193
<211> 28
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(28)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 193
ggcgguacgg ggagaguggg cuggggguc 28

<210> 194
<211> 22
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(22)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 194
gguacggggg gagugggcug gg 22

<210> 195
<211> 21
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(21)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 195
gguacggggg gagugggcug g 21

<210> 196
<211> 22
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(22)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 196
gguacgggga gagugggcug gg 22

<210> 197
<211> 25
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(25)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 197
ggcgguacgg ggggaguggg cuggg 25

<210> 198
<211> 25
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(25)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<400> 198
ggcgguacgg ggaguguggg uuggg 25

<210> 199
<211> 43
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(43)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 199
gggcacucag ccacaggugg cuuaauacug uaaagacgug ccc 43

<210> 200
<211> 48
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(48)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 200
ggagcgcacu cagccaccgg cuuaauaucc aauaggaacg uucgcucu 48

<210> 201
<211> 48
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(48)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 201
gggcacucag ccacagcucg guggcuuaau aucuauguga acgugccc 48

<210> 202
<211> 43
<212> RNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(43)
<223> all purines (A and G) are 2'-OH, all pyrimidines (C and U) are 2'-fluoro

<400> 202
gggcacucag ccaccuuggg cuuaauaccu aucggaugug ccc 43

<210> 203
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (2)..(2)
<223> n at position 2 is a phosphorothioate linker

<400> 203
ancaggcaag taattgggga gtgcgggcgg ggtgt 35

<210> 204
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (3)..(3)
<223> n at position 3 is a phosphorothioate linker

<400> 204
acnaggcaag uaauugggga gugcgggcgg ggugu 35

<210> 205
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (7)..(7)

<223> n at position 7 is a phosphorothioate linker

<400> 205
acaggcnaag uaauugggga gugcgggcgg ggugu 35

<210> 206
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (8)..(8)
<223> n at position 8 is a phosphorothioate linker

<400> 206
acaggcanag uaauugggga gugcgggcgg ggugu 35

<210> 207
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (9)..(9)
<223> n at position 9 is a phosphorothioate linker

<400> 207
acaggcaang uaauugggga gugcgggcgg ggugu 35

<210> 208
<211> 35
<212> DNA

<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (10)..(10)
<223> n at position 10 is a phosphorothioate linker

<400> 208
acaggcaagn uaauugggga gugcgggcgg ggugu 35

<210> 209
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (11)..(11)
<223> n at position 11 is a phosphorothioate linker

<400> 209
acaggcaagu naauugggga gugcgggcgg ggugu 35

<210> 210
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)

<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (12)..(12)
<223> n at position 12 is a phosphorothioate linker

<400> 210
acaggcaagu anauugggga gugcgggcgg ggugu 35

<210> 211
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (13)..(13)
<223> n at position 13 is a phosphorothioate linker

<400> 211
acaggcaagu aanuugggga gugcgggcgg ggugu 35

<210> 212
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (14)..(14)
<223> n at position 14 is a phosphorothioate linker

<400> 212

acaggcaagu aaunugggga gugcgggcgg ggugu 35

<210> 213
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (15)..(15)
<223> n at position 15 is a phosphorothioate linker

<400> 213
acaggcaagu aauunggggа gugcgggcgg ggugu 35

<210> 214
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (19)..(19)
<223> n at position 19 is a phosphorothioate linker

<400> 214
acaggcaagu aauuggggna gugcgggcgg ggugu 35

<210> 215
<211> 35
<212> DNA
<213> artificial

<220>

<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (20)..(20)
<223> n at position 20 is a phosphorothioate linker

<400> 215
acaggcaagu aauuggggan gugcgggcgg ggugu 35

<210> 216
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (21)..(21)
<223> n at position 21 is a phosphorothioate linker

<400> 216
acaggcaagu aauuggggag nugcgggcgg ggugu 35

<210> 217
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (22)..(22)
<223> n at position 22 is a phosphorothioate linker

<400> 217
acaggcaagu aauuggggag ungcgggcgg ggugu 35

<210> 218
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (23)..(23)
<223> n at position 23 is a phosphorothioate linker

<400> 218
acaggcaagu aauuggggag ugncgggcgg ggugu 35

<210> 219
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (27)..(27)
<223> n at position 27 is a phosphorothioate linker

<400> 219
acaggcaagu aauuggggag ugcgggncgg ggugu 35

<210> 220
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (28)..(28)
<223> n at position 28 is a phosphorothioate linker

<400> 220
acaggcaagu aauuggggag ugcgggcngg ggugu 35

<210> 221
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is a phosphorothioate linker

<400> 221
acaggcaagu aauuggggag ugcgggcgng ggugu 35

<210> 222
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (33)..(33)
<223> n at position 33 is a phosphorothioate linker

<400> 222
acaggcaagu aauuggggag ugcgggcggg gungu 35

<210> 223
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (34)..(34)
<223> n at position 34 is a phoshorothioate linker

<400> 223
acaggcaagu aauuggggag ugcgggcggg gugnu 35

<210> 224
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)

<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 224
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 225
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 1, wherein adenosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 225
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 226
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 3, wherein adenosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 226
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 227
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 4, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 227
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 228
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 5, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 228
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 229
<211> 35
<212> DNA

<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 7, wherein adenosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 229
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 230
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 8, wherein adenosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 230
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 231
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 9, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 231
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 232
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 11, wherein adenosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 232
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 233
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 12, wherein

adenosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 233
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 234
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 15, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 234
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 235
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 16, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature

<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 235
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 236
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 17, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 236
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 237
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 18, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 237

acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 238
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 19, wherein adenosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 238
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 239
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 20, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 239
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 240
<211> 35

<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 22, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 240
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 241
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 24, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 241
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 242
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 25, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 242
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 243
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 26, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 243
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 244
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)

<223> all purines (A and G) are deoxy, except at position 28, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 244
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 245
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 29, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 245
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 246
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 30 wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>

<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 246
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 247
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 31 wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 247
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 248
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 33 wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 248
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 249
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at positions 1 and 3, wherein adenosine is 2'-O-methyl and position 33 wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 249
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 250
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at positions 7 and 8, wherein adenosine is 2'-O-methyl and position 15 wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 250
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 251
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (4)..(4)
<223> n at position 4 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 251
acanggcaag uaauugggga gugcgggcgg ggugun 36

<210> 252
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (5)..(5)
<223> n at position 5 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 252
acagngcaag uaauugggga gugcgggcgg ggugun 36

<210> 253
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (6)..(6)
<223> n at position 6 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 253
acaggncaag uaauugggga gugcgggcgg ggugun 36

<210> 254
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (16)..(16)
<223> n at position 16 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)

<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 254
acaggcaagu aauugnggga gugcgggcgg ggugun 36

<210> 255
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (17)..(17)
<223> n at position 17 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 255
acaggcaagu aauuggngga gugcgggcgg ggugun 36

<210> 256
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (18)..(18)
<223> n at position 18 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 256
acaggcaagu aauugggnga gugcgggcgg ggugun 36

<210> 257
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (24)..(24)
<223> n at position 24 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 257
acaggcaagu aauuggggag ugcngggcgg ggugun 36

<210> 258
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature

<222> (25)..(25)
<223> n at position 25 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 258
acaggcaagu aauuggggag ugcgnggcgg ggugun 36

<210> 259
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (26)..(26)
<223> n at position 26 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 259
acaggcaagu aauuggggag ugcggngcgg ggugun 36

<210> 260
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (30)..(30)
<223> n at position 30 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 260
acaggcaagu aauuggggag ugcgggcggn ggugun 36

<210> 261
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (31)..(31)
<223> n at position 31 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 261
acaggcaagu aauuggggag ugcgggcggg ngugun 36

<210> 262
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base

<222> (1)..(35)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (32)..(32)
<223> n at position 32 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 262
acaggcaagu aauuggggag ugcgggcggg gnugun 36

<210> 263
<211> 41
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(40)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (12)..(12)
<223> n at position 12 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (21)..(21)
<223> n at position 21 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (23)..(23)
<223> n at position 23 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (25)..(25)
<223> n at position 25 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (27)..(27)

<223> n at position 27 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (32)..(32)
<223> n at position 32 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (41)..(41)
<223> n at position 41 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 263
acaggcaagu anauugggga ngnungncgg gncggggugu n 41

<210> 264
<211> 37
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(36)
<223> all purines (A and G) are deoxy, all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (4)..(4)
<223> n at position 4 is a PEG spacer

<220>
<221> misc_feature
<222> (33)..(33)
<223> n at position 33 is a PEG spacer

<220>
<221> misc_feature
<222> (37)..(37)
<223> n at position 37 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 264
acanggcaag uaauugggga gugcgggcgg ggnugun 37

<210> 265
<211> 39
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(38)
<223> all purines (A and G) are deoxy, except at positions 2 and 38, wherein guanosine is 2'-O-methyl; all pyrmidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (5)..(5)
<223> n at position 5 is a PEG spacer

<220>
<221> misc_feature
<222> (34)..(34)
<223> n at position 34 is a PEG spacer

<220>
<221> misc_feature
<222> (39)..(39)
<223> n at position 39 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 265
cgcanggcaa guaauugggg agugcgggcg gggnugcgn 39

<210> 266
<211> 29
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(28)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 266
ggcaaguaau uggggagugc gggcggggn 29

<210> 267

<211> 29
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(28)
<223> all purines (A and G) are deoxy, except at positions 4 ad 5, wherein adenosine is 2'-O-methyl, and position 12 wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 267
ggcaaguaau uggggagugc gggcggggn 29

<210> 268
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (9)..(9)
<223> n at position 9 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (18)..(18)
<223> n at position 18 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (20)..(20)
<223> n at position 20 is a phosphorothioate linker

<220>

<221> misc_feature
<222> (22)..(22)
<223> n at position 22 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (24)..(24)
<223> n at position 24 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 268
ggcaaguana uuggggangn ungncgggnc ggggn 35

<210> 269
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at positions 4 and 5, wherein adenosine is 2'-O-methyl, and position 13, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (9)..(9)
<223> n at position 9 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (18)..(18)
<223> n at position 18 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (20)..(20)
<223> n at position 20 is a phosphorothioate linker

<220>

<221> misc_feature
<222> (22)..(22)
<223> n at position 22 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (24)..(24)
<223> n at position 24 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 269
ggcaaguana uuggggangn ungncgggnc ggggn 35

<210> 270
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 1 wherein adenosine is 2'-O-methyl, and position 33, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 270
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 271
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at positions 8 and 11 wherein adenosine is 2'-O-methyl, and position 9, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 271
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 272
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at position 19 wherein adenosine is 2'-O-methyl, and positions 18, 20, and 22, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 272
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 273
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>

<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at positions 26, 28, and 29, wherein guanosine is 2'-O-methyl; all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 273
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 274
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at positions 1, 8, 11, and 19, wherein adenosine is 2'-O-methyl, and positions 9, 18, 20, 22, 26, 28, 29, and 33, wherein guanosine is 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 274
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 275
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>

<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, except at positions 16, 17, 24, 25, 30, and 31, wherein guanosine is 2'-O-methyl; all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 275
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 276
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are 2'-O-methyl, except at positions 3, 7, and 12, wherein adenosine is deoxy; and positions 4, 5, and 15, wherein guanosine is deoxy

<220>
<221> modified_base
<222> (1)..(34)
<223> all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 276
acaggcaagu aauuggggag ugcgggcggg gugun 35

<210> 277
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>

<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (4)..(4)
<223> n at position 4 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 277
acangcaagu aauuggggag ugcgggcggg gugun 35

<210> 278
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (5)..(5)
<223> n at position 5 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 278
acagncaagu aauuggggag ugcgggcggg gugun 35

<210> 279
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (4)..(5)
<223> n at positions 4 and 5 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 279
acanncaagu aauuggggag ugcgggcggg gugun 35

<210> 280
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (9)..(9)
<223> n at position 9 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 280
acaggcaanu aauuggggag ugcgggcggg gugun 35

<210> 281
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (15)..(15)
<223> n at position 15 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 281
acaggcaagu aauungggag ugcgggcggg gugun 35

<210> 282
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (16)..(16)
<223> n at position 16 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 282
acaggcaagu aauugnggag ugcgggcggg gugun 35

<210> 283

<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (17)..(17)
<223> n at position 17 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 283
acaggcaagu aauuggngag ugcgggcggg gugun 35

<210> 284
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (18)..(18)
<223> n at position 18 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 284
acaggcaagu aauugggnag ugcgggcggg gugun 35

<210> 285
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (15)..(16)
<223> n at positions 15 and 16 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 285
acaggcaagu aauunnggag ugcgggcggg gugun 35

<210> 286
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (16)..(17)
<223> n at positions 16 and 17 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 286
acaggcaagu aauugnngag ugcgggcggg gugun 35

<210> 287
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (17)..(18)
<223> n at positions 17 and 18 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 287
acaggcaagu aauuggnnag ugcgggcggg gugun 35

<210> 288
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (15)..(18)
<223> n at positions 15, 16, 17, and 18 are deoxy inosine

<220>
<221> misc_feature

<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 288
acaggcaagu aauunnnnag ugcgggcggg gugun 35

<210> 289
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (20)..(20)
<223> n at position 20 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 289
acaggcaagu aauuggggan ugcgggcggg gugun 35

<210> 290
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (22)..(22)
<223> n at position 22 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 290
acaggcaagu aauuggggag uncgggcggg gugun 35

<210> 291
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (24)..(24)
<223> n at position 24 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 291
acaggcaagu aauuggggag ugcnggcggg gugun 35

<210> 292
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>

<221> misc_feature
<222> (25)..(25)
<223> n at position 25 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 292
acaggcaagu aauuggggag ugcgngcggg gugun 35

<210> 293
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (26)..(26)
<223> n at position 26 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 293
acaggcaagu aauuggggag ugcggncggg gugun 35

<210> 294
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are

2'-O-methyl

<220>
<221> misc_feature
<222> (24)..(25)
<223> n at positions 24 and 25 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 294
acaggcaagu aauuggggag ugcnngcggg gugun 35

<210> 295
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (25)..(26)
<223> n at positions 25 and 26 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 295
acaggcaagu aauuggggag ugcgnncggg gugun 35

<210> 296
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>

<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (24)..(26)
<223> n at positions 24, 25, and 26 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 296
acaggcaagu aauuggggag ugcnnncggg gugun 35

<210> 297
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (28)..(28)
<223> n at position 28 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 297
acaggcaagu aauuggggag ugcgggcngg gugun 35

<210> 298
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 298
acaggcaagu aauuggggag ugcgggcgng gugun 35

<210> 299
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (30)..(30)
<223> n at position 30 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 299
acaggcaagu aauuggggag ugcgggcggn gugun 35

<210> 300
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (31)..(31)
<223> n at position 31 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 300
acaggcaagu aauuggggag ugcgggcggg nugun 35

<210> 301
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (28)..(29)
<223> n at positions 28 and 29 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 301
acaggcaagu aauuggggag ugcgggcnng gugun 35

<210> 302

<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (29)..(30)
<223> n at positions 29 and 30 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 302
acaggcaagu aauuggggag ugcgggcgnn gugun 35

<210> 303
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (30)..(31)
<223> n at positions 30 and 31 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 303
acaggcaagu aauuggggag ugcgggcggn nugun 35

<210> 304
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (28)..(31)
<223> n at positions 28, 29, 30 and 31 are deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 304
acaggcaagu aauuggggag ugcgggcnnn nugun 35

<210> 305
<211> 35
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(34)
<223> all purines (A and G) are deoxy, all pyrimidines (C and U) are 2'-O-methyl

<220>
<221> misc_feature
<222> (33)..(33)
<223> n at position 33 is deoxy inosine

<220>
<221> misc_feature
<222> (35)..(35)
<223> n at position 35 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 305
acaggcaagu aauuggggag ugcgggcggg gunun 35

<210> 306
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, except at positions 17, 18, 25, 26, 31 and 32, wherein guanosine is 2'-O-methyl; all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (3)..(3)
<223> n at position 3 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 306
acnaggcaag uaauugggga gugcgggcgg ggugun 36

<210> 307
<211> 37
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(36)
<223> all purines (A and G) are deoxy, except at positions 18, 19, 26, 27, 32, and 33, wherein guanosine is 2'-O-methyl; all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (4)..(4)
<223> n at position 4 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (6)..(6)
<223> n at position 6 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (37)..(37)
<223> n at position 37 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 307
acangngcaa guaauugggg agugcgggcg gggugun 37

<210> 308
<211> 41
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(40)
<223> all purines (A and G) are deoxy, except at positions 22, 23, 30, 31, 36 and 37, wherein guanosine is 2'-O-methyl; all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (7)..(7)
<223> n at position 7 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (9)..(9)
<223> n at position 9 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (11)..(11)
<223> n at position 11 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (14)..(14)
<223> n at position 14 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (16)..(16)
<223> n at position 16 is a phosphorothioate linker

<220>

<221> misc_feature
<222> (20)..(20)
<223> n at position 20 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (41)..(41)
<223> n at position 41 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 308
acaggcnana ngunanauun ggggagugcg ggcggggugu n 41

<210> 309
<211> 39
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(38)
<223> all purines (A and G) are deoxy, except at positions 16, 17, 28, 29, 34 and 35, wherein guanosine is 2'-O-methyl; all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (18)..(18)
<223> n at position 18 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (20)..(20)
<223> n at position 20 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (22)..(22)
<223> n at position 22 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (25)..(25)
<223> n at position 25 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (39)..(39)
<223> n at position 39 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 309

acaggcaagu aauugggngn angungcggg cggggugun 39

<210> 310
<211> 38
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(37)
<223> all purines (A and G) are deoxy, except at positions 16, 17, 24, 25, 33, and 34, wherein guanosine is 2'-O-methyl; all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (26)..(26)
<223> n at position 26 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (31)..(31)
<223> n at position 31 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (38)..(38)
<223> n at position 38 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 310
acaggcaagu aauuggggag ugcggngcng ngggugun 38

<210> 311
<211> 36
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(35)
<223> all purines (A and G) are deoxy, except at positions 16, 17, 24,

25, 30 and 31, wherein guanosine is 2'-O-methyl; all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (33)..(33)
<223> n at position 33 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (36)..(36)
<223> n at position 36 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 311
acaggcaagu aauuggggag ugcgggcggg gungun 36

<210> 312
<211> 52
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(51)
<223> all purines (A and G) are deoxy, except at positions 25, 26, 37, 38, 46 and 47, wherein guanosine is 2'-O-methyl; all pyrimidines are 2'-O-methyl

<220>
<221> misc_feature
<222> (3)..(3)
<223> n at position 3 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (5)..(5)
<223> n at position 5 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (7)..(7)
<223> n at position 7 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (10)..(10)
<223> n at position 10 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (12)..(12)

<223> n at position 12 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (14)..(14)
<223> n at position 14 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (17)..(17)
<223> n at position 17 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (19)..(19)
<223> n at position 19 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (23)..(23)
<223> n at position 23 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (27)..(27)
<223> n at position 27 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (31)..(31)
<223> n at position 31 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (34)..(34)
<223> n at position 34 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (39)..(39)
<223> n at position 39 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (42)..(42)
<223> n at position 42 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (44)..(44)
<223> n at position 44 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (49)..(49)
<223> n at position 49 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (52)..(52)
<223> n at position 52 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 312
acnangngcn anangunana uungggngna ngungcggng cngngggung un 52

<210> 313
<211> 39
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(38)
<223> all purines (A and G) are 2'-O-methyl, except at positions 4, 9, and 15, wherein adenosine is deoxy, and positions 5, 6, and 19, wherein guanosine is deoxy; all pyrimdines are 2'-O-methyl

<220>
<221> misc_feature
<222> (3)..(3)
<223> n at position 3 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (8)..(8)
<223> n at position 8 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (14)..(14)
<223> n at position 14 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (18)..(18)
<223> n at position 18 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (39)..(39)
<223> n at position 39 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 313
acnaggcnaa guanauungg ggagugcggg cggggugun 39

<210> 314
<211> 43
<212> DNA
<213> artificial

<220>
<223> synthetic aptamer

<220>
<221> modified_base
<222> (1)..(42)
<223> all purines (A and G) are 2'-O-methyl, except at positions 4, 9, and 15, and 25 wherein adenosine is deoxy, and positions 5, 6, and 19, 23, 27, and 30 wherein guanosine is deoxy; all pyrimdines are 2'-O-methyl

<220>
<221> misc_feature
<222> (3)..(3)
<223> n at position 3 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (8)..(8)
<223> n at position 8 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (14)..(14)
<223> n at position 14 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (18)..(18)
<223> n at position 18 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (22)..(22)
<223> n at position 22 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (24)..(24)
<223> n at position 24 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (26)..(26)
<223> n at position 26 is a phosphorothioate linker

<220>

<221> misc_feature
<222> (29)..(29)
<223> n at position 29 is a phosphorothioate linker

<220>
<221> misc_feature
<222> (43)..(43)
<223> n at position 43 is an inverted orientation deoxy thymidine (3'-3' linked)

<400> 314
acnaggcnaa guanauungg gngnangung cgggcggggu gun 43

<210> 315
<211> 335
<212> PRT
<213> Mus musculus

<400> 315

Met Cys Pro Gln Lys Leu Thr Ile Ser Trp Phe Ala Ile Val Leu Leu
1 5 10 15

Val Ser Pro Leu Met Ala Met Trp Glu Leu Glu Lys Asp Val Tyr Val
20 25 30

Val Glu Val Asp Trp Thr Pro Asp Ala Pro Gly Glu Thr Val Asn Leu
35 40 45

Thr Cys Asp Thr Pro Glu Glu Asp Asp Ile Thr Trp Thr Ser Asp Gln
50 55 60

Arg His Gly Val Ile Gly Ser Gly Lys Thr Leu Thr Ile Thr Val Lys
65 70 75 80

Glu Phe Leu Asp Ala Gly Gln Tyr Thr Cys His Lys Gly Gly Glu Thr
85 90 95

Leu Ser His Ser His Leu Leu Leu His Lys Lys Glu Asn Gly Ile Trp
100 105 110

Ser Thr Glu Ile Leu Lys Asn Phe Lys Asn Lys Thr Phe Leu Lys Cys
115 120 125

Glu Ala Pro Asn Tyr Ser Gly Arg Phe Thr Cys Ser Trp Leu Val Gln
130 135 140

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Arg Asn Met Asp Leu Lys Phe Asn Ile Lys Ser Ser Ser Ser Ser Pro
145                 150                 155                 160

Asp Ser Arg Ala Val Thr Cys Gly Met Ala Ser Leu Ser Ala Glu Lys
                165                 170                 175

Val Thr Leu Asp Gln Arg Asp Tyr Glu Lys Tyr Ser Val Ser Cys Gln
            180                 185                 190

Glu Asp Val Thr Cys Pro Thr Ala Glu Glu Thr Leu Pro Ile Glu Leu
        195                 200                 205

Ala Leu Glu Ala Arg Gln Gln Asn Lys Tyr Glu Asn Tyr Ser Thr Ser
    210                 215                 220

Phe Phe Ile Arg Asp Ile Ile Lys Pro Asp Pro Pro Lys Asn Leu Gln
225                 230                 235                 240

Met Lys Pro Leu Lys Asn Ser Gln Val Glu Val Ser Trp Glu Tyr Pro
                245                 250                 255

Asp Ser Trp Ser Thr Pro His Ser Tyr Phe Ser Leu Lys Phe Phe Val
            260                 265                 270

Arg Ile Gln Arg Lys Lys Glu Lys Met Lys Glu Thr Glu Glu Gly Cys
        275                 280                 285

Asn Gln Lys Gly Ala Phe Leu Val Glu Lys Thr Ser Thr Glu Val Gln
    290                 295                 300

Cys Lys Gly Gly Asn Val Cys Val Gln Ala Gln Asp Arg Tyr Tyr Asn
305                 310                 315                 320

Ser Ser Cys Ser Lys Trp Ala Cys Val Pro Cys Arg Val Arg Ser
                325                 330                 335
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<210> 316
<211> 196
<212> PRT
<213> mus musculus

<400> 316

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Met Leu Asp Cys Arg Ala Val Ile Met Leu Trp Leu Leu Pro Trp Val
1               5                   10                  15
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Thr Gln Gly Leu Ala Val Pro Arg Ser Ser Ser Pro Asp Trp Ala Gln
20 25 30

Cys Gln Gln Leu Ser Arg Asn Leu Cys Met Leu Ala Trp Asn Ala His
35 40 45

Ala Pro Ala Gly His Met Asn Leu Leu Arg Glu Glu Glu Asp Glu Glu
50 55 60

Thr Lys Asn Asn Val Pro Arg Ile Gln Cys Glu Asp Gly Cys Asp Pro
65 70 75 80

Gln Gly Leu Lys Asp Asn Ser Gln Phe Cys Leu Gln Arg Ile Arg Gln
85 90 95

Gly Leu Ala Phe Tyr Lys His Leu Leu Asp Ser Asp Ile Phe Lys Gly
100 105 110

Glu Pro Ala Leu Leu Pro Asp Ser Pro Met Glu Gln Leu His Thr Ser
115 120 125

Leu Leu Gly Leu Ser Gln Leu Leu Gln Pro Glu Asp His Pro Arg Glu
130 135 140

Thr Gln Gln Met Pro Ser Leu Ser Ser Ser Gln Gln Trp Gln Arg Pro
145 150 155 160

Leu Leu Arg Ser Lys Ile Leu Arg Ser Leu Gln Ala Phe Leu Ala Ile
165 170 175

Ala Ala Arg Val Phe Ala His Gly Ala Ala Thr Leu Thr Glu Pro Leu
180 185 190

Val Pro Thr Ala
195

<210> 317
<211> 215
<212> PRT
<213> mus musculus

<400> 317

Met Cys Gln Ser Arg Tyr Leu Leu Phe Leu Ala Thr Leu Ala Leu Leu
1 5 10 15

Asn His Leu Ser Leu Ala Arg Val Ile Pro Val Ser Gly Pro Ala Arg
20 25 30

Cys Leu Ser Gln Ser Arg Asn Leu Leu Lys Thr Thr Asp Asp Met Val
35 40 45

Lys Thr Ala Arg Glu Lys Leu Lys His Tyr Ser Cys Thr Ala Glu Asp
50 55 60

Ile Asp His Glu Asp Ile Thr Arg Asp Gln Thr Ser Thr Leu Lys Thr
65 70 75 80

Cys Leu Pro Leu Glu Leu His Lys Asn Glu Ser Cys Leu Ala Thr Arg
85 90 95

Glu Thr Ser Ser Thr Thr Arg Gly Ser Cys Leu Pro Pro Gln Lys Thr
100 105 110

Ser Leu Met Met Thr Leu Cys Leu Gly Ser Ile Tyr Glu Asp Leu Lys
115 120 125

Met Tyr Gln Thr Glu Phe Gln Ala Ile Asn Ala Ala Leu Gln Asn His
130 135 140

Asn His Gln Gln Ile Ile Leu Asp Lys Gly Met Leu Val Ala Ile Asp
145 150 155 160

Glu Leu Met Gln Ser Leu Asn His Asn Gly Glu Thr Leu Arg Gln Lys
165 170 175

Pro Pro Val Gly Glu Ala Asp Pro Tyr Arg Val Lys Met Lys Leu Cys
180 185 190

Ile Leu Leu His Ala Phe Ser Thr Arg Val Val Thr Ile Asn Arg Val
195 200 205

Met Gly Tyr Leu Ser Ser Ala
210 215