[0009] In one aspect, the present disclosure refers to an saRNA, wherein the saRNA comprises as an antisense sequence GACCAGUGACAAUGACCGCUU. In another aspect, the present disclosure refers to a pharmaceutical composition comprising the saRNA as disclosed herein and at least one pharmaceutically acceptable carrier. One example discloses a pharmaceutical composition comprising a saRNA that targets a C/EBPα transcript and at least one pharmaceutically acceptable carrier.

[0010] Another example discloses a method of modulating the metabolic pathway of a subject comprising administering a saRNA that targets a C/EBPα transcript to said subject.

[0011] Another example discloses a method of inhibiting the proliferation of hyperproliferative cells comprising contacting the cells with a saRNA that targets a C/EBPα transcript.

[0012] Another example discloses a method of treating or preventing hyperproliferative disorders comprising administering a saRNA that targets a C/EBPα transcript to a subject with said hyperproliferative disorder.

[0013] Another example discloses a method of regulating epithelial-mesenchymal transition of a cell comprising contact the cell with a saRNA that targets a C/EBPα transcript.

[0014] Yet another example discloses a method of regulating stem cell differentiation and pluripotency comprising contact said stem cell with a saRNA that targets a C/EBPα transcript.

[0059] FIG. 43 Western blots. A-B: Western Blot of HepG2 cells transfected with C/EBPα-saRNA to study the ratio of C/EBPα and C/EBPβ protein isoforms.

[0060] FIG. 44: Gene expression. A-C: Relative expression of C/EBP family members after transfection of C/EBPα-saRNA and C/EBPα-siRNA.

[0061] FIG. 45: C/EBPα expression levels in U87-fLuc cells 4 days after transfection with C/EBPα-saRNA (CAW1) or transduction with C/EBPα-saRNA in a miRNA design cloned into clinical retroviral replicating vector (miCAW1). Scrambled siRNA (con) and retrovirus vector delivering a small inhibitory hairpin against firefly Luciferase (mifLuc) used as negative controls.

[0062] FIG. 46: C/EBPα-AW1 miRNA insert sequence (SEQ ID NO: 383) and common miR-30 context (SEQ ID NO: 384).

[0063] FIG. 47: Serum albumin levels in cirrhotic patients treated with MTL-501 (C/EBPα-saRNA-dendrimers).

[0064] Fig. 48: White blood cell count in cirrhotic patients treated with MTL-501 (C/EBPα-saRNA-dendrimers).

[0065] Fig. 49: Expression in DU145 cells. A-C show CEBPA, ALB, p21 mRNA expression levels in DU145 cells with 50 nM C/EBPα-saRNA transfection. D-F show CEBPA, ALB, p21 mRNA expression levels in DU145 cells with 10 nM C/EBPα-saRNA transfection.

[0066] Fig. 50 Expression in DU145 cells. A-B show CEBPA and ecCEBPA mRNA expression levels in DU145 cells with 50nM C/EBPα-saRNA transfection. C-D show CEBPA and ecCEBPA mRNA expression levels in DU145 cells with 10nM C/EBPα-saRNA transfection. E shows ESTS of CEBPA mRNA, AW665812 levels in DU145 cells with 50nM C/EBPα-saRNA transfection.

[0067] Fig. 51 shows CEBPA, ALB, p21 relative expression levels in DU145 cells after transfection with modified C/EBPα-saRNA.

[0068] Fig. 52: Relative Expression. A-D show CEBPA mRNA relative expression levels in HepG2 cells with normal transfection (T), reverse transfection (RT), one dose (1), or two doses (2) of 50 nM C/EBPα-saRNA transfection.

[0069] Fig. 53: Proliferation and time study. A shows reduced proliferation for CEBPA-saRNA treated HepG2 cells. B-G show CEBPA, p21 and albumin mRNA levels in HepG2 cells at different time points after C/EBPα-saRNA transfection.

albumin level increased to normal range. The serum albumin level maintained at normal range until day 34.

[00497] In another study, C/EBPα-saRNA-dendrimer was used to increase white blood cell count in cirrhotic patients. Three doses of C/EBPα-saRNA-dendrimer MTL-501 were given to cirrhotic patients in day 1, day 3, and day 5 at about 0.5mg/kg. White blood cell count of said patients were measured as shown in Fig. 48. A single dose given to Patient 1 contained 10mg C/EBPα-saRNA and 30mg dendrimer. A single dose given to Patient 3 contained 20mg C/EBPα-saRNA and 60mg dendrimer. For both patients, an increase in white blood count was observed after first dose at day 2 and a bigger increase was observed at day 9 after 3 doses of C/EBPα-saRNA-dendrimer MTL-501 treatment.

[00498] The human study results suggest that C/EBPα-saRNA-dendrimer may be used as a therapeutic agent to treat patients with liver diseases and to increase white blood cell counts in patients in need thereof.

Example 14. Strand selection/identification and cleavage studies

[00499] Inverted abasic modifications at 5' terminus have been shown to prevent loading into the guide position in Ago2 complex. Antisense strand (AS) and sense strand (SS) of C/EBPA-saRNA were blocked with an inverted abasic modification at 5' end (**b**) and C/EBPA mRNA expression was measured and the impact of blocking AS and/or SS strands on C/EBPA mRNA expression was determined. All saRNA were synthesized and annealed in water. RP-HPLC has 90% purity. Sequences of the oligonucleotide samples were shown in the following table.

Table 12. Sequences of oligonucleotides used in the studies

Oligo ID	Sequence (SS on top)	SEQ ID No.	Notes
NC-500000	5'-ACUACUGAGUGACAGUAGAUU-3' 3'-UUUGAUGACUCACUGUCAUCU-5	33 255	Non-specific 'scramble' (negative control)
CEBPA-AW01-5000	5'-CGGUCAUUGUCACUGGUCAUU-3' 3'-UUGCCAGUAACAGUGACCAGU-5'	256 257	'AW1' (positive control)
CEBPA-AW01-5000	5'-bCGGUCAUUGUCACUGGUCAUU-3 3'-UUGCCAGUAACAGUGACCAGU-5'	258 257	Inverted abasic modification on SS only
CEBPA-AW01-5000	5'-CGGUCAUUGUCACUGGUCAUU-3' 3'-UUGCCAGUAACAGUGACCAGUb-5	256 259	Inverted abasic modification on AS only
CEBPA-AW01-5000	5'-bCGGUCAUUGUCACUGGUCAUU-3 3'-UUGCCAGUAACAGUGACCAGUb-5	258 259	Inverted abasic modification on both SS and AS (negative control)
CEBPA-AW01-5005	5'-CGGUCAUUCAGACUGGUCAUU-3' 3'-UUGCCAGUAAGUCUGACCAGU-5'	260 261	Mutated central three base pairs

[00500] DU145 cells were reverse transfected with 50nM oligonucleotide samples at seeding, forward transfected 24 hours later, and harvested at 72 hours. C/EBPA, ALB, and p21

Nucleic acid/Lipofectamine Complex Formation

[00521] All reagents were brought to room temperature before proceeding. 1.5ul /3ul (for 24well/6well plate) Lipofectamine 2000 was added to 50ul/125ul Optimem per transfection (for 24well/6well plate). saRNA was added to 50ul/125 Optimem (for 24well/6well plate) for a final concentration of 50nM. Diluted Lipofectamine and saRNAs were combined, mixed gently, and incubated for 15 minutes at room temperature.

Transfecting DU145 Cells

[00522] Cells were detached with Trypsin-EDTA, and excess amount of full media was added thereafter to deactivate trypsin. If necessary the cell number is determined using a hemocytometer. Cells were plated at the appropriate seeding density. Nucleic acid/Lipofectamine complexation was gently added to cells by dispensing as droplets and swirling the addition with the tip immersed in the buffer. 24 hours later, cells should be around the 40%-50% confluency. Cells were retransfected 24 hours later, following same procedure for nucleic acid/Lipofectamine complexation but the complex was added to attached cells freshly fed with 400ul growth media – a forward transfection. Cells were retransfected again 24 hours later, using the same forward transfection technique used in the previous step. RNA extraction was performed 24 hours after third transfection.

Example 17. Modification screen of C/EBPα-saRNA

[00523] Modification screen studies were conducted with C/EBPα-saRNA having various modifications. Sequences of the oligonucleotide samples were shown in the following table. DU145 cells were reverse transfected with 50 nM oligonucleotides at seeding, forward transfected 24 hours later, and harvested at 72 hours. CEBPA, ALB, p21 relative expression levels were shown in Fig. 51. 2'O-Me modifications were better tolerated on SS. Mismatch in SS opposite 5' of AS increased potency of the saRNA.

Table 15-1 Sense sequences (m means 2'OMe modified)

Oligo ID	SS sequence (5'→ 3')	SEQ ID No	Notes
NC-500000	ACUACUGAGUGACAGUAGAUU	33	Unmod non-specific control
NC-500001	ACUACUGAGUGACAGUAGAUmU	262	2x OMe non-specific control
CEBPA-AW01-1	CGGUCAUUGUCACUGGUCAUU	256	Unmod AW1

500000			
CEBPA-AW01 500001	CGGUCAUUGUCACUGGUCAUmU	249	2x OMe in vivo AW1
CEBPA-AW01 500004	CmGGmUCmAUmUGmUCmACmUGmGUC mAmUmU	263	SS: 11x OMe AS: 2x OMe
CEBPA-AW01 500005	CmGGmUCmAUmUGmUCmACmUGmGUC mAmUmU	263	SS: 11x OMe AS: 11x OMe
CEBPA-AW01 500006	CmGGUCAmUUmGUCACmUGmGUCAmUm U	264	SS: 7x OMe AS: 7x OMe
CEBPA-AW01 500007	mCGGmUmCAmUmUGmUmCAmCmUGGm UmCAmUmU	265	SS: 13x OMe AS: 5x OMe
CEBPA-AW01 500008	CGGmUCAmUmUGmUCACmUGGmUCAmU mU	266	SS: 8x OMe AS: 5x OMe
CEBPA-AW01 500009	CGGUCmAUUGUCACUmGGUCAmUmU	267	SS: 4x OMe AS: 4x OMe
CEBPA-AW01 500010	CGGmUCmAUUGUCACmUmGGUCAmUmU	268	SS: 6x OMe AS: 2x OMe
CEBPA-AW01 500102	CGGUCAUUGUCACUGGUCUmUmU	269	SS: 2x Ome + mismatch opposite 5' of AS AS: 2x Ome
CEBPA-AW01 500108	CGGmUCAmUmUGmUCACmUGGmUCUmU mU	270	SS: 8x Ome + mismatch opposite 5' of AS AS: 5x Ome

Table 15-2 Antisense sequences (m means 2'OMe modified)

Oligo ID	AS sequence (5'→3')	SEQ ID N	Notes
NC-500000	UCUACUGUCACUCAGUAGUUU	255	Unmod non-specific control
NC-500001	UCUACUGUCACUCAGUAGUUmU	271	2x OMe non-specific control
CEBPA-AW01 500000	UGACCAGUGACAAUGACCGUU	257	Unmod AW1
CEBPA-AW01 500001	UGACCAGUGACAAUGACCGUmU	272	2x OMe in vivo AW1
CEBPA-AW01 500004	UGACCAGUGACAAUGACCGmUmU	273	SS: 11x OMe AS: 2x OMe
CEBPA-AW01 500005	mUGmACCmAGmUGmACmAAmUGmACC mGmUmU	274	SS: 11x OMe AS: 11x OMe
CEBPA-AW01 500006	UmGACCmAGUmGACAAmUGACCmGmU mU	275	SS: 7x OMe AS: 7x OMe
CEBPA-AW01 500007	mUGACCAGmUGACAAmUGACCGmUmU	276	SS: 13x OMe AS: 5x OMe
CEBPA-AW01 500008	mUGACCAGmUGACAAmUGACCGmUmU	276	SS: 8x OMe AS: 5x OMe
CEBPA-AW01 500009	UGACCmAGUGACAAUmGACCGmUmU	277	SS: 4x OMe AS: 4x OMe
CEBPA-AW01 500010	UGACCAGUGACAAUGACCGmUmU	273	SS: 6x OMe AS: 2x OMe
CEBPA-AW01 500102	UGACCAGUGACAAUGACCGmUmU	273	SS: 2x Ome + mismatch opposite 5'

			of AS AS: 2x Ome
CEBPA-AW01 500108	mUGACCAGmUGACAAmUGACCGmUmU	276	SS: 8x Ome + mismatch opposite 5' of AS AS: 5x Ome

Example 18. C/EBPα-saRNA *in vitro* studies

A). HepG2 cells

[00524] HepG2 cells were transfected with C/EBPα-saRNA using various methods. Expression of CEBPA transcript in HepG2 cells relative to untransfected HepG2 was measured thereinafter. Normal transfection (T), reserves transfection (RT), one dose (1), two doses (2) at 50 nM final concentration of saRNA were performed. Cells were harvested at 24h, 48h and 72 h. Results in Fig. 52A-D confirm reverse transfection (RT) is optimal compared to normal transfection (T). Optimal effect of saRNA was detected at 72 hours using both reverse transcription or normal transcription. Double transfection (2 doses, 2^{nd} transfection 24 hours subsequent to 1^{st} transfection) resulted in at least 4 fold increase.

B) Time course studies

[00525] HepG2, DU145 and AML12 cells were transfected with C/EBPα-saRNA (CEBPA-AW01-500000) for time course studies. Cells were seeded at 1.3 x 105 cells/well (8 x 104 cells for DU145) in a 24-well plate and reverse transfected, and then forward transfected 24 hours later. Medium was changed 4 hours after each transfection. Cells were harvested at 24, 48, and 72 hours post-seeding. CEBPA, ALB, Cyclin, and p21 mRNA levels were measured.

C). Comparison of HepG2, DU145 and AML12 with CEBPA-saRNA

[00526] HepG2 and AML12 seeding was 1.2 x 10^5/well (24 well plate format). DU145 seeding was 0.8x10^5/well (24 well plate format). Reverse transfection was performed at Day 0, forward transfection at Day 1. 24 hour time point = Day 3. 48 hour time point = Day 4. 72 hour time point = Day 5. 96 hour time point = Day 6. 50 nM of saRNA (scramble or CEBPA) was transfected. Media was changed every 24 hour. qPCR was performed to measure relative quantitation (baseline = untransfected). CEBPA, p21 and albumin expressions were measured. HepG2 data were shown in Fig. 53A-G, DU145 in Fig. 54A-g, and AML12 in Fig. 55A-G.

Example 19. Formulated C/ EBPα-saRNA *in vitro* and *in vivo* studies

A). Formulated CEBPA-saRNA *in vitro* transfection analysis

[00560] The results suggest that repeated treatments of C/ EPBa-saRNA may have a positive impact on the regeneration of fibrotic liver in the rats after liver resection, and it will decrease the surgical mortality in cirrhotic animals.

Hypothesis around CEBPA treatment

[00561] Genes involved in HCC include CEBPA, CEBPB and HNF4. Not willing to be bound to any theory, as illustrated in Fig. 64, CEBPA is involved in not only HCC cancer including differentiated cancer (60%) and undifferentiated cancer (40%) but also liver failure causing failed production of albumin and coagulation factors. Fig. 64 also included a patient stratification for various therapies. Child-Pugh is a scoring system for liver cirrhosis. A is healthy, C is very unhealthy. Differentiation refers to the HCC tumor. % are estimates of proportions of stated differentiation relative to advanced HCC patient population. CEBPA-saRNA may be used in distinct proliferative conditions such as in HCC with low CEBPA expression and in HCC with high CEBPA following abrogation of CEBPB-LIP-20KD. In the meantime, CEBPA-saRNA may be used to assist liver regeneration following major liver resection. It is capable to be clinically useful in stopping proliferation or enhancing proliferation, according to the context. Therefore, CEBPA may be used as supportive care as it improved regeneration rate following removal of 70% of rat livers.

Example 23. Additional saRNA sequences

[00562] Nucleotide walk around bioinformatics hotspots (AW1, AW2, PR2) was conducted. The saRNA sequences were prescreened for cross reactivity and minimized potential off-target effects. Sequences of the saRNAs were shown in Table 18.

Table 18-1 Sense sequences (lower case means 2'O-Me modification)

Duplex ID/Target	Name	Sense-ID	Sense sequence	SEQ ID NO
XD-03287 CEBPA	CEBPA-AW01-500000			
XD-03291 scrambel	NC-500000			
XD-03300 CEBPA	CEBPA-AW01-420000	X09312	GUCACUGGUCAGCUCCAGCUU	278
XD-03301 CEBPA	CEBPA-AW01-460000	X09314	CAUUGUCACUGGUCAGCUCUU	279
XD-03302	CEBPA-AW01-	X09316	GCGGUCAUUGUCACUGGUCUU	280

CEBPA	510000			
XD-03303 CEBPA	CEBPA-AW01-520000	X09318	GGCGGUCAUUGUCACUGGUUU	281
XD-03304 CEBPA	CEBPA-AW01-530000	X09320	AGGCGGUCAUUGUCACUGGUU	282
XD-03305 CEBPA	CEBPA-AW01-540000	X09322	CAGGCGGUCAUUGUCACUGUU	283
XD-03306 CEBPA	CEBPA-AW01-550000	X09324	GCAGGCGGUCAUUGUCACUUU	284
XD-03307 CEBPA	CEBPA-AW01-560000	X09326	CGCAGGCGGUCAUUGUCACUU	285
XD-03308 CEBPA	CEBPA-AW01-570000	X09328	GCGCAGGCGGUCAUUGUCAUU	286
XD-03309 CEBPA	CEBPA-AW01-580000	X09330	UGCGCAGGCGGUCAUUGUCUU	287
XD-03310 CEBPA	CEBPA-AW01-590000	X09332	UUGCGCAGGCGGUCAUUGUUU	288
XD-03311 CEBPA	CEBPA-AW02-400000	X09334	AUUCAUCCUCCUCGCGGGGUU	289
XD-03312 CEBPA	CEBPA-AW02-410000	X09336	GAUUCAUCCUCCUCGCGGGUU	290
XD-03313 CEBPA	CEBPA-AW02-420000	X09338	GGAUUCAUCCUCCUCGCGGUU	291
XD-03314 CEBPA	CEBPA-AW02-430000	X09340	AGGAUUCAUCCUCCUCGCGUU	292
XD-03315 CEBPA	CEBPA-AW02-440000	X09342	AAGGAUUCAUCCUCCUCGCUU	293
XD-03316 CEBPA	CEBPA-AW02-450000	X09344	AAAGGAUUCAUCCUCCUCGUU	294
XD-03317 CEBPA	CEBPA-AW02-460000	X09346	UGAAAGGAUUCAUCCUCCUUU	295
XD-03318 CEBPA	CEBPA-AW02-480000	X09348	CUGAAAGGAUUCAUCCUCCUU	296
XD-03319 CEBPA	CEBPA-AW02-490000	X09350	GCUGAAAGGAUUCAUCCUCUU	297
XD-03320 CEBPA	CEBPA-AW02-500000	X09352	AGCUGAAAGGAUUCAUCCUUU	298
XD-03321 CEBPA	CEBPA-AW02-510000	X09354	CAGCUGAAAGGAUUCAUCCUU	299
XD-03322 CEBPA	CEBPA-AW02-520000	X09356	CCAGCUGAAAGGAUUCAUCUU	300
XD-03323 CEBPA	CEBPA-AW02-530000	X09358	GCCAGCUGAAAGGAUUCAUUU	301
XD-03324 CEBPA	CEBPA-AW02-540000	X09360	CGCCAGCUGAAAGGAUUCAUU	302
XD-03325 CEBPA	CEBPA-AW02-550000	X09362	GCGCCAGCUGAAAGGAUUCUU	303
XD-03326 CEBPA	CEBPA-AW02-560000	X09364	AGCGCCAGCUGAAAGGAUUUU	304
XD-03327 CEBPA	CEBPA-AW02-570000	X09366	CAGCGCCAGCUGAAAGGAUUU	305
XD-03328 CEBPA	CEBPA-AW02-580000	X09368	CCAGCGCCAGCUGAAAGGAUU	306
XD-03329 CEBPA	CEBPA-AW02-590000	X09370	GCCAGCGCCAGCUGAAAGGUU	307

XD-03330 CEBPA	CEBPA-AW02-600000	X09372	GGCCAGCGCCAGCUGAAAGUU	308
XD-03331 CEBPA	CEBPA-PR02-400000	X09374	CUUCAUCCUCCUCGCGGGGUU	309
XD-03332 CEBPA	CEBPA-PR02-420000	X09376	GGCUUCAUCCUCCUCGCGGUU	310
XD-03333 CEBPA	CEBPA-PR02-500000	X09378	AGCUGCUUGGCUUCAUCCUUU	311
XD-03334 CEBPA	CEBPA-PR02-540000	X09380	CGCCAGCUGCUUGGCUUCAUU	312
XD-03335 CEBPA	CEBPA-PR02-560000	X09382	AGCGCCAGCUGCUUGGCUUUU	313
XD-03292 Fluc	Fluc MiNA modified	X09208	CuUACGcUGAGUACUUCGAsusu	314
XD-00033 AHA1	EEL, transfection control	X00122	GGAuGAAGuGGAGAuuAGudTsdT	315

Table 18-2 Antisense sequences (lower case means 2'O-Me modification)

Duplex ID/Target	Name	Antisense-ID	Antisense sequence	SEQ ID NO
XD-03287 CEBPA	CEBPA-AW01-500000		positive control	
XD-03291 scrambel	NC-500000		scrambled control	
XD-03300 CEBPA	CEBPA-AW01-420000	X09313	GCUGGAGCUGACCAGUGACUU	316
XD-03301 CEBPA	CEBPA-AW01-460000	X09315	GAGCUGACCAGUGACAAUGUU	317
XD-03302 CEBPA	CEBPA-AW01-510000	X09317	GACCAGUGACAAUGACCGCUU	318
XD-03303 CEBPA	CEBPA-AW01-520000	X09319	ACCAGUGACAAUGACCGCCUU	319
XD-03304 CEBPA	CEBPA-AW01-530000	X09321	CCAGUGACAAUGACCGCCUUU	320
XD-03305 CEBPA	CEBPA-AW01-540000	X09323	CAGUGACAAUGACCGCCUGUU	321
XD-03306 CEBPA	CEBPA-AW01-550000	X09325	AGUGACAAUGACCGCCUGCUU	322
XD-03307 CEBPA	CEBPA-AW01-560000	X09327	GUGACAAUGACCGCCUGCGUU	323
XD-03308 CEBPA	CEBPA-AW01-570000	X09329	UGACAAUGACCGCCUGCGCUU	324
XD-03309 CEBPA	CEBPA-AW01-580000	X09331	GACAAUGACCGCCUGCGCAUU	325

XD-03310 CEBPA	CEBPA-AW01-590000	X09333	ACAAUGACCGCCUGCGCAAUU	326
XD-03311 CEBPA	CEBPA-AW02-400000	X09335	CCCCGCGAGGAGGAUGAAUUU	327
XD-03312 CEBPA	CEBPA-AW02-410000	X09337	CCCGCGAGGAGGAUGAAUCUU	328
XD-03313 CEBPA	CEBPA-AW02-420000	X09339	CCGCGAGGAGGAUGAAUCCUU	329
XD-03314 CEBPA	CEBPA-AW02-430000	X09341	CGCGAGGAGGAUGAAUCCUUU	330
XD-03315 CEBPA	CEBPA-AW02-440000	X09343	GCGAGGAGGAUGAAUCCUUUU	331
XD-03316 CEBPA	CEBPA-AW02-450000	X09345	CGAGGAGGAUGAAUCCUUUUU	332
XD-03317 CEBPA	CEBPA-AW02-460000	X09347	AGGAGGAUGAAUCCUUUCAUU	333
XD-03318 CEBPA	CEBPA-AW02-480000	X09349	GGAGGAUGAAUCCUUUCAGUU	334
XD-03319 CEBPA	CEBPA-AW02-490000	X09351	GAGGAUGAAUCCUUUCAGCUU	335
XD-03320 CEBPA	CEBPA-AW02-500000	X09353	AGGAUGAAUCCUUUCAGCUUU	336
XD-03321 CEBPA	CEBPA-AW02-510000	X09355	GGAUGAAUCCUUUCAGCUGUU	337
XD-03322 CEBPA	CEBPA-AW02-520000	X09357	GAUGAAUCCUUUCAGCUGGUU	338
XD-03323 CEBPA	CEBPA-AW02-530000	X09359	AUGAAUCCUUUCAGCUGGCUU	339
XD-03324 CEBPA	CEBPA-AW02-540000	X09361	UGAAUCCUUUCAGCUGGCGUU	340
XD-03325 CEBPA	CEBPA-AW02-550000	X09363	GAAUCCUUUCAGCUGGCGCUU	341
XD-03326 CEBPA	CEBPA-AW02-560000	X09365	AAUCCUUUCAGCUGGCGCUUU	342
XD-03327 CEBPA	CEBPA-AW02-570000	X09367	AUCCUUUCAGCUGGCGCUGUU	343
XD-03328 CEBPA	CEBPA-AW02-580000	X09369	UCCUUUCAGCUGGCGCUGGUU	344
XD-03329 CEBPA	CEBPA-AW02-590000	X09371	CCUUUCAGCUGGCGCUGGCUU	345
XD-03330 CEBPA	CEBPA-AW02-600000	X09373	CUUUCAGCUGGCGCUGGCCUU	346

XD-03331 CEBPA	CEBPA-PR02-400000	X09375	CCCCGCGAGGAGGAUGAAGUU	347
XD-03332 CEBPA	CEBPA-PR02-420000	X09377	CCGCGAGGAGGAUGAAGCCUU	348
XD-03333 CEBPA	CEBPA-PR02-500000	X09379	AGGAUGAAGCCAAGCAGCUUU	349
XD-03334 CEBPA	CEBPA-PR02-540000	X09381	UGAAGCCAAGCAGCUGGCGUU	350
XD-03335 CEBPA	CEBPA-PR02-560000	X09383	AAGCCAAGCAGCUGGCGCUUU	351
XD-03292 Fluc	Fluc MiNA modified	X09209	UcGAAGuAcUCAGcGUAAgsusu	352
XD-00033 AHA1	EEL, transfection control	X00123	ACuAAUCUCcACUUcAUCCdTsdT	353

[00563] The saRNAs were screened with a bDNA assay system in quadruplicate in HepG2 cells, wherein CEPBPA mRNA (target), albumin mRNA (downstream), and p21 mRNA (downstream) levels were measured. The results were shown in Fig. 65A-65C. They were also screened in DU145 cells, wherein CEBPA mRNA (target) and p21 mRNA (downstream) levels were measured. All cells were reverse transfected at 0hr and forward transfected at 24hr followed by harvest at 72hr. The results were shown in Fig. 65D-65E. Two concentrations of the saRNAs were used: 8nM and 50nM.

[00564] Dose response studies of some saRNAs were conducted with bDNA assay system in quadruplicate in HepG2 and DU145 cells. CEBPA mRNA (Fig. 66A), albumin mRNA (Fig. 66B), and p21 mRNA (Fig. 66C) levels in HepG2 cells were measured. Results were normalized to GAPDH and GAPDH mRNA levels in HepG2 cells were shown in Fig. 66D. CEBPA mRNA (Fig. 66E) and p21 mRNA (Fig. 66F) levels in DU145 cells were measured. Results were normalized to GAPDH and GAPDH mRNA levels in DU145 cells were shown in Fig. 66G. All cells were reverse transfected at 0hr and forward transfected at 24hr followed by harvest at 72hr. Four concentrations of the saRNA were used: 1.25 nM, 2.5 nM, 5 nM and 10 nM.

[00565] HepG2 cells were reverse transfected with 50nM CEBPA-AW01-510000 (XD-03302) at seeding, forward transfected 24 hours later, and harvested at 72 hours. Activation of ALB, CEBPA, ecCEBPA, and STAT3 were observed as shown in Fig. 67.

[00566] The saRNA sequences in Table 18 were modified, for example but not limited to, with 2'O-Me modifications, inverted abasic modifications as shown in Table 19. HepG2 and DU145 cells were transfected with the chemically modified saRNA sequences at various

concentrations (5nM and 10nM) and CEBPA mRNA, p21 mRNA, albumin mRNA, and AFP mRNA levels were measured with bDNA assays. Results were shown in the tables (Tables 20-26) below and Fig. 68A-68E and 69A-69B. Expression in mock-treated cells = 1. Dark color in the figures was parental saRNAs. Light color in the figures was negative controls. DU145 cells were transfected with Aha1 siRNA and Aha mRNA levels were measured as transfection control (Fig. 69C).

Table 19-1 CEBPA saRNA modifications – sense sequences (lower case means 2'O-Me modification)

Target	Duplex ID	Sense ID	Sense sequence	SEQ ID No.	Notes
CEBPA	XD-03287				Positive control
CEBPA	XD-03302	X09316	GCGGUCAUUGUCACUGGUCUU	280	
CEBPA	XD-03317	X09346	UGAAAGGAUUCAUCCUCCUUU	295	
CEBPA	XD-03318	X09348	CUGAAAGGAUUCAUCCUCCUU	296	
CEBPA	XD-03330	X09372	GGCCAGCGCCAGCUGAAAGUU	308	
Fluc	XD-03292	X09208	CuUACGcUGAGUACUUCGAsusu	314	Fluc MiNA modified
AHA1	XD-00033	X00122	GGAuGAAGuGGAGAuuAGudTsdT	315	EEL, transfection control
CEBPA	XD-03923	X11262	(invabasic)CGGUCAUUGUCACUGGUCAUU	258	XD-03287_IA
CEBPA	XD-03924	X11263	(invabasic)GCGGUCAUUGUCACUGGUCUU	354	XD-03302_IA
CEBPA	XD-03925	X11264	(invabasic)UGAAAGGAUUCAUCCUCCUUU	355	XD-03317_IA
CEBPA	XD-03926	X11265	(invabasic)CUGAAAGGAUUCAUCCUCCUU	356	XD-03318_IA
CEBPA	XD-03927	X11266	(invabasic)GGCCAGCGCCAGCUGAAAGUU	357	XD-03330_IA
CEBPA	XD-03928	X11267	(invabasic)CgGuCaUuGuCaCuGgUCauu	358	XD-03287_IA_MiNA
CEBPA	XD-03929	X11268	(invabasic)GCgGuCaUuGuCaCuGgUCuu	359	XD-03302_IA_MiNA
CEBPA	XD-03930	X11269	(invabasic)UgAaAgGaUuCAuCCuCCuuu	360	XD-03317_IA_MiNA
CEBPA	XD-03931	X11270	(invabasic)CuGaAaGgAuuCAuCCuCCuu	361	XD-03318_IA_MiNA
CEBPA	XD-03932	X11271	(invabasic)GgCCAgCgCCAgCuGaAaGuu	362	XD-03330_IA_MiNA
CEBPA	XD-03933	X11272	(invabasic)cgGuCAUUGuCACuGGUCAuu	363	XD-03287_IA_AXO1
CEBPA	XD-03934	X11273	(invabasic)gcGgUCAUUgUCAcUGGUCuu	364	XD-03302_IA_AXO1

CEBPA	XD-03935	X11274	(invabasic)ugAaAGGAUuCAUcCUCCUuu	365	XD-03317_IA_AXO1
CEBPA	XD-03936	X11275	(invabasic)cuGaAAGGAuUCAuCCUCCuu	366	XD-03318_IA_AXO1
CEBPA	XD-03937	X11276	(invabasic)ggCcAGCGCcAGCuGAAAGuu	367	XD-03330_IA_AXO1
CEBPA	XD-03938	X11277	(invabasic)cgGucAuuGucAcuGGucAuu	368	XD-03287_IA_AXO2
CEBPA	XD-03939	X11278	(invabasic)gcGGucAuuGucAcuGGucuu	369	XD-03302_IA_AXO2
CEBPA	XD-03940	X11279	(invabasic)ugAAAGGAuucAuccuccuuu	370	XD-03317_IA_AXO2
CEBPA	XD-03941	X11280	(invabasic)cuGAAAGGAuucAuccuccuu	371	XD-03318_IA_AXO2
CEBPA	XD-03942	X11281	(invabasic)ggccAGcGccAGcuGAAAGuu	372	XD-03330_IA_AXO2

Table 19-2 CEBPA saRNA modifications – antisense sequences (lower case means 2'O-Me modification)

Target	Duplex ID	Antisense ID	Antisense sequence	SEQ ID No.	Notes
CEBPA	XD-03287				Positive control
CEBPA	XD-03302	X09317	GACCAGUGACAAUGACCGCUU	318	
CEBPA	XD-03317	X09347	AGGAGGAUGAAUCCUUUCAUU	333	
CEBPA	XD-03318	X09349	GGAGGAUGAAUCCUUUCAGUU	334	
CEBPA	XD-03330	X09373	CUUUCAGCUGGCGCUGGCCUU	346	
Fluc	XD-03292	X09209	UcGAAGuAcUCAGcGUAAgsusu	352	Fluc MiNA modified
AHA1	XD-00033	X00123	ACuAAUCUCcACUUcAUCCdTsdT	353	EEL, transfection control
CEBPA	XD-03923	X09199	UGACCAGUGACAAUGACCGUU	257	XD-03287_IA
CEBPA	XD-03924	X09317	GACCAGUGACAAUGACCGCUU	318	XD-03302_IA
CEBPA	XD-03925	X09347	AGGAGGAUGAAUCCUUUCAUU	333	XD-03317_IA
CEBPA	XD-03926	X09349	GGAGGAUGAAUCCUUUCAGUU	334	XD-03318_IA
CEBPA	XD-03927	X09373	CUUUCAGCUGGCGCUGGCCUU	346	XD-03330_IA
CEBPA	XD-03928	X11282	UGACCAGUGACAAUGACCGuu	373	XD-03287_IA_MiNA
CEBPA	XD-03929	X11283	GACCAGUGACAAUGACCGCuu	374	XD-03302_IA_MiNA
CEBPA	XD-03930	X11284	AGGAGGAUGAAUCCUUUCAuu	375	XD-03317_IA_MiNA

CEBPA	XD-03931	X11285	GGAGGAUGAAUCCUUUCAGuu	376	XD-03318_IA_MiNA
CEBPA	XD-03932	X11286	CUUUCAGCUGGCGCUGGCCuu	377	XD-03330_IA_MiNA
CEBPA	XD-03933	X11282	UGACCAGUGACAAUGACCGuu	373	XD-03287_IA_AXO1
CEBPA	XD-03934	X11283	GACCAGUGACAAUGACCGCuu	374	XD-03302_IA_AXO1
CEBPA	XD-03935	X11284	AGGAGGAUGAAUCCUUUCAuu	375	XD-03317_IA_AXO1
CEBPA	XD-03936	X11285	GGAGGAUGAAUCCUUUCAGuu	376	XD-03318_IA_AXO1
CEBPA	XD-03937	X11286	CUUUCAGCUGGCGCUGGCCuu	377	XD-03330_IA_AXO1
CEBPA	XD-03938	X11287	UGACcAGUGAcAAUGACCGuu	378	XD-03287_IA_AXO2
CEBPA	XD-03939	X11288	GACcAGUGAcAAUGACCGCuu	379	XD-03302_IA_AXO2
CEBPA	XD-03940	X11289	AGGAGGAUGAAUCCUUUcAuu	380	XD-03317_IA_AXO2
CEBPA	XD-03941	X11290	GGAGGAUGAAUCCUUUcAGuu	381	XD-03318_IA_AXO2
CEBPA	XD-03942	X11291	CUUUcAGCUGGCGCUGGCCuu	382	XD-03330_IA_AXO2

Table 20 CEBPA mRNA levels in HepG2 cells

Name	Relative mRNA CEBPA 5nM	SD	Relative mRNA CEBPA 10nM	SD
XD-03287	1.00	0.08	0.71	0.09
XD-03923	0.99	0.11	0.73	0.04
XD-03928	0.94	0.04	0.59	0.10
XD-03933	1.03	0.05	0.58	0.15
XD-03938	0.94	0.08	0.83	0.34
XD-03302	1.68	0.13	1.50	0.20
XD-03924	1.75	0.10	1.37	0.10
XD-03929	1.57	0.31	1.27	0.15
XD-03934	1.91	0.72	1.31	0.12
XD-03939	1.04	0.21	0.76	0.02
XD-03317	1.12	0.02	0.84	0.04
XD-03925	1.10	0.04	0.83	0.04
XD-03930	1.10	0.14	0.80	0.08
XD-03935	1.13	0.08	0.83	0.10
XD-03940	1.17	0.05	0.69	0.04
XD-03318	1.45	0.26	0.86	0.05

5.Luc + AW51	8	56231	0.00014	0.99
5.Luc + AW51	3	140513	0.00002	0.15
5.Luc + AW51	5	33520	0.00015	1.03
6.Luc + Scrmbl	2	107694	0.00002	0.13
6.Luc + Scrmbl	21	92261	0.00023	1.58
6.Luc + Scrmbl	13	69692	0.00019	1.29
7.POS-Luc	154	110747	0.00139	9.64
7.POS-Luc	83	37131	0.00224	15.50
7.POS-Luc	169	54175	0.00312	21.63

[00570] Furthermore, peripheral blood mononuclear cell (PBMC) cytokine assays were conducted to measure immunocompetence. *In vitro* cytokine production by peripheral blood mononuclear cells (PBMCs) was measured as an indicator of immunocompetence. PBMC assays were carried out for various modifications of AW50 and AW51. Whole blood from 2 anonymous donors were obtained and pre-screened for infectious agents. Human peripheral blood mononuclear cells were isolated by centrifugation and plated in at ~100,000 cells per well. Transfection of saRNA was carried out using DOTAP. Supernatants were harvested ~20 hours after transfection and immediately assayed for IFN-α and TNF-α production by ELISA. Each treatment was analyzed in duplicate for all two donors. The sequences of the saRNAs were shown in Table 28 below.

Table 28-1 Sense sequence (lower case means 2'O-Me modification)

Duplex-ID	Notes	Sense-ID	Sense Sequence	SEQ ID No.
XD-03287	positive control			
XD-03318		X09348	CUGAAAGGAUUCAUCCUCCUU	296
XD-03928	XD-03287_IA_MiNA	X11267	(invabasic)CgGuCaUuGuCaCuGgUCauu	358
XD-03929	XD-03302_IA_MiNA	X11268	(invabasic)GCgGuCaUuGuCaCuGgUCuu	359
XD-03933	XD-03287_IA_AXO1	X11272	(invabasic)cgGuCAUUGuCACuGGUCAuu	363
XD-03934	XD-03302_IA_AXO1	X11273	(invabasic)gcGgUCAUUgUCAcUGGUCuu	364

Table 28-2 Anti-sense sequence (lower case means 2'O-Me modification)

Duplex-ID	Antisense-ID	Antisense Sequence	SEQ ID No.
XD-03287			
XD-03318	X09349	GGAGGAUGAAUCCUUUCAGUU	334
XD-03928	X11282	UGACCAGUGACAAUGACCGuu	373
XD-03929	X11283	GACCAGUGACAAUGACCGCuu	374
XD-03933	X11282	UGACCAGUGACAAUGACCGuu	373

XD-03934	X11283	GACCAGUGACAAUGACCGCuu	374

[00571] In PBMC assay for TNF-α response, freshly isolated PBMCs were transfected with DOTAP (ds-RNAs). Blunt end 25 mer RNA (transfected), CpG-motive single strand oligonucleotide (direct incubation), chol-conjugated siRNA (direct incubation) were used as positive controls. Incubation time was 20 hrs. ELISA for hsTNF-α was performed. Results were shown in Fig. 72A and Table 29.

Table 29-1

	133 nM transfection							
	medium	mock	XD-03287	XD-03928	XD-03933	XD-03929	XD-03934	XD-03318
Donor A	-0.36	-0.69	0.36	-0.93	-1.95	1.66	0.08	-0.89
Donor B	-2.32	-1.35	-1.55	-1.56	-1.06	-1.44	0.12	-0.62
Standard dev								
Donor A	0.01	1.17	2.76	0.70	0.56	0.43	0.81	0.09
Donor B	0.62	0.28	0.40	0.76	0.43	0.06	0.03	1.05

Table 29-2

			500 nM direct incubation	
	blunt end		CpG	chol
	pos. Ctrl	neg. ctrl	pos. Ctrl	pos. Ctrl
Donor A	0.45	-0.79	2.00	12.63
Donor B	2.43	-0.58	11.18	14.36
Standard dev				
Donor A	0.08	0.98	2.01	1.86
Donor B	1.48	0.92	2.43	0.56

[00572] In PBMC assay for INF-α response, freshly isolated PBMCs were transfected with Geneporter-2 control (ds-RNAs). Blunt end 25 mer RNA (transfected), CpG-motive single strand oligonucleotide (direct incubation), chol-conjugated siRNA (direct incubation) were used as positive controls. Incubation time was 20 hrs. ELISA for hsINF-α was performed. Results were shown in Fig. 72B and Table 30.

Table 30-1

	133 nM transfection							
	medium	mock	XD-03287	XD-03928	XD-03933	XD-03929	XD-03934	XD-03318

Claims

1. An saRNA, wherein the saRNA comprises as an antisense sequence GACCAGUGACAAUGACCGCUU.

2. A pharmaceutical composition comprising the saRNA of claim 1 and at least one pharmaceutically acceptable carrier.

3. The saRNA or composition of claim 1 or 2, wherein the saRNA comprises one or more modifications to a sugar, a nucleobase, and/or an internucleoside linkage, or wherein the saRNA does not comprise one or more modifications to a sugar, a nucleobase, and/or an internucleoside linkage.

4. The saRNA or composition of any one of claims 1-3, wherein the saRNA is double stranded.

5. The saRNA or composition of claim 4, wherein the saRNA consists of antisense and sense strands stably base-paired together with a number of unpaired nucleotides at the 3' end of each strand forming 3' overhangs.

6. The saRNA or composition of claim 5, wherein the number of unpaired nucleotides forming the 3' overhang of each strand is 1 to 3 nucleotides.

7. The saRNA or composition of any one of claims 1-6, wherein the saRNA comprises as a sense sequence GCGGUCAUUGUCACUGGUCUU, wherein the sense sequence does not comprise one or more modifications to a sugar, a nucleobase, and/or an internucleoside linkage.

8. The saRNA or composition of claim 7, wherein the saRNA comprises a duplex, the duplex comprising as a sense sequence GCGGUCAUUGUCACUGGUCUU; and as an antisense sequence GACCAGUGACAAUGACCGCUU, wherein the sense and antisense sequences do not comprise one or more modifications to a sugar, a nucleobase, and/or an internucleoside linkage.

9. The saRNA or composition of any one of claims 1-6, wherein the saRNA comprises as an antisense sequence, GACCAGUGACAAUGACCGCuu , wherein lower case letters indicate 2'O-Me modification.

10. The saRNA or composition of claim 9, wherein the saRNA comprises a duplex comprising as a sense sequence (invabasic)gcGgUCAUUgUCAcUGGUCuu; and as an antisense

sequence GACCAGUGACAAUGACCGCuu, wherein lower case letters indicate 2'O-Me modification and “invabasic” refers to an inverted abasic modification at the 5’ end.