

ENGINEERED GENETIC MODULATORS SYNERGISTIC TRANSCRIPTION FACTORS

CROSS-REFERENCE TO RELATED APPLICATIONS

- 5 **[0001]** The present application claims the benefit of U.S. Provisional Application No. 62/740,156, filed October 2, 2018, the disclosure of which is hereby incorporated by reference in its entirety.

TECHNICAL FIELD

- 10 **[0002]** The present disclosure is in the field of compositions and methods for modulating gene expression using genetic modulators comprising two or more artificial transcription factors.

BACKGROUND

- 15 **[0003]** Repression or activation of disease-associated genes has been accomplished through the use of engineered transcription factors. Methods of designing and using engineered zinc finger transcription factors (ZFP-TF) are well documented (see for example U.S. Patent No. 6,534,261), and both transcription activator like effector transcription factors (TALE-TF) and clustered regularly interspaced short palindromic repeat Cas based transcription factors (CRISPR-Cas-TF) have also been described (see review Kabadi and Gersbach (2014) *Methods* 69(2): 188-197). For example, engineered TFs that repress gene expression (repressors) have also been shown to be effective in treating trinucleotide disorders such as Huntingtin's disease (HD) (*see, e.g.*, U.S. Patent No. 8,956,828 and U.S. Patent Publication No. 2015/0335708) and tauopathies such as Alzheimer's disease (AD) (*see*, U.S. Publication No. 20180153921).
- 25 **[0004]** However, there remains a need for additional methods and compositions that provide enhanced activity and/or specificity for modulation of gene expression.

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SUMMARY

- [0005]** Disclosed herein are genetic modulators comprising two or more artificial transcription factors and methods for making and using these genetic modulators the treatment and/or prevention of diseases. In particular, genetic

CLAIMS

What is claimed:

1. A composition comprising two or more different artificial transcription factors,

wherein each artificial transcription factor comprises a DNA-binding domain and ~~functional~~ a transcriptional repressor domain,

wherein each DNA-binding domain comprises a zinc finger protein (ZFP),

wherein the two or more different artificial transcription factors bind to at least two different target sites in a target endogenous gene in the genome of a eukaryotic cell, the two different target sites being separated by a gap of 80 to 800 base pairs,

wherein the central position in the gap is within 600 base pairs from the transcription start site of the gene, and

wherein the artificial transcription factors synergistically ~~modulate~~ repress gene expression in a eukaryotic cell.

2. The composition of claim 1, wherein the eukaryotic cell is isolated or is in a living subject.

~~3. The composition of any of the preceding claims for use in the *ex vivo* or *in vivo* treatment of a disease or disorder, wherein the disease or disorder is HD, a prion disease, Parkinson's Disease, dementia with Lewy bodies (DLB), amyotrophic lateral sclerosis (ALS), and/or a tauopathy.~~

43. The composition of any of the preceding claims wherein the synergistic modulation is at least ~~about~~ 2 fold as compared to individual transcription factors.

54. The composition of any of the preceding claims, wherein each artificial transcription factor comprises ~~the~~ a DNA-binding domain that binds to a target site of 12 or more nucleotides.

~~6. The composition of any of the preceding claims, wherein the DNA-binding domain of each transcription factor comprises a zinc finger protein (ZFP), TAL-effector domain, and/or a sgRNA of a CRISPR/Cas system.~~

~~7. The composition of any of the preceding claims, wherein the functional domain comprises a transcriptional activation domain, a transcriptional repression domain, a domain from a DNMT protein such as DNMT1, DNMT3A, DNMT3B, DNMT3L, a histone deacetylase (HDAC), a histone acetyltransferase (HAT), a histone methylase, or an enzyme that sumolyates or biotinylates a histone and/or other enzyme domain that allows post translation histone modification regulated gene repression.~~

85. The composition of any of the preceding claims, wherein the two or more artificial transcription factors:

(i) bind to any target site of at least 12 nucleotides in a selected target gene, ~~optionally two or more of the artificial transcription factors same, different and/or overlapping target sites;~~

~~(ii) bind to target sites within 10,000 or more base pairs of each other;~~

(iii) bind to target sites within 0 to 300 base pairs on either side of the transcription start site (TSS) of the target gene to be modulated; and/or

(iii~~v~~) bind to the sense and/or anti-sense strand in a double stranded target.

96. The composition of any of the preceding claims, wherein the target gene is a tau (*MAPT*) gene, a ~~*HTT*~~ gene, a mutant ~~*HTT*~~ gene, a mutant *C9orf72* gene, a *SNCA* gene, a prion gene, a *SMA* gene, an *ATXN2* gene, an *ATXN3* gene, a *PRP* gene, an *Ube3a-ATS* encoding gene, a *DUX4* gene, an *PGRN* gene, a *MECP2* gene, an *FMR1* gene, a *CDKL5* gene, or a *LRKK2* gene.

~~107.~~ The composition of any of the preceding claims, wherein the two or more artificial transcription factors ~~are gene repressors, optionally repressors that repress~~ expression of the target gene by at least 50% to 100% (or any value therebetween) as compared to wild-type expression levels and/or as compared to expression levels using a single artificial transcription factor.

~~11.~~ ~~The composition of any of the preceding claims, wherein the two or more artificial transcription factors are gene activators, optionally activators that activate expression of the target gene by 1 to 5 fold or more as compared to wild-type expression levels and/or expression levels when the gene is modulated by a single genetic modulator.~~

~~812.~~ The composition of any of the preceding claims, wherein the activity of the functional domain is regulated by an exogenous small molecule or ligand such that

interaction with the cell's transcription machinery will not take place in the absence of the exogenous ligand.

139. A pharmaceutical composition comprising the composition of any of the preceding claims.

10. Use of the composition of any of the preceding claims in the manufacture of a medicament for *ex vivo* or *in vivo* treatment of a disease or disorder, wherein the disease or disorder is Huntington's disease (HD), Alzheimer's disease (AD), a prion disease, Parkinson's Disease, dementia with Lewy bodies (DLB), amyotrophic lateral sclerosis (ALS), and/or a tauopathy.

1411. ~~The composition of any of the preceding claims~~ The use of claim 10, wherein the artificial transcription factors are to be provided to the subject using one or more polynucleotides, optionally using one or more viral or non-viral vectors carrying sequences encoding one or more of the artificial transcription factors, optionally wherein the viral vectors comprise an adenovirus vector, a lentiviral vector (LV) and/or adenovirus associated viral vector (AAV) and the non-viral vectors optionally comprise a plasmid and/or single- or multi-cistronic mRNA.

1512. ~~The composition of any of the preceding claims~~ The use of claim 10 or 11, wherein the composition treats a CNS disease or disorder such as a tauopathy by repressing *MAPT* gene expression; prion diseases by repressing prions; Parkinson's Disease by repressing α -synuclein; ALS by repressing mutant *C9orf72* gene expression; HD by repressing gene ~~mHtt~~ mHTT expression, by reducing gene

expression for a period of ~~about~~ 4 weeks, ~~about~~ 3 months, ~~about~~ 6 months to ~~about~~ a year or more in the brain of subject,

13. The use of any one of claims 10 to 12, wherein ~~optionally by administering~~ the composition is to be administered to the brain of the subject, including to the frontal cortical lobe such as the prefrontal cortex, parietal cortical lobe, occipital cortical lobe; to the temporal cortical lobe such as the entorhinal cortex, to the hippocampus, to the brain stem, to the striatum, to the thalamus, to the midbrain, to the cerebellum and/or to the spinal cord such as the lumbar, thoracic and/or cervical regions of the spinal cord.

~~16~~14. The composition of any of the preceding claims The use of any one of claims 10 to 13, wherein the composition is to be administered to the subject via intravenous, intramuscular, intracerebroventricular, intrathecal, intracranial, mucosal, oral, intravenous, orbital (retro-orbital (RO)), and/or intracisternal administration.

~~17~~15. The composition The use of any one of ~~of the preceding claims~~ 10 to 14, wherein the composition is to be delivered using

(i) an adeno-associated virus (AAV) vector at ~~about~~ 10,000 - 500,000 vector genome/cell, optionally delivered at a dose of ~~about~~ 10,000 to 100,000, or from ~~about~~ 100,000 to 250,000, or from ~~about~~ 250,000 to 500,000 vector genomes (VG)/cell, optionally at a fixed volume of ~~about~~ 1-300 μ L to the brain parenchyma at ~~about~~ 1E11-1E14 VG/mL and/or at a fixed volume of ~~about~~ 0.5-10 mL to the CSF at ~~about~~ 1E11-1E14 VG/mL;

(ii) a lentiviral vector at MOI between ~~about~~ 250 and 1,000;

- (iii) a plasmid vector at ~~about~~ 0.01-1,000 ng/100,000 cells; and/or
- (iv) mRNA at ~~about~~ 0.01-3000 ng/100,000 cells.

~~18~~16. The composition of any of the preceding claims, wherein gene expression is reduced in the cell as compared to controls not receiving the genetic modulators as described herein by at least 30%, or 40%, preferably by at least 50%, even more preferably by at least 70%, or by at least 80%, or by 90%, or by greater than 90%.

~~19~~17. The composition of any of the preceding claims, wherein the cell is a neuron, optionally an HD or AD neuron.

~~20~~18. The ~~composition~~ use of any of the preceding claims, wherein the composition is to be administered once or administered multiple times.

~~21~~19. Use of the ~~The~~ composition of any of the preceding claims ~~for use in the~~ manufacture of a medicament for ~~in~~ reducing biomarkers, pathogenic species and/or symptoms of a disease or disorder, ~~optionally~~ wherein neurotoxicity, gliosis, dystrophic neurites, spine loss, excitotoxicity, cortical and hippocampal shrinkage, dendritic tau accumulation, cognitive deficits, motor deficits, dystrophic neurites associated with amyloid β plaques, tau pathogenic species, mHtt aggregates, hyperphosphorylated tau, soluble tau, granular tau, tau aggregation, and/or neurofibrillary tangles (NFTs) are reduced.

~~22~~20. A cell comprising the composition of any of the preceding claims, wherein sequences encoding one or more of the artificial transcription factors are stably

integrated into the genome and/or one or more sequences encoding the artificial transcription factors are maintained episomally, optionally wherein stable integration is targeted integration mediated by a nuclease.

2321. A kit comprising one or more compositions and/or one or more cells of any of the preceding claims, optionally further comprising reagents and/or instructions for use.

2422. ~~Two or more of the A method of making a composition comprising synergistic artificial transcription factors of any of the preceding claims or the coding sequences of the factors for use as a medicament, the method comprising:~~
~~screening individual and combinations of two or more artificial transcription factors targeted to a selected gene for their effect on gene expression; and~~
~~identifying synergistic combinations of the artificial ZFP TFs.~~

25. ~~The method of claim 24, wherein the two or more artificial transcription factors:~~

~~(i) bind to target sites and/or comprise functional domains that are 1 to 600 base pairs apart;~~

~~(ii) bind to target sites that are approximately 1 to 80; 160 to 220; 260 to 400; or 500 to 600 base pairs apart;~~

~~(iii) comprise functional domains that are separated from each other by approximately 1 to 80; 260 to 400; or 500 to 600 base pairs apart;~~

~~(iv) bind to target sites that are within about 400 base pairs on either side of the transcription start site (TSS); and/or~~

~~(v) bind to the same antisense (-) or sense (+) strand or to different strands in either orientation).~~

~~26. The method of claim 24 or claim 25, wherein the synergistic artificial TFs are at least 2 fold more active in modulating a target gene than the individual TFs.~~