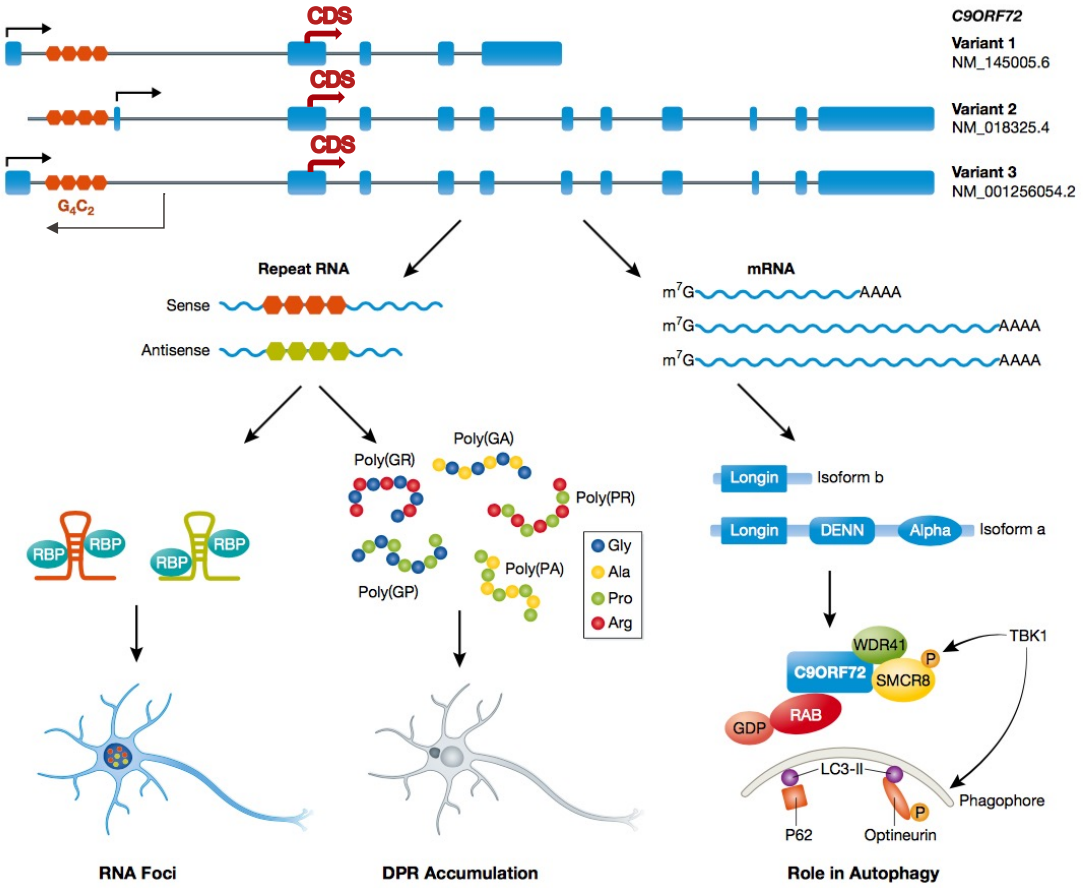
ALS: C9orf72

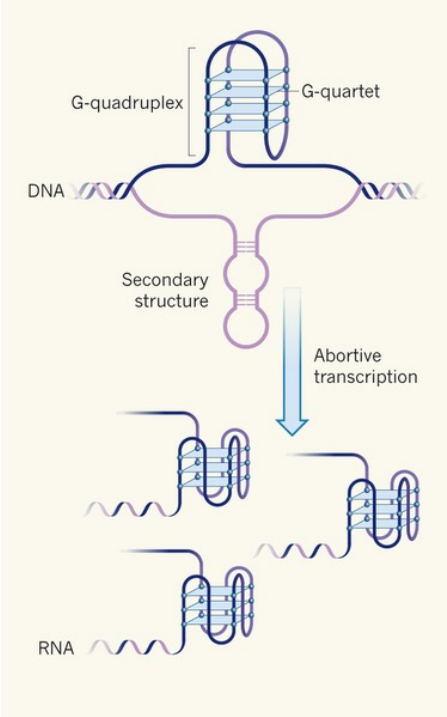
Main pathogenic pathways

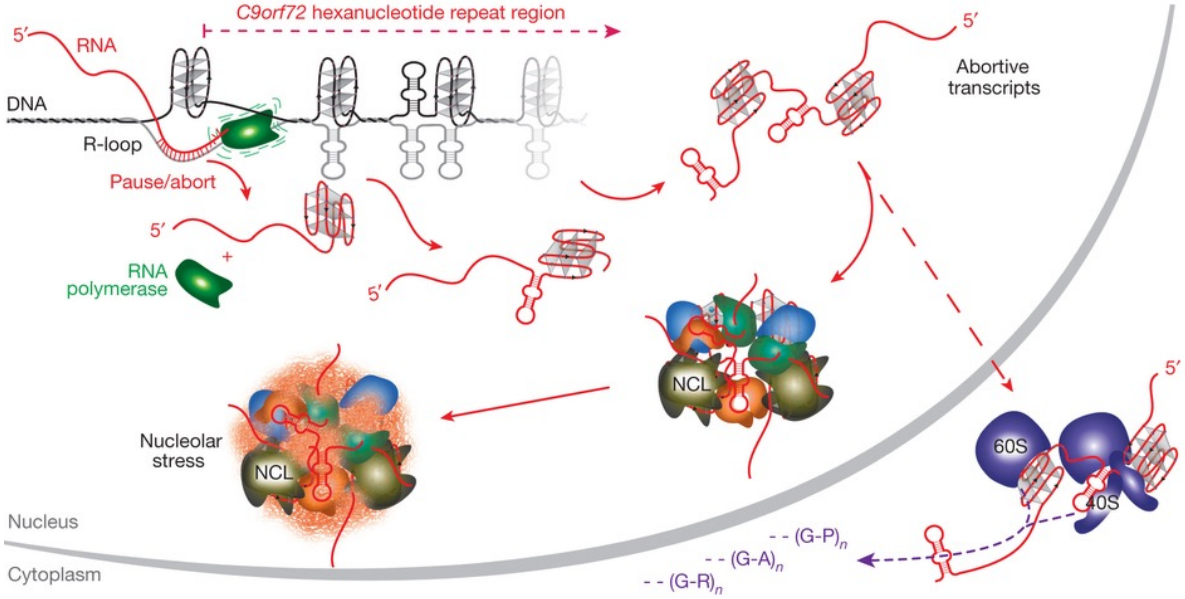
C9orf72 pathology



C9orf72: G-quadruplex structures

- Hexanucleotide repeat expansion $[GGGGCC]_n$ generates a **G-quadruplex structure** (DNA and RNA)
- Abortive transcription (reduced C9orf72 expression)
- Nucleolar stress
- Aberrant dipeptide expression (RAN translation – *RAN = Repeat-associated non-ATG*)



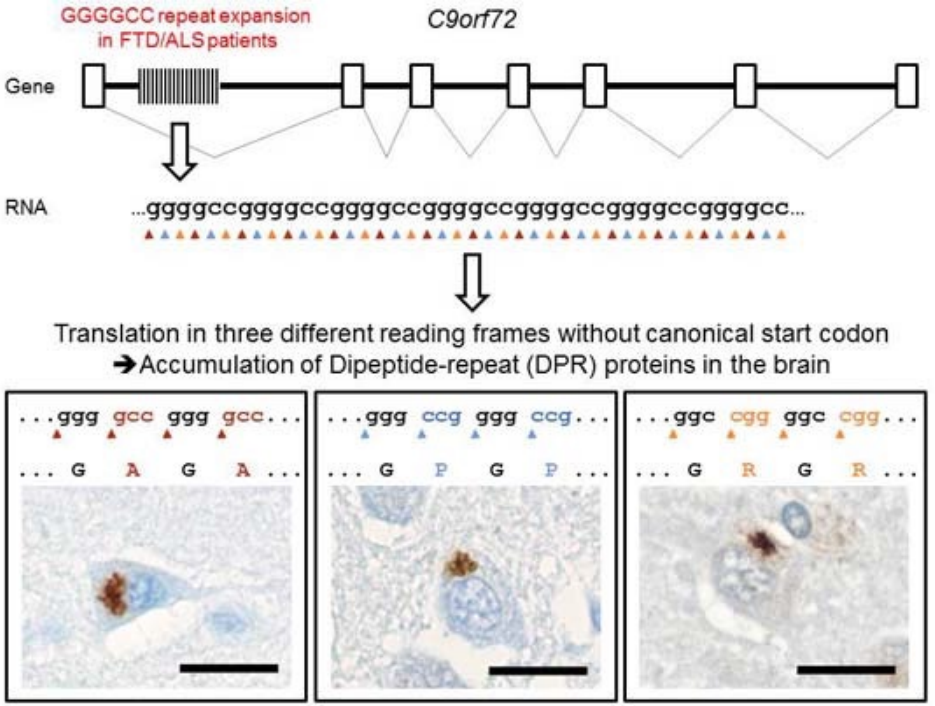


Haeusler AR et al, Nature 2014

ALS: Dipeptide pathology

C9orf72: 'RAN' (Repeat Associated Non-AUG) translation

- Accumulation of the $[GGGGCC]_n$ repeats correlates with neuronal accumulation of **aggregating dipeptide-repeat proteins** (glycine-alanine, glycine-proline or glycine-arginine)
- Repeats also lead to **reduced expression of C9orf72** (unknown function, possibly related to endosomal processing and autophagy)

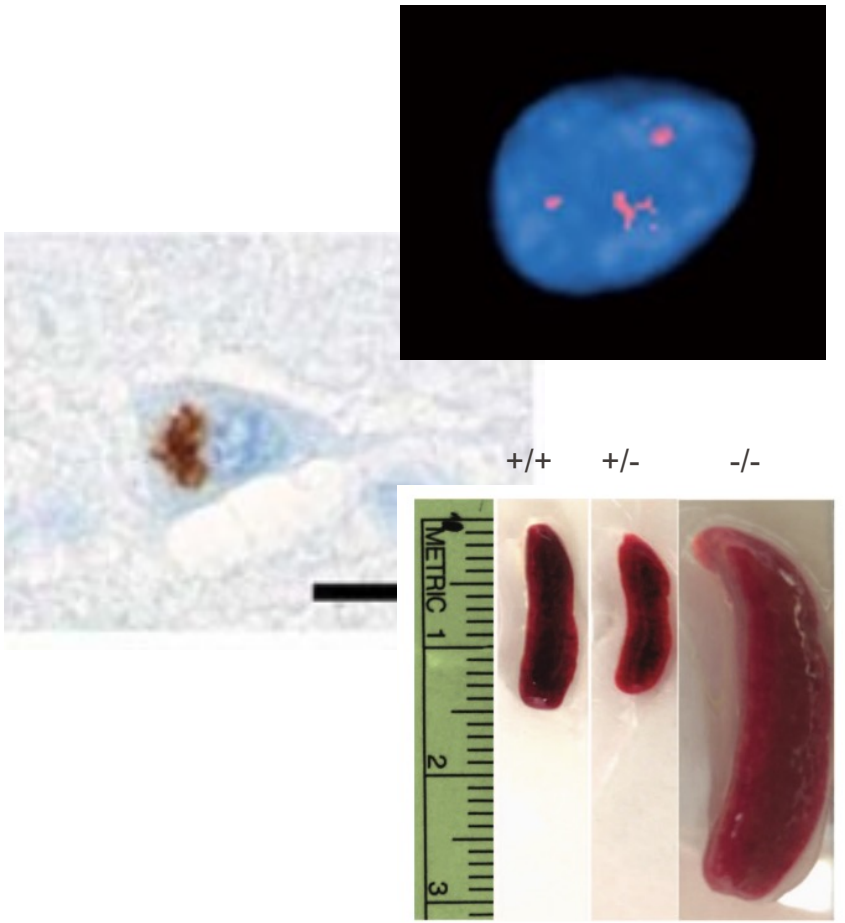


- Mori K et al, Science 2013

ALS: C9orf72

The following mechanisms have been proposed to explain the C9orf72 pathology:

1. r(GGGGCC) RNA is toxic (RNA foci).
2. RAN translation of poly[PR], poly[GR], poly[GP] or poly[GA] dipeptides leads to expression of toxic species in neurons.
3. The presence of r(GGGGCC) in the C9orf72 gene reduces expression and activity of the C9orf72 protein (loss of function). C9orf72 appears to upregulate cell autophagy and control inflammation.



- EMBO J. 2020;39(4):e100574.
- Nature 585, pp. 96–101 (2020)

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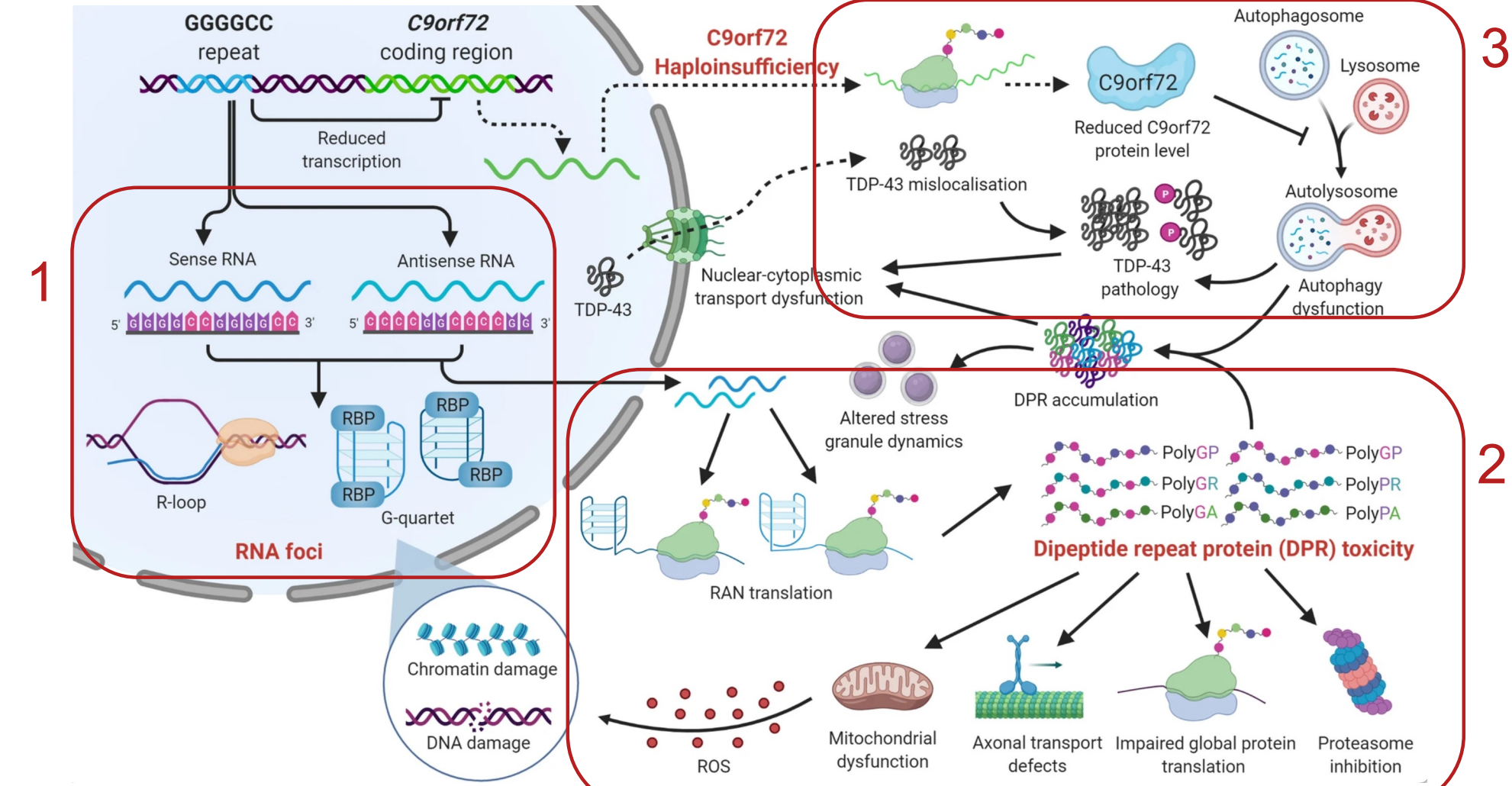
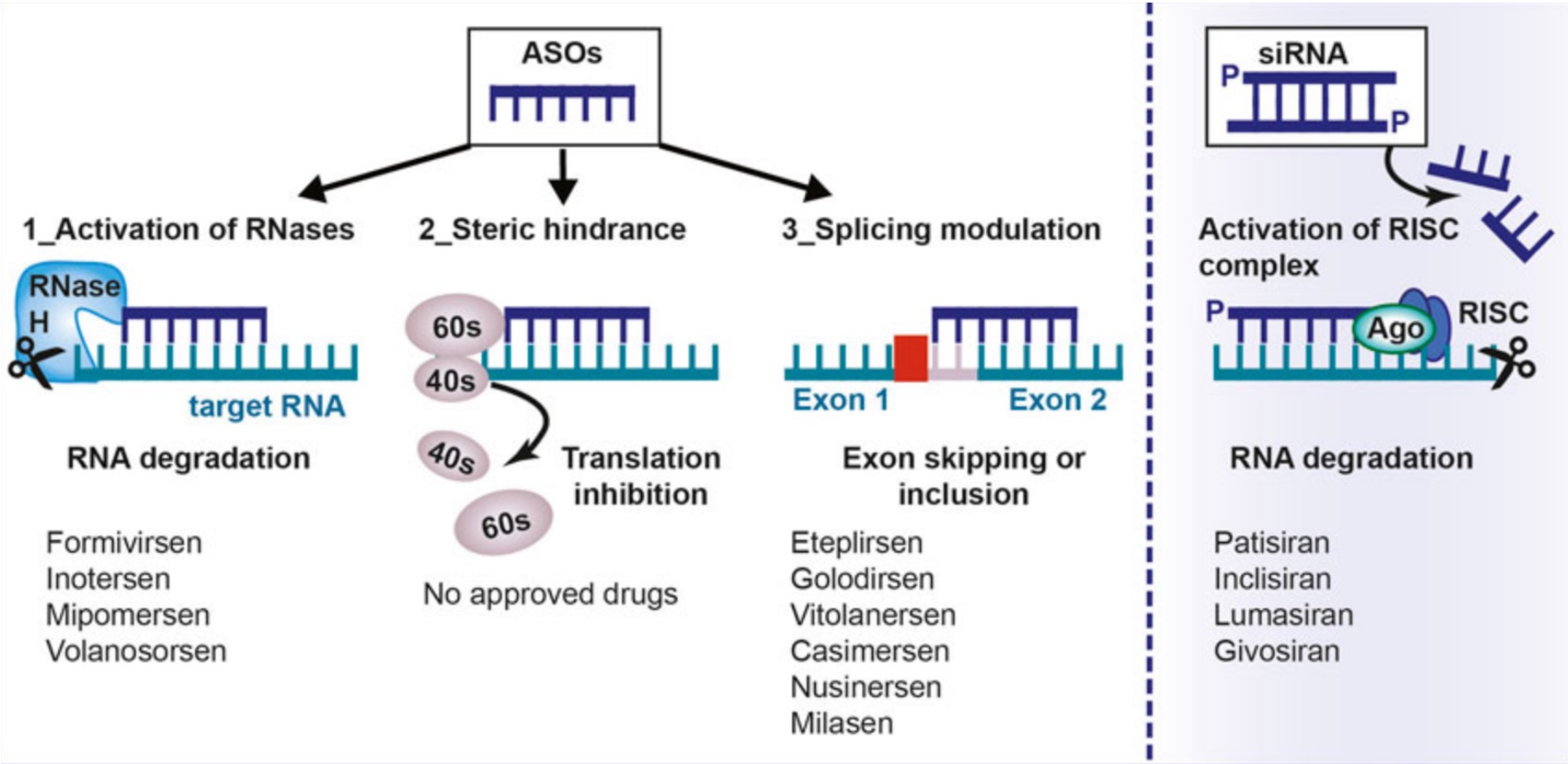


Molecular Neurodegeneration volume 17, Article number: 22 (2022)

Is it possible to target C9orf72 toxicity with an antisense oligonucleotide?



ALS: C9orf72

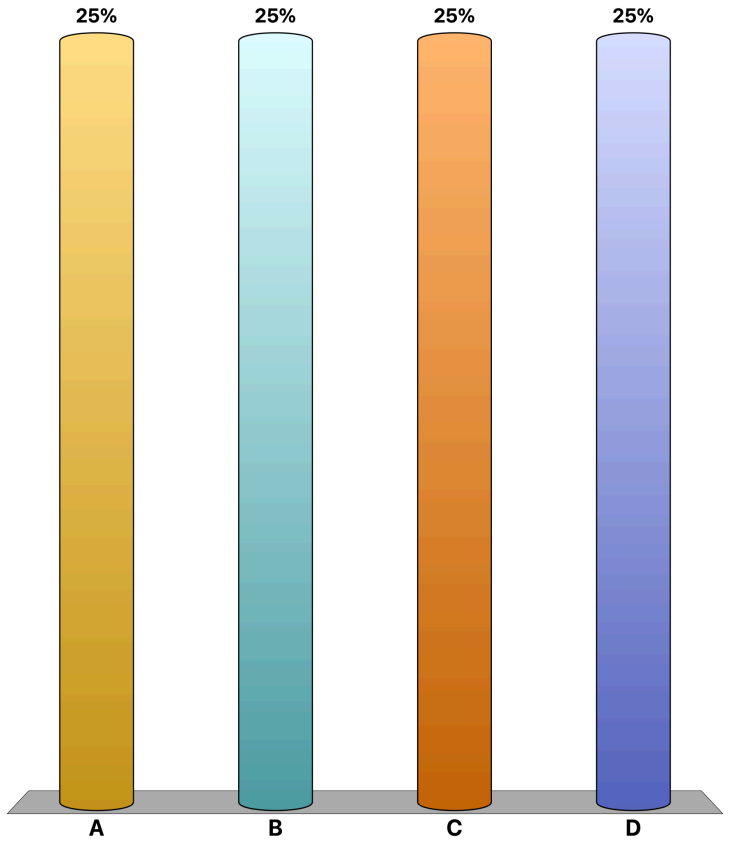
In your opinion, which approach has the best chance to provide therapeutic efficacy ?

A. Suppress toxic RNA

B. Silence the production of toxic dipeptides (RAN translation)

C. Restore physiological C9orf72 expression

D. A combination of two or more of the treatments above



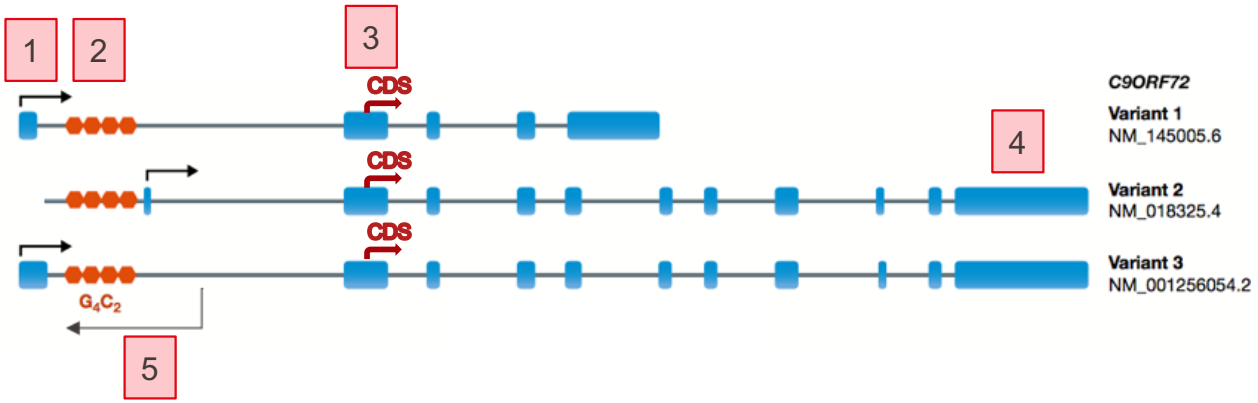
Complete the following table, indicating the suitable treatment(s) for each toxic activity of C9orf72:

Treatments	Cause of toxicity:	RNA foci	Dipeptide accumulation	Loss of C9orf72 activity
	Small RNA for RNAi			
	Compound inducing autophagy			
	Antibody			
	Gene editing to reduce the number of GGGGCC repeats			
	Small RNA for exon skipping (splicing modifier)			

C9orf72 pathology: exercise

You plan to design a small RNA to oppose C9orf72 toxicity.

Where in the precursor mRNA do you think that the small RNA should bind (multiple options are possible) ?



0% A. 1

0% B. 2

0% C. 3

0% D. 4

0% E. 5