The Science of RNAi

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RNA Interference Mechanism of Action

- Naturally-occurring mechanism used to regulate gene expression, provides a rapid, targeted and sustained approach for programmable silencing of gene expression.[1-6]
- Improved stability imparted by the double-stranded structure and chemical modifications (Enhanced Stabilization Chemistry). [1-7]
- The guide strand remains bound to the enzyme complex (RISC) between cleavage cycles; a single siRNA bound to RISC can therefore cleave multiple mRNAs during its lifetime. [1,5,8]
- Have minimal number of phosphorothioate (PS) modifications. Lower PS content reduces the likelihood of non-specific protein binding and off-target effects such as a pro-inflammatory response. [10-17]

PS, phosphorothioate; RISC, RNA induced silencing complex; RNA, ribonucleic acid; siRNA, small interfering RNA

1. Khvorova, A. *N Engl J Med.* 2017; 376(1): 4-7; **2.** Adams, D. *Amyloid* 2023; 30(1):1-9; **3.** Jay, PY. *Int J Cardiovasc Sci*. 2022; 35(5):665-7; **4**. Keam, SJ. *Drugs* 2022; 82: 1419–1425; **5.** Niemietz, C. *Molecules* 2015; 20:17944–75.; **6.** Raal, FJ. *N Engl J Med.* 2020; 382(16):1520-30; **7.** Alnylam Pharmaceuticals. US prescribing information: AMVUTTRA® (vutrisiran) injection, for subcutaneous use. 2022. Available from: https://www.alnylam.com/sites/default/files/pdfs/amvuttra-us-prescribing-information.pdf (accessed March 21, 2023); **8.** Hutvagner, G. *Science* 2002;297:2056–60; **9.** LEQVIO™ US Prescribing Information; **10.** Anderson, BA. *Nucleic Acids Res.* 2021; 49(16): 9026-9041; **11.** Crooke, ST. *J Biol Chem.* 2021; 296: 100416. doi: 10.1016/j.jbc.2021.100416; **12.** Shen, W. *Nat Biotechnol.* 2019; 37(6): 640-650; **13.** Flierl U. *J Exp Med.* 2015; 212(2): 129-37; **14.** Frazier, KS. *Toxicol Pathol.* 2015; 43(1): 78-89; **15.** Roberts, TC. *Nat Rev Drug Discov.* 2020;19(10): 673-694 **16.** Friedrich, M. *BioDrugs.* 2022; 36(5): 549-571; **17.** Diep, JK. *Br J Clin Pharmacol.* 2022; 88(12): 5389-5398.

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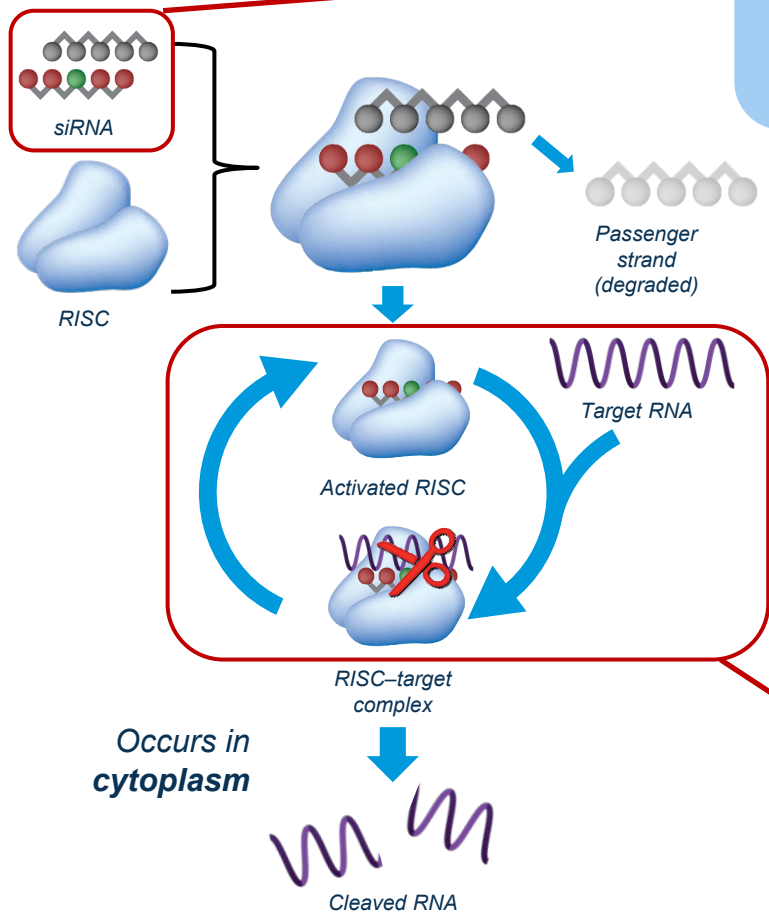
RNAi Therapeutics and RISC-mediated RNA Cleavage



- Enhanced metabolic stability[1,2]
- Minimal number of PS modifications (which are known to increase the possibility of non-specific protein binding and off-target effects).[3-10]

The guide strand remains bound to the enzyme complex (RISC) between cleavage cycles.[2,11,12]

- *A single siRNA bound to RISC can cleave multiple mRNAs during its lifetime*

- Based on Nobel Prize-winning scientific discovery[2,12]
- Leveraging the naturally-occurring mechanism for regulation of gene expression[1,2,13]
- Providing rapid, targeted, and sustained silencing of the disease-causing protein[1,2,12-15]

mRNA, messenger RNA; PS, phosphorothioate; RISC, RNA induced silencing complex; RNA, ribonucleic acid; RNAi, ribonucleic acid interference; siRNA, small interfering RNA

1. Adams, D. *Amyloid* 2022; 23: 1-9. doi: 10.1080/13506129.2022.2091985.; **2.** Khvorova, A. *N Engl J Med.* 2017; 376(1): 4-7.; 5; **3.** Anderson, BA. *Nucleic Acids Res.* 2021; 49(16): 9026-9041; **4.** Crooke, ST. *J Biol Chem.* 2021; 296: 100416. doi: 10.1016/j.jbc.2021.100416; **5.** Shen, W. *Nat Biotechnol.* 2019; 37(6): 640-650; **6.** Flierl, U. *J Exp Med.* 2015; 212(2): 129-37; **7.** Frazier, KS. *Toxicol Pathol*. 2015; 43(1): 78-89; **8.** Roberts, TC. *Nat Rev Drug Discov*. 2020;19(10): 673-694 **9.** Friedrich, M. *BioDrugs.* 2022; 36(5): 549-571; **10.** Diep, JK. *Br J Clin Pharmacol.* 2022; 88(12): 5389-5398; **11.** Hutvagner, G. *Science* 2002; 297:2056–60 **12.** Niemietz, C. *Molecules* 2015;20:17944–75 **13.** Jay, PY. *Int J Cardiovasc Sci*. 2022; 35(5):665-7;**14.** Raal, FJ. *N Engl J Med.* 2020; 382(16):1520-30; **15.** Keam, SJ. *Drugs* 2022; 82: 1419–142

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