

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization

International Bureau

(43) International Publication Date
15 February 2024 (15.02.2024)



(10) International Publication Number
WO 2024/033465 A1

(51) International Patent Classification:
C12N 15/113 (2010.01)

(21) International Application Number:
PCT/EP2023/072151

(22) International Filing Date:
10 August 2023 (10.08.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
22189636.8 10 August 2022 (10.08.2022) EP

(71) Applicant: **BOEHRINGER INGELHEIM INTERNATIONAL GMBH** [DE/DE]; Binger Str. 173, 55216 Ingelheim am Rhein (DE).

(72) Inventors: **WEISS, Linus**; c/o Boehringer Ingelheim International GmbH, Global Patents, Binger Strasse 173, 55216 Ingelheim am Rhein (DE). **FISCHER, Simon**; c/o Boehringer Ingelheim International GmbH, Global Patents, Binger Strasse 173, 55216 Ingelheim am Rhein (DE). **SCHMIEDER-TODTENHAUPT, Valerie**; c/o Boehringer Ingelheim International GmbH, Global Patents, Binger Strasse 173, 55216 Ingelheim am Rhein (DE).

(74) Agent: **WALLINGER RICKER SCHLOTTER TOST-MANN**; Patent- und Rechtsanwälte, Partnerschaft mbB, Zweibrückenstraße 5-7, 80331 Munich (DE).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: ARTIFICIAL MIRNAS TARGETING MULTIPLE HYDROLASES

(57) Abstract: The present invention relates to an artificial microRNA (artificial miRNA or amiRNA) comprising an artificial miRNA guide strand sequence targeting two or more mammalian hydrolases acting on ester bonds, wherein the two or more hydrolases are preferably endogenous hydrolases of a Chinese hamster ovary (CHO) cell. The invention further relates to a mammalian expression vector comprising a polynucleotide sequence encoding said artificial miRNA and to a mammalian cell (e.g., a CHO cell) comprising said artificial miRNA. The invention further relates to the use of said mammalian cell for producing a protein of interest and to the use of the artificial miRNA for preventing expression of at least two endogenous hydrolases in the mammalian cell and/or hydrolase activity associated with a recombinant protein produced therein. Moreover, provided is a method for manufacturing a protein of interest comprising introducing said artificial miRNA into a mammalian cell and to a composition comprising a recombinant protein of interest and optionally polysorbate, wherein the recombinant protein of interest is obtained by said method.



WO 2024/033465 A1