

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

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
13 July 2023 (13.07.2023)



(10) International Publication Number
WO 2023/133250 A1

(51) International Patent Classification:
G01N 33/68 (2006.01)

(21) International Application Number:
PCT/US2023/010286

(22) International Filing Date:
06 January 2023 (06.01.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/297,822 10 January 2022 (10.01.2022) US
63/426,199 17 November 2022 (17.11.2022) US
63/433,106 16 December 2022 (16.12.2022) US

(71) Applicant: **REGENERON PHARMACEUTICALS, INC.** [US/US]; 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(72) Inventors: **ZHANG, Sisi**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **ZHAO, Bo**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **XIAO, Hui**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US). **LI, Ning**; c/o Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Road, Tarrytown, New York 10591 (US).

(74) Agent: **CAPLAN, Jonathan S.**; Kramer Levin Naftalis & Frankel LLP, 1177 Avenue of the Americas, New York, New York 10036 (US).

(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, 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, 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).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))



WO 2023/133250 A1

(54) Title: IMPROVED SEQUENCE VARIANCE ANALYSIS BY PROTEOMINER

(57) Abstract: The present invention provides methods and systems to identify host cell protein (HCP) impurities in a sample containing high-abundance proteins. The HCP impurities can be enriched using interacting peptide ligands which have been attached to solid support. The HCP impurities can be eluted from the solid support. The isolated HCP impurities can be subjected to limited digestion to generate components of the isolated HCP impurities which can subsequently be identified using a mass spectrometer. The present invention also provides methods and systems to identify sequence variant (SV) peptides or proteins in a sample containing high-abundance proteins. The SV peptides or proteins can be enriched using interacting peptide ligands which have been attached to solid support. The SV peptides or proteins can be eluted from the solid support. The isolated SV peptides or proteins can be subjected to full or limited digestion to generate components of the isolated SV peptides or proteins which can subsequently be identified using a mass spectrometer.