A standard numbering scheme for class C β-lactamases

Andrew R Mack [1,6], Melissa D Barnes, [2,6] Magdalena A Taracila [2,6], Andrea M Hujer [2,6], Kristine M Hujer [2,6], Gabriel Cabot [24,25], Michael Feldgarden [7], Daniel H Haft [7], William Klimke [7], Focco van den Akker [4], Alejandro J Vila [12,14-17], Andrea Smania [18,19], Shozeb Haider [31]. Krisztina M Papp-Wallace [2,4,5,6], Patricia A Bradford [8], Gian Maria Rossolini [20,21], Jean-Denis Docquier [32], Jean-Marie Frère [22], Moreno Galleni [22], Nancy D Hanson [33], Antonio Oliver [24,25], Patrick Plésiat [26,27,28], Laurent Poirel [29,30], Patrice Nordmann [29,30], Timothy G Palzkill [9,10], George A Jacoby [23], Karen Bush [11], Robert A Bonomo [1-6,12,13,‡].

Departments of [1] Molecular Biology and Microbiology, [2] Medicine, [3] Pharmacology, [4] Biochemistry, and [5] Proteomics and Bioinformatics, Case Western Reserve University, Cleveland, OH, USA.

[6] Research Service, Louis Stokes Cleveland Department of Veterans Affairs, Cleveland, OH, USA.

[7] National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD, 20894,USA.

[8] Antimicrobial Development Specialists, LLC, Nyack, NY, USA.

[9] Department of Pharmacology and Chemical Biology and [10] Verna Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA.

[11] Department of Biology, Indiana University Bloomington, Bloomington, IN, USA.

[12] CWRU-Cleveland VAMC Center for Antimicrobial Resistance and Epidemiology (Case VA CARES), Cleveland, OH, USA.

[13] Geriatric Research Education and Clinical Centers (GRECC), Louis Stokes Cleveland Department of Veterans Affairs, Cleveland, OH, USA.

[14] Instituto de Biología Molecular y Celular de Rosario (IBR, CONICET-UNR), Ocampo y Esmeralda, Rosario, Argentina.

[15] Plataforma Argentina de Biología Estructural y Metabolómica PLABEM, Buenos Aires, Argentina.

[16] Plataforma Argentina de Biología Estructural y Metabolómica PLABEM, Buenos Aires, Argentina.

[17] Área Biofísica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, Argentina.

[18] Centro de Investigaciones en Química Biológica de Córodoba (CIQUIBIC), CONICET, Universidad Nacional de Córdoba, Córdoba, Argentina.

[19] Departamento de Química Biológica Ranwel Caputto, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina.

[20] Department of Experimental and Clinical Medicine, University of Florence, Florence, Italy.

[21] Microbiology and Virology Unit, Florence Careggi University Hospital, Florence, Italy.

[22] Centre for Protein Engineering (CIP), University of Liège, Liège, Belgium.

[23] Lahey Hospital and Medical Center, Burlington, Massachusetts, USA.

[24] Red Española de Investigación en Patología Infecciosa (REIPI), Instituto de Salud Carlos III, Madrid, Spain.

[25] Servicio de Microbiología, Hospital Universitario Son Espases, Instituto de Investigación Sanitaria Illes Balears (IdISBa), Palma de Mallorca, Spain.

[26] Laboratoire de Bactériologie, Centre Hospitalier Régional Universitaire, Besançon, France.

[27] UMR6249 CNRS Chrono-Environnement, Université de Bourgogne Franche-Comté, Besançon, France.

[28] Centre National de Référence de la résistance aux antibiotiques, Besançon, France.

[29] Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland.

[30] Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland.

[31] UCL School of Pharmacy, University College London, London, United Kingdom.

[32] Department of Medical Biotechnology, University of Siena, Siena, Italy.

[33] Department of Microbiology and Immunology, Creighton School of Medicine, Omaha, NE, USA.

Corresponding Author:

‡ Robert A. Bonomo, MD, Medical Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, 10701 East Blvd., Cleveland, OH 44106. P: 216-791-3800, x 4645. Email: robert.bonomo@va.gov.

Abstract (Re-write to 75 words)

Unlike classes A and B, a standardized amino acid numbering scheme has not been proposed for the class C (AmpC) β-lactamases, which complicates communication in the field. Here, we propose a scheme developed through a collaborative approach that considers both sequence and structure, preserves traditional numbering of catalytically important residues (Ser^{64}, Lys^{67}, Tyr^{150}, and Lys^{315}), is adaptable to new variants or enzymes yet to be discovered, and includes a variation for genetic and epidemiological applications.

An urgent need exists to address current inconsistencies in the numbering of amino acid residues among class C β-lactamases, both within families and across the class. Established conventions in the field define three common features shared among the serine-type β-lactamases. In the class C β-lactamases, also known as AmpC β-lactamases, these features occur at recognizable conserved motifs: $S^{64}XXK$ at the active site; $Y^{150}XN$; and $K^{315}(S/T)G$ (1–4). These designations align with the amino acid sequence of the mature form of both the P99 AmpC of the *Enterobacter cloacae* complex (NCBI Accession WP_049134845.1), originally characterized in an *Enterobacter hormaechei* strain, and *Escherichia coli* K-12 AmpC (NCBI Accession WP_001336292.1). While *E. coli* AmpC has historical significance as both the first β-lactamase reported (5) and the first class C β-lactamase sequenced, P99 maintains identical numbering of conserved motifs while the mature form begins with a natural residue one (6). Many other class C β lactamases, however, possess insertions and deletions that shift the numbering of the conserved residues, significantly complicating both nomenclature and comparisons between enzymes.

For this report, we analyzed 155 unique AmpC structures deposited in the Protein Data Bank (including 142 supported by 66 publications), and found that 129 β-lactamase structures identify the catalytic serine as Ser^{64} (123 naturally and 6 with alignment), 10 number from the beginning of the precursor form with the signal peptide included, and the remaining 16 number from the beginning of the mature form, but do not identify the catalytic serine as Ser^{64} (of which 8 are not associated with a publication). Additionally, based on a literature search of PubMed, we found consistency is lacking for numbering within the various families of class C β-lactamases. As an example, since the term PDC (*Pseudomonas*-derived cephalosporinase) was coined in 2009 for the chromosomal AmpC of *Pseudomonas aeruginosa*, three different approaches have been used to number amino acid residues in this β-lactamase (7). These approaches include: *i*) direct numbering of residues beginning with the N-terminus of the precursor protein (7); *ii*) direct numbering of residues beginning with the N-terminus of the mature protein (8); and *iii*) alignment-based numbering designed to maintain the conventional assignment of conserved residues and to simplify numbering for comparisons across families (9). Unfortunately, it can be unclear to readers which

of the various schemes is being used in a given publication. As a result, authors may sometimes find choosing a numbering scheme and numerically designating a given residue problematic. Comparing findings from multiple publications may be made unnecessarily difficult; resolving ambiguity in assignment may be extremely challenging. For example, a reference to Gly at position 183 in PDC could refer to a site that is described as having a clinically relevant mutation if numbering begins with Met[1] of the precursor form, but would refer to a different glycine, 26 residues away, using alignment based numbering (10, 11).

To address this growing concern, we propose a numbering scheme to use consistently when referring to crystallographically equivalent positions in the mature form of any class C β-lactamase. We suggest the acronym "*SANC*" to name the scheme, for Structural Alignment-based Numbering of class C β-lactamases, or else the simpler term "structural position." In developing this numbering scheme, we adapted the approaches used by Ambler et al. for the class A β-lactamases (12) and Galleni et al. for the class B β-lactamases (13). We conducted an amino acid alignment of 32 AmpC β-lactamases, both chromosomal and plasmid encoded (**Supplemental Material**) and identified characteristic differences from P99 for each enzyme (**Table 1** Sequences were obtained from the National Center for Biotechnology Information Protein Database (14) and signal peptide cleavage sites were determined using Uniprot (or SignalP 5.0 for entries not present in Uniprot) (15, 16). Mature protein sequences were aligned using the MUSCLE algorithm (17) with default settings.

Consensus secondary structure (defined as a majority of structures in agreement for a given amino acid position) was determined based on comparisons of a representative structure of each of the ten AmpC β-lactamases for which one or more structures are available in the Protein Data Bank, specifically: ACT-1 (PDB: 2ZC7), ADC-7 (PDB: 4U0T), CMY-2 (PDB: 1ZC2), *E. coli* K12 AmpC (PDB: 2BLS), FOX-4 (PDB: 5CGS), MOX-1 (PDB: 3W8K), *Mycobacterium smegmatis* AmpC (PDB: 5E2H), PDC-1 (PDB: 4GZB), and TRU-1 (PDB: 6FM6). The consensus agrees with the secondary structure (or lack thereof) of P99 for just over 90% of residues. This consensus was used to annotate secondary structure, including stripes to indicate residues with an even split between two secondary structure types, and helix numbers on

the alignment. Finally, a simple literature survey was conducted to determine residues belonging in either the consensus portion or fullest likely extent of the Ω-loop or R2-loop, both of which are also annotated on the alignment. By including this structural information, we hope to both better correlate the numbering system with well-known structural features and to provide additional points of reference for those just beginning to work with AmpC structures.

The exact position of one insertion and one deletion within the alignment were manually adjusted (residue 203a by MUSCLE became 204a by structure to preserve a β-turn and the deletion of residue 247 by MUSCLE became a deletion of residue 245 by structure to preserve an α-helix) to ensure they occurred in structurally reasonable areas of both the consensus structure and ten source structures.

Amino acid numbering was based on *E. cloacae* complex P99 while preserving the conventional numbering of the following residues: Ser^{64}, Lys^{67}, Tyr^{150}, and Lys^{315}. Insertions relative to P99 were addressed by appending lowercase letter(s) to the number of the amino acid immediately preceding the insertion (e.g., 125a in PDC-1). Deletions relative to P99 were skipped, resulting in "ghost residues" (e.g., ACC-1 has residues G115 and L117 with a deleted residue at 116). For mature enzymes with more C-terminal amino acid residues than P99, additional residues are assigned numbers in numerical order beginning with 362. For mature enzymes with more N-terminal amino acid residues than P99, the first additional residue is numbered 0 and subsequent residues are numbered by appending a lowercase letter to zero while moving in an N-terminal direction (e.g., 0 and 0a for BUT-1 and *Edwardsiella* AmpC). Signal peptide residues are assigned negative numbers, beginning with -1 for the residue adjacent to the cleavage site and proceeding in the N-terminal direction until all residues are numbered. Multiple sequence alignments are not considered for the signal peptide regions. **Figure 1** illustrates these principles with several examples.

Amino acid positions should be provided under both a family-specific, precursor-based scheme (precursor numbering) and the alignment based scheme (*SANC*) at first mention of a given residue in a publication. Authors are free to choose their favored convention for subsequent mentions, but as a general

suggestion we encourage the use of *SANC* for biochemical and structural publications and precursor numbering for genetic and epidemiological publications.

Providing numbering under both schemes is essential to our proposal. Structural numbering maintains continuity with the conventional assignment of the catalytic serine as Ser[64] and the majority of existing literature on class C β-lactamase structure and function while precursor numbering enables direct gene translation and simplifies interpretation of sequencing results, particularly within a single family. Utilizing this hybrid approach, an initial description of a typical PDC variant might read "PDC-221 differs from PDC-1 (GenBank AAG07497.1) by a single amino acid substitution, E247K, occurring at SANC position 219."

In the supplementary materials, we provide a table featuring a multiple sequence alignment of 32 class C β-lactamases with column headers indicating the appropriate number to be used at each position. The spreadsheet also features a text-based alignment of the structures used in determining the consensus secondary structure. Separately, we provide a protein profile hidden Markov model (HMM) which implements the SANC scheme, built from the multiple sequence alignment using HMMER (http://hmmer.org). Alignments of the HMM to class C β-lactamases can be expected to produce correct SANC assignments when results of the search are examined. We suggest using the HMM, rather than examinations by eye, to make position assignments under this scheme for novel AmpC enzymes that may be discovered in the future. Finally, basic instructions for using our HMM with the HMMER software are also included with the supplementary materials.

For the specific case of PDC variants, a database utilizing the three numbering schemes (SANC and both family-specific precursor and mature form numbering) is freely available at https://arpbigidisba.com/pseudomonas-aeruginosa-derived-cephalosporinase-pdc-database/

Acknowledgements

Class C β-Lactamase	NCBI Accession	Insertions and Deletions Relative to *E. cloacae* P99
ACC-1	WP_032491956.1	-116, +204a, +247a, -289, -290, +362, +363
ACT-1	WP_063857727.1	-361
ADC-7	WP_063857816.1	+0, +204a, -245, -304, -305, -306, +362
ADC-8	WP_004923134.1	+0d, +0c, +0b, +0a, +0, -245, +362, +363, +364, +365, +366, +367
AQU-1	WP_099156042.1	-1, -2, +204a, -243, -245, -301, -302, +362
B. multivorans AmpC1	WP_012218336.1	+204a, -245, +362, +363, +364
BUT-1	WP_104531863.1	+0a, +0
CepH	WP_063843234.1	-1, -2, +204a, -243, -245, +362
CepS	WP_063843235.1	-1, -2, +204a, -243, -245, +362

CFE-1	WP_032490699.1	None
CMA-1	WP_032974004.1	-1, -2, -3, -4, -5, -6, -116, +204a, -245, +362
CMH-1	WP_063859580.1	None
CMY-2	WP_000976514.1	None
CSA-1	WP_007888761.1	-1, -2, -3, -4, -5, -6, -116, +204a, -245, +362
DHA-1	WP_004236386.1	-1, -2, -3, -4, -301
E. coli K-12 AmpC	WP_001336292.1	-1, -2, -3
EC-5	WP_001443153.1	-1, -2, -3
Edwardsiella AmpC	WP_041692555.1	+0a, +0
FOX-4	WP_032489727.1	-1, -2, +204a, -243, -245, +362
LHK-1	WP_081666691.1	-1, -2, -3, -4, +204a, -245
LRA-10	WP_099982803.1	-1, -126, +204a, -245, -361
LRA-18	WP_099982801.1	-1, -245, -311, +362, +363, +364, +365
M. smegmatis AmpC	WP_011729443.1	-1, -2, -3, -4, -5, -6, +204a, -245, -305, -306, +362
MIR-1	WP_032489464.1	None
MOX-1	WP_032489888.1	+0, +204a, -243, -245, -301, -302, -303, +362
OCH-1	WP_040129485.1	+0, +204a, -245, +362, +363, +364
PAC-1	WP_034051940.1	-1, -2, -3, -4, -5, -116, +204a, -245, +362
PDC-1	WP_003101289.1	+125a, +204a, -245, +362, +363, +364, +365, +366, +367, +368, +369, +370
SRT-1	WP_063864749.1	-1, -2, -3, -4, -5, -6, -116, +204a, -245, +362, +363

		-1, -2, -3, -4, -5, -6, -116, +204a, -245, +362,
SST-1	WP_063864750.1	+363
TRU-1	WP_042027926.1	-1, -2, +204a, -243, -245, +362

Table 1: Insertions and deletions present in the AmpC enzymes examined when compared to *E. cloacae* P99. Minus indicates a deletion and plus indicates an insertion. Appended letters indicate an insertion follows a given residue number.

```
SANC Position        -20        -10      -1 0 +1          10        20        30        40        50        60        70        80        90        100
                                          ||||||
P99    -20         MMRKSLCCALLLGISCSALA-----TPVSEKQLAEVVANTITPLMKAQSVPGMAVAVIYQGKPHYYTFGKADIAANKPVTPQTLFELGSISKTFTGVLGGDAIARGEISLDDAVTRYWPQLTGKQWQGI  104
ADC-7  -23          MRFKKISCLLLSPLFIFSTSIYA----DNTPKDQEIKKLVDQNFKPLLEKYDVPGMAVGVIQNNKKYEMYYGLQSVQDKKAVNSNTIFELGSVSKLFTATAGGYAKNKGKISFDDTPGKYWKELKNTPIDQV  104
ADC-8  -26       MMKDILGNLDNVPFKIMTGCIAGLLSCGTVAQSTVQQSIQQSVDRHFKPLMAQYQISGMAIAVSINGQHYYQNYGVASKQTEQNVSEHTLFELGSVSKLFNATLTGYAQAQGQLKLSDHPAQYFPELKNTAVNQA  104
BUT-1  -20          MMKKTLCCALVLSASFSAFA---AQKTLSDKQLEDVVNQTLKPMITAQAIPGMAVAVIYQGKPHYFTYGVADIAKKQPVTTQTIFELGSVSKTFTGVLGGDIVARGEVKLSDPATKYWPELTGKQWQGI  104
PDC-1  -26        MRDTRFPCLCGIAASTLLFATTPAIA-----GEAPADRLKALVDAAVQPVMKANDIPGLAVAISLKGEPHYFSYGLASKEDGRRVTPETLFEIGSVSKTFTATLAGYALTQDKMRLDDRASQHWPALQGSRFDGI  104

SANC Position        110       120   125a  130       140       150       160       170       180       190       200 204a 210       220       230
P99    105   RMLDLATYTAGGLPLQVPDEV-TDNASLLRFYQNWQPQWKPGTTRLYANASIGLFGALAVKPSGMPYEQAMTTRVLKPLKLDHTWINVPKAEEAHYAWGYR-DGKAVRVSPGMLDAQAYGVKTNVQDMANWVMAN  237
ADC-7  105   NLLQLATYTSGNLALQFPDEV-QTDQQVLTFFKDWKPKNPIGEYRQYSNPSIGLFGKVVALSMNKPFDQVLEKTIFPALGLKHSYVNVPKTQMQNYAFGYNQENQPIRVNPGPLDAPAYGVKSTLPDMLSFIHAN  237
ADC-8  105   TLLNLGTYTAGGFPLQFPEQI-KTTQDMTQYFQHWQPKAAPRRIREYSNPSIGLMGYVTALAMKNSYSNLIENTLFPALGMHHSYINVPAAQMSNYAWGYQ-ADQQAIRVSPGMFDAEAYGIKSNTADMLKFLDAQ  237
BUT-1  105   TLLDLATYTAGGLPLQVPDEV-DNQAALLKFYQNWQPAWAPGTRRQYANSSIGLFGALAVKPSGMTFNDAMRQRVLQPLNLKHTWLTVPASEENHYAWGYR-DGKAMHVGPGMLDTEAYGVKSTIEDMASWVQYN  237
PDC-1  105   SLLDLATYTAGGLPLQFPDSVQKDQAQIRDYYRQWQPTYAPGSQRLYSNPSIGLFGYLAARSLGQPFERLMEQQVFPALGLEQTHLDVPEAALAQYAQGYGKDDRPLRVGPGPLDAEGYGVKTSAADLLRFVDAN  237

SANC Position        240 245   250       260       270       280       290       300 304 310       320       330       340       350       360       370
                                                                               305
                                                                               306
P99    238   MAPENVADASLKQGIALAQSRYWRIGSMYQGLGWEMLNWPVEANTVVEGSDSKVALAPLPVAEVNPPAPPVKASWVHKTGSTGGFGSYVAFIPEKQIGIVMLANTSYPNPARVEAAYHILEALQ--------  361
ADC-7  238   LNPQKYP-TDIQRAINETHQGRYQVNTMYQALGWEEFSYPATLQTLLDSNSEQIVMKPNKVTAISK---EPSVKMYHKTGSTSGFGTYVVFIPKENIGLVMLTNKRIPNEERIKAAYVVLNAIKK--------  362
ADC-8  238   INPQNLK-PTLRKAIQTTQMGYFRVGQMRQGLGWEQYTYPVSLTTTLLAGNSAKMALQPQPVTGISKPITAPQQALLNKTGATNGFSAYVVVIPSQKIGLVMLANRNFPNDARVKAAYATLQQILNADIQK---  367
BUT-1  238   MNPQQVKQPTLQKGLELAQSRYWRSGSMYQGLGWEMLNWPVAAATVINGSDNKVALAASPVTAIEPPVAPVKASLVHKTGSTNGFGAYVAFIPEKQIGIVMLANKMYPNTERVKAANTLLNTLQ--------  361
PDC-1  238   LHPERLD-RPWAQALDATHRGYYKVGDMTQGLGWEAYDWPISLKRLQAGNSTPMALQPHRIARLPAPQALEGQRLLNKTGSTNGFGAYVAFVPGRDLGLVILANRNYPNAERVKIAYAILSGLEQQGKVPLKR  370
```

Figure 1: Using an alignment to assign SANC-based amino acid residue numbers. Positions corresponding to insertions and deletions are indicated in bold. ADC-7 adds residues 0, 204a, and 262 and deletes residues 245 and 304-306. ADC-8 adds residues 0-0d and 262-267 and deletes residue 245. BUT-1 adds residue 0. PDC-1 adds residues 125a, 204a, and 362-370 and deletes residue 245. For reference, signal sequences are highlighted in yellow, $S^{64}XXK$ in green, $Y^{150}XN$ in blue, and $K^{315}(S/T)G$ in red.

References

1. Bush K. 2013. The ABCD's of β-lactamase nomenclature. Journal of Infection and Chemotherapy 19:549–559.

2. Jacoby GA. 2009. AmpC β-lactamases. Clin Microbiol Rev 22:161–182.

3. Oefner C, D'Arcy A, Daly JJ, Gubernator K, Charnas RL, Heinze I, Hubschwerlen C, Winkler FK. 1990. Refined crystal structure of β-lactamase from *Citrobacter freundii* indicates a mechanism for β-lactam hydrolysis. Nature 343:284–288.

4. Lobkovsky E, Moews PC, Liu H, Zhao H, Frere JM, Knox JR. 1993. Evolution of an enzyme activity: crystallographic structure at 2-A resolution of cephalosporinase from the ampC gene of *Enterobacter cloacae* P99 and comparison with a class A penicillinase. PNAS 90:11257–11261.

5. Abraham EP, Chain E. 1940. An enzyme from bacteria able to destroy penicillin. Nature 146:837.

6. Galleni M, Lamotte-Brasseur J, Raquet X, Dubus A, Monnaie D, Knox JR, Frère J-M. 1995. The enigmatic catalytic mechanism of active-site serine β-lactamases. Biochemical Pharmacology 49:1171–1178.

7. Rodríguez-Martínez J-M, Poirel L, Nordmann P. 2009. Extended-spectrum cephalosporinases in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 53:1766–1771.

8. Berrazeg M, Jeannot K, Enguéné VYN, Broutin I, Loeffert S, Fournier D, Plésiat P. 2015. Mutations in β-lactamase AmpC increase resistance of *Pseudomonas aeruginosa* isolates to antipseudomonal cephalosporins. Antimicrob Agents Chemother 59:6248–6255.

9. Drawz SM, Taracila M, Caselli E, Prati F, Bonomo RA. 2011. Exploring sequence requirements for C_3/C_4 carboxylate recognition in the *Pseudomonas aeruginosa* cephalosporinase: Insights into plasticity of the AmpC β-lactamase. Protein Sci 20:941–958.

10. MacVane SH, Pandey R, Steed LL, Kreiswirth BN, Chen L. 2017. Emergence of ceftolozane-tazobactam-resistant *Pseudomonas aeruginosa* during treatment is mediated by a single AmpC structural mutation. Antimicrob Agents Chemother 61:e01183-17.

11. Cabot G, Bruchmann S, Mulet X, Zamorano L, Moyà B, Juan C, Haussler S, Oliver A. 2014. *Pseudomonas aeruginosa* ceftolozane-tazobactam resistance development requires multiple Mutations leading to overexpression and structural modification of AmpC. Antimicrob Agents Chemother 58:3091–3099.

12. Ambler RP, Coulson AF, Frère JM, Ghuysen JM, Joris B, Forsman M, Levesque RC, Tiraby G, Waley SG. 1991. A standard numbering scheme for the class A β-lactamases. Biochem J 276 (Pt 1):269–270.

13. Galleni M, Lamotte-Brasseur J, Rossolini GM, Spencer J, Dideberg O, Frère J-M, The Metallo-β-Lactamase Working Group. 2001. Standard numbering scheme for class B β-lactamases. Antimicrobial Agents and Chemotherapy 45:660–663.

14. NCBI Resource Coordinators. 2018. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 46:D8–D13.

15. The UniProt Consortium. 2019. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Research 47:D506–D515.

16. Almagro Armenteros JJ, Tsirigos KD, Sønderby CK, Petersen TN, Winther O, Brunak S, von Heijne G, Nielsen H. 2019. SignalP 5.0 improves signal peptide predictions using deep neural networks. Nat Biotechnol 37:420–423.

17. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797.