# Aminoacyl-tRNA synthetases as therapeutic targets

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Abstract | Aminoacyl-tRNA synthetases (ARSs) are essential enzymes for protein synthesis with evolutionarily conserved enzymatic mechanisms. Despite their similarity across organisms, scientists have been able to generate effective anti-infective agents based on the structural differences in the catalytic clefts of ARSs from pathogens and humans. However, recent genomic, proteomic and functionomic advances have unveiled unexpected disease-associated mutations and altered expression, secretion and interactions in human ARSs, revealing hidden biological functions beyond their catalytic roles in protein synthesis. These studies have also brought to light their potential as a rich and unexplored source for new therapeutic targets and agents through multiple avenues, including direct targeting of the catalytic sites, controlling disease-associated protein–protein interactions and developing novel biologics from the secreted ARS proteins or their parts. This Review addresses the emerging biology and therapeutic applications of human ARSs in diseases including autoimmune and rare diseases, and cancer.

Aminoacyl-tRNA synthetases (ARSs) are a family of 20 essential enzymes (one for each amino acid) that ligate amino acids to their corresponding tRNAs in protein synthesis (FIG. 1a). Each ARS protein and the encoding gene are designated as XRS and *XARS*, respectively, where X designates the single-letter amino acid code of the cognate amino acid. For example, KRS designates the lysyl-tRNA synthetase and *KARS* is the gene encoding it. ARSs can be grouped into class I and class II, which are characterized by fundamental structural differences and enzyme kinetics (FIG. 1b). In mammals, ARSs are also classified into free and complex-bound forms, and the latter are components of the multi-tRNA synthetase complex (MSC), which includes three ARS-interacting multi-functional proteins (AIMPs), named AIMP1, AIMP2 and AIMP3 (FIG. 1c). AIMPs have a scaffolding role in the assembly of the MSC and participate in diverse signalling pathways upon stresses and stimuli[1]. Although the structure and function of the MSC are not yet fully understood, it seems to contribute to system control and homeostasis in higher-order eukaryotes. Hereafter, we use the term ARSN to refer to the group constituted by AIMPs and the entire family of ARSs, including their MSC-component and free forms.

Structural differences in the catalytic sites of pathogenic and human ARSs have led to the development of several successful anti-infective agents[2–4] (BOX 1). However, human ARSs are generally not considered suitable drug targets for non-infectious diseases because of concerns that inhibitory agents would block translation in normal cells. Yet, an accumulating body of evidence suggests that human ARSs are, in fact, worthy of investigation in drug development. In this Review, we discuss both the well-known catalytic activity and the recently unveiled functions of ARSN, as well as emerging opportunities for exploiting them in the discovery of novel therapeutics.

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https://doi.org/10.1038/s41573-019-0026-3

## The physiological role of ARSs

***Protein synthesis.*** The charging of tRNAs with their cognate amino acid is a high-fidelity process in which an ARS specifically recognizes the identity site in its corresponding tRNAs (FIG. 1a). The ARS activates the correct amino acid by ATP hydrolysis, which forms an aminoacyl adenylate (aa-AMP) and releases a pyrophosphate. The amino acid is then transferred to the cognate tRNA, which contains an anticodon loop complimentary to the mRNA codon that encodes the amino acid and an acceptor stem where the matched amino acid is ligated[5] (FIG. 1a). The aminoacylated tRNA then enters the ribosome for protein synthesis. Although the initial interaction between ARS and tRNA is somewhat non-specific, the interaction between the ARS and the tRNA anticodon stem-loop or variable arm that follows is rapid and specific. The ARS and tRNA then undergo a slow conformational change whereby aa-AMP transfers the amino acid to the tRNA acceptor stem[6]. The specifics of this interaction vary depending upon the ARS and tRNA involved.

The high fidelity of tRNA charging is ensured not only by the specific recognition of its cognate tRNA by the ARS but also by its proofreading capability, which

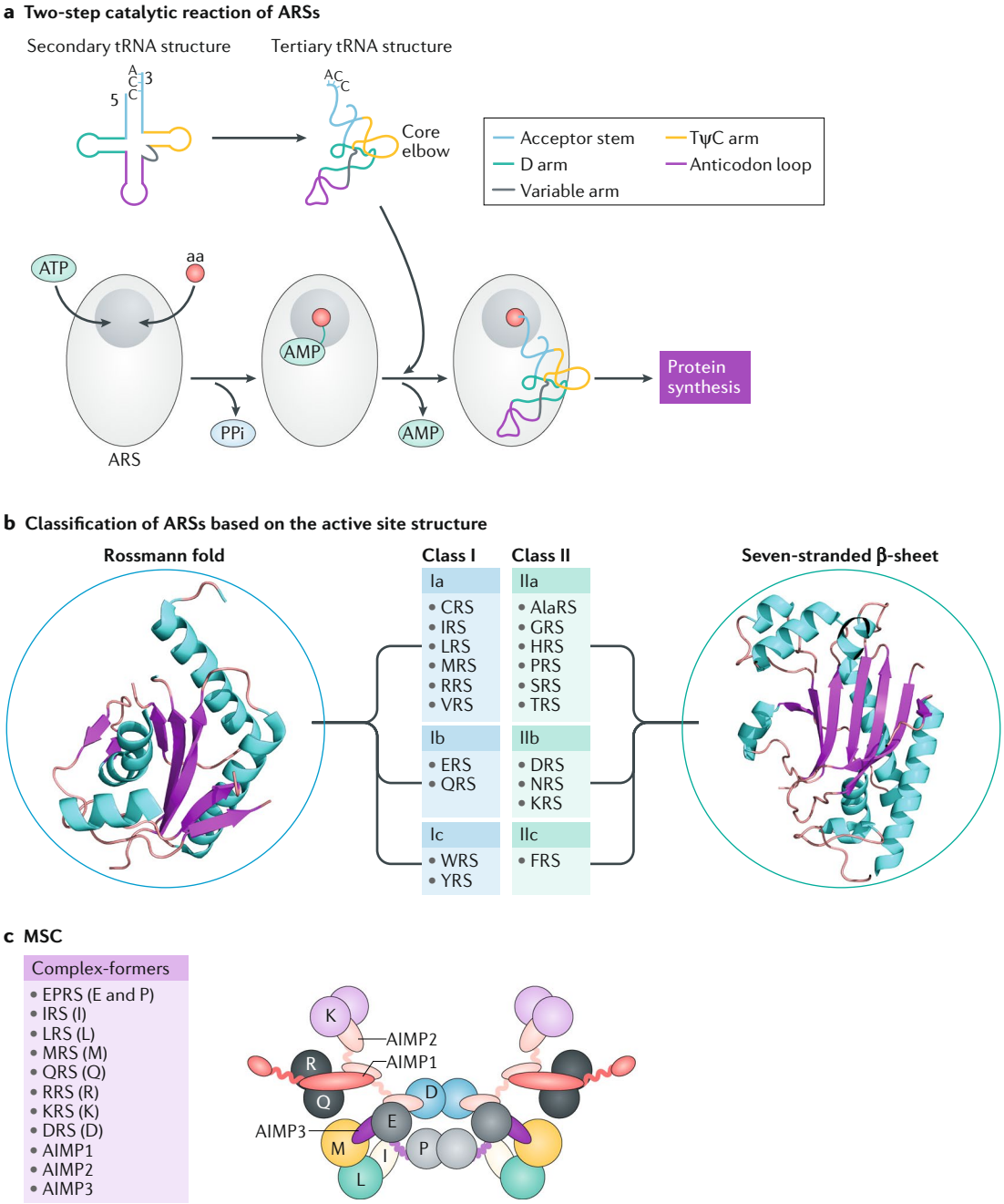


Fig. 1 | **Catalysis and architecture of the ARS catalytic site. a** | Two-step catalytic reaction of aminoacyl-tRNA synthetases (ARSs). ARSs use an amino acid (aa), ATP and tRNA as substrates to produce aa-tRNA (lower part). The upper part shows the secondary (left) and tertiary (right) structures of tRNA. The secondary tRNA structure consists of an anticodon loop, D and TψC arms, a variable arm and an acceptor stem to which the aa is ligated[5]. tRNAs adopt an L-shaped tertiary structure where the anticodon and the acceptor stem-loops are on opposite ends and the D and TψC arms form a core elbow[5]. **b** | Classification of human ARSs based on active site structure. Class I ARSs harbour a Rossmann fold (left), and class II ARSs contain a seven-stranded β-sheet flanked by α-helices (right). Each class divides further into three subclasses, a, b and c, according to their tendency to recognize amino acids that are chemically related. **c** | Schematic of the mammalian multi-tRNA synthetase complex (MSC). Eight ARSs and three ARS-interacting multi-functional proteins (AIMPs) form the MSC. The cartoon represents the recently published model of the MSC[33], to which leucyl-tRNA synthetase (LRS) and isoleucyl-tRNA synthetase (IRS) were added on the basis of interactions reported elsewhere[188,189]. It is expected that some proteins exist as homodimers (lysyl-tRNA synthetase (KRS), aspartyl-tRNA synthetase (DRS) and prolyl-tRNA synthetase (PRS)) and others as two units of monomers. AlaRS, alanyl-tRNA synthetase; CRS, cysteinyl-tRNA synthetase; EPRS, glutamyl-prolyl-tRNA synthetase; ERS, glutamyl-tRNA synthetase; FRS, phenylalanyl-tRNA synthetase; GRS, glycyl-tRNA synthetase; HRS, histidyl-tRNA synthetase; MRS, methionyl-tRNA synthetase; NRS, asparaginyl-tRNA synthetase; QRS, glutaminyl-tRNA synthetase; RRS, arginyl-tRNA synthetase; SRS, seryl-tRNA synthetase; TRS, tyrosyl-tRNA synthetase; VRS, valyl-tRNA synthetase; WRS, tryptophanyl-tRNA synthetase; YRS, tyrosyl-tRNA synthetase.

Polyketide
A type of secondary metabolite that either contains alternating carbonyl and methylene groups or is derived from precursors that contain such alternating groups.

prevents misaminoacylation by removing an incorrect amino acid from the aa-AMP or mischarged tRNA[7]. Inaccurate amino acid selection by ARS occurs more frequently than inaccurate tRNA selection because of the limited structural diversity of the amino acid binding area in ARSs[8]. The editing activity is of high functional importance, as shown by a study in which a missense mutation in the editing domain of the alanyl-tRNA synthetase (AlaRS) of mice resulted in an intracellular accumulation of misfolded proteins leading to neurodegeneration[9].

### Box 1 | ARSs and infectious diseases

Aminoacyl-tRNA synthetases (ARSs) represent validated targets for therapeutic strategies against infectious diseases whereby microbial and parasitic infections are controlled by inhibition of the mRNA translation machinery[180]. Because ARS catalytic activities are required for both pathogens and their hosts, finding therapeutic agents that selectively target the pathogenic ARSs is crucial for the successful development of antibiotics. A few natural compounds have been identified that bind pathogen ARSs with high selectivity. For example, the pseudomonic acid mupirocin, which is produced by *Pseudomonas fluorescens*, specifically inhibits the isoleucyl-tRNA synthetase (IRS) of Gram-positive pathogens[167]. The $K_i$ for mupirocin varies between 6 nM for susceptible *Staphylococcus aureus* and 2.73 μM for resistant eukaryotes. Among the IRS residues important for the mupirocin interaction, only two residues differ among species: the His581 and Leu583 residues in the Rossmann fold of the mupirocin-sensitive *Thermus thermophilus* IRS on the basis of the crystal structure[181]. In mupirocin-resistant eukaryotic IRS, the His581 and Leu583 residues are replaced with other residues, demonstrating that a difference in only a few residues can impart selectivity despite an otherwise high degree of sequence and structural homology in the catalytic core. Indolmycin, a natural tryptophan analogue isolated from *Streptomyces griseus*, is highly selective towards bacterial tryptophanyl-tRNA synthetase (WRS)[167], with half-maximal inhibitory concentration ($IC_{50}$) values of 9.25 nM against *Escherichia coli* WRS and of 4.04 mM against eukaryotic WRS. The related compounds TAK-083 and chuangxinmycin also possess selective inhibitory activities against bacterial WRS. Preliminary clinical studies of chuangxinmycin have suggested a promising degree of efficacy for treating septicaemia and urinary and biliary infections caused by *E. coli*[167]. Cladosporin is a natural polyketide that mimics ATP and can be isolated from several fungal genera, including *Aspergillus*, *Penicillium* and *Cladosporium*[167], and has a broad affinity for bacterial, fungal and malarial lysyl-tRNA synthetase (KRS). Its $IC_{50}$ value for *Plasmodium falciparum* KRS is 61 nM, and it has little affinity for human KRS, even at 20 μM (REF.[182]). The key residues responsible for its selectivity are Val324 and Ser340 in the *P. falciparum* KRS, as replacement of these two residues in human KRS with Gln and Thr, respectively, led to a change in sensitivity. The crystal structures of *P. falciparum* KRS and cladosporin further support the notion that Ser344, Gly554 and Gly556 are critical for fine fitting because these residues in other class II ARSs are too large or small to mediate the cladosporin interaction[183]. Interestingly, cladosporin induces overall structural stabilization of *P. falciparum* KRS in the presence of Lys, which has not been observed for human KRS[183]. This finding suggests another mechanism for inhibitor selectivity to ARSs that is not mediated solely by differences in amino acid sequence. Borrelidin is a macrolide compound that inhibits the catalytic activity of tyrosyl-tRNA synthetase (TRS)[136]. Borrelidin is less selective and has off-target effects on formin-binding protein 21 (FBP21) when used at a high dose[136,184,185]. Borrelidin seems to alter the FBP21-mediated splicing of vascular endothelial growth factor, extending the possibility to modulate other splicing steps. Other borrelidin derivatives have been generated on the basis of structure–activity relationships to reduce toxicity. Modifications at the carboxyl group at C22 have been shown to lower in vitro cytotoxicity in two independent studies[184,186,187]. A borrelidin analogue that bears the CH2SPh moiety through a triazole linkage showed potent antimalarial activity with at least 20,000-fold selectivity against the human diploid embryonic cell line MRC-5 (REF.[187]). Compared with borrelidin, BC196 showed 12-fold increased selectivity to *P. falciparum* TRS over the human enzyme and did not inhibit human TRS at the tested dose range in vitro. BC220 revealed a strikingly enhanced level of selectivity in that a 4,000-fold lower concentration was required to kill *P. falciparum* than that required to kill human HEK293 cells. BC220 completely cleared parasitaemia in a mouse model of malaria infection, with an efficacy comparable to that of chloroquine. These results also show that targeting the active site of ARSs can be a promising approach for developing effective anti-infective agents with high efficacy and selectivity.

***The architecture of active sites.*** As previously mentioned, ARSs fall into two families, class I and class II[10] (FIG. 1b). Class I ARSs are usually monomeric and harbour a Rossmann fold comprising a five-stranded parallel β-sheet connected by α-helices. Class II ARSs are dimeric or multimeric and adopt a seven-stranded β-sheet conformation flanked by α-helices[7]. The Rossmann fold of class I ARSs contains two conserved sequence motifs, HIGH (His-Ile-Gly-His) and KMSKS (Lys-Met-Ser-Lys-Ser), which mediate its interaction with ATP[11]. The HIGH motif correctly positions the adenine base of ATP and interacts with phosphates. The second Lys of the KMSKS motif stabilizes the transition state of the aminoacylation step[12]. Recognition and binding of amino acids take place in the catalytic site when the KMSKS motif is open. After the ARS binds to ATP and aa-AMP is formed, the KMSKS loop closes[6,13,14]. In class II ARSs, the catalytic domain features three conserved motifs. The highly conserved Arg and Phe in motif 2, and Arg in motif 3 have critical roles in catalytic activity: motif 3 binds ATP, and motif 2 is involved in the coupling of ATP to the amino acid and transfer of the amino acid to the 3′-tRNA[7]. The interaction between class II ARSs and their substrates also induces a conformation change, but because of the substantial diversity within the subclasses, the crucial catalytic interactions are specific to individual ARSs[7].

Three members of the class I ARSs, leucyl-tRNA synthetase (LRS), isoleucyl-tRNA synthetase (IRS) and valyl-tRNA synthetase (VRS), contain an editing domain, connective polypeptide 1 (CP1), which is inserted into the Rossmann fold[15]. By contrast, the editing domain in class II ARSs is idiosyncratic in both structure and position, and hence the editing domains of tyrosyl-tRNA synthetase (TRS), prolyl-tRNA synthetase (PRS) and phenylalanyl-tRNA synthetase (FRS) are designated N2, INS and B3-B4, respectively[7,8]. The relative position of the synthetic and editing domains suggests that a conformational change mediates tRNA translocation between the domains, which was demonstrated in ARSs from the subclass Ia[16]. Interestingly, AlaRS contains a unique C-Ala domain tethered to the C-terminal end of the editing domain. The C-Ala domain recognizes the elbow of alanine tRNA ($tRNA^{Ala}$) (FIG. 1a) such that the editing and catalytic domains simultaneously bind to the $tRNA^{Ala}$ acceptor stem and cooperatively edit and aminoacylate[17]. In addition to the catalytic domain, all ARSs contain an α-helical anticodon-binding domain (ABD)[8]. The tRNA anticodon loop associates with the ABD of ARSs except for some of the class Ia enzymes[16], and the acceptor stem associates with the catalytic domain[7,18].

***Species-specific structural diversity.*** The catalytic domain of ARSs is well conserved, with structural and functional differences more pronounced between classes than between species[19,20]. In class I ARSs, protein sequences are phylogenetically well conserved among ARSs in the same subclass (such as Ia, Ib and Ic),

implying a common ancestor[21]. The editing domains of class I ARSs are also homologous in structure and sequence. The CP1 editing domains of LRS, VRS and IRS are highly conserved from *Escherichia coli* to humans[7,19], the INS domain is present in prokaryotic but absent in eukaryotic PRS, and the N2 editing domain of TRS is well conserved among bacteria and eukaryotes but lacking in archaea[19,22]. Intriguingly, the editing domain of the bacterial FRS does not share any obvious sequence similarity with its archaeal and eukaryotic counterparts[23]. Although the catalytic core has remained relatively well conserved, it has acquired new sequence motifs and appended domains that have expanded its functional capacity[20,24].

## The evolution of ARSs

Whereas the catalytic mechanism of ARSs is well understood, their regulatory activities beyond protein synthesis have only recently come to light[1,2,20]. ARS evolution was likely first driven by the need for increased catalytic fidelity and then by acquisition of new functions to meet the demands of higher eukaryotes for rigorous control over systems such as stress responses and cell proliferation.

***Catalytic evolution.*** The functional units of ARSs likely evolved in a step-wise fashion, starting with an ancient core enzyme that activated the amino acid and mediated docking of RNA oligonucleotides for aminoacylation[5,25]. RNA oligonucleotides probably originated in a primitive acceptor stem form that evolved to become the current tRNA structure through the addition of anticodon stem and loop parts (FIG. 1a). Through co-evolution with tRNAs, ARSs acquired ABDs to accommodate additional tRNA interactions and incorporated editing domains[5]. Given that misaminoacylation can be detrimental to the organism, editing domains may have emerged before the divergence of three kingdoms from the last universal common ancestor[20].

***Non-catalytic evolution.*** ARS proteins participate in many types of protein–protein interactions (PPIs), which have evolved through the incorporation of additional motifs and domains at multiple structural sites[20,24] (FIG. 2a). These additions include the N-terminal helix appendix, leucine zipper (LZ), endothelial monocyte activating polypeptide II (EMAPII), glutathione *S*-transferase (GST) and WHEP domains. Some ARSs are associated with *trans*-acting factors (AIMP1, AIMP2 and AIMP3 for higher eukaryotes) to accommodate tighter and more diverse PPIs. The emergence of additional domains coincided with an expansion of ARS function beyond the catalytic role in protein synthesis.

The N-helix is attached to the ABD of eukaryotic aspartyl-tRNA synthetase (DRS) and KRS, and binds to the elbow region of the tRNA to enhance binding specificity. In addition to its role in catalysis, the N-helix of KRS helps to package HIV by delivering $tRNA^{Lys3}$, which is one of the isoacceptors of $tRNA^{Lys}$, into the virion[26]. It also enables KRS to translocate to the plasma membrane, where it promotes laminin-mediated cell migration by interacting with and stabilizing the 67 kDa laminin receptor (67LR; also known as 40S ribosomal protein SA)[27,28]. The LZ domain is a helical motif that contains a Leu residue at every fourth position of the heptad repeat. The LZ domain is present in arginyl-tRNA synthetase (RRS), AIMP1, and AIMP2 from insects to humans and participates in assembling these proteins into the MSC[29]. The EMAPII domain, which is located in the C-terminal region of AIMP1, is found in tyrosyl-tRNA synthetase (YRS) from insects to humans, as well as in the *Caenorhabditis elegans* methionyl-tRNA synthetase (MRS) and in yeast ARS cofactor 1 protein (Arc1p), a yeast orthologue of AIMP1 (REF.[24]). EMAPII facilitates the interaction with tRNA[24] and is secreted in the control of immune responses[30,31] and angiogenesis[32]. The GST domain is embedded in VRS, cysteinyl-tRNA synthetase (CRS), Arc1p, MRS, AIMP3, AIMP2 and glutamyl-prolyl-tRNA synthetase (EPRS)[20]. In the last four, the GST domain forms a heterotetramer that participates in the assembly of the MSC[33].

The WHEP domain, which is found in tryptophanyl-tRNA synthetase (WRS), histidyl-tRNA synthetase (HRS), EPRS, MRS and glycyl-tRNA synthetase (GRS), is involved in diverse interactions with other proteins[24]. The human EPRS contains three WHEP domains involved in the formation of the interferon-γ (IFNγ)-activated inhibitor of translation (GAIT) complex, which controls translation of vascular endothelial growth factor A (*VEGFA*)[34,35]. The WHEP domain of WRS interacts with the catalytic subunit of DNA-dependent protein kinase (DNA-PKc) and poly(ADP-ribose) polymerase 1 (PARP1), which activate p53 in the nucleus[36]. When secreted, the WHEP-domain-containing N-terminal 154 amino acids of WRS induce infection-dependent macrophage activation by interacting with the heterodimeric complex formed by Toll-like receptor 4 (TLR4) and myeloid differentiation factor 2 (MD2; also known as LY96)[37]. Although the WHEP domains share the common helix-turn-helix structure, their sequences are highly diversified, perhaps to endow them with the capability of interacting specifically with multiple partners.

Several ARSs contain a uniquely attached sequence motif (UNE) (FIG. 2a). Whereas UNE-F, UNE-N and UNE-Q, where F, N and Q represent the corresponding ARSs, are all involved in tRNA binding, other UNEs seem to mediate specific PPIs. For example, $UNE\text{-}I_2$, the second UNE domain of IRS, is required for its association with the MSC[24] and may be involved in other functions. In the mammalian LRS, the C-terminal region that harbours the variable C terminus (VC) and UNE-L domains interact with RAS-related GTP-binding protein D (RAGD) GTPase when regulating mechanistic target of rapamycin complex 1 (mTORC1)[38], while in yeast, the CP1 domain of LRS binds to GTPase[39]. In zebrafish, the UNE-S of seryl-tRNA synthetase (SRS) contains a nuclear localization signal that moves SRS into the nucleus, where it attenuates *VEGFA* expression[40].

***Functional control through structural metamorphosis.*** ARSs can further expand their functional repertoires through structural changes such as alternative splicing, post-transcriptional and translational modifications and

Rossmann fold
A super-secondary structure composed of a series of alternating β-strand and α-helical segments that commonly appears in a variety of nucleotide binding proteins.

WHEP domains
Helix-turn-helix domains whose name comes from the first letters of tryptophanyl-tRNA synthetase (WRS), histidyl-tRNA synthetase (HRS) and glutamyl-prolyl-tRNA synthetase (EPRS), in which WHEP domains were first discovered.

Isoacceptors
In the context of this review, the different tRNA species that bind to alternate codons for the same amino acid residue.

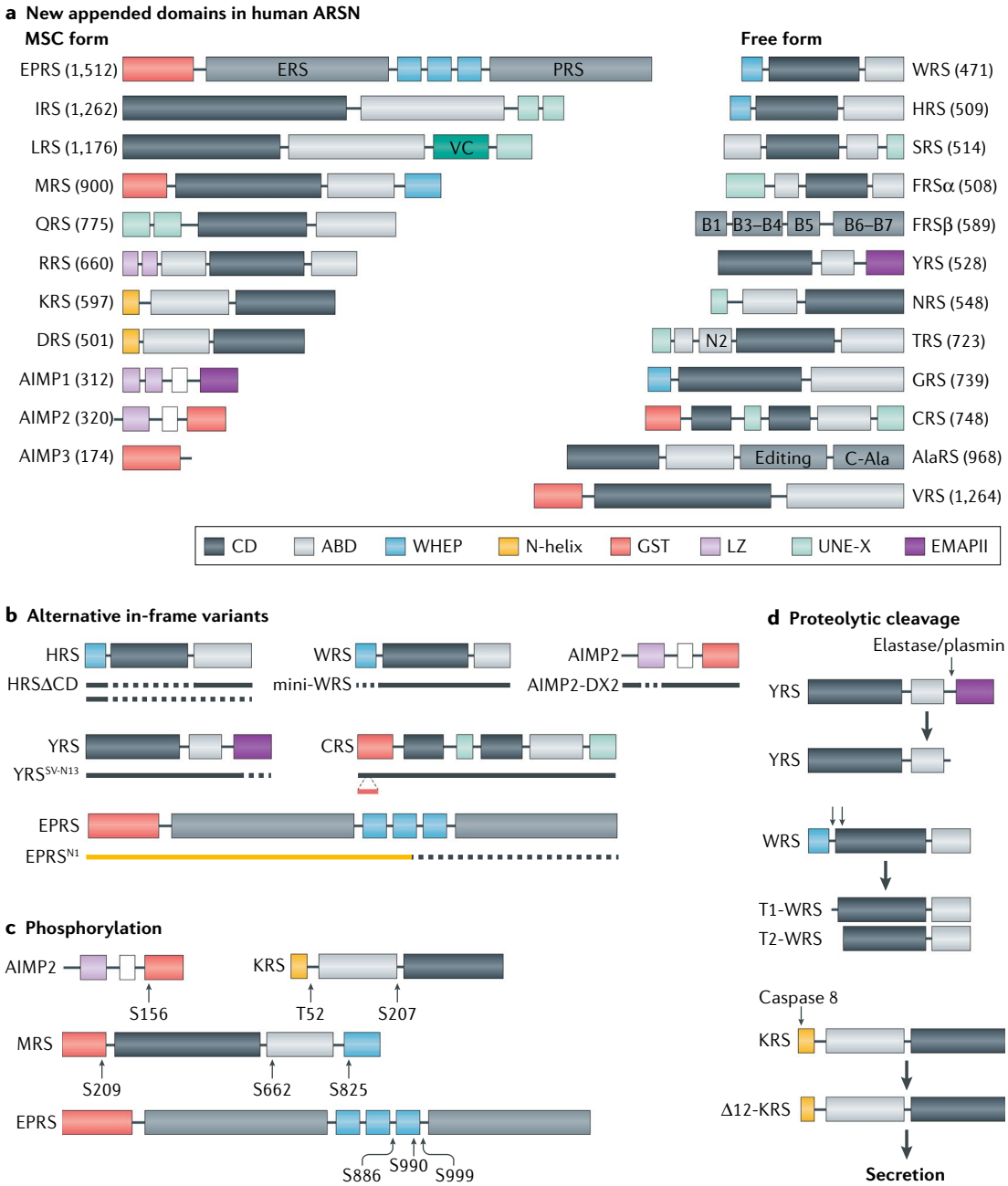


Fig. 2 | **The metamorphosis of ARSs for functional expansion. a** | Domains of human ARSN appended during evolution. Uniquely attached sequence motif (UNE) domains attached to aminoacyl-tRNA synthetases (ARSs) are designated as UNE-X according to the substrate amino acids. WHEP and endothelial monocyte activating polypeptide II (EMAPII) domains are found only in ARSN but no other proteins. The total number of amino acids in each ARSN is indicated in parentheses. **b** | Alternative in-frame variants of ARSN. Among the splicing and post-transcriptional variants of ARSN identified to date[41–47], representative forms whose functions were reported are presented on the basis of the modified domains. Solid lines and dotted lines indicate translated and deleted sequences, respectively. The inserted sequence in cysteinyl-tRNA synthetase (CRS) is highlighted in orange. Whereas the variants of histidyl-tRNA synthetase (HRS), tryptophanyl-tRNA synthetase (WRS), tyrosyl-tRNA synthetase (YRS), CRS and ARS-interacting multi-functional protein 2 (AIMP2) are generated by alternative splicing, the C-terminal truncated glutamyl-prolyl-tRNA synthetase (EPRS), EPRS$^{N1}$, depicted in yellow, is produced by polyadenylation-directed stop codon replacement after transcription. **c**,**d** | Representative post-translational modification of ARSN. Phosphorylation sites of AIMP2, methionyl-tRNA synthetase (MRS), lysyl-tRNA synthetase (KRS) and EPRS domains (part **c**) and the proteolytic cleavage sites of YRS, WRS and KRS (part **d**) are shown, with the involved proteases indicated at the cleavage sites. ABD, anticodon-binding domain; AlaRS, alanyl-tRNA synthetase; CD, catalytic domain; DRS, aspartyl-tRNA synthetase; ERS, glutamyl-tRNA synthetase; FRS, phenylalanyl-tRNA synthetase; GRS, glycyl-tRNA synthetase; GST, glutathione *S*-transferase; IRS, isoleucyl-tRNA synthetase; LRS, leucyl-tRNA synthetase; LZ, leucine zipper; MSC, multi-tRNA synthetase complex; NRS, asparaginyl-tRNA synthetase; PRS, prolyl-tRNA synthetase; QRS, glutaminyl-tRNA synthetase; RRS, arginyl-tRNA synthetase; SRS, seryl-tRNA synthetase; TRS, tyrosyl-tRNA synthetase; VRS, valyl-tRNA synthetase.

proteolytic cleavage[24,32]. Numerous splicing variants of ARSs have been reported[41–46], such as the HRS splicing variant HRSΔCD, which comprises the N-terminal WHEP domain fused to the C-terminal ABD[43] (FIG. 2b). The absence of the catalytic domain induces a prominent conformational change that converts the native homodimer into a monomer, thereby loosening the connection of the WHEP and ABD domains. A splicing variant of WRS in which the N-terminal WHEP domain is truncated has been reported to function as an angiostatic factor upon secretion[45]. The C-terminal EMAPII-truncated YRS domain, $YRS^{SV-N13}$, stimulates megakaryopoiesis[44], and the inserted peptide in the splicing variant of CRS mediates interaction with elongation factor 1γ (REF.[46]). The LZ-deleted splicing variant of AIMP2, designated as AIMP2-DX2, interferes with anti-proliferative signalling of AIMP2 (discussed further below).

The N-terminal truncated EPRS fragment, $EPRS^{N1}$ (N1 indicates the first N terminus-containing truncated form), can be generated by post-transcriptional modification, in which an alternative polyadenylation event within the *EPRS* mRNA recodes a Tyr codon to a stop codon, which results in deletion of the entire PRS and partial WHEP domains (FIG. 2b). $EPRS^{N1}$ can associate with *VEGFA* transcript but not with other GAIT complex constituents; therefore, $EPRS^{N1}$ shields *VEGFA* mRNA from the GAIT complex and blocks the repression of VEGFA translation[47].

Post-translational modification is another mechanism of ARS functional diversification. The cell regulatory activities of the MSC components are controlled by specific phosphorylation events (FIG. 2c). For example, phosphorylation of the S156 residue (pS156) of AIMP2 induces its translocation to the nucleus, where it increases transforming growth factor-β (TGFβ) signalling through its interaction with SMURF2 (REF.[48]). Phosphorylation of KRS at the T52 and S207 residues induces a conformational change that dissociates KRS from the MSC and induces their translocation to the plasma membrane and nucleus, respectively[28,49]. Once localized to the plasma membrane, pT52-KRS promotes cell migration through its association with 67LR. Nuclear-localized pS207-KRS produces the second messenger diadenosine tetraphosphate, which activates the transcription factor microphthalmia-associated transcription factor (MITF). Interestingly, pT52-KRS and pS207-KRS can collaborate to enhance the dissemination of colon cancer spheroids in 3D gels via facilitating cell migration and gene transcription, respectively, to remodel the microenvionment[50]. In macrophages, IFNγ induces sequential phosphorylation at the S886 and S999 residues of EPRS, which releases it from the MSC to form a new GAIT complex with other inflammation-suppressing cellular factors[34]. Upon insulin stimulation in adipocytes, EPRS is phosphorylated by S6K1, inducing its interaction with fatty acid transport protein 1, transporting it to the plasma membrane and enhancing long-chain fatty acid uptake[51]. EPRS is also phosphorylated at S990 upon RNA virus infection, which protects the mitochondrial antiviral signalling protein from ubiquitylation and induces an immune response[52]. In MRS, phosphorylation mediated by GCN2 (also known as E2AK4) of the S662 residue upon ultraviolet irradiation prevents protein synthesis by ablating tRNA binding capability[53]. Similarly, oxidative stress can induce MRS dual phosphorylation at S209 and S825, which lowers $tRNA^{Met}$ specificity and increases Met misincorporation into nascent proteins[54]. Because Met residues on the surface of proteins can scavenge reactive oxygen species, Met mistranslation works as a protective mechanism[55].

The regulatory activities of ARSs can also be controlled by proteolytic cleavage (FIG. 2d). The secreted forms of YRS and WRS, for example, are cleaved by elastase or plasmin[30,56,57]. Removal of the EMAPII domain from YRS by proteolysis exposes an ELR cytokine motif that interacts with CXC-chemokine receptor 1 (CXCR1) and/or CXCR2, enabling the 'mini-YRS' product to promote cell migration and angiogenesis[32,58]. The truncated forms of WRS produced by these N-terminal cleavages are designated T1-WRS, for the 70-amino acid deletion, and T2-WRS, for the 93-amino acid deletion. They both acquire angiostatic activity upon interaction with vascular endothelial cadherin (VE-cadherin) of endothelial cells[57]. In another example, human KRS forms an anti-parallel homodimer where the N-terminal end of one KRS interacts with the C-terminal end of the other and vice versa. Caspase 8-mediated cleavage of the 12 N-terminal amino acids of one subunit exposes the PDZ-binding motif at the C terminus of the other subunit, facilitating interaction with syntenin for incorporation into secreted exosomes[59].

Although the catalytic and non-catalytic activities of ARSs seem to operate with little connection, the two activities can be mutually influenced in some cases. The Ala734Glu AlaRS mutant — which charges a Ser to $tRNA^{Ala}$ instead of an Ala — interacts with ANKRD16, a vertebrate-specific protein that contains ankyrin repeats, via the catalytic domain and ABD[60]. ANKRD16 helps pre-transfer editing by sequestering misactivated Ser and prevents protein aggregation and cell death. For the case of T2-WRS and YRS, amino acid binding pockets are used to develop multifunctionality, such as interaction of T2-WRS with two Trp residues of VE-cadherin to ensure angiostasis[61] and the interaction of YRS with a Tyr analogue of resveratrol that redirects YRS to bind PARP1 to activate it for the DNA damage response[62].

## ARSs in disease

The involvement of cytosolic and mitochondrial ARSs in various human diseases is well documented. Although all ARSs participate in protein synthesis, their pathological connection to human diseases seems to involve structural and functional variations that affect catalytic and non-catalytic activities and can result from misexpression, copy number variation, mutations and genetic variants, and aberrant molecular interactions and secretion.

***Pathological expression.*** The expression profiles of ARSs can be a useful prognostic tool for cancers, as they correlate with overall patient survival for each type of cancer listed in the Human Protein Atlas database[63]. High expression of ARSs usually correlates with shorter patient survival, although the opposite is true for ovarian (WRS and AIMP3), renal (AIMP2), cervical

Angiostatic factor
A substance that inhibits angiogenesis.

Megakaryopoiesis
A complex process in the bone marrow that ends with platelet formation from commitment of pluripotent haematopoietic stem cells.

PDZ-binding motif
A specific C-terminal motif that is usually approximately four or five residues in length and interacts with PDZ domains that are found in anchoring proteins.

(AIMP3 and FRSα) and stomach (FRSα) cancers, which may in part be due to the role of these ARSs in suppressing cell proliferation[36,42,64–66]. Expression of GRS has been proposed as an indicator of unfavourable outcomes for patients with renal, urothelial, liver, breast and endometrial cancers[67]. Increased MRS expression in the neoplastic region of lung tissue correlates with a poor prognosis, including advanced-stage cancer and shorter disease-free survival (DFS)[68]. MRS overexpression has also been reported in other types of cancers including malignant fibrous histiocytomas, lipoma, osteosarcomas, malignant gliomas and glioblastomas[1,69–72]. In prostate cancer, high expression levels of AlaRS, FRSα, GRS, NRS, TRS, HRS and WRS suggest a link between their function and androgen response[73]. Co-expression and colocalization of TRS with VEGF and mucin 1 (MUC-1, which is a Thr-enriched protein) are strongly associated with advanced-stage epithelial ovarian cancer[74] and poor survival of patients with pancreatic cancer[75], respectively.

WRS is significantly overexpressed in oral squamous cell carcinoma (OSCC)[76,77]. IFNγ signalling is one of the most significantly altered pathways in OSCC[77], and interestingly, IFNγ regulates expression, splicing, localization and secretion of WRS[45,56,78,79]. Moreover, WRS expression positively correlates with OSCC tumour stage and invasion, and WRS induces OSCC cell migration. Two tandem promoters and five spliced transcripts produce a full-length WRS and a truncated form with an N-terminal 47-amino acid deletion ('mini-WRS')[45,56,78,79] (FIG. 2b). The full-length and mini forms of WRS oppose each other in the control of angiogenesis[56] and immune stimulation[37], which suggests that caution is necessary in interpreting the relationship between WRS forms and underlying mechanisms in OSCC.

***Disease-specific mutations.*** According to the Open Targets Platform database[80], mutations in ARSs and AIMPs correlate with numerous genetic diseases (TABLE 1). Missense mutations in *KARS* have been linked to autosomal recessive non-syndromic sensorineural deafness[81], possibly by decreasing aminoacylation in cells of the inner ear. Mutations in ARSs are also implicated in hypomyelinating leukodystrophy (HLD)[82]. The Online Mendelian Inheritance in Man (OMIM) database indicates that HLD is genetically heterogenous, with 17 forms of HLD linked to various mutations. On the basis of the location of the mutations (TABLE 1) and according to mechanistic studies[83,84], the affected catalytic activity of ARSs and subsequent reductions in cellular translational activity are generally considered an underlying cause of this disease. The HLD3, HLD9, HLD15 and HLD17 forms of HLD share a similar phenotype and are caused by mutations in *AIMP1, RARS*, *EPRS* and *AIMP2*, respectively[83–87]. Other HLD-associated mutations may also have a role in HLD by affecting less-understood aspects of ARS and AIMP function. For instance, AIMP1 maintains the normal phosphorylation levels of neurofilament light domain (NFL), which is required for the structural integrity of neurons[88]. The AIMP1 mutation identified in HLD3 causes C-terminal truncations through frameshift. The expression of a green fluorescent protein (GFP)-tagged, C-terminal truncated form of AIMP1 that lacks the EMAPII domain maintained its interaction with NFL, resulting in aggregation and punctate formation in cells[89]. Competition between the mutant-mediated interaction and its wild-type counterpart may be the underlying mechanism for AIMP-related HLD pathology.

Early infantile epileptic encephalopathy has been linked to a compound heterozygous missense mutation and a homozygous mutation in the *AARS* gene that reduces the catalytic activity of AlaRS[90]. Mutations in *MARS* and *LARS* are involved in acute infantile liver failure with multi-organ phenotypes[91,92]. Heterozygous MRS mutants have significantly reduced catalytic activity. By contrast, LRS mutations are located in the CP1 domain, which suggests that the disease may be mediated by misaminoacylation or dysfunction of an mTORC1-dependent pathway such as autophagy[91,92]. *IARS* mutations have been identified in patients with infantile hepatopathy[93], and Usher syndrome — a rare genetic disorder characterized by combined hearing loss and visual impairment — is associated with mutation in HRS that results in reduced catalytic activity[94]. Two compound heterozygous mutations in the *QARS* gene have been reported in patients with diffuse cerebral-cerebellar atrophy[95], a disease that is usually associated with defects in gene transcription or protein translation. The mutations p.Arg403Trp and p.Arg515Trp completely disrupt QRS catalytic activity.

Most reports on disease-related ARS mutations describe aminoacylation defects as the underlying cause of disease phenotypes on the basis of the location of mutation sites, a reduced level of the affected ARSs or affected aminoacylation activity. Two recent reports on *FARSB* mutations[96,97], however, raise the importance of discrimination between aminoacylation activity and total protein synthesis. Reduced amount or stability of FRSβ mutants affected the level of FRSα, the binding partner of FRSβ, and the aminoacylation rate of Phe[96,97] but did not reduce total protein synthesis or primary cell proliferation[96]. This finding suggests that more caution and in-depth analysis are required to interpret the mode of action of disease-associated ARS mutations. Researchers have also identified numerous mutations in mitochondrial ARSs[98,99]. Many of them manifest as neurodegenerative disorders that sporadically affect other organs such as skeletal muscle, kidney, lung and heart[98,99].

***Mutation-associated interactions.*** Whereas most of the disease-causing mutations in ARSs described above are autosomal recessive, and their impaired aminoacylation activity is proposed as the link for the phenotypes, other disease-associated ARS mutations are autosomal dominant and therefore can potentially be targeted to control diseases. For instance, autosomal dominant mutations in GRS, YRS, AlaRS, KRS, HRS and MRS are associated with Charcot–Marie–Tooth (CMT) disease (TABLE 1). Intriguingly, only 20% of the CMT-related mutations affect catalytic activity[100]. *GARS* mutations are tightly linked to CMT type 2D (CMT2D) and its subtype, distal spinal muscular atrophy V (dSMA-V)[101,102].

Hypomyelinating leukodystrophy
(HLD). An autosomal recessive neurodegenerative disorder characterized by infant or childhood onset of progressive motor decline.

Frameshift
A shift of translation from one reading frame to another, generally caused by an addition or deletion in the nucleic acid sequence.

Early infantile epileptic encephalopathy
A debilitating progressive neurological disorder that involves intractable seizures and severe mental retardation.

Compound heterozygous missense mutation
A condition in which a gene has two different point mutations resulting in single amino acid change in both alleles.

Charcot–Marie–Tooth (CMT) disease
One of the hereditary motor and sensory neuropathies, a group of varied inherited disorders of the peripheral nervous system characterized by progressive loss of muscle tissue and touch sensation across various parts of the body.

Table 1 | **Disease-related mutations of human cytosolic ARSN**

| Mutation | Location of mutation | Zygotes | Diseases[a] |
|---|---|---|---|
| ***AlaRS*** | | | |
| p.Asn71Tyr or p.Gly102Arg | CD | Heterozygous | CMT or dHMN[190–192] |
| p.Arg329His | ABD | Heterozygous | CMT or dHMN |
| p.Tyr652Ile, p.Glu688Gly, p.Arg729Trp or p.Glu778Ala | Editing domain | Heterozygous | CMT or dHMN |
| p.Asp893Asn, p.Gly931Ser or p.Lys967Met | C-Ala | Heterozygous | CMT or dHMN |
| p.Lys81Thr and p.Arg751Gly | CD or editing domain | Compound heterozygous | EIEE29 (REFS[90,193]) |
| p.Tyr690LeufsX3 and p.Gly913Asp | Editing domain or C-Ala | Compound heterozygous | EIEE29 (REFS[90,193]) |
| p.Arg751Gly | Editing domain | Homozygous | EIEE29 (REFS[90,193]) |
| ***CRS*** | | | |
| Translocation t(2;11)(p23;p15) with ALK | NA | NA | Inflammatory myofibroblastic tumours[32] |
| 11p15.5–p15.4 locus deletion | NA | NA | Wilms tumour and embryonal rhabdomyosarcoma, adrenocortical carcinoma and lung, ovarian and breast cancers[32] |
| ***DRS*** | | | |
| p.Met256Leu | CD | Homozygous | Hypomyelination with brainstem and spinal cord involvement and leg spasticity[139] |
| p.Arg487Cys | CD | Homozygous | As above |
| p.Ala274Val and p.Asp367Tyr | CD | Compound heterozygous | As above |
| p.Arg460His and p.Arg494Gly | CD | Compound heterozygous | As above |
| p.Pro464Lue and p.Arg494Cys | CD | Compound heterozygous | As above |
| ***EPRS*** | | | |
| p.Arg339X and p.Pro1115Arg | ERS CD | Compound heterozygous | HLD[84] |
| p.Pro1160Ser and p.Thr1223LeufsX3 | PRS CD | Compound heterozygous | HLD[84] |
| p.Pro1115Arg | PRS CD | Homozygous | HLD[84] |
| p.Met1126Thr | PRS CD | Homozygous | HLD[84] |
| ***FRSB*** | | | |
| p.Thr256Met and p.His496LysfsX14 | B3-B4 or B6-B7 | Compound heterozygous | Similar to ILFS2 (REF.[97]) |
| c.848þ1G>A and p.Arg305Gln | B3-B4 or B5 | Compound heterozygous | Hypotonia and interstitial lung disease with cholesterol pneumonitis, etc.[96] |
| p.Arg401Gln and p.Thr461Pro | B6-B7 or B6-B7 | Compound heterozygous | As above |
| p.Phe252Ser and p.Arg401Gln | B3-B4 or B6-B7 | Compound heterozygous | As above |
| p.Cys76Arg and p.Lys262Glu | B1 or B3-B4 | Compound heterozygous | As above |
| ***GRS***[b] | | | |
| p.Ala57Val | WHEP | Heterozygous | CMT or HMN5A[102,105] |
| p.Glu71Gly, p.Leu129Pro, p.Asp146Asn, p.Asp146Tyr, p.Cys157Arg[c], p.Ser211Phe, p.Leu218Gln, p.Pro234LysTyr[c], p.Met238Arg, p.Gly240Arg, p.Pro244Leu, p.Glu279Asp, p.Ile280Phe, p.His418Arg, p.Asp500Asn or p.Gly526Arg | CD | Heterozygous | CMT or HMN5A[102,105] |
| p.Ser581Leu or p.Gly598Ala | ABD | Heterozygous | CMT or HMN5A[102,105] |
| ***HRS*** | | | |
| p.Ala5Glu | WHEP | Heterozygous | CMT[102,194] |
| p.Thr132Ile, p.Pro134His, p.Arg137Gln, p.Asp175Glu, p.Gly205Gln, p.Asp364Tyr or p.Lys376Arg | CD | Heterozygous | CMT[102,194] |

Table 1 (cont.) | **Disease-related mutations of human cytosolic ARSN**

| Mutation | Location of mutation | Zygotes | Diseases[a] |
|---|---|---|---|
| ***HRS (cont.)*** | | | |
| p.Pro505Ser | ABD | Heterozygous | CMT[102,194] |
| p.Tyr454Ser | Between CD and ABD | Homozygous | Usher syndrome 3B[94] |
| ***IRS*** | | | |
| p.Arg418X and p.Ile1174Asn | CD or UNE-I | Compound heterozygous | GRIDHH[93] |
| p.Arg254X and p.Pro437Leu | CD | Compound heterozygous | GRIDHH[93] |
| p.Val370Gly and p.Asn992Asp | CD or ABD | Compound heterozygous | GRIDHH[93] |
| ***KRS***[d] | | | |
| p.Tyr173His | ABD | Homozygous | Deafness, autosomal recessive 89 (REF.[81]) |
| p.Asp377Asn | CD | Homozygous | Deafness, autosomal recessive 89 (REF.[81]) |
| p.Leu133His and p.Tyr173SerfsX7 | ABD | Compound heterozygous | CMT[195] |
| p.Ile302Met | CD | Homozygous | CMT[195] |
| ***LRS*** | | | |
| p.Lys82Arg | CD | Homozygous | ILFS1 (REF.[91]) |
| p.Tyr373Cys | Editing domain | Homozygous | ILFS1 (REF.[91]) |
| p.Ala504Val and p.Asn614Lys | Editing domain or CD | Compound heterozygous | ILFS1 (REF.[91]) |
| ***MRS*** | | | |
| p.Arg618Cys | CD | Heterozygous | CMT[102] |
| p.Pro800Thr | WHEP | Heterozygous | CMT[102] |
| p.Leu71CysfsX33 | GST | Heterozygous | Gastric and colon cancer[32] |
| p.Phe370Leu and p.Ile523Thr | CD | Compound heterozygous | ILFS2 (REF.[92]) |
| p.Ala393Thr, p.Ser567Leu or p.Asp605Val | CD | Homozygous | PAP[196] |
| p.Ser567Leu and p.Ala393Thr | CD | Compound heterozygous | PAP[196] |
| p.Tyr344Cys | CD | Heterozygous | PAP[196] |
| 12q13–q15 locus amplification | NA | NA | Malignant fibrous histiocytomas, sarcomas, malignant gliomas and glioblastomas[32] |
| ***QRS*** | | | |
| p.Gly45Val and p.Arg403Trp or p.Tyr57His and p.Arg515Trp | UNE-Q or CD | Compound heterozygous | MSCCA[95] |
| ***RRS*** | | | |
| p.Asp2Gly and c.45 + 1G>T | LZ | Compound heterozygous | HLD[83,85] |
| p. Asp2Gly and p.Cys32TrpfsX39 | LZ | Compound heterozygous | HLD[83,85] |
| p.0? and p.Arg512Gln | LZ/ABD | Compound heterozygous | HLD[83,85] |
| p.Asp2Gly | LZ | Homozygous | HLD[83,85] |
| p.Ser456Leu and p.Tyr616LeufsX6 | ABD | Compound heterozygous | HLD[83,85] |
| ***SRS*** | | | |
| p.Asp172Asn | CD | Homozygous | NEDMAS[197] |
| ***VRS*** | | | |
| c.1577 −2A>G and p.Met1064Ile | CD or ABD | Compound heterozygous | Progressive microcephaly[198] |
| ***YRS*** | | | |
| p.Asp81Ile, p.Gly41Arg, p.Val153_Val156del or p.Glu196Lys | CD | Heterozygous | CMT[102] |
| ***AIMP1*** | | | |
| p.Gln98ValfsX30 | EMAPII | Homozygous | PMLD[e][87] |
| p.Val176Gly and p.Gly299Arg | EMAPII | Homozygous | ARIDWN[87] |
| p.Glu39X | LZ | Homozygous | Cortical neurodegenerative disease with infantile onset[87] |

Table 1 (cont.) | **Disease-related mutations of human cytosolic ARSN**

| Mutation | Location of mutation | Zygotes | Diseases[a] |
|---|---|---|---|
| ***AIMP2*** | | | |
| p.Tyr35X | Upstream of LZ | Homozygous | HLD[86] |
| ***AIMP3*** | | | |
| p.Thr35Ala, p.Ser40Ala, p.Glu76Ala, p.Thr80Pro, p.Ser87Ala, p.Arg144Ala or p.Val106Ala | GST | Heterozygous | Chronic myeloid leukaemia[32] |

ABD, anticodon-binding domain; AIMP, aminoacyl-tRNA synthetase (ARS)-interacting multi-functional protein; AlaRS, alanyl-tRNA synthetase; ARIDWN, autosomal recessive intellectual disability without neurodegeneration; CD, catalytic domain; CMT, Charcot–Marie–Tooth; CRS, cysteinyl-tRNA synthetase; dHMN, distal hereditary motor neuropathy (a variant of CMT); DRS, aspartyl-tRNA synthetase; EIEE29, early infantile epileptic encephalopathy 29; EMAPII, endothelial monocyte activating polypeptide II; EPRS, glutamyl-prolyl-tRNA synthetase; ERS, glutamyl-tRNA synthetase; FRSβ, phenylalanyl-tRNA synthetase-β; GRIDHH, growth retardation, intellectual developmental disorder, hypotonia and hepatopathy; GRS, glycyl-tRNA synthetase; GST, glutathione S-transferase; HLD, hypomyelinating leukodystrophy; HMN5A, neuropathy distal hereditary motor 5A; HRS, histidyl-tRNA synthetase; ILFS1, infantile liver failure syndrome type 1; ILFS2, ILSF1 with interstitial lung diseases; IRS, isoleucyl-tRNA synthetase; KRS, lysyl-tRNA synthetase; LRS, leucyl-tRNA synthetase; LZ, leucine zipper; MRS, methionyl-tRNA synthetase; MSCCA, microcephaly, progressive with seizures and cerebral and cerebellar atrophy; NA, not available; NEDMAS, neurodevelopmental disorder with microcephaly, ataxia and seizures; PAP, pulmonary alveolar proteinosis; PMLD, Pelizaeus–Merzbacher (PMD)-like disease; PRS, prolyl-tRNA synthetase; QRS, glutaminyl-tRNA synthetase; RRS, arginyl-tRNA synthetase; SRS, seryl-tRNA synthetase; UNE, uniquely attached sequence motif; VRS, valyl-tRNA synthetase; YRS, tyrosyl-tRNA synthetase. [a]The nomenclature of diseases was presented as designated in the published paper or in the Open Targets Platform database. [b]The positions of mutations are based on NP_001303701.1 (isoform 2), which is for cytosolic *GARS*. [c]These mutations were first identified in mice. [d]The positions of these mutations in *KARS* are based on isoform 1 (mitochondrial form), which correspond to p.Tyr145His, p.Asp349Asn, p.Leu105His, p.Tyr145SerfsX7 and p.Ile274Met on isoform 2 (cytosolic form). Both isoforms are generated by splicing of the chromosomal *KARS* gene; the original papers that reported these mutations did not distinguish which isoform was associated with the diseases. Either or both isoform(s) could be the disease-associated mutant(s). [e]Pelizaeus–Merzbacher disease (PMD) is designated as HLD1 and is caused by mutations in X-linked proteolipid protein 1. Other HLD types similar to classical PMD but not associated with proteolipid protein 1 mutations are called PMLD.

The five different mutations in *GARS* found in CMT2D, including the mouse form (Pro234LysTyr), are dispersed near the dimeric interface and induce a conformational change opening a new protein surface[103] (FIG. 3a; upper left panel). Because the GRS mutants associate more strongly with neuropilin 1 (NRP1) than the wild-type protein, they outcompete VEGFA for NRP1 binding. Therefore, as VEGFA–NRP1 signalling is necessary for the caudal migration of facial motor neurons from the rhombomere during embryonic development[104], GRS mutants could induce the disease phenotype by disrupting NRP1 signalling. The GRS mutants Pro234LysTyr, Ser581Leu and Gly598Ala can also bind to and enhance the activity of histone deacetylase 6 (HDAC6) on α-tubulin[105]. The removal of acetylation from α-tubulin significantly impairs axonal transport in CMT2D Pro234LysTyr mice, explaining why patients who harbour the Ser581Leu and Gly598Ala mutations suffer from more severe distal weakness and wasting in their lower limbs than those patients with other mutations[105]. Consistent with these motor neuron defects, the GRS Cys201Arg mutant in mice (Cys157Arg in humans) interacts aberrantly with tyrosine kinase receptors, causing developmental sensory deficits[106]. Gain-of-function mutations in other ARSs may explain similar neomorphic transformation and disease-causing interactions.

Mutations present in ARS-interacting proteins have also been associated with disease-associated gain-of-interaction phenotypes. For instance, the superoxide dismutase 1 (SOD1) mutant protein that is found in patients with amyotrophic lateral sclerosis can bind to KRS2 (REF.[107]), the mitochondrial KRS, leading to misfolding, aggregation and eventual degradation of KRS2 and impaired mitochondrial translation[108] (FIG. 3a; upper right panel). In another interesting case, MRS gains the ability to interact with cyclin-dependent kinase 4 (CDK4). This interaction results in an accelerated cell cycle when tumour suppressor $p16^{INK4a}$ (encoded by *CDKN2A*) is inactivated by gene deletion or by a mutation that renders it inactive[109] (FIG. 3a; lower left panel).

Loss-of-interaction mutations in ARSs or ARS-interacting proteins also can be pathological. For instance, parkin is a multi-functional E3 ligase that polyubiquitylates its substrates, targeting them for proteasome-mediated degradation[110]. Loss-of-function mutations in parkin are considered the cause of Parkinson disease[111]. Parkin mutants lose the ability to bind and ubiquitylate AIMP2, which results in the accumulation of AIMP2 in Lewy-body inclusions in the substantia nigra[112] (FIG. 3a; lower right panel). AIMP2 overexpression in mice induced age-dependent, progressive loss of dopaminergic neurons[111,113].

***Disease-specific variant production.*** The splicing variant of AIMP2, AIMP2-DX2, is highly expressed in cancers[42]. AIMP2-DX2 lacks the LZ domain, so it cannot be incorporated into the MSC[42]. It can, however, compete with AIMP2 for binding to cellular target proteins through its retained GST domain, which interferes with the tumour-suppressing activities of AIMP2 (REFS[32,42,114,115]). AIMP2-DX2 expression is induced by carcinogenic stresses (FIG. 3b), and its expression levels are inversely correlated with overall survival and DFS[42,115]. Interestingly, AIMP2-DX2 gains the ability to interact with $p14^{ARF}$ (also encoded by *CDKN2A*) through a unique sequence at the junction of exon 1 and exon 3 in AIMP2-DX2 (REF.[116]). The AIMP2-DX2-specific interaction with $p14^{ARF}$ and the subsequent inactivation of $p14^{ARF}$ are critical for tumour growth in KRAS-driven lung cancers, and overexpression of AIMP2-DX2 induces small-cell lung cancer in the AIMP2-DX2 transgenic mouse model[116].

The presence of anti-ARS autoantibodies is a consistent feature of autoimmune diseases such as interstitial lung disease, dermatomyositis and arthritis,

Rhombomere
Any of the nine segments of the embryonic neural tube.

Lewy-body inclusions
Abnormal aggregates of protein that develop inside nerve cells contributing to disorders including Parkinson disease.

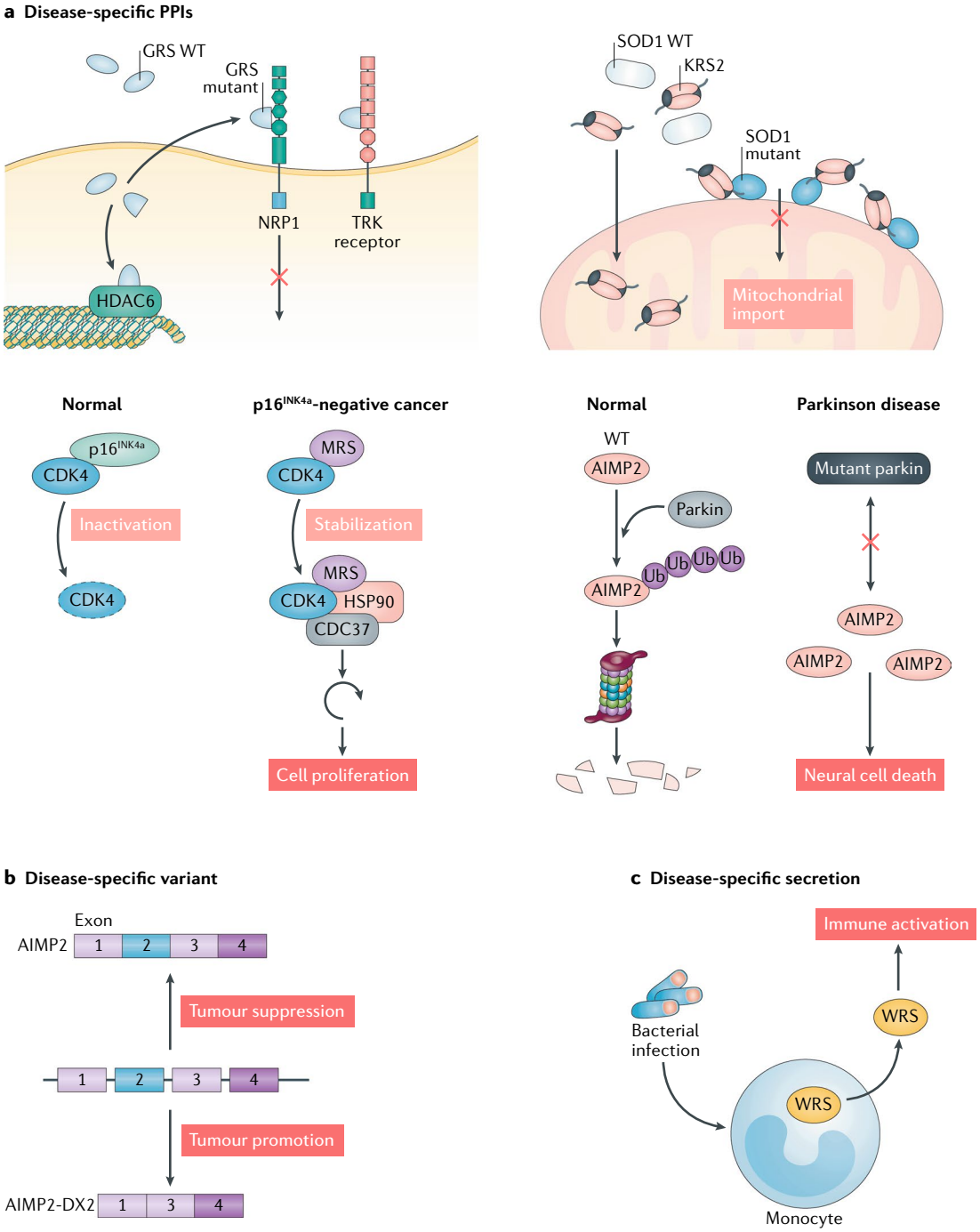


Fig. 3 | **Disease specificity of ARSN. a** | Disease-specific effects of protein–protein interactions (PPIs). Four different cases have been identified. Glycyl-tRNA synthetase (GRS; upper left) and lysyl-tRNA synthetase 2 (KRS2; upper right) are representative examples showing that mutations in ARSN or the binding partner superoxide dismutase 1 (SOD1) lead to a novel binding ability (please see main text for further details). In the case of methionyl-tRNA synthetase (MRS; lower left), the absence or inactivation of the disease-associated factor ($p16^{INK4a}$) by deletion, mutation or hypermethylation enables MRS to associate with cyclin-dependent kinase 4 (CDK4) in a disease-specific manner. A mutation of the disease-associated factor (parkin) disrupts its ability to bind to ARS-interacting multi-functional protein 2 (AIMP2), resulting in neural cell death (lower right). **b** | Disease-specific variant formation of AIMP2. AIMP2-DX2 is an exon 2-deleted splice variant of AIMP2 that is specifically induced in cancer. Continuous exposure to the chemical carcinogen benzo(a)pyrene diol epoxide induces the normal lung cell line WI-26 to express AIMP2-DX2 as a result of the A152G mutation in the exon 2 region of *AIMP2* mRNA, which inactivates the normal splicing process[42]. AIMP2 serves as a tumour suppressor; however, AIMP2-DX2 compromises the activities of AIMP2 by competing with its interaction partners, promoting tumorigenesis. AIMP2-DX2 also interacts with $p14^{ARF}$, regardless of the presence of AIMP2. **c** | Disease-specific secretion of tryptophanyl-tRNA synthetase (WRS). Full-length WRS is rapidly secreted from monocytes upon bacterial infection to prime innate immunity. HDAC6, histone deacetylase 6; NRP1, neuropilin 1; TRK, tropomyosin receptor kinase; WT, wild type.

which are collectively termed antisynthetase syndrome (ASS)[117,118]. The anti-JO-1 autoantibody that recognizes the N-terminal WHEP domain of HRS has been identified most frequently among patients with ASS[119]. Interestingly, two splice variants of HRS, $HRS^{WHEP}$ (containing only the WHEP domain)[41] and HRSΔCD[43], also react with anti-JO-1 patient sera, and $HRS^{WHEP}$ is upregulated in dermatomyositis muscle biopsy samples.

***Disease-specific secretion.*** Upon infection, WRS is promptly secreted from monocytes for the priming of innate immunity[37] (FIG. 3c). The N-terminal 154-amino acid peptide that contains the WHEP domain could trigger immune stimulation against infection. Infection-induced WRS secretion and activity suggest its potential not only as a therapeutic immune-stimulatory agent but also as a novel biomarker for early diagnosis of sepsis because WRS levels are consistently and significantly increased in the serum of patients with sepsis[37].

Secreted ARSs also have critical roles in the tumour microenvironment. GRS is specifically secreted from tumour-associated macrophages and induces tumour cell death through interaction with K-cadherin (also known as CDH6), resulting in deactivation of the ERK pathway[120]. As mentioned above, patients with ASS produce autoantibodies against the secreted ARSs (HRS, IRS, TRS, NRS, AlaRS, FRS, GRS and YRS)[121]. However, whether these pathological autoantibodies are generated by uncontrolled secretion or necrotic cell lysis is unknown. Depending on the particular pathophysiological mechanism of the secreted ARSs, they may represent either an agent or a target for exploration of novel therapies.

## Multiple routes for drug development

***Targeting catalytic sites.*** The catalytic sites of ARSs exhibit several features that can provide therapeutic specificity (TABLE 2). First, pathogenic and human ARSs possess differences in sequence, structure and topology that are sufficient for the selection or design of pathogen-specific drugs. The antibacterial drug mupirocin specifically inhibits *E. coli* IRS over the rat enzyme with a more than 8,000-fold increase in selectivity[3,122] (BOX 1). The oxaborole AN2690 (approved for human topical use under the name of Tavaborole[3,123]), which targets the fungal LRS in the treatment of nail onychomycosis[124], is the first drug to target an ARS editing site. The boron atom of AN2690 forms a covalent adduct with $tRNA^{Leu}$ that inhibits the further catalytic turnover of the fungal LRS[125]. Interestingly, AN2690 does not discriminate between the human and fungal LRS in vitro[126]. Its selectivity for the fungal form may be due to inefficient penetration into human cells or steric hindrance of the human LRS within the MSC[2,127]. AN2690 effectively eliminated fungal infection in two clinical trials (NCT01270971 and NCT01302119) with only 4% incidence of minor treatment-emergent adverse effects such as skin irritation[123]. Oxaborole and benzoxaborole are under development for oral use to treat bacterial or protozoan infections[128–130] (TABLE 2). GSK3036656 was designed to enhance specificity and safety of early compounds that targeted the LRS of *Mycobacterium tuberculosis*[129]. It exhibits excellent antibacterial activity with a minimum inhibitory concentration of 80 nM, which is an approximately ninefold improvement in specificity over protein synthesis in the human HepG2 cell line, and showed superior tolerability and in vivo efficacy. The clinical phase I study (NCT03075410) was completed in 2018 (data not posted yet), and the clinical phase II study (NCT03557281) has been launched. AN3365 is a benzoxaborole derivative that targets the LRS of multidrug-resistant Gram-negative bacteria[130]. The clinical phase II trial (NCT01381549) for complicated urinary tract infection was terminated owing to emergence of AN3365 resistance despite observed therapeutic responses.

Other interesting catalytic inhibitors are bicyclic azetidines, which are aminoacylation inhibitors of *Plasmodium falciparum* FRS[131]. These compounds were optimized to improve specificity, solubility and bioavailability and are curative in animal models at a single dose[131]. Most of all, they showed in vivo efficacy against all parasite life stages[131]. Information about the clinical trial initiation is not yet available, but these novel inhibitors are expected to provide promising therapeutics to treat malaria.

The second feature that may lend ARSs to pharmacological approaches is the potential to target ARSs involved in specific pathologies, such as PRS in fibrosis, on the basis of their amino acid composition. For example, because collagen is enriched in trimeric Gly-Pro-hydroxy-Pro repeats, catalytic inhibition of human PRS can specifically suppress enhanced collagen synthesis in fibrotic tissues while having little effect on the synthesis of other proteins or normal cells. In fact, a catalytic inhibitor of PRS, halofuginone, specifically affects the synthesis of a dipeptide containing, but not lacking, Pro[132], exerting potent anti-fibrotic activity in a multiple sclerosis mouse model[132–134] (FIG. 4a; left). Although halofuginone received US Food and Drug Administration (FDA) orphan drug designation for the treatment of scleroderma[3], it has not been approved for human treatment owing to its poorly understood toxicity. It is worth noting that another specific human PRS inhibitor, DWN12088, recently developed by Daewoong Pharmaceuticals, has shown excellent efficacy against heart fibrosis with significantly improved safety compared with halofuginone[135], suggesting that toxicity in halofuginone may result from either off-target activities or its chemical properties rather than from its direct inhibition of PRS catalytic activity. In an in vivo study of a C57Bl/6 mouse model of induced cardiac fibrosis, 2-week oral treatment with DWN12088 markedly reduced cardiac fibrosis, left ventricle thickness and accumulation of collagen fibres. Similarly, the macrolide compound borrelidin catalytically inhibits TRS[136] (FIG. 4a; right), resulting in specific inhibition of MUC1, which is 28–35% Thr in its amino acids composition, without affecting global translation[75]. MUC1 is clinically important because it promotes migration of pancreatic cancer and melanoma cells[137]. Borrelidin inhibits spontaneous lung metastasis of melanoma cells in vivo[138] and

Scleroderma
A rare autoimmune connective tissue disease that can affect skin, joints, tendons and internal organs.

growth of *P. falciparum*[3]. Despite these promising results, its toxicity in humans must be overcome for it to be a viable therapeutic. To this end, several approaches to increase the specificity of borrelidin for parasitic TRS are underway (BOX 1).

The third feature of ARSs that makes them attractive for pharmacological intervention is the ability of normal cells to withstand significant suppression of ARSs with little effect on global translation. In the various cases of ARS mutations in HLD and other rare diseases, parents or siblings who were heterozygous for one of the ARS mutations do not display the disease phenotype[90,92,95,96,139]. Moreover, *Mars*-knockdown mice generated by insertion of a gene-trap cassette did not exhibit any detectable phenotypes such as a reduction in global translation or body weight during 1 year of monitoring[109]. Equally, reduced levels and aminoacylation activity of FRS have little influence on protein synthesis[96]. It is possible that cells can tolerate ARS downregulation because they require only low levels of ARS for translation under physiological conditions[96,109]. If so, it may be possible to identify drugs that specifically target hyperproliferative cells such as cancer cells because of their high demand for protein synthesis[140].

This feature of ARSs could also be explained by the concept of non-oncogene addiction[141,142], the observation that tumour cells are more dependent than normal cells on the function of certain non-tumorigenic molecules. Molecules involved in general protein synthesis may fit into this category, as supported by several marketed drugs, such as rapamycin (an mTOR inhibitor) and bortezomib (a proteasome inhibitor)[141,142].

***Targeting disease-associated protein–protein interactions.*** Whereas targeting the catalytic sites of ARSs could provide a novel therapeutic space for controlling translation, the targeting of specific, disease-associated PPIs could intervene in pathological progression without altering the catalytic activities of ARSs (TABLE 2). For instance, the interaction of LRS with RAGD can promote pathogeneses associated with the mTORC1 pathway[38]. mTORC1 senses intracellular amino acids and is critically implicated in diverse human diseases including cancer, obesity, diabetes and neurodegeneration[143]. LRS directly binds to RAGD and functions as a GTPase-activating protein in RAGD-mediated, amino acid-induced mTORC1 activation[38] (FIG. 4b; left). Specifically, the pyrazolone compound BC-LI-0186 intervenes in the interaction of LRS and RAGD and inhibits Leu-dependent mTORC1 downstream signalling with a half-maximal inhibitory concentration ($IC_{50}$) of 81.4 nM and has little effect on the catalytic activity of LRS[143]. Structural analyses have revealed that a BC-LI-0186 derivative could directly interact with the VC region of LRS, which is the binding site for RAGD. BC-LI-0186 effectively inhibited cell proliferation and induced cell death of isogenic HCT116 colon cancer cells, regardless of their sensitivity to rapamycin. Moreover, BC-LI-0186 significantly reduced the tumour volume of rapamycin-resistant HCT116 cell xenografts in vivo[143].

KRS promotes cancer metastasis through its interaction with cell membrane-localized 67LR[27]. The compound BC-K-YH16899, which inhibits the KRS–67LR interaction through binding to the N-terminal extension and ABD of KRS, suppresses cancer metastasis without influencing the catalytic activity of KRS[28] (FIG. 4b; right). BC-K-YH16899 reduced cancer metastasis to the brain and bones by approximately 50% of radiance in the animal model transplanted with the fluorescence-expressing A549 lung cancer cells[28]. It also decreased numbers of metastasized lung nodules by 60–70% in an orthotopic mouse model transplanted with 4T1 breast cancer cell lines, as well as in the spontaneous MMTV-PyVT breast cancer model[28]. Efforts to optimize BC-K-YH16899 to generate compounds with enhanced affinity and efficacy are underway[144]. Similarly, SLCB050, a specific inhibitor of the interaction between AIMP2-DX2 and $p14^{ARF}$, suppresses proliferation of $p14^{ARF}$-positive lung cancer cells[116]. The level of AIMP2-DX2 expression was high in the lungs of mice that carried the oncogenic KRAS-G12D mutation. Combination therapy with SLCB050 and adriamycin in AIMP2-DX2 and KRAS double-transgenic mice showed synergistic antitumour activity, suggesting the potential of simultaneously targeting AIMP2-DX2 and KRAS[116].

***Therapeutic applications of secreted ARSs.*** The secreted forms of ARSs control diverse cellular processes, such as immunity, angiogenesis, tissue regeneration and metabolism, which could be exploited for the development of novel therapies[145] (TABLE 2). For instance, aTyr Pharma (San Diego, USA) has developed several approaches to use fragments of secreted HRS, which are currently being assessed in clinical trials. One of their candidate molecules, ATYR1923 (iMod.Fc), is a therapeutic version of $HRS^{WHEP}$ fused to a crystallizable fragment (Fc) domain and is under development for treatment of multiple pulmonary diseases[146]. An Fc domain enables recycling of the Fc-fused protein via the neonatal Fc receptor in endothelial cells to increase systemic exposure. Intravenous administration of only 0.4 mg per kg of iMod.Fc outperforms commercially available drugs such as pirfenidone and TFGβ antibody in the bleomycin-induced fibrosis model[147]. aTyr Pharma has completed a phase I study (ACTRN12617001446358)[148] and is preparing for a first-in-patient multiple-ascending phase Ib/IIa clinical trial.

GRS also represents a promising candidate for an anticancer agent. Macrophage-secreted GRS induces cell death in ERK-activated cancer cells through its interaction with K-cadherin[120] (FIG. 4c; left) and intraperitoneal-injected, purified GRS potently suppresses in vivo tumour growth from transplanted renal carcinoma cells[120]. However, small peptides offer advantages over purified proteins in improving pharmacological properties[149] and synthesis costs[150]. Thus, information about the domain of GRS responsible for its anticancer activity is critical to the design and development of a GRS-derived therapeutic peptide.

Evidence is mounting for the potential therapeutic utility of systemically administered, purified AIMP1 (REFS[151,152]). Intravenous injection of AIMP1 significantly

Table 2 | **Therapeutics and biomarkers under development derived from ARSN biology**

| Agent or biomarker | Mechanism of action or indicator | Stage | Potential indications |
|---|---|---|---|
| ***AlaRS*** | | | |
| A5 and A3 compounds | Catalytic inhibitor | Hit | Malaria[199] |
| ***ERS*** | | | |
| Microcin C[a] analogues specific to ERS | Catalytic inhibitor | Hit | Bacterial infection[200] |
| ***FRS*** | | | |
| TCMDC series compounds from GlaxoSmithKline's library | Hypothesized inhibitor[b] | Hit | Malaria[199] |
| Bicyclic azetidines (BRD3316 and BRD7929) | Catalytic inhibitor | Preclinical | Malaria[131] |
| ***GRS*** | | | |
| GRS peptide | Functional mimetics | Candidate | Renal cancer[160] |
| ***HRS*** | | | |
| ATYR1923 (iMod.Fc) | Functional mimetics | Clinical | Multiple pulmonary disease[148] |
| ***IRS*** | | | |
| TCMDC series compounds from GlaxoSmithKline's library | Hypothesized inhibitor[b] | Hit | Malaria[199] |
| PLD-118; a cispentacin[a] analogue | Catalytic inhibitor | Preclinical | *Candida* spp. infection[201] |
| ***KRS*** | | | |
| Cladosporin[a] analogues | Catalytic inhibitor | Lead | Malaria[202] |
| BC-K-YH16899 and derivatives | PPI inhibitor (KRS–67LR) | Lead | Cancer metastasis[28] |
| Antibody | Blocking membrane KRS | Candidate | Inflammation[160] |
| ***LRS*** | | | |
| BC-LI-0186 and derivatives | PPI inhibitor (LRS–RAGD) | Lead | Rapamycin-resistant cancer and neural disorders[143,160] |
| Leucyladenylate sulfamates | Catalytic inhibitor | Hit | Cancer[109,135,203] |
| Microcin C[a] analogues specific to LRS | Catalytic inhibitor | Hit | Bacterial infection[200] |
| GSK2251052 (AN3365) | Editing inhibitor | Clinical | Hospital-acquired Gram-negative bacterial infection[130] |
| GSK3036656 | Editing inhibitor | Clinical | Tuberculosis[129] |
| AN6426 | Editing inhibitor | Lead | *Cryptosporidium* spp. and *Toxoplasma* spp. infection[128] |
| AN6426 and AN8432 | Editing inhibitor | Lead | Malaria[204] |
| ***MRS*** | | | |
| FSMO compound | PPI inhibitor (MRS–CDK4) | Hit | $p16^{INK4a}$-negative cancer[109] |
| 1,3-Oxazines, benzoxazines and quinoline scaffolds | Catalytic inhibitor | Hit | Cancer[205] |
| TCMDC series compounds from GlaxoSmithKline's library | Hypothesized inhibitor[b] | Hit | Malaria[199] |
| REP3123, REP8839 and C1-C8 compound | Catalytic inhibitor | Hit | Malaria[199] |
| CRS3123 (REP3123) | Catalytic inhibitor | Clinical | *Clostridium difficile* infection[206–208,c] |
| Met mimics | Catalytic inhibitor | Candidate | Bacterial infection[d] |
| Cytological biomarker | Differential expression in cancer | Clinical | Cancer[e] |
| ***NRS*** | | | |
| Tirandamycins[a] and congeners of the depsipeptide[a], adipostatins[a], variolin B[a] and rishirilide B[a] | Catalytic inhibitor | Lead | Lymphatic filariasis[209] |
| ***PRS*** | | | |
| DWN12088 | Catalytic inhibitor | Clinical | Fibrosis[135,160,f] |
| T-3833261 | Catalytic inhibitor | Lead | Scleroderma[210] |

Table 2 (cont.) | **Therapeutics and biomarkers under development derived from ARS biology**

| Agent or biomarker | Mechanism of action or indicator | Stage | Potential indications |
|---|---|---|---|
| ***QRS*** | | | |
| Microcin C[a] and its analogues | Catalytic inhibitor | Hit | Bacterial infection[167,200] |
| ***SRS*** | | | |
| Albomycin[a] and analogues albomycin δ2 (SB-217452) | Catalytic inhibitor | Lead | Bacterial infection[211] |
| ***TRS*** | | | |
| BC220 and borrelidin derivatives | Catalytic inhibitor | Lead | Malaria[184,186,187] |
| ***WRS*** | | | |
| Indolmycin[a] derivatives (TAK-083) | Catalytic inhibitor | Preclinical | Gastritis and gastric ulcers from *Helicobacter pylori* infection[4,167] |
| Chuangxinmycin[a] | Catalytic inhibitor | Clinical (preliminary) | Bacterial infection[4,167] |
| Blood biomarker | High blood level in patients with sepsis | Clinical | Sepsis[g] |
| ***YRS*** | | | |
| SB-219383[a] and derivatives | Catalytic inhibitor | Lead | Bacterial infection[167,212] |
| ***AIMP1*** | | | |
| GPM1 | Functional mimetic | Lead | Systemic lupus erythematosus[168] |
| AIMP1 peptide | Functional mimetic | Candidate | Alopecia[160] |
| Antibody (atliximab) | Neutralization of secreted AIMP1 | Preclinical | Rheumatoid arthritis[158] |
| ***AIMP2-DX2*** | | | |
| SLCB050 | PPI inhibitor (AIMP2-DX2 and $p14^{ARF}$) | Lead | KRAS-positive cancer[116] |
| BC-DXI series compounds | PPI inhibitor | Lead | Colon cancer[160] |

67LR, 67 kDa laminin receptor; AIMP, aminoacyl-tRNA synthetase (ARS)-interacting multi-functional protein; AlaRS, alanyl-tRNA synthetase; ARS, aminoacyl-tRNA synthetase; CDK4, cyclin-dependent kinase 4; ERS, glutamyl-tRNA synthetase; FRS, phenylalanyl-tRNA synthetase; GRS, glycyl-tRNA synthetase; HRS, histidyl-tRNA synthetase; IRS, isoleucyl-tRNA synthetase; KRS, lysyl-tRNA synthetase; LRS, leucyl-tRNA synthetase; MRS, methionyl-tRNA synthetase; NRS, asparaginyl-tRNA synthetase; PPI, protein–protein interaction; PRS, prolyl-tRNA synthetase; QRS, glutaminyl-tRNA synthetase; RAGD, RAS-related GTP-binding protein D; SRS, seryl-tRNA synthetase; TCMDC, Tres Cantos Medicines Development Campus; TRS, tyrosyl-tRNA synthetase; WRS, tryptophanyl-tRNA synthetase; YRS, tyrosyl-tRNA synthetase. [a]These inhibitors are natural products. [b]Suggested by in silico analysis but not proved by in vitro or in vivo experiments. [c–g]Each clinical pipeline was developed by the National Institute of Allergy and Infectious Diseases[c], Bioxiness Pharmaceuticals[d], Oncotag Diagnostics[e], Daewoong Pharmaceuticals[f] and JW Bioscience[g]. The phase I clinical trials of CRS3123 (NCT01551004 and NCT02106338)[c] can be found on the Clinical Trials website. The clinical trials of DWN12088[f] and for MRS[e] and WRS[g] diagnostics are being prepared.

reduced the growth of NUGC-3 stomach cancer cells in a mouse xenograft model[152]. Moreover, administration of AIMP1 in combination with paclitaxel synergistically decreased tumour volume of NUGC-3 cells by more than 80% in the mouse xenograft model[152]. AIMP1 exerts bi-phasic activities towards angiogenesis, promoting the migration of endothelial cells at low dose but inducing their apoptosis at high dose[145,153]. It is also enriched in pancreatic α-cells and undergoes low glucose-stimulated secretion into the circulation, where it exerts a glucagon-like hormonal activity[154]. Exogenous infusion of purified AIMP1 maintains glucose homeostasis by increasing glucose and glucagon levels[154]. Topical treatment of AIMP1 enhances fibroblast proliferation, immune cell infiltration and collagen production, all of which promote wound healing[155] (FIG. 4c; right). AIMP1 also increases bone marrow-derived mesenchymal stem cell proliferation[156]. Interestingly, multiple regions of the polypeptide are involved in these diverse extracellular activities of AIMP1, suggesting that different AIMP1-derived peptides could be designed for different therapeutic indications. For instance, the AIMP1 46-amino acid and 146-amino acid N-terminal peptides facilitate mesenchymal stem cell and fibroblast proliferation, respectively; the middle domain (amino acids 101–170) inhibits angiogenesis[31]; and EMAPII can provoke an immune reaction[31]. Interestingly, the 101–192 amino acids region binds the CD23 receptor, which is critical for TNF secretion in immune cells[157]. Atliximab, a humanized chimeric antibody against AIMP1, recognizes the 101–192 amino acids epitope region and neutralizes AIMP1 cytokine activity. The antibody has shown therapeutic efficacy in a collagen-induced arthritis mouse model, significantly attenuating disease severity as well as TNF secretion[158]. Indeed, high levels of AIMP1 have been observed in the peripheral blood and synovial fluid of patients with rheumatoid arthritis[158].

***Exploration of ARSs for drug development.*** Mupirocin was FDA-approved for the treatment of Gram-positive bacterial skin infection in 1987, and AN2690 was FDA-approved for the topical treatment of onychomycosis in 2014. Halofuginone has been used in Europe as a feed additive to prevent coccidiosis in poultry since

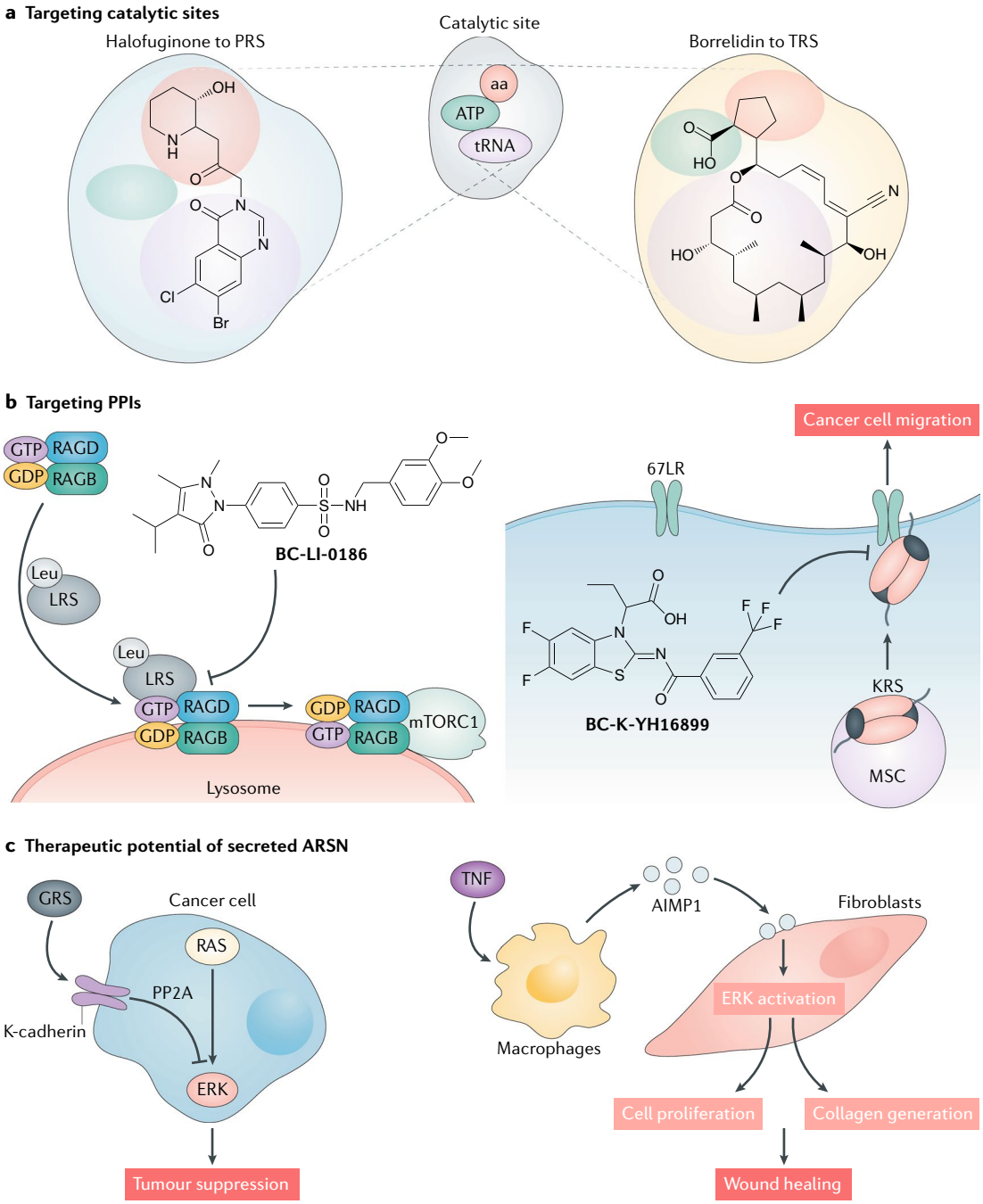


Fig. 4 | **Multiple routes for drug development from unique ARS activities. a** | The chemical structures of halofuginone and borrelidin, which are representative catalytic inhibitors of prolyl-tRNA synthetase (PRS) and tyrosyl-tRNA synthetase (TRS), respectively, are presented. The catalytic sites consist of the binding sites for amino acid (aa), ATP and the acceptor stem of tRNA. Halofuginone is a dual-site inhibitor for the aa and tRNA binding sites of PRS, and borrelidin occupies all the three substrate-binding sites of TRS as well as an auxiliary site created upon induced-fit inhibitor binding. **b** | BC-LI-0186 is a compound that specifically intervenes in the interaction between leucyl-tRNA synthetase (LRS) and RAS-related GTP-binding protein D (RAGD; left). Owing to the chemical intervention, RAGD cannot be converted into GDP-RAGD and interact with mechanistic target of rapamycin complex 1 (mTORC1) either; therefore, the mTORC1 pathway is inhibited. A laminin signal induces lysyl-tRNA synthetase (KRS) translocation from the cytosolic multi-tRNA synthetase complex (MSC) to the plasma membrane, where it interacts with 67 kDa laminin receptor (67LR) to promote cancer cell migration. BC-K-YH16899 (right) inhibits metastasis by specifically blocking the KRS–67LR interaction. **c** | Secreted glycyl-tRNA synthetase (GRS) inhibits the ERK pathway by binding to K-cadherin on the cancer cell membrane and subsequently releasing bound phosphatase 2A (PP2A; left), leading to specific cancer cell death. Aminoacyl-tRNA synthetase (ARS)-interacting multi-functional protein 1 (AIMP1) is secreted from macrophages in the damaged tissue. Its binding to fibroblasts stimulates ERK signal activation, fibroblast proliferation and collagen production that facilitates wound healing (right). PPIs, protein–protein interactions.

1999 (REFS[3,159]). All three of these approved ARS-targeted drugs are catalytic (synthetic or editing site) inhibitors, which suggests that defining safety windows in drug development for ARS aminoacylation inhibitors is not an overriding concern.

A challenge remains for the application of ARS catalytic inhibitors in non-infectious indications, in which the cells to be controlled and protected originate from the same species. Here, we have reviewed in depth the theoretical potential of ARSs as druggable targets for cancer and other non-infectious diseases; however, achieving this goal will demand further investigation. Companies such as aTyr Pharma, Pangu BioPharma, Curebio and Daewoong Pharmaceuticals and nonprofit organizations such as Biocon[160] are actively developing drugs that target ARSs and AIMPs, with several candidates in or expected to enter preclinical and clinical stages of testing.

A sensitive catalytic assay is essential for developing ARS catalytic inhibitors. A radiometric assay that uses radiolabelled amino acids has been the standard method for measuring the catalytic activity of ARSs[161] because of its high sensitivity, specificity and reliability. This protocol, however, is labour-intensive and limited by its use of a radioisotope; thus, spectrophotometric, fluorescent and luminescent assays have been proposed as alternatives[162–166]. Notably, a luminescent assay based on in vitro translation using rabbit reticulocyte lysate has been recently shown to be applicable to high-throughput screening (HTS) with an acceptable coefficient of variation and Z′ factor. This innovative approach will be useful to identify specific ARS inhibitors by HTS[166].

Historically, many ARS inhibitors and modulators have been derived from natural products and food sources[4,62,167]. Thus, strategies to modify and optimize natural inhibitors may be effective for generating an ARS-specific compound library to screen for catalytic inhibitors. Designing compounds that mimic ARS substrates or occupy substrate-binding sites is another promising approach. The binding pockets for ATP, amino acids and tRNA substrates that are necessary for aminoacylation are located in close proximity to one another (FIG. 4a); therefore, strategies to simultaneously target more than two substrate-binding sites with one compound may be feasible[3]. Another strategy to develop catalytic inhibitors is to design compounds that bind to the ARS sites involved in editing, of which AN2690 is the best example.

Cladosporin and resveratrol are representative single-active-site inhibitors that mimic ATP and amino acid, respectively. Mupirocin is an example of an inhibitor that mimics aa-AMP, occupying dual-active sites. Halofuginone is another dual-active-site inhibitor that binds to the amino acid and tRNA binding sites. To our knowledge, no catalytic inhibitors that mimic tRNA have been identified. Borrelidin occupies all three of the substrate-binding sites as well as an auxiliary site[136] and exerts the most potent inhibitory effect compared with other catalytic inhibitors[136], suggesting a correlation between the number of binding sites that a compound occupies and its inhibitory activity. The synthesis of compounds that occupy three or more sites may be difficult owing to required compound complexity but would generate the most powerful drug. The relationship between multi-site inhibition and side effects awaits further investigation.

ARS-mediated and/or AIMP-mediated PPIs represent another attractive target space. The development of screening assays has been a hurdle in the identification of PPI inhibitors because of the need to monitor specific interactions between binding partners. The yeast two-hybrid assay[28] and the enzyme-linked immunosorbent assay[116,168] have been applied for this purpose, and recently, a luciferase assay was developed for HTS to monitor the interaction between AIMP2-DX2 and its binding partners that is generally applicable to all ARS-mediated PPI targets[169]. In this assay, NanoLuc luciferase is split into two fragments, each of which is fused to target proteins. Interactions between the target proteins reconstitute luciferase activity. Thus, compounds that perturb the PPI can be selected on the basis of reduced luminescence.

To target intracellular ARSs, the optimal compounds must be capable of penetrating the cell. However, most clinical-stage PPI-inhibiting compounds are large and hydrophobic[170]. Therefore, to perturb broad interaction interfaces, cell-penetrating antibody technology[171,172] could be considered.

## Perspectives

The ARSN research field is advancing on two tracks: basic research and drug and diagnostic development. On the basic side, researchers are working to elucidate the roles and mechanisms of ARSs in diverse biological and pathological processes. Whereas reports of disease-associated mutations in cytosolic ARSs and AIMPs (TABLE 1) and splicing variants[41–46] are plentiful, the understanding of their pathological mechanisms is poor. Progress on understanding the molecular structure of ARSs necessary for the rational design of drugs that specifically target each of the ARSN is being made rapidly. Since the first ARS structure was published in 1976 (REF.[173]), the number of ARSN structures in the Protein Data Bank has grown exponentially[3].

On the therapy side, the diversity of ways in which ARSs may impact human pathologies suggests that multiple therapeutic modalities, including chemical compounds, small interfering RNAs, antibodies, peptides, gene therapy and combination therapies, can be explored as effective strategies. In addition, new technologies such as proteolysis-targeting chimaeras (PROTACs) that are being tested in clinical trials for induction of target-protein degradation[174] could be valuable for targeting the destruction of gain-of-function molecules such as AIMP2-DX2 in cancer, AIMP2 in Parkinson disease and GRS in CMT.

Owing to the positioning of novel action mechanism and potency[175], ARSN-targeting and ARSN-derived therapeutics would provide a wide choice for combined treatment with currently used therapies with the expectation of synergy. A few previous studies show possible examples of combination therapies of ARSN with genotoxic drugs[116,152], and understanding the mechanism

Coefficient of variation
A standardized measure of dispersion of a probability distribution or frequency distribution, defined as the ratio of the standard deviation to the mean.

Z′ factor
A measure of statistical effect size proposed for use in high-throughput screening.

NanoLuc luciferase
An engineered small luciferase derived from a deep sea luminous shrimp, which reveals stable, bright and sustained luminescence.

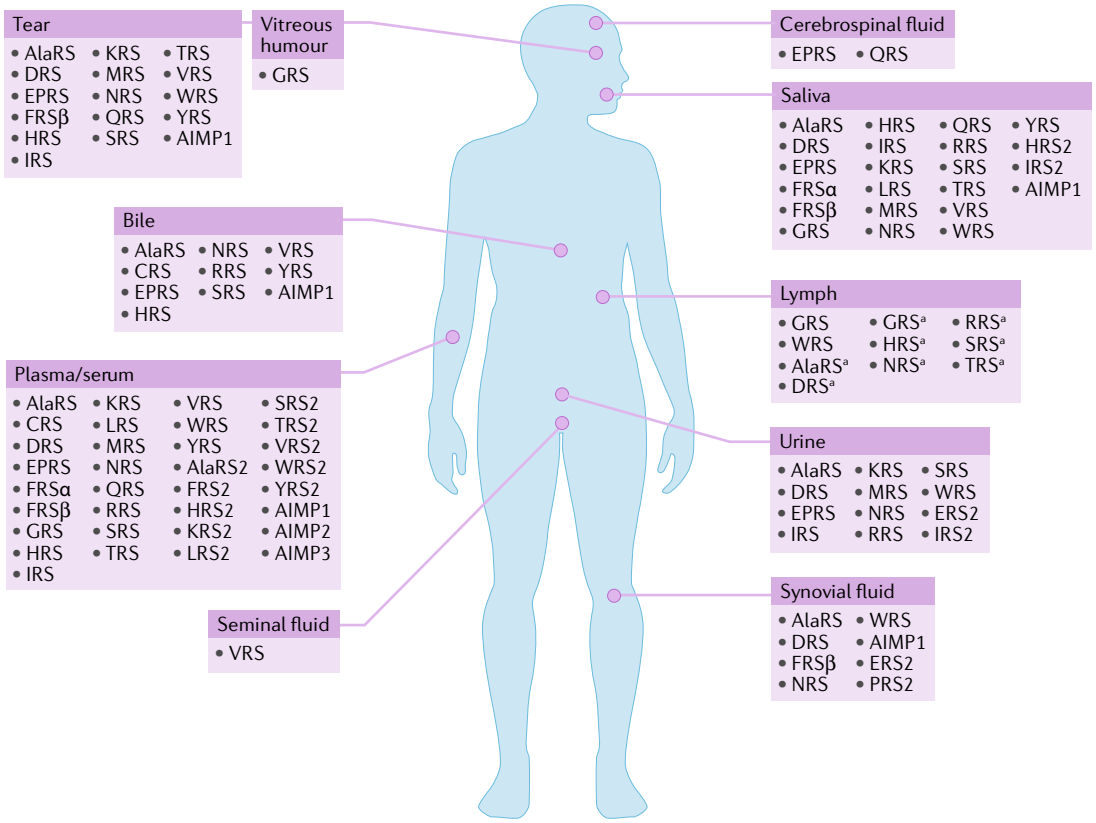


Fig. 5 | **ARSN in human body fluids.** The diagram shows ARSN proteins and peptides detected in human body fluids. Information was collected from original papers (Supplementary Table 1) and several databases, including the Exosome protein, RNA and lipid database, the Plasma Proteome Database, Max-Planck Unified Proteome Database, the Urine Proteomics database and the Urinary Protein Biomarker Database. Mitochondrial aminoacyl-tRNA synthetases (ARSs) are designated with a terminal 2. AIMP, ARS-interacting multi-functional protein; AlaRS, alanyl-tRNA synthetase; CRS, cysteinyl-tRNA synthetase; DRS, aspartyl-tRNA synthetase; EPRS, glutamyl-prolyl-tRNA synthetase; ERS, glutamyl-tRNA synthetase; FRS, phenylalanyl-tRNA synthetase; GRS, glycyl-tRNA synthetase; HRS, histidyl-tRNA synthetase; IRS, isoleucyl-tRNA synthetase; KRS, lysyl-tRNA synthetase; LRS, leucyl-tRNA synthetase; MRS, methionyl-tRNA synthetase; NRS, asparaginyl-tRNA synthetase; PRS, prolyl-tRNA synthetase; QRS, glutaminyl-tRNA synthetase; RRS, arginyl-tRNA synthetase; SRS, seryl-tRNA synthetase; TRS, tyrosyl-tRNA synthetase; VRS, valyl-tRNA synthetase; WRS, tryptophanyl-tRNA synthetase; YRS, tyrosyl-tRNA synthetase. [a]ARSN identified from mice infected with *Bacillus anthracis*.

of drug synergy will further guide the exploration of drug combinations.

As shown in the history of drug development, drug resistance is a major problem facing current cancer and infectious therapies[176,177]. Owing to the short history of ARS drug development for non-infectious diseases, drug resistance to ARS inhibition has only been reported in the case of ARS antibiotics. Resistance to mupirocin and other bacterial ARS-targeting compounds was acquired by mutation, gene duplication or horizontal gene transfer[178]. In humans, ARSN are unique as drug targets in the sense that the function of each ARS is not replaceable by other factors. Thus, alternate cellular mechanisms and pathways to circumvent pharmacological activities targeting each ARS would be unlikely.

In addition, the characteristics and behaviour of ARSN suggest their utility as biomarkers for various diseases, as shown in the case of MRS and WRS, which are currently being applied for cancer and sepsis diagnostics, respectively (TABLE 2). Although ARSN are ubiquitous and constitutively expressed, which are generally considered weaknesses for biomarkers, their cellular levels and extracellular secretion seem to be under tight control depending on stimuli and cellular context[37,59,120,145,179]. As the profiles of different ARSN levels present in body fluids may reflect the type and status of pathophysiological conditions (FIG. 5; Supplementary Table 1), the secretory behaviour of individual or ARSN profiles as a whole could be used to monitor health and disease.

ARSs are crucial catalysts for building proteins in all living organisms. However, their functions have expanded throughout evolution such that each ARS has a unique role in building, maintaining and controlling higher-order multicellular systems in a manner that coordinates with their catalytic activity. Their extraordinary diversity of roles can be intelligently reconnoitred to develop novel therapeutics and biomarkers to enable precision and preventive medicine.

Published online 9 May 2019

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**Acknowledgements**
This work was supported by NRF-M3A6A4-2010-0029785 (S.K.), NRF-2015M3A6A4065724 (N.H.K.) and NRF-2017M3A9F7079378 (N.H.K.) from the National Research Foundation, the Ministry of Science and ICT (MSIT) of Korea and by the US National Institutes of Health (NIH) P01 HL029582 (P.L.F.). The authors thank B. S. Kang (Kyungbuk University) for drawing the architecture of class I and class II catalytic sites. They also thank J. Y. Lee (Buck Institute) for collecting data for secreted ARSN in human body fluids.

**Author contributions**
P.L.F. provided substantial contribution to discussion of the content and reviewed and edited the manuscript before submission. N.H.K and S.K. contributed equally to all aspects of the article.

**Competing interests**
S.K. has financial interest in aTyr and Curebio, and N.H.K. has financial interest in Oncotag Diagnostics, although none specifically related to this Review.

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**Supplementary information**
Supplementary information is available for this paper at https://doi.org/10.1038/s41573-019-0026-3.

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