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Drug resistance without drug selection: polymorphism in *leuS* confers reduced susceptibility to GSK2251052 in a clinical isolate of *Staphylococcus aureus*

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Running title: Reduced susceptibility to GSK'052 in *S. aureus*

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Abstract

GSK2251052 is a broad-spectrum antibacterial inhibitor of leucyl tRNA-synthetase (LeuRS) that has been evaluated in Phase II clinical trials. Here we report the identification of a clinical isolate of *Staphylococcus aureus* that exhibits reduced susceptibility to GSK2251052 without prior exposure to the compound, and demonstrate that this phenotype is attributable to a single amino acid polymorphism (P_{329}) within the editing domain of LeuRS.

Text

GSK2251052 (GSK'052) is a novel, broad-spectrum antibacterial agent that selectively inhibits bacterial leucyl tRNA-synthetase (LeuRS) [(1)]. Although this compound appears to possess many of the requisite properties of an antibacterial drug for treating infection in humans, it also has the undesirable feature of rapidly selecting resistance in bacteria; in Phase II clinical trials involving adult subjects suffering complicated urinary tract infections, resistance to GSK'052 developed within two days of administration in three of 14 patients [(1) (2)]. Here we report that, in addition to arising rapidly in bacteria under selection, reduced susceptibility to GSK'052 is pre-existing amongst clinical isolates of *Staphylococcus aureus* that have not been exposed to the drug, a phenomenon that is the result of polymorphism in the drug target.

GSK'052 was obtained by chemical synthesis, according to established methodology [(3)]. To evaluate the susceptibility of staphylococcal strains to this compound, a small panel of *S. aureus* blood culture isolates (*n*= 52) was tested using the micro-broth dilution method, according to CLSI guidelines [(4)]. These isolates were recovered from patients at the Erasmus MC University Medical Center Rotterdam (Netherlands) between November 2009 and May 2010, and therefore originate from a country in which GSK'052 has never been trialled, and during a period that predates the clinical evaluation of GSK'052 [(2)]. Consequently, it may be stated with some confidence that these isolates have never been exposed to this compound in the clinic. GSK'052 exhibited a minimum inhibitory concentration (MIC) of 2-4 mg/L against all isolates, with the exception of one (strain 1372) for which the MIC was 16 mg/L. This degree of reduced susceptibility to GSK'052 is equivalent to that exhibited by a resistant *Escherichia coli* strain selected in a patient upon administration of GSK'052 in the Phase II clinical trial, and which was associated with microbiological failure [(2)].

To determine the mechanism for reduced susceptibility to GSK'052 in *S. aureus* 1372, we proceeded on the basis that this phenotype was likely the result of polymorphism in the drug target (LeuRS), and subjected the entire *leuS* gene from this strain to PCR amplification and DNA sequence determination. This revealed that, in comparison to the *leuS* gene of the fully GSK'052-susceptible laboratory strain *S. aureus* SH1000 [5][6], $leuS_{1372}$ encodes a protein containing four amino acid polymorphisms ($T_{311}I$, $S_{329}P$, $A_{553}G$ and $F_{734}Y$; SH1000 residue shown first in each case).

To establish whether one or more of these polymorphisms account for the reduced susceptibility to GSK'052, $leuS_{1372}$ and $leuS_{SH1000}$ were PCR-amplified using oligonucleotide primers ATCGTTATGTCGACTTTTTTATTGAATAGGAGGA and TGCTTAGTGGATCCATTTCAAAGTCCTCCTTAAA (engineered restriction sites underlined) and introduced into the staphylococcal expression vector, pLOW [7], for ectopic expression in *S. aureus* SH1000. Strain SH1000 (pLOW: $leuS_{1372}$) exhibited a substantial reduction in susceptibility to GSK'052 (MIC 64 mg/L) compared to SH1000 (pLOW: $leuS_{SH1000}$) (MIC 16 mg/L), confirming that the reduced susceptibility of *S. aureus* 1372 to GSK'052 is indeed a consequence of polymorphism in LeuRS.

Of the four amino acid polymorphisms in $LeuRS_{1372}$, two (I_{311} and G_{553}) are also found encoded in the *leuS* gene of strains of *S. aureus* whose genome sequences have been deposited in the public databases (strains MRSA252 [GenBank accession no: BX571856] and Mu50 [GenBank accession no: BA000017]). When we tested these strains, neither was more resistant to GSK'052 than SH1000, implying that neither of these polymorphisms participates in reduced susceptibility to GSK'052. Of the remaining two polymorphisms in $LeuRS_{1372}$, we considered P_{329} the most likely candidate for mediating the relative insensitivity of the enzyme to GSK'052, since it resides within the editing domain, a region of the protein containing the majority of amino acid substitutions identified in the LeuRS of clinical isolates of *E. coli* in which resistance to GSK'052 has evolved [2] (Figure 1). To test this, the substitution $S_{329}P$ was engineered into pLOW: $leuS_{SH1000}$ using the Q5 Site-Directed Mutagenesis Kit (New England Biolabs, MA, USA) and oligonucleotide primers TTATGTATTACCAACATATGGTACTG (engineered

mutation underlined) and TCAGCAATCCAAATTTGTAC. Introduction of this construct into SH1000 resulted in a strain exhibiting the same degree of reduced susceptibility to GSK'052 (MIC 64 mg/L) as SH1000 (pLOW: *leuS*$_{1372}$), thereby confirming that the polymorphism P_{329} in LeuRS is responsible for the decreased susceptibility of strain 1372 to GSK'052.

With a view to understanding how this polymorphism negatively impacts the activity of GSK'052 against LeuRS, we examined the published crystal structure of *Thermus thermophilus* LeuRS bound to the parent compound (AN2690) of GSK'052 (PDB ID: 2V0C). AN2690 forms an adduct with $tRNA^{leu}$ that becomes trapped in the editing site of the enzyme [8], with residues lying in close proximity to the P_{329} polymorphism participating in binding the tRNA portion of this adduct. In particular, the preceding residue (L_{329}, *T. thermophilus* numbering) forms two hydrogen bonds with nucleotide A76 of the $tRNA^{leu}$ [8]. The presence of a conformationally-rigid proline adjacent to this position would likely serve to constrain the protein backbone, thereby restricting the conformation of this leucine residue and potentially impairing its ability to make these hydrogen bonds; loss of one or more hydrogen bonding contacts would reduce the affinity of the enzyme for the $tRNA^{leu}$: drug adduct, and thereby lead to reduced susceptibility to the compound.

In conclusion, we have shown that a polymorphism (P_{329}) in the LeuRS enzyme of a clinical isolate of *S. aureus* mediates reduced susceptibility to GSK'052. Whilst our results do not at this stage enable informed speculation regarding the prevalence of, or the underlying reason(s) for, this polymorphism, it is clear that its presence is in no part attributable to selection by GSK'052. The identification of a clinical *S. aureus* isolate that exhibits uniform reduced susceptibility, at the level of the drug target, to an experimental antibacterial drug with which it has never before been challenged, although a phenomenon that has been reported previously [9], is apparently rare or infrequently documented. Our findings raise the possibility that polymorphisms associated with reduced susceptibility to GSK'052 also exist in strains of other bacterial pathogens, and underscore the utility of assessing the

activity of antibacterial drug candidates against clinical isolates as part of preclinical evaluation to identify any pre-existing mechanisms mediating reduced susceptibility.

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Transparency declaration

None to declare.

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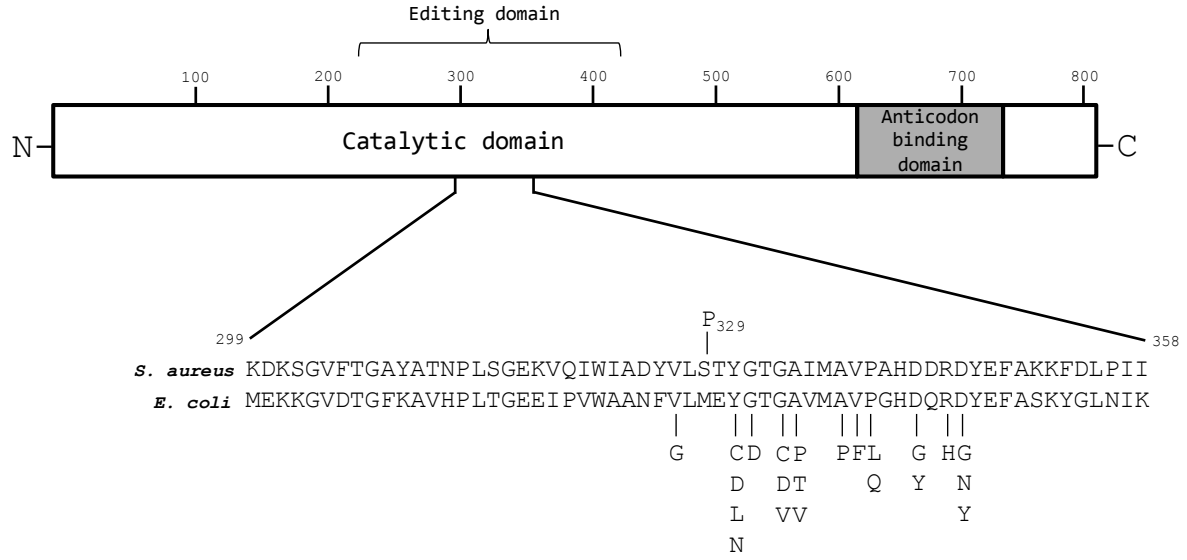

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150 **Figure 1. Schematic of the LeuRS protein, with a close-up on part of the editing domain, showing the**
151 **amino acid substitutions (within this region) that mediate reduced susceptibility to GSK'052 in *S.***
152 ***aureus* (this study) and *E. coli* [2].** Residue numbering corresponds to the *S. aureus* sequence, and
153 amino acid substitutions are denoted above and below the sequence alignment for *S. aureus* and *E.*
154 *coli*, respectively.

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