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Targeting evolution of antibiotic resistance by SOS response inhibition

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ABSTRACT

Antibiotic resistance is acquired in response to antibiotic therapy by activating SOS-depended mutagenesis and horizontal gene transfer pathways. Compounds able to inhibit SOS response are extremely important to develop new combinatorial strategies aimed to block mutagenesis. The regulators of homologous recombination involved in the processes of DNA repair should be considered as potential targets for blocking. This review highlights the current knowledge of the protein targets for the evolution of antibiotic resistance and the inhibitory effects of some new compounds on this pathway.

Contents

1. Introduction

To overcome the ability of bacteria to reduce their susceptibility to antimicrobial drugs is becoming a global emergency. Most of the currently used antibiotics promote genetic instability or increased mutagenesis in bacteria. Even antibiotics that do not act directly on DNA, may indirectly increase the rate of mutagenesis. In the recent years it has become clear that the spread of resistance determinants can be combated not only with the help of antibiotics, but also using the strategy of blocking the evolution of resistance [1]. Resistance may develop through several main pathways that involve mutagenesis, recombination, and horizontal gene transfer (HGT). Antibiotics can affect mutagenesis by a wide variety of mechanisms, such as oxidative metabolism response, general stress responses, SOS response [2]. HGT also includes processes such as conjugation, transformation, and transduction [3]. All these processes have been covered extensively in the literature. Meanwhile, the pathways partially overlap with each other, due to the fact that one system can activate or suppress another. Interestingly, some proteins, in particular such as SOS-controlled proteins or the mediators of recombination, can also be incorporated simultaneously in different metabolic perturbations, even within the same bacterial strain. Undoubtedly, such mediator proteins represent an appealing target for blocking the evolution of resistance. At the same time, depending on the strain, the significance of modulatory effects of such proteins can vary or even be completely abolished. Hence, the extent of usefulness of such multiple-target

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inhibitors always remains rather uncertain. The principal aim of this review is to highlight several potential targets based on their specific function, and discuss current knowledge, taking into account all the side effects.

2. Mutagenesis and resistance

Modern antibiotics are developed for targeting key cellular processes such as biosynthesis of proteins and components of cell membranes, DNA replication and repair [1]. Most of the strategies to overcome acquired resistance usually include chemical modifications of previously known antibiotics. In spite of many antibiotics undergoing the third or fourth cycle of modifications, the number of newly developed antibiotics has been gradually decreasing in recent years. Moreover, the tolerance to antibiotic treatment is gradually diminishing over time due to evolutionary adaptation of bacteria [4]. The evolution of bacterial resistance is driven by genetic variation with the subsequent selection of resistant variants. In addition, bacteria have developed a complex regulating evolutionary adaptation by acquiring resistance genes mainly through conjugation or - to a lesser extent, through natural transformation and transduction [5,6]. While transformation are considered as less important recent studies suggest their role may be larger than previously thought [7].

In the absence of the above-mentioned conjugation, bacterial cells can develop antibiotic resistance by activating the mechanisms of the cellular SOS response, related induced mutagenesis, and genome rearrangement [2,8]. The SOS system is regulated by LexA promotor binding repressor protein. LexA protein cleaves itself after binding to the RecA filament, therefore reducing LexA protein level in the cell and activating more than 40 genes including *recA* gene [9]. SOS response proteins are involved in all the aspects of cell metabolism [10–12]. Depending on their function, each of the proteins is expressed in a specific sequence. In addition, the mutagenesis is triggered in the late stages of the SOS response. PolV polymerase is known to drive SOS-dependent mutagenesis in *E.coli* [13,14]. PolV polymerase belongs to the class of "error prone" polymerases, which are known by their low accuracy of DNA synthesis. The incorporation of an erroneous nucleotide into the DNA strand by PolV is a source of induced mutagenesis. PolV polymerase forms a Mutasome complex with RecA, which probably imposes some restrictions on the potential variability of recombinases during selection [15,16]. The Mutasome repairs a significant part of the damage at single-stranded gaps as an alternative pathway to homologous recombination [17,18]. Replication of damaged DNA also termed as translesion synthesis (TLS) involves PolV polymerase that bypass DNA lesions. In addition to being a component of TLS, PolV is also an antagonist RecA-dependent recombination. The balance between these two strategies is very important. In the absence of the SOS response, TLS accounts to 1–2% of the events. Whereas under stress TLS increases up to 40% according to the TLS mechanism [19]. The ratio can also shift strongly towards the TLS mechanism, if recombination is performed by the specific RecA variants that are partially deficient in strand invasion but proficient in polymerization onto ssDNA. At the same time, the increase in mutations is extremely deleterious for bacteria. Bacteria population size drops even more with the suppression of recombination. Moreover, if the size of the bacterial population is significantly reduced due to random genes drift, there is a risk of fixation of moderately disadvantageous mutations. Since recombination is interfering with harmful mutations, it sets the limits for "mutational catastrophe" [20].

Despite the fact that PolV polymerase (UmuD2C) is the general source of mutants for evolutionary selection, there are some other pathways for induced mutagenesis. Another "error prone" *E.coli* Pol IV polymerase, although not forming a mutasome with the RecA protein, is expressed during the SOS response [21,22]. Both polymerases belong to the "Y" family of polymerases and have been found in most bacterial species [23]. Despite the significant diversity of the "Y" family, most polymerases share a similar 30-residue sequence at the C-terminal. The sequence of this short motive has similarities to the N-terminus of the RecA protein, which in its turn is involved in the creation of an inter-monomeric interface during filament formation [24].

Similar families of polymerases have taken on the function of induced mutagenesis in at least some other groups of bacteria. In the bacterium *Mycobacterium tuberculosis*, the function of acquiring evolutionary resistance is assigned to DnaE2 polymerase, which belongs to the "C" family of polymerases [25]. DnaE2 polymerase is expressed in response to DNA damage and is a component of the SOS regulon of *M. tuberculosis* bacteria, while members of the Y polymerase family are expressed by the LexA-independent mechanism.

Table 1
Inhibitors of the proteins involved in the SOS-response.

Protein	Inhibitor	Proposed mechanism of action	Ref.
SSB	Small molecules	Disrupt SSB protein interfaces	[27]
RecBCD	sulfanyltriazolobenzimidazole NSAC1003	Acts on RecB ATP-binding site	[29]
PolV	RecAD112R/N113R	Acts on UmuD ATP-binding site	[16]
LexA	5-amino-1-(carbamoylmethyl)-1H-1,2,3-triazole-4-carboxamide	This effect appears specific for the self-cleavage activity of LexA	[33,34]
	Boron-containing compounds	interact with the catalytic Ser-119 (act as inhibitors of LexA self-cleavage)	[31]
RecA	peptide (N-terminal helix)	N-terminal helix disrupt protein interfaces	[77]
	compounds, suramin-like agents	ATPase inhibitors	[70]
	2-amino-4,6-diarylpyridine	ATPase inhibitors	[72]
	compounds, 33 unique scaffold groups	inhibitors with varied specificity for RecA conformation	[63]
	suramin	disassemble RecA-single-stranded DNA filaments	[61]
	Zinc acetate	inhibitor of LexA cleavage	[69]
	Compounds (A03, A10)	disrupt ssDNA binding	[76]
	epiphorellic acid/divaricatic, perlatolic, alpha-collatolic, lobaric, lichesterinic, protolichesterinic	binds the ssDNA binding site inhibitors for ATP binding site	[62]
	peptide 4E1 (RecX)	RecX-like disassemble RecA-single-stranded DNA filaments	[85]

3. Targets

The above-presented examples of mutagenic polymerases could be regarded as potential targets for inhibiting antibiotic resistance development (Table 1). The RecA surface, defined by residues 112–117, directly interacts (Fig. 1) with the amino acid residues on the surfaces of the UmuC subunit of PolV. The *E.coli* RecAD112R/N113R mutant protein exhibits an extremely reduced capacity for PolV activation and SOS mutagenesis [16]. Despite significant progress in understanding the functioning of PolV, the lack of confirmed

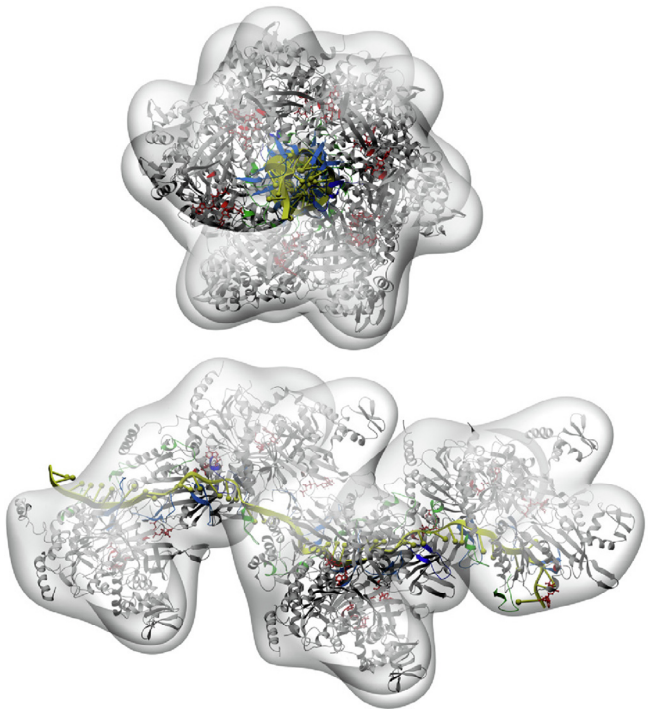

Fig. 1. Surface view of two turns of RecA filaments built on a single-strand DNA with a regular ATP-binding geometry. The filaments are represented in gray. The DNA strand is in yellow. Regions of the protein that cover the DNA strand are represented in blue, ATP – red, LexA – green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3D structure of polymerase complicates the assessment of the possibilities of using inhibitors. Therefore, the strategy might be expanded to the search for more universal targets into other critical proteins from bacterial metabolism.

There are several examples of this approach. The small molecules inhibiting the SSB protein, (it regulates replication and recombination) have been discovered recently [26,27]. Another attempt has been made to obtain a compound for inhibiting *E.coli* RecBCD protein, the multiple activities of which are involved in the initial steps of double-strand break repair and recombination. RecBCD binds to double strand DNA end, unwinds the DNA and load RecA onto generated single strand DNA [28]. It was shown that a small molecule NSAC1003, a sulfanyltriazolobenzimidazole binds to the RecB and RecD ATP-binding sites. Molecule NSAC1003 induces RecBCD to cut DNA at novel positions [29].

Blocking LexA repressor may be regarded as an opportunity [30,31]. For example, numerous studies on the use of *in vivo* uncleavable variants of the LexA repressor successfully led to the blocking of the bacterial SOS response and mutagenesis [1,32]. In another approach screening compounds for LexA self-cleavage blocking revealed an active substance 5-amino-1-(carbamoylmethyl)-1H-1,2,3-triazole-4-carboxamide [33]. This work led to the identification of an analogue with improved activity and an expanded spectrum of applications [34].

Perhaps, LexA proteolysis inhibition approach may not be ideal for a number of reasons, since the basal levels of recombinase and mutagenic polymerase are always maintained in the bacteria (Fig. 2). In some bacteria, such as *D. radiodurans*, LexA protein has lost its function as SOS response regulator. Moreover, *S. pneumoniae*, *S. thermophilus*, *L. pneumophila* bacteria neither have LexA protein, nor the classic SOS response [35–37]. Finally, LexA protein does not regulate gene conjugation and transformation systems, and therefore does not prevent antibiotic resistance acquired through horizontal transmission. For example, *S. pneumonia*, which lacks an SOS-like response mechanism, instead uses the competence regulatory cascade to control a defense to mitomycin C [7].

Horizontal gene transfer is mainly represented by conjugation [38–42] and to a lesser extent by the natural transformation of exogenous DNA into the chromosome, but plasmid transformation and conjugation are driven by different exchange mechanisms. The comparative genomic analysis of enterohemorrhagic strain O157: H7 and laboratory strain K12 has shown that the pathogenic strain O157 contains over a thousand adopted genes distributed over the chromosome. Up to 30% of the genes in pathogenic strains O157 may have been acquired through horizontal gene transfer. Therefore, the mechanism of antibiotic resistance evolution covers the entire spectrum of scenarios and depends on many factors. In general, if to compare the contributions of each pathway, events of horizontal gene transfer should be prevalent in those cases, when the antibiotic was encountered before and the natural mechanisms of microbial adaptation have already essentially evolved [43,3]. The use of the modification of chemical compounds offers the advantage over the mutagenic pathway of adaptation in this case. It is worth noticing, that in some bacteria a direct relationship is formed between SOS-mutator phenotype and horizontal gene transfer [44]. For example, many antibiotics induce a SOS response, which in turn leads to the mobilization of integrative conjugation elements that serve as transporters of antibiotic resistance genes [5,45,46].

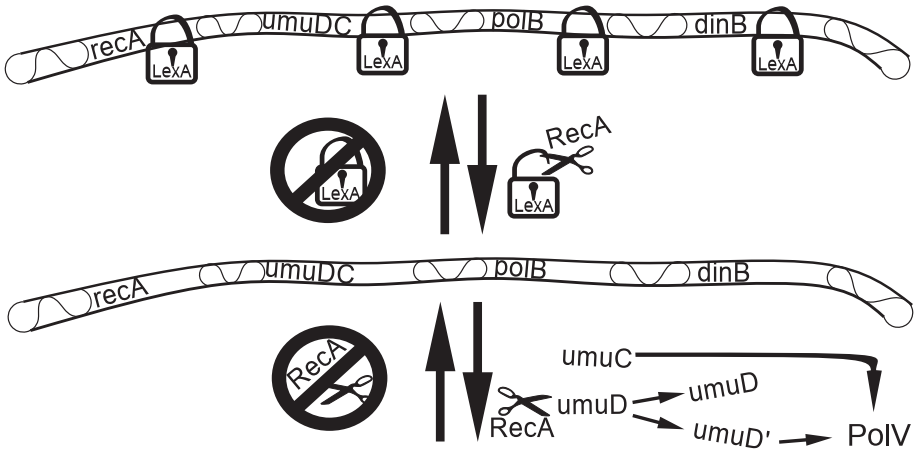

Fig. 2. SOS response inhibition stages.

Such a close integration raises the possibility of searching for the essential targets, that could simultaneously block mutagenesis and horizontal gene transfer pathways. Conjugation is the most studied and at the same time the most widespread variant of horizontal gene transfer. It has been found in both Gram-positive and Gram-negative bacteria. The RecA protein is involved in many processes of DNA repair including integration of the acquired genes into the chromosome of the recipient bacterium. During Hfr (high frequency of recombination) chromosomal DNA transfer the donor DNA incorporation into the recipient chromosome is promoted by the RecA protein. The RecA protein gets involves only at the final phase of donor DNA incorporation into the recipient's chromosome, but not the initial step of DNA transfer through conjugative pili. A limited number of conjugation-specific inhibitors offer an opportunity for the search of inhibitors for the steps associated with the RecA involvement i.e specific blockers of recombinant steps controlled by RecA. Some studies have shown, that the conjugation of *E. cloacae* and *E. coli* can be effectively blocked by using zinc–containing compounds known to inhibit RecA activity [45].

Another widespread pathway of antibiotic resistance transfer is the one through natural transformation either of plasmid or chromosome. Plasmid transformation proceeds according to the RecA-independent mechanism. However, the persistent presence of such an R-plasmid in the bacterial line requires the selective pressure of the medium, i.e. the presence of an antibiotic. Otherwise, some R plasmids may turn out unstable [47]. For instance, in some species of *Pseudomonas*, plasmids extinct due to purifying selection, fitness costs, incompatibility properties [48,49].

Chromosomal transformation involves the incorporation of the resistance gene into the chromosome through homologous recombination performed by the RecA protein in the presence of other regulatory proteins. In this case, the acquired resistance gene is permanently fixed in the bacterial population. For example, the majority of resistance genes of a *Neisseria gonorrhoeae* pathogens have a chromosomal localization and persist for a long time [50,51].

Undoubtedly, regulators of homologous recombination involved in the processes of chromosomal transformation should be considered as potential targets for blocking. Several studies have resulted in publishing a detailed analysis of transformation factors on *Bacillus subtilus*, *Neisseria meningitidis*, *Streptococcus pneumoniae* and others bacteria [52–54]. *B. subtilus* were tested for their ability to maintain chromosomal or plasmid transformations in the absence of genes important for DNA metabolism such as *recA*, *recX*, *dprA*, and others [55]. As expected, the deletion of the *recA* gene completely prevented the natural chromosomal transformation of DNA, but did not affect the efficiency of plasmid transformation. The deletion of the *recX* or *dprA* genes led to the suppression of both chromosomal transformation and plasmid transformation. Interestingly, the products of all three genes are known to interact with each other *in vitro* systems. Each of these gene's products can serve as a potential target for suppressing the transformation of these bacilli. Unfortunately, the functional roles of these genes are not consistent across strains. The deletion of the *recX* gene in *E. coli* does not affect either the efficiency of horizontal transfer or the SOS response. In contrast, a *recX* deletion in *Pseudomonas aeruginosa* is lethal [56]. The *dprA* gene seems to be a more appealing target, although until now its role has been little studied in gram-negative bacteria. For example, this DprA/Smf protein has never been purified from *E. coli*, and its function has not been established *in vivo* [57]. In Gram-positive bacteria, DprA plays a more significant role. The resulting structure of the DprA dimeric protein from *S. pneumoniae* offers direct opportunities for the search for specific inhibitors [53]. The involvement of RecA protein in the process of natural transformation significantly expands its appeal as a potential target for antibiotic therapy. At least, this approach would be reasonable in the case of many Gram-positive bacteria, where transformation is extremely widespread.

Recently attempts have been made to consider inhibitors of the SOS response not only as evolutionary blockers, but also as enhancer or adjuvant for the antibiotic efficiency. In cases where the SOS response is a trigger in a specific antibacterial mechanism, its suppression removes the barrier, making the cell more vulnerable. In particular, for *E. coli* the combined utility of a RecA inhibitor and antibiotics of the quinolone or beta-lactam class can dramatically reduce the effective concentration of the latter in several times [58,59]. The resistance is overcome when the final target of an antibiotic is directly or indirectly the bacterial DNA. In other bacteria, such as *Vibrio cholerae*, the SOS response is induced by the action of most widespread antibiotics used, including chloramphenicol and tetracyclines [60]. The treatment of the SOS response, in these cases, turns the evolutionary blockers into universal adjuvant for antibiotic. Since high concentrations of most antibiotics have high toxicity, this area of research seems promising.

4. The RecA protein inhibitors

The RecA protein is involved in chromosomal transformation, conjugation, and also is known as an inducer of the bacterial SOS response. Therefore, RecA is an ideal target in the search for compounds able to block of these processes [61,62]. The structure and functions of the RecA protein are highly conservative among bacteria, including pathogenic ones. Recently, many attempts have been made to search for RecA protein inhibitors [63,64].

Inhibitor search strategies can be arbitrarily divided into two categories. Within the first category, natural or synthetically synthesized compounds are searched or screened for the desired inhibition activity of the RecA protein. The second group of strategies implies that some peptides or proteins are screened for the ability to disrupt or modulate the interaction of the RecA protein monomers during filamentation. Due to their higher specificity combined with lesser toxicity and fewer side effects, small peptides or proteins, appear to be better candidates than low molecular weight organic compounds [65–67].

Among successful examples of the first strategy are the discovery of polysulfated and polysulfonated naphthyl compounds, including natural phenol (curcumin), suramin, bis-ANS, and congo red dye as the RecA protein inhibitors [68]. For suramin, zinc acetate, bis-ANS, and congo red, it has been shown that they block the ATPase activity of the RecA protein [69,70]. The proposed mechanism of action of curcumin has not yet conclusively determined. Among the compounds exhibiting inhibitory activity against RecA were some nucleotide analogs [71]. Small organic molecules based on 2-amino-4,6-diarylpyridine, 1,2,4-oxadiazole, quinazolinone, benzimidazole and diazepinone also have been investigated [72]. An interesting group of flavonoid natural compounds reducing the expression of *recA*, *lexA*, and *umuC* genes has been discovered in plant roots [73]. Later, it was shown that the SOS response can be blocked by p-coumaric acid [74]. There has been only one study, in which the RecA protein and SOS response were successfully suppressed by phthalocyanine tetrasulfonate *in vivo* [75]. Unfortunately, all of these compounds have lower specificity and may have potentially high toxicity with a wide range of side effects. For example, ATPase inhibitors with low specificity may interact with the ATP binding center of RecA and some human ATPases sharing similar ATP binding structures.

In any approach, the danger of finding common inhibitors for homologous proteins with similar functions between bacterial and human shall be considered. The Rad51 protein is a eukaryotic homologue of *E. coli* RecA. Rad51 is similar to RecA, both biochemically and structurally: it forms a right-twisted filament, hydro-

lyzes ATP and exchanges homologous DNA strands. It is not surprising that two of the three effective small inhibitors of the Rad51 eukaryotic recombinase also display a strong specificity for the RecA protein [76]. This example demonstrates that no screening method guarantees exceptional selectivity. Thus, the development of new blocking methods is largely driven by improvement of specificity and the necessity to overcome the cross-interaction.

Initially, the second strategy of using peptides as blocking agents was implemented by selecting amino acid sequences from the central RecA subdomain involved in the formation of the intermolecular interface of RecA monomers. These peptides have been able to inhibit the activity of the RecA protein at the stage of the helical ssDNA filament formation [77]. However, weak conformational stability with IC50 = 35–500 μM is not practical for future use [77]. Other constructed variants with lower the IC50 to ≈3 μM, require the formation of disulfide bonds for stabilization of active conformation, which makes it impractical because of blocking by glutathione in bacteria. In addition, the published IC50 value of ≈3 μM is likely to have been underestimated due to the reduced RecA concentration used in the study. The actual IC50 values for these peptides are likely to be ≈9 μM. Monomeric peptides generally do not have sufficient conformational stability, therefore they have low efficiency as inhibitors of certain biochemical reactions [77].

The RecX protein is one of the natural regulators of the RecA protein activity [78–82]. It is directly involved in interactions with the complex of the RecA protein and ssDNA. α-helical regions of the RecX protein were selected based on the structure of the RecA-RecX-ssDNA protein complex [83], using the Molsoft ICM Pro software package. These regions are involved in the interaction with both the RecA protein and ssDNA. To ensure the high conformational stability of a short peptide, the SEQOPT method of constructing alpha-helical peptides with global optimization of amino acid sequences was used [84]. The resulting peptide 4E1 has the same ability as the natural RecX protein to quickly disassemble RecA protein filaments [85]. Therefore, it completely suppresses the activation of the bacterial SOS response and inhibits bacterial adaptation to the effect of antibiotics. The use of this or similar peptides enables to control induced mutagenesis at several levels. At first, the peptide binding in the filament groove disassembles the nucleoprotein complex and shields the interaction of LexA with the nucleoprotein groove. At second, the peptide blocks the binding and processing site of the UmuD subunit of PolV polymerase leading to the abolition of PolV dependent mutagenesis even without turning off SOS response.

The further study of the structure of the complex of RecA filament with other filament binding proteins like UmuD protein or mCI may lead to designing of a short protein inhibitor of the RecA protein and SOS response [86].

It is worth to mention, an approach should be developed in which a transmitter fragment designed to penetrate the bacterial wall does not impair the basic properties of the active part of the peptide. An important assumption when using a peptide tag transporting across the cell membrane is that the existing variety of bacterial strains is likely to limit its potential use. It seems likely that in the paradigm of using peptide-based blockers, firmicutes that are capable of absorbing tens of amino acids long peptides without requiring a specific "tag" peptide will be of most interest. Since firmicutes include a significant number of pathogenic forms and the pathway of natural chromosomal transformation is a common characteristic for them, it can be expected that in this setting the method can find especially broad application.

In any case, the use of SOS response blockers will be most relevant in conjunction with the use of new modified antibiotics, the resistance to which are initially absent in the arsenal of natural populations. The combined approach will selectively inhibit any RecA-dependent evolutionary pathway, leaving only RecA-independent horizontal gene transfer pathways beyond the boundaries.

In summary, we can conclude that although it is impossible to find some universal method for suppressing simultaneously all the pathways of evolutionary adaptation, the creation of blockers having a broad range of targets, seems conceptually realistic.

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CRediT authorship contribution statement

Yakimov Alexander: Writing - review & editing, Visualization. **Bakhlanova Irina:** Writing - review & editing. **Baitin Dmitry:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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