mechanism may help to address the increase or antimicrobial resistance as bacteria become resistant to conventional antimicrobial drugs over time.

Earlier attempts to characterize the mechanism of Pol II inhibition by the ABU by comparison of whole genome sequences of *E. coli* 83972 and the uropathogenic strain, *E.coli* CFT073 have failed to identify specific factors associated with this (I. Amibite et al. Pathogens, 2016, 5, 49; doi:10.3390), indicating that this is not a simple matter. However, as a result of the serendipitous occurrence of attenuated Pol II inhibitory activity in a re-isolate, obtained from a patient inoculated with a prototype ABU, the applicants have been able to make a significant breakthrough in identification of the factors which may be useful in immunosuppression.

Summary

There is provided an inhibitor of host RNA polymerase II (pol II inhibitor) for use in therapy, wherein said inhibitor is a moiety which targets a protein selected from PAF1C or CDK12. According to a first aspect, there is provided an inhibitor of RNA polymerase II for use in therapy, wherein said inhibitor is selected a moiety which targets a protein selected from cyclin kinase 12 (CDK12) or its recruiting protein PAF1C.

Moieties which target these proteins may be known in the art or may be identified or designed using conventional screening methods. They may for example comprise antibodies or binding fragments thereof, aptamers or small molecules, which bind to CDK12 or PAF1C so as to inhibit RNA polymerase II in a host cell.

Specific examples of such moieties may be obtained from bacteria, such as the commensal bacteria or asymptomatic carriers such as asymptomatic bacteriuria.

As a result of the isolation of a spontaneous, loss of function mutant of the ABU strain *E. coli* 83972, the applicants have identified bacterial proteins able to act as direct inhibitors Pol II phosphorylation in infected hosts. Mutations were localized by comparative genome sequencing of the *E. coli* 83972 WT and mutant strain and identified sequence variants were systematically introduced into the *E. coli* 83972 WT strain for analyses of effects on Pol II phosphorylation.

In a particular example, the inhibitor is a polypeptide expressed by a gene selected from IldD, IldR, nlpD or rfaH of a bacterial species, or a variant of said polypeptide.

The TATA box binding protein Sigma S was shown to bind human TATA box DNA and to competitively inhibit the binding of human TBP, thus incapacitating the Pol II pre-initiation complex. Furthermore, NlpD, which regulates Sigma S expression, stimulated the

degradation of PAF1C, which recruits the kinase CDK12 to the Pol II phosphorylation complex. Both proteins entered host cells. The results identify a novel mechanism used by bacteria to regulate fundamental host cell functions, such as the transcriptional activity at sites of infection.

In a particular example, the inhibitor is a bacterial Sigma S protein or a variant thereof, or an active fragment of either of these. An example of such a protein is SEQ ID NO 2 or a truncated form of SEQ ID NO 2.

MSQNTLKVHDLNEDAEFDENGVEVFDEKALVEQEPSDNDLAEEELLSQGATQRVLD
ATQLYLGEIGYSPLLTAEEEVYFARRALRGDVASRRRMIESNLRLVVKIARRYGNRG
LALLDLIEEGNLGLIRAVEKFDPERGFRFSTYATW**WIRQTIERAIMNQTRTIRLPIHI**
VKELNVYLRTARELSHKLDHEPSAEEIAEQLDKPVDDVSRMLRLNERITSVDTPLGG
DSEKALLDILADEKENGPEDTTQDDDMKQSIVKWLFELNAKQREVLARRFGLLGYE
AATLEDVGREIGLTRERVRQIQVEGLRRLREILQTQGLNIEALFRE (SEQ ID NO 1)

MFRQGITGRSHLMSQNTLKVHDLNEDAEFDENGVEVFDEKALVEEEPSDNDLAEEE
LLSQGATQRVLDATQLYLGEIGYSPLLTAEEEVYFARRALRGDVASRRRMIESNLRL
VVKIARRYGNRGLALLDLIEEGNLGLIRAVEKFDPERGFRFSTYATW**WIRQTIERAI**
MNQTRTIRLPIHIVKELNVYLRTARELSHKLDHEPSAEEIAEQLDKPVDDVSRMLR
LNERITSVDTPLGGDSEKALLDILADEKENGPEDTTQDDDMKQSIVKWLFELNAKQR
EVLARRFGLLGYEAATLEDVGREIGLTRERVRQIQVEGLRRLREILQTQGLNIEALFR
E (SEQ ID NO 2)

As used herein, the term 'fragment' refers to any portion of the given amino acid sequence which will shows Pol II inhibitory activity. Fragments may comprise more than one portion from within the full-length protein, joined together. Portions will suitably comprise at least 5 and preferably at least 10 consecutive amino acids from the basic sequence.

Suitable fragments will include deletion mutants comprising at least 10 amino acids, for instance at least 20, more suitably at least 50 amino acids in length or analogous synthetic peptides with similar structures. They include small regions from the protein or combinations of these.

In a particular example, the fragment will be a peptide which inhibits binding of TBP to Sigma S, for example a fragment comprising amino acids 149-183 of SEQ ID NO 2, shown in bold type in the above sequence, which forms SEQ ID NO 3.

Certain such pol II inhibitors will be novel and these form a part of the disclosure.

They may be used in a range of therapeutic applications as discussed above, to suppress protein expression. In particular, they may be used as immunosuppressant, anti-inflammatory or anti-infection (such as antibacterial) agents.

The expression "variant" refers to proteins or polypeptides having a similar biological function but in which the amino acid sequence differs from the base sequence from which it is derived in that one or more amino acids within the sequence are substituted for other amino acids. Amino acid substitutions may be regarded as "conservative" where an amino acid is replaced with a different amino acid with broadly similar properties. Non-conservative substitutions are where amino acids are replaced with amino acids of a different type.

By "conservative substitution" is meant the substitution of an amino acid by another amino acid of the same class, in which the classes are defined as follows:

Class	Amino acid examples
Nonpolar:	A, V, L, I, P, M, F, W
Uncharged polar:	G, S, T, C, Y, N, Q
Acidic:	D, E
Basic:	K, R, H.

As is well known to those skilled in the art, altering the primary structure of a polypeptide by a conservative substitution may not significantly alter the activity of that polypeptide because the side-chain of the amino acid which is inserted into the sequence may be able to form similar bonds and contacts as the side chain of the amino acid which has been substituted out. This is so even when the substitution is in a region which is critical in determining the peptide's conformation.

Non-conservative substitutions are possible provided that these do not interrupt the function of the DNA binding domain polypeptides. Broadly speaking, fewer non-conservative substitutions will be possible without altering the biological activity of the polypeptides.

Determination of the effect of any substitution (and, indeed, of any amino acid deletion or insertion) is wholly within the routine capabilities of the skilled person, who can readily determine whether a variant polypeptide retains the fundamental properties and activity of the basic polypeptide. For example, when determining whether a variant of the polypeptide falls within the scope of the disclosure, the skilled person will determine whether complexes comprising the variant retain biological activity (e.g tumour cell death) of complexes formed with unfolded forms of the native protein and the polypeptide has at least 60%, preferably at least 70%, more preferably at least 80%, yet more preferably 90%, 95%, 96%, 97%, 98%, 99% or 100% of the native protein.

Variants of the polypeptide may comprise or consist essentially of an amino acid sequence with at least 70% identity, for example at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 96%, 97%, 98% or 99% identity to a native polypeptide sequence. The level of sequence identity is suitably determined using the BLASTP computer program with the native polypeptide sequences as the base sequence. This means that native polypeptide sequences form the sequence against which the percentage identity is determined. The BLAST software is publicly available at http://blast.ncbi.nlm.nih.gov/Blast.cgi (accessible on 13 October 2016).

In a particular example, the inhibitor is a polypeptide expressed by a gene selected from IldD, IldR, nlpD or rfaH of a bacterial species. The bacterial species is suitably a commensal bacteria or an asymptomatic carrier such as asymptomatic bacteriuria (ABU). In particular, the bacteria is an *E.coli* strain, such as *E. coli* 83972.

In a particular example also, the inhibitor is a polypeptide expressed by an IldD, Ildr or nldD gene and secreted by the bacteria.

In some examples, the inhibitor is of low molecular weight for instance less than 3kDa. It is suitably resistant to Proteinase K.

In other examples, the inhibitor is a larger protein, for example of about 40kDa.

Such inhibitors may be obtained by culturing the bacteria in an appropriate culture medium such as RPMI. RNA polymerase II activity, which may be tested using appropriate testing methods such as those exemplified hereinafter, may be found in the supernatant, suggesting that the factor responsible is secreted by the bacteria. Suitably the supernatant is separated from the bacteria for example using centrifugation as a preliminary step and then

subjected to analysis to confirm RNA polymerase II inhibitor activity. Filtration of the supernatant for example using centrifugal or flow-through filters suitable for separating proteins can be used to remove non-active fractions which tend to be high molecular weight components, thus concentrating the inhibitor activity.

The inhibitor may be purified from the concentrate using conventional methods and the amino acid sequence determined, also using conventional methods. When this has been done, the inhibitor may be produced synthetically, for example using recombinant DNA technology. In another aspect, there is provided a method for preparing an inhibitor as defined herein which comprises culturing suitable bacteria in a culture medium, and isolating a factor having RNA polymerase II inhibitor activity from the supernatant.

For use in modulating the immune system, the pol II inhibitor of the disclosure is suitably formulated into a pharmaceutical composition in which it is combined with a pharmaceutically acceptable carrier. Such compositions form a part of the disclosure. In another aspect, there is provided a pharmaceutical composition comprising an inhibitor of RNA polymerase II as defined herein in combination with a pharmaceutically acceptable carrier.

The composition may be in a form suitable for oral administration, for example as a tablet or capsule, for parenteral injection (including intravesical, intravenous, subcutaneous, intramuscular, intravascular or infusion) as a sterile solution, suspension or emulsion, for topical administration as an ointment or cream or for rectal administration as a suppository.

In general the above compositions may be prepared in a conventional manner using conventional excipients.

Methods of using the RNA polymerase II inhibitor will depend upon a variety of factors such as the disease being treated and the nature of the particular inhibitor being used. These will be determined in accordance with clinical practice.

The disclosure provides a method of treating a patient in need therefore with an effective dosage of an inhibitor of RNA polymerase II as described above. Suitable dosages will be determined in accordance with normal clinical practice, but will generally be in the range approximately from 0.01 g/kg to 25 g/kg but with considerable variation between individuals and disease conditions.

As reported hereinafter, the mechanism of Pol II inhibition by the ABU strain was further investigated, but using re-isolates of the prototype ABU strain from inoculated patients, which were screened for attenuation of Pol II inhibitory activity. An attenuated re isolate SN25, was subjected to whole genome sequencing and genomic differences between the ancestor and progeny were identified. The variant positions in the chromosome were

subsequently mutated in *E. coli* 83972, by homologous recombination and the mutants were screened for effects on Pol II Ser 2 phosphorylation. The genes lldD and lldR, whose products are involved in aerobic L-lactate metabolism, were shown to significantly affect Pol II phosphorylation. To confirm these effects, a biochemical approach was used. Supernatant of bacteria incubated in tissue culture medium were shown to contain inhibitory activity and was further fractionated to define the molecules responsible for these effects. Weak acids of <3 kD molecular weight, formic and acetic, were identified to have inhibitory effect of Pol II phosphorylation. In addition, Pol II phosphorylation was affected by deletion of nlpD, which encodes a lipoprotein with a potential function in cell wall formation and rfaH and cysE with products of both genes involved in biofilm formation. In another aspect, there is provided an inhibitor of RNA polymerase II which comprises an isolated polypeptide expressed by a gene selected from IldD, IldR, nlpD or rfaH of a bacterial species, or a variant of said protein.

In particular, the applicants have identified a new mechanism by which bacterial proteins disturb the formation of the Pol II complex and attenuate the Pol II activation in human cells. The bacterial TATA box binding protein, encoded by rpoS and its transcriptional regulator Nlpd as bacterial genes responsible for this effect through competitive inhibition of the human TATA box binding protein TBP and a reduction in PAF1C, CDK 9 and CDK12. The findings define a new, potent mechanism for cross-regulation of the transcriptional machinery between eukaryotic and prokaryotic cells. Without being bound by theory, the findings may illustrate a new general mechanism of bacterial adaptation and survival in infected hosts. In another aspect, there is provided a bacterial protein selected from Sigma S protein or NlpD protein or an active fragment thereof for use in therapy.

The encounters between bacteria and host cells are guided by specific molecular interactions. Pathogen attack is often executed by virulence factors via receptor-mediated interactions, involving conserved receptors like the TLRs and pathogen specific recognition mechanisms with specificity for unique sets of virulence factors. Adhesive interactions determine the tissue specificity and site of infection. Exotoxins bind cell surface receptors, such as GM1 (cholera toxin) or Gb3 (Shiga toxin) and after internalization, the toxins interfere with key cellular functions. Endotoxins activate TLR4 signaling cascades and by

engaging specific co-receptors, virulence factors determine adaptor protein usage and transcription factor recruitment. Here we propose that bacteria export molecules that enter host cells and attenuate the transcriptional machinery, by competing with molecules involved in the assembly of the Pol II complex and its phosphorylation at Ser2.

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NlpD being potentially inserted into membrane of the host cell before reaching its targets inside the cell.

NlpD also exerts its effect through transcriptional control of rpoS and rpoS-dependent genes. The transcription of rpoS is regulated from a common nlpD promoter and from additional sites within the nlpD ORF. Sigma S is an important regulator of more than 20 stationary phase genes and operons such as genes required for multiple-stress resistance. In addition, as NlpD and Sigma S proteins are encoded by the same gene cluster and are derived from the same polycystronic RNA, direct interactions cannot be excluded. It can be hypothesized that NlpD allosterically modifies Sigma S, facilitating its transport and rendering it active in binding and melting of host cell DNA.

In a particular example, the inhibitor comprises a bacterial NplD protein, or a variant thereof, or an active fragment of either of these. Examples of such proteins are of SEQ ID NO 4.

Without being bound by theory, it is possible that disruption of the pre-initiation complex by Sigma S might be a key step, affecting downstream transcriptional activity. The applicants have shown that bacterial RpoS competes with human TBP for binding to TATA box DNA. By dislodging TBP from its binding site, RpoS may thus prevent pre-initiation complex formation and therefore the binding of Pol II to specific promoters. A general effect on gene expression is supported by a clinical study, showing reduced expression of >60% of all genes in circulating blood cells in patients inoculated with *E. coli* 83972. Despite the inhibition of a large number of genes, effects were much less pronounced than when a pharmacological inhibitor was used, however, suggesting specificity. In eukaryotic promoters, PIC formation and binding of general transcription factor II B (TFIIB) recruits Pol II to the promoter. II B (TFIIB) consists of 4 functional domains – N-terminal Zn ribbon, B reader, B linker and Core domain. Core domain in eukaryotes is required to stabilize the TBP-DNA complex and the Zn ribbon recruits RNA Pol II.

Amino acid analysis reveals that Sigma S shows homology to the core domain of TFIIB (38% over the stretch of 35 AA) but no homology with the Zn ribbon, potentially explaining why Sigma S binds DNA but fails to recruit eukaryotic Pol II. The findings suggest an interesting evolutionary model, where maintaining the basic organization of gene

expression and Pol II machinery constituents from bacteria to humans offers a mechanism for coordinate regulation of gene expression between eucaryotes and procaryotes.

To summarize, the applicants obtained a set of data supporting the hypothesis that both bacterial proteins NlpD and Sigma S are internalized by the human host cells. These include experiments on whole cell lysates of cells infected with ABU, co-immunoprecipitation of Pol-II, Sigma S and Nlp D, as well as immunofluorescence studies of ABU infected kidney cells (Figures 6A-6D). With immunofluorescence, some background staining is observed for NlpD and RpoS in uninfected control, which might be explained by the relative homology between NlpD and RpoS with some human proteins. NlpD is homologous to human protein, membrane-spanning 4-domains subfamily A member 15 (with 37% homology and 53% similarity over the stretch of 47 amino acids) as well to human protein GREB1-like protein isoform X4, Growth Regulation By Estrogen In Breast Cancer 1 (25% homology and 40% similarity, 116 AA). Likewise, Sigma S is homologous to two human proteins, circulating B cell antibody heavy chain variable region (31% homology and 54% similarity, 45 AA) and as discussed earlier to transcription factor II B (38% homology and 48% similarity, 35 AA).

The disclosure will now be particularly described by way of example with reference to the accompanying Figures in which:

Figure 1 illustrates inhibition of eukaryotic RNA Pol II phosphorylation by 83972 ABU strain: **A.** Cells were infected in suspension, labelled for Pol II-Ser2 with fluorescently labeled AB and run on cell flow cytometer. The distribution of phosphorylated Pol II fluorescence was analyzed. Infection with the AU strain decreases host Pol II phosphorylation; B Pol II phosphorylation in control and ABU infected cells, visualized by laser-scanning, slides were mounted and imaged. Nuclei were counterstained with DRAQ5 and fluorescence intensity was quantified by ImageJ software 1.46r.

C. Mean value of Pol II phosphorylation in ABU infected cells and uninfected control cells. One representative experiment is shown, measured with flow cytometry as shown in A and one was quantified by confocal microscopy, using ImageJ software 1.46r. Means of two experiments. **D-E** shows how a particular re-isolate, designated SN25, has lost the inhibitory effect on RNA Pol II phosphorylation. Results show loss of Pol II phosphorylation repression by reisolate strain SN25 as compared to ancestor strain 83972 in A-C. Error bars show

10. An inhibitor according to claim 9 which is a protein of SEQ ID NO 4.

11. An inhibitor according to any one of claims 2 to 6 wherein the inhibitor is secreted by the bacteria.

12. An inhibitor according to claim 9 which is of less than 3kDa in molecular weight.

13. An inhibitor according to any one of claims 2 to 12 which is synthetic.

14. An inhibitor according to any one of the preceding claims wherein the therapy is immunosuppression, anti-inflammatory or anti-infection therapy.

15. A method for preparing an inhibitor according to claim 11 or claim 12 which comprises culturing suitable bacteria in a culture medium, and isolating a factor having RNA polymerase II inhibitor activity from the supernatant.

16. A pharmaceutical composition comprising an inhibitor of RNA polymerase II according to any one of claims 1 to 14 in combination with a pharmaceutically acceptable carrier.

17. An inhibitor of RNA polymerase II which comprises an isolated polypeptide expressed by a gene selected from IldD, IldR, nlpD or rfaH of a bacterial species, or a variant of said protein.

18. An inhibitor according to claim 17 wherein the bacteria are commensal bacteria or an asymptomatic carrier.

19. An inhibitor according to claim 18 wherein the bacteria are asymptomatic bacteriuria (ABU).

20. An inhibitor according to any one of claims 17 to 19 wherein the bacteria is an *E.coli* strain.

21. An inhibitor according to claim 20 wherein the bacterial strain is *E. coli* 83972.

22. An inhibitor according to any one of claims 17 to 21 wherein the inhibitor is a protein secreted by the bacteria.

23. An inhibitor according to claim 20 which is of less than 3kDa in molecular weight.

24. An inhibitor according to any one of claims 17 to 23 which is synthetic.

25. A bacterial protein selected from Sigma S protein or NlpD protein or an active fragment thereof for use in therapy.

26. A bacterial protein according to claim 25 wherein the Sigma S protein is of SEQ ID NO 1 or SEQ ID NO 2, or a fragment of SEQ ID NO 3, and the NlpD protein is of SEQ ID NO 4.