polymorphisms are associated with susceptibility to acute pyelonephritis (APN) and *Irf3*$^{-/-}$ mice develop acute, septic infections accompanied by massive tissue damage. Tissue pathology is driven by the closely related transcription factor *Irf7*, which regulates the expression of proinflammatory, tissue destructive genes. Attenuating *IRF7* promoter polymorphisms are negatively associated with APN susceptibility in children but specific molecular determinants of pathology, downstream of *Irf7*, remain to be defined.

Around 10% of expressed human genes are involved in transcription and mechanisms of transcriptional regulation have been extensively characterized. Innate immunity and inflammation is regulated by multiple gene sets that control transcriptional ''modules'' and functional programs (Medzhitov and Horning, 2009). The constitutively expressed Class I transcription factors, are activated by post-translational modifications during the initial phase of innate immune activation and include IRF-3 and NF-κB. The Class II category, which requires *de novo* synthesis, regulates subsequent waves of gene expression and may in some cases, stably reprogram transcription. This category includes IRF-7 and different *c-Fos* and *c-Jun* related C/EBP isoforms, which also interact with CREB and NF-κB, in infected cells.

Summary

During an investigation into the inherent, gene expression differences distinguishing highly infection-prone individuals from asymptomatic bacterial carriers, the applicants have surprisingly identified c-MYC as a key transcriptional regulator. Specifically, the applicants identified MYC and MYCN as major regulators of mucosal innate immune responses, downstream of *IRF7*. By genome-wide RNA sequencing, an "immunological disequilibrium" was detected in patients prone to acute

pyelonephritis (APN), associated with an elevated MYC and TCR expression but an unresponsive innate immune system. In contrast, asymptomatic carriers showed an innate immune response profile with modest activation of *IRF7*-dependent genes and low MYC expression.

The genetic basis for this discrepancy was examined in the murine pyelonephritis model, where *Irf7* was identified as a key regulator of MYC expression, with pronounced inhibition of MYC in *Irf7*$^{-/-}$ mice that were protected from pathology.

According to one aspect of the present disclosure there is provided a therapeutic agent comprising a bacteria which expresses an inhibitor of MYC activity or a extract or product obtainable therefrom, for use in the prevention or treatment of disease wherein MYC levels are elevated.

The applicants have found that certain bacteria, in particular certain uropathogenic bacteria inhibit MYC activity in a host, and that this is caused by expression of a bacterial factor, which appears to be secreted by the bacteria, and thus is present in supernatants. Thus in a particular embodiment, the therapeutic agent comprises an extract of product obtainable from bacteria, which comprises or is derived from a supernatant produced during culture of the bacteria.

Furthermore, they have identified that the mechanism by which the bacteria and in particular the factor obtainable from the bacteria exerts its effect. The identified mechanism has been found to comprises a direct inhibitory effect of bacteria on mucosal MYC expression, due to a new class of virulence factors encoded on PAI I in uro-pathogenic *E. coli* strains. The findings suggest a scenario where mucosal MYC expression is regulated downstream of *Irf3* and *Irf7*, which are major determinants of disease severity and susceptibility in infected kidneys and that

MYC is targeted by specific bacterial genes on pathogenicity islands that are shared by uropathogenic *E. coli* strains.

Furthermore, the applicants have found that MYC may be degraded by a second strategy, in which activation of CK1α1 And IFNβ dependent mechanisms of phosphorylation and proteasomal degradation by the host is triggered.

The isolated factors are novel and so these form a further example of the disclosure.

Thus according to a second aspect of the present disclosure there is provided an inhibitor of MYC activity comprising a factor obtainable from a bacteria. In particular, the bacteria is a pathogenic bacteria, such as a uropathogenic E. coli. Particular examples of such uropathogenic E. coli is selected from CFT073 and 536.

As used herein, the expression 'inhibitor of MYC activity' refers to any entity which controls, regulates or limits the activity of the MYC transcription factor, in particular *in vivo* in mammals. This may be achieved for example by direct inhibition of the MYC protein, by downregulation of the c-Myc gene *in vivo*, or by interfering with any pathway in which MYC is involved.

Bacteria can evolve by modification of the pre-existing genome sequence, acquisition or loss of genetic information. Gene acquisition by horizontal gene transfer, involving mobile genetic elements like plasmids or genomic islands (GEIs), increases the capacity of bacteria to evolve and adapt to new environments.

The pathogenic properties of bacteria are correlated to the expression of virulence- or disease-associated factors, that are

For administration to patients, the inhibitor or bacteria is suitably administered in the form of a pharmaceutical composition, which further comprise a pharmaceutically acceptable carrier. Such compositions form a further example of the disclosure.

Thus according to a third aspect of the present disclosure there is provided a pharmaceutical composition comprising a therapeutic agent as defined herein, or an isolated inhibitor as defined herein, in combination with a pharmaceutically acceptable carrier.

Suitable pharmaceutical compositions will be in either solid or liquid form. They may be adapted for administration by any convenient route, such as parenteral, oral or topical administration or for administration by inhalation or insufflation. The pharmaceutical acceptable carrier may include diluents or excipients which are physiologically tolerable and compatible with the active ingredient.

Parenteral compositions are prepared for injection, for example either subcutaneously or intravenously. They may be liquid solutions or suspensions, or they may be in the form of a solid that is suitable for solution in, or suspension in, liquid prior to injection. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH-buffering agents, and the like.

Oral formulations will be in the form of solids or liquids, and may be solutions, syrups, suspensions, tablets, pills, capsules, sustained-release formulations, or powders. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like.

Topical formulations will generally take the form of suppositories or intranasal aerosols. For suppositories, traditional binders and excipients may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient.

The disclosure provides a method of treating or preventing a disease in which MYC levels are elevated, said method comprising administering to a patient in need thereof, an effective amount of an inhibitor as described above, or a bacteria which expresses said inhibitor, or a pharmaceutical composition comprising either of these.

The amount of inhibitor administered will vary in accordance with normal clinical practice, and will depending upon factors such as the nature of the reagent being used, the size and health of the patient, the nature of the condition being treated etc. in accordance with normal clinical practice. Typically, a dosage in the range of from 1µg-50mg/Kg for instance from 2-20 mg/Kg, such as from 5-15 mg/Kg would be expected to produce a suitable effect.

In particular, the disease in which MYC levels are elevated and which is therefore susceptible to treatment using the method of the disclosure is cancer. Examples of such cancers may include cervical cancer, colon cancer, breast cancer, lung cancer, stomach cancer, kidney cancer, bladder cancer, bowel cancer, mouth cancer or cancer of the gastrointestinal track. In a particular embodiment, the cancer may be associated with a mucosal or epithelial surface, such as cervical cancer, lung cancer, kidney cancer, bladder cancer, bowel cancer, mouth cancer or cancer of the gastrointestinal track.

The pleiotropic MYC transcription factors regulate cellular life and increased MYC activity is associated with oncogenic

transformation. To reiterate, the applicants have made the unexpected observation that the c-MYC family of transcription factors regulate innate immune responses to bacterial infection in a tissue specific manner, involving the mucosal barrier. They first detected elevated MYC transcript levels in children prone to acute pyelonephritis, which is a common and severe bacterial infection of the kidneys. The applicants also identified *Irf3* and *Irf7* as transcriptional regulators of *Myc* expression in the murine acute pyelonephritis model and characterized the profile of c-MYC dependent genes involved in tissue repair. However in accordance with the disclosure, they have shown that C-MYC expression is inhibited by uropathogenic *E. coli* infection, through a new class of bacterial virulence factors.

These findings are unexpected; effects of c-MYC on host susceptibility to bacterial infection have not been described previously. Different mycobacterial species induce MYC expression via ERK1/2 and JNK, leading to TNF-α and IL-6 activation and suppression of intracellular mycobacterial growth and a MYC-dependent global metabolic transcriptome has been detected in activated, primary T lymphocytes. In addition, as c-MYC plays an important role in T cell priming during viral or a bacterial infection, effects on the specific immune response have been extensively documented. Wang et al. (2011) Immunity, 2011. **35**(6): p. 871-82 showed that T cell activation leads to increased glucose, glutamine, and mitochondrial oxidative metabolisms that are essential for growth and proliferation.

In addition to MYC, MYCN was differentially activated in the APN and primary ABU patients. Previously, the amplification of MYCN in neuroblastoma cell lines has been shown to affect both immune cell recruitment and chemokine expression and an inverse correlation between *MYCN* RNA levels and monocyte chemoattractant protein-1/CC chemokine ligand 2 (MCP-1/CCL2) has been observed.

17. An inhibitor according to claim 16 which comprises SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4 or an active fragment thereof.

18. An inhibitor according to claim 15 which is a miRNA molecule.

19. A pharmaceutical composition comprising a therapeutic agent according to any one of claims 1 to 9, or an isolated inhibitor according to any one of claims 10 to 18, in combination with a pharmaceutically acceptable carrier.