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(54) Title: ANTISENSE OLIGOMERS FOR CONTROLLING CANDIDA ALBICANS INFECTIONS

(57) Abstract: The present disclosure relates to the use of antisense oligomers in the treatment or therapy of *Candida albicans* infections. The present disclosure further describes the use of antisense oligomers in antisense therapy to inhibit the morphological transition of *Candida albicans* from yeast to filamentous form.

D E S C R I P T I O N

ANTISENSE OLIGOMERS FOR CONTROLLING *CANDIDA ALBICANS* INFECTIONS

Technical Field

[0001] The present disclosure relates to the use of antisense oligomers in the treatment or therapy of *Candida albicans* infections. In particular, the use of antisense oligomers to target specific genes involved in the morphological transition of *Candida albicans* from yeast to filamentous form.

[0002] The present disclosure further describes the use of antisense oligomers in antisense therapy to inhibit the morphological transition of *Candida albicans* from yeast to filamentous form.

Background

[0003] *Candida* species normally exist as commensal microorganisms but they can also act as opportunistic pathogens with the ability to cause superficial and systemic infections. The incidence of *Candida* infections has increased remarkably in the last few years due to the rise of the elderly population and the number of immunocompromised patients, as well as the widespread use of indwelling medical devices. *Candida albicans* infections remain as the most prevalent of all *Candida* species infections; approximately 47% in all *Candida* infections are caused by *C. albicans*.

[0004] As a dimorphic fungus, the ability of *C. albicans* to change from commensal to pathogenic is primarily due to its ability to morphologically switch between yeast and hyphal forms, a property that is central to its ability to penetrate human body tissues and escape the host´s immune system. This ability contributes strongly to its pathogenicity. *Candida albicans* is responsible for causing about 400,000 deaths each year and one of its most problematic virulent factor is its ability to develop filaments.

[0005] In the last few years, important technical advances have facilitated the investigation of the molecular biology of *C. albicans´* morphological transition from yeast

to filamentous form. These advances include the availability of genomic and transcriptomic sequence data essential for identifying and characterizing the genes involved in *C. albicans´* dimorphism. Several works have demonstrated the importance of *EFG1*, *HWP1* and *HYR1* genes as inducers of *C. albicans* filamentation [1].

[0006] Despite extensive research dedicated to the development of new therapeutic strategies, there are only a limited number of drugs available to fight superficial and invasive *Candida* infections. Indeed, only four molecular classes that target three distinct fungal metabolic pathways are currently used in clinics to treat *Candida* related systemic infections: polyenes, azoles, and echinocandins. Several other classes such as morpholines and allylamines are only used as topical agents due to either poor efficacy or severe adverse effects when administered systemically [2]. The increase in *Candida* multi-drug resistance, the scarcity of new drugs and the high plasticity of *Candida* species´ transition switch from commensal to pathogenic fungi had led to the increase in the number of cases of candidiasis. In spite of the advances in therapies over the last few years, the rate of development of antifungal drugs continue to lag behind rate of fungal infections. Furthermore, most of these compounds have limited potential as systemic agents due to issues of toxicity.

[0007] These factors constitute a clinical problem, resulting in high morbidity and mortality (30-37%) as well as higher economic costs associated to prolonged hospital stays of patients. Average costs associated with candidemia among hospitalized patients range from €5700 - €85000 per episode. These evidence highlight the need for new strategies to manage *C. albicans* infections.

[0008] Antisense therapy (AST) holds great promise for the treatment of many human diseases. The concept underlying AST is relatively straightforward: the use of a complementary sequence to a specific mRNA that can inhibit expression of the latter and induce a 'blockage' in translation of the DNA to protein. Antisense oligomers (ASO) are short strands of nucleic acids that are complementary to the target mRNA [16]. ASO generally compose of short sequences with 13-25 nucleotides of unmodified or chemically modified molecules that are gene specific. Moreover, in recent years, ASOs chemical modifications have been developed to enhance nuclease resistance, to prolong

tissue half-live, affinity and potency, and to reduce specific toxicity. Currently there are three generations of ASOs. The first-generation contains phosphorothioate backbone modification and is characterized by the substitution of non-bridging phosphate oxygen with sulphur atoms. The second-generation was developed to enhance nuclease resistance and increase-binding affinity for target mRNA. For this 2'-O-methyl and 2'-O-methoexyethyl sugar modifications are added to the OH group in the 2´position of the nucleotide. Numerous studies have documented the use of AST as biochemical tools for studying target human proteins [3]. This methodology has been proposed as an alternative to antibiotic treatments of bacteria infections. Fomivirsen (brand name Vitarvene), an S-oligo, is the only FDA-approved antisense therapeutic that targets a microorganism. Fomivirsen was approved in 1998 for treatment of cytomegalovirus-induced retinitis. Earlier work targeting bacteria found that modified ASOs inhibits growth of *Salmonella*, *Listeria*, *Brucella*, *Pseudomonas*, *Staphylococcus*, *Streptococcus* and *Escherichia* species [4]. Despite the fact that the development in ASO for use in controlling bacteria growth has been ongoing for the last decade, antisense based applications for use in controlling *C. albicans* growth is scarce and poorly exploited [5].

[0009] Document WO2014144024 (A1) discloses the method of producing fragments of the *Candida* cell Hyhr1 surface protein. The document further discloses the use of the protein for therapy and immunization against fungal infections.

[0010] Document AU2016244238 (A1) discloses the use of Hyhr1 surface protein as a target for immunization (active and passive) and as a prophylactic therapy for disseminate candidiasis.

[0011] Document US2019030141 (A1) discloses fragments of the *Candida* cell surface proteins Als3 and Hyr1 and combinations thereof for use in in immunization.

[0012] Document CN107304429 (A) discloses the use of inhibitors such as small molecule compounds to down-regulate Nuo2 protein or genes that inhibit virulence factor-related genes (*ALS3*, *HWP1* and/or *ECE1* gene expression).

[0013] Document US2017298349 (A1) discloses intergenic non-coding RNA molecules that regulate the expression of *HWP1* and *ALS3* of *Candida*. The document further

discloses use of the non-coding RNA molecules and complementary molecules thereof in modulating *HWP1* or *ALS3* expression, as well as modulate the adherence, yeast-to-hyphal transition, or biofilm development of *Candida*. The aim of which is to prevent or treat candidiasis.

[0014] Document US2005244861 (A1) discloses nucleic acids required for the regulation of *HWP1* expression in *C. albicans* and the use of these nucleic acids in identifying agents which inhibit the expression of *HWP1*.

[0015] Document CN1730654 (A) discloses the use of an ASO sequence for use against *C. albicans* infection. Specifically, the document discloses an ASO sequence against the core pseudoknot of *C. albicans* type I introne ribozyme thus strongly repressing the shearing reactivity of the *C. albicans*.

[0016] These facts are disclosed in order to illustrate the technical problem addressed by the present disclosure.

General Description

[0017] This disclosure describes the use of ASOs to target specific genes involved in the morphological transition of *C. albicans* from yeast to filamentous form. The present disclosure further describes the use of ASOs in AST to inhibit the morphological transition of *C. albicans* from yeast to filamentous form.

[0018] ASOs are synthetic oligomers that silence expression of specific genes. This specificity confers an advantage over broad-spectrum antifungals by avoiding unintended effects on other commensal *Candida* species and, by minimizing the possibility of cross resistance. Furthermore, the sequence specificity and the short length of ASOs also pose little risk to human gene expression. Another important advantage of this approach is the ability to nearly eliminating or significantly reducing the time required for discovering new antifungals thus broadening the range of potentially available targets to any gene with a known base sequence in any yeast, for example *C. albicans.*

[0019] So far, there are no identified, designed and synthesized ASOs to target non-essential genes required for *C. albicans* virulence, namely genes involved in the morphological transition from yeast to filamentous form. The application of ASOs against a group of genes involved in one of most problematic virulence factor of *C. albicans* strains enable us to not only develop new nano-drugs specific for this problematic yeast but develop new strategies to control candidiasis. The present disclosure intends to identify short sequence ASOs that are able to specifically hybridize with the mRNA of three important regulator genes (*EFG1*, *HWP1* and *HYR1*) of *C. albicans* filamentation and to block their molecular function. This allows for the development of nano-drugs to be used singly or in combination to control *C. albicans´* invasiveness and pathogenicity.

[0020] An aspect of the present disclosure relates to an isolated oligomer comprising: at least a sequence selected from a list consisting in the following sequences:

SEQ ID No. 7 - for *EFG1*: 5'-mG mG mC mA TACCGTTA mU mU mG mU-3';

SEQ ID No. 8 - for *HWP1*: 5' mUmGATAACATGTAATAAGmCmG 3';

SEQ ID No. 9 - for *HYR1*: 5' mGmGmU TGA GAG TAmA mGmC 3'; or combinations thereof; or a variant of said sequence which is at least 95% identical to the selected sequence, based on the identity of all the nucleotides of said sequence, as a *C. albicans* filamentation blocker.

[0021] Another aspect of the present disclosure relates to an isolated oligomer comprising: at least a sequence selected from a list consisting in the following sequences: SEQ ID 7; SEQ ID 8; SEQ ID 9; or combinations thereof; or a variant of said sequence which is at least 95% identical to the selected sequence, based on the identity of all the nucleotides of said sequence, for use in medicine, preferably for use in the treatment or therapy of *C. albicans* related infections. In particular, for use in the treatment or therapy of vaginal infection and/or oral infections.

[0022] In an embodiment, the sequence is at least 96% identical to the selected sequence, based on the identity of all the nucleotides of said sequence; preferably 97%; 98%; 99% or identical.

[0023] Another aspect of the present disclosure relates to a composition comprising a combination of at least two isolated oligomers comprising: at least a sequence selected from a list consisting in the following sequences: SEQ ID 7; SEQ ID 8; SEQ ID 9; or combinations thereof; or a variant of said sequence which is at least 95% identical to the selected sequence, based on the identity of all the nucleotides of said sequence, as a controlling *C. albicans* filamentation blocker.

[0024] In an embodiment, the sequence is at least 96% identical to the selected sequence, based on the identity of all the nucleotides of said sequence; preferably 97%; 98%; 99% or identical.

[0025] In an embodiment, the combination of isolated oligomers is selected from the following combinations:

SEQ. ID. 7 and SEQ. ID. 8;

SEQ. ID. 7 and SEQ. ID. 9;

SEQ. ID. 8 and SEQ. ID. 9;

SEQ. ID. 7; SEQ. ID. 8 and SEQ. ID. 9.

[0026] In an embodiment, the composition may be a coating composition.

[0027] Another aspect of the present disclosure relates to an article comprising the isolated oligomer or the composition described in the present subject-matter, preferably a coating composition.

[0028] In an embodiment, the article may be a medical device, in particular a patch, a catheter, a stent, a contact lens or a pacemaker, among others.

[0029] In an embodiment, the article may be an intravaginal tampon, a sanitary napkin, or panty liners.

[0030] In one embodiment, ASOs targeting non-essential genes required for virulence, such as those that confer invasiveness and increases *C. albicans* pathogenicity were synthesized. It is considered that if in a pathogenic microorganism, the genetic sequence of a particular gene is known as a determinant agent of infection, synthesizing a strand

of nucleic acid that will bind to the mRNA produced and inactivating it, in its translation into protein, it will be possible to control its virulence.

[0031] In another embodiment, cocktails of potential ASOs based on AST that are able to control *C. albicans´* morphological transition from yeast to filamentous form thus control the invasiveness of this microorganism were generated.

[0032] In one embodiment, the hybridization ability of ASOs with *C. albicans* cells were functionally analyzed. The ASOs´ ability to inhibit the target genes´ expression and their ability to reduce *C. albicans´* filamentation in different human body fluids were also functionally analyzed. These analyses were done using individual ASOs and combinations of ASOs.

Brief Description of the Drawings

[0033] The following figures provide preferred embodiments for illustrating the description and should not be seen as limiting the scope of invention.

[0034] **Figure 1** shows the antisense oligomers sequences according to the description.

[0035] **Figure 2** shows the anti-*EFG1* and anti-*HWP1* oligomers sensitivity and specificity against *Candida* species determined by fluorescence in situ hybridization assays.

[0036] **Figure 3** shows the cytotoxicity effect of 40 nM of anti-*EFG1,* anti-*HWP1* and anti-*HYR1* oligomers against 3T3 cell line (Fibroblast cells, Embryonic tissue, Mouse from CCL3, American Type Culture Collection).

[0037] **Figure 4** shows the effects of anti-*EFG1,* anti-*HWP1* and anti-*HYR1* oligomers on *C. albicans* filamentation.

[0038] **Figure 5** shows the effect of 40 nM of anti-*EFG1* on *C. albicans* filamentation, on *EFG1* gene expression and on efg1p translation at 24 h of incubation.

[0039] **Figure 6** shows the results related to different combinations with 40 nM of each oligomer (anti-*EFG1,* anti-*HWP1* and anti-*HYR1*) on *C. albicans* filamentation during 24 h of incubation.

[0040] **Figure 7** shows the performance of anti-*EFG1* oligomer on different human body fluids: artificial saliva (AS) and urine (AU) during 24 h and blood at 48 h of incubation.

Detailed Description

[0041] The present disclosure relates to the use of ASOs to target specific genes involved in the morphological transition of *C. albicans* from yeast to filamentous form.

[0042] The present disclosure further describes the use of ASO in AST to inhibit the morphological transition of *C. albicans* from yeast to filamentous form.

[0043] In one embodiment, ASOs targeting the three different genes were designed and synthetized to ensure the total blockade of *C. albicans* filamentation. The target regions were selected from each gene taking into account its high specificity against *C. albicans* genome and lower specificity against *Homo sapiens* genome. The regions selected were (5'-ACAATAACGGTATGCC-3'**), (**5' CGCTTATTACATGTTATCA 3') and (5' GCTTACTCTCAACC 3') for *EFG1*, *HWP1* and *HYR1*, respectively.

[0044] In an embodiment, the target regions selected for methylation were:

SEQ ID No. 1 - for *EFG1*: 5'-^{47}ACAATAACGGTATGCC^{62}-3';

SEQ ID No. 2 - for *HWP1*: 5' ^{33}CGCTTATTACATGTTATCA^{51} 3';

SEQ ID No. 3 - for *HYR1*: 5' ^{36}GCTTACTCTCAACC^{49} 3'.

[0045] In one embodiment, for each sequence of the target regions selected the reverse complement was determined in order to design the respective ASOs. The sequences determined were (5' GGCATACCGTTATTGT 3'), (5'TGATAACATGTAATAAGCG3') and (5'GGTTGAGAGTAAGC 3`) for *EFG1*, *HWP1* and *HYR1*, respectively.

[0046] The reverse complement sequences determined for methylation were:

SEQ ID No. 4 - for *EFG1*: 5' GGCATACCGTTATTGT 3';

SEQ ID No. 5 - for *HWP1*: 5'TGATAACATGTAATAAGCG3';

SEQ ID No. 6 - for *HYR1*: 5'GGTTGAGAGTAAGC 3'.

[0047] In one embodiment, in order to increase the ASOs hit-rate, part of the oligonucleotides belonging to each selected sequence were chemically modified based on second generation nucleic acid mimics design (2'-*O*-methyl).

[0048] In another embodiment, once it has been demonstrated that the inclusion of the two or more modifications in each end of the nucleic acid mimics increase its stability in human serum, antisense oligomers were designed and synthesized.

[0049] In one embodiment, anti-*EFG1* oligomer was designed and synthetized with four 2'-*O*-methyl chemical modifications (5'-mG mG mC mA TACCGTTA mU mU mG mU-3'). Anti-*HWP1* oligomer was designed and synthetized with two chemical modifications (5' mUmGATAACATGTAATAAGmCmG 3'). Anti-*HYR1* oligomer was designed and synthetized with three chemical modifications (5' mGmGmU TGA GAG TAmA mGmC 3').

[0050] In an embodiment, the methylated sequences were:

SEQ ID No. 7 - for *EFG1*: 5'-mG mG mC mA TACCGTTA mU mU mG mU-3';

SEQ ID No. 8 - for *HWP1*: 5' mUmGATAACATGTAATAAGmCmG 3';

SEQ ID No. 9 - for *HYR1*: 5' mGmGmU TGA GAG TAmA mGmC 3'.

[0051] Methods for the alignment of sequences for comparison are well known in the art, such methods include BLAST and FASTA. The BLAST algorithm (Altschul et al. (1990) J Mol Biol 215: 403-10) calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (NCBI). The sequence identity values, which are indicated in the present subject matter as a percentage were determined over the entire amino acid sequence, using BLAST with the default parameters.

[0052] In an embodiment, **figure 1** shows the ASOs sequences for *EFG1* (anti-*EFG1*), *HWP1* (anti-*HWP1*) and *HYR1* (anti-*HYR1*) genes of *C. albicans* and the respective 2'-*O*-methyl chemical modifications insertions. Figure 1B summarizes the characteristics of the three ASOs sequences in terms of its size, melting temperature and percentage of guanine and cytosine (GC).

[0053] In an embodiment, **figure 2** shows Anti-*EFG1* and anti-*HWP1* oligomers sensitivity and specificity against *Candida* species determined by fluorescence in situ hybridization assays. Figure 2A summarizes the intensity of fluorescence obtained after 2 h of hybridization at 37°C for different *Candida* species tested. Figure 2B shows the fluorescence images of *Candida* species hybridization with anti-*EFG1* and anti-*HWP1* labelled with red fluorescein (56-FAM) and green fluorescein (TYE 563).

[0054] In an embodiment, **figure 3** shows the cytotoxicity effects of 40 nM of Anti-*EFG1,* anti-*HWP1* and anti-*HYR1* oligomers against 3T3 cell line (Fibroblast cells, Embryonic tissue, Mouse from CCL3, American Type Culture Collection). These were measured using MTS kit (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega). The error bars represent standard deviation.

[0055] In an embodiment, **figure 4** shows the effects of anti-*EFG1,* anti-*HWP1* and anti-*HYR1* oligomers on *C. albicans* filamentation. Figure 4A shows the effect of 40 nM of each oligomer in terms of percentage of *C. albicans* filamentation inhibition. Figure 4B shows the fluorescence images of *C. albicans* filamentation reduction when treated with the ASOs for 24 h. The control is related to *C. albicans* cultured in same conditions in absence of the oligomers. The error bars represent standard deviation. Statistical differences among the different time point tested ($P<0.05$) are marked with *.

[0056] In an embodiment, **figure 5A** shows the effect of 40 nM of anti-*EFG1* on *C. albicans* filamentation ability after 24 h of incubation. The effect on *EFG1* gene expression is measured by qRT-PCR (as shown in Figure 5B) and on efg1p translation obtained by nanoLC-MS/MS analysis (as shown in Figure 5C). The error bars represent standard deviation. Statistical differences between *C. albicans* treated with anti-*EFG1* oligomer and untreated ($P<0.05$) are marked with *.

[0057] In an embodiment, **figure 6** shows the results related with different combinations with 40 nM of each oligomer (anti-*EFG1,* anti-*HWP1* and anti-*HYR1*). Figure 6A shows the cytotoxicity effect against 3T3 cell line. This is measured using MTS kit. Figure 6B shows the effect on *C. albicans* filamentation (% of inhibition). Figure 6C shows the fluorescence images of *C. albicans* filamentation reduction at 8 h and 24 h of

incubation. The error bars represent standard derivation. Statistical differences between *C. albicans* treated with mixed ASOs and untreated (P<0.05) are marked with *.

[0058] In an embodiment, **figure 7** shows the performance of anti-*EFG1* oligomer on human body fluids (AS-artificial saliva; AU-artificial urine and blood). **Figure 7A** shows anti-*EFG1* oligomer effect against *C. albicans* filamentation. **Figure 7B** shows anti-*EFG1* oligomer effect against *EFG1* gene expression.

[0059] The above described embodiments are combinable.

[0060] The term "comprising" whenever used in this document is intended to indicate the presence of stated features, integers, steps, components, but not to preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

[0061] The following claims further set out particular embodiments of the disclosure.

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C L A I M S

1. An isolated oligomer comprising:

   at least a sequence selected from a list consisting the following sequences: SEQ ID 7; SEQ ID 8; SEQ ID 9; or combinations thereof;

   or a variant of said sequence which is at least 95% identical to the selected sequence, based on the identity of all the nucleotides of said sequence, as a *C. albicans* filamentation blocker.

2. An isolated oligomer comprising:

   at least a sequence selected from a list consisting the following sequences: SEQ ID 7; SEQ ID 8; SEQ ID 9; or combinations thereof;

   or a variant of said sequence which is at least 95% identical to the selected sequence, based on the identity of all the nucleotides of said sequence, for use in medicine.

3. The isolated oligomer according to the previous claim for use in the treatment or therapy of *C. albicans* related infections.

4. The isolated oligomer according to the previous claim for use in the treatment or therapy of vaginal infection and/ or oral infections.

5. The isolated oligomer according to the previous claims wherein the sequence is at least 96% identical to the selected sequence, based on the identity of all the nucleotides of said sequence; preferably 97%; 98%; 99% or identical.

6. A composition comprising a combination of at least two isolated oligomers comprising:

   at least a sequence selected from a list consisting the following sequences: SEQ ID 7; SEQ ID 8; SEQ ID 9; or combinations thereof;

or a variant of said sequence which is at least 95% identical to the selected sequence, based on the identity of all the nucleotides of said sequence, as a controlling *C. albicans* filamentation blocker.

7. The composition according to the previous claims wherein the sequence is at least 96% identical to the selected sequence, based on the identity of all the nucleotides of said sequence; preferably 97%; 98%; 99% or identical.

8. The composition according to any of the previous claims 6-7 wherein the combination of isolated oligomers is selected from the following combinations:

SEQ. ID. 7 and SEQ. ID. 8;

SEQ. ID. 7 and SEQ. ID. 9;

SEQ. ID. 8 and SEQ. ID. 9;

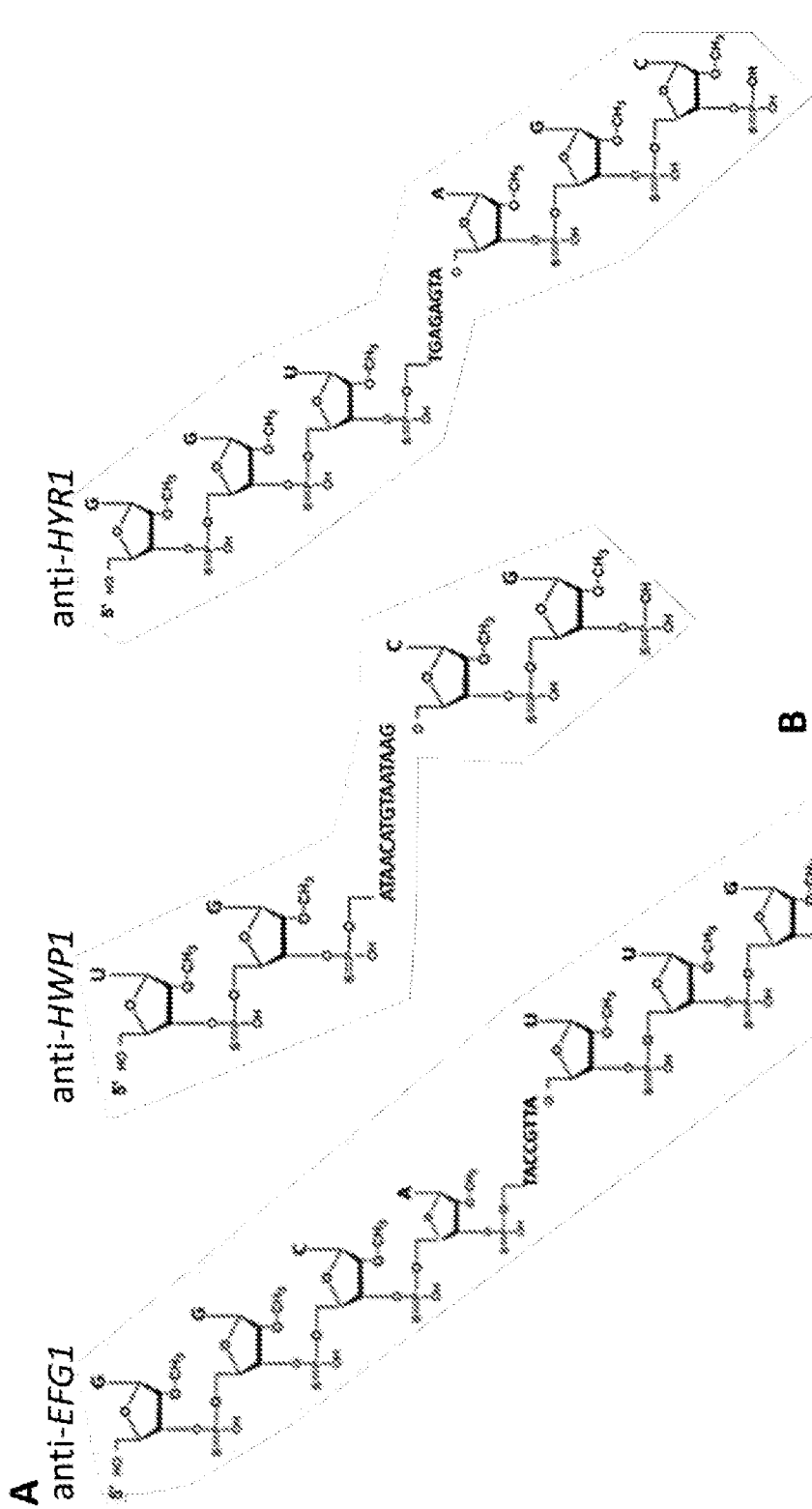
SEQ. ID. 7; SEQ. ID. 8 and SEQ. ID. 9.

9. The composition according to any of the previous claims 6-8 wherein the composition is a coating composition.

10. An article comprising the composition described in any of the previous claims, preferably a coating composition.

11. The article according to the previous claim wherein the article is a medical device, in particular a patch, a catheter, a stent, a contact lens or a pacemaker.

12. The article according to the previous claim wherein the article is an intravaginal tampon, a sanitary napkin, or panty liners.



B

ASO	Size	Melting Temperature (°C)	% of GC
anti-*EFG1*	16	41.1	43.8
anti-*HWP1*	19	41	43.8
anti-*HYR1*	14	42.4	50

Fig. 1

Species	Strains	Origin	anti-*EFG1*	anti-*HWP1*
Candida albicans	SC5314	Reference	+++	+++
	324LA/94	Oral cavity	+++	++
	569322	Vaginal	++	+
	541863	Urinary tract	+	+
	557834	Vaginal	+	++
	545547	Urinary tract	++	++
	547096	Urinary tract	+	-
	552401	Urinary tract	+	++
	575541	Urinary tract	+	+
	568426	Expectoration	+	-
	HLC52 (*efg1/efg1*)		-	
	Δhwp1/hwp1			-
Candida parapsilosis	ATCC 22019	Reference	-	-
Candida glabrata	ATCC 2001	Reference	-	-
Candida tropicalis	ATCC 750	Reference	-	-
Saccharomyces cerevisiae	BY4741	Reference	-	-

- negative signal; + positive signal with different **intensities:** +++ high; ++ moderate and + low

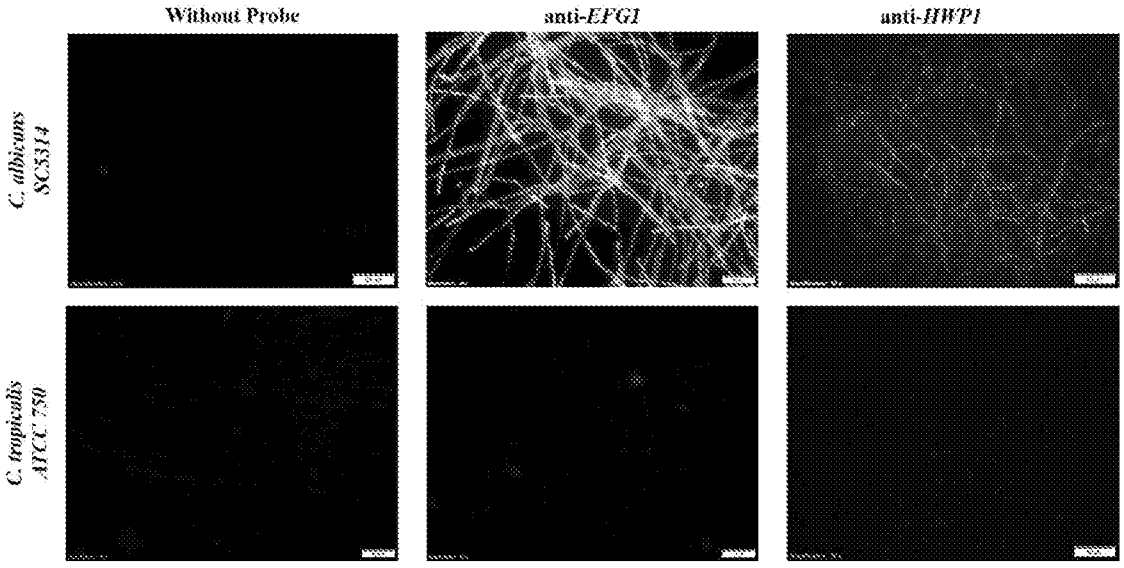


Fig. 2

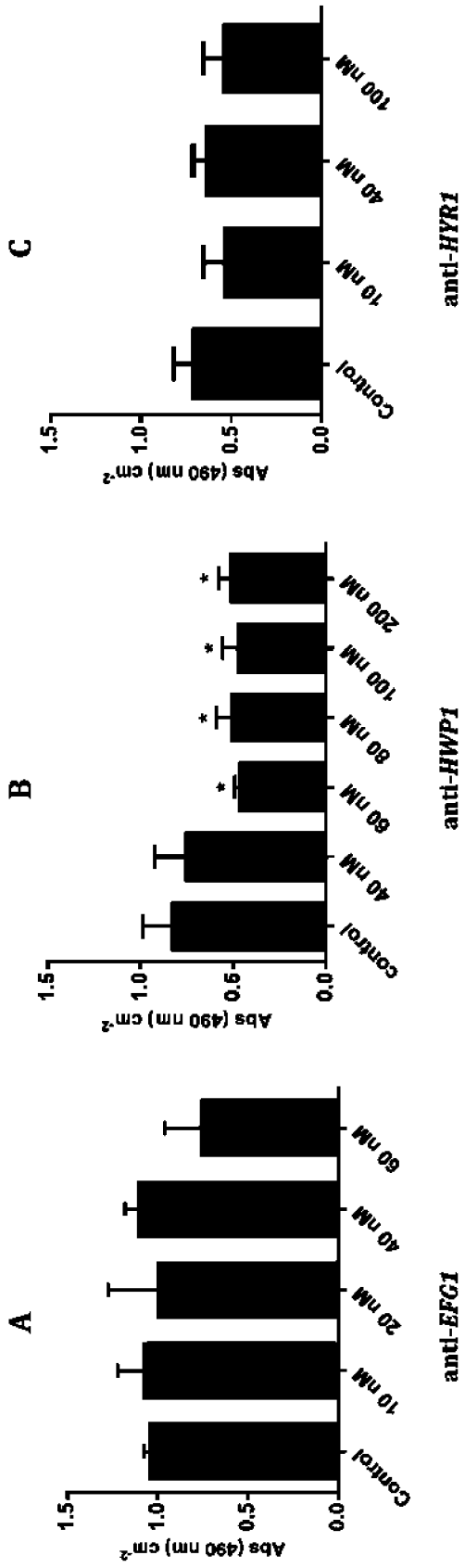

A
anti-EFG1
Abs (490 nm) cm-2
1.5
1.0
0.5
0.0
control
10 nM
20 nM
40 nM
60 nM
B
anti-HWP1
Abs (490 nm) cm-2
1.5
1.0
0.5
0.0
control
40 nM
60 nM
80 nM
100 nM
200 nM
*
C
anti-HYR1
Abs (490 nm) cm-2
1.5
1.0
0.5
0.0
control
10 nM
40 nM
100 nM

Fig. 3

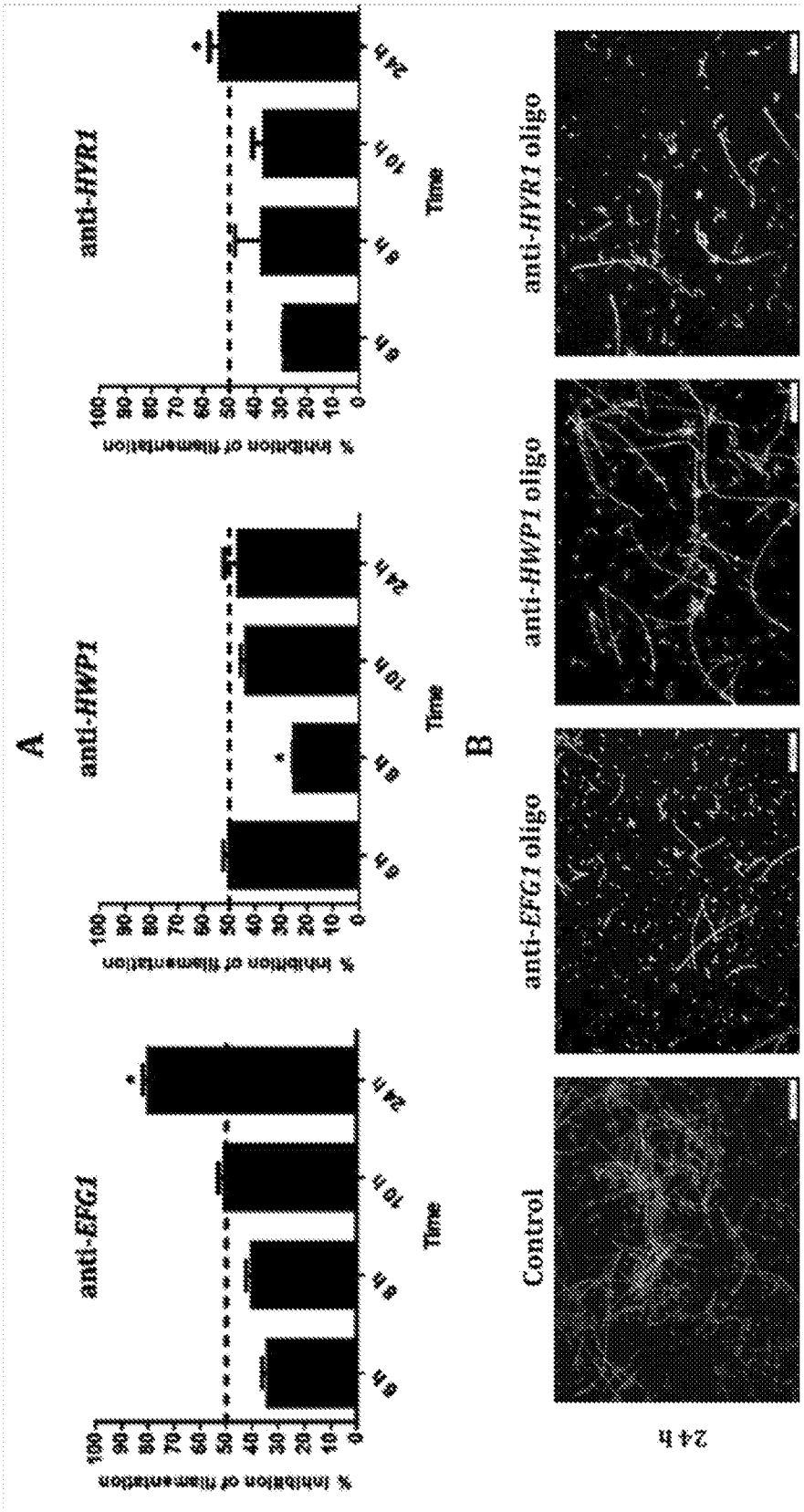

A
anti-EFG1
anti-HWP1
anti-HYR1
% inhibition of filamentation
Time
B
Control
anti-EFG1 oligo
anti-HWP1 oligo
anti-HYR1 oligo
24h

Fig. 4

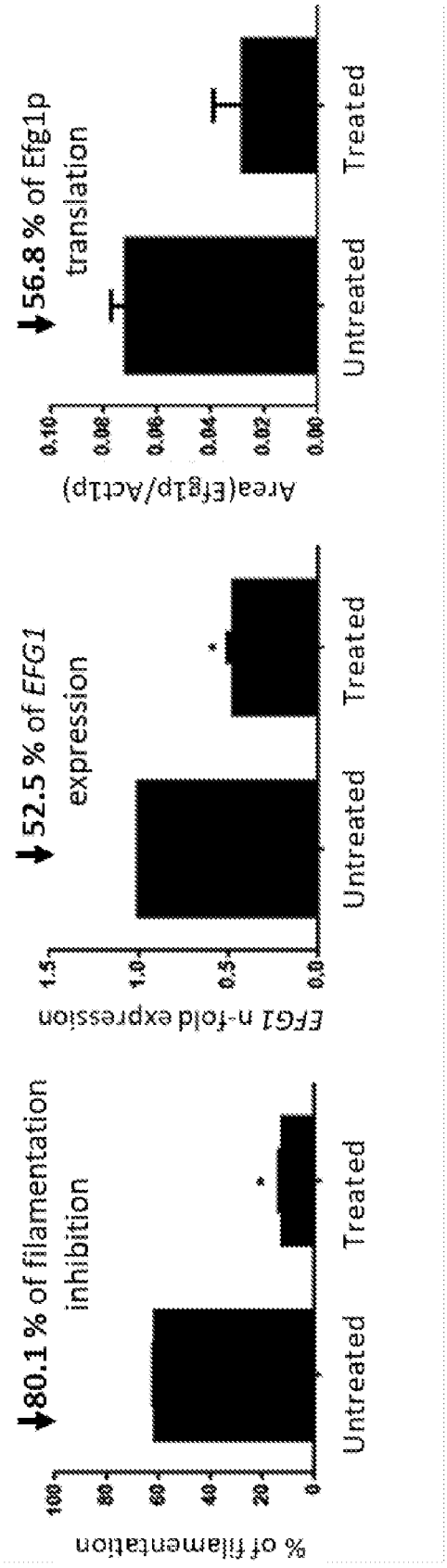

% of filamentation
100
80
60
40
20
0
80.1 % of filamentation inhibition
Untreated
Treated
*
EFG1 n-fold expression
1.5
1.0
0.5
0.0
52.5 % of EFG1 expression
Untreated
Treated
*
Area(Efg1p/Act1p)
0.10
0.08
0.06
0.04
0.02
0.00
56.8 % of Efg1p translation
Untreated
Treated

Fig. 5

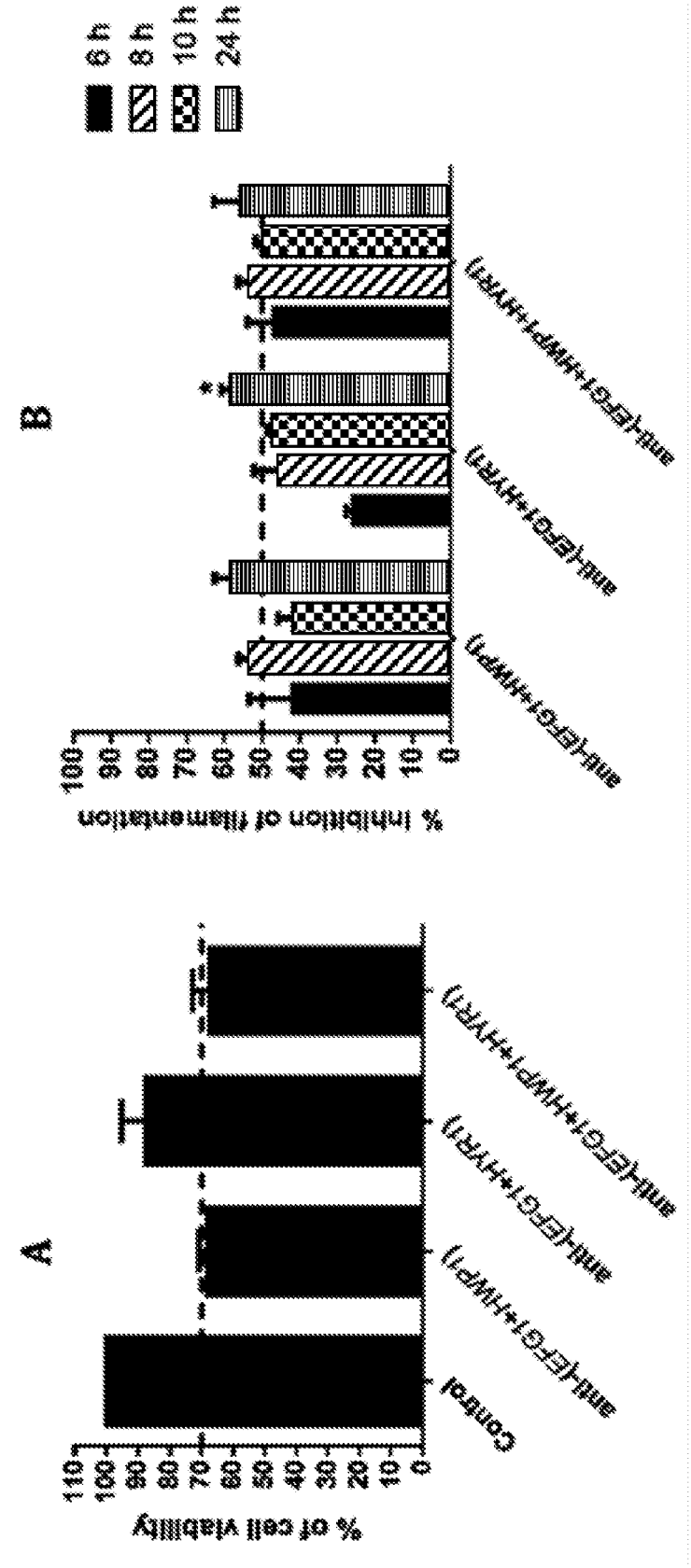

B
6 h
8 h
10 h
24 h
anti-(EFG1+HWP1+HYR1)
anti-(EFG1+HYR1)
anti-(EFG1+HWP1)
% inhibition of filamentation
A
anti-(EFG1+HWP1+HYR1)
anti-(EFG1+HYR1)
anti-(EFG1+HWP1)
Control
% of cell viability

Fig. 6 A-B

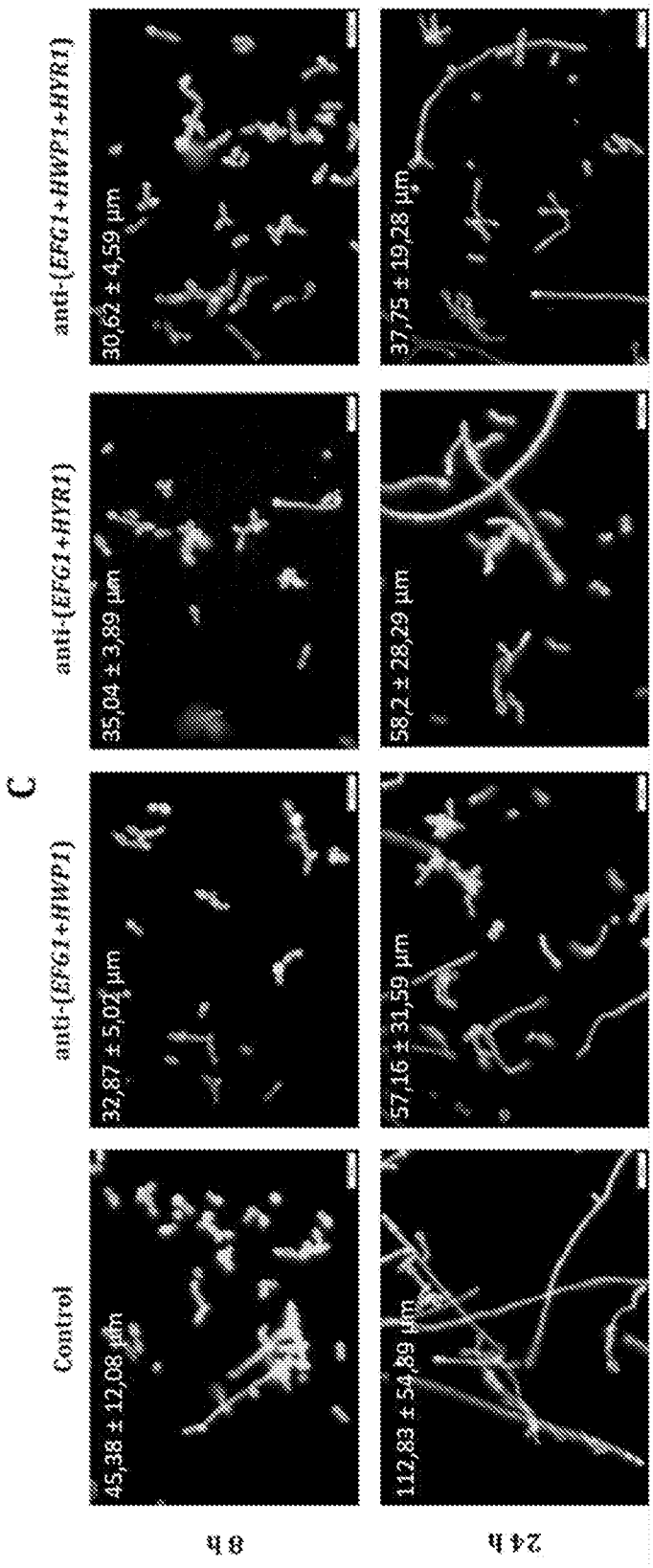

C
Control
anti-{EFG1+HWP1}
anti-{EFG1+HYR1}
anti-{EFG1+HWP1+HYR1}
8 h
24 h
45,38 ± 12,08 µm
32,87 ± 5,02 µm
35,04 ± 3,89 µm
30,62 ± 4,59 µm
112,83 ± 54,89 µm
57,16 ± 31,59 µm
58,2 ± 28,29 µm
37,75 ± 19,28 µm

Fig. 6 C

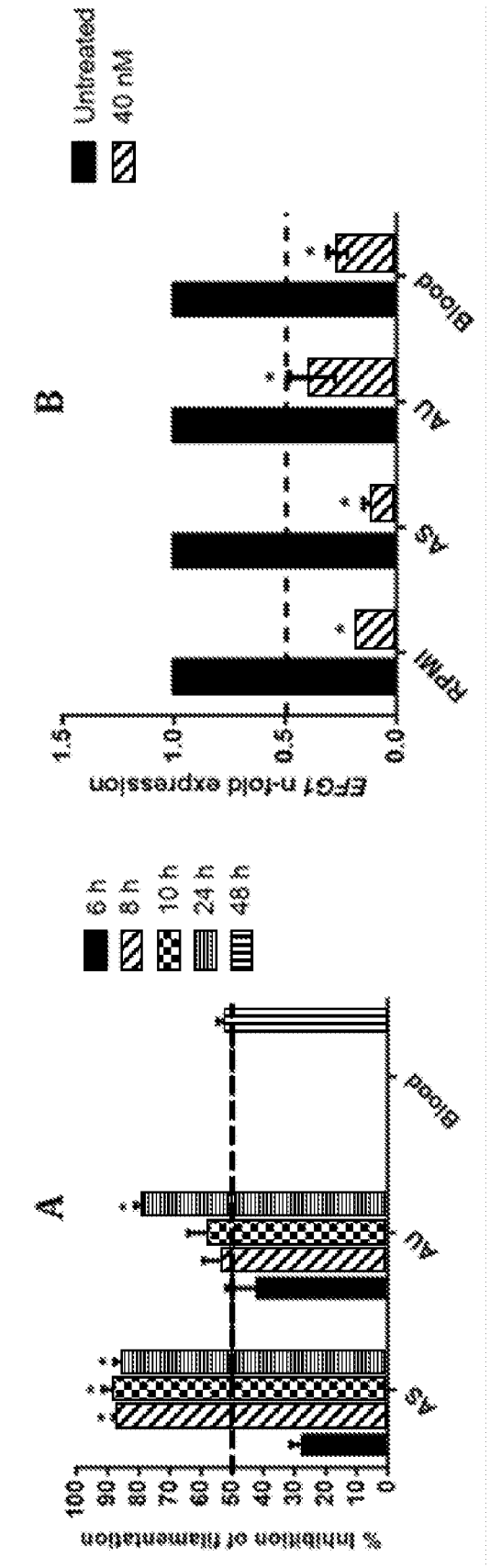

B
Untreated
40 nM
Blood
AU
AS
RPMI
1.5
1.0
0.5
0.0
EFG1 n-fold expression
A
6 h
8 h
10 h
24 h
48 h
Blood
AU
AS
100
90
80
70
60
50
40
30
20
10
0
% inhibition of filamentation

Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/051552

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/712 A61K31/7125
ADD. A61P31/10 A61F13/20 A61F2/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K A61F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 92/03455 A1 (ISIS PHARMACEUTICS INC [US]) 5 March 1992 (1992-03-05) the whole document	1-12
A	WO 2012/126899 A2 (UNIV LEUVEN KATH [BE]; BINK ANNA [BE] ET AL.) 27 September 2012 (2012-09-27) the whole document	1-12
A	MARYAM MOAZENI ET AL: "RNA-Mediated Gene Silencing in: Inhibition of Hyphae Formation by Use of RNAi Technology", MYCOPATHOLOGIA, vol. 174, no. 3, 7 April 2012 (2012-04-07) , pages 177-185, XP035081954, ISSN: 1573-0832, DOI: 10.1007/S11046-012-9539-6 the whole document	1-12

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 June 2020	16/06/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Andres, Serge

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2020/051552

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SOHN K ET AL: "EFG1 is a major regulator of cell wall dynamics in Candida Albicans as revealed by DNA microarrays", MOLECULAR MICROBIOLOGY, vol. 47, no. 1, 2003, pages 89-102, XP002331978, ISSN: 0950-382X, DOI: 10.1046/J.1365-2958.2003.03300.X the whole document -----	1-12
T	ARAUJO DANIELA ET AL: "Application of 2 '-OMethylRNA' Antisense Oligomer to Control Candida albicans EFG1 Virulence Determinant", MOLECULAR THERAPY-NUCLEIC ACIDS, vol. 18, 6 December 2019 (2019-12-06), pages 508-517, XP055701462, -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2020/051552

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		US 2014079741 A1	20-03-2014
		WO 2012126899 A2	27-09-2012