Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
In recent decades, RNA-based therapeutics have transitioned from a near impossibility to a compelling treatment alternative for genetic disorders and infectious diseases. The mRNA vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are truly groundbreaking, and new adaptations are already being proposed to fight other microbes. Unfortunately, the potential of RNA-based therapeutics to treat human fungal infections has remained mostly absent from the conversation, despite the fact that invasive fungal infections kill as many per year as tuberculosis and even more than malaria. Here, we argue that RNA-based therapeutics should be investigated for the treatment of human fungal infections and discuss several major roadblocks and potential circumventions that may allow for the realization of RNA-based therapies against human fungal pathogens.

Emerging fungal pathogens and rising resistance rates necessitate new therapeutic approaches

**Fungi** (see Glossary) remain important global pathogens of organisms across the kingdoms Plantae and Animalia [1]. Emerging outbreaks of chytrid fungi are devastating amphibian populations worldwide, and fungi like *Magnaporthe oryzae* and *Botrytis cinerea* routinely hinder crop production [2]. In humans, *Cryptococcus gattii* has proven to be a dangerous, infectious pathogen of immunocompetent individuals with poorly defined risk factors [3,4], *Aspergillus fumigatus* remains an ever-present danger to the immunocompromised [5], and *Candida auris* is threatening patients in hospitals with exceedingly high rates of antifungal resistance (Figure 1, Key figure) [6,7]. More generally, invasive fungal infections caused by the genera *Aspergillus*, *Candida*, *Pneumocystis*, and *Cryptococcus* are estimated to kill upwards of 1.5 million people per year [8]. In the clinic, the rise of antifungal resistance is further complicating treatment of these difficult infections.

Existing therapeutic options for systemic human fungal infections now center on five classes of drugs, the azoles, echinocandins, polyenes, allylamines, and antimetabolites, which have been extensively reviewed previously [9,10]. Although effective in many instances, a combination of high toxicity and increasing resistance limits their therapeutic efficacy [11]. The limited scope of available antifungals necessitates the development of an expanded repertoire of new drugs [9]. Numerous strategies are being undertaken to develop novel antifungals, including modifications to existing drugs, combination therapies, and altogether new approaches. One strategy that has received only cursory attention thus far is the use of RNA-based therapeutics to target human fungal pathogens.

**RNA-based therapeutics are proving useful against both genetic disorders and infectious diseases**

Traditional treatment approaches often rely on the use of small molecules to induce a therapeutic effect against target proteins [12–14]. Unfortunately, this approach has some underlying...
limitations, including few 'druggable' target proteins and a tremendous workload to screen for strong affinity drugs [14–16]. Nucleic-acid 'biologics' have emerged as a compelling alternative to address many of these limitations [13,14]. The variety of RNA species belonging to this group offers options that not only influence the activity of a target protein but can also modulate the genetic information, transcription, and even translation of the target gene (Figure 2).

The most notable, although not first, success of RNA-based therapeutics came as the mRNA vaccines against SARS-CoV-2 (Figure 2A). mRNA therapeutics have been investigated widely over the past 30 years, including for immunization, cancer treatments, and even as protein substitution therapies [14,17]. Development of functional mRNA therapeutics was initially hampered by a variety of issues regarding administration, translational regulation, stability, and immunogenicity, but these obstacles were mostly resolved by progress in RNA modifications, transfection technologies, and expression regulation systems [13,14,17]. It is no wonder then, that many mRNA-based therapeutics are now being investigated in clinical trials [14]. The mRNA therapeutics provide an intriguing example of how RNA biologics can be employed to enhance an existing host response against an invading microbe.

In addition to the mRNA-based approaches, a number of reports have described manipulation of target expression at the transcriptional or translational level by the employment of small single- or double-stranded RNAs, like short activating RNAs (sARNAs), antisense oligonucleotides (ASOs), small interfering RNAs (siRNAs), and microRNA (miRNA) mimics. All of these have proven to be useful tools for regulating expression [14], but siRNAs and miRNA mimics may represent the most interesting options in fighting fungal infections by inducing an immune response or directly targeting the pathogen.

miRNAs and small interfering RNAs are core molecules of the well-conserved RNA interference (RNAi) pathway, which regulates gene expression by post-transcriptional gene silencing (Figure 2B). After processing of long double-stranded precursor molecules, the resulting small RNAs are loaded into an Argonaute (AGO)-containing RNA-induced silencing complex [14,18–20]. The activated complex is then directed to a target mRNA, resulting in a block of translation. Complete hybridization of siRNAs leads to a cleavage of the target transcript by AGO, whereas partial hybridization by miRNAs prompts steric hindrance of the translation process and eventual mRNA degradation [14,18,19]. The RNAi pathway has been frequently investigated for therapeutic use and numerous clinical trials have reached varying stages of success in treating a wide range of diseases, including viral infections and cancers [14,18,21].

Using RNAi to fight fungal pathogens has advanced more quickly in the agricultural sector [22]. Phytopathogenic fungi release extracellular small RNAs that exploit the RNAi machinery of their host plant to weaken host defense, while the host is able to send short RNA molecules to silence virulence genes of the pathogen in return [22]. This molecular interplay termed cross-kingdom RNA interference was leveraged to limit pathogen infection by silencing virulence genes with small RNAs generated from either transgenic plants expressing these small RNAs (host-induced gene silencing) or by spray-application of RNA onto the host plant (spray-induced gene silencing) [22]. The success of this procedure depends on the RNA uptake efficiency of the pathogen as not all plant pests efficiently import RNA [23]. Nonetheless, this example demonstrates that RNA-based applications are a feasible way to fight fungal pathogens and that the spray-induced gene silencing principle might be adaptable as nasal sprays or salves to treat human fungal pathogens in the future [24].

Despite bearing strong similarities to siRNAs and miRNAs, saRNAs induce, rather than repress, gene expression (Figure 2C) [25–27]. RNA activation occurs when an AGO protein transports
saRNAs into the nucleus to interact with the target promoter sequence or transcript [14,25,28]. This mechanism was anticipated to be an excellent therapeutic possibility for applications where a disease-suppressed gene needs to be increased in expression [25]. In fact, this proved successful in a recent Phase I clinical trial for the saRNA MTL-CEBPA, which treats advanced hepatocellular carcinoma [29]. In this case, the researchers selected and validated specific candidate-binding RNAs that activated the target gene CEBPA (CCAAT/enhancer-binding protein alpha), then incorporated a 5′ inverted abasic modification to the sense strand and 2′-O-methyl moieties on the antisense strand, and finally encapsulated the RNA in liposomal nanoparticles called SMARTICLES to improve delivery and prevent immune activation [26,30]. Building on studies like these, a situation could easily be imagined in which a saRNA might be leveraged to promote host defense against a fungal pathogen.

RNA aptamers offer a completely different mode of action and are designed to directly bind a target molecule instead of modulating transcription or translation (Figure 2D). These single-stranded oligonucleotides can be easily selected using approaches like the ‘systematic evolution of ligands by exponential enrichment’ (SELEX) technology. Over the years, this approach has provided an efficient way of screening extensive libraries of nucleic acids for their ligand capability in a high-throughput manner [31]. The evolved DNA and RNA aptamers generally bind to their target in a picomolar or nanomolar range, respectively, and bear various advantages over conventional antibodies [32]. Only one aptamer drug has been approved for therapeutic use – for the treatment of age-related macular degeneration (Pegaptanib); however, numerous clinical trials are now underway [14,32]. Development of aptamer-based diagnostics to identify pathogens or pathogen-secreted toxins are also in various stages of commercial availability, for instance, to find mycotoxins such as aflatoxins or ochratoxin A in food [32,33].

Other RNA-based approaches have also been considered for the treatment of a broad spectrum of diseases. Short, single-stranded ASOs consisting of either deoxy- or ribonucleotides are capable of binding a target mRNA (seemingly) unassisted where they can exert a variety of effects [14,34]. Early variants of antisense oligonucleotides consisted of DNA and were designed to interact with mRNA to form RNase H–degradable DNA–RNA hybrids [14,34]. The RNA-based ASOs added more mechanisms of regulation to our therapeutic toolkit, for example, translation impedance by hybridization close to the translation start site, masking of upstream open reading frames, and modulation of transcript splicing to correct faulty protein production [14,34].

The most recent addition to RNA therapeutics is represented by the clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (Cas) system. CRISPR/Cas offers the possibility to precisely modify genetic information to directly tackle the cause of a variety of diseases [35] by employing guide RNAs. The therapeutic use of this system is currently limited to ex vivo approaches, which are conducted by retransplantation of genetically modified patient cells [14,35]. Several studies as well as clinical trials are now investigating the potential of this system as a treatment for different cancer variants, HIV, diabetes, and other severe (genetic) diseases [35–39].

Considering the progress in the development of RNA therapeutics, it appears to be only a matter of time before some, if not all, of these RNA-based tools are employed in antifungal therapies.

The current roadblocks to fighting human fungal pathogens with RNA
Significant hurdles exist in adapting these existing strategies for the treatment of eukaryotic fungal pathogens with RNA-based therapeutics (see Outstanding questions). In fact, many of the same problems that hinder current efforts to treat genetic disorders and cancers, including rapid sequence-specific suppression of gene expression.

RNA modifications: post-transcriptional alterations to RNA molecules that often convey a change in function, immune reactivity, or localization.

RNase H: type H endoribonuclease; it recognizes and degrades DNA–RNA hybrids.

Short activating RNAs (saRNAs): small, double-stranded RNAs that induce transcription by targeting gene promoters.

Small interfering RNAs (siRNAs): small, double-stranded noncoding RNAs, typically 20–27 nucleotides, that operate within the RNA interference pathway to silence gene expression.

Spore: a sexual or asexual reproductive unit particularly suited to surviving harsh conditions and adapted for efficient dispersal.
therapeutic degradation and difficulties in promoting RNA escape from the endosome, will also make treatment of fungal infections more difficult [14,40–42].

The first challenge is to define appropriate targets for RNA-based therapies that will specifically inhibit the fungal pathogen without detrimentally altering the host or commensal organisms (Figure 3). Studies of model organisms such as Saccharomyces cerevisiae and Neurospora crassa have provided a wealth of knowledge into RNA regulation in fungi, yet our understanding of many of these same systems in fungal pathogens such as Candida albicans and A. fumigatus is still lagging.

We do know that there can be differences between RNA regulatory pathways in pathogens and non-pathogens. For example, the model filamentous fungus Aspergillus nidulans has a functional...
RNAi system, as does its close relative, \textit{A. fumigatus}; however, several RNAi proteins in \textit{A. nidulans} are truncated compared with those of \textit{A. fumigatus} \cite{43}. The practical importance of these differences in RNAi functionality remain incompletely described in these systems. \textit{A. nidulans} is not the only example of a modified RNAi system. In fact, the model yeast \textit{S. cerevisiae} has actually lost the RNAi machinery in favor of maintaining a killer virus that provides a growth advantage \cite{44}. Other yeasts, such as \textit{C. albicans} and \textit{Cryptococcus neoformans}, typically have a functional RNAi system \cite{45}, but examples of RNAi loss do exist in pathogenic yeast as well \cite{46}. Studies across the fungal kingdom have revealed that the RNAi system appears relatively plastic and can be repurposed or supplemented with additional proteins to provide additional functionality \cite{47,48}. The biological importance and conservation of the RNAi system in many fungal pathogens, including organisms such as \textit{Mucor circinelloides} \cite{49} and \textit{C. neoformans} \cite{46}, suggests that it may be a suitable target to exploit for RNA-based therapeutics in the future.

Production of fungal long noncoding RNAs and their downstream regulatory functions also provide an attractive ingress for RNA-based therapeutic intervention. Long noncoding RNAs are

**Figure 2.** Common mechanisms of RNA-based therapeutics. Listed are four mechanistic examples of common approaches applied in RNA-based therapeutics. RNA-based therapeutics are currently used, in a number of forms, to introduce an mRNA for translation of a specific product, to modify gene expression, and even inhibit protein activity by direct binding. Panel (A) shows an example of specific translation in which an exogenously delivered mRNA can be used to produce a therapeutic protein, such as the spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Panel (B) depicts post-transcriptional modulation by small interfering RNAs (siRNAs), microRNA (miRNA) mimics, or antisense oligonucleotides (ASOs). Panel (C) shows how RNA-based therapeutics can be used to promote gene expression with short activating RNAs (saRNAs) or modify genes with clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9. Finally, Panel (D) depicts how RNA aptamers can be used as therapeutics to trigger inhibition or degradation of a target protein. Together, these provide just a few examples of how RNA-based therapeutics are revolutionizing treatment of disease. The figure was created with BioRender.com.
known to be highly sequence divergent, even between closely related species, making them intriguing targets for sequence-specific RNA-based drugs. Additionally, these long noncoding RNAs often exhibit important regulatory functions despite frequently being transcribed at low copy numbers per cell. Although poorly studied so far in human pathogens, there are some hints that long noncoding RNAs can contribute significantly to regulation of fungal pathogenesis. As just one example, the long noncoding RNA RZE1 of *C. neoformans* was previously shown to be a potent regulator of the morphological switch between yeast and hyphae—a feature with obvious importance for virulence [50]. Efforts are now underway in many organisms to better define the long noncoding RNA repertoire and uncover the diversity of regulatory cascades that remain hidden in this varied set of pathogens. Clearly, more mechanistic research into fungal RNA
biology is essential to expand our understanding of appropriate targets and determine the potential of existing classes of RNA for future RNA-based therapeutics.

The second major roadblock to delivery of antifungal RNAs is the diverse cell wall and capsule structures of fungal pathogens. Fungi come in many forms, ranging from yeasts to hyphae, to impermeable, stress-resistant spores. As one example, the hyphal cell wall of A. fumigatus, composed primarily of positively charged galactosaminogalactan, rapidly binds free, negatively charged nucleic acids and provides a strong barrier against nucleic acid uptake [51]. Ultimately, some RNA does enter fungal hyphae, and these small RNAs have a limited ability to silence gene expression. In addition to the cell wall as a barrier, some fungi promote formation of even more complicated 3D structures in the host, such as nodules, biofilms on catheters, or even granulomas composed of host immune cells. These forms remain clinically challenging even under the best circumstances. Delivery of RNA into fungal pathogens may limit the effectiveness of RNA therapeutics to a subset of fungal pathogens that readily take up RNA, as in plant fungal pathogens [23].

The third set of obstacles relates to difficulties in delivering RNA into specific human sites of infection without stimulating an unwanted immune reaction. Fungal infections can spread throughout the human body, but most commonly begin on the skin, lungs, and gastrointestinal tract (Figure 1). In addition to getting an RNA therapeutic specifically to the site of infection, the RNA must be modified or protected to prevent activation of sensitive host pathogen recognition receptors such as Toll-like receptor (TLR) 3, retinoic acid-inducible gene I (RIG-I), and melanoma differentiation-associated protein 5 (MDA-5), which typically recognize double-stranded RNA molecules to promote an aggressive inflammatory response [52]. Delivery of a therapeutically active RNA into a pathogen at the site of infection without triggering an overreactive immune response will be challenging, yet advances in RNA delivery, modification, and synthesis suggest that there is, in fact, a path forward for treating human fungal disease with RNA-based drugs.

**The way forward in fighting human fungal pathogens with RNA**

Despite the challenges, RNA-based therapeutics do offer unique advantages for the treatment of fungal infections. In particular, RNA is easy to synthesize, fast to adapt to evolving pathogens or after development of antifungal resistance, and it can offer specificity against targets at the sequence level, where variation can exist even in highly conserved pathways. Although barriers are in place, numerous innovative biochemical and biological solutions are already in development in related systems as described earlier – solutions that might be adapted for the treatment of fungal infections. Replacement of uridine with modified N1-methyl-pseudouridine in the mRNA vaccines against SARS-CoV-2 limits recognition by host nucleic acid sensors and aberrant activation of host inflammatory cascades [53–55]; the nucleic acid phosphate backbone is modifiable with 2′-O-methylation to minimize immunogenicity [52]; and advances in RNA synthesis and in vitro transcription can limit production of unwanted immunogenic byproducts such as double-stranded RNA [56]. Combined, these developments seem likely to change the way we treat all sorts of diseases in the future, but additional research is required to see how they can be applied to the treatment of human fungal pathogens.

The fungal cell wall remains a formidable obstacle. To facilitate delivery of RNA into fungal pathogens, new approaches will have to be developed to cross this barrier. The protection of RNA in modified liposomes and nanoparticles, or conjugation to peptide aptamers and antibodies, has improved delivery to both mammalian cells [14] and bacterial pathogens [57,58], but the utility of these approaches in crossing more complicated fungal cell walls remains unclear. Synthesized nanoparticles coupled to RNA can be produced in a variety of potential formulations [59] but few
studies exist testing these particles for uptake by fungi. Decoration of nanoparticles with protein receptors or viral proteins is another option that has been considered but only a few extracellular mycoviruses are known at this time that might be leveraged for these purposes [60]. In other systems, researchers are turning to naturally evolved delivery mechanisms for inspiration, such as extracellular vesicles. New approaches using engineered extracellular vesicles are compelling [61], but additional study is required to identify their full potential as therapeutics. We do know that the host produces extracellular vesicles in response to infection with A. fumigatus, C. albicans, and C. neoformans that can modulate both fungal and host behavior [62–64]. Finally, coupling RNA-based therapeutics to enzymes or compounds that can degrade the cell wall may serve as an additional strategy for delivery.

If delivery of an RNA into fungi proves too challenging, alternative host-directed strategies could still be advantageous. Vaccines have been attempted against many fungal pathogens with little success [65,66], but the rapid advances in mRNA vaccines could provide a new tactic [67]. Alternatively, manipulation of the host to recruit additional immune cells, or modulate behavior, remain interesting [68]; however, we still have much to learn about the immunological response to fungal pathogens in humans before these host-directed strategies will come to fruition. A recent report investigated RNA tertiary structures, namely, group II intron ribozymes, as potential target sites for chemical antifungal therapeutics. Target specificity and catalytic activity inhibition of the ribozyme was demonstrated, which suppressed growth of the pathogen Candida parapsilosis [69]. This finding may offer new opportunities for RNA-based treatments by enlarging the repertoire of possible target sites in fighting fungal infections.

Concluding remarks
RNA-based therapeutics are rapidly changing the way we treat disease, from siRNAs-based drugs to treat liver diseases to mRNA vaccines that provide protection against SARS-CoV-2. As these treatment options mature, opportunities will become available to extrapolate these advances to the treatment of human fungal infections. We hope that by bringing attention to the possibility of using RNA-based therapeutics to treat fungal infections, the fungal research community and clinical infrastructure will be poised to adapt and quickly advance these gains into meaningful treatment options for this often devastating, but certainly under-studied class of difficult-to-treat infections.

Acknowledgments
This work was supported by the Federal Ministry for Education and Research (BMBF: https://www.bmbf.de), Germany, Project FKZ 01K12012 ‘RFIN – RNA-Biologie von Pilzinfektionen’. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. We would like to thank Axel Brakhage, Amelia Barber, Corissa Visser, and Pamela Baumann for fruitful discussions regarding this manuscript.

Declaration of interests
There are no interests to declare.

References
1. Rhodes, J. (2019) Rapid worldwide emergence of pathogenic fungi. Cell Host Microbe 26, 12–14
2. Fisher, M.C. et al. (2020) Threats posed by the fungal kingdom to humans, wildlife, and agriculture. mBio 11, e00449-20
3. Chen, S.C. et al. (2014) Cryptococcus gattii infections. Clin. Microbiol. Rev. 27, 980–1024
4. Sajio, T. et al. (2014) Anti-granulocyte-macrophage colony-stimulating factor autoantibodies are a risk factor for central nervous system infection by Cryptococcus gattii in otherwise immunocompetent patients. mBio 5, e00912-14
5. Latge, J.P. and Chanios, G. (2019) Aspergillus fumigatus and aspergillosis in 2019. Clin. Microbiol. Rev. 33, e00140-18
6. Spivak, E.S. and Hanson, K.E. (2018) Candida auris: an emerging fungal pathogen. J. Clin. Microbiol. 56, e01588-17
7. Munoz, J.F. et al. (2018) Genomic insights into multidrug-resistance, mating and virulence in Cryptococcus auris and related emerging species. Nat. Commun. 9, 5346
8. Brown, G.D. et al. (2012) Hidden killers: human fungal infections. Sci. Transit. Med. 4, 165v13
9. Geijten, T.J. et al. (2020) Aspiring antifungals: review of current antifungal pipeline developments. J. Fungi (Basel) 6, 28
10. Pound, M.W. et al. (2011) Overview of treatment options for invasive fungal infections. Med. Mycol. 49, 561–580

Outstanding questions
How can we select appropriate targets for RNA-based therapeutics against human fungal pathogens?
Will promoting a more robust immune response, or production of additional immune effector cells, serve as a host-directed strategy towards treating fungal infections?
How can the targeting of RNA therapeutics be improved to direct treatment to specific tissues, organs, or pathogen cells?
How do we facilitate RNA passage across the diverse fungal cell wall structures?
Will strategies used to make RNA immunologically inert still allow these modified RNAs to inhibit/modulate fungal gene expression pathways?
Can RNA-based therapeutics be coupled to, or loaded into, nanoparticles or extracellular vesicles to promote delivery into fungal pathogens?
Is it possible to adapt existing strategies designed and optimized for genetic diseases, cancers, or viral infections to target specific fungi instead?
Will facilitating endosomal escape of RNA from fungal endosomes or endosome-like structures prove as difficult as it has for human cells?
Can RNA-based therapeutics be made cost-effective enough for low-income countries?
Will we be able to design robust RNA-based therapeutics against currently unculturable pathogens from genome sequences alone, for example, Pneumocystis jiroveci?
How will the high adaptability of RNA-based therapeutics impact the drug approval process? Does every sequence/nucleotide change justify a revisiting of the clinical trials for a previously approved pharmaceutical?
How fast can fungal pathogens evolve new strategies to circumvent the effect of RNA-based therapeutics?
38. Ma, S. et al. (2018) β Cell replacement after gene editing of a neonatal diabetes-causing mutation at the insulin locus. Stem Cell Rep. 11, 1407–1417
39. Su, S. et al. (2018) CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. Sci. Rep. 6, 20070
40. Agrawal, S. (2020) RNA therapeutics are stepping out of the maze. Trends Mol. Med. 26, 1061–1064
41. Wang, F. et al. (2020) RNA therapeutics on the rise. Nat. Rev. Drug Discov. 19, 441–442
42. Dowdy, S.F. (2017) Overcoming cellular barriers for RNA therapeutics. Nat. Biotechnol. 35, 222–229
43. Hammond, T.M. et al. (2008) RNA silencing gene truncation in the filamentous fungus Aspergillus nidulans. Experientia. 7, 333–349
44. Drinnenberg, I.A. et al. (2011) Compatibility with killer explains the rise of RNA-deficient fungi. Science 333, 1592
45. Bernstein, D.A. et al. (2012) Candida albicans Dicer (Dcr1) is required for efficient ribosomal and spliceosomal RNA maturation. Proc. Natl. Acad. Sci. U. S. A. 109, 523–528
46. Ferretzaki, M. et al. (2016) Gene network polymorphism illuminates loss and retention of novel RNA silencing components in the cryptic pathogen species complex. PLoS Genet. 12, e100568
47. Canovas-Marquez, J.T. et al. (2021) A rhizobacteria II involved in virulence of Mucorales fungi has evolved to cut exclusively native, single-stranded RNA. Nucleic Acids Res. 49, 5294–5307
48. Lee, H.C. et al. (2016) Diverse pathways generate microRNA-like RNAs and Dicer-independent small interfering RNAs in fungi. Mol. Cell 38, 801–814
49. Calk, S. et al. (2014) Antifungal drug resistance evolved via RNA-dependent epimutations. Nature 513, 555–558
50. Checko, N. et al. (2015) The InrRNA R2E1 controls cryptococcal morphological transition. PLoS Genet. 11, e1005692
51. Jochl, C. et al. (2009) Development-dependent scavenging of nucleic acids in the filamentous fungus Aspergillus fumigatus. RNA Biol. 6, 179–186
52. Karko, K. et al. (2005) Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity 23, 165–175
53. Corbett, K.S. et al. (2020) SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature 586, 567–571
54. Karko, K. et al. (2012) Increased erythropoiesis in mice injected with submicrogram quantities of pseudouridine-containing mRNA encoding erythropoietin. Mol. Ther. 20, 948–953
55. Anderson, B.R. et al. (2010) Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. Nucleic Acids Res. 38, 5884–5892
56. Nelson, J. et al. (2020) Impact of mRNA chemistry and manufacturing process on innate immune activation. Sci. Adv. 6, eaaz935
57. Howard, J.J. et al. (2017) Inhibition of Pseudomonas aeruginosa by peptide-conjugated phosphorodiamidate morpholino oligomers. Antibiotics. Agents Chemotherapy. 61, e01559–15
58. Mountal, D.A. et al. (2021) Peptide-conjugated phosphorodiamidate morpholino oligomers retain activity against multidrug-resistant Pseudomonas aeruginosa in vitro and in vivo. mBio 12, e02411–20
59. Wilter, K.H. and Wolfram, J. (2021) Extracellular vesicles versus synthetic nanoparticles for drug delivery. Nat. Rev. Mater. 6, 103–109
60. Yu, X. et al. (2013) Extracellular transmission of a DNA mycovirus and its use as a natural fungicide. Proc. Natl. Acad. Sci. U. S. A. 110, 1452–1457
61. Zhang, X. et al. (2020) Programmable extracellular vesicles for macromolecule delivery and genome modifications. Dev. Cell 784–801
62. Shopova, I.A. et al. (2020) Human neutrophils produce antifungal extracellular vesicles against Aspergillus fumigatus. mBio 11, e02659–20
63. Halder, L.D. et al. (2020) Immune modulation by complement receptor-3-dependent human monocyte TGF-beta-1 transporting vesicles. Nat. Commun. 11, 2352
64. Zhang, L. et al. (2021) Cryptococcus neoformans – infected macrophages release proinflammatory extracellular vesicles: insight into their components by multi-omics. mBio 12, e02279–21
65. Santos, E. and Levitz, S.M. (2014) Fungal vaccines and immunotherapeutics. Cold Spring Harb. Perspect. Med. 4, a019711
66. Nami, S. et al. (2019) Fungal vaccines, mechanism of actions and immunology: A comprehensive review. Biomed. Pharmacother. 109, 333–344

67. Sandbrink, J.B. and Shattock, R.J. (2020) RNA vaccines: a suitable platform for tackling emerging pandemics? Front. Immunol. 11, 608460

68. de Candia, P. et al. (2016) Extracellular RNAs: a secret arm of immune system regulation. J. Biol. Chem. 291, 7221–7228

69. Fedorova, O. et al. (2018) Small molecules that target group II introns are potent antifungal agents. Nat. Chem. Biol. 14, 1073–1078