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APTORUM

Aptorum: developing novel mechanisms for the treatment of infectious diseases

Aptorum is developing novel drugs that tackle bacteria and viruses. Acting as the commercialization platform in partnership with major academic and research institutions, the company has in-licensed a number of infectious-disease-targeted compounds and methods from Hong Kong academic institutions and through the company's research and development team continue to advance them toward clinical development. Aptorum is now looking to develop further projects with collaborators globally.

The rise of drug-resistant bacteria and viruses is neutering humanity's main weapons against public health threats, creating a need for novel approaches against pathogens including influenza and bacteria. Aptorum is positioned to help humanity fight back. Having scoured academia for novel technologies, the company is building an arsenal of drug candidates that could usher in a new era of infectious disease treatments.

It is clear that the current arsenal of anti-infective agents has significant existing and emerging limitations. Seasonal influenza, for example, is prevented by vaccines and treated with antivirals but nonetheless causes 3–5 million cases of severe illness annually. Up to 650,000 people die globally from infections caused by flu viruses every year[1].

To exacerbate the situation, influenza A viruses have developed resistance to multiple classes of antivirals[2]. One class of antivirals—adamantanes—is no longer recommended for use by the US Centers for Disease Control and Prevention owing to the prevalence of resistant viral strains, and there is also evidence that the efficacy of the cornerstone of antiviral treatment is waning.

Oseltamivir, better known as Tamiflu, has been at the forefront of efforts to treat seasonal and pandemic influenza for years but has been rendered ineffective by subtypes of the viruses. During the 2008–2009 flu season, almost all the strains of H1N1 circulating in the US were resistant to the drug. Around the same time, Norwegian authorities found that 75% of strains of circulating influenza A were resistant to oseltamivir[3]. Multiple other European health authorities reported high rates of resistance.

The emergence of resistant strains stems from the targeting of single vulnerabilities in infectious disease agents that mutate rapidly. A similar situation, in which drug-induced selection pressure speeds up the prevalence of resistant strains, is happening in critical pathogenic bacteria. Notably, *Staphylococcus aureus* has become resistant to first-line therapeutics. People infected with the resistant strain, dubbed MRSA (methicillin-resistant *S. aureus*), are estimated to be 64% more likely to die than those fighting a nonresistant *S. aureus* strain[4].

Governments have responded to such infectious disease threats by proposing and offering incentives to encourage research and development (R&D). The success of these initiatives will depend on the ability of researchers to come up with novel ways to tackle bacteria and viruses. Aptorum is striving to meet that challenge.

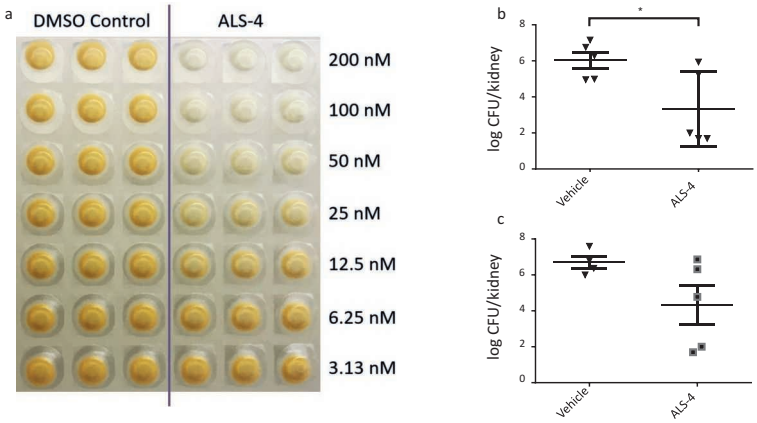


Fig. 1 | In vitro pigment inhibition by ALS-4. a, ALS-4 is intended to inhibit *Staphylococcus aureus* pigment production with a half-maximal inhibitory concentration (IC_{50}) of 20 nM. **b**, ALS-4 is observed to reduce bacterial load in mice with acute treatment. In the experimental setting, ALS-4 was administered twice daily for 7 days at a concentration of 1 mM with an inoculum containing 5×10^6 CFU per mouse; the first injection took place 30 minutes after infection. **c**, ALS-4 is also observed to reduce bacterial load in mice with delayed treatment. In the experimental setting, ALS-4 was administered twice daily for 7 days at a concentration of 1 mM with an inoculum containing 2×10^7 CFU per mouse; the first injection took place 11 days after infection.

Licensing technology to fight disease

Aptorum's efforts to develop novel drugs that tackle bacteria and viruses have been boosted by its presence in Hong Kong. As a succession of infectious disease outbreaks ranging from severe acute respiratory syndrome to swine flu have swept through Hong Kong, the region's academic institutions have stepped up their research into the control of viruses and bacteria.

The research has led to novel approaches to the treatment of infectious diseases, but academic institutions are typically limited in infrastructure to take programs into clinical stage and commercialization.

Under the leadership of Ian Huen, the CEO of Aptorum Group Limited, the company has exclusively licensed a number of novel compounds and methods for the treatment of certain infectious diseases from Hong Kong academic institutions and has built an R&D team capable of advancing them into clinical development.

The business model is exemplified by Aptorum's programs based on chemical genetics research performed at The University of Hong Kong. Using chemicals to systematically probe pathogenic pathways, researchers at the University identified novel small molecules capable of disrupting biological processes essential to the proliferation and virulence of infectious diseases. This concept has been recognized in the global scientific community and won Richard Kao (the inventor of ALS-4) first place in the Innovation Academy category at the International Conference on Prevention & Infection Control in 2017.

Aptorum licensed intellectual property stemming from the work done at the University in an attempt to further develop and commercialize these technologies.

A novel approach to treating MRSA

ALS-4, one of Aptorum's three lead candidates, illustrates the potential of chemical genetics and the business model that brought the approach out of academia. To the best of the company's knowledge, the candidate is a new treatment for infections caused by *S. aureus* including MRSA that has a completely different mechanism of action than existing antibiotics, most of which have been rendered ineffective by resistant strains.

ALS-4 is a small drug molecule that appears to target the products produced by bacterial genes that facilitate the successful colonization and survival of the bacterium in the body or that cause damage to the body's systems. These products of bacterial genes are referred to as 'virulence expression'. Targeting bacterial virulence is an alternative approach to antimicrobial therapy that offers promising opportunities to overcome the emergence and increasing prevalence of antibiotic-resistant bacteria.

ALS-4 is being developed to achieve this outcome by suppressing one of the enzymes involved in the production of staphyloxanthin, a pigment that acts as both a virulence factor and a defense mechanism against the reactive oxygen species and neutrophil-based killing mechanisms used by host immune cells to attack pathogenic bacteria.

Animal studies of strains of the bacteria that lack the pigment show that when deprived of staphyloxanthin, MRSA is more vulnerable to immune attacks and less virulent. These modified strains cause lower bacterial loads than the pigmented, wild-type form of the pathogen.

This body of evidence led Aptorum to identify the inhibition of staphyloxanthin as a potential targeted, nonantibiotic approach to the treatment of MRSA infections and to the development of a drug, ALS-4, with this mechanism.

The nonantibiotic nature of ALS-4 gives it an edge over existing treatments for MRSA. Notably, ALS-4 does not apply selection pressure—and by extension does not promote drug resistance—as it only weakens bacteria[5]. In contrast, today's antibiotics continuously select for resistant strains by killing all bacteria that are vulnerable to their mechanism of attack.

Another benefit of ALS-4 stems from its targeted mechanism of action. As staphyloxanthin is specific to *S. aureus*, ALS-4's mechanism of action is designed to only affect that pathogenic species of bacteria. As a result, other species of bacteria, some of which perform essential roles in the human body, are expected to be unaffected by ALS-4.

Aptorum has generated preclinical data to back up these hypotheses. The data show that ALS-4 inhibits production of the staphyloxanthin pigment and reduces bacterial load in mice infected with MRSA. In generating the data, Aptorum has shown preclinical proof of concept for its hypothesis that blocking the production of staphyloxanthin could stop MRSA from colonizing the body and trigger the removal of already-established communities of pathogenic cells (**Fig. 1**).

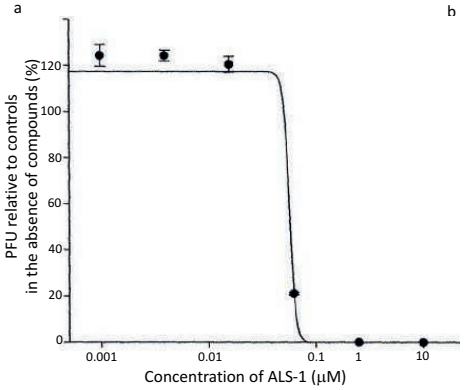
Those positive early findings have encouraged Aptorum to take ALS-4 through the final steps in lead optimization and the company aims to move the candidate into investigational new drug (IND)-enabling studies later in 2019 or early 2020.

Avoiding drug resistance to Tamiflu

ALS-1, another of Aptorum's lead candidates, is intended to treat infections caused by influenza A virus. Similar to the established antiviral oseltamivir, ALS-1 is designed to exploit vulnerability in the influenza virus. However, ALS-1 has a different mechanism of action than current antivirals, suggesting that it could be free from the resistance that blights existing products.

Whereas oseltamivir inhibits the neuraminidase enzyme, ALS-1 targets nucleoprotein, an abundant protein that performs multiple roles essential to the structure and function of the influenza virus.

a



b

Compound or drug	Virus strain	IC_{50} (μM)
ALS-1	H1N1 (A/WSN/33)	0.069 ± 0.003
ALS-1	H3N2 (clinical isolate)	0.16 ± 0.01
ALS-1	H5N1 (A/Vietnam/1194/04)	0.33 ± 0.04
Oseltamivir	H1N1 (A/WSN/33)	>100
Peramivir	H1N1 (A/WSN/33)	7.9 ± 3.0
Zanamivir	H1N1 (A/WSN/33)	0.36 ± 0.58
Ribavirin	H1N1 (A/WSN/33)	33 ± 13
Amantadine	H1N1 (A/WSN/33)	>100
Rimantadine	H1N1 (A/WSN/33)	>22

Fig. 2 | ALS-1 is shown to cause a greater reduction in the number of infectious virus particles of human H1N1, H3N2 and H5N1 influenza viruses. a, The half-maximal inhibitory concentration (IC_{50}) of ALS-1 for H1N1 is 0.01–0.1 μM. **b**, ALS-1 demonstrated a lower IC_{50} in H1N1 (A/WSN/33) influenza viruses than approved antiviral drugs including oseltamivir (Tamiflu), peramivir (Rapivab) and zanamivir (Relenza). ALS-1 also demonstrated its effectiveness against H3N2 (clinical isolated) and H5N1 (A/Vietnam/1194/04) influenza viruses. PFU, plaque-forming unit. Part **a** adapted from ref. [6]. Data in part **b** from ref. [7].

The abundance and importance of nucleoprotein to the influenza virus render it an interesting target for teams seeking to develop alternative antivirals that will be free from the resistance problems that affect existing drugs. Recognizing that, Aptorum used its forward chemical genetics approach to show that nucleoprotein is druggable, laying the groundwork for the development of ALS-1.

This work led to in vitro and animal studies that found drugs targeting the nucleoprotein can cause the cessation of viral replication. In in vitro testing, ALS-1 was more potent at inhibiting the replication of human H1N1, H3N2 and H5N1 influenza viruses than oseltamivir. The tests showed ALS-1 had a lower half-maximal inhibitory concentration (IC_{50}) than oseltamivir, indicating it inhibited 50% of the viruses at lower concentrations than the established antiviral drug (**Fig. 2**).

Animal studies have provided further evidence that targeting nucleoprotein is an effective strategy. Without treatment, all mice infected with a highly pathogenic strain of H5N1 died within one week. All the mice treated with a nucleoprotein inhibitor survived. Subsequent tests on lung tissue revealed a tenfold drop in viral load in mice treated with the nucleoprotein inhibitor compared with in the control mice.

Having demonstrated preclinical proof of concept, Aptorum is now conducting lead optimization and subsequently preparing to move into IND-enabling studies in 2020. The timeline should position Aptorum to file to commence a phase 1 clinical trial for ALS-1 in humans in 2020 or 2021.

Building a broad inventory of assets

ALS-1 and ALS-4 are two of Aptorum's most advanced candidates but are far from the totality of its assets. Aptorum also has candidates under development targeting women's health (such as a nonhormonal approach to endometriosis) and neurodegenerative diseases, among others.

In light of growing evidence of the role gut microbiota play in various disease pathways, Aptorum is also actively developing drugs aimed to act locally in the gut to treat systemic disease with minimal toxicity.

The company also anticipates generating more candidates through its newly established subsidiary Smart Pharmaceutical, which is conducting drug discovery for rare diseases based on existing approved drugs (drug repurposing). Smart Pharmaceutical employs a novel drug discovery and modelling platform to accelerate the commercialization of therapeutics for rare diseases. Smart Pharmaceutical aims to provide five to ten potential candidates per year for rare diseases with streamlined paths to market over the next five years.

These activities are advancing in parallel with the pursuit of additional technologies. Aptorum is now looking to develop further projects with collaborators globally.

The expanded strategies set Aptorum up to work with collaborators worldwide to advance therapeutic candidates into the clinical phase and potentially onto the market. Aptorum's work with ALS-1, ALS-4 and other assets with roots in Hong Kong shows it can serve as a diverse platform between academia and commercial development.

Through these activities, Aptorum will cement its position at the forefront of efforts to tackle some of the biggest public health challenges facing countries around the world. Aptorum put itself in that position by investing in ALS-1 and ALS-4, novel candidates that could revitalize humanity's defenses against serious infectious diseases.

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