A pan-serotype dengue virus inhibitor targeting the NS3–NS4B interaction

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1. 簡述論文的概要以及重大發現

登革熱病毒的複製合成與登革熱的非結構蛋白 (no structure protein) 息息相關，而其中的非結構蛋白 NS3 和 NS4B 之間的交互作用被認為是能做為治療登革熱的研究方向。

本研究根據舊有的文獻合成出新的化合物-- JNJ-A07，該化合物能藉由阻斷 NS3 和 NS4B 之間的交互作用來抑制登革熱病毒複製合成，達到抗病毒作用，且不容易產生抗藥性，而此結果也證實阻斷 NS3 和 NS4B 之間的交互作用是個值得作為登革熱抑制劑的研究方向。

2. 對論文內容的提問

雖然作者提到說登革熱病毒需要有三個基因位點突變才能完全抑制化合物 (JNJ-A07) 的抗病毒能力，但是在 Extended Data Fig. 3e, 3f 中可以看到當有一個基因位點突變，化合物 (JNJ-A07) 的抗病毒能力就會大幅下降，那麼不容易產生抗藥性這個說法就值得再探討。

3. 論文的缺點與評論

此研究藉由體外和體內測試得到了正向的結果，證實化合物 (JNJ-A07) 作為登革熱抑制劑是可行的，但是否容易產生抗藥性仍需要進一步研究。

Article

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Suzanne J. F. Kaptein[1], Olivia Goethals[2], Dominik Kiemel[3], Arnaud Marchand[4], Bart Kesteleyn[5], Jean-François Bonfanti[6,11], Dorothée Bardiot[4], Bart Stoops[5], Tim H. M. Jonckers[5], Kai Dallmeier[1], Peggy Geluykens[5,12], Kim Thys[5], Marjolein Crabbe[5], Laurent Chatel-Chaix[3,13], Max Münster[3], Gilles Querat[7], Franck Touret[7], Xavier de Lamballerie[7], Pierre Raboisson[5,14], Kenny Simmen[5], Patrick Chaltin[4,8], Ralf Bartenschlager[3,9], Marnix Van Loock[2] ✉ & Johan Neyts[1,10] ✉

Dengue virus causes approximately 96 million symptomatic infections annually, manifesting as dengue fever or occasionally as severe dengue[1,2]. There are no antiviral agents available to prevent or treat dengue. Here, we describe a highly potent dengue virus inhibitor (JNJ-A07) that exerts nanomolar to picomolar activity against a panel of 21 clinical isolates that represent the natural genetic diversity of known genotypes and serotypes. The molecule has a high barrier to resistance and prevents the formation of the viral replication complex by blocking the interaction between two viral proteins (NS3 and NS4B), thus revealing a previously undescribed mechanism of antiviral action. JNJ-A07 has a favourable pharmacokinetic profile that results in outstanding efficacy against dengue virus infection in mouse infection models. Delaying start of treatment until peak viraemia results in a rapid and significant reduction in viral load. An analogue is currently in further development.

Dengue is currently considered one of the top ten global health threats[1]. Annually, an estimated 96 million individuals develop dengue disease[2], which is probably an underestimation[3–5]. The incidence has increased approximately 30-fold over the past 50 years. The virus is endemic in 128 countries in (sub-)tropical regions, with an estimated 3.9 billion people at risk of infection. A recent study[6] predicts an increase to 6.1 billion people at risk by 2080. The upsurge is driven by factors such as rapid urbanization and the sustained spread of the mosquito vectors[6–8]. Dengue virus (DENV) has four serotypes (further classified into genotypes), which are increasingly co-circulating in endemic regions. A second infection with a different serotype increases the risk of severe dengue[9,10]. The vaccine Dengvaxia, which is approved in a number of countries for individuals aged ≥9 years, is only recommended for those with previous dengue exposure[11–13]. There are no antiviral agents for the prevention or treatment of dengue, and the development of pan-serotype DENV inhibitors has proven challenging[14,15].

JNJ-A07 is a highly potent DENV inhibitor

Following a large-scale cell-based anti-DENV-2 screen[16], a hit was identified and optimized (a total of approximately 2,000 analogues were synthesized). JNJ-A07 is a representative analogue (Fig. 1a) with nanomolar to picomolar antiviral potency in various cell lines and high selectivity (Table 1, Extended Data Fig. 1). JNJ-A07 is also active in primary immature dendritic cells, which may be the initial target cells of the virus[17]. Potent pan-genotype and pan-serotype activities (nanomolar to picomolar potencies) were demonstrated against a panel of 21 clinical isolates covering all available genotypes within the 4 serotypes[18] (Table 1). No marked antiviral activity was detected against other flaviviruses or against a selection of other RNA and DNA viruses (Extended Data Fig. 2).

JNJ-A07 targets the DENV NS4B protein

The addition of JNJ-A07 to infected cultures could be delayed without loss of antiviral potency as long as intracellular viral RNA synthesis had not been initiated to a detectable level (at 10 h after infection; Extended Data Fig. 3a, b). When the inhibitor was added after onset of viral RNA synthesis, a gradual loss of its antiviral activity was noted, which suggests that there is an interaction with the viral RNA replication machinery. A similar pattern was observed with the nucleoside analogue 7-deaza-2′-*C*-methyladenosine (7DMA), a broad-spectrum RNA virus inhibitor. To identify the molecular target, drug-resistant variants were selected by passaging DENV-2 in the presence of gradually increasing concentrations of JNJ-A07 (Extended Data Fig. 3c). This proved difficult in two independent efforts (A and B). As shown in the dynamics of appearance of mutations (Extended Data Fig. 3d, e), a decrease in susceptibility to the drug (32-fold) was first observed at

[1]Laboratory of Virology and Chemotherapy, Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, KU Leuven, Leuven, Belgium. [2]Janssen Global Public Health, Janssen Pharmaceutica, Beerse, Belgium. [3]Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg, Germany. [4]Cistim Leuven, Leuven, Belgium. [5]Janssen Research & Development, Janssen Pharmaceutica, Beerse, Belgium. [6]Janssen Infectious Diseases Discovery, Janssen-Cilag, Val de Reuil, France. [7]Unité des Virus Émergents (UVE), Aix-Marseille Univ, IRD 190 Inserm 1207, IHU Méditerranée Infection, Marseille, France. [8]Centre for Drug Design and Discovery (CD3), KU Leuven, Leuven, Belgium. [9]German Center for Infection Research, Heidelberg Partner Site, Heidelberg, Germany. [10]Global Virus Network (GVN), Baltimore, MD, USA. [11]Present address: Galapagos, Romainville, France. [12]Present address: Charles River Beerse, Discovery, Beerse, Belgium. [13]Present address: Institut National de la Recherche Scientifique, Centre Armand-Frappier Santé Biotechnologie, Quebec, Quebec, Canada. [14]Present address: Aligos, Leuven, Belgium. ✉e-mail: mvloock@its.jnj.com; johan.neyts@kuleuven.be