Comment on: Concerns of using sialidase fusion protein as an experimental drug to combat seasonal and pandemic influenza

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Sir,

Zhang1 questioned the validity of developing a sialidase fusion protein (DAS181) as an experimental drug against influenza. He raised two main concerns—first, that sialidase treatment would result in a greater chance of bacterial infection by exposing novel cryptic binding sites to bacterial pathogens and second, that influenza virus (IFV) receptors may be different from sialic acids (SA); thus, sialidase treatment may be ineffective. Although we agree with Zhang that the safety and efficacy of any novel therapeutic candidate, including DAS181, needs to be evaluated cautiously, statements made by him on sialidase treatment would increase colonization of respiratory epithelial cells and basal cells in the control tissue; it was modestly increased only in the goblet cells after DAS181 treatment (Figure 1). Consistent with our observation, the matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) glycan profile analysis of the human bronchial epithelium also revealed abundant Galβ1-4GlcNAc structures in the absence of sialidase.5 Galβ1-3GalNAc expression was low and restricted to goblet cells and basal cells in the control tissue; it was modestly increased only in the goblet cells after DAS181 treatment (Figure 1). Noticeably, mass spectrometric analysis has also revealed numerous Galβ1-4GlcNAc structures in the normal lung tissue (http://www.functionalglycomics.org/glycomics/publicdata/glycoprofiling.jsp?species=Human). Together, these studies demonstrate that the inner polysaccharide structures exposed by desialylation already exist on the normal respiratory surface. As a result, sialidase treatment is unlikely to introduce de novo bacterial adhesion sites.

We conducted further studies to evaluate whether sialidase treatment would increase colonization of respiratory epithelial cells by S. pneumoniae, Haemophilus influenzae or Pseudomonas aeruginosa. DAS181 treatment of A549 cells did not increase cell adhesion by these bacteria over a broad range of bacterial inputs (L. M. Aschenbrenner and F. Fang, unpublished results). In the well-differentiated human airway epithelium (HAE) cultures, DAS181 treatment did not increase adhesion of S. pneumoniae (Figure 2) and did not result in any cytopathic effect. Together, our findings support previous observations, indicating that removing SA alone does not have a significant impact on bacterial cell adhesion.

Zhang also raised concerns over the entry of IFV into desialylated cells by citing isolated reports that investigate aspects of IFV entry in cell culture models. Evidence accumulated over the past six decades has indisputably demonstrated the key role of SA as the IFV receptor.6,7 The purpose of these cited reports was to investigate other aspects of IFV entry. None of them disputed the role of SA in promoting IFV infection. For example, although Stray et al.8 demonstrated non-SA-dependent entry of IFV into cells at high doses, they also demonstrated 90% to 99.999% reduction of IFV multicycle infectivity in desialylated Madin-Darby canine kidney (MDCK) cells. Chu and Whittaker9 showed that glycoproteins which contain sialylated N-linked glycans were critical for productive entry of IFV into cells. These results in fact support the role of sialylated glycans as the receptors of IFV.

DAS181 (FluidaseTM) is being developed as a potential broad-spectrum prophylactic and therapeutic agent for seasonal inflammation and denudation and thus can potentially prevent secondary bacterial infection.

Zhang suggested that sialidase increases S. pneumoniae adherence by exposing cryptic receptors. SA is usually connected via α2-6 or α2-3 linkage to either Galβ1-4GlcNAc or Galβ1-3GalNAc. Thus, the inner polysaccharide or ‘cryptic’ fragments exposed after sialidase treatment would be predominantly Galβ1-4GlcNAc and Galβ1-3GalNAc. Using an established methodology, we stained normal and DAS181-treated human tracheal tissue sections with lectins. We observed that DAS181 treatment abolished SA binding by Sambucus nigra agglutinin (SNA) (which binds SAα2-6). Importantly, Galβ1-4GlcNAc [bound by Datura stramonium agglutinin (DSA)] was abundantly present on the ciliated surface of tracheal epithelium in the native state as well as following DAS181 treatment (Figure 1). Consistent with our observation, the matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) glycan profile analysis of the human bronchial epithelium also revealed abundant Galβ1-4GlcNAc structures in the absence of sialidase.5 Galβ1-3GalNAc expression was low and restricted to goblet cells and basal cells in the control tissue; it was modestly increased only in the goblet cells after DAS181 treatment (Figure 1).

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We conducted further studies to evaluate whether sialidase treatment would increase colonization of respiratory epithelial cells by S. pneumoniae, Haemophilus influenzae or Pseudomonas aeruginosa. DAS181 treatment of A549 cells did not increase cell adhesion by these bacteria over a broad range of bacterial inputs (L. M. Aschenbrenner and F. Fang, unpublished results). In the well-differentiated human airway epithelium (HAE) cultures, DAS181 treatment did not increase adhesion of S. pneumoniae (Figure 2) and did not result in any cytopathic effect. Together, our findings support previous observations, indicating that removing SA alone does not have a significant impact on bacterial cell adhesion.

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DAS181 (FluidaseTM) is being developed as a potential broad-spectrum prophylactic and therapeutic agent for seasonal
influenza-like illness and for pandemic influenza. We have reported results that demonstrate potent in vitro and in vivo activities of DAS181 against a broad panel of IFV strains, including the highly pathogenic H5N1 strain. In order to fully address the legitimate questions on drug safety, in the ongoing Phase 1 clinical trial, we are closely monitoring the status of bacterial colonization among an extensive panel of safety parameters.

Preparing the world to safeguard itself against an influenza pandemic poses daunting challenges to the scientific community and industry alike. We genuinely welcome constructive scientific input from our colleagues and focus our effort on the important task in front of us.

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**Transparency declarations**

Letters to the Editor

Sir,

In my Leading article, I discussed 12 papers that studied bacterial infection. In their commentary on my article, Nicholls et al. chose to address only one of them. Studies by several groups using different animal models have demonstrated that influenza virus infection contributes significantly to secondary bacterial pneumonia and that neuraminidase activity in influenza viruses is a predictor of mortality from secondary bacterial pneumonia. Nicholls et al. provided data on sialidase treatment and "This action of NA also promotes adherence and invasion of Streptococcus pneumoniae, because cleavage of sialic acid from the surface of host cells exposes cryptic receptors for S. pneumoniae. Bacteria that can successfully invade the lower respiratory tract typically express NA for this purpose."

The fact that influenza A viruses, including recent H3N2 clinical isolates, can infect desialylated cells clearly demonstrates that sialic acid-independent entry can occur, but this should not be misconstrued as 'influenza virus (IFV) receptors may be different from sialic acids (SA)'.

Nicholls et al. provided data on sialidase treatment and bacterial adherence to address some of the concerns I raised. However, I have some specific concerns regarding this. Figure 1 in their comment has no quantification and there is a clear increase of DSA and PNA binding after DAS181 treatment. To describe each of these as a 'modest' increase is subjective and debatable; indeed, the MALDI-MS data are not quantitative and the relative amounts were not disclosed. The statement by Nicholls et al. that DAS181 treatment did not affect "influenza virus (IFV) receptors may be different from sialic acids (SA)".

I believe that the concerns raised in my original article remain and warrant further consideration and investigation to determine their ramifications.

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References


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