Correction for Haid et al., “Identification of a Human Respiratory Syncytial Virus Cell Entry Inhibitor by Using a Novel Lentiviral Pseudotype System”

Sibylle Haid,^a Christina Grethe,^a Dorothea Bankwitz,^a Thomas Grunwald,^b Thomas Pietschmann^a

Division of Experimental Virology, TWINCORE Centre for Experimental and Clinical Infection Research, Hannover, Germany^a; Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany^b

Volume 90, no. 6, p. 3065–3073, 2016, <https://doi.org/10.1128/JVI.03074-15>. We recently uncovered a systemic error in our report describing lentiviral pseudoparticles decorated with functional respiratory syncytial virus (RSV) envelope proteins. It has been described that lengthy cytoplasmic tails of viral envelope proteins can preclude efficient lentiviral pseudotyping. Considering this, we initially created a full-length and a C-terminally truncated variant of the RSV F protein. Initial experiments comparing these constructs did not reveal gross differences in lentiviral pseudotyping between full-length and truncated RSV F proteins. Now we have uncovered an early mix-up between these constructs. Consequently, all experimental data presented in Fig. 1 to 4 were obtained with a truncated RSV F protein, which we now designate RSV F trc. Moreover, RSV F-derived constructs such as RSV F K394R (Fig. 2 and 4) and RSV F K272E (Fig. 2 and 4) were created in the context of this truncation mutant and are now designated RSV F trc K394R and RSV F trc K272E.

Page 3068, Fig. 1: We have now compared lentiviral pseudotyping between full-length RSV F and the RSV F trc constructs and summarize these novel data in Fig. 1 given below. The figure below complements the published data by providing a comparison of the functioning of RSV F trc and RSV F in lentiviral pseudotyping. Our comparison revealed a trend that RSV F trc constructs produce slightly higher titers of lentiviral pseudotypes than RSV F (Fig. 1B and C). This trend was independent of RSV F constructs being expressed alone or in combination with additional RSV envelope proteins, including small hydrophobic (SH) proteins and glycoprotein G (G) (Fig. 1B). Moreover, this trend was also seen for RSV F constructs carrying a single point mutation (K272E) conferring resistance to palivizumab, an F protein-targeting monoclonal antibody, or resistance to a membrane fusion inhibitor (K394R; bis-hydrochloride monohydrate [BMS-433771]) (Fig. 1C). Since the truncation does not affect the ectodomain of the RSV F protein, we assumed that the function of the truncation mutant RSV F trc closely phenocopies the behavior of the wild-type RSV F in terms of entry factor usage and inhibition by antibodies and small molecules. To support this, we confirmed that both proteins share comparable susceptibility to palivizumab (Fig. 1D) and the BMS-433771 membrane fusion inhibitor (Fig. 1E).

Page 3070, Fig. 3A: In the displayed experiment, we used heparin at a concentration of 1 μ g/ml, not 5 μ g/ml. This error has no influence on the general conclusion derived from these data.

Citation Haid S, Grethe C, Bankwitz D, Grunwald T, Pietschmann T. 2020. Correction for Haid et al., “Identification of a human respiratory syncytial virus cell entry inhibitor by using a novel lentiviral pseudotype system.” *J Virol* 94:e01690-19. <https://doi.org/10.1128/JVI.01690-19>.

Copyright © 2020 American Society for Microbiology. All Rights Reserved.

Address correspondence to Thomas Pietschmann, thomas.pietschmann@twincore.de.

Published 1 July 2020

FIG 1 Comparison of phenotypic properties of RSV F and RSV F trc constructs in lentiviral pseudotyping and RSV infection assays. (A) Schematic drawing of expression constructs highlighting the site of truncation and the amino acids deleted in the RSV F trc construct. (B) Efficiency of lentiviral pseudotyping as measured by transduction efficiency of a luciferase-expressing lentiviral vector upon transient transfection of given envelope protein expression constructs. Lentiviral particles were used to infect A549 ($n = 11$), HEp-2 ($n = 11$), or Huh-7.5 ($n = 13$) cell lines. Lentiviral particles prepared after transfection of an empty expression vector (black bar) served to control for nonspecific transduction of the luciferase reporter construct. (C) RSV F protein-derived constructs with given resistance mutations were examined in the context of the full-length and the truncated RSV F expression construct ($n = 4$ for all cell lines). Data in panels B and C were log10 transformed, followed by multiple t-testing corrected for multiple comparisons using the Holm-Sidak method. Assay controls (VSV-G and empty vector) were not included in the statistical analysis. (D) Palivizumab, an F protein-targeting monoclonal antibody, dose-dependently inhibits cell entry of RSV F and RSV F trc containing lentiviral pseudotypes ($n = 5$). (E) BMS-433771, an RSV membrane fusion inhibitor, dose-dependently prevents infection by RSV F and RSV F trc carrying lentiviral pseudotypes ($n = 4$). The K394R resistance mutation prevents inhibition by BMS-433771 when introduced into the RSV F full-length and the RSV R trc backbone. Data presented in panels D and E are expressed relative to control infections performed in the absence of inhibitors. Colored dotted lines indicate the background of the respective pseudotype assays as determined in uninfected control cells. Note that the pseudotypes differ in infectious titer so that the background is more or less separated from the control infections conducted in absence of inhibitors. (D) Black dotted line, background of VSV-G pseudotypes; dark blue dotted line, background of RSV F; dark green dotted line, background of RSV F trc pseudotypes; bright blue or bright green dotted line, background of RSV F full length plus G and plus SH pseudotypes or RSV F trc plus G and plus SH, respectively. (E) Black dotted line, background of VSV-G pseudotypes; dark blue or dark green dotted line, background of RSV F or RSV F trc pseudotypes; bright blue or bright green dotted line, background of RSV F K394R and RSV F trc K394R pseudotypes.