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1   **Single-chain variable fragment antibody constructs neutralize measles virus infection *in vitro* and *in***
2   ***vivo***

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24 **One-Sentence Summary:** Targeting the measles F protein in its prefusion state neutralizes *in vitro*
25 and *in vivo* infection.

26 **Key words:** Viral fusion; neutralizing epitopes; antiviral activity.

27   Despite the availability of an effective measles virus (MeV) vaccine and efforts to increase
28 vaccine coverage by the WHO, UNICEF, and their partners, MeV has not been eradicated, and the
29 estimated global measles death rose from 89,780 in 2016 to 207,500 in 2019¹. Because there is an
30 effective measles vaccine, antiviral development for measles has not been prioritized, but recent
31 outbreaks have highlighted the need for drugs to prevent transmission in unvaccinated populations
32 and to protect and treat immunocompromised individuals. We identified several neutralizing mouse
33 monoclonal antibodies (mAbs) that target the MeV fusion (F) protein in its prefusion state² and
34 inhibit fusion and viral infection. We engineered a single-chain variable fragment (scFv) from the
35 most potent anti-MeV F mAb. The scFv retains the ability to inhibit fusion and prevents infection *in*
36 *vitro*, and intranasal administration of the scFv antibody construct prevents infection *in vivo*.

37   MeV infects activated SLAM/CD150-expressing immune cells in the respiratory tract, from
38 which the virus invades immune tissues³. Upon attachment of MeV to cell surface receptors, entry is
39 mediated by the MeV receptor-binding (H) and F proteins, which comprise the H/F viral fusion
40 complex on the surface of the virus³⁻⁵ (Fig. 1A). Viral infection can be blocked at several steps during
41 entry. MAbs that block H-receptor interaction and thereby interfere with entry have been described⁶.
42 Entry can also be inhibited by interfering with the refolding step whereby the F protein attains a
43 stable postfusion state – the process that drives membrane fusion during viral entry. This step relies
44 on interaction between the complementary heptad repeat (HR) regions at the N- and C-termini of the
45 protein (HRN and HRC). In previous studies, we demonstrated the potential of HR-targeting peptides
46 as antiviral agents *in vivo*⁶. Several mAbs with neutralizing activity against MeV F have been

47 identified^{7,8}, and for one of these mAbs (mAb 186), neutralization potency correlated with
48 recognition of the prefusion state of F⁹. However, mutation at F position 70 resulted in a naturally
49 occurring mAb 186 escape mutant^{7,8} (Fig. 1A and SFIGs. 1-2); the IC323 strain has a K at F position 70,
50 while the F of the circulating strain G954 has an R at this position and is not recognized by mAb 186
51 (see SFIGs. 1-3).

52 We assessed viral neutralization by two mouse mAbs that target the prefusion F protein (77.4
53 and Y503, SFIG. 3H-K). From the most potent antibody – 77.4 – we engineered an scFv by fusing the
54 variable regions of the heavy (VH) and light chains (VL) of mAb 77.4 (SFIG. 4A). The scFv construct was
55 expressed in eukaryotic cells and affinity purified using a 6x histidine tag (see SI). The neutralizing
56 activity of the scFv is shown in Fig. 1B-C (62.5 µg/ml or 2280 nM scFv is shown in 1C; additional data
57 are shown in SFIG. 4). The scFv inhibited viral infection (Fig. 1B-C) and specifically bound to MeV F
58 (SFIG. 4E-F). Finally, we assessed whether the scFv blocked cell-cell fusion mediated by either MeV
59 IC323 or G954 H/F fusion complexes using a beta-galactosidase (β-Gal) complementation assay⁶ that
60 measures the fusion of cells that express viral envelope glycoproteins (MeV IC323 or G954 H/F) with
61 cells that express SLAM (Fig. 1D). The scFv inhibited cell fusion mediated by both the IC323 and G954
62 H/F fusion complexes (Fig. 1D).

63 To assess anti-MeV prophylaxis by the scFv *in vivo*, we used cotton rats as an infection model.
64 The scFv antibody construct was administered intranasally 24 and 12 h before infection. Four days
65 after infection, the animals were euthanized, and lung viral titers were measured. Treatment with
66 scFv significantly reduced lung viral titers compared with those of untreated controls (Fig. 1E). We
67 assessed three doses (0.25, 0.5 and 1 mg/ml) and observed that the two highest doses of scFv
68 completely inhibited lung infection. The inhibition of infection *in vivo* after intranasal administration
69 of scFv suggests that targeting the prefusion state of MeV F protein is a feasible anti-MeV strategy.

70 Next, we investigated the possibility of synergy between the scFv construct, which targets the
71 early prefusion state of F, and HR-targeting antiviral peptides (HRC4) that target a later step, the

refolding of the F protein after activation (Fig. 1F-G). Fusion of cells that express viral envelope glycoproteins with cells that express Nectin-4, a second MeV receptor, was quantified as shown in Fig. 1D. The results were analyzed with Combenefit software to obtain the additive curve based on the curves of the two inhibitors alone and to compare this with the result of the inhibitors added together (see also SFig. 5), revealing significant synergy between scFv and HRC4 for inhibiting viral fusion. Similar results were obtained when we assessed the combined effects of mAb 77.4 and HRC4 on viral fusion (see SFig. 6).

Grafting the variable region to a single-chain antibody would reduce the size of the inhibitor and may permit the construct to cross the blood-brain barrier to address central nervous system manifestations of disease. Intranasal administration of the scFv construct was sufficient to neutralize MeV infection, suggesting that these modifications may be tolerated without loss of efficacy. Targeting viral entry is an ideal approach to prevent transmission of respiratory viruses¹⁰. Future strategies will entail either combination of the two entry inhibitors or conjugation of HRC4 and scFv (or a single-chain antibody) into a single bifunctional inhibitor.

Figure 1 – A single-chain variable fragment (scFv) derived from mAb 77.4 inhibits MeV infection *in vitro* and *in vivo*. (A) Measles virus (MeV) fusion (F) protein in PyMOL (Schrödinger) using the crystal structure of MeV prefusion F ectodomain² (PDBID: 5YXW). (B-C) **scFv inhibits MeV infection:** Vero-SLAM cells treated with the indicated concentration of scFv (or not treated) were infected with MeV IC323-EGFP at a multiplicity of infection of 0.001. Images were obtained using epifluorescence microscopy 48 h after infection (scale bar= 200 μm). (D) **scFv inhibits MeV H/F-mediated fusion:** Fusion of MeV G954 or IC323 H/F-coexpressing cells with SLAM-bearing cells in the presence of the scFv at the indicated concentrations was quantified at 6 h. The results are presented as the percent reduction in luminescence (Y-axis) compared to no treatment. Each bar represents the mean (± standard error) of results from at least 3 separate experiments. (E) **scFv blocks MeV *in vivo*:** Cotton rats were infected intranasally with 10⁵ TCID₅₀/mL MeV WTFb. Animals received either the indicated amount of the mAb 77.4-derived scFv construct (n=4) or vehicle (n=8) 24 h and 12 h prior to infection. Animals were euthanized 5 days after infection. The results are presented as TCID₅₀/g of lung tissue. Statistical analyses were performed using the Mann-Whitney U test. (F-G) **The mAb 77.4-derived scFv and HRC4 peptide inhibitor were synergistic in fusion assays.** The effects of mAb 77.4-derived scFv and the HRC4 inhibitor on MeV H/F-mediated cell fusion were assessed and analyzed using Combenefit software. (F) Loewe synergy and antagonism response surface for scFv and HRC4 at the indicated concentrations. (G) Multicolor synergism response surface in comparison to the additive outcome. Data are from three independent experiments.

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