Abstract: Exploring The Potential Of Engineered Antibodies To Prevent HIV Cell-Free And Cell-Cell Transmission

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Broadly neutralizing antibodies (bNAbs), isolated from some HIV-infected individuals, can prevent infection in animal models and are being evaluated for the prevention and treatment of HIV infection in humans. To further explore the potential of bNAbs, we designed and expressed single-chain variable fragments (scFv) that artificially link the variable regions of antibody heavy and light chains and which may have advantages due to their smaller size. We tested individual scFv derived from bNAbs targeting the major sites of vulnerability on the HIV-1 envelope glycoprotein, namely CAP256-VRC26.25 (V2 apex), PGT121 (N332-supersite), 3BNC117 (CD4bs), and 10E8v4 (MPER) using a pseudovirus neutralization assay. Each scFv retained good neutralizing activity against a large multi-subtype virus panel, although there was variable loss of function compared to the parental IgG antibodies. For CAP256-VRC26.25 (hereafter CAP256.25), there was a significant loss of potency (138-fold), in part related to differential interaction with charged amino acids in the V2 epitope at residues 166 and 169. There was also a reduction in potency for the 3BNC117 scFv (13-fold) among viruses lacking the N276 glycan. Similarly, a reduction in potency for the PGT121 scFv (4-fold) was found among viruses lacking an N332 glycan and in viruses with a longer V1 loop. This variation at key residues affected scFv neutralization more than the matched IgGs, suggesting that scFv interacted with their epitopes in subtly different ways. Remarkably, the scFv of 10E8v4 maintained breadth of 100% in our panel with only a minor reduction in potency. Overall, these scFv of clinically relevant bNAbs retained significant neutralizing activity, indicating that they may be suitable for passive immunization to prevent HIV-1 infection.

HIV envelope diversity represents a significant challenge to the efficacy of bNAbs and it is widely acknowledged that double or triple combinations will be needed to ensure complete coverage. To assess whether this would also apply to scFv, we tested five scFv of bNAbs in equimolar combinations against a small multiclade panel of viruses. This included the four bNAbs described above plus 8ANC195, an antibody that targets the gp120-gp41 interface. Similar to IgG, experimental combinations of two and three scFv showed a significant improvement in potency and breadth compared to single scFv. Using the Loewe additive model, combination titres were predicted using single scFv data and compared to the

experimental combination data. Most combinations followed this additive model of potency and no significant antagonism was observed for any combination of two or three scFv. Low levels of synergy were observed within several combinations specifically those containing CAP256.25 and 10E8v4 scFv. This was shown by the improved potency of the experimental titres compared to the predicted titres based on this model. These data and model were used to extrapolate and predict neutralization titres for a larger 45-virus panel. At therapeutic levels (1µg/mL), 100% coverage was reached for one of the dual and three of the triple combinations. These combinations always contained 10E8v4 scFv but never 8ANC195. Moreover, the geometric mean potency for the best triple combination consisting of CAP256.25, 10E8v4, and 3BNC117, was significantly improved to 0.047µg/mL compared to the most potent single scFv (0.12µg/mL, $p<0.0001$). These results show that combinations of scFv generally follow an additive model of potency, with modest levels of synergy and that their breadth and potency are significantly improved when applied in combinations.

Transmission of HIV through cell-associated HIV particles may play an important role in sexual and perinatal transmission. bNAbs have been shown to have reduced activity in this mode of transmission, which could limit their ability to prevent infection. Furthermore, cell-associated virus is thought to play an important role in maintaining HIV infection by establishing and maintaining the HIV reservoir. To explore the ability of smaller molecules to prevent this mode of transmission, we tested the scFv and IgG of four bNAbs, namely CAP256.25, PGT121, 3BNC117, and 10E8v4, in a cell-free and cell-cell transmission assay. We found that the IgG lost potency against cell-associated virus compared to the neutralization of free virus, with a 9-fold geometric mean potency difference for all IgG. This potency loss was most pronounced in PGT121 IgG (19-fold) and 3BNC117 IgG (15-fold) but was also noted for CAP256.25 IgG (3.4-fold) and 10Ev4 IgG (4.9-fold). However, the scFv neutralized both cell-free and cell-associated viruses with similar potency (geometric mean potency difference between 1.3 and 2.3 fold). Thus, despite the differential potency losses between IgG and scFv for three of the four bNAbs in the free virus assay, scFv retain an advantage in blocking this mode of transmission. The net result was that 3BNC117 and PGT121 IgG and scFv neutralized cell-associated virus with similar potency, though the CAP256.25 scFv was slightly less potent than the IgG (13-fold). In contrast, 10E8v4 was slightly better at preventing cell-cell transmission as an scFv than its IgG counterpart (2-fold), possibly due to the location of its epitope close to

the cell membrane. These data demonstrated that scFv may show potential for the prevention of cell-associated virus neutralization, as their small size likely allows them to better diffuse between cells as well as into the mucosal tissues where cell-cell transmission occurs.

In conclusion, the scFv of HIV-directed bNAbs retained much of their function in both cell-free neutralization and cell-cell neutralization assays. Furthermore, they showed the expected level of activity when tested in combination and in some cases displayed modest synergy. Due to their smaller size, scFv are more easily incorporated into novel expression vectors and could be particularly useful for vectored immunoprophylaxis (VIP) for the prevention of HIV infection. Taken together, the findings from this thesis support the further research and development of scFv derived from HIV bNAbs for potential use in clinical applications.