

Novel approaches in Immunotherapeutics (including bnAbs and anti-inflammatory mediators)

**PE39**

**Potent and broad neutralizing activity of small antibody fragments targeting CD4i (CD4-induced) epitope**

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**Background:** CD4-induced (CD4i) epitope is exposed on the surface of trimeric HIV-1 envelope glycoprotein (Env) after conformational changes of gp120 by binding to CD4. The CD4i epitope is highly conserved because the N-terminal region of CCR5 binds to this epitope. Therefore, the CD4i epitope is a favorable target for antibodies to neutralize a broad range of HIV-1 strains. However, most of primary HIV-1 isolates are resistant to anti-CD4i antibodies because the CD4i epitope is hidden inside trimeric Env before binding to CD4. In this study, we aim at developing more potent anti-CD4i neutralizing antibody than the original IgG form by constructing antigen-binding fragment (Fab) and single-chain variable fragment (scFv).

**Methods:** We constructed six Fabs and three scFvs from monoclonal antibodies (mAb) targeting CD4i epitope (16B2, 17B11, 4E9C, 5D6, 25C4b and 12G10). These anti-CD4i Fabs and scFvs were examined for their abilities to bind trimeric Env by flow cytometry. Neutralizing activities of these antibody fragments were examined by infection of TZM-bl cells with the pseudoviruses with various sensitivities to neutralizing antibodies, which were categorized to very high (tier 1A), above-average (tier 1B), moderate (tier 2), and low (tier 3).

**Results:** Three anti-CD4i scFvs (16B2, 4E9C and 25C4b) efficiently bound trimeric Env of HIV-1JRFL without sCD4, while the addition of sCD4 was necessary for the binding of the corresponding anti-CD4i IgG antibodies to Env. In addition, the binding activities of these scFvs were significantly higher than those of the corresponding anti-CD4i Fabs. These three scFvs neutralized tier 2, and tier 3 clade B viruses which were resistant to the corresponding IgGs, and the neutralizing activities were significantly higher than those of the corresponding Fabs. Moreover these scFvs effectively neutralized non-clade B viruses, including clade A, C, and CRF01\_AE.

**Conclusions:** Taken together, the anti-CD4i scFvs are accessible to CD4i epitope hidden inside trimeric Env before binding to CD4, and effectively neutralize multi-clade HIV-1. The small fragment of anti-CD4i antibodies will be useful for a potent and broadly neutralization of HIV-1.