DEVELOPING STRATEGIES TO IMAGE HIV IN VIVO: COMBINING THE SARCOPHAGINE CHELATOR MECOSAR TO 3BNC117 DOES NOT AFFECT HIV BINDING OR NEUTRALISATION

Authors:
McMahon JH[1], Tumpach C[2], Lange JL[3], Roche M[2], Alt K[3], Zerbato JM[2], Chang J[2], Zia N[4], Roney J[1], Caskey M[5], Nussenzweig M[5,6], Scott A[7], Donnelly PS[4], Hagemeyer CE[3], Lewin SR[1,2]

[1]Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, Australia
[2]The Peter Doherty Institute for Infection and Immunity, University of Melbourne and Royal Melbourne Hospital, Melbourne, Australia
[3]Australian Centre for Blood Diseases, Monash University, Melbourne, Australia
[4]School of Chemistry, Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Melbourne, Australia
[5]Laboratory of Molecular Immunology, The Rockefeller University, New York, New York
[6]Howard Hughes Medical Institute, The Rockefeller University, New York, New York
[7]Olivia Newton John Cancer Research Institute, Austin Health and Latrobe University, Melbourne, Australia

Background:
Non-invasive methods to detect and quantify HIV persistence in tissue and assess cure-focused interventions in HIV-infected individuals on antiretroviral therapy (ART) are needed. Infusing radiolabelled broadly neutralising antibodies (bNAbs) targeting HIV envelope (Env) then scanning with positron emission tomography (PET) identified affected tissues sites in a macaque model. Prior to a clinical trial, binding of chelator modified bNAb to Env needs to be confirmed *in vitro*.

Methods:
The bNAb 3BNC117 was reacted with different molar ratios (5x, 10x, 15x, 20x) of the copper chelator MeCOSar-NHS then assessed by size exclusion chromatography (SEC) and liquid chromatography-mass spectrometry (LC-MS) and the optimal molar ratio selected. Unlabelled and MeCOSar-modified 3BNC117 were assessed for neutralisation of reporter viruses pseudotyped with 3 subtype B Env strains in JC53 cells, and in 2 binding assays: 1) ELISA to immobilised Env (gp140) and 2) to surface Env on human embryonic kidney cells transfected with an Env expression plasmid. The 50% inhibitory concentration, colorimetric absorbance and flow cytometry were compared for unlabeled and MeCOSar-modified 3BNC117 respectively.

Results:
Different molar ratios of MeCOSar bound to 3BNC117 yielded SEC with similar elution profiles to IgG and unmodified 3BNC117. The predominant peak for unmodified 3BNC117 mass on LC-MS was 151467 Dalton (Da). The 10x molar ratio demonstrated addition of 1-3 MeCOSar (410 Da each) per 3BNC117 and was selected for further characterisation. Unlabeled and MeCOSar-modified 3BNC117 had comparable levels of binding to immobilized gp140; binding to Env expressed on

the surface of 293T cells; and neutralisation of reporter viruses pseudotyped with 3 different Envs.

Conclusions:
The copper chelator MeCOSar conjugates to 3BNC117 and does not interfere with binding to HIV Env or neutralisation *in vitro.* MeCOSar is appropriate to combine with 3BNC117 and tightly binds the radioisotope copper-64. This construct is ideally suited to continue development for a clinical trial using PET to image persistent HIV.

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