High Resolution Crystal Structure of KD-247, a Humanized Antibody that Inhibits HIV Entry

Karen A. Kirby[1], Jennifer L. Moran[1], Bruno Marchand[1], Kazuhisa Yoshimura[2], Toshio Murakami[3], Shuzo Matsushita[2], and Stefan G. Sarafianos[1]

[1]Department of Molecular Microbiology & Immunology, University of Missouri, School of Medicine, Columbia, MO USA

[2]Division of Clinical Retrovirology and Infectious Diseases, Center for AIDS Research, Kumamoto University, Kumamoto, Japan

[3]The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan

Highly active antiretroviral therapy (HAART) has been very efficient in reducing the rate of mortality of human immunodeficiency virus type 1 (HIV-1) infected patients. However, resistance to clinically used drugs inevitably develops and impairs the potency of these drugs. There is also no vaccine available to prevent the spread of the virus. Our collaborators, Dr. Shuzo Matsushita and his colleagues have developed a monoclonal antibody, KD-247, that is currently in Phase Ib clinical trials for the treatment of HIV-1 infections. KD-247 blocks virus entry into host cells by binding to the V3 loop of the surface glycoprotein of HIV. It is the first humanized antibody shown to neutralize a wide range of subtype B HIV viruses (Matsushita *et al*. *Hum. Antibodies,* **14**, 81) and to prevent HIV infection in cell culture and in a chimpanzee model (Eda *et al. J. Virol*., **80**, 5563). KD-247 reacts exclusively with subtype B viruses (Eda *et al. J. Virol*., **80**, 5552). In order to understand the molecular basis of this specificity we have solved the crystal structure of KD-247 at 1.5 Å resolution, the highest resolution structure for any humanized antibody reported to date. The present structure reveals in atomic detail the molecular boundaries of a pocket formed by the antigen-binding region of the antibody. Molecular docking experiments of a pre-existing structure of the V3-loop target at the presumed binding pocket on KD-247 suggest possible molecular interactions involved in HIV resistance to KD-247 and clade B specificity. A G314E V3 loop mutation that has been reported to confer

resistance to KD-247 (Yoshimura *et al*., *AIDS* **20**, 2065) appears to result in steric interactions between the tip of the V3 loop and residues of the heavy chain of KD-247. Further, Arg315, a residue critical for clade B specificity, appears to form extensive interactions with multiple residues of KD-247. Analysis of these interactions has provided insights into the design of second-generation antibodies with broader subtype specificity and improved ability to evade resistance mutations. This work is a product of collaborations between the University of Missouri and researchers at Kumamoto University, an academic institution in Japan, and the Chemo-Sero-Therapeutic Research Institute, an industrial partner in Japan, working to commercialize the antibody and further its progress in clinical trials.