

Progressed Research Projects Supported by MHLW (Ministry of Health, Labour and Welfare)

Development of New Anti-HIV Drug and Immunotherapy

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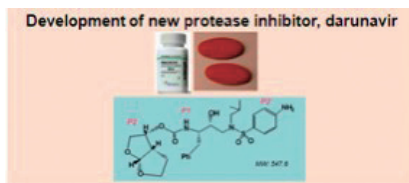
Since 1985, when AZT was introduced into clinical practice, more than 20 products have been approved and commercialized worldwide as anti-HIV-1 treatment. The drugs are in five categories, according to their various modes of action and chemical structures, such as nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), entry inhibitors (EIs), and integrase inhibitors (IIs). The current standard treatment for AIDS is highly active antiretroviral therapy (HAART), a combination regimen including drugs with different modes of action. However, during long-term treatment with anti-HIV-1 drugs, patients can develop a resistant strain of the virus, and require a new drug to counter the mutation.

On the other hand, immunotherapy is a candidate of new therapy for resistant strain of HIV. HIV-1-specific cytotoxic T cells (CTL) and neutralizing antibodies are well known to control HIV-1 replication. However, approaches using these immunological agents have not been succeeded in the control of HIV-1 in HIV-1-infected patients.

Our research team (Masafumi Takiguchi, Shinichi Oka, Hiroaki Mitsuya, Masanori Baba, Masao Matsuoka, Syuzo Matsushita) recently developed the studies of anti-HIV drug and immunotherapy. Especially, Dr. Mitsuya developed new protease inhibitors, darunavir. Other approaches are also under clinical trials. Near future, some of them will be approved and contribute as the therapy for HIV.

1. Development of New Anti-HIV Drug

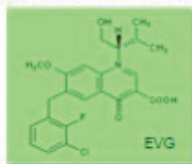
Dr. Mitsuya recently focused on a group of protease inhibitors (PIs) containing 3(R),3a(S),6a(R)-bis-tetrahydrofuranyl urethane (bis-THF) in collaboration with Professor Arun Ghosh of Purdue University (Ghosh et al., Acc Chem Res 2008). It is of note that in June 2006, darunavir, a bis-THF-containing PI, was approved by the US Food and Drug Administration as a therapeutic for treatment of individuals who harbor multi-drug-resistant HIV variants and do not respond to previously existing regimens. Continuing further collaboration with Dr. Ghosh, Dr. Mitsuya is developing more effective PIs containing unique active components. More interestingly, Dr. Mitsuya has most recently identified that a group of agents block dimerization of HIV protease subunits, an essential step for the acquisition of the proteolytic activity of HIV protease.



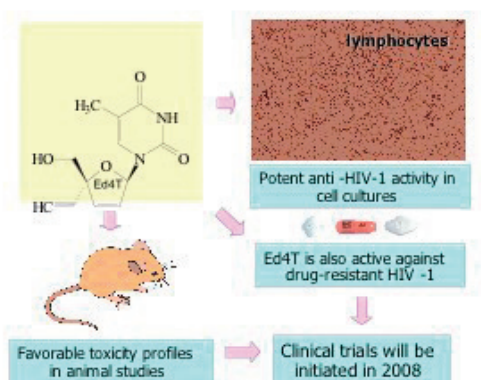
Emergence of resistant virus is a serious obstacle for therapy to HIV-1 infected individuals. In addition to reverse transcriptase and protease, development of drugs to new targets is an attractive way is for successful treatment to overcome the resistant viruses. A viral enzyme, integrase, integrates its proviral DNA into the host genome, which is specific to retrovirus. Therefore, the viral integrase is an ideal target for anti-virus therapy. Dr. Matsuoka developed a new drug, elvitegravir (EVG), which potently inhibits integration and replication of HIV-1. EVG inhibits replication of both wild-type HIV-1 and drug-resistant variants. Detailed analyses reveal that EVG binds to catalytic site of integrase, and blocks integration. EVG inhibits not only HIV-1, but also other retroviruses, including HIV-2, simian immunodeficiency virus, and murine leukemia virus. At present, EVG is in phase 3 clinical trial in USA.

4'-Ethynyl-d4T (Ed4T) is a thymidine analogue that blocks HIV-1 reverse transcriptase, the enzyme

Development of integration inhibitor, emtricitabine (EVG)

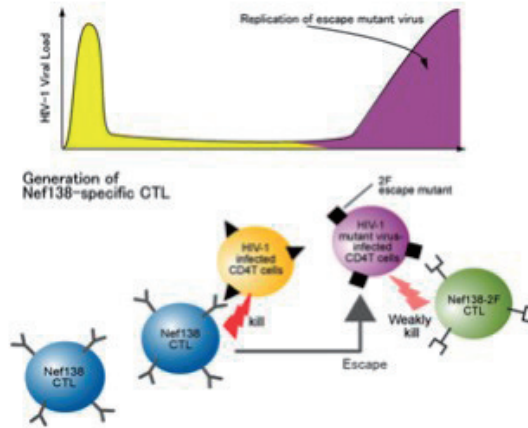


that is essential for viral replication. The compound was discovered and developed jointly by Professor Masanori Baba of Kagoshima University and Professor Hiromichi Tanaka of Showa University in Japan, and Professor Yung-Chi Cheng of Yale University School of Medicine, U.S.A. Pharmacological studies have demonstrated that Ed4T has more potent anti-HIV-1 activity than the existing NRTI and is active against those viruses that are resistant to the existing NRTIs and NNRTIs. Furthermore, Ed4T does not affect DNA synthesis in mitochondria, a toxic side effect of some nucleotide analogues. These findings suggested that Ed4T might offer unique therapeutic advantages over existing anti-HIV-1 drugs. Phase I clinical trials of Ed4T will be initiated in the first year of 2008 by Oncolys BioPharma, Inc. of Tokyo, the pharmaceutical company which has obtained the global exclusive right for clinical and business development of Ed4T.



2. Development of Immunotherapy

Cytotoxic T lymphocytes (CTLs) play an important role in the control of HIV-1 replication during acute and chronic phases of an HIV-1 infection. However, CTLs cannot completely eradicate HIV-1 because HIV-1 escapes from the host immune system by various mechanisms including mutations of immunodominant CTL epitopes. Indeed, HIV-1-specific CTLs have weak ability to kill HIV-1-infected CD4 T cells. Dr. Takiguchi and Dr. Oka found 3 HIV-1-specific CTLs having strong ability to suppress HIV-1 replication in vitro. However, out of these 3 CTLs, Nef138- and Pol283-specific CTLs selected escape mutants in vivo, indicating that these CTLs have strong ability to control HIV-1 in vivo. Nef138-specific CTLs selected 2F mutant. Interestingly the donors who selected the 2F mutant again elicit CTLs specific for the 2F mutant. However, the CTLs specific for the 2F mutant had weak ability to suppress replication of the 2F mutant virus. The CTLs having high affinity for the escape mutant epitope are expected to control the escape mutant virus. The immunotherapy to elicit such CTLs is now under investigation.



HIV-1-specific CTLs and escape HIV-1 mutant

Dr. Matsushita produced a high-affinity humanized monoclonal antibody (mAb) KD-247 against an envelope protein (gp120) of HIV-1. KD-247 is reactive against 47% of the subtype B viruses which are prevalent in USA, Japan and Europe. KD-247 was obtained by transferring CDRs (antigen binding domain of the antibody) of murine mAb C25 to a gene of human mAb. The humanized mAb effectively neutralized primary HIV-1 subtype B, R5 and X4/R5 viruses, and suppressed the ex vivo generation of primary HIV-1 in PBMC culture from individuals seropositive for HIV-1, that had quasispecies. While sterile protection was obtained by a single administration of a high-dose of the antibody transfer, partial protection required substantially low levels of the antibody in a highly pathogenic HIV monkey model. A Phase 1b clinical trial of KD-247 is currently underway in multiple sites in USA.

We induced a neutralization escape mutant at passage 8 in the culture where HIV-1JRFL was propagating in the presence of KD-247 (1 mg/ml) and we found an amino acid substitution, Gly to Glu at position 314 (G314E), in the V3-tip. Neutralization resistance of the G314E mutant to KD247 was confirmed by a neutralization assay using a pseudotyped virus with G314E mutation. Unexpectedly, this mutant virus was sensitive to CCR5 inhibitors, RANTES, rsCD4 and anti-CCR5 mAb compared with wild type virus. We also evaluated the anti-HIV-1 interactions between KD-247 and various CCR5 inhibitors in vitro. Combinations of KD-247 with the CCR5 inhibitors showed highly synergistic interactions. These results may have an implication of future passive immunotherapy using KD-247 in combination with CCR5 inhibitors.

