Editorial

Is cyclin-dependent kinase 9 a novel specific molecular target of adult T-cell leukemia/lymphoma?

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Adult T-cell leukemia/ lymphoma (ATL/ATL) is an aggressive form of CD4+ T-cell leukemia/lymphoma. The primary cause of this fatal disease is infection by human T-cell leukemia virus type I (HTLV-I) (1-3). ATL was first identified in Japan by Takatsuki *et al.* in 1977 (4). The Japanese Lymphoma Study Group proposed four subtypes of ATL such as acute, lymphoma, chronic, and smoldering in 1991 (5). Up to now, though there are several new therapies have been developed, no curative regimen is accepted for the treatment of ATL. The median survival time of acute and lymphoma subtypes of ATL patients without receiving an allogeneic hematopoietic stem cell transplantation was found to be 7.7 months (6). These data were obtained based on a survey of the diagnosed ATL patients from 2000 to 2009. There are several new treatment options available including multi-agent chemotherapy, and monoclonal antibody but the overall prognosis of ATL is still poor. Among the other cause, resistance to the conventional high-dose chemotherapy is one of the main factors associated with its poor prognosis.

Narita *et al.* identify a specific sensitivity of ATL cells to CDK9 inhibition (7). BAY 1143572, an inhibitor specific to CDK9 (Bayer AG Pharmaceuticals Division, Berlin, Germany), inhibits the cell proliferation and induces apoptosis in ATL-derived and HTLV-I-immortalized cell lines dose-dependently. BAY 1143572, a selective inhibitor of PTEFb/CDK9, inhibits the phosphorylation of RNAPII at a Ser2 site, and thereby inhibits the transcription of c-Myc and Mcl-1 in ATL-derived or HTLV-I-immortalized cell lines. BAY 1143572 also inhibits the growth and induces the apoptosis in T-ALL lines dose-dependently in association with phosphorylation of RNAPII at a Ser2 site in all T-ALL lines tested as well as the inhibition of c-Myc and Mcl-1.

The authors also found that BAY 1143572 inhibits the growth of primary ATL cells freshly isolated from diagnosed ATL patients. Initially, growths of CD4-positive cells from healthy individuals were also interfered with BAY 1143572 but a slight increase in the cell viability was observed with increasing concentration of the chemical, in contrast. In Western blotting, essentially the same results were noticed, namely BAY 1143572 inhibition of RNAPII phosphorylation at a Ser2 site. However, it is important to recognize that inhibitory effects are variable among patients' samples; rather weak or no effect was seen in cells isolated from some acute type ATL patients. The final observation of prolongation of survival time of BAY-treated ATL mouse group compared with the untreated controls confirms the anti-ATL activity of BAY 114372. Intraperitoneal inoculation of leukemic cells of a cell clone initially established from an acute type ATL patient, S-YU BAY into NOG mice resulted in the development of intraperitoneal masses in the mesentery within three to four weeks after inoculation. In macro- and microscopic observations of NOG mice, liver tissues, and bone marrow were aggressively infiltrated by ATL cells in control mice whereas those from mice orally treated with BAY 1143572 were histologically intact. Thereby, it is concluded that initial treatment with BAY 1143572 significantly decreased

the liver and bone marrow infiltration of ATL cells upon inoculation. The serum level of human sIL2R also significantly reduced after treatment with BAY 1143572, reflecting the reduced cancer activity and tumor burden in ATL cells-inoculated mice.

Cyclin-dependent kinase 9 (CDK9) is a member of serine/threonine kinase family that forms a positive transcription elongation factor b (P-TEFB) complexes. P-TEFB plays an important role in stimulating and regulating the gene transcription elongation of most protein-coding genes by phosphorylating the C-terminal domain of RNA polymerase II (8). CDKs are essential in mammalian cell biology. Dysfunction of CDK9-related signaling pathways is related to developing and maintaining of several human malignancies (9). It has been also reported that the replication program of numerous viral agents, including HTLV-I, is regulated by CDK-9 related pathway (10,11). Therefore, it has been hypothesized that CDK9 inhibitors might be a novel therapeutic target for the treatment of ATL.

Narita *et al*. have identified a specific sensitivity of ATL cells to a selective inhibitor of CDK9/P-TEFB, BAY 1143572 and evaluated the therapeutic effect of this inhibitory compound to ATL with poor prognosis (7). However, some major drawbacks appear in this study. The study found that in addition to inhibition of cell proliferation and induction of apoptosis of ATL-derived or HTLV-I-transformed cell lines and primary ATL cells, BAY 1143572 also inhibits the proliferation of CD4-positive cells from healthy volunteers. Median inhibitory concentrations (IC50) of BAY 1143572 in ATL-derived or HTLV-I–transformed lines (n=8), primary ATL cells (n=11), and CD4-positive cells from healthy volunteers (n=5) were 0.535, 0.30, and 0.36 μM, respectively. There is essentially no difference in terms of selectivity (IC50) though normal cells seem to show more resistance to the drug at higher concentration ranges. In *in vivo* study, an isolated leukemic cell clone from an ATL patient was injected intraperitoneally (ip) into NOG mice to develop ATL model and to evaluate the effect of BAY 1143572. As previously reported, NOG mouse is a novel recipient of leukemic cells, hematopoietic stem cells and all blood cells (12). Investigators did not rule out the effect of BAY 1143572 *in vivo* whether it was patient specific or not since one ATL patient sample was used for this study. Previously, we established very simple and useful mouse model to study pathophysiology of primary ATL cells by using the same NOG mice (13). All peripheral blood samples freshly isolated from various subtypes of ATL patients tested were readily transplanted in mice. Furthermore, the IC50 values of BAY 1143572 in S-YU cells were much higher than those in primary ATL cells in vitro. The authors state that in human ATL patients, the antitumor effect of BAY 1143572 could be observed much more clearly than in ATL mice (7). Hence, the anti-ATL effect of BAY 1143572 is expected to be analyzed further in different subtypes of primary leukemic cells freshly isolated from ATL patients by using NOG mice. Also, authors did not show IC50 of BAY 1143572 on mouse cells. Above drawbacks will limit the use of BAY 1143572 for the treatment of ATL and indicates that further research for more specific and safer CDK9 inhibitors is necessary.

Besides therapeutic aspects of CDK9 in ATL, this paper also highlighted a possible role of CDK9 in ATL leukemogenesis. The disease develops after a long latent period of infection of HTLV-I. The study shows that the reason behind this long latency is due to accumulation of multiple genetic events in the HTLV-I-infected cells. However, the definitive molecular mechanism behind this leukemogenesis and late development of ATL is not fully understood. The viral gene *Tax* is considered to play a crucial role in this HTLV-I-induced transformation through transactivation of the HTLV-I long terminal repeat. At the same time, the other cellular genes such as those encoding IL-2, and the α-chain of the IL-2 receptor (IL-2Rα) (CD25, Tac) are involved in the T-cell activation and growth (14). Induction of other cellular genes by Tax is mediated through the activation of transcription factor NF-κB. The malignant ATL cells of all phases express a high level of IL-2Rα. However, even though the *Tax* protein plays a central role in leukemogenesis, it is not detected in all ATL patients (15). A second HTLV-I specific gene called HBZ gene has been discovered recently associated with HTLV-I pathogenesis (16). *HBZ* gene seems to be active in ATL cells even at late stages. Hence, it is possible that *HBZ* gene is crucial for maintenance of the leukemic state through replacing the role of Tax protein which is required for the early proliferation of ATL cells.

The fundamental treatment for ATL consists of combination chemotherapy and allogeneic hematopoietic stem cell transplantation, if applicable (17). A combination therapy using interferon-α /zidovudine has been approved outside Japan as a therapeutic option for acute, chronic, and smoldering-type ATLs (18). Previously, we were able to show the anti-ATL effect of ritonavir, an HIV protease inhibitor, on primary ATL cells by using the same NOG

mice (13). The study shows that this drug induces apoptosis and inhibits transcriptional activation of NF-κB in malignant/HTTLV-I-infected cells. Furthermore, ritonavir inhibits the expression of the target molecules such as NF-κB, Bcl-xL, survivin, c-Myc, and cyclin D2. Ritonavir-treated NOG mice also show a reduction of tumor growth and infiltration in various organs very efficiently. The drug dose used was the same dose as used for treatment of patients with AIDS. Unfortunately, no further clinical studies have been conducted against ATL with this drug thereafter.

Because of its crucial role in cell survival, CDK9 was actually put forth as a potential therapeutic target in a number of malignancies such as hepatocellular carcinoma, ovarian cancer, and hematological malignancies (19,20). With an aim to seek for cancer species in which CDKs play an active role, screening of more than 1,500 CDKs inhibitors against 78 cancer cell lines was performed to systematically uncover genotype-specific vulnerabilities (21). As a result, Brägelmann *et al.* identified a specific sensitivity of BRD4-NUT-rearranged NUT midline carcinoma (NMC) cells to CDK9 inhibition. CDK9 inhibition apparently affects transcriptional elongation, de-regulates MYC signaling, and induces apoptosis by suppressing anti-apoptotic MCL1. These results suggest that CDK9 could be a promising target in NMC. Progress of systematic genomic profiling of tumors and targeted therapeutics seems to open a new path for the treatment of cancer patients (21).

Apart from CDK inhibitors, several new candidate molecules have been developed as anti-ATL reagents. Among others, attempts have been made to target surface antigens such as CCR4 and CD30 on ATL cells with novel monoclonal antibodies. In particular, the CCR4 monoclonal antibody Mogamulizumab (Potelligeo) was successfully introduced in the clinical settings by a group of one of the coauthors of this paper, and promising results were obtained (22). Mogamulizumab show strong ADCC on target ATL cells by efficient and antigen-specific activation of NK cells due to increased binding to FcγRIIIa.

Currently, the leading role in cancer therapy is played by combination therapy. In addition to the original standard care for cancer patients including chemotherapy, surgery, radiation therapy, a therapy that combines various regimen has been attempted. Besides already mentioned antibody medicine, various immunotherapy methods are becoming available (23). These include adoptive immunotherapy using immune cells such as NK cells, dendritic cells, killer T-cells with or without genetic modification like CAR-T cells, and immune checkpoint inhibitors. More recently, oncolytic virus therapy, which many infectious viruses preferentially infect cancer cells and destroy the target cancer cells, has attracted attention (24). Over 3 decades ago, we showed that human immunodeficiency virus type 1 (HIV-1), a causative agent of AIDS, preferentially infects and destroys ATL cells very efficiently (25). Selective killing of cancer cells by the virus is a phenomenon commonly seen by infection with conventional viruses which are not as pathogenic as HIV.

In conclusion, present CDK9-specific inhibitor or its successor is expected to become a powerful tool to treat malignancy such as ATL. Especially, their combination with various immuno-therapeutic medicines and oncolytic viruses will be of particular interest.

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