Annexin V-FITC/APC and propidium iodide. Protein levels were analyzed by immunoblotting. For combination studies, cells were pretreated 2h with BAY 43-9006 before the BH3-mimetic GX15–070/Obatoclax exposure.
Results: In all CLL samples tested, including those bearing adverse cytogenetic alterations, BAY 43-9006 induced apoptosis in a dose dependent manner, with a mean lethal dose 50 (LD_{50}) of 9.77±2.33 μM at 24 h and of 7.31±2.28 μM at 48 h. Importantly, the sensitivity of mononuclear cells from healthy donors to BAY 43-9006 was significantly lower when compared with CLL cells, both in T lymphocytes (LD_{50} = 22.33±2.39 μM) and B lymphocytes (LD_{50} = 60.15±4.75 μM). Noteworthy, we demonstrated that the coculture with HS-5 protected CLL cells from spontaneous apoptosis but not from BAY 43-9006-induced apoptosis, indicating that this kinase inhibitor could overcome the protective effect mediated by the stromal microenvironment in CLL. Moreover, western blot analysis showed that BAY 43-9006-induced apoptosis was accompanied by a dose-dependent decrease on the antiapoptotic MCL1 protein. Based on this finding, we demonstrated a strong synergism when combining BAY 43–9006 with the BH3-mimetic GX15–070/Obatoclax in CLL cells.
Conclusions: Our results support the use of multikinase inhibitors, alone or in combination, as a novel therapeutic strategy able to overcome the microenvironment in CLL.
Grants: Ministerio de Ciencia e Innovación, SAF09/9503; Redes Temáticas de Investigación Cooperativa de Cáncer (RTICC), Instituto de Salud Carlos III, RED 2006−20–014.

167 POSTER

Effects of a novel, investigational 17,20-lyase inhibitor, TAK-700, on androgen synthesis in male rats: in vivo and in vitro specificity studies

T. Hara[1], M. Yamaoka[1], T. Hitaka[2], T. Kaku[3], A. Tasaka[3], M. Kusaka[1]. *[1]Takeda Pharmaceutical Company Limited, Pharmacology Research Laboratories, Tsukuba, Japan; [2]Takeda Pharmaceutical Company Limited, Medicinal Chemistry Research Laboratories, Tsukuba, Japan; [3]Takeda Pharmaceutical Company Limited, Environment and Safety Department, Tsukuba, Japan*

Background: Recent studies suggest that residual adrenal androgen after castration could be responsible for the progression of castration-resistant prostate cancer (CRPC). To develop an effective therapy for CRPC that involves inhibition of adrenal and testicular androgen production, we searched for a novel, non-steroidal and selective inhibitor of 17,20-lyase, a key enzyme in androgen synthesis. We identified TAK-700 as an investigational clinical candidate, and this molecule is currently undergoing clinical assessment in a phase 2 clinical trial.
Materials and Methods: We assessed the activity and specificity of TAK-700 on androgen production *in vitro* in rat testicular and adrenal cells, as well as the *in vivo* effects of TAK-700 at 30 mg/kg on androgen synthesis in human chorionic gonadotropin (hCG)-injected and adrenocorticotropic hormone (ACTH)-injected hypophysectomized rats. The effects of repeated dosing with TAK-700 1,800 mg/kg/day on the mass of prostate and seminal vesicles were also assessed in normal rats.
Results: TAK-700 suppressed production of testosterone and androstenedione in rat testicular cells, with IC_{50} values of 640 and 210 nmol/L, respectively. In rat adrenal cells, the IC_{50} value of corticosterone was >200,000 nmol/L and that of aldosterone was 35,000 nmol/L. In agreement with these *in vitro* results, a single oral administration of TAK-700 at 30 mg/kg significantly reduced serum testosterone and androstenedione levels in hCG-injected hypophysectomized rats by 90% compared with baseline levels. In contrast, corticosterone and aldosterone levels in ACTH-injected hypophysectomized rats did not significantly change after single oral administration of TAK-700 at concentrations up to 300 mg/kg. Serum testosterone levels in intact male rats were also significantly reduced by single oral administration of TAK-700 ⩾100 mg/kg. Furthermore, in normal male rats, the mass of prostate and seminal vesicles was significantly decreased by repeated dosing with TAK-700 at 1,800 mg/kg/day.
Conclusions: TAK-700 suppresses the androgen-synthesis pathway with high specificity, which in turn decreases the mass of androgen-dependent organs in male rats. Our data suggest that TAK-700 is an attractive agent for further investigation in androgen-driven progression of prostate cancer.

168 POSTER

MiR-155 expression in acute leukemia cells treated with the multikinase inhibitor sorafenib

M. Goetz[1], C. Schult[1], M. Dahlhaus[1], K. Fischer[1], M. Freund[1], C. Junghanss[1]. *[1]University of Rostock, Hematology/Oncology, Rostock, Germany*

Background: The MicroRNA-155 (miR-155) is involved in lymphomagenesis and is upregulated in FLT3-ITD positive acute myeloid leukemia (AML). Little is known about the miR-155 expression in acute lymphoblastic leukemia (ALL) cells. Sorafenib (Nexavar®) is a multikinase inhibitor targeting FLT3 and other receptor tyrosine kinases. It has been approved for treatment of renal cell and hepatocellular carcinoma. In a previous study we have demonstrated the antiproliferative effect of Sorafenib on ALL cells in vitro. Here we investigated the expression of miR-155 in different AML, ALL and lymphoma cell lines. We then aimed at detecting effects of Sorafenib on leukemia cell proliferation and on the expression of miR-155 in vitro.
Material and Methods: ALL and AML cell lines as well as lymphoma cell lines with different cytogenetics and phenotypes were analyzed (n = 11). MiR-155 expression was measured using stem-loop RT-PCR and data was compared to miR-155 expression in hematopoietic stem cells. The ALL cell line SEM and the AML cell line MV4−11 (FLT3-ITD positive) were incubated with Sorafenib at concentrations of 7.3 μM and 0.73 μM for 96 h. Cell number, apoptosis, necrosis, metabolic activity and expression of miR-155 were detected at 6 h, 24 h, 48 h, 72 h and 96 h. Cells were counted by microscopy, apoptotic and necrotic rate were measured by flow cytometry and metabolic activity was detected by WST-1 testing. Results were compared to controls incubated with DMSO only.
Results: We found miR-155 to be upregulated in SEM and MV4−11 compared to other AML and ALL cell lines. Very low miR-155 expression was detected in the Burkitt lymphoma cell line DOGKIT. Sorafenib treatment inhibited the proliferation of SEM and MV4−11 significantly. Whereas in SEM cells apoptosis and necrosis rates were found to be upregulated only by high concentrations of Sorafenib (10% vs. 45% after 96h respectively), the apoptotic and necrotic rates in MV4−11 cells increased with both concentrations significantly (up to 26% and 64% after 96 h respectively). Besides the metabolic activity was decreased significantly following treatment with Sorafenib. In SEM cells the expression rates of miR-155 increased during the 96h treatment with Sorafenib up to 2-fold compared to the controls. However, kinase inhibition with Sorafenib did not influence the expression of miR-155 in MV4−11.
Conclusions: Expression of miR-155 is deregulated in acute leukemia and high grade lymphoma cell lines. Sorafenib has antiproliferative effects on AML and ALL cells in vitro. Expression of miR-155 in FLT3-ITD positive AML cells seems not to be influenced by Sorafenib, but is slightly induced in the ALL cell line SEM.

169 POSTER

Identifying molecular hallmarks of sensitivity to the anticancer effects of statins in breast tumour cells

C.A. Goard[1], M.L. Gauthier[1], L.Z. Penn[1]. *[1]Ontario Cancer Institute of Princess Margaret Hospital & University of Toronto, Dept. of Medical Biophysics, Toronto, Canada*

There is an urgent need for novel, effective anticancer therapeutics to improve outcome and quality of life in breast cancer patients. Recent evidence suggests that statins, drugs commonly used to lower blood cholesterol, can fill this gap. By inhibiting the rate-limiting enzyme of the mevalonate (MVA) pathway, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), statins have been shown to trigger tumour-specific apoptosis in a variety of malignancies. Preclinical, epidemiological and clinical data all suggest that statins may be particularly useful in the prevention and treatment of breast cancer. While certain breast tumour cell lines are highly sensitive to the antiproliferative effects of statins, others do not exhibit such a strong cytotoxic response, reflective of the heterogeneity of this disease. Recent work in our lab has demonstrated in multiple myeloma that statin-sensitive tumour cells, which readily undergo apoptosis upon treatment with lovastatin, harbour a dysregulated MVA pathway. Advancing statins to breast cancer patient care requires a similar understanding of how to identify statin-sensitive breast tumours, and how to combine statins with other therapeutics to efficiently eradicate the tumour. These questions will be addressed by discovering patterns of gene and protein expression in breast cancer cells associated with their sensitivity to the anticancer effects of statins. A panel of over 20 heterogeneous breast cancer cell lines was assessed for sensitivity to the antiproliferative effects of lovastatin and fluvastatin by MTT assay, and clear delineations between relatively statin-sensitive and -insensitive cell lines emerged. Interestingly, estrogen receptor expression seemed to be associated with resistance to statin therapy, while statin sensitivity did not appear to segregate with several other common histological or molecular features or with rate of proliferation. Importantly, preliminary data suggest that dysregulation of the MVA pathway is also involved in statin sensitivity in breast cancer. Since statins are FDA-approved drugs, they can be fast-tracked to patient care to improve patient outcome in the near future. This research will help us and others design clinical trials of treatments including statins in a carefully-chosen subset of breast cancer patients harbouring molecular hallmarks of statin sensitivity.