163 POSTER

Effect of a novel, investigational 17,20-lyase inhibitor, TAK-700, on enzyme activity and serum androgen levels in human H295R cells and cynomolgus monkeys

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Background: Residual adrenal androgen after castration has been suggested to be responsible for the progression of castration-resistant prostate cancer (CRPC). Consequently, we identified the investigational agent TAK-700, a novel, selective, and potent inhibitor of 17,20-lyase, a key enzyme in androgen synthesis, as a clinical candidate.
Materials and Methods: We investigated the inhibitory effects of TAK-700, versus ketoconazole, on the androgen-synthesis enzymes 17,20-lyase and 17-hydroxylase, and the cortisol-synthesis enzyme 11-hydroxylase, and on intracellular androgen production in monkey adrenal and human H295R cells. We then assessed the effects of TAK-700 at 7.5 and 15 mg/kg twice daily (BID) for 7 days on serum androgen levels in castrated and gonadally intact cynomolgus monkeys. The toxicity profile of TAK-700 was also assessed using *in vitro* screening and toxicology studies in monkeys (4-week oral administration of TAK-700 at 0.8–100 mg/kg/day).
Results: TAK-700 inhibited human and monkey 17,20-lyase activities (IC_{50} = 140 and 27 nM, respectively; versus 110 nM and 750 nM with ketoconazole). TAK-700 was less effective against monkey 17α-hydroxylase (IC_{50} 38 nM) and monkey 11-hydroxylase (IC_{50} >10 μM). This specificity for 17,20-lyase was seen in TAK-700 inhibition of dehydroepiandrosterone (DHEA) and cortisol production in human H295R cells (IC_{50} 37 vs 999 nM). TAK-700 showed ~27-fold selectivity for DHEA, versus ~1.5-fold selectivity with ketoconazole. *In vivo* studies in cynomolgus monkeys showed that oral TAK-700 rapidly suppressed serum levels of DHEA, testosterone, and cortisol in castrated and gonadally intact monkeys. Consistent with the *in vitro* data, TAK-700 showed specificity for DHEA versus cortisol suppression; mean DHEA levels decreased to 9.0% and 6.9% of baseline levels in castrated monkeys treated with TAK-700 at 7.5 and 15 mg/kg, respectively, whereas mean cortisol levels decreased to 26.0% and 17.3% of baseline levels, respectively. The preclinical toxicity profile is encouraging, with no hit in MDS *in vitro* screening, and no adverse effects in monkeys administered TAK-700 at 0.8−4 mg/kg/day.
Conclusion: TAK-700 potently and selectively inhibits 17,20-lyase activity and DHEA production in castrated and gonadally intact monkeys. Together with the encouraging toxicity profile, TAK-700 is an attractive agent for further investigation as an effective treatment for CRPC.

164 POSTER

The SEPT9_v1 first 25 amino acids fragment suppresses tumor growth through disruption of hypoxia-inducible factor 1 alpha (HIF-1 alpha) nuclear translocation

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Chronic hypoxia is associated with tumor progression and angiogenesis. The major mediator of the hypoxic response is hypoxia-inducible factor 1 (HIF-1). We have previously described a novel association between HIF-1α, the oxygen-regulated subunit, and SEPT9_v1, a family member of the mammalian septins. This interaction increases HIF-1α protein stability, HIF-1α expression and HIF-1 transcriptional activity *in vitro*, and promotes proliferation, tumor growth and angiogenesis *in vivo*. The first 25 amino acids of the SEPT9_v1 protein (N_{25}) are uniquely different from any other member of the overall septin family and contain bipartite nuclear signal. The SEPT9_v1 N_{25} terminus was found critical for HIF-1 activation by SEPT9_v1 but was not required for their interaction. In this work, we show that expression of the SEPT9_v1-N_{25} free fragment induced a significant dose dependent inhibition of HIF-1 transcriptional activity but did not affect HIF-1α protein expression levels or stability. *In vivo* studies showed that SEPT9_v1- N_{25} inhibits proliferation and tumor growth. Under hypoxia, HIF-1α nuclear translocation was decreased in PC-3 cells expressing the SEPT9_v1-N_{25} compared to control cells. We found that both HIF-1α and the SEPT9_v1 proteins directly interact with the nuclear transport adaptor protein importin-α, and this interaction is disrupted in the presence of SEPT9_v1-N_{25}. Moreover, the interaction between HIF-1α and importin-α is significantly reduced in cells knocked-down to SEPT9_v1. These results imply that SEPT9_v1 increases HIF-1α interaction with importin-α to facilitate its nuclear translocation, while SEPT9_v1-N_{25} polypeptide interrupts this dual interaction with importin-α to inhibit HIF-1α nuclear translocation. Altogether, we suggest that SEPT9_v1-N_{25} can be used to inhibit tumor growth through interfering with SEPT9_v1/importin-a-dependent HIF-1a nuclear translocation.

165 POSTER

JNJ-26483327, a second-generation spectrum-targeted protein tyrosine kinase inhibitor with superior characteristics in advanced cancer models

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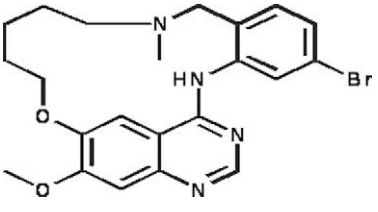
Protein tyrosine kinases play crucial roles in the regulation of cancer cell growth, survival and interactions with the tumour microenvironment resulting in cancer progression. For example, when activated, they stimulate tumour cell migration leading to invasion of surrounding tissues and distant metastasis, the major cause of death from cancer.
We designed JNJ-26483327, a macrocyclic inhibitor of EGFR, Her-2 and Her-4, Src family kinases Lyn, Yes, Fyn, Lck and Src, as well as Ret, Ack1, RIPK2, Brk and VEGFR3 with nanomolar activity. We then tested this compound in a series of *in vitro* experiments and *in vivo* models of human cancer growth and progression to establish its therapeutic potential in a preclinical setting.
JNJ-26483327 inhibits (1) EGFR phosphorylation in Western blotting analysis, (2) migration in the scratch wound assay and (3) growth *in vitro* and *in vivo* in EGFR-driven cancer cell lines, (4) reduces lymphangiogenesis in the Xenopus tadpole model, (5) has anti-angiogenic activity in a transgenic zebrafish model, (6) produces a dose-dependent therapeutic effect on both tumour size and bone erosion in a bone metastasis model, (7) significantly diminishes spontaneous paw lifting as an indicator of pain in a bone pain model, and (8) considerably reduces tumour size and increases survival in a mouse intracranial tumour model mimicking brain metastasis. More recently, we found that JNJ-26483327 is comparatively insensitive to competition by ATP as evaluated by the INTANA titration assay and shows unique activity in *in vitro* and *in vivo* models of human Ras-mutant and PI 3-kinase-activated cancers.
As a result of our pre-clinical studies, which have demonstrated characteristics superior to those of existing drugs targeting the EGFR, JNJ-26483327 is undergoing clinical evaluation.



166 POSTER

BAY 43-9006/Sorafenib overcomes the protective effect of stroma and synergizes with the BH3-mimetic GX15–070/Obatoclax in chronic lymphocytic leukemia cells

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Background: Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western countries. Despite the development of several new therapeutic strategies, it remains an incurable disease. Kinase inhibitors have emerged in the last years as an important class of antitumoral agents. Among them, BAY 43-9006/Sorafenib (Bayer) is a multikinase inhibitor that has shown activity against several solid tumors and hematological malignancies. Our purpose was to establish the molecular mechanisms related with BAY 43-9006-induced cytotoxicity in CLL cells, together with the analysis of its combination with other antitumoral agents.
Materials and Methods: Primary cells from 38 CLL patients and from 3 healthy donors were incubated with different doses of BAY 43-9006. The stromal cell line HS-5 was used to mimic the tumoral microenvironment. Cell viability was assessed by flow cytometry labelling of cells with