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MLN8054, an Aurora A Kinase inhibitor, demonstrates potent anti-tumor activity in disseminated tumor models

M. Zhang, M. Meetze, J. Huck, M. Silva, T. Sells, C. Claiborne, M. Manfredi. *Millennium Pharmaceuticals Inc., USA*

MLN8054 is a potent, selective, and orally available small molecule inhibitor of Aurora A kinase that is currently being investigated in phase I clinical trials. Preclinically, MLN8054 has broad-based anti-tumor activity against multiple subcutaneously grown human xenograft models including those of colon, lung, prostate, and ovarian. Moreover, results from in vivo pharmacodynamic studies demonstrated target inhibition at efficacious doses, including mitotic accumulation and inhibition of Aurora A autophosphorylation on Thr288. Here we demonstrate that MLN8054 has potent anti-tumor activity in advanced models of prostate bone metastasis and disseminated Non-Hodgkin's lymphoma. MLN8054 dosed orally for 21 days inhibited growth of the OCI-Ly3 diffuse large B-cell lymphoma model that had been inoculated intravenously. Tumor growth inhibition (TGI), as measured by a decrease in bioluminescent signal, was 74.5 % and 99.5 % when dosed at 10 mg/kg twice a day (BID) and for 30 mg/kg once a day (QD) respectively. Both doses were well tolerated with less than 5% body weight loss observed. MLN8054 also had potent anti-tumor activity in the CWR22Rv1 prostate bone model. Tumor cells were implanted into the intraosseous space in the tibia and growth was tracked using bioluminescent imaging and MRI. Bone destruction was determined by X-ray and micro-CT analysis. MLN8054 dosed for a total of 42 days at 10 and 30 mg/kg BID significantly inhibited growth with TGIs of 74.5 % and 94.5 % respectively as determined by bioluminescent imaging. TGI based on MRI was similar. MLN8054 also protected against osteolytic bone destruction. MLN8054 dosed at 30 mg/kg BID resulted in almost complete protection of bone loss while the 10 mg/kg BID dose protected partially. These data further support the broad anti-tumor activity of MLN8054 by extending previous findings into models that may be more relevant to human disease.

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AKT as a molecular target of fenretinide activity in glioblastoma in vitro

A. Gasperi-Campani[1,2], D. Baiocchi[1], G. Marti[1], L. Roncuzzi[3]. *[1]University Of Bologna, Department Of Experimental Pathology, Bologna, Italy; [2]University Of Bologna, Interdepartmentale Center For The Research On Cancer, Bologna, Italy; [3]Istituti Ortopedici Rizzoli, Laboratory "Fisiopatologia Degli Impianti Ortopedici", Bologna, Italy*

Glioblastoma multiforme is the most aggressive of the primary central nervous system tumours, with a median patient's survival of less than 1 year. In this tumour, a constitutive activation of the serine/threonine protein kinase Akt/PKB pathway is associated with increase in tumour grade, decreased level of apoptosis and adverse clinical outcome. Fenretinide, a synthetic derivative of retinoic acid, is a modulator of cell proliferation and inductor of apoptosis *in vitro*; it is a very promising agent because of easy administration, long-term tolerability and low incidence of long-term side effects. It is currently under evaluation in clinical trials as a chemopreventive agent against a variety of cancers. Fenretinide has been shown to inhibit glioblastoma cells *in vitro,* but the mechanism of its antiproliferative action remains elusive. The present study was designed to investigate the role of Akt/PKB in the molecular mechanism of action of fenretinide in human glioblastoma *in vitro*, and for this purpose CRS-A2 and A-172 cell lines were chosen, which do highly express Akt/PKB. The dose- and time-dependent significance of cell survival inhibition was determined by Trypan Blue exclusion test. Apoptosis was checked by DNA fragmentation and caspase induction. Protein expression was evaluated by Western blotting analysis. Results show that the antiproliferative activity of Fenretinide in human glioblastoma *in vitro*, at pharmacologically achievable doses, is correlated with a downregulation of Akt protein expression as well as an inhibition of constitutively active Akt phosphorylation. In addition, the drug induced a down-regulation of cyclin D1/Cdk4 and a decrease of $p21^{CIP1}$ protein expression. These events preceded activation of caspases, proteolysis of the nuclear enzyme poly(ADP-ribose)polymerase (PARP) and DNA fragmentation in CRS-A2 glioma cells. No induction of apoptosis was evident in A-172 glioblastoma cells. Our data identified in the Akt/PKB pathway a new molecular target of fenretinide activity and provides a molecular rationale for therapeutic strategies in human glioblastomas.
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Phase I study of NGR-TNF, a novel vascular targeting agent, in patients with refractory solid tumours (EORTC 16041)

C. Van Herpen[1], W. Fiedler[2], S. Toma[3], S. Marreaud[4], H. Van Laarhoven[5], P. Lasch[2], J. Bogaerts[4], A. Heerschap[5], C. Bordignon[3], C. Punt[1]. *[1]Radboud University Nijmegen Medical Centre, Department of Medical Oncology, Nijmegen, The Netherlands; [2]University Hospital Hamburg-Eppendorf, Department of Medicine II, Hamburg, Germany; [3]MolMed, Milano, Italy; [4]EORTC, Brussel, Belgium; [5]Radboud University Nijmegen Medical Centre, Department of Radiology, Nijmegen, The Netherlands*

Background: NGR-TNF is a novel agent exploiting a tumour homing peptide (cNGRCG) that selectively targets CD13 that is expressed on the neovasculature of solid tumors. Preclinical data show that its antitumour activity is achieved by a change of vascular permeability (at low doses) and damage of tumour-associated blood vessels (at high doses). This phase I study is being conducted to assess its safety, PK, PD, maximum tolerated dose (MTD), and optimal biological dose in patients (pts) with advanced solid tumours.
Methods: NGR-TNF was administered once every 3 weeks by a 20 min IV infusion to cohorts of 3–6 pts. The starting dose was 0.2 $\mu g/m^2$. Dose escalation was performed with a doubling of the dose until grade 2 toxicity was observed; thereafter a modified Fibonacci schedule was used. PK and PD analysis in blood was performed during the first 4 cycles. DCE-MRI was performed in cycle 1 at baseline and 2 hours after start of the infusion to document modification of the vessel permeability. Anti-tumour activity was assessed by CT scan every 2 cycles.
Results: 34 patients have been treated across 10 dose levels (0.2, 0.4, 0.8, 1.3, 1.95, 2.6, 3.46, 4.6, 6.1, and 8.1 $\mu g/m^2$). Out of 30 patients with available data, the most frequently reported drug related adverse events (AEs) were chills (n = 23), fever (n = 12), fatigue (n = 10), bronchospasm (n = 2 of which one is not related), hypotension (n = 2), and nausea (n = 13). Dose limiting toxicity was observed in only one pt (grade 3 bronchospasm at 1.3 $\mu g/m^2$ after the first infusion). Because 4/16 pts experienced grade 2 chills, we decided to prolong the infusion time to 60 min. After that, only 1/12 pts experienced grade 2 chills. Analysis for the plasma levels of sTNF-RI and sTNF-RII showed a lower peak level for the 60 min compared with the 20 min infusion. None of 14 pts showed an increase of anti-NGR-hTNF antibodies after treatment. NGR-hTNF induced an increase in MIP-1 beta and MCP-1 circulating levels. Preliminary results obtained with DCE-MRI showed changes in vascular parameters (kep and Ktrans) in some pts, possibly reflecting the biological activity of NGR-TNF. SD was observed in 11/28 (39%) of pts, with a median duration of 11 weeks (range 5–36).
Conclusions: NGR-TNF is well tolerated at the dose levels explored, and some biological activity was observed by DCE-MRI. The MTD has not yet been reached, and dose escalation is continuing.

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Histone deacetylase inhibitors reactivate MEIS2 in synovial sarcoma

J. Lubieniecka, T. Nielsen. *University of British Columbia, Vancouver, Canada*

Synovial sarcoma (SS) is a soft tissue malignancy affecting young adults for which no effective systemic therapy currently exists. SS bears t(X;18) producing the SYT-SSX fusion oncoprotein, a composite transcriptional cofactor which drives oncogenesis by incompletely understood mechanisms. Evidence to date suggests that SYT-SSX modulates chromatin structure thereby altering expression of multiple genes. Because SS has histology resembling undifferentiated mesenchyme, we have investigated gene expression profiles of primary SS, in comparison with other sarcomas, for downregulation of genes driving mesenchymal differentiation and identified several candidates. In separate studies we and others have found that histone deacetylase inhibitors (HDACi), drugs which are thought to reverse gene silencing by promoting histone acetylation and chromatin relaxation, halt proliferation and induce apoptosis in monolayer, three-dimensional spheroid cell culture and xenograft models of SS. For this reason, we investigated the effect of a HDACi, depsipeptide (FK228, NSC 630176), on expression of MEIS2, a gene essential for limb development and differentiation which is downregulated in primary SS. SS cells were treated with 0.5, 1 and 5 ng/μl depsipeptide for 6, 12, 24 and 48h. MEIS2 expression was assessed by qPCR and promoter acetylation of the gene was investigated by chromatin immunoprecipitation (ChIP) assay using antibodies against acetylated histones H3 and H4. By 24h, the expression of MEIS2 increased 9, 11 and 13 fold with 0.5, 1 and 5 ng/μl depsipeptide treatment, respectively, and increased even more after 48h treatment. The results of ChIP assay showed increased histone H3/H4 acetylation of the MEIS2 promoter after 24h treatment with 0.5 ng/μl and higher doses of depsipeptide. The observed activation of MEIS2 immediately preceded cell