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

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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

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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)

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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

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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

apoptosis. Our results suggest that MEIS2 is direct target of HDACi in SS and supports a mechanism of cell killing through re-activation of repressed genes driving mesenchymal differentiation.

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Localization of human TACC3 to centrosomes is mediated by phosphorylation on serine 558 by aurora a; a novel pharmacodynamic method for measuring aurora a activity

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Aurora A is a serine/threonine protein kinase essential for normal mitotic progression. Aberrant increased expression of Aurora A, which occurs frequently in human cancers; results in mitotic spindle defects, chromosome instability and possibly tumorigenesis. Aurora A localizes to centrosomes and the proximal mitotic spindle during cell division and phosphorylates a variety of microtubule-associated proteins, including TACC3. TACC3 forms a complex at the centrosomes with ch-TOG where it modulates microtubule stabilization of the mitotic spindle. Recent studies identified a conserved serine in Xenopus (Ser626) and Drosophila (Ser863) TACC3 orthologs that is phosphorylated by Aurora A. We demonstrate that this conserved serine on human TACC3 (Ser558) is also phosphorylated by Aurora A *in vitro*. Moreover, phosphorylation of TACC3 by Aurora A is essential for its' proper localization to centrosomes and proximal mitotic spindles. Exogenously expressed wild type TACC3, but not Ser558 to Ala558 mutant, localize to centrosomes in cultured human tumor cells. Inhibition of Aurora A in cultured human tumor cells with the selective small molecule inhibitor MLN8054 results in mislocalization of endogenous TACC3 away from centrosomes in a dose-dependent manner. Furthermore, oral administration of MLN8054 to mice bearing human tumor xenografts also disrupted TACC3 localization to centrosomes detected in tumor sections. In summary, this work introduces a novel pharmacodynamic method for measuring Aurora A activity by quantifying the loss of TACC3 from centrosomes and proximal mitotic spindles.

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The anticancer agent, ECO-4601, is a potent inhibitor of the Ras-mitogen-activated protein kinase pathway

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Background: ECO-4601 (MW 462), a farnesylated dibenzodiazepinone, is a promising new chemical entity discovered through Ecopia's Decipher® technology, a proprietary drug discovery platform. The compound was shown to have a broad cytotoxic activity (low μM range) when tested in the NCI 60 cell line panel. Antitumor evaluation in human glioma, breast and prostate xenografts indicated that ECO-4601 had potent antitumor activity (EORTC-NCI-AACR 2005, Abstract #2910). Although the compound binds the peripheral benzodiazepine receptor (PBR) (AACR 2006, Abstract # 5896), transcriptome analysis and antitumor data suggest that other mechanisms are involved. Related to its farnesylated moiety, the effect of ECO-4601 was assessed on the Ras signaling pathway.
Material and Methods: We first verified if ECO-4601 interfered with Ras processing by monitoring farnesyltransferase (FPTase) and geranylgeranyl transferase (GGPTase I) activities. Downstream Ras signaling events, such as Raf-1 and ERK1/2 phosphorylation, were also evaluated by immunoblots in prostate (PC-3), breast (MCF7 and MDA-MB-231) and glioma (U-87 MG) cell lines. Cells were treated with 10 μM ECO-4601 for 30 min, 1, 4 and 6h. Subsequently, half of the treated cells were exposed to EGF (50 ng/ml) for 10 min.
Results: No mobility shift of either HDJ2 or Rap1A (specific surrogate markers of FPTase and GGPTase I, respectively) were observed in PC-3 or MCF7 cells exposed to ECO-4601 for up to 48h. In contrast, a strong inhibition of EGF-induced phosphorylation of *c-Raf-1* and *ERK1/2* in the four cell lines tested was shown. This effect was time dependent with *complete inhibition* of protein phosphorylation within 6 h.
Conclusions: Our data suggest that ECO-4601 is a potent inhibitor of the Ras-mitogen-activated protein kinase pathway. The inhibitory activity appears to be prior to Raf-1 phosphorylation and post prenylation. ECO-4601 is presently being tested in a Phase I clinical trials against solid tumors.

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Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma

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Background: Sorafenib is an oral multi-kinase inhibitor with anti-angiogenic and anti-proliferative activity that targets the Raf/MEK/ERK pathway at the level of Raf kinase and receptor tyrosine kinases VEGFR-1/-2/-3 and PDGFR-. Sorafenib has demonstrated efficacy against several tumor types, including hepatocellular carcinoma (HCC), in Phase I/II trials. This Phase I trial was conducted to evaluate the pharmacokinetics (PK), safety and tolerability, and preliminary efficacy of sorafenib in Japanese HCC patients with underlying liver dysfunction.
Material and Methods: Patients with histologically confirmed, unresectable HCC, Child–Pugh status A or B, and adequate organ functions were treated. A single dose of sorafenib was administered, followed by a 7-day wash-out period. After the wash-out period, patients received either sorafenib 200 mg (Cohort 1) or 400 mg (Cohort 2) twice daily (bid) for 28 days (Cycle 1). From Cycle 2 onwards, patients continued sorafenib until disease progression or intolerable toxicity. The tolerability at each dose level was correlated with Child–Pugh class. Efficacy was evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST).
Results: A total of 27 patients were enrolled; 24 patients were evaluable for PK, tolerability, and efficacy. Despite a relatively high level of interpatient variability in PK, both AUC_{0-12} and C_{max} at steady state were slightly lower in Child–Pugh B patients. Common adverse drug toxicities included elevated lipase (85.2%), rash/desquamation (40.7%) and hand–foot skin reaction (HFSR; 33.3%). A dose-limiting toxicity of HFSR was observed in one patient (Cohort 2). One patient (4%) achieved a partial response (PR), 20 (83%) had stable disease, and three (13%) had progressive disease. The patient who experienced PR continued sorafenib treatment for $\geqslant$1 year. For the 27 patients, median progression-free survival was 4.9 months and median overall survival was 15.6 months.
Conclusions: Sorafenib demonstrated favorable tolerability in Japanese HCC patients. There were no clinically relevant differences in PK and safety between Child–Pugh A and B patients. Further studies of sorafenib are warranted in HCC, based on its encouraging safety profile and preliminary anti-tumor activity.

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COX-2 expression by Wnt signaling activation decides radiosensitivity of head and neck cancer in association with regulation of Ku 70/80

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Background: Ku70/80 is an important molecule in repair of DNA damage by irradiation to head and neck cancer. However, little is known of molecular mechanisms regulating Ku 70/80 expression. We examined role of Wnt signaling and Ku70/80 expression and their associations with cyclooxygenase-2 (COX-2) in the radioresistance mechanism of head and neck cancer.
Material and Methods: COX-2, β-catennin, and Ku 70/80 changes before and after irradiation to a head and neck cancer cell line, AMC-HN3, having moderate radiosensitivity, were examined by immunflurescence, western blotting, and real-time PCR in a condition of pretreatment with by 1 μM (2′Z,3′E)-6-bromoindirubin-3′-oxime (BIO). By BIO treatment following 4-Gy irradiation, viability change and radiosensitivity of the cancer cells were analyzed by FACS and clonogenic assays. Radiosensitivity of the cancer cells after transfection with COX-2 siRNA or celecoxib, a selective COX-2 inhibitor was observed by the change of Ku70/80 by western blotting.
Results: Activation of Wnt signaling pathway, increased β-catennin level, by BIO treatment resulted in the increased expression of COX-2 and Ku 70/80, increasing radioresistance of the irradiated cancer cells. COX-2 suppression by siRNA or celecoxib induced no significant change Ku70/80 level in the condition of Wnt signaling activation by pretreatment of BIO and recovered the radiosensitivity of the cancer cells, suggesting that COX-2 may play a role in the radioresistance mechanism by Ku70/80 induction.
Conclusions: COX-2 expression by Wnt signaling activation is a key molecule in regulating Ku70/80 induced by radiation and thus, in the radioresistant mechanism. This may include a therapeutic implication that the suppression of Ku70/80 or COX-2 contributes to increasing the radiosensitivity of head and neck cancer.