By the means of quantitative real-time PCR, we measured the expression levels of 23 genes involved in apoptosis, proliferation and endoplasmic reticulum stress in Jurkat cells RNA, extracted from ionomycin/PMA treated and non-treated cells. The expression data were normalized to the expression levels of four housekeeping genes and cDNA concentration. Our preliminary results show that in the Jurkat cells, in the absence of exogenous SCF (c-kit ligand), ionomycin/PMA treatment down-regulates the expression c-kit receptor and induces the moderate up-regulation of both pro-apoptotic and pro-survival genes. The increased expression of IL-2, NFkB, JNK, ERK, XBP and GADD34 genes, together with the down-regulation of c-kit, show that the ionomycin/PMA treatment induces the proliferation, inflammation and differentiation processes, independently from c-kit activation.
According to our data, the up-regulation of the genes involved in Jurkat cells proliferation and endoplasmic reticulum stress, does not disturb the balance between pro- and anti-apoptotic Bcl-2 family genes upon the ionomycin/PMA treatment.

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New curcumin analogues show enhanced antitumour activity in malignant melanoma cells

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Background: Malignant Melanoma (MM) is one of the fastest growing cancer in western populations with the incidence having tripled in the last decades. Chemotherapy, immunotherapy and vaccines are still unsatisfactory thus new approaches for MM treatment are urgently needed. Curcumin, a natural spice extracted from the root of *Curcuma longa* L. and largely used in oriental cuisine and medicine, has recently been described as potential anticancer agent. We tested several curcumin-related compounds for their capability to inhibit cell growth on primary MM cell lines.
Material and Methods: Viability and antiproliferative assays together with dose and time-response assays have been carried out on MM cell lines to compare antitumour activity of curcumin to that of six related biphenyls. Cultured fibroblasts from healthy donors have been used as controls. DNA fragmentation with ELISA and TUNEL assays have been performed to assess apoptosis triggered by some of the treatments.
Results: Curcumin, a natural compound already known for its antitumour activity, showed to be a potent antiproliferative agent on our MM cells. We tested six curcumin-related hydroxylated biphenyls (D2-D7) on MM cells to assess their potential antitumour activity in comparison with that of curcumin: IC50 values established after 5 days of treatments showed the α,β-unsaturated keton (D6) the most efficient at concentrations around 1−2 μM, much lower than the IC50 values calculated for curcumin (about 10 μM). Fibroblasts proliferation rate was not affected in the same conditions. Wash-out experiments further demonstrated that the D6 action was more powerful and rapid in arresting MM cells growth than that of curcumin, giving rise to irreversible effects after only 2−4 hours of co-culture with MM cells. Clonogenic assays were performed to measure long-term effects of D6 on permanent cell growth arrest and cell death, showing a dose-dependent reduction in MM colony formation. ELISA and TUNEL assays on some of the MM cell lines allowed the detection of oligonucleosomes in the cytoplasm and apoptotic bodies in the nucleus, showing involvement of apoptosis in D6 activity.
Conclusions: Our results indicate this compound as good lead to develop new therapeutic agents against MM. D6 activity should be further investigated on in vivo melanoma models to assess the real anticancer effectiveness on such tumour.

Aurora kinase

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MLN8054, a selective inhibitor of Aurora A kinase: final results of a phase I clinical trial

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Background: MLN8054 is an oral, selective, small-molecule inhibitor of Aurora A kinase. This phase I clinical trial examined the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of MLN8054 administered over two weeks in a 28-day cycle.
Materials and Methods: Patients (pts) with advanced solid tumors at 2 centers were enrolled. Cohorts of 3−6 pts received successively increasing doses until dose-limiting toxicity (DLT) was seen in $\geqslant$2 pts. The first 2 cohorts received 10 and 20 mg once daily (QD) on days 1−5 and 8−12. Subsequent cohorts were treated on days 1−14 with 25, 35, 45, 55, 60, 70, and 80 mg/day in four divided doses (QID) with the largest dose at night to mitigate against benzodiazepine-like effects, such as somnolence. Starting at the 45 mg dose level, oral methylphenidate (MP) 5−15 mg was also permitted during daytime dosing. Serial blood samples were collected to estimate PK. Skin and tumor biopsies were obtained before and after dosing to assess accumulation of mitotic cells as a measure of PD effects.
Results: Of the 44 pts enrolled, 43 were treated with MLN8054. Pts received a median of 1 cycle (range, 1−10). DLT included reversible Grade 3 benzodiazepine-like effects, primarily somnolence (n = 3), and reversible Grade 3 liver function test (LFT) elevations (n = 2). Dose-escalation was stopped at 80 mg/day because of DLTs of somnolence despite prophylactic therapy with MP (1 pt), and LFT elevation (1 pt). Grade 2 neutropenia and alopecia (1 pt) and mucositis (1 pt) were first observed at the highest dose level of 80 mg. Mean exposure levels were roughly linear with dose. The terminal half-life was 30−40 hours. Among skin biopsies evaluable pre- and post-treatment in 40 pts, there was sporadic evidence of accumulation of mitotic cells in basal epithelium within 24 hours after the first daily dose or at steady-state. Among tumor biopsy specimens evaluable pre- and post-treatment in 14 pts, there was evidence of Aurora A inhibition as measured by multiple mechanistic PD markers, especially at the higher doses.
Conclusions: MLN8054 dosing for up to 14 days of a 28-day cycle was feasible. Somnolence and LFT elevation were dose-limiting ahead of clinical anti-proliferative effects. Skin and tumor biopsy findings supported Aurora A kinase inhibition. MLN8054 has been replaced in clinical trials by MLN8237, a more potent second-generation Aurora A kinase inhibitor.

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Phase I study of the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of MLN8237, a selective Aurora A kinase inhibitor, in the United States

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Background: Preclinical studies suggest the selective Aurora A kinase inhibitor, MLN8237, is more potent than MLN8054 and less likely to cause benzodiazepine-like effects. This ongoing phase I clinical trial examined the safety, PK, and PD of MLN8237.
Materials and Methods: MLN8237 was given orally once daily (QD) for 7 days in 21-day cycles. Cohorts of 3 patients (pts) with advanced solid tumors were enrolled to increasing dose cohorts (5, 10, 20, 40, 80, and 150 mg/day) until dose-limiting toxicity (DLT) was seen in $\geqslant$2 of 6 pts. Serial blood samples were collected to estimate PK. PD effects on Aurora A kinase were inferred from accumulation of mitotic cells in the basal epithelial layer of skin biopsies.

Results: All 23 pts enrolled by 15-Apr-08 received ⩾1 dose of MLN8237, with a median of 2 cycles (range, 1−11+), and 9 (39%) pts were still on study. No DLTs were observed for 5−80 mg. At 80 mg 2 pts experienced G1−2 neutropenia on days 8 or 15. At 150 mg, 3 of 6 pts had DLTs: (a) prolonged G3−4 neutropenia requiring delay of cycle 2 at a reduced dose of 80 mg; (b) G3 mucositis/oral candidiasis requiring hospitalization and dose reduction to 80 mg for cycle 2; (c) G3 somnolence with concurrent initiation of a long-acting opiate and confusion/agitation on day 2 (MLN8237 was discontinued). Alopecia was seen in 1 pt at 80 mg and 2 pts at 150 mg. MLN8237 was rapidly absorbed (mean T_{max}, 1−4 h). Mean $AUC_{0-24\,h}$ and C_{max} increased with dose. At 150 mg, mean steady-state C_{max} was 4.6 μM and mean steady-state $C_{24\,h}$ was ⩾1 μM, the estimated efficacious exposure in preclinical models. Day 7 concentrations were ⩾1 μM for ⩾12 h in 1 of 3 pts at 80 mg (C_{max}, ~4 μM) and 4 of 6 pts at 150 mg (C_{max}, ~5 μM). Mean $t_{1/2}$ was 30−40 h for 5 to 80 mg, and 15 h at 150 mg. One pt had preliminary evidence of antitumor activity after 2 cycles (cycle 1, 150 mg; cycle 2, 80 mg) in platinum-refractory, radiation-resistant, metastatic ovarian cancer. 4 pts received 6−13+ cycles. Effects of MLN8237 in serial skin biopsies will be presented to support proof-of-mechanism.
Conclusions: Dosing with MLN8237 for 7 days in 21-day cycles was well-tolerated. Anti-proliferative clinical effects of MLN8237 were first noted at 80 mg and DLTs were seen at 150 mg. Clinically significant benzodiazepine-like side effects were not observed, except when MLN8237 was administered with long-acting opiates. Planned dose groups include 110 mg QD and 70–100 mg twice daily. Lower doses over 14−21 days will be evaluated.

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Phase I and pharmacokinetic study of MLN8054, a selective inhibitor of Aurora A kinase

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Background: MLN8054 is a selective small-molecule inhibitor of Aurora A kinase. This phase I clinical trial examined the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of MLN8054.
Materials and Methods: MLN8054 was given orally for 7−21 consecutive days followed by 14 days break (21−35 day cycles). Cohorts of 3−6 patients (pts) with advanced solid tumors at 3 centers in the US were enrolled in escalating cohorts until dose-limiting toxicity (DLT) was seen in ⩾2 of 6 pts. Serial blood samples were collected to estimate PK. The PD effects of MLN8054 on Aurora A kinase were inferred from accumulation of mitotic cells in basal epithelium in 2−3 mm skin biopsies obtained before and after dosing.
Results: 61 pts were treated (38 men, 23 women, median age 60). Tumor types included gastrointestinal (30), lung (9), genitourinary (8), sarcoma (8), breast (3), and other (3). Dose levels evaluated were 5, 10, 20, 30 and 40 mg/day in single daily doses (QD) for 7 days; 25, 35, 45 and 55 mg/day in four divided doses (QID) for 7 days; and 55, 60, 70 and 80 mg/day in QID doses for 7−21 days plus methylphenidate (MP) 5−10 mg with daytime doses to reduce somnolence. Pts received a median of 2 cycles (range, 1−14). Maximum tolerated doses were 30 mg/day for QD dosing, 45 mg/day for QID dosing, and 60 mg/day for QID dosing plus MP. Reversible Grade 3 benzodiazepine-like effects, especially somnolence, were the DLTs in all dosing permutations, usually starting in the first week of dosing. No dose studied was associated with significant myelosuppression or mucositis. MLN8054 was rapidly absorbed and exposure was dose-proportional. Terminal half-life was 30−40 hours. Skin biopsies were evaluable both pre- and post-treatment in 52 pts. Accumulation of mitotic cells was observed within 24 hours after either the first or last daily dose; the level of this effect varied by pt. While no RECIST responses were seen, 3 pts had stable disease for more than 6 cycles.
Conclusions: MLN8054 dosing for up to 21 days of a 35-day cycle was feasible. Despite divided daily dosing and MP, reversible benzodiazepine-like effects, especially somnolence, continued to be dose-limiting. PD studies suggested that Aurora A was inhibited in the skin of some pts. Studies of MLN8237, a second-generation Aurora A kinase inhibitor, are ongoing.

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Preliminary results of a Phase I accelerated dose-escalation, pharmacokinetic and pharmacodynamic study of PF-03814735, an oral Aurora kinase A and B inhibitor, in patients with advanced solid tumors

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Background: Aurora kinases are a family of kinases that are key regulators of mitosis and cytokinesis, and have been associated with carcinogenesis. Aurora kinase A is commonly amplified in solid tumors and has been established as an oncogene. Aurora B over-expression in tumors leads to defects in mitosis and is associated with increased invasiveness. PF-03814735 is a novel oral ATP-competitive, reversible inhibitor of Aurora A and B kinases with a broad spectrum of preclinical antitumor activity.
Material and Methods: This is an ongoing dose-escalation study to identify the Maximum Tolerated Dose (MTD) and Recommended Phase II Dose, to assess the pharmacokinetics (PK), and to obtain proof-of-mechanism (by assessment of pH3 inhibition in tumor biopsies and FDG-PET) with PF-03814735 administered daily for 5 or 10 consecutive days q3w.
Results: In the 5-day schedule, 25 patients received a median of 2 cycles (1−8) across 7 dose levels from 5–100 mg/day. The most common primary diagnoses in this cohort were non-small cell lung cancer, colorectal cancer and malignant melanoma. Dose-limiting febrile neutropenia was observed in 2/7 patients treated at 100 mg/day. The most commonly observed treatment-related adverse events were mild to moderate diarrhea (44%), vomiting, anorexia, fatigue, (25% each) and nausea (20%). MTD expansion for safety and pharmacodynamics is currently ongoing. No objective response has been observed so far. Serum exposure of PF-03814735 (Cmax and AUC) increased in a dose-proportional manner at all dose levels tested, indicating a linear PK at least up to 100 mg/day. After a single dose, the total clearance of PF-03814735 is 1.25±0.39 L/h and the median t1/2 was 20.2 h. Dose escalation on the 10-day treatment schedule is ongoing. Three patients have been treated at 40 mg/day, and 2 patients have been treated at the next dose level (50 mg/day).
Conclusions: For the 5-day treatment schedule, the MTD was defined as 80 mg/day. The effects of PF-03814735 on tumor metabolism and pH3 levels are currently being evaluated. The 10 day schedule is open for accrual, and results will be reported.

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Phase 1 trial of SNS-314, a novel selective inhibitor of Aurora kinases A, B, and C, in advanced solid tumor patients

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Background: The Aurora Kinases are a family of serine/threonine kinases (Aurora kinases (AK) A, B, and C) that play a key role in orderly progression through mitosis and have been implicated in a wide range of human tumors. Elevated expression levels of AKs have been detected in a high percentage of melanoma, colon, breast, ovarian, gastric, and pancreatic tumors, and in a subset of these tumors the AURKA locus (20q13) is amplified. SNS-314, a novel aminothiazole-derived urea, is a selective inhibitor of AKs A, B, and C with IC50 values in the low nanomolar range.
Methods: The trial is a standard 3+3 phase 1 dose escalation study design. Patients (pts) with advanced solid tumors were treated with SNS-314 given as a three hour IV infusion once weekly X 3 (28 day cycle). Primary endpoints of the study are: safety, tolerability, and DLT assessment. Secondary endpoints of the study include: pharmacokinetic (PK) evaluation on Days 1 and 15 and pharmacodynamic (PD) evaluation. PD evaluation assesses histone H3 phosphorylation (pHH3) in cells obtained by punch skin biopsies.
Results: A total of 19 pts (10M/9F) have been enrolled into 5 cohorts: median age = 59 (range 38−66). The initial dose was 30 mg/m^2 with subsequent dose escalation doubling until first observation of clinically significant ⩾Grade 2 related toxicity then according to a modified Fibonacci schema. No dose limiting hematological or non-hematological toxicities