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Regulation of matrix metalloproteinase 3 by tumor-associated mutant E-cadherin variants

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Background: Tumor progression is characterized by loss of cell adhesion and increase of invasion and metastasis. The cell adhesion molecule E-cadherin is frequently down-regulated or mutated in tumors. In addition to down-regulation of cell adhesion, degradation of the extracellular matrix by matrix metalloproteinases is necessary for tumor cell spread. To investigate a possible link between E-cadherin and matrix metalloproteinase 3 (MMP-3), we examined expression of MMP-3 in human MDA-MB-435S cells stably transfected with wild-type (*wt*) or three different tumor-associated mutant E-cadherin variants with alterations in exons 8 or 9, originally identified in gastric carcinoma patients.
Materials and Methods: MMP-3 expression in lysates and supernatants of MDA-MB-435S transfectants was determined by Western blot analysis. The effect of NNGH or MMP-3 specific siRNA on cell motility was assayed by time lapse laser scanning microscopy.
Results: In the presence of *wt* E-cadherin, the MMP-3 protein level was decreased in cellular lysates and in the supernatant where a secreted form of the protein is detectable. Down-regulation of MMP-3 was not found in MDA-MB-435S transfectants expressing mutant E-cadherin variants which indicates that E-cadherin mutations interfere with the MMP-3 suppressing function of E-cadherin. We have previously found that cell motility is enhanced by expression of the mutant E-cadherin variants used in this study. Here, we found that application of the synthetic inhibitor of MMP-3 NNGH and small interfering RNA (siRNA) directed against MMP-3 reduce mutant E-cadherin-enhanced cell motility.
Conclusions: Taken together, our results point to a functional link between MMP-3 and E-cadherin. MMP-3 is differentially regulated by expression of *wt* or mutant E-cadherin. On the other hand, MMP-3 plays a role in the enhancement of cell motility by mutant E-cadherin. Both observations may be highly relevant for tumor progression and metastasis since they concern degradation of the extracellular matrix and tumor cell spread.

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Clinical significance of insulin-like growth factor-binding protein-3 expression in advanced gastric cancer

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Insulin-like growth factor (IGF) family proteins play a pivotal role in regulating cell growth and apoptosis in normal and tumor tissues, and their m itogenic and antiapoptotic activities are mainly modulated by a family of high-affinity insulin-like growth factor binding proteins (IGFBPs). Among them, IGFBP-3 is the major binding protein of IGFs and modulates the bioactivity of IGFs. Even though it is well known that IGFBP-3 plays an important role in cell proliferation, the expression of IGFBP-3 and its significance in advanced gastric cancer (AGC) samples are unknown. This study explored IGFBP-3 expression in tumor samples using tissue microarray technique from 227 patients to determine if the expression status of IGFBP-3 influences the prognosis of patients with AGC who underwent curative resection followed by adjuvant chemoradiation therapy (Park et. al., *Ann Oncol* 14:1373–7, 2003). Two-sided statistical analyses were performed to correlate the clinical parameters and the prognostic effect with the IGFBP-3 expression level in this cohort. Reduced IGFBP-3 expression was found in 121 (53.3%) of 227 samples, and it was more frequent in diffuse type than in intestinal type (P=0.01). However, down-expression of IGFBP-3 was not associated with the other clinicopathological parameters tested, such as age, sex, histology, Bormann type, tumor location and staging. In a univariate analysis, patients with decreased IGFBP-3 expression had shorter overall (P=0.03 by log-rank test) and disease free (P=0.05 by log-rank test) survival rates than did patients with normal IGFBP-3 expression. In subgroup analysis, s ignificant statistical correlation between IGFBP-3 expression and disease-specific survival was noted on ly in patients with stage I or II disease. This study demonstrates that reduced expression of IGFBP-3 is a frequent event in advanced gastric cancer and correlates with the disease-specific survival probability of patients, especially patients with stage I or II disease. These results suggest that IGFBP-3 functions as a tumor suppressor and plays an important role in determining biological aggressiveness especially in early stage AGC. Thus, IGFBP-3 is a good target to develop the new treatment strategies for AGC.

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Potent antiproliferative effects of Src kinase inhibition in a model of neuropeptide-induced androgen-independent prostate cancer

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Background: Cells with neuroendocrine (NE) differentiation are found in cancer of the prostate (CaP) and may facilitate the transition to androgen independence by supplying alternate growth factors. Androgen deprivation further induces a subpopulation of androgen-independent NE cells by transdifferentiation. Androgen receptor (AR)- and Src kinase-mediated signalling may participate in this NE differentiation.
Materials and Methods: The neuropeptide gastrin-releasing peptide (GRP) was minimally expressed in androgen-sensitive LNCaP cells. LNCaP-GRP clones demonstrated androgen- and anchorage-independent growth, developed orthotopic tumours in castrated nude mice and activated prostate-specific antigen (PSA) with nuclear localisation of AR. Mouse xenografts were recultured (LNCaP-Pro) and grown in androgen-free soft agar or tissue culture either alone, with synthetic androgen R1881 or with inhibitors including: GRP monoclonal antibody 2A11, 10 μM antiandrogen bicalutamide (bic), Src kinase inhibitors AZM475271, AZD0530 (AstraZeneca, 5 μM) and PP2 (EMD Biosciences, 10 μM).
Results: Growth of LNCaP-GRP cells in castrated mice with activation of PSA and nuclear localisation of AR suggested GRP activation of AR. Androgen-depleted soft agar colony counts are presented. Compared with controls LNCaP-Pro colony formation was not stimulated by R1881. Incubation of LNCaP-Pro cells with GRP 2A11 antibody or bic caused partial inhibition of colony formation; however, when both were used in combination, significant growth reduction ($p<0.05$) resulted compared with control. R1881 mostly reversed the GRP 2A11 inhibition of colony formation. The Src kinase inhibitors AZM475271 and PP2 showed greatest colony formation inhibition ($p<0.05$) [Figure]. To date, AZD0530 has only been tested *in vitro*, but has shown significant growth inhibition compared with untreated or bic-treated cells.
Conclusions: Our model of neuropeptide-mediated androgen-independent CaP growth is dependent on both GRP and AR. Importantly, we find a role for Src kinase inhibition in this model, which may have therapeutic implications for patients with androgen-independent CaP.
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Reduction of in vitro metastatic potential of tamoxifen-resistant breast cancer cells following inhibition of Src kinase activity by AZD0530

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Background: Src kinase plays a central role in many growth factor signalling pathways regulating a diverse array of cellular functions including proliferation, metastasis, invasion and cell survival. Recent studies have demonstrated that Src activity is frequently elevated in human tumours and appears to correlate with disease stage. We have previously shown that, upon acquisition of tamoxifen resistance, MCF7 cells display increased levels of EGFR and a more aggressive phenotype *in vitro*. Since tumours with elevated EGFR signalling have been reported to have elevated levels of Src activity, we investigated the expression and role of Src in our MCF7 tumour model of endocrine resistance (Tam-R cells).
Materials and Methods: Src expression was measured by RT-PCR and Western blotting; Src activity was determined by Western blotting using a phospho-specific Src antibody. To obtain the optical density (OD), Western blots were scanned and analysed using a densitometer. Modulation of Src activity in Tam-R cells was achieved using the potent and selective Src kinase inhibitor AZD0530 (AstraZeneca). Cellular invasion was assessed by seeding 5×10^4 cells onto a Matrigel-coated porous membrane in the presence or absence of 1 μM AZD0530. After 3 days, cells that had migrated to the underside of the membrane were fixed, stained with DAPI and counted using a fluorescence microscope. Cell migration was determined by seeding cells onto a fibronectin-coated, porous surface and allowing migration to occur for 24 h. Migrated cells were then visualised by staining with crystal violet and counted. Changes in FAK activity were determined by Western blotting following treatment of cells with or without AZD0530.

Results: No significant change was observed in Src mRNA levels and a small increase (~1.9 fold) in Src protein level was observed in Tam-R cells compared with their non-resistant counterparts (wtMCF7). Src kinase activity was significantly elevated in Tam-R cells (20.5±3.8 [SD]-fold increase in Tam-R cells vs wtMCF7). Treatment of Tam-R cells with the Src kinase inhibitor AZD0530 significantly reduced the amount of Y418-phosphorylated Src detectable, measured as OD (mean expression of Src ± SD was 33.08±5.04 [control] and 9.13±3.86 [+AZD0530], $p<0.02$), but had no effect on total Src levels. AZD0530-mediated Src inhibition significantly reduced both cellular invasion (mean invading cells/field ±SD: 50±6.4 [control] and 17±1.4 [+AZD0530], $p<0.05$) and motility (mean migrating cells/field ± SD: 35.2±7.3 [control] and 12.57±4.32 [+AZD0530], $p<0.05$) in Tam-R cells. Further studies revealed that inhibition of Src by AZD0530 reduced basal levels of activated FAK and paxillin, and inhibited both basal and TGFα-stimulated MAPK activity (ERK1/2).
Conclusion: Src kinase plays a central role in mediating the enhanced *in vitro* metastatic phenotype of endocrine-resistant breast cancer cells and thus presents a potential target for future therapies.

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Mitogen-activated protein kinase phosphatase-1 (MKP-1) expression correlates with responsiveness of human tumor xenografts to receptor tyrosine kinase inhibition

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Background: The benefit of chemotherapies in non-small-cell lung cancer (NSCLC) remains modest. Frequent overexpression of the epidermal growth factor receptor (EGFR) in NSCLC raises the opportunity to improve chemotherapy by combination with antiEGFR compounds. Gefitinib is a selective EGFR-tyrosine kinase inhibitor recently introduced to clinical practice.
Purpose: In this study, we investigated the efficacy of gefitinib using 5 NSCLC human xenografts, and searched for molecular determinants of the response to RTK's inhibitors in these human tumor models.
Material and Methods: Five NSCLC tumors were obtained from patients cared at the Curie Institute and Marie-Lannelongue Hospital, and were established as xenografts by subcutaneous implantation of fresh tumor fragments into nude swiss mice. The growth of each tumor was measured twice weekly with a caliper. Gefitinib was given at 120 mg/kg, daily per os for 2 weeks. Individual tumor growth inhibition was calculated as a ratio between the RTV of individuals divided by the mean relative tumor volume (RTV) of controls at the same day where the optimal effect is obtained. A percentage of individuals displaying drug-responses was calculated for each treatment. Expression of EGFR and mitogen-activated protein kinase phosphatase-1(MAPK phosphatase-1) was determined by real-time RT-PCR.
Results: Gefitinib, significantly inhibited the growth of 4 of the 5 different tumors in a dose-dependent manner, regardless of histological type. Overall, gefitinib produced a significant number of responses in NSCLC xenografts (41/70 mice). The response was independent of EGFR expression, but dependent on the NSCLC tumor, some being sensitive (50% complete regression), and others resistant to gefitinib. Low MKP-1 mRNA levels were found in gefitinib-responsive tumors as opposed to the high MKP-1 expression in resistant tumors.
Conclusion: this study has confirmed the therapeutic potential of receptor tyrosine kinase inhibition with gefitinib in a subset of NSCLC tumors. Optimal therapeutic use of such inhibitors requires a previous knowledge of determinants of tumor response. This study identifies MKP-1 as a potential marker of NSCLC response to EGFR inhibition.

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A phase II study of erlotinib in combination with cisplatin in patients with recurrent or metastatic squamous cell cancer of the head and neck (PHL-002e//NCIC CTG IND.157)

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Background: The epidermal growth factor receptor (EGFR) is a molecular target of interest in recurrent or metastatic squamous cell cancer of the head & neck (RMHNC). Erlotinib is an oral, selective EGFR tyrosine kinase inhibitor. Prolonged disease stabilization in refractory RMHNC patients has been reported with single agent erlotinib (Soulieres et al, J Clin Oncol 2004). In vivo, erlotinib combined with cisplatin produced additive antitumor effects without increased toxicity (Pollack et al, J Pharm Exp Ther 1999). The RP2D of erlotinib+cisplatin in RMHNC patients was determined in the phase I portion of this study & then tested in this phase II cohort to determine efficacy & toxicity.
Patients and Methods: Patients ECOG PS 0–2 with no prior chemotherapy for RMHNC & measurable disease were treated with erlotinib 100 mg po/g-tube daily continuously & after a 1 week run-in then started cisplatin 75 mg/m^2 IV Q3weeks in a 2-stage phase II trial. Investigations included PK, scans, tumor & skin biopsies & assessment of EGFR status in archival tumor slides/blocks. The primary endpoint was ORR determined by RECIST criteria.
Results: Thirty-five patients have been enrolled at the phase 2 dose, and 31 (25 male: 6 female) have baseline data available as of April 2003. The median age was 57 yrs (25–81), ECOG 0:1:2=7:18:6, prior radiation 31, prior chemotherapy 5, and sites of disease=nodes (13), lung (13), liver (3) & other (27). There were 4 deaths unrelated to study treatment (3 PD & 1 hemorrhage). Follow-up toxicity data is complete on 87 cycles in 26 pts (AEs reported regardless of attribution). Grade 3 or 4 AEs occurred in <10% of cycles except lymphopenia (21%), pain (17%) & hyponatremia (11%). The most common AEs of any grade were as follows (% of pts). Hematological: anemia 92%, lymphopenia 77%, thrombocytopenia 27% & neutropenia 19%. Nonhematological: fatigue 88%, pain 85%, rash 69%, nausea 58%, vomiting 46%, anorexia 42%, dyspnea 42%, diarrhea 38% & sensory neuropathy 38%. Biochemical: hypomagnesemia 77%, hypoalbumin 77%, hyponatremia 58%, hyperglycemia 54%, creatinine 54% & hypocalcemia 38%. Seven objective responses have been observed (1 CR, 6 PR [1 unconfirmed], 12 SD & 6 PD) in 25 evaluable pts. Four pts will be inevaluable and 2 are too early to assess. Intention-to-treat ORR 24% (7/29). Baseline EGFR status, PK and correlative studies are pending.
Conclusions: This schedule of erlotinib+cisplatin has antitumor activity comparable to standard cisplatin-based combination chemotherapy regimens in RMHNC, and may have a more favorable toxicity profile. Accrual will continue to a total of 37 evaluable pts.

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AP23573, an mTOR inhibitor, administered IV daily × 5 every other week in patients with refractory or advanced malignancies – a phase I, pharmacokinetic (PK), and pharmacodynamic (PD) study

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Background: AP23573, a novel non-prodrug rapamycin analog that inhibits mTOR, has demonstrated potent inhibition of proliferation *in vitro* in several human tumor cell lines and elicited antitumor activity *in vivo* in multiple xenograft models.
Materials and Methods: This phase I trial uses an accelerated titration scheme to determine the safety, tolerability and maximum tolerated dose (MTD) of AP23573 administered as 30-minute IV infusion daily × 5 days every 2 weeks on a 4-week cycle. Secondary objectives include characterization of the PK profile and potential PD markers in peripheral blood mononuclear cells (PBMCs) and skin biopsies, as well as a description of antitumor activity.
Results: To date, twenty-two pts (11M/11F), median age 54.5 yrs (range 23–71 yrs) have received AP23573 doses ranging from 3 to 28 mg in 5 dose level cohorts (total number of cycles 76.5; median cycles, 3/pt). Severe (grade 3) oral mucositis was the dose-limiting toxicity recorded in two pts at the 28 mg dose level, and by definition, this dose level exceeds the MTD. Therefore, additional pts have been entered at 18.75 mg, the MTD identified for this trial. Side effects deemed related to study drug for first cycle also have included reversible: grade (gr) 1 or 2 mucositis, fatigue, nausea, rash, anemia, and neutropenia; and gr 1 diarrhea, hyperlipidemias and thrombocytopenia. PK analyses (doses 3 to 18.75 mg) suggest a median estimated AP23573 half-life of 47.5 hours (range 29 to 63 hours). AUC and Cmax increase with the dose but are not dose-proportional. PD analyses (doses 3 to 28 mg) indicate rapid (within 1 hr) and generally prolonged inhibition of mTOR activity with > 80% decrease in phosphorylated 4EBP1 levels in PBMCs. Of the 16 pts evaluated, minor responses have been observed in two pts: a pt with metastatic renal cell cancer dosed at 6.25 mg for 9 months (28% overall tumor reduction), and a pt with imatinib-refractory GIST receiving 12.5 mg for > 4 months. Additionally, one pt with metastatic uterine sarcoma receiving 3 mg has stable disease lasting > 12 months. Furthermore, 9 additional pts have had stable disease lasting 2 to > 6 months.
Conclusions: AP23573 can be administered safely using this schedule, and has exhibited antitumor activity as well as evidence of a substantial PD effect. Patient enrollment at the MTD is nearly complete. Furthermore, pt dosing is ongoing as is additional evaluation of potential PD markers in PBMCs and skin biopsies.