cells compared to its resistant derivative cells, under 5-FU or TRAIL conditions. In contrast, D59 cells were sensitized by both molecules exposure simultaneously, witch increased level of apoptosis in the two cells. These results indicated that the function of the death receptors was partially unaffected in the D59 cells. So, we next investigated if modulation of the different death receptors could be involved in 5-FU apoptosis pathway. Although 5-FU treatment did not modulate mRNA of the DRs and using flow cytometry analyzes, we showed that 5-FU induction seem to increase the pro-apoptotic DR5 expression level on HCT116 cell lines whereas only the DcR1 seem to be regulated on D59 cells. In addition, siRNA–mediated down regulation of DcR1, were found to sensitize resistant colon cancer cells to the chemotherapeutic agent. In contrast, siRNA targeted of DR5 on HCT116 cells, rendered resistant these cells to apoptosis induced by 5-FU. Our work demonstrates that change in surface expression of death receptors might be a key determinant in acquired resistance to 5-FU and use of DcR1 targeted therapy might be a good strategy to overcome 5-FU chemoresistance.

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Do not die, become senescent: a new type of cellular resistance induced by topoisomerase II inhibitors in tumor cells with functional p53

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Cellular senescence is one of the mechanisms which prevents the development of cancer by eliminating cells which acquired potentially deleterious DNA mutations. Recent studies show that treatment of tumor cells with anticancer agents leads to permanent growth arrest with phenotypic features of senescent cells. In this study, we characterized effects induced by three different DNA topoisomerase II inhibitors (m-AMSA, ICRF-187 and triazoloacridone C-1305) in human lung carcinoma A549 cells. At minimal effective concentrations, all studied drugs induced permanent growth arrest in A549 cells which was accompanied by morphological and biochemical features of senescent cells, such as flat cell morphology, expanded lysosomal compartment and increased activity of senescence-associated form of β-galactosidase. Flow cytometry analysis showed that the majority of drug-treated cells arrested in G2/M and a substantial fraction of cells entered polyploidy. Expression of p53 and p21 dramatically increased in drug-treated cells as revealed by Western blot analysis. At the same time, expression of mitotic regulators, cyclin B1 and cdk1, as well as topoisomerase IIα and PARP-1 decreased to undetectable levels at drug exposure times longer than 72h.
It is believed that tumor cells which become senescent after exposure to antitumor agents are not able to regain the proliferative potential. However, after prolonged post-incubation of A549 cells (1–2 weeks) treated with minimal effective doses of topoisomerase II inhibitors, a small fraction of cells re-started cell proliferation. Interestingly, both the fraction of cells which were able to proliferate after drug treatment and the time required for proliferation recovery was different for studied topoisomerase II inhibitors. We have also shown that topoisomerase II inhibitors induced senescent phenotype followed by proliferation recovery only in cells with functional p53. In tumor cells in which p53 gene was inactivated, exposure to topoisomerase II inhibitors led to mitotic catastrophe and cell death.
Together, we propose that induction of long-term growth arrest in tumor cells by topoisomerase II inhibitors corresponds to a new type of resistance mechanism which is characteristic for tumor cells with functional p53 pathway. Further characterization of its molecular mechanisms could be important given that induction of drug-induced premature senescence is proposed to represent an alternative approach to treat human cancers.

Monoclonal antibodies and targeted toxins/nuclides

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In vitro and in vivo inhibition of functional responses at insulin-like growth factor-1/insulin hybrid receptors by h7C10, a novel humanized anti-IGF-1R monoclonal antibody

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Background: a novel monoclonal antibody (Mab, 7C10) was raised against the human insulin-like growth factor-1 receptor (IGF-1R); both murine and humanized (h7C10) Mabs exhibited potent inhibition of tumor growth in animal models (Goetsch et al., 2005). Further evaluation of their inhibitory activity at hybrid receptors (Hybrid-Rs) composed of the hetero-tetrameric association between IGF-1R and insulin receptor (IR) was performed using both *in vitro* approaches and *in vivo* animal models. Importance of Hybrid-Rs has repeatedly been reported as playing a potential role in various diseases including cancer.
Materials and Methods: R- mouse fibroblasts were stably co-transfected to express either IGF-1R or IR alone or both IGF-1R and IR, thereby expressing Hybrid-Rs. Pharmacological and biochemical *in vitro* assays were set-up to evaluate Mab activities such as [125I]IGF-1 and [125I]insulin competition binding to immuno-captured cell lysates, western blot analyses using specific antibodies to detect IGF-1R, IR or Hybrid-Rs phosphorylation, down-regulation of IGF-1R and Hybrid-Rs expression. *In vivo* experiments were performed in a xenograft model of MDA-MB-231, a non-estrogen dependent tumor cell line expressing comparable levels of IGF-1R and IR randomly assembled in Hybrid-Rs (Pandini et al., 1999), to compare the anti-tumor activity of h7C10 (binding to IGF-1R+Hybrid-Rs) with IR3 and 47–9 Mabs recognizing selectively IGF-1R and IR+Hybrid-Rs respectively.
Results: potent and full inhibition of [125I]IGF-1 binding was observed for 7C10 and h7C10 at both IGF-1R and Hybrid-Rs with affinities in the nanomolar range. On the other hand, [125I]insulin could not be displaced by these Mabs from its cognate receptor. Potent and efficacious inhibition of both IGF-1 and IGF-2-mediated IGF-1R and Hybrid-Rs phosphorylation was demonstrated. The response was similar to the control Mab 47–9. No modulation of the insulin- or IGF-2-mediated IR phosphorylation status was observed. Ligand-independent down-regulation of both IGF-1R and Hybrid-Rs was obtained upon long-term (24 hours) association with 7C10 Mab or its humanized form. Significant inhibition of the *in vivo* growth of MDA-MB-231 cells was observed with h7C10. Comparison between the *in vivo* activity of h7C10 with the one of IR3 and 47–9 Mabs showed that h7C10 had a significantly higher activity than that observed for the two other antibodies in the MDA-MB-231 model.
Conclusion: the herewith data clearly demonstrate that 7C10 and h7C10 selectively and efficaciously bind to both IGF-1R and Hybrid-Rs without affecting IR. They inhibit as well the functional signaling of IGF-1R/IR Hybrid-Rs regardless the activating ligand as well as mediate their down-regulation. These potent inhibitory properties are likely to participate in their *in vivo* anti-tumoral activities in xenograft models expressing both IGF-1R and Hybrid-Rs and may be of potential interest for therapeutic applications for the humanized Mab.

References

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A phase I trial incorporating the pharmacodynamic (PD) study of circulating tumour cells (CTC) of CP-751,871 (C), a monoclonal antibody against the insulin-like growth factor 1 receptor (IGF-1R), in combination with docetaxel (D) in patients (p) with advanced cancer

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Background: C is the first specific, fully human, monoclonal antibody to target IGF-1R in clinical trials. It potently inhibits IGF-1R signaling, enhancing D antitumor activity. This trial investigated the safety, feasibility, dose limiting toxicity (DLT), PK and antitumor activity of D administered with C every 3 weeks. PD studies evaluated CTC counts pre- and post-treatment and IGF-1R expression in CTC.
Methods: The C doses tested were 0.1, 0.4, 0.8, 1.5, 3.0, 6.0 and 10 mg/kg in sequential cohorts of 3–6 p. D was fixed at 75 mg/m^2. P achieving disease control continued on C alone if experiencing D toxicity.
Results: 27 p (26 male) have received 173 courses of C with D. 11 p received 6 or more courses of the combination. A further 34 courses of C alone have been administered. No grade (Gd) 3/4 toxicities has been attributed to C to date with the observed toxicities being attributable to D. Gd 3/4 toxicities were neutropenia (22/27 p) and neutropenic fever in 3/27 p. Gd 3 diarrhea was reported in 4 p, but this was easily controlled with antidiarrheals. Transient mainly Gd 1/2 hyperglycaemia was noted largely on day 1, following steroid premedication (20 p), but no significant C related hyperglycemia has been observed without steroids except for 1 p with Gd 2 hyperglycemia on C alone. An MTD has not been reached. Serial echocardiograms demonstrated no cardiac toxicity. Of 21 castration resistant prostate cancer (CRPC) p treated, 7 have had a confirmed PR, with 1 further unconfirmed PR. Six p have disease stabilization for >6 months (median number of courses: 10; range: 7–16). 10 p have maintained PR or SD with C alone for 1–7 courses. IGF-1R expression

in CTC was detected in 11/19 p. CTC IGF-1R was undetectable following treatment with C at doses above 3 mg/kg.
Conclusions: This combination is safe and feasible with no significant toxicity attributed to C and encouraging antitumor activity in CRPC.

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Denosumab is a selective inhibitor of human receptor activator of NF-kB ligand that blocks osteoclast formation in vitro and in vivo

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Introduction: Receptor activator of NF-κB ligand (RANKL), a member of the TNF superfamily, is an essential mediator of osteoclast formation, function, and survival. Increased osteoclast activity is critical in the pathogenesis of diseases that result from excessive bone resorption, including cancer-related bone metastasis and multiple myeloma. Denosumab is a fully human monoclonal antibody to RANKL that is in clinical trials for the treatment of bone disorders associated with pathologically increased bone resorption. Here we describe the results of studies that characterized the RANKL-binding properties of denosumab and evaluated its effects on osteoclast function in vitro and in vivo.
Methods: Denosumab binding to human RANKL (huRANKL) was determined by flow cytometry and ELISA, and the binding affinity was measured using BIAcore and a kinetic exclusion assay. The effects of denosumab on osteoclast formation in vitro were assessed using the mouse RAW 264.7 cell line. To evaluate the effect of denosumab on osteoclast function in vivo, mice were administered soluble huRANKL (twice daily at 1.0 mg/kg/day for 5 days), which produced hypercalcemia due to increased bone resorption. Concurrent with the first huRANKL dose, mice were treated with vehicle, another RANKL inhibitor, OPG-Fc (3 mg/kg), or various single doses of denosumab (1, 3, or 10 mg/kg).
Results: Binding assays showed that denosumab bound both soluble and membrane-bound forms of huRANKL. Moreover, denosumab binding to either form of huRANKL was inhibited by excess huRANKL, but not by TNF-α, TNF-β, TRAIL, or CD40 Ligand. Using BIAcore methods and a kinetic exclusion assay, the dissociation constants of denosumab were calculated to be 9.5×10^{-11} M and 3×10^{-12} M, respectively. Denosumab neutralized the ability of soluble huRANKL to stimulate the differentiation of RAW 264.7 cells into osteoclasts in vitro (IC_{50} of 1.64 ng/ml vs OPG-Fc IC_{50} of 1.15 ng/ml). Administration of either denosumab or OPG-Fc delayed the development of hypercalcemia in huRANKL-treated mice, indicating that denosumab neutralized the activity of soluble huRANKL in vivo. Denosumab caused dose-dependent suppression of hypercalcemia in this model.
Conclusion: These data demonstrate that denosumab binds human RANKL with high affinity and does not bind TNF-α, TNF-β, TRAIL, or CD40 ligand, thereby inhibiting osteoclast function in vitro and in vivo.

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Multi-targeted inhibition of the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR) pathways: a phase I study of cetuximab (C), erlotinib (E), and bevacizumab (B) in patients with solid tumors

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Background: Complex interrelationships exist between the EGFR and VEGFR pathways. EGFR activation elicits cell proliferation, and downstream effects increase expression of VEGF. In renal cell carcinoma, mutations increase hypoxia inducible factor-1alpha, stimulating VEGF and transforming growth factor expression. Moreover, there is additive tumor inhibition from combined EGFR targeting with C, and a tyrosine kinase inhibitor. To maximally inhibit EGFR, and then inhibit downstream VEGFR activity, this phase I study was initiated to determine the maximum tolerated dose (MTD) of E with a fixed dose of C, and then the MTD of B with combined E and C in patients with advanced malignancies.
Methods: Patients with advanced malignancies likely to express EGFR were entered in part 1 to daily oral E (starting at 100 mg, planned initially to increase to 150 mg), with fixed dose C (400 mg/m^2 loading and 250 mg/m^2 IV weekly). Once the MTD was determined for E in combination C, part 2 incorporated the addition of escalating doses of B (5 mg/kg IV every 2 weeks, to increase to 10 mg/kg) to the combination of E and C.
Results: 30 patients were entered and received 113 courses over 4 dose levels. In part 1 grade 3 rash occurred in 2 patients at E at 100 mg daily, and the MTD of E for this combination was 50 mg daily with standard dose C (11 patients treated). Other adverse events included rash, diarrhea, fatigue, and hypomagnesemia. Part 2: B at 5 mg/kg IV q14 days can be added to the MTD of E with C, with additional non-dose limiting toxicities of headache, proteinuria, and hypertension. There is one partial response in a patient with renal cell carcinoma. Durable stable disease has been observed in 4 patients for 7 (head and neck sqaumous cell); 10+, 12, and 12+ (renal cell) months.
Conclusions: The MTD for E combined with standard C is 50 mg daily. B at 5 mg/kg can be combined safely with this combination and dose escalation is ongoing.

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Efficacy evaluation of the humanized anti-EGFR MAb h-R3 (nimotuzumab) in combination with radiotherapy in the treatment of patients with unresectable squamous cell carcinomas of the head and neck

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Background: The incidence of head and neck tumors is worldwide increasing. High levels of Epidermal Growth Factor Receptor (EGFR) are associated with malignant transformation of squamous cells and are observed in head and neck squamous cell carcinomas (SCCHN). There is evidence of a relationship between EGFR expression and tumour cell proliferation, metastases development and radiation resistance.
Material and Methods: h-R3 (nimotuzumab) is a humanized monoclonal antibody (mAb), with high affinity and specificity to the EGFR. In *vitro* as well as *in vivo*, h-R3 demonstrated a remarkable anti-proliferative, pro-apoptotic and anti-angiogenic effect.
In order to assess the efficacy of h-R3 in combination with radiotherapy in the treatment of advanced SCCHN patients, a controlled, double blinded, Phase II clinical trial was conducted. Patients received 6 weekly infusions of a placebo or h-R3 at the dose of 200 mg. Immunohistochemical evaluation of EGFR expression in tumours was done before trial inclusion. A second biopsy was taken after the 4th dose of the mAb or placebo.
Results: Thus far, 72 evaluable patients, median age 66, with documented unresectable SCCHN have been randomly assigned to groups A or B. Ionizing radiation was delivered in doses of 2 Gy to a total dose of 66−70 Gy. Fifty-seven patients (79%) had either T3 or T4 at presentation. The most common toxicities were fatigue, anemia, fever, hypotension and cephalea. These events were classified as mild or moderate, according to the NCI-CTC scale. None of the patients had skin rash or allergic reactions. Seven patient developed grade 3 adverse events consisting in fatigue, anemia and peripheral arterial ischemia. The most frequent radiation associated toxicities were mucositis, radiodermitis and dysphagia. Objective response (complete or partial response) was achieved in 70 % of the patients, in spite of the treatment group. With a median follow up time of 23 months, the median survival is 16.50 months for all patients treated with mAb or placebo. Pre-treatment tumor biopsies as well as second biopsies were taken to compare h-R3 and placebo impact on the EGFR signal transduction cascade, proliferative activity and angiogenesis. Trial blinding will be open once 84 patients had been recruited.
Conclusions: Nimotuzumab is well tolerated. Preliminary efficacy, safety and pharmacodynamic results per treatment group are intended to be presented at the meeting.

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Strictly target cell-dependent activation of T cells by bispecific single-chain antibody constructs of the BiTE class

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Background: Bispecific antibodies have been extensively studied in vitro and in vivo for their use in redirected tumor cell lysis. A particular challenge of bispecific antibody constructs recognizing the CD3 signaling complex is a controlled polyclonal activation of T cells that, ideally, is entirely dependent on the presence of target cells. If this is not the case, systemic production of inflammatory cytokines and secondary endothelial reactions may occur as side effects, as are observed with the murine anti-human