concentration-dependent manner without receptor level alteration. Proliferation assays using HER1-, HER2- and non-overexpressing human breast carcinoma cells revealed that treatment with either D-69491 or D-70166 inhibited cell growth to similar extent. Independent of HER1 and HER2 expression levels, D-70166 inhibited proliferation of the human breast carcinoma cells MDA-MB-468, MDA-MB-453, SK-BR-3, MDA-MB-231 and MCF-7 with IC_{50} concentrations ranging between 0,86 and 2,85 μM and average maximal growth inhibition of 79% to control cells. This suggests that in addition to HER2- and HER1-driven cell growth inhibition, D-70166 exhibited unspecific cytotoxicity. Whereas the growth inhibitory effects of D-69491 on SK-BR-3, MDA-MB-453 and MDA-MB-231 cells were comparable to the effects observed after D-70166 treatment, D-69491 achieved lower inhibitory responses on MCF-7 cells and higher responses on HER1-overexpressing MDA-MB-468. Our *in vitro* data demonstrated that both D-70166 and D-69491 are potential clinical candidates, which target EGFR as well as HER2 tyrosine kinase activities.

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The potential role of STI 571 in the treatment of head and neck cancer

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The complete response of head and neck cancer to systemic therapies is often disappointing. The novel agent STI 571 (2-phenyl aminopyrimidine derivative) is designed to be effective against CML via inhibition of Bcr-Abl kinase. However the drug is known to inhibit other tyrosine kinases, including PDGFR and c-kit. There is evidence that c-kit is expressed in certain head and neck tumours including 90% of adenoid cystic carcinomas (ACC). The aim of this study is to provide pre-clinical data on the response of a panel of head and neck squamous cell carcinoma (SCC) cell lines along with primary explanted tumour cell cultures (adenoid cystic and SCC) to STI 571. We have also explored the interaction of STI 571 when given in combination with commonly used chemotherapuetic agents.. STI 571 alone shows significant growth inhibition against in both SCC cell lines and primary cultures. In SCC cell lines STI 571 was also found to be synergistic with several agents and antagonistic with gemcitabine. These Gemcytabine results were mirrored in ACC primary cultures, and a degree of synergy with other drugs was also observed. The growth inhibitory effect of STI 571 in ACC can be explained by inhibition of the c-kit receptor kinase expressed in these tumours. However this cannot explain the toxicity seen in c-kit –ve SCC cell cultures. It is proposed that this effect is mediated via an as yet unidentified kinase pathway. Likewise the reported synergy and antagonism may well be due to inhibition of other kinases. Studies are ongoing to further establish the role of these kinases in the toxicity of STI571. Furthermore work is ongoing to explore the possible role of STI571 in the treatment of c-kit +ve ACC's, both as a single agent and in combination. Clinical studies are planned to follow this.

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Cellular responses to DNA topoisomerase I poisons and the TOR kinase inhibitor, rapamycin

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Genetic analyses of cellular responses to DNA topoisomerase I (Top1) poisons in the budding yeast Saccharomyces cerevisiae suggest common pathways regulate the cytotoxicity of rapamycin, a Tor kinase inhibitor. Eukaryotic topoisomerase I plays an important role in DNA replication and recombination. Camptothecin (CPT) targets the enzyme by reversibly stabilizing a covalent Top1-DNA intermediate. During S-phase, these complexes are converted into irreversible DNA lesions as a consequence of collisions with advancing replication forks. To define cellular factors that recognize or repair Top1-induced lesions, conditional tah mutants were isolated with enhanced sensitivity to top1T722A at 35oC. The self-poisoning top1T722A mutant is a CPT mimetic that avoids issues of drug transport. Nine TAH genes (including CDC45, DPB11, DOA4, TAH11 and SLA1) were identified that protect cells from top1T722A-induced DNA damage. These mutants were hypersensitive to hydroxyurea and exhibited terminal phenotypes consistent with S-phase induced DNA lesions. Remarkably, the majority of tah mutants were also hypersensitive to rapamycin. This macrocyclic antibiotic targets the PI3-related TOR kinase (mTOR in mammalian cells, Tor1 and Tor2 in yeast) and induces cell cycle arrest in G1 phase. Yeast and mammalian Tor kinases regulate protein translation and cell cycle progression in response to growth signals and nutrient deprivation. Rapamycin has demonstrated surprising antitumor activity in clinical trials, consistent with recent reports of rapamycin-induced apoptosis observed in p53 null cells. The enhanced sensitivity of yeast tah mutants to CPT and rapamycin suggest specific alterations in S-phase potentiate the cytotoxicity of both agents. The ability of extragenic repressors of doa4-10 to suppress top1T722A- or rapamycin-induced lethality provides further support for similar mechanisms of drug action, which may be investigated in yeast. Supported by NIH grants CA23099, CA58755, CA77776 and ALSAC.

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A phase I study of ZD 1839 (Iressa) in combination with oxaliplatin, 5-fluorouracil (5-FU) and leucovorin (LV) in advanced solid malignancies

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Iressa is an oral small molecule tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR-TKI). Preclinical studies demonstrated promising anti-tumor effects using Iressa alone or in combination with chemotherapy agents in a variety of epithelial tumors. We report the results of a phase I dose-escalation study that investigates the tolerability and clinical biology of Iressa in combination with oxaliplatin, and 5-FU/LV. A sequential dose escalation of Iressa and oxaliplatin was performed. From July 2001 to April 2002, 16 patients (10 men: 6 women, median age 50.5 years, range 31-61 years) were treated. The median number of prior chemotherapy regimens was 1.5 (range 0-3). Twelve patients had stage IV adenocarcinoma of the colon, 1 patient an adenocarcinoma of unknown primary, 1 patient a squamous cell carcinoma of unknown primary, and 1 patient a basosquamous cell carcinoma of unknown primary. Three dose levels were tested. A total of 102 cycles were administered (range 1-8). A dose-limiting toxicity was seen at the second dose level (catheter-related bacteremia). One patient at the third dose level experienced a DLT with grade 3 nausea, diarrhea, and hypokalemia requiring hospitalization for intravenous hydration. This dose level (Iressa 500 mg daily, with a standard dose and a every two week schedule of oxaliplatin, 5-FU/LV) established the phase II recommended dose (MTDs) for Iressa and oxaliplatin combined with 5-FU/LV. Additional grade 3/4 toxicities were neutropenia without fever (4). Grade 2 toxicities consisted of acneiform rash (6), vomiting (5), abdominal pain (4), diarrhea (4), nausea (3), fatigue (2), mucositis (2), thrombocytopenia (2), anemia (1), anorexia (1), and personality/behavioral change (1). Grade 1 toxicities included nausea (11), sensory neuropathy (8), fatigue (7), vomiting (7), anorexia (6), diarrhea (6), abdominal pain (1), ALT elevation (1), AST elevation (1), and fever (1). Three patients with colorectal cancer had a partial response. Nine patients had stable disease after a minimum of 4 cycles of treatment. Pharmacokinetic and biological endpoint studies are ongoing. Currently, a phase II study of this regimen in colorectal cancer is in progress and these results will be presented.

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Antitumor activity of the EGFR/TK inhibitor Tarceva™ (erlotinib, OSI-774) in tumor models

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Tarceva™ (OSI-774) is a potent, orally bioavailable, small molecule inhibitor of EGFR (HER1, erbB1) tyrosine kinase. Tarceva™ inhibits phosphorylation of the EGFR tyrosine kinase domain, thereby blocking key signal transduction molecules downstream from the receptor. Currently, Tarceva™ is in advanced clinical trials for several solid tumors, including NSCLC and pancreatic cancer. Treatment of tumor-bearing animals with Tarceva™ results in significant tumor growth inhibition (TGI) and regression in a variety of *in vivo* models of cancer. In the A431 human epidermoid xenograft model (high EGFR expression), Tarceva(TM) treatment causes tumor regression. Treatment of mice bearing H460a and A549 human NSCLC tumor xenografts (moderate EGFR expression) results in approximately 70% TGI. Tarceva™-induced tumor growth inhibition in animal models is dose-dependent, correlates with circulating levels of drug and with inhibition of phosphorylation of EGFR *in vivo*. In addition, using immunohistochemistry, we have evaluated the ability of Tarceva™ to inhibit cell proliferation and induce apoptosis in tumor cells, as well as studied its effect on tumor angiogenesis. Finally, we have studied the characteristic skin lesions observed in Tarceva™-treated

tumor-bearing athymic nude mice by histological analysis. In toto, the data reveal that inhibition of the EGFR pathway with Tarceva™ provides a multipronged approach for the treatment of solid tumors by inhibiting tumor growth, survival and angiogenesis.

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EKB-569, an irreversible inhibitor of the epidermal growth factor receptor: Phase 1 trial results in patients with advanced solid tumors

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EKB-569 is a potent, selective, low molecular weight, irreversible inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase. The drug inhibits the growth of tumor cells that overexpress EGFR or HER2 *in vitro* and *in vivo*. Patients (pts) with advanced solid tumor malignancies that are known to overexpress EGFR were enrolled onto a phase 1, open-label study to assess the safety, tolerability, and pharmacokinetics (PK) of EKB-569. In part 1 of the study, EKB-569 was administered orally once daily for 14 days of a 28-day treatment cycle (intermittent dosage schedule). In part 2 of the study, EKB-569 was administered orally once daily for 28 days of a 28-day treatment cycle (continuous dosage schedule). Treatment cycles were continued as long as EKB-569 was tolerated, until disease progression. For part 1 of the study, enrollment and treatment are completed; 30 pts were treated with 25 mg (7 pts), 50 mg (3 pts), 75 mg (13 pts), or 125 mg (7 pts) of EKB-569. Part 2 of the study is ongoing; 29 pts were treated with 25 mg (4 pts), 50 mg (7 pts), 75 mg (13 pts), or 100 mg (5 pts) of EKB-569. The most frequently occurring tumor types for part 1 or part 2 included colorectal, non-small-cell lung, breast, renal, and head and neck. The most frequently reported adverse events for part 1 or part 2 were diarrhea, rash, nausea, asthenia, stomatitis, vomiting, anorexia, dry skin, and dehydration. These were generally mild and reversible. Dose-limiting toxicity was grade 3 diarrhea at the 125-mg dose level in part 1 and at the 100-mg dose level in part 2, so the maximum tolerated dose was 75 mg/day EKB-569 for both parts. Two patients in part 1 and 1 patient in part 2 completed at least 6 months of treatment. Paired skin biopsy samples collected before and after EKB-569 treatment will be analyzed for phospho-EGFR levels. Pharmacokinetic assessments were performed on day 1 and day 14. For patients in part 1 of the study, concentrations in plasma on day 14 were approximately 1.5-fold higher than those on day 1. For day 14, 125 mg EKB-569, Cmax was 100 23 ng/mL (mean SD) and tmax was 6.8 1.5 h (mean SD). Distribution was extensive with mean Vss/F of 2000 to 4700 L. Mean oral clearance ranged from 73 to 155 L/h, and half-life was approximately 22 h. Additional PK and safety data will be presented. EKB-569 had an acceptable safety and PK profile, was generally well tolerated, and offers a promising targeted approach for the treatment of solid tumors.

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17AAG low target binding affinity and potent whole cell potency: finding an explanation

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The ansamycin geldanamycin (GM) and its derivative, 17AAG now in early clinical trial in cancer patients, have potent activity against several cancer cells at nanomolar concentrations. The main target of these drugs is the molecular chaperone Hsp90. Contrary to the high anti-tumor potency the affinity of these drugs for the chaperone was determined to be around 1uM. We propose that this discordance can partly be explained by the chemical characteristics of the ansamycins. GM and 17AAG are hydrophobic in nature and therefore, highly water insoluble. Upon addition to media they accumulate into cells, resulting in higher intracellular concentrations than expected. We conclude that the real potency of ansamycins correlates with their Hsp90 binding affinity and is in the low micromolar concentration range. We suggest that in the clinic micromolar amounts of 17AAG at the site of the tumor will be necessary to see anti-tumor effects in patients comparable to ones achieved in tissue culture settings.

Regulatory affairs

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The cancer therapy evaluation program initiatives for enhancing industry collaborations

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The Cancer Therapy Evaluation Program (CTEP) of the Division of Cancer Treatment and Diagnosis, National Cancer Institute has recently instituted and/or revised a number of initiatives designed to enhance CTEP's interactions with Industry Collaborators. These initiatives are in response to the concerns voiced by Collaborators considering collaborations with CTEP, including intellectual property issues, access to data, combination studies, indemnification, and others. In response to concerns regarding intellectual property issues, CTEP has developed a document entitled Intellectual Property Option to Collaborators (the Option) which offers rights of first negotiation to a license to any invention by an extramural investigator to the Collaborators whose agent was used for clinical trials which resulted in an invention. This option is now in place for all Phase 1 and 2 contracts, as well as all Cancer Center grants, Cooperative Group and Cancer Trials Support Unit agreements. A modification of the'Option' for studies involving the combination of proprietary agents is currently under way. Further, this has been extended to cover preclinical studies under CTEP-sponsored agreements. Revisions have been made to electronic data submission contracts to permit Collaborators frequent access to data from CTEP-sponsored Phase 1 and 2 studies to address industry needs for rapid data access. Expedited adverse events are now reported electronically via a web-based system (AdEERS). AdEERS is currently being modified to allow Collaborators simultaneous access to safety reports. More detailed information on these and other initiatives will be presented.

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The Adverse Event Expedited Reporting System (AdEERS) of the cancer therapy evaluation program

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The Cancer Therapy Evaluation Program (CTEP) of the Division of Cancer Treatment and Diagnosis (DCTD), NCI has developed and implemented a web-based system, the Adverse Event Expedited Reporting System (AdEERS), for the electronic reporting of expedited adverse events for all clinical trials using a CTEP-sponsored investigational agent. AdEERS is designed to support the classification, retrieval, and evaluation of adverse event information using the standard language of MedDRA (Medical Dictionary for Regulatory Activities terminology). In addition, AdEERS is integrated into the existing CTEP Enterprise applications including the Common Toxicity Criteria (CTC, v2.0) and the Clinical Data Update System (CDUS). AdEERS provides an assessment section to help users determine whether the event requires expedited reporting based on the expectedness of the event and the NCI Adverse Event Reporting Guidelines. Two pathways of submission of expedited reports are supported by AdEERS - central processing as used by the Cooperative Groups/Consortia and non-central processing used by single institutions. The security of AdEERS is ensured using SSL 128 bit encryption. A random ticket number is automatically generated for each report. Users can access a particular report by using a combination of the ticket number, the patient identifier, and the CTEP protocol number. The system can be accessed using industry standard browsers on systems running Windows, Netscape v4.x and above or Internet Explorer v5.x and above. AdEERS was implemented on January 1, 2001 for all CTEP-sponsored investigational agent studies. In CY 2001, CTEP received approximately 3900 reports. AdEERS is being piloted with the FDA and has been demonstrated to other potential users at NIH. The development of xml tags is in process and should facilitate the transfer of AdEERS data to the FDA, pharmaceutical collaborators, and others.