prior therapies, and 5 patients had more than two prior therapies. Median number of metastatic sites were 2 (range 1–5). Six patients were entered at dose level 0 and 6 patient entered at dose level 1 (Table).
**Results:** 9 of 12 patients have completed 12 weeks of therapy. Two partial responses have been observed. Six patients had stable disease. One patient has had progressive disease. The remaining patients are too early for evaluation. Non-hematologic toxicity was generally well tolerated. Hematologic toxicity at dose level 0 consisted of 3 patients with Grade 3/4 neutropenia and 1 patient with Grade 3 thrombocytopenia. Hematologic toxicity at dose level 1 consisted of 2 patients with Grade 2/3 neutropenia.
**Conclusion:** The completion of our phase I experience will determine an MTD.

Study design:

| Protocol stage | Dose level | No. of patients | Interferon dosage | Thalidomide dosage | Capecitabine dosage (days 1–14, Wks 1+2) | Gemcitabine dose levels (Day 1, Wk 1 and Day 8, Wk 2) |
|---|---|---|---|---|---|---|
| 1 | −1 | 3 | 1 mlu bid | 200 to 400 mg | 1000 mg/m$^2$ | 200 |
| 1 | 0 | 3 | 1 mlu bid | 200 to 400 mg | 1000 mg/m$^2$ | 400 |

**277** POSTER

**Analysis of c-kit expression in small cell lung cancer patients and its clinical implications**

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**Background:** c-Kit is a growth factor receptor with tyrosine kinase activity that play an important role in the biology of cancer and its expression has been documented in several malignancies. The aim of this study was to determine the incidence and role of CD117 overexpression as a predictive or prognostic marker in patients with small cell lung cancer (SCLC).
**Patients and Methods:** We performed a retrospective study with 51 patients diagnosed of SCLC between January 2000 to July 2003. Relevant clinical patient information was obtained from extensive chart review (age at diagnosis, performance status – PS, weight loss, tumor stage and response to therapy). c-kit expression was analysed in paraffin-embedded tumor tissues immunohistochemically with commercial antibodies and we obtain successful results in 40 patients (39 males and 1 female) with a mean age of 62.4 years.
**Results:** Positive c-kit expression was observed in 32.5% patients. At the time of diagnosis 27 patients presented extended disease (ED) while 13 patients limited disease (LD). c-kit expression was observed in 46.2% in LD and 25.9% in EE although this difference was not significant (p=0.21). Patients with a PS between 0–2 represented 85.7% of the c-kit positive group and 90% in the c-kit negative one. All patients were formers o actual smokers. Weight loss was present in 52.3% of the patients at diagnosis.
36 patients received as a median 4 cycles of chemotherapy as first-line treatment (78.6% vs 83.3% in c-kit positive and negative group). The most used schedule was platinum-salts + etopiside and the most common second-line agent was topotecan. Radiotherapy of the primary tumor was administered in 42.9% of the patients in the c-kit positive group and in 30% in the negative one.
Comparing the c-kit positive group vs the negative one, was observed Complete Response in 30.8% vs 7.4% of the patients while Partial Response in 15.4% vs 11.1% (with 7.7% vs 11.1% of patients who achieved PR>80%). Stable Disease was observed in 15.4% vs 14.8% and Disease Progression in 30.8% vs 51.9% of the patients. In patients with c-kit expression, the median survival was 16.01 months vs 7.6 months in the c-kit-negative population (p=0.093).
**Conclusions:** c-Kit is expressed in one third of the patients with SCLC. Our findings do not suggest a significant association between c-kit expression and survival. However more studies are needed to define its possible prognostic value.

# Monoclonal antibodies and targeted toxins/nuclides

**278** POSTER

**Immunoassay and mass spectrometry analysis of specific EGFr phospho-tyrosines; effects of panitumumab (ABX: EGF), a fully humanized anti-EGFr monoclonal antibody, and the kinase inhibitor AG1478**

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**Background:** There is a clinical need to verify and stratify patient response rate with bio- and surrogate markers. Over expression of the epidermal growth factor receptor (EGFr/ErbB1/HER1) and its ligands have been correlated with aggressiveness and poor prognosis in various tumors such as colon, breast and prostate cancer and treatment with antagonists of this receptor have been beneficial to some patients. We have previously shown, using mass spectrometry, seven ligand induced EGFr phosphorylation sites (J Am Soc Mass Spectrom. **14**:1022–1031) and quantified their phosphate content upon ligand (EGF, TGFα (and inhibitor (AG1478) addition. We have extended these results to include inhibition by panitumumab, a high affinity (KD=$5\times10^{-11}$) fully human monoclonal antibody to EGFr. In addition, we report the development of an immunoassay platform to measure specific phosphotyrosines of the EGFr.
**Material and Methods:** Proliferating cell cultures of A-431, SK-MES, H1299, H2126, or xenografts (A431 or SK-MES) were pretreated with inhibitors before addition of ligand (EGF or TGFα (. Thereafter cells or xenograft tissue were immunoassayed using a BioVeris (Igen) platform. For LC tandem mass spectrometry, EGFr was immunoprecipitated and isolated by SDS-PAGE before analysis.
**Results:** The phosphate content of eight sites (T669, Y992, Y1045, Y1068, Y1086, S1142, Y1148, and Y1173) was measured by mass spectrometry upon ligand addition. Some sites exhibit large dynamic ranges (Y1045, Y1068, Y1086, Y1173) in their phosphate content, while other sites reach a plateau (Y992, S1142, Y1148). Decreases in phosphate content were seen with the addition of the EGFr inhibitor panitumumab similar to what was reported for the kinase inhibitor AG1478. The measurement of the phosphate content at several Tyr residues was adapted to a BioVeris immunoassay platform. Using this high through-put methodology we were able to detect increases in phosphate content with ligand addition and decreases in phosphate content upon inhibitor (panitumumab, AG1478) treatment of cells and xenograft tumors. An immunoassay using only monoclonal antibodies was also developed measuring phosphate content at Y1068.
**Conclusion:** We have identified specific Tyr residues that may serve as potential markers of the EGFr responsiveness to panitumumab administration. Phosphorylation induced by ligands or a decrease in phosphorylation by inhibitors (panitumumab, AG1478) can be monitored by measuring phosphate content at these ligand induced sites using a high through-put immunoassay. These experiments will allow us to investigate more precisely, the mechanism of action of anti-EGFr therapy in patients.

**279** POSTER

**Correlation of acne rash and tumor response with cetuximab monotherapy in patients with colorectal cancer refractory to both irinotecan and oxaliplatin**

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**Background:** Cetuximab (Erbitux™), an IgG1 monoclonal antibody, has demonstrated activity in patients with epidermal growth factor receptor (EGFR)-expressing colorectal cancer (CRC) both as a single agent and in combination with irinotecan. Cetuximab binds specifically to the EGFR preventing homo- and heterodimerization and signal transduction. This large phase II study was designed to explore the activity of cetuximab in patients with metastatic CRC, with no clear treatment alternative.
**Methods:** Patients with metastatic EGFR+ CRC were eligible to enroll in this study if they have failed at least two prior chemotherapy regimens, containing irinotecan, oxaliplatin and a fluoropyrimidine. Patients were to receive cetuximab monotherapy at an initial dose of 400 mg/m$^2$, followed

by weekly doses at 250 mg/m$^2$, until disease progression or unacceptable toxicity. Response was assessed by a modified version of WHO criteria.
**Results:** From 08/02 through 04/03 a total of 344 patients were enrolled and treated on this study. The median age 59 years; 54% were male, and all patients had an ECOG performance status of either 0 (42%) or 1 (58%). Patients had received a median 4 regimens of prior therapy for CRC (range 2–9 regimens). Median cetuximab therapy was 9 doses (range 1–52+ doses). Partial responses were observed in 12% of patients (95% CI 9–16%) and the median survival time was 6.7 months (95% CI 5.9–7.8 mo). The most common toxicity of cetuximab was an acne-like skin rash (86% any grade, 5% grade 3, no grade 4). The correlations between acne-like skin rash and tumor response and survival were investigated, and the results are as follows:

| Acne-like rash | None (N=49) | Grade 1 (N=140) | Grade 2 (N=140) | Grade 3 (N=17) | p-value* |
|---|---|---|---|---|---|
| Response rate (%) | 2 | 6 | 20 | 29 | <0.001 |
| Median survival (mo) | 2.4 | 4.6 | 9.1 | 13.2 | <0.001 |

* Grades 0/1 vs. Grades 2/3 (Fisher's exact or log-rank test, as appropriate)

**Conclusion:** Patients with grade *2 acne-like skin rash had a statistically significant improvement in tumor response and overall survival. This trend was observed across the patient characteristic classes of age, gender, ECOG performance status, and EGFR status.

**280** POSTER

**Updated results of the phase I study of SS1(dsFv)PE38 for targeted therapy of mesothelin expressing cancers**

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**Background:** Mesothelin is a 40-kDa cell surface glycoprotein whose expression is normally limited to mesothelial cells lining the pleura, peritoneum and pericardium. However, it is highly expressed in several solid tumors including the vast majority of epithelial mesotheliomas (MM), ovarian cancer (OC) and pancreatic cancer (PC). To target these mesothelin positive cancers we developed the immunotoxin – SS1(dsFv)PE38 (SS1P), consisting of the anti-mesothelin Fv linked to a truncated Pseudomonas exotoxin that mediates cell killing. Based on the pre-clinical activity of SS1P, including cytotoxic activity against tumor cells obtained directly from patients, a phase I clinical trial of SS1P was initiated.
**Methods:** Eligible patients (pts) had previously treated MM, OC and PC, tumor mesothelin expression as determined by immunohistochemistry and ECOG PS 0–2. SS1P was administered intravenously over 30 minutes every other day (QOD) for 6 or 3 doses.
**Results:** A total of 23 pts (8 peritoneal MM; 4 pleural MM; 1 inguinal MM; 8 OC; 2 PC) have been treated to date. On the QOD × 6 schedule 17 pts were treated at 4 dose levels (8, 12, 18 and 25 μg/kg/dose) and the maximum tolerated dose (MTD) of SS1P was 18 μg/kg/dose. Dose limiting toxicities (DLT's) included Grade 3 urticaria (1 pt) and vascular leak syndrome (VLS) (2 pts). Since the DLT's on the SS1P QOD × 6 schedule were seen in pts who had received more than 4 doses of SS1P, the protocol was amended to treat pts QOD × 3 doses to allow further dose escalation. Six pts have been treated on the QOD × 3 schedule (3 pts at 25 μg/kg/dose; 3 pts at(with no DLT's. Dose escalation is ongoing and the next cohort of pts will be treated at 45 μg/kg/dose. Pharmacokinetic (PK) analysis shows dose dependent increase in the SS1P AUC, with peak SS1P concentration of 411 ng/ml and SS1P half-life of 13 hr at the 35 μg/kg/dose level. Of the 21 evaluable pts treated, 11 had stable disease; 2 had minor response and 8 had progressive disease. One pt with OC had complete resolution of abdominal and pelvic ascites lasting 6 months; 1 pt with peritoneal MM has had complete resolution of abdominal ascites lasting > 3 yrs. and has required no further treatment.
**Conclusions:** SS1P is well tolerated and shows promising clinical activity including resolution of ascites and stable disease in several pts. PK analysis demonstrates high SS1P blood levels, prolonged half-life and dose dependent increase in AUC. Dose escalation on the QOD × 3 schedule is ongoing. Since greater than 90% of mesotheliomas and pancreatic cancer highly express mesothelin and SS1P shows activity in the ongoing Phase I study, Phase II clinical trials are being planned for mesotheliomas and pancreatic cancer.

**281** POSTER

**Phase I study of intravenous (IV) CI-1033 in patients with advanced solid tumors**

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**Background:** CI-1033 is a pan-erbB tyrosine kinase inhibitor that has undergone phase I and II evaluation as an oral agent. This study was undertaken to establish the safety, pharmacokinetic (PK) profiles, and feasibility of administering CI-1033 intravenously.
**Methods:** Fifty-three patients (pts) with advanced nonhematologic malignancies received IV CI-1033 as 30 min infusions (10–500 mg/dose) on a 3-day/week (MWF) schedule. Pts were initially treated 4 out of every 6 weeks; later, the protocol was modified to allow 3 days/week dosing without interruption. PK samples were collected on Days 1 and 8 and evaluated using compartmental analysis.
**Results:** 31M/22F, median KPS 90 (range, 70–100), median age 64 (23–78). Tumors: lung (14), colorectal (10), mesothelioma (10), melanoma (5); unknown primary, breast, sarcoma, and H&N (2 each); other solid tumors (7). Dose levels evaluated (#pts): 10mg (5), 20mg (3), 30mg (6), 45mg (5), 67.5mg (3), 100mg (7), 150mg (8), 225mg (7), 337.5mg (7), and 500mg (3). The most common treatment related grade (Gr) 1–2 adverse events (AEs) were rash (38% of pts), stomatitis (14%), nausea (17%), vomiting (17%), and diarrhea (13%). Most common Gr 3 AEs were hypersensitivity reaction (7.5%), rash (3.8%) and diarrhea (3.8%). No Gr 4 toxicities were observed. The maximum administered dose was 500mg, at which level 2 of 3 pts had dose limiting toxicities (DLTs) – 1 pt with Gr 3 myalgia and 1 pt with Gr 3 syncope. Subsequently, 3 additional pts were entered at the next lower dose level (337.5mg), 2 of which encountered DLTs – 1 pt with Gr 3 hypersensitivity and 1 pt with Gr 3 diarrhea. Consequently, the next lower dose level (225mg) was declared the maximum tolerated dose (MTD).
The initial CI-1033 distribution phase had a 2 to 3 minute half-life while the terminal elimination phase half-life was approx 3 hrs. Central volume of distribution (18.5 liters) approximated extravascular water volume. Systemic clearance was rapid at 3 L/min. Systemic exposure was dose proportional with bi-phasic elimination and was not dependent upon age, gender, race, renal function, body weight or surface area.
Although no confirmed objective responses were seen, 10 of the 53 (19%) patients had disease stabilization at their first efficacy evaluation visit (after 6 to 8 weeks of treatment), with 2 of these 10 pts also having minor responses. Tumors demonstrating disease stabilization with IV CI-1033 included cancers of the lung, colon, breast, thyroid, and H&N, as well as mesothelioma, sarcoma, and melanoma.
**Conclusions:** CI-1033 was safely given intravenously up to 225mg/dose on a 3 days/week schedule, with evidence of antitumor activity in a variety of tumors. DLTs were hypersensitivity, vomiting, and diarrhea. Administering CI-1033 intravenously is practical and may prove to be an important complementary regimen to oral CI-1033 dosing.

**282** POSTER

**Use of the humanized anti-EGFR antibody h-R3 and radiotherapy for the treatment of patients with high-grade astrocytic tumors**

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The current standard of care for patients with high-grade glioma is resection followed by radiotherapy. For anaplastic astrocytomas and glioblastome multiforme patients, the cure rate is low with standard local treatment and they are appropriate candidates for clinical trials designed to improve local control by adding newer forms of treatment. During the last 10 years Epidermal Growth Factor Receptor (EGFR) has become one of the most widely explored targets for anticancer drugs. Elevated levels of EGFR are associated with malignant transformation of neoplastic cells and are observed in several cancer types including high-grade astrocytic tumours. h-R3 is a humanized monoclonal antibody that recognized the EGFR external domain with high affinity. In advanced head and neck cancer patients, overall survival and response rate have significantly increased after the use of the antibody doses above 200 mg. In order to further evaluate the safety and preliminary efficacy of h-R3, we conducted a Phase I/II trial using h-R3 in combination with radiotherapy in 24 high-grade astrocytic tumors patients. The primary endpoint of the trial was s afety of h-R3 when used at multiple doses in combination with radiation.