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In human tumor xenografts the resistance to ET-743 or to cisplatin can be overcome by giving the two drugs in combination

M. D'Incalci[1], T. Colombo[1], G. Giavazzi[1], I. Nicoletti[1], E. Erba[1], P. Ubezio[1], D. Meco[2], R. Riccardi[2], J. Jimeno[3], G. Faircloth[4]. [1]*Istituto di Ricerche Farmacologiche "Mario Negri", Department of Oncology, Milan, Italy;* [2]*Policlinico Universitario A. Gemelli, Rome, Italy;* [3]*PharmaMar SA, Scientific Development, Madrid, Spain;* [4]*PharmaMar USA Inc., Preclinical R & D, Boston, USA*

ET-743 is a marine-derived antitumour agent isolated from Ecteinascidia turbinata. ET-743 binds to the minor groove of the DNA bending the DNA towards the major groove. In cancer cell lines growing *in vitro* the combination of ET-743 and cisplatin showed an additive or slightly synergistic effect evaluated by isobologram analysis. Instead, in several xenografts the combination was clearly synergistic. Specially, in xenografts that are partially sensitive to both drugs such as H&N (FADU), H-187 melanoma and TE 671 rhabdomyosarcoma, the effect of the combination was significantly better than the one achieved with each drug alone at the optimal dosage-schedule. In xenografts which were resistant either to ET-743 or to cisplatin such as A 2780 (1 A9), SKOV-3, HOC-8 ovarian cancer, LX1 non-small cell lung cancer and SKN-DZ neuroblastoma, the potentiation was clear-cut, suggesting that the combination can overcome the resistance mechanisms. The combination was also tested in the murine M5076 (M5) ovarian reticular cell sarcoma and a subline made resistant to cisplatin by repeated *in vivo* treatments (M5/DDP). Both in M5 and in M5/DDP the combination of ET743 and cisplatin produced a significant increase of the antitumor and antimetastatic effect that were markedly greater than those of each drug alone, although the effects were less marked in the latter resistant tumor. No consistent differences in the antitumor activity were observed when the two drugs were given simultaneously or when cisplatin was given first followed by ET-743 after 1h or in the opposite sequence. The toxicity of the combination, assessed by determining the weight loss, was moderate and reversible but greater than that of each drug alone given at the same dosage. In conclusion these data strongly support the development of the combination of ET-743 and cisplatin in clinic, also for tumors that are not very sensitive to ET-743 or cisplatin given alone.

Prodrugs

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Pharmacological study of CT-2103 (XyotaxTM), a poly(L-glutamic acid)-paclitaxel conjugate administered every 3 weeks or every 2 weeks in a phase I study

A.V. Boddy[1], R. Todd[1], M. Verrill[1], J. Sludden[1], K. Fishwick[1], L. Robson[2], J. Cassidy[3], D. Bisset[3], P.D. Garzone[4], A.H. Calvert[1]. [1]*University of Newcastle, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom;* [2]*Cancer Research UK, Clinical Projects, London, United Kingdom;* [3]*Aberdeen Royal Infirmary, Oncology, Aberdeen, United Kingdom;* [4]*Cell Therapeutics Inc, Seattle, USA*

CT-2103 is a water-soluble poly (L-glutamic acid)-paclitaxel conjugate, which has preferential tumour distribution and demonstrated antitumour activity in mice. In a two centre, Phase I, dose escalation study CT-2103 was administered as a thirty minute infusion every three weeks (Phase Ia). When the maximum tolerated dose (MTD) and dose-limiting toxicity (DLT) were established, a two-weekly schedule was employed (Phase Ib) with further investigation of the pharmacokinetic profile. Nineteen patients were entered into Phase Ia (30 to 720mg/m^2 CT-2103). Three patients have been treated on Phase Ib (480 mg/m^2 CT-2103, 177mg/m^2 paclitaxel equivalents). Plasma levels of CT-2103 and unconjugated paclitaxel were measured during cycles 1 and 2 of both schedules. In the initial dose-escalation (Phase Ia), DLT was seen in two of six patients (1 neutropaenia, 1 motor neuropathy) at 720mg/m^2 (266mg/m^2 paclitaxel equivalent). 630mg/m^2 (233mg/m^2 paclitaxel equivalent) was subsequently established as the MTD on a 3-weekly schedule. Grade 4 neutropaenia was observed at the 720 and 630mg/m^2 dose levels. Only one other grade 1 sensory neuropathy was observed. Three patients experienced hypersensitivity reactions to treatment. CT-2103 has a long plasma half-life of up to 162hr. The patients at 630 or 720mg/m^2 had prolonged elevated plasma concentrations of paclitaxel (0.35$\pm$0.17μM at 24 hr). A confirmed partial response has been seen after 2 cycles in a patient with mesothelioma at 480mg/m^2 (176mg/m^2 paclitaxel equivalent). This persisted until 2 months following completion of six cycles of treatment. In the Phase Ib study, plasma concentrations of CT-2103 and of paclitaxel were similar to those seen in Phase Ia at the same dose level. Neither CT-2103 nor paclitaxel accumulated in plasma following the second cycle of administration 2 weeks later. Clinical development with CT-2103 is proceeding.

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Enzyme-Catalyzed Therapeutic Activation (ECTA) NB1011 (Thymectacin™) selectively targets thymidylate synthase (TS) - overexpressing tumor cells: preclinical and phase I clinical results

M. Pegram[1], N. Ku[1], M. Shepard[2], L. Speid[2], H.-J. Lenz[3]. [1]*University of California Los Angeles, School of Medicine, Los Angeles, USA;* [2]*NewBiotics, Inc., San Diego, USA;* [3]*University of Southern California, School of Medicine, Los Angeles, USA*

Resistance to fluoropyrimidine cancer chemotherapy is associated with an increase in expression of TS. NB1011 is a pronucleotide analog converted to (E)-5-(2-bromovinyl)-2'-deoxyuridine-5'-monophosphate (BVdUMP), a known substrate for TS. Enzymatic activation of BVdUMP by TS results in liberation of cytotoxic product(s) resulting in preferential cytotoxicity to TS-overexpressing tumor cells and human colorectal and breast carcinoma xenografts. This activity is associated with induction of p21 and BAX, and is associated with arrest in cell cycle progression (Neuteboom, et al., Mol Cancer Therap 1: 377-384, 2002). To test the hypothesis that NB1011 may be useful in the treatment of fluoropyrimidine-resistant cancers, we have initiated a phase I dose-escalation clinical trial for patients with advanced colorectal cancer with fluoropyrimidine failure within the prior 6 months. The primary endpoint of the study is assessment of safety and pharmacokinetics. Secondary endpoint is assessment of objective clinical response. Thus far, 15 subjects have been dosed with NB1011 (dose range 200mg/m^2 – 1250mg/m^2) given intravenously over 1 hour daily X 5 days (repeat cycles every 28 days for patients with stable or responsive disease). Over this dose range, plasma pharmacokinetics appear linear with mean cycle 1 Cmax = 3495ng/mL at 200mg/m^2 and 10,469ng/mL at 800mg/m^2. Mean AUC at 200mg/m^2 is 2499ng-h/mL and is 9429 ng-h/mL at 800mg/m^2. The half life of NB1011 is just under 1 hour; however half life of metabolite BVDU is ~12 hours with mean BVDU day 1 Cmax = 9190 ng/mL and AUC = 13932 ng-h/mL at 800mg/m^2 dose level. NB1011 is clinically well tolerated at all dose levels tested, without significant hematologic toxicity. The maximum tolerated dose has not yet been established. There have been 7 serious adverse event reports, including: ascites, respiratory distress, vomiting, possible bowel obstruction, surgical procedure (transurethral prostate resection), hyperglycemia, and allergic reaction. One possible DLT event was recorded (pneumonia) at the 800mg/m^2 dose level; however, cohort expansion at this level showed no further DLT events. Four patients with stable disease received multiple cycles of NB1011: one received 5 cycles at 200mg/m^2; two received 2 cycles at 800mg/m^2; one received 3 cycles at 800mg/m^2. Ten patients have discontinued secondary to disease progression; 5 patients remain active on study as of 13/6/2002. NB1011 dose escalation is continuing with cohorts of 3 patients per dose level; updated data will be presented.

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Phase 2 Study of TLK286 (GST P1-1 Activated Glutathione Analog) in patients with platinum and paclitaxel refractory/resistant advanced epithelial ovarian cancer

J.J. Kavanagh[1], A. Kudelka[1], D.R. Spriggs[2], M.A. Bookman[3], L. Lewis[1], C. Maack[4], J. Dombroski[4], J. Macpherson[4], W.D. Henner[4], G.L. Brown[4]. [1]*MD Anderson Cancer Center, Houston, USA;* [2]*Memorial Sloan Kettering Cancer Center, New York, USA;* [3]*Fox Chase Cancer Center, Philadelphia, USA;* [4]*Telik, Inc., South San Francisco, USA*

Objectives: To determine the objective tumor response rate (ORR), disease stabilization rate, safety and survival of patients with platinum and paclitaxel refractory or resistant ovarian cancer treated with TLK286.
Methods: TLK286 is a glutathione analog activated in cancer cells by glutathione S-transferase P1-1, resulting in apoptosis through the stress kinase pathway. Patients with ovarian cancer resistant or refractory to paclitaxel and platinum-based chemotherapy were to be enrolled in this multicenter, Fleming two-stage design study. TLK286 was given intravenously at 1,000 mg/m^2 once every 3 weeks until tumor progression or unacceptable toxicities. ORR was assessed by RECIST. Toxicity was graded by NCI-CTC. Survival was estimated by Kaplan-Meier Analysis.
Results: Thirty-six patients, ECOG 0-1, prior chemotherapy regimens, median 3 (range 1-7), were enrolled between June 2001 and March 2002. A to-

tal of 117+ cycles (median 2, mean 3.2, range 1-14+) were administered at 99% of the specified dose intensity. All patients had failed prior therapy with paclitaxel and platinum. 20/36 (55%) had failed one to three additional salvage therapies, including 12/36 (33%) Doxil, 10/36 (28%) topotecan, 4/36 (11%) gemcitabine, and 1/36 (3%) docetaxel therapy. The most common possibly drug-related toxicities (< Grade 2) were: fatigue, nausea, and anemia. There were no Grade 4 toxicities, no Grade 3 myelosuppression or thrombocytopenia and no cumulative toxicities. At interim analysis, ORR was seen in 4/31 patients (13%), 1 CR (3%), 3 PRs (10%), 12 SDs (39%) and 15 PDs (48%). The ORR was 15% in 2nd-line patients. The disease stabilization rate (CR+PR+SD) was 52% (16/31). The longest duration of therapy is in the CR patient, progression-free for 14+ cycles (12+ months). Tumor responses have been accompanied by declines in CA125 and symptom improvement. Three patients have died due to disease progression. Median survival exceeds 10 months.

Conclusions: TLK286 has significant single-agent antitumor activity in platinum and paclitaxel refractory or resistant ovarian cancer and is well tolerated. Median survival has not yet been reached but exceeds 10 months. Patient follow-up for response and survival is ongoing. Objective tumor responses including a durable complete response in bulky disease and improved survival in this heavily pretreated population are encouraging and warrant future studies of TLK286 in ovarian cancer.

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Phase II Study of TLK286 (Glutathione Analog Activated by GST P1-1) in refractory colorectal cancer

L.S. Rosen[1], T. Dragovich[2], H.J. Lenz[3], E. Lin[4], L. Wiggins[1], G.L. Brown[5], C. Maack[5], J. Macpherson[5], J. Dombroski[5], W.D. Henner[5]. *[1]UCLA Jonsson Cancer Center, Department of Medicine, Los Angeles, USA; [2]Arizona Cancer Center, Tucson, USA; [3]USC/Norris Comprehensive Cancer Center, Los Angeles, USA; [4]MD Anderson Cancer Center, Houston, USA; [5]Telik, Inc, South San Francisco, USA*

Introduction: TLK286 is a novel glutathione analog, activated by the enzyme glutathione-S transferase P1-1 (GST P1-1). GST P1-1 is overexpressed in many types of human malignancies and is implicated in resistance to several classes of anticancer therapies. Following activation of TLK286 by GST P1-1, apoptosis is induced through the stress response pathway.

Methods: Up to 75 evaluable patients with colorectal cancer who had failed prior 5-fluorouracil, leucovorin and irinotecan chemotherapy and any amount of cytostatic agents were to be enrolled in this multicenter, open label, single arm study. TLK286 was administered at 1000 mg/m^2 every 3 weeks until tumor progression or unacceptable toxicities. Objective response rate (ORR) was determined by RECIST criteria. Time to progression (TTP) and survival were estimated by Kaplan-Meier analysis. Adverse events (AEs) were graded by the NCI-CTC.

Results: 73 patients (35 M/22F) median age 66 (range 29-81), ECOG median 1 (range 0-1), median number of prior chemotherapy regimens 2 (range 1-5) were treated with a total of 196+ TLK286 treatments (median 2, mean 2.7, range 1-8+). The target dose of TLK286 was maintained in 94% of cycles. Most frequent AEs were (Grade 1-2): fatigue (24%), nausea (15%), vomiting (7%), hematuria (9%), urinary frequency (9%) and anemia (9%). There was one Grade 4 AE reported at day 21 (ANC 476/mm3) in a patient with progressive disease and underlying bone marrow disorder. Grade 3 AEs were infrequent (7% of patients). As of interim analysis, 36 of 73 patients were evaluable for tumor response. Five patients (14%) had stable disease (SD) as best response. Median duration of SD was 167 days (range 120-219+ days). In patients with SD there were declines in the CEA tumor marker (median decrease 42%, range 6-70%) These CEA declines have not translated into objective responses. There have been 19 deaths reported due to progressive disease. At interim analysis, estimated median survival (Kaplan-Meier) was 172 days (range 30-219+ days).

Conclusions: TLK286 was well tolerated in heavily pre-treated patients with refractory colorectal cancer. There were no objective tumor responses in advanced colorectal cancer when TLK286 was administered as a single agent once every 3 weeks. Analysis of subpopulations suggests that investigation of a more intensive dose schedule or use of TLK286 in combination therapy in colorectal cancer is warranted.

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Enhanced antitumor activity of TLK286 in combination with carboplatin, doxorubicin and docetaxel in human ovarian and breast cancer cell lines

J. Keck[1], F. Meng[1], E. Grossman[1], D. Cai[1], G. Brown[2], S. Schow[3], H. Xu[1]. *[1]Biology; [2]Clinical; [3]Chemistry, Telik, Inc., South San Francisco, USA*

TLK286 is a novel glutathione analog that is activated by the enzyme glutathione-S transferase P1-1 (GST P1-1). GST P1-1 is constitutively expressed in many cancers and overexpressed following treatment with chemotherapeutic agents. Following activation of TLK286 by GST P1-1, apoptosis is induced through the stress response signaling pathway. TLK286 is being evaluated in phase 2 clinical trials in ovarian, breast, non-small cell lung and colorectal cancers and has shown significant single agent antitumor activity and improvement in survival in patients with lung and ovarian cancers. Since the side effect profile observed with TLK286 is non-overlapping with standard chemotherapeutics, we have tested TLK286 *in vitro* in combination with docetaxel, doxorubicin and carboplatin, respectively. Ovarian cancer cell line OVCAR3 was incubated with TLK286 alone and in combination with doxorubicin or carboplatin for approximately three cell doublings and viability was determined using the Wst-1 assay. In various study designs (fixed and variable ratios), we have consistently observed marked enhancement of cytotoxicity when TLK286 was combined with either doxorubicin or carboplatin compared to either agent alone. The results are particularly significant for TLK286 and carboplatin, with maximum or near maximum activity observed under all conditions examined. TLK286 was tested in combination with docetaxel in the breast cancer cell line MCF-7. MCF-7 cells were treated with the single drugs or in combination for approximately 1 doubling time, and the cells were then labeled with BrdU overnight. The incorporation of BrdU, which reflects the extent of cell proliferation, was determined using ELISA. The drug combination, at various concentrations and ratios, was more effective at inhibiting cell proliferation than the single drugs. Analyses using the combination index method indicate synergies between low concentrations of carboplatin, doxorubicin or docetaxel and variable concentrations of TLK286. The results suggest that TLK286 shows enhanced cytotoxicity towards ovarian and breast cancer cells when used in combination with carboplatin, doxorubicin or docetaxel.

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Ethanolatoamine platinum chelates as prodrugs which are selectively activated in slightly acidic environment

C. Baumgartner[1], M. Fremuth[1], M. Galanski[1], A. Küng[1], M.A. Jakupec[1], B.K. Keppler[1]. *[1]Inst. of Inorganic Chemistry, Vienna University, Wien, Austria; [2]Faustus Forschungs Compagnie Translational Cancer R, Leipzig, Germany*

Dichloroplatinum(II) and tetrachloroplatinum(IV) complexes with two hydroxyethylamine ligands in cis-configuration undergo intramolecular ligand exchange reactions in aqueous solution. NMR studies have shown that the hydroxyethylamine ligands are able to chelate platinum, thereby forming cyclic ethanolatoamine platinum species under proton and chloride abstraction in a pH-dependent equilibrium reaction (Fig. 1).

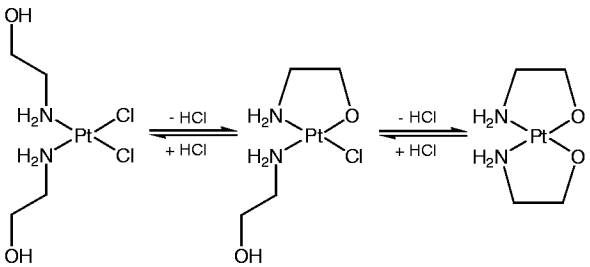

Figure 1. Intramolecular ligand exchange reactions of dichlorobis(2-hydroxyethylamine) platinum(II) (left) resulting in ethanolatoamine chelates.

The chelates are stable in slightly basic solution, whereas in acidic solution the rings open by protonization of ethanolate oxygen. Since rupture of the ethanolatoamine rings increases the reactivity towards biological targets, it should be possible to administer the less reactive chelates as prodrugs, which are then selectively activated within the acidic microenvironment found in many solid tumors. In order to evaluate this concept both ring-closed and ring-opened dichloroplatinum(II) and tetrachloroplatinum(IV)