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.

101

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.

102

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.

103

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).

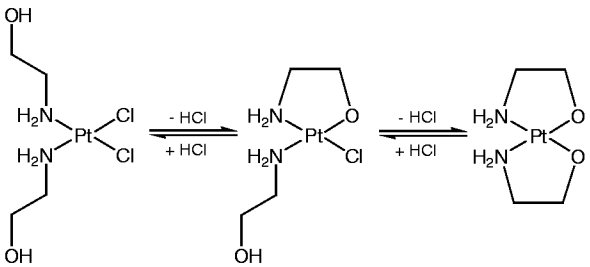

Figure 1. Intramolecular ligand exchange reactions of dichlorobis(2-hydroxyethylamine) platinum(II) (left) resulting in ethanolatoamine chelates.

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)

species have been prepared and their reactions with GMP have been studied in model experiments by means of capillary zone electrophoresis (CZE). Kinetics of these reactions have been investigated by CZE applying diode array detection (CZE-DAD), and the reaction products have been characterized by CZE coupled to a mass spectrometer via an electrospray ionization interface (CZE-ESI-MS). Formation of monoadducts and bisadducts with GMP could be demonstrated and the expected increase in GMP binding in acidic solution has been confirmed. Binding of dichlorobis(2-hydroxyethylamine)platinum(II) proceeds more than six times faster at pH 6.0 (half time 4.5 $\pm$ 0.7 h) than at pH 7.4 (half time 28.5 $\pm$ 2.1 h) in chloride-free phosphate-buffered solution. In the presence of 100 mM chloride the half times are 11.0 $\pm$ 0.3 h and 40.5 $\pm$ 3.5 h, respectively. Evaluation of the concept in human tumor cell lines cultured under normal vs. acidic conditions (with or without hypoxia) is ongoing and results will be presented.

104

Enzyme-mediated insolubilization therapy

A.I. Kassis, A.M. Kirichian, K. Wang, S.J. Adelstein. *Harvard Medical School, Radiology/Nuclear Medicine, Boston, USA*

We have developed a new strategy that aims to concentrate therapeutic radionuclides (energetic β-particle emitters, e.g. iodine-131, and alpha-particle emitters, e.g. astatine-211) within solid tumors. This approach, which we have named EMIT (Enzyme-Mediated Insolubilization Therapy), is a method for enzyme-dependent, site-specific, *in vivo* precipitation of a radioactive molecule (from a water-soluble precursor) within the extracellular space of solid tumors. The prodrug, ammonium 2-(2'-phosphoryloxyphenyl)-6-iodo-4-(3H)-quinazolinone, labeled with iodine-125 (125IPD) and its authentic compound labeled with iodine-127 (IPD) have been synthesized, purified, and characterized. The prodrug is water soluble and non-fluorescent. In the presence of alkaline phosphatase (ALP), 125IPD and IPD are hydrolyzed, respectively, to 125I-labeled 2-(2'-hydroxyphenyl)-6-iodo-4-(3H)-quinazolinone (125ID) and its 127I-labeled derivative (ID), iodinated molecules that are water insoluble and fluorescent. Fluorescence microscopy and autoradiography demonstrate that the *in vitro* incubation of 125IPD/IPD with ALP-expressing confluent/clustered tumor cells leads to the hydrolysis of the prodrug and its entrapment. Biodistribution studies in mice injected intravenously with 125IPD or 125ID show that neither compound is retained by normal tissues and organs. In addition, when the mice are initially injected subcutaneously with ALP and then intravenously with 125IPD, radioactivity is localized only in the ALP-rich regions. Finally, our results also indicate 125ID remains indefinitely within the tissues where it is produced. We believe that EMIT is a strategy that will lead to the active and specific concentration and entrapment of therapeutic radionuclides within solid tumors, the consequent protracted irradiation of tumor cells within the range of the emitted particles, and the effective therapy of solid tumors.

105

Enzymatic activation of prodrugs by prostate-specific membrane antigen (PSMA)

A. Mhaka, S. Janssen, A. Gady, M. Rosen, S. Denmeade. *The Johns Hopkins University, Oncology, Baltimore, USA*

PSMA is a 100 kDa type II transmembrane glycoprotein that possesses a number of characteristics that make it a suitable target for prostate-specific therapy. PSMA expression is highly restricted to prostate tissue with strongest expression in both primary and metastatic prostate cancers and PSMA expression is upregulated upon androgen withdrawal. The extracellular domain of PSMA is accessible to agents in the extracellular peritumoral fluid thus making it possible to target prodrugs for enzymatic activation. Two discrete enzymatic functions for PSMA have been described. PSMA possesses the hydrolytic properties of an N-acetylated a-linked acidic dipeptidase (NAALADase) and is able to hydrolyze the neuropeptide N-acetyl-l-aspartyl-l-glutamate (NAAG). In addition, PSMA also functions as a pteroyl poly-g-glutamyl carboxypeptidase (folate hydrolase) and is able to progressively hydrolyze g-glutamyl linkages of both poly-g-glutamated folates and methotrexate analogs with varying length glutamate chains. In order to develop prodrugs that can be activated within prostate cancers by PSMA's enzymatic activity, a PSMA specific peptide carrier is required. In the present study a number of peptide substrates for PSMA were screened in order to identify specific and efficient substrates for the NAALADase and/or folate hydrolase activity of PSMA. A series of substrates were synthesized in which the amino acid portion consisted primarily of alpha or gamma carboxy-linked aspartic and/or glutamic acids of varying chain was coupled to the 4-N[N-2,4diamino-6-pteridinyl-methyl)-N-methylamino-benzoate](APA) portion of methotrexate. These substrates were then characterized on the basis of rates of PSMA hydrolysis and stability in human serum. Gamma-linked substrates were hydrolyzed by PSMA but were relatively unstable in human serum. Only one alpha-linked dipeptide substrate (APA-Asp-Glu) was both hydrolyzed by PSMA and stable to hydrolysis in human serum. Substrates that combined both alpha and gamma linked Asp and Glu residues were both hydrolyzed by PSMA and stable to hydrolysis in human serum. These substrates are currently being used to develop prodrugs out of the potent natural product thapsigargin (TG). TG induces proliferation independent apoptosis in all cell types. TG prodrugs that are specifically activated by PSMA represent a novel therapy that could be given to men with prostate cancer while avoiding significant systemic toxicity.

106

Elaboration of synergy between the prodrugs TST220 and TST334 and conventional chemotherapeutics

T. Borgford, C. Braun, A. Purac, S. Purac, J. Rathmann, W. Khuu, E. Amandoron, D. Stoll. *Twinstrand Therapeutics Inc., Burnaby, Canada; [2]Twinstrand Therapeutics Inc., Burnaby, Canada*

TST220 and TST334 are prodrugs designed for activation by matrix metalloproteinases 2 (MMP2) and 9 (MMP9). TST220 contains a protease-sensitive cleavage site of 23 amino acid residues whereas the recognition sequence in TST334 is 8 residues. The activated TST220 and TST334 are cytotoxics with IC_{50} prodrug/IC_{50} drug ratios, *in vitro*, of 25 and 1200 respectively. They induce cell death via a pro-apoptotic pathway and exhibit anti-tumor activities in both human xenograft and murine tumor models. Preliminary combination studies indicated a strong synergy between the prodrugs and the conventional cytotoxic drug adriamycin (doxorubicin). Moreover, the two prodrugs showed evidence of efficacy in the treatment of adriamycin-resistant tumours. In this study, drug synergy and efficacy against drug-resistant tumors was elaborated in a P388 animal model. Subcutaneous tumors were initiated in BDF1 mice and the animals then treated i.v. with 5 injections of an adriamycin/prodrug combination or saline (control) at 4-6 day intervals. In low-dose monotherapy, TST220 (15 μg/kg) and TST 334 (200 μg/kg) produced roughly 1 day delays in tumor growth. Adriamycin (at 5 mg/kg) produced a modest 3 day delay. However, the combination of either prodrug with adriamycin (i.e., at the same concentrations) produced delays in tumor growth of >17 days (combination delay a minimum estimate due to the death of the control group). TST220 and TST334 showed significant efficacy in the treatment of P388Adr, an adriamycin-resistant variant of P388. Three intravenous injections of prodrug (monotherapy) at 4 day intervals resulted in on average a 5 day delay in tumour growth. Preliminary results of synergy between TST prodrugs and other conventional agents is also reported. Molecules of TST class do not cause genetic damage nor do they cause bone marrow suppression. In proposed human trials TST prodrugs are expected to potentiate the activity of adriamycin without exacerbating side effects.

106A

Phase 2 study of TLK286 (GST P1-1 activated glutathione analog) in patients with non-small cell lung cancer who failed prior platinum-based regimens

V. Papadimitrakopoulou[1], R. Figlin[2], L. Garland[3], D. Von Hoff[3], M. Kris[4], M. Purdom[1], G.L. Brown[5], C. Maack[5], J. Macpherson[5], W.D. Henner[5]. *[1]MD Anderson Cancer Center, Houston, USA; [2]UCLA Jonsson Cancer Center, Los Angeles, USA; [3]Arizona Cancer Center, Tucson, USA; [4]Memorial Sloan Kettering Cancer Center, New York, USA; [5]Telik, Inc., South San Francisco, USA*

Introduction: 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 including non-small cell lung cancer (NSCLC) and is overexpressed following treatment with platinum-based regimens. Following activation of TLK286 by GST P1-1, apoptosis is induced through the stress response pathway.
Methods: Up to 55 Stage IIIB or IV NSCLC patients who had failed prior platinum-based therapy (up to two cytotoxic regimens) and may have had prior adjuvant therapy and cytostatic agents such as EGFR tyrosine kinase inhibitors were to be enrolled in this multicenter single-arm study. Patients received TLK286 at 1000 mg/m^2 once every 3 weeks until tumor progression or unacceptable toxicities. Adverse events were graded by NCI-CTC, objective tumor response was measured by RECIST, and survival was estimated by Kaplan-Meier.