FUTURE ONCOLOGY
TECHNOLOGY, PRODUCTS, MARKETS AND SERVICE OPPORTUNITIES
A NEW MEDICINE PUBLICATION FEBRUARY 28, 2006 VOLUME 8, NUMBER 8/9/10

MEETING COVERAGE

NOVEL ANTICANCER AGENTS/ FORMULATIONS IN PHASE I MONOTHERAPY CLINICAL TRIALS

FROM THE 2005 MEETINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, THE NATIONAL CANCER INSTITUTE AND THE EUROPEAN SOCIETY FOR RESEARCH AND TREATMENT OF CANCER (AACR-NCI-EORTC), AND THE AMERICAN SOCIETY OF HEMATOLOGY (ASH)

In Volume 8, issues 6/7 and in this current issue, FUTURE ONCOLOGY has profiled 93 anticancer drugs in monotherapy phase I clinical trials ongoing worldwide, acting by a variety of mechanisms spanning the gamut from novel cytotoxics and formulations of cytotoxic drugs to molecularly targeted agents and immunotherapeutics/vaccines. These agents were selected because they are in active development with results from preclinical studies and clinical trials reported in at least four major oncology-related meetings in 2005. A complete description of every aspect of these agents and their targets may be found on NEW MEDICINE's Oncology KnowledgeBASE (nm/OK) subscription-based resource located at www.mnok.net.

MEETING COVERAGE

NOVEL ANTICANCER AGENTS/ FORMULATIONS IN PHASE I MONOTHERAPY CLINICAL TRIALS

FROM THE 2005 MEETINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, THE NATIONAL CANCER INSTITUTE AND THE EUROPEAN SOCIETY FOR RESEARCH AND TREATMENT OF CANCER (AACR-NCI-EORTC), AND THE AMERICAN SOCIETY OF HEMATOLOGY (ASH)

This triple issue completes a review by FUTURE ONCOLOGY of novel anticancer agents in phase I monotherapy clinical trials about which results were reported, from preclinical studies or clinical trials, at several major meeting that took place in 2005. In total, 93 agents were profiled acting on a variety of mechanisms. Depending on results from phase I monotherapy clinical trials, many of these agents will be entered in phase II monotherapy trials, and more importantly, in phase I combination trials with various agents that demonstrated synergy based on preclinical evaluations.

Although the anticancer agents described here have been classified in distinct categories based on their mechanism of action, such classification is rather arbitrary as most of these agents are involved in multiple activities. In this issue, selected anticancer drugs are described acting as:

- cell-cycle regulators/inhibitors
- spindle poisons
- traditional cytotoxics/apoptosis inducers
- heat-shock protein (Hsp) inhibitors
- angiogenesis inhibitors
- aurora kinase inhibitors
- epidermal growth factor receptor (EGFr) inhibitors

The complete list of anticancer agents in this report is presented in Exhibit 1. Selected targets addressed by agents described here are presented in Exhibit 2. Comprehensive profiles of all of these agents and classifications along multiple mechanisms of action are incorporated in NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), residing at www.nmok.net.

CYTOTOXIC AGENTS/TARGETED CYTOTOXICS/ CELL-CYCLE MODULATORS/APOPTOSIS INDUCERS/ IMMUNOCONJUGATES

Generally, cytotoxics remain a popular target of drug development in oncology. However, traditional cytotoxics are being replaced by novel synthetic analogs with more attractive safety and efficacy profiles. In addition, drug delivery systems are being incorporated in existing drugs to improve their behavior in the host. One relatively new approach in drug delivery, based on advances in nanotechnology, promises to further enhance the utility of cytotoxic agents.

Cell-cycle Modulators

The cell cycle is the ultimate decision-making step in cell proliferation. Intervening and blocking the cell cycle deprives cells from dividing and multiplying. Control of the cell cycle is critical in preventing proliferation of cancer cells.

Most cytotoxic agents interfere, one way or another, with a cell's ability to proliferate. Their selectivity to cancer cells stems from the fact these cells, with the exception of a few other normal cell types, are the only rapidly dividing cells in the host and, therefore, most likely to be vulnerable to cell-cycle toxins. Traditional cytotoxics interfere mechanistically with the cell cycle by damaging the cells' DNA preventing it from being used in ensuing cell populations, by damaging the structures needed to support the process of constructing new cells, or by depriving the cell from vital biochemical processes. In contrast, novel cell-cycle modulators act at the molecular level, interfering with signaling that takes the cell-cycle process through its paces. This anticancer approach owes its existence to the discovery and cloning of cell-cycle regulating proteins that shed new light on the process of normal cycling and cancer-cell proliferation.

Cell-cycle regulators/inhibitors are experiencing a comeback with various novel agents entering phase I monotherapy clinical trials, among them several inhibitors of cyclin-dependent kinases (CDK). CDK are essential for regulation of the cell cycle in proliferating cells, playing key roles in cell-cycle progression. Many malignancies are characterized by defects in cell-cycle regulation caused by hyperactivation of CDK. Aberrant expression or altered activity of distinct CDK complexes results in the escape of cells from cell-cycle control leading to malignant transformation. Although a number of CDK inhibitors were tested in the clinic in the past, many lacked specificity for CDK, often targeting many unrelated kinases, increasing the likelihood of toxicity. Identifying highly selective small molecule inhibitors targeting CDK is currently a major focus in cancer research.

AG-024322, under development by Pfizer, is a multitargeted pan-cyclin-dependent kinase inhibitor displaying potent inhibition of CDK1, CDK2, and CDK4. AG-024322 potently inhibits these CDK, selectively over a number of other kinases. AG-024322 also inhibits Rb phosphorylation in cells. Aberrant phosphorylation of tumor suppressor Rb, that occurs through the amplification of cyclin-dependent kinase 4 (cycD1-CDK4), or loss of p16ink4a, leads to disruption of Rb function and compromises its growth-inhibitory abilities. Potent antiproliferative activity with AE-024322 was demonstrated in multiple human tumor cell lines; AG-024322 causes arrest at multiple stages

of the cell cycle, is cytotoxic, and induces cell death by apoptosis in a time- and dose-dependent manner.

AG-024322 displays broad spectrum antitumor activity and clear target modulation *in vivo*, with the extent of target modulation correlating with drug exposure in the pharmacodynamic model. In cellular assays, AG-024322 inhibits phosphorylation of CDK substrates, causing induction of cell-cycle arrest, broad spectrum antiproliferative activity, and induction of apoptosis. *In vivo*, AG-024322 inhibits growth of established human tumor xenografts of various origins. Significant antitumor efficacy was observed in 8/9 tumor models tested, with tumor growth inhibition ranging from 32% to 86.4%. In addition, the antitumor effects of AG-024322 were dose-dependent. In the MV522 tumor model, AG-024322 causes a 65% inhibition at MTD, 52% at 1/2 of MTD and only slight antitumor activity at 1/4 of MTD. Direct evidence that AG-024322 antitumor activity correlates with target modulation was obtained by monitoring phosphorylation status of the substrate Rb protein in tumor tissue; dose-dependent reduction in the level of phospho-Rb795 was strongly correlated with dose-dependent inhibition of tumor growth. In addition, treatment with AG-024322 causes dose-dependent apoptosis in tumors *in vivo* (Zhang CC, etal, AACR05, Abs. 4413, and AACR05, Abs. 4415).

A multicenter (n=4), phase I clinical trial (protocol ID: A7091001; NCT00147485) with AG-024322 was initiated in December 2004, in patients with advanced solid tumors and certain types of non-Hodgkin's lymphoma (NHL). The trial's primary objectives are to test the safety of AG-024322, identify the appropriate dose to be used in future clinical trials, and assess the drug's pharmacokinetics (PK). According to the protocol, AG-024322 is administered intravenously (IV), daily, for 5 days, every 3 weeks. Expected total enrollment is 50 patients.

AT7519, under development by Astex Therapeutics (Cambridge, UK), is a novel CDK inhibitor discovered and developed internally using Pyramid, Astex's fragment-based drug discovery platform. Pyramid combines novel and proprietary small molecule fragment libraries with computational chemistry and structural biology techniques, including X-ray crystallography and NMR spectroscopy, to identify drug fragments bound to target proteins. These fragments are then rapidly transformed, using efficient medicinal chemistry, into potent and selective lead compounds. AT7519 is one compound from a series of CDK inhibitors; additional molecules with distinct biologic profiles are also progressing through preclinical development.

A dose-escalation, phase I clinical trial of AT7519 was initiated in October 2005, in the USA and the UK, designed to evaluate the safety and tolerability of the compound administered IV in patients with advanced solid tumors. The trial is being conducted at the Arizona Cancer Center (Tucson, AZ) under the direction of Daniel Von Hoff, MD, and Daruka Mahadevan, MD, and at the Northern Institute for Cancer Research at the University of Newcastle upon Tyne, in the UK, under the direction of Professor Hilary Calvert, MD, who is the international coordinating PI. The IND to initiate phase I clinical trials with AT7519 was approved by the FDA in June 2005. This agent progressed from first synthesis to regulatory approval in just 14 months. IND approval for AT7519 closely followed Clinical Trial Authorization (CTA) by the UK Medicines and Healthcare Products Regulatory Agency (MHRA).

Currently, Astex is using pharmacodynamic biomarker assays as exploratory endpoints in early phase trials of AT7519 that will also be used to support development of other compounds arising from the CDK and other in-house programs. Pharmacodynamic biomarkers were validated preclinically as exploratory endpoints in clinical trials. In these studies AT7519's efficacy was associated with concomitant downregulation in the tumor of markers of CDK inhibition, such as phospho-nucleophosmin (pNPM) and phospho-protein phosphatase 1α (pPP1α), and upregulation of markers of apoptosis such as cleaved poly-ADP ribose polymerase (PARP) and activated caspase 3. Downregulation of several markers was demonstrated in skin samples from tumor-bearing mice. Therefore, it is possible to monitor compound activity in skin punch biopsies obtained from patients in clinical trials in order to establish minimum biologically effective doses (Squires MS, etal, AACR-NCI-EORTC05, Abs. B194).

In December 2005, Novartis entered into an option for a global license of AT7519. Under terms of the agreement, Astex is responsible for the ongoing clinical development of AT7519 until the completion of phase II trials when Novartis may assume responsibility for further development by exercising its licensing option.

RO4584820, under development by Roche, is a novel small molecule selective CDK inhibitor (CDKi) with potent *in vivo* antitumor activity that correlates with inhibition of phosphorylation (hypophosphorylation) of pRb. This drug, 2, 4-diamino-5-oxo-pyrimidine, significantly inhibited growth of a variety of tumor types both *in vitro* and *in vivo*.

In cell-free assays, RO4584820 is a potent and selective ATP-competitive inhibitor of cycB-CDK1, cycE-CDK2, and cycD1-CDK4, and not against a panel of 19 other kinases. It strongly inhibits all these three kinases and, in cellular assays, effectively inhibits proliferation of tumor cell lines. *In vitro*, it effectively inhibits proliferation of tumor cell lines independent of histologic type, and MDR or p53 status, as manifested by cell-cycle block at G1 and G2 and induction of apoptosis, and also reduces phosphorylation of pRb in cells at specific cyc-CDK phosphorylation sites. *In vivo*, it has shown antitumor activity in all models tested to date. It was efficacious when dosed orally at or below MTD in 6/6 established tumor models in rodents with either continuous daily dosing or intermittent once weekly dosing. Also, it was equally active when dosed once a week IV in 5/5 established tumor models. It also inhibited

pRb phosphorylation in human peripheral blood mononuclear cells (PBMC) treated *ex vivo*, thus indicating a potential pharmacodynamic biomarker for clinical use (DePinto WE, etal, AACR05, Abs. 4410, and DePinto WE and Yin X, AACR-NCI-EORTC05, Abs. A76).

Considerable flexibility in dosing schedule and route of administration was demonstrated with this drug in the HCT116 human xenograft model of colon cancer. Similar tumor growth inhibition between 87% and 92% was observed after either oral or IV dosing. In the syngeneic, orthotopic MTLn3 rat mammary tumor model, antitumor activity (92% to 96%) was demonstrated with once weekly oral or IV administration. Antitumor activity was observed in a broad range of tumor models from histologically distinct tumor types. IV or oral administration inhibited tumor growth by 61% to 99% in the H460a non-small cell lung cancer (nsclc), Lox melanoma, MDA-MB-435 breast, and DU145 prostate cancer xenograft models. At therapeutic doses, the drug was well tolerated in mice and rats, with neither overt nor histopathologic signs of toxicity. Histologic evaluation of tumors resected from animals treated for three weeks revealed decreased overall cellularity and mitotic figures (Packman K, etal, AACR-NCI-EORTC05, Abs. A80).

A dose-escalation, phase I clinical trial of RO4584820 was initiated in May 2005 at the University of Colorado Comprehensive Cancer Center (Denver, CO), in patients with advanced solid tumors, including breast, colorectal and small cell lung cancer (sclc). According to the protocol, the drug is administered IV on days 1 and 8 of a 21-day cycle. Initial dose is 8.6 mg/m^2. As of early 2006, 8 patients had been enrolled at this center. The drug has been well tolerated to date. A clinical trial with this drug is also ongoing at M. D. Anderson Cancer Center (MDACC; Houston, TX), under PI Vali Papadimitrakopoulou, MD.

Spindle Poisons

The success of anticancer agents paclitaxel and docetaxel (Taxotere; sanofi-aventis) has spurred intensive development of spindle poisons. Currently, over 100 agents are in various stages of development, with over 60 having entered clinical trials.

BI 2536, under development by Boehringer Ingelheim, is a potent and highly selective inhibitor of polo-like kinase 1 (Plk1) required for mitotic progression and accurate spindle assembly. Plk1 is a member of the polo-like kinase (Plk) family of mitosis control genes. Blocking Plk1 in cancer cells prevents mitosis and proliferation. Because Plk inhibitor drugs specifically target dividing cells, they may avoid the undesirable toxicities of such agents as the taxanes. BI 2536, a mitotic regulator, is a mechanistically novel targeted anticancer agent that induces mitotic arrest with subsequent apoptosis in a wide range of cultured human cancer cells at low nanomolar concentrations.

BI 2536 induces mitotic arrest with subsequent apoptosis in a wide range of cultured human cancer cells at low nanomolar concentrations. BI 2536, administered IV to BomTac:NMRI-Foxn1nu mice grafted with HCT 116 colon carcinoma, NCI-H460 or A549 lung carcinoma, BxPC-3 pancreatic carcinoma or MDA-MB-453 breast carcinoma cells was well tolerated in multiple cycles of once or twice weekly treatment schedules, and efficacious causing significant tumor growth delay or regression. Treated versus control (T/C) values ranged between 0.3% and 31%. Regression of large tumors (average tumor volume at start of treatment=0.5 cm^3) was observed in the HCT 116 colorectal carcinoma model. According to biomarker analyses of sectioned tumors, therapeutic doses of BI 2536 caused a large increase in the score for mitotic figures in xenografted NCI-H460 lung tumors with a peak at 24 hours post administration. At 48 hours post administration *in vivo*, BI 2536 induced first a mitotic block followed by a surge of apoptosis (Baum A, etal, AACR-NCI-EORTC05, Abs. C191).

A multicenter (n=4), open label, dose-escalation, phase I clinical trial (protocol ID: CDR0000446176; BOEH-BI-1216.3; UNMC-16005, NCT00243087) with BI 2536 was initiated in 2005, in the USA, in patients with recurrent or refractory NHL, including adult T-cell leukemia or lymphoma, and anaplastic large cell lymphoma (ALCL). The trial's primary objectives are to determine MTD, safety and tolerability and its secondary objectives are to determine PK and, preliminarily, antitumor activity. According to the protocol, patients are treated with BI 2536 IV over 1 hour on day 1. Courses repeat every 21 days in the absence of disease progression or unacceptable toxicity. Cohorts of 3 to 6 patients are treated with escalating doses of BI 2536 until MTD is determined. An additional 24 patients are then to be treated at MTD. After completion of treatment, patients are followed periodically until disease progression or initiation of another cancer treatment. A maximum of 50 patients will be accrued for this trial, being conducted at Lombardi Comprehensive Cancer Center at Georgetown University Medical Center (Washington, DC), under PI Bruce D. Cheson, MD; Eppley Cancer Center at the University of Nebraska Medical Center (Omaha, NB), under PI Julie M. Vose, MD, who is also Study Chair; James P. Wilmot Cancer Center at University of Rochester Medical Center, in NY, under PI Jonathan W. Friedberg, MD; and MDACC, under PI Anas Younes, MD.

A dose-escalation phase I clinical trial with IV BI 2536 was also initiated at the Klinik für Tumorbiologie (Freiburg, Germany), under PI Klaus B. Mross, MD, in patients with advanced or metastatic solid tumors. Trial objectives are assessment of MTD and overall safety, PK, and preliminary efficacy. According to the protocol, sequential cohorts of 3 to 6 patients were administered a single infusion of BI 2536 per treatment course according to a toxicity-guided dose-escalation design. Further treatment courses were administered in the absence of disease progression or persisting toxicity, after a 3-week observation period. A total of 21 patients were treated at doses of 25, 50, 100, 200 and 250 mg. Reversible Grade 3 neutropenia in 9/21 (43%) patients

was the main drug-related toxicity experienced by 4/6 patients in the 200 mg and 5/6 in the 250 mg dose cohorts. DLT consisting of neutropenic infection occurred in 2/6 patients at 250 mg. MTD was defined as 200 mg. Other common (>20%) drug-related adverse events of mild-to-moderate intensity (Grade 2) consisted of nausea (8/21), fatigue (6/21) and anorexia (5/21). Generally, BI 2536 exhibited a favorable PK and safety profile at the tested dose and schedule. There were no drug-related adverse events resulting in trial discontinuation. A median of 4 courses (maximum=10) was administered without evidence of accumulating toxicity. There was 1 PR in a patient with metastatic squamous cell head and neck cancer treated at 250 mg. Neutropenia, as a mechanism-related toxicity, indicates target inhibition *in vivo* (Mross K, etal, AACR-NCI-EORTC05, Abs. B219).

C242-DM4 (huC242-DM4), under development by ImmunoGen (Cambridge, MA), in collaboration with Takeda Pharmaceutical, is a tumor-activated prodrug (TAP) conjugate consisting of humanized monoclonal antibody (MAb) huC242, and the maytansinoid prodrug DM4. C242 is directed against CanAg antigen, a mucin-type tumor-associated glycoprotein. ImmunoGen has incorporated certain design modifications that include fine tuning the number of methyl groups on either side of the bond to keep DM4, the cell-killing agent, firmly attached to the antibody until the TAP compound binds to and enters a cancer cell.

TAP conjugates kill not only target antigen-positive cells, but also neighboring antigen-negative cells through a bystander mechanism referred to as target cell-activated killing of proximal cells (TKPC). The ability of a drug conjugate to induce TKPC is largely determined by the nature of the linker between antibody and drug. Conjugates linked via a reducible disulfide bond were capable of exerting TKPC, while similar equally potent conjugates linked via a nonreducible thioether bond were incapable of TKPC (Kovtun Y, etal, AACR-NCI-EORTC05, Abs. A71).

Comparison of biodistribution and PK profiles of radiolabeled huC242 antibody and huC242-DM4 conjugate indicates that properties of the huC242 antibody were unaltered by conjugation with DM4. Both antibody and conjugate were biphasically cleared from blood, with terminal phase beginning about 24 hours after administration. Distribution pattern was also similar, with a majority of the injected dose remaining in the bloodstream throughout the study. According to PK evaluation of the conjugate, terminal half-life (t1/2) was between 5 to 6 days. Clearance of the MAb component of the conjugate was 2-fold lower than that measured for the intact conjugate. Linkage between drug and antibody was stable. According to analysis of tissue distribution of huC242-[3H]DM4 upon administration to SCID mice bearing CanAg-positive COLO 205 xenografts, the construct was detectable in tumor as early as 2 hours post administration. By 24 hours, accumulation in tumor exceeded that in blood, and levels in tumor remained higher than levels in blood for the 11-day duration of the study. Total accumulation of DM4 in tumor accounted for approximately 6% of injected dose. Accumulation was observed in bile, suggesting that the liver plays an important role in elimination of DM4 from the host. Excretion in urine accounted for only 8% of injected dose over 11 days. The observed stability of huC242-DM4 in circulation, and its ability to deliver drug to targeted tumor tissue, are consistent with *in vivo* efficacy studies that found the compound to be effective against xenograft tumors at doses well below MTD (Mayo M, etal, AACR-NCI-EORTC05, Abs. A69).

In preclinical studies, huC242-DM4 was found to be significantly more active than cantuzumab mertansine in which huC242 is conjugated to DM1, an earlier version of a maytansinoid prodrug, with comparable tolerability. Treatment with huC242-DM4 resulted in CR in several tumor xenograft models even with single IV administration at doses far below MTD. For example, treatment of SCID mice bearing measurable N87 human gastric tumor xenografts, with a single dose of HuC242-DM4, resulted in CR; huC42-DM4 was also curative in mice bearing HT29 and COLO 205 human colon tumor xenografts, at doses that were nontoxic. Disulfide linkage of huC242-DM4 was approximately 2-fold more stable during circulation in mice than that of cantuzumab mertansine. While huC242-DM4 exhibited increased stability and efficacy *in vivo* compared to cantuzumab mertansine, preliminary acute toxicity studies suggest that the two conjugates have similar tolerability in normal mice. The resulting increased therapeutic window for huC242-DM4 provides a preclinical rationale for its clinical evaluation in patients with CanAg-positive tumors, such as colorectal, pancreatic, gastric, and esophageal cancer (Lutz R, etal, AACR05, Abs. 1429).

In June 2005, patient dosing with huC242-DM4 was initiated in a dose-escalation, phase I clinical trial, at the Cancer Therapy and Research Center (CTRC; San Antonio, TX), under PI Anthony W. Tolcher, MD. In this trial, huC242-DM4 is administered once every 3 weeks to patients with refractory CanAg-expressing tumors. This trial's primary objective is to evaluate safety and PK, and establish MTD. Once MTD is defined, additional patients will be enrolled with tumors that consistently and intensely express CanAg to gain further experience regarding this treatment in these patients.

TPI-287, under development by Tapestry Pharmaceuticals (Boulder, CO), is a third generation taxane analog modified from paclitaxel, designed to circumvent resistance associated with mutant tubulin and multidrug resistance 1 (MDR1), two principle mechanisms by which cancer cells develop resistance to spindle poisons. *In vitro*, activity with TPI-287 was seen across multiple cell lines, including taxane-sensitive and taxane-resistant ones. Taxane-sensitive cell lines in which TPI-287 shows activity include those derived from breast and uterine cancer,

and sclc. Taxane-resistant cell lines in which TPI-287 shows activity include those derived from breast, colon, prostate, and pancreatic cancer. *In vivo*, the drug reduces the rate of tumor growth when compared to paclitaxel in both taxane-resistant and taxane-sensitive breast cancer xenografts, and is also superior to docetaxel in both prostate cancer and nsclc xenografts. Furthermore, TPI-287 has comparable activity to SN-38, a prodrug of irinotecan, in colon cancer.

TPI-287 is more potent than paclitaxel or other tubulin-binding agents. Also, TPI-287 is not a substrate for the MDR drug-efflux pump. According to *in vitro* cytotoxicity data, TPI-287 is more active than paclitaxel by a factor of 18- to 100-fold in tumor cell lines expressing the MDR phenotype. In the KBV1 (MDR+) tumor xenograft model, tumor growth inhibition indexes were 0% in controls, 43% with paclitaxel (43%), and 40%, 72%, and 85% with TPI-287. Cell permeability studies support ability of this novel taxane analog to circumvent the MDR pathway (Jones M, etal, AACR-NCI-EORTC05, Abs. B232).

In a series of *in vitro* and *in vivo* human tumor models, the IC_{50} of TPI-287 was up to 2 orders of magnitude below that observed with reference compounds in 4 tumor cell lines, MCF-7/NCI-ADR, HCT-15, HepG2, DU-145, and MES-SA/Dx5, characterized by the expression of MDR1 P-glycoprotein (P-gp) drug efflux pump, and in tumor xenograft mammary MCF-7/NCI-ADR, prostate PC-3, colon HCT-15, and NSCLC MV522 models grown in athymic mice models. TPI-287 dosing, either once or twice every 7 days for 4 days, was selected in anticipation of future clinical regimens. T/C was 0.56 for TPI 287 versus 0.73 for paclitaxel in the MCF-7/NCI-ADR model, 0.10 for TPI-287 versus 0.81 for docetaxel in the PC-3 model, 0.17 for TPI-287 versus 0.21 in the colon HCT-15 model, and 0.49 for TPI-287 versus 0.93 for docetaxel in NSCLC MV522 model. Therefore, TPI-287 is a potent antitumor agent, with demonstrated activity in both wild type (wt) and drug-resistant human tumor cell lines. Computational modeling of the taxane binding site predicts a unique binding confirmation for TPI-287 between the M-loop and GTP regions of mutant β-tubulin isoform 1. Previous cytotoxicity observations support the original modeling study hypothesis that TPI-287 activity may be attributed to a flexible mechanism of β-tubulin binding, as well as an ability to circumvent the drug efflux pumps (Helson L, etal, AACR05, Abs. 3411).

TPI-287 was 4- to 200-fold more potent than paclitaxel in the ovarian mutant tubulin MDR1-negative and in MDR1-positive cell lines, while displaying equivalent potency to paclitaxel in other MDR1-negative cell lines. Exposure of NCI-AR paclitaxel-resistant breast tumor cells to TPI-287 at a 25% inhibitory concentration did not sensitize the cells to paclitaxel, suggesting that this analog was circumventing rather than reversing MDR1. Cytotoxic advantage of TPI-287 over paclitaxel in tumor cells is independent of the expression of MDR1 and of mutant tubulin-associated resistance, a phenomenon that may be caused by different binding sites on β-tubulin for paclitaxel and TPI-287 (Helson L, etal, ASCO04, Abs. 3114).

Two phase I clinical trials testing different dosing schedules of TPI-287 have been initiated. An open label, multicenter, dose-escalation, phase I clinical trial (protocol ID: TPI 287-01, NCT00113724) was initiated in May 2005, designed to evaluate safety and PK profile of TPI-287 in eligible patients with recurrent and/or refractory malignancies. The trial, to enroll approximately 48 patients, is being conducted at 2 clinical sites, the Lombardi Comprehensive Cancer Center at Georgetown University, under PI, John Marshall, MD, and Westchester Medical Center (Valhalla, NY), under PI Tauseef Ahmed, MD. Based on preclinical evaluation in dogs, the starting dose is 7 mg/m^2. Escalation is performed in 3-patient cohorts at each dose level, with the drug being administered every 7 days.

A nonrandomized, open label, dose-escalation, phase I clinical trial (protocol ID: TPI 287-02, NCT00256191) was also initiated in November 2005, in patients with advanced malignancies. The primary objective is to determine MTD of a single IV administration of TPI-287, once weekly in each 21-day treatment cycle. Like the ongoing every 7-day phase I clinical trial, this trial is evaluating safety and PK profile of the compound in eligible patients with recurrent and/or refractory malignancies. As a result of the experience in the first phase I trial, administration in the second trial began at 56 mg/m^2, which is the dose used in the fourth cohort of the every 7-day trial. Cohorts in this new trial consist of a single patient until there is initial evidence of Grade 2 drug-related toxicities, at which point that cohort and all future cohorts will enroll at least 3 patients at this dose level. The trial is being conducted at the Rocky Mountain Cancer Center (Denver, CO), under PI, Allen Cohn, MD, and at the Sheba Medical Center (Tel Aviv, Israel), under PI Yaacov Baram, MD. Treatment of the fifth cohort is ongoing at a dose of 85 mg/m^2.

Tapestry expects to evaluate TPI-287 in phase II clinical trials in multiple tumor types beginning in the second half of 2006. Additional preclinical experiments studying viability of TPI 287 in an oral formulation are ongoing, and Tapestry will make a decision about whether to initiate clinical trials with an oral form of the drug sometime in 2006.

TTI-237, under development by Wyeth, is a potent, synthetic small molecule promoter of tubulin polymerization with a unique mechanism of action combining properties of a vinca-site and a taxoid-site ligand, making it unique among microtubule-active compounds. TTI-237 is not derived from natural products.

TTI-237 promotes tubulin polymerization in a dose-dependent manner through a novel mechanism of action. It does not bind competitively with paclitaxel, a microtubule-stabilizing agent. Instead, it inhibits binding of vinblastine, a microtubule-destabilizing agent. TTI-237 is

Exhibit 1
Anticancer Agents in Phase I Monotherapy Clinical Trials with Recently Reported Preclinical or Clinical Findings

Developer ❑ Affiliate(s)	Generic Name ❑ Number ❑ Brand Name	Description ❑ Administration Route	Development Status ❑ Indication(s)
Agensys ❑ U California Los Angeles, Abgenix	AGS-PSCA	Fully human high affinity, IgG1κ monoclonal antibody (MAb) targeting prostate cancer stem cell antigen (PSCA) ❑ IV	Phase I (begin 11/05)➢USA ❑ advanced or hormone-refractory prostate cancer (HRPC)
Allos Therapeutics ❑ Cancer Research Technology, U Colorado, U Salford	RH1, RH-1	Novel targeted diaziridinylbenzoquinone cytotoxic prodrug bioactivated by the enzyme DT-diaphorase (DTD), which is overexpressed in many tumors relative to normal tissue ❑ IV	Phase I (begin 9/03, ongoing 2/06) ➢Europe (UK) ❑ advanced, refractory solid tumors
Alza ❑ Chong Kun Dang Pharmaceutical	CKD-602, S-CKD602, AP-30	Stealth liposome formulation of CKD-602, a camptothecin derivative topo I inhibitor marketed abroad ❑ IV	Phase I (begin 9/03, ongoing 2/06) ➢USA ❑ advanced, refractory solid tumors
Array BioPharma	ARRY-334543	Novel, potent, orally active, small molecule, dual inhibitor of epidermal growth factor receptor (EGFr) and HEr2 kinases ❑ PO	Phase I (begin 2/06)➢Canada ❑ advanced solid tumors or hematologic malignancies
Astex Therapeutics ❑ Novartis	AT7519	Novel cyclin dependent kinase (CDK) inhibitor ❑ IV	Phase I (begin 10/05)➢USA, Europe (UK) ❑ advanced solid tumors
AstraZeneca	AZD1152	Prodrug of AZD1152 hydroxy-QPA, a specific inhibitor of Aurora kinase enzyme activity, with selectivity for Aurora B ❑ IV	Phase I (ongoing 2/06)➢Europe ❑ advanced solid tumors
AstraZeneca ❑ Array BioPharma	AZD6244, ARRY-142886	Selective, orally active inhibitor of MAPK/ERK/kinase (MEK) ❑ PO	Phase Ia (begin 6/04, completed 12/05)➢USA; phase Ib (ongoing 2/06)➢USA ❑ advanced, refractory solid tumors
Boehringer Ingelheim	BI 2536	Potent and highly selective inhibitor of polo-like kinase 1 (Plk1) required for mitotic progression and accurate spindle assembly ❑ IV	Phase I (begin 05)➢USA ❑ advanced, refractory or relapsed non-Hodgkin's lymphoma (NHL); phase I (ongoing 06)➢Europe (Germany) ❑ advanced or metastatic solid tumors
Boehringer Ingelheim	BIBF 1120	Potent, orally available inhibitor of vascular endothelial growth factor receptor (VEGFr), fibroblast growth factor receptor (FGFr), and platelet-derived growth factor receptor (PDGFr) tyrosine kinases ❑ PO	Phase I (completed 11/04)➢ Europe (Germany, UK) ❑ refractory solid tumors; phase I (completed 05)➢Europe (Germany) ❑ advanced colorectal cancer
Boehringer Ingelheim	BIBW 2992	Highly potent, orallly available selective and irreversible inhibitor of intracellular EGFr and HEr2 tyrosine kinase activity ❑ PO	Phase I (completed 05)➢Europe (Netherlands), phase I (completed 05)➢USA, phase I (ongoing 2/06)➢Europe (UK) ❑ advanced solid tumors
Callisto Pharmaceuticals ❑ AnorMED	Azaspirane ❑ Atiprimod	Macrophage-targeting oral cytokine inhibitor with antiangiogenic properties ❑ PO	Phase I/IIa (begin 5/04, ongoing 2/06)➢USA ❑ multiple myeloma; phase I/II (begin 3/05, ongoing 2/06)➢USA ❑ advanced solid tumors or hematologic malignancies; phase I (planned 2/06) ➢USA ❑ myelodysplastic syndrome (MDS)

— continued on next page

Chiron	CHIR258, CHR258LC, CHIR 258	Orally active small molecule benzimidazole-quinoline with potent inhibitory activity against certain growth factor receptor tyrosine kinases important in tumor growth and angiogenesis ❑ PO	Phase I (begin 1/04)➢USA ❑ advanced solid tumors; phase I (begin 6/05, ongoing 2/06)➢USA, UK ❑ refractory or relapsed multiple myeloma; phase I (begin 9/04, ongoing 2/06)➢Europe (UK) ❑ relapsed or refractory acute myelogenous leukemia (AML)
Chiron ❑ Xoma, Abgenix	Anti-CD40mAb, CHIR-12.12	Recombinant MAb targeting B-cell hematologic malignancies with a dual mechanism of action; upon binding to certain tumor cells, it mediates killing of CD40-expressing tumors by immune effector cells and prevents CD40 ligand-mediated growth and survival ❑ injection	Phase I (begin 4/05, ongoing 2/06) ➢USA ❑ relapsed or refractory chronic lymphocytic leukemia (CLL); phase I (begin 12/05) ➢USA ❑ relapsed multiple myeloma
Conforma Therapeutics ❑ Memorial Sloan-Kettering Cancer Center (MSKCC), Duke U, Pfizer, Burnham Institute, Harvard U	CNF-101, CNF1010	Heat shock protein-90 (hsp90)-directed drug based on a new proprietary form of the geldanamycin derivative 17-AAG, in a novel, optimized formulation ❑ IV	Phase I (begin 8/04, ongoing 2/06) ➢USA ❑ advanced solid tumors; phase I (begin 8/04, ongoing 2/06) ➢USA ❑ refractory or relapsed chronic myelogenous leukemia (CML)
Cougar Biotechnology ❑ Emory U School of Medicine	Noscapine ❑ CB3304	Orally active alkaloid derived from opium that targets and alters microtubule dynamics, blocks mitosis, and causes apoptosis ❑ PO	Phase I/II (begin 3/00, ongoing 2/06)➢USA ❑ relapsed or refractory NHL or CLL
EntreMed	ENMD-1198	New chemical entity based on the antitumor and antiangiogenic agent 2-methoxyestradiol (2-ME2), with a reduced rate of metabolism and improved PK and antitumor activity in animal models ❑ PO	Phase I (planned 2/06)➢USA ❑ solid tumors
Exelixis ❑ GlaxoSmithKline, Symphony Evolution	XL647	Potent simultaneous inhibitor of receptor tyrosine kinases (RTK) EGFr, HEr2, VEGFr, and EphB4 ❑ PO	Phase I (begin 6/04, ongoing 2/06) ➢USA ❑ advanced or metastatic solid tumors
Exelixis, GlaxoSmithKline	XL880	Novel, orally administered small molecule drug that significantly inhibits Met and VEGFr2 ❑ PO	Phase I (begin 3/05, ongoing 11/05) ➢USA ❑ advanced solid tumors
Gemin X Biotechnologies	Obatoclax ❑ GX15-070, GX015-070	Small molecule inhibitor of the BH3-binding groove of the Bcl-2 family of proteins that are frequently overexpressed in malignancy ❑ IV	Phase I (begin 11/04, ongoing 2/06)➢USA ❑ solid tumors, CLL; phase I/II (begin 1/05, ongoing 2/06)➢USA, Canada ❑ refractory CLL
Genentech ❑ Amgen, U Pittsburgh	PRO1762, TRAIL/Apo2L, Apo2L/TRAIL, AMG 951	Soluble type II transmembrane protein detected in most tissues that binds to at least 4 distinct receptors found on many tumor cells, and signals these cells to destroy themselves through apoptosis ❑ IV, intralesional	Phase I (begin 8/04, ongoing 2/06) ➢USA ❑ advanced or metastatic solid tumors, or advanced NHL
Hana Biosciences ❑ Dana-Farber Cancer Institute (DFCI)	Talotrexin ammonium ❑ PT-523, PT523, NSC 712783 ❑ Talopterin	Novel multitargeted antifolate that is a water-soluble, nonpolyglutamatable, type-B analog of aminopterin ❑ IV	Phase I (begin 3/04, ongoing 2/06) ➢USA ❑ advanced, recurrent solid tumors; phase I/II (begin 7/05, ongoing 2/06)➢USA ❑ relapsed or refractory acute lymphoblastic leukemia (ALL); phase I/II (ongoing 1/06) USA, Europe (Russia) ❑ relapsed or refractory non-small cell lung cancer (nsclc); phase I/II (planned 2/06)➢USA ❑ cervical cancer

— continued on next page

ImmunoGen ❑ Takeda Pharmaceutical, Pfizer	HuC242-DM4	Tumor-activated prodrug (TAP) conjugate consisting of humanized MAb huC242 and the maytansinoid prodrug DM4, directed against CanAg antigen, a mucin-type tumor-associated glycoprotein ❑ IV	Phase I (begin 6/05, ongoing 2/06) ➢USA ❑ refractory solid tumors
Infinity Pharmaceuticals	IPI-504	Novel, water-soluble targeted anticancer agent that preferentially induces death of cancer cells through inhibition of the Hsp-90 complex ❑ IV	Phase I (begin 6/05, ongoing 1/06) ➢USA ❑ refractory or relapsed multiple myeloma; phase I (begin 12/05)➢USA ❑ refractory gastrointestinal stromal tumors (GIST)
Ipsen ❑ Spirogen, Cancer Research UK	BN2629, BN-2629, SP-2001 SJG-136, SJG136, NCI 694501	Novel rationally designed DNA minor groove interstrand cross-linking agent spanning 6 base pairs of DNA with potent and broad spectrum antitumor activity ❑ IV	Phase I (begin 5/04, ongoing 11/05) ➢USA, Europe (UK) ❑ advanced, refractory solid tumors
Kosan Biosciences ❑ National Cancer Institute (NCI)	KOS-1022, 17-DMAG, NSC707545	Second generation water-soluble benzoquinone ansamycin that is a highly potent Hsp90 inhibitor, with good oral bioavailability ❑ IV, PO	Phase I (begin 6/04, ongoing 2/06) ➢USA (IV), phase I (begin 7/04, ongoing 2/06)➢USA (IV); phase I (begin 1/06)➢USA (PO) ❑ inoperable or metastatic solid tumors, or lymphoma; phase I (begin 5/05)➢USA (IV) ❑ advanced ALL, AML or CML; phase I (begin 7/04, ongoing 2/06)➢USA (IV), phase I/II (begin 1/05, ongoing 2/06) ➢Europe (UK) ❑ inoperable or metastatic solid tumors
Merck ❑ Vertex Pharmaceuticals	VX-680, MK0457	Small molecule inhibitor of Aurora and FLT-3 kinases ❑ PO	Phase I (begin 1/05, ongoing 2/06) ➢USA ❑ advanced, refractory solid tumors; phase I (begin 6/05, ongoing 2/06)➢USA ❑ relapsed or refractory AML, MDS, ALL, and CML in blast crisis (CML-BC); phase I (begin 12/04, ongoing 1/06) ➢USA ❑ advanced, refractory colorectal cancer
Millennium Pharmaceuticals	MLN8054	Orally bioavailable, potent, selective small molecule Aurora A kinase inhibitor ❑ PO	Phase I (begin 10/05)➢USA ❑ advanced or metastatic solid tumors, or advanced lymphoma
OSI Pharmaceuticals	OSI-930	Oral, small molecule, receptor tyrosine kinase (RTK) inhibitor that primarily targets c-kit, VEGFr2, and PDGFrβ ❑ PO	Phase I (begin 2/05, ongoing 2/06) ➢USA ❑ healthy volunteers
Pfizer ❑ OSI Pharmaceuticals	CP-868,596	Potent angiogenesis inhibitor targeting PDGFrβ kinase ❑ PO	Phase I (begin 03, ongoing 2/06) ➢USA ❑ advanced solid tumors
Pfizer	AG-024322	Inhibitor of cyclin-dependent knases (CDK) 1, 2 and 4 ❑ IV	Phase I (begin 12/04, ongoing 2/06) ➢USA ❑ advanced solid tumors or NHL
Pfizer Global Research and Development	PD0325901	Significantly more potent analog of CI-1040 with an improved pharmaceutical profile and exquisite specificity MEK and, particularly, MEK1 ❑ PO	Phase I/II (begin 2/04, ongoing 2/06) ➢USA ❑ advanced, refractory solid tumors
PharmaMar	ES-285, ES285 ❑ Spisulosine	Sphingosine-like compound derived from the edible Arctic clam Mactromeris (Spisula) polynyma, that inhibits cell migration, adhesion, and metastasis ❑ IV	Phase I (begin 6/03, ongoing 10/05) ➢Europe (Spain, Netherlands) ❑ advanced, refractory solid tumors
PharmaMar	PM-10450, PM00104 ❑ Zalypsis	Novel chemical entity related to the marine natural compounds Jorumycin and the family of Renieramycins, obtained from mollusks and sponges, respectively ❑ IV	Phase I (begin 1/05, ongoing 2/06) ➢Europe (Spain) ❑ advanced solid tumors or lymphoma

— continued on next page

Reata Pharmaceuticals ❑ U Texas Southwestern Medical Center, U Texas MDACC, Dartmouth College	RTA 401 (CDDO) and RTA 402 (CDDO-Me)	Novel synthetic oleanane triterpenoid with potent cytotoxic activity in a wide variety of cancer cell types ❑ PO, IV	Phase I (begin 10/05)➢USA ❑ relapsed or refractory leukemia
Reata Pharmaceuticals ❑ U Texas Southwestern Medical Center, U Texas MDACC, Roswell Park Cancer Institute	RTA 744 (IV), WP744 and RTA 769 (oral)	Topoisomerase II inhibitors belonging to a novel class of anthracycline derivatives that efficiently cross the blood-brain barrier for the treatment of brain cancer, as well as hematologic malignancies and other solid tumors ❑ PO, IV	Phase I (begin 9/05)➢USA ❑ advanced or refractory primary brain cancer
Roche	RO4584820, CDKi	Small molecule that selectively inhibits CDK 1, 2, and 4, as well as Rb phosphorylation, causing G1/G2 arrest, and inducing apoptosis ❑ PO	Phase I (ongoing 2/06)➢USA ❑ solid tumors
Santaris Pharma	SPC2996	Inhibits synthesis of Bcl-2, a key sensor protein that protects cells against apoptosis ❑ IV	Phase I (begin 5/05, ongoing 2/06) ➢Europe (Denmark, France, UK) ❑ advanced CLL
Seattle Genetics	SGN-40, SGN40 (anti-huCD40 MAb)	High-affinity, rapidly internalizing, humanized MAb targeting CD40 antigen, which is highly expressed on most B-lineage hematologic malignancies ❑ IV	Phase I (begin 3/04, ongoing 2/06) ➢USA ❑ refractory or recurrent multiple myeloma; phase I (begin 12/04, ongoing 2/06)➢USA ❑ refractory or recurrent NHL; phase I/II (begin 12/05)➢USA ❑ relapsed or refractory CLL
Serono ❑ ZymoGenetics	TACI-Ig	Soluble fusion protein that links the extracellular portion of the transmembrane activator and CAML-interactor (TACI) receptor to the Fc portion of human immunoglobulin (Ig) ❑ IV, subcutaneous (SC)	IND (approved 10/04)➢USA ❑ B-cell hematologic malignancy, phase I/II (begin 10/04, ongoing 2/06)➢Europe (France) ❑ advanced, refractory or relapsed, multiple myeloma
Servier Group ❑ Université René Descartes	S23906-1, S23906	Potent novel benzoacronycine derivative antitumor agent acting through alkylation of the N2 position of guanines in DNA ❑ IV	Phase I (ongoing 2/06) Europe (France) ❑ advanced solid tumors or leukemia
Tapestry Pharmaceuticals	NBT-287, TPI-287	Third generation taxane analog modified from paclitaxel, designed to circumvent mutant tubulin resistance and multidrug resistance 1 (MDR1) ❑ IV, PO	Phase I (begin 5/05, ongoing 2/06)➢USA, phase I (begin 11/05)➢USA ❑ advanced, recurrent or refractory solid tumors
Wyeth Pharmaceuticals	TTI-237	Potent, synthetic small molecule promoter of tubulin polymerization with a unique mechanism of action combining properties of vinca-site and taxoid-site ligands ❑ PO, IV	Phase I (begin 8/05, ongoing 2/06) ➢USA, phase I (begin 5/05, ongoing 2/06)➢USA ❑ advanced solid tumors
Ziopharm Oncology ❑ M. D. Anderson Cancer Center, Texas A&M U	ZIO-101	Arsenic atom, complexed to organic molecules, that is water soluble, more potent and less toxic than inorganic arsenic ❑ IV	Phase I (begin 5/05, ongoing 2/06) ➢USA ❑ hematologic malignancies; phase I (begin 5/05, ongoing 2/06)vadvanced or metastatic solid tumors; phase I/II (begin 1/06) ➢USA, Canada, Europe (UK) ❑ advanced, refractory multiple myeloma

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), January 2006

potent against various tumor cell lines, including those that are resistant to paclitaxel because of elevated P-gp expression. TTI-237 possesses favorable pharmaceutical properties and is active in several tumor xenograft models when dosed orally or IV (Zhang N, etal, AACR-NCI-EORTC05, Abs. 229).

An open label, multicenter (n=3), dose-escalation, phase I clinical trial (protocol ID: 3162K1-101, NCT00195247) of TTI-237, administered IV every 3 weeks in patients with advanced solid tumors, was initiated in May 2005 to determine safety, tolerability, and MTD, as well as PK and antitumor activity. Expected total enrollment is 45 patients. Among participating centers is the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University (Baltimore, MD), under PI Julie R. Brahmer, MD. In this phase I clinical trial (protocol ID: CDR0000428448; UCLA-0412089-01; W-AR-3162K1-101-US; NCT00112814), also initiated in June 2005 at Jonsson Comprehensive Cancer Center at UCLA, under PI Carolyn Britten, MD, patients are treated with TTI-237 IV over 1 hour on day 1. Treatment repeats every 21 days for up to 6 courses in the absence of disease progression or unacceptable toxicity. Responders or those with stable disease at the end of 6 courses may be treated with additional courses at the discretion of the investigator. Cohorts of 1 to 6 patients are treated with escalating doses of TTI-237 until MTD is determined. An additional 6 to 10 patients are treated at MTD. After completion of treatment, patients are followed at 30 days.

Another multicenter (n=3) dose-escalation, phase I clinical trial (protocol ID: 3162K1-100, NCT00195325) of TTI-237, administered IV once weekly in patients with advanced solid tumors, was initiated in August 2005 to determine safety, tolerability, and MTD, as well as PK and antitumor activity. Expected total enrollment is 45 patients. One participating center is Vanderbilt University (Nashville, TN), under PI Albert C. Lockhart, MD.

Other Cytotoxics/Apoptosis Inducers

Many of the agents described here act on a variety of distinct mechanisms of action but with cytoxicity as the ultimate result.

Azaspirane (Atiprimod), under development by Callisto Pharmaceuticals (New York, NY), is a macrophage-targeting oral cytokine inhibitor with antiangiogenic properties. Callisto is currently evaluating Atiprimod in solid tumors and hematologic malignancies, primarily in multiple myeloma, and also plans to evaluate Atiprimod in patients with myelodysplastic syndrome (MDS). Atiprimod significantly inhibits tumor-cell growth and induces apoptosis in drug-sensitive and drug-resistant human multiple myeloma cell lines.

In preclinical studies, Atiprimod downregulated genes involved in growth and cell-cycle control, adhesion, and cell-signaling pathways; upregulated genes implicated in apoptotic cascades; and negatively regulated signal transduction. In a preclinical *in vitro* study, Atiprimod decreased cell survival of both IL-6-independent (OPM1) as well as dependent (INA-6) multiple myeloma cells. *In vivo*, Atiprimod was evaluated in 3 different animal models of human multiple myeloma. In SCID mice bearing SC OPM1 cells, treated IP with Atiprimod or vehicle alone, on alternate days for 2 weeks, tumor inhibition was dose-dependent, ranging from 28% to 60%. Treatment with Atiprimod reduced human paraprotein in mouse sera, whereas IgGκ continued to rise in mice treated with vehicle alone (Neri EP, etal, ASCO05, Abs. 6603, and Neri P, etal, ASH05, Abs. 249).

Neither IL-6, insulin growth factor 1 (IGF-1), nor adherence of multiple myeloma cells to bone marrow stromal cells (BMSC), protected against Atiprimod-induced apoptosis. Apoptosis induced by Atiprimod was augmented by conventional agents such as dexamethasone, doxorubicin, or melphalan, and novel agents such as arsenic trioxide. Atiprimod inhibited Stat3 and Akt, but not Erk1/2, phosphorylation triggered by IL-6, and also inhibited IκBα and NFκB p65 phosphorylation triggered by tumor necrosis factor α (TNF-α). Importantly, Atiprimod inhibited both IL-6 and VEGF secretion in BMSC triggered by multiple myeloma cell binding, and also inhibited angiogenesis in human umbilical vein cells (HUVEC) (Anderson KC, etal, ASH05, Abs. 1182).

In May 2004, a multicenter phase I/II clinical trial (protocol ID: CP-101, NCT00086216) was initiated with Atiprimod at MDACC, under PI Moshe Talpaz, MD, St. Vincent's Comprehensive Cancer Center (New York, NY), Roswell Park Cancer Institute (Buffalo, NY), and Dana-Farber Cancer Institute (DFCI; Boston, MA), under PI Nikhil C. Munshi, MD, in patients with refractory or relapsed multiple myeloma. Primary objectives are to evaluate safety and to identify MTD. Each cycle of treatment consists of 14 consecutive days of oral Atiprimod followed by 14 consecutive days without treatment.

In this trial, cohorts of 3 patients were treated at 30, 60, 90, 120 mg/day, and 2 patients were enrolled at the 180 mg/day level. No cohorts were expanded because of DLT. Median number of cycles was 2 (range=1-5). In an interim analysis of this trial, 12/14 enrolled patients were evaluable. These patients had been previously treated with a median of 4 (range=3-7) lines of therapy; median duration from initial treatment to entry in this trial was 36 months (range=19-76). Common Grade 1 toxicities included diarrhea, liver enzyme elevation, and dyspepsia. There were 2 Grade 2 toxicities, a neutropenia at the 90 mg/day level, and diarrhea at the 120 mg/day level. There was 1 Grade 3 transaminase elevation during the second treatment cycle that resolved on its own during the 14-day period off treatment. After the first 14 days of treatment, a transient but clear reduction of M proteins (30% and 80%) was noted in 2 patients with rapidly rising serum M proteins prior to enrollment; also, 2 patients treated at higher dose levels reported subjective improvement in bone pain. Atiprimod was generally well tolerated in this heavily pretreated group. MTD has not been reached. Although no responses were observed to date, clinical activity is not expected until higher dose levels (240 mg/day, 300 mg/day, and 360 mg/day) are evaluated. After MTD is established, Atiprimod combinations should be considered based on *in*

vitro assessment of synergy with other active agents (Wang M, etal, ASH05, Abs. 111).

A phase I/II clinical trial (protocol ID: CP-102, NCT00214838) with Atiprimod was initiated in March 2005, in patients with advanced solid tumors or hematologic malignancies, at MDACC under PI Razelle Kurzrock, MD. The trial's primary objective is to identify MTD and evaluate safety of Atiprimod in patients with advanced cancer. Secondary objectives include PK analysis and efficacy. The trial is also comparing PK of Atiprimod tablets versus capsules at the starting dose, with the intent of switching to capsules for the dose-escalation process if capsules pose no safety issues. Expected total enrollment is 34 patients.

In February 2006, Callisto announced its commitment to initiate a new phase I/II clinical trial of Atiprimod in patients with advanced carcinoid cancer, to open at several new sites in mid-2006, based on encouraging clinical results that showed a clear response in a patient with advanced carcinoid cancer plus additional encouraging clinical data in other patients with carcinoid tumors. This activity of Atiprimod in carcinoid tumors was uncovered from the ongoing phase I/II trial (protocol ID: CP-102, NCT00214838). Because of the response seen in this trial, Callisto is now focusing on a range of other types of liver cancer, and other metastatic diseases in addition to carcinoids.

CB3304 (noscapine), under development by Cougar Biotechnology (Los Angeles, CA), is an orally active alkaloid derived from opium that targets and alters microtubule dynamics, blocks mitosis, and causes apoptosis. This tubulin-binding agent increases the time cellular microtubules spend idle in a paused state, arresting mammalian cells in mitosis. Its mechanism of action appears similar to that of vincristine. Noscapine is a commonly used antitussive agent commercially available in Europe, Asia, and South America.

In June 2004, Cougar Biotechnology licensed worldwide, exclusive rights to an intellectual property portfolio for noscapine and analogs from Emory University (Atlanta, GA). Under terms of the agreement, Emory received an upfront payment, and is eligible for milestone payments as compounds progress through clinical development, as well as royalties on sales. As part of this licensing agreement, Cougar is sponsoring additional preclinical research studies of noscapine at Emory.

Noscapine is well tolerated at even higher doses in humans than in animal models, including pregnant women. Basis for tumor selectivity arises because of accumulation of genotoxic amounts of DNA as a result of multiple rounds of DNA synthesis without cytokinesis during which the cytoplasm is cleaved into the daughter cells. This leads to apoptosis in tumors attributed to mutational lesions and compromised checkpoints. Nontumorigenic normal cells halt the cell cycle until the drug is metabolized and/or secreted and then resume normal cell cycles. *In vivo*, no toxicity was detected in hematopoietic tissue, gut, spleen, and long nerves that are common targets of antimicrotubule cancer drugs. Tumor suppressive doses of noscapine in mice do not affect overall numbers of B cells, T cells, or NK cells. Mice undergoing noscapine treatment mounted potent T-cell-mediated immune responses to an acute lymphocytic choriomeningitis (LCMV) infection. Level of CD8 T-cell expansion and ability of CD8 T cells to produce effector molecules including granzyme B and IFN-epsilon, and TNF-α cytokines, was unaltered (Aneja R, etal, AACR05, Abs. B230).

Murine melanoma B16LS9 cells treated with noscapine do not arrest in mitosis but rather become polyploid prior to cell death, whereas primary melanocytes reversibly arrest in mitosis and resume a normal cell cycle after noscapine removal. In an *in vivo* model of melanoma, treatment with noscapine inhibited tumor volume by 85% on day 17 when compared with untreated animals, without evidence of toxicity to the spleen, liver, duodenum, bone marrow, or peripheral blood. This inhibition was greater than that of paclitaxel alone, and similar to inhibition when noscapine was combined with paclitaxel. Noscapine also significantly inhibited melanoma progression by 83% on day 18 when delivered in drinking water (Landen JW, etal, Cancer Res, 15 Jul 2002;62 (14):4109-14).

Orally administered CB3304 exhibits potent antitumor activity against NHL and multiple myeloma cell lines *in vitro*, and *in vivo* in SCID-beige murine xenograft models. According to investigators at Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY), and Weill Presbyterian Hospital (New York, NY), noscapine's potency increases with increased duration of exposure in B- and T-cell lymphoma, and multiple myeloma. Across all cell lines studied, increasing duration of exposure by only a couple of days resulted in multiple log decreases in IC_{50}. Combining noscapine with doxorubicin and 4-hydroxycyclophosphamide *in vitro* resulted in cytotoxicity that was at least additive or better. Toxicity was much greater when noscapine was administered intraperitoneally (IP); 60% of animals died as a result of toxicity in the 600 mg/kg IP cohort, while none died from toxicity when the drug was administered by oral gavage. According to preliminary efficacy experiments, by day 15, tumor growth delays were 30% to 50% compared to control tumor volume in animals treated with noscapine at doses of 600 and 400 mg/kg by oral gavage. A linear dose-response relationship was observed in the activation of caspase 3 by noscapine, which was strongly related to duration of drug exposure. Noscapine may represent a new well tolerated oral treatment option for NHL and multiple myeloma. Preclinical antitumor activity has been demonstrated in models of glioblastoma multiforme (GBM), lymphoma, breast and bladder cancer, and melanoma (O'Connor O, etal, ASH05, 2427).

Noscapine inhibited proliferation of both paclitaxel-sensitive and paclitaxel-resistant human ovarian carcinoma

cell lines (1A9, 1A9PTX10 and 1A9PTX22), which harbor α-tubulin mutations that impair paclitaxel-tubulin interaction. These cells undergo apoptotic death upon noscapine treatment, accompanied by activation of the c-Jun NH2-terminal kinases (JNK). Inhibition of JNK activity by treatment with antisense oligonucleotide, or transfection with dominant-negative JNK, blocks noscapine-induced apoptosis. The JNK pathway, therefore, plays an essential role in microtubule inhibitor-induced apoptosis (Zhou J, etal, J Biol Chem, 18 Oct 2002;277 (42):39777-85).

In an open label, dose-escalation, phase I/II clinical trial (protocol ID: 0C-99-16; NCT00183950), initiated in March 2000 at the University of Southern California (USC) Norris Comprehensive Cancer Center and Hospital (Los Angeles, CA), under PI Anil Tulpule, MD, cohorts of patients with relapsed/refractory NHL or chronic lymphocytic leukemia (CLL)/small lymphocytic leukemia (SLL) are being treated at 3 different daily dose levels of 1 g, 2 g, and 3 g. At each dose level, the drug is being administered orally on a 3 times-a-day schedule. Among 12 patients [CLL/SLL=4, mantle-cell lymphoma (MCL)=2, follicular Grade III lymphoma=1, diffuse large cell (DLC) NHL=4, and lymphoplasmacytic low grade lymphoma=1] enrolled, treated for 49 days, 10 were evaluable for response. There was 1 PR in a patient with Grade III follicular NHL, lasting 56+ months. Disease stabilized in a patient with MCL for 30 days, and in a patient with DLC for 77 days; disease progressed in the remaining 7 patients. Noscapine was well tolerated, with no Grade 3 or 4 hematologic toxicities. There was one incident of Grade 3 neurotoxicity consisting of a depressed level of consciousness at the 3 g dose level (Tulpule A, etal, ASH05, Abs. 3341).

ES-285 (Spisulosine), under development by PharmaMar (Madrid, Spain), is a sphingosine-like synthetic small molecule originally found in the edible Arctic clam *Mactromeris (Spisula) polynyma*. ES-285 inhibits growth of human tumor xenografts *in vivo*, and causes loss of actin stress fibers, cell-cycle arrest at G2/M, and apoptosis *in vitro*. As a result, ES-285 inhibits cell migration, adhesion, and metastasis.

ES-285 is similar to some of the second messengers used by human cells in their internal signaling processes. Its mechanism of action includes inhibition of key molecules regulating the intracellular actin fibers of the cytoskeleton. These novel mechanism of action of ES-285 differentiates it from other antitumor agents in that it disrupts the cytoskeleton of cancer cells. Molecular studies suggest that ES-285 may achieve this by targeting the activity of the GTP-binding protein Rho, a crucial factor for the formation of cytoskeletal fibers, cell migration, cell adhesion, and tumor-cell proliferation, via endothelial cell differentiation gene (EDG) receptor (i.e. LPA and S1P) signaling. ES285 activates Erk, and stress-fiber formation and mobilizes calcium in NIH-3T3 cells, in a similar fashion as LPA or S1P. However, ES285 is not a complete agonist of these bioactive lipids (Salcedo M, etal, AACR-NCI-EORTC03, Abs. C24). This drug acts by a new mechanism, not as yet exploited in cancer chemotherapy.

Scientists at the Institute of Cancer Research (Sutton, Surrey, UK) used cDNA gene expression microarrays to investigate the mechanism of action of ES-285. Cellular and molecular response to ES-285 was compared with those of structurally related compounds, including safingol, sphingosine and ES-427, an inactive analog of ES-285, together with compounds known to modulate Rho/ROCK signaling, including FTI-277, GGTI-298 and Y-27632. ES-285 exhibited antiproliferative activity against a panel of 10 cancer cell lines *in vitro* at nanomolar concentrations. In HT29 colon cancer cells, treatment with ES-285 was associated with loss of actin stress fibers. The transcriptional response to ES-285 is distinct from, but overlaps with that induced by structurally related compounds and inhibitors of Rho/ROCK signaling (Baird R, etal, AACR05, Abs. 4111).

A phase I clinical program was initiated involving four trials using different schedules of administration of ES-285. One of these trials, conducted at the Institut Gustave Roussy (Villejuif, France) and Institut Catala de Oncologia (Barcelona, Spain), assessed a 3-hour infusion of ES-285, administered every 3 weeks, in patients with advanced solid tumors. Primary objectives were to evaluate safety and tolerability of ES-285, and to define DLT and MTD. Secondary objectives were preliminary PK and efficacy. According to the protocol, the starting dose was 4 mg/m^2 with cohorts of 3 to 6 patients treated at increasing doses of 8, 16, 32, 64, 128, and 256 mg/m^2. A 200 mg/m^2 dose was used in 12 additional patients in the expanded cohort. PK sampling was performed during the first infusion. Among 40 patients enrolled in this trial, DLT was observed at the two higher dose levels, in 2/2 patients at the 256 mg/m^2 dose and 2/19 patients at the 200 mg/m^2 dose. DLT occurred in 1/7 patients during dose escalation, and 1/12 during the expanded cohort. DLT involved asymptomatic Grade 3/4 reversible transaminases elevation and Grade 3 self-limiting (24 hours) confusional syndrome. Other toxicities included phlebitis and pyrexia as well as headache appearing early post infusion. There was one unconfirmed PR after 2 infusions in a patient with metastatic melanoma treated at the 128 mg/m^2 dose. ES-285 has a long half-life (range=37-66 hours) and is distributed widely. Because ES-285 exhibits a wide (supraphysiologic) volume of distribution, most of the drug is out of the circulation. ES-285 PK appears to be dose linear in this schedule (Armand JP, etal, AACR-NCI-EORTC05, Abs. C70).

A dose-escalation, PK and pharmacodynamic phase I clinical trial of ES-285 is also being conducted at Royal Marsden Hospital (London, UK) and Erasmus Medical Centre (Rotterdam, The Netherlands), in patients with advanced solid tumors, to determine safety, tolerability and MTD of ES-285 administered as a 24-hour IV infusion on a 21-day cycle. Pharmacodynamic studies include genomic profiling of blood samples and skin biopsies taken

pre and post infusion. According to the protocol, sequential cohorts of 3 to 4 patients were treated at doses of 4, 8, 16, 32, 64, 128 and 256 mg/m^2. According to interim results, 25 patients were treated with a median of 2 cycles; 6 patients were treated with 4 cycles. There were no CR or PR. At the 256 mg/m^2 dose level, 2/4 patients experienced DLT, manifested as asymptomatic Grade 3 rises in liver transaminases (AST, ALT), peaking on day 3 after infusion and resolving over 2 to 3 weeks. In addition, one of these patients experienced Grade 3 confusion and ataxia on day 4 which resolved on day 5. Other side effects that were self-limiting, included phlebitis, fever, fatigue, nausea, vomiting and renal impairment. At the 128 mg/m^2 dose level, ES-285 has a long half-life (>30 hours), and a large volume of distribution. MTD on this schedule has been established at 256 mg/m^2. The 200 mg/m^2 dose is currently being explored in order to identify a recommended dose for phase II studies. Genomic translational studies are ongoing to further explore mechanism of action and pharmacodynamic endpoints for use in future clinical trials (Baird R, etal, AACR-NCI-EORTC05, Abs. B98).

PM00104 (Zalypsis), also under development by PharmaMar, is a novel chemical entity related to the marine natural compounds *Jorumycin* and the family of *Renieramycins*, obtained from mollusks and sponges, respectively. Zalypsis binds to DNA and is cytotoxic; however, it does not activate the 'DNA damage checkpoint' response. Thus, Zalypsis has cytotoxic effects dependent on DNA binding that are not associated with DNA damage.

PM00104 is antiproliferative *in vitro* against representative lines of solid tumors such as bladder, gastric, kidney, pancreatic, prostate, and thyroid cancer, melanoma and sarcoma, and hematologic malignancies such as leukemia and lymphoma. PM00104 also demonstrated *in vitro* antitumor activity, although to a lesser extent, in representative cell lines of breast, colon, lung, and prostate (DU-145) cancer, sarcoma (SK-LMS-1 and SW-684), and lymphoma (U937). *In vitro* activity was not observed, however, in a representative ovarian cell line (SK-OV-3). A panel of 6 human tumor types, including breast, colon, gastric, ovarian, prostate, and renal cancer, was used to investigate PM00104's activity *in vivo* in xenografts grown in athymic mice. PM00104 was administered as a single IV bolus injection. A statistically significant nanomolar antitumor activity was noted against breast, gastric, and renal malignancies but only micromolar activity against a colon tumor cell line. PM00104 significantly inhibited prostate tumors, but was inactive against ovarian tumors. In human gastric (MRI-H-254) and renal (MRI-H-121) tumor xenografts, PM00104, administered as a single bolus IV injection, resulted in profound and statistically significant tumor inhibition, which commenced approximately one week after treatment and lasted for at least 10 days with a high degree of statistical significance. In contrast, antitumor effect of a similar dose of PM00104 injection in the human colon model (HT-29) was not significant. Xenografts of other tumor types in which PM00104 showed potent *in vitro* antitumor activity, such as prostate cancer (PC-3) did result in statistically significant tumor inhibition that lasted at least one week after treatment. In all these cases, treatment with PM00104 was well tolerated by tumor-bearing animals (Elices M, etal, AACR05, Abs. 5882; Elices M, etal, ASCO05, Abs. 623; and Elices M, etal, AACR-NCI-EORTC05, Abs. B216).

Zalypsis showed promising PK characteristics with reasonable Cmax, plasma clearance, and t1/2 values in mice and beagle dogs. Following IV dosing, elimination kinetics of Zalypsis were biexponential; t1/2) was ~7 hours in mice and ~10 hours in dogs (Yin J, etal, AACR-NCI-EORTC05, Abs. B163).

In January 2005, a multicenter, open label, dose-escalation, phase I clinical trial was initiated with Zalypsis in Spain, in patients with advanced solid tumors or lymphoma. Zalypsis is administered as a 1-hour IV infusion every 3 weeks.

PT-523 (Talopterin), under development by Hana Biosciences (South San Francisco, CA), is a novel multi-targeted antifolate anticancer drug that is a water-soluble, nonpolyglutamatable, type-B analog of aminopterin. PT-523 was acquired from DFCI in 2003. PT-523 was developed at DFCI and the NCI as part of a program to develop therapies with improved efficacy and tolerability. Potential advantages of PT-523 include increased targeting to tumor cells, better tolerability, and a superior resistance profile over existing therapies.

Talotrexin is 10-fold more efficiently transported by the membrane-bound transporter replication factor C (RFC) into cells, 10-fold more tightly bound to the target enzyme, dihydrofolate reductase (DHFR), and 10- to 100-fold more efficacious in a wide variety of tumor cell lines and animal models compared to methotrexate. Animal studies with PT-523, conducted at the Free University (Amsterdam, The Netherlands) indicate that it may also be more potent than methotrexate against resistant tumors.

In a preclinical study with rats and dogs, toxicities included gastrointestinal effects, hematologic and histopathologic changes, and body weight loss. Dogs were more sensitive to the toxic effects of PT523 than rats (Noker PE, etal, AACR02, Abs. 5420).

According to researchers at DFCI, there was optimal synergistic inhibition of the human acute lymphoblastic T-cell leukemia (CEM) cell line growth by PT-523 when combined with cisplatin or topotecan at dose ratios of 1:2 and 2:1, respectively. By comparison, growth inhibition with PT-523 in combination with doxorubicin, carboplatin, or docetaxel was only additive (Wright JE, etal, AACR01, Abs. 445).

In March 2004, a nonrandomized, open label, uncontrolled, phase I clinical trial (protocol ID: 02-000, DFCI Legacy-03-183, CTEP Grant No.- UO1-CA62490-09, NSC No.- 712783, NCT00088023) was initiated at DFCI, under

PI Joseph P. Eder, MD, as well as at Beth Israel Deaconess Medical Center, and Massachusetts General Hospital, in Boston, to determine safety of a short IV infusion of PT-523 in patients with solid tumors who have failed curative or survival-prolonging therapy or for whom no such therapies exist. Primary objectives of this trial are to evaluate safety of PT-523 when administered on days 1, 8, and 15 of a 28-day cycle and to establish MTD and DLT. Secondary objectives of this trial are to determine PK, and to evaluate preliminary efficacy. Total expected enrollment is 30.

An open label, dose escalation, phase I clinical trial (protocol ID: CDR0000400150, DFCI-02000, NCI-6400, DFCI-IRB-03183, HANABIO-DFCI-02000, NCT00098514) was initiated at DFCI, in patients with advanced or recurrent solid tumors to determine MTD, safety, DLT, PK, and antitumor efficacy of PT-523. Patients are treated with PT-523 IV over 5 minutes on day 1 or days 1 and 8 or days 1, 8, and 15. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Cohorts of 1 to 6 patients are treated with escalating doses of PT-523 until MTD is determined. An additional 6 to10 patients are then treated at MTD. Patients are followed at 1 month. A total of 20 to 40 patients will be accrued for this trial. Joseph Paul Eder, MD, is Study Chair.

An open label, randomized, multicenter, phase I/II clinical trial (protocol ID: HBS103, NCT00129558) was initiated in July 2005, with PT-523 as a single agent in adult patients with relapsed or refractory acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), chronic myelocytic leukemia in blast crisis (CML-BC), and high risk MDS. The phase I portion of this trial will determine dose, safety, tolerability and PK of talotrexin, while the phase II component will only enroll patients with relapsed or refractory ALL to determine the efficacy of talotrexin, as measured by a CR rate. Francis Giles, MD, at MDACC, is the PI.

A randomized, open label, multicenter, phase I/II trial (protocol ID: HBS101, HBS101.00, NCT00112060) was initiated with IV PT-523 in patients with histologically or cytologically confirmed Stage IIIb nsclc with malignant effusion, Stage IV nsclc, or recurrent nsclc refractory to two lines of standard chemotherapy and/or an EGFr inhibitor. In the phase I portion, PT-523 is administered as a 5-minute IV infusion on days 1 and 8 of a 21-day cycle to a total a 20 to 40 patients. In the phase II portion, the primary endpoint is overall survival (OS). Secondary endpoints include overall response rate, time-to-progression (TTP), and progression-free survival (PFS). Patients continue treatment until disease progression or intolerable toxicity and are followed monthly for up to 18 months. Participating institutions include the Sylvester Comprehensive Cancer Center, Duke University Medical Center (Durham, NC), Ireland Cancer Center (Cleveland, OH), University of Chicago, Case Western University Hospitals of Cleveland, Pulmonology Research Center (St. Petersburg, Russia), St. Petersburg Pavlov State Medical University, and St. Petersburg Oncology Center, also in Russia. Primary objectives are to determine MTD and recommended phase II dose. PK and exploratory analyses for safety and efficacy are also being performed. Patients are first administered PT-523 at a dose of 13.5 mg/m^2, based on the dose of an ongoing phase I clinical trial (protocol ID: NCT00098514) in patients with solid tumors. Dose escalation is based on the modified Fibonacci sequence. A total of 3 patients are treated at each dose level in absence of DLT. Intrapatient dose escalation is allowed if enrolled patients do not develop DLT at lower dose levels. All patients are treated with folic acid and B12 vitamin supplementation. Safety assessments are performed by standard laboratory and clinical DLT definitions. Response is based on response evaluation criteria in solid tumors (RECIST) every 2 cycles.

According to an interim report, among 9 heavily pretreated patients treated with a total of 23 cycles of talotrexin (median 2, range=1-5) at doses of 27 mg/m^2 to 108 mg/m^2 per cycle, no DLT was observed, permitting higher doses. There was one PR and disease stabilized in 4 patients. There was 1 incident of Grade 3 neutropenia without fever. A patient stopped treatment because of progressive disease, and there was 1 death deemed unrelated to talotrexin. Talotrexin at the tested doses in this heavily pretreated patient population was well tolerated (Caio M, etal, AACR-NCI-EORTC05, Abs. B113). According to a further interim analysis, among 15 patients treated with a total of 50 cycles of talotrexin (median 3, range=1-8) at doses of 27 mg/m^2 to 270 mg/m^2 per cycle, 13 patients who had failed previous platinum-based regimens had evaluable CT scans. Among these patients, talotrexin was well tolerated over multiple cycles of therapy, with mucositis, febrile neutropenia, and thrombocytopenia as the most commonly reported adverse events. MTD has not yet been determined. Among 15 evaluable patients there were 2 PR and disease stabilized in 8, for a clinical benefit of 67% after 2 or more cycles of talotrexin at doses below MTD.

RH1, under development by Allos Therapeutics (Westminster, CO), is a novel targeted cytotoxic prodrug, bioactivated by the enzyme DT-diaphorase (DTD), also referred to as NAD(P)H:quinone oxidoreductase (NQO1), overexpressed in many tumors relative to normal tissue. RH1, a water-soluble synthetic analog of 2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone (MeDZQ), is a better substrate for recombinant human NQO1 than the parent compound. MeDZQ was identified as a potential antitumor agent based on its high rate of bioactivation by human NQO1, and its selective cytotoxicity to tumor cells containing elevated NQO1, such as nsclc. RH1 is more cytotoxic to human H460 nsclc and HT29 colon tumor cells expressing elevated NQO1 activity than to cells deficient in NQO1 activity, and may be an effective NQO1-directed antitumor agent (Winski SL, etal, Clin Cancer Res, Dec 1998;4 (12):3083-8).

In December 2004, Allos Therapeutics acquired an exclusive, worldwide license from the University of Colorado, the University of Salford, in the UK, and Cancer Research Technology (CRC; London, UK), to develop and commercialize RH1. In January 2005, investigators at Christie Hospital NHS Trust initiated a project (protocol ID: N0063030548), to perform synthesis and biologic studies on RH1.

In September 2003, a multicenter, open label, dose-escalation, phase I clinical trial (protocol ID: PH1/089) was initiated at Christie Hospital (Manchester, UK), under Trial Chair Malcolm Ranson, MD, with RH1 in patients with advanced solid tumors refractory to other chemotherapy regimens. Up to 40 patients were to be enrolled in this trial to test safety, tolerability, PK, and pharmacodynamics of escalating doses of RH1, and establish MTD, and DLT. Patient DTD enzyme levels are being measured to correlate with drug efficacy. Patients are treated with escalating doses of RH1 (40-1905 μg/m²/day), administered as a 10- to 30-minute IV infusion, daily for 5 days for 3 weeks, for up to 6 cycles. Other participating institutions include Southampton General Hospital (Southampton, UK), and Paterson Institute for Cancer Research (Manchester, UK).

According to an interim analysis from this phase I trial, involving 15 patients, RH1 was generally well tolerated at doses up to 1430 μg/m²/day. Clinically significant drug-related toxicities included bone marrow suppression and mild-to-moderate phlebitis. DLT, presenting as Grade 3 thrombocytopenia with hemorrhage and Grade 3 anemia in one patient, and Grade 3 neutropenic sepsis/chest infection in another, occurred in 2/2 patients treated daily at 1905 μg/m². There have been no objective tumor responses to date. An expanded patient cohort is being evaluated at a daily dose of 1430 μg/m². Plasma PK analysis on days 1 and 5 in cycle 1 shows rapid plasma clearance of RH1 (t1/2=17 minutes) with AUC increasing proportionately with dose.

Dose-related increases were observed in DNA interstrand crosslinking in peripheral blood lymphocytes. DNA crosslinking studies are being extended to tumor samples. To detect polymorphism in the NQO1 gene responsible for inactivation of DTD, restriction fragment length polymorphism (RFLP) analysis on peripheral blood DNA has been performed (Danson S, etal, AACR-NCI-EORTC05, Abs. C205).

RTA 401 (CDDO) and RTA 402 (CDDO-Me), under development by Reata Pharmaceuticals (Dallas, TX), are novel synthetic oleanane triterpenoids with potent cytotoxic activity in a wide variety of cancer cell types. Triterpenoids that are steroid-like in their structure, are widely used in Asian countries for medicinal purposes. Modified, synthetic triterpenoid compounds, such as CDDO, exhibit greatly increased activity as anticancer and anti-inflammatory agents. In all preclinical studies, RTA 401, administered IV, and its analogs were well tolerated at therapeutic doses. An orally bioavailable analog of RTA 401, designated RTA 402 (CDDO-Me), is completing final preclinical development with an IND filing slated for the end of 2006.

In October 2005, Reata signed a CRADA with the NCI, under which the two organizations are collaborating in the clinical development of RTA 401. NCI had completed much of the preclinical development of RTA 401 under the Rapid Access to Intervention Development (RAID) program.

RTA 401 and other compounds in the RTA 400 series show potent cytotoxic activity in a wide variety of cancer cell types in multiple animal models of cancer, and in cancer-cell samples from patients with treatment-resistant malignancies. RTA 401 treatment disrupts oxidation/reduction balance in cancer cells, depleting glutathione and triggering a rapid increase in levels of reactive oxygen species. This disruption of redox balance produces a generalized effect on cell signaling pathways, leading to cell death by both apoptotic and nonapoptotic mechanisms. Importantly, RTA 401 is significantly more toxic to cancer cells than to normal cells from the same tissue types. Analogs of RTA 401 have also shown potent activity in several of these systems, confirming the opportunity for development of second generation products in this class. In all preclinical studies, RTA 401 and its analogs were well tolerated at therapeutic doses.

CDDO compounds block synthesis of two key enzymes, inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2), involved in inflammation and carcinogenesis. The inflammatory and carcinogenesis pathways are linked because the same key enzymes in the inflammatory pathway, activated for tissue repair, are also overactivated in cancer. CDDO compounds are believed to block production of mediators that trigger the carcinogenic cascade in colon carcinogenesis. Using two independent, well defined mouse genetic models of colon cancer, SMAD3 knockouts and APC/MIN mutant, it was demonstrated *in vivo* that CDDO causes a highly significant decrease in the incidence of polyp formation and colon cancer, with a corresponding increase in survival time.

RTA 401 is synergistic with TRAIL, in TRAIL-resistant breast cancer cells, both *in vivo* and *in vitro*. In a murine model, RTA 401 potently inhibited both HEr2-positive and HEr2-negative human breast tumors. RTA 401 also exhibits significant activity in animal models of leukemia, ovarian cancer, glioma, and melanoma; RTA 401 was particularly effective against patient-derived drug-resistant leukemia and myeloma cells. Importantly, RTA 401 was also synergistic with bortezomib (Velcade; Millennium Pharmaceuticals) in myeloma cell samples from patients who did not respond to standard therapy and from patients who did not respond to bortezomib therapy.

Among CDDO's several biologic activities is induction of apoptosis in many cancer cell lines. To identify potential protein targets, immobilized biotinylated-CDDO was used

to screen the proteome of a human lymphoma cell line (U937) sensitive to CDDO-induced apoptosis. Tubulin was identified as one of several putative targets of CDDO. CDDO selectively binds to tubulin, disrupting microtubules both *in vivo* and *in vitro*. CDDO inhibits tubulin polymerization *in vitro*, possibly through interactions with a hydrophobic site on β-tubulin. CDDO-tubulin interaction may also involve a reversible 1,4-addition with a protein sulfhydryl group. Unlike other known spindle poisons, CDDO does not result in a temporal increase in the mitotic index. Rather, CDDO appears to initiate apoptosis early in M phase (Couch RD, etal, Mol Pharmacol, 11 Jan 2006; epub ahead of print).

In a preclinical study, scientists at MDACC and Texas A&M University (Houston, TX) explored the effect of HEr2 overexpression on sensitivity of breast cancer cells to growth-inhibitory effects of CDDO *in vitro* and of CDDO and CDDO-Me (RTA 402) *in vivo* in a xenograft model of breast cancer. CDDO at low concentrations preferentially suppressed cell growth and colony formation in soft agar assays, while growth-inhibitory effects at high concentrations did not correlate with HEr2 expression levels. CDDO dose-dependently inhibited phosphorylation of HEr2 in HEr2-overexpressing cells, and diminished HEr2 kinase activity *in vitro*. CDDO induced transactivation of the nuclear receptor peroxisome proliferator-activated receptor-epsilon (PPArepsilon) in both vector control and HEr2-transfected MCF-7 cells. According to dose-response studies, growth inhibition at lower concentrations of CDDO correlated with induction of caveolin-1, which inhibits breast cancer cell growth. CDDO also reduced cyclin D1 mRNA and protein expression.

In *in vivo* studies liposomally encapsulated CDDO completely abrogated growth of the highly tumorigenic MCF7/HEr2 cells in a xenograft model of breast cancer. Similar potency was observed with CDDO-Me in the MCF7/HEr2 xenograft model, with even greater reduction of tumor weight. These results indicate the therapeutic potential of CDDO and CDDO-Me in breast cancer (Konopleva M, etal, AACR-NCI-EORTC05, Abs. C208).

Another preclinical study conducted at MDACC, examined effects of CDDO-Me on CD34+ AML progenitor cells *in vitro*. CDDO-Me induced apoptosis in all but 1/10 AML samples. In this series of primary AML samples, CDDO-Me inhibited Erk phosphorylation in 5/10 samples; Erk was expressed and phosphorylated in all patient samples studied. CDDO-Me also induced apoptosis in 4/5 samples without decreasing pErk levels, suggesting that pErk is not the sole target of the compound. CDDO-Me induced phosphorylation of p38 in AML-derived U937 cells; pretreatment of U937 cells with a p38 inhibitor protected cells from the cytotoxic effects of CDDO-Me. These findings suggest a role for p38 in CDDO-Me-induced apoptosis. In preliminary studies, CDDO-Me induced p38 phosphorylation in 7/8 primary AML samples. Because CDDO-Me treatment shifts cell signaling away from cytoprotective pathways, it may be effective in the treatment of AML (Konopleva M, etal, Leukemia, Aug 2005;19(8):1350-4).

RTA 401/402 may also be applicable for the treatment/prevention of graft versus host disease (GvHD) and mucositis. CDDO enhanced efficacy of allogeneic bone marrow transplantation (BMT) by decreasing acute GvHD in mice. In a preclinical study, scientists at the University of Nevada School of Medicine (Reno, NV) and MDACC, examined effects of CDDO on both human and murine T-cell mitogen responses *in vitro*. CDDO significantly inhibited mitogen responsiveness of both human and murine T cells *in vitro* with evidence of cell-cycle arrest of the human T cells. To test effects of CDDO on acute GvHD induction and progression, lethally irradiated C57BL/6 mice were treated with 10 million bone marrow cells and 40 million spleen cells from fully MHC-mismatched BALB/c donors. All control mice succumbed rapidly from acute GvHD. In contrast, survival improved in mice treated with CDDO twice daily from days 0 to 3 following BMT. Body weights from treated mice also were significantly increased compared to untreated controls. Timing of CDDO administration was a critical factor for protection from GvHD that was only possible when CDDO was administered early after BMT. No adverse toxicities or effects on donor myeloid reconstitution were observed in mice treated with bone marrow cells alone and continuously administered CDDO (Murphy W, etal, AACR-NCI-EORTC05, Abs. C33).

In three preclinical studies, conducted in the golden Syrian hamster model, RTA 402 also exhibited significant antimucositis activity. Predosing produced the best results, while administration immediately after radiation on day 0 or when dosing was initiated several days after radiation (days 3 or 6) did not improve mucositis scores (Meyer J, etal, AACR-NCI-EORTC05, Abs. A180).

In October 2005, a dose-finding, phase I clinical trial of RTA 401 was initiated in patients with relapsed and refractory leukemia and high risk MDS, at MDACC (protocol ID: 2005-0469), under PI Hagop Kantajarian, MD. Primary objectives are to determine DLT, MTD, recommended phase II dose, and to characterize PK of RTA 401 in plasma and blood cellular elements, following its administration as a continuous IV infusion for 5 days. Secondary objectives are to determine clinical activity, *in vivo* molecular and biologic effects, and to correlate biologic activity. Total expected enrollment is 35 patients.

RTA 744 (WP774) and RTA 769, also under development by Reata Pharmaceuticals, are part of a novel class of anthracycline derivatives that efficiently cross the blood-brain barrier (BBB) and show significant potential for the treatment of primary and secondary brain cancer, as well as other solid tumors and hematologic malignancies. RTA 744 and RTA 769 are part of a portfolio of novel topoisomerase II inhibitors, licensed by Reata from the University of Texas Southwestern Medical Center (Dallas, TX) and MDACC that induce cancer cell death by binding to DNA

and inhibiting its synthesis. These agents exhibit potent activity in drug-resistant cell lines, cross the BBB, and produce a survival benefit in one of the most clinically relevant models of GBM. RTA 744 is administered IV, while RTA 769 is orally available.

RTA 744 was active against a diverse panel of cancer cell lines in *in vitro* cytotoxicity assays. Most importantly, RTA 744 is at least 50-fold more potent than doxorubicin against multidrug-resistant tumor cells with the MDR1 or MRP1 phenotype. RTA 744 is also active in *in vivo* models of cancer, including a highly rigorous orthotopic model of GBM, in xenograft models of GBM and breast cancer, and in a murine model of leukemia. Administered orally, RTA 769 also significantly extended survival in the orthotopic model of GBM and the murine leukemia model.

RTA 744 was designed to circumvent P-gp and MRP1-mediated cellular efflux while possessing enhanced activity in cells overexpressing Bcl-2 and its homolog Bcl-XL. RTA 744 demonstrated enhanced activity against cell lines resistant to doxorubicin and other anticancer agents, including GBM cell lines *in vitro* and *in vivo*. Also, brain tumor concentrations were >6-fold those measured in the surrounding CNS tissue, suggesting that RTA 744 may be an excellent new agent for the treatment of brain tumors. In a human xenograft murine model, following IP administration, more frequent administration of the same dose resulted in less weight loss with more tumor growth inhibition (Thapar NC, etal, AACR-NCI-EORTC05, Abs. A222).

Extensive PK and toxicology studies were completed with RTA 744 in murine models. RTA 744 crosses the BBB and reaches therapeutic concentrations in under an hour. Most importantly, the drug partitions preferentially into tumor tissue achieving intratumoral concentrations more than 70-fold higher than the concentrations in contralateral brain tissues. RTA 744 and 769 were well tolerated in preclinical studies. Preliminary toxicology studies indicate that RTA 744 has a tolerability profile similar to that of marketed anthracyclines. RTA 769 also shows favorable PK properties including oral bioavailability.

Investigators at MDACC tested RTA 744 activity against a panel of doxorubicin-sensitive and resistant human bladder cancer cell lines. RTA 744 was 3-fold to 5-fold more cytotoxic than doxorubicin against human bladder cancer cell lines UM-UC-6, UM-UC-2, UM-UC-3, and UM-UC-14. RTA 744 was 78-fold more cytotoxic and an effective inducer of apoptosis in the doxorubicin-resistant variant UM-UC-6dox. RTA 744-induced cell growth inhibition and apoptosis appear to result from cell-cycle arrest at G2/M phase. Uptake of RTA 744 was much faster than doxorubicin in both the sensitive and resistant cell lines UM-UC-6 and UM-UC-6dox. The cellular localization of RTA 744 was similar to that of doxorubicin, but the nuclear concentration of RTA 744 was much higher (Lee S, etal, AACR05, Abs. 2389).

Initially, clinical development is focusing on high grade glioma, because 90% and 100% of GBM overexpressed P-gp and MRP1, respectively. *In vivo* tests of RTA 744 and RTA 769 against GBM have shown a survival benefit in one of the most clinically relevant orthotopic models of the disease, in which human tumors are implanted directly into the brains of mice, placing such tumors in the natural setting and requiring compounds to cross the BBB in order to be effective. Treatment with RTA 744 and RTA 769 in this setting extended survival significantly. Other indications in development include certain malignancies that metastasize to the CNS, and drug-resistant phenotypes found in many other types of malignancies, including ovarian and lung cancer, and certain types of leukemia.

A dose-finding, PK phase I clinical trial of IV RTA 744 in patients with recurrent or refractory primary brain cancer, including anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic mixed oligoastrocytoma, GBM, or gliosarcoma, being treated concurrently with (group B) or without (group A) enzyme-inducing anticonvulsants, was initiated in September 2005, at MDACC (protocol ID: 2005-0188), under PI Charles Conrad, MD. The trial, to enroll 28 patients, will determine the safe dose for this drug, and provide additional information on its efficacy and side effects. The trial will also investigate the effects of anticonvulsant drugs on treatment with this agent, and characterize the multiple dose PK of RTA-774 in patients enrolled into group A and group B. In addition, the trial will document any potential antitumor activity of RTA-774 in patients with measurable disease, and correlate PK information with clinical (efficacy and safety) responses, as a possible aid in selecting appropriate doses for later studies.

S23906, under development by Servier (Neuilly-Sur-Seine, France), a benzoacronycine derivative, is a novel potent antitumor agent acting through alkylation of the N2 position of guanines in DNA. It is an alkylating minor groove binder that destabilizes local DNA base pairing; its reactivity towards DNA can be modulated by glutathione (GSH). The acridone alkaloid acronycine, first isolated from *Acronychia baueri Schott (Rutaceae)* in 1948, is an antitumor alkaloid with poor water solubility and low potency. The modest antitumor activity of this compound was significantly improved by the total synthesis of original analogs resulting in the selection of S23906-1 (Guilbaud N, etal, Anticancer Drugs, Jun 2002;13(5):445-9).

Nucleotide excision repair (NER) plays a pivotal role in the cellular sensitivity to S23906 because loss of NER function is associated with an almost 45-fold increased sensitivity to this agent. This is not restricted to a single NER factor but was observed for all 3 NER sub-pathways including transcription-coupled repair, global genome repair and the common NER repair pathway. These results indicate that NER status is likely to become an important factor in the clinical response to S23906. NER profiling revealed important differences in the activity spectra of S23906 compared with both cisplatin and with other clinically active

monofunctional agents. Unrepaired S23906-DNA adducts are rapidly converted into secondary DNA damage including double strand DNA breaks, most likely as a result of collision between S23906-DNA lesions and advancing replication fork or RNA polymerase. Loss of p53, Bax and mismatch repair function are associated with modest resistance to S23906 while loss of PARP has no influence. These findings confirm that S23906 possesses a unique mechanism of action and suggest that NER plays an unprecedented role in the repair of S23906-induced DNA lesions (Poindessous V, etal, AACR-NCI-EORTC05, Abs. A207).

S23906 is in phase I clinical trials in Europe.

S-CKD-602, under development by Alza (Mountain View, CA), a Johnson & Johnson company, is a Stealth liposome formulation of CKD-602, a camptothecin derivative topoisomerase I inhibitor marketed abroad by Chong Kun Dang Pharmaceutical (Seoul, South Korea). In the Stealth liposomal formulation, drug-loaded liposomes are coated with polyethylene glycol (PEG) molecules. Stealth liposomal formulations are designed to prolong drug circulation time, increase tumor delivery, and improve therapeutic index.

Disposition of Stealth liposome-encapsulated drug is dictated by the composition of the liposome, which alters the PK profile of the drug. In order to achieve antitumor effects, active drug in the liposomal formulation must be released into the tumor extracellular fluid (TECF). In a study comparing plasma and tumor disposition of drug after S-CKD602 and non-liposomal CKD-602 administration to mice bearing A375 human melanoma xenografts, the drug was detectable in TECF from 10 minutes to 2 hours after CKD-602, and from 10 minutes to 75 hours after S-CKD602 administration. Therefore, the sum total plasma exposure after S-CKD602 is 25-fold greater than that after non-liposomal CKD-602. S-CKD602 provides PK advantages in plasma, tumor, and TECF compared to non-liposomal CKD-602 at 1/30th of the dose, which is consistent with the improved antitumor efficacy of S-CKD602 in preclinical studies (Zamboni WC, etal, AACR-NCI-EORTC05, Abs. B173).

Stealth CKD-602 represents an improvement in therapeutic index compared to free CKD-602 and topotecan in human tumor xenografts. Compared to free drug, S-CKD602 prolongs drug half-life in plasma and improves antitumor efficacy in many human tumor xenograft models, such as ES-2 ovary, A375 melanoma, HT-29 colon, and H82 small-cell lung cancers. When tumor-bearing athymic nude mice with SC tumors approximately 100-200 mm^3 in size, were treated with S-CKD602 or free CKD-602 IV, S-CKD602 significantly enhanced antitumor efficacy over that of free CKD-602 in these models, with ES-2 ovarian and A375 melanoma xenografts being slightly more sensitive to S-CKD602 than HT-29 colon and H82 sclc xenografts. Antitumor activity was also observed in other human xenograft models, including B585 and MCF7 breast, and Capan-2 pancreas. The therapeutic index, defined as the ratio of MTD to the minimum efficacious drug dose of S-CKD602 versus free CKD-602 and topotecan, was compared in both A375 and HT-29 human xenograft models in athymic nude mice. S-CKD602 was administered IV once weekly for 3 weeks. Free CKD-602 and topotecan were administered intraperitoneally (IP) once every 4 days for 4 treatments, the optimal dosing schedule. Tumor regression was used as a criterion for antitumor efficacy, and either animal death or >15% weight loss for apparent toxicity.

In the A375 xenograft model the therapeutic indices were >10, ~6 and ≤2 for S-CKD602, free CKD-602 and topotecan, respectively. In the HT-29 xenograft model, the corresponding therapeutic indices were ~3, <1 and <1, respectively. Influence of S-CKD602 dosing schedules on the therapeutic indices was also investigated. In the A375 human melanoma xenograft model, a once weekly dosing schedule resulted in a greater therapeutic index than a twice weekly schedule, and both schedules were superior to the once every 2 weeks schedule. Weekly administration of S-CKD602 greatly enhanced the therapeutic effectiveness of free CKD-602 in both sensitive (A375 melanoma) and resistant (HT-29 colon) human tumor xenograft models, and was also superior to topotecan (Yu NY, etal, AACR05, Abs. 2396, and Yu NY, etal, AACR04, Abs. 3069).

A phase I clinical trial (protocol ID: 03-052, NCT0017728) of S-CDK602 was initiated in September 2003, at the University of Pittsburgh Cancer Institute (UPCI), under PI Ramesh Ramanathan, MD, in patients with advanced, refractory solid tumors. According to the protocol, S-CKD602 is administered on day 1 of a 21-day cycle. PK blood sampling is performed during the first dose (cycle), predose, end of the infusion, and at 2, 4, 8, 24, 48, 72, 96 hours, and on days 8, and 15 after drug administration, and 24-hour urine collections are made on days 1, 2, 3, and 4. Trial goals are to determine MTD of S-CKD602 when administered every three weeks, incidence and severity of toxicity of this regimen, and PK of CKD-602 following administration of S-CKD602. In addition, investigators plan to perform 'metabolic genotyping analyses' to determine how the genes in patients' blood affect how S-CKD602 is metabolized in the body. Expected total enrollment is 70 patients.

SJG-136, under development by Ipsen (Paris, France), in collaboration with Spirogen (London, UK), is a novel rationally designed DNA minor groove interstrand crosslinking agent spanning 6 base pairs of DNA with potent and broad spectrum antitumor activity. SJG-136, a pyrrolo[2,1-c][1,4]benzodiazepine dimer, selectively crosslinks guanine residues located on opposite strands of DNA.

The pyrrolobenzodiazepines (PBD) are a family of naturally occurring antitumor antibiotics isolated from various Streptomyces species. PBD units can be joined together to create PBD dimers capable of forming covalent sequence-selective DNA interstrand crosslinks. SJG-136 is

a rationally designed PBD dimer with a preference for binding to purine-GATC-pyrimidine sequences. It forms DNA crosslinks via covalent binding of guanine residues in the minor groove in a sequence-specific manner. SJG-136 interferes either with regulatory proteins that control gene expression, or with transcription elongation. By contrast, molecules that bind to DNA non-covalently do not inhibit transcription elongation. They cannot block the progress of polymerases, as these enzymes push them out of the groove as they progress. Most significantly, recent experiments have demonstrated that Spirogen's molecules are capable of penetrating both cellular and nuclear membranes, a feature that differentiates them from competitive molecules.

Instead of addressing proteins associated with key gene regulation pathways, as is the case with many drug development strategies, Spirogen's goal is to regulate expression of the gene directly, either upwards or downwards as appropriate for the disease state. This is achieved by binding small molecule drugs to specific DNA sequences of the disease-causing genes. Binding to the coding region (to prevent transcription elongation), or to a transcription factor binding site downregulates gene expression. Conversely, binding to a suppressor protein binding site upregulates expression. As this approach is specific for a particular gene, it has the advantage of modulating the activity of the relevant gene without affecting the majority of proteins necessary for normal cellular functions, minimizing a drug's side effects.

Spirogen's gene targeting agents are based on molecules that are able to block transcription in a highly sequence-selective fashion (Puvvada MS, etal, Biochemistry, 4 Mar 1997;36(9):2478-84). Although these molecules fit snugly in the minor groove without causing any distortion to the DNA helix, they are apparently capable of efficiently blocking the progress of RNA polymerase as it moves along the helix. Also, DNA adducts that form in cancer cells treated with Spirogen's drugs appear to be remarkably resistant to repair compared to those of other classes of DNA-interactive agents, suggesting potential use in the treatment of drug-resistant disease.

To discover SJC-136, Spirogen investigators combined solid phase chemistry and combinatorial technology to produce large libraries of DNA-interactive agents, with some containing >1 million compounds. To identify a molecule that binds selectively to the target DNA sequence, in a process similar to 'natural selection', the library was challenged with a fragment of DNA containing the sequence of interest. This approach contrasts with that involving building molecules by the traditional stepwise approach, a time consuming method hampered by a significant degree of trial and error.

According to investigators at the Royal Free and University College Medical School (London, UK), SJG-136 demonstrated potent activity in the NCI's anticancer drug screen. Although the agent is similar to other DNA binding agents, its pattern of activity does not fit within the clusters of any known agents, suggesting that SJG-136 possesses a distinct mechanism of action. SJG-136 produces DNA interstrand crosslinks between 2 N-2 guanine positions on opposite strands separated by 2 base pairs. In human tumor cell lines, crosslinks form rapidly (within 1 hour) and persist (over a 24 hour period) compared with those produced by conventional crosslinking agents such as nitrogen mustard (Hartley JA, etal, Cancer Res, 15 Sep 2004;64 (18):6693-9).

In vitro testing in the NCI's 60-human cell line screen, SJG-136 demonstrated a broad pattern of antitumor activity in subnanomolar concentrations. When SJG-136 is administered to mice at MTD, high peak plasma concentrations occur after 30 minutes. Moreover, the PBD dimer binds only moderately to proteins (65-75%), revealing why plasma concentrations achieved in the mouse are substantially higher than those required to elicit an antitumor response *in vitro* (Wilkinson GP, etal, Invest New Drugs, Aug 2004;22 (3):231-40).

SJG-136 was evaluated for *in vivo* efficacy in 10 tumor models selected on the basis of sensitivity of cells grown in the hollow fiber, and in *in vitro* time course assays, including LOX IMVI and UACC-62 (melanoma), OVCAR-3 and OVCAR-5 (ovarian carcinoma), MDA-MB-435 (breast carcinoma), SF-295 and C-6 (glioma), LS-174T (colon carcinoma), HL-60 TB (promyelocytic leukemia), and NCI-H522 (lung carcinoma). SJG-136 is active against small and large xenografts reducing tumor mass in all 10 models. In addition, significant growth delays occurred in 9 models, cell kill in 6 models, and there were tumor-free responses in 6 models. SJG-136 is active following IV bolus injections, as well as by 5-day continuous infusions. Of all of the schedules tested, bolus administrations for 5 consecutive days conferred the greatest efficacy (Alley MC, etal, Cancer Res, 15 Sep 2004;64(18):6700-6).

SJG-136 induces apoptosis in all tumor cells derived from patients with B-cell CLL. The drug's cytotoxicity is undiminished in B-CLL cells derived from patients treated previously, those with unmutated VH genes, and those with p53 mutations. Furthermore, SJG-136-induced apoptosis is associated with activation of caspase-3 that could be partially abrogated by the caspase-9 inhibitor Z-LEHD-FMK. Furthermore, SJG-136 does not trigger phosphorylation of p53 or the upregulation of GADD45 expression in B-CLL, suggesting that SJG-136 crosslinking adducts are not subject to p53-mediated DNA excision repair mechanisms in B-CLL cells (Pepper CJ, etal, Cancer Res, 15 Sep 2004;64 (18):6750-5).

A phase I clinical trial (protocol ID: 1089464) with SJG-136 was initiated in Europe, in May 2004, sponsored by Cancer Research UK, and funded by Ipsen, to recruit 20 to 30 patients with a variety of advanced, refractory solid tumors. The drug is being administered IV once every 3 weeks. The aim of this initial trial is to determine the dose of SP-2001 that can be administered safely to patients.

In a phase I clinical trial, being conducted at Vanderbilt-Ingram Cancer Center (Nashville, TN), patients with refractory solid tumors were administered SJG-136 IV over 20 minutes, daily, for 5 consecutive days, every 21 days, at a starting daily dose of 6 μg/m^2. Blood and urine samples were obtained for PK assessment on days 1 and 5 of cycle 1. In consenting patients, biopsies were obtained prior to day 1 and on day 4 of cycle 1 to quantify drug-DNA adduct formation and profile gene expression. PBMC were also collected for pharmacogenomic studies. Among 7 patients enrolled, the median number of prior regimens was 3 (range=1-5). Daily dose levels tested (μg/m^2) and number of patients treated at each dose level were 6 (n=1), 12 (n=1), 24 (n=3), 48 (n=2). In cycle 1 DLT in the form of Grade 3 soft tissue edema, dyspnea, and fatigue was observed in 1 patient at the daily dose of 48 μg/m^2. Similar toxicities occurred during cycle 2 in the lead patient treated daily with 24 μg/m^2. Both episodes were preceded by a precipitous drop in serum albumin (mean=37%) not associated with proteinuria. Symptoms resolved over 2 to 3 weeks following treatment discontinuation and aggressive diuresis. Investigations are underway to determine whether edema is secondary to hypoalbuminemia or whether both are attributable to a disturbance in sodium homeostasis. Other non-DLT included Grade 2 myalgias and increases in transaminases and alkaline phosphatase. No significant myelosuppression was observed. Preliminary PK assessment at 24 μg/m^2/day indicate a t1/2 of 113 minutes on day 1. Although no objective responses have been observed, disease stabilized in 2 patients with heavily pretreated colorectal cancer by radiographic criteria accompanied by 25%-30% decreases in CEA, and in 1 patient with malignant melanoma lasting for 3 months. DLT of SJG-136, administered daily for 5 days, is soft tissue edema leading to dyspnea and fatigue. The mechanism(s) underlying this toxicity are under investigation. Additional patients are being enrolled at a daily dose of 24 μg/m^2 to determine whether this represents MTD and recommended phase II dose (Puzanov I, etal, AACR-NCI-EORTC05, Abs. B117).

ZIO-101 (S-dimethylarsino-glutathione), under development by Ziopharm Oncology (New York, NY), is a novel organic arsenic synthesized by conjugating dimethylarsenic to glutathione. It is water soluble, and more potent and less toxic than inorganic arsenic. Also, it appears that ZIO-101 induces apoptosis by mechanisms different than arsenic trioxide and can kill arsenic trioxide-resistant cells.

In September 2004, Ziopharm completed a worldwide licensing agreement with MDACC and Texas A&M University for this new class of organic arsenicals to be developed for treatment of a wide range of malignancies.

Clinical use of inorganic arsenics, like arsenic trioxide, that are potent anticancer agents, is limited by severe toxicity at high doses. Inorganic arsenic therapy is highly effective in treating acute promyelocytic leukemia (APL), a rare form of leukemia. However, treatment with inorganic arsenics is limited against other malignancies, including solid tumors that require higher doses. Organic arsenics, in contrast, are substantially less toxic. ZIO-101 induces reactive oxygen species in treated cells leading to G2/M-arrest and apoptosis via cleavage of PARP and caspase-9 while Bcl-2, BL-XL and Bax are unaffected. ZIO-101 is active against various solid tumors in *in vitro* and *in vivo* models, and is considerably less toxic than arsenic trioxide in human cells. Clonogenic assays of arsenic trioxide and ZIO-101 against normal and leukemia stem cells indicate a more favorable toxicity profile for ZIO-101. Furthermore, the 50% lethal dose (LD_{50}) is about 50-fold higher in animals. Additionally, at equimolar arsenic concentrations, intracellular concentration of atomic arsenic with ZIO-101 is 15-fold higher than with arsenic trioxide, resulting in dramatically more mitochondrial damage and greater apoptosis induction in cancer cells. A large dose increase, possible with ZIO-101, is expected to take advantage of arsenic's ability to kill cancer cells specifically by causing cell-cycle arrest and cell death rather than cell differentiation, as is the case with inorganic arsenic. A higher dose, coupled with less toxicity, is expected to provide a wider therapeutic window and an incremental benefit, or treatment alternative, for patients with multiple myeloma.

At the 7th annual meeting of New Trends in the Treatment of Acute Leukemia that took place in September 11-14, 2004, in Dubrovnic, Croatia, MDACC researchers reported that based on results from a mouse trial, ZIO-101 may be administered at doses 30- to 50-fold higher than possible with inorganic arsenic without any severe side effects. Also, according to further testing of ZIO-101 in dogs, there was no evidence of heart damage or other severe toxicity. In animal trials, it was possible to deliver 5- to 10-fold more ZIO-101 than arsenic trioxide without cardiac damage. Other side effects of inorganic arsenics, including liver damage, and bone marrow and skin toxicities, are also much less prominent with ZIO-101. As of January 2006, there have been no drug-related toxicities in human clinical trials at doses considerably higher compared to arsenic trioxide. Results from clinical trials to date have confirmed safety and activity as demonstrated in preclinical animal studies.

ZIO-101 is being clinically investigated in both solid tumors and hematologic malignancies. In April 2005, the FDA approved Ziopharm's IND and, in May 2005, Ziopharm initiated a dose-escalation, phase I clinical trial of ZIO-101, at MDACC, under PI Steven M. Kornblau, MD, to enroll up to 40 patients with various blood and bone marrow malignancies. This trial was designed to determine MTD of ZIO-101.

In May 2005, Ziopharm also initiated a phase I clinical trial to assess safety and dosing of ZIO-101 in patients with various solid tumors. This trial, being conducted at MDACC, under PI Luis H. Camacho, MD, is to enroll up to 40 patients to determine MTD of ZIO-101. Originally, patients

were first treated with ZIO-101 at doses approximately 14 times higher than the FDA-approved starting dose of inorganic arsenic trioxide. In November 2005, ZIO-101 demonstrated safety at doses approximately 25 times higher than the currently approved dose for arsenic trioxide in this trial that treated 11 patients to date. The starting dose was 78 mg/m²/day, administered IV for 5 days (about 14-fold higher than arsenic trioxide) with 40% dose increases after successive cohorts of 3 subjects. Therapy is well tolerated, with rapid dose increases. There was 1 CR of a brain metastasis and disease stabilized overall in a patient with rapidly progressing metastatic renal cell cancer (Camacho L, etal, AACR-NCI-EORTC05, Abs. C90). As of January 2006, this phase I clinical trial is in cohort 5 with dosing about 45 times higher than the approved dose of arsenic trioxide.

TARGETED AGENTS (REGULATORY AGENTS)/ CYTOSTATICS/ANGIOGENESIS INHIBITORS

Targeted agents described here exclude cytotoxic drug linked to moieties that carry such drugs to their intended target. Rather, the agents described here interfere with specific genes, proteins or pathways to prevent signals associated with the establishment and/or progression of cancer. Most of these agents are cytostatic in nature and most are expected to be eventually studied in the clinic in combination with more traditional cytotoxics.

Angiogenesis Inhibitors

Inhibition of angiogenesis is an approach confirmed to work in malignancy, in animal models and human clinical trials. Successful market introduction of bevacizumab (Avastin; Genentech) has further validated this anticancer approach. However, an angiogenesis inhibitor that effectively treats/prevents malignancy as monotherapy has yet to emerge. Currently, over 130 angiogenesis inhibitors are in various stages of development, with over 70 having entered clinical trials.

BIBF 1120, under development by Boehringer Ingelheim (Vienna, Austria and Biberach, Germany), is a potent, orally available small molecule inhibitor of vascular endothelial growth factor receptor (VEGFr), fibroblast growth factor receptor (FGFr), and platelet-derived growth factor receptor (PDGFr) tyrosine kinases. The drug, referred to as a triple angiokinase, is an indolinone-type small molecule that inhibits VEGFr, PDGFr, and FGFr kinases at concentrations in the low nanomolar range, induces apoptosis in endothelial cells, and exhibits excellent single agent antitumor activity in preclinical tumor models with a clear reduction in tumor vessel density. BIBF 1120 also inhibits members of the Src family of tyrosine kinases (Src, Lck, Lyn).

The human nsclc cell line NCI-H460 was used to evaluate antitumor effects of BIBF 1120 in combination with taxanes, *in vitro*, in tumor cells and HUVEC, and *in vivo*, in xenografts growing in nude mice. Treatment involved suboptimal doses of either BIBF 1120 or paclitaxel alone, or in combination. Treatment of VEGF-stimulated HUVEC with a combination of BIBF 1120 plus paclitaxel *in vitro* inhibited proliferation much more strongly (92%) than treatment with either agent alone (24% and 17% inhibition, respectively). The fraction of apoptotic HUVEC increased from 25% in cultures treated with either drug alone to ~50% with the combination. Combined treatment with BIBF 1120 and paclitaxel inhibited NCI-H460 cell proliferation by 94%, whereas single agent treatment was much less effective (10% and 42% inhibition, respectively). *In vivo*, the combination of BIBF 1120 with docetaxel showed clear antitumor efficacy with a T/C ratio of 27% at dose levels at which single agent treatments were ineffective. Enhanced *in vivo* efficacy was accompanied by an increase in tumor-cell apoptosis. Therefore, combining BIBF 1120 with taxanes strongly impacts proliferation and survival of tumor and endothelial cells *in vitro*, with promising activity *in vivo* supporting further clinical evaluation of BIBF 1120 in combination with taxanes (Hilberg F, etal, AACR-NCI-EORTC05, Abs. A19).

Effects of BIBF 1120 on tumor vasculature were assessed in SC human tumor xenograft models in nude mice bearing established head and neck carcinoma (FaDu, n=7) and colon carcinoma (HT-29, n=8) tumors treated with daily oral doses of BIBF 1120 for 3 consecutive days. Tumor volume, perfusion, permeability, and relative blood volume (rBV) were determined by morphologic and dynamic contrast enhanced (DCE) MRI using gadolinium-based contrast agents. Untreated tumor-bearing mice (n=8 for both tumor models) served as controls. In FaDu xenografts, tumor growth was not affected by 3 days of treatment with BIBF 1120, but the value of the transfer constant (Ktrans) between blood plasma and extravascular extracellular space was reduced by 80%, resulting in ~75% lower Ktrans values compared with controls on day 3. BIBF 1120 treatment also reduced rBV by ~55%, resulting in ~50% lower rBV compared with control tumors on day 3. Similarly to FaDu xenografts, there was no difference in tumor volumes, Ktrans, and rBV in the HT-29 tumor-bearing groups prior to treatment. Ktrans and rBV values in untreated HT-29 xenografts were lower (Ktrans ~15% and rBV ~50%) than those in FaDu tumors, suggesting a lower grade of tumor perfusion and vascular permeability in this xenograft model. Tumor growth was again not affected by 3 days of treatment with BIBF 1120, but Ktrans values were reduced by ~40%, resulting in about ~50% lower Ktrans values compared with control tumors on day 3. BIBF 1120 treatment also reduced rBV by ~40%, resulting in ~25% lower rBV compared with control tumors on day 3. Therefore, BIBF 1120 treatment has significant impact on tumor vasculature in both models, resulting in a substantial decrease in tumor perfusion. These changes can be detected by DCE-MRI prior to changes in tumor growth rate. Differences in magnitude of this antivascular effect between these models may be explained by different levels

of tumor perfusion and vascular permeability prior to treatment (Krssák M, etal, AACR-NCI-EORTC05, Abs. Abs. A22).

A multicenter, dose-escalation, phase I clinical trial of BIBF 1120 was performed at the University of Freiburg, in Germany, under PI K. Mross, MD, in patients with chemotherapy-refractory, advanced solid tumors, to determine this treatment's safety, efficacy, and PK. According to the protocol, patients are administered BIBF 1120 orally in the form of capsules, daily for 4 weeks, with a one week break, repeated until disease progression. Patients are followed weekly in the first 5 weeks, and subsequently twice monthly. Treatment success is reviewed every 4 weeks by DCE MRI to determine tumor size, blood vessel permeability, and tumor density. MTD for BIBF 1120 was evaluated during this phase I trial in 61 patients with various advanced solid tumors.

According to an interim report, patients were administered a fixed oral dose of BIBF 1120 (50 mg/day) continuously for 28 days, followed by 1 week rest. Doses were escalated until DLT. Consecutive treatment cycles were allowed in the absence of progressive disease and persistent toxicity. Among 25 patients enrolled, 23 were administered at least 1 treatment cycle; 3 patients were excluded during the first cycle as a result of early progression (n=2) or noncompliance (n=1). Doses were escalated following an accelerated titration scheme from 50 to 450 mg/day, delivered at 50 mg (n=2), 100 mg (n=1), 200 mg (n=8), 250 mg (n= 6), 300 mg (n=5), and 450 mg (n=3). The most frequently observed drug-related adverse events were nausea, vomiting, diarrhea, abdominal pain, and elevation of hepatic enzymes. Liver enzyme elevations were dose limiting (Grade 3) in 1/8 patients at 200 mg/day, in 2/5 patients at 300 mg/day, and in 2/3 patients at 450 mg/day. Among 25 patients, 13 were treated for more than 2 cycles. Disease stabilized in 10 patients, for 2 months (n=1), 3 months (n=5), 4 months (n=2), 5 months (n=1), and 7 months (n=1). Treatment is continuing in 3 patients (+7, +8, +14 months). There was 1 CR of pulmonary metastases at the 200 mg/day dose level. DCE-MRI measurements reflected a decrease in permeability and blood flow in all evaluable patients. PK evaluation indicate that BIBF 1120 exposure (AUC) increases with dose with moderate to high variability. Maximum measured plasma concentrations were attained approximately 3 hours following intake. BIBF 1120 was distributed out of the blood, and demonstrated a high clearance corresponding to a mean t1/2 of ~13 hours. Steady state was attained within 9 days. BIBF 1120 was well tolerated in this trial. Adverse events were mostly gastrointestinal, and of mild to moderate intensity. Asymptomatic elevations of liver enzymes comprised DLT. MTD of BIBF 1120 was 250 mg/day administered once daily. In this trial, a significant number of patients experienced durable SD (Mross K, etal, EORTC-NCI-AACR04, Abs. 135).

In a subgroup analysis of the phase I clinical trials program, safety, PK and pharmacodynamic effects of BIBF 1120 were evaluated in 30 patients with advanced colorectal cancer. Treatment consisted of 28 days of BIBF 1120 followed by 7 days off. The dose was first escalated with once daily dosing until DLT; MTD was 250 mg daily. In a second cohort, twice daily dosing was explored to further increase drug exposure at MTD of 250 mg twice daily. In all patients additional treatment courses were allowed in the absence of progressive disease or persistent toxicity. PK profiles were obtained at the beginning and at the end of the first course. DCE MRI was performed at baseline, on days 2/3, days 29/30, and after each further course. Among 30 patients included in this subgroup analysis, 14 were treated with BIBF 1120 at 50 mg to 450 mg once daily and 16 at 150 mg to 250 mg, twice daily. Patients were treated for a median of 2.5 courses (range=1-13); 15/30 (50%) patients were treated for >2 courses. There was 1 PR at the 250 mg twice daily regimen. Median TTP was 65 days in patients treated once daily and 107 days in patients treated twice daily with BIBF 1120. The most frequent drug-related adverse events were Grade 1/2 nausea (n=16/4), vomiting (n=14/2) and diarrhea (n=7/5). Drug-related toxicities ≥Grade 3 were seen in 3 patients, involving a decrease in CD4 lymphocytes in 2 patients, and elevation of hepatic enzymes in 2 patients; Grade 1 drug-related hypertension was seen in 2 patients. ALT and/or AST ≥Grade 3 occurred in 4/14 patients treated with once daily BIBF 1120. There were no >Grade 2 increases in ALT/AST in the 16 patients treated with twice daily BIBF 1120. Maximum BIBF 1120 plasma concentrations were reached ~ 3 hours after drug intake. BIBF 1120 was extensively distributed out of the blood and showed a high clearance with mean t1/2 of ~ 15 hours. According to DCE-MRI, blood flow and permeability in target lesions were reduced by >40% in 15/21 evaluable patients. A reduction >40% from baseline in Ktrans was positively associated with nonprogressing disease. BIBF 1120 was generally well tolerated with primary evidence of antitumor activity in patients with advanced colorectal cancer. DCE-MRI results suggest an antiangiogenic effect of BIBF 1120 that correlated with stabilization of disease (Mross K, etal, AACR-NCI-EORTC05, Abs. A1).

In a phase I clinical trial, initiated in 2003 at Gray Cancer Institute/Mount Vernon Hospital (Northwood, Middlesex, UK), in patients with a variety of advanced solid tumors, BIBF 1120 was administered as a continuous once daily dosing starting at 100 mg/day; dose was doubled in successive cohorts until incidence of Grade 2 drug-related toxicities. Thereafter, escalation steps by no more than 50% were allowed. All patients underwent PK sampling. DCE-MRI trials were performed at baseline, and on days 2, 28, and 56 to assess functional tumor change following treatment. Among 39 treated patients, 37 who had been treated for 21 days were evaluable for toxicity. Median duration of treatment was 2 months (range=8 days to 13 months). Patients were treated at 5 dose levels, including 100 mg/day (n=6), 200 mg/day (n=6), 300 mg/day (n=6), 400 mg/day (n=14), and 450 mg/day (n=5). Most common toxicities were Grade 2 nausea, vomiting, diarrhea, abdom-

inal pain, and fatigue; Grade 3 asymptomatic, reversible elevation in liver enzymes occurred in 10 patients at ≥200 mg/day. MTD was identified as 400 mg/day, with 2 patients experiencing dose-limiting liver enzyme elevation at 450 mg/day, which returned to baseline within 2 weeks after stopping treatment. Among 29 patients assessable for response, who underwent 8 weeks' treatment, disease stabilized in 10 (ovarian, cervical, colon, prostate, and renal cancer, GIST, nsclc, and leiomyomatosis) for 4 to 11 months. A significant prolongation of PSA doubling time from 2 months to 10 months was observed in a patient with prostate cancer, and disease stabilized for 11 months in a patient with renal cancer. According to PK analysis Cmax and AUC values rose in concert with dose increase, displaying moderate to high interpatient variability. Cmax values reached ~3 hours after dosing. Mean t1/2 ranged from 7.5 to 14.0 hours. A total of 28 patients underwent DCE-MRI. BIBF 1120 was well tolerated in patients with advanced malignancy. This trial is ongoing, with patients being treated twice daily (Lee CP, etal, ASCO05, Abs. 3054).

CHIR258 (CHIR258LC), under development by Chiron (Emeryville, CA), a benzimidazole-quinoline, is an orally active small molecule that exhibits potent inhibitory activity against selected growth factor receptor tyrosine kinases (RTK), including VEGF, PDGF, bFGF that are important in tumor growth and angiogenesis. PK values of CHIR258, investigated in mice, rats, dogs, and monkeys, were favorable (Vora J, etal, AACR03, Abs. 3783).

CHIR258 inhibits fibroblast growth factor receptor 3 (FGFr3). Ectopic expression of FGFr3 in multiple myeloma is a consequence of the t(4:14) translocation that occurs uniquely in a subset (15%) of patients with this malignancy. Inhibition of activated FGFr3 in multiple myeloma cells induces apoptosis, validating FGFr3 as a therapeutic target in t(4;14) multiple myeloma, and supporting the case for clinical development of FGFr3 inhibitors for the treatment of patients with poor prognosis multiple myeloma. CHIR258 potently inhibits FGFr3 *in vitro*, and selectively inhibits growth of B9 cells and human myeloma cell lines expressing wt or activated mutant FGFr3. In responsive cell lines, CHIR258 induced cytostatic and cytotoxic effects. Importantly, addition of interleukin-6 (IL-6), IGF-1, or co-culture on stroma did not confer resistance to CHIR258. In primary myeloma cells from patients harboring t(4;14), CHIR-258 inhibited downstream Erk1/2 phosphorylation with an associated cytotoxic response. Also, therapeutic efficacy of CHIR258 was demonstrated in a xenograft mouse model of multiple myeloma expressing FGFr3 (Trudel S, etal, Blood, 1 April 2005;105(7):2941-2948).

In *in vivo* preclinical models, CHIR258 inhibited both the target receptors and the downstream signaling molecule, Erk (MAPK), in a dose- and time-dependent manner, as well as VEGFr1, VEGFr2, FGFr and PDGFrβ RTK (Wiesmann M, etal, AACR03, Abs. R4702, Heise C, etal, AACR03, Abs. 1006, Lee SH, etal, AACR03, Abs. 4703).

In vitro, 3 human cancer cell lines, two AML cell lines, and a human colon cancer cell line (KM12L4a), were at least 10-fold more sensitive to the antiproliferative effects of CHIR258 than other cell lines tested. *In vivo* daily oral dosing of CHIR258 results in significant antitumor activity in a broad range of human and murine tumor models. Established tumor xenografts of prostate, colon, ovarian and hematologically derived cancer cells all respond to treatment in a dose-dependent manner. *In vivo* activity ranges from growth inhibition to stable disease and tumor regression. According to tissue concentration studies, CHIR258 is retained in the tumor at levels up to 300-fold higher than in plasma at 24 hours after dosing. Various standard drug combinations with CHIR258 were effective in the KM12L4a colon tumor model, and synergistic and greater than additive effects were seen with trastuzumab combined with CHIR258 in the erbB2-overexpressing SKOV3ip1 ovarian tumor model. Additionally, tumor responses and regressions were significantly improved over each single agent treatment in the A431 epidermoid tumor model when CHIR258 was combined with ZD1839 (Wiesmann M, etal, AACR03, Abs. R4702, Heise C, etal, AACR03, Abs. 1006, Lee SH, etal, AACR03, Abs. 4703).

CHIR258 is being clinically investigated in both solid tumors and hematologic malignancies. Initially a 7 days on/7 days off treatment schedule was used, with daily administration tried subsequently. In a phase I clinical trial, conducted at Royal Marsden Hospital, and Beatson Oncology Center (Glasgow, UK), CHIR-258 was administered intermittently, as repeated single daily doses for 7 days followed by a 7-day washout, to adult patients with advanced solid tumors enrolled in groups of 3 to 6. The protocol was later amended to explore continuous dosing in an expanded patient cohort. Among 20 enrolled patients (median prior regimens=1, range=0-5), treated in 4 intermittent dosing cohorts (25, 50, 75 and 100 mg/day) and 1 continuous dosing cohort (100 mg/day), DLT consisted of Grade 3 hypertension in 1 patient with pre-existing hypertension, treated with continuous dosing. Other drug-related toxicities include mild-to-moderate nausea and vomiting, diarrhea, fatigue, anemia, headache and transient pruritic rash. Consistent with preclinical observations that the drug preferentially accumulates in tumors, inhibition of pErk at 100 mg/day was observed in 2/3 evaluable patients at 4 and 24 hours following dosing, despite time-dependent reduction of plasma exposure. Disease stabilized in 3 patients with parotid and renal cancer and imatinib-refractory GIST, for 4+ months; in the patient with GIST a reactive increase was observed in metabolic uptake in the PET obtained after the 7-day washout compared to that after 7-day dosing. Biologic activity of CHIR258 at tolerable doses has been noted; accrual to the continuous dosing group continues to define the recommended dose (Sarker D, etal, ASCO05, Abs. 3044).

In a dose-escalation, phase I clinical trial being conducted in the UK, CHIR258 was administered orally to adult patients with relapsed/refractory AML on a 7 days on/7

Exhibit 2
Molecular Targets of Anticancer Agents in Phase I Monotherapy Clinical Trials

Developer	Generic Name ❑ Number ❑ Brand	Target
Merck	VX-680, MK0457	Aurora FLT3
Millennium Pharmaceuticals	MLN8054	Aurora A
AstraZeneca	AZD1152	Aurora B
Gemin X Biotechnologies	Obatoclax ❑ GX15-070, GX015-070	Bap31 B-cell lymphoma 2 (Bcl-2)
Santaris Pharma	SPC2996	Bcl-2
Serono	TACI-Ig	B-lymphocyte stimulator (BLyS)
ImmunoGen	huC242-DM4	CanAg
Chiron	Anti-CD40mAb, CHIR-12.12	CD40
Seattle Genetics	SGN-40, SGN40 (anti-huCD40 MAb)	CD40
Astex Therapeutics	AT7519	Cyclin-dependent kinase (CDK)
Pfizer	AG-024322	CDK1 CDK2 CDK4
Roche	CDKi	CDK1 CDK2 CDK3
Hana Biosciences	Talotrexin ammonium ❑ PT-523, PT523, NSC 712783 ❑ Talopterin	Dihydrofolate reductase (DHFR)
Allos Therapeutics	RH1, RH-1	DT-diaphorase (DTD), NAD(P)H dehydrogenase, quinone 1 (NQO1)
Array BioPharma	ARRY-334543	Epidermal growth factor (EGF) receptor (EGFr, ErbB-1, ErbB1, HEr-1, HEr1) HEr2 (HEr-2/neu, ErbB-2, c-erbB-2, ErbB2)
Conforma Therapeutics	CNF-101, CNF1010	Heat-shock protein 90 (hsp90)
Exelixis	XL647	EGFr HEr2 Vascular endothelial growth factor (VEGF) receptor (VEGFr) Ephrin B4 (EphB4)
Boehringer Ingelheim	BIBW 2992	EGFr, HEr2
Infinity Pharmaceuticals	IPI-504	Hsp90
Kosan Biosciences	KOS-1022, 17-DMAG, NSC707545	Hsp90
AstraZeneca	AZD6244, ARRY-142886	Mitogen-activated protein kinase (MAPK)/ERK/kinase (MEK)
Exelixis	XL880	Met [hepatocyte growth factor receptor (HGFr) /c-Met] VEGFr2 (FLK1, Flk-1, KDR)
Pfizer	PD0325901	MEK 1
Boehringer Ingelheim	BI 2536	Polo-like kinase 1 (Plk1)
Agensys	AGS-PSCA	Prostate stem cell antigen (PSCA)
Boehringer Ingelheim	BIBF 1120	VEGFr Src-kinase family (SKF) FGFr PDGFrB
Chiron	CHIR258, CHR258LC, CHIR 258	VEGFr Fibroblast growth factor receptor 3 (FGFr3) c-Kit PDGFrB
OSI Pharmaceuticals	OSI-930	VEGFr2 c-Kit PDGFrB
UCB Pharma	CMC-544	CD22

days off schedule followed by continuous daily dosing for 28 days (1 cycle). Subsequent 28-day cycles of continuous dosing were permitted. As of May 2005, 8 patients (median prior relapses=1, range=1-3; FAB subtypes M1=1, M2=2, M5=1, M6=2, unclassifiable=1, no information=1) were treated in 2 (50 mg and 100 mg) cohorts of 3 to 6 patients; 5 patients remain on trial (median number of treatment cycles=3; range=1-11). There were no DLT to date. Dose escalation to 200 mg was tolerated and accrual continues. Drug-related adverse events include fatigue, anorexia, nausea and vomiting, and headache and were generally mild (Grade1/2). Among the 8 patients treated, there was 1 FLT-3 internal tandem duplication (ITD) mutation, and 7 were wt for ITD and D835. A patient with AML-M6 with wt FLT-3 continues with SD after 11 cycles of therapy; in this patient, dose was escalated from 50 mg to 100 mg after 8 cycles. Near complete clearing of blasts from marrow and peripheral blood was observed in the patient with FLT-3 ITD mutation treated at the 100 mg dose; this patient later died from a presumed fungal infection, not attributed to CHIR258. Plasma exposure and Cmax increased proportionally across the dose range; Cmax at the 100 mg dose level is in the range where antitumor activity was seen in a preclinical tumor model for FLT-3 ITD AML (MV4;11). In this trial, CHIR-258 was active in relapsed refractory patients with AML, both with a FLT-3 mutation and wt FLT-3. Accrual continues at the 200 mg dose (Morgan GJ, etal, ASH05, Abs. 2794).

In June 2005, Chiron and the Multiple Myeloma Research Consortium (MMRC; New Canaan, CT) initiated a phase I clinical trial (protocol ID: CHIR-258-003, NCT00243763) to evaluate safety, tolerability, PK, and pharmacodynamic profiles of CHIR258 in patients with multiple myeloma. CHIR258 is the first drug candidate to undergo clinical trial through the MMRC, a nonprofit organization that integrates leading academic institutions with the goal of accelerating drug development in multiple myeloma. MMRC member institutions, expected to enroll patients in the trial, are DFCI, H. Lee Moffitt Cancer Center and Research Institute (Tampa, FL), the Mayo Clinic (Rochester, MN), and Emory University. A phase I clinical trial (protocol ID: N0258156386) was also initiated in June 2005, at Royal Marsden Hospital, Glasgow Royal Infirmary, Christie Hospital, and St. Bartholomew's Hospital, to determine MTD, DLT, and safety of CHIR-258 when administered twice daily to patients with refractory or relapsed multiple myeloma.

CP-868,596, under development by Pfizer, is a potent inhibitor of PDGFrβ kinase targeting tumor-derived angiogenesis. It is more than 450-fold selective for PDGFrβ versus other angiogenic receptors. PDGF and its receptor play an important role in angiogenesis, modulation of tumor interstitial fluid pressure (IFP), and influence cell growth, migration, and survival through signal-transduction pathways.

To support development of this compound, multiple models were used to characterize its *in vivo* activity, including an *ex vivo* measure of inhibition of PDGFrβ phosphorylation in tumors as a biochemical endpoint, and multiple models of tumor growth inhibition as functional endpoints. A U87MG human GBM *ex vivo* model was used to demonstrate that CP-868,596 inhibits PDGFrβ phosphorylation in tumors after oral administration. Inhibition of PDGFr phosphorylation in tumors correlates with plasma levels and tumor levels. *In vivo*, the antitumor efficacy of CP-868,596 was evaluated in a number of human tumor xenografts in athymic mice, including H460 human lung carcinoma, Colo205 and LS174T human colon carcinoma, and U87MG human GBM. Once daily oral dosing for 10 days inhibited tumor growth. When oral CP-868,596 was dosed in combination with docetaxel in MDA-MB-231 human breast xenografts, and with gemcitabine, in H460 human lung xenografts, combinations resulted in additive or synergistic activity. Phospho-PDGFrβ and phospho-SHP2 in peripheral blood leukocytes (PBL) were evaluated in treated mice and normal human volunteers as potential biomarkers to support the clinical development of CP-868,596. Comparable reduction in phosphorylation of both biomarkers was observed in murine PBL, murine tumor xenografts, and human PBL with exposure to compound. These data demonstrate that CP-868,596, one of the most selective PDGFr inhibitors published to date, inhibits phosphorylation of PDGFrα in tumors resulting in significant tumor growth inhibition in multiple human xenograft models (Whalen PM, etal, AACR-NCI-EORTC05, Abs. B12).

A multicenter, open label, dose-escalation, phase I clinical trial (protocol ID: AD5301001;PPFIZER004) was initiated in 2003 to evaluate safety and tolerability of continuous daily oral dosing of CP-868,596 in patients with advanced solid tumors, and, if feasible, establish MTD, including MTD with empty stomach and MTD with food, and the recommended phase II dose, and characterize PK of single and multiple oral dosing of CP-868,596 when administered with empty stomach and with food. Secondary objectives are to evaluate the relationship of drug-related adverse events to PK parameters of exposure, quantify PDGFr phosphorylation in PBL following multiple oral dosing of CP-868,596, evaluate the relationship of changes in this biomarker to the time course of CP-868,596 serum concentration and, if feasible, define a pharmacodynamic-based dose, and document any preliminary evidence of antitumor activity. The trial is being conducted at Fox Chase Cancer Center (Philadelphia, PA), under PI Nancy Lewis, MD, and at DFCI, Beth Israel Deaconess Medical Center and Norris Cotton Cancer Center (Lebanon, NH).

In this trial, patient cohorts were first treated at doses of 100, 200, 280 and 340 mg 4 times daily, administered on an empty stomach. Prevalence of persistent Grade 1 nausea and vomiting led to amendments that added a cohort of 60 mg twice daily administered on an empty stomach, in addition to 'with food' cohorts. Safety assessments included adverse events, clinical laboratory tests, and ECG monitoring. PK blood samples were collected after a single dose and at steady state. Analysis of PK exposure-liver

function tests (LFT) elevation was performed to assess relationship between drug exposure and liver enzyme abnormalities. Among 33 patients enrolled in the trial, data is currently available on 31 patients. The most common treatment-related adverse events were nausea (59.0%), vomiting (54.5%) and diarrhea (27.7%) and most toxicities were Grade 1/2. DLT included nausea and vomiting (n=2), elevated ALT/GGT (n=1/1), and elevated Mg (n=1). MTD with co-administration of antiemetics, on an empty stomach, was reached at 200 mg/day. CP-868,596 was rapidly absorbed after oral administration on an empty stomach with co-adminstration of antiemetics. Mean t1/2 ranged from 11.6 to16.8 hours and was similar across all dose levels. Food caused an approximate 60% drop in Cmax and AUC. However, mean steady state systemic concentration at 60 mg twice daily with food exceeded the predicted efficacious exposure based on preclinical *in vivo* models. Analysis of PK exposure-LFT elevation suggests a rise in LFT with increased PK exposure, likely related to CP-868,596. No objective responses have been observed to date. Daily administration of CP-868,596 appears to be safe and well tolerated. Mean steady state systemic exposure exceeds the predicted human efficacious exposure concentration in all dose cohorts. Dose escalation is ongoing in the 'with food' cohorts (Lewis N, etal, AACR-NCI-EORTC05, Abs. A228).

ENMD-1198 (2-methoxyestra-1, 3, 5, (10) 16-tetraene-3-carboxamide), under development by EntreMed (Rockville, MD), is an analog of 2-methoxyestradiol (2-ME), with significant *in vitro* and *in vivo* antitumor and antiangiogenic activities, microtubule-destabilizing characteristics, and causes HIF-1α inhibition. 2-ME2, an endogenous metabolite of estradiol-17β, inhibits tumor angiogenesis while also exerting potent cytotoxic effects on various cancer cells. HIF-1α is overexpressed in more than 70% of human malignancies, including breast, prostate, brain, lung, and head and neck cancer, and overexpression correlates with tumor aggressiveness, metastases, and poor prognosis.

In order to improve bioavailability and increase activity, a series of 2ME2 analogs modified at position 3 on the A-ring and position 17 on the D-ring were designed and synthesized. These analogs retain antitumor and antiangiogenic activities *in vitro*, and demonstrate improved oral PK parameters in preclinical models. Up to 3 lead analogs (883, 900, and 5171) were selected for further preclinical studies. Similar to 2ME2, these 3 analogs show a broad range of antiproliferative activity, good oral bioavailability in mice, and decreased metabolism by rat and human hepatocyes *in vitro*. The lead analogs are microtubule-destabilizing agents causing a reduction of HIF-1α protein *in vitro*. Interestingly, *in vivo* oral delivery of 883 and 5171, but not 900, resulted in significantly improved MST in a metastatic Lewis lung carcinoma model compared to vehicle-treated mice. Significantly reduced tumor volumes were observed following treatment with all 3 analogs in an MDA MB 231 orthotopic model (Burke P, etal, AACR05, Abs. 5847).

A lead compound, ENMD-1198, was subsequently selected for further development. ENMD-1198 is orally active and tolerated by mice and rats when administered at efficacious doses. Tumor volume was reduced by 77% and 93% in a mouse orthotopic human breast carcinoma model when animals were treated with 200 mg/kg/day and 400 mg/kg/day, respectively.

To support IND development, acute dose range finding and chronic multiple dose studies were performed with ENMD-1198 in rats and dogs for up to 28 days. Organs affected by ENMD-1198 were those with cell populations of high mitotic potential, including bone marrow, lymph nodes, spleen, thymus, gastrointestinal tract, and testes. Liver abnormalities were also regularly reported, including increases in organ weight, elevations in LFT, and histopathology, that partially or fully recovered after a 14-day drug-free period. At a given dose, higher exposures were achieved in dogs compared to rats, and in female compared to male rats. On day 28, Cmax and AUC values of ENMD-1198 were comparable to day 1 values in both species. ENMD-1198 is tolerated at higher levels in mice and dogs than in rats. While ENMD-1198 shows different tolerability in mice and rats, a therapeutic window was demonstrated in both species using xenograft tumor models. Toxicities with ENMD-1198 are consistent with its microtubule destabilizing activities (LaVallee T, etal, AACR-NCI-EORTC05, Abs. B240).

In October 2005, positive preclinical results for ENMD-1198 were presented at the International Tumor Metabolism Summit held in Genova, Italy. Oral administration of ENMD-1198 showed pronounced *in vivo* antitumor activity in preclinical models of human cancer. Oral daily treatment with ENMD-1198 in an orthotopic MDA MB 231 breast cancer model led to disruption of microtubules within tumor cells, and a substantial decrease in cells staining positively for HIF-1α. Protein levels for two additional transcription factors, NFκB and Stat3, known to modulate HIF-1α protein levels *in vitro*, were also reduced following daily oral ENMD-1198 treatment, as determined by decreased nuclear staining for active transcription factor proteins. In addition to their relationship to HIF-1α protein, all 3 transcription factors are known to regulate multiple genes and their proteins that contribute to tumor growth and progression.

Serum proteins regulated by HIF-1α, NFκB, and Stat3 were also reduced substantially following oral administration of ENMD-1198 in a preclinical orthotopic breast cancer model. Results from several studies demonstrate substantially (40% to 100%) decreased plasma or serum levels of human VEGF compared to control animals following therapy with ENMD-1198. Serum levels of human IL-6, which is modulated by NFκB, were also decreased significantly (62% to 96%) with ENMD-1198. Overexpression of IL-6 is associated with higher morbidity in breast cancer, bone metastases, increased aromatase synthesis, and increased cancer drug resistance. Tumor levels of a third

tumor protein regulated by HIF-1α, carbonic anhydrase IX (CA IX), as determined by decreased cytoplasmic staining on tumor cells, were also decreased, consistent with HIF-1α inhibition.

2-ME activates the p38 MAPK and JNK pathways and induces apoptosis in cells. Expression of Smad7, an adaptor molecule required to activate p38 MAPK in the transforming growth factor β (TGFβ) signaling pathway, is also required for 2-ME-induced p38 activation and apoptosis in human prostate cancer cells (PC-3U). PC-3U/AS-S7 cells stably transfected with an antisense Smad7 construct, or PC-3U cells transiently transfected with short interfering RNA (siRNA) for Smad7, were protected against 2-ME-induced apoptosis. Smad7 was shown by both antisense and siRNA techniques to affect levels of β catenin, implicated in the regulation of apoptosis. Moreover, Smad7 was found to be important for the basal expression of Bim, a pro-apoptotic Bcl-2 family member, and for 2-ME-induced expression of Bim. These results suggest that expression of Smad7 is crucial for 2-ME-induced apoptosis in human prostate cancer cells (Davoodpour P, etal, J Biol Chem, 15 Apr 2005;280(15):14773-9).

In November 2005, the FDA accepted EntreMed's IND application for ENMD-1198, to enter into phase I clinical trials in 2006.

OSI-930, under development by OSI Pharmaceuticals is an oral, small molecule, kinase inhibitor that primarily targets RTK, c-kit, VEGFr2 (KDR) and PDGFrβ. OSI-930 potently inhibits these closely related kinases in cell-based assays. OSI-930 has good oral bioavailability in mice and lengthened plasma exposure, which permitted single daily dosing in xenograft efficacy studies.

In a preclinical study, dose-dependent tumor growth inhibition was observed in a number of models, including SW48 colon carcinoma and NCI-H526 and NCI-H209 sclc, with maximal effects on tumor growth seen with daily dosing of OSI-930. This regimen was well tolerated with minimal body weight loss when treatment lasted up to 38 consecutive days. In xenograft models, OSI-930 was effective in delaying tumor growth in sclc (NCI-H209, WBA), colorectal carcinoma (HT29, HCT-116, LS180, DLD-1, COLO 205, SW48), head and neck carcinoma (KB), gastric carcinoma (NCI-SNU-5), GBM (U251), and renal cell carcinoma (SN12C). In the most sensitive of these models (WBA, U251, NCI-SNU-5 and KB), OSI-930 affected tumor regression and durable cures. These findings indicate that OSI-930 may have broad clinical use (Winski S, etal, EORTC-NCI-AACR04, Abs. 329).

Cisplatin and etoposide were selected for preclinical combination studies with OSI-930 in the NCI-H526 sclc model. These agents were administered IV on day 1 and IP on days 1 to 3, respectively, followed by maintenance therapy of either vehicle on days 8 to 36 or OSI-930 PO on days 8 to 59. The combination of cisplatin and etoposide induced tumor regression; addition of maintenance OSI-930 was effective in delaying tumor growth by 29 days over vehicle maintenance. Log cell kill index was 3 times greater; there was one 1 durable cure in the group treated with OSI-930. A regimen comprising 5-FU and oxaliplatin, similar to the 5-FU, oxaliplatin, and leucovorin (FOLFOX) regimen approved for first line use in colon cancer, was selected for combination studies with OSI-930 in 2 human colorectal cancer models, SW48 and COLO 205. 5-FU was administered IV on days 1 to 5, oxaliplatin IP on day 1, and vehicle or OSI-930 PO, as maintenance therapy, on days 8 to 21. Treatment with maintenance OSI-930 resulted in enhanced tumor growth inhibition (TGI) and growth delay (GD) compared to vehicle maintenance in both tumor models; in SW48, TGI of 84.4% compared to 68.9%, and GD was 6.8 days over vehicle, and in COLO 205, TGI of 70.8% compared to 28.4%, and GD was 12.4 days over vehicle. In a separate COLO 205 study, OSI-930 administered PO either concomitantly on days 1 to 14, or as maintenance therapy on days 8 to 21, improved tumor growth delay by 2- to 3-fold, and increased TGI by 15 to 20% compared to control. Both schedules were well tolerated, with body weight loss in both groups being <5% (Srebernak M, etal, AACR05, Abs. 677).

OSI-930 inhibits a common downstream p70 S6K marker in both PBMC and tumors, which may be useful as a pharmacodynamic marker for evaluating effects of OSI-930 in clinical trials (Biesecker G, etal, AACR-NCI-EORTC05, Abs. B166).

In February 2005, OSI Pharmaceuticals initiated a single center, open label, vehicle-controlled, phase I clinical trial of OSI-930 in healthy volunteers to determine safety and efficacy of single oral doses of OSI-930. This trial was to enroll up to 35 subjects in the USA. The trial includes extensive PK sampling and pharmacodynamic evaluation, to facilitate design of an optimal clinical program.

XL880, under development by Exelixis (South San Francisco, CA), is a novel, orally administered, small molecule drug that significantly inhibits the Met and VEGFr2 RTK, which play synergistic roles in promoting tumor growth and angiogenesis. In addition, XL880 exhibits potent activity against other RTK implicated in various malignancies, including Kit, PDGFr, FLT3, and Tie-2.

XL880 is particularly potent against Met, potentially making this compound a first-in-class therapy. Activating mutations of Met have been identified in hereditary and sporadic papillary renal cell carcinoma, gastric, hepatocellular, head and neck, and ovarian cancer, sclc, and glioma, indications for which XL880 demonstrated dose-dependent growth inhibition. Biochemically, XL880 inhibits Met virtually irreversibly. It binds to the molecule, not covalently, but in such a way that the target activation arm moves toward and folds over it and virtually holds the inhibitor in place so that it essentially never comes off again. Even long after the drug has disappeared from plasma it is exerting a pharmacodynamic effect, inhibiting its target in the tumor.

In March 2005, Exelixis initiated an open label, single and repeat dose-escalation, phase I clinical trial (protocol ID: XL880-001, NCT00105924), being conducted at the Barbara Ann Karmanos Cancer Institute at Wayne State University (Cleveland, OH), to evaluate safety, tolerability, and PK profile of XL880 in patients with advanced solid tumors for whom there are no available therapies known to prolong survival. Patricia M. LoRusso, DO, is the PI. Participating institutions include DFCI/Harvard Medical School. According to the protocol, XL880 was administered orally in successive cohorts treated with a single dose on day 1 with PK sampling, followed by 5 continuous daily doses starting on day 4 with additional PK sampling. Patients continued treatment for 5 days, followed by a break, with cycles repeated every 14 days. Cohorts proceed to 100% escalation until >Grade 2 toxicity is observed. Escalations can be modified contingent on observed events by continuous re-assessment. A total of 9 patients with colon cancer (n=3), melanoma (n=2), renal cell cancer (n=2), papillary kidney cancer (n=1) and neuroendocrine cancer (n=1), have been treated across dose levels 0.1, 0.2, and 0.4 mg/kg. No Grade 1/4 toxicities have been observed. MTD has not yet been reached. According to plasma PK, systemic drug exposure and peak plasma values increase with increasing dose. Following 5 consecutive daily doses, these values were both approximately 2.5-fold higher than following a single XL880 dose, suggesting possible drug accumulation with repeat dosing. Mean t1/2 was ~ 60 hours, and appeared to be unaffected by dose or duration of treatment (LoRusso P, etal, AACR-NCI-EORTC05, Abs. A245).

Aurora Kinase Inhibitors

The Aurora kinases are a family of serine-threonine kinases that participate in several stages of mitosis including chromosomal segregation and cytokinesis, the final stages of cell division. The Aurora kinase family comprises Aurora A, B and C. Both Aurora A and B kinases are overexpressed in a wide variety of human malignancies and are viewed as potential therapeutic targets in oncology.

Aberrant expression of Aurora kinases can result directly in the malignant transformation of normal cells. Overexpression of Aurora kinases can cause cells to rapidly develop an abnormal number of chromosomes. Elevated levels of Aurora kinases are frequently associated with such human malignancies as breast, bladder, colon, ovarian, head and neck, and pancreatic cancer. Aurora kinases downregulate p53; amplification of Aurora genes is associated with progression and/or poor prognosis in certain types of cancer. Amplified Aurora A in tumors may act as an oncogene, transforming cells when ectopically expressed *in vitro*. Inhibition of Aurora kinases arrests cell division and promotes apoptosis and, therefore, inhibitors of Aurora kinases offer considerable potential for targeted intervention in a variety of tumors.

In late 2002, Vertex was the first to publish the 3-dimensional x-ray crystal structure of Aurora A kinase (Cheetham GM, etal, J Biol Chem, 8 Nov 2002;277(45):42419-22), a scientific advance that enabled the design and optimization of multiple classes of small molecule aurora kinase inhibitors.

Investigators at Royal Free Hampstead NHS Trust (London, UK), under PI Dr. El Sheikh Soha, are currently studying expression of Aurora enzymes and activated forms in colon adenoma, and colon cancer, compared to normal tissue, to establish any utility of these enzymes as a diagnostic/prognostic marker, by correlating their expression with patient outcome.

AZD1152, under development by AstraZeneca, is a novel acetanilide substituted pyrazole-aminoquinazoline prodrug that is converted rapidly to the active drug, AZD1152 hydroxy-QPA, in human plasma. AZD1152 hydroxy-QPA is a specific inhibitor of Aurora kinase enzyme activity, with selectivity for Aurora B and C and specificity over a panel of 50 other kinases tested. AZD1152 hydroxy-QPA's novel mechanism of action affects chromosome alignment and segregation, inhibits cell division, leads to a reduction in cell viability, and induces apoptosis *in vitro*. AZD1152 is a dihydrogen phosphate prodrug with striking solubility (>10 mg/ml) in simple pH-adjusted aqueous vehicles making it suitable for parenteral administration.

AstraZeneca investigators discovered AZD1152 as part of a program to identify small molecule inhibitors of Aurora kinases for clinical evaluation. The pyrazole acetanilide and quinazoline C-6 and C-7 substituents were found to be critical in obtaining high levels of cellular potency, and in optimizing physicochemical and PK properties. The active drug, AZD1152 hydroxy-QPA, is a potent inhibitor of Aurora B and C kinases but a weaker inhibitor of Aurora A kinase, exerting its activity through ATP-competitive and reversible inhibition of the target enzyme. In a panel of serine-threonine and tyrosine kinase enzymes it is highly specific for Aurora B kinase and against a large range of other kinases including CDK2 and EGFr. In human plasma AZD1152 is converted completely to AZD1152 hydroxy-QPA, which has excellent PK properties in a range of preclinical animal species leading to high plasma exposure in a dose-dependent manner following administration of the prodrug. In human cancer xenograft models grown in immunocompromised rodents, AZD1152 causes pharmacodynamic changes resulting in durable antitumor growth inhibition at well tolerated doses (Foote KM, etal, AACR-NCI-EORTC05, Abs. C271).

AZD1152 exerts potent antitumor activity *in vivo.* In athymic nude rodents bearing a range of human tumor xenografts, administration of AZD1152 by either IV bolus or SC mini pump infusion, resulted in a reduction of phosphorylation of the Aurora B substrate histone H3 at serine 10, increased the proportion of cells with polyploid DNA, and enhanced apoptosis, consistent with the pharmacology of inhibition of Aurora B kinase observed *in vitro*. AZD1152 was evaluated for antitumor efficacy in a panel of human colorectal (SW620, HCT116, Colo205) and lung

(A549, Calu-6) cancer xenografts. When AZD1152 was dosed for 48-hours using SC osmotic mini pumps, a statistically significant inhibition of tumor growth was observed in all xenograft models with mean tumor inhibition ranging in individual models between 69% to 100%. The greatest response was observed in the Colo205 model, with evidence of tumor regression, which lasted for 2 weeks post dosing. Impact of dosing schedules on the activity of AZD1152 was investigated further in nude rats bearing SW620 tumors. Antitumor efficacy was achieved by dosing AZD1152 either at high doses for short durations, or at lower doses for longer periods. All schedules with AZD1152 appeared to be well tolerated. Myelosuppression was observed transiently, with full bone marrow recovery occurring in all treated animals (Wilkinson RW, etal, AACR-NCI-EORTC05, Abs. B214).

When AZD1152 was infused into rodents bearing human tumor xenografts, including colon, lung, breast and cervical cancer, suppression of histone H3 phosphorylation and durable antitumor effects at well tolerated doses were observed, with phenotypes consistent with inhibition of cellular Aurora B kinase activity. Phenotypically these treated tumor cells do not arrest in mitosis or exhibit delayed progression through the cell cycle but progress with normal kinetics through aberrant mitoses, characterized by improper chromosome alignment and segregation. Cells fail to divide yielding polyploid cells leading to a reduction in cell viability and induction of apoptosis. In contrast to the phenotypic changes observed in tumor cells, normal cells (e.g. HUVEC) restrain cell cycle progression and arrest in a pseudo G1 state with 4N DNA in the presence of AZD1152 hydroxy-QPA. The unique phenotype induced by AZD1152 hydroxy-QPA in tumor cells distinguishes it from the effects of classic antimitotic agents. This may in part be explained by the ability of AZ1152 hydroxy-QPA, via inhibition of cellular Aurora B kinase activity, to abrogate the mitotic spindle checkpoint. Treatment of tumor cells *in vitro* with AZ1152 hydroxy-QPA overcomes mitotic arrest induced by spindle damage and accelerates exit from mitosis in this setting, probably as a consequence of the loss of a key checkpoint regulator, BubR1, from the kinetochores of mitotic chromosomes (Keen N, etal, AACR-NCI-EORTC05, Abs. B220).

AZD1152 entered a phase I clinical trial in 2005.

MK0457 (VX-680), under development by Merck, in collaboration with Vertex Pharmaceuticals, is a small molecule inhibitor of Aurora and FLT-3 kinases. VX-680 is a potent and selective small molecule inhibitor of Aurora kinases inducing accumulation of cells with >4N DNA content followed by cell death. VX-680 blocks cell-cycle progression and induces apoptosis in a diverse range of human tumor types and profoundly inhibits tumor growth in a variety of *in vivo* xenograft models, leading to regression of leukemia, and colon and pancreatic tumors at well tolerated doses (Harrington EA, etal, Nat Med, Mar 2004;10(3):262-7).

Integrity of p53 function may govern response to VX-680. VX-680 induces endoreduplication and apoptosis preferentially in p53 and p21Waf1/Cip1-deficient cells. While cells with intact p53 function exposed to VX-680 undergo G2/M arrest, those with compromised p53 function are more likely to undergo endoreduplication followed by eventual apoptosis (Gizatullin F, etal, AACR05, Abs. 456).

Leukemia, lymphoma and colorectal tumor cell lines are particularly sensitive to the cytotoxic effects of VX-680. VX-680 is also potent against primary AML cells from patients refractory to standard treatments, including those with activating FLT-3 mutations, and can completely inhibit colony formation. *In vivo*, inhibition of tumor growth is complemented by a reduction in phosphorylation of histone H3, a key Aurora substrate, and a significant increase in apoptosis within the tumor tissue. VX-680 dramatically increases median survival time (MST), and induces sustained remission in a murine leukemia model of BAF3 cells transduced with an activating human FLT-3 mutation (Harrington E, etal, AACR04, Abs. LB-238).

In June 2004, Vertex Pharmaceuticals and Merck entered into a global collaboration to develop and commercialize VX-680 for the treatment of cancer. Under the agreement, Merck is responsible for clinical development and worldwide commercialization of VX-680 and will pay Vertex development milestones and royalties on product sales. In addition, the companies are conducting a joint research program to characterize VX-680's activity across a broad range of cancer types and to identify follow on drug candidates directed at Aurora kinases, using molecular profiling approaches and microarray technologies pioneered by Merck.

In December 2004, an open label, phase I clinical trial (protocol ID: 2004_095, NCT00099346) was initiated with MK0457 in patients with advanced colorectal cancer and other solid tumors to assess the drug's safety and efficacy in this setting. The trial is being conducted in two parts. Part I is enrolling patients with advanced solid tumors while only patients with colorectal cancer will be eligible to participate in part II.

In January 2005, an open label, dose-escalation, phase I clinical trial (protocol ID: 2005_005, NCT00104351) with VX-680 was initiated in patients with solid tumors at two treatment centers. The trial will evaluate the safety and tolerability of VX-680 when administered in multiple cycles to patients refractory to prior chemotherapy treatment.

In June 2005, Merck and Vertex initiated a 2-part, open label, dose-escalation phase I clinical trial (protocol ID: 2005_033, NCT00111683) with VX-680 designed to evaluate the safety and tolerability of VX-680 administered in a 5-day treatment cycle in patients with relapsed or refractory hematologic malignancies, including AML, MDS, ALL, and CML-BC.

In December 2005, Vertex reported that in a phase I clinical trial in patients with solid tumors, dosing with VX-680

(MK-0457) demonstrated activity on a clinically relevant biomarker, triggering achievement of a milestone under its contract with Merck. In addition, Merck has selected a follow-on compound for development from a joint Aurora kinase research program that is part of the collaboration. In conjunction with this progress, Vertex will receive two milestone payments from Merck totaling $19.5 million.

MLN8054, under development by Millennium Pharmaceuticals (Cambridge, MA) is an orally active, potent, selective, small molecule Aurora A kinase inhibitor. The *in vivo* efficacy of MLN8054 was studied in a number of human tumor models in immunocompromised mice. Dose-dependent tumor growth inhibition was observed after once or twice daily oral administration in multiple SC xenograft models, including colon and prostate cancer. At the end of the 21-day treatment period, tumor growth inhibition ranged from 47% to 96%. In some models, growth delay persisted for up to 30 days after treatment ceased. Micro-PET studies demonstrated that MLN8054 reduced glucose uptake after repeat dosing. Data that MLN8054 may be effective across many different malignancies (Zhang M, etal, AACR-NCI-EORTC05, Abs. B199).

MLN8054 inhibited Aurora A and Aurora B in cultured human tumor cells in micromolar concentrations; Aurora A was inhibited with an IC_{50} of 0.034 μM and Aurora B with an IC_{50} of 5.700 μM. Tumor cells treated with lower concentrations of MLN8054 for 24 and 48 hours, accumulated in mitosis and presented with abnormal mitotic spindles. Treatment with higher concentrations resulted in loss of phosphorylation of histone H3 on Ser 10, and induced formation of multinucleated cells, phenotypes consistent with Aurora B inhibition (Hoar K, etal, AACR-NCI-EORTC05, Abs. C40).

Treatment of tumor cells with MLN8054 induced mitotic spindle defects, mitotic accumulation, and inhibition of cell proliferation, resulting in apoptosis in human xenograft models. Growth of several human tumor xenografts in nude mice including those of colon, prostate, and lung cancer was dramatically inhibited after oral administration of MLN8054 at doses that were well tolerated. Moreover, tumor growth inhibition was sustained after discontinuing MLN8054 treatment (Manfredi MG, etal, AACR-NCI-EORTC05, Abs. B202).

Inhibition of tumor growth by MLN8054 in the HCT-116 xenograft model by daily oral administration was dose dependent. Consistent with Aurora A kinase inhibition, a single dose of MLN8054 increased the mitotic index in HCT-116 tumors. At MTD, the mitotic index increased beginning 4 hours post dosing as indicated by an increase in 2 mitotic markers, phospho-histoneH3 (pHisH3) and MPM2. Within 24 hours after dosing, the mitotic index began to decline. Moreover, in a dose-response study, MTD resulted in a longer duration of mitotic arrest, peaking between 8 and 24 hours, than the lowest dose group that peaked at 4 hours. Repeat dosing of MLN8054 resulted in a significant increase in apoptotic cells starting 3 days after initiating dosing (Burenkova O, etal, AACR-NCI-EORTC05, Abs. B200).

An open label, dose-escalation, single group assignment, safety/efficacy, phase I clinical trial (protocol ID: C10001; NCT0024930), was initiated in October 2005, at the Sarah Cannon Research Institute (Nashville, TN), under PI Howard A Burris, MD, in patients with histologically or cytologically confirmed metastatic and/or advanced solid tumors (including lymphoma) refractory to or ineligible for standard treatment.

Epidermal Growth Factor Receptor (EGFr) Inhibitors

Approval of several anticancer agents inhibiting EGFr has spurred development of many novel inhibitors of the EGF family of RTK. Approximately 30 separate agents are in various stages of development addressing this family of molecular targets.

ARRY-33454, under development by Array BioPharma (Boulder, CO), is a novel, small molecule, orally active, potent, reversible, ATP-competitive, dual inhibitor of EGFr and ErbB2 (HEr2) kinases. ARRY-334543 potently inhibits substrate phosphorylation in cell-based assays using tumor cells that overexpress EGFr (A431) or erbB2 (BT-474). ARRY-334543 was shown to be highly selective for EGFr/erbB2, but did not show significant activity when screened against a panel of 104 other kinases. In murine xenograft models ARRY-334543 demonstrated significant dose-related tumor growth inhibition in A431-derived tumors when administered orally, twice daily, for 21 days (Miknis G, etal, AACR05, Abs. 3399).

In a preclinical study, the *in vivo* efficacy of ARRY-33454 was evaluated in several human tumor xenograft models. Dose-dependent inhibition of tumor growth was observed in the A431 epidermoid carcinoma model that overexpresses EGFr; twice daily dosing resulted in inhibition of 57% and 80% of tumors, respectively. Activity of ARRY-334543 was also evaluated in a panel of nsclc cell lines, including those with both wt (A549, Calu-3) and mutant (H1650; exon 19 deletion) EGFr. Dose-dependent inhibition of tumor growth was observed in the A549 model and significant growth inhibition was observed at twice daily doses in the Calu-3 model, with tumor regression seen in 8/8 animals, and in the H1650 model, with regressions seen in 2/8 animals. In nude and SCID mice bearing MDA-MB-453 and BT-474 tumors that overexpress Her2, dose-dependent inhibition of tumor growth was observed in the MDA-MB-453 model, and significant tumor regressions in 6/8 animals were observed in the BT-474 model. Therefore, ARRY-334543 has broad *in vivo* antitumor activity in EGFr- and ErbB2–dependent models. Efficacy was noted in both wt and mutant EGFr xenograft models (Pheneger T, etal, AACR-NCI-EORTC05, Abs. A247).

An open label, multiple dose phase I clinical trial (protocol ID: ARRY-0501, NCT00278902) was initiated in January

2006, at British Columbia Cancer Agency (Vancouver, BC, Canada), to assess safety, tolerability and PK of PO ARRY-334543, administered on a daily regimen, in patients with advanced solid tumors or hematologic malignancies.

BIBW 2992, under development by Boehringer Ingelheim, is a highly potent, orally available selective and irreversible inhibitor of intracellular EGFr and HEr2 tyrosine kinase activity. It is active in the subnanomolar range, and profoundly inhibits EGF-induced receptor phosphorylation and cellular proliferation in the low nanomolar range. BIBW 2992 was selected from compounds produced by a chemical synthesis program that designed potent and drug-like dual EGFr/HEr2 inhibitors.

Although IBW 2992 is a potent EGFr blocker, it inhibits little if at all many other RTK classes and signaling pathways. The compound blocks EGF-stimulated EGFr phosphorylation in A431 cells and, similarly, at nanomolar concentrations, causes dephosphorylation of constitutively active HEr2 in NCI-N87 and BT-474 cells, inhibiting proliferation of these cells. In animals, BIBW 2992 exhibited acceptable tolerability and suitable PK. Potent and long lasting growth suppression, or even tumor regression, were observed in human cancer xenograft models, including A431 squamous cell, MDA-MB-453 breast, NCI-N87 gastric, and SKOV-3 ovarian carcinoma. In the A431 model, antitumor activity is correlated with a decrease in pEGFr and pAkt biomarkers. A distinctive feature of BIBW 2992 in cell culture studies is a long lasting kinase inhibition of up to 24 hours after brief exposure, which is in line with the observed antitumor activity even with intermittent dose schedules (Solca F, etal, AACR-NCI-EORTC05, Abs. A244).

The inhibitory activity of BIBW 2992 was investigated in 3 nsclc cell lines representing different mutational status of the EGFr, to assess this drug's activity against cancer cells with wt and mutated EGFr. Presence of specific gain-of-function mutations within the tyrosine kinase domain of EGFr, in a subgroup of patients with nsclc, has been associated with increased sensitivity to treatment with gefitinib (Iressa; AstraZeneca) and erlotinib (Tarceva; OSI Pharmaceuticals). In particular, the L858R point mutation (exon 21) as well as deletion/insertion mutations in the ELREA sequence (exon 19) account for the majority of gefitinib responders. Recently, T790M, a secondary point mutation was also described in exon 20, associated with acquired resistance to gefitinib or erlotinib (Pao W, etal, PLoS Med, Mar 2005;2(3):e73). This mutation is analogous to the T315I mutation identified in patients with CML who relapse under imatinib treatment. The sensitivity of BIBW in cell lines H1666 that expresses a wt receptor, H3255 that harbors the activating mutation L858R, and H1975 that expresses both the activating L858R as well as the gate-keeper T790M mutations, was assessed in two *in vitro* assay systems. BIBW 2992 potently inhibited EGFr phosphorylation in all 3 cell lines. These observations are in line with recently published data suggesting that irreversible inhibitors of EGFr may circumvent acquired resistance in contrast to reversible inhibitors such as gefitinib (Kwak EL, etal, PNAS USA, 24 May 2005;102(21):7665-70), thus inhibiting proliferation and EGF-induced EGFr phosphorylation in cell lines expressing double mutant EGF receptors. BIBW 2992 may prove effective in the treatment of patients with nsclc harboring activating mutations in the EGFr gene, as well as in those resistant to gefitinib or erlotinib because of the acquired T790M mutation. Furthermore, BIBW 2992, which also inhibits HEr2 kinase activity, offers additional therapeutic potential in tumors where HEr2 plays a central role (Solca F, etal, AACR-NCI-EORTC05, Abs. A242).

As of December 2005, 3 different dose schedules have been investigated in dose-escalation phase I clinical trials, chronic daily dosing, 14 days on and 14 days off daily dosing, and 21 days of daily dosing with 7 days off.

A dose-escalation phase I clinical trial of BIBW 2992 in patients with advanced solid tumors was conducted at Northern Centre for Cancer Treatment (Newcastle upon Tyne, UK) and Royal Marsden Hospital. BIBW 2992 was administered orally as a continuous once daily dose starting, at 10 mg daily and doubled in successive cohorts until drug-related toxicity ≥Grade 2. Thereafter, escalation steps of no more than 50% were allowed. All patients underwent PK sampling and pre and post treatment skin biopsies. DNA sequencing of tumor cell EGFr and HEr2 was performed in patients with objective responses. Among 18 enrolled patients, 15 who completed ≥28 days of treatment were evaluable for toxicity. Duration of treatment ranged from 4 days to 10 months. There were 2 cases of DLT. One patient with HEr2+ breast cancer previously treated with trastuzumab and lapatinib, treated at 30 mg, developed increasing dyspnea on day 4 associated with radiologic interstitial changes; this patient fully recovered after drug discontinuation. At 40 mg, there was one DLT, a Grade 3 acneiform skin rash, causing dose reduction to 20 mg/day. The 40 mg dose level was expanded to 6 patients. Thus far, no other DLT have been observed at the daily 40 mg dose and further dose escalation is planned. Other toxicities were mild (Grade 1/2) including nausea, diarrhea, mucositis and fatigue. There was 1 confirmed PR in a female patient with adenocarcinoma of the lung treated at 10 mg daily who remained in the trial beyond 40 weeks. This patient's tumor cells have a complex heterozygous EGFr mutation including a deletion and missense mutations of 4-amino acids (wt: KELREATSP-KANKEILD; mutated: KEP----SPRANKEILD) in the kinase domain, but a wt HEr2 domain. Disease stabilized and tumor marker response was observed in one patient with cervical cancer and another with colorectal cancer for 6 and 4 months, respectively. BIBW 2992 shows promising activity with an acceptable toxicity profile. The maintained PR achieved in lung adenocarcinoma indicates effective mutated EGFr kinase modulation at the lowest evaluated dose level (Plummer R, etal, AACR-NCI-EORTC05, Abs. A105).

Principal objectives of a dose-escalation phase I clinical trial in patients with advanced solid tumors, conducted in the Netherlands, at Erasmus Medical Center and the University Medical Center Groningen, were to determine MTD and DLT of BIBW 2992 administered once daily for 14 days followed by 14 days off treatment, characterize the drug's safety and tolerability including acute and chronic toxicities, characterize single and multiple dose PK of the drug, and seek preliminary evidence of antitumor activity. To date, 34 patients have been enrolled at dose levels of 10 mg (n=3), 20 mg (n=3), 30 mg (n=3), 45 mg (n=3), 70 mg (n=14), 100 mg (n=2) and 85 mg (n=6). At 100 mg, DLT, consisting of Grade 3 skin rash and Grade 3 diarrhea despite adequate treatment with loperamide, occurred in two consecutive patients. At the next lower dose level of 70 mg only one episode of DLT, consisting of Grade 3 nausea, diarrhea and ALAT elevation, occurred among 6 patients treated, prompting evaluation of the intermediate dose level of 85 mg. At this dose level DLT were seen in 2 patients, consisting of one episode of Grade 3 diarrhea, and one episode of Grade 2 diarrhea lasting for more than 7 days, despite adequate treatment in both cases. In accordance with the protocol an additional 8 patients were enrolled at the 70 mg dose level. In this group of patients diarrhea was controllable using loperamide on indication, whereas skin toxicity could either be prevented or treated with PO minocycline (100 mg). No additional DLT were seen, and the dose of 70 mg BIBW 2992 administered once daily PO for 14 days, followed by 14 days off, was established as the recommended phase II dose. There were no CR or PR but disease stabilized for 4 or more courses in 7 patients (Eskens FA, etal, AACR-NCI-EORTC05, Abs. A235).

Patients with refractory solid tumors historically known to express EGFr and/or HEr2, were enrolled into a dose-escalation phase I clinical trial, conducted at Fox Chase Cancer Center, and Lombardi Comprehensive Cancer Center (protocol ID: 03-371), under PI John Marshall, MD, and treated with oral BIBW 2992 for 21 consecutive days followed by a 7 day washout. A rapid dose-escalation scheme was used with each 3-patient cohort. If DLT was observed in a single patient, cohorts were expanded using a traditional 3+3 trial design. After reaching DLT, 18 patients were treated at MTD. PK and skin biopsies were performed in all patients at baseline and on day 21, and tumor biopsies in appropriate patients were obtained pre and post treatment for the final dose level. A total of 42 evaluable patients were enrolled and treated with daily doses ranging from 10 to 65 mg. Tumor types included colon (n=10), breast (n=8) and ovarian (n=2) cancer; 3 each thyroid, pancreatic, esophageal, prostate, and head and neck cancer, and nsclc; and 1 each gastric, endometrial, and hepatocellular cancer and cholangiocarcinoma. Principal toxicities, typical with RTK inhibitors, were diarrhea, skin rash, pruritus, mucositis, and nausea and vomiting, with severity related to dose. Although the dose was escalated to 65 mg per day, it was deemed unacceptable for chronic dosing based upon 2 occurrences of DLT including skin rash (n=1) and mucositis (n=1). The 55 mg dose level was chosen as the recommended phase II dose based on its acceptable toxicity profile using chronic administration. No objective responses have been observed thus far, but disease stabilized in 15/35 (43%) evaluable patients who were treated with 3 or more courses. BIBW 2992 was well tolerated at 55 mg per day using a 21-day on, 7-day off treatment schedule but the actual dose may eventually be closer to 40 mg daily on this schedule (Marshall JL, etal, AACR-NCI-EORTC05, Abs. B161).

The two phase I clinical trials, conducted in the Netherlands and in the USA, investigated, among other parameters, PK of BIBW 2992 and incidence of skin reactions, in patients with various refractory advanced solid tumors, known to express EGFr and/or HEr2. A total of 54 patients from both trials were included into an interim PK analysis. Exploring two different dosing schedules, BIBW 2992 was administered orally continuously, once daily for either 14 or 21 days followed by a 14-day or 7-day washout period. The starting dose of 10 mg/day was doubled in successive cohorts until the occurrence of ≥Grade 3 drug-related toxicities. From then on doses were increased by a maximum of 50%. In terms of PK, absorption of BIBW 2992 was moderately fast with median time to maximum plasma concentration (Tmax) values between 1 to 5 hours post administration. Generally, Tmax and exposure of BIBW 2992 increased with dose. Interpatient variability in Cmax and AUC values (on days 1 and steady state) was high, but within the expected range for an orally administered anticancer drug. BIBW 2992 exhibited a high tissue distribution and moderate apparent total body clearance. Mean t1/2 ranged between 13 to 50 hours. BIBW 2992 exposure increased with dose on day 1 and at steady state. High interpatient variability in all PK parameters was found and reasons for this needs to be evaluated during further clinical trials. Skin reactions typical for EGFr-inhibitors were also observed (Stopfer P, etal, AACR-NCI-EORTC05, Abs. B172).

XL647, under development by Exelixis, is a potent simultaneous inhibitor of RTK EGFr, HEr2, VEGFr, and EphB4. XL647 has good oral bioavailability and shows sustained inhibition of target RTK *in vivo* following a single oral dose. Preclinically, in models of major tumor types, including human breast, lung, colon, and prostate cancer, XL647 demonstrated potent inhibition of tumor growth, and caused tumor regression in one model. Consistent with its spectrum of activity, tumors from XL647-treated animals exhibit significant decreases in both tumor vascularity and tumor cell proliferation, and an increase in tumor cell death.

A nonrandomized, dose-escalation, open label, uncontrolled, single agent, phase I clinical trial (protocol ID: XL647-001, NCT00086528) was initiated in June 2004, to assess pharmacodynamic effects of XL647 in plasma and peripheral blood cells. This trial's primary objective is to

evaluate safety and tolerability of PO XL647 administered as a single dose, and as repeat doses (up to 11 different dose levels) in patients with advanced solid tumors. Secondary objectives are to evaluate plasma PK, and estimate renal elimination of XL647 in this setting. In addition, subjects may be eligible to enter a treatment extension period, to assess long term safety and tolerability, and tumor response after repeat administration of XL647. First, patients are administered a single dose, and the compound is allowed to wash out for a couple of days. Then, patients are administered 5 sequential daily doses, which may be repeated every 2 weeks for as long as the disease does not progress. Approximately 66 patients with advanced or metastatic solid tumors are to enroll in this trial being conducted at Stanford University Medical Center (Stanford, CA), under PI Branimir I Sikic, MD, and at Mayo Clinic, under PI Alex A Adjei, MD, PhD.

In this phase I clinical trial, tumor imaging was performed at baseline, after the first 3 or 4 cycles and then after every 4 cycles. Patients with stable or improving disease were allowed to continue treatment. Among 28 patients treated across 8 dose levels (0.06, 0.12, 0.19, 0.28, 0.39, 0.78, 1.56, 3.12 mg/kg), an asymptomatic QTc prolongation on ECG occurred in one patient treated with 3.12 mg/kg. MTD has not yet been reached and dose escalation continues. Additional schedules (continuous) are also under investigation. According to preliminary PK analysis, XL647 shows approximately dose-proportional exposure with a mean time to Cmax of approximately 6 to 9 hours, and an elimination t1/2 of approximately 70 hours. There was 1 PR in a patient with nsclc treated in cohort 1, and disease stabilized for prolonged periods (>3 months) in 7 patients (nsclc=2, chordoma=2, adenoid cystic carcinoma=1, adrenocortical carcinoma=1, colorectal cancer=1) (Wakalee H, etal, AACR-NCI-EORTC05, Abs. A261).

XL647 is expected to enter initially into phase II clinical trials in patients with nsclc and breast cancer, tumors where the kinases inhibited by XL647 are known to play a role. Additionally, Exelixis is considering combination trials of XL647 with other anticancer treatments to test the ability of the combination therapy to prolong PFS.

Heat-shock Protein 90 (Hsp90) Inhibitors

Heat shock protein 90 (Hsp 90) is a molecular chaperone that maintains the stability of numerous client proteins. In malignancy, Hsp 90 maintains conformation, stability and function of key oncogenic client proteins such as c-Raf, HEr2 and Cdk4, involved in signal transduction pathways leading to proliferation, cell-cycle progression and apoptosis, as well as other processes of the malignant phenotype such as invasion, angiogenesis and metastasis (Maloney A, and Workman P, Expert Opin Biol Ther, Jan 2002;2(1):3-24). Because Hsp90 is required for the correct folding, stability and function of a range of oncoproteins that are mutated or overexpressed in cancer, Hsp90 inhibitors may provide a simultaneous attack on multiple oncogenic pathways. By depleting the levels of multiple oncoproteins in cancer cells and blocking a wide range of oncogenic pathways, Hsp90 inhibitors could address all hallmark characteristics of cancer (Workman P, Cancer Detect Prev 2002;26(6):405-10).

The first Hsp90 inhibitor to enter clinical development in the late 1990s, is 17-allylamino, 17-demethoxygeldanamycin (17-AAG), a derivative of the natural product geldanamycin. Despite its potent biologic activity, this natural product proved difficult to formulate as a drug; early efforts to formulate 17-AAG required use of such undesirable formulation components as organic solvents. Next generation synthetic Hsp90 inhibitors designed to overcome the limitations of 17-AAG are in early clinical development.

Novel, synthetic inhibitors may also overcome drug-resistance associated with 17-AAG treatment. Investigators at Conforma Therapeutics (San Diego, CA) report that next generation synthetic Hsp90 inhibitors may have broader applications against tumors with acquired MDR1, and in malignancies located in organs protected by P-gp, such as the adrenal glands, brain, and testis (Zhang H, etal, AACR-NCI-EORTC05, Abs. C39).

It appears that 17-AAG and other ansamycin drugs are highly sensitive P-gp substrates. P-gp is a major contributor to drug resistance commonly observed in heavily pretreated patients. The new generation of potent, synthetic, Hsp90 inhibitors is largely independent of MDR. When activity of a panel of ansamycin and synthetic compounds was tested in a variety of cell lines expressing P-gp or MRP1 at various levels, overexpression of P-gp significantly increased the IC_{50} of 17-AAG and 17-DMAG in client protein degradation, biomarker secretion and cytotoxicity assays by >500 fold. There was an inverse correlation between P-gp level and the cytotoxicity of 17-AAG. These phenomena were reversed by co-administration of P-gp or MRP1 inhibitors, which significantly increased 17-AAG's potency. By contrast, most synthetic analogs were not affected by either P-gp or MRP1 expression. Furthermore, synthetic Hsp90 inhibitors were considerably more active against adrenocortical carcinoma, a tumor that naturally expresses P-gp.

CNF-1010, under development by Conforma Therapeutics, is an Hsp90-targeted drug based on a lipid formulation of 17-AAG. Unlike 17-AAG, CNF1010 does not require co-administration of cremaphore or organic solvents such as DMSO. CNF-1010 is being clinically investigated in the treatment of both hematologic malignancies and solid tumors.

A multicenter, open label, nonrandomized, dose-finding, phase I clinical trial (protocol ID: CDR0000407499, UCLA-0408048-01, CTC-CNF1010, CTC-CNF1010-CML-04001, NCT00100997) with CNF1010 was initiated in August 2004, in patients with imatinib-resistant Philadelphia chromosome (Ph)-positive (Ph+) CML. Primary objectives are to determine MTD, DLT, and PK of

this drug and its primary metabolite 17 AAG in these patients. Secondary objectives are to determine hematologic response rate, in terms of WBC, and platelet count, and blast cells in peripheral blood, and cytogenic response rate, in terms of Ph+ progenitor cells in the bone marrow. The effect of this drug on pharmacodynamic markers, such as CRKL (a substrate of Bcr-Abl) phosphorylation, Bcr-Abl kinase activity, and Bcr-Abl, Raf kinase and Hsp70 expression, is also being assessed. According to the protocol, patients are treated with CNF1010 IV once over 15 minutes or 1 hour, depending on the dose administered, on days 1, 4, 8, 11, 15, 18, 22, and 25. Treatment repeats every 28 days for up to 3 courses in the absence of unacceptable toxicity or disease progression. Eligible patients may be treated with additional courses of CNF1010 at the discretion of the investigator. Cohorts of 3 to 6 patients are treated with escalating doses of CNF1010 until MTD is reached. Up to 10 additional patients are treated at MTD. Patients are followed for 1 month. Approximately 40 patients will be accrued for this trial being conducted in the USA, coordinated by the Jonsson Comprehensive Cancer Center at UCLA, under PI Charles Sawyers, MD.

A multicenter open label, dose-finding, phase I clinical trial with CNF1010, was also initiated in August 2004, in the USA, in patients with advanced solid tumors, designed to investigate safety, tolerability, PK, and pharmacodynamic profile of CNF1010. In this trial, patients with advanced solid tumors were treated with CNF1010 as a 1-hour IV infusion, twice weekly for 3 weeks out of 4, starting at a dose of 6 mg/m^2. Doses were escalated sequentially in single patients (6 and 12 mg/m^2) and in 3- to 6-patient cohorts (>25 mg/m^2) according to a modified Fibonacci scheme. Plasma PK profiles were obtained on days 1 and 18. Hsp90 was measured in PBMC and HEr2 ectodomain (HEr2-ECD) in plasma. A total of 27 patients with colorectal (n=8), pancreatic (n=5), and ovarian (n=2) cancer, melanoma (n=5), and other malignancies (n=7), were treated with a median of 2 courses (range=1-7). There was no DLT. There was one death at the at 175 mg/m^2 dose level, but its relation to the drug was unclear. Grade 1/2 GI toxicities predominated. There were Grade 3, but no Grade 4, reversible hepatic enzyme elevations (n=2) and fatigue (n=2). There were no hematologic, renal, cardiovascular, or neurologic toxicities. Plasma 17-AAG PK appeared dose-proportional; t1/2 of 5.2 hours was dose-independent and unchanged after repeated dosing. Post treatment increases in Hsp70 were observed in PBMC, and decreases in plasma HEr2-ECD were observed at doses ≥83 mg/m^2. There was 1 minor tumor regression in a patients with duodenal cancer (83 mg/m^2), and 1 with melanoma (175 mg/m^2). CNF1010 administered twice weekly was well tolerated at doses ≥175 mg/m^2. Dose escalation continues (Dragovich T, etal, AACR-NCI-EORTC05, Abs. C75). Participating institutions include Arizona Cancer Center (Tucson and Scottsdale, AZ), University of Alabama at Birmingham, and Mayo Clinic.

IPI-504, under development by Infinity Pharmaceuticals (Cambridge, MA), is a novel, water-soluble, targeted anticancer drug that preferentially induces death of cancer cells through inhibition of the Hsp90 complex. IPI-504 and related compounds were created through the application of a proprietary platform, diversity-oriented synthesis (DOS). DOS is a combinatorial synthetic approach that creates molecules with complex, 3-dimensional structures with multiple and completely controllable stereocenters. DOS-created molecules exhibit all the hallmarks of biologically active molecules previously only available from natural sources.

IPI-504, a chemical reduction product of 17-AAG, exists as a hydrochloride salt, which is soluble in water. IPI-504 has with 17-AAG, existing in a pH and enzyme-mediated dynamic redox equilibrium. When IPI-504 or 17-AAG is administered to mice, rats, or monkeys, a dynamic equilibrium occurs *in vivo* in which both species are present in plasma as well as tissue. Thus, IPI-504 possesses all of the properties of Hsp90 inhibitors and the biologic activity of 17-AAG without its formulation liabilities (Jagannath S, etal, ASH05, Abs. 2560).

Up to three areas were explored with IPI-504 including *in vitro* and *in vivo* interconversion of IPI-504 with 17-AAG, pharmacodynamics and selective tumor tissue uptake of IPI-504, and scientific rationale and *in vivo* efficacy of IPI-504 in human xenograft and orthotopic models of multiple myeloma. IPI-504, or 17-AAG, administered to mice, rats, or monkeys, produces a dynamic equilibrium *in vivo*, in which both species are present in plasma as well as tissue. This equilibrium is a balance of IPI-504 oxidation and 17-AAG enzymatic reduction. IPI-504 and 17-AAG are rapidly cleared from plasma within 4 to 6 hours, but are selectively retained in tumor tissue at pharmacologically active concentrations for up to 48 hours. Because of the 48-hour tumor tissue retention of IPI-504 and its active metabolites, tumor-bearing animals could be treated 2 to 4 times a week (Sydor, etal, AACR05, Abs. 6160).

IPI-504 has demonstrated activity as a single agent, and in combination with other anticancer agents, in models of a wide variety of hematologic malignancies as well as solid tumors. When examined in a preclinical xenograft model, IPI-504 reduced relapsed refractory multiple myeloma tumor growth by 71% relative to controls. Furthermore, *in vivo* reduction in tumor growth was also observed in several other human xenograft models, including models of breast, prostate, and ovarian cancer.

IPI504 inhibits and degrades Kit in imatinib-resistant GIST. Because Hsp90 protects Kit oncoproteins from proteasome-mediated degradation, inhibition of Hsp90 by IPI504 was evaluated in imatinib-sensitive (GIST882) and resistant GIST cell lines characterized by secondary kinase domain mutations (GIST48 and GIST430) and by loss of Kit expression (GIST62). IPI504 inhibited both imatinib-sensitive and resistant Kit oncoproteins. Inhibition of downstream signaling intermediates AKT and S6 was seen in the Kit-positive GIST (GIST882, GIST430

and GIST48) cells but not in Kit-negative GIST62 cells, suggesting that IPI504 effects depend on Kit. Likewise, IPI504 inhibited cell proliferation in GIST48 and GIST430 by 88% and 34%, respectively, but not in GIST62 (12%). IPI504 induced apoptosis in Kit-positive GIST. Synergistic effects for the combination of IPI504 and imatinib were not seen in any of the GIST cell lines. Therefore, HSP90-mediated stabilization of Kit oncoproteins is crucial for expression and activation of oncogenic Kit in GIST. Hsp90 inhibition by IPI-504 has strong antiproliferative and proapoptotic effects in imatinib-resistant GIST at clinically achievable doses. Hsp90 inhibitors abrogate oncogenic Kit signaling irrespective of the underlying imatinib-resistance mutations, and may thus overcome the problem of genomic heterogeneity in imatinib-resistant GIST (Bauer S, etal, AACR-NCI-EORTC05, Abs. C49).

GIST may serve as a bellwether of IPI-504's activity, because its mechanism of action may also render it effective in breast cancer resistant to trastuzumab, lung cancer resistant to erlotinib, and multiple myeloma resistant to bortezomib.

A multicenter, open label, dose-escalation, safety assessment, phase I clinical trial (protocol ID: IPI-504-01, NCT00113204) was initiated in June 2005, to evaluate potential antitumor activity and tolerability of various doses of IPI-504 in patients with relapsed or refractory multiple myeloma. The trial is being performed at St. Vincent's Comprehensive Cancer Center (New York, NY) under PI Sundar Jagannath, MD. Additional sites include Hackensack University Medical Center, and Jerome Lipper Multiple Myeloma Center at DFCI. Primary objectives are to determine safety and MTD of IPI-504, and the recommended dose for subsequent clinical trials. Secondary objectives are to examine PK parameters, evaluate potential antitumor activity with standard markers of disease progression, and examine pharmacodynamic markers of biologic activity. Total expected enrollment is 40 patients.

In this phase I clinical trial, escalated doses of IPI-504 were infused in 250cc of normal saline over 30 minutes on days 1, 4, 8 and 11 of a 21-day cycle. Patients are being treated with one cycle. An accelerated titration design was used during the first three dose levels. According to preliminary results the *in vivo* dynamic equilibrium of IPI-504 and 17-AAG is detectable in human plasma PK samples, and the water-based formulation is well tolerated. Clinical evaluation of this agent is ongoing to define safety, tolerability and potential activity in this setting. These results may help clarify the effects of Hsp90 inhibition using a water-soluble, ansamycin-based Hsp90 inhibitor without the confounding effects of DMSO-based formulations (Jagannath S, etal, ASH05, Abs. 2560).

In January 2006, Infinity initiated an open label, dose-escalation, phase I clinical trial (protocol ID: NCT00276302) to evaluate safety, PK, and potential efficacy of IPI-504, in patients with gastrointestinal stromal tumors (GIST) resistant to imatinib. This trial is being conducted at DFCI under PI George Demetri, MD. Expected enrollment is 40 patients.

KOS-1022 (17-dimethylaminoethylamino-17-demethoxy-geldanamycin or 17-DMAG), under development by Kosan Biosciences (Hayward, CA), is a second generation, highly potent and water-soluble benzoquinone ansamycin with good oral bioavailability. Kosan is also developing KOS-953, a proprietary formulation of 17-AAG. Kosan is clinically evaluating KOS-953, in clinical trials in combination with trastuzumab in patients with Her2-positive breast cancer, and in patients with relapsed refractory multiple myeloma, both as single agent and in combination with bortezomib. Kosan may initially seek approval for KOS-953, in combination with bortezomib, to treat relapsed refractory multiple myeloma. In 2004, Kosan obtained orphan drug designation for KOS-953 from both the FDA and the European Medicines Evaluation Agency (EMEA) for treatment of multiple myeloma as well as CML.

To assess the sensitivity of testicular teratoma to KOS-1022, investigators at the NCI treated GCT27 cells, derived from human testicular teratoma, and NCCIT cells, derived from human mediastinal teratoma cell lines, with KOS-1022, with untreated cells serving as controls. Compared to baseline, untreated GCT27 cells increased in number by 87%, while cells exposed to KOS-1022 decreased dose-dependently by 22%, 52%, and 53%. Under the same conditions, the numbers of untreated NCCIT cells increased by 47%, while those exposed to KOS-1022 decreased dose-dependently by 68%, 66%, and 71%. In this model, findings suggest that the effects of KOS-1022 on teratoma are cytotoxic. These data support current phase I clinical testing of KOS-1022 in solid tumors, and suggest KOS-1022 may be an effective therapeutic adjunct for regionally metastasized germ cell tumors containing teratomatous elements. Further testing is underway to determine the minimum effective *in vitro* cytotoxic dose in the tested cell lines, and to determine if these effects persist in animal models. Additional studies are underway to determine which Hsp90 client proteins are most sensitive to inhibition in these cell lines, and identify the likely mechanism of Hsp90 sensitivity (Williams CR, etal, AACR05, Abs. 616).

Hsp90 inhibition was evaluated in Akt-expressing multiple myeloma cell lines. Inhibition of Akt kinase, a mediator of tumor proliferation, leads to induction of apoptosis in multiple myeloma. Because Hsp90 is involved in the refolding of proteins destabilized by stress, including Akt, Hsp90 inhibitors exhibit *in vitro* and *in vivo* activity in multiple myeloma. Therefore, combining agents that target both of the Hsp90 and Akt dysregulated pathways in multiple myeloma, interacting at the level of Akt, may result in a synergistic cytotoxic activity. Multiple myeloma cell lines OPM2 with high level of Akt activity and multiple dexamethasone-sensitive MM.1S, dexamethasone-resistant MM.1R, and plasma cell leukemia cell line OPM1 with lower Akt activity, were exposed to serial dilutions of Akt

inhibitor perifosine (KRX-0401; Keryx) and KOS-1022, alone and in combination. Perifosine induced dose-dependent inhibition of proliferation in all cell lines tested, inducing 49% inhibition compared to control and 60% inhibition in MM.1S cells. Perifosine induced more significant apoptosis in OPM2 cells, with 51% apoptosis as compared to 14.7% in MM.1S cells. Dose-dependent inhibition of proliferation and induction of apoptosis was seen with KOS-1022 in all cell lines tested inducing 40% inhibition as compared to control and 56% inhibition in MM.1S. There was no differential response to KOS-1022 in cell lines tested. Combination of perifosine and KOS-1022 resulted in a significant 76% inhibition of proliferation. Apoptosis induction rose from 13.9% with perifosine alone and 3.1% with KOS-1022 alone, to 47.9% with the combination of the two agents. Based on these results, targeting both the PI3kinase pathway and the Hsp response may constitute an attractive treatment approach for relapsed/refractory multiple myeloma where drug resistance is often a major problem. Furthermore, the differential activity noted among higher Akt activity and lower Akt activity raises the possibility of tailoring therapy based on Akt expression levels (Huston A, etal, ASH05, Abs. 1592).

IV KOS-1022 is currently being evaluated in a company-sponsored phase I clinical trial in patients with hematologic malignancies as well as in multiple phase I clinical trials in patients with advanced solid tumors sponsored by the NCI under a Cooperative Research and Development Agreement (CRADA) between Kosan and the NCI Cancer Therapy Evaluation Program (CTEP). Because of the complementary nature of oral and IV KOS-1022, Kosan plans to simultaneously develop both formulations.

Two phase I clinical trials are ongoing, evaluating IV KOS-1022 in patients with advanced solid tumors or lymphoma using different dosing schedules. A phase I clinical trial (protocol ID: CDR0000377488; NCI-04-C-0218; NCI-6544; 040218; NCT00086008), initiated in June 2004 and sponsored by the NCI, is determining MTD, DLT, and toxicity profile of KOS-1022 administered to patients with metastatic or inoperable solid tumors or advanced lymphoma for which standard curative or palliative measures do not exist. Secondary objectives include characterizing effects of KOS-1022 on client proteins, estimating drug dose at which some client protein effects are noted in a majority of patients, and evaluating expression of CAIR-1/BAG-3, a putative geldanamycin resistance protein, and its relationship to client protein degradation. KOS-1022 is infused IV for 1 hour, twice weekly, for 4 weeks, unless precluded by excessive toxicity. Treatment may continue beyond 4 weeks in responding patients. Patient response to the drug is evaluated 8 weeks post treatment. Target trial enrollment is 40 patients.

Another phase I clinical trial (protocol ID: CDR0000378189; PCI-03-153; NCI-6548; PCI-IRB-0404083; NCT00089271), sponsored by the NCI, was initiated in July 2004 to determine MTD, effectiveness, safety, and toxicity of KOS-1022 in patients with metastatic or inoperable solid tumors or lymphoma. In this trial, patients are administered IV KOS-1022 over 1 hour on days 1 to 3, or 1 to 5, with courses repeating every 21 days in absence of disease progression or unacceptable toxicity. Cohorts of 1 to 2 patients are treated with accelerated escalating doses of KOS-1022 until at least 1 of 2 experience DLT. Cohorts are then expanded to 3 to 6 patients, administered escalating doses until MTD is determined. A total of 3 to 60 patients will be accrued for this trial being conducted at Hillman Cancer Center at the University of Pittsburgh Cancer Institute (Pittsburgh, PA), with Ramesh K. Ramanathan, MD as Trial Chair. Additional trial sites include the Ireland Cancer Center, and Mayo Clinic.

A phase I clinical trial (protocol ID: CDR0000378288; MSKCC-04053; NCI-6542; NCT00089362), initiated in July 2004 at MSKCC, under PI David B. Solit, MD, is slated to determine MTD, toxicity, and effectiveness of KOS-1022 in patients with metastatic or inoperable solid tumors. Secondary objectives include assessment of any effect of this drug on expression of Hsp90 client proteins. Patients are administered KOS-1022 IV over 1 hour on days 1, 8, and 15, with courses repeating every 28 days in absence of disease progression or unacceptable toxicity. A total of 3 to 30 patients will be accrued for this trial.

A multicenter, open label, dose-escalation, phase I/II clinical trial (protocol ID: N0258156412; CDR0000442402; ICR-PH1/102; NCI-6547; NCT00248521) was initiated in January 2005, at Royal Marsden NHS Foundation Trust, under PI Ian R. Judson, MD, in patients with inoperable, metastatic solid tumors. Primary objectives are to determine MTD, DLT, recommended phase II dose, and assess the feasibility, safety, and toxicity profile of KOS-1022 in this setting. Secondary objectives are to determine PK, and tumor response. According to the protocol, patients are treated with KOS-1022 IV over 1 hour on days 1, 8, 15, and 22. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Cohorts of 3 to 6 patients are treated with escalating doses of KOS-1022 until MTD is determined, and then an additional 10 patients are treated at MTD. After completion of trial treatment, patients are followed for 28 days. Approximately 25 to 35 patients will be accrued for this trial. Participating institutions include Belfast City Hospital in Northern Ireland.

In June 2005, Kosan Biosciences initiated a company-sponsored, multicenter, dose-escalation, phase I clinical trial of KOS-1022 in patients with advanced hematologic malignancies. The rationale for testing KOS-1022 in hematologic malignancies is particularly compelling, because in preclinical tests KOS-1022 reduced the levels of several client proteins, such as FLT-3, Akt, and Bcr-Abl, which are important targets in leukemia. This phase I clinical trial will assess the safety, PK, and pharmacodynamics of escalating doses of KOS-1022 in patients with AML, ALL, or CML. According to the phase I trial design, KOS-1022 is administered as a 1-hour infusion on a twice weekly schedule, every 3 weeks. In addition, pharmacodynamics

of KOS-1022 in leukemic blast cells will be investigated in order to determine the biologic activity of KOS-1022 against client proteins critical to the progression of leukemia.

A multicenter phase I clinical trial was initiated in January 2006, with an oral formulation of KOS-1022 at the University of Colorado Health Science Center and Presbyterian Hospital Medical Center at the University of Pennsylvania (Philadelphia, PA). KOS-1022 is the first Hsp90 inhibitor to be administered orally to patients with cancer. This trial is evaluating safety, PK, pharmacodynamics, and bioavailability of escalated doses of KOS-1022 in patients with advanced solid tumors, as well as assessing any preliminary evidence of antitumor activity.

Other Targeted Therapeutics

AGS-PSCA, under development by Agensys (Santa Monica, CA), is a fully human high affinity, IgG1κ MAb targeting prostate stem cell antigen (PSCA). AGS-PSCA was generated using XenoMouse technology through a licensing agreement with Abgenix (Freemont, CA). Generation, development, and manufacturing of AGS-PSCA were carried out at Agensys.

AGS-PSCA silences expression of PSCA, a glycosyl phosphotidylinositol anchored cell-surface antigen that is expressed in approximately 80% of prostate, pancreatic and bladder tumors. PSCA regulates cell motility, migration and proliferation. AGS-PSCA appears to affect both tumor cell growth and migration, leading to a significant impact on all clinically relevant endpoints in xenograft models, including tumor growth, metastasis formation, overall health, and prolongation of survival. In addition to AGS-PSCA's significant efficacy when administrated as monotherapy, enhanced activity is observed when it is combined with other anticancer drugs.

AGS-PSCA has a half-life of approximately 7 days in SCID mice where it has been shown to significantly inhibit growth of SC established LAPC9 androgen-independent tumors, and LAPC9 androgen-dependent tumors implanted orthopically, resulting in an increased lifespan. In pancreatic xenograft models, AGS-PSCA significantly inhibited primary tumor growth, local invasion and metastases to distant organs. Enhanced antitumor activity of AGS-PSCA was demonstrated when the antibody was administered with docetaxel or gemcitabine in the prostate and pancreatic tumor models, respectively. According to preclinical toxicology and PK *in vivo*, all administered antibody doses were well tolerated and the drug had a long half-life in serum. The safety profile together with strong preclinical efficacy data provide the rationale for developing AGS-PSCA as monotherapy and in combination with chemotherapeutic agents for treating relevant malignancies (Gudas JM, etal, AACR-NCI-EORTC05, Abs. A53 and Gudas JM, etal, AACR05, Abs. 695).

In November 2005, Agensys initiated an open label, dose escalation, phase I clinical trial (protocol ID: J0554) of AGS-PSCA in patients with advanced prostate cancer, being conducted at MSKCC and Kimmel Cancer Center at Johns Hopkins, under PI Michael Carducci, MD. The trial is designed to evaluate safety and PK profile of a multiple dose regimen of AGS-PSCA. Up to 24 patients with hormone-refractory prostate cancer (HRPC) will be enrolled and treated with a single infusion of AGS-PSCA, every 3 weeks during weeks 1, 4, 7, and 10. Following the fourth infusion, during weeks 12 through 14, scans are being performed to determine tumor status. Responders may be candidates for extended therapy.

AZD6244 (ARRY-142886), under development by AstraZeneca, in collaboration with Array BioPharma, is a non-ATP-competitive, selective, orally active inhibitor of MAPK/Erk/kinase (MEK), a critical cellular hyperproliferation pathway in human cancer cells. AZD6244 is a highly potent and selective noncompetitive inhibitor of MEK1/2. In preclinical studies, efficacy of this agent was demonstrated in cellular and *in vivo* tumor models of melanoma and pancreatic, colon, lung, and breast cancer. No significant adverse effects were observed in early toxicity testing.

AZD6244 belongs to a new class of ATP noncompetitive MEK1/2 inhibitors, including 4-(4-bromo-2-fluorophenylamino)-1-methyl-pyridin-2(1H)ones. These agents are highly selective potent inhibitors of MEK with cellular IC_{50} as low as 2 nM in mechanistic assays, and 11 nM in functional assays. This class of MEK inhibitors exhibit favorable characteristics such as low hepatic clearance, high permeability, and excellent bioavailability in rodents (Wallace EM, etal, AACR-NCI-EORTC05, Abs. B77).

To investigate *in vivo* potential mechanisms of AZD6244 on inhibition of tumor growth, and correlate these with changes in PK and pharmacodynamic biomarkers, 4 human tumor xenografts with different responses to AZD6244 in mice, Colo205 (mutant B-raf; tumor regression), Calu-6 (mutant K-ras; complete growth suppression), SW620 (mutant K-ras; growth delay) and PC3 (insensitive) were selected and treated with a single dose of AZD6244, in combination with ID 5-bromo-2-deoxyuridine (BrdU), a synthetic thymidine analog. Changes in tumor histology were also examined in selected xenografts.

Inhibition of Erk1/2 phosphorylation by AZD624 was rapid in all tumor types with an inverse relationship with plasma drug concentrations. Following single doses of AZD6244, an increase in cleaved caspase 3 was detected in both Colo205 and Calu-6 tumors, reaching a peak at approximately 8 hours post dosing. There was no change in BrdU incorporation in these tumors. In contrast, in SW620 tumors, induction of cleaved caspase 3 was very modest, whereas there was a clear decrease in BrdU incorporation following a single dose of AZD6244. Multiple doses of AZD6244 induced a morphology change in the SW620 model to a more differentiated phenotype. There was no change in BrdU incorporation or cleaved caspase 3 in PC3 tumors following a single dose of AZD6244. Therefore, treatment with AZD6244 can be antiproliferative, proapoptotic, and may induce differentiation depending

on the tumor type (Logie A, etal, AACR-NCI-EORTC05, Abs. A240).

When PK activity AZD6244 was assessed *in vitro*, alone and in combination with gefitinib, an ATP-competitive inhibitor of EGFr, synergy was observed with the combination in cell lines sensitive to either drug alone, but no activity was detected in cell lines that are insensitive to either agent alone. This combination was also evaluated *in vivo* in LoVo human colorectal tumor xenografts that carry a mutant K-ras allele (Gly13Asp). In this model, when gefitinib was administered daily, or AZD6244 twice daily, as monotherapies, tumor growth was inhibited by 40% and 35%, respectively; when combined, tumor growth was inhibited by 75%. *Ex vivo* tissue from these xenografts confirmed that the primary pharmacologic activities of gefitinib and AZD6244 are inhibition of EGFr and ErK1/2 phosphorylation, as was observed in tumors treated with the combination regimen (Cockerill M, etal, AACR-NCI-EORTC05, Abs. A110).

In preclinical studies, in murine xenograft models, AZD6244 inhibited tumor growth the HT29 human colon carcinoma model, as well as in other xenograft models including MIA PaCa2, A549, Colon26, PANC-1, LoVo, Calu-6, HCT116, MDA-MB-231, ZR-75-1 and LOX (Lee P, etal, AACR04, Abs. 3890). AZD6244 also prevented elevated basal phosphorylation of Erk in various human carcinoma cell lines expressing mutant Ras or B-Raf, including HT-29, Malme-3M, SK-MEL-28, BxPC3, and MIA PaCa2, and prevented EGF-induced Erk phosphorylation in A431 cells and TPA-induced Erk phosphorylation in human whole blood. AZD6244 did not prevent phosphorylation of MEK1/2. Rather, cells exposed to this drug went into G1/S cell-cycle arrest, resulting in inhibition of cell proliferation, and in some cell lines, induction of apoptosis. ARRY-142886 was highly potent in inhibiting proliferation of HT-29, Malme-3M, SK-MEL-28, MIA PaCa2, and SK-MEL-2 cells. AZD6244 induced apoptosis of Malme-3M cells and SK-MEL-2 cells, both melanoma cell lines, while cell viability of the normal counterpart Malme-3 cells was not affected. Therefore, AZD6244 is not a general growth inhibitor but specifically targets cells with activated MEK, which arises from either Ras or B-Raf mutations (Yeh T, etal, AACR04, Abs. 3889).

In June 2004, Array BioPharma initiated an open label, dose-escalation, phase I clinical trial (protocol ID: ARRY-0401, NCT00085787), at the University of Colorado Cancer Center, Anschutz Cancer Center (Aurora, CO), Mayo Clinic, and Fox Chase Cancer Center, designed to establish MTD, safety, PK profile, and biologic activity of AZD6244 following daily oral administration in patients with advanced cancer. In addition, the trial is designed to examine patients for indications of biologic activity using tumor biomarkers and pharmacodynamic measurements. The phase Ia portion of this trial was completed and the phase Ib portion was ongoing as of January 2006. Phase Ib is assessing a constellation of biomarkers in tumor tissue and normal tissue from patients with melanoma and other malignancies. In this trial, 23 patients with refractory solid tumors were enrolled and dosed with PO AZD6244, twice daily, for 28-day cycles. A total of 50 cycles (median cycles per patient=2) were administered at twice daily doses ranging from 50 mg to 300 mg. The most common adverse events included rash, fatigue, edema, hypoxia, increased LFT, and diarrhea. DLT included hypoxia (n=1) at the 200 mg twice daily dose level, and diarrhea and skin rash (n=1) and skin rash (n=1) at the 300 mg twice daily dose. MTD was established at 200 mg twice daily. Tmax of a single dose of AZD6244 was 1.3 to 2.6 hours, t1/2 was 7 to 12 hours, and plasma concentration exposure increased with increasing dose. The best clinical response was stable disease, with 4 patients (3/7 with melanoma and 1/1 with nsclc) remaining in the trial for >4 cycles, and 2 patients with melanoma for 8 and 10 cycles, respectively (Chow LQM, etal, AACR-NCI-EORTC05, Abs. C162).

GX15-070 (obatoclax), under development by Gemin X Biotechnologies (Montreal, Canada), is a small molecule inhibitor of the BH3-binding groove of the Bcl-2 family of proteins that are frequently overexpressed in malignancy. Gemin X has identified a cellular protein, Bap31, which is a Bcl-2 regulated component of the apoptosis pathway. Bap31 is a component of a Bcl-2/Bcl-XL-regulated caspase activation complex at the endoplasmic reticulum. Bap31 plays an important role in the activation of caspases, and undergoes detectable changes in structure in response to internal apoptotic signals, including oncogenic stress. Modulation of Bap31 confers chemoresistance by affecting apoptosis independent of p53 that is often mutated or lost in drug-resistant malignancies. When Bcl-2 is present within the complex, apoptosis triggered by activated oncogenes such as E1A is impaired. However, if the Bcl-2/Bap31 complex is disrupted by Bcl-2 inhibitors, apoptosis is reinstated and the cancer cell dies. Gemin X has developed several assays, using Bcl-2 family proteins and Bap 31, to identify drugs that affect the Bcl-2 checkpoint resulting in apoptosis.

GX15, a naturally occurring substance that is cytotoxic in several cancer cell lines, was identified by screening libraries of small molecule compounds that may improve efficacy of standard chemotherapy by influencing Bap31. Structure-activity-relationship (SAR) studies were conducted by preparing semisynthetic derivatives from the natural product and by total synthesis. The resulting GX15 family of small molecule compounds, including GX15-003 (GX015-003) and GX15-070 (GX015-070), effectively block the action of Bcl-2 by inhibiting Bap31, thus reinstating apoptosis in cancer cells. Also, GX15-070 administered as a single agent inhibits tumor growth in mouse models of melanoma as well as of breast, cervical, prostate, and colon cancer.

GX15-070MS induced apoptosis in CLL B cells in a time and dose-dependent manner, independent of IgVH gene mutational status or ZAP-70 expression. CLL B cells

express high levels of antiapoptotic proteins of the Bcl-2 family, such as Mcl-1, Bcl-xL, and Bcl-2, which enhance resistance of CLL cells to spontaneous and/or drug-induced apoptosis primarily by interacting with, and antagonizing the activity of mitochondria membrane proapoptotic proteins such as Bax and Bik. GX15-070MS inhibits Bcl-2 family member proteins, inducing apoptosis in CLL B cells both *in vitro* and *in vivo* at dose ranges that are substantially below those estimated to induce toxicity in preclinical animal models (Castro JE, etal, ASCO05, Abs. 3167).

In a preclinical study, GX15-070 induced apoptosis *in vitro* in primary cells from patients with MCL as well as in MCL cells lines. Susceptibility of MCL cells correlated with expression of Bcl-2, Mcl-1, and Bcl-xL, both at the protein and mRNA levels. When evaluated in MCL, in combination with bortezomib, preincubation of MCL cells with GX15-070 before bortezomib addition induced a synergistic cytotoxic effect. This combination reduced Mcl-1 levels, displaced Bak from Mcl-1 sequestration, and reduced the bortezomib dose by 5- to 20-fold *in vitro*. Upregulation of proapoptotic BH3-only protein Noxa by bortezomib was further increased when this drug was combined with GX15-070. Findings suggest that the combination of GX15-070 and bortezomib may represent new therapeutic approach for treatment of MCL (Galan PP, etal, ASH05, Abs. 1490).

GX15-070 potently induces apoptosis in myeloid leukemia cells via disruption of Mcl-1/Bak dimerization, and activation of the intrinsic apoptotic cascade. GX15-070 inhibited cell growth of HL-60, U937, OCI-AML3, and KG-1 cell lines. Overexpression of Bcl-2 or Bcl-xL did not confer resistance to GX15-070. MDR-overexpressing HL-60/Dox cells were similarly sensitive compared to their non-MDR counterpart, and specific MDR inhibitor PSC 833 did not enhance GX15-070-induced apoptosis. GX15-070 inhibited Mcl-1/Bak heterodimerization in HL-60 cells but did not affect Bax/Bcl-2 heterodimerization. This was associated with cytosolic release of cytochrome c, followed by an increase in annexin positivity, and a decrease in mitochondrial inner membrane potential with ~64% of cells losing membrane potential at 72 hours. GX15-070 did not affect cell-cycle distribution of HL-60 cells. GX15-070 induced apoptosis in CD34+ progenitor cells in 6/7 primary AML samples (Contractor R, etal, ASH05, Abs. 3372).

In a preclinical study, GX15-070 was tested against the MMRC 12 validation cell line panel consisting of 12 standardized and annotated human myeloma cell lines (HMCL); expression of Bcl-2, Mcl-1, and/or Bcl-xL was confirmed in 8 of these HMCL. Basal levels of Bcl-2 and Mcl-1 were relatively similar across all HMCL evaluated, whereas levels of Bcl-xL were variable. GX15-070 inhibited viability of all 12 myeloma cell lines; sensitivity did not correlate with level of Bcl-2, Mcl-1 or Bcl-xL expression. Cytotoxicity to normal human blood lymphocytes, stroma, and bone marrow colony-forming units (CFU) was not observed at concentrations effectively cytotoxic to HMCL. In time-course experiments, apoptosis and cleavage of caspase 3 began by 12 hours and continued to increase over a 96-hour period. Co-culture with human bone marrow stroma cells (BMSC) failed to protect HMCL from GX15-070-induced cytotoxicity. Potent multiple myeloma growth factors, IL-6 and IGF-1 did not confer resistance to GX15-070. Although overexpression of Bcl-2 attenuates PS-341-induced cell death, combination of PS-341 and GX15-070 did not reveal synergistic interaction in preliminary tests (Stewart A, etal, ASH05, Abs. 1572).

An open label, dose-escalation, phase I clinical trial (protocol ID: 2004-139) with GX15-070, was initiated in November 2004, at the Lombardi Comprehensive Cancer Center, under PI John Marshall, MD. This trial, the first involving a small molecule inhibitor of Bcl-2, is designed to evaluate safety and tolerability of multiple doses of GX15-070 in patients with solid tumors. The protocol will include extensive PK sampling and pharmacodynamic evaluation. As reported in May 2005, among 5 treated patients, disease progressed in 3 after 2 cycles, and stabilized in 1 at 6 weeks; 1 potential responder was not yet evaluable (McGreivy JS, etal, ASCO05, Abs. 3180).

An open label, dose-escalation, phase I/II clinical trial (protocol ID: 04-137) was initiated in January 2005, at the University of California San Diego (UCSD), to administer multiple doses of GX15-070 to patients with CLL. GX15-070 is being administered once every 3 weeks to patients with intermediate risk or high risk disease, refractory to alkylating drugs, fludarabine-based regimens, and, in some cases also alemtuzumab (Campath; Schering). Administration of GX15-070 has been well tolerated; there was no Grade 3/4 toxicity.

According to interim results, reported in December 2005, from the phase I/II clinical trial (protocol ID: 04-137) of GX15-070, in patients with CLL, the drug was generally well tolerated, was biologically active, and resulted in clinical improvement. In this trial, 12 patients were treated with doses ranging from 3.5 mg/m^2 to 14 mg/m^2 using a 1-hour infusion, and 3 patients at 20 mg/m^2 using a 3-hour infusion. Single intrapatient dose escalation was allowed. Participating institutions include MDACC, UCSD, Princess Margaret Hospital (Toronto, Canada, and Georgetown University Hospital.

The most frequent adverse events were somnolence Grade 1 (70%) or 2 (5%) and euphoria Grade 1 (65%) or 2 (15%), occurring during or shortly following drug infusion; there was no correlation with dose level or frequency. Other adverse events reported in ≥25% of patients were transient O2 desaturation (31%), AST increase (30%), and fatigue (25%). There have been no DLT to date. According to PK analysis t1/2 was 39.0 hours. Pharmacodynamic activity has been noted across all dose levels. There was a reduction of peripheral lymphocyte counts (12% to 79%) in 6/7 patients with lymphocytosis at baseline. Induction of apoptosis was monitored quantitatively with serial determinations of plasma concentration of histone-oligonucleo-

somal DNA (ODNA) complexes. An early release of ODNA occurred 1 to 6 hours after start of infusion. A secondary increase peaked with a noticeable lag time from peak plasma GX15-070 concentration, 24 to 168 hours after start of infusion. Best clinical responses include an unconfirmed PR in 1/15 patient, and SD lasting ≥6 weeks in 7/15. Among 12 patients with a baseline platelet count <150,000 mm^3, 4 sustained elevations of platelet counts by ≥50%, including 2 patients improving from 70,000 mm^3 to 144,000 mm^3, and from 47,000 mm^3 to 105,000 mm^3. Also, hemoglobin (Hb) levels in 2/4 patients who were anemic at baseline were elevated from 8.7 to 11.3 g/dl, and from 7.9 to 13.9 g/dl in a patient who was transfusion dependent. Single agent GX15-070 has dose-dependent biologic activity in patients with previously treated CLL with documented induction of apoptosis and improvement in hematologic parameters at well tolerated doses (O'Brien S, etal, ASH05, Abs. 446).

In updated results from this trial, a total of 25 patients with Stage I/IV CLL were enrolled, who had failed a median of 4 prior types of treatment. Of these, 12 patients were treated with GX15-070 at doses of 3.5-14 mg/m^2 administered as a 1-hour infusion, and 13 patients at 20 to 40 mg/m^2 via a 3-hour infusion. To date, there is 1 PR and 9 SD. Up to 4/14 patients with low platelet counts experienced sustained increases of more than 50% after treatment, while 3/11 anemic patients showed sustained hemoglobin elevations, with 2 achieving transfusion independence. DLT consisted of somnolence, ataxia, and dysphoria, which were rapidly reversed. MTD using a 3-hour infusion schedule, once every 3 weeks, was 28 mg/m^2. Pharmacodynamic activity was frequently noted, with apoptosis evidenced by an increase in ODNA levels with peak levels correlating with dose and AUC. Additionally, 18/25 patients showed an average reduction of 29% in peripheral lymphocyte counts.

PD0325901, under development by Pfizer, is a significantly more potent analog of CI-1040 with an improved pharmaceutical profile that is noncompetitive with ATP and exquisitely specific for MEK and, particularly MEK1. The structural basis for the activity of CI-1040-like MEK inhibitors was elucidated by determination of the *de novo* crystal structure of human MEK1. In contrast to all other known protein kinase-inhibitor co-complex structures, CI-1040-like MEK1 inhibitors achieve exquisite selectivity and potency by virtue of their interaction with both the protein and ATP to lock the enzyme in an inactive conformation.

PD0325901 is roughly 500-fold more potent than CI-1040 with respect to its cellular effects on phosphorylation of Erk1 and Erk2. *In vivo*, a single oral dose of PD0325901 suppressed phosphorylation of Erk by >50% at 24 hours post dosing. In comparison, CI-1040 at a much higher dose could only inhibit pErk levels for roughly 8 hours, returning to control levels by 24 hours after treatment. Efficacy comparisons revealed that the dose required to produce a 70% incidence of complete tumor responses (C26 model) was 25 mg/kg/day for PD0325901 compared to 900 mg/kg/day for CI-1040. This improved anticancer activity of PD0325901 compared to CI-1040, may be attributable to several pharmacologic factors, including not only a longer duration of MEK inhibition, but also improved solubility, improved bioavailability, and greater potency of MEK inhibition. In addition, PD0325901 exhibits improved metabolic stability compared to CI-1040. The 40-fold higher concentration of the carboxylic acid metabolite relative to parent compound that was observed in patients treated with CI-1040 in a phase 1 trial, may have prevented sufficient drug exposure (Sebolt-Leopold JS, etal, AACR-NCI-EORTC03, Plenary Session).

PD0325901 treatment of mice bearing established melanoma and breast cancer xenografts resulted in complete inhibition of SkMel28 (V599E B-Raf, MEK inhibitor sensitive) tumor growth for the duration of treatment. Mutational activation of N-Ras or B-Raf occurs in a significant proportion of human melanoma, and the mutual exclusivity of the mutations suggests that constitutive activation of the Ras MAPK pathway is a key event in tumor development. Treatment with PD0325901 was well tolerated with no treatment-induced deaths or weight loss. Growth inhibition was accompanied by downregulation of P-MAPK and D-cyclins, Rb hypophosphorylation, and a decline in proliferative index as determined by Ki67 staining. Although discontinuation of therapy was followed by tumor regrowth within two weeks, tumors retained sensitivity to PD0325901 on rechallenge. No significant increase in the apoptotic index was observed but tumor regression and CR were observed following prolonged treatment.

Consistent with *in vitro* data, SkMel31 tumors (wt Ras/Raf) were less sensitive to PD0325901, with treatment resulting in a dose-dependent tumor growth delay but not complete growth inhibition. Furthermore, BT-474 xenograft tumors (wt Ras/Raf, HEr2 amplified in breast cancer, MEK inhibitor resistant) were completely refractory to this agent. PD0325901 caused inactivation of MAPK in the BT-474 tumors but little change in cyclin D1 expression, no hypophosphorylation of Rb, and no change in proliferative index. These findings suggest that in tumors with activating B-Raf mutation, growth is dependent on MEK-MAP kinase, whereas in breast tumors with wt Ras and Raf, tumor growth is independent of the MEK-MAP kinase pathway. This data provide a rationale for clinical trials of PD0325901 in patients whose tumors harbor activating B-Raf mutations and suggest that clinical trials of such agents in tumor types with a low frequency of B-Raf mutation be enriched for patients with activation of this pathway (Pratilas C, etal, AACR05, Abs. 5285).

Investigators at MSKCC used FLT (3'-deoxy-3'-(18)F-fluorothymidine), a novel PET tracer that is preferentially taken up by dividing cells, to assess if changes in FLT uptake can be employed as a marker of antiproliferative activity. Tumor cells with B-Raf mutations demonstrate

enhanced and selective sensitivity to inhibitors of MEK. This MEK dependency was observed in B-raf mutant cells regardless of tissue lineage, and correlated with both downregulation of cyclin D1 protein expression and induction of G1 arrest. Because MEK inhibition potently induces a G1 arrest in sensitive but not in resistant tumors, FLT may represent an ideal noninvasive early marker of activity for this class of agents. To test this approach, mice with established SKMEL28 and BT-474 xenografts were randomized to treatment with PD0325901 or vehicle only as control. Mice were imaged with both FLT and FDG PET pretreatment and then weekly thereafter. In vehicle-treated mice, an increase in FLT and FDG uptake was observed over time, which paralleled continued tumor growth. However, in SKMEL28-bearing mice, FLT uptake in the tumor declined sharply after one week of treatment with PD0325901; this decline in FLT uptake was accompanied by downregulation of pErk and Ki67 in tumors. The change in FDG uptake in PD0325901-treated mice was less pronounced and more delayed paralleling the gradual regression of the tumor. In contrast, while PD0325901 potently downregulated Erk activity in BT-474 tumors, treatment had no effect on tumor growth, FLT or FDG uptake in this PD0325901-resistant tumor. These data support use of FLT PET as a predictive marker of response in clinical trials of agents that inhibit MEK kinase (Solit DB, etal, AACR-NCI-EORTC05, Abs. C274).

A multicenter (n=19), open label, phase I/II clinical trial (protocol ID: A4581001; NCT00147550) of oral PD0325901, in patients with advanced solid tumors including breast and colorectal cancer, nsclc or melanoma, was initiated in February 2004 to determine this drug's safety profile including DLT, and determine the objective response rate. Secondary objectives include TTP, OS, and QoL. Patients are treated with oral PD0325901 once daily for 21 consecutive days followed by a 1-week holiday. Treatment cycles repeat every 28 days. Adult patients eligible for enrollment must have tumor accessible for biopsy and be willing to undergo baseline and post treatment biopsy. One of the centers participating in this trial is the Ireland Cancer Center. Total expected enrollment in the complete phase I/II trial is 310 patients.

PRO1762, TNF-related apoptosis-inducing ligand (TRAIL)/APO2L, under development by Genentech (South San Francisco, CA), is a soluble type II transmembrane protein detected in most tissues that binds to at least 4 distinct receptors found on many tumor cells, and signals these cells to enter apoptosis. PRO1762 selectively induces apoptosis in many types of tumor cells, but not in most normal cells, by activating the apoptotic caspase cascade independently of the p53 tumor suppressor gene (Ashkenazi A, EORTC-NCI-AACR04, Abs. 462). Apo2L/TRAIL triggers apoptosis through death receptors 4 (DR4) and 5 (DR5).

PRO176 may be effective as second line treatment of tumors that have acquired resistance to conventional therapy, and for augmenting the efficacy of current first line treatments in several types of cancer. Although the majority of thoracic cancer cells express adequate levels of functional TRAIL receptors DR4/DR5, they frequently exhibit resistance to the cytotoxic effect of this ligand. Chemotherapeutic agents have been shown to sensitize refractory cancer cells to TRAIL. Investigators at the NCI treated DR4/DR5-positive cultured cancer cells of the lung (H322, H460), esophagus (TE2, TE12), and pleura (H513, H211) with either Apo2L/TRAIL alone or in combination with paclitaxel followed by Apo2L/TRAIL. Paclitaxel treatment, while having no effect on baseline DR4/DR5 expression, significantly potentiated the cytotoxic effect of Apo2L/TRAIL as indicated by 5- to 20-fold reduction of the TRAIL IC50 values. Paclitaxel-mediated enhancement of Apo2L/TRAIL sensitivity was brief and only observed within 6 hours following drug treatment. While either Apo2L/TRAIL alone or paclitaxel alone induced only 10% to 20% cell death, 70% to 90% of cancer cells treated with the combinations underwent apoptosis 48 hours after the onset of drug exposure. Enhanced cytotoxicity and apoptosis of thoracic cancer cells by the sequential paclitaxel-Apo2L/TRAIL treatment were almost completely abrogated either by Bcl-2 overexpression or by the selective caspase 9 inhibitor (LEDH-fmk) stressing the essential role of the mitochondrial apoptosis-inducing pathway. Moreover, significant increase of caspase 8 (2- to 3-fold) or of caspase 9 (1.5- to 2.5-fold) activity following combination, but not individual pacitaxel or Apo2L/TRAIL exposure, was completely inhibited by Bcl-2 overexpression, implying that robust activation of the apical caspase 8 and the caspase cascade was mediated by the mitochondria-dependent positive feedback loop. Paclitaxel, at clinically achievable conditions, synergizes with Apo2L/TRAIL in a schedule-dependent fashion to induce profound apoptosis of cultured thoracic cancer cells via a process that requires the intrinsic mitochondria-dependent death signaling cascade. These findings provide rationale for development of TRAIL-based molecular therapy in combination with paclitaxel for enhanced cytotoxic efficacy for thoracic malignancies (Ziauddin MF, etal, AACR05, Abs. 5340).

As of February 2006, a phase I clinical trial was to be initiated at the University of Colorado Comprehensive Cancer Center, to investigate PRO1762 in patients with advanced solid tumors, including colorectal, ovarian, and breast cancer, GBM, melanoma, and lymphoma. The trial is being conducted in two stages. In stage 1, PRO1762 is infused at doses of 1 to 20 mg/kg on day 1 of cycle 1 every 28 days, and then on day 1 of a 14-day cycle for cycles 2 to 8. In stage 2, the drug is administered on day 1 of a 14-day cycle for cycles 1 to 8.

An open label, dose-escalation phase I clinical trial, initiated in December 2005 at MSKCC, under PI Roy Herbst, MD, PhD, is investigating the safety and PK of IV PRO1762 in patients with advanced or metastatic solid tumors, or NHL. The objectives of the trial are to determine the safety, and tolerability of multiple doses of PRO1762 adminis-

tered IV over 1 hour, establish MTD, characterize the the drug's PK, determine the relationship between the formation of antibodies to PRO1762 and treatment-emergent hepatotoxicity, preliminarily assess the drug's efficacy, and identify any biologic markers that may act as an indicator of PRO1762 activity. Expected enrollment is 65 patients.

SPC2996, under development by Santaris Pharma (Hørsholm, Denmark), acts by inhibiting the synthesis of Bcl-2. SPC2996, a new chemical entity, is composed of a short single chain of nucleotides including locked nucleic acids (LNA), a conformational analog of RNA, which confers very high specific RNA binding activity. SPC2996 was selected from a small library of LNA versions of Genasense (Genta) on the basis of biochemical and functional assays *in vitro* and in cell cultures. SPC2996 exhibits superior activity to Genasense *in vitro* with respect to downregulation of Bcl-2 mRNA and protein. Cells treated with the compound also show strong induction of apoptosis and inhibition of proliferation. In *in vivo* testing in mouse models of cancer, conducted in collaboration with the University of Vienna, in Austria, SPC2996 exhibited promising tumor growth inhibition both as a single agent and in combination with chemotherapy. SPC2996 is particularly effective in CLL, because Bcl-2 is highly overexpressed in CLL cells and appears to play a role in pathogenesis of the disease.

SPC2996 completed preclinical toxicology studies in rodents and monkeys. No clinical, hematologic, biochemical or pathologic adverse effects were observed at clinical relevant doses in either species. Notably, even very high plasma levels of the drug did not produce clinical signs of the acute toxicities reported for phosphorothioate oligonucleotides, such as dose-dependent prolongation of aPTT, and complement activation.

SPC2996 downregulated Bcl-2 in cellular studies and inhibited growth of human xenotransplanted tumor cells in mice. Compared to phosphorothioates and siRNA, SPC2996 was substantially more resistant to nuclease degradation in plasma. In biodistribution studies in rodents, SPC2996 was distributed to many tissues including the bone marrow. IV SPC2996 was safe and downregulated Bcl-2 in cynomolgus primates (Westergaard M, etal, AACR-NCI-EORTC05, Abs. A41).

In June 2005, a multicenter (n=9), international, open label, dose-escalation, phase I/II clinical trial (protocol SPC2996-101; NCT00285103) of SPC2996 in the treatment of CLL, was initiated in Denmark, the UK, and France. Professor Bertrand Coiffier, Centre Hospitalier Lyon Sud, in France, is the trial's international PI. This phase I/II clinical trial will evaluate safety and efficacy of repeated doses of SPC2996 in patients with CLL refractory to chemotherapy. Primary objective is to detect changed in Bcl-2 mRNA levels from day 0 to day 13. Secondary objectives are to detect changes in Bcl-2 protein expression from day 0 to day 14, changes in Bcl-2 mRNA levels during the trial, tumor responses, and CD5+CD20+ cells in peripheral blood, and to determine TTP, time-to-response, duration of response, time to next anti-CLL therapy, PK, adverse events, MTD, biochemistry, hematology, values of complement, T-cell, normal B-cell, and NK-cell values, and ECG parameters. In November 2005, the FDA also approved an IND for this trial in the USA, adding another center, Holden Comprehensive Cancer Center, at the University of Iowa (Iowa City, IA), under PI James Wooldridge, MD. Expected total enrollment in this trial is 45 patients.

IMMUNOTHERAPY/VACCINES

The incredible commercial success of rituximab (Rituxan; Biogen Idec), has spurred development of various novel B-cell targeting immunotherapies. Among validated targets for immunotherapy of hematologic malignancies are those belonging to the tumor necrosis factor receptor (TNFr) superfamily, including CD40, Fas, transmembrane activator and calcium-modulator and cyclophilin ligand (CAML) interactor (TACI), B-lymphocyte stimulator (BlyS), etc.

One TNFr member, CD40, is a 45 kDa integral membrane glycoprotein found on the surface of B lymphocytes, thymic epithelial cells, dendritic cells (DC), and some carcinoma cells, including urinary bladder transitional carcinoma cells. The vast majority of B-cell malignancies express CD40. This receptor, along with the cognate ligand (CD154), mediates cell survival and proliferation of normal B cells and can regulate apoptosis as well as survival in transformed cells. CD40 antigen is highly expressed on most B-lineage hematologic malignancies including multiple myeloma, NHL, CLL, Hodgkin's disease, hairy cell leukemia (HCL), and ALL. Both *in vitro* and *in vivo* preclinical studies support the development of anti-CD40 immunotherapy for NHL. CD40 is also found on many types of solid tumors, including lung, gastric, ovarian and bladder cancer.

CHIR-12.12 (anti-CD40mAb), under development by Chiron, is a recombinant human MAb targeting B-cell hematologic malignancies. CHIR-12.12 is a non-internalizing anti-CD40 MAb that mediates antitumor activity by at least two mechanisms of action, by blocking CD40L-induced cytokine production mediating survival signals, and by inducing tumor cell lysis by antibody-dependent cellular cytotoxicity (ADCC). CHIR-12.12 blocks CD40L binding to CD40 and inhibits CD40L-induced proliferation and survival of normal human B cells, primary CLL cells, and primary NHL cells.

CHIR-12.12 blocks tumor stimulatory CD40/CD40L interaction and mediates ADCC even in the presence of low expression of the target antigen (Weng WK, etal, ASH04, Abs 3279). CHIR-12.12 appears to be broadly applicable in various hematologic malignancies, including multiple myeloma, CLL and lymphoma, as monotherapy, and in combination with other anticancer agents.

As shown *in vitro* in cell lines, *in vivo* in animal models, and *ex vivo* in patient cells, CHIR-12.12 binds to tumor cells that express CD40 and antagonizes CD40 ligand-mediated growth and survival of malignant B cells. CHIR-12.12 is well tolerated at a range of doses in cynomolgus monkeys. Reduction in B-cell counts was observed as early as 4 hours after the first dosing and at all subsequent time points throughout the study, while there was no change in T-cell counts (CD3+, CD4+ and CD8+ cells). No significant treatment related adverse events were seen in any organs (Ursula B, etal, ASH04, Abs. 3282).

Combination of rituximab and CHIR-12.12 in a SC xenograft model of a Burkitt's lymphoma cell line (Namalwa) resistant to rituximab, produces synergistic antitumor activity that rises from 77% to 83% with escalating doses of CHIR-12.12. *In vitro*, CHIR-12.12 mediates stronger target cell lysis than rituximab (31.43% versus 14.15%) in this cell line; combining the drugs does not enhance ADCC killing. When each drug is administered alone *in vivo*, CHIR-12.12 induces 60% tumor growth inhibition whereas rituximab does not inhibit Namalwa tumor growth (Long L, etal, ASH04, Abs. 3281).

CHIR-12.12 and rituximab were compared for their relative ADCC activity against a variety of malignant human B-cell lines expressing both CD40 and CD20 antigens, including two lymphoma cell lines (Daudi, Namalwa), two multiple myeloma cell lines (ARH77, IM-9), a B-ALL cell line (CCRF-SB), and a B-CLL cell line (EHEB). All cell lines expressed both CD20 and CD40 antigens, but the number of cell-surface CD20 molecules per cell were 2.6- to 30.8-fold higher than CD40 ones. Despite the greater number of CD20 receptors, treatment with CHIR-12.12 resulted in greater maximum cell lysis and a lower ED_{50} than rituximab in all target cell lines. ADCC activity of rituximab is known to correlate with the FcγrIIIa genotype of effector cells, resulting in greater cell lysis in the homozygous valine (V/V) or heterozygous valine/phenylalanine (V/F) polymorphisms at aa158 than in the homozygous F/F polymorphism. In Daudi lymphoma target cells and effector NK cells purified from human donors expressing all three polymorphisms, CHIR-12.12 induced potent ADCC with NK cells of all three genotypes. Also, CHIR-12.12 bound the F allele with a 4.6-fold higher affinity than rituximab. Therefore, CHIR-12.12 is a more potent ADCC mediator than rituximab, even in human NK cells of the aa158 F/F genotype (Luqman M, etal, ASH05, Abs. 1472).

Addition of CHIR-12.12 to primary CLL cells inhibited CD40L-mediated production of a variety of cytokines, including IL-10, TNF-α, IL-8, GM-CSF, IL-6, MCP-1, and MIP-1β. Cytokine production by primary CLL cells cultured with CHIR-12.12 alone in the absence of CD40L, did not exceed levels produced by CLL cells cultured in medium, suggesting that CHIR-12.12 is a potent antagonist of CD40L-mediated cytokine production by primary CLL cells and shows no agonistic activity by itself. In addition, CHIR-12.12 exhibited greater ADCC than rituximab against CLL cells from all patients tested. CHIR-12.12 was 10-fold more potent than rituximab. The greater ADCC potency and efficacy of CHIR-12.12 was not dependent on a higher density of cell surface CD40 molecules, as there were 1.3- to 14-fold higher numbers of CD20 than CD40 molecules on the cell surface. Antibody internalization studies show that upon binding to CD40 at 37°C, CHIR-12.12 remains uniformly distributed on the cell surface, even after 3 hours. In contrast, after binding at 37ºC, rituximab is redistributed into caps and internalized. These data suggest that the potent ADCC activity of CHIR-12.12 may be partly related to its ability to remain on the surface of target cells uniformly, allowing optimal interaction with effector cells (Tong X, etal, ASH05, Abs. 2964).

CHIR-12.12 has potent activity against human multiple myeloma cells *in vitro* and in xenograft models *in vivo*. CHIR-12.12 induces lysis of KMS-12-BM cells by ADCC in a dose-dependent manner. In orthotopic and SC KMS-12-BM xenograft models, CHIR-12.12 significantly prolonged MST of tumor-bearing mice in a dose-dependent manner. CHIR-12.12 significantly inhibited tumor growth in the staged SC model. Bortezomib and melphalan/prednisone did not inhibit KMS-12-BM tumor growth at the doses and schedules reported in other human multiple myeloma xenograft models. Significantly increased levels of cleaved PARP were detected in KMS-12-BM SC tumors 7 days after the initiation of CHIR-12.12 treatment, suggesting CHIR-12.12-induced cell death (Long L, etal, ASH05, Abs. 3470).

CHIR-12.12 induces a maximum specific lysis of 64% in the human multiple myeloma cell line IM-9, which expresses both CD40 and CD20, in a dose-dependent manner. In comparison, rituximab produces only a 45% maximum lysis in this cell line, which actually contains more CD20 molecules, the known target for rituximab. In an IM-9 xenograft model, IV CHIR-12.12 significantly escalated survival (from 60% to 80%) in a dose-dependent manner. CHIR-12.12 administered SC also significantly inhibits tumor growth with escalating doses from 17% to 44%; in comparison, bortezomib inhibits tumor growth by 30% (Long L, etal, ASH04, Abs. 4888).

In culture, treatment with CHIR-12.12 inhibits prolongation of survival of primary CLL cells induced by soluble human CD40L. Additionally, CHIR-12.12 treatment results in induction of cleaved caspase 3 and PARP, and reduction of XIAP, Mcl-1, and Bcl-xl expression, ultimately leading to CLL cell apoptosis (Cherukuri A, etal, ASH05, Abs. 2965).

A multicenter (n=3), open label, dose-finding, phase I clinical trial (protocol ID: C12001, NCT00108108) was initiated in April 2005, to determine MTD, safety, and activity of CHIR-12.12 in patients with relapsed or refractory CLL. Translational medicine will be used to monitor biomarkers in real time. The trial is expected to enroll up to 40 patients in the USA at Thorton Hospital and Perlman Cancer Center at UCSD (La Jolla, CA), Sidney Kimmel Cancer Center at Johns Hopkins, and MDACC.

In October 2005, a multicenter (n=4) single agent, open label, phase I clinical trial (protocol ID: C12101, NCT00231166) of CHIR-12.12 was initiated at Fred Hutchinson Cancer Research Center (Seattle, WA) in patients with relapsed previously treated multiple myeloma, to evaluate this drug's safety, tolerability, and PK. Translational medicine will be used to monitor biomarkers and allow correlation of these markers with response to CHIR-12.12 therapy, guiding dose regimen and selection of responsive patient populations. The trial is expected to enroll up to 40 patients.

SGN-40, under development by Seattle Genetics (Bothell, WA), is a high affinity, rapidly internalizing, humanized MAb targeting CD40. SGN-40 has demonstrated potent *in vitro* and *in vivo* efficacy against cell lines expressing CD40.

Currently, there are 3 open label, multidose, single arm, phase I clinical trials of SGN-40 in patients with relapsed or refractory multiple myeloma, NHL, and CLL, designed to evaluate the safety, antitumor activity and PK profile of escalating doses of SGN-40. According to the protocol, patients are treated with multiple doses of SGN-40 over 5 weeks and are then followed for at least 8 weeks. Patients who experience a clinical benefit are eligible for a second cycle of therapy.

Patients in all trials are currently being treated by an amended protocol that uses an intrapatient dose-escalating approach during the first two weeks of treatment to attenuate potential cytokine release, which was observed in several patients treated under the original protocol. Thus far, SGN-40 has been well tolerated using this strategy and dose escalation is ongoing in these trials. Generally, based on preliminary data from these phase I trials, SGN-40 is well tolerated and exhibits antitumor activity. In the NHL trial, objective responses were observed following one cycle of SGN-40 therapy.

In a phase I clinical trial, being conducted at the Cleveland Clinic Taussig Cancer Center, Institute for Myeloma & Bone Cancer Research (Los Angeles, CA), Weill Medical College of Cornell University (New York, NY), and DFCI, SGN-40 is being evaluated in patients with relapsed and/or refractory multiple myeloma. This trial is designed to evaluate safety, PK, immunogenicity, and antitumor activity, and establish MTD. The original protocol called for cohorts of patients to be treated with four weekly infusions of 0.5, 1.0, 2.0, 4.0, 8.0, or 16.0 mg/kg; 16 patients were treated at doses ranging from 0.5 to 4.0 mg/kg/week for four weeks. Enrollment of new patients was temporarily halted after 2/3 patients developed severe headaches and aseptic meningitis following the first dose at 4 mg/kg. Grade 1 headaches were seen after the first dose in 3/6 patients at 2 mg/kg, but no patients at lower doses reported headaches. It was determined that this drug-related event is a first-dose effect, not seen clinically after second or subsequent SGN-40 infusions. SGN-40 appears to trigger cytokine release, and TNF-α levels in the plasma are elevated following the first infusion only.

In this trial, initial PK data indicated that t1/2 of SGN-40 depends on the administered dose; after the first and third infusions t1/2 mean values at 0.5 mg/kg were 0.9 and 1.3 days, at 1.0 mg/kg were 1.7 and 2.6 days, and at 2.0 mg/kg were 2.9 and 4.2 days, respectively. This is consistent with results in nonhuman primates, in which t1/2 was relatively short at low doses. These data suggest that there is a rapid elimination pathway and/or redistribution volume that has not been saturated at the doses used to date. Therefore, higher doses are required to saturate the elimination pathway. Although human anti-human antibodies (HAHA) have not yet been measured, preliminary analysis suggests that if antibodies were formed, they did not significantly affect PK. Even at the low doses tested thus far, there is preliminary evidence for antitumor activity; serum and/or urine M-protein declined in 4/16 patients during treatment. Of note, the most profound B-cell depletion during therapy was observed in these 4 patients, an expected consequence of SGN-40 activity. This trial's protocol has been amended to include a drug-loading period that should eliminate first dose cytokine release syndrome. Dose escalation is ongoing under the revised protocol, and no headaches have been seen in the first cohort at a peak dose of 3 mg/kg (Hussein MA, etal, ASH05, Abs. 2572).

In this ongoing phase I trial, among 23 enrolled patients, 7 were treated under the amended dosing schedule, 3 at doses up to 3 mg/kg/week and 4 at doses up to 4 mg/kg/week. Patients in this trial were heavily pretreated with a median of more than 5 prior therapies. SGN-40 was well tolerated at both dose levels using the intrapatient dose escalation schedule. Overall, disease stabilized in 2 patients at the conclusion of the first cycle and M-protein levels dropped during therapy in another 4 patients, although no patients have met criteria for objective response. One patient with stable disease advanced to a second cycle of therapy after clinical improvement.

An open label, phase I clinical trial (protocol ID: SG040-0002, NCT00103779), initiated in December 2004, is administering SGN-40 to patients with refractory or recurrent B-lineage NHL, including follicular, mantle cell, diffuse large B cell, small lymphocytic cell, and marginal zone NHL. The trial will enroll up to 21 patients into cohorts of 3. In the first cohort, patients are administered 2 mg/kg/week of SGN-40 for four weeks, with doses escalating in successive increments to 2, 4, 8, and 16 mg/kg, or until MTD. Participating centers include Stanford University, University of Miami, Weill Medical College of Cornell University, University of Alabama at Birmingham, and MDACC. Although headaches were not seen in the NHL trial, the administration schedule was amended in accordance with the changes made to the multiple myeloma clinical trial.

In this trial, 4/6 patients treated at 2 mg/kg/week in cohort 1, completed the planned 4 weekly infusions. No Grade 4 toxicity was observed. There were 2 Grade 3 tox-

icities, unilateral conjunctivitis and ipsilateral loss of vision, both in a patient with pre-existing macular degeneration. Grade 2 loss of balance was also seen in the same patient. Imaging studies with CT and MRI did not reveal stroke or leptomeningeal disease nor were there inflammatory or malignant cells in the cerebrospinal fluid (CSF). All these adverse events resolved over six weeks, with vision returning to baseline acuity. The only other severe adverse event reported was a case of deep venous thrombosis (DVT) in the setting of bulky ipsilateral pelvic adenopathy, which was considered to be unrelated to SGN-40. Dose escalation was temporarily suspended to evaluate these unexplained toxicities and because of the report of severe headaches in the multiple myeloma trial. Although none of the patients in the NHL trial developed headaches or clinical symptoms, plasma cytokine levels (TNF-α, IL-6) did increase significantly in some patients following the first infusion but not subsequent ones. Disease stabilized in one patient at the 2 mg/kg dose level, as determined by serial CT scans, and in another patient with subjective improvement in symptoms during therapy but whose disease progressed based on CT scans 4 weeks after completing therapy (Advani RH, etal, ASH05, Abs. 1504).

According to more mature data, among 12 patients with NHL enrolled in this trial, 6 were treated at 2 mg/kg/week using the original schedule, and 6 at doses up to 3 mg/kg/week on the amended dosing schedule. These patients had been treated with a median of 3.5 prior therapies. There were 2 PR at the 3 mg/kg/week dose level after 36 days on the trial. Both of these patients were treated with a second cycle of therapy. One responding patient, who was restaged after the second cycle, continues to respond at day 79. The other responding patient has not yet been restaged. SGN-40 was well tolerated under the amended administration schedule at up to 3 mg/kg/week with mild adverse events consistent with antibody administration.

In July 2005, Seattle Genetics initiated a multicenter (n=3), single agent, phase I/II clinical trial (protocol ID: SG040-0003; NCT00283101) of SGN-40 in patients with relapsed or refractory CLL. The trial, to evaluate tolerability, PK profile, and antitumor activity of a multidose regimen of SGN-40, will enroll approximately 20 patients at the University of Alabama at Birmingham, under PI Andres Forero, MD, University of Miami, Sylvester Comprehensive Cancer Center, under PI Mark Goodman, MD, and Weill Medical College, under PI Richard Furman, MD. A minimum of 3 patients will be entered into each dose-level cohort and treated for 5 weeks. Escalation to the next cohort will occur when 3 patients have treated by at least one infusion at the highest scheduled dose level, and at least one patient has completed the entire 5-week dosing schedule. Cohorts will be enrolled at a maximal dose level of 3, 4, 6, or 8 mg/kg/week for 5 weeks. Responding patients will be treated with 4 additional infusions every two weeks at the maximal dose for each specific cohort.

In November 2005, SGN-40 was granted orphan drug designation for CLL by the FDA. In August 2004, the FDA had also granted SGN-40 orphan drug designation for the treatment of multiple myeloma.

TACI-Ig

TACI-Ig, under development by Zymogenetics (Seattle, WA), in collaboration with Serono (Geneva, Switzerland), is a soluble fusion protein that links the extracellular portion of the TACI receptor to the Fc portion of human immunoglobulin (Ig). TACI is a cell-surface receptor found on B lymphocytes. By using the receptor portion of TACI responsible for binding growth factors, the antagonist protein TACI-Ig was created that binds to B-lymphocyte stimulator (BlyS) and a proliferation-inducing ligand (APRIL), two cytokines belonging to the TNF family of cytokines that stimulate B-cell growth and production of antibodies, including harmful autoantibodies, which cause certain autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE).

TACI-Ig 'mops up' excess BLyS and APRIL in the blood. By preventing binding of growth factors to B cells, TACI-Ig regulates development of mature B-cell production of antibodies. For instance, few mature B cells and reduced levels of circulating antibody are found in TACI transgenic mice, i.e. mice that have been genetically engineered to overexpress a soluble form of the TACI receptor. Similar results were observed in normal mice treated with soluble TACI-Ig receptor. TACI-Ig is being investigated in clinical trials in autoimmune disorders, including RA and SLE, and in B-cell hematologic malignancies. Preliminary findings from these trials indicate that TACI-Ig is well tolerated with no serious adverse events observed in any of trial participants.

In October 2004, an open label, dose-escalation, phase I/II clinical trial was initiated at the Centre Hospitalier Universitaire de Montpellier, in France, to evaluate the safety and PK of multiple doses of TACI-Ig, in patients with refractory or relapsed multiple myeloma, or refractory Waldenström's macroglobulinemia. The aim is to determine tolerability, PK, pharmacodynamics and biologic activity of TACI-Ig. The trial follows a classical Simon 2-stage design to determine MTD as well as the optimal biologic dose of TACI-Ig in this setting. Eligible patients are enrolled in sequential cohorts and treated with 5 weekly SC injections of TACI-Ig at 2, 4, 7 or 10 mg/kg. Patients with at least stable disease after the first cycle may be treated with 2 additional treatment cycles. PK is assessed after the 1st and 5th dosing. Usual safety parameters are assessed, including measurement of potential anti-TACI-Ig antibodies. The biologic activity assessment comprises M protein, β 2-microglobulin, soluble syndecan-1, lymphocyte subpopulation counts (by flow cytometric analysis), polyclonal Ig, serum and urinary free light chains and C-reactive protein (CRP). Evaluation of response is assessed at the end of cycles 1 and 3.

According to preliminary results involving the first 3 cohorts that enrolled 6 patients with multiple myeloma and 3 patients with Waldenström's macroglobulinemia, no DLT was observed or serious adverse events related to the drug; 1 case of mild injection-site erythema is the only drug-related toxicity reported to date. Among 7 evaluable patients, disease stabilized in 2 with multiple myeloma and 2 with Waldenström's macroglobulinemia at the end of the first treatment cycle, and in 2 of these 4 patients stable disease was maintained through the end of the third cycle, while disease progressed in 3 patients with multiple myeloma and 1 patient with Waldenström's macroglobulinemia, after the first cycle. Polyclonal Ig in 6/9 patients (multiple myeloma=5) and soluble syndecan-1 in 2/5 patients with multiple myeloma decreased during treatment, while CRP levels were not affected. Treatment with TACI-Ig was well tolerated at the dose levels tested so far. A biologic response in accordance with the expected TACI-Ig mode of action is observed in this heavily treated population with refractory disease. Accrual of patients at higher dose levels is ongoing (Rossi J-F, etal, ASH05, Abs. 2566).

In January 2006, preliminary results were reported from a randomized, placebo-controlled, double blind, phase Ib clinical trial of TACI-Ig in patients with RA. This trial's primary objective was to determine the safety and tolerability of TACI-Ig, and examine the relationship between TACI-Ig dose and schedule with markers of biologic activity and disease activity. TACI-Ig appeared to be safe and well tolerated across the full range of dose levels and schedules tested in this trial, and clear biologic responses were observed, which appeared to correlate with clinical benefit. The trial enrolled 73 adult patients with moderate to severe RA after failure of other non-biologic therapies. No antibodies to TACI-Ig were formed in any patient treated with TACI-Ig. Schedule and dose-dependent reductions of IgM, IgA and IgG and of rheumatoid factor, a biologic marker of disease were noted, and in a cohort of 19 patients who were treated with seven doses of TACI-Ig over a 3-month period, there were positive trends on some disease activity measures such as ACR 20 and DAS 28. Final results from this trial are expected to be released in mid-2006. A trial in patients with SLE patients is expected to finish in the first half of 2006, with full study results to be presented at a scientific meeting in the second half of 2006.

In addition to the multiple myeloma trial, being conducted in France, phase Ib trials have also been initiated in NHL and CLL at Mayo Clinic.

INDEX OF COMPANIES & INSTITUTIONS

INDEX OF COMPANIES & INSTITUTIONS

FUTURE ONCOLOGY

PUBLISHED BY **NEW MEDICINE, INC.**

PUBLISHER AND EDITOR: **Katie Siafaca**
VICE PRESIDENT-OPERATIONS: **Beth Schweitzer**
EDITOR: **Adele Simon**
DESIGN & PRODUCTION: **Jill Burch**

NEW MEDICINE, INC. MAILING ADDRESS:

P.O. Box 909 ■ Lake Forest, CA 92609
Tel: 949. 830. 0448 ■ Fax: 949. 830. 0887

e-mail: info@newmedinc.com
www:http://www.newmedinc.com
www:http://www.oncologyknowledgebase.com
www:http://www.nmok.net

SUBSCRIPTION INFORMATION:

- FUTURE ONCOLOGY (ISSN 1082-331X) is published as 12 issues (several double issues) per subscription period, with a free index listing companies/institutions and subjects covered.
- A one-volume subscription to the electronic version is US $840 and to a hard copy version, sent first class to U.S. addresses, is US $1040, and sent air mail to foreign addresses, US $1,100.
- Volumes V3, V4, V5, V6, and V7 are $2,400 (U.S.) and $2,460 (outside the U.S.), either electronic or hard copy versions.
- Volumes V6 and V7 are $1,400 (U.S.) and $1,460 (outside the U.S.).
- Additional subscriptions sent to the same location are $390 each.
- Payment must accompany your order; checks must be drawn on a U.S. bank. (A purchase order number is acceptable; however, the subscription will not begin until payment is received.) Make checks payable to NEW MEDICINE. Payment may also be made by AMERICAN EXPRESS, VISA, or MASTERCARD and wire transfer; please call 949. 830. 0448 for details.