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MEETING COVERAGE

PHASE I MONOTHERAPY CLINICAL TRIALS IN ONCOLOGY

FROM THE 2005 MEETINGS OF THE AMERICAN ASSOCIATION OF CANCER RESEARCH (AACR) AND THE AMERICAN ASSOCIATION OF CLINICAL ONCOLOGY (ASCO)

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Phase I monotherapy clinical trials are critical in establishing safe dose and schedule of a novel investigational agent/formulation before efficacy may be determined. In addition, phase I combination clinical trials are conducted to evaluate safe doses and schedules of approved drugs in combination with each other or with novel agents. This report only covers novel, 'first-in-humans' agents in phase I monotherapy trials about which new clinical or preclinical data was reported at 2005 meetings of the American Association of Cancer Research (AACR05) and the American Association of Clinical Oncology (ASCO05).

Although often, before they are studied in patients, novel agents are evaluated in healthy volunteers to establish their basic safety profile, this practice is uncommon in the oncology sector because of the inherent toxicity of most of these agents. Most phase I clinical trials in oncology recruit patients with advanced disease. Therefore, participation in clinical trials, in general, is a leap of faith by patients with late/terminal stage disease and few options. Of course, there is an outside chance that a phase I drug would provide a cure, but this is a rare unexplained phenomenon occurring in 1 or 2 cases among the 30-50 patients usually entering a phase I monotherapy trial.

There are many controversies regarding how oncology drugs are evaluated in humans, especially now that advanced science has resulted in identifying distinct attributes in tumors that may require highly targeted individualized treatments. The whole phase I, II, III, IV scheme may be reconsidered in favor of a better means of determining an agent's viability early on.

PHASE I MONOTHERAPY CLINICAL TRIALS

Phase I monotherapy trials are the first hurdle that needs to be overcome before a novel agent enters a rigorous development cycle. These trials use dose escalation to establish a safe dose associated with optimal pharmacology for further treatment. Phase I monotherapy clinical trials are being conducted to (Exhibit 1):

- characterize and quantify toxicity
- establish a safe dose for future trials by determining an agent's dose-limiting toxicity (DLT) and maximum tolerated dose (MTD)
- evaluate a drug's pharmacokinetics (PK) and pharmacodynamics
- assess antitumor activity, including complete responses (CR), partial responses (PR), or stable disease (SD), although absence of such activity during phase I trials does not imply drug failure
- experiment with and/or identify surrogate markers/assays that may reveal information on a drug's activity

Patient Selection

The first step in initiating a clinical trial is patient selection, usually based on preclinical results regarding the agent's activity in certain malignancies, i.e., solid tumors. This selection may be based more and more often on certain characteristics of the patient's disease, such as a certain molecular marker expressed by the tumor. Eligible patients may be determined by expected longevity, gender or age, disease stage, performance status, previous exposure to any type of anticancer treatment, and previous response to such treatment, among others. Usually, patients entering phase I clinical trials have been heavily pretreated for advanced disease.

It is anticipated that patient selection would become more targeted as more is known about the nature of each patient's disease. Already, phase I clinical trials have been or are being conducted in patient populations selected because their tumors express a specific marker such as a receptor or an antigen. As more is learned about the significance of these targets, enrollment in phase I clinical trials will become more specific, encompass more homogeneous patient groups, and be more decisive as to viability of the early stage agent under investigation.

Trial Initiation

In the USA, an Investigational New Drug (IND) application must be filed with the FDA and an approval secured from the agency before clinical trials may be initiated. The IND delineates goals of the clinical trial program, specific human testing being proposed, and expected risks [21 CFR 312.23(a)(8)].

IND applications for phase I trials are supported by an extensive preclinical database of drug evaluations *in silico*, *in vitro*, *ex vivo,* and *in vivo* in animal models. These preclinical studies are designed to allow selection of a safe starting dose for humans, gain an understanding of which organs may be the targets of toxicity, estimate the margin of safety between a clinical and a toxic dose, and predict PK and pharmacodynamic parameters. Although much time and resources, translated in large expenditures, are invested in preclinical evaluations, fewer than 10% of IND for new molecular entities (NME) progress beyond the investigational stage. Animal testing does not always predict performance in humans, and potentially effective candidates may not be developed because of resource constraints at the preclinical phase.

Currently, the FDA is proposing a wider use of limited exploratory IND investigations in humans that can be initiated with less, or different, preclinical support than is required for traditional IND trials because exploratory IND

trials present fewer potential risks to human subjects than do traditional phase I trials. In April 2005, FDA's Center for Drug Evaluation and Research (CDER) published a draft guidance document, "Guidance for Industry, Investigators, and Reviewers Exploratory IND Studies" on exploratory IND studies presenting its views on a more flexible approach to early phase I clinical trials. Such limited approaches are particularly suited in development of drugs that treat serious, life-threatening diseases, because they may help identify promising candidates faster and more precisely, particularly in cases where patients are enrolled in a trial with therapeutic intent.

Usually, to ensure rapid patient accrual, particularly when rare malignancies are concerned, phase I clinical trials may involve many centers and even foreign locations. At each center, an Institutional Review Board (IRB), an independent body comprised of medical, scientific, and non-scientific members whose responsibility is to ensure protection of the rights, safety, and well being of patients or subjects involved in a trial, must approve initiation of the trial, and provide continual review of a trial's protocol and of methods and materials used in obtaining and documenting informed consent.

Outside the USA, most other countries also have strict regulations governing human clinical trials in place. As an example, the European Commission's Directive 2001/20/EC, aiming at streamlining performance of clinical trials across the European Union (EU), that was officially adopted in April 2001, with compliance becoming mandatory in May 2004, has intensified standards and increased the administrative burden of carrying out clinical trials in the EU. Subsequently, this directive was fortified by the Good Clinical Practice Directive that further increased regulatory requirements.

Dosing and Treatment Schedules

The starting dose is determined based on preclinical experiments in mice and, sometimes, larger animals such as dogs or primates. The human dose is 10% of the dose that leads to death of 10% of rodents treated with the drug, or 33% of the toxic dose low (TDL) in larger animals such as dogs.

The dosing schedule is based on the type and mechanism of action of the drug. Depending on their mechanism of action and inherent characteristics, anticancer drugs may be administered via various routes, with the most common being intravenously (IV), subcutaneously (SC), or orally (PO), on an intermittent or continuous basis, in predetermined maximum numbers of cycles, or chronically as a maintenance treatment.

Dose escalation may conform to various schemes. The two major components of dose escalation are cohort size and dose increment. Typically, cohort sizes involved 3 patients. However, novel approaches to evaluate patient status use various assays that are more likely to require a larger cohort; more recently designed trials are using larger 6-patient cohorts.

FDA's new suggestions regarding phase I clinical trial dosing may change the way dose is determined for first-in-human agents. For instance, an exploratory IND requires a lesser or different preclinical support, because studies based on such IND involve administering either subtherapeutic doses of a product, or doses expected to produce a pharmacologic, but not a toxic, effect, thus exposing human subjects to a lesser potential risk than that anticipated in traditional phase I trials that seek, for example, to establish DLT and MTD.

Microdosing/phase 0 clinical trials are being considered as an alternative in establishing accurate dose-related PK data. In microdosing, exquisitely sensitive analytical technologies are used to track sub-pharmacologic doses of new drugs administered to humans at a very early stage in the drug development process. This allows information in humans to be determined much earlier than ever possible, providing drug developers with a means to select only the most promising drug candidates for further, more expensive clinical trials.

Microdosing exposes healthy volunteers to 1/100th of the dose calculated to yield a pharmacologic effect with a maximum dose of <100 µg. Microdosing studies are designed to evaluate PK or image specific targets, but not to induce pharmacologic effects, limiting any potential risk to humans. To establish a margin of safety, the sponsor must demonstrate that a large multiple of the proposed human dose does not induce adverse effects in experimental animals. Scaling from animals to humans based on body surface area can be used to select the dose for the clinical trial.

Microdosing is expected to reduce drug development time and costs. One proponent of microdosing is Xceleron (Gaithersburg, MD) that uses ultra sensitive accelerator mass spectrometry (AMS) as the enabling technology for human microdosing studies.

Personalized dosing departs from the traditional 'one-size-fits-all' approach to therapy, allowing physicians to optimize therapeutic benefit of certain anticancer drugs for each patient. Optimizing treatment regimens through individualized dosing may be a practical evolution towards the way patients may be treated with certain drugs in the future.

In September 2005, Xanthus Life Sciences (Cambridge, MA) received a grant valued at up to $2.3 million from the National Cancer Institute (NCI) to develop its proprietary personalized dosing (ParaMetabolic) technology to improve the way cancer drugs are dosed.

Personalized dosing based on ParaMetabolics works by integrating all sources of variability in an individual to seek to optimize the dose of a drug. In this way, patients are treated with a dose tailored to their requirements that is neither too low nor too high, thus reducing chances that the drug will be ineffective or cause unnecessary side effects.

In the Xanthus' process for determining an individual's optimal dose, a patient is administered an initial dose of drug, and then the amount of drug remaining in this patient is measured together with patient-specific biomarkers of drug exposure. This data is then analyzed with proprietary software that determines a score for each patient. The score correlates to the dose that best meets a patient's individual characteristics while minimizing risk of side effects. Xanthus' technology takes into account not only the genetic makeup of the individual, but also other factors that govern responses to therapy, such as effects of concurrent drug therapies, diet, metabolic rate, major organ function, and blood flow.

Exhibit 1
Phase I Clinical Trial Terminology

Term	Definition ❑ Description
Dose-limiting toxicity (DLT)	Dose-limiting toxicity (DLT) is defined as the dose resulting in a side effect that is serious enough to prevent any further increase in dose or treatment intensity. DLT is usually characterized as >Grade 3 toxicity.
Maximum tolerated dose (MTD)	Maximum tolerated dose (MTD) refers to the highest effective concentration of a drug predicted to produce a minimum toxic effect. MTD is often defined as the dose preceeding that at which 30% of patients experience DLT.
Optimal biologic dose (OBD)	The optimal biologic dose (OBD) is the dose that produces the optimal desired host response for the parameters deemed important for that agent. OBD is used in agents that may be effective within a large range of doses without exhibiting DLT.
Pharmacokinetics (PK)	Pharmacokinetics (PK) is the rate of quantitative uptake of a drug by the host, and its biotransformation, distribution, metabolism, and elimination from the body; PK describes the host's effect on a compound.
Pharmacodynamics	Pharmacodynamics is the study of reactions between drugs and organisms, including pharmacologic, biochemical, physiologic, and therapeutic effects.
Response	Although responses are not part of outcomes assessed in phase I monotherapy trials, they are often reported. Responses may be complete (CR) or partial (PR), or disease may stabilize (SD) or progress (PD).
Surrogate markers	Phase I monotherapy trials may also provide an opportunity to identify/evaluate surrogate markers of response or toxicity

Informed Consent and Patient Recruitment

The very nature of phase I clinical trials implies little chance of a curative outcome. Therefore, it has been often suggested that patients enrolling in such trials lack the necessary information to make informed decisions and are vulnerable to being coerced into participating.

In a study to assess the decision-making process of patients entering phase I clinical trials, 142 English-speaking, adult patients from 5 centers who enrolled in a phase I cancer trial, were surveyed just after consenting. Overall, 81% of patients were aware of hospice, but only 6% seriously considered hospice for themselves at time of enrollment. Although 84% were aware of the option of palliative care alone, only 10% seriously considered that option, while only 7% considered no treatment at all. The majority of patients (69%) were aware of other clinical trials, but only 29% seriously considered such an option for themselves. Among all patients, 75% felt they understood the risks and benefits of enrolling in a phase I clinical trial very well, and 25% somewhat well. In making an informed decision, the most useful information for 62% of the patients was whether the agent killed cancer cells; only 12% considered the drug's side effect profile as an important factor. Also, 24% would not participate if the drug caused temporary mental impairment, while only 10% would not participate if the experimental drug had a 10% chance of death. Regarding pressure, 78% of patients said they felt moderate or a lot of pressure to participate in the phase I trial because of progressive disease (PD), while 7% felt either a moderate or a lot of pressure from their own families or from researchers to participate. This survey concluded that patients entering phase I trials are aware of alternatives, but do not consider them seriously for themselves. Key to their decision-making was the fact that the agent under investigation could kill cancer cells, and not any risk inherent in the treatment. Any pressure to participate in the phase I trial came from their disease and not from other people (Agrawal M, etal, ASCO05, Abs. 6014).

The elderly represent a group particularly underserved in regards to participation in phase I clinical trials. The impact of participation of elderly patients in phase I clinical trials is being investigated both in terms of determining availability of such options to the elderly as well as addressing informed consent issues, and likelihood of higher toxicity. A better understanding of the needs of this group of patients may improve treatment outcomes.

Analysis of elderly patients' participation in phase I clinical trials at Case Comprehensive Cancer Center (Cleveland, OH) and University of Pittsburgh Cancer Institute, was performed to compare demographics, disease staging information, baseline laboratory values, chemotherapy treatment information, and recorded toxicities in those <65 and ≥65 years-of-age (elderly). Nearly

40% of patients recruited to phase I trials at these two centers were elderly, a higher proportion than what has been reported in phase II and III trials in oncology. Elderly patients represented 38% (73/192) of all patients at Case and 37% (81/218) at Pittsburgh; patients 65-75 years-of-age represented 33% and 29%, and patients >75 years-of-age accounted for 5% and 8%, respectively. Univariate analyses revealed no statistically significant differences between the two age groups in terms of baseline demographics (sex, race, performance status) or baseline laboratory values (CBC, LFT, albumin, creatinine). For patients with nonhematologic malignancies on chemotherapeutic trials (Case=137, Pittsburgh=131), Grade 3 toxicity occurred in 65% (89/137) and 58% (76/131) of patients, respectively, while Grade 3 hematologic toxicity was observed in 41% (56/137) and 33% (43 /131), respectively. Grade 3/4 hematologic or nonhematologic toxicity was not statistically different in the two groups at either institution. This finding was not anticipated, and the reasons are being evaluated. Thus, for the select group of patients with near normal organ function and a good performance status (required for patients enrolling in phase I trials), older age does not appear to be associated with increased toxicity. Further analyses are underway to better characterize the impact of enrolling elderly patients in trials of novel cancer agents and to analyze available PK data (Savvides P, etal, ASCO05, Abs. 8133).

Investigators at the University of Chicago conducted a subset analysis of an existing database of results from surveys and semi-structured face-to-face interviews of patients with advanced cancer participating in phase I clinical trials, regarding their informed consent and decisions to enroll in a clinical trial. Survival data was also collected. Among 76/212 patients ≥65 years-of-age enrolled in phase I trials, the median age was 71 (range=65-82). Regarding informed consent based on understanding the research purpose of a phase I trial, younger patients were more likely to identify toxicity as the outcome measurement (80% versus 20%). Older patients were more likely not to know the trial's purpose. Older patients also reported not being given alternative care options, including palliative care (40% versus 11%). Older patients were less worried about dying (28% versus 13%).

There were significant differences in survival after enrollment. Median survival time (MST) of older patients was 10.8 (range=0.9-48.7) months, compared to 6.4 months for younger patients (range=0.2 to 35.9) months. Older patients, while sharing many demographic characteristics with younger patients, survived significantly longer than younger subjects. Survival advantage of the elderly patients in this highly selected population is an interesting and unexpected finding, and suggests a significant selection bias of elderly patients with advanced cancer entering early phase trials (Teitelbaum UR, etal, ASCO05, Abs. 8109).

Phase I clinical trials are also an appropriate treatment option for children with malignancies refractory to or recurrent after conventional treatment. It is estimated that in the USA, between 500 and 600 children <15 years-of age are diagnosed with cancer annually. Children constitute a unique subpopulation of patients with cancer. Unfortunately, most cancer therapeutics are being developed for adults.

In order to determine availability of such therapies, investigators at the University of Texas Southwestern Medical Center (Dallas, TX) prospectively monitored all patients between January 1, 2002 through December 1, 2004 who were evaluated for enrollment in a phase I or II trial. There were 61 episodes in which a patient met eligibility criteria. Of these patients, 22 children were successfully enrolled in a research protocol, 13 (phase I=10, phase II=3) in an NCI-sponsored Children's Oncology Group (COG) trial, and 9 in an institutional phase I or II trial. In this group, 39 (64%) children did not enroll in a clinical trial; 23 (38%) were treated with chemotherapy at the investigator's discretion, 12 (20%) were treated no further, three (5%) were treated with radiation therapy, and 1 with surgery alone. Of the patients not enrolled on an investigational trial, 27 (70%) were the result of a trial not being available, in 6 cases, the parents refused enrollment, and in 6 others, the physician chose an alternative therapy. Although only 10 patients were enrolled in COG phase I trials during this 3-year period, 6 have been enrolled since 2004. There are many reasons for failure to enroll eligible patients in phase I and phase II trials. One of the biggest obstacles is lack of available open trials, hindering development of potentially efficacious therapies for children with malignancies. Although recent changes in enrollment procedures may have had a positive impact, potentially eligible children may still be denied trial entry because of lack of trials (Aquino VM, etal, ASCO05, Abs. 8531).

Phase I Clinical Trial Reporting

In view that at least 1 in 3 drugs fail during phase I clinical trials, it is not surprising that only about 1 in 3 phase I clinical trials presented at ASCO97 were published in peer-reviewed journals (Camacho LH, etal, Cancer, 22 August 2005, epub).

To begin with, only 54% of the 275 abstracts submitted describing phase I trials were selected for presentation at ASCO97. Abstracts involving novel agents were more likely to be selected for presentation (68%) than those reporting non-novel compounds (38%). More of the presented abstracts (72%) were subsequently published, compared with 62% of those not presented. The overall publication rate was 67% at 7.5 years, with the median from presentation to publication being 3.4 years. Major obstacles to publication were lack of time and investigator relocation.

The sheer volume of phase I clinical trials in oncology is overwhelming. Thousands of monotherapy phase I clinical trials of novel anticancer agents have been conducted in the last 5 years, not taking into account adjunct therapies

that treat complications of the disease or its treatment. As the number of potential new anticancer drugs and protocols expand, communication of results of clinical trials in the oncology field has become even more challenging. Because publication of clinical trial results in peer-reviewed journals is the only means currently to accurately disseminate data to professionals, widespread underreporting of phase I clinical trials delays scientific progress, and may ultimately have an adverse effect on patient care.

Decision to Advance Development of Agents Based on Phase I Clinical Trials

Although phase I monotherapy trials are being conducted to primarily assess DLT and MTD, it is becoming commonplace to look for early indications of anticancer activity. Of course, an early indication of anticancer activity has many ramifications. It provides confidence in proceeding with further clinical development of the drug. For small developers, it may mean additional funding or lucrative collaborations. For investigators, it may result in faster future trial recruitment. However, it is unclear whether such conclusions may be based exclusively on data collected in a phase I trial.

A study was conducted to assess significance of responses reported in single agent phase I trials of investigational anticancer agents. The objective of this study was ascertain if there have been approved and/or effective agents that showed little to no activity in the phase I setting. An analysis of publicly available literature was conducted on a mix of 14 currently marketed and investigational agents. For each phase I single agent trial, the number of patients, and the CR, PR, minor response (MR), and SD rate, as reported by investigators, based on Response Evaluation Criteria in Solid Tumors (RECIST) or World Health Organization (WHO) criteria, were calculated and compared. Among all agents analyzed, the CR rate was 36%, the PR 50%, and disease stabilized as best response in at least 1 patient in 14% of the agents. Of the 7 approved agents with an available complete dataset, a response (CR or PR) was observed in 4, an MR/SD in 1, and no activity as best response for a later approved indication was observed in 2. Of the 6 approved drugs with phase I single agent trials in advanced solid tumors, a response in several tumor types was observed in 3, a response in only the tumor type which was later approved, was observed in 1, and no response in any tumor type was observed with 2 agents, bevacizumab (Avastin; Genentech) and cetuximab (Erbitux; ImClone Systems), which are approved and active in treating colorectal cancer. CR are uncommon in phase I single agent trials of investigational anticancer agents. For those agents where responses are seen, continued development usually proceeds in indications where responses were reported. However, a lack of response should not necessarily deter continued development of what may prove to be a promising anticancer agent in future trials (Manela J, etal, ASCO05, Abs. 6049).

Benefits of Phase I Monotherapy Trials in Oncology

When phase I clinical trials are discussed and compared, it should be noted that these trials do not describe first-in-human monotherapy trials of novel agents. In some phase I clinical trials, novel agents are combined with other novel agents or with approved anticancer drugs. Phase I clinical trials are also undertaken to evaluate combinations of approved drugs. This report only covers single agent phase I clinical trials. These trials may involve drugs acting on various mechanisms, some well understood, such as cytotoxicity, and others more obscure such as signal transaction modulation, angiogenesis inhibition or immunotherapy. Novel cytotoxic agents/formulations are the most suited to be evaluated in phase I clinical trials because the goal is mostly to assess toxicity and establish MTD. The value of phase I clinical trials is murkier when regulatory/immunotherapy agents are the targets of such trials.

In a analysis of 460 phase I clinical trials (both monotherapy and combination therapy), conducted by the Cancer Therapy Evaluation Program (CTEP) of the NCI between 1991 and 2002, involving 11,935 patients (all participants were assessed for toxicity and 10,402 for response), the overall response rate (ORR) was 10.6%, with considerable variation among trials (see Exhibit 2). As expected, response rates were higher among patients participating in combination trials that included FDA-approved agents. ORR in phase I trials of single investigational chemotherapeutic agents that represented only 20% of all trials, was 4.4%; toxicity-related death rate was 0.57%. Response rate in trials that included at least one anticancer agent approved by the FDA that constituted 46.3% of all trials, was 17.8%. Overall, disease stabilized in an additional 34.1% of participants in all phase I clinical trials. The overall death rate attributable to toxic events was 0.49%. Among 3,465 participants for whom data on patient-specific Grade 4 toxicity was available, 14.3% experienced at least one episode of Grade 4 toxicity (Horstmann E, etal, NEJM, 3 Mar 2005;352(9):895-904).

If one is to include SD as a beneficial outcome, these phase I clinical trials produced a response of 44.7%, and monotherapy phase I trials resulted in an almost identical response rate of 42.5% (see Exhibit 3). Significance of this result is not obvious. However, in treatment of gastrointestinal stromal tumors (GIST) with an extremely commercially successful approved anticancer agent, imatinib mesylate (Gleevec; Novartis), there was significant clinical improvement, and prolonged progression-free survival (PFS) in patients with SD, a common treatment result in approximately 25%-28% of treated patients. It is, therefore, possible that SD is a desirable outcome and results of phase I clinical trials of novel agents involving SD are of significant value.

NOVEL ANTICANCER AGENTS/FORMULATIONS IN PHASE I MONOTHERAPY TRIALS

This review describes 49 anticancer agents that have either been recently evaluated in phase I monotherapy clinical trials, or are being currently evaluated in such trials. In some cases, these drugs are being evaluated with radiotherapy as radiosensitizers and, in others, they are in phase I/II clinical trials. In some cases, initiation of phase I clinical trials is planned in the near future. The selected drugs are either truly novel agents being administered to patients for the first time ever, or older agents that have returned to the clinic because of new information regarding improvements in their deployment.

Exhibit 4 describes all the agents covered in this review.

Cytotoxic Agents, Targeted Cytotoxics, Apoptosis Inducers, and Immunoconjugates

Cytotoxic agents are experiencing a kind of rebirth, with new novel agents and imaginative formulations and drug delivery approaches breathing new life in long ignored generic drugs. Generally, a higher rate of response is observed in phase I monotherapy trials of cytotoxic agents (Exhibits 3 and 5).

AQ4N (banoxantrone), under development by KuDOS Pharmaceuticals (Cambridge, UK) as Hypoxin, is a novel drug targeting the treatment-resistant hypoxic fraction of tumors. AQ4N is a highly soluble targeted prodrug activated in hypoxic cells to AQ4, a topoisomerase II inhibitor with radiosensitizing properties, preferentially in conditions of low oxygen tension. AQ4N also enhances effects of several chemotherapeutics (cyclophosphamide, cisplatin, thiotepa, dimethyl xanthone acetic acid) *in vivo*.

It appears that AQ4N is not a substrate for MDR, which is one of the major barriers to effectiveness of anthraquinones and other cytotoxic agents. AQ4N is also effective under conditions of hypoxia, another well known characteristic of treatment-resistant tumors. Taken together, these features indicate significant clinical potential for AQ4N, both as monotherapy and in combination with established anticancer regimens (Albertella MR, etal, AACR05, Abs. 525).

In an inventive approach, scientists at the University of Manchester, in the UK, employed adenoviral delivery of inducible nitric oxide synthase (NOSII) to hypoxic tumor cells to enhance cytotoxicity of AQ4N in combination with radiotherapy. Because NOSII, commonly overexpressed in tumors, plays a role in AQ4N bioactivation, NOSII gene therapy is expected to sensitize hypoxic tumor cells to AQ4N, and also generate nitric oxide (NO) to act as a potent radiosensitizer. HT1080 tumor cells were infected with an engineered adenovirus, Ad NOSII, or AdLacZ, and then exposed to AQ4N and irradiated. HT1080 cells infected with Ad NOSII exhibited ~8-fold higher NOS activity. AQ4N toxicity to Ad LacZ infected cells increased with decreasing levels of O_2. This toxicity was enhanced by Ad NOSII infection, evidence of AQ4N bioactivation, and not NO-produced toxicity. When AQ4N was combined with radiation, AdNOSII-infected cells were dramatically sensitized in 1% O_2 and anoxia. At 1% O_2, this increase in sensitization appears to be through NOS-mediated NO release, that acts as a radiosensitizer. These preliminary results show that NOSII gene therapy can enhance hypoxic tumor response to AQ4N chemoradiotherapy (Cowen RL, etal, AACR05, Abs. 1348).

A phase I clinical trial was initiated by BTG in January 2001, to determine MTD of AQ4N administered IV in combination with fractionated radiotherapy in patients with advanced esophageal carcinoma. This trial, conducted at Leicester Royal Infirmary and the University of Bradford, in the UK, and Churchill Hospital (Oxford, UK), was closed in December 2004. A total of 22 patients (adenocarcinoma=14, squamous carcinoma=8) suitable for palliative radiotherapy were treated in this trial. AQ4N was administered as a 30-minute IV infusion on days 1 and 14, with the first radiotherapy fraction administered 6 hours after the second dose of AQ4N. The total radiotherapy dose was 20 Gy in 5 daily fractions. AQ4N dose was escalated from 22.5 mg/m^2 to 447 mg/m^2. Sequential plasma samples were taken on day 1. Biopsies of tumor and normal esophageal tissue were taken 24 to 48 hours after the first dose, and tissue concentrations of AQ4N and AQ4 were measured in 3 patients treated with 447 mg/m^2. There were no drug-related deaths; 1 patient was not evaluable. There were 16 episodes in 10 patients of Grade 3 or 4 toxicity (probably drug-related) including lymphopenia (n=9), hyponatremia (n=3), fatigue (n=3), and hyperuricemia (n=1). None was clinically significant. PK studies showed a predictable dose-related increase in AUC in excess of those seen in mice at therapeutic doses. Based on calculated concentrations of AQ4 in tumor and normal tissue, tumor-tissue concentrations were greater than those in normal tissue, with a >3.7-fold mean ratio. Tumor concentrations of the active AQ4 are well above IC_{50} values needed *in vitro*. AQ4N is well tolerated, without serious toxicity. DLT and MTD were not reached in this trial, but a dose for future development was chosen before toxicity occurred using PK targeting and from a demonstration of the preferential conversion to AQ4 in tumor tissue. No excess normal tissue radiation-related morbidity was observed (Benghiat A, etal, ASCO05, Abs. 2062, and Benghiat A, etal, ASCO04, Abs. 2091).

In December 2003, Novacea (South San Francisco, CA) licensed from KuDOS the North American rights to develop and commercialize AQ4N in all indications in exchange for development milestones and royalty payments. KuDOS will continue to develop AQ4N in Europe and other markets outside North America.

In September 2004, Novacea enrolled the first of 45 patients in an open label, multidose, phase I clinical trial (protocol ID: 021-001, NCT00090727) with AQ4N in patients with advanced solid tumors or non-Hodgkin's lymphoma (NHL). Primary objectives of the trial are to

determine safety and tolerance of multiple weekly IV doses of AQ4N, as well as to obtain MTD. Kyriakos P Papdopoulos, MD, of the Cancer Therapy & Research Center (CTRC; San Antonio, TX), is the PI.

In April 2005, Novacea initiated a multicenter, open label, dose-escalation, phase I/II clinical trial to test AQ4N in up to 55 patients with B-cell neoplasms, including NHL and chronic lymphocytic leukemia (CLL). Primary objectives of this trial are to establish the drug's MTD, determine its PK profile, and evaluate its safety and tolerability. In addition, the trial will evaluate evidence of antitumor activity as measured by ORR.

C-1311 (Symadex), under development by Xanthus Life Sciences (Cambridge, MA), is a substituted imidazoacridone designed to have similar or improved efficacy in comparison to mitoxantrone, but with reduced side effects such as cardiac and hematologic toxicities. Symadex induces DNA damage, leading to apoptosis and G2/M blockade. However, although the drug is nominally classified as a more traditional topoisomerase II inhibitor and DNA intercalator, it exerts a profound, if pleiotropic effect, on major gene targets.

In a preclinical study, cells were grown in the presence of Symadex, and then harvested along with untreated control fractions. Using bioarrays, genes in colorectal cell lines HT29 and HCT116, were considered to be differentially expressed if a change from baseline could be demonstrated as significant. At each time point tested, a cohesive set of 35 differentially downregulated genes were identified in the HT29 cells affecting DNA replication, cell proliferation, signaling, and focal adhesion ontologies. Among these, there was significant downregulation of >50 times of COLIA2, IBSP, RB1, RPL4, LIMS2, SYNE1, and of >20 times of CSRP3, IL1RL1, IL7R, ITGA9, FOXF2, MAPT, and PECAM1. By contrast, the effect on HCT116 was more muted, but broader, exhibiting downregulation of 110 genes (2-5 fold difference over control), affecting DNA replication, DNA and RNA repair, cell-cycle checkpoints, and mitotic arrest. These downregulated genes were clustered around the CDC2, E2F4, MYC, and TP53 regulatory networks. This experiment links downregulation of distinctive sets of genes with the biochemical mode of action of Symadex in two carcinoma cell lines with divergent patterns of regulatory gene expression (Cole J, etal, AACR05, Abs. 55).

In another preclinical study, C-1311 was combined with anticancer drugs irinotecan, oxaliplatin, 5-FU, amonafide L-malate (Xanafide; Xanthus Life Sciences), and etoposide. Combinations were used to treat HT29 and HCT116 cell lines *in vitro*. Agents were individually tested using 5-day exposures to determine their GI50. C-1311 was the most active agent in both cell lines. Agents were then combined using fixed GI_{50} with variable concentrations of the paired drug to allow testing of various drug ratios. Combinations of imidazoacridone with irinotecan, oxaliplatin, and etoposide were not synergistic in either cell line. Combinations of GI_{50} C-1311 with varying concentrations of Xanafide or 5-FU were also not synergistic. However, combinations of GI_{50} Xanafide or 5-FU with varying concentrations of C-1311 were highly synergistic. At nontherapeutic doses of C-1311, Xanafide and 5-FU-induced growth inhibition increased from the expected 50% to >80%. Therefore, combinations of C-1311 with Xanafide or 5-FU may be active regimens in treatment of colon cancer. Observed synergy of C-1311 and 5-FU is particularly interesting, as it may also apply to a combination of C-1311 with capecitabine (Xeloda; Roche) for treatment of colorectal cancer; 5-FU was used as a surrogate because capecitabine is not activated *in vitro* (Paterson J, etal, AACR05, Abs. 4987).

C-1311 is being evaluated in two phase I/II clinical trials, testing different dosing schedules, in 15-20 patients with advanced solid tumors. The primary goal of the trials is to determine the optimal dosing schedule to be used in phase II clinical trials, and to evaluate the PK profile of Symadex in order to understand whether individuals vary significantly in their ability to metabolize the drug.

In June 2004, a dose-escalation, phase I clinical trial was initiated with Symadex, administered by a once weekly IV infusion schedule, every 3 out of 4 weeks, to patients with advanced solid tumors. The dose is increased as each patient is added to the trial until MTD is reached, when additional patients will be treated to establish a dose for use in subsequent trials.

In August 2004, a second dose-escalation, phase I clinical trial (protocol ID: N0123164256) with Symadex was initiated at Leicester General Hospital NHS Trust, in the UK, under PI Ann Thomas, MD, to test a daily dosing regimen. In this trial, patients are treated with 1-hour daily infusions, for 5 consecutive days, every 3 weeks. The dose is increased as each cohort of patients is added until MTD is reached, at which point additional patients are treated at a dose level under MTD. This trial was closed in July 2005.

CHR-2797, under development by Chroma Therapeutics (Oxon, UK), is a novel, synthetic, orally active metalloenzyme inhibitor with pleiotropic activity against a range of human malignancies. A substantial body of evidence indicates that molecular targets underpinning these pleiotropic effects are intracellular aminopeptidases. Both CHR-2797 and an active metabolite, CHR-79888, are pharmacologically active, and may exert their antiproliferative effect by disruption of the turnover of cell-cycle intermediates. CHR-2797 shows selective antiproliferative activity against transformed cells over normal cells.

CHR-2797 strongly inhibits lung tumor burden and spontaneous lung metastases of MDA-MB 435 mammary tumors in murine xenograft models. Orally administered CHR-2797 inhibited lung tumor burden by 98%, affecting both colony size and number. The inhibitory effect of CHR-2797 on spontaneous metastasis was also investigated in

MDA-MB 435 xenografts in nude mice. Oral CHR-2797 profoundly inhibited number and size of tumors on the lung surface. Longer term administration of CHR-2797 to nude mice was well tolerated (Hooftman LW Sr, etal, AACR05, Abs. 637).

Results show that orally administered CHR-2797 strongly inhibits increases in tumor burden, tumor weight, and colony number, in a dose-dependent fashion, when administered to rats in syngeneic HOSP1 P cancer models. HOSP1 P cells were injected IV and localized to the lungs. By day 2, all injected cells were extravasated or cleared from circulation. Once daily oral administration was initiated 2 days after tumor cell inoculation and continued throughout the experiment. A total of 3 cohorts of at least 8 animals per group were administered three dose levels. A clear dose-response relationship was observed for inhibition of tumor burden, tumor weight, and colony number. In the third dose group, statistically significant differences were seen for all 3 parameters, whereas at the second dose, decreases in tumor burden and colony number were significant. At the first dose level, only reduction in tumor burden was significant compared to controls. A similar effect was shown when HOSP1 P tumors were grown subcutaneously. In this experiment, two other dose levels commenced when the tumors became palpable. There was significant inhibition of tumor growth (with both doses) and dose dependence (Eccles S, etal, AACR05, Abs. 5850).

Exhibit 2
Overall Responses in Phase I Clinical Trials

Treatment Regimen	Patients (#)	ORR (#)	ORR (%)	ORR+SD (#)	ORR+SD (%)
Cytotoxics					
Novel/Single	2,341	103	4.4	1,058	45.2
Novel/Multiple	273	32	11.8	107	39.3
Novel+Approved	2,251	369	16.4	1,074	47.7
Approved Combo	792	217	27.4	432	54.6
Subtotal	5,657	721	12.8	2,672	47.2
Immunotherapy					
Novel/Single	203	23	11.4	95	46.9
Novel/Multiple	651	46	7.0	191	29.3
Novel+Approved	392	102	26.0	207	52.7
Subtotal	1,246	171	13.7	493	39.5
Regulation					
Novel/Single	1,347	43	3.2	572	42.5
Novel/Multiple	81	6	7.4	28	34.6
Novel+Approved	935	108	11.6	458	49.0
Subtotal	2,363	158	6.7	1,059	44.8
Angiogenesis Inhibition					
Novel/Single	335	13	3.9	117	34.9
Novel+Approved	135	20	14.8	70	51.8
Subtotal	470	33	7.0	187	39.8
Vaccine					
Novel/Single	265	9	3.4	75	28.3
Novel/Multiple	198	2	1.0	72	36.4
Novel+Approved	111	6	5.4	28	25.2
Subtotal	574	17	3.0	175	30.5
Gene Therapy					
Novel/Single	89	3	3.4	30	33.7
Novel+Approved	3	0	0.0	0	0.0
Subtotal	92	3		3	3.3
All Trials					
Total	10,402	1,103	10.6	4,588	44.1

Legend: ORR=CR+PR

Source: Horstmann E, etal, Risks and benefits of phase I oncology trials from 1991 through 2002, NEJM, 3 Mar 2005;352(9):895-904

A phase I clinical trial was initiated in October 2004 at Royal Marsden NHS Foundation Trust (Sutton, Surrey, UK), under PI Johann De Bonoto, MD, in patients with refractory, advanced or metastatic solid tumors, to assess the toxicity profile and PK and establish safety and tolerability of CHR-2797.

CMC-544, under development by Wyeth Research (Pearl River, NY), in collaboration with UCB Pharma (Brussels, Belgium), is a cytotoxic immunoconjugate comprising a humanized MAb targeting CD22 conjugated by an

acid-labile linker to to the antibiotic calicheamicinDMH (calichDMH), capable of depleting malignant B cells. Calicheamicin is a potent cytotoxic natural product that binds DNA in the minor groove and causes double strand DNA breaks. Antibody-targeted chemotherapy using tumor-targeted immunoconjugates of the cytotoxic agent calicheamicin, is a clinically validated strategy for the treatment of acute myeloid leukemia (AML). The approved cytotoxic Mylotarg, also developed by Wyeth, is such an agent.

CD22 was chosen as the B-lymphoid lineage-specific antigen, after CD19, CD20, and CD22 were studied extensively as potential targets for therapeutic applications of immunotoxins. In order to determine which one of these three antigens is most suitable for antibody-targeted calicheamicin therapy, MAb BU12 (murine anti-CD19 MAb), rituximab (chimeric anti-CD20 MAb), and m5/44 (murine anti-CD22 MAb) were conjugated to a hindered disulfide derivative of N-acetyl gamma calicheamicin. The antitumor activity of these conjugates was evaluated against three human B-cell lymphoma lines (BCL), Ramos, Raji, and RL. Each of these three MAb bound to their respective antigens on the surface of BCL and was modulated, indicative of their potential internalization. Immunoconjugates of these MAb, prepared by covalently linking calicheamicin via either acid-labile or acid-resistant linkers, potentently inhibited BCL growth *in vitro*. Immunoconjugates with acid-labile linkers were more potent than those with acid-stable linkers. Also, conjugates targeted to either CD19 or CD22 were more potent than those targeted to CD20 in inhibiting BCL growth *in vitro*. In contrast, unconjugated MAb to CD19 or CD22 had no effect on BCL growth *in vitro*, whereas anti-CD20 MAb, at concentrations >1 μg/ml, caused a 30% inhibition *in vitro* BCL growth. When examined for their effects on growth of established SC BCL xenografts in nude mice, calicheamicin conjugated to anti-CD22 was by far the most efficacious conjugate against each of the three BCL xenografts studied. Calicheamicin conjugated to rituximab caused significant inhibition of BCL growth, but was less effective than the anti-CD22 or anti-CD19 MAb conjugates. Interestingly, anti-CD19 conjugates of calicheamicin, while effective *in vitro* against both Raji and Ramos BCL and against Raji BCL xenografts, had no effect on growth of Ramos BCL xenografts *in vivo*. Reasons underlying this lack of antitumor activity have not been elucidated (DiJoseph JF, etal, ASH04, Abs. 2490).

Scientists at Wyeth also investigated preclinical antitumor activity of a combination of CMC-544 and rituximab (Rituxan; Biogen Idec) both *in vitro* and *in vivo*. CMC-544 inhibited *in vitro* growth of 3 CD20+, CD22+ B-lymphoma cells (Ramos, Raji, or Daudi) with subnanomolar IC_{50}. Rituximab alone caused a weak (20%) inhibition of B-lymphoma cell growth. Cytotoxic activity of CMC-544 was significantly enhanced by up to 4-fold by addition of rituximab, suggestive of supra-additive cytotoxic activity of the combination of CMC-544 and rituximab. In xenograft models, although each compound by itself caused significant inhibition of tumor growth, the effect of CMC-544 was maintained longer than that of rituximab. In contrast, combination of CMC-544 and rituximab completely suppressed tumor growth. In the disseminated B-cell lymphoma model, up to 60% of the CMC-544-treated mice and 20% of the rituximab-treated mice survived for 125 days. In contrast, 90% of mice treated with the combination of CMC-544 and rituximab survived for 125 days (DiJoseph JF, etal, AACR05, Abs. 700).

A multicenter, international, open label, phase I clinical trial (protocol ID: 3129K1-100-WW, NCT00073749) was initiated in February 2004, to evaluate safety, tolerability, and PK of CMC-544 as monotherapy in patients with B-cell NHL. CMC-544 is being administered IV approximately once every 21 days (±2 days) for at least 4 doses unless there is evidence of PD. Dose escalation decisions are based on toxicity assessed in the first 21 days after the first dose. Patients may be treated with additional doses of CMC-544, beyond the first 4 doses, if eligible. The trial is taking place in the USA, at Robert H. Lurie Comprehensive Cancer Center at Northwestern University (Chicago, IL), Cleveland Clinic Taussig Cancer Center, and Fox Chase Cancer Center (Philadelphia, PA), and in Europe, at Hopital Saint-Louis (Paris, France), Universitaetsklinikum Bonn and Klinikum Grosshadern der Ludwig-Maximilians Universitaet Muenchen, in Germany, Johannes Gutenberg University (Mainz, Germany), Hospital Clinic de Barcelona in Spain, Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland), and Saint Bartholomew's Hospital (London, UK).

CYC682, under development by Cyclacel (Dundee, UK), in collaboration with Sankyo Pharma (Parsippany, NJ), is a prodrug that is converted by intestinal and plasma amidases to the active agent CNDAC [1-(2-C-cyano-2-deoxy-β-D-arabino-pentafuranosyl)-cytosine], and intracellularly activated by deoxycytidine kinase to CNDAC triphosphate. The drug's mechanism of action includes inhibition of DNA polymerase, cessation of DNA strand elongation, and DNA strand breakage.

In evaluating CYC682, in addition to other objectives, investigators are also identifying biomarkers for use in clinical trials with CYC682. Nucleoside kinases, like deoxycytidine kinase, are needed to activate CYC682, while CYC682 can be deactivated by phosphatases and deaminases, such as cytidine deaminase. It is, therefore, hypothesized that CYC682 will be more potent in combating tumors that contain a greater proportion of nucleoside kinases relative to phosphatases and deaminases.

CYC682 completed two phase I clinical trials in the USA involving 88 patients with a variety of tumors. According to results, CYC682 is well tolerated in man with myelosuppression as the DLT. SD was observed in 17 patients after CYC682 treatment, including 1 patient with ovarian cancer who experienced a minor response (MR)

and 1 patient with a GIST whose disease stabilized for over 90 weeks after failing multiple therapies.

An open label, phase Ib clinical trial (protocol ID: CYC682-03-03) of CYC682 was approved in May 2004 (IND #53,748), and initiated in September 2004, at Fox Chase Cancer Center under protocol ID: PCYCLAC001, in patients with refractory solid tumors or lymphoma. The trial's primary objective is to evaluate the safety profile of escalating doses of CYC682. The drug is being administered in 14-day cycles, every 3 weeks, until evidence of disease progression. Secondary objectives are to analyze PK of CYC682 and its metabolite, to investigate use of biomarkers to predict tumor sensitivity, and to assess tumor response. The trial is taking place at two clinical centers in the USA.

In a previous phase I clinical trial involving administration of CYC682 on a 5 days/week, for 4 weeks every 6 weeks schedule, MTD was determined at 40 mg/m^2/day, with neutropenia as the principal toxicity. To maximize plasma exposure, a twice daily oral administration was evaluated on days 1-14 of a 21-day cycle, with determination of MTD and recommended phase II dose as objectives. Dose levels of 20, 40, 66, and 100 mg/m^2/day were explored. Blood samples for PK analysis of CYC682 and CNDAC were drawn on days 1 and 14 of the first cycle and on day 14 of the third cycle. A total of 15 patients were administered 22 cycles of CYC682; principal tumor types were breast cancer (n=2), nsclc (n=3), ovarian cancer (n=2), and colon cancer (n=2). All patients but 1 had been pretreated with numerous chemotherapy regimens (median=4, range=1-6). DLT of febrile neutropenia and thrombocytopenia occurred in 2/6 patients at dose level 3; MTD attributable to febrile neutropenia was observed in 3/3 patients at dose level 4. Expanded enrollment is ongoing at 66 mg/m^2/day. Nonhematologic toxicities including diarrhea have been observed, but are manageable. PK data based on 12 patients shows a moderate correlation between CNDAC exposure and CYC682 dose. Terminal half-life in dose level 3 is 2.3 ±0.9 hours. MTD for this schedule is 100 mg/m^2/day, with febrile neutropenia as DLT (Tolcher A, etal, ASCO05, Abs. 2026).

DOXO-EMCH, a (6-maleimidocaproyl) hydrazone of doxorubicin under development by the Aventis Pharma Deutschland (Hattersheim, Germany) unit of sanofi-aventis, in collaboration with the Hospital for Tumor Biology at the University Freiburg in Germany, is an albumin-binding prodrug of doxorubicin with acid-sensitive properties, developed to bind rapidly and selectively to the cysteine-34 position of endogenous albumin after IV administration. As a result of incorporation of an acid-sensitive carboxylic hydrazone bond between the drug and the spacer molecule, albumin-bound doxorubicin is released in the acidic environment of the tumor cell. DOXO-EMCH was developed in 1999, by a research group at the Hospital for Tumor Biology, working in the area of 'macromolecular prodrugs' by chemically modifying doxorubicin so that after IV administration, it bound quickly and selectively to albumin, a protein present in abundance in the blood, which also accumulates in solid tumors and enters tumor cells by endocytosis. Because serum albumin accumulates in solid tumors, coupling an anticancer prodrug to albumin delivers it to its target site, preventing its diffusion into healthy tissue. The prodrug is inactive in circulation. Doxorubicin is released from this prodrug only after it enters the acid environment of tumor tissue and tumor cells.

In animal models, DOXO-EMCH was released in tumor cells, acting as an efficient prodrug that was significantly more efficacious than free doxorubicin. Toxicity studies with DOXO-EMCH in mice, rats, or dogs did not identify any other special toxicity when compared to toxicity data for doxorubicin. Preclinical tolerance for DOXO-EMCH was considerably higher in mice, rats, and dogs compared to doxorubicin (Kratz F, etal, AACR05, Abs. 4192). Also, DOXO-EMCH was associated with a reduced incidence of immunosuppression, inflammation of the mucous membranes, vomiting, and hair loss.

A phase I clinical trial was initiated in August 2003, at the University of Freiburg, under PI Clemens Unger, MD. The objective of this trial is to evaluate the safety profile and PK of DOXO-EMCH in order to assess MTD, evaluate DLT, and preliminarily assess antitumor activity. Cohorts of 3-6 patients with advanced cancer were treated with an IV infusion of DOXO-EMCH over 30 minutes once every 3 weeks at dose levels of 20, 40, 80, 135, 150, 180, and 200 mg/m^2 doxorubicin equivalent. Assessment of tumor size was performed before and after every second cycle. Among 28 patients treated with DOXO-EMCH, no DLT was observed with up to 180 mg/m^2 doxorubicin equivalent doses. Grade 3 somnolence was observed at 200 mg/m^2 in 2/6 patients, but the causal relationship to the treatment remained unclear. Disease stabilized in 8/24 evaluable patients for up to 6 cycles. Drug half-life (t1/2) was ~21 hours. IV of DOXO-EMCH every 3 weeks was well tolerated up to 180 mg/m^2, demonstrating potential antitumor efficacy. Expansion at the dose of 260 mg/m^2 is ongoing in order to determine a safe dose recommendation for phase II trials (Gmehling D, etal, AACR05, Abs. 3987, and Drews J, etal, ASCO04, Abs. 2125).

DTS-201 (CPI-004 Na), under development by Diatos (Paris, France), is a prodrug of doxorubicin belonging to the family of tumor-selective enzyme prodrugs. DTS-201 incorporates the company's Tumor Selective Prodrug (TSP) technology, comprising a 4-amino acid peptide complex (tetrapeptide) conjugated to a cytotoxic agent whose activity is inhibited when conjugated to the peptide. DTS-201 is stable and inactive in human blood, and not taken up by tissues after injection in the mouse. Preclinical studies indicate that the peptide is cleaved by endopeptidases released by cancer cells when prodrug molecules reach the vicinity of a tumor, thereby freeing the cytotoxic compound.

To date, two oligopeptidases involved in the reactivation of the prodrug have been identified, CD10 (CALLA, neprilysin) and TOP (Thimet oligopeptidase), which are overexpressed in some solid tumors. Single and repeated dose administration studies with DTS-201 were conducted in the mouse, rat, and dog. All the clinical signs observed were identical to those already described for doxorubicin, but were noticed at much higher doses indicating that the prodrug reduced systemic toxicity. Doses of DTS-201 up to 8 times the doxorubicin dose were administered without inducing significant cardiomopathy following 7 repeated injections in the rat. To test whether the prodrug DTS-201 is effectively cleaved to an active form *in vivo*, efficacy of DTS-201 and doxorubicin was compared using two tumor xenograft models, exhibiting high (H1299 human colon cancer) or low (PC-3 human prostate carcinoma) CD10 immunostaining intensity. The superior antitumor potency of DTS-201 in the H1299 tumor compared to the PC3 tumor, confirms the role of CD10 in activation of the prodrug.

On the other hand, immunohistochemistry (IHC) analysis of a large panel of human tumor biopsies suggest that CD10 and TOP are both expressed in 50% of prostate tumors and in 98% of breast tumors. In order to reinforce the development rationale of this new peptide-conjugated cytotoxic agent, expression of CD10 and TOP will be evaluated in the tumors of every patient involved in clinical trials with DTS-201 (Delord JP, etal, AACR05, Abs. 4125).

An IND to initiate a phase I/II clinical trial was approved in December 2004 at three oncology centers in France and Belgium to assess safety and PK of DTS-201 in patients with advanced or metastatic tumors.

FFC11 [tris-(8-quinolinolato)gallium(III) complex], under development by Faustus Forshung (Leipzig, Germany), is an orally bioavailable gallium complex with antitumor activity. The drug inhibits ribonucleotide reductase, and induces S phase arrest and apoptosis. In preclinical models, FFC11 was a more potent anticancer agent than gallium nitrate. Also, FFC11 was effective against a model of tumor-associated hypercalcemia.

In a completed phase I clinical trial, FFC 11 was administered orally for 14 days followed by a 14-day recovery period representing one cycle of therapy, at an initial dose of 30 mg/m^2, escalated by 100%, based on an accelerated dose-titration scheme. The accelerated phase is completed when DLT occurs in 1 patient, or when 2 patients experience Grade 2 toxicities. Further dose escalation then continues based on a modified Fibonacci scheme. Tumor response is being assessed using RECIST criteria. PK parameters were estimated assuming a one-compartment model. A total of 7 patients with parotid gland, stomach, renal cell cancer (n=4), and ovarian cancer were entered at dose levels of 30 mg/m^2, 60 mg/m^2, 120 mg/m^2, 240 mg/m^2, and 480 mg/m^2. Most patients were heavily pretreated. Adverse events for which a relationship to the drug could not definitely be ruled out were Grade 2 neutropenia (n=1) and anemia (4=2) at dose level 1; Grade 1 stomatitis and conjunctivitis (n=1) at dose level 2; Grade 1 dizziness, headache and acne (n=1) at dose level 4; and Grade 1 fatigue and Grade 3 diarrhea, in 1 patient, at dose level 5; the latter represented a DLT. Further enrollment on this dose level was terminated because of feasibility considerations. There was an unconfirmed PR in 1/4 patients with renal cell carcinoma (RCC), after 8 weeks, and SD in a second patient with RCC lasting 29 weeks. Peak plasma levels were reached 5 to 7 hours after intake. PK analysis revealed a long terminal half-life of 28 hours. FFC11 was well tolerated, with some preliminary evidence of efficacy in RCC. The trial was conducted by the Central European Society for Anticancer Drug Research (CESAR)-EWIV. FFC11 will be developed further after production of higher dose tablets (Dittrich C, etal, ASCO05, Abs. 3205).

FFC14A (KP1019), also under development by Faustus Forschung, was selected from >100 ruthenium compounds for clinical development because of its activity in colorectal cancer models, and in a variety of freshly explanted human tumors including primary chemoresistant specimens. Ruthenium interferes with iron-dependent enzymes, and may cause cellular damage by substitution and release of iron.

FFC14A uses the natural iron transport protein transferrin (Tf) to target tumor cells overexpressing transferrin receptors *in vivo*. FFC14A binds to transferrin and albumin, and is taken up preferentially by tumor cells via overexpressed Tf receptors. The drug accumulates in the target tissue, where it induces apoptosis and a change in mitochondrial membrane potential. After intracellular release from Tf, FFC14A is activated by reduction, forms DNA crosslinks, interferes with the mitochondrial electron transport chain, and, finally, induces apoptosis.

In a multicenter, dose-escalation, phase I clinical trial, FFC14A was administered IV at an infusion rate of 10 ml/minute, starting at a total dose of 25 mg, twice weekly, over 3 weeks, followed by 2 weeks off therapy. Dose was escalated by 100% until occurrence of toxicity or of DLT, when further dose escalation was based on a modified Fibonacci scheme. PK profiles were obtained after the first and fifth application within the first treatment cycle. Ruthenium was measured in plasma and ultrafiltrated plasma, and PK parameters were estimated assuming a 2-compartment model. A total of 8 patients were entered at the 25 mg, 50 mg (n=2), 100 mg, 200 mg, 400 mg, and 600 mg (n=2) dose levels. Malignancies included bladder, liver, endometrial, tongue, and colon (n=3) cancer, and melanoma of the chorioidea. Most patients were heavily pretreated. The first patient treated with 25 mg of FFC14A tolerated 6 applications well without any side effects. Generally, FFC14A was well tolerated in the doses studied. Adverse events observed in some patients, for which a relationship to the drug could not definitely be ruled out, were predominantly Grade 1; DLT was not

Exhibit 3
Responses in Monotherapy Phase I Clinical Trials

	Trials (#)	Patients (#)	CR Patients (#)	CR (%)	PR Patients (#)	PR (%)	ORR Patients (#)	ORR (%)	SD Patients (#)	SD (%)	ORR+SD Patients (#)	ORR+ SD (%)
Cytotoxics	92	2,341	35	1.5	68	2.9	103	4.4	955	40.8	1,058	45.2
Immunotherapy	13	203	6	3.0	17	8.4	23	11.4	72	35.5	95	46.9
Regulation	51	1,347	9	0.7	34	2.5	43	3.2	529	39.3	572	42.5
Angiogenesis	11	335	2	0.6	11	3.3	13	3.9	104	31.0	117	34.9
Vaccine	15	265	8	3.0	1	0.4	9	3.4	66	24.9	75	28.3
Gene Transfer	7	89	0	0.0	3	3.4	3	3.4	27	30.3	30	33.7
Total	189	4,580	61	1.3	134	2.9	194	4.2	1,753	38.3	1,948	42.5

Legend: ORR=CR+PR
Source: Horstmann E, etal, Risks and benefits of phase 1 oncology trials from 1991 through 2002, NEJM, 3 Mar 2005;352(9):895-904

observed. Dose escalation was terminated because of feasibility. Disease stabilized after the first treatment cycle in 5 patients with confirmed PD at trial entry. According to RECIST criteria, SD lasted between 8 to 10 weeks. In terms of PK, FFC 14A was extensively protein bound, with a long terminal half-life (range=105-284 hours), a low total clearance, and a small volume of distribution. The dose-proportional increase of ruthenium AUC and Cmax indicates linear PK. The trial was conducted by the Anticancer Drug Research CESAR-EWIV group. Participating institutions included University of Essen Medical School and University of Bonn in Germany, and University of Vienna in Austria (Dittrich C, etal, AACR05, Abs. 472, and Scheulen ME, etal, ASCO04, Abs. 2101). This trial was completed in October 2005.

In December 2004, Bioaccelerate (New York, NY) and Faustus Forschung entered into an agreement to codevelop 8 new anticancer drug candidates, 5 of which are in clinical and 3 in preclinical development. Under terms of agreement Bioaccelerate will fund further development of the portfolio through to commercialization. The agreement will leverage both parties' extensive experience in oncology development, with Faustus using its facilities for development work.

KOS-1584 (9,10 didehydroepothilone D), under development by Kosan Biosciences (Hayward, CA) in collaboration with Roche, is a second generation epothilone. It is a rationally designed novel and potent analog of KOS-862 (epothilone D), a polyketide that inhibits cancer cell growth *in vitro* by a mechanism similar to that of paclitaxel, but with improved potency and PK properties. KOS-1584 was identified as part of a systematic effort to identify a new generation of epothilones that possess greater potency with improved pharmacologic properties.

In preclinical studies, KOS-1584 effectively induced tubulin polymerization in a cell-based assay using MCF-7, a breast cancer cell line, arresting cells at G2/M phase of the cell cycle. Potent antiproliferative activity against a broad range of tumor cell lines was observed *in vitro*, including hematologic and solid tumor cell lines, such as leukemia, and breast, colon, lung, and ovarian cancer, with an average IC_{50} that was 3-12 times more potent compared to KOS-862 in the same experiment. The *in vivo* anticancer activity of KOS-1584 has been studied in several animal models, including hollow fiber models of A549 lung adenocarcinoma, HT-29 colorectal carcinoma, SKOV3 ovarian adenocarcinoma, MV522 human lung xenograft models in nude mice, and HCT-116 human colorectal xenograft model in nude rats. KOS-1584 significantly inhibited tumor-cell growth in the hollow fiber models, and reduced tumor size in MV522 xenograft model in mice and HCT-116 xenograft model in rats; antitumor effects were observed at 3-5 mg/kg, compared to 30 mg/kg with KOS-862. In addition, KOS-1584 was selected for development on the basis of its desirable PK properties. Allometric scaling, using the PK of KOS-1584 in the mouse, rat, dog, and monkey, predicted significantly improved PK properties in humans. Consistent with improved potency, severely toxic doses in the rat and dog were 3-16 fold lower comparing the two epothilones with identical schedules. KOS-1584 is being evaluated in clinical testing using a cyclodextrin formulation (Zhou Y, etal, AACR05, Abs. 2535).

In December 2004, Kosan Biosciences initiated a phase I clinical trial program to evaluate KOS-1584 in patients with advanced malignancies. This first trial, a dose-escalation, phase I trial to establish MTD, PK, pharmacodynamics, safety, and potential clinical benefit of the drug when administered as an IV infusion every 3 weeks, was initiated at Arthur James Cancer Center at Ohio State University, under PI Miguel Villalona, MD.

IpdR, under development by Hana Biosciences (South San Francisco, CA), is a novel, orally available, halogenated deoxythymidine (dThd) analog and prodrug of IUdR. IUdR is selectively incorporated into tumor-cell DNA result-

ing in radiosensitization. High grade brain tumors usually treated with radiotherapy are ideal targets for this therapeutic approach.

In a preclinical study, the effect of IPdR was tested at various doses with or without radiation on growth of U87 glioblastoma multiforme (GBM) tumors in xenograft models. The exponentially growing U87 cells (3x106) were implanted SC into the right hind leg of athymic mice, and the animals were treated with IPdR. In a dose-dependent manner, IPdR sensitized glioblastoma xenografts to radiation, and also inhibited glioblastoma growth as monotherapy. Results demonstrate a dose-response relationship such that IPdR acts synergistically with radiation to inhibit tumor growth and alone IPdR (500 mg/kg/day) has the same effect on tumor growth as radiation (2 Gy/day) alone (Pei L, etal, AACR05, Abs. LB-242).

In September 2005, a phase I clinical trial was initiated with IPdR in colorectal, gastric, pancreatic, and liver cancer, to establish safety, dose, and preliminary efficacy of IPdR in combination with radiation therapy. As of September 2005, a phase I/II clinical trial of IPdR in GBM was being planned.

MPC-2130, under development by Myriad Pharmaceuticals (Salt Lake City, UT) is a small molecule drug discovered through an extensive medicinal chemistry effort. The original lead compound, from which MPC-2130 was derived, was discovered in a yeast-based high throughput screen. Several indicators of apoptotic cell death are observed in tumor cells treated with MPC-2130, including phosphatidylserine flipping to the outer cell membrane, release of cytochrome C from the mitochondria, caspase activation, cell condensation, and DNA fragmentation. Also, MPC-2130 is equipotent in inducing apoptosis in cancer cell lines, regardless of expression levels for the multidrug resistance ABC transporters MDR-1 (Pgp-1), MRP-1, and BCRP-1.

In preclinical evaluations of the drug's proapoptotic activity in tissue culture models using multiple solid and hematopoietic derived cell lines, solid tumor lines responsive to MPC-2130 include ovarian cancer cell line OVCAR-3, and prostate cancer cell lines LNCaP and PC-3. Responsive bone marrow-derived tumors include T-cell lines (H9, Molt-4, and Jurkat), as well as Burkitt's lymphoma (Daudi and Ramos).

The ability of MPC-2130 to inhibit growth of OVCAR-3 and LNCaP tumor lines was investigated in SC implanted cancer cells into athymic nude mice. In OVCAR-3 xenografts, IV MPC-2130 significantly increased survival when compared to vehicle. Significant inhibition of the growth of OVCAR-3 xenografts was observed in animals treated with MPC-2130 when compared to vehicle treatment alone. Also, MPC-2130 significantly inhibited growth of the LNCaP xenografts when compared to animals treated with vehicle alone. Terminal elimination half-life was approximately 6.0 hours (Pleiman C, etal, AACR05, Abs. 641).

An open label, dose-escalation, multiple dose, phase I clinical trial was initated in September 2005 at M. D. Anderson Cancer Center (Houston, TX), under PI Francis J Giles, MD, to determine safety, tolerability, MTD, and PK of MPC-2130 administered as a daily IV infusion for 5 days, repeated every 21 days, in patients with refractory malignancies. The trial's primary objective is to find the highest tolerable dose of MPC-2130 that can be administered to patients with resistant disease, and to establish MTD of MPC-2130 as a single agent in this setting. Secondary objectives are to observe for any evidence of antitumor activity of MPC-2130, and characterize the PK profile of MPC-2130. A total of 20 patients will be enrolled this trial.

MPC-6827, under development by Myriad Pharmaceuticals, in collaboration with Maxim Pharmaceuticals (San Diego, CA), is a small molecule inhibitor of microtubule formation that is not a substrate for multidrug resistance (MDR) pumps. MPC-6827 is being developed for treatment of primary or metastatic tumors, including those of the central nervous system (CNS) that have progressed despite best standard treatment.

ADME properties suggest that MPC-6827 may be well suited for treatment of primary or metastatic tumors arising within the CNS. Distribution of this drug into the brain was evaluated in mice by treating them with a single IV dose of MPC-6827. The time to maximum drug concentration of MPC-6827 was just three minutes in both blood plasma and brain tissue, indicating that MPC-6827 distributed rapidly into the CNS. Brain concentration of MPC-6827 was approximately 15 times higher than plasma concentration. Concentration of MPC-6827 in the brain was 825 times greater than that required to activate caspases and induce apoptosis in cancer cells *in vitro*. Importantly, MPC-6827 was cleared from the brain at a similar rate as from the blood. Primary metabolic products of MPC-6827, i. e., the demethyl metabolite and its glucuronide and sulfate conjugates, were all found predominantly in urine. MPC-6827 is distributed rapidly and extensively into the CNS, exhibiting 14-fold higher brain exposure relative to plasma and elimination half-life similar to plasma. In addition, MPC-6827 is not a substrate for the main MDR pumps (Jessing K, etal, AACR05, Abs. 3413).

The high brain concentration was achieved at a safe therapeutic dose for treatment of peripheral tumors in mice. Importantly however, it is theorized that a much lower dose in humans should result in brain concentrations of MPC-6827 sufficient for antitumor activity without peripheral toxicity. Temozolomide (Temodal; Schering Plough), exhibiting the highest brain penetration percentage of any drug currently used in treating brain cancer, reaches a peak brain concentration level that is just 29% of the blood plasma concentration. The strong and selective brain penetration of MPC-6827 suggests a special opportunity to study its antitumor activity in patients with primary brain tumors and brain metastases resistant to current treatment approaches.

In order to determine the molecular target of MPC-6827, three structurally related photoaffinity and radiolabeled homologs of MPC-6827 were synthesized. All three were found to bind a 55 kDa protein, competing with MPC-6827, paclitaxel, and colchicine, but not vincristine. MPC-6827 effectively inhibited polymerization of tubulin *in vitro* and disrupted formation of microtubules, but not actin filaments in intact A549, NIH-3T3, NRK, CHO-K1, and MDCK cells. Treatment of either MCF-7 or Jurkat cells led to pronounced G2/M cell-cycle arrest, followed by nearly complete apoptotic response. Unlike tubulin binding vinca alkaloids (vincristine, vinblastine, and navelbine), and taxanes (paclitaxel and docetaxel), MPC-6827 was equipotent for induction of apoptosis in cancer cell lines, regardless of the expression levels for the MDR ABC transporters MDR-1 (Pgp-1), MRP-1, and BCRP-1. These studies demonstrate that MPC-6827 acts through tubulin, a well validated drug target, and might be effective in treatment of MDR malignancies (Baichwal V, etal, AACR05, Abs. 3416).

An open label, dose-escalating, multiple dose, phase I clinical trial was initiated in March 2005 at M. D. Anderson Cancer Center, under PI Razelle Kurzrock, MD, to determine safety, tolerability, MTD, and PK of MPC-6827 administered as a 1-hour IV infusion once weekly for 3 weeks, repeated every 28 days, in patients with refractory solid tumors. The trial will also measure tumor biomarkers, when appropriate and microtubule disruption in peripheral blood white blood cells (WBC). A total of 30 patients will be enrolled this trial.

In March 2005, Myriad Genetics initiated a phase I clinical trial under an FDA-approved IND, to evaluate potential of MPC-6827 in treating metastatic brain cancer by achieving sufficient therapeutic concentrations in the brain to treat tumors without significant systemic exposure or toxicity. This phase I trial is being conducted at Memorial Sloan-Kettering Cancer Center (New York, NY), under PI Lauren E. Abrey, MD.

MST-997 (TL-909) under development by Wyeth, in collaboration with Taxolog (Fairfield, NJ), is a novel taxane with greater apparent potency in preclinical models than paclitaxel. Also, the drug exhibits antitumor activity in cells that express low to moderate MDR1 levels. PK and toxicity profiles also appear improved relative to conventional taxanes.

MST-997 was cytotoxic to human colon cell lines HCT116, HT-29, and drug resistant DLD-1, and the PANC-1 human prostate cell line, with IC_{50} of 0.7 nM, 1.1 nM, 1.0 nM, and 1.5 nM, respectively, compared to values of 2.2 nM, 2.1 nM, >10.0 nM, and 4.1, respectively, for paclitaxel, and 0.6 nM, 0.9 nM, 6.3 nM, and 1.8, respectively, for docetaxel. Incubation of HCT116 cells with varying concentrations of MST-997, caused the numbers of cells in the G1 phase of the cell cycle to decrease, and the number of cells in S and G2/M phases of the cell cycle to increase with increasing concentrations of MST-997, indicating a block in the G2/M phase of the cell cycle. Treatment of cells with MST-997 caused extensive microtubule bundling comparable to treatment with paclitaxel and docetaxel, and there was evidence of nuclear fragmentation and multinucleation, at low concentrations of MST-997. Therefore, TL-909 appears to be a more potent *in vitro* cytotoxic agent in paclitaxel-resistant cells compared to paclitaxel and docetaxel, and exerts effects similar to these taxanes on cell cycle and microtubules (Longley RE, etal, AACR04, Abs. LB-90).

A dose-escalation, phase I clinical trial (protocol ID: 3161K1-100-WW, 3161K1-100, NCT00088647) was initiated in July 2004, at New York University School of Medicine, under PI Howard Hochster, MD, at Cleveland Clinical Taussing Cancer Center, under PI Ronald Bukowski, MD, and at Vanderbilt-Ingram Cancer Center, under PI Albert C. Lockhart, MD, to evaluate safety, tolerability, and determine MTD of IV MST-997, formulated in intralipid 20%, administered without premedications on a weekly schedule to patients with advanced solid tumors, including breast cancer and nsclc.

In this phase I clinical trial, dose escalation of MST-997 proceeded using accelerated dose titration with 1 or 2 patients treated at initial dose levels. Cohorts were expanded to 3 subjects when Grade 2 adverse events were observed. Planned dose levels are 5, 10, 20, 30, and 40 mg/m^2, with PK analysis performed following the first drug dose. Among 9 patients with nsclc, carcinoid tumor, sarcoma, and bladder, ovarian, pancreatic, biliary, and thyroid cancer, treated at dose levels of 5 to 30 mg/m^2, 1 patient developed DLT (Grade 3 fatigue) at the 30 mg/m^2 dose level. Other Grade 3 adverse events that did not meet the criteria for a DLT include neutropenia (n=1) and pain (n=1). There were no CR or PR. Disease stabilized (per modified RECIST criteria) in 3 patients with pancreatic (with unconfirmed decrease in Ca 19-9) and ovarian cancer, and carcinoid tumor. Terminal half-life of MST-997 was long, with values ranging from 13 to 71 hours; Cmax and AUC values appeared to increase with dose level. MST-997 administered in an intralipid formulation appears well tolerated when on a weekly schedule in doses up to 30 mg/m^2. Dose escalation is ongoing, with a confirmatory MTD cohort planned. Additional phase I trials with MST-997 are also planned (Lockhart AC, etal, ASCO05, Abs. 2106).

SB-743921, under development by Cytokinetics (South San Francisco, CA) in collaboration with GlaxoSmithKline, is a novel, small molecule inhibitor of kinesin spindle protein (KSP). This antimitotic is expected to be more specific than other spindle poisons and, therefore, cause fewer side effects than existing therapeutics.

An open label, nonrandomized, phase I clinical trial (protocol ID: 743921/001, NCT00136513), was initiated in May 2004 at the University of Wisconsin Comprehensive Cancer Center and University of Pittsburgh Cancer Institute

Exhibit 4
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)
Antisoma ❑ Archemix, Raylo Chemicals	AS1411 (formerly AGRO100)	G-rich oligonucleotide that binds to nucleolin ❑ continuous IV (CIV)	Phase I (begin 9/03, closed 7/04) ➢USA ❑ advanced, metastatic cancer; phase I (begin 9/05) ➢USA ❑ renal cell carinoma (RCC), non-small cell lung cancer (nsclc)
Aphton ❑ Protein Design Labs, Celltrion	HuABL-364, IGN311 (previously SMART ABL-364)	Humanized IgG1 monoclonal antibody (MAb) directed against Lewis y carbohydrate antigen ❑ IV	Phase I (begin 12/02, completed 9/04)➢Europe (Germany); phase I/II (begin 7/05)➢USA ❑ epithelial cancer
Argos Therapeutics ❑ Duke U, DC Bio		Personalized cancer vaccine composed of dendritic cells (DC) taken from the patient, pulsed with amplified mRNA from the patient's tumor and injected back into the patient ❑ IV, intradermal (ID)	Phase I (completed 01)➢USA ❑ metastatic, hormone-refractory, prostate cancer; phase I (begin 4/05)➢Europe (Germany) ❑ metastatic (Stage IV) melanoma; phase I/II (begin 6/04, ongoing 4/05)➢USA, Canada ❑ metastatic kidney cancer, first line
AstraZeneca	AZD0530	Highly selective dual-specific Src/Abl non-receptor tyrosine kinase inhibitor with anti-invasion activity in a wide range of tumors ❑ PO	Phase I (ongoing 4/05)➢Europe ❑ advanced solid tumors
Attenuon ❑ U Michigan	ATN-224	Second generation copper binding tetrathiomolybdate (TM); small molecule drug that causes tumor cells to decrease production of several factors involved in multiple tumor progression pathways, including CuZn superoxide dismutase (SOD1), leading to apoptotic cell death ❑ PO	Phase I (begin 5/04, ongoing 9/05) ➢Europe (UK) ❑ advanced, refractory solid tumors; phase I (ongoing 9/05)➢USA ❑ advanced hematologic malignancies
Bayer	BAY 57-9352	Inhibitor of vascular endothelial growth factor receptor 2 (VEGFr2) and platelet-derived growth factor receptor β (PDGFrβ) ❑ PO	Phase I (begin 7/03, ongoing 10/05) ➢Europe (Germany) ❑ advanced, refractory solid tumors
Bristol-Myers Squibb	BMS-599626	Pyrrolo[2,1-f][1,2,4]triazine dual HEr1/HEr2 kinase inhibitor ❑ PO	Phase I (begin 11/04, ongoing 9/05) ➢USA ❑ refractory solid tumors; phase I (begin 3/04, ongoing 9/05) ➢USA ❑ refractory, metastatic solid tumors overexpressing HEr2/neu
Celera Genomics Group	CRA-024781, CG-781	Novel histone deacetylase (HDAC) inhibitor ❑ IV, PO	Phase I (begin 7/05, ongoing 10/05)➢USA ❑ cancer
Celgene	CC-8490, SPC8490	Selective estrogen receptor (Er) modulator (SERM) targeting Erα with antitumor activity independent Er binding ❑ PO	Phase I/II (begin 10/03, completed 6/05)➢USA ❑ malignant glioma
Chemokine Therapeutics ❑ National Cancer Institute (NCI)	CTCE-9908	Antagonist of SDF-1 receptors developed using rational drug design ❑ subcutaneous (SC)	Phase I (begin 12/03, completed 4/05)➢Europe (UK) ❑ nsclc
Chroma Therapeutics ❑ Cancer Research Technology, U Virginia	CHR-2797, CHR2797	Novel, synthetic, orally active metalloenzyme inhibitor with pleiotropic activity against a range of human malignancies, based on inhibition of intracellular aminopeptidases ❑ PO	Phase I (begin 10/04, ongoing 9/05) ➢Europe (UK) ❑ refractory advanced or metastatic solid tumors

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Cylene Pharmaceuticals ❑ U Arizona	CX-3543	Cationic porphyrin; small molecule compound designed to interact with a structurally defined cluster targeting the c-Myc/VEGF quadrome ❑ PO	Phase I (begin 7/05)➢USA ❑ advanced solid tumors or lymphoma
Cytokinetics ❑ GlaxoSmithKline	SB-743921	Novel small molecule inhibitor of kinesin spindle protein (KSP) expected to be more specific than other spindle poisons causing fewer side effects ❑ PO	Phase I (begin 5/04, ongoing 9/05) ➢USA ❑ advanced solid tumors
Diatos	Doxorubicin ❑ DTS-201, CPI-004 Na	Doxorubicin conjugated to a proprietary prodrug peptide ❑ IV	IND (approved 12/04)➢Europe (Belgium, France)
Eisai	E7820, NSC 719239	Aromatic sulfonamide derivative that, by inhibiting alpha-2 integrin, exerts antiangiogenic activity by blocking endothelial cell proliferation and tube formation ❑ PO	Phase I (begin 1/04, ongoing 4/05) ➢USA ❑ metastatic or relapsed solid tumors or lymphoma
Faustus Forschungs Compagnie Translational Cancer Research ❑ Bioaccelerate Holdings	FFC14A, KP1019, FFC 14a	Novel ruthenium(III) complex targeting tumor cells overexpressing transferrin receptors ❑ IV	Phase I (begin 04, ongoing 5/05) ➢Europe (Germany, Austria) ❑ advanced solid tumors
Faustus Forschungs Compagnie Translational Cancer Research ❑ Bioaccelerate Holdings	FFC11, KP46	Orally bioavailable gallium complex that exerts its antitumor activity by inhibition of ribonucleotide reductase, induction of S phase arrest, and apoptosis ❑ PO	Phase I (completed 05)➢Europe (Austria, Germany) ❑ advanced, refractory solid tumors
Geron ❑ Transgenomic, U Texas Southwestern Medical Center (UTSW), U California, U Colorado, Sirna Therapeutics	GRN163L, formerly GRN719	Phosphoramidate antisense oligonucleotide targeting the RNA component (hTR) of telomerase with the addition of a lipid to enhance cellular uptake ❑ IV	Phase I/II (begin 7/05)➢USA ❑ advanced, relapsed or refractory chronic lymphocytic leukemia (CLL)
GlobeImmune ❑ U Colorado Health Sciences Center	GI-4000, GI-4014, GI-4015 and GI-4016 ❑ Tarmogen	Nonpathogenic, heat-killed recombinant Saccharomyces cerevisiae (baker's yeast) genetically engineered to express protein antigens within their cell walls that generate potent T-cell immune responses against cells expressing mutant Ras ❑ injection	Phase I (begin 5/04, ongoing 8/05) ➢USA ❑ advanced, metastatic solid tumors expressing ras mutations
Hana Biosciences ❑ State U New York, Yale U	IPdR	Novel, orally available, halogenated deoxythymidine (dThd) analog and prodrug of IUdR ❑ PO	Phase I (ongoing 9/04)➢USA; phase I (begin 9/05)➢USA ❑ solid tumors
Human Genome Sciences ❑ Cambridge Antibody Technology, Kirin, DakoCytomation, Medarex	HGS-TR2J, HGS-ETR2, KMTR2	Fully human TNF-related apoptosis-inducing ligand receptor 2 MAb (TRAIL-r2) that agonizes TRAIL-r2, blocking tumor growth and inducing apoptosis ❑ IV	Phase I (begin 8/04)➢Canada; phase I (ongoing 8/05)➢USA ❑ advanced, refractory solid tumors
Innate Pharma	BromoHydrin PyroPhosphate (BrHPP) ❑ Phosphostim	Small molecule agonist of Vγ9Vδ2 (γδ) T lymphocytes, a critical peripheral blood lymphocyte (PBL) subset ❑ IV	Phase I (ongoing 9/05)➢Europe ❑ solid tumors
Kirin	KRN951	Quinoline-urea derivative that potently inhibits VEGF-stimulated phosphorylation of VEGFr-1, -2 and -3, resulting in strong antiangiogenic activity ❑ PO	Phase I (ongoing 10/05)➢Japan ❑ solid tumors
Kosan Biosciences ❑ Roche, Memorial Sloan-Kettering Institute for Cancer Research, Stanford U, Harvard College, Eli Lilly	Epothilone D ❑ KOS-1584, KOS 1584, Kos 1584	Second generation epothilone, is a polyketide that inhibits cancer cell growth *in vitro* by a mechanism similar to that of paclitaxel ❑ IV	Phase I (begin 12/04, ongoing 10/05)➢USA ❑ advanced solid tumors

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Kyowa Pharmaceuticals (Kyowa Hakko Kogyo)	KW-2871	Chimeric IgG1k MAb that binds to ganglioside GD3 synthase, a cell-surface antigen expressed in neuro-ectodermal tumors, including in about 90% of malignant melanoma cells	Phase I/IIa (begin 6/02, completed 5/05)➢USA; phase I/II (begin 9/05) ➢USA ❑ inoperable, metastatic melanoma (Stage IV)
KuDOS Pharmaceuticals ❑ BTG, U London, Novacea	Banoxantrone ❑ AQ4N ❑ Hypoxin	Selective hypoxic cell cytotoxin designed to be relatively nontoxic until selectively activated within hypoxic cells of solid tumors by enzyme-mediated reduction; when activated it kills preferentially reservoirs of hypoxic cells and immediately adjacent cells ❑ IV	Phase I (begin 1/01, completed 12/04)➢Europe (UK) (multi-modality) ❑ esophageal carcinoma; phase I (begin 9/04, ongoing 9/05) ➢USA ❑ advanced solid tumors or non-Hodgkin's lymphoma (NHL); phase I/II (begin 3/05, ongoing 9/05) ➢USA ❑ NHL, (CLL)
MethylGene ❑ Taiho Pharmaceutical, Idera Pharmaceuticals	MGCD0103	Orally active small molecule, isotype-selective histone deacetylase (HDAC) inhibitor, resulting in an activity profile genotypically, pheno-typically, and functionally distinct from indiscriminate HDAC inhibitors ❑ PO	Phase I (begin 4/04, ongoing 9/05) ➢USA, Canada ❑ advanced, refractory solid tumors; phase I (begin 12/04, ongoing 9/05) ➢Canada, USA ❑ advanced, refractory hematologic malignancies
Myriad Genetics	MPC-2130 (formerly MPI-176716)	Small molecule drug that induces apoptotic cell death in tissue culture models using multiple solid and hematopoietic derived cell lines regardless of the expression levels for the multidrug resistance ABC transporters MDR-1 (Pgp-1), MRP-1, and BCRP-1 ❑ IV	Phase I (begin 9/05)➢USA ❑ refractory hematologic malignancies
Myriad Genetics ❑ Maxim Pharmaceuticals	MPC-6827	Lead compound within the MX90745 series of small molecule inhibitors of microtubule formation; not a substrate for multidrug resistance (MDR) pumps ❑ IV	Phase I (begin 3/05, ongoing 10/05)➢USA ❑refractory solid tumors; phase I (begin 3/05, ongoing 10/05)➢USA ❑ cancer metastasized to the brain
NeoPharm ❑ Georgetown U	LErafAON, LErafAON-ETU	Liposome-encapsulated antisense phosphorothioate oligonucleotide (LE-AON) that inhibits c-Raf-1 mRNA to control tumor-cell growth ❑ IV	Phase I (begin 3/01, completed 12/04)➢USA (multimodality), phase I (begin 3/01, completed 10/04)➢USA, phase I (begin 11/04, ongoing 7/05)➢USA ❑ advanced, radiation-resistant solid tumors
Novartis	AEE788, NVP-AEE788	Pyrrolo-pyrimidine dual EGFr and VEGFr2 tyrosine kinase inhibitor ❑ PO	Phase I (begin 7/03, ongoing 11/05) ➢USA, Europe (Belgium, Spain) ❑ advanced solid tumors or lymphoma; phase I (closed 2/05) ➢USA ❑ liver metastases of colorectal cancer; phase I/II (begin 6/04, ongoing 11/05)➢USA, phase I/II (begin 3/05, ongoing 11/05)➢USA (combination) ❑ glioblastoma multiforme (GBM)
Novartis	LBH 589, LBH589	Novel hydroxamic acid analog HDAC inhibitor ❑ IV, PO	Phase Ia (ongoing 9/05)➢USA, Europe (Germany) ❑ refractory solid tumors
Onconova Therapeutics ❑ Temple U	ON 01910.Na, ON01910.Na, ON01910	Novel anticancer agent acting as a dual kinase inhibitor affecting cyclin dependent kinase (cdk1), polo-like kinase 1 (PLK1), VEGFr and PDGFr ❑ IV	Phase I (begin 7/04, ongoing 9/05) ➢USA ❑ locally advanced or metastatic solid tumors
OXiGENE ❑ Arizona State U	Oxi 4503, Oxi-4503, OXi4503, CA1P, CA-1-P	Diphosphate prodrug form of combretastatin A1 (CA1P), a water-soluble ortho-quinone prodrug with antitumor and antivascular effects ❑ IV	Phase I (begin 4/05, ongoing 9/05) ➢Europe (UK) ❑ advanced solid tumors

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Pfizer	SU014813	Broad-spectrum receptor tyrosine kinase (RTK) inhibitor, similar to SU011248, that inhibits ligand-dependent and independent proliferation, migration and tube formation of endothelial cells and/or tumor cells expressing Flk-1/KDR (VEGFr2), PDGFrα and ß, c-Kit, and Flt-3 ❑ PO	Phase I (ongoing 9/05)➢Europe (Germany, the Netherlands ❑ advanced, refractory solid tumors
PSMA Development ❑ Memorial Sloan-Kettering Cancer Center, Progenics Pharmaceuticals, Cytogen		Vaccine comprising highly purified recombinant soluble human PSMA (rsPSMA) protein combined with an adjuvant ❑ SC	Phase I (begin 12/02, completed 05)➢USA ❑ newly diagnosed or recurrent prostate cancer
Raven Biotechnologies	RAV12	Chimeric form of glycotope-specific IgG1 MAb KID3 with potent anti-proliferative activity ❑ injection	Phase I (begin 1/05, ongoing 9/05) ➢USA ❑ advanced gastrointestinal adenocarcinoma
Sankyo ❑ Cyclacel	CYC682 (formerly CS-682)	Orally available prodrug that is converted by intestinal and plasma amidases to the active agent CNDAC [1-(2-C-cyano-2-deoxy-β-D-arabino-pentafuranosyl)-cytosine] and intracellularly activated by deoxycytidine kinase to CNDAC triphosphate; inhibits DNA polymerase, DNA strand elongation, and DNA strand breakage ❑ PO	Phase I (begin 2/97, completed 02) ➢USA ❑ refractory solid tumors; phase Ib (begin 9/04, ongoing 8/05) ➢USA ❑ refractory solid tumors or lymphoma
sanofi-aventis ❑ Ajinomoto	AVE8062A, AVE8062 (formerly AC-7700)	Synthetic, water-soluble analog of combretastatin A4 with angiotoxic properties ❑ IV	Phase I (completed 6/03)➢USA, Europe, Japan, phase I (begin 3/02, ongoing 7/05)➢USA, Europe (Switzerland) ❑ advanced solid tumors
sanofi-aventis	DOXO-EMCH (6-maleimidocaproyl) hydrazone)	Albumin-binding prodrug of doxorubicin ❑ IV	Phase I (begin 8/03, ongoing 9/05) ➢Europe (Germany) ❑ solid tumors
sanofi-aventis	Thioxanthone ❑ SR271425, SR-271425, BCN326862, WIN71425	Third-generation thioxanthone with broad preclinical activity ❑ IV, PO	Phase I (ongoing 8/05)➢USA ❑ refractory solid tumors
Schering	ZK-CDK, ZK-304709, ZK 304709	Multitarget tumor growth inhibitor (MTGI), blocking cdk2, cdk1 and cdk4 to induce tumor-cell apoptosis, and VEGFr 1, 2, and 3, and PDGFrβ to block angiogenesis ❑ PO	Phase I (begin 5/04, ongoing 8/05) ➢Europe ❑ solid tumors
Sunesis Pharmaceuticals ❑ Dainippon Pharmaceutical	SNS-595, SPC-595 (formerly AG-7352)	Novel cell-cycle modulator member of the class of compounds known as naphthyridines ❑ IV	Phase I (begin 6/04, ongoing 9/05) ➢USA, phase I (begin 10/04, ongoing 9/05)➢USA ❑ advanced solid tumors
UCB Pharma ❑ Wyeth	CMC-544	Immunocongugate comprising a MAb targeting CD22, conjugated to the enedyine antibiotic calicheamicin ❑ IV	Phase I (begin 6/04, ongoing 8/05) ➢USA, Europe (France, Germany, Spain, Switzerland, UK) ❑ B-cell NHL
Wyeth ❑ Taxolog	MST-997, TL-909	Taxane analog ❑ IV	Phase I (begin 7/04, ongoing 9/05) ➢USA ❑ advanced solid tumors
Vaxon Biotech	VX-001	Vaccine containing hTERT572Y, a single optimized cryptic peptide that targets tumors expressing the telomerase antigen ❑ IV	Phase I (completed 12/04) ➢Europe (Greece) ❑ advanced, refractory solid tumors
Xanthus Life Sciences ❑ BTG, Gdansk U Technology	Imidazoacridone ❑ C-1311, C1311 ❑ Symadex	Substituted imidazoacridone designed to have similar or improved efficacy in comparison to mitoxantrone, but with reduced side effects, including cardiac and hematologic toxicities ❑ IV, PO	Phase I (begin 6/04, ongoing 8/05) ➢USA; phase I (begin 10/04, ongoing 8/05)➢USA ❑ solid tumors

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Xenova Group ❑ Auckland Cancer Research Laboratory	XR5942/XR5944 series (formerly MLN944)	Orally available bisphenazine with a unique mechanism of action ❑ PO	Phase I (begin 7/03, completed 3/05)➢Europe (UK) ❑ solid tumors
ZymoGenetics ❑ Novo Nordisk	Recombinant human Interleukin-21 (rhIL-21)	Stimulates classes of immune cells involved in the body's ability to fight malignant or infected cells ❑ IV	Phase I (begin 5/04, ongoing 7/05)➢USA ❑ metastatic kidney cancer and metastatic melanoma

Source: NEW MEDICINE's Oncology KnowledgeBASE (nm|OK), October 31, 2005

(Pittsburgh, PA), to determine MTD, tolerability, and PK profile of SB-743921 administered IV over 60 minutes every 21 days to patients with a variety of advanced solid tumors that had relapsed or were refractory to previous treatment with a variety of standard chemotherapeutic regimens including irinotecan, topotecan, gemcitabine, paclitaxel, vinblastine, cyclophosphamide, and others. According to interim results, a total of 11 patients with solid tumors were treated at doses of 2-8 mg/m^2. Common tumor types included nsclc (n=2), hepatocellular carcinoma (n=2), and colorectal cancer (n=2). Prolonged (>/=5 days) Grade 4 neutropenia was observed in 1 patient at 8 mg/m^2. Among 6 patients in this cohort, 3 experienced DLT, including prolonged Grade 4 neutropenia (n=2), Grade 3 elevated ALT/AST (n=1), and Grade 3 elevated bilirubin (n=1). The most common toxicities, all Grade 1, observed during the trial included headaches (n=3), chills (n=3), nausea (n=3), myalgia (n=2), and injection site reactions (n=2). Grade 3 toxicities included neutropenia (n=1), acute abdominal pain (n=1), ileus (n=1), and fatigue (n=1). This is the first clinical trial of SB-743921 in humans. MTD at 8 mg/m^2 every 21 days caused prolonged neutropenia and hepatic lab abnormalities. Further dose exploration continues at 6 mg/m^2 to determine MTD (Holen KD, etal, ASCO05, Abs. 2010).

According to more mature results from this phase I clinical trial, presented at ASCO05, 20 patients with solid tumors (colon=5, nsclc=4, hepatocellular=2, ovarian=2, other=7) progressing on standard therapy, or for which there is no standard therapy, were treated with this regimen; up to 2 patients are treated at each dose level, with a starting dose of 2 mg/m^2. Doses are escalated by 100% until Grade 2 toxicity is observed in at least 2 patients, or non dose-limiting Grade 3/4 toxicity or DLT is observed in 1 patient. A total of 3 patients per cohort are treated at subsequent dose levels, with dose increments of 33%-50% until MTD is established. All patients undergo physical/neurologic examinations at baseline, and are examined for ECOG status, vital signs, and disease status. In addition, patients undergo a 12-lead ECG, and are assessed for clinical chemistry and hematology. Prior to each cycle, patients are examined for cancer-related signs and symptoms and ECOG performance, and undergo physical/neurologic examinations. Vital signs and body weight are also monitored. Clinical chemistry and hematology tests are performed weekly, and more frequently if warranted. Standard PK assessments are evaluated following cycle 1. Noncompartmental analysis of concentration-time data is performed using standard methods. Blood samples for PK assessments are collected at pre-dose on cycle 1 at 15, 30, and 45 minutes; 1 hour (immediately at end of infusion); and 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 36, and 48 hours after start of infusion.

At 5 mg/m^2, out of 2/6 patients with DLT, there was 1 Grade 3 febrile neutropenia and 1 Grade 3 febrile neutropenia with infection. At 6 mg/m^2, out of 2/3 patients with DLT, there was 1 prolonged Grade 4 neutropenia (25 days) and 1 Grade 3 hyponatremia. At 8 mg/m^2, out of 4/6 patients with DLT, 2 experienced prolonged Grade 4 neutropenia, 1 Grade 3 elevated transaminases, and 1 Grade 3 hyperbilirubinemia. Neurotoxicity, mucositis, thrombocytopenia, alopecia, and nausea/vomiting requiring premedication have not been observed. A potential phase II dose of 4 mg/m^2 is being evaluated in this trial. SB-743921 has an acceptable tolerability profile on a once every 21 days schedule.

SR271425, under development by sanofi-aventis, is a third generation thioxanthone with broad preclinical activity. SR271425 was equally active by the IV and oral routes of administration, although the oral route requires approximately a 30% higher dose.

A dose-escalation, phase I clinical trial (Sanofi-Synthelabo protocol: DFI4072) of a single dose, 1-hour IV schedule of SR271425, is being evaluated in patients with refractory solid tumors at Karmanos Cancer Institute, under PI Pat LoRusso, MD.

In another a dose-escalation, phase I trial, being conducted at the Cancer Therapy and Research Center (CTRC), Institute for Drug Development (IDD; San Antonio, TX), Brooke Army Medical Center (BAMC; Fort Sam Houston, TX), and Vanderbilt-Ingram Cancer Center (Nashville, TN), a single IV dose of SR271425 is administered over 1 hour, weekly, for 2 weeks, followed by a 1-week rest. The trial's objectives are to determine tolerability, toxicities, MTD, and recommended phase II dose, and to assess its PK profile of SR271425 with this treatment schedule. Of note, in the rabbit model, QTc prolongation, related to Cmax, was reported at doses >660 mg/m^2. Therefore, all patients enrolled in this trial are undergoing cardiology assessment with serial ECG, which

are assessed by a central reviewer. Among 17 patients treated at 5 dose levels ranging from 64 to 675 mg/m²/week, Grade 1/2 toxicities included QTc prolongation, nausea/vomiting/constipation, and fatigue. Following weekly dosing, PK of SR271425 was consistent with that observed previously in a single dose ascending trial with this agent. As would be predicted from the drugs short half-life of 6.7 hours, no systemic accumulation was observed. Disease stabilized in 3 patients. Preliminary data on this ongoing trial suggests that SR271425 administered at split, weekly doses will likely allow greater cumulative exposure without significant toxicity (Calvo E, etal, ASCO05, Abs. 3095).

XR5944, an orally available bisphenazine under development by Xenova (Slough, UK), recently acquired by Celtic Pharma Development UK, a subsidiary of Bermuda-based Celtic Pharma Holdings, is a DNA bisintercalator that binds through the major groove of DNA, which may explain the novel mechanism action and potent activity of the compound (Yang D, etal, AACR-NCI-EORTC03, Abs. A58 and Clin Cancer Res, 1 Dec 2003;9(16).

Despite structural similarity of XR5944 with its parent monophenazine carboxamide and acridine carboxamide anticancer compounds, it appears to work by a distinct mechanism of inhibiting DNA transcription rather than the expected mechanism of topoisomerase I and II inhibition. The specific binding site of XR5944 is recognized by a number of important transcription factors. DNA binding of the AP-1 proteins to the AP-1 site is significantly inhibited by XR5944 in a dose-dependent manner (Punchihewa C, etal, AACR05, Abs. 2355).

A multicenter (n=3), open label, dose-escalation, phase I clinical trial of XR5944 was initiated in July 2003, in the UK, in patients with advanced solid tumors, to evaluate the drug's safety and tolerability, and PK. In this trial, XR5944 is administered as a single 30-minute IV infusion repeated once every 21 or 28 days. The starting dose selected for this trial was 3.6 mg/m², which is a no observable adverse effect level (NOAEL) in the most sensitive species (Beagle dogs) and is just below one-tenth of the MTD in the rat. According to the dose-escalation scheme used in this trial, 2 patients are enrolled at each level until ≥Grade 2 drug-related toxicity is observed. After occurrence of such toxicity, cohorts of 3 to 6 patients are evaluated at each dose level. According to interim results, a total of 9 patients were enrolled in this trial, 2 each at dose levels of 3.6, 7.2, 14, and 24 mg/m², and 1 of 2 planned patients at the 36 mg/m² dose level. No drug-related toxicity was observed to date. Participating centers include Tayside Cancer Center, Ninewells Hospital (Dundee, UK), Grampian Universities NHS Trust (Foresthill, UK), and St. Luke's Cancer Center (Guildford, UK) (Cooper M, etal, ASCO04, Abs. 2100).

This phase I clinical trial of XR5944 in solid tumors was being finalized as of March 2005. MTD, based on initial data from the 27 patients enrolled in this trial, is 24 mg/m²; DLT was mucositis and neutropenia. No objective responses were reported, but disease stabilized in 4/27 (14.8%) patients. When final data is available later in 2005, Xenova will review future development plans for this compound.

Hormone Modulators

CC-8490, a selective estrogen receptor modulator (SErM) under development by Celgene (San Diego, CA), is a novel benzopyranone that, in xenograft models, blocks glioma cell proliferation and induces apoptosis. SErM are structurally diverse compounds that can bind to one or both of the known estrogen receptors, Erα and Erβ. These compounds have tissue-specific effects, and are relatively nontoxic and orally bioavailable. SErM are known for their antiestrogenic activity in estrogen-stimulated Er-expressing cancer cells. Benzopyranones are a series of proprietary SErM that were originally optimized for Erα selectivity in bone and breast cell lines.

CC-8490 exhibits antiproliferative activity in both Er-expressing and non-Er-expressing tumors and cell lines, suggesting that it may function via an Er-independent mechanism. Based on this information, several of the most sensitive cell lines were chosen to explore combination treatments with benzopyranones and the standard of care for glioma. Based on known mechanisms of action, CC-8490 does not block PKC activity *in vitro*, release of glutamic acid from C6 glioma cells is marginally decreased, no significant inhibition is observed in either the HMVEC migration assay, or A172 glioma cell invasion assay, and tubulin polymerization is not affected. A structurally similar benzopyranone was evaluated against the NCI-60 cell line database, and was found to have no significant correlation with known drugs. Data suggests that benzopyranones have a novel antiproliferative mechanism in non-Er-expressing cancer cells (Fultz KE, etal, AACR05, Abs. 627).

CC-8490 and its close analogs were tested in a panel of 15 cancer cell lines representing 9 different tumor types including glioblastoma multiforme (GBM), leukemia, nsclc, and breast, colon, prostate, pancreatic, ovarian, and uterine cancer. These SErM inhibited proliferation of these cancer cells lines, and also a MOR phenotype (MESSA-dx5) of a uterine carcinoma cell line (MES-SA) with IC_{50} values in a low micromolar range. CC-8490 and its analogs were also tested in a panel of SCID mouse xenograft models of human cancer cell lines including GBM, nsclc, and colon and breast cancer. Once daily intraperitoneal (IP) administration of CC-8490 and its analog Cpd-A to SCID mice with established U87 GBM tumors, resulted in dose-dependent inhibition of tumor growth with tumor growth delay (TGD) of 2 and 5 days for CC-8490, and 3 and 7 days for Cpd-A. In the NCI-H460 (nsclc) xenograft model, treatment with CC-8490 and its analogs Cpd-A, Cpd-B, and Cpd-C, inhibited tumor growth in a similar fashion as docetaxel administered IV at MTD. These compounds were well tolerated as measured by loss of body weight. Similar tumor growth inhibition with CC-

8490 and its analogs was seen in HT-29 (colon cancer) and MDA-MB-231 (breast cancer) xenograft models. Therefore, CC-8490 and its close analogs have potent *in vitro* and *in vivo* anticancer activity against a variety of human cancer models (Narla RK, etal, AACR05, Abs. 5891).

A phase I/II clinical trial (protocol ID: 040035, 04-C-0035, NCT00071864, NCT00074646) of CC-8490 was initiated in October 2003 in patients with histologically proven supratentorial malignant glioma including GBM, gliosarcoma, anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic mixed oligoastrocytoma, and unspecified malignant glioma/astrocytoma. Also included is radiographically diagnosed, infiltrating brain stem glioma not amenable to biopsy. Other CNS tumor histologies are excluded in this trial. A total of 35 patients were to be enrolled in this trial, conducted by the NCI, which was completed in June 2005.

Histone Deacetylase Inhibitors (HDAC)

Histone deacetylases (HDAC) comprise a family of enzymes involved in the epigenetic regulation of gene expression. HDAC keep histones in a hypoacetylated, positively charged state so that they tightly bind the negatively charged phosphate backbone of DNA, preventing gene transcription. Transcription factor complexes must gain access to the DNA to allow gene expression, which is normally achieved by histone acetyl-transferases, the natural counterparts of HDAC.

The balance between transcriptional activity and gene silencing is often disturbed in tumors. Deacetylation of histones by HDAC inactivates tumor suppressor genes, leading to neoplastic transformation. Thus, inhibition of these enzymes may restore normal cell growth control. HDAC inhibition may be antiproliferative, activating tumor suppressor genes such as the cell-cycle inhibitor p21WAF1/CIP1, and inducing differentiation and/or apoptosis. There is growing experimental evidence for this hypothesis, and a number of HDAC inhibitors are currently in phase I/II clinical trials.

CRA-024781 (CG-781), under development by Celera Genomics (South San Francisco, CA), is a novel HDAC inhibitor displaying potent activity against recombinant human HDAC enzymes *in vitro* in an array of human cancer cell lines. Examination of CRA-024781 *in vivo* reveals significant antitumor activity against different tumor xenografts. Statistically significant tumor growth inhibition at 80+% and 40+% has been achieved with CG-781 against HCT 116 and DLD-1, respectively. Antitumor activity was observed with different dosing regimens, and also with either IV or IP delivery. According to pharmacodynamic evaluation of circulating blood cells and tumor samples, protein acetylation correlates with drug exposure. Gene expression profiling over various doses and time points identified a robust set of genes whose expression in tumors is affected by treatment with CRA-024781. Similar analysis of whole blood samples from the same mice also provided a set of marker genes that-track with efficacy (Cao ZA, etal, AACR05, Abs. 1801).

In order to identify tissue types that were most sensitive to effects of HDAC inhibition, tissue distribution of the acetylated tubulin, a pharmacodynamic marker of HDAC inhibition, was examined in female Balb/c mice treated with the CRA-024781. According to this experiment, measurement of tubulin and histone acetylation may be used to monitor the pharmacodynamic effects of CRA-024781 *in vivo* (Balasubramanian S, etal, AACR05, Abs. 4168).

In July 2005, Celera initiated a dose-escalation, phase I clinical trial of CRA-024781 in patients with refractory solid tumors, being conducted at the University of Chicago Hospitals, under PI Samir Undevia, MD. Objectives of the trial are to determine MTD and to evaluate safety and PK of CRA-024781. Up to 40 patients will be enrolled in this trial.

LBH589, under development by Novartis, is a novel cinnamic acid hydroxamate that inhibits HDAC activity, as well as the hERG channel implicated in cardiac arrhythmias. LBH589 activates p21, and inhibits proliferation of tumor cell lines at nanomolar concentrations. LBH589's antiangiogenesis activity is attributable to inhibition of histone deacetylase 6 (HDAC6), a class II HDAC capable of removing acetyl group from lysine residues on both histone and non-histone proteins. In mammalian cells, the primary subcellular location of HDAC6 is in the cytoplasm, and this determines its potential involvement as a deacetylase for cytoplasmic non-histone proteins. HDAC6 is a microtubule-associated deacetylase able to regulate microtubule acetylation and chemotactic cell motility.

Small interference RNA (siRNA) was used to inhibit HDAC6 expression in a human RCC line that lacks functional von Hippel-Lindau gene expression, and has constitutive HIF-1α protein expression. Inhibition of HDAC6 by siRNA increased the acetylation of HIF-1α, decreased its stability and, subsequently, inhibited downstream VEGF expression. By using nanomolar range concentration of hydroxamic acid HDAC inhibitors LAQ824 and LBH589, a direct correlation was observed between HIF-1α inhibition and increase of α-tubulin acetylation, a marker for HDAC6 inhibition. In contrast, non-hydroxamic HDAC inhibitors that do not increase tubulin acetylation did not have inhibitory effect on HIF-1α. In endothelial cells (HUVEC), LBH589 also inhibited HIF-1α-induced CXCR4 expression, and Matrigel invasion induced by VEGF and SDF-1. These results suggest that the antiangiogenic activity of hydroxamic acid HDAC inhibitors is mediated through inhibition of HDAC6 (Qian DZ, etal, AACR05, Abs. 1812). LBH589 is being evaluated in both IV and oral routes of administration.

In a phase Ia clinical trial, LBH589 is administered as a 30-minute IV infusion employing two dosing regimens. Patients with advanced refractory solid tumors are treated with LBH589, once daily for 3 consecutive days for the first two weeks of a 3-week cycle on days 1-3, and days 8-10,

Exhibit 5
Responses in Phase I Clinical Trials Involving Regulatory/Antiangiogenesis Agents

Treatment Regimen	Trials (#)	Patients (#)	CR (%)	CR (#)	PR (%)	PR (#)	ORR (%)	CR+PR (#)	SD (%)	SD (#)	ORR+ SD (#)	ORR+ SD (%)
Regulatory Agents												
Novel/Single	51	1,347	0.7	9	2.5	34	3.2	43	39.3	529	572	42.5
Multiple Novel	7	81	1.2	1	6.2	5	7.4	6	27.2	22	28	34.6
Novel + Approved	61	935	2.1	20	9.5	89	11.6	108	37.4	350	458	49.0
Subtotal	119	2,363	1.3	30	5.4	128	6.7	158	38.1	901	1,059	44.8
Angiogenesis Inhibitors												
Novel/Single	11	335	0.6	2	3.3	11	3.9	13	31.0	104	117	34.9
Multiple Novel	9	135	5.2	7	9.6	13	14.8	20	37.0	50	70	51.8
Subtotal	20	470	1.9	9	5.1	24	7.0	33	32.7	154	187	39.8

Legend: ORR=CR+PR

Source: Horstmann E, etal, Risks and benefits of phase 1 oncology trials from 1991 through 2002, NEJM, 3 Mar 2005;352(9):895-904

repeated after 21 days (protocol ID: 2101), while those with hematologic malignancies are treated on days 1-3 and days 15-17, repeated after 28 days (protocol ID: 2102). Patients with impaired heart function are excluded from this trial, and drugs known to prolong QT interval were prohibited. These trials are ongoing at the CTRC Institute for Drug Development (IDD) and at the University of Mainz, in Germany.

According to an interim analysis from these phase I clinical trials, reported in 2004, 13 (RCC=4, nsclc=2, sarcoma=2, liver cancer=1, colon=1, others=3) were enrolled in both arms. Of these, 11 were enrolled in arm 1, treated at dose levels 1.2 mg/m^2 (n=2), 2.4 mg/m^2 (n=3), 4.8 mg/m^2 (n=3), and 7.2 mg/m^2 (n=3). In arm 2, 2 patients were treated at dose levels 2.4 mg/m^2 (n=1), and 4.8 mg/m^2 (n=1). There was one DLT (prolonged Grade 2 thrombocytopenia) at the 7.2 mg/m^2 dose level in arm 1. Other toxicities included Grade 3 neutropenia (n=1) and hypoglycemia (n=1), Grade 2 thrombocytopenia (n=2), and anemia (n=2). There were no abnormalities in >100 ECG. Disease stabilized in 6/13 patients (RCC=3, sarcoma=1, nsclc=1, and liver cancer=1). To determine if LBH589A increases histone acetylation, Western blots were performed on total cell lysates from peripheral blood lymphocytes. Increased acetylation was observed in 1/2 at 2.4 mg/m^2, and in 2/3 at 4.8 mg/m^2 up to 24 hours after the third dose. Onset of acetylation was rapid (1 hour) and, in 1 patient at the 4.8 mg/m^2 dose level, lasted up to 7 days after the first dose. LBH589A administered IV appears to be well tolerated with consistent pharmacodynamic effects. Additional evaluation continues at the 7.2 mg/m^2 dose level for arm 1, and at the 9.6 mg/m^2 dose level for arm 2 (Beck J, etal, ASCO04, Abs. 3025).

Cardiac monitoring is an integral part of the clinical evaluation of LBH589. Serial digital ECG were performed at baseline (6 ECG), and on days of dosing. A total of 1475 post-dose ECG were performed in 45 patients treated at 10 daily dose levels, ranging from 1.2 mg/m^2 to 20.0 mg/m^2. Central tendency analysis showed no change in QTcF on day 1; however, on day 3 of dosing, there was a dose-related increase in QTcF of at most 20 milliseconds. Frequency of outliers, QTcF >500 milliseconds and/or >60 milliseconds change from baseline, was 28% (n=12) and occurred over a broad dose range, from 4.8 mg/m^2 to 20.0 mg/m^2. Among 12 patients tested, outlier ECG occurred primarily on day 3 (n=7) or later (n=3) on days 4 or 5; in only 2 patients outlier ECG were detected on day 1, and 6 patients (14%) had QTc >500 milliseconds. Frequency of QTcF >500 milliseconds increased with dose. At doses of 9 mg/m^2, QTcF was >500 milliseconds in only 1/33 patient, while at doses >9 mg/m^2, 5/12 patients had QTcF >500 milliseconds. T wave and ST-T wave changes, primarily T wave flattening were frequently observed and were nonspecific. LBH589 induces dose-related increases in QTcF of ≤20 milliseconds at doses up to 20.0 mg/m^2. Cardiac repolarization changes were delayed until day 3 perhaps because of a different mechanism affecting the hERG channel (Fischer T, etal, ASCO05, Abs. 3106).

A phase I clinical trial, being conducted at Duke University (Durham, NC), CTRC, and Johannes-Gutenberg University (Mainz, Germany), is testing safety and tolerability of oral LBH589B in patients with advanced solid tumors or lymphoma. According to the protocol, LBH589B is administered PO in 2 dosing schedules, MWF of a 28-day cycle (arm 1), or a planned continuous daily dosing (arm 2). Among 9 patients (RCC=3, prostate cancer=2, RCC/pelvis cancer=1, colon cancer= 1, other=2) treated in arm 1 at 2 dose levels at 15 mg/day (n=3) and 30 mg/day (n=6), were no DLT observed to date. Toxicities included Grade 3 (n=1) and Grade 2 (n=4) diarrhea, Grade 3 (n=1) and Grade 2 (n=1) thrombocytopenia, Grade 2 fatigue (n=4), Grade 2 weakness (n=1), Grade 2 anorexia (n=2),

Grade 2 nausea (n=3), and Grade 2 vomiting (n=1). Out of 250 analyzed ECG, an increase in QTcF from baseline of 50-60 milliseconds (none with QTcF >500 milliseconds) was observed in 2 patients, dosed at 30 mg. In this trial, LBH589B administered orally was well tolerated, with consistent pharmacodynamic effects and PK. The drug's terminal half-life averaged 16.5 hours. Comparison of AUC with IV administration indicated 17% of absolute bioavailability. Additional evaluation is continuing at 30 mg/day, and further dose escalation is planned (Beck J, etal, ASCO05, Abs. 3148).

MGCD0103, an orally active small molecule under development by MethylGene (Montreal, Canada), is an isotype-selective HDAC inhibitor. Its activity profile is genotypically, phenotypically, and functionally distinct from indiscriminate HDAC inhibitors.

MethylGene developed novel methods to monitor the pharmacodynamic effect of MGCD0103 in clinical samples. A whole cell enzyme assay using intact white cells from patients in the phase I clinical trial was used to monitor enzyme inhibitory activity of MGCD0103, while histone acetylation in these cells was analyzed by Western blotting, enzyme-linked immunosorbent assay (ELISA) and fluorescence-based (FACS) cytometry. FACS and ELISA measure histone acetylation with improved sensitivity compared to immunoblotting. In 3 of the first 7 patients for whom comparative data is available, HDAC enzyme inhibition correlated positively with histone acetylation induction in peripheral white cells. HDAC enzyme inhibition and induction of histone acetylation in patients treated with MGCD0103 were well correlated. In patients with >20% total enzyme inhibition, induction of histone acetylation was 50%, while among patients with >50% enzyme inhibition, induction of histone acetylation was 67% (Kalita A, etal, ASCO05, Abs. 9631).

Using this whole cell HDAC enzyme assay, it was shown that when inhibitory activities of MGCD0103, MS-275, and SAHA are compared, MGCD0103 appears to be the most potent inhibitor across various cancer cell lines. MGCD0103 can inhibit, in a dose-dependent manner, whole cell HDAC activity in human peripheral white blood cells *ex vivo*, and in white blood cells isolated from mice treated *in vivo*. *In vivo*, HDAC inhibitory activity of MGCD0103 correlates with plasma accumulation of MGCD0103 and induction of histone acetylation in white blood cells in treated mice. This whole cell enzyme assay is being used to monitor the pharmacodynamic effect of MGCD0103 in patients in phase I clinical trials (Bonfils C, etal, AACR05, Abs. 606).

In April 2004, two multicenter, open label, dose-escalation, single agent, phase I clinical trials were initiated with MGCD0103 in the USA and Canada, in patients with advanced, refractory solid tumors, or NHL. Eric Rowinsky, MD, of the Institute for Drug Development (IDD) is the PI of the phase I trial being conducted in the USA.

The primary purpose of these two trials is to determine safety, MTD, DLT, PK, and pharmacodynamic properties of MGCD0103 in this setting, and check for response. Available peripheral blood samples are also being evaluated for histone H3 and H4 acetylation, and inhibition of total cellular HDAC activity. A total of 33 eligible patients were administered oral doses of MGCD0103 ranging from 12.5 to 36 mg/m^2, either daily for 14 days, every 3 weeks, or 3 times per week for 2 weeks, every 3 weeks. In the daily dosing schedule trial, among 12 enrolled patients, DLT was fatigue; MTD was 12.5 mg/m^2. In the 3 times weekly trial, a total of 46 cycles were administered to 21 patients in 4 cohorts (12.5, 20, 27, or 36 mg/m^2). MGCD0103 was safe at doses up to, and including 36 mg/m^2, 3 times a week in 3-week cycles. The once daily dosing phase I clinical trial was closed in May 2005, after enrolling 12 patients.

Initial results indicate that, in the majority of patients in both trials, histone acetylation increased and/or total cellular HDAC activity decreased based on MethylGene's proprietary whole cell assay. MGCD0103 is orally available, and preliminary PK indicates that the Cmax and AUC are generally dose-proportional. Mean terminal plasma half-life of 9.1 hours was seen across dose cohorts. Moreover, initial pharmacodynamic data suggests that, in some patients, inhibition of HDAC activity in whole cells extended beyond the plasma half-life of the drug, lasting as long as 48 hours post drug administration. According to preliminary efficacy results, disease stabilized in 1 patient with previously treated thymic carcinoma who was administered 7 cycles of MGCD0103 on the daily dosing schedule. Disease also stabilized in a previously treated, second patient with RCC, treated with for 6 cycles on the 3 times per week schedule. The most common adverse events observed in this trial were fatigue, nausea, and anorexia, the majority of which were ≤Grade 2. No significant myelosuppression was observed in patients on either trial. All patients are also monitored by ECG both prior to and post initial administration of MGCD0103. No clinically significant ECG abnormalities have been reported to date (Gelmon K, etal, ASCO05, Abs. 3147). MethylGene expects enrollment for the 3 times weekly trial to be completed soon, with additional results becoming available by year-end 2005. Preliminary data suggest that absence of hematologic side effects and ECG abnormalities, coupled with a favorable half-life, positively differentiate MethylGene's compound from some of the other HDAC inhibitors in clinical trials.

In December 2004, MethylGene initiated the first of two dose-escalating, phase I clinical trials with MGCD0103 in refractory hematologic malignancies. Both trials are to enroll between 18 and 25 patients to determine safety and tolerability of MGCD0103 in patients with such hematologic malignancies as AML, chronic myeloid leukemia (CML), and advanced myelodysplastic syndrome (MDS). For the first trial, MGCD0103 is administered orally 3 times

weekly every 21 days. This trial is being conducted at the Princess Margaret Hospital (Toronto, Canada) and University of Texas M. D. Anderson Cancer Center.

A second phase I clinical trial in hematologic malignancies is scheduled to be initiated in 2005. For this trial, MGCD0103 will be administered orally twice weekly, every 21 days. This trial will be conducted at University of Chicago (Chicago, IL), with a second site still to be confirmed.

Regulatory/Targeted Agents, Cell-Cycle Modulators, Angiogenesis Inhibitors, and Vascular Targeting Agents (VTA)

The majority of novel agents currently entering monotherapy phase I clinical trials are therapeutics targeting markers/pathways putatively implicated in the malignant phenotype, and in disease progression and metastasis. Despite strong association of the targeted markers with some aspect of malignancy and specificity of the agents addressing these targets, results of phase I monotherapy trials with these agents are below par when compared with those associated with cytotoxic drugs and immunotherapies/vaccines (Exhibits 3 and 5).

The agents described in this group address a variety of targets signaling pathways (Exhibit 6). The most common targets are various members of the vascular endothelial growth factor receptor (VEGFr) and epidermal growth factor receptor (EGFr) families. However, many others are also being addressed by novel agents; over 1,200 targets have been investigated preclinically and clinically to date.

AEE788, a pyrrolo-pyrimidine under development by Novartis, is a novel multitargeted dual EGFr and VEGFr2 tyrosine kinase inhibitor. In preclinical evaluations, treatment with AEE788 was effective in a variety of settings, as monotherapy and in combination regimens.

Investigators at the Institute of Cancer Research (London, UK), discovered that combinations of AEE788 with tamoxifen or letrozole in breast cancer overexpressing EGFr and/or HEr2, provide superior antitumor activity as compared with that of single agent strategies (Hauge Evans A, etal, AACR05, Abs. 3408).

Investigators at M. D. Anderson Cancer Center have studied effects of AEE788 treatment in a variety of preclinical settings. Concomitant blockade of EGFr and VEGFr signaling pathways by AEE788 inhibited growth of cutaneous squamous cell carcinoma cells and human cutaneous cancer xenografts in nude mice (Younes MN, etal, AACR05, Abs. 2034). Combining AEE788 with paclitaxel significantly inhibited experimental human follicular thyroid carcinoma (FTC) bone metastasis in the human WRO FTC cell line and in mice xenografted with this cell line (Younes MN, etal, AACR05, Abs. 2022). Blockade of EGFr and VEGFr signaling pathways by AEE788, also combined with paclitaxel, induced apoptosis of tumor-associated endothelial cells, and was associated with inhibition of progressive growth of human prostate cancer cells implanted in the prostate of nude mice (Yazici S, etal, AACR05, Abs. 2020). Treatment of human prostate cancer cells growing in the bone of nude mice with AEE788, combined with paclitaxel, significantly increased the ratio of apoptotic endothelial cells, which correlated with better treatment outcome, inhibiting progressive tumor growth, and preserving bone structure in a model of human prostate cancer metastasized to the bone. These results suggest that AEE788, in combination with paclitaxel, should be tested clinically (Kim SJ, etal, AACR05, Abs. 2987).

An international, multicenter, dose-escalation, phase I clinical trial (protocol ID: CAEE788A2101) was initiated in July 2003, to assess safety, PK, pharmacodynamics, MTD/DLT, and optimal biologic dose of AEE788 in patients with advanced solid tumors. Participating institutions include IDD/CTRC, Vall d'Hebron University Hospital (Barcelona, Spain), and University Hospital Gasthuisberg (Leuven, Belgium). According to the protocol, doses were escalated in a standard phase I design with 3-6 patients/cohort. Safety monitoring included extensive cardiac assessment. All but one patient were naïve to EGFr and VEGFr inhibitory therapy. Pharmacodynamic markers were analyzed in pre and post treatment skin and tumor biopsies. A 24-hour PK profile was obtained on days 1, 15, and 28, with trough sampling on days 8 and 22. Each cycle is 28 days. A total of 69 patients (breast cancer=10, colon cancer=9, medullary thyroid cancer=7, head and neck cancer=6, melanoma=4, nsclc=4, RCC=3, angiosarcoma=3, other tumor types=23), were treated with once daily AEE788 at doses of 25 (n=5), 50 (n=6), 100 n=(5), 150 (n=5), 225 (n=6), 300 (n=8), 400 (n=14), 450 (n=5), 500 (n=6), and 550 mg (n=9) per day. DLT represented by Grade 3 diarrhea was seen in 2 patients at both 500 and 550 mg. Most other adverse events were mild to moderate; 3 patients (500 mg=2 and 550 mg=1) withdrew from the trial after <1 cycle because of adverse events. The most common adverse events, observed in >20% of patients, were diarrhea (67%), fatigue/asthenia (51%), anorexia (49%), rash (43%), nausea (42%), and vomiting (28%). Grade 3 LFT elevation was seen in 6 patients; LFT of one patient remained normal when rechallenged at a lower dose. There was no QTcF >500 milliseconds, with no change of QTcF= 60 milliseconds in over 1,980 post-treatment ECG. Serum concentrations of parent (AEE788) and active metabolite (AQM674) increased with dose and dose duration. Exposure of AEE788 increases more than proportionately with increased dose, while AQM674 exposure increases proportionally. The metabolite/parent ratio (range=0.2-2) appears to decline with dose and duration to an average of 0.3-0.4 at steady state by day 15. A PR was seen in 1 patient with angiosarcoma at 400 mg, and disease stabilized in 28 patients in 2 cycles (range=2-10). Median number of cycles of AEE788 delivered was 2 (range=<1-10). AEE788 is well tolerated. DLT dose levels were defined at 500 and 550 mg (Martinelli E, etal, ASCO05, Abs. 3039).

In this phase I clinical trial, AEE788 and AQM674 concentrations varied widely and increased with dose and duration. IC_{50} values were estimated for pEGFr (42 nM), pMAPK (38 nM), and Ki67 (7 nM) in skin biopsies, and pEGFr (28 nM), and pMAPK (22 nM) in tumor biopsies. Treatment with AEE788 resulted in a dose-dependent inhibition of EGFr signaling as observed in skin and tumor biopsies; profound receptor inhibition was achieved with a dose of >300 mg/day. As expected, inhibition of endothelial pMAPK and Ki67 occurs at higher doses than EGFr inhibition which occurs at >300 mg (Baselga J, etal, ASCO05, Abs. 3028).

AS1411 (formerly AGRO100), under development by Antisoma (London, UK), is a G-rich oligonucleotide that binds to nucleolin, a protein found on tumor cell surfaces, involved in cancer cell proliferation. The drug was originally developed by Aptamera, which was acquired by Antisoma in February 2005. AS1411 is an aptamer, an oligonucleotide that can fold into a stable, 3-dimensional structure capable of interacting with particular target proteins. AS1411 binds with high specificity to the protein nucleolin, is internalized, and induces apoptosis in a range of malignancies.

AS1411 is the first nucleic acid aptamer ever tested for treatment of cancer in humans. In a phase I clinical trial, initiated in September 2003 at the James Graham Brown Cancer Center at the University of Louisville in KY, AS1411 was administered as a continuous IV (CIV) infusion over 96 hours to patients with metastatic cancer. The first dose level started at 1 mg/kg/day; patients were enrolled in cohorts of 3. If no toxicity was observed 28 days after treatment, the dose of AS1411 was doubled. Enrollment in this trial was completed in June 2004. In dose level 1, AS1411 (1 mg/kg) was administered daily for 4 days. If no toxicity was observed 28 days after treatment, the dose of AS1411 was increased up to 10 mg/kg/day over 7 days. Responses were assessed every 28 days by using RECIST criteria. From September 2003 through July 2004, 17 patients (renal cancer=3, colon cancer=3, pancreatic can- cer=2, and 1 patient each with lung, gastric, cervical, and prostate cancer, and melanoma, lymphoma, synovial sarcoma, and hemangiopericytoma) with objectively documented progressive metastatic disease after multiple therapies, were enrolled. All patients are evaluable for toxicity and response. Up to 22 treatment cycles were administered (1-2 cycles/patient). AS1411 was remarkably well tolerated, with no toxicity of any type related to drug administration in any of the patients. Disease stabilized in 8 (50%) patients at 2 months after treatment, who remained stable for 2 to 9 months before progression. A near CR has been maintained in 1 patient for more than 6 months. No toxicity of any type related to drug adminis- tration has been observed. AS1411 was present in the serum during the entire infusion, and the drug's concentration was related to the dose (Laber DA, etal, ASCO05, Abs. 3064).

Among 3 patients with RCC, disease stabilized in 1 for 9 months, and then progressed. A second patient was still stable >10 months after start of therapy, and the third patient, with a large abdominal tumor that grew after removal of a cancerous kidney, maintained a near CR 10 months after treatment, with tumor reduction in size from around 19 cm before treatment to <1 cm as of April 2005.

Antisoma is planning a program of trials to thoroughly evaluate the efficacy of AS1411, building on promising signs of anticancer activity. In September 2005, the company reopened the phase I trial (protocol ID: APT-1-0603) of AS1411 to enroll 40 patients with RCC and nsclc. The reopened trial is taking place at various centers in the USA, led by the Brown Cancer Center, under PI Damian Laber, MD. Patients are administered a 168-hour CIV of AS1411 from days 1 to 7.

In August 2005, AS1411 was granted 'orphan drug' designation by the FDA for treatment of kidney cancer, and in August 2004, for treatment of pancreatic cancer.

ATN-224, under development by Attenuon (San Diego, CA), is an orally administered small molecule drug that is a second generation analog of the copper binding drug tetrathiomolybdate (TM) with antitumor and antiangiogenic properties. ATN-224 inhibits CuZn superoxide dismutase (SOD1) by depleting it of copper, thereby interrupting several pathways important for tumor progression. Inhibition of SOD1 in endothelial cells leads to downregulation of several signaling pathways including those mediated by ERK1/2, FAK and Src, leading to inhibition of endothelial cell proliferation and induction of apoptosis. Thus, ATN-224 has potential to affect multiple cellular compartments within a tumor. ATN-224 has been shown to enhance activity of multiple chemotherapies in animal models of breast and prostate cancer, and melanoma.

In preclinical models, ATN-224 inhibits tumor angiogenesis and tumor-cell growth. Antiangiogenic activity of ATN-224 is mediated through its ability to inhibit SOD1. Different tumor cell lines exhibit differential sensitivity to ATN-224. Inhibition of SOD1 activity in red blood cells and/or induction of apoptosis in tumor cells from patients with hematologic malignancies are being investigated as possible bio-markers to optimize the dose of ATN-224 in the clinic (Juarez JC, etal, AACR05, Abs. 5897).

In May 2004, a phase I clinical trial of ATN-224 was initiated in patients with solid tumors by Cancer Research UK under a collaboration with Attenuon. Professor Adrian is the PI of the trial being conducted at the Churchill Hospital (Oxford, UK). Up to 16 patients with solid tumors refractory to standard treatment, or for whom no conventional treatment exists, are to be enrolled in this trial to evaluate safety and tolerability of orally administered ATN-224, determine optimal daily dose, and MTD. The trial's primary objectives are to determine the time course of suppression of serum caeruloplasmin (Cp), a surrogate for copper, and the dose of ATN-224 required to achieve Cp levels within the target range. Secondary objectives are to

investigate PK of ATN-224, and to document possible antitumor activity.

A phase I clinical trial in advanced hematologic malignancies was also ongoing as of September 2005.

AVE8062A, under development by sanofi-aventis, is a novel combretastatin derivative that inhibits microtubule polymerization through reversible binding at the colchicine site. This VTA is highly cytotoxic to endothelial and tumor cells *in vitro*, and causes selective vascular shutdown and tumor regression *in vivo* across a broad spectrum of human tumor xenograft models, including those resistant to docetaxel.

In tumor-bearing mice, combinations of AVE8062A with cisplatin, carboplatin, doxorubicin, or vinorelbine were synergistic, with a large therapeutic index for combinations of AVE8062A with platinum-based drugs and doxorubicin. Combination of AVE8062A with docetaxel was also found to be synergistic, with a large therapeutic index; synergy could be expected with a 97.5% probability, with dosages as low as 13% of the highest nontoxic dose (HNTD) of AVE8062A (Lejeune P, etal, AACR05, Abs. 3425).

A dose-finding, phase I clinical trial (Aventis protocol ID: AVE8062A/1002) with AVE8062A, administered as a 30-minute IV infusion every 3 weeks in patients with advanced solid tumors, was initiated in March 2002 at the Barbara Ann Karmanos Cancer Institute/Wayne State University (Detroit, MI), under PI Pat LoRusso, MD. Additional participating centers include Istituto Oncologico della Svizzera Italiana (Bellinzona, Switzerland), Southern Europe New Drug Organization (SENDO; Milan, Italy), Institute of Drug Development (IDD), and Ospedale S. Giovanni (Bellinzona, Switzerland). This trial's regimen was decided based on cardiotoxicity encountered in the phase I clinical trial testing three different administration schedules, every 21 days, daily for 5 days, and weekly. In the daily for 5 days and weekly trials, occurrence of 4 potentially drug-related vascular events, i.e. myocardial ischemia (MI), transient asymptomatic hypotension, transient cerebral ischemia, and asymptomatic ventricular tachycardia without residual clinical deficits, led to a voluntary interruption of all trials. In 1 patient experiencing MI, the Cmax of AVE8062A (805 ng/mL) was the highest determined and close to the free drug level at which cardiac damage was observed in dogs. No vascular event was observed in the every 21 days schedule up to a dose of 22 mg/m^2 thus this trial was resumed after restricting eligibility criteria and increasing cardiovascular monitoring.

In this new phase I clinical trial, AVE8062 was administered as a 30-minute IV infusion every 3 weeks. Patients were monitored with continuous 24-hour ECG Holter, Holter CABPM (continuous ambulatory blood pressure monitoring), serial CPK, troponin, ECG, ventriculographies, and echocardiograms. DCE-MRI for blood flow analysis in suitable patients, treated at doses >15.5 mg/m^2 was to be performed before treatment and 4 hours post-treatment. All 23 patients (colorectal cancer=7, ovarian cancer=3, kidney cancer=3, sarcoma=2, and others=8) out of 24 patients treated, were evaluable for DLT determination in the first cycle. All in all, 5 dose levels from 6 to 22 mg/m^2 were delivered in 42 cycles. No vascular, nonhematologic, nor hematologic DLT were reported. Grade 1 nonhematologic toxicity was observed in 3 patients (diarrhea=1, cutaneous rash=1, nausea=3). No significant modifications of vascular parameters were reported. The half-life of AVE8062 and RPR258063 were 22 minutes and 7 hours, respectively. RPR258063 Cmax values did not exceed 300 ng/mL. Therefore, AVE8062 may be administered safely on an every 3 weeks schedule up to a dose of 22 mg/m^2 which remains below the RPR258063 concentration at which myocardial toxicities were reported in dogs (Sessa C, etal, AACR05, Abs. 5827).

AZD0530, under development by AstraZeneca, is a highly selective dual-specific Src/Abl nonreceptor tyrosine kinase inhibitor with anti-invasion activity in a wide range of tumors. For a detail review of this drug and the c-SRC signal transduction pathway see FO, pp 1821.

BAY 57-9352, under development by Bayer (West Haven, CT), is a novel orally active inhibitor of angiogenesis by blocking VEGFr2 and platelet-derived growth factor receptor β (PDGFrβ) tyrosine kinases. As a single agent, it exhibits a broad spectrum of activity in multiple human tumor xenograft models of breast, colon, prostate and lung cancer.

BAY 57-9352 was discovered as part of a continuing effort by Bayer to identify novel receptor tyrosine kinase inhibitors. An initial set of 2-carboxamidopyrido phthalazines was found to be highly potent VEGFr2 inhibitors in both biochemical and cellular assays. During the course of the program, various core changes were explored, resulting in novel furo- and thieno-pyridazines. These efforts led to identification of BAY 57-9352, which combines potent VEGFr2 inhibition with attractive PK, as well as CYP450 inhibition profiles. BAY 57-9352 inhibits angiogenesis and tumor growth in breast, colon, and lung tumor xenograft models (Dixon JA, etal, AACR05, Abs. 3877).

In preclinical studies, BAY 57-9352 also enhanced anticancer activity of various cytotoxic agents. Combination of BAY 57-9352 with capecitabine or paclitaxel was at least as effective as the individual agents administered alone, when evaluated in SC Colo-205 human colorectal cancer or H460 human nsclc xenograft models implanted in female athymic (NCr-nu/nu) mice. As monotherapy, both BAY 57-9352 and capecitabine exhibited significant antitumor activity after once daily administration in the Colo-205 model with an average tumor growth delay of 16 and 30 days. Combination of capecitabine with BAY 57-9352 caused an average tumor growth delay of 40 days with no toxicity. In the H460 model, BAY 57-9352 and paclitaxel exhibited antitumor activity as single agents with a tumor growth delay of 6 days and 10 days. When combined, these

Exhibit 6
Molecular Targets of Drugs in Phase I Clinical Trials

Developer ❑ Affiliates		
Onconova Therapeutics	ON 01910.Na, ON01910.Na, ON01910 ❑ ON 01910.Na	CDC2/CDK1 Polo-like kinase 1 (Plk1)
AVI BioPharma	AVI-4126 ❑ Oncomyc-NG (Restin-NG)	c-myc
Cylene Pharmaceuticals	CX-3543	c-myc VEGF, VEGF-A, VEGFA
Schering	ZK-CDK, ZK-304709, ZK 304709 ❑ Multi-target Tumor Growth Inhibitor (MTGI)	CDK1 CDK2 CDK4 VEGFr1 VEGFr2, VEGFr-2/FLK1, Flk-1/KDR VEGFr3, VEGFr-3/(FLT4, Flt-4)/PCL PDGFrB, PDGFr
Sankyo	CYC682 (formerly CS-682)	DNA polymerase
Bristol-Myers Squibb	BMS-599626	EGFr, ErbB-1, ErbB1, HEr-1, HEr1 HEr2/neu ErbB-2, c-erbB-2, ErbB2
Novartis	AEE788, NVP-AEE788 .	EGFr, ErbB-1, ErbB1, HEr-1, HEr1 VEGFr2, VEGFr-2/FLK1, Flk-1/KDR
Celgene	CC-8490, SPC8490	Estrogen receptor α (Erα)
MethylGene	MGCD0103	HDAC
Celera Genomics Group	CRA-024781, CG-781	HDAC
Novartis	LBH 589, LBH589	HDAC
Geron	GRN163L, formerly GRN719	hTR mRNA Telomerase
Raven Biotechnologies	RAV12	Insulin-like growth factor 1 receptor (IGF1r, IGF-1r)
Eisai	E7820, NSC 719239	Integrin α2
Aphton	HuABL-364, IGN311 (previously SMART ABL-364)	Lewis y antigen
Aptamera	AS1411 (formerly AGRO100)	Nucleolin
NeoPharm	LErafAON, LErafAON-ETU	RAF
GlobeImmune	GI-4000, GI-4014, GI-4015 and GI-4016 ❑ Tarmogen	Ras
Chemokine Therapeutics	CTCE-9908	Stromal derived factor 1 (SDF-1)
Attenuon	ATN-224	Superoxide dismutase 1, soluble (SOD1)
Faustus Forschungs Compagnie Translational Cancer Research	FFC14A, KP1019, FFC 14a	Transferrin
Human Genome Sciences	HGS-TR2J, HGS-ETR2, KMTR2	Tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor 2 (TRAIL-r2, TRAILr2)
Pfizer	SU014813	VEGFr2, VEGFr-2 (FLK1, Flk-1)/KDR PDGFrB, PDGFr PDGFrA c-Kit Fms-like tyrosine kinase 3 (FLT3, FLT-3)
Bayer	BAY 57-9352	VEGFr2, VEGFr-2/FLK1, Flk-1/KDR PDGFrB, PDGFr
UCB Pharma	CMC-544	CD22

Source: New Medicine's Oncology KnowledgeBASE (nm|OK), October 2005

agents produced a tumor growth delay of 14 days with acceptable toxicity (Chang Y, etal, AACR05, Abs. 2030).

In September 2003, a phase I clinical trial with oral BAY 57-9352 was initiated at Klinik für Tumorbiologie at the University of Freiburg, in Germany, in patients with refractory solid tumors. According to the protocol, BAY 57-9352 is administered daily orally in form of a solution, in repeated cycles of 14 days on and 7 days off. Patients are monitored weekly in the first 5 weeks, and every 2 weeks thereafter. Treatment success is reviewed every 4 weeks by means of a DCE-MRI to assess tumor size, and blood vessel tumor density, and permeability.

BMS-599626, under development by Bristol-Myers Squibb, is an orally available dual HEr1/HEr2 kinase inhibitor. BMS-599626 emerged as the lead candidate from a series of novel pyrrolotriazine analogs that were optimized for inhibition of both HEr1 and HEr2 and of proliferation of tumor cells that depend on HEr1/HEr2 signaling. Medicinal chemistry at Bristol-Myers Squibb identified the pyrrolo[2,1-f][1,2,4]triazine nucleus as a novel template for a variety of ATP-competitive kinase inhibitors. Extensive homology of catalytic sequences of HEr1 and HEr2 receptors allowed design of inhibitors that occupy the ATP binding pockets of both receptor kinases. BMS-599626 was selected for clinical evaluation based on its potent and selective kinase inhibition profile and excellent antitumor efficacy in xenograft models with HEr1 and/or HEr2 overexpression (Gavai AV, etal, AACR05, Abs. 2539).

BMS-599626 inhibits proliferation of tumor cell lines that express moderate to high level of each or both of HEr1 and HEr2. In addition to inhibiting HEr1 and HEr2 kinase activity, BMS-599626 also inhibits formation of HEr1/HEr2 heterodimers. BMS-599626 is highly selective for HEr1 and HEr2, with no significant inhibition or interaction with >100 diverse protein kinases tested. According to pharmacogenomic analyses, HEr2 expression is the predominant predictor of sensitivity of breast tumor cell lines to inhibition by BMS-599626, further supporting this agent's selectivity. BMS-599626 exhibits significant antitumor efficacy in multiple HEr1- and HEr2-dependent xenograft models, and tumor growth inhibition correlates with inhibition of receptor signaling. BMS-599626 has excellent oral bioavailability in multiple species, and efficacy in xenograft models was achieved with once daily oral dosing (Wong TW, AACR05, Abs. 3395).

In March 2004, a nonrandomized, open label, dose comparison, parallel assignment, safety, phase I clinical trial (protocol ID: CA181-002, NCT00095537) was initiated at Arizona Cancer Center (Tucson and Scottsdale, AZ) and at the University of California Los Angeles (UCLA), to identify the highest oral dose of BMS-599626 that can be administered safely on a daily schedule of 21 days, with a 7-day rest period, in patients with cancer who no longer benefit from other commonly used treatments. Primary objectives are to determine MTD, biologically active doses, and recommended phase II dose. Secondary objectives are to evaluate effect of BMS-599626 on biomarkers and predictive markers of HEr1 and HEr2, ascertain preliminary evidence of antitumor activity, and evaluate effect of gastric acid modifying agents on systemic exposure of BMS-599626. Total expected enrollment is 60.

Initial eligibility requirements for trial participation included tumor expression of HEr2 by IHC. After 6 patients were enrolled, this criterion was modified to include all patients regardless of HEr2 status. A total of 7 patients were treated by 3 dose levels of 100, 200, and 320 mg/day. As of November 2004, safety and PK data were available for 6 patients treated with 100 mg (n=3) and 200 mg (n=3). No DLT was observed during cycle 1. Possibly drug-related Grade 1 or 2 adverse events included diarrhea (n=1), nausea (n=3), vomiting (n=1), rash (n=1), fatigue (n=3), musculoskeletal pain/cramp (n=3), and cough (n=3). Terminal half-life was about 20 hours. Exposure increase was linear from 100 mg to 200 mg dose levels. To date, BMS-599626 has been well tolerated, and has a favorable PK profile for daily dosing. Dose escalation is ongoing. HEr2 positivity will be reinstated as an eligibility criterion once MTD is established for further examination in a dose expansion cohort (Garland LL, etal, ASCO05, Abs. 3152).

In another dose-escalation, phase I clinical trial, being conducted in Europe at Institut Gustav Roussy (Villejuif, France) and Vall d'Hebron University Hospital, between May 2004 and November 2004, 13 patients with refractory metastatic solid tumors expressing HEr1 or HEr2 by IHC (FISH for breast), were administered BMS-599626 by daily oral dosing. Dosing was initiated at 100 mg/day, and escalated in subsequent cohorts based on a modified Fibonacci scheme. Data from a prior trial in healthy volunteers, which evaluated single dose PK in 18 subjects, 6 at each dose level (10 mg, 30 mg, and 100 mg) are also included. A large translational ancillary study included skin biopsies (baseline and day 8) and analysis of biomarkers of HEr1 and HEr2. Cohort level 5 reached a dose level of 660 mg. As of November 2004, Grade 1 or 2 adverse events at least possibly related to BMS-599626 included diarrhea (n=3), anorexia (n=1), dyspnea (n=1), sweating (n=1), and rash (n=2). All 3 patients at 480 mg developed Grade 1 skin rash. There was one adverse event in the healthy volunteer trial, represented by transient Grade 2 ALT elevation. Fresh tumor sampling before and during treatment was performed in 3 of 13 patients. Terminal half-life was about 17 hours. BMS-599626 was well tolerated. MTD has not yet been reached (Soria JC, etal, ASCO05, Abs. 3109).

In November 2004, a multicenter, open label, dose-escalation, single agent, phase I clinical trial (protocol ID: CDR0000389510, UCLA-0404066-01, BMS-CA181002, NCT00093730) was initiated at the Jonsson Comprehensive Cancer Center at UCLA, under Study Chair Mark D. Pegram, MD, to determine side effects and best dose of BMS-599626 in patients with metastatic HEr2/neu-overexpressing primary solid tumors. Primary objectives are to determine MTD, biologically active dose,

and recommended phase II dose. Secondary objectives are to determine safety and tolerability, PK, effect on biomarkers and predictive markers of HEr1 and HEr2 in skin and tumors, evaluate tumor metabolic activity, and determine antitumor activity. Patients are treated with oral BMS-599626 once daily on days 1-21. Courses repeat every 28 days in the absence of disease progression or unacceptable toxicity. Cohorts of 3-6 patients are treated with escalating doses of BMS-599626 until MTD is determined. Once the MTD is determined, 20 patients are to be treated at that dose level.

CTCE-9908, an analog of stromal cell-derived factor (SDF-1) and antagonist of SDF-1 receptors, was developed by scientists at Chemokine Therapeutics (Vancouver, BC, Canada) using rational drug design. CTCE-9908 belongs to a family of chemokines that regulate the immune system to prevent cancer growth and metastasis. CTCE-9908 binds competitively to receptors on cancer cells, preventing their interaction with the chemokine SDF-1, which is produced naturally in organs such as bone marrow, liver, and lungs, and is an important regulator of stem cells. SDF-1 acts on receptors, which are expressed in both stem cells and various common cancer cells. Presence of these receptors on cancer cells allows them to migrate from the original cancer site to new sites that are rich in SDF-1, where they develop new blood vessels and form metastases. By limiting transport of malignant cells throughout the body, as well their subsequent attachment in a remote site, treatment with CTCE-9908 significantly curtails new tumor formation. CTCE-9908 may also potentially be used in combination with antineoplastic agents and surgical modalities.

According to results of a preclinical study, CTCE-9908 dramatically inhibited spread of cancer to lung tissue in mice with osteosarcoma. In treated mice, the number of visible lung nodules decreased by 67% when compared to controls. In controls, the average number of surface lung metastases was 9.9, compared to 3.4 nodules in mice treated with CTCE-9908 (Su Young Kim, etal, AACR05, Abs. 256).

The ability of CTCE-9908 to block CXCr4 specific for SDF-1, may also improve effectiveness of chemotherapy at both killing cancer cells and preventing surviving cancer cells from metastasizing by inhibiting angiogenesis. Blocking CXCr4 may work independently of the VEGF mechanism and provide a new avenue for cancer drug development. In a preclinical study, CXCr4 neutralization suppressed growth of tumors derived from Colon38 and PancO2 cells *in vivo* but not *in vitro*. This attenuation of tumor growth appears to be independent of the expression of CXCr4 by cancer cells themselves, as PancO2 cells do not express CXCr4. Furthermore, CD31-positive tumor capillaries were reduced to 45%, and intratumoral blood flow was decreased to 65% by CXCr4 neutralization. VEGF concentration in tumors was not affected by neutralization of CXCr4. These results suggest that the antiangiogenic effects of CXCr4 blockade are related to a reduction in establishment of tumor endothelium, independently of VEGF inhibition. Therefore, the SDF-1/CXCr4 pathway may be a general target for anticancer strategies and blocking this target may be cooperatively effective in combination with other antiangiogenic strategies such as VEGF blockade (Keisuke T, etal, AACR05, Abs. 2350).

A randomized, double blind, dose-escalation, phase I clinical trial, initiated in December 2003, to evaluate safety and PK profile of CTCE-9908 in patients with nsclc, has been completed.

In July 2005, the FDA granted 'orphan drug' designation to CTCE-9908 for treatment of osteogenic sarcoma. Chemokine has been awarded by the USPTO several patents relating to a broad range of composition of matter claims, and to methods by which CTCE-9908 treats cancer.

CX-3543, a cationic porphyrin under development by Cylene Pharmaceuticals (San Diego, CA), in collaboration with the University of Arizona (Tucson, AZ), is a synthetic small molecule targeting a G-quadruplex DNA motif (quadrome) found in the promoter regions of many well characterized oncogenes. This quadruplex motif is grouped into clusters related by sequence, structure, function, and other features that regulate oncogene expression. These structures that regulate transcription of certain oncogenes may be targeted with small molecules, leading to the selective suppression of oncogene expression (Siddiqui-Jain etal, PNAS 2002;99:11593–11598).

According to molecular selectivity assays, CX-3543 targets the quadruplex structures found in the promoter regions of several major oncogenes, including VEGF, PDGF, HIF-1α, H-ras, and c-myc, among others. In *in vitro* tests, CX-3543 did not significantly inhibit liver cytochrome P450 enzymes, exhibited very low metabolism when incubated with human hepatocytes, and did not have any mutagenic effects. PK properties of CX-3543 are favorable in mice, rats, and dogs. The agent is well tolerated in murine xenograft models dosed at levels substantially higher than needed for an antitumor effect. Moreover, CX-3543 inhibits xenograft tumor growth *in vivo* in murine models of pancreatic cancer (MiaPaCa), refractory prostate cancer (PC3), and colorectal cancer (HCT-116) (Lim JK, etal ASCO05, Abs. 3206).

In July 2005, Cylene Pharmaceuticals initiated a phase I clinical trial with CX-3543 to determine safety and tolerability, characterize PK profile, define DLT and MTD, and select the appropriate dose for phase II trials. This phase I trial, being conducted at the Cancer Therapy and Research Center and at the Mayo Clinic (Scottsdale, AZ), is to enroll 36 patients with solid tumors likely to be driven by the relevant oncogenes, such as colorectal, renal, lung, prostate, ovarian, pancreatic, and breast cancer, and lymphoma.

E7820, under development by Eisai (Tokyo, Japan), is an aromatic sulfonamide derivative that, by inhibiting α-2 integrin, exerts antiangiogenic activity by blocking endothelial cell proliferation and tube formation.

E7820 may disrupt signaling associated with microfilaments and microtubules in the non-canonical Wnt pathway that regulates migration and differentiation. E7820 demonstrated a differential cytotoxicity profile in the 60-cell line panel of the NCI Anticancer Drug Screen, and its biologic response profile was similar to that of chloroquinoxaline sulfonamide (CQS) by COMPARE analysis; CQS is a topoisomerase IIα and IIβ poison, which was evaluated in a phase I clinical trial.

In *in vivo* studies, performed by investigators at the NCI, E7820 significantly inhibited growth of HCT116 human colon tumor xenografts at a variety of doses. Thus, in order to explore mechanisms underlying activity of E7820, transcriptional profiles were generated for HCT-116 cells treated with E7820, CQS, and etoposide, another known topoisomerase II inhibitor. DKK-1, a gene whose protein inhibits Wnt signaling, was induced by E7820 and CQS, but not etoposide, indicating the possibility that β-catenin translocation to the nucleus would be inhibited. However, E7820 and CQS treatment augmented β-catenin translocation in HCT-116 cells. Moreover, lack of inhibition of translocation was also confirmed in the APC mutant colon cell line SW480. It is notable that there was no change in protein expression of the β-catenin target genes, cyclin D and c-myc, but both p53 protein levels and nuclear translocation were increased by treatment with E7820 and CQS. Also, there was no difference in E7820-induced cytotoxicity in HCT-116 cell lines expressing exclusively wild-type or mutant β-catenin.

These findings suggest that DKK-1 involvement in E7820 cytotoxicity might be through a noncanonical Wnt pathway linked to cytoskeletal regulation, corroborated further by the observation that E7820 and CQS induced actin and actin-binding genes; increased actin fibers were evident as early as 2 hours after exposure, as well as double nuclei. In comparison, HCT-116 cells treated with actin inhibitors, dolastatin 11 and jaskaplinolide, displayed characteristic aggregation post-treatment. Because actin regulates cytokinetic events, the mitotic spindle and nuclei were examined in drug-treated cells. Immunofluorescent staining for β-tubulin revealed structures consistent with monopolar spindles in cells treated with these agents (Mertins SD, etal, AACR05, Abs. 2307).

A nonrandomized, open label, uncontrolled, single group assignment, phase I clinical trial (protocol ID: E7820-A001-102, NCT00078637) of E7820 in patients with metastatic and/or recurrent solid tumors or lymphoma was initiated in January 2004, in the USA, to determine the appropriate dose and evaluate safety of this treatment. This trial uses a standard dose-escalation design to evaluate safety and to determine MTD of daily oral administration of E7820. Secondary objectives include characterization of the PK profile, assessment of pharmacodynamic markers, such as levels in plasma and serum of DCE-MRI, VEGF, and bFGF, and changes in α-2 integrin expression in circulating platelets, and preliminary exploration of antitumor activity.

Among 18 patients treated with E7820 at doses ranging from 10 to 100 mg/day, no DLT or serious drug-related adverse events were observed. Patients were treated with a median of 3 cycles (range=1-8). Side effects include Grade 1/2 fatigue, anorexia, constipation, diarrhea, nausea, vomiting, dizziness, maculopapular rash, mucositis, liver enzymes elevation, paresthesia, and fever. There were no objective responses, but disease stabilized in 5 patients lasting from 3 to 7+ months, and 4 patients continued therapy for 4+ cycles. The drug's half-life was between 5.7 to 11.9 hours. Cmax at doses above 70 mg/day approaches concentrations active *in vivo* in preclinical models. E7820 at doses up to 100 mg/day has an excellent safety profile in this setting. Enrollment to higher dose levels is ongoing (Mita MM, ASCO05, Abs. 3082).

GRN163L, under development by Geron (Menlo Park, CA), is a lipid-conjugated thio-phosphoramidate (N3'-P5') oligonucleotide that is a specific telomerase inhibitor for treatment of cancer. Telomerase is ribonucleoprotein, an enzyme, that synthesizes new 5'-d(TTAGGG)-3' hexameric repeats (telomeres) at the 3' ends of eukaryotic chromosomes. Without telomerase, telomeres shorten to a critical length, resulting in chromosomal instability, and cell-cycle arrest or death. Each time a normal cell divides, telomeres shorten, and having reached a certain critical length after generally 60 to 100 cell divisions, cells enter a nondividing state called replicative senescence.

Human telomerase is repressed in most normal somatic cells, is transiently inducible in certain stem or progenitor cells, and is constitutively activated in germline and tumor cells. Reactivation of telomerase is a very common feature of human malignancies, with high telomerase activity detectable in 85%-90% of primary tumors, but not in most normal tissues. Abnormal telomerase upregulation has been associated with cell immortality, and although not sufficient in itself to induce neoplasia, is thought to be essential in maintaining the proliferative capacity of tumor cells.

GRN163L binds tightly to the template region of the RNA component of telomerase, blocking the active site of the enzyme. GRN163L inhibition of telomerase activity slows tumor-cell growth and progression *in vitro,* and inhibits tumor growth in various *in vivo* tumor models, including myeloma, and hepatocellular, ovarian, and lung cancer. Additionally, GRN163L in combination with various cytotoxics, including melphalan, bortezomib (Velcade; Millennium Pharmaceuticals), and paclitaxel was more effective than any agent alone in selected tumor models, including melanoma, myeloma, and ovarian carcinoma (Tressler RJ, etal, AACR05, Abs. 4326).

In preclinical safety studies, the toxicity profile of GRN163L generally resembles that typically observed with the oligonucleotide class of molecules, with a clear relationship between plasma concentration and acute toxic effects, readily managed by dose and infusion time. ADME and pharmacodynamic studies support an inter-

mittent treatment schedule at drug levels that should achieve efficacious concentrations of GRN163L in patients with cancer.

GRN163L, is the successor to GRN163. In a preclinical study, in which cells were treated with both GRN163 and GRN163L oligonucleotides, including a mismatch control, with or without a transfection enhancer reagent, GRN163L inhibited telomerase activity effectively in a dose-dependent manner, even without a transfection reagent. The IC_{50} values for GRN163 in various cell lines were on average, 7-fold higher than for GRN163L. GRN163L inhibition of telomerase activity resulted in a more rapid loss of telomeres and cell growth than GRN163. For the first time, it was shown that lipid modification enhanced potency of the novel GRN163 telomerase inhibitor (Herbert B, etal, Oncogene, 23 May 2005;10(1038);1208760). GRN163L was more active than GRN163 in inhibiting telomerase in 14 different tumor cell lines, representing 9 different human malignancies including lung, breast, prostate, liver, and early stage breast cancer. GRN163L-treated cells exhibited growth inhibition and apoptosis after only 20 cell divisions. In another experiment, a single tail vein injection of fluorescently labeled versions of GRN163L and GRN163 into mice harboring SC human prostate cancer, resulted in greater tumor cell uptake and greater telomerase inhibition by GRN163L, compared to the nonlipidated drug.

In a preclinical study to assess the consequences of telomerase inhibition by GRN163L in breast cancer cells, involving a variety of breast cancer cell lines representing different tumor subtypes and genetic backgrounds, MDA-MB-231, MCF-7, SK-BR-3, 21NT, HCC1937, HCC1937+wtBRCA1, and tumorigenic human mammary epithelial (HME) cells lines were treated every 3 days with GRN163L during the course of a long term experiment. GRN163L was effective in inhibiting telomerase activity in a dose-dependent fashion in these cell lines; however, effective inhibitory concentrations differed within the breast cancer cell line panel. Interestingly, optimal endpoints of cell growth inhibition and apoptosis did not correlate with what was predicted based on initial telomere length. The cell lines in this study all had similar initial telomere lengths, but telomerase inhibition did not always result in a decrease in cell growth as predicted. However, those cells that exhibited telomerase inhibition yet continued to grow did exhibit changes in tumorigenicity (Gryaznov S, etal, AACR05, Abs. 583).

In another preclinical study, GRN163L reduced tumor volume in a human ovarian carcinoma xenograft model. Treatment of a panel of ovarian carcinoma cell lines with GRN163L also inhibited telomerase activity by >95% and induced apoptosis. When efficacy of GRN163L alone was compared with that of a combination of GRN163L and paclitaxel, tumor mass was reduced 90% with GRN163L alone, 80% with paclitaxel alone, and 96% with the combination (Chin A, etal, AACR05, Abs. 2839).

Systemic daily treatment with GRN163L also reduced tumor burden by 70% in the CAG multiple myeloma xenograft model (Wang, E, etal, Blood, 1 Jan 2004;103(1);258-66). In this model, GRN163L was administered alone or in combination with either a single dose of melphalan, or 9 doses of bortezomib. Tumor mass was reduced 56% with GRN163L alone, 60% with melphalan alone, and 89% with the combination of GRN163L and melphalan. At the dose used in this study, bortezomib alone showed no efficacy, but combination of GRN163L with bortezomib reduced tumor mass by 68% compared to controls. These data illustrates increased efficacy of GRN163L in combination with cytotoxics over either agent alone (Chin A, etal, AACR05, Abs. 2840).

In July 2005, Geron initiated a dose-escalation, sequential cohort, phase I/II clinical trial (protocol ID: GRN163L CP04-151, NCT00124189), primarily designed to demonstrate safety and tolerability, and establish DLT and MTD of GRN163L administered IV on a weekly basis to patients with refractory or relapsed CLL. Secondary objectives include PK profile of GRN163L measured for 2 cycles; pharmacodynamic markers of GRN163L activity measured in each cycle; and preliminary antineoplastic activity. The trial, expected to enroll 48 patients, is ongoing at two clinical sites, North Shore University Hospital, (Manhasset, NY), under PI Steven Allen, MD, and Long Island Jewish Medical Center (New Hyde Park, NY), 11040), under PI Kanti Rai, MD. CLL provides a unique opportunity to measure both magnitude and time course of telomerase inhibition in tumor cells achieved at various doses of GRN163L. By serially assessing effects of the drug on the target enzyme in CLL cells, insights will be gained regarding dose and dosing interval that optimally inhibit telomerase activity in the tumor. In this way, PK and pharmacodynamic parameters can be correlated with any observed reduction in patients' tumor burden.

HGS-ETR2 (HGS-TR2J, or KMTR-2), under development by Human Genome Sciences (HGS; Gaithersburg, MD), is a tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor 2 MAb (TRAILr2 MAb), a fully human MAb agonist of TRAILr2 that blocks tumor growth and induces apoptosis. This agent is being developed in collaboration with Cambridge Antibody Technology (Cambridge, UK).

TRAIL death receptors TRAILr1 and TRAIr2 are abundantly expressed on the cell surface of a majority of human tumor cells. Binding of agonist MAb or the ligand TRAIL, to either functional death receptor activates a protease-mediated signaling cascade that culminates in apoptosis. HGS-ETR2 actively and rapidly stimulates the TRAIL receptor pathway, leading to significant inhibition of tumor growth in human tumor cell lines *in vitro* and *in vivo*. In *in vitro* experiments, HGS-ETR2 was shown to bind specifically to human TRAILr2. *In vivo*, HGS-ETR2 exhibits antitumor activity in a wide range of human tumor xenograft models, both as a single agent and in com-

bination with commonly used chemotherapeutics. Also, sensitivity to HGS-ETR2 extends beyond the requirement for cell-surface receptor expression.

Human tumor cell lines from different tumor types expressing TRAILr2 on their cell surface displayed moderate to significant sensitivity to HGS-ETR2, ranging from 50% to 90% cell death. In addition, in several different human tumor cell lines, HGS-ETR2 enhanced cytotoxic activity of various chemotherapeutic agents. Importantly, some combinations elicited a synergistic effect on cell viability. Pre-established nsclc and colon adenocarcinoma xenografts rapidly regressed after treatment with HGS-ETR2. Tumor volume was significantly reduced (90%-75%) after the first dose of HGS-ETR2, and inhibition of tumor growth persisted throughout duration of treatment, with tumors completely eliminated in several animals. Rapid decrease in tumor volume was associated with a concomitant increase in the number of apoptotic cells in the tumor, and an increase in levels of active caspases, an indicator of TRAILr pathway activity. In addition, TRAILr2 expression appeared to fluctuate in xenografts as determined by IHC (Humphreys RC, etal, AACR05, Abs. 4388).

Clinical development of HGS-ETR2 began in July 2003, in the UK, with an open label, dose-escalation, phase I clinical trial (protocol ID: N0258127572), initiated at Royal Marsden Hospital (Sutton, Surrey, UK), under PI Johann de Bono, MD, and at Northern Institute for Cancer Research (Newcastle-upon-Tyne, UK). According to the protocol, patients with advanced solid tumors were treated with HGS-ETR2 IV once every 21 days. Primary objectives of this trial were to assess this agent's safety and tolerability, as well as PK and disease response. According to an interim analysis, HGS-ETR2 was administered by a 30-minute IV infusion every 3 weeks at a dose of 0.1 and 0.3 mg/kg (n=4 each) and at 1 mg/kg by a 2-hour infusion (n=6). A total of 14 patients were treated with 34 doses (range=1-8 doses per patient) of HGS-ETR2. Disease stabilized in 1 patient with rapidly progressing metastatic chondrosarcoma, treated with 8 courses of HGS-ETR2.

HGS-ETR2 was well tolerated with minimal toxicity; 1 patient treated with 1 mg/kg developed Grade 3 asymptomatic rise in serum amylase, detected on day 15 of course 1, which resolved to Grade 2 on day 23, and to baseline by day 43. This event may have been related to concurrent administration of ciprofloxacin. Up to 5 patients were treated at this dose level without DLT or serious adverse events. Preliminary PK results are consistent with a two-compartment model with first order elimination from the central compartment. At 0.3 mg/kg, terminal half-life ranged from 10.03 to 14.98 days (de Bono J, etal, EORTC-NCI-AACR04, Abs. 197).

This phase I trial was completed in August 2004. Among 22 patients treated with 73 courses of HGS-ETR2 over 5 dose levels ranging from 0.1 to 10 mg/kg, the majority (20/22) were treated with at least 2 courses (range=1-13). With the exception of the Grade 3 asymptomatic elevation of serum amylase in a patient treated with concomitant ciprofloxacin, there were no other instances of DLT. A patient with hepatocellular carcinoma and extensive hepatic metastases experienced Grade 3 transient transaminitis, which subsequently resolved with the patient remaining on treatment. To date, disease stabilized for 2 cycles in 7 patients. HGS-ETR2 PK are linear across a 100-fold dose range. Human antihuman antibodies (HAHA) formation has not been detected. Based on results from this trial, HGS-ETR2 can be safely administered IV every 3 weeks at doses that reach plasma concentrations associated with activity in preclinical models (Pacey S, etal, ASCO05, Abs. 3055).

In an open label, dose-escalation, phase I clinical trial being conducted at the Institute for Drug Development (IDD) and Stanford University School of Medicine (Stanford, CA), patients with advanced, relapsed or refractory solid tumors previously treated with chemotherapy, radiotherapy, and/or hormone therapy, are being enrolled into 5 cohorts (0.1, 0.3, 1.0, 3.0, or 10.0 mg/kg) and treated with HGS-ETR2 administered IV every 14 days. The primary objective of this trial is to evaluate safety and tolerability and PK of HGS-ETR2 in this every 2 weeks dosing scheme. According to an interim report, the drug was well tolerated, with minimal toxicity in 19 patients treated with a combined total of 89 courses of HGS-ETR2 over 4 dose levels (0.1-3.0 mg/kg), every 14 days. Among 11 patients, treated with 44 courses at dose levels 0.1 and 0.3 mg/kg, events possibly related to the drug include Grade 2 fatigue (n=2), and Grade 1 anxiety (n=1), confused state (n=1), hypertension (n=1), pyrexia (n=1), and stomatitis (n=1). There were no DLT, and MTD has not been reached. Disease stabilized in 5 patients. Preliminary data indicate that PK of HGS-ETR2 is dose-proportional up to 1.0 mg/kg, with a terminal half-life of 5 days. The trial continues to enroll patients at higher dose levels (Tolcher AW, etal, AACR05, Abs. 543).

KRN951, under development by Kirin (Tokyo, Japan), is a novel small molecule inhibitor of receptor tyrosine kinases VEGFr1, 2, and 3. In preclinical models, KRN951 was shown to be a potent angiogenesis inhibitor with a broad spectrum of antitumor activity against human tumor xenografts in animal models.

Antitumor efficacy of KRN951 against peritoneal carcinomatosis was evaluated in a rat syngeneic model using RCN-9, a rat colon cancer cell line. Once daily oral administration of KRN951 at 1.0 and 3.0 mg/kg for 10 days starting from day 4 significantly reduced the number of tumor nodules (65.3% and 32.3%, respectively) compared with control. This reduction was associated with suppression of tumor angiogenesis on mesenteries. KRN951 also inhibited accumulation of malignant ascites at both doses; virtually no ascites developed in the group treated with KRN951 at 3 mg/kg. Surprisingly, KRN951 administration initiated on day 14, when well established tumor nodules and accumulation of malignant ascites were present, resulted in a

similar significant antitumor activity including apparent loss of malignant ascites on day 25 at the end of the study, while half of the control rats were dead by day 25. These observations suggest that KRN951 plays both a therapeutic as well as preventive role in this setting. Using the same model, effects of KRN951 were also evaluated on rat survival time. Continuous treatment with KRN951 initiated on day 0 at 1.0 mg/kg almost doubled MST to 53.5 days in the treated animals compared to 27.5 days in controls. Treatment initiated on day 14 at 1.0 mg/kg also significantly prolonged MST to 34.5 days compared to 22.5 days in controls. According to these findings KRN951 may be of value in treating patients with peritoneal cancer with ascites (Taguchi E, etal, AACR05, Abs. 5836).

KRN951 is being evaluated in a phase I clinical trial in Japan.

LErafAON-ETU, under development by NeoPharm (Lake Forest, IL), is an easy-to-use (ETU), liquid, liposomal formulation of an antisense oligonucleotide complementary to the c-raf mRNA sequence. The lipid component consists of a novel, positively charged, synthetic cardiolipin (PCL-2), based on NeoLipid technology comprising a cationic liposome composed of dimethyldioctadecyl ammonium bromide, phosphatidylcholine, and cholesterol. NeoLipid formulations are designed to produce a small and homogenous drug particle size that may be readily reconstituted from a lyophilized (freeze-dried) form. In addition, liposomal formulation eliminates the need for extensive phosphorothioate modification; thus, only the 3'- and 5'- termini of the LERαfAON-ETU oligonucleotide are modified.

In November 2004, NeoPharm initiated a dose-escalation, phase I clinical trial (protocol ID: LERαfAON-ETU-104, NCT00100672) at the Arizona Cancer Center (Phoenix, AZ), under PI Michael Gordon, MD, designed to determine DLT and MTD of LERαfAON-ETU in patients with advanced cancer. According to the protocol, the drug is administered as a 60-minute IV infusion once weekly for 3 consecutive weeks, constituting one treatment cycle. Patients may be treated with repeated cycles until disease progression or unacceptable toxicity. Dose levels of 7.5, 15, 30, 60, 120, 240, and 480 mg/m^2 or higher are to be evaluated, depending on tolerability of LERαfAON-ETU. An accelerated dosing strategy will be implemented after safety and tolerability of the initial dose level has been established in 3 patients. In this design, a single patient is treated at each subsequent dose level until a Grade ≥2 adverse event, a pre-infusion platelet count of <75,000/mm^3, or an increase in the serum creatinine level to ≥1.5 x the upper limit of normal, occurs. At such time, this cohort will be expanded, and a standard dose-escalation scheme will be employed, with 3-6 patients in each cohort dependent upon occurrence of DLT. This accelerated dose-escalation scheme should reach potentially therapeutic doses more rapidly than a standard design. Infusion has been completed in 3 patients at the initial dose of 7.5 mg/m^2, and dose escalation continues (Steinberg JL, etal, ASCO05, Abs. 3214).

ON01910.Na, under development by Onconova Therapeutics (Lawrenceville, NJ), is a novel anticancer agent acting as a dual kinase inhibitor affecting cyclin-dependent kinase 1 (CDK1), polo-like kinase 1 (PLK1), VEGFr, and PDGFr.

Antitumor effects of ON01910.Na are negatively affected by binding of this agent to human serum albumin (HSA). Investigators at Mount Sinai School of Medicine (New York, NY) and Temple University (Philadelphia, PA), examined interaction of ON01910.Na with HSA *in vitro* as a function of cell kill effect against Daudi human lymphoma cells. In the presence of HSA at a concentration of 2.5 g/dl, chosen because of its proximity to physiologic conditions, >90% of ON01910.Na was bound to HSA, rendering the drug hardly cytotoxic, with only a 10% cell kill effect. Addition of warfarin, ibuprofen, and acetylsalicylic acid into the ON01910.Na/HSA mixture resulted in restoration of cell kill effects to 30%, 10%, and 37% of controls. At a higher concentration of ON01910.Na, which produces nearly total cell kill without HSA, but 30% cell kill in the presence of HSA, warfarin, ibuprofen, and acetylsalicylic acid restored cell kill effects of ON01910.Na to 90%, 70%, and 100%. These data indicate that HSA-bound ON01910.Na is biologically inactive. Warfarin almost completely and ibuprofen partially restored HSA's inactivating effect on ON01910.Na. Acetylsalicylic acid may release ON01910.Na from HSA *in vivo* and improve its biologic activity in the clinical setting (Ohnuma T, etal, AACR05, Abs. 4103).

In July 2004, Onconova Therapeutics initiated enrollment in a single agent, dose-escalation, phase I clinical trial of ON01910.Na in patients with advanced solid tumors. This trial marks this agent's first use in humans, and is designed to measure safety of ON01910.Na when administered IV twice per week, for 3 weeks, with 10 days off. This trial, being conducted at the Johns Hopkins Kimmel Cancer Center (Baltimore, MD), is to enroll up to 30 patients. Trial objectives are to determine DLT, MTD, recommended phase II dose, plasma PK, and responses by objective measurements.

OXi4503 (CAP1), under development by OXiGENE (Waltham, MA), is the first in a new class of compounds known as ortho-quinone prodrugs, which display a novel cytotoxic effect in addition to their proven vascular targeting capabilities mediated by their action on the tubulin cytoskeleton. Unlike antiangiogenesis agents that focus on preventing new tumor blood vessels from forming, ortho-quinone prodrugs act as VTA attacking existing blood vessel structures in the central regions of solid tumors, and also exert a cytotoxic effect that could destroy the outside rim of cells residing next to, and dependent on, normal tissue blood vessels.

In August 2005, in a presentation at the 9th International Workshop on the Tumor Microenvironment

Meeting held at Christ Church College, in Oxford, UK, OXiGENE presented data supporting a separate and distinct mechanism of action for OXi4503 as compared to CA4P, from a study conducted by Peter Wardman, PhD, and colleagues at the Gray Cancer Institute (Northwood, Middlesex, UK). Results suggest fundamental differences between pharmacologic properties of CA1 and CA4, including formation of a reactive quinone and hydroxyl radicals that merit further investigation as the possible basis for their different activities *in vivo*.

Investigators at the Royal Free and University College Medical School (London, UK) evaluated therapeutic efficacy of CA1P as a potential candidate for combination trials. CA1P was administered IP to nude mice bearing either SC or orthotopic SW1222 colorectal xenografts with metastatic deposits in the liver. Tumors were removed at selected time points, and a range of tumor parameters were studied in order to ascertain any changes related to vascular shutdown, such as blood vessel status, hypoxia, and perfusion. Untreated tumors were mostly viable, with perfused blood vessels throughout, and only small areas of hypoxia. In the SC animal model, tumor perfusion ceased in all but the tumor periphery by 40 minutes post CA1P treatment, widespread hypoxia developed by 1 hour, the center of the tumor became necrotic by 6 hours and, by 24 hours, only a thin outer rim of tumor cells survived. These effects were similar to those achieved with CA4P, but were more reproducible and more dramatic.

Results with orthotopic tumors were similar to those seen in the SC model. Central perfusion ceased in most of the tumor deposits by 1 hour after treatment, with no effect on normal liver. By 24 hours, there was no resumption of perfusion within the tumor, and many of the deposits exhibited extensive central necrosis. In preliminary experiments, groups of 6 mice were administered either no treatment, or CA1P IP on days 1, 8, and 21. CA1P alone significantly inhibited tumor growth compared with control, which was not the case when CA4P was used as a single agent (Begent R, etal, AACR05, Abs. 3016).

In April 2005, a 2-center, dose-escalation, phase I clinical trial (protocol ID: N0143162638) of OXi4503 was initiated at Christie Hospital (Manchester, UK), under PI Pat Price, MD, and Mount Vernon Hospital (Northwood, Middlesex, UK) under PI Gordon Rustin, MD, in patients with advanced solid tumors. OXi4503 is administered IV 3 times weekly. The objective of the trial is to establish MTD of this regimen. Up to 40 patients will be enrolled in this trial.

RAV12, under development by Raven Biotechnologies (South San Francisco, CA), is a high affinity IgG1 chimeric MAb that recognizes RAAG12, a primate-specific N-linked carbohydrate epitope (glycotope) expressed by >90% of gastric, colon, and pancreatic adenocarcinomas, and in smaller proportions by RCC and prostate, ovarian and breast carcinomas. In addition to its prevalent expression in many tumor types, RAAG12 is also expressed by normal nonkeratinizing epithelia, including some ductal, stratified squamous, and gastrointestinal (GI) epithelia. RAV12 was constructed based on its murine precursor, KID3, which was generated by immunization of mice with a Raven proprietary human kidney progenitor cell line as the antigen source. Like KID3, RAV12 exhibits cytotoxic activity *in vitro* against COLO 205 human colon tumor-derived cell line, which expresses high levels of the RAV12 epitope. RAV12 is highly efficacious in colon, gastric, and pancreatic tumor xenografts *in vivo*.

In vitro, within hours after RAV12 treatment, cells increase in volume, followed by bursting of the plasma membrane and release of lactate dehydrogenase (LDH), consistent with a cytotoxic mechanism of action. Cytotoxic activity was correlated with high and uniform antigen expression levels, antigen cross-linking, and internalization of RAV12. RAV12 exhibits potent antitumor activity in murine tumor xenograft models. RAV12 reduced the average size of four different human GI tumor-cell xenografts grown beneath the renal capsule of mice by more than 90% at the end of the 2-week dose period, and eliminated detectable subrenal capsule tumor xenografts, with no tumor regrowth seen 2 weeks following cessation of dosing. In SC tumor xenografts, the minimal efficacious dose of IV RAV12, dosed twice weekly, was 3 mg/kg in the SNU-16 gastric tumor xenograft model, conferring 70%-82% tumor growth inhibition. Although RAAG12 is expressed by normal epithelia, RAV12 was well tolerated in a repeat dose toxicology study in cynomolgus monkeys, in which a no observed adverse affect level (NOAEL) was established supporting the proposed starting dose for the phase I trial (Loo D, etal, AACR05, Abs. 558).

In preclinical tests, RAV12 accelerated desensitization of Akt/PKB pathway associated with insulin-like growth factor 1 receptor (IGF1r) signaling in COLO205 cancer cells. IGF1r, responsible for promoting cell survival and preventing apoptosis, is widely overexpressed in various tumor-derived cell lines. The RAV12 epitope was found on the IGF1r of COLO201, COLO205, and SNU-16 cells. Treatment of COLO205 cells with RAV12 causes significantly accelerated IGF1r phosphorylation and desensitization, as well as significant changes in some of the major downstream signaling components of IGF1r. RAV12 treatment preferentially activated Akt/PKB (40-fold increase) and p42/MAPK (1.5-fold increase) through the insulin receptor substrate-1 (IRS-1). Activation of these components could be selectively attenuated by various IGF1r inhibitors (Li J and Li R, AACR05, Abs. LB-12).

In January 2005, after obtaining IND clearance by the FDA in December 2004, Raven began enrollment in a multicenter, open label, dose-escalation, phase I/IIa clinical trial (protocol ID: RAVENBIO-RV-2004-002, SCMP-REF-MAL-63, SCMP-TSN-04-094, NCT00101972) of RAV12 in patients with advanced GI malignancies. The trial will enroll 30 patients with colon, stomach, or pancreatic cancer, or other target antigen-positive adenocarcinomas,

including breast, lung, and prostate cancer. The dose range being evaluated in a phase I clinical trial ranges between 3 and 10 mg/kg. The dose range being evaluated in this trial ranges between 3 and 10 mg/kg. The trial is designed to measure safety and tolerability of RAV12. Howard Burris, MD, of the Sarah Cannon Research Institute (Nashville, TN) is the PI.

SNS-595, under development by Sunesis Pharmaceuticals (South San Francisco, CA), is a novel naphthyridine analog that induces a G2 cell-cycle arrest *in vitro*, and exhibits broad activity in tumor xenografts and drug-resistant tumor models *in vivo*.

SNS-595 acts during the S-phase of the cell cycle, causing cell-cycle arrest, which leads to apoptosis, mediated through both p53-independent and dependent mechanisms. To confirm biologic activities observed in cell culture *in vivo*, tumor homogenates were analyzed after a single administration of SNS-595 to mice bearing advanced HCT-116 tumors. Within 24 hours of SNS-595 administration, modulation of pharmacodynamic markers was detected consistent with G2 cell-cycle arrest (cyclin B and cdc 2) and apoptosis (p53, p21, and caspase-3). Pharmacodynamic effects in tumors correlated with tumor exposure to SNS-595 and, when dosed on a weekly schedule, contributed to significant growth delay of SC-implanted HCT116 tumors. In addition to a weekly schedule, less frequent schedules were studied in mice bearing SC Colo-26 tumors, a highly metastatic syngeneic colorectal tumor model refractory to many of the major classes of cytotoxic drugs. SNS-595 was highly active on all schedules with CR and cures seen when dosed at MTD. When dosed at half the MTD, weekly and biweekly regimens still resulted in cures, but a reduction of the curative effect was apparent with the least frequent regimen (Hoch U, etal, AACR05, Abs. 2277).

Kinetics and mechanism of apoptosis was characterized in SNS-595-treated cells and compared to activities of 8 clinically relevant cytotoxics, including cisplatin, docetaxel, etoposide, gemcitabine, doxorubicin, irinotecan, bleomycin, and mitomycin C. The relationship between cell cycle and apoptosis was studied in both asynchronous and synchronous cell populations using several markers of p53-dependent and independent pathways including p53, p73, c-Abl, and p21. In an asynchronous cell population, SNS-595 caused half maximal caspase-3 activation within 5 hours of exposure, 2 times faster than the other cytotoxics studied. In cells synchronized at G1/S and treated with SNS-595, a steep increase in caspase-3 activation was observed as the cells entered S phase. SNS-595 did not activate caspase-3 during M or G1 phases of the cell cycle, nor was caspase-3 activated in noncycling cells. Results are consistent with cell-cycle analysis, indicating an S-phase lag, S-phase checkpoint activation, and G2 arrest following SNS-595 treatment. Analysis of the signaling pathways indicate that apoptosis is stimulated by SNS-595 through p53-independent and dependent mechanisms. In contrast to comparator compounds, SNS-595 stimulates p21 expression and caspase-3 activation rapidly (within 30 minutes) after p53 phosphorylation. Thus, SNS-595 stimulates the apoptotic cascade and subsequent cell death only when dosed during DNA synthesis; apoptosis follows stimulation of the p53, p73, and cell-cycle checkpoint pathways (Hyde J, etal, AACR05, Abs. 2285).

Cell-cycle progression was analyzed using both DNA content and cell-cycle markers cyclin A, B, and E. In asynchronous populations, SNS-595 treatment caused a full G2 arrest in all cell lines tested, accompanied by rapid apoptosis as determined by DNA fragmentation. In synchronized cell populations treated with SNS-595, cells cycled normally until they reached S phase, which took 30% longer than in untreated cells. Checkpoint markers (chk kinases, cdc25 phosphatases, cdc2, and p21) appeared rapidly upon entering S phase, and cells eventually reached a sustained and irreversible arrest with 4N DNA content. SNS-595 action is distinct from the other G2 arrestors tested in that it causes p21 expression early in S phase, and a significant S-phase lag. SNS-595 also differs from other S-phase active compounds in that it causes a definitive arrest at G2, as opposed to a varied G1/S/G2 arrest profile (Hyde J, etal, AACR05, Abs. 2293).

A multicenter (n=4), dose-escalation, phase I clinical trial was initiated in June 2004, to examine safety, tolerability, and PK of SNS-595 in order to establish optimal dosing regimens for use in phase II clinical trials. In this trial, SNS-595 is being administered to patients with advanced solid tumors, as a single dose without premedication, every 3 weeks as a 10-minute IV infusion, followed by a 21-day observation period for up to 6 treatment cycles. Cohorts of 3 to 6 patients were accrued at doses based on a modified Fibonacci scheme. As of December 2004, 16 patients were treated in the first 5 cohorts at doses starting at 3 mg/m^2 and advancing to 48 mg/m^2. Tumor types included lung cancer (n=4), adenocarcinoma of unknown primary (n=4), RCC (n=3), and ovarian cancer, melanoma, bladder cancer, sarcoma, and cholangiocarcinoma (n=1 each). No DLT was observed. Transient Grade 4 hematologic toxicity occurred in 2 of 3 patients in cohort 5 treated at the 48 mg/m^2 dose. Nonhematologic toxicities were all Grade 1/2. AUC increased proportionally with dose. Terminal half-life is approximately 19 hours. In this phase I trial, SNS-595 was well tolerated, with consistent and predictable PK effects. Dose escalation is continuing (Advani R, etal, ASCO05, Abs. 2099).

In October 2004, a second, open label, multicenter (n=4), dose-escalation, phase I clinical trial was initiated, designed to examine weekly dosing regimens of SNS-595 for safety, tolerability, and PK, in patients with advanced solid tumors. Over a 28-day cycle, SNS-595 is being administered weekly on days 0, 7, and 14, followed by a 14-day observation period. Patients participating in the trial are eligible for up to 6 cycles of treatment.

SU014813, under development by Pfizer (La Jolla, CA), is a novel, orally active, multireceptor RTK inhibitor with potent antiangiogenic and antitumor activity. It is a broad spectrum RTK inhibitor similar to Sugen's (San Francisco, CA) agent SU011248. It inhibits ligand-dependent and independent proliferation, migration, and tube formation of endothelial cells and/or tumor cells expressing VEGFr2, PDGFrα and β, c-Kit, and Flt-3.

SU014813 inhibits VEGF and Flt-3 receptor tyrosine phosphorylation *in vivo* in the A375 human melanoma and the MV4;11 human leukemia xenograft models in mice, respectively. It also significantly inhibits VEGF-induced skin vascular permeability, and partially inhibits c-Kit-driven pigmentation of newly regrown hair in mice. SU014813 dose dependently delays growth of diverse xenograft tumors in mice including Colo205, C6, MV522, and LLC, and also blocks MV4;11 cell engraftment and bone marrow myeloproliferation in mice. Furthermore, administration of SU014813 in combination with docetaxel significantly enhanced inhibition of primary tumor growth and survival of the LLC-bearing mice as compared to administration of either agent alone. SU014813 is well tolerated in preclinical efficacy studies (Hu-Lowe DD, etal, AACR05, Abs. 2031).

A multicenter, dose-escalation, phase I clinical trial is being conducted in Europe in patients with advanced refractory solid tumors. One of the participating centers is VU Medical Center (Amsterdam, the Netherlands), under PI E. Boven, MD.

ZK-304709 (ZK-CDK), also referred to as the Multitarget Tumor Growth Inhibitor (MTGI), under development by Schering (Berlin, Germany), is a pyrimidine-based nanomolar inhibitor of CDK1, 2, 4 and 7, VEGFr1, 2, and 3, and PDGFβ. ZK-CDK is a novel, chemically synthesized small molecule ATP-competitive kinase inhibitor that is unique in that it combines inhibition of tumor-cell growth with inhibition of tumor angiogenesis in one single molecule.

ZK-304709 in the nanomolar range potently inhibits proliferation of various human tumor cells. It blocks cell-cycle progression consistent with CDK inhibition. In tumor-cell lysates, 1 micromolar of ZK-304709 was sufficient to inhibit phosphorylation of retinoblastoma (Rb) protein in MCF7 cells. Inhibition of VEGF-induced vascular permeability in nude mice indicates that ZK-304709 blocks the VEGF-RTK system *in vivo*. Oral treatment of MaTu human estrogen-independent xenografts resulted in inhibition of phosphorylation of intratumoral Rb protein, induction of massive apoptosis, and tumor regression. ZK-304709 quantitatively inhibits the relevant pathways *in vivo*. Furthermore, ZK-304709 was well tolerated by nude mice at therapeutic doses. The multitarget mechanism of action of ZK-304709 results in highly efficacious inhibition of growth of human tumor xenografts (Siemeister G, etal, AACR05, Abs. 5842).

A dose-escalation, phase I clinical trial with ZK 304709 was initiated in May 2004, in Europe, in patients with advanced solid tumors.

Immunotherapy/Vaccines

Numerous immunotherapy/vaccine approaches are in development with over 150 in ongoing clinical trials. In addition, many vaccines, mostly based on autologous/individualized approaches using *ex vivo* processing, are being developed by institutions often without commercial backing.

Immunotherapy is an established management approach for several immunogenic tumors, including RCC and melanoma. It is, therefore, not surprising that responses in phase I clinical trials with novel single agent immunotherapeutics are the highest compared to those achieved with any other novel anticancer drugs (Exhibit 3 and 7).

GI-4000, under development by GlobeImmune (Aurora, CO), is based on the company's Tarmogen vaccine technology involving whole nonpathogenic heat-killed recombinant Saccharomyces cerevisiae (baker's yeast) genetically engineered to express one or more protein antigens within their cell walls. Tarmogens generate potent T-cell immune responses against cells expressing target antigens, and are not neutralized by the host immune system. They are simple to manufacture, and can be rapidly engineered against new antigen and disease targets.

Recombinant yeast represents a novel vector for generating antigen-specific immune responses. GI-4000 generates potent T-cell immune responses against cells expressing mutant Ras. GI-4000 comprises GI-4014, GI-4015, and GI-4016 recombinant yeast vectors, each expressing a truncated and modified human Ras protein containing 1 of the 3 most common mutations at codon 12, G12V, G12C, or G12D, and the two most common mutations at codon 61, Q61R, and Q61L. A number of tumors express activating, transforming mutations at codons 12 and 61 of the ras oncogene. These mutations in Ras oncoproteins are attractive targets for cancer immunotherapy. Early data in humans suggest that, even at the lowest dose, these vectors generate mutation-specific cellular responses with an acceptable safety profile.

Whole heat-inactivated recombinant S. cerevisiae expressing mutated Ras proteins induce protective cellular immunity, as well as CR of established, carcinogen-induced, ras mutation-bearing lung tumors in mice. Therapeutic immunization with the whole recombinant yeast causes complete regression of established ras mutation-bearing lung tumors in a dose-dependent, antigen-specific manner (Lu Y, etal, Cancer Res, 1 Aug 2004;64(15):5084-8).

A multicenter (n=4), open label, dose-escalation, phase I clinical trial was initiated in May 2004, to evaluate safety, immunogenicity, and clinical benefit of GI-4000 in patients with metastatic colorectal or pancreatic cancer or

nsclc, refractory to at least first line chemotherapy. A requirement for enrollment is that a patient's tumor sample may be subjected to genomic sequencing of the K-, H- and N-ras genes. Patients whose tumors contain one of the target mutations, are treated SC with 5 weekly doses with the appropriate vaccine construct, and are followed for an additional 56 days for safety, immunogenicity and tumor response.

Among 32 patients evaluated, 9 had ras mutations in their tumors, with 7 matching those targeted by the GI-4000 constructs. Mutation-specific T-cell responses were confirmed by proliferation and cytokine secretion assays in 2/3 patients treated with a low dose of the drug. There were no serious treatment-related adverse events; one patient experienced possibly treatment-related mild fever and malaise. Based on these early results, it appears that recombinant yeast represents a novel vector for generating antigen-specific immune responses. Even at the lowest dose, these vectors generate mutation-specific cellular responses with an acceptable safety profile (Cohn A, etal, ASCO05, Abs. 2571). This trial is to enroll 20-25 patients. Bert O'Neil, MD, at the University of North Carolina-Chapel Hill (UNC-CH), Lineberger Cancer Center, is the PI. Other participating sites include Duke University Comprehensive Cancer Center, University of Colorado (Aurora, CO), and the Seattle Cancer Care Alliance, under PI Anthony Back, MD.

IGN311, under development by Igeneon (Wien, Austria), a subsidiary of Aphton (Philadelphia, PA), is a humanized IgG1 MAb directed against Lewis y carbohydrate antigen, a hexasaccharide selectively expressed on tumors of epithelial cell origin. This blood group-related antigen is associated with 60% to 90% of human malignancies of epithelial cell origin including breast, colon, gastric, and lung cancer. IGN311 was humanized using technology developed by Protein Design Labs (PDL; Freemont, CA).

In an open label, single treatment arm, dose-escalation, phase I clinical trial, initiated at the Augsburg Central Clinic, in Germany, in December 2002, IGN311 was administered to 10 evaluable patients with colorectal cancer (n=7), gastric cancer (n=1), pancreatic cancer (n=1), and breast cancer (n=1), expressing Lewis y antigen. Patients were administered an IV infusion of IGN311, at 50 mg (n=3), 100 mg (n=3), or 200 mg (n=4) on days 1 and 15 of a 43-day protocol. The primary objective of the trial was to deduce the drug's safety and tolerability. In the 200 mg cohort, Grade 3 vomiting and skin reaction was observed in 1 patient. In addition, 4 serious disease-related adverse events occurred, and 2 patients died before protocol completion because of tumor progression (Oruzio DV, etal, ASCO04, Abs. 2624).

According to final results from this phase I clinical trial, reported in July 2005, 12 patients with breast, colorectal, gastric, and pancreatic cancer were enrolled and treated with IV IGN311, administered in escalating doses of 50 mg (n=3), 100 mg (n=3), and 200 mg (n=6). No adverse events related to trial medication were seen in the 50 mg and 100 mg cohort, and only 1 adverse event (vomiting and nausea) was observed in the 200 mg arm. IGN311 was generally well tolerated, with no observed hematologic toxicities. Complement-dependent cytotoxicity (CDC), i.e., the capacity of the antibody to destroy tumor cells, was significantly induced by IGN311 infusions, and was correlated with IGN311 concentration in blood. In this phase I trial, IGN311 demonstrated favorable safety and tolerability, and advantageous PK with a serum terminal half-life of ~21 days. Data from the trial also indicate efficacy of IGN311 against Lewis y-positive tumor cells circulating in peripheral blood; the number of such cells was significantly decreased in those patients who presented with detectable levels of such cells prior to first infusion.

IGN311 demonstrates characteristics of a connective antibody, which may be naturally present in the immunologic network of ~60 % of patients. Despite detection of type I anti-idiotypic antibodies (anti-Id) in 7 of 11 (60%) patients shortly after first infusion of IGN311 in the phase I clinical trial, the drug turned out to have a very favorable serum terminal half-life of 21 days. However, IGN311 titer was decreased in patients with anti-Id responses. The anti-Id antibody response in one of the patients was of IgG class only. An increase in anti-Id titers was seen only after the first infusion, and these titers were not boostable. Repeated infusions of IGN311 in the patient with the highest anti-Id titers confirmed that neutralization of biologic activity of IGN311 was seen only at the onset of treatment, when anti-Id antibodies were still detectable. CDC and ADCC activity correlated with PK data. Detection of both IGN311 and anti-Id antibodies in the same serum sample, indicate low affinity interactions between the two counterparts, which was confirmed by absence of complement activation in such samples. Presence of anti-Id antibodies of this type did not correlate with clinical side effects (Waxenecker G, etal, AACR05, Abs. 692).

In July 2005, enrollment was initiated in a new open label, phase I/II trial of IGN311. Trial endpoints are to evaluate ability of IGN311 to effectively reduce the number of Lewis y-positive tumor cells, and to decrease volume of pleural effusions or ascites, in patients with epithelial malignancies. In addition, the trial will evaluate safety, tolerability, and PK of IGN311. This trial is to enroll up to 24 patients.

Interleukin-21 (IL-21), under development by ZymoGenetics (Seattle, WA), is recombinant human IL-21 (rhIL-21), a cytokine produced by activated CD4+ T cells that participates in growth, survival, and activation of several hematopoietic cell types. IL-21 was identified by ZymoGenetics scientists as a ligand of a new member of the Class I cytokine receptor family, designated interleukin 21 receptor (IL-21r). IL-21r, a key immune system component, is expressed in bone marrow cells and in defined subsets of B cells, T cells, and NK cells. IL-21 binds to cells expressing IL-21r on their surface, activating

Exhibit 7
Responses in Phase I Clinical Trials Involving Immunomodulators/Vaccines

Treatment Regimen	Trials (#)	Patients (#)	CR (#)	CR (%)	PR (#)	PR (%)	ORR (#)	ORR (%)	SD (#)	SD (%)	ORR+ SD (#)	ORR+ SD (%)
Immunomodulators												
Novel/Single	13	203	6	3.0	17	8.4	23	11.4	72	35.5	95	46.9
Multiple Novel	28	651	14	2.2	31	4.8	46	7.0	145	22.3	191	29.3
Novel + Approved	19	392	22	5.6	80	20.4	102	26.0	105	26.7	207	52.7
Subtotal	60	1,246	42	3.4	128	10.3	171	13.7	322	25.8	493	39.5
Vaccines												
Novel/Single	15	265	8	3.0	1	0.4	9	3.4	66	24.9	75	28.3
Multiple Novel	7	198	2	1.0	0	0.0	2	1.0	70	35.4	72	36.4
Novel + Approved	6	111	3	2.7	3	2.7	6	5.4	22	19.8	28	25.2
Subtotal	28	574	13	2.3	4	0.7	17	3.0	158	27.5	175	30.5

Legend: ORR=CR+PR

Source: Horstmann E, etal, Risks and benefits of phase 1 oncology trials from 1991 through 2002, NEJM, 3 Mar 2005;352(9):895-904

cell division. IL-21 is structurally similar to interleukin 2 (IL-2), with homology to IL-4 and IL-15. Based on its mechanism of action and its effectiveness in inhibiting tumor growth in animal models of metastatic cancer, IL-21 is being developed as an immunotherapy for treatment of solid tumors, and potentially hematologic malignancies and viral infections.

Adjuvant treatment with rIL-21 may also enhance MAb-based therapy by improving antibody-dependent cellular cytoxicity (ADCC) (Krejsa C, etal, ASCO05, Abs. 2567). Because IL-21 augments ADCC *in vitro*, it is also expected to affect rituximab-mediated B-cell depletion *in vivo*. Combined treatment with rituximab and rIL-21 resulted in depletion of more B cells compared to rituximab alone. Thus, rIL-21 may potentially improve rituximab efficacy through dual actions of enhanced ADCC and B-cell depletion in lymphoid tissues (Hughes SD, etal, ASCO05, Abs. 2568).

A dose escalation, open label, phase I clinical trial (protocol ID: 494C10, NCT00095108) of rhIL-21 was initiated in May 2004, at Providence Portland Medical Center (Portland, OR), University of Michigan (Ann Arbor, MI), and University of Washington (Seattle, WA), to evaluate safety and PK of rhIL-21 in treatment of metastatic melanoma and RCC. This trial consists of 2 parts. Part A will determine how high a dose of IL-21 may be safely administered to patients with this type of malignancies, while in part B, additional patients will be treated at the dose selected in part A. Part A may involve up to 7 different dose groups. According to the protocol, 2 cycles of IL-21 are being administered IV. In cycle 1, IL-21 is administered for 5 days, followed by a rest period of 9 to 16 days. In cycle 2, IL-21 is administered for 5 days, with a follow-up period of 28 days from the final dose. IL-21 is administered to cohorts of 3-6 patients as a single daily bolus IV at each dose level. Doses are escalated by half-log increments per cohort. Doses ranging from 3-1000 µg/kg/day for 5 days are planned. This trial's primary objective is to determine MTD. Secondary objectives are to determine PK, immunogenicity, clinical or biologic parameters that may correlate with efficacy, and antitumor effect.

To date, 12 patients enrolled in this trial, 8 with metastatic melanoma and 4 with metastatic RCC. Grade 2 adverse events, possibly related to trial drug, include rash at 10 µg/kg (n=1) and at 30 µg/kg (n=4); headache (n=1) and hyperhidrosis (n=1) at 10 µg/kg; and fatigue (n=2), fever (n=2), anemia (n=1), and anorexia (n=1) at 30 µg/kg. There was 1 Grade 3 ALT (single day), and 2 Grade 3 lymphopenia (transient) at 30 µg/kg. Among the 12 patients treated with IL-21 in this trial, there was 1 PR, in a patient with RCC, and 2 PD at 3 µg/kg/day. At 10 µg/kg/day, disease stabilized in 2 patients with melanoma, and progressed in 1. At 30 µg/kg/day, disease stabilized in 4 patients (RCC=2, and melanoma=2) and progressed in 2.

The PR in a patient with RCC after 2 treatment cycles at a dose of 3 µg/kg/day, was maintained for 7+ months. This patient tolerated well all four 5-day cycles, with no Grade 3/4 toxicities. Also, at 30 µg/kg/day, disease stabilized in a patient with melanoma after 2 cycles. Although the first 3 cycles were well tolerated, this patient developed acute hepatic injury by the fifth day, with transient Grade 4 AST, ALT, and bilirubin with hepatic necrosis. However, hepatic function recovered during follow-up. CTL and NK cell numbers decreased during dose intervals, and rebounded during the recovery period. Monocytes increased during dose administration. NK cells expressed more CD25 on their surface.

IL-21 is well tolerated as an outpatient treatment, with reversible observed toxicities. No anti-IL-21 antibodies were detected. Dose-related thrombocytopenia and increased

soluble CD25, IL-15, and IL-18 levels suggest modulation of innate and cellular immunity (Curti BD, etal, ASCO05, Abs. 2502).

In October 2005, the FDA granted 'orphan drug' designation to IL-21 for treatment of advanced or aggressive malignant melanoma.

KW-2871, under development by Kyowa Pharmaceuticals (Tokyo, Japan), is an IgG1κ chimeric MAb targeting ganglioside antigen GD3, expressed on neuroectodermal tumors including melanoma, glioma and neuroblastoma. KW-2871 mediates ADCC.

In a phase I clinical trial, conducted at the University of Alabama (Birmingham, AL), KW-2871 was administered to 17 patients with inoperable Stage IV melanoma, to evaluate the safety, tolerance and PK of this drug. All patients were administered a test dose of 10 mg/m^2 IV. Patients were then stratified into 4 cohorts and treated with 20, 40, 80 and 120 mg/m^2 at a 2-week interval without premedication. No DLT were observed in the 20 and 40 mg/m^2 cohorts. DLT, manifested as Grade 3 urticaria, seizures, edema of the larynx, and chest tightness, occurred in the first two patients treated with 80 mg/m^2. No patients were enrolled in the 120 mg/m^2 cohort. Rather a fifth cohort with 6 patients was then opened at a dose of 60 mg/m^2 and this dose level, associated with Grade 3 urticaria, laryngeal edema, and chest tightness, exceeded MTD. There was no myelosuppression associated with KW-2871. MTD was established at 40 mg/m^2 without premedication. At this dose mean terminal half-life β was 13±6 days (range=8-21 days). Disease stabilized in 2 patients treated at 40 mg/m2, lasting 8 and 3 months. Antibody development against KW-2871 was not detected in this trial (Shah J, etal, ASCO05, Abs. 2556).

A second dose-escalation, phase I/II clinical trial (protocol ID: 2871-US-002, NCT00199342) was initiated in September 2005, to help identify a higher MTD to maximize potential efficacy of KW-2871 in patients with advanced melanoma. This trial is to evaluate doses of 60, 80, and 100 mg/m^2, administered at 14-day intervals with a premedication regimen comprising ranitidine, diphenhydramine, and dexamethasone. Primary objectives are to determine MTD and a recommended phase II dose. Secondary objectives are to characterize the safety and tolerability profile of KW-2871, obtain a preliminary assessment of the drug's antineoplastic activity as measured by the number of objective antitumor responses, duration of response, time-to-progression (TTP) and survival in this patient population, and characterize the PK profile of KW-2871 when administered with this specified premedication regimen. Total expected enrollment for both phase I and phase II portions is 48 patients.

The phase I portion of this trial, being conducted at a single center, at the University of Alabama at Birmingham, under PI Andres Forero, MD, is to enroll up to 18 patients with histologically documented Stage IV melanoma that is not amenable to surgical resection or other therapies. Premedication is administered to all patients 30 minutes prior to infusion of any dose of KW-2871. When MTD is determined, 30 patients will be enrolled at MDT in the phase II portion of this trial. If no MTD is identified after treatment with the 100 mg/m^2 dose level, the dose administered in phase II will be 100 mg/m^2. If MTD is determined as 60 mg/m^2, the trial will be closed to further patient accrual and will not proceed to phase II.

Phosphostim [BromoHydrin PyroPhosphate (BrHPP)], under development by Innate Pharma (Marseille, France), is a novel cancer immunotherapy approach that relies on stimulation of nonconventional lymphocytes (NCL) such as NK cells and Vg9Vd2 (gd) T cells *in vivo*. These T lymphocytes, a critical peripheral blood lymphocyte (PBL) subset, are directly cytotoxic against several tumor types, including RCC. Phosphostim, in combination with low dose IL-2, is used to selectively expand gd T lymphocytes *in vivo*.

A phase I clinical trial was initiated at Centre René Gauducheau (Nantes, France) and Hospital St. Louis (Paris, France) to define DLT, characterize PK and pharmacodynamics, and recommend a dose for phase II clinical trials in patients with advanced solid tumors. A single, 1-hour IV infusion of BrHPP was administered alone during cycle 1, combined with low dose SC IL-2 (1 μg/m^2) on days 1 to 7. In subsequent cycles, on days 1 every 3 weeks, BrHPP dosage was escalated in cohorts of 3 patients extended to 6 if 1/3 patients on any dose level experienced DLT. A total of 18 patients (metastatic RCC=10, colon=1, breast=2, ovarian=1, gastric=1, esophageal cancer=3) were enrolled at 4 dose levels; 4 patients were treated at 200 mg/m^2, 3 at 600 mg/m^2, 6 at 1200 mg/m^2, and 5 at 1800 mg/m^2. At 1800 mg/m^2 during the first cycle, 2 out of 5 patients experienced completely reversible Grade 3 hypotension (n=1) and Grade 3 hyperthermia (n=1), fulfilling DLT criteria, and suggesting a cytokine release syndrome immediately following first infusion. At lower doses treatment was well tolerated; the most frequent adverse events were mild fever, chills, and abdominal pain, without exacerbation in the IL-2 combined cycles. BrHPP alone did not induce significant γδ T-lymphocyte expansion, whereas addition of SC IL-2 increased γδ in all tested doses at a 5- to 50-fold expansion rate. In this trial, BrHPP in combination with low dose SC IL-2 was safe, well tolerated, and induced a potent γδ T-lymphocyte expansion *in vivo*. Clinical activity will be evaluated in phase II clinical trials (Bennouna J, etal, ASCO05, Abs. 2536).

PSMA-based vaccine, under development by PSMA Development, a joint venture between Cytogen (Princeton, NJ) and Progenics Pharmaceuticals (Tarrytown, NY), is a recombinant soluble human prostate-specific membrane antigen (rsPSMA) comprising the entire extracellular domain of PSMA, produced via mammalian cell culture and purified to homogeneity.

PSMA is an attractive self-antigen abundantly expressed on the surface of prostate cancer cells and on neovasculature of a wide array of other tumors, but it is not widely expressed in normal extraprostatic tissues. In prostate cancer, PSMA expression increases with disease progression, becoming highest in high grade, metastatic, hormone-refractory disease.

The main goal of active immunization with rsPSMA is to overcome tolerance to PSMA in order to reject tumor cells expressing PSMA. This PSMA vaccine and others based on PSMA are being developed under an approximately $1 million grant awarded to Progenics Pharmaceuticals in October 2002, by the NCI for the development of novel immunotherapies for prostate cancer. The funding comes from four Phase I Small Business Innovation Research (SBIR) grants for therapies that use components of the immune system to target cancer cells. Funding is intended to support development of MAb and therapeutic vaccines involving PSMA. Two of the grants support production and preclinical development of lead vaccine candidates, rsPSMA and a viral vector vaccine. The projects seek to optimize these two vaccines, first individually and then eventually in novel 'prime-boost' combinations in which the immune system is 'primed' with a first vaccine and then 'boosted' with a second vaccine in a manner that induces an optimal balance of killer T cells and MAb capable of eliminating PSMA-expressing prostate cancer cells. The other two grants support preclinical development of anti-PSMA MAb both in unlabeled form and labeled with α and β-emitting radioisotopes, also for the treatment of prostate cancer.

In addition to evaluating rsPSMA as monotherapy, PSMA Development, in collaboration with AlphaVax (RTP, NC), are investigating rsPSMA immunogen in prime-boost regimens in combination with non-replicating vaccine replicon particles (VRP) based on the alphavirus, Venezuelan equine encephalitis virus. VRP infect cells in the lymph nodes driving production of large quantities of heterologous antigen. Robust immune responses may be further amplified by VRP-induced cell apoptosis leading to cross priming. When used as single immunogens, VRP encoding full length human PSMA, elicit vigorous, durable Th1-biased cellular and humoral responses in normal BALB/c mice, whereas monotherapy with rsPSMA vaccines induces high levels of Th2-biased antibodies. Evaluations of these two constructs are now proceeding in human HLA-A2 transgenic mice, a more pertinent model for quantification of human Class I-restricted cellular responses, and prime-boost regimens (Gardner JP, etal, ASCO05, Abs. 2572) Based on these translational findings, rsPSMA protein vaccine has been advanced into a dose-escalating phase I clinical trial in advanced prostate cancer, while the VRP-PSMA vaccine is in late stage preclinical testing

A dose-escalation, phase I clinical trial was initiated at Memorial Sloan-Kettering Cancer Center, in December 2002, to evaluate the safety, tolerability, and immune-stimulating properties rsPSMA protein, formulated with Alhydrogel adjuvant, in patients with either newly diagnosed or recurrent prostate cancer. This vaccine was administered to 14 patients with rising PSA following surgery or radiation, in 4 SC immunizations over a period of 8 weeks with either 50 or 250 μg of rsPSMA with Alhydrogel over 7 weeks. Patients were monitored for safety, PSMA-specific immune responses and serum PSA.

The vaccine was generally well tolerated with no observed DLT. Sera from 6 patients treated at the 50 μg dose level did not demonstrate serologic reactivity by ELISA, while those of 2 patients, among the 8 patients treated at the 250 μg level, of rsPSMA, showed high titers of IgG antibody by week 7. IgG MAb titers peaked at week 13 and remained measurable through 38 weeks. IgG antibody preferentially recognized native, dimeric PSMA. Minimal IgM responses were observed. This vaccine was generally well tolerated. There appeared to be a relationship between vaccine dose and antibody titer. Antibodies recognized native PSMA and persisted for >30 weeks after final vaccination. Although preliminary, the data suggest that this is a strategy worthy of additional pursuit (Slovin SF,etal, ASCOPC05, Abs. 259). This trial was completed in 2005.

RNA-loaded autologous dendritic cell (DC) vaccine, under development by Argos Therapeutics (Durham, NC), is a personalized vaccine that targets tumor antigens unique to each patient, bypassing the requirement to isolate such antigens, or know their identity. To create the personalized vaccine, DC generated from the patient's peripheral blood monocytic cells (PBMC) are transfected with amplified mRNA from the patient's tumor, making it specific to each person's cancer. The vaccine is then returned to the patient. By eliciting a potent T-cell-mediated antitumor immunity the vaccine is expected to stimulate the immune system to fight the disease. This strategy is applicable to all malignancies, allowing expansion into new indications without the need to reinvent manufacturing process each time. With this approach, a large number of vaccine doses may be prepared from a single manufacturing run from a small tumor specimen.

This RNA-based method for transfecting DC that are antigen presenting cells stimulating cell-mediated immunity by effects on both CD4+ and CD8+ T-cells, may use the full repertoire of antigens derived from the patient's own tumors. RNA transcripts of defined antigens to certain malignancies and infectious diseases may also be produced, thereby offering even greater flexibility to induce the most potent immune response possible. This technology offers a unique and proprietary ability to produce a nearly limitless supply of individualized (autologous) vaccine for potentially every patient.

A phase I clinical trial was conducted at Duke University Medical Center to evaluate feasibility, safety, and efficacy of this approach in inducing tumor-specific T-cell responses in subjects with metastatic RCC. Renal tumor

RNA-transfected DC were administered to 10 patients with no evidence of DLT or vaccine-related adverse effects including autoimmunity. In 6/7 evaluable patients, expansion of tumor-specific T cells was detected after immunization. The vaccine-induced T-cell reactivity was directed against a broad set of RCC-associated antigens, including telomerase reverse transcriptase, G250, and oncofetal antigen, but not against self-antigens expressed by normal renal tissues. Although most patients underwent secondary therapies after vaccination, tumor-related mortality of these patients was unexpectedly low with only 3 of 10 patients dying from RCC after a mean follow-up of 19.8 months. These data provide a scientific rationale for continued clinical investigation of this polyvalent vaccine strategy in the treatment of metastatic RCC and, potentially, other malignancies (Su Z, etal, Cancer Res, 1 May 2003;63(9):2127-33; comment in Cancer Res, 1 Jul 2004;64(13):4685; author reply 4685).

Based on these encouraging results, a multicenter (n=5), single arm, open label, phase I/II clinical trial was initiated in June 2004, in the USA and Canada, to evaluate an RNA-loaded autologous DC vaccine in patients newly diagnosed with metastatic RCC. One of the trial's objectives was to demonstrate the safety and commercial feasibility of processing DC at a central manufacturing facility and delivering it to multiple clinical sites. Among sites participating in this trial are Roswell Park Cancer Institute (Buffalo, NY), University of California Irvine, Lineberger Cancer Center at the University of North Carolina, Jewish General Hospital at McGill University (Montreal, Canada), and Princess Margaret Hospital at the University of Toronto, under PI Jennifer Knox, MD.

In this trial, tumor RNA is isolated from nephrectomy specimens, and DC are generated from monocytes harvested by leukapheresis. Vaccine is administered by an intradermally injection of $1.2x10^7$ cells every 2 weeks for 5 weeks during induction, followed by treatment every 4 weeks for 5 weeks and boosters ever 3 months until progression. As of December 2004, 22/26 patients were enrolled and treated with 47 vaccine doses. Disease had metastasized to the lung in 33% of patients, to the liver in 19%, and the mediastinum in 19%. Among 14 patients who underwent nephrectomy and leukapheresis, and were administered the first vaccine dose, 1 withdrew consent, disease progressed in 1 prior to restaging, and in 8 patients who completed induction, immune monitoring, and restaging, disease stabilized in 7 at 3 months and progressed in 1. The remaining 6 patients were not immunized because of rapidly progressing disease (n=2), inoperable tumor (n=1), and failure to obtain RNA (n=3). Vaccine-related adverse events include injection site redness/itching, mild flu-like symptoms, headache and joint pain. This interim data suggest that supplying multiple sites with quality controlled autologous tumor mRNA transfected DC vaccine from a central production facility is feasible in this setting. An average of 13 vaccines were generated per patient (Knox JJ, etal, ASCO05, Abs. 4710).

In April 2005, an investigator-sponsored phase I clinical trial was initiated at the University Hospital of Erlangen, in Germany, under PI Gerold Schuler, MD, using Argos' personalized RNA-loaded autologous DC constructed from the patient's own tumor RNA and immune cells, to treat metastatic melanoma. Argos is applying the same technology and process in this vaccine that was employed in the vaccine to treat metastatic RCC.

In 2005, Argos Therapeutics entered into a joint licensing agreement with DC Bio (Toronto, Canada), a privately-held biotechnology company, to further advance its RNA-loaded DC vaccine technology. This alliance leverages the companies, expertise to accelerate development of personalized immunotherapy treatment for such malignancies as RCC, and chronic lymphocytic leukemia (CLL), and infectious diseases such as human immunodeficiency virus (HIV). Under the agreement, Argos granted rights to DC Bio to use its technology platform for select disease indications in Canada. Additionally, Argos received an ownership interest in DC Bio, as well as rights to DC Bio inventions outside of Canada and other considerations, including royalties on sales of products by DC Bio in Canada.

Argos Therapeutics, in conjunction with DC Bio, are also planning to initiate an investigator-sponsored phase I/II clinical trial in chronic lymphocytic leukemia (CLL) during the first quarter of 2006 using Argos's proprietary RNA-loaded DC vaccine technology. The trial will be conducted at McMaster University (Hamilton, Ontario, Canada), under PI Graeme Fraser, MD. McMaster University received a $573,000 grant from the Ontario Cancer Research Network (OCRN) to conduct this trial.

VX-001, under development by Vaxon Biotech (Paris, France), is a vaccine containing hTERT572Y, a single optimized cryptic peptide homologous to TERT that targets tumors expressing the telomerase antigen and induces efficient antitumoral T-cell cytotoxic immunity but not autoreactivity *in vivo* in HLA-A*0201 transgenic mice, healthy blood donors, and patients with prostate cancer (Gross DA, etal, J Clin Invest, Feb 2004;113(3):425-33). This antigen is overexpressed in a wide variety of tumors, including colon, breast, prostate and lung cancer. VX-001 is based on proprietary identification and optimization methods used to engineer the cryptic peptide so that it binds to antigen-presenting cells and stimulates T-cell immunity.

A phase I clinical trial was conducted at Heraklion University General Hospital, in Greece, under PI Vassilis Georgoulias, MD, and Genimatas General Hospital (Athens, Greece), to evaluate safety and immunogenicity VX-001 in HLA-A*0201 patients with refractory and progressing advanced malignancies. This trial enrolled 19 patients with advanced stage metastatic cancer of different types, which had progressed despite chemotherapy. According to the protocol, two SC injections of optimized TERT572Y peptide were followed by 4 injections of native

TERT572 peptide, at 3-week intervals. Peptides were injected emulsified in Montanide ISA51. Patients were vaccinated with escalated doses of peptide ranging from 2 to 6 mg. Among 14 patients who completed the trial, disease stabilized in 4, for a median of 10 (range=9-12) months. In addition TERT572Y-specific CTL were detected in the peripheral blood of 13/14 evaluable patients, as early as 3 weeks after the second vaccine injection. These CTL were fully functional and killed TERT-overexpressing tumor cells. There was only Grade 1 toxicity, affecting 13/19 patients. Overall, treatment with VX-001 was well tolerated, with no significant side effects and no evidence of autoimmunity (Kosmatopoulos K, etal, ASCO05, Abs. 2579). This trial was completed in December 2004.

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