CYTOTOXIC / CYTOSTATIC AGENTS

A.I. Docetaxel (Taxotere®)

Docetaxel is a semi-synthetic member of the taxoid family originally synthesized from 10-deacetylbaccatin III, a non-cytotoxic precursor extracted from the needles of Taxus baccata and esterified with a chemically synthesized side chain. Docetaxel promotes assembly and stabilization of microtubules, altering the physiologic equilibrium between free tubulin dimers and tubulin microtubules. These actions result in blocking cell cycle traverse in the mitotic phases, thereby inhibiting cellular replication.

Docetaxel has a broad spectrum of anti-neoplastic activity in vitro and in vivo. In addition, clinical studies of this agent have demonstrated anti-neoplastic activity in a variety of tumors that include breast, non-small-cell and small-cell lung, ovarian, head and neck, and stomach cancer.

Pharmacokinetic analysis performed in early phase I studies using 1-6 hours i.v. infusions every 3 weeks of docetaxel indicated that its elimination and distribution are linear. Increases in AUC or in peak concentrations were observed with increases in docetaxel doses from 20 to 115 mg/m². Total plasma clearance was independent of dose. Its pharmacological behavior was best fitted by a three-compartment pharmacokinetic model with α, ß and γ half-lifes of 4 minutes, 36 minutes, and 11.1 hours, respectively. However, when administered as a 24-hour continuous i.v. infusion or as a brief, daily infusion for 5 days, clearance was increased relative to short (1-6 h) exposure schedules. A two- rather than a three compartment model provided a better fit of the plasma concentration data in the schedules achieving more prolonged exposure.

Following intravenous administration, docetaxel is heavily (98%) bound to plasma proteins. Elimination occurs principally by hepatic metabolism and biliary excretion. Up to 75-80% of radio-labeled docetaxel is recovered in the bile and feces, whereas less than 10% is excreted unchanged in the urine Cytochrome P-450 enzymes are responsible for the metabolism of docetaxel, specifically the CYP-3A subfamily.

Pharmacokinetic analysis of 24 phase II studies of 640 patients receiving docetaxel over 1 hour every 3 weeks, using four randomized limited-sampling schedules, confirmed the pharmacokinetic results of phase I trials and have permitted the evaluation of several prognostic factors as predictors of docetaxel-induced toxicity. Clearance, area under the plasma concentration *versus* time curve (AUC), and the duration of exposure over 0.20 mmol/l (0.16 mg/ml) were all independent predictors for grade 4 neutropenia. Cumulative dose was the strongest predictor of the time to onset of fluid retention, however, measures of drug exposure were also covariates with independent predictive power. Patients with concomitant elevations in hepatic enzymes (transaminases >1.5 x ULN and alkaline phosphatase >2.5 x ULN) in the above studies had a 27% reduction in docetaxel clearance, predicting for a 1.5-fold increase in the odds of febrile neutropenia. This finding motivated a specific safety analysis conducted in 1366 patients from the entire docetaxel phase II clinical data base. Fifty-four patients (4%) who met the criteria for concomitant elevation in hepatic enzymes had a threefold higher incidence of febrile neutropenia during course 1 than 1312 patients with normal enzymes (22.6% vs. 6.2%, $p<0.001$). Other safety parameters (severe infections, mucositis, and toxic death) were also markedly impaired in these patients.

The results of pharmacokinetics studies in animals indicate that docetaxel exhibits multi-phasic plasma kinetics, good tissue distribution, primarily metabolism and biliary excretion, and extensive excretion in the feces. After i.v. administration, docetaxel is distributed to all tissues and organs with the exception of the brain. It is highly bio-available in tumor tissue. It crosses the blood-placental barrier and has been detected in maternal milk. It is eliminated very rapidly, although at a slower rate from tumor tissue than from normal tissue. Monoxygenase enzymes, in particular Cytochrome P-450-3A, play a leading role in docetaxel metabolism. Docetaxel binds strongly to plasma proteins in all species studied, including humans.

The dose limiting toxicity for docetaxel is neutropenia with 76% of patients with normal liver function (normal bilirubin, transaminases <1.5 x ULN, and alkaline phosphatase <2.5 x ULN) having grade 4 neutropenia when docetaxel is used as a single agent at a dose of 100 mg/m² administered over

1 hour every 3 weeks. Other toxicities include hypersensitivity reactions (with recommended pre-medication) in 15.7% of patients (severe 0.9%), skin toxicity in 58.5% (severe 5.6%), and fluid retention (with recommended pre-medication) in 48.5% (severe 5.2%).

Additionally, neurotoxicity, particularly neurosensory, myalgia, diarrhea, stomatitis, nausea, vomiting, asthenia or fatigue and neutropenic infection and sepsis as well as typhlitis have been reported in patients treated with docetaxel.

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A.II. Pemetrexed (Alimta®)

Pemetrexed is a new antifolate drug exerting its inhibitory activity at several steps in folate metabolism. It inhibits several key enzymes in the pathways of the de novo pyrimidine and purine biosynthesis, i.e. thymidilate synthase (TS), but also dihydrofolate reductase (DHFR), glycinamide ribonucleotide formyltransferase (GARFT) or aminoimidazole carboxamide ribonucleotide formyl transferase (AICARFT), thus inhibiting DNA and RNA synthesis. These key enzymes provide continuous supply of nucleotides, the structural elements of DNA and RNA. Antifolates impede the synthesis of nucleotides, especially in rapidly growing cells, such as most of the cancerous cells, inducing their growth arrest and consecutive death. Pemetrexed is transported via the reduced folate carrier into the cell where it undergoes poly-glutamation catalyzed by folylpolyglutamate synthase (FPGS). The latter reaction increases the potency of the substance 60-fold in its inhibition of TS and more than 140-fold in that of GARFT.

Plasma homocysteine levels have been identified to most accurately reflect the functional folate status of patients with elevated levels being associated with hematotoxicity and mucositis. Reduced functional availability of folate or vitamin B12 is reflected by an increase in the plasma homocysteine level. The supplementation with folate and vitamin B12 in all clinical trials with pemetrexed as of December 1999 has significantly reduced most severe toxicities without impacting the anti-tumor activity of the drug.

Phase I-studies of different schedules have identified 600 mg/m² pemetrexed over 10 minutes i.v., q 3 weeks, as the preferable schedule to be used for further clinical development. Because of toxicities, the dose was reduced to 500 mg/m² for further clinical testing prior to mandatory vitamin supplementation.

Pharmacokinetic studies of the drug determined terminal half-life (t½) to be 2.5 – 4.0 hrs. The pharmacokinetic behavior is linear with regard to dose and area under the plasma concentration *versus* time curve (AUC) as well to dose and peak plasma concentration (C_{max}). 80% of the drug is cleared unchanged via renal excretion.

Pemetrexed has shown clinical activity as a single agent in multiple tumors. Percentages of activity are as follows: non-small cell lung cancer (NSCLC) 5-23%, pancreas cancer 6%, colorectal cancer 88%, mesothelioma 14%, cervix cancer 25%, head and neck cancer 26%, gastric cancer 24% and bladder cancer 32%. The addition of pemetrexed to cisplatin in patients with advanced mesothelioma has yielded significantly better results with regard to the overall response rate ($p<0.001$), the time to progression ($p<0.001$) and the median survival ($p=0.020$) in comparison to cisplatin single agent therapy.

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A.III. ZK 219477

ZK 219477 is a fully synthetic analogue of epothilone B, a naturally occurring cytotoxic substance which have been isolated from the myxobacterial strain sporangium cellulosum. ZK 219477 inhibits microtubule de-polymerization; as a consequence, the cell cycle is blocked in the G2/M phase and the cell undergoes apoptosis. This mechanism of action is similar to that of taxanes (paclitaxel and docetaxel). In contrast to taxanes, epothilones retain activity against multi-drug resistant tumors. Epothilones have the potential for considerable anti-tumor activity against a wide range of malignancies.

ZK 219477 has shown significant anti-tumor activity in various preclinical models. In cell culture experiments, ZK 219477 inhibited the growth of almost every tumor cell line (e.g. breast, lung, prostate, vulva, ovary, pancreas and stomach cancer, melanoma, leukemia, including cell lines resistant to other chemotherapeutics) tested so far at concentrations mostly below one nano-molar. ZK 219477 is not recognized by cellular efflux mechanisms. Experiments performed with solid human tumors xenografted in immunodeficient mice, showed that ZK 219477 has strong growth inhibiting activity in a broad range of tumors, including major tumor entities (breast, ovarian, prostate, and lung cancer) as well as tumors less amenable to chemotherapy (melanoma, glioma, cholangiocarcinoma, and pancreatic cancer).

In pharmacological experiments, ZK 219477 has shown an improved side effect profile (with respect to body weight gain and survival) compared with natural epothilone B, and increased efficacy compared with partially synthetic epothilone derivatives and natural epothilone D.

Safety pharmacology studies did not indicate relevant effects on the cardiovascular system, gastrointestinal system, renal system and isolated organs (trachea, ileum, uterus). The available data suggest that ZK 219477 has some potential to interfere with pulmonary function which, however, is not expected to represent a serious risk for patients at the envisaged human doses. In addition, CNS stimulation and gait alterations were noted in mice at doses of 3.13 mg/kg b.w. (ca. 10 mg/m² body surface area) and higher. Clonic convulsions were observed during the toxicological dose-range-finding study in rats, however only at the high, lethal dose of 10 mg/kg (60 mg/m²). The findings are consistent with the ability of ZK 219477 and/or its metabolites to cross the blood-brain barrier. However, based on the results from animal studies, major CNS effects should not occur during treatment of humans.

ZK 219477, which shows high serum protein binding, was rapidly and extensively distributed in almost all tissues in rats. The blood-brain barrier was permeated by ^{3}H-ZK 219477 and high concentrations were also present in the CNS. After single intravenous administration, the excretion was almost complete within 7 days with the biliary route as the major path. After two weeks, minor parts of radioactivity were still present in the animal. There is no indication for melanin binding.

ZK 219477 is metabolized by a non-cytochrome P450 dependent pathway (hydrolytic cleavage of the lactone ring presumably by esterases), and also by CYP enzymes. Comparable metabolic pathways were observed in vitro (animal and human liver microsomes) and in vivo (plasma, feces and urine). A phase II metabolism was not observed.

The total body clearance (CL_T) of ZK 219477 in rats and monkeys was 150 mL/min/kg and 20 mL/min/kg, respectively. In rats (377 mL/kg) and monkeys (80-360 mL/kg), a high volume of distribution was observed. The terminal half-life ($t_{1/2}$) in rats was estimated to be 8-10 hours, in monkeys in the range of 50-150 hours.

In acute toxicity studies in rats, the lethal dose (LD)50 of ZK 219477 after i.v. administration was >24 mg/m². The minimal LD was 48 mg/m².

Sub-chronic systemic toxicity studies performed in rats and monkeys with a schedule of six administrations, once every three weeks, revealed a maximum tolerated dose (MTD) of 1.2 mg/kg (appr. 7.2 mg/m²) in rats and 0.5 mg/kg (appr. 6 mg/m²) in monkeys. Clear compound-related, dose-dependent clinical findings were observed in rats beginning at 7.2 mg/m² (thinning of fur, reduced food consumption and body weight gain) and monkeys beginning at 2.0 mg/m² (diarrhea and reduced food consumption). In both species, transient changes in hematology and biochemistry parameters occurred 3-6 days after the administration but were reversible until the next treatment.

Preliminary results of chronic toxicity studies (6 and 12 months in rats and monkeys, respectively, with administration once weekly or once every three weeks) indicate that in addition to the findings in the sub-chronic studies, auto-mutilation (considered as a sign of peripheral neuropathy), an increase in thinning of fur and indications for renal toxicity (increased incidence of chronic progressive nephropathy) are compound-related changes in the rat.

According to the results of the systemic toxicity studies, the following organs are considered major target organs of toxicity for ZK 219477: hematopoietic system, gastrointestinal tract, reproductive organs, liver, kidneys and possibly peripheral nervous system. With the exception of the kidneys and the peripheral nervous system, all other organs consist of rapidly dividing tissues and the observed effects are considered to be related to the pharmacodynamic properties of ZK 219477 as a cytotoxic agent.

No evidence of CNS damage was noted during the toxicological studies except in a preliminary rat study at the LD of 10 mg/kg (approx. 60 mg/m²) where clonic convulsions occurred.

ZK 219477 induced genome mutations on mammalian cells in vitro and in vivo and therefore has to be considered as mutagenic. However, mutations caused by direct DNA-damage were not observed in the Ames-test and chromosomal aberration test in vitro. Since genome mutations may be involved in carcinogenesis, ZK 219477 is assumed to be potentially carcinogenic.

The local tolerability of the formulation of ZK 219477 used for the clinical trials was investigated in studies using the intravenous (i.v.), paravenous, intraarterial (i.a.) and intramuscular (i.m.) route of administration. After i.v. and i.a. application, marginal signs of transient irritation were reported. Slight to mild transient irritation was seen after i.m. application while slight to moderate irritation was noted after paravenous administration.

Two phase I clinical studies in patients with various advanced solid tumors are ongoing: One phase I-study is an investigation of the safety, tolerability, and pharmacokinetics (PK) of ZK 219477 when administered to patients with advanced solid tumors as an i.v. infusion for 30 minutes once every three weeks, in order to determine the maximum tolerated dose (MTD), the dose-limiting toxicities (DLT), and the PK profile of the substance. Dose escalation proceeded according to a modified Fibonacci series. The study, in which 52 patients have been treated, has recently been completed. The MTD has been established at 22 mg/m². DLT at MTD level was grade 3 peripheral neuropathy observed in one out of six patients. The most frequent toxicities considered to be drug-related were nausea (25%), peripheral sensory neuropathy (25%), vomiting (11.5%), neuropathy peripheral (11.5%), ataxia (11.5%; 7.7% peripheral origin and 3.8% central origin), leukopenia (9.6%), arthralgia (9.6%), and neuropathic pain (9.6%). First indications of anti-tumor activity were observed, including a partial response in a patient with breast cancer with a duration of six months. PK data indicate a dose-proportional increasing of systemic exposure; there were no indications of accumulation. A total of 20 out of 52 patients displayed peripheral neuropathy during the study (recorded as peripheral sensory neuropathy, neuropathy peripheral, paresthesia, neuropathic pain, and polyneuropathy).

The design and objectives of the other phase I-study are similar. The difference between the two phase I-studies is in the administration schedule. A weekly administration is being investigated, which consists of a three-week period of treatment followed by one week of recovery. So far, twenty-two patients were treated. The most frequent adverse events of all grades considered to be drug-related were, in percentage of patients: peripheral neuropathy (31 %; 4% grade 3), anemia (27%), nausea (22%), vomiting (18%), increase in GGT (9%; 4% grade 4), constipation (9%), pain (9%),

hypaesthesia (9%) and abdominal pain (20%). Drug-related toxicities of grade 3 observed in single cases were thrombocytopenia, fatigue, and diarrhea.

Nausea/vomiting were mostly of lower grade, although no prophylactic anti-emetics were used, and amenable to usual anti-emetic treatment. Low-grade hematological toxicities were reported

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B. (ANTI-)HORMONE AGENTS

B.I. ZK PRA 230211

ZK PRA 230211 is a strong and pure antagonist of the progesterone receptor (PR). Blocking the PR with ZK PRA 230211 demonstrated a strong inhibition of tumor growth in various preclinical murine as well as human xenograft breast cancer models with superior results compared to aromatase inhibitors (AIs) and anti-estrogens. Furthermore, synergistic or additive effects of ZK PRA 230211 in combination with other administered endocrine agents could be shown. In addition, induction of programmed cell death (apoptosis), a mechanism of action distinctly different from those of other endocrine agents, could be demonstrated.

For the in vitro characterization of ZK PRA 230211, the human breast cancer cell line T47D was used. ZK PRA 230211 induced a complete inhibition of cell growth being superior with regard to potency and efficacy to other progesterone antagonists tested.

ZK PRA 230211 in comparison with anti-estrogens was tested in vivo in the NMU- or DMBA-induced (autochthonous) rat mammary carcinomas and the syngenic murine MXT breast cancer model. In therapeutic experiments, performed with all 3 models, ZK PRA 230211 demonstrated a strong growth inhibition of established tumors. The effects were superior or comparable to that of other anti-estrogens i.e. tamoxifen. The combination with tamoxifen showed some additive effects on tumor growth inhibition.

In addition, experiments with the human breast cancer lines (T47D, MCF7) implanted in immunodeficient scid mice (xenografts) were performed. In both xenograft models, T47D and MCF7, an anti-tumor activity of ZK PRA 230211 was observed.

Furthermore to the effect on tumor growth, ZK PRA 230211 is unique because it does not just stop the cell from growing and dividing, it appears to prompt the cell to undergo apoptosis.

ZK PRA 230211 has a very favorable profile both in vitro and in vivo. In trans-activation assays, ZK PRA 230211 shows no glucocorticoid, estrogenic, and anti-estrogenic activities. Only marginal anti-glucocorticoid activity was observed in vitro. With regard to androgenicity, ZK PRA 230211 exhibits in vitro a mixed antagonistic/agonistic activity with more pronounced antagonistic activity.

The same general properties were observed in in vivo experiments. In rats, ZK PRA 230211 showed marginal anti-glucocorticoid activity and a weak glucocorticoid activity at a very high dose. ZK PRA 230211 did not exhibit an estrogenic activity in ovariectomized rats. In male rats, ZK PRA 230211 showed an anti-androgenic activity and no androgenic activity.

The overall results of the safety pharmacology studies addressing the impact of ZK PRA 230211 on vital organs [central nervous system, cardiovascular system (including QT prolongation), respiratory system] as well as on renal function, gastrointestinal system, isolated organs and on human platelets tested in vitro and in mice, rats, rabbits and dogs showed no adverse effects.

In systemic tolerance studies in female rats with a treatment duration of up to 26 weeks and daily i.g. administration of ZK PRA 230211, no clear-cut signs for toxicity occurred up to the highest tested dose of 100 mg/kg.

ZK PRA 230211 treatment in rats did provoke a response of organs of the genital tract as well as the endocrine system at the low dose of 4 mg/kg and higher, which was related to the pharmacodynamic effect of ZK PRA 230211 and was induced by an unopposed estrogen action. Similar effects on endocrine and genital system were noted in female mini-pigs in systemic tolerance studies with repeated i.g. administration of ZK PRA 230211 over a period of up to 26 weeks at the low dose of 4 mg/kg and higher.

Evaluation of the data on mutagenicity testing does not indicate that ZK PRA 230211 is a mutagen.

The parent substance is highly bound to serum proteins of animals and humans in vitro without concentration dependent alterations. However, ZK PRA 230211 and its metabolites do not exhibit detectable binding affinity to human sex hormone binding globulin (SHBG) and cortisol binding globulin (CBG).

In vitro biotransformation of ZK PRA 230211 was studied in liver microsomal preparations of rat, dog, monkey, rabbit, mini-pig and human. Human liver microsomes and cryopreserved human hepatocytes catalyzed the formation of three main metabolites (ZK 208000 (M2), ZK 208099 (M3) and ZK 371014 (M4)).

Following oral administration to rats, ZK PRA 230211 was predominantly metabolized to M3, whereas in mini-pigs the metabolites M2 and M4 were predominantly formed. After oral administration of ZK PRA 230211 to female mouse and rabbits, all three major human metabolites (M2, M3, M4) were detected in serum at relevant amounts. Pharmacological in vitro and in vivo experiments demonstrated that both dia-stereoisomers of M4 (ZK 190542 and ZK 190543) exhibited anti-progestogenic potency. Therefore, these metabolites may also contribute to the pharmacological profile in humans.

The metabolite ZK 208000 (M2) represents a mixture of two dia-stereoisomers. The employed LC-MS/MS method did not separate the individual dia-stereoisomers, thus both dia-stereoisomers were always quantified in the preclinical and clinical studies. Similarly, the putative two dia-stereoisomers of the mixture of ZK 371014 (ZK 190542 and ZK 190543) were always quantified together in all preclinical and clinical studies. Preliminary data indicate that the dia-stereoisomere ZK 190543 is mainly formed in mini-pigs and rats, whereas both dia-stereoisomers can be detected in human serum.

ZK 230211 is predominantly metabolized by CYP3A4, and only to a minor extent by CYP2D6 and CYP2C19. In in vitro studies, ZK 230211 inhibited CYP2C9 (Ki=1.4 μM) and to a lesser extent CYP2D6 (Ki=16 μM) and CYP2C19 (Ki=17 μM).

Preliminary data of in vitro inhibition studies in human liver microsomes to investigate the metabolic drug-drug interaction potential between ZK PRA 230211 administered in combination with either tamoxifen, anastrozole or exemestane suggest that all three drug substances inhibit the metabolism of ZK PRA 230211 only moderately indicating a low interaction potential with the pharmacokinetics of ZK PRA 230211. Furthermore, in vitro data suggest no or only weak interaction potential of ZK PRA 230211 with the pharmacokinetics of tamoxifen (e.g. increase of tamoxifen serum concentrations) and anastrozole when administered together with ZK PRA 230211 at therapeutic doses.

In a placebo-controlled, double-blind and randomized study, tolerability and pharmacokinetics of ZK PRA 230211 after oral administration of increasing single doses was evaluated in healthy postmenopausal women volunteers showing that ZK PRA 230211 was well tolerated up to single oral

doses of 200 mg. No clinical relevant findings were observed for the laboratory examinations, vital signs measurements and ECG and no relevant nor serious adverse events (SAE) occurred.

In a randomized, placebo-controlled study in healthy postmenopausal women volunteers ZK PRA 230211 was administered orally at daily doses from 5 to 100 mg for 28 days. ZK PRA 230211 was well tolerated by all volunteers and no clinically relevant findings were observed. The most common adverse events (AEs) assessed to be at least possible related were headache, hot flashes, sweating increased, breast pain, constipation and hematuria.

Following single oral administration with escalating doses from 1 to 200 mg in 74 postmenopausal women, a more than dose-proportional increase in the systemic exposure of ZK PRA 230211 was observed between 1 and 5 mg, however linear pharmacokinetics between 5 and 100 mg. No further increase in the systemic exposure of ZK PRA 230211 was observed after the 100 mg dose. A saturation of one enzymatic pathway during the first pass is discussed for the non-linearity up to 5 mg or slightly above, whereas saturation of absorption occurs at doses above 100 mg.

Following single or repeated oral administration, serum levels of ZK PRA 230211 increased rapidly reaching maximum concentrations after 1.5-3 h post dose. Thereafter, a steep decline of the parent compound was observed in serum within the first 4-6 hours followed by a long terminal half-life of about 2-3 days. After repeated oral administration, ZK PRA 230211 accumulates 2.5-fold in serum. The pharmacokinetics of the parent compound seems to be linear with respect to time dependency in the investigated dose range of 5-100 mg.

Following daily oral doses for 28 days of 5 to 100 mg to 32 postmenopausal women, linear pharmacokinetics were observed in the dose range of 5-100 mg. ZK PRA 230211 was eliminated from serum with a terminal half-life of approx. 2-3 days. Steady state was reached approximately two weeks after start of treatment at the latest.

Steady-state data on the metabolites indicate a 3-fold higher exposure of ZK 371014 and a 15-fold higher serum concentration of ZK 208000.

Based on the currently available data from two placebo-controlled phase I studies with single oral administration (1,5,25,50,100 and 200 mg) of ZK PRA 230211 and with repeated oral administration of ZK PRA 230211 over 4 weeks (5,25,50,100 mg) in healthy postmenopausal women, ZK PRA 230211 was well tolerated and no relevant nor SAEs occurred. No changes in standard laboratory parameters were observed and no indication for a significant anti-glucocorticoid effect was shown. The most commonly AEs reported in both trials were headaches, hot flushes, increased sweating, nausea, vomiting and gastrointestinal pain. These AEs are comparable to those seen with anti-estrogens and AIs after short term treatment.

Contrary to the predecessor compounds, onapristone and ZK 137316 (successor to onapristone), ZK PRA 230211 did not reveal any signs of hepatic toxicity in humans. This can be explained by the change in the chemical structure of ZK PRA 230211 in comparison to onapristone and ZK 137316.

The effective therapeutic dose of ZK PRA 230211 in patients was calculated by estimation of the systemic exposure achieved in the pivotal pharmacological rat model resulting in maximum efficacy. The corresponding human dose in the multiple phase I study was selected based on the systemic exposure that was estimated in rats. The data suggest that daily oral doses of 50 mg to patients will be sufficient to achieve this pharmacologically relevant systemic exposure. The results of the phase I study (repeated dosing over 4 weeks) demonstrated that daily doses of 100 mg were well tolerated, at doses higher than 100 mg (phase I study with increasing single doses up to 200 mg) saturation of absorption was observed.

Whereas all currently available endocrine treatments including anti-estrogen (AE), selective estrogen receptor down-regulators (SERD) and aromatase inhibitors (AI) target estrogen and the estrogen receptor, ZK PRA 230211 is a highly selective and pure antagonist of the progesterone receptor (PR). ZK PRA 230211, the follow-up compound of onapristone, has shown significant anti-tumor activity in various preclinical models. Based on these results, ZK PRA 230211 could provide an innovative and successful extension to the current available treatment modalities.

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B.II. Melatonin

Melatonin (N-acetyl-5-methoxytrptamine) is produced primarily by the pineal gland in humans, in a circadian fashion with serotonin as the immediate precursor. Melatonin production is stimulated by darkness and suppressed by light. Production increases in the evening and peaks between 2 and 4 a.m. then gradually declines. It does not vary with the menstrual cycle in premenopausal women. Melatonin is metabolized by the liver (90% at first pass) and mainly excreted in the urine. Bioavailability of oral melatonin varies, but doses of 1-5 mg generally result in supraphysiologic concentrations (10-100 times the usual peak) within one hour after ingestion, followed by a decline to baseline within 4 to 8 hours. Melatonin's half-life after oral administration varies between 30-60 minutes. Selective beta-adrenergic blockers (e.g. atenolol, propranolol) have been shown to significantly reduce melatonin release which is stimulated by norepinephrine effects on beta receptors.

Melatonin has been extensively studied with no significant adverse effects reported and, in the U.S., is widely available over the counter as a supplement. At doses of 0.1-5 mg, some studies have shown an increase in sleep propensity and sleep quality, although they have not been consistent. Even with doses as high as 1 gram per day, no significant adverse effects were noted, except increased drowsiness. A placebo-controlled, double-blind trial of healthy adult men was specifically designed to assess the toxicological effects of oral melatonin; after 28 days of 10mg melatonin daily, no evidence for toxicity was reported. Longer-term trials have also been conducted with melatonin administered daily over six months, again without any significant adverse effects. The optimal dose of melatonin for either the therapeutic or the preventive setting is currently not known. However, aiming at a nocturnal high level by giving melatonin prior to bedtime appears physiologically more useful compared to a 24-hour dose level because, ultimately, it is the level of melatonin at night that has been linked with cancer risk.

Melatonin has been used extensively in cancer patients, both alone and in combination with chemotherapy, with no significant adverse effects reported thus far.

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C. SIGNAL TRANSDUCTION INHIBITORS (STIs)

C.I. Cetuximab (Erbitux®)

The epidermal growth factor receptor (EGFR) is a commonly expressed transmembrane glycoprotein of the tyrosine kinase growth factor receptor family. EGFR is expressed in many normal human tissues, and activation of these proto-oncogenes results in over-expression in many types of human tumor. As a transmembrane glycoprotein, the extra-cellular domain of the EGFR is a ligand-binding site for transforming growth factor alpha (TGFα), epidermal growth factor (EGF) and other factors. Upon ligand binding, the intracellular domain of the EGFR is activated, thereby triggering cellular mechanisms that regulate cell growth, inhibition of tumor cell invasion and angiogenesis In vitro analysis using cells that express high numbers of EGFRs and produce ligands for these receptors has shown that the EGFR may be activated through an autocrine pathway, thereby leading to the proliferation of cells in culture. In order to inhibit proliferation of EGFR-rich cells, antagonists to EGFR have been produced that block the ligand-binding site. In this capacity, monoclonal antibodies to EGFR have been shown to inhibit the proliferation of cells that produce both TGFα and EGF. An antagonist directed against the ligand-binding site of EGFR offers an interesting approach to the therapy of cancers involving up-regulated EGFR-dependent pathways. Among cancers that over-express EGFR are colorectal (72%), head and neck (92%), pancreatic (95%), ovarian (35-70%), renal cell (50-90%), non-small cell lung cancer (40-80%), and gliomas (40-50%). The prognosis for many of these malignancies is poor if not diagnosed at an early stage, and therapy for advanced disease is limited.

The effects of EGFR blockade on cell cycle progression have been investigated in several human cell types, including DiFi colon adenocarcinoma cells, non-transformed breast epithelial MCF1OA cells, A431 squamous epithelial cancer cells, and DU145 prostatic cancer cells. These studies suggest that blocking EGFR with monoclonal antibodies leads to cell cycle arrest in G1, which is accompanied by a decrease in cyclin-dependent kinase 2 activity, and an increase in the expression of cyclin-dependent kinase inhibitor p27KIP1. In addition to inducing G1-phase arrest, EGFR blockade has also been shown to lead to cell death via apoptosis in DiFi colon adenocarcinoma cells.

Cetuximab, a chimerized antibody of the IgG1 subclass, was originally derived from a mouse myeloma cell line. The chimerization process resulted in an antibody with binding affinity to EGFR greater than the natural ligand EGF. Cetuximab blocks binding of EGF and TGFα to the EGFR and inhibits ligand-induced activation of this tyrosine kinase receptor. Cetuximab also stimulates EGFR internalization, effectively removing the receptor from the cell surface for interaction with ligand. Cetuximab was created by chimerization of the murine monoclonal antibody M225 developed at the University of California, San Diego, USA. It was genetically engineered by cloning the heavy and light chains of M225 and adapting them for expression together with the constant regions of the human kappa light chain and human gamma 1 heavy chain.

In an in vitro study performed to establish the biological activity of cetuximab and its specificity for human EGFR as compared to a murine antibody directed at EGFR (M225), both cetuximab and M225 inhibited cell growth to a similar extent, i.e. 30% of the control.

A series of immunohistochemical studies performed to characterize the binding of cetuximab to human and animal tissues demonstrated that cetuximab reacted positively and specifically with epithelium of human placenta. Specific staining was observed also in normal epithelia of skin, digestive tract, urogenital system and tonsillar crypts, and in squamous cell cancer and large cell cancer of the lung. Specific staining was absent in cancers originating from other organs, in melanomas, and in lymphoid tumors.

When the clinical development of cetuximab started, the goal was to administer doses of the antibody that would be safe and would maintain serum cetuximab concentrations greater than those needed to saturate the binding of tumor-associated EGFR in murine models (approximately 20 nM). As early clinical development proceeded, this criterion for dose selection was revised based on a hypothesis that non-tumor-associated EGFR binding in patients (especially liver and skin) might represent a large sink for cetuximab which could limit availability of the antibody to tumor associated receptors. An extension of this hypothesis is that non-tumor binding of cetuximab and subsequent receptor internalization represented a major route of elimination for cetuximab that would theoretically become saturated. Thus at same point, systemic clearance of cetuximab may become stable which might be detected by estimation of patient serum pharmacokinetic parameters (total body clearance and half-life). Based on this hypothesis, the target criteria for dose selection were revised to identify a dose at which systemic clearance (as determined by serum pharmacokinetics) became stable.

The initial clinical development program of cetuximab included 14 studies 13 of which contributed to the pharmacokinetic database. Across all dose-ranging studies, as the dose of cetuximab was increased from 5 to 500 mg/m², a trend to decreasing cetuximab clearance was reported. At doses of >200 mg/m², the clearance of cetuximab from the body appeared to level off and remained at approximately 0.02 L/h/m² through the highest dose tested of 500 mg/m². Estimates of mean serum cetuximab terminal half-life increased from 14 to 97 hours over the dose range of 5 to 300 mg/m², after which the half-life appeared to plateau. The mean serum cetuximab volume of distribution in the steady state was independent of cetuximab dose and ranged from 1.96 to 2.52 L/m², suggesting that cetuximab distributes into a volume equal to or slightly greater than that of the vascular space. Based on all of the above and the finding of increased incidence of skin toxicity at 500 mg/m², the cetuximab regimen chosen for phase II trials was an initial dose of 400 mg/m² followed by repeated weekly doses of 250 mg/m². With this regimen, it was proposed that EGFR occupancy and pharmacological activity would be sustained.

When given as mono-therapy in patients with EGFR-expression solid tumors at an initial dose of 400 mg/m² followed by 2-weekly doses of 250 mg/m² (n=7), the mean plasma peak concentration (C_{max}) for cetuximab in week 3 was 153 µg/mL (range: 112 to 225 µg/mL). The mean half-life of elimination in week 3 was 119 hours (range: 82 to 188 hours). The mean volume of distribution at steady state in week 3 was 3.6 L (range: 2.2 to 4.5 L). Concomitant administration of irinotecan (350 mg/m²) in week 4 did not change the pharmacokinetic characteristics of cetuximab.

A total of 614 patients treated with cetuximab were tested for the presence of anti-cetuximab antibodies by analyzing baseline and post-baseline sera. The overall incidence of an anti-cetuximab immune response in these patients was 3.7%. When it occurred, the anti-cetuximab response was generally found to be weak. The anti-cetuximab antibodies from 2 patients with the highest reactivity (4670 and 6516 ng/mL) were tested in an in vitro assay for neutralizing activity. The tested sera did not interfere with the ability of cetuximab to inhibit proliferation in a cetuximab-sensitive cell line, suggesting that the antibodies in these sera were non-neutralizing. Levels of reactivity in sera from other patients were not high enough to perform this type of analysis. In order to determine the specificity of the antibody response, sera from 15 patients who had a positive anti-cetuximab response were further studied in a double antigen radiometric assay using unlabeled cetuximab as a competitor. This analysis demonstrated that sera from 14 of the 15 patients contained cetuximab-specific antibodies.

Cetuximab clinical trials began in 1994. Cetuximab has been administered in phase I-III clinical trials as a single agent or in combination with chemotherapy and/or radiation therapy. These trials have demonstrated anti-tumor activity in metastatic colorectal cancer (mCRC), squamous cell cancer of the head and neck, non-small-cell lung cancer, and pancreatic cancer.

In single-agent studies, 233 patients (82.9%) had a cetuximab-related adverse event (AE) of any grade. In combination with chemotherapy, 861 patients (87.3%) had a related AE of any grade. In combination with radiation therapy, 190 patients (92.2%) had a related AE of any grade.

Skin reactions are the most common AEs associated with cetuximab. They usually present as an acne-like rash or less frequently, as nail disorders. Acne-like rash usually occurs in the first 3 weeks of treatment on the face, upper chest and back, but occasionally extends to the extremities. It occurs as

multiple follicular or pustular lesions characterized histologically as lymphocytic perifolliculitis or suppurative superficial folliculitis. It tends to resolve without sequelae over time following cessation of therapy. In patients who have received cetuximab in doses lower than 100 mg/m², the acne-like rash has been reported infrequently and has been restricted to grades 1 and 2. Several therapeutic interventions have been attempted, including oral and topical antibiotics, topical steroids, and rarely, oral steroids. The value of these measures is unknown since definite clinical trials have not been performed. The etiology of the acne-like rash is believed to be the result of cetuximab binding to the EGFR in the epidermis. Recent trials have shown that the occurrence of acne-like skin reactions were correlated with better efficacy outcomes (response, time to progressive disease, survival).

Another typical but less frequently reported AE is nail disorder which presents as pain, tenderness and fissuring of the distal finger tufts to different degrees. The patients developed paronychial inflammation with associated swelling of the lateral nail folds of the toes and fingers. The most commonly affected digits are the great toes and thumbs. From investigator reports, it is known that nail disorders may persist for up to 3 months after discontinuation of cetuximab.

Grade 3 or 4 hypersensitivity reactions (including allergic and anaphylactic reactions) characterized by the rapid onset of airway obstruction (bronchospasm, stridor, hoarseness), urticaria, and/or hypotension, have been observed in 2.2% patients treated with cetuximab. Approximately 80% of all allergic/hypersensitivity reactions occurred during the first infusion of cetuximab and were observed during or within 1 hour of the completion of the infusion. Prior to the first administration of cetuximab, patients must be pre-medicated with an antihistamine. This pre-medication is also recommended prior to all subsequent infusions of cetuximab as approximately 15% of patients experienced their first severe allergic/hypersensitivity reaction during later infusions. In studies with cetuximab to date, patients who experienced severe reactions received standard treatment, and all but 1 patient recovered without sequelae and were withdrawn from the studies concerned. One patient died due to angioedema following the end of cetuximab infusion, which was recorded as being the result of a grade 4 reaction. The occurrence of allergic/hypersensitivity reactions does not appear to be related to single-drug therapy or combination therapy, underlying disease, or previous exposure to murine monoclonal antibodies. Mild to moderate allergic/hypersensitivity reactions can generally be managed by slowing the infusion rate of cetuximab.

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C.II. Bevacizumab (Avastin®)

A large body of scientific data supports the conclusion that the growth of solid tumors is dependent on angiogenesis, the formation of new blood vessels, to nourish the tumor. Delivery of oxygen and nutrients by the new vessels is a rate-limiting step for tumor cell proliferation and thus a target for anti-tumor therapy. Recognition that angiogenesis is crucial to tumor growth has led to identification of angiogenic factors responsible for stimulating new blood vessel formation.

Recent work has indicated that vascular endothelial growth factor (VEGF; also known as vascular permeability factor or VPF), an endothelial cell–specific mitogen, is one of the most important regulators of angiogenesis. VEGF is a highly conserved, homodimeric glycoprotein whose dominant iso-form has a molecular mass of ~45,000 daltons. Although other angiogenic factors have been identified, VEGF is the most potent and specific, with a well-defined role in normal and pathologic angiogenesis. VEGF stimulates proliferation of vascular endothelial cells, with a mean effective dose (the dose resulting in 50% of maximal achieved proliferation) of 10–50 pM. Two VEGF receptors have been identified: fms-like tyrosine kinase-1 (Flt-1) and kinase insert domain-containing receptor (KDR). These receptors are found almost exclusivly on vascular endothelial cells. VEGF gene expression is substantially increased in the majority of human tumors when compared with the surrounding tumor-free tissues. Direct evidence for a role of VEGF in tumorigenesis has been provided by studies showing that a murine anti-VEGF neutralizing antibody, alone or in combination with cytotoxic chemotherapy, is able to inhibit the in vivo growth of a variety of human tumor cell lines derived from breast, colon, ovary, lung, glia, and other tissues.

Bevacizumab is a recombinant humanized anti-VEGF monoclonal antibody composed of human IgG1 framework regions and antigen-binding complementarity-determining regions from a murine monoclonal antibody (muMAb VEGF A.4.6.1) that blocks binding of human VEGF to its receptors. Approximately 93% of the amino acid sequence, including most of the antibody framework, is derived from human IgG1, and ~7% of the sequence is derived from the murine antibody. Bevacizumab has a molecular mass of ~149,000 daltons and is glycosylated.

muMAb VEGF A.4.6.1, which belongs to the IgG1 isotype, was found to consistently and potently neutralize the biologic activities of human VEGF, including the endothelial cell mitogenic activity, the vascular permeability-enhancing activity, and the angiogenic properties in the chick chorioallantoic membrane. This antibody has been shown to recognize all iso-forms of VEGF, with a Kd of $\sim 8\times10^{-10}$ M. muMAb VEGF A.4.6.1 is specific for VEGF; it fails to recognize other peptide growth factors tested (fibroblast growth factor, epidermal growth factor, hepatocyte growth factor, platelet-derived growth factor, and nerve growth factor).

In situ hybridization studies have demonstrated that VEGF mRNA is markedly up-regulated in the vast majority of human tumors examined to date, when compared with the surrounding tumor-free tissues. These tumors include lung, thyroid, breast, gastrointestinal tract, kidney/bladder, ovary, uterine/cervix, and endometrial carcinomas; angiosarcoma; germ cell tumors; and several intracranial tumors, including glioblastoma multiforme, and sporadic and von Hippel-Lindau syndrome–associated capillary hemangioblastoma. Only sections of lobular carcinoma of the breast and papillary carcinoma of the bladder fail to show significant VEGF mRNA expression. The expression of VEGF in glioblastoma multiforme and other tumors with significant necrosis is highest in hypoxic tumor cells adjacent to necrotic areas.

A correlation has been noted between the degree of tumor vascularization and the level of VEGF mRNA expression. In virtually all specimens examined, VEGF mRNA is expressed in tumor cells but not in endothelial cells. In contrast, as previously noted, mRNAs for the two VEGF receptors, Flt-1 and KDR, are up-regulated in endothelial cells associated with the tumor. These findings are consistent with the hypothesis that VEGF is primarily a paracrine mediator. Freeman et al. have

suggested that lymphocytes infiltrating the tumor may constitute an additional source of VEGF, which contributes to tumor angiogenesis.

In a study published by Kim et al., muMAb VEGF A.4.6.1 was found to exert a potent inhibitory effect on the growth of three human tumor cell lines injected subcutaneously in nude mice: the SK-LMS-1 leiomyosarcoma, G55 glioblastoma multiforme, and A673 rhabdomyosarcoma cell lines. Growth inhibition ranged from 70% to >95%. A maximal effect was observed with a dose of 5 mg/kg administered intra-peritoneally twice weekly. In agreement with the hypothesis that inhibition of neo-vascularization is the mechanism of tumor suppression, the density of blood vessels was significantly lower in sections of tumors from antibody-treated animals compared with control. Neither the antibodies nor VEGF had any effect on the in vitro growth of the tumor cells. These findings provided a direct demonstration that inhibition of the action of endogenous VEGF may result in suppression of tumor growth in vivo.

To evaluate the biologic activity of anti-VEGF antibodies in combination with cytotoxic chemotherapy, a series of studies were performed in animal tumor models. With the combination of anti-VEGF antibodies plus cytotoxic chemotherapy, a striking inhibitory effect was observed in animal models of a subcutaneous human lung cancer tumor (Calu-6 cell line). The combination of anti-VEGF treatment and cisplatin resulted in markedly enhanced biologic activity against tumors compared with the activity of either agent alone. Similarly, Borgström et al. have shown that a combination treatment including muMAb VEGF A.4.6.1 and doxorubicin results in significantly increased efficacy relative to either agent alone and in some cases leads to complete regression of tumors derived from MCF-7 breast carcinoma cells in nude mice.

Intra-vital video-microscopy techniques have allowed a more direct verification of the hypothesis that anti-VEGF antibodies block tumor angiogenesis. Thus, inhibition of VEGF-induced angiogenesis caused a dramatic change in the growth characteristics of the A673 cell line, from a rapidly growing malignancy to a dormant tumor seedling. Very similar findings were subsequently obtained with other tumor cells lines, including the D-145 prostatic carcinoma and the MCF-7 breast carcinoma cell lines. Intra-vital fluorescence microscopy and video imaging analysis have also been applied to address the important issue of the effects of VEGF on permeability and other properties of tumor vessels. Treatment of three different human tumor cell lines (U87 glioblastoma, P-MEL melanoma, and LS174T colon adenocarcinoma) with muMAb VEGF A.4.6.1, initiated after the tumor xenografts were established, resulted in time-dependent reductions in vascular permeability. A regression of blood vessels was observed after repeated administrations of muMAb VEGF A.4.6.1. These findings led to the suggestion that some tumor vessels may require constant stimulation with VEGF in order to maintain not only their proliferative properties but also some key morphologic features.

Bevacizumab pharmacokinetic studies were conducted in mice, rats, and cynomolgus monkeys. Bevacizumab was cleared slowly from the serum, with a terminal elimination half-life of 1–2 weeks in all species tested. A tissue distribution study in male rabbits using ^{125}I-labeled bevacizumab indicated that bevacizumab distributes to various highly perfused tissues (kidney, testes, spleen, heart, and lung). Minimal localization was observed in the liver.

The safety and pharmacokinetics of the combination of bevacizumab and anti-neoplastic agents were evaluated in two studies in cynomolgus monkeys. The first study evaluated the combination of bevacizumab with paclitaxel/cisplatin, and the second study evaluated the combination of bevacizumab with irinotecan/5-fluorouracil (5-FU)/leucovorin (IFL). In the first study, bevacizumab (10 mg/kg) or vehicle was administered i.v. twice weekly for 18 days, and on the last day of dosing (day 18), animals received either a single i.v. dose of paclitaxel (4 mg/kg) and cisplatin (1 mg/kg) or a single dose of saline. There was no difference in the pharmacokinetics of bevacizumab between the groups that received bevacizumab alone or in combination with paclitaxel/cisplatin. In addition, bevacizumab did not affect the pharmacokinetics of cisplatin or paclitaxel. In the second study, all animals received IFL i.v. at doses of 100 or 125 mg/m² (irinotecan) and 500 mg/m² (5-FU and leucovorin) every 7 days for two doses. The antineoplastics were administered either by themselves (n=7) or in combination with bevacizumab at 10 mg/kg i.v. every 7 days for two doses (n=5). Bevacizumab did not appear to affect the pharmacokinetics of irinotecan or 5-FU.

Since serum VEGF concentrations increased in subjects receiving bevacizumab in the initial clinical trial (AVF0737g), the pharmacokinetics of serum rhVEGF165, when administered alone or concomitantly with bevacizumab, were investigated in rats following single-dose administration. rhVEGF165 clearance decreased an average of 3.4-fold when administered concomitantly with bevacizumab. These results support the hypothesis that the rise in VEGF concentrations observed in bevacizumab-treated subjects can be attributed, at least in part, to a decrease in VEGF clearance following complexation of VEGF to the antibody.

The safety of the IFL regimen in combination with bevacizumab was assessed in cynomolgus monkeys. Male monkeys were treated i.v. once weekly with IFL alone or IFL in combination with 10 mg/kg bevacizumab. The co-administration of bevacizumab with IFL did not alter the magnitude of the effects related to treatment with the anti-neoplastic therapy regimen.

Treatment with bevacizumab resulted in a delay in the rate of wound healing in rabbits. It is possible that treatment of human patients with bevacizumab may adversely impact wound healing.

A number of studies were undertaken to address adverse events observed in clinical trials of bevacizumab. Proteinuria was observed in a small number of subjects in phase II trials and was studied further in rabbit models of renal dysfunction. Bevacizumab treatment did not alter the magnitude of renal glomerular injury induced by bovine serum albumin (BSA) or renal tubular damage induced by cisplatin. Several thrombotic episodes were also observed in clinical trials of bevacizumab, and a rabbit model of venous thrombosis was utilized to investigate this particular adverse event. Treatment with bevacizumab did not alter the rate of clot formation or any other hematologic parameters compared with treatment with bevacizumab vehicle. Additionally, data from toxicology studies in normal animals and from the renal dysfunction model studies indicate that bevacizumab treatment does not affect hematology parameters.

In clinical pharmacokinetic studies, bevacizumab was administered either as a single agent or in combination with various anti-neoplastic agents. In these studies, bevacizumab doses ranged from 1 to 20 mg/kg administered at a frequency ranging from weekly to every 3 weeks. A population analysis, using a nonlinear mixed-effect model, was conducted combining data from 491 subjects across all eight trials to estimate the pharmacokinetic parameters and evaluate the potential effect of covariates that may affect bevacizumab pharmacokinetics. Bevacizumab concentration–time profiles were well described using a two-compartment model.

Of all the covariates tested, body weight and sex showed the highest correlation with bevacizumab pharmacokinetics. Bevacizumab clearance and volume of distribution (V_d) increased with increasing body weight. After adjusting for body weight, bevacizumab clearance was 26% faster, and V_d was 22% larger in men than in women. The initial (α) half-life was 1.4 days for both sexes, and the terminal (β) half-life was 19 and 20 days in men and women, respectively. In subjects with low serum albumin levels (<29 g/dL) and high alkaline phosphatase levels (≥484 U/L) (both markers of disease severity), bevacizumab clearance was approximately 20% faster than in subjects with median laboratory values. Consistent with those for other IgG1s, the pharmacokinetics of bevacizumab were characterized by slow clearance and a long terminal half-life, which enables administration every 2–3 weeks.

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C.III. Panitumumab

Panitumumab is a high-affinity ($Kd=5x10^{-11}$ M) fully human immunoglobulin (Ig) IgG2 monoclonal antibody that targets the human epidermal growth factor receptor (EGFR or erbB1), leading to inhibition of EGFR activation. EGFR is a 170,000-dalton transmembrane glycoprotein that promotes cell growth in a variety of normal and transformed tissues and is expressed in several solid tumors, including kidney, lung, prostate, breast, ovarian, head and neck, and colorectal tumors.

Panitumumab blocks EGFR binding of the ligands epidermal growth factor (EGF), transforming growth factor-alpha (TGFα), amphiregulin, betacellulin, epiregulin, and heparin-binding EGF. Autocrine or paracrine stimulation of EGFR by its ligands may have a critical role in the progression of tumors expressing this receptor; thus, an antibody that blocks ligand binding to EGFR may inhibit tumor cell survival, proliferation, and metastasis.

In vitro studies demonstrated that panitumumab inhibited EGF binding to A431 cells and A431 tumor growth in a dose-dependent manner. Panitumumab alone (mono-therapy) prevented A431 xenograft tumor formation and eradicated established xenograft tumors; inhibited the growth of epidermoid, breast, renal, pancreatic, prostate, and ovarian carcinomas that expressed EGFR at levels ranging from 11,000 to $1600x10^3$ EGFR molecules/cell; and prolonged the survival of mice bearing metastatic human breast carcinoma. These results indicate that panitumumab has potential as a therapeutic agent in the treatment of multiple EGFR-expressing human solid tumors.

When panitumumab was combined with chemotherapeutic agents, eradication and inhibition of the growth of human epidermoid, ovarian, breast, and pancreatic carcinoma cells in vitro and A431 epidermoid, DU145 prostate, and A549 lung xenografts in vivo were observed. In addition, panitumumab when administered to mice in combination with a multi-kinase inhibitor, AMG 706, resulted in significantly greater inhibition of A431 epidermoid, HT29 colon, and Calu6 non-small cell lung xenografts than either agent alone.

Toxicology studies conducted in cynomolgus monkeys administered i.v. doses ranging from 0.3 to 30 mg/kg panitumumab once weekly demonstrated that the primary treatment-related effects were skin rash and diarrhea. Based on the distribution of EGFR in normal human tissues (in particular, in the skin and the gastrointestinal tract), these are expected "on-target" effects of panitumumab. Reproductive toxicology studies in female cynomolgus monkeys indicated that fetal abortions occurred in all dose groups. Based on these findings, panitumumab should not be administered to pregnant women, and women of child-bearing potential should continue to use contraception during panitumumab therapy and for 6 months after the last dose administered. In the toxicology studies, panitumumab exhibited non-linear pharmacokinetics. As the dose increased, exposure to panitumumab increased more than dose proportionally. This non-linear clearance may be the result of progressive saturation of a specific EGFR sink. Pharmacokinetic modeling also suggests that, in addition to the EGFR sink, a linear clearance pathway is involved, presumably by the reticulo-endothelial system (RES).

Data were available for 646 subjects participating in clinical studies. Doses administered initially ranged from 0.01 mg/kg/week to 5.0 mg/kg/week. Dose escalation was not taken to the maximum tolerated dose. The dose that achieved the optimal trough concentration felt to be clinically relevant to drug activity and efficacy was 2.5 mg/kg once weekly; the serum half-life of panitumumab at this dose level is approximately 6 days. Additional cohorts were tested at 6.0 mg/kg once every 2 weeks and 9.0 mg/kg once every 3 weeks; preliminary data showed that the trough concentrations for these less-frequent dose schedules were similar to those for the dose of 2.5 mg/kg administered once weekly. Panitumumab exhibits non-linear pharmacokinetics in humans; the clearance decreases with increasing dose and approaches the clearance value for endogenous IgG2, The pharmacokinetics of panitumumab are stable over time, consistent with the absence of immunogenicity.

The most common drug-related adverse event reported in clinical studies was skin rash. The incidence of rash was dose dependent. Diarrhea was observed in approximately 25% of subjects receiving panitumumab mono-therapy at dose of 2.5 mg/kg. Three infusion-associated adverse events have been reported, so far.

The fully human construct of panitumumab is anticipated to result in an improved safety profile versus that of antibodies with a chimeric construct, with retention of efficacy. Data for both safety and efficacy support this hypothesis. Panitumumab, because of its fully human construct, is typically administered without the use of pre-medication, unlike chimeric antibodies that require pre-medication

as prophylaxis for infusion reactions. Consistent with this hypothesis, the incidence of panitumumab-related infusion reactions is uncommon (<1%), and panitumumab administration has not resulted in a human anti-human antibody (HAHA) response in subjects tested.

Evidence of single-agent, panitumumab-induced objective clinical response (partial response by Response Evaluation Criteria in Solid Tumors [RECIST]) has been observed. Interim results (un-audited data) of the phase II study of panitumumab alone in subjects with metastatic colorectal cancer (mCRC) who had been previously treated with two or more prior chemotherapy regimens demonstrated a 10% objective response rate (15 of 148 subjects treated) with a median duration of response of 5.2 months. Objective responses were also seen in a small number of subjects with previously treated renal cell cancer (RCC). Additional studies in mCRC, non-small cell lung cancer (NSCLC), and hormone-resistant prostate cancer are ongoing.

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C.IV. Erlotinib (Tarceva®)

The development of rationally designed molecules (tyrosine kinase inhibitors or TKIs) targeting the intra-cellular domain of epidermal growth factor receptor (EGFR) is a new approach in the treatment of cancer. Among these, erlotinib is a quinazoline derivative, which reversibly inhibits the kinase activity of EGFR. It has shown in vitro and in vivo activity in preclinical trials in multiple human cancer cell lines, including ovarian, head and neck, and non-small cell lung cancer (NSCLC).

Recent data have shown that mutations in the ATP-binding site of the *egfr* gene predict sensitivity of NSCLC patients to gefitinib, another member of the TKI family like erlotinib. In two retrospective analyses involving 16 and 66 patients with refractory NSCLC treated with gefitinib, 8/9 and 5/5 responders carried a mutation in the EGFR ATP-binding site, whereas 0/8 and 0/5 of the non-responders had such alterations. In the first report, cell lines were transfected with such mutations, and mutant strains showed equivalent sensitivity to gefitinib concentrations 10-fold lower than parental cell lines. Parental EGFR was inhibited by 50% at a gefitinib concentration of 0.1 μM and was completely inhibited by a concentration of 2.0 μM, whereas the inhibitory concentration (IC)50 values for the mutant EGFR were 0.015 μM and 0.2 μM, respectively. This phenomenon is not agent-specific, given the fact that it has been also documented in NSCLC patients treated with erlotinib.

In another report, modifications of EGFR serum values during treatment for NSCLC seemed to reflect gefitinib activity; responding patients had decreasing EGFR serum levels compared with refractory patients, where an increment from baseline was consistently observed.

The number of dinucleotide (CA) repeats in intron 1 of the *egfr* gene has been associated with EGFR transcription efficiency, and EGFR expression in patients with breast cancer. Preliminary reports indicate that they may also be predictive of sensitivity to EGFR-targeted agents (erlotinib among others).

Erlotinib has been evaluated in phase I studies using different doses and schedules It is well tolerated, with a toxicity profile consisting of skin rash and mild diarrhea. It is usually given orally as a daily dose of 150 mg on an un-interrupted schedule. The U.S. Food and Drug Administration (FDA) has granted orphan drug status for erlotinib in patients with relapsed, chemo- and radiotherapy-refractory glioblastoma multiforme. Two large, multinational, randomized phase III clinical trials in patients with NSCLC comparing standard chemotherapy regimens (cisplatin plus gemcitabine, and carboplatin plus paclitaxel with or without erlotinib) failed to demonstrate an advantage of the combination approach with regard to response or survival. In contrast, erlotinib has been the first EGFR-targeted therapy to show significant prolongation of survival in a placebo-controlled maintenance trial in patients with NSCLC following first-line or second-line chemotherapy.

In 2004, erlotinib has been approved by the FDA for treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen. Safety and efficacy were demonstrated in a 731 patient double-blind, multi-national, randomized trial comparing erlotinib at 150 mg po daily to placebo. Survival was significantly prolonged on the erlotinib arm with a median overall survival of 6.7 months and 4.7 months in the erlotinib and placebo groups, respectively. The adjusted hazard ratio (HR) for death in the erlotinib group relative to the placebo group was 0.73, p=0.001. Progression-free survival (PFS) was significantly prolonged on the erlotinib arm with a median PFS of 9.9 weeks vs. 7.9 weeks in erlotinib and placebo groups, respectively. The adjusted HR for progression was 0.61, p<0.001. The most common adverse reactions in patients receiving erlotinib were diarrhea and rash. Grade 3/4 rash and diarrhea occurred in 9% and 6%, respectively. Rash and diarrhea each resulted in study discontinuation in 1% of erlotinib-treated patients. Only 6% and 1% of patients required dose reductions for rash and diarrhea, respectively. The median time to onset of rash was 8 days; the median time to onset of diarrhea was 12 days.

Serious Interstitial Lung Disease (ILD), including fatalities, in patients receiving erlotinib for treatment of NSCLC or other advanced solid tumors has been reported occasionally. In the randomized single-agent study, the incidence of ILD (0.8%) was the same in both the placebo and erlotinib groups. The overall incidence in erlotinib-treated patients from all studies (including uncontrolled studies and studies with concurrent chemotherapy) was approximately 0.6%. Reported diagnoses in patients suspected of having ILD include pneumonitis, interstitial pneumonia, interstitial lung disease, obliterative bronchiolitis, pulmonary fibrosis, Acute Respiratory Distress Syndrome and lung infiltration. Symptoms started between 5 days to more than 9 months (median 47 days) after initiating erlotinib therapy. Most of the cases were associated with confounding or contributing factors such as concomitant/prior chemotherapy, prior radiotherapy, pre-existing parenchymal lung disease, metastatic lung disease, or pulmonary infections. In the event of the onset of new or progressive, un-explained pulmonary symptoms such as dyspnea, cough, and fever, erlotinib therapy should be interrupted pending diagnostic evaluation. If ILD is diagnosed, erlotinib should be discontinued and appropriate treatment instituted as necessary. Although interstitial lung disease is well recognized it is still an uncommon adverse event seen in patients treated with single agent gefitinib.

These preliminary results highlight the relevance of targeting EGFR pathway with erlotinib, and also support a relatively favorable toxicity profile that will allow the administration of this agent in a population possibly with a poor performance status.

Treatment with tyrosine kinase inhibitors and other agents interacting with EGFR is associated with a characteristic acneiform cutaneous reaction that is dose dependant and often occurs within the first two weeks after the start of treatment. The most frequent characteristics of the cutaneous toxicity are macular rash and acneiform folliculitis (acne/papulo-pustular rash). In phase II studies of erlotinib in patients with lung, ovarian and head and neck cancer, there was a statistically significant association between the development of rash/acne (papulo-pustular rash) and improved overall survival of patients. A combined analysis of the data showed that patients who developed rash/acne (papulo-pustular rash) of any grade had a statistically significant longer median survival, implying that this particular cutaneous toxicity might serve as a clinical surrogate marker of efficacy. However, none of those reports has analyzed time to rash/acne (papulo-pustular rash) occurrence, this idiosyncrasy of such reaction is assumed rather than proven, and the possibility that this constitutes a bias (a higher toxicity due to a longer administration secondary to a longer survival) has not been ruled out.

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C.V. Sorafenib (Nexavar®)

The Raf family of serine/threonine-specific protein kinases comprises three members: A-Raf, B-Raf, and Raf-1. Experimental evidence supports a direct role for Raf-1 kinase in the development and maintenance of human malignancies. For instance, the Raf kinases are the direct down-stream mediators of the Ras proteins, whose oncogenic version is associated with approximately 30% of human solid tumor types. The potential for Raf-1 to play a broad role in tumorigenesis is evidenced by its ability to become activated by either protein kinase Cα (PKCα) or the anti-apoptotic protein Bcl-2 in a Ras-independent manner. Furthermore, Raf mutations have been identified in a range of human tumors. Especially, mutations of the B-Raf gene were detected in a wide range of human tumors and in 66% of malignant melanomas. Independent of its mutation status, Raf is also activated in tumor cells containing enhanced growth factor signaling pathways, such as those induced by mutant or constitutively expressed EGF receptor family members. In addition, analysis of the transcriptional program induced by Raf in epithelial cells revealed an autocrine activation of the epidermal growth factor receptor to be responsible for the ability of Raf activation to protect transformed cells from apoptosis. Therefore, the collective evidence suggests that Raf is a viable anti-cancer drug target for various solid tumors including pancreatic and prostate cancer as well as non-small cell lung cancer (NSCLC).

The targeting of molecular abnormalities in human cancer may provide an opportunity to improve the selectivity of cancer therapy.

Sorafenib (BAY 43-9006) is a novel potent and selective small-molecule inhibitor of Raf kinase in vitro, in cells, and in vivo, with significant dose-dependent anti-tumor activity in four different human tumor xenografts. Additionally, sorafenib is also a potent inhibitor of vascular endothelial growth factor receptor 2 (VEGFR 2) (with an inhibitory concentration (IC)50 of 90 nM). Anti-cancer activity was observed in cancers that have Ras mutations, as well as in cancers without Ras mutations. Anti-tumor activity was also demonstrated in tumor models that express wild type Ras, but over-express growth factor receptors.

This suggests a potential use of this compound in a large spectrum of cancer types, including tumors with a variety of molecular etiologies, all of which have not yet been defined. This activity is cytostatic in nature, and is maintained upon continuation of dosing. In xenograft models sorafenib demonstrated significant anti-tumor activity also against large (400 mg–1 g) colon and ovarian tumors, producing some tumor regressions during the dosing period.

Data from the clinical mass balance study have shown that, on average, less than 20% of the administered dose is excreted in the urine. This is in contrast to preclinical data showing that less than 10% of sorafenib is excreted by the kidneys. Thus, even in the presence of complete shut-down of

renal clearance of the drug, it is expected that a small increase in exposure (e.g. 20 – 40%) may be observed and one which is within the realm of the sizeable inter-patient variability that has been observed in phase I trials is likely to result. Consistent with this, evaluation of preliminary safety data from the phase I database indicates that the incidence of toxicities of grade 3 or greater severity or serious adverse events (SAEs) appear to be similar in patients with creatinine clearance values of 30-50 mL/min and 50-80 mL/min as that with patients with normal creatinine clearance (0,80 mL/min).

Four phase I studies, each with different schedules, were conducted in Canada, Germany, Belgium and USA. More than 170 patients have received sorafenib in phase I. Treatment regimens varied from 50 mg every fifth day to 800 mg twice daily (bid). In these studies, over 67 patients were treated at a dose level of 400 mg bid or greater. The protocol-defined maximum tolerated dose (MTD) for these studies was observed at 400-600 mg bid. At least 22 unique patients were started at 400 mg bid as their initial therapeutic dose, 13 of which have been treated at 400 mg bid continuously. The 400 mg bid dose was well tolerated, with one SAE (pancreatitis) considered possibly study drug-related. At the 200 mg bid dose, two patients with solid malignancies experienced drug-related SAEs: one patient experienced elevated PT-INR while on warfarin, and one patient experienced grade 3 diarrhea. Two patients with acute leukemia also experienced SAEs considered possibly drug-related: acute myocardial infarction resulting in death (200 mg bid) and brain stem stroke (100 mg bid). Based on the combined safety and tolerability experience with these schedules, a dosing regimen of 400 mg bid continuously is recommended for evaluations.

To date, over 350 patients have been treated at the recommended phase II dose of 400 mg bid in two ongoing phase II studies. The most prominent dose limiting toxicities (DLTs) seen have been grade 3 diarrhea and grade 3 hand and foot syndrome. Diarrhea has resolved within several days of discontinuation of dosing. The hand and foot syndrome resolved with discontinuation of sorafenib. Symptoms have also been successfully treated with non-steroidal anti-inflammatory agents and urea-containing cream. There have been no cases of myelosuppression observed.

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C.VI. Sunitinib (Sutent®)

RTKs are transmembrane proteins containing extra-cellular ligand-binding domains and intra-cellular catalytic domains. They are activated following binding of their cognate ligands. Much of the specificity in signal transduction stems from the specific interaction of RTKs with their ligands, with both receptors and ligands having defined patterns of expression. Many of the processes involved in tumor growth, progression, and metastasis are mediated by signaling molecules acting down-stream from activated RTKs. In particular, several members of the split-kinase domain family of RTKs are implicated in deregulated/autocrine proliferation and survival of solid and hematological cancer cells. These include the platelet-derived growth factor (PDGF) receptors (PDGFRα and ß); vascular endothelial growth factor (VEGF) receptor types 1,2 and 3 (VEGFR1, 2 and 3); stem cell factor receptor (KIT); Fms-like tyrosine kinase-3 (FLT-3); and glial cell-line derived neurotrophic factor receptor (RET). In addition, RTKs such as the PDGFRs and VEGFRs are implicated in tumor-dependent angiogenesis

These split-kinase domain RTKs are expressed on solid tumor cells and may be activated by autocrine loops or mutations, leading to cancer cell growth and survival (eg, PDGFR in gliomas, VEGFR in melanoma, KIT in SCLC, FLT-3 in acute myeloid leukemia (AML), and RET in thyroid carcinoma). In particular, some solid tumors are driven by constitutively activated versions of these receptors or their ligands, such as KIT juxtamembrane and PDGFRα activation loop mutations in gastrointestinal stromal tumors (GISTs), and collagen IA1/PDGF-B fusion proteins in dermato-fibrosarcoma protuberans. Mutations of these split-kinase domain RTKs have been particularly evident in hematopoietic malignances. For example, in AML, mutations in FLT-3 result in de-regulation of kinase activity and associated with poor prognoses. Also, activating mutations in KIT have been detected in blasts from 5% of AML subjects. In CMML subjects, PDGFRβ is constitutively activated by enforced dimerization mediated by fusion with the TEL transcription factor. Hence, although the pathogenesis of solid tumors and hematological malignancies is complex, there is good rationale that inhibition of these split-kinase domain RTK targets may result in direct effects against cancer cells expressing them.

In addition to their direct role in tumor cell growth and survival, several split-kinase domain RTKs, most notably VEGFR and PDGFRβ, play prominent roles in tumor neo-angiogenesis. VEGF produced by the tumor cells and associated stromal cells acts on endothelial cells, directly promoting their proliferation, migration, invasion, and survival, all critical facets of angiogenesis. PDGFRβ is expressed on pericytes (smooth muscle cells that provide mechanical support to vasculature) and is also expressed on tumor neo-vasculatur. In addition, PDGFRβ is expressed on fibroblasts in the tumor stromal compartment, which is an important source of VEGF and other growth factors. Recent data suggest that combined pharmacological disruption of PDGFRβ and VEGFR2 (also known as FLK-1 in mice and KDR in humans) signaling results in profound anti-angiogenic effects in tumors

The signaling cascades generated by the split-kinase domain RTKs directly and indirectly regulate tumor growth, survival, and angiogenesis. Inhibiting these targets in concert might be expected to result in broad anti-tumoral efficacy. Sunitinib is a potent and selective inhibitor of these RTK targets in vitro and in vivo, and exhibits potent anti-tumor efficacy in diverse mouse tumor models.

Sunitinib (SU011248) is a small molecule that inhibits receptor tyrosine kinases (RTKs) involved in tumor proliferation and angiogenesis, specifically the PDGFR, VEGFR, KIT, FLT-3, and RET. The non-clinical data from in vitro and in vivo studies indicate that sunitinib selectively inhibits the class 3 and class 5 split-kinase domain RTKs, including the PDGFR and VEGFR, KIT, and FLT-3; that sunitinib is active in vivo, inhibiting target RTK phosphorylation, VEGF-induced vascular permeability, and tumor growth in all rodent tumor models tested; that the plasma concentration of sunitinib required for activity against VEGFR2 and PDGFRß in tumors is at least 50 ng/mL, with slightly lower concentrations required to inhibit FLT-3-ITD and slightly higher concentrations required to inhibit KIT; and that full efficacy in mouse xenograft tumor models is achieved when the relevant target RTK(s) (ie, the RTK driving tumor cell proliferation or survival, or VEGFR and PDGFR for tumor-dependent angiogenesis) are inhibited for at least 12 hours of a 24-hour dosing interval.

Non-clinical pharmacokinetic characteristics after intravenous administration include a moderate to high systemic clearance and a large volume of distribution in all species, and a prolonged plasma elimination half-life in monkeys. Following repeated oral dosing in rats and monkeys, accumulation of sunitinib and SU012662 (its major active metabolite) occurred in plasma and an apparent steady state was reached in most animals within 28 days. With repeated administration of sunitinib to female monkeys for two 4-week treatment cycles separated by a 2-week rest period, exposure (area under the plasma concentration *versus* time curve (AUC) and plasma peak concentration (C_{max})) in the second cycle was not different from the first, indicating that continued plasma accumulation does not occur with subsequent cycles. In the monkey study, tissue retention of sunitinib plus SU012662 relative to plasma (≥13-fold that of plasma concentrations) was measured after each cycle, 24 hours after administration of the last dose. After a 2-week rest period, the combined tissue levels of sunitinib and SU012662 were not higher than 0.4 µg/g, which is less than 1% to 5% of those levels observed 1 day after dosing. When comparing plasma and tissue levels of sunitinib and SU012662 at 24 hours after the last dose, the plasma levels are a good indicator of tissue exposure.

The in vitro bio-transformation of sunitinib is similar across species except in the dog. The major circulating metabolite in all species is SU012662. Because sunitinib is primarily metabolized in human liver microsomes by Cytochrome P450 (CYP) iso-form 3A4 (CYP3A4), there is a potential for concomitantly administered CYP3A4 inducers and/or inhibitors to affect the pharmacokinetics of sunitinib.

In vitro studies on metabolism enzymology have been conducted in human liver microsomes, human hepatocytes, and expressed human CYP enzymes. Studies in human hepatocytes indicated that neither sunitinib nor SU012662 induced CYP3A4. At target plasma concentrations (at least 50 ng/mL sunitinib plus SU012662), the calculated Ki values for all CYP450 iso-forms tested (CYP1A, CYP2D6, CYP3A4, CYP2C9, CYP2E1, CYP4A9/11, and CYP2C19) indicated that sunitinib and SU012662 are unlikely to have any clinically relevant drug-drug interactions with drugs that may be metabolized by these enzymes.

The acute toxicity of sunitinib is low. Monkeys given oral doses of sunitinib in acute and repeated-dose studies had reduced body weight and food consumption, emesis, diarrhea, pale skin, decreased activity, hunched posture, hypothermia, incidence of QTc prolongation and decreased heart rate, discolored mouth or gums and lip or mouth lesions, slight increases in liver transaminases (without histological correlate), and decreases in red blood cell, white blood cell, reticulocyte and platelet counts. The primary target organs of sunitinib were the hemo-lymphopoietic system (bone marrow hypo-cellularity, and lymphoid depletion of thymus, spleen, and lymph node), the exocrine pancreas (acinar cell degranulation with single cell necrosis in rats), the adrenal gland (cortical congestion and hemorrhage in rats and monkeys, with necrosis followed by fibrosis in the rat), the salivary gland (acinar hypertrophy), and the gastrointestinal system (emesis and diarrhea in monkeys, and single incidences of intestinal necrosis associated with erosion/ulceration). All findings were reversible or reversing following termination of treatment.

Sunitinib was not mutagenic in bacteria using metabolic activation. Sunitinib did not induce structural chromosome aberrations in human peripheral blood lymphocyte cells in vitro, however, polyploidy (numerical chromosome aberrations) was observed in human peripheral blood lymphocytes in vitro, in the presence and absence of metabolic activation. In addition, sunitinib was not clastogenic in rat bone marrow in vivo. Overall, sunitinib was considered non-genotoxic in vitro or in vivo.

The carcinogenic risk of sunitinib to humans is unknown, as carcinogenicity studies have not been conducted. Sunitinib may impair fertility in humans.

So far, 1563 subjects have been exposed to at least one dose of sunitinib in 34 completed or ongoing clinical trials (366 in phase I studies, 497 in phase II studies, and 700 in phase III studies).

Clinical studies were conducted globally to determine the maximum tolerated dose (MTD), pharmacokinetics (PK) and pharmacodynamics (PD) of sunitinib in healthy subjects and patients with advanced malignant disease. PK data were collected in 15 clinical phase I and II studies with sunitinib - including 8 studies in healthy subjects, 1 study in patients with AML, and in 6 studies in patients with solid malignant tumors.

In human subjects, the results of clinical pharmacology studies demonstrate that C_{max} and AUC increased in a proportional manner after single doses of 50 to 350 mg, as well as after multiple doses of 25 to 100 mg. The terminal elimination half-lifes range from about 40 to 60 hours for sunitinib and 80 to 110 hours for SU012662. As expected by the prolonged half-lifes and in comparison with day 1 values, the sunitinib $AUC_{(O-24)}$ after 4 weeks of dosing increased 2.5- to 3.5-fold, while the metabolite $AUC_{(O-24)}$ increased 4- to 12-fold. Concurrent administration of sunitinib with the potent CYP3A4 inhibitor, ketoconazole, resulted in a mean 59% and 74% increase in sunitinib C_{max} and AUC, respectively sunitinib and a small decreases to SU012662 (29% and 12%, respectively). Concurrent administration of sunitinib with the potent CYP3A4 inducer, rifampin resulted in a mean 56% and 78% decrease in sunitinib C_{max} and AUC, respectively. SU012662 showed a 137% increase in C_{max} and 27% increase in AUC with concurrent rifampin administration. These results indicate that the safety and efficacy profile of sunitinib may be altered by co-administration with potent inhibitors or inducers of CYP3A4.

Dose-limiting toxicities (DLTs) observed in phase I studies during cycle 1 were generally non-hematological in nature. Fatigue was the primary DLT in subjects receiving sunitinib treatment in repeating cycles and the most commonly reported adverse event (AE) (experienced by approximately 64% of solid tumor subjects overall). The MTD has been defined as 50 mg on both schedules 4/2 and 2/2 (4 or 2 weeks on treatment followed by 2 weeks off) in phase I and II clinical studies in subjects with advanced solid tumors, although other doses and regimens, including continuous daily dosing, are under investigation. Intra-subject dose reduction in 12.5-mg decrements may be required depending on the type and severity of toxicity encoutered.

Among sunitinib-exposed subjects, the most commonly reported all-causality AEs (experienced by at least 20% of the patients) of any severity grade were fatigue, nausea, diarrhea, skin discoloration, anorexia, vomiting, abdominal pain, constipation, dyspepsia, dysgeusia, stomatitis, and headache. Exposure to sunitinib did not pose a major risk of significant clinical cardiac toxicity for subjects with solid malignant tumors.

In a few subjects, rapid destruction of bulky solid tumors by sunitinib resulted in other complications, such as pneumothorax, intestinal fistulae, or intestinal perforation.

Efficacy of sunitinib was demonstrated in a randomized, placebo-controlled phase III clinical study of sunitinib in patients (N=312) with GIST following failure of imatinib mesylate treatment due to resistance or intolerance. The difference in time to progression (TTP) between the treatment arms was statistically and clinically significant with median TTP 27.3 vs 6.4 weeks for the sunitinib and placebo arms, respectively (hazard ratio 0.329; 95% CI: 0.233 - 0.466, $p<0.001$). The secondary efficacy endpoints also identified advantage for treatment with sunitinib. Overall survival (OS) was statistically superior for the sunitinib arm (hazard ratio 0.491; 95% CI: 0.290 - 0.831, $p=0.007$) and partial responses (PRs) were observed in 7.2% of the sunitinib-treated patients compared to no responses in the control arm (95% CI of the difference: 3.34 to 10.18, $p=0.006$). Results for patients (N=55) in the supportive Study RTKC-0511-013 were consistent with the pivotal study, with the median TTP 34 weeks (95% CI: 22.0 to 46.0) and PRs observed in 9.1 % of the patients.

Efficacy of sunitinib has also been demonstrated in patients with cytokine-refractory metastatic renal cell carcinoma (mRCC). The overall response rate (ORR) in the pivotal trial (N=106) using the investigator assessments was 43.4% (95% CI: 33.8 to 53.4) and progression-free survival (PFS) was 35 weeks (95% CI: 23.9 -45.1). In the supportive trial (N=63), the ORR using the investigator assessments was 36.5% (95% CI: 24.7 to 49.6).

OveraII, the AEs reported in clinical studies are manageable and reversible, and objective tumor responses and prolonged median TTP/PFS have been observed in GIST and mRCC in phase II and III clinical studies. Median OS was also prolonged in the GIST phase III study as compared to placebo. In addition, PRs and stable disease have been observed in a variety of tumor types in other phase I and II clinical studies, including metastatic breast cancer and NSCLC.

Ongoing sunitinib studies in subjects with mRCC include a phase III study of sunitinib versus interferon-alfa as first-line treatment, a phase II study in mRCC patients previously treated with bevacizumab, and a phase I/II study of sunitinib in combination with gefitinib in subjects with advanced mRCC.

Clinical development includes evaluation in other tumor types, including breast cancer, NSCLC, colorectal cancer, and prostate cancer. An ongoing study is being conducted in subjects with metastatic NSCLC who had previously received platin-based chemotherapy. Additional studies in NSCLC includes a phase II study of sunitinib as consolidation following carboplatin/paclitaxel given as first line treatment and a phase II combination of sunitinib and erlotinib as second-line treatment; and phase I combinations with carboplatin/paclitaxel and cisplatin/gemcitabine. A phase II study in colorectal cancer has also been completed enrolling subjects with metastatic colorectal cancer who have previously received treatment with fluoropyrimdine, irinotecan, and oxaliplatin; half of the patients had also previously received bevacizumab. A total of 40 bevacizumab-naive patients and 42 patients previously treated with bevacizumab were enrolled. One PR was observed in a patient previously treated with bevacizumab. One phase I/II study of sunitinib in combination with docetaxel and prednisone is ongoing in patients with chemotherapy-naive metastatic hormone refractory prostate cancer. Further development include phase I studies of sunitinib in combination with chemotherapy comprising docetaxel, gemcitabine, capecitabine, FOLFOX, FOLFIRI, carboplatin/paclitaxel, cisplatin/gemcitabine and irinotecan.

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C.VII. RAD001 (Certican®)

RAD001 is a macrolide, derivative of rapamycin, which is being developed as an anti-proliferative drug for oral administration with therapeutic applications as an immunosuppressant in solid organ transplantation and autoimmune diseases as weIl as anti-cancer agent.

At the cellular and molecular level, RAD001 acts by selectively inhibiting mTOR (mammalian target of rapamycin), an intra-cellular protein kinase implicated in the control of cellular proliferation of activated T-lymphocytes (immunosuppression indication) and neoplastic cells (cancer indication). mTOR is a ubiquitous protein kinase implicated in cell cycle control and specifically in the progression of cells from the G1 to S phase.

RAD001 inhibits the proliferation of a range of human tumor cell lines in vitro including lines originating from lung, breast, prostate, colon, melanoma and glioblastoma, inhibitory concentration (IC)50 range from sub/low nM to μM. RAD001 also inhibits the proliferation of human umbilical vein endothelial cells (HUVECs) in vitro, being particularly potent against vascular endothelial growth factor (VEGF)-induced proliferation suggesting that RAD001 may aIso act as an anti-angiogenic agent. Potent inhibition of osteoclast formation and activity has also been demonstrated.

The potential of RAD001 as an anti-cancer agent was shown in rodent models. RAD001 is orally bio-available, residing longer in tumor tissue in a s.c. mouse xenograft model, and demonstrating high tumor penetration in a rat pancreatic tumor model. In normal mice and rats, low level brain penetration is observed. The pharmacokinetic profile of RAD001 indicates sufficient tumor penetration, above that needed to inhibit the proliferation of endothelial cells and tumor cell lines deemed sensitive to RAD001 in vitro. RAD001 administered daily p.o. was a potent inhibitor of tumor growth, at well-tolerated doses, in 11 different mouse xenograft models (including pancreatic, colon, epidermoid, lung and melanoma) and two syngeneic models (rat pancreatic, mouse orthotopic melanoma). These models included tumor cell lines considered sensitive and "relatively resistant" in vitro.

In general, RAD001 was better tolerated in mouse xenograft models than standard cytotoxic agents (i.e. doxorubicin and 5-fluorouracil), while possessing similar anti-tumor activity. Additionally, activity in a VEGF-impregnated s.c. implant model of angiogenesis and reduced vascularity (vessel density) of RAD001-treated tumors (murine melanoma) provided evidence of in-vivo effects on angiogenesis.

Prolonged in-activation (up to 72 hr) of p70S6K1 in tumors and skin and (up to 7 days) in peripheral blood mononucleocytes was observed following a single administration of a RAD001 dose that is efficacious with intermittent (weekly) regimens in the rat pancreatic tumor model. These data indicate the possibility of using skin or blood sampling for biomarker assessment when establishing RAD001 dosing schedules in the clinic, and demonstrate the potential for intermittent dosing schedules. Although, exactly which molecular determinants predict responsiveness of tumor cells to RAD001 is still unclear, molecular analysis has revealed that relative sensitivity to RAD001 in vitro correlates with the degree of phosphorylation (activation) of the AKT/PKB protein kinase and the S6 ribosomal protein; in some cases (i.e. GBM) there is also a correlation with PTEN status. Furthermore, inhibitory effects of RAD001 on the p70S6K1 and 4E-BP1 pathways do not necessarily predict anti-proliferative response to RAD001. Interestingly, RAD001 treatment induces a decline in ^{18}F-deoxyglucose (FDG) uptake in two RAD001-sensitive mouse tumor models (orthotopic murine melanoma and lung xenograft) as determined by positron emission tomography (PET).

When combined with other signal transduction modifiers, a superiority of the combination compared to either agent alone was demonstrated in vitro (imatinib, letrozole, gefitinib) or in a lung xenograft mouse model. In the latter, no tolerability issues occurred. No tolerability problems were observed when combining high-dose imatinib with RAD001 in athymic mice. In a model of radiation-induced brain damage, RAD001 did not exacerbate gross neuro-motor toxicity resulting from locally-applied radiation.

All significant adverse effects observed in toxicity studies with RAD001 in mice, rats, monkeys and mini-pigs were consistent with its anticipated pharmacological action as an anti-proliferative and immunosuppressant agent, and at least in part reversible after a 2- or 4-week recovery period with the exception of the changes in the male reproductive organs, most notably testes. RAD001 caused adverse effects on spermatogenesis in male rats, probably related to a hormonal effect. There was, however, no evidence that the effect on male spermatogenesis adversely influenced matemal or fetal parameters when males were mated to untreated females. In reproduction studies, RAD001 was toxic to the conceptus. In pre- and post-natal development study in rats, however, RAD001 did not show a

specific toxic potential. Ocular effects observed in rats were not observed in other species and are considered to be species-specific. Experiments in rats show that RAD001 dose/schedule impacts its immunosuppressive properties. In rats, a weekly regimen of RAD001, shown to be efficacious in the rat pancreatic carcinoma model, was insufficient to produce prolonged immunosuppression as determined by antibody response to a T cell-dependent antigen. This indicates that longer treatment intervals may at least partially dissociate the anti-tumor effects of RAD001 from its immunosuppressive effects in experimental systems. RAD001 has been shown to be devoid of an oncogenic or genotoxic potential in animal studies. RAD001 was devoid of relevant effects on vital functions including the cardiovascular, respiratory and nervous systems, and had no influence on QT interval prolongation. Furthermore, RAD001 showed no antigenic potential. Although RAD001 passes the blood-brain barrier, there was no indication of relevant changes in the behavior of rodents. Based on these findings, the potential of RAD001 to affect vital functions in patients is considered to be low.

Clinical experience with RAD001 in oncology is based on ongoing phase I studies of single-agent RAD001 and phase Ib studies of RAD001 in combination with systemic anti-cancer agents (imatinib, paclitaxel, gemcitabine and letrozole).

The pharmacokinetic characteristics of RAD001 have been extensively investigated in the context of the drug's development as an immunosuppressant in solid organ transplantation where RAD001 is administered twice daily as a part of an immunosuppressant, multi-drug regimen consistently including cyclosporin A and glucocorticoids. Recent phase I studies in oncology patients provide steady state pharmacokinetics for both the weekly and daily schedules at varying dose levels in patients with advanced cancers.

RAD001 is rapidly absorbed after oral administration, with a median time to peak blood levels (t_{max}) of 1-2 hours post-dose. The extent of absorption is estimated at above 11%. The area under the blood concentration *versus* time curve (AUC) is dose-proportional over the dose range tested while maximum blood concentration (C_{max}) appears to plateau at the dose levels higher than 20 mg. The terminal half-life in cancer patients averaged 30 hours, which is similar to that in healthy subjects. Inter-patient variability is moderate with the coefficient of variation (CV) of approximately 50%. A high-fat meal altered the absorption of RAD001 with a 1.3 hour delay in t_{max}, a 60% reduction in C_{max} and a 16% reduction in AUC. In whole blood, approximately 80% of RAD001 is contained in red blood cells. Of that contained in plasma, 74% is protein-bound. The apparent distribution volume (Vz/F) after a single dose was 4.7 L/kg. RAD001 is eliminated by metabolism, mainly by hydroxylation, then excreted into the feces (>80%). The parent compound is the main circulating component in the blood. RAD001 is a substrate of both the CYP3A iso-enzyme and the P-glycoprotein (P-gp). Strong inhibitors of CYP3A (azole antifungals, cyclosporin, erythromycin) have been shown to reduce the clearance of RAD001 thereby increasing RAD001 blood levels. Rifampin, a strong inducer of CYP3A, increases the clearance of RAD001, thereby reducing RAD001 blood levels. cyclosporin A, a CYP3A4 and P-gp inhibitor, increases AUC by between 2-3 times. Similarly, imatinib, a CYP3A4 and P-gp substrate, an average 3.7-fold increase in the AUC of RAD001 was observed. RAD001 itself does not appear to have an enzyme-inducing/inhibiting effect at investigated doses.

Pharmacokinetic drug-drug interactions with anti-cancer agents are evaluated in ongoing phase 1b studies. Based on currently available results, gemcitabine and paclitaxel did not alter RAD001 pharmacokinetics to a clinically relevant extent whereas imatinib notably increased RAD001 exposure with a mean increase in AUC by a multiple of 3.7 for RAD001 administered weekly; two-fold for RAD001 administered daily. Exposure to RAD001 in the presence of letrozole did not exceed that in mono-therapy. Co-administration of RAD001 did not influence pharmacokinetics of gemcitabine, imatinib, or letrozole. Exposure to paclitaxel in the presence of RAD001 was slightly decreased (average by 23%).

RAD001 pharmacokinetics in transplant patients was investigated in special populations such as subjects with hepatic or renal impairment, various ethnic groups and pediatric renal transplant patients. In subjects with mild-moderate hepatic impairment, mean AUC to RAD001 is increased by 3-fold whilst renal impairment does not affect the pharmacokinetics of RAD001. Age, weight (both over the adult range) and gender do not affect the pharmacokinetics of RAD001 to a clinically relevant extent.

Also, pharmacokinetics does not alter in Japanese or Asian ethnicity whereas black patients have 21% higher clearance compared with non-blacks.

Phase I clinical studies of RAD001 as a single agent explore two regimens: weekly dosing (range 5-70 mg) and daily dosing (5-10 mg). Preliminary data are available for 84 patients, 74 advanced cancer patients, and 10 patients with newly-diagnosed prostate carcinoma. At the weekly schedule, dose-limiting toxicity (DLT) in the first four weeks of treatment has been observed at 50 mg (1/12) and 70 mg (3/14) and at the daily dosage, DLT has been observed at 10 mg only (1/12). DLT was principally grade 3 stomatitis. Other DLTs were grade 3 fatigue and neutropenia.

Apparent adverse drug reactions (ADR) include rash (approx. 40%), stomatitis/mucositis and fatigue (30% each), headache (20%), nausea, vomiting, diarrhea (10% each).

Reduced blood cell counts at the initiation of treatment are frequent, but remain mostly within normal range or limited to grade 1 although a grade 3 neutropenia was dose-limiting in one patient (as was a grade 3 thrombocytopenia in a patient receiving RAD001 with letrozole where a pharmacodynamic interaction is unlikely). This suggests that some patients may be particularly sensitive to the myelosuppressive effect of RAD001 making it necessary to monitor carefully blood cell counts at initiation of treatment.

Hyperlipidemia had been reported as ADR in 10% of patients although review of the laboratory values suggests that as many a quarter of patients develop grade 1-2 hyperlipidemia on treatment, mostly hypercholesterolemia.

Infectious episodes have not been more frequent than might have been expected. Herpes infections (zoster, labialis), observed in 5 patients on the mono-therapy were not severe.

Pharmacokinetic/pharmacodynamic modeling based on inhibition in a peripheral biomarker (S6 kinase inhibition in peripheral blood mononuclear cells) suggests that 5-10 mg daily should be adequate a dose to produce a high-degree of sustained target inhibition. With the weekly dosing, the degree of inhibition will be contrasted with its duration.

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C.VIII. AZD2171

AZD2171 has been developed as a potent inhibitor of the tyrosine kinase activity of vascular endothelial growth factor (VEGF) receptor 2 (VEGFR2; also called kinase insert domain-containing receptor [KDR]), VEGF receptor 1 (VEGFR1 also called fms-like tyrosine kinase-1 [Flt-1]) and

VEGF receptor 3 (VEGFR3, also called fms-like tyrosine kinase-4 [Flt-4]), and of VEGF-driven human umbilical vein endothelial cell (HUVEC) proliferation. AZD2171 is expected, at chronic oral dosing, to inhibit VEGF driven angiogenesis and, as a consequence, constrain tumor growth. Since angiogenesis is necessary for the growth and metastasis of most tumors and VEGF is believed to have a pivotal role in this process, AZD2171 treatment may have broad-spectrum clinical utility.

Following a single dose, AZD2171 is orally available with maximum plasma (peak) drug concentration after single dose administration (C_{max}) ranging from 1 to 8 hours post dosing. Concentrations declined in an apparent bi-exponential manner thereafter, with a $t_{1/2\lambda z}$ ranging from 12.5 to 35.4 hours.

Steady-state plasma concentrations were predicted by the single dose pharmacokinetics (PK) with the grand arithmetic mean temporal change parameter value being 1.07. This result supports no time dependent changes in PK.

Dose-proportionate increases in C_{max}, maximum (peak) steady state drug concentration in plasma during dosing interval ($C_{ss,max}$), area under the plasma concentration *versus* time curve from zero to infinity ($AUC_{(0-\infty)}$) and AUC_{ss} provide no evidence to reject linear PK for single and multiple doses ranging from 0.5 to 60 mg.

Following multiple oral doses of AZD2171 20 mg, the unbound minimum (trough) steady state drug concentration in plasma during dosing interval ($C_{ss,min}$) is 4.5 times greater than the human umbilical vein endothelial cell proliferation inhibitory concentration (IC)50. The PK profile of AZD2171 is supportive of once daily oral dosing.

Serial assessments of blood flow and vascular permeability in liver metastases were carried out by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). Across the dose range studied (0.5-60 mg), there was a strong negative association between AZD2171 exposure and percentage change from baseline in the DCE-MRI parameter, iAUC60 (a measure of both blood flow and vascular permeability). In the randomized cohort expansion part of the study, significant decreases from baseline were seen at 20, 30 and 45 mg. Within this range, the difference between the doses was not statistically significant.

Initial biomarker assessments show increases in VEGF at all doses. Time-dependent reductions in VEGFR-2 have been documented at doses of 10 mg and higher. Dose-dependent reductions in VEGFR-2 have also been seen at doses of up to and including 20 mg. Decreases in sVEGFR-2 levels may be a surrogate for decreased angiogenesis and changes in VEGF could potentially be indicative of acute vascular effects.

Response data from 63 patients assessed by Response Evaluation Criteria in Solid Tumors (RECIST) indicate two confirmed partial responses (PR) in prostate cancer (45 mg) and renal cell cancer (60 mg), 23 patients with stable disease, including two un-confirmed PRs in hepatocellular cancer (30 mg and 45 mg), one un-confirmed PR in soft tissue carcinoma (60 mg) and eight confirmed minor responses (MR) in soft tissue sarcoma (60 mg), head and neck and hepatocellular cancer (45 mg); lung, stomach and breast cancer (30 mg); colorectal cancer and breast cancer (20 mg), and 38 patients with progressive disease.

Preliminary response data showed one confirmed PR observed in a patient with mesothelioma treated with AZD2171 45 mg in combination with gefitinib 250 mg. Six patients had a RECIST best response of stable disease (SD), including one patient with non-small cell lung cancer who had a confirmed MR with sustained decreases from baseline ranging from 25% to 30% over 5 months following treatment with AZD2171 30 mg in combination with gefitinib 250 mg.

The maximum tolerated dose (MTD) for AZD2171 was defined as 45 mg from the dose escalation part of the study exploring three doses 20 mg, 30 mg and 45 mg in a randomized cohort expansion design. The MTD for the acute myeloid leukemia study was defined as 30 mg. The MTD in another study was 20 mg; the MTD in combination with 250 mg gefitinib was 30 mg. In combination with 500 mg gefitinib, 30 mg was well tolerated.

The most commonly reported adverse events (AEs) in the AZD2171 mono-therapy studies were fatigue, diarrhea, nausea, hypertension, dysphonia (hoarseness), anorexia, headache and vomiting. Of the commonly occurring AEs, a dose relationship was suggested for diarrhea, dysphonia (hoarseness),

headache and hypertension. However, with the exception of hypertension, the majority of these events were of Common Toxicity Criteria (CTC) grade 1 or 2.

The most commonly reported AEs were diarrhea, hypertension, anorexia, fatigue and rash. Hypertension is an expected pharmacological effect of agents that inhibit VEGF and is one of the most common AEs reported with AZD2171. In AZD2171 studies, dose-related increases in blood pressure were observed at AZD2171 doses of 20 mg and above. There were a total of 13 serious adverse events (SAEs) reports of hypertension from 12 patients that were possibly related to AZD2171 treatment. In addition, there were four reports of hypertensive crisis and one report of malignant hypertension with retinopathy. A hypertension management protocol was implemented for all new AZD2171 studies in March 2005. This approach has been successful in reducing the number of hypertension grade 3 AEs and following its introduction only two hypertension SAE reports have been received.

Muscle weakness, dry mouth and oral mucosal inflammation have also been observed in AZD2171 studies.

There were no observed dose-related changes in creatinine or creatinine clearance across the three mono-therapy studies and gefitinib combination study. 6 patients who received doses of AZD2171 30 mg and above developed proteinurea (urine protein analysis ++ or higher) on two or more consecutive visits; this number constitutes 7% of all patients (i.e., across all doses). In another study, four patients (17%) developed proteinuria on two or more consecutive visits

Dose-related increases in thyroid stimulating hormone (TSH) and decreases in total thyroxine were observed at doses at and above 30 mg, but were most marked at 60 mg. No AEs of clinical hypothyroidism were reported.

Dose-related increases in hemoglobin, hematocrit and erythrocytes have been observed at AZD2171 doses of ≥20 mg. All but two remained within the normal range and clinical symptoms were associated with these dose related changes.

So far, over 260 patients have received AZD2171 across seven phase I studies, including four mono-therapy studies in patients with advanced solid tumors, acute myeloid leukemia and prostate cancer and three studies of AZD2171 in combination with the following agents gefitinib, modified oxaliplatin, leucovorin and 5-fluorouracil (mFOLFOX6), pemetrexed, docetaxel, irinotecan with and without cetuximab and carboplatin/paclitaxel and capecitabine. In addition, a National Cancer Institute of Canada (NCIC) placebo-controlled phase II/III study of AZD2171 in combination with carboplatin and paclitaxel for the treatment of patients with advanced non- small cell lung cancer has recently started, as has a phase II program of approximately 20 studies in collaboration with the National Cancer Institute (NCI).

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