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Review paper

E7070: a novel synthetic sulfonamide targeting the cell cycle progression for the treatment of cancer

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Cell cycle regulation and cell growth are interesting targets in the search for new antitumor agents as these processes are highly disturbed in malignant cells. E7070 is a novel synthetic sulfonamide that targets the G_1 phase of the cell cycle and is currently in clinical development for the treatment of solid tumors. The potential antitumor activity of the compound was discovered through optimization of the structure–activity relationships of a series of sulfonamide structures. E7070 causes a blockade in the G_1/S transition through inhibition of the activation of both cyclin-dependent kinase 2 and cyclin E. Preclinical studies with E7070 showed activity in multiple tumor types, most prominently in colon and lung cancer. A phase I clinical program was conducted with E7070 evaluating different treatment regimens. Dose-limiting toxicities were hematological, including neutropenia and thrombocytopenia. Preliminary results of phase II studies demonstrated limited antitumor activity following treatment with E7070 as single agent in heavily pretreated patients with non-small cell lung and colon cancer. Studies evaluating the activity of E7070 in combination with other chemotherapeutic agents are being conducted. [© 2002 Lippincott Williams & Wilkins.]

Key words: Cell cycle inhibitor, E7010, E7070, sulfonamide.

Introduction

Although significant advances have been made over the past decades in the treatment of various types of cancer, improvements in tumor response rates and survival of patients with advanced cancer are still desperately needed. In the continuous search for new anticancer agents, cell cycle regulation is an interesting target as this is highly disturbed in malignant cells.[1–3] In normal cells, cell growth is strictly controlled by a variety of molecular mechanisms that prevent the occurrence of invasive and unlimited growth.[1–3] In cancer cells, however, mutations have occurred in genes governing the cell cycle regulation. Therefore, agents that inhibit or modulate the cell cycle progression are considered potential anticancer drug candidates.[1,3]

Many important genes (for instance *ras* and p53) that are associated with the regulation of the G_1 phase appeared to be involved in, for example, proliferation, oncogenic transformation and programmed cell death (apoptosis).[1,2] At the moment, several agents that affect the progression through the G_1 phase of the cell cycle or the transition of the G_1 to the S phase are being clinically evaluated, such as the cyclin-dependent kinase inhibitor flavoperidol.[1]

E7070 [*N*-(3-chloro-7-indolyl)-1,4-benzenedisulfonamide, Figure 1] is one of the compounds targeting the G_1 phase of the cell cycle and is currently being clinically evaluated for its therapeutic benefits. In this paper, the discovery and the mode of action of E7070 in relation to the cell cycle will be described, as well as the (preliminary) results of the preclinical and clinical studies with the compound.

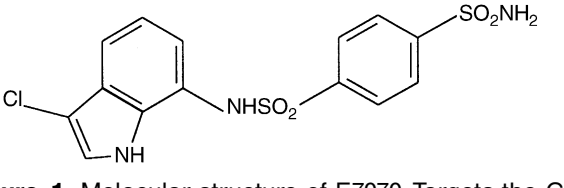

Figure 1. Molecular structure of E7070. Targets the G_1 phase of the cell cycle.

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A brief description of the cell cycle

The cell cycle consists of several sequential phases, which are schematically depicted in Figure 2. In brief, from the quiescent stage in G_0, the cells prepare for DNA replication in the G_1 phase, the S phase is the period of DNA synthesis, in the G_2 phase the cells prepare for mitosis, and in the mitotic phase (M phase) the cell division occurs and two identical daughter cells are generated. The cell cycle progression is subject to strict regulations and during the cell cycle a complex signal system operates to decide a cell's fate: quiescence, differentiation, proliferation or apoptosis.[1,3]

The processes in the cycle must occur in a specified sequence, i.e. one phase must be fully completed before progression to the subsequent phase. For example, DNA synthesis must be finished before mitosis in order to ensure that the cell cycle yields two identical daughter cells.[1] The progress of a cell through the phases of its growth cycle is governed by a system consisting mainly of two components: the cyclin-dependent kinases (CDKs) and the cyclins.[3] As their name implies, the CDKs require association with cyclins to exert activity. A variety of CDK–cyclin complexes are formed at distinct moments in the cell cycle (Figure 2) and each complex has different activity. The levels of CDK are relatively constant throughout the cell cycle, while the levels of the multiple cyclins vary substantially and therefore the effect of the CDK–cyclin complexes is mainly determined by the available cyclins.[3] In the early stages of the cell cycle, cyclin D is synthesized, followed by an induction of cyclin E in the mid/late G_1. Cyclin E associates with CDK2 and this complex is essential for transition to the S phase,[4] where cyclin A is generated followed by an emerge of cyclin B in the M phase.

The central event in the cell cycle is the progression beyond the so-called restriction point, in the late G_1 phase.[1–4] At this point, it is decided whether the conditions in terms of available nutrients and growth factors are sufficient for the cells to complete a full cycle. If the criteria are not met, the cells exit from the cycle, and become quiescent and re-enter G_0. However, when the conditions are sufficient, the cells commit themselves to the cycle and enter the S phase. The decision at the restriction point is governed by the retinoblastoma protein, pRb. When pRb is unphosphorylated or hypophosphorylated, pRb blocks the transition through the restriction point. The G_1 cyclins, cyclin D and E, are mainly responsible for pRb phosphorylation.[3]

Although the restriction point is the most crucial decision point, several other checkpoints operate during the subsequent steps in the cell cycle to detect problems at all stages and to halt the cell cycle progression whenever necessary.[1] For example, progression through mitosis is prevented in case of errors in the replicated DNA and the mitotic checkpoint subsequently ensures correct spindle assembly. When the cell cycle is not arrested at these checkpoints although irregularities have occurred, the risk of a malignant cell increases. Besides the regulation by CDK–cyclin complexes, the cell cycle is

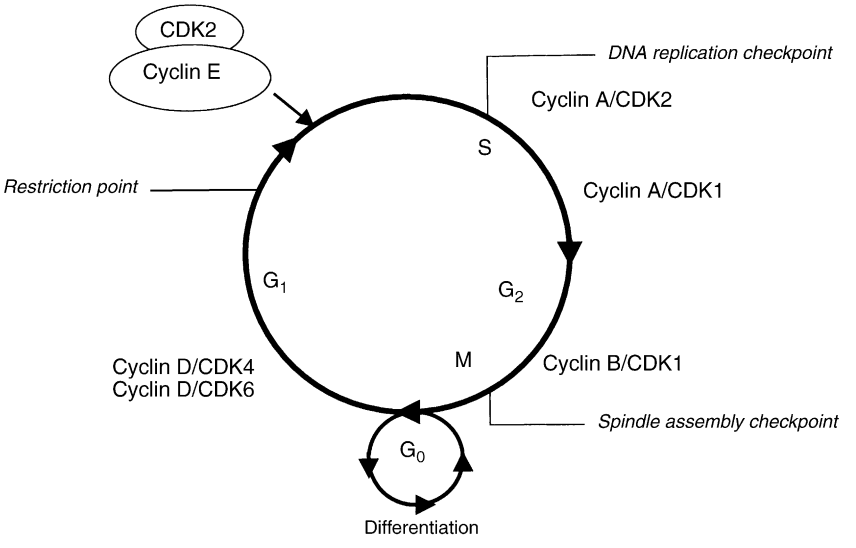

Figure 2. A schematic representation of the cell cycle.

further regulated by various endogenous proteins such as the CDK inhibitors. These can inhibit cell cycle progression in the G_1 phase and have been shown to cause G_1 arrest in older cells and contact-inhibited or growth-inhibited cells.[1]

In the malignant cell, the regulation of the G_1 phase appears to be distorted. The CDK inhibitors have been found to be absent, mutated or silenced and can therefore no longer exert their suppressive action on cell cycle progression. On the other hand, cyclins appear to be overexpressed in several cancer cells. Furthermore, in almost all cancerous cells mutations occur that eliminate the restriction point function and allow the cells to enter the subsequent phases inappropriately.[3,4] Ultimately, these processes are likely to result in tumorigenesis and tumor progression. Therefore, agents that target the G_1 phase of the distorted cell cycle are thought to provide a new therapeutic opportunity for the treatment of cancer.[1]

Sulfonamides as potential anticancer agents

In an early report in 1992, Yoshino *et al.* described the synthesis and screening of a new series of chemical structures with potential cytotoxic properties.[5] As a lead compound they used the sulfonamide moiety because it was described for the antibacterial sulfonamide sulfadiazine, that it accumulates in s.c. implanted murine tumors after i.p. administration. Furthermore, the sulfonamide compounds possess a variety of other biological activities, such as inhibition of carbonic anhydrase, release of insulin and diuretic effects.[6] Modifications were made to a basic benzenesulfonamide structure and the benefit of these modifications in terms of *in vitro* and *in vivo* antiproliferative activity was evaluated.[5] One compound *N*-[2-[(4-hydroxyphenyl)amino]-3-pyridinyl]-4-methoxy-benzenesulfonamide, which was designated E7010 (Figure 3), was found to be inhibitory active against colon 38 murine adenocarcinoma cells and KB human nasopharynx carcinoma cells as well as *in vivo* models of colon 38, with IC_{50} values ranging between 0.29 and 0.38 μg/ml.[5] Based on this activity and the structural novelty, E7010 was selected for further evaluation. Studies on its mechanism of action indicated that E7010 causes cell cycle arrest in the M phase resulting from the inhibition of tubulin polymerization.[7] An antivascular effect of E7010 on the tumor cells could not be demonstrated.[8] E7010 was shown to bind preferentially to the colchicine binding site of β_3-tubulin.[7,9] After showing potent activity in rodent tumors and human xenografts,[10] E7010 was investigated as an orally administered agent in a phase I clinical trial in two different treatment regimens: a single administration and a 5-day repeated administration in one treatment course.[11] The dose-limiting toxicities were neurologic in the single-dose administration, and peripheral neuropathy and intestinal paralysis in the 5-day repeated schedule. Hematological toxicity was mild in both schedules. Pharmacokinetics were linear in both treatment regimens and no accumulation occurred in the 5-day schedule. With the single-dose schedule, response was observed as a 75% reduction in the spinal cord metastases in a patient with uterine sarcoma and a minor response in a pulmonary adenocarcinoma patient.[11] In the 5-day repeated dose study, decreases in the tumor markers were observed in patients with gastric cancer and recurrent uterine cervical carcinoma.[11] A later study by Funahashi *et al.* investigated the effect of E7010 on liver metastases and the lifespan of mice in an orthotopic colon transplantation model. In this model, E7010 suppressed tumor growth of the primary murine colon tumor, but growth of the hepatic metastases was inhibited even more effectively.[12]

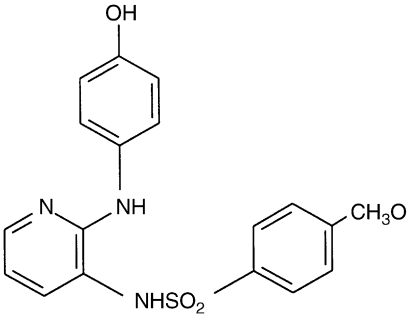

Figure 3. Molecular structure of E7010. Targets the M phase of the cell cycle.

E7070 development and mechanism of action

E7010 was the first sulfonamide that was discovered as an anticancer agent through optimization of the structure–activity relationships of the sulfonamides. However, the search for other potentially active anticancer drugs was continued with more than 300 structurally related compounds using multiple antitumor screening assays.[13,14] This process resulted in the identification of a class of antitumor sulfonamides that target the G_1 phase of the cell

cycle but not the M phase, as was seen with E7010.[14] These compounds induced a decrease in the proportion of cells in the S phase and an accumulation of cells in the G_1 phase in a dose-dependent manner.[14]

In addition to a different mode of action, this class demonstrated *in vitro* activity against different tumor types. The M phase targeting sulfonamides almost equally suppressed growth in KB (human nasopharynx), colon 38 murine adenocarcinoma and P388 (murine leukemia), whereas the G_1-targeting compounds were more potent towards colon 38 rather than KB and P388 cells.[13]

E7070 appeared to be the most potent compound of this class of G_1 phase targeting compounds.[14] Watanabe *et al.* studied the mechanism of action of E7070 using human colon cancer cells (HCT116). They demonstrated that the cell cycle arrest in the G_1/S phase was caused by inhibition of the activation through phosphorylation of CDK2 and of the expression of cyclin E.[15] Furthermore, they found that E7070 caused an upregulation of p53 and p21, resulting in apoptotic cell death.[15] Fukuoka *et al.* continued the studies on the mechanism of antitumor activity of E7070 using human non-small cell lung cancer A549 cells. They demonstrated that E7070 not only caused an inhibition of the G_1/S transition in these cells, but also a blockade of the G_2/M transition and an abrogation of the S phase progression.[16] Furthermore, E7070 caused an inhibition of the phosphorylation of pRb, suppressed the cdk2 activity with the induction of p21 and p53 proteins, and decreased the expression of cyclin A, B1, cdk2 and cdk4 proteins. These effects were absent in A549/ER cells, which were made resistant to E7070.[16] Ozawa *et al.* demonstrated in human colon cancer cells that the proportion of accumulating cells in the G_1 phase increases with both the time and concentration at which the cells are exposed.[17,18]

Tsukuhara *et al.* attempted to elucidate the precise mode of action of E7070 using normal cells of two different yeast species as model organisms, in order to search for possible targets of E7070.[19] In the presence of E7070, the number of yeast cells in the G_1 phase increased and the entry in the S phase was significantly inhibited. Furthermore, their experiments demonstrated that E7070 inhibits the transport of the amino acid leucine and of uracil into the cell, most likely through a direct inhibition of their corresponding transporters.[19] As a result, E7070 causes a nutritional starvation of the cell, which has been shown to be an effective strategy for the treatment of cancer.[19]

Pre-clinical studies with E7070

In the screening assays that were conducted with the sulfonamides, E7070 showed potent activity in *in vitro* models with colon 38 murine adenocarcinoma and P388 murine leukemia, but was less active against human nasopharynx cells.[14] Ozawa *et al.* further explored the pre-clinical antitumor profile of E7070 and found antiproliferative activity of the compound in a variety of tumors, with HCT116 colon carcinoma being the most sensitive.[17] Furthermore, the cytotoxicity increased with prolonged exposure. The *in vivo* antitumor spectrum of E7070 was studied using several colon and lung cancer xenografts. E7070 showed tumor growth suppression as well as a decrease in tumor volume in three colon cancer types and two lung cancer types of these xenografts. Furthermore, in the HCT116 xenograft model, E7070 was more active than mitomycin C and irinotecan. In addition, activity was observed in HCT15 xenografts which are considerably resistant to drugs such as paclitaxel and doxorubicin. As both HCT15 and HCT116 cells highly express P-glycoprotein (P-gp) and multidrug resistance protein (MRP), these results indicate that E7070 efficacy is independent of the multidrug resistance phenotype. Furthermore, a cell's sensitivity to E7070 does not seem to be related to the status of either *ras* or p53.[17] Evaluation of different dosing regimens in HCT116 and LX-1 lung cancer xenografts confirmed that prolonged administration would be beneficial for activity.[17]

Antitumor activity of E7070 has also been studied in combination with other anticancer agents. In human tumor xenograft models with colorectal cancer HCT15, a synergistic effect was observed of the combination with irinotecan.[20] This result was further confirmed in SW620, another type of human colon cancer xenografts. Combinations with other anticancer agents, such as 5-fluorouracil, paclitaxel and gemcitabine, yielded additive effects and antagonism was not observed.[20]

Clinical studies with E7070

The positive results from the preclinical studies led to the initiation of a phase I clinical program with E7070. Four dose-escalation studies have been conducted with different treatment regimens to determine the maximum tolerated dose and the dose-limiting toxicities in cancer patients.[21–25] The evaluated schedules were: (i) a 1-h infusion, every 3

weeks; (ii) a daily × 5, 1-h infusion, every 3 weeks; (iii) a weekly × 4, 1-h infusion, every 6 weeks; and (iv) a continuous 120-h infusion, every 3 weeks. The main results of the phase I studies are summarized in Table 1. The dose-limiting toxicities were consistent across the different treatment schedules, being neutropenia and thrombocytopenia.[21–25]

The final results of two studies have been published so far;[21,22] the results of the other two schedules are still preliminary.[23–25] In the 1-h infusion study,[21] 22 patients were assessable for tumor response and 14 patients had stable disease lasting for 2–13 months. These patients had a variety of tumor types, including metastatic melanoma and colon cancer with liver metastases.[21] In the study evaluating a daily × 5, 1-h infusion (n=33),[22] a partial response in a heavily pretreated patient with breast cancer lasting for 12 months was reported. Furthermore, three patients with soft tissue sarcomas and colorectal cancer experienced disease stabilization during 12–18 weeks. In five additional patients minor antitumor activity was noted.[22] In the study evaluating a continuous 120-h infusion, no objective responses were documented; however, three patients had a stabilization of disease.[23] A patient with a uterine adenocarcinoma showed a partial response after treatment with E7070 in the weekly × 4, 1-h infusion schedule.[24] In addition, five patients experienced disease stabilization lasting between 2.5 and 16.8 months.[25]

During the four studies, pharmacokinetic sampling was performed in all patients to assess the pharmacokinetic profile of the drug in cancer patients. Noncompartmental analyses revealed the non-linear, dose-dependent pharmacokinetics of E7070, characterized by a decreased clearance of the compound and a more than proportionate increase in total exposure with increasing doses.[21–25] A population pharmacokinetic model has been developed to describe the pharmacokinetics of E7070 in all four treatment schedules.[26] The model comprised three compartments and saturable distribution to one of the peripheral compartments (Figure 4). The nonlinear pharmacokinetics were further characterized by saturable elimination from the central compartment, which was parallel to an additional linear pathway of elimination (Figure 4).[26] Important pharmacokinetic parameters such as volume of distribution and maximum elimination capacity of the drug appeared to be related to body surface area (BSA), indicating that BSA-guided dosing is a rational approach for E7070.[26] Analyses of the relations between the pharmacokinetics and pharmacodynamics of E7070 revealed that both neutropenia

Table 1. Overview of the phase I studies with E7070

Schedule	No. of patients	Dose range	Recommended phase II dose	DLT	Other toxicities	Best response	Reference
DX1	40	50–1000 mg/m^2	700 mg/m^2	neutropenia, thrombocytopenia	acne-like skin eruption, mucositis, conjunctivitis, nausea, fatigue, alopecia	SD lasting longer than 6 months	21
DX5	35	10–200 mg/m^2/day	130 mg/m^2/day	neutropenia, thrombocytopenia	alopecia, skin folliculitis	PR in heavily pretreated breast cancer; SD in three patients with soft tissue sarcoma and colon cancer	22
WX4	43	40–500 mg/m^2/week	400 mg/m^2/week	neutropenia, thrombocytopenia, stomatitis	diarrhea	PR in one patient (4.6 months); SD in five patients (up to 16.8 months)	24
CIV	26	6–200 mg/m^2/day	130 mg/m^2/day	neutropenia, thrombocytopenia, stomatitis	nausea, asthenia, alopecia	SD in three patients	23

DX1: daily × 1, 1-h infusion every 3 weeks; DX5: daily × 5, 1-h infusion every 3 weeks; WX4: weekly × 4, 1-h infusion every 6 weeks; CIV: continuous 120-h infusion, every 3 weeks. DLT: dose-limiting toxicity; SD: stable disease; PR: partial response.

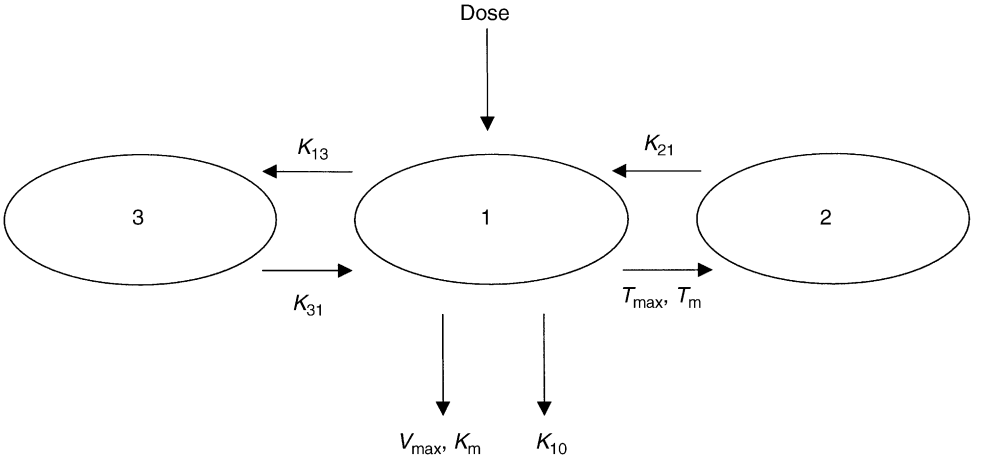

Figure 4. Schematic representation of the population pharmacokinetic model developed for E7070.[27]

and thrombocytopenia are correlated to the degree of exposure to E7070, defined as area under the plasma concentration–time curve (AUC).[27]

The major pathway of elimination of E7070 is metabolism. The urinary excretion of the unchanged compound calculated over the first 24 h accounted for only 1.4% of the dose after a 1-h infusion[21] and for less than 0.5% of the dose in the daily × 5 schedule.[22] To obtain further insight into the metabolic pathway a mass balance study was conducted where radiolabeled E7070 was administered to patients, and radioactivity, E7070 and a known metabolite were determined in plasma, urine and feces.[28] The results showed that E7070 is extensively metabolized, although the metabolites remain to be identified. It was suggested that the extensive metabolism of E7070 could be a physiological explanation for the parallel linear and saturable elimination in the population pharmacokinetic model, as it is likely that some metabolites are formed through saturable enzymatic processes whereas others are formed linearly.[26] The mass balance study also demonstrated high red blood cell binding of E7070 and the binding was saturable at clinically relevant concentrations.[28] This was proposed as a plausible explanation for the observed saturable transport in the population pharmacokinetic model.[26]

Currently, phase II clinical studies are conducted evaluating the antitumor activity of E7070 in several tumor types. So far, preliminary results of two studies with single-agent E7070 have been published.[29,30] In both studies, patients were randomized to the two treatment regimens selected from the phase I studies: 700 mg/m^2 administered as a daily × 1, 1-h infusion every 3 weeks (DX1) or 130 mg/m^2 as a daily × 5, 1-h infusion, every 3 weeks (DX5). The first study evaluated the activity of E7070 as second-line therapy in patients with progressive colorectal cancer which was refractory or resistant to treatment with 5-fluorouracil.[29] In general, the treatment was well tolerated. Forty-four patients were evaluable for response after two treatment courses. Two patients in both the DX1 (n=21) and DX5 (n=23) schedule showed a partial response, stable disease was observed in 14 and 13 patients, respectively. Progression-free survival rates for the respective treatment schedules were 25 and 26% at 4 months, and 10 and 13% at 6 months. Overall, the objective response rates in this tumor type were low and, in contrast to the results of the preclinical studies, there was no clear difference between the two treatment regimens and therefore the more convenient DX1 schedule is preferred for further clinical investigation.[29] The second phase II study involved the second-line treatment of patients with non-small cell lung cancer who have failed platinum-based chemotherapy.[30] E7070 was well tolerated in this patient group. The response to treatment in both regimens was low with one (out of 15 patients) partial response in the DX1 group and none (in total 13 patients) in the DX5 schedule. In the respective treatment groups, seven and three patients had stable disease, and seven and 10 patients were progressive under treatment. Again, no marked differences existed between the schedules. In this study, cell cycle analyses were performed in tumor biopsies before and after treatment with E7070 as a pharmacodynamic marker of activity. Biopsies of three patients were available for these analyses and in two an increased number of cells in the apoptotic fraction was observed, with a decrease in the number of cells in the G_1 phase.

However, none of these patients experienced an objective response.[30] Further clinical efficacy studies with E7070 will focus on combinations with approved anticancer drugs.[30]

Although E7010 (Figure 1) and E7070 (Figure 3) were derived from a single lead structure,[13] marked differences exist between the two compounds. E7010 was shown to affect the M phase of the cell cycle resulting from an inhibition of the tubulin polymerization, whereas E7070 blocks cell cycle progression in the G_1/S phase. DNA micro-arrays have been applied to further study the mechanism of action of both compounds in a subclonal colon cancer cell line HCT116-C9.[31] E7010 induced changes in the transcript levels of 50 genes (34 induced and 16 repressed), including genes involved in cytoskeletal organization, transcriptional regulation and signal transduction. On the other hand, E7070 affected the expression of 154 genes (15 induced and 139 repressed), but only two of them were also altered by E7010. Alterations due to E7070 were mainly observed in genes involved in cell cycle progression and cellular metabolism.[31] Apparently, the substituents to the basic sulfonamide structure cause a marked difference between the mode of action of E7010 and E7070. This different mechanism of action is likely to affect their pattern of *in vitro* antitumor activity as it has been reported that agents with a similar mode of action show a comparable pattern of antitumor activity.[18] Indeed, antitumor activity in different tumor types has been demonstrated for the two compounds.[13,18] The similarity in the antitumor spectrum of E7010 and E7070 was evaluated using the COMPARE algorithm of the National Cancer Institute. The correlation coefficient between the activity of the two agents was only 0.44, which emphasizes their different mode of action. In contrast, for instance, the topoisomerase II inhibitors doxorubicin and etoposide showed a high correlation of 0.77.[18]

In clinical trials, E7010 was administered orally, the pharmacokinetics were dose independent and the dose-limiting toxicities were neurological.[11] In contrast, E7070 was administered to patients as i.v. infusions, and severe neutropenia and thrombocytopenia were dose limiting.[21–25] Furthermore, E7070 showed dose-dependent pharmacokinetics, and it was suggested that this non-linear profile could be caused by saturation of metabolic enzymes and saturable binding to red blood cells. In summary, the structural differences between E7010 and E7070 lead to markedly different behavior of the two compounds.

Discussion

Sulfonamides are well known for their variety in pharmacological activities, such as antibacterial, diuretic and insulin releasing. The first suggestion of possible antitumor activity of sulfonamides was obtained with the antibacterial sulfonamide sulfadiazine, which appeared to accumulate in murine tumors after i.p. administration.[5] With the sulfonamide moiety as a lead compound, over 300 related molecules were synthesized, and these were tested for their *in vitro* and *in vivo* antiproliferative activity. Initially, E7010 was selected for further development as an anticancer agent but currently the main focus is on E7070.

E7070 is one of the new drug candidates that mainly target cell cycle regulation in the G_1 phase although the precise mechanism through which it exerts its antitumor activity is not completely elucidated. In the phase I studies, classical toxicity parameters have been used to establish a dose recommendation for phase II trials. For cell cycle affecting agents such as E7070, measurement of biological markers could offer a contribution to more rationally recommend dosages.[32,33] For example, inhibition of the cell cycle progression or phosphorylation of pRb could serve as markers for biological activity. Once the relation between these biological effects and actual antitumor activity are validated, they could be incorporated in clinical trials.[21,32,33] For E7070, this was done in a phase II trial where cell cycle analyses were used to assess the fraction of apoptotic cells before and after treatment in tumor biopsies.[30] The preliminary results of two patients demonstrated cell cycle arrest in tumor tissue but this did not correlate with objective response to treatment, which is a prerequisite for the successful application of a pharmacodynamic endpoint.

Preclinical studies have indicated that prolonged treatment could enhance the antitumor activity of E7070. However, pharmacokinetic research in the phase I studies revealed the relatively long half-life of E7070,[21–25] indicating that protracted infusions such as the 120-h infusion are probably not required for activity. Currently, a single 1-h infusion and a daily $\times$ 5, 1-h infusion are being compared in terms of safety and efficacy in the phase II studies.

So far, limited antitumor activity of E7070 has been observed in patients. However, mostly heavily treated patients have been studied thus far. In these patient populations low response rates are expected. The phase II studies could not reveal a beneficial effect of the fractionated daily $\times$ 5 schedule on the efficacy of E7070, as compared to the single dose regimen.

Mature results of the ongoing phase II studies with E7070 should reveal its actual activity in cancer patients, as well as the optimal treatment schedule. In addition to the studies evaluating E7070 as a single agent, combinations with other, licensed, chemotherapeutics are being investigated which will hopefully lead to promising effective combinations.[20,30]

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