be separated into pure enantiomers, one of which is less potent on calcium channels and the cardiovascular system [3–5], a novel class of antitumour drugs might emerge.

The idea for this new approach originated in our finding that the calcium channel blocker niguldipine is also a potent calmodulin antagonist [6]. Moreover, niguldipine and its optical antipode B859–35 were equipotent in inhibiting the calmodulin-dependent enzymes phosphodiesterase (concentration producing 50% inhibition [IC_{50}] 0.5 μmol), calcium-transporting ATPase and myosin light-chain kinase (for methods see reference [7]). Studies of the fluorescent probes 9-anthroylcholine bromide and 2-*p*-toluidinylnaphthalene-6-sulphonic acid [8] revealed that binding of niguldipine and B859-35 to calmodulin occurred, which provides the molecular basis for inhibition of calmodulin-dependent cellular functions. Both B859-35 and niguldipine exhibited identical binding characteristics when interacting with calmodulin, which is consistent with the observed inhibition of calmodulin-dependent enzymes.

There has been much debate that treatment of tumours may be a therapeutical implication for calmodulin antagonists [9]. This hypothesis and the discovery of the calmodulin antagonistic properties of B859-35 initiated our research into the effects of B859-35 on proliferation of the following human cell lines: ZR-75-1 and MCF-7 (mammary tumours), A-549 (lung tumour), Molt-4 and HL-60 (leukaemia) and FL (amnion cell line derived from normal tissue). Growth of tumour cells measured after incubation for 6 days in Richter's IMEM-ZO and 2% fetal calf serum with the respective drug was determined by total DNA content [10]. Our experiments showed that B859-35, less potent on calcium-channels than its optical antipode, had the same potency of inhibition as niguldipine for a given tumour cell line. This finding is also valid for the enantiomers of isradipine. However, the anti-proliferative potency of B859-35 (IC_{50} 0.3 μmol) on the mammary tumour cell line ZR-75-1 is about 100 times greater than that of isradipine. In addition, B859-35 showed selectivity for the investigated cell line and the following order of decreasing potency was assessed: ZR-75-1 > MCF-7 > A-549 > FL. Significantly, no effect has been observed on Molt-4 and HL-60 in the concentration range 0.01–10 μmol. Neither isradipine (IC_{50} 25 μmol), diltiazem (IC_{50} 30 μmol) nor nitrendipine (IC_{50} 22 μmol) showed selectivity for any of the cell lines, as is also the case for the unspecific antineoplastic drug doxorubicin.

The high potency of B859-35 to suppress tumour cell growth in contrast to its low toxicity, due to low affinity for calcium channels, favours its potential use in the treatment of tumours. Calmodulin antagonism may be involved in inhibition of cell proliferation since both effects showed no stereospecificity for the optical antipodes B859-35 and niguldipine and occurred in the same concentration range (0.3–0.5 μmol). The results argue against the possibility that calcium channel blockade might be the rationale for arrest of tumour cell growth.

1. Yanovich S, Preston L. Effects of verapamil on daunomycin cellular retention and cytotoxicity in P388 leukemic cells. *Cancer Res* 1984, **44**, 1743–1747.
2. Godfraind T. Classification of calcium antagonists. *Am J Cardiol* 1987, **59**, 11B–23B.
3. Hof RP, Hof A, Ruegg UT, Cook NS, Vogel A. Stereoselectivity at the calcium channel: different profiles of hemodynamic activity of the enantiomers of the dihydropyridine derivative PN200-110. *J Cardiovasc Pharmacol* 1986, **8**, 221–228.
4. Boer R, Grasseger A, Schudt C, Glossmann H. (+)-Niguldipine binds with high affinity to Ca^{2+} channels and to a subtype of α_1-adrenoceptors. *Eur J Pharmacol* 1989, **172**, 131–146.
5. Sanders KH, Boer R, Eltze M, Galvan M. Differential vascular and cardiac effects of the enantiomers of three dihydropyridine calcium antagonists, niguldipine, nitrendipine and felodipine. *Arch Pharmacol* 1988, Suppl 338, R36.
6. Drozd MPR, Gietzen K. Effects of the novel dihydropyridine derivative niguldipine on the cytoplasmic free calcium concentration of mouse thymocytes. *Biochem Pharmacol* 1990, **40**, 955–959.
7. Gietzen K. Comparison of the calmodulin antagonists compound 48/80 and calmidazolium. *Biochem J* 1983, **216**, 611–616.
8. Lugnier C, Follenius A, Gerard D, Stoclet JC. Bepridil and flunarizine as calmodulin inhibitors. *Eur J Pharmacol* 1984, **98**, 157–158.
9. Gietzen K. Pharmacological regulation of the activity of calmodulin. In: Bader H, Gietzen K, Rosenthal J, Rüdel R, Wolf HU, eds. *Intracellular Calcium Regulation*. Manchester, University Press, 1986, 405–423.
10. Burton DA. Studies of conditions and mechanism of the diphenylamine reaction for the colorimetric determination of deoxyribonucleic acid. *Biochem J* 1956, **62**, 315–323.

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Amonafide in Metastatic Colorectal Carcinoma

Werner Scheithauer, Gabriela Kornek, Karin Haider and Dieter Depisch

AMONAFIDE (nafidimide, NSC 308847) is a new imide derivative of naphthalic acid with DNA intercalative properties [1]. Preclinical data in murine leukaemia and solid tumour models suggested significant activity [2] that, with a reversible toxicity profile, encouraged evaluation of amonafide in clinical trials. Reasonable tolerance with myelosuppression as the dose-limiting toxicity was found in phase I/II trials [3–5]. Antitumour effects were observed in breast [4], non-small cell lung [3] and prostate cancer [3, 5]. We have investigated the antitumour activity of amonafide in patients with advanced metastatic colorectal cancer.

Patients with progressive, histologically confirmed metastatic colorectal cancer, with bidimensionally measurable disease and with an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or under were eligible. Pretreatment laboratory values had to indicate adequate bone marrow (white cells 4000/μl or higher, platelets 100 000/μl or higher), renal (creatinine 1.5 mg/dl or less, or creatinine clearance 75 ml/min or less) and hepatic function (bilirubin 1.5 mg/dl or less, aspartate aminotransferase twice normal or less). All patients gave informed consent.

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Amonafide (Knoll, Ludwigshafen, West Germany) was given in a starting dose of 800 mg/m^2 over 3 h through a free-flowing intravenous line by infusion pump. The schedule was a single dose every 28 days. A 100 mg/m^2 dose escalation was allowed at the investigator's discretion when myelotoxocity was absent or minimal.

14 patients entered the study (Table 1), all were evaluable for response and toxicity. 4 patients showed no change in disease status with a median duration of 16 weeks (range 12–20). The other 10 patients had progressive disease (95% CI 0–23%). All previously untreated patients, except 1 with tumour-related, rapidly deteriorating liver function, were crossed over to 5-FU/leucovorin with or without cisplatin chemotherapy. Of these 2 responded, 2 did not respond, and 3 had stable disease. At the time of analysis, 8 of the patients had died. The median survival time was 24 weeks (8 to over 41).

When amonafide was administered at 800 mg/m^2, 29 of the 31 treatment cycles were assessable for haematological toxicity. Granulocytopenia with neutrophil counts below 1000/μl was observed in only 1 patient. The median nadir neutrophil count was 3527/μl (972–10 665). The median platelet count was 252 000/μl (81 000–568 000). In 5 patients the dose was escalated to 900 mg/m^2. 2 had WHO grade 3 granulocytopenia. The median nadir granulocyte and platelet counts at this dose level (8 out of 8 assessable treatment cycles) were 1834/μl (528–6794) and 236 000/μl (71 000–439 000). Leukopenia was more frequent and more severe in patients who had received previous chemotherapy than in those who had not, although there was no clear correlation between the degree of myelosuppression and the extent of previous anti-cancer therapy. Non-haematological side effects were generally mild and included nausea/vomiting in 5 patients, diarrhoea in 2, local irritation at the injection site in 1, and reversible acute toxicity during the drug infusion such as headache, dizziness, diaphoresis and tinnitus in 7 patients. These symptoms were promptly ameliorated by an increase in the duration of infusion and/or paracetamol.

Amonafide in the dose and schedule chosen was not active against colorectal cancer. In retrospect, the modest degree of myelosuppression observed in this study indicated that a higher (ie, 900 mg/m^2) starting dose can be safely administered in previously untreated subjects. We cannot exclude that such an approach in all our patients or use of a more intensive schedule [5] might have resulted in a different outcome. However, the short duration of stable disease in 4 cases and rapid tumour progress in the remaining patients indicated that a significant therapeutic value of amonafide in colorectal cancer is unlikely. The occurrence of objective responses in patients crossed over to 5-FU/leucovorin chemotherapy supported the safety and feasibility of the approach of using new agents in previously untreated colorectal cancer.

Table 1. Patients' characteristics

	Number
Median age (range 45–72)	60
M/F	7/7
ECOG performance status	
0	4
1	7
2	3
Previous chemotherapy	
5-FU/leucovorin ± cisplatin	6
None	8
Number of metastatic sites	
1	5
≥ 2	9
Site of metastases	
Liver	7
Lung	6
Abdominal/pelvic mass	6
Other	5

5-FU = 5-fluorouracil.

1. Brana MF, Castellano JM, Roldan CM. Synthesis and mode(s) of action of a new series of imide derivatives of 3-nitro-1,8-naphthalic acid. *Cancer Chemother Pharmacol* 1980, **4**, 61–66.
2. National Cancer Institute Clinical Brochure. *Amonafide (Benzisoquinolinedione) NSC* 308847. 1984, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.
3. Saez R, Craig JB, Kuhn JG, *et al.* Phase I clinical investigation of amonafide. *J Clin Oncol* 1989, **7**, 1351–1358.
4. Costanza ME, Korzun AH, Henderson IC, Rice MA, Wood WC, Norton L. Amonafide: An active agent in metastatic breast cancer (CALGB 8642). *Proc Am Soc Clin Oncol* 1990, **9**, 31 (abstr).
5. Craig J, Crawford E. Phase II trial of amonafide in advanced prostate cancer: A Southwest Oncology Group study. *Proc Am Soc Clin Oncol* 1989, **8**, 147 (abstr).

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Transient Proteinuria during Interleukin-2 Therapy

Sylvie Bastuji-Garin, Olivier Chosidow, Philippe Lang, Jean-Claude Roujeau and Jean Revuz

IMMUNOTHERAPY WITH recombinant interleukin-2 (rIL-2) may result in regression of metastatic malignant melanoma [1]. However, toxic effects have been frequently found [2]: influenza-like symptoms, nausea, diarrhoea, erythema rash, hepatic dysfunction, haematological toxicity, oliguria, weight gain and sometimes mild reversible nephrotoxicity (i.e. increased levels of creatinine and urea nitrogen). A rIL-2 associated nephrotic syndrome has been recently described in a patient treated for malignant haemangioepithelioma [3]. We report a patient with nephrotic syndrome proteinuria occurring during rIL-2 therapy.

A 41-year-old woman was initially treated with rIL-2 for a metastatic melanoma. In our regimen, rIL-2 was given for three courses of 5, 4 and 3 consecutive days. During courses, rIL-2 (Roussel-Uclaf) was continuously infused at a daily dose of

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