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# Phase I trial of fluorouracil modulation by N-phosphonacetyl-L-aspartate and 6-methylmercaptopurine ribonucleoside

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**Abstract** Inhibition of pyrimidine and purine synthesis has been demonstrated to potentiate 5-fluorouracil (5-FU) activity in preclinical models. Low-dose phosphonacetyl-L-aspartate (PALA) potentiates the incorporation of 5-FU into RNA, without detectably increasing its toxicity. 6-Methylmercaptopurine riboside (MMPR) results in inhibition of purine biosynthesis with elevation of phosphoribosyl pyrophosphate (PRPP), which in turn is believed to increase the phosphorylation and intracellular retention of 5-FU. We conducted a phase I clinical trial to determine the maximum tolerated dose of 5-FU in combination with low-dose PALA and a biochemically-optimized dose of MMPR. The regimen consisted of PALA 250 mg/m$^2$ given on day 1, followed 24 h later by MMPR 150 mg/m$^2$, and escalating doses of 5-FU from 1625 to 2600 mg/m$^2$ by 24 h continuous infusion. This regimen was repeated weekly. A group of 29 patients with a diagnosis of malignant solid tumor were entered; their median performance status was 1. The dose-limiting toxicity was mucositis, while other gastrointestinal toxicity was minimal. Two patients also experienced ischemic chest pain during the 5-FU infusion. The maximum tolerated dose of 5-FU in this combination was 2600 mg/m$^2$. Several responses were observed including a complete remission in a previously treated breast cancer patient and two partial responses in breast and colon cancer. MMPR pharmacokinetics were obtained from urine analyses in 21 patients on this trial; there was no correlation between the pharmacokinetics of MMPR and the toxicity observed. This regimen was well tolerated and phase II trials are warranted using PALA 250 mg/m$^2$, MMPR 150 mg/m$^2$, and 5-FU 2300 mg/m$^2$ by continuous infusion over 24 h.

**Key words** 5-Fluorouracil · Phosphonacetyl-L-aspartate · b-Methylmercaptopurine riboside · Combination treatment · Pharmacokinetics

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## Introduction

5-Fluorouracil (5-FU) has been widely used in the treatment of solid tumors for over 30 years, with limited but reproducible efficacy. Its low therapeutic index and poor selectivity for tumor over normal tissue has stimulated research towards a strategy aimed at modulation of its intracellular action [1,2]. 5-FU has several mechanisms of action that may contribute to its cytotoxicity, including inhibition of RNA processing by incorporation of fluorouridine triphosphate, and inhibition of thymidylate synthase which results in inhibition of DNA synthesis, and indirectly, fragmentation of DNA. Alteration of the intracellular biochemical microenvironment has the potential to increase the efficacy of 5-FU, and so to enhance its antitumor effect and its selectivity.

Phosphonacetyl-L-aspartate (PALA) is a transition state analog inhibitor of the enzyme aspartate transcarbamylase (ACTase), which catalyzes an early step in *de novo* pyrimidine synthesis. PALA depletes pyrimidine nucleotide pools in susceptible cell lines. The rationale of combining PALA with 5-FU is based upon observations demonstrating increased 5-FU incorporation into tumor cell RNA in murine and human tumor cell lines [3,4]. Casper et al. demonstrated that a low, nontoxic dose of PALA was sufficient to inhibit whole body pyrimidine synthesis without clinical toxicity. In a phase I trial, with this regimen of low-dose PALA, with weekly infusional 5-FU, high doses of 5-FU were

well tolerated and several responses were observed [5]. In a phase II trial in colorectal cancer at Fox Chase Cancer Center [6], 3 complete remissions and 13 partial remissions were observed among 37 evaluable patients, for a total response of 43%. The median survival of 17 months was comparable to that of other modulation regimens.

Intracellular retention of 5-FU is accomplished by phosphorylation by orotate phosphoribosyl transferase, the extent of which depends on the availability of phosphoribosylpyrophosphate (PRPP). Since most of the supply of PRPP is destined for purine synthesis, inhibition of purine synthetic pathways makes available greater amounts of the high energy cofactor. 6-Methylmercaptopurine riboside (MMPR) is a thiopurine which inhibits purine biosynthesis at the level of phosphoribosyl aminotransferase. MMPR is phosphorylated by adenosine kinase to MMPR-P, the monophosphate, which inhibits phosphoribosyl aminotransferase [7–9]. The enzyme catalyzes the first committed reaction in purine biosynthesis resulting in lowering of adenine and guanine nucleotide pools, and a marked elevation in PRPP. Both in vitro and in vivo studies have demonstrated that MMPR may increase PRPP levels by as much as 15-fold, resulting in increased phosphorylation and intracellular retention of 5-FU. This effect enhances 5-FU incorporation into RNA [10,11].

While clinical studies of MMPR have been performed on both weekly [12] and 5-day dose schedules [13], we recently reported the optimal dose and timing of MMPR administration [14]. An MMPR dose of 150 mg/m$^2$ provided a significant increase in the tumor PRPP level lasting 12–24 h in more than 50% of the patients from whom sequential tumor biopsy specimens were studied. The time-course indicated that MMPR should be administered at the beginning of the 24–h infusion of 5-FU. In addition, this biochemically active dose displayed less toxicity than the 225 mg/m$^2$ MMPR dose, thus allowing a greater dose of the active drug, 5-FU [14].

The objectives of the present clinical trial were to determine the maximum tolerated dose of 5-FU in the combination of PALA 250 mg/m$^2$ followed after 24 h by MMPR 150 mg/m$^2$ and 5-FU, and to describe the pharmacokinetics of MMPR and 5-FU in this combination.

## Materials and methods

Patients eligible for this study had a histological diagnosis of malignant solid tumor and had exhausted the conventional therapeutic options for their disease, or had a disease for which no established treatment exists. They were required to have recovered from all toxic effects of prior treatment and to be older than 18 years of age, with Eastern Cooperative Oncology Group performance status of 0–2. Patients had an adequate bone marrow function (WBC $> 4000/mm^3$ and platelets $> 100{,}000/mm^3$), adequate liver function (serum bilirubin $< 1.5$ mg/dl) and adequate renal function (creatinine $< 1.5$ mg/dl). All patients gave written informed consent in accordance with federal, state and institutional guidelines.

Prior to therapy a medical history, physical examination, complete blood count, biochemical profile, urinalysis, chest X-radiograph and appropriate scans were performed. Patients were monitored with complete blood counts weekly, and with monthly clinical examination and biochemical profile. Doses were reduced if necessary. Based on the level of toxicity (grade 1 gastrointestinal toxicity or grade 2 toxicity of any other type) on the day of the treatment, the week's dose was held. Following resolution of symptoms the therapy was reinstituted at the lower dose. The dose modification for toxicity was based on the worst level experienced. For grade 1 and 2 toxicity, a 25% dose reduction was used. For grade 3 and 4 toxicity, a 50% dose reduction was used. If the reduced dose was well tolerated for a minimum of 4 weeks an intermediate dose escalation could be taken. Doses were not escalated within patients. Only the dose of 5-FU was modified; those of PALA and MMPR were unchanged. Results are reported using the Common Toxicity Criteria (Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Md, 1988). Patients with measurable disease were evaluated every 8 weeks and those whose disease had stabilized or reduced in size were continued on therapy. Response criteria were standard [15]. The maximum tolerated dose was defined as a dose of the drug that would produce predictable and reversible toxicity, and would not be incapacitating nor interfere with the patient's wellbeing and general activity.

### Treatment plan

Patients were admitted to Mary S. Schinagl Clinical Studies Unit at Fox Chase Cancer Center for a period of 24 to 48 h surrounding the initial dose of therapy. PALA was supplied by the National Cancer Institute as the disodium salt in 10–ml ampules containing 100 mg/ml in water for injection. The PALA was further diluted in 5% dextrose or 0.9% sodium chloride and administered at 250 mg/m$^2$ as a rapid infusion over 10 min on day 1. The MMPR was supplied by the National Cancer Institute in 15-mg vials of lyophilized drug, MMPR was reconstituted with 0.9% sodium chloride, and administered at 150 mg/m$^2$ by intravenous infusion over 10 min on day 2, immediately preceding the 24-h infusion of 5-FU. The 5-F-U was commercially available in 500 mg/10-ml ampules, and was reconstituted in 500 ml of 0.9% sodium chloride or 5% dextrose. 5-FU was administered as a constant infusion over 24 h. There were three levels of dose escalation of 5-FU: 1625 mg/m$^2$, 2000 mg/m$^2$ and 2600 mg/m$^2$. This regimen was repeated weekly.

### Pharmacokinetic sampling of 5-FU and MMPR.

#### *5-Fluorouracil*

Plasma samples, for the determination of steady-state 5-FU concentrations were taken at 12 and 24 h after the beginning of the 24-h 5-FU infusion. Steady-state 5-FU levels were calculated from the mean of the two determinations.

The analytical method for 5-FU was essentially asdescribed by Stetson et al. [16] and is briefly described below. Plasma samples (1 ml) in 17 $\times$ 100 mm snap-top polypropylene tubes (Baxter, Scientific Products Division, McGraw Park, Ill. ) were prepared for extraction by the addition of 125 $\mu$ l water for the plasma blank, or 25 $\mu$ l of the appropriate 5-FU standard and 100 $\mu$ l of the internal standard, 5-chlorouracil (10 $\mu$g/ml) dissolved in water. Patient samples were prepared by adding 25 $\mu$ l water and 100 $\mu$ l of 5-chlorouracil solution. After briefly vortex-mixing the samples, 2 ml of saturated ammonium sulfate solution and 100 $\mu$ l of 1.0 *M* ammonium phosphate were added and the samples were again

vortex-mixed. This was followed by the addition of 8 ml of ethyl acetate and rocking the samples for 15 min. The upper organic layer was transferred to a glass conical tube and 400 µl 0.5 *M* potassium hydroxide was added. After vortex-mixing for 15 min, the organic and aqueous layers were separated by centrifugation at 100 *g* for 10 min. The upper organic layer was aspirated and discarded; 20 µ l of the aqueous layer was taken for analysis by HPLC.

The chromatographic system consisted of a Hewlett-Packard (Palo Alto, Calif.) HP-1090 Series A liquid chromatograph equipped with an autoinjector/autosampler and an HP1040A diode-array detector. The column effluent was monitored at 254 nm. The chromatograph was operated with an HP-85B personal computer, and the data were processed with a DPU multichannel integrator. Chromatography was performed on a Spherisorb ODS-2 C18 reverse-phase analytical column, 5 µm, 250 × 4.6 mm ID (Alltech Associates, Deerfield, Ill.) preceded by a 15 × 3.2 mm, 7-µm Newguard C18 guard column (Applied Biosystems, San Jose, Calif.). The isocratic mobile phase consisted of a 95% 0.05 *M* ammonium phosphate, pH 6.8, and 5% methanol (v/v) at a flow rate of 1 ml/min. The standard curve was plotted as the peak-height ratio of 5-FU to 5-chlorouracil versus concentration of 5-FU. The linear regression was calculated by the method of least squares and was unweighted.

*MMPR*

For the determination of MMPR pharmacokinetics, serial blood and urine samples were obtained following the 10-min MMPR infusion. Plasma samples were collected at time 0 (predose), and at frequent intervals. Urine samples were collected at 4-h intervals between 0 and 24 h after the start of the MMPR infusion.

Plasma and urine samples were analyzed for the concentration of MMPR by a reverse-phase HPLC method developed in our laboratory [17]. Plasma samples were prepared for analysis by the addition of internal standard (6-dimethylaminopurine 9-riboside) followed by extraction using disposable C18 cartridges. Urine samples (1 ml) were prepared for analysis by filtration through a Millipore centrifuge filter (0.22 µm Durapore). The HPLC injector volume was 25 µl.

Steady-state plasma 5-FU concentration ($C_{ss}$) was determined by averaging the plasma concentrations at 12 and 24 h from the start of the 5-FU infusion. 5-FU total body clearance ($Cl_{tot}$) was calculated from infusion rate/$C_{ss}$ [18].

An estimate of plasma MMPR elimination was obtained from urine data by plotting the amount of drug remaining to be excreted against time on semilogarithmic paper. The amount remaining to be excreted (ARE) was calculated from the difference between the total amount excreted from time 0 to infinity ($A_\infty$) and the amount excreted up to the end of the time interval ($Ae_t$) as previously described [19].

## Results

A group of 29 patients were entered at the three dose levels of 5-FU; their demographic characteristics are summarized in Table 1. The most common diagnoses were colorectal and breast cancer. The patients were minimally symptomatic from their tumor; the median performance status was 1. The majority had been treated with previous chemotherapy and/or radiation. All patients in this part of the study received a constant dose of 150 mg/m$^2$ MMPR based on the biochemical measurements in tumor tissue previously reported. This dose represents the optimal modulatory dose of MMPR in the combination [14].

**Table 1** Patient characteristics

| | |
|---|---|
| Patients | |
| No. entered | 29 |
| Male/female | 14/15 |
| Median age (range) (years) | 55 (32–82) |
| ECOG performance status | |
| 0 | 6 |
| 1 | 23 |
| Prior treatment | |
| Chemotherapy | 11 |
| Radiation | 3 |
| Chemotherapy/radiation | 10 |
| Biological-chemotherapy | 1 |
| None | 4 |
| Primary sites | |
| Colorectal | 13 |
| Breast | 5 |
| Lung | 3 |
| Pancreas | 3 |
| Unknown | 3 |
| Endometrial | 1 |
| Cholangiocarcinoma | 1 |

Three dose levels of 5-FU were studied: 1625 mg/m$^2$, 2000 mg/m$^2$ and 2600 mg/m$^2$. We did not escalate above 2600 mg/m$^2$, since higher doses had proven prohibitively toxic in a previous study, even without MMPR [6]. The clinical toxicities experienced by patients are shown in Table 2. Gastrointestinal toxicity, primarily mucositis, was dose limiting at 5-FU dose of 2600 mg/m$^2$. A single episode of grade II diarrhoea was observed at the 5-FU dose of 2600 mg/m$^2$. Diarrhoea was not dose limiting in this combination.

The gastrointestinal toxicities were easily controlled by the initial dose reduction of 5-FU by 25%; most patients were then able to reescalate to the intermediate dose of 2300 mg/m$^2$ per week for further cycles of this combination. Based on these findings, 2300 mg/m$^2$ of 5-FU by continuous infusion over 24 h was established as a recommended phase II dose in combination with 250 mg/m$^2$ PALA and at 150 mg/m$^2$ MMPR. Hand-foot syndrome and grade II leukopenia were observed in four patients at the higher dose levels; they did not require any dose modification.

The most important toxicity with this regimen was cardiac. One patient each at the 2000- and 2600-mg/m$^2$ 5-FU dose level complained of chest pain during the infusion of 5-FU. These patients had no prior history of ischemic heart disease. These complaints were associated with electrocardiographic evidence of myocardial injury. The symptoms and electrocardiographic changes resolved with interruption of the 5-FU infusion and administration of calcium channel blockers and nitrates. A third patient complained of supraventricular tachycardia, but this did not seem to be related to the chemotherapy regimen and there was no dose modification of the 5-FU.

**Table 2** Clinical toxic effects of grade 2 or greater

| 5-Fu dose (mg m$^2$) | Total number of patients | Number of patients with grade 2 or greater toxity | | | | | |
|---|---|---|---|---|---|---|---|
| | | Hand/foot | Mucositis | Diarrhoea | Leukopenia | Neurologic | Cardiac |
| 1625 | 6 | – | 1 | – | – | – | – |
| 2000 | 7 | – | 2 | 0 | 1 | 1 | 1 |
| 2600 | 16 | 4 | 4 | 1 | 1 | – | 1 |

**Table 3** Pharmacokinetics of MMPR at 150 mg/m$^2$

| 5-FU dose (mg/m$^2$) | Number of patients | $K_e$ ($h^{-1}$) | | $t_{1/2}$ (h) | | Dose excreted (%) | |
|---|---|---|---|---|---|---|---|
| | | Mean | (SD) | Mean | (Range) | Mean | (Range) |
| 1625 | 4 | 0.1143 | 0.0413 | 6.85 | 4.5–10.9 | 2.2 | 1.1–3.1 |
| 2000 | 6 | 0.1495 | 0.0633 | 6.62 | 3.3–18.8 | 1.9 | 0.6–4.4 |
| 2600 | 11 | 0.1333 | 0.0318 | 5.427 | 3.4–7.2 | 3.1 | 1.3–8.5 |
| 1300 | 5 | 0.1374 | 0.0254 | 5.16 | 3.9–6 | 2.0 | 1.5–2.7 |

**Table 4** Pharmacokinetics of 5-FU and MMPR at the dose level of 2600 mg/m$^2$ 5-FU

| Patient number | 5-FU | | MMPR | | |
|---|---|---|---|---|---|
| | $C_{ss}$ (μg/ml) | $Cl_{tot}$ (ml/min/m$^2$) | Ke ($h^{-1}$) | $t_{1/2}$ (h) | Dose excreted (%) |
| 1 | 1.373 | 1331 | 0.1250 | 5.5 | 2.1 |
| 2 | 1.315 | 1403 | 0.1402 | 4.9 | 4.2 |
| 3 | 0.624 | 2908 | 0.1171 | 5.9 | 1.4 |
| 4 | 1.420 | 1289 | 0.1184 | 5.8 | 3.0 |
| 5 | 1.270 | 1422 | 0.1693 | 4.1 | 2.9 |
| 6 | 1.156 | 1572 | 0.1397 | 5.0 | 1.3 |
| 7[a] | 0.429 | 4170 | – | – | – |

[a] There were no MMPR pharmacokinetic data for this patient

Several responses were observed in this study. The majority of patients had stabilization of their disease for a minimum of 8 week. Objective responses were identified in patients with breast and colorectal cancer (response rate 10.3%). Of the five patients with breast cancer, two responded. One patient had complete remission lasting 71 weeks and one had a partial response. A patient with colorectal cancer had a partial remission. All of these responses were observed at the 2600 mg/m$^2$ dose level. There were also three minor responses observed in two patients with colorectal cancer and one with breast cancer. All responders had previously been treated with chemotherapy and/or radiotherapy.

MMPR pharmacokinetics were derived from urine analyses in 21 patients. This indirect method of pharmacokinetic analysis was required because of the accumulation of MMPR and its 5-monophosphate in red cells [20], with the result that the slightest hemolysis would produce wide swings in measured plasma MMPR levels. The pharmacokinetic results are summarized in Table 3. Less than 9% of the dose of MMPR was recovered intact in the urine in the 24-h period following MMPR dosing. Estimates of plasma MMPR half-life, obtained from ARE plots of urine data, ranged from 3.3 to 18.8 h and did not vary with dose. There was no correlation between the pharmacokinetic parameters derived for MMPR and the toxicity observed.

The pharmacokinetic parameters, steady-state concentration, and total exposure to 5-FU were studied in seven patients at the 2600 mg/m$^2$ dose level, and these data are summarized in Table 4 along with the MMPR pharmacokinetic data obtained from these patients. Total body clearance of 5-FU was 1422 ml/min per m$^2$ in the only patient reported with a grade 2 diarrhoea. As described in a previous pharmacodynamic model, diarrhoea was more likely to occur if the $Cl_{tot}$ 5-FU was less than 1400 ml/min per m$^2$ [21].

## Discussion

In single-institution trials, regimens designed to modulate the activity of 5-FU through biochemical manipulations continue to produce high response rates and prolonged survival. The only intervention that has been supported by large-scale trials to date is that of the addition of leucovorin to enhance 5-FU thymidylate synthase inhibition [22–24].

We have performed a series of trials based on the preclinical in vivo studies of Martin et al. [25]. It is hypothesized that RNA incorporation is also an important mechanism of 5-FU action, and that this incorporation may be enhanced by manipulation of nucleotide pathways. The current study was designed to integrate the purine synthesis inhibitor MMPR into a regimen of PALA and 5-FU which had previously yielded substantial response rates in a phase II trial [6]. In the initial phase of the study, we set out to determine the optimal dose and schedule of MMPR for use in the combination [14]. As previously described, on biochemical and clinical grounds, the recommended dose of MMPR was 150 mg/m$^2$, to be administered as a bolus at the beginning of the 24-h infusion of 5-FU. In this part of the study, we sought to determine the recommended phase II dose of 5-FU in the combination, to allow broader disease-specific testing.

The results revealed that at 2600 mg/m$^2$ of 5-FU, moderate stomatitis required dose reduction in some patients. Since this dose is that which is maximally tolerated even in the absence of MMPR, further dose escalation was not undertaken. 5-FU by 24-h continuous infusion weekly has been shown to be tolerated in previous studies at doses ranging from 2600 to 3400 mg/m$^2$ [26]. More recently Haas et al. [27] reported severe dose-limiting toxicity at doses of 5-FU ranging from 2800 to 3250 mg/m$^2$ administered by 24-h continuous infusion. Analysis of the median dose tolerated by patients at the 2600 mg/m$^2$ level in this trial, revealed a recommended phase II 5-FU dose of 2300 mg/m$^2$ in the combination. Stomatitis has not previously been a pronounced toxicity of the PALA/5-FU combination; it is possible that the addition of MMRP resulted in more stomatitis than diarrhoea. It is likely, however, that phase II trials will identify both forms of gastrointestinal toxicity as dose limiting.

Of some concern in this study was the occurrence of chest pain in two patients. The symptoms were suggestive of coronary artery spasm, and electrocardiographic changes supported this conclusion. While of no consequence in the patients treated in this study, this is an important side effect that may be more serious in patients with preexisting myocardial disease. The incidence of cardiac toxicity observed in this trial in 2 of 29 patients or (7%) is in the range of the expected toxicity observed in patients treated with high-dose continuous infusion of 5-FU [28].

The responses observed in this trial are of interest. Those in colorectal cancer are not perhaps surprising: PALA/5-FU is an active combination in this disease, and some response would be expected. Those in breast cancer, however, were of interest. The role of 5-FU in metastatic breast cancer is unknown, though it forms a component of the most commonly used combinations. We recently performed a phase II trial of PALA/5-FU in refractory breast cancer: a very low level of activity was observed (R. Scher, personal communication). The observation of responses with MMPR in this combination may prompt a re-evaluation of the regimen in this disease.

Previous studies have not adequately characterized the pharmacokinetics of MMPR and the variability of our plasma data also precluded a reliable analysis. Therefore, we sought to infer plasma pharmacokinetic behavior from a consideration of urinary elimination over time. Though urinary elimination of the parent compound represents a minor fraction of MMPR disposition (range 0.6–8.5% of administered dose), the plots of ARE versus time were reproducibly derived. From a sample of 26 patients, the elimination constant showed relatively little variability. The calculated half-life was about 6 h. A previous pharmacokinetic study in cancer patients, using $^{35}$S-MMPR, revealed an average half-life for plasma radioactivity of 5 days [19]. The same study showed that the majority of radioactivity in RBCs was in the form of MMPR-P and that the RBC to plasma ratio of radioactivity was 40:1, showing that RBCs concentrate MMPR in the form of MMPR-P. In addition, about 50% of the radioactivity was excreted in the urine of these patients in 5 days, and $> 90\%$ was in the form of MMPR-P. These findings (a 5-day plasma half-life and 50% urinary excretion in 5 days) suggest that all of the administered radioactivity is excreted in urine, with the majority of the dose eliminated in the form of MMPR-P. In our study, measurements of plasma MMPR levels were inconclusive. We did notice that in plasma samples that came from hemolyzed blood samples the measured MMPR levels were wildly variable, suggesting that liberated MMPR-P was dephosphorylated, presumably due to plasma phosphatases.

In an attempt to derive some information about the plasma pharmacokinetics of MMPR, urinary excretion plots of ARE versus time were constructed. From these plots, an estimate of plasma MMPR half-life of about 6 h was calculated with about 3% of the MMPR dose excreted unchanged in the urine over 24 h. This finding is entirely consistent with the findings of Loo et al [20] who showed that over the first day most of the radioactivity is excreted as MMPR, and after the initial 24 h the drug is excreted in the urine in the form of MMPR-P. Thus, the half-life of MMPR calculated from urine data is probably representative of the terminal half-life of MMPR in plasma. Unfortunately, very little other information on the pharmacokinetics

of MMPR, like volume of distribution or clearance, can be deduced with this method.

This regimen of PALA/MMPR/5-FU represents the sequential construction of a modulation regimen targeted to the RNA mechanism of 5-FU cytotoxicity, though an action at thymidylate synthase is not excluded. The role of PALA in this combination is being tested in phase II and III cooperative group trials. The further development of the regimen in colorectal cancer and possibly in breast cancer is indicated.

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