From 5 Fluorouracil to the New Oral Fluoropyrimidines: A Brief Overview of Four Decades of Clinical Investigations

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5-Fluorouracil has been in continuous clinical use for over 40 years. The drug was synthesized as an anti-cancer agent in 1957 by Dr. Charles Heidelberger and associates at the University of Wisconsin in Madison [1]. 5FU is an analog of uracil, an anti-metabolite that exerts its anti-tumor activity on cells during the S-phase of the cell cycle. Intracellularly, 5FU is converted into several nucleotides, with a number of metabolic routes; in one, it transforms into fluorodeoxyuridine monophosphate (FDUMP), which binds to the target enzyme, thymidylate synthase, thus inhibiting the synthesis of DNA. Alternatively, 5FU may convert into fluorouridine triphosphate, which incorporates into RNA, making this molecule ineffective [2]. 5FU has shown anti-tumor activity in a wide range of solid tumors, mainly breast, colorectal, gastric and head and neck primaries.

There have been several milestones along the developmental history of 5FU that should be emphasized. One, of course, is our ability to biomodulate the activity of 5FU, defining biomodulation as the concomitant administration of 5FU with another drug, not necessarily a cytotoxic agent, in order to improve the therapeutic index of 5FU. In the last 20 years or so, many potential biomodulators have been used, including thymidine, allopurinol, methotrexate, PALA, levamisole and others. Leucovorin or folinic acid is, however, the prototype biomodulator [3]. LCV creates a stable, irreversible ternary complex with both FDUMP and thymidylate synthase, and thus enhances the anti-tumor activity of 5FU. In a pioneer clinical trial carried out by a group of French investigators and published in 1982, a group of 30 patients suffering from advanced colorectal cancer were treated with 5FU as a rapid IV infusion, 370 mg/m^2/day for 5 days every 28 days concomitantly with LCV 200 mg/m^2 per dose. Sixteen of the patients had not been exposed to prior chemotherapy and their response rate to 5FU and leucovorin reached 56%. Equally impressive was the response rate among 14 patients who had been previously treated with 5FU, reaching 21% [4].

Many Phase II clinical trials followed this work and were able to confirm higher than expected response rates to 5FU alone when LCV was added. The next methodological step was to carry out Phase III trials comparing the combination of 5FU and LCV with 5FU alone. Several trials were conducted, the majority of which showed higher response rates to the combination; nonetheless, these trials failed to show an advantage in median overall survival for the 5FU and LCV treated patients [5]. Results of a meta-analysis including close to 1,400 patients with advanced colorectal cancer who were treated with 5FU with or without the addition of LCV were published in 1992. The overall response rate for the subgroup of patients who received 5FU and LCV reached 23% as compared to a response rate of only 11% with 5FU alone ($P = < 10^{-7}$); however, again, there was no difference in median survival between both groups [6]. Only when patients were stratified by the presence or absence of measurable disease was a small, non-significant advantage obtained for patients without measurable disease treated with 5FU and the biomodulator, probably indicating that 5FU and LCV are more effective in colorectal cancer patients with less bulky disease.

Along its four decades of clinical development, 5FU has been given in a myriad of doses and schedules of administration. These can be grossly arranged into two major subgroups: the first, where 5FU is given as a rapid intravenous infusion – the "push" category; and the second where 5FU is given as a prolonged intravenous infusion – the "continuous infusion" category. The definition of a "continuous infusion" is very broad indeed and varies from 24 hours to up to 10 weeks or more [7]. As will be noted further on, continuous infusion schedules today tend to range mostly between 24 and 48 to 72 hours [8,9].

The initial question the clinician should ask is whether there is any therapeutic advantage of one category over its counter-part. In an early comparative trial, Lockich et al. [10] treated a cohort of advanced colorectal cancer patients with either continuous 5FU at a dose of 300 mg/m^2/day for 70 consecutive days, or with rapidly administered 5FU, 500 mg/m^2/daily for 5 days every 5 weeks. The response rate favored the former subgroup, 30% vs. 7%. This was confirmed in another trial by Seifert et al. [11], where continuous 5FU was given over 5 consecutive days only. More recently, De Gramont and collaborators [12] compared a "combined" 5FU program, 400

5FU = 5-fluorouracil
LCV = leucovorin

mg/m^2 by IV push followed by 600 mg/m^2 as a 24 hour infusion on days 1 and 2, with leucovorin, every 2 weeks, with a "standard" push schedule of 5FU and leucovorin as given by the Mayo Clinic daily for 5 days every 28 days. The response rate favored the "combined" arm, 32%, as compared to 14% for the push program.

The next question is whether this gain in anti-tumor activity for the protracted infusion programs comes at the expense of an increased toxicity. Paradoxically, the toxicity of 5FU is not increased by its protracted administration. If anything, the pattern of toxicity is changed by this form of administration; while the major, dose-limiting toxicities of push 5FU are hematological (leukopenia) and gastrointestinal (diarrhea), the major adverse effects for the continuous infusion programs include stomatitis, and, prominently, a form of skin toxicity known as the hand-foot syndrome or palmo-plantar erythrodysesthesia. Characterized by an erythematous rash on the extremities, it can progress to desquamation that may be severe, with marked discomfort, pain and functional impairment. This, however, is promptly reversible upon discontinuation of the drug [13]. Hematological toxicity during continuous infusion of 5FU is conspicuously low [13].

One has to take into consideration that in order to administer a continuous infusion program of 5FU, a number of logistical problems need to be resolved. These include the necessity to implant permanent venous access devices, and the use of portable infusion pumps. These, of course, interfere, to a certain degree, with the patient's quality of life. It is mainly for this reason that major efforts have been invested in recent years in developing oral analogs of 5FU that can be given over protracted schedules in order to mimic a continuous intravenous infusion of 5FU while at the same time overcoming the technological issues associated with its administration.

The original oral fluoropyrimidine to enter clinical trials was ftorafur or tegafur, synthesized in the 1960s in the former Soviet Union. Ftorafur has an excellent bioavailability and is, in fact, a prodrug of 5FU; it is converted into 5FU in the liver by the liver microsomes. Unfortunately, extensive clinical tests both in the Soviet Union and in the United States showed only marginal clinical activity even when given intravenously [14].

UFT, or uracil-ftorafur, is a second-generation oral fluoropyrimidine synthesized in Japan in the late 1970s by adding uracil to ftorafur at a molar ratio of 4:1. The mechanism of action of UFT is based upon the fact that uracil is a potent inhibitor of dihydropyrimidine-dehydrogenase, the main catabolic enzyme of 5FU. By inhibiting DPD, more 5FU is available for its anti-tumor activity. Japanese clinicians have built an extensive database on UFT in a wide range of solid tumors, and have shown activity in cancers of the head and neck area, stomach, breast and large bowel, among others. Utilizing a protracted schedule of administration typically lasting 4 weeks with three daily doses, the drug has usually been well tolerated. Gastrointestinal symptoms (nausea, diarrhea) are the most frequently encountered, but only in a minority of patients and of mild to moderate magnitude [15].

Formal early clinical trials with UFT were carried out in the West in the early 1990s. These trials showed a schedule-dependent toxicity to the drug. When it was given at higher doses for 5 days, the dose-limiting toxicity was myelosuppression. For more prolonged schedules with lower daily doses, the most prominent toxicity was diarrhea, and leukopenia was rarely observed [16].

The protracted schedule of administration was selected for further exploration. This included the administration of UFT as a single agent or with low or high doses of orally administered leucovorin. A number of phase II clinical trials clearly demonstrated anti-tumor activity of UFT in colorectal cancer with a compiled mean response rate of over 30% in almost 600 patients participating in those trials [17]. Pazdur and collaborators [18] at the MD Anderson Cancer Center conducted a representative trial in which UFT was given at a dose of 300 mg/m^2 per day in three divided doses 8 hours apart, daily for 28 days, every 5 weeks, together with leucovorin, 150 mg daily, orally as well. The response rate in 38 patients with metastatic colorectal cancer reached 42%.

Interestingly, when these clinical investigators attempted to moderately increase the daily dose of UFT by an approximate 15%, from 300 to 350 mg/m^2 per day, unacceptable toxicity ensued in most patients in the form of severe diarrhea, indicating that UFT may have a narrow safety margin. Special attention should be paid both by the clinician and by a compliant patient to the development of diarrhea. If low grade, a temporal, brief discontinuation of the drug will bring prompt resolution, otherwise diarrhea can become severe and require hospitalization and substantial supportive care.

Preliminary results of an ongoing Phase III clinical trial comparing oral UFT and LCV with a standard Mayo Clinic intravenous 5FU and LCV program were presented recently at the Annual Meeting of the American Society of Clinical Oncology [19]. More than 800 patients with metastatic colorectal cancer entered this trial, and while response rates appear similar for both programs, the oral one has been, to date, better tolerated. UFT and LCV thus represent a total oral program as an alternative to the more traditional intravenous 5FU regimens. In a further step, UFT and LCV are being explored in the adjuvant setting against IV 5FU and LCV by the NSABP group (National Surgical Adjuvant Breast [and colon] Program).

A number of third-generation oral fluoropyrimidines are now undergoing clinical investigation as well. These include the S1 compound, capecitabine and eniluracil, and they are briefly discussed here.

The S1 compound, also synthesized in Japan, includes three different agents: ftorafur, chlorohydroxy dihydropyridine (a potent inhibitor of the catabolic enzyme DPD), and a third substance, oxonic acid, which makes this compound unique

UFT = uracil-ftorafur
DPD = dihydropyrimidine-dehydrogenase

among the oral fluoropyrimidines as it acts by inhibiting the phosphorylation of 5FU in the intestinal mucosa, thereby decreasing the incidence of gastrointestinal toxicity.

Early clinical trials with S1 have been carried out in Japan and in the West. Preliminary results show anti-tumor activity in tumor sites such as breast, head and neck and colon and rectum. Diarrhea has occurred in a proportion of the treated patients [20].

Capecitabine, an oral carbamate derivative, is an interesting and unique compound as it undergoes a 3-step enzymatic activation, the last of which occurs selectively within the tumor tissue itself. The drug passes intact through the bowel and reaches the liver where it is converted first into 5-deoxyfluorocytidine by a carboxylesterase and then to 5-deoxfluorouridine which reaches the tumor where it is transformed into its active form, 5FU, by thymidine phosphorylase. It has been demonstrated *in vitro* that for a wide variety of solid tumors, the intratumoral concentration of thymidine phosphorylase is substantially higher than in the surrounding healthy tissues. *In vivo*, in a clinical setting, a group of 19 patients with colorectal primaries, who were candidates for surgical resection of the primary tumor and/or hepatic metastases, were given capecitabine for 5 to 7 days prior to surgery. When the tumor tissue was analyzed for its thymidine phosphorylase content, the levels both in the primary tumor and in the metastasis were substantially higher than in the surrounding peritumoral tissue [21].

Several schedules of administration were tested in the frame of Phase II clinical trials, where capecitabine was given for 2 weeks in a row every 3 weeks with or without LCV or where it was given continuously for longer periods. All three schedules resulted in similar response rates in patients with advanced colorectal cancer (range 21–24%), and the 2 week schedule of capecitabine alone was selected for further exploration [22].

Recently, two large clinical trials with a similar design were carried out – one in Western Europe and Israel and the other in the Americas. More than 600 patients with metastatic colon cancer were included in these trials where capecitabine was given at a dose of 2,500 mg/m^2/day in two daily doses 12 hours apart for 14 days every 3 weeks, and compared with a Mayo Clinic type of intravenous 5FU and LCV regimen. The response rate to capecitabine was higher, 27% vs. 18%. There was no difference in median survival for both treatment arms, however capecitabine was better tolerated, resulting in a lower hospitalization rate than for patients receiving 5FU and LCV. The main toxicity of capecitabine was the hand and foot syndrome [23].

Capecitabine has been shown to have an emerging role in breast cancer as well. Blum et al. [24] recently built a database for capecitabine in a group of 162 patients with advanced breast cancer previously exposed to anthracyclines and taxanes. The response rate to capecitabine as a single agent reached 19%. The authors were able to identify a subgroup of 42 patients who were considered to have disease resistant to both doxorubicin and paclitaxel and achieved an encouraging 26% response rate while on capecitabine.

Another small series including 42 breast cancer patients previously treated with anthracyclines were randomized to either single-agent capecitabine or paclitaxel. The response rate to the oral fluoropyrimidine was higher than to the taxane (36 vs. 26%), with an identical median duration of response of 10 months for both [25].

O'Shaughnessy [26] recently reported a clinical trial carried out in postmenopausal patients, 55 years and older, with previously untreated metastatic breast cancer who were randomized to either single-agent capecitabine or to a standard CMF program (cyclophosphamide, methotrexate, 5FU). Both the response rate and the median duration of response slightly favored the capecitabine arm. Given the demonstrated anti-tumor activity of capecitabine as a single agent and in view of its favorable toxicity pattern, a number of clinical trials are now underway combining capecitabine with other cytotoxic agents such as paclitaxel and docetaxel, irinotecan, etc.

Finally, the last compound to be discussed is eniluracil, the most potent inhibitor of DPD in clinical use. In fact, a standard dose of 5FU when given with eniluracil will result in lethal toxicity. Eniluracil improves the oral bioavailability of 5FU; it prolongs its plasma half-life, and increases its area under the curve. In Phase I clinical trials, the maximal tolerated dose of oral 5FU, when given with eniluracil, reached 1.35 mg/m^2 per dose, given twice daily for 28 consecutive days. Phase II clinical trials were undertaken with a dose of 5FU ranging between 1.0 and 1.15 mg/m^2, with eniluracil 10 mg/m^2. Activity has been demonstrated in breast and colon tumors [27].

Progress has indeed taken place with the use of 5FU and other fluoropyrimidinated agents. We have come a long way since the introduction of 5FU into clinical use as a single agent given as a rapid IV infusion, with only marginal anti-tumor activity. The new oral fluoropyrmidines appear to have an improved therapeutic index over 5FU as they have a more favorable toxicity profile. Their clinical use in a variety of solid tumors is increasing and indeed may become standard care in the very near future. These are exciting times with the clinical development of novel cytotoxic agents unfolding, such as the incorporation of irinotecan and oxaliplatin into the treatment of large bowel tumors in addition to 5FU and its derivatives. The continued search for novel concepts and new venues should eventually result in a better outcome for the cancer patient.

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Capsule

Gene therapy holds promise for treatment of diabetes

Two new animal studies suggest that delivery of insulin genes using gene therapy may be better able to mimic the endogenous secretion profiles of insulin from pancreatic cells than conventional pharmacological approaches.

The long-term aim of both research groups is to replace insulin secretion in patients with diabetes, though the two teams took very different approaches to try to match insulin release with blood glucose concentrations. In the first study, Ji-Won Yoon (University of Calgary, Alberta, Canada) and colleagues inserted the DNA coding for a genetically engineered insulin analogue into an adeno-associated virus, and put the entire DNA construct under the control of the promotor region of the L-type pyruvate-kinase gene found in liver cells. When they injected the virus through the portal vein of either rats made diabetic by injection of the beta-cell toxin streptozotocin, or autoimmune diabetic mice, they found that the viral genome became incorporated exclusively in the liver cells and secreted the insulin analogue into the animals' bloodstream for up to 8 months (*Nature* 2000; 408:48). In the second study, Timothy Kieffer (University of Alberta, Edmonton, Canada) and co-workers opted for a different approach to obtain insulin secretion in response to meals. They targeted naturally existing meal-responsive endocrine cells for insulin replacement. The cells in question, K-cells, are located predominately in the upper gastrointestinal tract and secrete the hormone glucose-dependent insulinotropic polypeptide (GIP) immediately after a meal. Indeed, the secretion patterns of GIP and insulin in humans can be virtually superimposed, since GIP normally acts as an anticipatory signal to beta-cells that a meal containing glucose is being absorbed from the gut. The researchers created a strain of transgenic mouse which releases human insulin from K-cells in the duodenum and stomach. Unlike control animals, these mice did not develop diabetes after treatment with streptozotocm.

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