The Effect of Leucovorin on the Therapeutic Index of Fluorouracil in Cancer Patients

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Fluorouracil has been in clinical use as an anticancer drug for 30 years. Although this drug has a broad spectrum of anticancer activity, including significant activity against the common solid tumors of the gastrointestinal system, only a minority of patients treated with fluorouracil experience an objective response to therapy. Furthermore, in randomized clinical trials completed to date, it has not been possible to demonstrate that fluorouracil therapy significantly prolongs the life span of patients with advanced cancer.

Recent laboratory studies have indicated that leucovorin can enhance the cytotoxicity of fluorouracil in vitro, evidently by enhancing inhibition of the key enzyme, thymidylate synthetase, by the fluorouracil metabolite, FdUMP (fluorodeoxyuridine monophosphate; a stable inactive FdUMP-reduced folate-thymidylate synthetase complex is formed). Pilot, uncontrolled studies of leucovorin-fluorouracil combinations have suggested that leucovorin may significantly increase both the clinical efficacy and the clinical toxicity of fluorouracil in cancer patients. These findings have led to the initiation of several randomized, controlled studies of leucovorin plus fluorouracil versus fluorouracil alone in the treatment of patients with advanced colorectal cancer. Three of these studies have recently completed patient accrual, and the preliminary results of each of the three studies indicate that leucovorin-fluorouracil combinations will have a better therapeutic index than fluorouracil used alone in this disease. Further follow-up of these studies will be needed to determine whether leucovorin-fluorouracil combination therapy will prolong the life span of patients with colorectal cancer.

INTRODUCTION

The modern era of cancer chemotherapy began at Yale more than 40 years ago, when Goodman, Gilman, and Doherty found that nitrogen mustard therapy produced substantial palliation in patients with Hodgkin's lymphoma [1]. Since then, major advances have been made in the chemotherapy of a number of forms of cancer. Unfortunately, effective chemotherapy regimens are not yet available for some of the most common forms of cancer, including most forms of lung cancer and the most common cancers of the gastrointestinal system.

Fluorouracil was discovered by Heidelberger and colleagues at the University of Wisconsin some 30 years ago [2]. Although fluorouracil does not have a high level of anticancer activity, it does have a broad spectrum of activity against human solid tumors. In particular, fluorouracil is generally regarded as the most active single agent currently available for the chemotherapy of adenocarcinomas of the colon, rectum, and pancreas. Colorectal cancer and pancreatic cancer are the two most common gastrointestinal cancers in the United States and are the second and fifth leading causes of cancer death. In 1987, about 60,000 patients will die of colorectal cancer and about

Abbreviations: FdUMP: fluorodeoxyuridine monophosphate FdUTP: fluorodeoxyuridine triphosphate PALA: phosphono-N-acetyl-L-aspartic acid TS: thymidylate synthetase

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24,000 patients will die of pancreatic cancer in the U.S. [3]. Fluorouracil also has significant clinical activity against several other forms of cancer and is included in the standard treatment regimens for breast cancer and gastric cancer.

In the chemotherapy of colorectal cancer, fluorouracil, used alone, continues to be the standard against which newer chemotherapeutic regimens must be compared. Although objective response rates of 8 to 85 percent have been reported in colorectal cancer patients treated with fluorouracil [4], it is generally accepted that standard fluorouracil treatment regimens will produce a 15–20 percent objective response rate in patients receiving fluorouracil as their first chemotherapy following diagnosis of advanced colorectal cancer. Since such a small proportion of patients experience a clear benefit, it is not surprising that randomized studies have never shown a statistically significant survival benefit attributable to fluorouracil therapy for this disease (although there may be a significant survival benefit for those patients who do respond). The best survival results in a randomized trial of fluorouracil in patients with colorectal cancer were obtained by Ansfield and colleagues [5]; in that study, there was a trend to improved survival in patients who received a relatively aggressive (and more toxic) fluorouracil treatment regimen, but this trend did not achieve statistical significance at the \( p \leq .05 \) level.

A great deal of clinical research has been directed toward finding ways to increase the level of anticancer activity of this drug. Other drugs have been administered in attempts to (1) increase the formation of active metabolites of fluorouracil; (2) decrease the rate of catabolism of fluorouracil; or (3) decrease the level of normal uracil metabolites, which compete with fluorouracil metabolites and lessen their effectiveness. Other drugs utilized in these past clinical studies have included methotrexate, phosphono-N-acetyl-L-aspartic acid (PALA), pyrazofurin, thymidine, and others [6]. The possible benefits of these approaches have yet to be demonstrated in definitive, controlled clinical trials.

**MECHANISMS OF FLUOROURACIL ACTION: EFFECTS OF LEUCOVORIN**

Three biochemical actions of fluorouracil are presently regarded as potentially important mechanisms of fluorouracil cytotoxicity. These include (1) conversion to fluorouridine triphosphate, which is incorporated into RNA and interferes with RNA function; (2) conversion to fluorodeoxyuridine monophosphate (FdUMP), which binds to and inhibits the enzyme thymidylate synthetase, thereby starving the cell for thymidylate acid, which is of critical importance for DNA repair and replication; and (3) conversion to fluorodeoxyuridine triphosphate (FdUTP), which may be incorporated into DNA and then undergo rapid removal from DNA, leaving breaks in the DNA (if a large number of breaks are generated, the DNA may be fragmented) [6–8]. There are additional, more subtle biochemical effects of fluorouracil which could also be important; these have been reviewed in detail previously [6] and will not be further discussed here.

The roles that each of these various biochemical effects play in the efficacy and toxicities of fluorouracil are not yet clear. However, studies conducted by Houghton and colleagues in human colorectal cancer xenografts maintained in immune-compromised mice [9], and studies conducted more recently by Rustum and colleagues on tumor biopsy specimens obtained from colorectal cancer patients treated with fluorouracil [10], have suggested that effective inhibition of thymidylate synthetase
Leucovorin appears selectively to enhance the inhibition of thymidylate synthetase in fluorouracil-treated cells. As noted above, the fluorouracil metabolite, FdUMP, binds to and inhibits thymidylate synthetase. This binding is rather weak and readily reversible. If, however, there are adequate intracellular levels of the reduced folate cofactor 5, 10-methylene-tetrahydrofolate, this cofactor will bind to the FdUMP-TS complex to produce a ternary complex which is extremely stable [11]; this action results in prolonged, effective inhibition of thymidylate synthetase. Figure 1 illustrates the thymidylate synthetase reaction, its inhibition by FdUMP, and the mechanism by which reduced folate can enhance FdUMP inhibition of TS. Ullman and colleagues [12] and Evans and colleagues [13] have shown that exposure of cancer cells in culture to increasing levels of reduced folate (leucovorin) renders these cells much more sensitive to the cytotoxicity of fluorouracil and other fluoropyrimidines, by allowing for more effective inhibition of thymidylate synthetase on exposure of these cells to fluoropyrimidines. Pharmacologic levels of leucovorin (10 micromolar or more) are required for optimal enhancement of fluorouracil cytotoxicity in vitro [13].

Past attempts to improve the clinical therapeutic index of fluorouracil by administration of a second drug have generally utilized drugs which non-selectively enhance all of the biochemical actions of fluorouracil. Assuming that the clinical efficacy of fluorouracil is due primarily to its effects on thymidylate synthetase, and that the other biochemical effects of fluorouracil may contribute to toxicity but not to efficacy, selective enhancement of thymidylate synthetase inhibition in fluorouracil-treated patients (as may be achieved with leucovorin) could be a more successful approach to improving the therapeutic index of this drug.

**LEUCOVORIN PLUS FLUOROURACIL: PILOT CLINICAL STUDIES**

As noted above, *in vitro* studies have suggested that extracellular levels of leucovorin should be 10 micromolar or greater at the time of cellular exposure to fluorouracil, for maximal enhancement of thymidylate synthetase inhibition and cytotoxicity. There-
fore, clinical studies of leucovorin plus fluorouracil in cancer patients have generally utilized large doses of leucovorin, to produce plasma levels of reduced folate of 10 micromolar or higher. The more commonly used leucovorin/fluorouracil treatment regimens, and aspects of folate pharmacology and pharmacokinetics in patients treated with these regimens, are presented in Table 1.

The first large pilot (uncontrolled) study of a leucovorin/fluorouracil treatment regimen in cancer patients was completed by Machover and colleagues in Villejuif, France [14]. Patients were treated daily for five days with a bolus intravenous injection of leucovorin, 200 mg/m², followed immediately by a fluorouracil intravenous infusion administered over fifteen minutes. Patients generally received 370 mg of fluorouracil per m² per day in the first course of therapy, with adjustment of the fluorouracil dosage in subsequent courses of therapy depending on tolerance. Courses were repeated at 28-day intervals. The primary toxicities observed using this treatment were oral mucositis and diarrhea, each of which occurred in 25–30 percent of treatment courses and each of which was severe (grade 3) in 3–4 percent of treatment courses which included fluorouracil at a dose of 370 mg/m²/day. Myelosuppression was less common, rarely severe (1–2 percent grade 3 or above neutropenia or thrombopenia at this dose), and rapidly reversible [14,17]. Rather impressive response rates were observed in this early, uncontrolled study (refer to Tables 2 and 3). A number of additional uncontrolled studies, utilizing this and other leucovorin/fluorouracil treatment regimens in several forms of cancer, have been reported in the past few years. The results of some of the larger published uncontrolled studies in patients with colorectal cancer, and in patients with other malignancies, are summarized in Tables 2 and 3, respectively. The tremendous variation in objective response rates observed in colorectal cancer patients treated with leucovorin/fluorouracil regimens is reminiscent of the 8 to 85 percent range in response rates reported in different studies of the efficacy of fluorouracil used as a single agent in colorectal cancer patients. Conclusions regarding

| Treatment Regimen | Folate Measured | Concentration (μM) | Half-Times (minutes) | Reference |
|------------------|-----------------|--------------------|----------------------|-----------|
| 200 mg/m² intravenous bolus | Leucovorin | 43<sup>a</sup> | 20 122 — — | [14] |
| | 5-Methyl-tetrahydrofolate (metabolite) | 2<sup>a</sup> | 362 — — | |
| 500 mg/m²/day intravenous infusion | Leucovorin | 4.5<sup>b</sup> | — — 53<sup>c</sup> | [15] |
| | 5-Methyl-tetrahydrofolate (metabolite) | 4.7<sup>b</sup> | — — 167<sup>c</sup> | |
| 500 mg/m²/two-hour intravenous infusion | Leucovorin | 111<sup>d</sup> | — — 414<sup>d</sup> | [10,16] |
| | 5-Methyl-tetrahydrofolate | 12<sup>d</sup> | — — 336 | |

<sup>a</sup>Peark concentrations (Regimens were designed to maintain 10 micromolar or higher leucovorin levels for one hour after fluorouracil injection.)
<sup>b</sup>Sate-state concentrations
<sup>c</sup>Post-infusion half-times
<sup>d</sup>Assay measured both d and l isomers. Only d isomer remained by one to two hours post-infusion.
| Investigators                   | No. of Patients | Patients with Prior Chemotherapy | Treatment Regimen (mg/m²)* | CR + PR* (%) |
|--------------------------------|----------------|----------------------------------|---------------------------|--------------|
| Machover et al. [14]           | 86             | 32/86                            | LV: 200/day × 5           | 33           |
|                                |                |                                  | FU: 370/day × 5           |               |
| Bertrand et al. [18]           | 36             | 36/36                            | LV: 500/day × 6           | 9            |
|                                |                |                                  | FU: 370/day × 5           |               |
|                                |                |                                  | (24-hour infusion)        |               |
| Petrelli et al. [16]           | 24             | 20/24                            | LV: 500/week              | 38           |
|                                |                |                                  | FU: 600/week              |               |
|                                |                |                                  | (2-hour infusion)         |               |
| Zakem et al. [19]              | 29             | 14/29                            | LV: 500/week              | 48           |
|                                |                |                                  | FU: 600/week              |               |
|                                |                |                                  | (2-hour infusion)         |               |
| Valone et al. [20]             | 40             | 40/40                            | LV: 200/day × 5           | 2            |
| Greene et al. [21]             | 25             | 15/25                            | LV: 60/day × 4            | 8            |
|                                |                |                                  | (24-hour infusion)        |               |
| Cunningham et al. [22]         | 36             | 11/36                            | LV: 60/day × 5            | 39           |
|                                |                |                                  | or 280–480/day × 5        |               |
|                                |                |                                  | or 60/day × 4             |               |
|                                |                |                                  | (24-hour infusion)        |               |
| Budd et al. [23]               | 62             | 0/62                             | LV: 200/day × 5           | 22           |
| Budd et al. [23]               | 63             | 0/63                             | LV: 375/day × 5           | 21           |
|                                |                |                                  | (24-hour infusion)        |               |
| Schmoll et al. [24]            | 68             | 0/68                             | LV: 1,200–1,600           | 18           |
| Sanzo et al. [25]              | 20             | Not given                        | LV: 500/week              | 35           |

*All dosages are expressed in milligrams per square meter of body surface area (mg/m²). All studies allowed for adjustment of fluorouracil dosage based on appropriate clinical parameters. LV is leucovorin; FU is fluorouracil. All drugs were given by intravenous bolus or brief infusion, unless otherwise indicated. Drug administration by continuous infusion is denoted here as 24-hour infusion, continuing for the number of days indicated.

*Objective (complete plus partial) response rate
| Investigators      | Type of Cancer | No. of Patients | Patients with Prior Chemotherapy | Treatment Regimen (mg/m²)* | CR + PR (%) |
|--------------------|----------------|----------------|---------------------------------|---------------------------|-------------|
| Machover et al. [14]| Gastric        | 27             | 1/27                            | 200/day × 5               | 48          |
| Arbuck et al. [26]  | Gastric        | 22             | 12/22                           | 500/week                  | 16          |
|                     |                |                |                                 | (2-hour infusion)         |             |
| Marini et al. [27]  | Breast         | 28             | 23/28                           | 200/day × 5               | 61          |
| Doroshow et al. [28]| Breast         | 24             | 24/24                           | 500/day × 6               | 17          |

*All dosages are expressed in milligrams per square meter of body surface area (mg/m²). All studies allowed for adjustment of fluorouracil dosage based on appropriate clinical parameters. LV is leucovorin; FU is fluorouracil. All drugs were given by intravenous bolus or brief infusion, unless otherwise indicated. Drug administration by continuous infusion is denoted here as 24-hour infusion, continuing for the number of days indicated.

*Objective (complete plus partial) response rate
the effect of leucovorin on the therapeutic index of fluorouracil in colorectal cancer cannot be drawn from these uncontrolled studies; however, the results of uncontrolled studies have been considered positive enough by clinical investigators to warrant proceeding to randomized clinical trials comparing several leucovorin/flourouracil treatment regimens to standard fluorouracil regimens in colorectal cancer patients (see the section following). The uncontrolled studies have also served to characterize the effects of leucovorin on the toxicities of fluorouracil. In brief, although patients treated with leucovorin/flourouracil combination regimens experience the same spectrum of toxicities as patients treated with fluorouracil alone, stomatitis and diarrhea appear to be more prominent in patients treated with leucovorin/flourouracil, while myelosuppression is more prominent in patients receiving fluorouracil alone.

Uncontrolled studies of leucovorin/flourouracil regimens have also been carried out in patients with several other forms of cancer. The results of published studies in patients with breast cancer and in patients with gastric cancer are presented in Table 3. The reported objective response rates in these patients are impressive by historical standards. Again, randomized controlled studies will be required to assess definitively the contribution of leucovorin to the therapeutic index of fluorouracil in these diseases.

**LEUCOVORIN PLUS FLUOROURACIL: RANDOMIZED CONTROLLED STUDIES**

Six randomized, controlled studies comparing leucovorin plus fluorouracil versus fluorouracil alone in the treatment of patients with advanced colorectal cancer have been initiated to date in the United States and Canada, and a seventh study is ongoing in Italy. The design and current status of these studies are summarized in Table 4. As noted, three studies have completed patient accrual.

Doroshow and colleagues at the City of Hope Medical Center have completed patient accrual to their trial, comparing moderate doses of fluorouracil used alone versus the same doses of fluorouracil administered together with infusion of a large dose of leucovorin [29]. As indicated in Table 5, patients receiving leucovorin plus fluorouracil experienced a substantially higher objective response rate (45 percent versus 15 percent), and a significantly longer median time to progression. There is a trend toward improved survival in the patients who received leucovorin plus fluorouracil, but this trend has not yet reached statistical significance. The strength of this trend may have been reduced by the study design, which allowed patients on the control arm to cross over to the leucovorin plus fluorouracil arm; most of the patients who were entered on the control arm did in fact eventually cross over to the experimental arm. Hematologic toxicity was mild on both arms of the study; stomatitis was significantly more severe in patients receiving leucovorin plus fluorouracil.

Petrelli and colleagues at Roswell Park Memorial Institute have completed patient accrual for their trial, comparing an aggressive loading dose fluorouracil alone control arm versus a weekly fluorouracil-high dose leucovorin experimental arm [30]. As noted in Table 5, patients treated with leucovorin plus fluorouracil experienced a significantly higher response rate (48 percent versus 11 percent) than patients treated with fluorouracil alone. To date, there is a slight trend to improved survival in the patients who received leucovorin plus fluorouracil, but it is unlikely that this trend will ever reach statistical significance in this small study. Diarrhea was more common in patients receiving leucovorin plus fluorouracil, while leukopenia was more common in patients receiving fluorouracil alone.
### TABLE 4
Leucovorin Plus Fluorouracil: Controlled Studies in Colorectal Cancer

| Investigators | Study Treatment Arms | Study Status |
|---------------|----------------------|--------------|
| Doroshow et al. [29] | Control: Fluorouracil 370 mg/m²/day × 5 days | Accrual complete (79 patients total) |
| | Experimental: Leucovorin 500 mg/m²/day × 6 (infusion) Fluorouracil 370 mg/m²/day × 5 days | |
| Petrelli et al. [30] | Control: Fluorouracil 450 mg/m²/day × 5 days, then 200 mg/m² alternate days to toxicity | Accrual complete (44 patients total) |
| | Experimental: Leucovorin 500 mg/m²/week (2-hour infusion) Fluorouracil 600 mg/m²/week | |
| Erlichman et al. [31] | Control: Fluorouracil 370 mg/m²/day × 5 days | Accrual complete (130 patients total) |
| | Experimental: Leucovorin 200 mg/m²/day × 5 days Fluorouracil 370 mg/m²/day × 5 days | |
| Northern California Oncology Group [32] | Control: Fluorouracil 12 mg/kg/day × 5 days, then 15 mg/kg/week | Ongoing; planned accruals, 70 patients (control) and 140 patients (experimental) |
| | Experimental: Leucovorin 200 mg/m²/day × 5 days Fluorouracil 400 mg/m²/day × 5 days | |
| Canobbio et al. [33] | Control: Fluorouracil 600 mg/m²/week | Ongoing (67 patients accrued) |
| | Experimental: Leucovorin 500 mg/m²/week (2-hour infusion) Fluorouracil 600 mg/m²/week | |
| GI Tumor Study Group [3] | Control: Fluorouracil 500 mg/m²/day × 5 days | Ongoing |
| | Experimental No. 1: Leucovorin 500 mg/m²/week (2-hour infusion) Fluorouracil 600 mg/m²/week | |
| | Experimental No. 2: Leucovorin 25 mg/m²/week (10-minute infusion) Fluorouracil 600 mg/m²/week | |
| North Central Cancer Treatment Group-Mayo [3] | Control: Fluorouracil 500 mg/m²/day × 5 days | Ongoing; planned accrual of 70 patients per arm, with extension to 140 patients per arm possible |
| | Experimental No. 1: Leucovorin 200 mg/m²/day × 5 days Fluorouracil 370 mg/m²/day × 5 days | |
| | Experimental No. 2: Leucovorin 20 mg/m²/day × 5 days Fluorouracil 425 mg/m²/day × 5 days | |

*Most studies allow dose adjustments in second and subsequent treatment courses, depending on toxicity.*

*These studies include additional arms which test other therapeutic approaches.*
Although Erlichman and colleagues (Princess Margaret Hospital, Toronto, and Tom Baker Cancer Clinic, Calgary) have completed patient accrual to their study, only partial results have been published to date [31]. This study compared moderate doses of fluorouracil used alone, versus the same doses of fluorouracil administered with high doses of leucovorin, in the first course of therapy. Fluorouracil dosages were adjusted in the second and subsequent courses of treatment, to achieve equal toxicity in the two treatment arms. In the interim report on the first 52 evaluable patients, objective responses were reported in ten patients on the leucovorin plus fluorouracil arm, while no patients had responded to fluorouracil alone. Crossover of patients was not allowed in this relatively large study, so the relative effects of the two treatment arms on patient survival should be clearly discernible. Additional results from this study should come out in the near future.

Accrual is ongoing in the Northern California Oncology Group study, which compares an aggressive loading dose-maintenance dose fluorouracil alone control arm with a commonly used leucovorin plus fluorouracil experimental arm [32]. To date, only interim toxicity results are available from this study; these results indicate that toxicities (particularly myelosuppression and diarrhea) are substantially more common and more severe in patients who are being treated on the fluorouracil alone control arm.

Preliminary results of the study being conducted in Italy by Canobbio and colleagues have recently been published [33]. This study compares weekly treatment with a modest dose of fluorouracil alone versus a weekly fluorouracil-high-dose leucovorin experimental arm. To date, objective responses have been observed in 1 of 23 evaluable patients on fluorouracil alone, and in 6 of 29 patients on leucovorin plus fluorouracil (4 percent vs. 21 percent, not statistically significant). Patients receiving leucovorin plus fluorouracil have experienced slightly more stomatitis, diarrhea, and leukopenia.
No results have been published to date from the ongoing North Central Cancer Treatment Group-Mayo Clinic study or from the ongoing GI Tumor Study Group study. In an effort to ensure definitive results, the North Central Cancer Treatment Group-Mayo Clinic consortium has recently decided to double the accrual in selected arms of this study, which will probably extend the patient accrual period for that study to 1990 or beyond.

CONCLUSIONS

Although it will be several years before the last of the randomized studies is concluded, all of the results which have come out of these studies to date indicate that leucovorin will be shown to significantly enhance the therapeutic index of fluorouracil in colorectal cancer. The improvement in therapeutic index could be substantial enough to permit demonstration of a statistically significant survival benefit in patients treated on the leucovorin/fluorouracil arm of one or more of these studies. An efficacy difference of this magnitude would most likely be evident in one of the studies comparing a leucovorin/fluorouracil regimen to a moderate-dose fluorouracil alone control arm (e.g., the Doroshow, Erlichman, and Canobbio studies), as it is clear that moderate doses of fluorouracil tend to be less active as well as less toxic than maximal tolerated doses of this drug. Studies comparing leucovorin/fluorouracil regimens to aggressive fluorouracil alone control arms will be less likely to show a clear efficacy difference, but should still show a therapeutic index advantage for leucovorin/fluorouracil (e.g., equal efficacy with less toxicity), if leucovorin does indeed enhance the therapeutic index of fluorouracil.

Assuming that leucovorin is shown to enhance the therapeutic index of fluorouracil in colorectal cancer, potentially fruitful avenues for future research will include addressing questions such as the following: What dose of leucovorin is optimal? Can other reduced folates (or folic acid itself) provide the same clinical results? Will leucovorin similarly enhance the therapeutic index of fluorouracil in other fluorouracil-responsive tumors? Will leucovorin expand the spectrum of anticancer activity of fluorouracil? How can leucovorin be used to best advantage in methotrexate/fluorouracil combinations? What role does folate polyglutamylation play in the leucovorin/fluorouracil interaction? Can leucovorin/fluorouracil be effective as adjuvant therapy, to prevent recurrence of colorectal cancer after surgery?

Finally, although it appears that leucovorin/fluorouracil regimens could represent a significant step forward in the treatment of colorectal cancer, it is clear that this is a modest step. Although the addition of leucovorin could significantly improve the therapeutic index of fluorouracil in advanced colorectal cancer and could lead to the first demonstration of a statistically significant survival benefit in patients receiving chemotherapy for this disease, it is clear that fewer than half of the patients treated will have an objective response; complete responses will still be rare, and response and survival durations will still be relatively brief. But the history of progress in modern cancer chemotherapy has been a history of modest steps like this; major advances have, in reality, been the result of a series of smaller steps. The symbolic importance of this advance would be the demonstration that we can make progress even in the treatment of this historically refractory form of cancer and that, given patience and hard work, there is every reason to believe that we will ultimately devise effective medical therapies for this cancer, and for other cancers that are currently refractory to our best treatments.
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