

Flavopiridol, A Novel Cyclin-Dependent Kinase Inhibitor, in Metastatic Renal Cancer: A University of Chicago Phase II Consortium Study

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Purpose: Flavopiridol is the first cyclin-dependent kinase (cdk) inhibitor to enter clinical trials. Serum levels of flavopiridol obtained during phase I studies were sufficient to inhibit in vitro cancer cell growth. Because responses were observed in kidney cancer patients in the phase I trials, we performed a phase II trial of flavopiridol in this patient population.

Patients and Methods: Thirty-five minimally pre-treated patients were accrued using a standard two-step mechanism. Flavopiridol (50 mg/m²/d) was administered by continuous infusion for 72 hours every 2 weeks, and response was evaluated every 8 weeks. Peripheral blood mononuclear cells (PBMCs) were collected at baseline, at completion of drug infusion, and on day 7 of the first therapy cycle, and cell cycle parameters after phytohemagglutinin and interleukin-2 stimulation were assessed.

Results: There were two objective responses (re-

sponse rate = 6%, 95% confidence interval, 1% to 20%). The most common toxicities were asthenia, occurring in 83% of patients (grade 3 or 4 in 9%), and diarrhea, occurring in 77% of patients (grade 3 or 4 in 20%). Also, nine patients (26%) experienced grade 3 or 4 vascular thrombotic events, including one myocardial infarction, two transient neurologic ischemic attacks, four deep venous thrombosis, and two pulmonary emboli. Cell cycle studies did not reveal any effect of flavopiridol on stimulated PBMCs.

Conclusion: Flavopiridol, at the dose and schedule administered in this trial, is ineffective in metastatic renal cancer. In addition to the diarrhea observed in phase I studies, we also observed a higher incidence of asthenia and serious vascular thrombotic events than expected.

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THE MAMMALIAN CELL cycle is driven by series of cyclins which bind and activate a number of cyclin-dependent kinases (cdks).¹ Negative control of cdk activity is in part accomplished by small protein inhibitors, including p21, p27, p15, and p16.¹ These proteins are frequently mutated or dysregulated in human malignancies. This observation, as well as the conservation of cdk inhibitors throughout evolutionary history, makes small molecule inhibitors of these enzymes attractive putative anticancer compounds. One of the first cdk inhibitors to undergo clinical trials is flavopiridol (NSC 649890). Initial in vitro studies revealed that it leads to growth inhibition, G2/M arrest, and cytotoxicity in breast carcinoma cells by inhibiting the cdc2 cdk.²⁻⁴ A subsequent study showed that flavopiridol also inhibits cdk2 and cdk4, which can lead to G1/S arrest under certain conditions.⁵ Although initial in vitro evaluation suggested that flavopiridol induces only cell cycle arrest in proliferating cells, additional publications revealed that it is cytotoxic to some resting carcinoma cells and can induce apoptosis in others.⁶⁻¹¹ Flavopiridol is also toxic to resting as well as proliferating endothelial cells in culture, suggesting that it may have some antiangiogenic properties as well.¹² Finally, preliminary data from our own laboratory suggests that flavopiridol may be equally effective in cells overexpressing the *MDR1* multidrug resistance gene,¹¹ a common abnormality in renal carcinoma.¹³

These in vitro evaluations, along with animal studies with rodents bearing murine and human tumor xenografts,^{6,10,14,15} suggested that prolonged exposure to flavopiridol was necessary to observe its therapeutic efficacy. Thus, two phase I studies evaluated a 72-hour continuous-infusion schedule.^{16,17} Although the dose-limiting toxicity was secretory diarrhea, other organ toxicity was mild and the diarrhea could be controlled with standard measures. The recommended phase II dose was 50 mg/m²/d for 72 hours every 2 weeks. At this dose, the median plasma flavopiridol concentration was 271 nmol/L, a concentration sufficient to inhibit cdk activity in vitro. Finally, in one of the phase I trials, 19 patients with refractory renal carcinoma were entered, and one partial and one minor response

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were observed.¹⁶ Thus, we initiated a phase II trial of flavopiridol in patients with refractory renal carcinoma.

PATIENTS AND METHODS

Patients

This study was conducted through the University of Chicago phase II consortium in collaboration with the M.D. Anderson Cancer Center. Patients were required to have metastatic or unresectable renal carcinoma clearly measurable on radiologic or physical examination. One prior immunotherapy regimen but no prior chemotherapy was allowed. The requirements for normal organ function included WBC $\geq 3,000/\mu\text{L}$, platelets $\geq 100,000/\mu\text{L}$, hemoglobin $\geq 9.0\text{ g/dL}$, total bilirubin $\leq 1.5\text{ mg/dL}$, transaminases ≤ 2.5 times the upper limit of normal, and creatinine $\leq 2.0\text{ mg/dL}$ (or estimated creatinine clearance $\geq 60\text{ mL/min}$). Patients were required to have a World Health Organization performance status of 0 to 2 and no other history of invasive malignancy in the past 5 years. CNS metastases were allowed, provided that patients had adequate local therapy and did not require systemic steroids. All patients signed a written informed consent approved by individual institutional review boards. Thirty-five patients were accrued from October 1997 to May 1998 through the following institutions: University of Chicago (14 patients); M.D. Anderson Cancer Center (eight patients); University of Illinois (six patients); Michiana Hematology/Oncology (four patients); Fort Wayne Medical Center (two patients); and Weiss Memorial Hospital (one patient).

Therapy and Dose Modifications

Flavopiridol was supplied by the Division of Cancer Treatment and Diagnosis, National Cancer Institute and was administered as a continuous 72-hour infusion via a central venous catheter at a dose of $50\text{ mg/m}^2/\text{d}$ every 2 weeks. Drug was reconstituted in benzyl alcohol preserved saline at a final concentration of 0.25 to 2.25 mg/mL in polyvinylchloride bags and administered via an ambulatory pump. Patients experiencing grade 3 or greater diarrhea had flavopiridol infusion halted, and loperamide or diphenoxylate and atropine therapy was initiated. If diarrhea continued, octreotide was begun. Any grade 3 or grade 4 toxicity led to dose reductions of 25% or 50%, respectively, in subsequent cycles.

Patient Follow-Up and Study End Points

The primary objective of this study was to determine the objective response rate of patients with metastatic renal carcinoma treated with flavopiridol. A secondary clinical objective was to determine flavopiridol's toxicity in this patient population. Response evaluations were performed every 8 weeks. Complete response was defined as complete disappearance of all radiologic and clinical signs of cancer maintained for at least 4 weeks. Partial response was defined as a 50% reduction from baseline in the sum of the products of two perpendicular diameters of all measured lesions, without the appearance of new lesions, also maintained for at least 4 weeks. Progressive disease was defined as 25% increase from baseline in the sum of the products of two perpendicular diameters of all measured lesions or the appearance of new lesions. All other situations were defined as stable disease. Patients discontinued therapy when there was disease progression, which included the need for palliative radiotherapy or other systemic antineoplastic therapy. Other withdrawal criteria included interruption of therapy for greater than 2 weeks for any cause, intolerable or unresolved adverse events, or patient decision.

Biologic Monitoring

Additional secondary objectives of this trial included pharmacokinetic monitoring and assessment of flavopiridol's effect on in vitro cell cycle parameters in stimulated peripheral-blood mononuclear cells (PBMCs). Pharmacokinetic monitoring and correlation with response and toxicity are objectives in ongoing trials of flavopiridol in colon cancer and non-Hodgkin's lymphoma as well and, therefore, will be reported separately. For the cell cycle study, PBMCs were isolated before beginning therapy, 71 hours after the start of infusion, and on day 7 of cycle 1 only. Collected cells were frozen for viability at -80°C in RPMI 1640 media with 10% fetal bovine serum and 10% dimethyl sulfoxide and then batch analyzed. To study the effect of flavopiridol on cell cycle kinetics, 1.0×10^6 PBMCs were grown in RPMI 1640 with 10% fetal bovine serum and 2 mmol/L L-glutamine and stimulated with phytohemagglutinin A (PHA) $10\text{ }\mu\text{g/mL}$ and interleukin-2 (IL-2) 8 ng/mL . After 72 or 96 hours of stimulation, bromodeoxyuridine (10 mmol/L) was added to the media. Cells were harvested, washed in phosphate buffered saline, and fixed with 4% paraformaldehyde. PBMCs were then stained with $10\text{ }\mu\text{g/mL}$ propidium iodide, $100\text{ }\mu\text{g/mL}$ RNase A and 0.1% Triton X-100 for 30 minutes at room temperature. Thereafter, cells were stained for 30 minutes with fluorescein isothiocyanate-conjugated antibody to bromodeoxyuridine. Cell cycle distributions and apoptotic fractions were determined using ModFit LT v2.0 software (Verity Software House, Inc, Topsham, ME) after analysis on a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ). Because no effects on cell cycle parameters were noted in the first six patients (see Results), additional patient samples were not analyzed.

Statistical Considerations

Accrual proceeded using a standard two-stage mechanism. The first stage was designed to accrue 14 patients, with a second stage designed to accrue an additional 21 patients if one or more patients responded in the first stage. The regimen would be considered worthy of further study if four or more of 35 patients ($>11\%$) experienced an objective response. This design yields a 0.91 or greater probability of accepting the regimen if the true response rate is at least 20% and a 0.91 or greater probability of rejecting the regimen if the true response rate is less than 5%. It also gives a 0.49 or greater probability of stopping early if the flavopiridol response rate in this population is less than 5%. Survival was assessed using the Kaplan-Meier method. All survival data was censored as of December 1, 1998.

RESULTS

Table 1 lists the patient characteristics. One response was observed in the first 14 patients. Thus, accrual proceeded through the second stage, and a total of 35 patients were entered. As a group, the enrolled patients were predicted to have a good prognosis.¹⁸ Thirty-one patients (89%) had a World Health Organization performance status of 0 or 1. The median time since diagnosis of metastatic disease was 14 months; 12 patients (34%) had only one site of metastatic disease, and 10 patients (29%) had lung only or lymph node only disease. One patient refused clinical and radiologic follow-up and was, therefore, not assessable for response but was assessable for toxicity. All other patients were considered assessable for toxicity and response. Among the

Table 1. Patient Characteristics

Characteristic	No. of Patients
Total	35
Patients eligible for response evaluation	34
Sex	
Male	24
Female	11
Age, years	
Median	58
Range	29-76
WHO performance status*	
0	19
1	12
2	4
Prior nephrectomy	22
Time since nephrectomy, months	
Median	22
Range	1-389
Time since metastatic disease diagnosis, months	
Median	14
Range	2-28
Prior therapy	
Immunotherapy	21
Radiotherapy	8
Resection metastatic site	8
No. of metastatic sites	
1	12
2	10
≥3	13
Sites of metastatic disease	
Lung only	7
Lymph node only	3
Liver (with or without other sites)	9
Bone (with or without other sites)	3

Abbreviation: WHO, World Health Organization.
 *Median performance status = 0.

34 patients assessable for response, there were two partial responders, for an overall response rate of 6% (95% confidence interval, 1% to 20%.) Fig 1 depicts event-free and overall survival with events defined as progressive disease or toxicity requiring discontinuation of therapy. The median event-free survival was 9 weeks, and the median overall survival was 48 weeks. As of this report, three patients remain on study at 31+, 32+, and 57+ weeks.

Table 2 lists the observed toxic events. There was essentially no myelosuppression and no evidence of renal, neurologic, or hepatic toxicity either. The most common toxicity was asthenia and/or fatigue, which occurred in 83% of patients. In the majority of patients, it was mild and did not influence therapy, but it was graded as severe by three patients. Diarrhea was also a common toxicity despite the use of prophylactic antidiarrheal medication at the first sign of loose stools and was considered severe in seven patients (20%). Twenty-four of 35 patients required some antidiar-

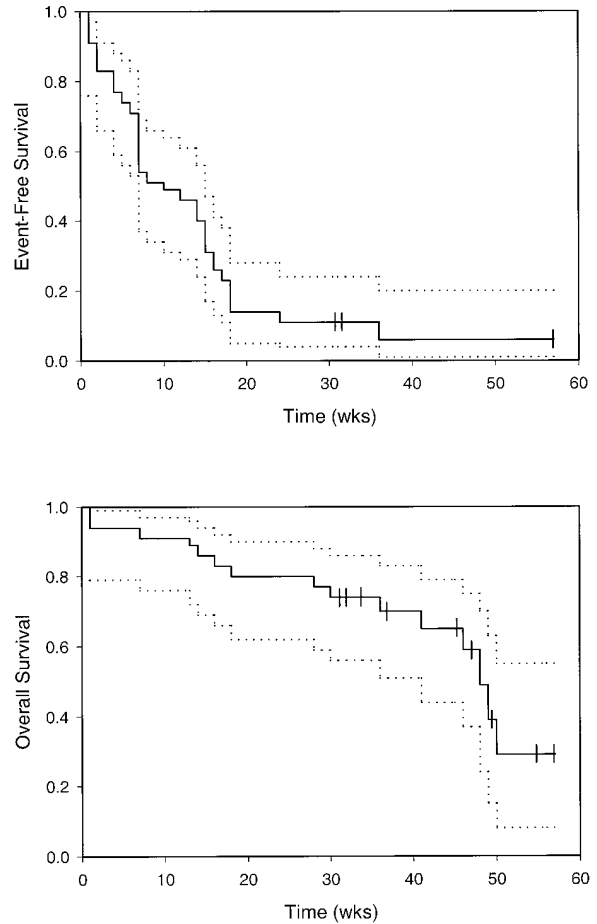


Fig 1. Overall and event free survival with events defined as progressive disease or toxicity requiring discontinuation of drug therapy. Dotted lines reflect 95% confidence intervals, and tick marks represent censored patients.

rehal medication during therapy. This included loperamide and/or diphenoxylate and atropine in 22 patients and additional octreotide in four patients. Seven patients required intravenous fluids for control of fluid and electrolyte abnormalities secondary to diarrhea. Nausea was also significant in a few patients. A number of patients were noted to have hyperglycemia during therapy. All patients with grade 2 or 3 hyperglycemia had known diabetes and the relationship to flavopiridol is questionable.

The most serious adverse events were arterial and venous thrombotic events. Three arterial events were noted. The first was a patient who experienced a nonfatal myocardial infarction on day 10 of the study and received no further flavopiridol. A second event occurred in a patient with a history of transient ischemic attacks, who was admitted on the last day of the third flavopiridol infusion with an episode of confusion that was similar to previous transient ischemic

Table 2. Toxicity

Event	Patients With Toxicity (no.)			
	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	0	0	0	0
Thrombocytopenia	6	0	0	0
Hemoglobin	17	11	0	0
Hyperglycemia	8	2	5	0
Asthenia/fatigue	17	9	3	0
Nausea/vomiting	9	6	1	0
Diarrhea	9	11	6	1
Arterial vascular	0	0	1	2
Venous vascular	0	0	4	2

NOTE. Other grade 3/4 toxicities included two episodes of peripheral edema, one episode of catheter related infection, and one episode of elevated alkaline phosphatase. All these events were interpreted as being most likely unrelated to flavopiridol.

attack episodes. Brain magnetic resonance imaging revealed only chronic small vessel disease. He was placed on warfarin, but experienced several additional transient confusional episodes that were not further evaluated. A final patient experienced transient vision loss and scotoma in one eye, with a normal ophthalmologic examination. This patient underwent a dose reduction for concomitant grade 3 diarrhea and was treated for an additional 6 months without recurrent symptoms. The relationship of each of these arterial events to flavopiridol was unclear.

Six patients experienced deep venous thromboses during therapy. Four patients had documented extremity thromboses, two in the lower extremities and two associated with a permanent intravenous access device in the upper extremities. One of the latter patients remained on study and developed a second thrombosis in the upper extremity despite the use of warfarin. Two patients experienced probable pulmonary emboli. One patient was diagnosed after a computed tomography scan of the chest performed for disease re-evaluation. The second was in a patient who developed sudden chest pain and shortness of breath on day 3 of therapy and died that same day with a cardiopulmonary arrest.

In vitro cell cycle studies were performed on stimulated PBMCs collected at baseline, 71 hours into the infusion, and on day 7 of cycle 1. It was hypothesized that cells collected at the end of the flavopiridol infusion would be inhibited from entering the cell cycle when stimulated by PHA and IL-2. There was no difference in cell cycle parameters of PHA/IL-2-stimulated lymphocytes collected at baseline or 72 or 96 hours after beginning the flavopiridol infusion in the first six enrolled patients. Thus, further patient samples were not analyzed.

DISCUSSION

We have conducted a phase II evaluation of the cdk inhibitor flavopiridol in patients with refractory renal carcinoma. This trial confirmed the phase I data; there was little hematologic, hepatic, or renal toxicity, but grade 3 or 4 diarrhea was common and occurred in 20% of patients. The diarrhea did not always respond to opiate antidiarrheal medications, and some patients required octreotide and subsequent flavopiridol dose reduction. Interestingly, the most common toxicity was asthenia/fatigue, which was experienced by 83% of patients. Although this was also noted in the phase I trial, it seemed to be more distressing to the presumably better functioning patients in this study. A serious potential toxicity that was not appreciated in the phase I study was arterial and venous thromboses. Although the arterial events were not considered drug related by the treating physicians and although deep venous thromboses and pulmonary emboli are certainly common in patients with metastatic renal carcinoma, the frequency with which these events were observed was greater than we experienced in other trials with renal cancer patients. For example, in ongoing phase I and II studies using continuous-infusion fluorouracil and gemcitabine, we have observed no catheter-associated thromboses in more than 70 renal carcinoma patients (unpublished data). Thus, additional clinical trials with flavopiridol may need to consider the etiology and prevention of these events. Different doses and/or schedules may also need to be considered to decrease the incidence of toxic events, especially if prolonged administration is anticipated (see below).

We attempted to detect the cell cycle inhibitory effect of flavopiridol by in vitro stimulation of PBMCs collected during drug infusion. The lack of observed effect may be caused by freezing the cells before analysis, but, more likely, it is because of the observation that cells need to be continuously exposed to flavopiridol in order for cell cycle arrest to become manifest.² Thus, cells that are exposed to 71 hours of flavopiridol and then undergo ex-vivo growth stimulation do not experience any permanent cell cycle perturbations.

Despite encouraging preclinical data and suggestive data from the phase I study, little activity, as determined by objective responses, was observed in this population. In vitro, flavopiridol causes growth arrest in almost all cell types, but apoptosis and cytotoxicity are rapidly induced in only a few, usually hematopoietic cell lines.^{2,6,8-11} In these cases, apoptosis occurs within 24 hours of flavopiridol exposure at doses less than 1 $\mu\text{mol/L}$.^{6,9} Although apoptosis can be observed in other cell types, it usually requires flavopiridol doses and exposure times that are above plasma

levels achieved in the phase I study at the recommended phase II dose. Thus, such cells, which likely includes most renal carcinomas, will experience only a transient cell cycle arrest. This would not be expected to lead to objective tumor shrinkage. Such an effect may lead to slowing of tumor growth, but this trial was not designed to detect this event. Although the prolonged survival in this patient cohort may be encouraging, this was a good prognosis cohort in whom a more prolonged survival would be expected even in the absence of therapy.¹⁸

Recently, it has been demonstrated that in vitro combination standard chemotherapy and flavopiridol increases apoptosis and may be synergistic.^{19,20} Whether flavopiridol could enhance the minimal activity of fluorouracil in renal cancer²¹ or the activity of IL-2, whose mechanism of action

is immune stimulation, remains to be determined. Thus, flavopiridol at this dose and schedule does not lead to significant objective responses in renal cancer patients. To determine whether prolonged administration at lower doses could lead to disease stabilization with minimal toxicity or whether combination therapy could be useful would require additional trials.

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REFERENCES

- Sherr CJ: Cancer cell cycles. *Science* 274:1672-1677, 1996
- Kaur G, Stetler-Stevenson M, Sebers S, et al: Growth inhibition and reversible cell cycle arrest of carcinoma cells by flavone L86-8275. *J Natl Cancer Inst* 84:1736-1740, 1992
- Losiewicz MD, Carlson BA, Kaur G, et al: Potent inhibition of cdc2 kinase activity by the flavonoid L86-8275. *Biochem Biophys Res Comm* 201:589-595, 1994
- Worland PJ, Kaur G, Stetler-Stevenson M, et al: Alteration of the phosphorylation state of p34^{cdc2} kinase by the flavone L86-8275 in breast carcinoma cells. *Biochem Pharm* 46:1831-1840, 1993
- Carlson BA, Dubay MM, Sausville EA, et al: Flavopiridol induces G1 arrest with inhibition of cyclin dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. *Cancer Res* 56:2973-2978, 1996
- Arguello F, Alexander M, Sterry JA, et al: Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity in vivo against human leukemia and lymphoma xenografts. *Blood* 91:2482-2490, 1998
- Bible KC, Kaufmann SH: Flavopiridol: A cytotoxic flavone that induces cell death in noncycling A549 human lung carcinoma cells. *Cancer Res* 56:4856-4861, 1996
- Konig A, Schwartz GK, Mohammad RM, et al: The novel cyclin-dependent kinase inhibitor flavopiridol downregulates Bcl-2 and induces growth arrest and apoptosis in chronic B-cell leukemia lines. *Blood* 90:4307-4312, 1997
- Parker BW, Kaur G, Nieves-Neira W, et al: Early induction of apoptosis in hematopoietic cell lines after exposure to flavopiridol. *Blood* 91:458-465, 1998
- Patel V, Senderowicz AM, Pinto D Jr, et al: Flavopiridol, a novel cyclin-dependent kinase inhibitor, suppresses the growth of head and neck squamous cell carcinomas by inducing apoptosis. *J Clin Invest* 102:1674-1681, 1998
- Chien M, Astumian M, Liebowitz D, et al: In vitro evaluation of flavopiridol, a novel cell cycle inhibitor, in bladder cancer. *Cancer Chem Pharm* 44:81-87, 1999
- Brusselbach S, Nettelbeck DM, Sedlacek HH, et al: Cell cycle-independent induction of apoptosis by the anti-tumor drug Flavopiridol in endothelial cells. *Int J Cancer* 77:146-152, 1998
- Kim WJ, Kakehi Y, Kinoshita H, et al: Expression patterns of multidrug-resistance (MDR1), multidrug resistance-associated protein (MRP), glutathione-S-transferase-pi (GST-pi) and DNA topoisomerase II (Topo II) genes in renal cell carcinomas and normal kidney. *J Urol* 156:506-511, 1996
- Sedlacek HH, Czech J, Naik R, et al: Flavopiridol (L86-8275, NSC-649890), a new kinase inhibitor for tumor therapy. *Int J Oncol* 9:1143-1168, 1996
- Drees M, Dengler W, Roth T, et al: Flavopiridol (L86-8275): Selective antitumor activity in vitro and activity in vivo for prostate carcinoma cells. *Clin Cancer Res* 3:273-279, 1997
- Senderowicz AM, Headlee D, Stinson SF, et al: Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms. *J Clin Oncol* 16:2986-2999, 1998
- Thomas J, Cleary J, Tutsch K, et al: Phase I clinical and pharmacokinetic trial of flavopiridol. *Proc Ann Meet Am Assoc Cancer Res* 38:1997 (abstr A1496)
- Elson PJ, Witte RS, Trump DL: Prognostic factors for survival in patients with recurrent or metastatic renal cell carcinoma. *Cancer Res* 48:7310-7313, 1988
- Schwartz GK, Farsi K, Maslak P, et al: Potentiation of apoptosis by flavopiridol in mitomycin-C treated gastric and breast cancer cells. *Clin Cancer Res* 3:1467-1472, 1997
- Bible KC, Kaufmann SH: Cytotoxic synergy between flavopiridol (NSC 649890, L86-8275) and various antineoplastic agents: The importance of sequence of administration. *Cancer Res* 57:3375-3380, 1997
- Motzer R, Vogelzang NJ: Chemotherapy for renal cell carcinoma, in Raghavan D, Scher H, Leibel S, et al (eds): *Principles and Practice of Genitourinary Oncology*. Philadelphia, PA, Lippincott, 1997, pp 885-896