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13. ABSTRACT (Maximum 200 Words) Huanglian is a botanical agent prepared as a tea form the roots of Coptis chinensis. We reported that huanglian potently inhibits the growth of a number cancer cells in vitro in a dose-dependent manner, with maximal inhibition at low micromolar concentrations (Li X. et al. <i>Molecular Pharmacology</i> , 58:1287-1293,2000). MCF-7 and MDA-468 breast cancer lines were particularly sensitive to huanglian. In addition, huanglian was also shown to enhance the effect of paclitaxel, supporting the future development of huanglian in combination with paclitaxel for the treatment of patients with metastatic breast cancer. The overall goal for this grant is to develop new therapeutic approaches in the treatment of patients with metastatic breast cancer based utilizing the Chinese botanical huanglian. The specific aims are to (1) conduct a phase I clinical trial of huanglian with both toxicity and efficacy endpoints, and (2) following the single agent trial, conduct a phase I/II clinical trial of huanglian in combination with paclitaxel in the treatment of patient with metastatic breast cancer. To date, we have entered 22 patients to the single agent trial of huanglian. We are currently evaluating a dose of 8.25 gm/day. We encouraged by preliminary evidence of clinical and biological activity.				
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Introduction

Huanglian is a botanical agent prepared as a tea from the roots of *Coptis chinensis*. In traditional Chinese medicine it has been used to treat inflammatory conditions ranging from gastroenteritis to acute febrile illnesses with no reported toxicity. We tested huanglian for activity against cancer at MSKCC. We reported that huanglian potently inhibits the growth of a number cancer cells in vitro in a dose-dependent manner, with maximal inhibition at low micromolar concentrations (Li X. et al. Molecular Pharmacology, 58:1287-1293, 2000). MCF-7 and MDA-468 breast cancer lines were particularly sensitive to huanglian. The activity of huanglian was greater than an equivalent concentration of its major component, berberine, suggesting that several components contribute to its anticancer effect. It was therefore decided to take whole huanglian to human trial, as a novel departure from the conventional approach in drug development in which a single active compound is selected and tested. In addition to single agent activity against breast cancer cell lines, huanglian was also shown to enhance the effect of paclitaxel, supporting the future development of huanglian in combination with paclitaxel for the treatment of patients with metastatic breast cancer.

Research Aims

The overall goal for this grant is to develop new therapeutic approaches in the treatment of patients with metastatic breast cancer based utilizing the Chinese botanical huanglian. The specific aims are to:

- 1) To conduct a phase I clinical trial of huanglian with both toxicity and efficacy endpoints.
- 2) Based on the results of the phase I clinical trial of single agent huanglian, conduct a phase I/II clinical trial of huanglian in combination with paclitaxel in the treatment of patients with metastatic breast cancer.

Reportable Outcomes

1. "A Phase I Study of the Chinese Herb Huanglian (*Coptis chinensis*) in Patients with Advanced Solid Tumors" (MSKCC Protocol Number 00-061A(6)) has now been open for the past year. The purpose of this study is to determine the optimal dose of huanglian for future phase II trials, especially for combination therapy. Patients with advanced solid tumors who have failed all conventional therapy or for which there is no conventional therapy are eligible for this study. Twenty-one patients have been registered to this study. One patient elected to withdraw consent after study registration and never received huanglian.

i) Study design, defining the MTD, and best response: The initial study design utilized a rapid dose escalation schedule of 1 patient/level and the huanglian dose was to be increased by 50% in successive cohorts. The starting dose of huanglian was 1 gm/day or one capsule (250 mg/tablet), p.o., 4x/day. At does level 3 (2.25 gm/day), one additional patient was added since the first patient developed progression of disease (POD) before

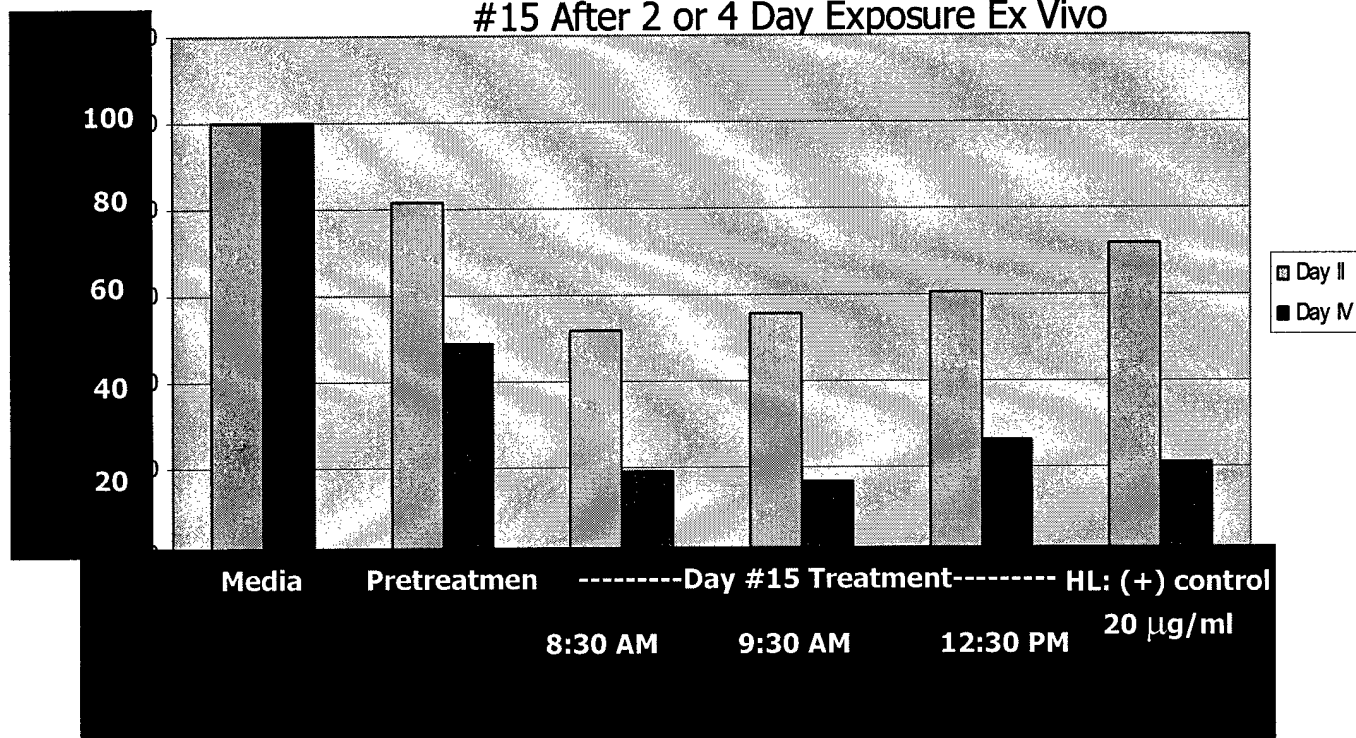
completing her assessment for toxicity. Using this study design, we safely escalated to a dose of 3.5 gm/day or 14 capsules in 4 divided doses. At a dose of 5.25 gm/day (21 capsules/day), one patient developed grade 3 diarrhea (DLT) and the cohort was expanded to 6 patients with no further DLTs noted. However, because of this toxicity, the study design now changed to a classic dose escalation schema of 3 to 6 patients/dose level and a 25% dose escalation in all successive cohorts. Utilizing this approach, we escalated to a dose of 6.56 gm/day (26 capsules/day) in 3 patients without DLT. In the next cohort of 8.25 gm/day (33 capsules/day), we again observed 1 patient with grade 3 diarrhea. This cohort has recently been expanded to 6 patients and therapy is still ongoing. These results are summarized in the table below, which also indicates stable disease as best response in several patients on the study, including 1 patient with metastatic breast cancer. Each of these patients had been progressing under observation or on therapy before entering the clinical trial with huanglian.

Cohort	Pt. #	Dose (gm/day)	Pill #	Toxicity	Best Response
1	1	1	4	0	Stable (colon): 6.4 months
2	1	1.5	6	0	Stable (neuro): 12.0 months
3	2	2.25	9	0	Stable (breast): 1 month
4	1	3.5	14	0	None
5	6	5.25	21	1: gr. 3 diarrhea	Stable (sarcoma): 6.5 months
6	3	6.56	26	0	Stable (renal): 8.0 months
7	6	8.25	33	1: gr 3 diarrhea	Stable (sarcoma): 4.5+ months

b) Pharmacokinetic (PK) and surrogate marker studies: Since berberine represents approximately 50% of the herb, we have been conducting PK measurements of berberine by HPLC. However, only very low levels of berberine (< 50 ng/ml) have been detected in the plasma of any patient on the study. We have also been testing plasma collected from patients on day #15 of therapy to determine if the patient's own plasma will inhibit cancer cell growth of the gastric cancer cell line MKN-74 ex vivo, as determined by SRB assays. For these studies, the patient's plasma is concentrated and the MKN-74 cells are exposed to the patient's own plasma for either 2 (II) or 4 (IV) days before the tumor cells are assayed for growth inhibition. As shown for the patient treated in cohort 3 (see graph below), the patient's own pretreatment plasma (day 0) was capable of inhibiting MKN-74 cell growth, even though the patient had been off all chemotherapy for at least 4 weeks. Three separate samples were then drawn on day 15 of treatment at 8:30 AM, 9:30 AM, and 12:30 PM. As shown, when the MKN-74 cells were exposed ex vivo to the patient's plasma for 2 (II) or 4 (IV) days, there was inhibition of cell growth in all three samples when compared to the patient's pretreatment plasma for both the 2 and the 4 day assay. The last lanes show the positive control of media "spiked" with huanglian (HL) at a concentration of 20 µg/ml and exposed to MKN-74 cells for both 2 and 4 days. This degree of growth suppression confirms the activity of the herb from the capsules.

Despite these promising results, this pattern of growth inhibition, which has varied from 0 to 60% in the patients on the study, has been highly variable from patient to patient. It has also shown no correlation to dose or to treatment response. Nevertheless, it does suggest some degree of biologic activity from the preparation under investigation. Since huanglian has also been shown to inhibit cyclin B1 protein expression in the same cell line, we have also performed western blots for cyclin B1 expression in the MKN-74 cells in these same samples. For these studies the cyclin B1 was not detectable at baseline and considered non-informative or showed no suppression in this ex vivo assay.

977984-HLTx ND Control
Inhibition of MKN-74 Cell Growth by SRB From Patient Plasma on Day #15 After 2 or 4 Day Exposure Ex Vivo



Conclusions and Future Plans:

i) The immediate plan is to complete this phase I study of huanglian, so as to define the MTD and then test this in phase II clinical trials. We are especially encouraged by stable disease in patients with advanced cancers (breast, renal, sarcoma, and neuroendocrine tumors) who were progressing either under observation or on chemotherapy. These patients had no other treatment options at the point of study entry.

It is conceivable that for huanglian we may not be able to define an MTD with standard grade 3 and 4 dose-limiting toxicity. Instead the highest non toxic dose may be

determined by the number of capsules consumed at any one time. In fact, if we do escalate to our next cohort, without a second DLT at the current dose level, patients will be taking 41 capsules/day, divided in 4 doses. In its current formulation, this may represent a pill count which will exceed that which a patient can take at any given time. The SRB assays on the MKN-74 cells from the patient's own plasma indicate a pharmacodynamic effect, even at doses of huanglian as low as 1.5 gm/day (cohort 2). Though this has not correlated to treatment response, and it has not achieved our target of 50% inhibition of cell growth in all patients, it does suggest that we are achieving plasma levels of huanglian that can induce some degree of a biologic effect. Without tumor biopsies to indicate biologic effects in the tumor, it is impossible to know what in fact is taking place in the tumor. However, it does suggest that we are obtaining plasma levels of some component of huanglian (other than berberine, which is non-measurable by HPLC) which is capable of inhibiting tumor growth *ex vivo*. Thus, in the case of huanglian it may be possible to select a dose that is considerably below the MTD for future clinical development.

ii) New 500 mg capsules: At our current dose level patients are taking 33 capsules in 4 divided doses. Though not in itself dose-limiting, the large number of capsules administered each day has resulted in symptoms of early satiety. Though patient compliance with taking this number of capsules each day has been excellent, we believe that, if further dose escalations are required, the capsule number may in itself become a dose-limiting issue, even in the absence of other dose-limiting toxicities. In order to address this, we are in the process of making 500 mg (rather than 250 mg) huanglian capsules for future clinical trials. Phoenix Laboratories, our outside contractor, has agreed to make these additional capsules at no cost to our institution. Without even a change in capsule size or formulation, we believe we can pack an additional 250 mg of huanglian into each capsule. We are in the process of identifying and testing several new lots of huanglian root for biological activity (SRB assay, cyclin B1 suppression) and chemical composition by HPLC. Once we identify a lot that meets our predetermined specification for drug development, we will begin the process of making 500 mg huanglian capsules.

iii) Once the phase I clinical trial of single agent huanglian is completed (2004), we will initiate our combination studies with paclitaxel in the treatment of metastatic breast cancer. For these next clinical studies we will be to take the highest non-toxic dose or that dose which gives us our greatest biologic effect *ex vivo*. This dose will then be used to conduct the phase I/II trial of huanglian and paclitaxel in 20 to 30 patients with metastatic breast cancer. This study is based on preclinical studies indicating that huanglian enhances the effect of paclitaxel *in vitro*. There is no evidence of any antagonism between huanglian and paclitaxel *in vitro*. Based on the current phase I trial of single agent huanglian, we also anticipate no serious or adverse events greater than that which is achieved with single agent paclitaxel alone. We anticipate starting this clinical trial within the next year.