USC Norris Comprehensive Cancer Center
Part of the Keck School of Medicine of USC

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Protocol Title: A Phase II Study of Intra-Arterial Chemotherapy with Cisplatin and Mitomycin-C in Patients with Hepatocellular Carcinoma

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PROTOCOL NUMBER:	3L-03-1
TITLE:	A Phase II study of intra-arterial chemotherapy with cisplatin and mitomycin-C in patients with Hepatocellular Carcinoma
SITE:	Liver
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STAGE:	Unresectable but confined to the liver
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TYPE:	Phase II
ARMS:	Single
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1.3.1 To examine associations of molecular markers that may predict response, survival and toxicity in patients with HCC receiving intra-arterial chemotherapy with cisplatin and mitomycin-C.

2.0 Background and Hypotheses

2.1 Incidence of Hepatocellular Carcinoma

Hepatocellular carcinoma causes about 1 million deaths annually, with most of these deaths occurring in the Far East and in sub-Saharan Africa. Accordingly, the disease is uncommon in the United States and Western Europe with an annual incidence of about 3 cases /100,000 population. The disease is predominantly found in males. There is evidence that Hepatitis B infection alone or in combination with other factors leads to most cases worldwide. Hepatitis C infection is also strongly associated with HCC. Additionally, about 80% of patients with HCC have cirrhosis. Other potential etologies that have been implicated in this disease include: aflatoxins produced by Aspergillus flavus and related fungi, long-term use of oral contraceptives and alcohol abuse, which is modestly associated with HCC but markedly increases the risk in patients with Hepatitis C infection.

2.2 Current Strategies for Treatment of Hepatocellular Carcinoma

Definitive treatment of hepatoma generally involves resection, local ablation or transplantation. Advanced stage or hepatic cirrhosis limit resection options, and donor liver supply restricts the transplantation option. Regional arterial therapy may offer palliation and life extension and also may be used in individual cases during the wait for a donor liver.

Intravenous chemotherapy usually results in low objective response rates, a median survival of 3 to 6 months, and a 2 year survival of 5% and has not shown a survival benefit over palliative care.[1] [2] [3] [4] [5] Older published intra-arterial chemotherapy experience tends to be similar to intravenous chemotherapy, with median survival time of 6 months, although longer survival times have been reported.[6] [7] [8] [9] Recently a study was done in patients done in patients with advanced hepatocellular carcinoma (patients in which surgery or locoregional treatments are not indicated as disease is not confined to the liver). Patients with Child-Pugh status A were randomized to receive sorafenib 400 mg bid or placebo. This trial was stopped at the interim analysis, as based on 321 deaths (sorafenib n=143, placebo n=178), the hazard ratio for overall survival (sorafenib vs. placebo) was 0.69 (95% CI: 0.55, 0.87; p=0.0006), representing a 44% improvement in overall survival. Median overall survival was 10.7 for sorafenib versus 7.9 months for placebo. Sorafenib is the first agent to demonstrate a statistically significant improvement in overall survival for patients with advanced hepatocellular carcinoma. This effect is clinically meaningful and establishes sorafenib as first-line treatment for these patients.[10]

Chemoembolization is the concurrent administration of drug and particle to increase drug dwell time within the target. Chemoembolization experiences have been reported using a variety of agents and catheter techniques. We have submitted a Phase II chemoebolization study in 85 hepatocellular carcinoma patients who were not considered to be operative candidates.[11] In this study the median survival time was 12.7 months.

The current treatment standard at USC is intra-arterial drug administration. Intra-arterial drug administration became more refined with the introduction of steerable micro-catheters that facilitate selective access through the femoral artery.[12] Placement of the catheter tip within selected intra-hepatic branches reduces the risk of non-target perfusion or embolization and enhances drug delivery to the target. Our current non-protocol experience suggests that we can expect response rates in the range of 50% and that toxicity requiring hospitalization is rare.

Intra-arterial chemotherapy is currently the standard of care in patients with unresectable hepatocellular carcinoma and disease confined to the liver. This is mainly due to the results of two studies in this patient population. In one study 80 patients were randomized to receive a) chemoembolization with an emulsion of cisplatin in lipiodol and gelatin-sponge particles through the hepatic artery or b) symptomatic treatment. The results showed a survival advantage for the chemoembolization group (1 year, 57%; 2 years, 31%; 3 years, 26%) over the symptomatic treatment group (1 year, 32%; 2 years, 11%; 3 years, 3%; P =.002). When adjustments for baseline variables that were prognostic on univariate analysis were made with a multivariate Cox model, the survival benefit of chemoembolization remained significant (relative risk of death, 0.49; 95% CI, 0.29-0.81; P =.006).[13] A study by Llovet et. al randomized patients to receive a) arterial embolization with gelatin sponge b) chemoembolization with gelatin sponge plus doxirubicin or c) conservative treatment (palliative care). This study was stopped when an interim analysis showed chemoembolization

had survival benefits compared with conservative treatment (hazard ratio of death 0.47 [95% CI 0.25-0.91], p=0.025). Survival probabilities at 1 year and 2 years were 75% and 50% for embolization; 82% and 63% for chemoembolization, and 63% and 27% for control (chemoembolization vs control p=0.009). Chemoembolization induced objective responses sustained for at least 6 months in 35% (14)of cases, and was associated with a significantly lower rate of portal-vein invasion than conservative treatment. Treatment allocation was the only variable independently related to survival (odds ratio 0.45 [95% CI 0.25-0.81], p=0.02).[14]

Although intra-arterial chemotherapy is standard of care in this patient population, no evidence indicating the best chemotherapy agents to use, thus regimens based on doxorubicin, mitomycin and cisplatin are commonly used as they have shown benefit. [13] [14] [15] The goal of this therapy is to shrink tumors enough that they may benefit from radiofrequency ablation or cryotherapy.

Percutaneous image-guided radiofrequency ablation (RFA) uses thermal energy for the local treatment of solid malignancies. It has been shown to be safe and effective for the treatment of hepatocellular carcinomas, particularly those less than 5 cm. It has been shown to be more effective than percutaneous alcohol injection for the treatment of small hepatocellular carcinomas < 3cm.

Percutaneous RFA is typically performed under conscious sedation. With direct image guidance by ultrasound or CT, a 12-14g multi-tine needle is introduced to the target tumor within the liver. The tines are deployed and their position ascertained with imaging. Radio-frequency energy is then applied according to a standard escalating protocol to produce an appropriate area of tissue necrosis. At the conclusion of the ablation program the needle tract is treated as well, if possible, to preclude tumor seeding.

A limitation of RFA treatment is the inability to reliably create a large enough area of complete thermal tumor destruction and to unequivocally include the actual tumor margins that may be inaccurately inferred from imaging. Tumor foci may survive RFA because of biophysical limitations such as perfusion-mediated tissue cooling which occurs near large vessels or because foci of tumor exist beyond the margin of burn. Additionally, RFA may fail due to multi-focal disease.

2.3 Current proposed therapy

In the current study intra-arterial chemotherapy will be administered twice at an 8 week interval (up to 4 times at physician discretion). Response to the initial chemotherapy will result in reduced volume, better-delineated margins and treatment of satellite foci.

2.4 Prognostic Index

A Prognostic Index (PI) for patients with hepatoma was developed from the USC chemoembolization study.[11] The Prognostic Index effectively identifies patient groups most likely to benefit from chemoembolization. Patients in the more favorable prognostic groups had higher objective

response rates, and responders appeared to live longer than non-responders.

The PI described is calculated by adding two points for elevated LDH and 1 point each for abnormal ALP (>200 u/L), abnormal AST (>100 u/L), and presence of ascites on baseline imaging. LDH, the dominant component of this index, is often cited as a prognostic indicator for outcome in malignant disease.[16] [17] [18] In hepatoma, one would expect increases in LDH both from the tumor and as a function of liver injury. When established prognostic systems were compared as predictors for this patient population, only the Okuda stage was significantly associated with survival. The superiority of the PI developed during this analysis is shown in Table 1 below. Both a lower p-value and a higher correlation coefficient are associated with the PI, demonstrating its superiority as a prognostic indicator for survival.

Responses were usually obtained for patients with PI scores between 0 and 2 and response in these groups was associated with meaningful extension of survival. Confirmation of the usefulness of the PI as a tool for prognostic evaluation of individual cases needs to be done with further studies of additional patients with hepatoma.

Table 1

Group	N	# Deaths	MST (mo.)	Estimated Survival (%)					p-value	RR
				1-yr	2-yr	3-yr	4-yr	5-yr		
All Patients	85	73	12.7	52	26	16	11	8		
PI – 0	24	19	25.9	85	58	39	24	14	<0.0001	1
PI - 1	20	17	13.2	56	13	11	11	11		1.8
PI - 2	10	10	15.3	55	25	5	0	0		1.3
PI - 3	10	9	5.4	15	-	-	-	-		4.3
PI – 4	11	11	2.9	0	0	0	0	0		10
PI - 5	6	6	2.3	0	0	0	0	0		9.5

2.5 Molecular Correlates

2.5.1 Apoptosis

By understanding the role that some major regulators of apoptosis play either at the commitment or execution phases of cell death in hepatocellular carcinoma tissue, we will be in a better position to design and explore new therapeutic modalities. The bcl-2 and ICE gene family products are intrinsic proteins regulating the decision of a cell to survive or die and executing part of the cell death process. Among the various bcl-like proteins, the effects and functions of the bcl-x and bax proteins in controlling apoptosis induced by cancer chemotherapy have been recently studied. In human cancer variant cell lines show differential expression of the bcl-xL protein. A preventive effect of bcl-xL on cell death induced by various cytotoxic drugs has been observed, with greater effects in cells containing the highest level of bcl-xL expression. Similarly, overexpression of bax in cancer cell lines sensitizes these cells to some cancer chemotherapy compounds. Modulation of apoptosis either negatively by bcl-xL or positively by bax resides downstream of the primary mechanism of action of anticancer drugs, suggesting that they act primarily as intrinsic control points following cytotoxic drug injuries.[19-21] We will test the hypothesis that gene expression levels of bcl-2, bcl-xL, bcl-xS and bax will predict response and survival in patients with hepatocellular carcinoma treated with intra-arterial mitomycin-C and cisplatin.

2.5.2 DNA repair

The presence of intact DNA repair is an important prognostic indicator for colorectal cancer. In studies of colorectal cancer cell lines containing DNA mismatch repair gene mutations (MMR), lack of response to chemotherapeutic agents in vitro is found in cell lines with microsatellite instability (MIN) which is the landmark for DNA mismatch repair gene defects, where tumors without MIN remain sensitive. For example, HCT 116 cells lack hMLH1 function, and are insensitive to 5-FU in vitro. When

chromosome 3, which contains hMLH1, is transferred to HCT116 cells, mismatch repair function is restored, and cells are inhibited by culture in 5-FU.[22] Colorectal cancer cell lines with MMR defects are resistant to methylating agents.[22] Lothe et al demonstrated, using PCR-based analysis of 7 microsatellite loci, that 17% of patients without and 31% of patients with a strong family history of cancer exhibited changes at one or more loci.[23] Patients with tumors expressing MIN exhibited increased survival when compared to patients with tumors lacking MIN. Recently, Garioboldi et al reported that human colon cancer cell line HCT116, which is defective in mismatch repair, is hypersensitive to mitomycin-c.[24] HCT116 cells that had undergone transfer with human chromosome 2 or 3 to correct the mismatch repair deficiency were 3- to 4-fold resistant to CPT-11. These results suggest that DNA mismatch repair is involved in processing CPT-induced DNA damage, and that mitomycin-c and its derivatives may be selective for tumors with mismatch repair deficiency. We will test the hypothesis that the DNA mismatch repair status will be a predictor of response and survival in patients with hepatocellular carcinoma treated with intra-arterial mitomycin-c and cisplatin.

Moreover, analysis of the 7 human colon carcinoma cell lines of the NCI Anticancer Drug Screen showed that defects in replicon elongation and G2 breakthrough capability correlate with sensitivity to mitomycin-c, suggesting that misrepair of damaged replicons and/or alterations in DNA damage checkpoints is critical to defining chemosensitivity to CPT-induced DNA damage.[25] Analysis of expression of different genes potentially involved in DNA repair and in cell responses to chemotherapy showed a significant correlation between topoisomerase I and ERCC1 expression indicating some regulatory relationship between these two genes.[26] ERCC1 is an essential enzyme for nucleotide excision repair. Recently, our group demonstrated that ERCC1 is a significant predictor of response to cisplatin in patients with gastric cancer.[27] DNA repair enzymes such as ERCC1 may play some role in repairing DNA damage induced by mitomycin-C and therefore may be a predictor of therapy efficacy.[28] We will test the hypothesis that ERCC1 gene expression will predict response and survival in patients with hepatocellular carcinoma treated with intra-arterial cisplatin and mitomycin-C.

2.5.3 p53 and p21

Clinically relevant exposures to platinum compounds will lead to a tumor injury response (TIR) that shares characteristics with the response produced by many other injurious agents. Although very little is known about the specifics of the signal transduction pathway activated by platinum injury, it is possible to identify the general nature of this response. It is thought that the capacity to induce growth arrest in response to injurious conditions arose from the advantages of delaying replication of defective DNA templates until damage has been repaired (G1/S arrest) and delaying the segregation of sister chromatids until DNA breaks have been corrected (G2/M). The function of p53 is to send cells with damaged DNA either into G1 arrest for repair or into programmed cell death through apoptosis.[29, 30] Neither G1 arrest nor the apoptotic program is initiated by drug treatment in cells with mutated p53 genes, suggesting that tumors with mutant p53

might be resistant to DNA damaging agents. *In vitro* studies confirmed that p53 is a major modulator of sensitivity to drugs such as 5-FU, etoposide, adriamycin and cisplatin.[31, 32] Tumors in mice expressing wt p53 contained a high proportion of apoptotic cells and regressed after radiation or adriamycin, whereas tumors expressing mutated p53 did not respond to treatment indicating the relevance of p53 as a predictor of response to chemotherapy.[33] p53 is not only an important predictive marker but also a possible therapeutic agent. In fact, Fujiwara and colleagues were able to increase the cellular sensitivity to cisplatin by transfection of wt p53 into tumor spheroids of human non-small cell lung cancer cells.[34] All these results have strengthened the image of p53 as a predictor of chemosensitivity and have obvious clinical relevance for GI cancer, which has up to 70% p53 mutations. Many of the effects of p53 such as G1 cell cycle arrest and apoptosis in most cases can be attributed to biological functions of downstream p53-regulated genes, such as WAF1/CIP1.[35] WAF1/CIP1 plays an important role in the p53 induced G1 arrest by coordinately inhibiting the functions of both CDKs and PCNA which arrest DNA replication while permitting active DNA repair.[29, 36] WAF1/CIP1 has been shown to be directly induced by wt p53 in vitro but its expression is lost when active p53 is absent.[37] While the presence of p53 mutations can be easily established, to date no easily measurable biochemical parameter has been identified that would be indicative of p53 function. WAF1/CIP1 is thought to be a direct mediator of p53 cell-cycle regulation activity and p53-mediated apoptosis.[36, 38] These findings suggest that WAF1/CIP1 expression levels can be used as a direct indicator of p53 functionality in cells. That is, low or absent expression of WAF1/CIP1 should indicate a lack of p53 function while partial expression of WAF1/CIP1 should indicate some retention of p53 activity.

These findings suggest that p21 level may be useful as an indicator of p53 function in cells and may be a link between p53 status and TS expression. If p21 expression is a reflection of the p53 function, low or absent expression of p21 may indicate a lack of p53 transcriptional activity, while partial expression of p2l should indicate some retention of p53 activity. p21 expression may not only help to identify p53 mutations which may have partial wild-type activity but may also help to identify tumors with false negative p53 staining due to deletion, non-sense and misplaced mutations. However, p21 may be regulated in a p53-independent manner through activation of the type II TGFβ receptor.[35] Therefore, p21 expression may occur in the face of mutant p53 through this p53-independent pathway. We will test the hypothesis that p53 status and p21 expression levels are predictors of response, tumor recurrence and survival in patients with hepatocellular carcinoma treated with intra-arterial cisplatin and mitomycin-C.

Cancer cells may also exhibit imbalance of cell cycle regulation and fail to arrest in G1 or G2 to prevent further lesions and/or to repair existing DNA damage. p53 and p21 play an important part in DNA damage checkpoint, cell cycle arrest and initiation of apoptosis. The relationship between chemosensitivity and p53 is currently considered from two mutually exclusive points of view: (1) wt p53 increases chemosensitivity due to apoptosis and (2) wt p53 decreases chemosensitivity due to growth arrest

and DNA repair. A p53-expressing adenovirus (Ad-p53) was used to directly evaluate effect of p53 on sensitivity to anticancer drugs. When p53 was expressed at sublethal levels, it sensitized cells to the DNA-damaging drugs such as adriamycin, cisplatin and mitomycin-C. This sensitization was observed in cancer cell lines regardless of endogenous p53 status. The degree of sensitization appeared to be greater in cancer cells with mutant p53, indicating that p53 may play an important role in chemoresistance.[39]

2.5.4 Ribonucleotide Reductase

Recent evidence suggests that in addition to its essential role in DNA synthesis, Ribonucleotide reductase (RR) plays a critical role in DNA repair and is induced by DNA damaging drugs.[40] Furthermore, Fan et al have demonstrated that overexpression of the M2 subunit plays an important role with ras in malignant progression.[41] M2 overexpression, as expected, produces resistance to hydroxyurea. Our own data suggest that M2 mRNA expression, measured by RT-PCR in tumors, is 10-20 fold higher than expression in adjacent normal tissue, indicating that RR may play a role in chemoresistance to DNA damaging drugs (unpublished data). In this study we will test whether the expression levels of RR will predict to resistance to intra-arterial cisplatin and mitomycin-C in for patients with hepatocellular carcinoma.

2.5.5 Metallothioneins

Elevation of glutathione (GSH) is widely observed in cellular resistance to platinum agents.[42] Previous studies have shown that sublines of human ovarian carcinoma cell line A2780, which exhibited low levels of resistance to oxaliplatin, showed elevated steady state levels of mRNA and activity of gamma-glutamyl transpeptidase (gamma-GT, EC 2.3.2.2), but not of gamma-glutamylcysteine synthetase (gamma-GCS, EC 6.3.2.2).[43, 44] Single exposures of cells to oxaliplatin induced a time- and concentration-dependent increase in the mRNA of gamma-GT, but not of gamma-GCS. Cisplatin also induced an elevation in gamma-GT mRNA, but to a lower degree. The gamma-GT enzyme activity increased corresponding to the elevation in mRNA expression. The gamma-GT-induced cells showed an increase in cellular GSH when incubated in medium containing GSH. The data suggest that a) single, brief exposures to pharmacologically relevant concentrations of platinum complexes induce elevation in mRNA of gamma-GT, b) elevation in gamma-GT mRNA translates into elevated gamma-GT activity and increase in GSH salvage, and c) the degree of induction of gamma-GT mRNA differs between platinum complexes. Elevation of glutathione (GSH) is commonly observed in cellular resistance to a number of anticancer agents. The most frequently reported changes in GSH metabolism that are associated with the elevated GSH levels are increased mRNA expression and activity of gamma-glutamyl cysteine synthetase (gamma GCS), the first enzyme of the GSH biosynthetic pathway. Sublines of the A2780 ovarian carcinoma cell line (C10 and C25) have been shown to be 8- and 12-fold resistant to oxaliplatin by repeatedly exposing the cells to increasing concentrations of the platinum agent. The GSH levels in C10 and C25 cell sublines are 3.1-and 3.8-fold higher than the parent A2780 cell line. The increase in GSH in C10 and C25 was

associated with an elevation in gamma GT mRNA (2.5- and 8-fold) and gamma GT activity (2.7- and 2.8-fold). No changes were observed in gamma GCS mRNA levels or activity. The data indicate that alterations in GSH metabolism leading to elevations in cellular GSH in A2780 ovarian carcinoma cells selected for low levels of resistance to oxaliplatin are mediated by gamma GT, the “salvage’ pathway, rather than an increase in GSH biosynthesis.[42, 44] In this study we will test whether mRNA levels of gamma glutamyl transpeptidase are predictive of response to intra-arterial cisplatin and mitomycin-C for patients with hepatocellular carcinoma.

2.5.6 GST-P1

Glutathione transferases consist of a super-family of phase II metabolic enzymes that catalyze the conjugation of reduced glutathione. The detoxifying character of these reactions is responsible for the protection of cellular macromolecules from damage caused by carcinogenic and cytotoxic agents.[45, 46] GSTP1-1 has been shown to be widely expressed in human epithelial tissues and to be over-expressed in several tumors including colon tumors.[47, 48] [49, 50] Increased levels in tumors may be in part responsible for the observed resistance to chemotherapy as it has been found in several tumors, but the mechanism still remains unknown.[51] Factors that influence the expression level of GSTP1 may become important tools to predict therapy response and survival of patients treated with certain drugs or drug combinations.

A G→A transition in exon 5 at nucleotide 313 leads to an amino acid exchange in the protein from isoleucin to valine, as previously reported by Board and colleagues.[52] *In-vitro* cDNA expression studies revealed an association between this amino acid change and a reduced activity level of the GSTP1 enzyme.[53] Recently it has been found that the 105Val allele variant of the GSTP1 gene at exon 5 is associated with a low GST enzyme activity in normal lung tissue and esophageal Barrett’s epithelium.[54, 55] Additionally, it has been shown that the 105Val allele is associated with increased risk for testicular, bladder cancer and esophageal carcinoma, but not for colon or breast cancer.[56] This Ile105Val substitution has been shown to be associated with better survival in women with breast cancer who received chemotherapy (cyclophosphamide, 5-FU, adriamycin).[57] Nishimura and collegues showed that the response rate of patients with head and neck cancer receiving platinum-based chemotherapy was significantly higher for patients with low GST protein expression.[58] Based on these encouraging data we genotyped 81 patients with advanced colorectal tumors that received combination chemotherapy of 5-FU/oxaliplatin. We found a significant association between survival and the Ile105Val polymorphism at exon 5 of the GSTP1 gene. Patients homozygous for the amino acid substitution had a significant survival benefit. According to previous *in-vitro* reports and studies in different human tissues the Val/Val genotype is associated with a lower GST enzyme activity compared to the heterozygous and the ILE/ILE genotype.[54, 57] Considering these results, patients with the VAL/VAL genotype and respectively a lower GST enzyme activity benefit from treatment with 5-FU/oxaliplatin compared to heterozygotes and the ILE/ILE genotype group. A lower GST enzyme activity is thought to be less efficient

in glutathione conjugation of drug intermediates, which leads to a longer and most likely more efficient exposure of the active drug to the tumor cell. This might explain the survival benefit for patients with two or at least one VAL allele compared to the ILE/ILE genotype. In this study we will test whether mRNa levels of GST-P1 and the 105Val allele variant of the GSTP1 gene at exon 5 are predictive of response to and survival after intra-arterial cisplatin and mitomycin-C for patients with hepatocellular carcinoma.

2.5.7 ERCC1 and XRCC1 polymorphisms determine cisplatin resistance

Surveillance and repair of DNA damage are essential for maintaining the integrity of the genetic information that is needed for normal development. Several multienzyme pathways, including the excision repair of damaged or missing bases, carry out DNA repair in mammals, including excision repair of cisplatin-induced damaged. Accumulating evidence suggests that increased repair of cisplatin-induced DNA damage by either increased levels of repair enzymes or polymorphic variants is associated with cisplatin resistance.

ERCC1 (excision repair cross complementation group 1) is an essential and highly conserved enzyme specific to the nucleotide excision repair (NER) pathway.[59] Defects in ERCC1 seem to be associated with the most severe DNA repair deficiency.[60] Studies have shown that increased ERCC1 mRNA levels are directly related to clinical resistance to cisplatin in both human ovarian and cervical cancer.[28, 61] Lenz and colleagues have shown that ERCC1 mRNA levels are also directly correlated to clinical resistance to combination 5-FU/cisplatin in gastric cancer patients and with response and survival in patients with metastatic colorectal cancer treated with combination 5FU/oxaliplatin.[27, 62] More recently, Lenz and colleagues have shown that a very common polymorphism at codon 118 (exon 4) in the ERCC1 gene, resulting in a single nucleotide C→T change, correlates with significantly different intratumoral levels of mRNA, response to 5FU/oxaliplatin and survival in patients with colorectal cancer (unpublished data). Patients with the T/T or C/T genotype were significantly more likely to have elevated ERCC1 mRNA levels than patients with the C/C genotype ($p = 0.049$). The median survival of patients with at least one copy of the T allele was 256 days compared to 531 days without ($p = 0.056$) (unpublished data).

XRCC1 is a DNA repair gene with a central role in single strand break repair, base excision repair and optimal activity of DNA ligase III. It has been shown to be essential for mammalian (murine) survival and cells lacking XRCC1 are extremely sensitive to ionizing radiation and alkylating agents and exhibit elevated spontaneous frequencies of chromosome aberrations.[63] Transfection of functional murine XRCC1 into EM9 cells efficiently corrected (94-100%) the high sister-chromatid-exchange defect.[64]

XRCC1 polymorphisms have been associated with different susceptibilities to DNA damaging agents and to the development of certain malignancies. Cigarette smokers with the polymorphic XRCC1 399Gln allele have been shown to have significantly more DNA adducts and sister chromatid

exchange (SCE) frequencies than smokers with the 399Arg/Arg genotype.[65] Polymorphisms in codon 399 have been associated with higher AFB1-adducts and GPA somatic mutations as well as with lung cancer risk, colon cancer risk in Egyptians, and prostate cancer risk. A polymorphism in exon 6 has been shown to have a protective effect against bladder cancer development.[66-68]

Based on these data, Lenz and colleagues investigated whether the XRCC1 allele 399 polymorphism correlated with response in 45 patients with colorectal cancer treated with a new platinum agent, oxaliplatin, combined with 5FU. The XRCC1 399 Arg/Arg. Arg/Gln and Gln/Gln polymorphisms were found in 18, 22 and 5 patients, respectively. The Arg/Arg polymorphism was strongly associated with chemotherapy response (observed in 5 of 6 responding patients); the Gln/Gln polymorphism was strongly associated with chemotherapy resistance (4 of 5 patients with the Gln/Gln genotype had disease progression) ($p = 0.0063$, 99% CI = 0.0043 – 0.0083, two-sided testing) (unpublished data). In this study we will test whether ERCC1 and XRCC1 polymorphisms are predictive of response to and survival after intra-arterial cisplatin and mitomycin-C for patients with hepatocellular carcinoma.

2.5.8 XPD

The XPD protein is thought to participate in transcription and nucleotide excision repair. Several polymorphisms in the XPD gene have been identified, though their functional status is still uncertain. Recently, a polymorphism in codon 751 of the XPD gene was shown to be associated with differential DNA repair proficiency.[69] This single nucleotide polymorphism (C→A) causes an amino acid change Lys→Gln at codon 751. Lymphocytes from individuals with the Lys/Lys genotype were shown to have higher number of chromatid aberrations after exposure to X-rays compared to those with the Gln allele, thus suggests a decreased ability for DNA repair. Increased DNA repair is a well-established mechanism for chemoresistance, especially platinum based compounds. Our hypothesis is that patients with the Lys allele will respond at a higher rate to platinum compounds compared to those without the Lys allele.[65, 69] We assessed the XPD codon 751 polymorphic status of 62 patients with metastatic colorectal cancer and determined their response to 5-FU/oxaliplatin treatment. The overall response rate was 16% (10/62). We found that 25% (5/20) of patients with the Lys/Lys genotype responded, compared to 13% (4/32) and 10% (1/10) of those with the Lys/Gln and Gln/Gln genotypes respectively ($p= 0.017$ Fisher's exact test). More significantly, of those with the Gln/Gln genotype, 50% (5/10) had progressive disease compared to 10% (2/20) and 6% (2/32) of patients with the Lys/Lys and Lys/Gln genotypes respectively. To our knowledge this was the first study that shows an association between the XPD polymorphism at codon 751 and clinical response to chemotherapy. It is our conclusion that genotyping patients for the XPD codon 751 polymorphism may be useful in predicting clinical response to platinum based chemotherapy. This may in turn aid in the selection of patients who will most likely benefit from the treatment, as well as avoiding the risk for toxic side effects for patients who are not likely to respond to the agent. In this study we will test whether XPD

polymorphisms are predictive of response to and survival after intra-arterial cisplatin and mitomycin-C for patients with hepatocellular carcinoma.

3.0 Drug Information

3.1 Cisplatin

3.1.1 Description

Cisplatin is a heavy metal complex containing an atom of platinum surrounded by two chloride molecules and two ammonia molecules in the cis configuration. It is soluble in water or saline.

3.1.2 Drug Administration

Patients will receive intravenous hydration pre and post the administration of cisplatin, consisting of at least one liter of NS 150 cc/hr. Prior to the cisplatin administration enough hydration should be given to establish a urine output of at least 75 cc/hr. Cisplatin will be administered as a 5 to 10 minute intra-arterial infusion diluted in 0.9% Sodium Chloride to a concentration of 1 mg/mL. Needles or intravenous sets containing aluminum parts that may come in contact with cisplatin should not be used for preparation or administration. Aluminum reacts with cisplatin, causing precipitate formation and a loss of potency.

3.1.3 Storage and Stability

Cisplatin is supplied as Platinol-AQ in 10 mg and 50 mg vials. It is stored at room temperature. Each vial is labeled with an expiration date.

3.1.4 Toxicity

Dose related and cumulative renal toxicity of cisplatin, but it may be ameliorated by vigorous hydration. Ototoxicity may occur and is manifested by tinnitus and/or hearing loss in the high frequency range. Nausea and vomiting are also frequently seen. Less frequent toxicities include hyperuricemia, hypomagnesemia, peripheral neuropathy, myelosuppression and anaphylactoid reactions.

3.1.5 Source of drug

This drug is commercially available.

3.2 Mitomycin-C

3.2.1 Description

Mitomycin-C exerts its cytotoxic activity by a mechanism similar to that of the alkylating agents. The drug is converted to an active compound that forms cross-links between strands of DNA, inhibiting DNA synthesis. Mitomycin also inhibits RNA and protein synthesis to a lesser extent.

3.2.2 Drug Administration

Mitomycin-C will be administered as a 5 to 10 minute intra-arterial infusion diluted in 0.9% Sodium Chloride to a concentration of .5 mg/mL.

3.2.3 Storage and Stability

Mitomycin-C is supplied in 5 mg, 20 mg and 40 mg vials. It is stored at room temperature. Each vial is labeled with an expiration date.

3.2.4 Toxicity

Thrombocytopenia and leukopenia may be delayed up to eight weeks and may be cumulative with succesive doses. Frequent side effects include nausea, vomiting, anorexia, diarrhea, reversible alopecia, and fever. Less frequent, but serious side effects include interstitial pneumonitis, septicemia and microangiopathic hemolytic anemia (characterized by thrombocytopenia, renal failure and hypertension).

3.2.5 Source of drug

This drug is commercially available.

4.0 Staging

This is a phase II study open for patients with hepatocellular carcinoma, with evident disease limited to the liver. Patients should be staged for their disease by the appropriate, TNM staging system (sixth edition), of the American Joint Committee on Cancer.

5.0 Eiligibility Criteria

5.1. Inclusion Criteria

5.1.1. Unresectable hepatocellular carcinoma (HCC), with evident disease limited to liver.

The diagnosis of hepatocellular carcinoma should be based on at least one of the following (a-c):

a. The presence of one or more liver lesions, measuring ≥ 2 cm, with characteristic arterial enhancement and venous washout in the setting of liver cirrhosis and/or hepatitis B or C infection

b. The presence of liver lesion(s) with AFP ≥ 400

c. Tissue confirmation in the absence of a and/or b

Tissue availability is desired and will be sought, but tissue availability is not mandated for accrual to the study

5.1.2. Patients must agree to have a 20 cc blood sample drawn in addition to routine labs at baseline.

5.1.3. Patients must have measurable disease. If prior radiation therapy was administered, measurable disease must be outside the radiation field.

5.1.4. Patients must be ≥18 years of age.

5.1.5. Patients must have a Zubrod performance status of 0-2.

5.1.6. Patients must have a predicted life expectancy of at least 12 weeks.

5.1.7. Patients must have a pre-treatment granulocyte count (i.e., segmented neutrophils + bands) of $\geq$ 1,500/mm^3, a hemoglobin level of $\geq$ 9 gm/dl, and platelet count $\geq$ 50,000/mm^3. The granulocyte requirement may be waived if in the investigator's opinion the lower count reflects hypersplenism with adequate bone marrow reserves.

5.1.8. Patients must have adequate renal function as documented by a calculated creatinine clearance > 50.

5.1.9. Patients must have adequate hepatic function as documented by a serum bilirubin $\leq$ 2x the institutional upper limit of normal, regardless of whether patients have liver involvement secondary to tumor. Patients may not have ascites or the ascites must be responsive to diuretics.

5.1.10. Patients must have signed the informed consent.

5.2 Exclusion Criteria

5.2.1 Patients who have received prior chemotherapy for unresectable disease

5.2.2 Patients with any active or uncontrolled infection, including known HIV infection. (Patients with active hepatitis B will be placed on lamivudine. Patients with active hepatitis C will be eligible if liver tests qualify (5.1.9)

5.2.3 Patients with psychiatric disorders that would interfere with consent or follow-up.

5.2.4 Patients with a history of prior malignancy, except for adequately treated basal cell or squamous cell skin cancer, in-situ cervical cancer, or other cancer for which the patient has been disease-free for at least five years.

5.2.5 Pregnant or lactating women. Men and women of reproductive potential may not participate unless they have agreed to use an effective contraceptive method.

5.2.6 Patients with any other severe concurrent disease, which in the judgment of the investigator, would make the patient inappropriate for entry into this study.

6.0 Stratification/Descriptive Factors

6.1 Two descriptive factors will be used in this analysis:

Institution: LAC/USC vs. Norris or University Hospital

Prognostic Index: The potential score ranges from 0 (most favorable) to 5 (least favorable).

Prognostic Index		
Parameter	Range	Points
LDH	>normal	2
ALP	> 200 u/L	1
AST	> 100	1
Ascites	present	1

The institutional stratification anticipates potential outcome impacts that may result from either institutional or socio-economic differences with respect to compliance and details of supportive care.

7.0 Outline of Study Treatment

7.1 Treatment Plan

All patients will receive at least two cycles of chemotherapy, and at most four cycles of IA chemotherapy at 8 week intervals. Patients will be evaluated for eligibility for other treatment options after 2, 3 and 4 cycles of chemotherapy. As soon as it is determined that it is in the patient's best interest to terminate IA chemo treatment and proceed with no further treatment or a different treatment, they will go off study.

7.1.1 IA treatment

Intra-arterial cisplatin 60 mg/m2 and mitomycin-C 12 mg/m2 every 8 weeks (+/- 2 weeks) with evaluation of response at week seven of each treatment cycle. Treatment beyond 2 cycles will be continued at the discretion of the physician with the option of lengthening treatment intervals.

Drug administration will be based on actual calculated body surface area.

All patients will be pre-medicated with Decadron and Kytril or Anzemet and Kytril or Anzemet will be repeated the next day.

Cisplatin and mitomycin will be infused intra-arterially over 30-90 minutes, either separately or mixed. Intravenous fluids will consist of 300 mL 3% NaCl initiated at the start of angiography followed by NS at 100 mL/hr. A minimum of 1 L NS will be administered. Subsequent hydration is at the discretion of the investigator.

7.1.2 Drug Doses, Schedule of Agents, Number of Courses

The length of the treatment cycle is 8 weeks, +/- 2 weeks. The doses of the drugs utilized, the schedule of the agent, and the number of courses, are outlined in Table 2 below.

Table 2

AGENT	DOSE	ROUTE	DAYS	COURSES
Cisplatin	60 mg/m^2	IA	1	2-4
Mitomycin-C	12 mg/m^2	IA	1	2-4

On each day of treatment patients will undergo an angiogram. A catheter will then be threaded into the appropriate vessel(s) and the cisplatin and mitomycin-C will be infused sequentially or together over 30-90 minutes.

7.2 Criteria for Removal from Treatment

7.2.1 Patients will be removed from protocol after disease progression.

7.2.2 Treatment will be discontinued if a patient continues to experience toxicities to the point that they go more than 12 weeks with no treatment.

7.2.3 A patient may always be removed from treatment whenever he or she wishes.

7.2.4 Patients will be removed from the protocol once it is determined that another therapy would be more beneficial to them (such as, but not limited to transplant, local regional therapy (such as RFA and cryo) or no further treatment),

7.3 Follow-up

All patients who go off study will be followed up for death and disease recurrence via telephone calls or review of medical records. This will be done every four months for two years, or more frequently at the investigator's discretion.

8.0 Toxicities To Be Monitored and Dosage Modifications

8.1 General Approach to Patient Experiencing Toxicity

All toxicities will be graded according to the NCI Common Toxicity Criteria (Version 3.0). A full list of toxicities can be found on http://ctep.info.nih.gov. If multiple toxicities are seen, the dose administered should be based on the most severe toxicity experienced.

8.2 Dose modification steps for mitomycin-C/cisplatin.

Dose Modification Steps				
Drug	Starting Dose 0	Dose Level –1	Dose Level –2	Dose Level –3
Mitomycin-C	12 mg/m^2	8 mg/m^2	5 mg/m^2	Off Study
Cisplatin	60 mg/m^2	40 mg/m^2	30 mg/m^2	Off Study

8.3 Toxicity Grade and Dose Modification

Prior to retreatment patients must recover granulocytes, platelet count, bilirubin and ascites status to eligibility level measured within one week of retreatement.

The following table describes the recommended dose modifications. Non-hematologic dose modifications should be based on the worst preceding toxicity. If either the cisplatin or the mitomycin-C is delayed, the other drug should be delayed as well, until toxicities have resolved and patients can start a new course of therapy.

Mitomycin-C and Cisplatin dose modifications

Toxicity **NCI Grade* (Value)**	Mitomycin-C	Cisplatin
Neutropenic fever (Grade 4 neutropenia & ≥ Grade 2 fever)	↓ 1 dose levels of mitomycin-C when resolved	↓ 1 dose level when resolved
Stomatitis / Mucositis 1 painless ulcers, mild sores 2 painful but can eat 3 painful, requiring IV hydration 4 severe ulceration, requiring TPN, intubation	-Maintain dose level -↓ 1 dose level -Delay dose, then ↓ 1 dose level, when resolved to ≤ Grade 2 -Delay dose, then ↓ 1 dose levels when resolved to ≤ Grade 2	-Maintain dose level -Maintain dose level - Delay dose, then ↓ 1 dose level, when resolved to ≤ Grade 2 -Delay dose, then ↓ 1 dose level when resolved to ≤ Grade 2
Renal Toxicity 1. 0-25% decrease of creatinine clearance 2. 26-50% decrease in creatinine clearance 3. 51% or more decrease in creatinine clearance 4. Creatinine clearance < 50	-Maintain dose level -Maintain dose level -Maintain dose level -Off Study	-Maintain dose level -↓ 1 dose level -Decrease -2 dose level -Off Study
Duodenal ulceration, gastric ulceration, gastritis, gastrointestinal bleeding (should be documented endoscopically) 1. grade 1 or 2 2. grade 3 or 4	Institute anti-ulcer therapy**	Institute anti-ulcer therapy**
Hepatic Toxicity AST, ALT and/or alkaline phosphatase*** 1. Grade 1 2. Grade 2 3. Grade 3 or more	 -Maintain dose level -↓ 1 dose level -Delay dose, then ↓ 1 dose level, when resolved to ≤ Grade 2	 -Maintain dose level -↓ 1 dose level -Delay dose, then ↓ 1 dose level, when resolved to ≤ Grade 2

Mitomycin-C and Cisplatin dose modifications

Toxicity NCI Grade* (Value)	Mitomycin-C	Cisplatin
* National Cancer Institute Common Toxicity Criteria version 3.0 ** Anti-ulcer therapy should consist of proton pump inhibitors or h2 blockers *** When a patient is experiencing toxicity in more than on of these lab values, treatment should be based on the highest grade toxicity.		

If a patient experiences unacceptable toxicities requiring a dose reduction at the start of a course and that course is completed with no further toxicities greater than grade 2, then the dose may be increased, at the investigator's discretion, one level at a time during an entire course, in the following courses.

For toxicities which are considered by the Investigator unlikely to develop into serious or life–threatening events (e.g. alopecia, altered taste etc.), treatment will be continued at the same dose without reduction or interruption.

For any event Grade 1-3 event which is apparent at baseline, the dose modifications will apply according to the corresponding shift in toxicity grade, if the investigator feels it is appropriate. (e.g. if a patient has grade 1 asthenia at baseline which increases to grade 2 during treatment, this will be considered as a shift of 1 grade and treated as a grade 1 toxicity for dose modification purposes).

A new course of treatment may begin when the granulocyte count is $\geq 1500/mm^3$ or baseline and the platelet count is $\geq 50{,}000/mm^3$, AST, ALT and alkaline phosphatase are resolved to Grade 2 or less and any other treatment-related toxicities are $\leq$ Grade 1. If after a one-week delay, all toxicities are $\leq$ Grade 1, then proceed with treatment at the dose level based on the preceding table. If toxicities are not resolved to $\leq$ Grade 1 after a one-week delay, then treatment will be held again, and the patient will be evaluated weekly. Treatment may continue, if clinically appropriate, once toxicity has resolved, but with a dose modification. If a patient continues to experience toxicities so that they go more than 12 weeks with no treatment, they will be taken off study.

8.4 Procedure related untoward outcomes.

Potential catheter related untoward events include hematoma at the arterial puncture sites, pseudoaneurysm formation, and embolic events. RFA untoward events include subcapsular hemorrhage, bileoma formation, and biliary duct stricture.

8.5 Adverse Events (AE)

Any untoward medical occurrence in a patient or clinical investigational subject administered a pharmaceutical product, which does not necessarily have to have a causal relationship with this treatment.

The Investigator is required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safety of the drug under investigation.

8.5.1 Types of Adverse Events

The term “adverse event” could include any of the following events, which develop or increase in severity during the course of the study:

(a) any signs or symptoms whether thought to be related or unrelated to the condition under study

(b) any clinically significant laboratory abnormality

(c) any abnormality detected during physical examination

Sign or Symptoms will be graded by the Investigator according to the NCI Common Toxicity Criteria, version 3.0.

8.5.2 Serious Adverse Events (SAE)

Any untoward medical occurrence that at any dose:

(a) results in death

(b) is life threatening (NOTE: The term “life threatening” in the definition of “serious” refers to an event in which the patient was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.)

(c) requires inpatient hospitalization or prolongation of existing hospitalization

(d) results in persistent or significant disability/incapacity

(e) is a congenital anomaly/birth defect

(f) is an important medical event, based upon appropriate medical judgment, that may jeopardize the patient or subject or may require medical or surgical intervention to prevent one of the other outcomes defining serious.

8.5.3 Adverse Event Reporting Obligations

Serious events, whether or not unexpected or considered to be associated with the use of the study drug, must be communicated, by telephone (323-865-0451), to USC immediately (within 24 hours) upon awareness of the event. The call then must be followed-up with a completed Serious Adverse Event Report and faxed within 48 hours of telephone contact, to USC (323-865-0089). The report must include, at least:

(a) site identifiers

(b) the patient identifiers

(c) description of the event

(d) the investigator's opinion of its causality/relationship to study drug

(e) onset date of adverse event

(f) date event became serious

(g) institutional PI's assessment of patient's recovery.

8.5.4 Follow-up of Adverse Events

All "serious" adverse events must be followed with appropriate medical management until resolved or stabilized for 30 days.

Phase II Reporting Guidelines

Unexpected Reaction		**Expected Reaction**	
Grades 1-3	**Grades 4 and 5**	**Grades 1-3**	**Grades 4 and 5**
Written report within 10 working days.	Report by phone to IDB within 24 hours. Written report to follow within 10 working days.	ADE reporting NOT required.	Written report within 10 working days. Grade 4 myelosuppression does not have to be reported as an ADE, but should be submitted as part of study results.

Reporting requirements and timing of reporting are dependent on the grade, attribution and prior experience [expected (known) or unexpected (unknown).

Attribution - Report only if the ADE has an attribution of possible, probable or definite relation to the investigational agent. An ADE is NOT required for adverse events with an attribution of unrelated or unlikely.

A list of expected adverse events can be found in Section 3.0.

9.0 Study Calendar

One cycle will consist of eight weeks (+/- 2 weeks). Patients will receive intra-arterial cisplatin and mitomycin-C on day one of each cycle. Patients will receive 2-4 cycles of IA chemotherapy. Patients will be evaluated for eligibility for other treatments after 2, 3 and 4 cycles of chemotherapy. As soon as it is determined that another therapy would be more beneficial to them (such as, but not limited to transplant and local regional therapy) they will go off study. All patients will be evaluated every two weeks, more frequently at the physician's discretion.

Parameter	**Pre-Rx[4]**	**Day 1 of Each Cycle**	**Days 14 and 28 of Each Cycle**	**Week 7 of Each Cycle**	**RFA[9]**	**Off study**
History & Physical Exam	X	X[10]				
Weight, Performance Status	X			X		X
WBC (differential), Hgb, Platelets	X	X	X	X		
Toxicity assessment	X	X	X	X		
Electrolytes, BUN, Cr	X	X		X		
Calculated creatinine clearance[5]	X			X[5]		
LFT (Alk Phos, ALT, AST, LDH, Total Bili)	X	X				
Hepatitis panel	X[6]					
Urinalysis	X					
AFP	X			X		
EKG	X	X[2]				
CXR (or chest CT)	X					
Pregnancy Test	X[8]					
Tumor Biopsy	X[3]					
Liver image (CT, MR or US) for tumor dimensions	X[1]			X		
Blood for Pharmacogenetics[7]	X					
Calculate Prognostic Index	X					

[1]Record tumor dimensions
[2]As clinically indicated.
[3]If paraffin embedded tissue section is not available and tissue is needed for diagnosis of disease
[4], All tests must be done within 30 days of the start of study treatment, with the exception of radiologic studies. Radiologic studies must be completed within 28 days before the start of therapy.
[5]Calculated Creatinine clearance, calculated with Cockcroft-Gault equation
Men=((140-age) x weight in kg) / (72 x serum creatinine)
Women=.85 x ((140-age) x weight in kg) / (72 x serum creatinine)
[6] Hepatitis panel will include HBsAb, HbsAg, HCAb, HB DNA by PCR, HC RNA by PCR (quantitative). Repeat RNA/DNA for elevations of AST/ALT. The panel will be obtained within 4 months of the study entry. HB DNA will be obtained only for HB+ patients, HC RNA for HC+ patients
[7] One purple top and one red top tube
[8] For women of child bearing potential
[9]Patients will be evaluated for eligibility for other treatments after 2, 3 and 4 cycles of chemotherapy. As soon as it is determined that another therapy would be more beneficial to them (such as, but not limited to transplant and local regional therapy) they will go off study. -
[10]Within 10 days of treatment cycle

10.0 Criteria for Evaluation and Endpoint Definitions

All patients who are registered will be accounted for in the report of the results. Patients who complete 1 cycle of chemotherapy and who are followed a minimum of 3 weeks after completion of the first cycle of chemotherapy or who experience dose limiting toxicity, will be evaluable for toxicity.

The outcome status (in terms of toxicity, response, reason off study, progression, and survival) of all eligible patients will be reported. Patients with measurable tumor who complete one cycle, or who terminate treatment for reasons of toxicity, or who progress prior to completion of one cycle, will be included in analysis of tumor response and time to progression, and in any decision to terminate the study early. All eligible patients who begin treatment will be included in the analysis of survival and time-to-failure. A modified Response Evaluation Criteria In Solid Tumors (RECIST) and the CTCAE version 3.0 toxicity criteria will be used. The modification to RECIST is that although all lesions will be followed, only the treated lesions will be included in the assessment of response. Additionally, all lesions will be evaluated according to the following criteria: Presence of arterial enhancement: Yes or No.

10.1 Measurability of tumor lesions at baseline.

At baseline, tumor lesions will be categorized as:

measurable:	Treated lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm with conventional techniques or as ≥ 10 mm with spiral CT scan

OR

non-measurable:	all other treated lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with spiral CT scan) and truly non-measurable lesions.

All measurements should be recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

Lesions that are considered as truly non-measurable include the following: bone lesions; leptomeningeal disease; ascites; pleural / pericardial effusion; inflammatory breast disease; lymphangitis cutis / pulmonis; abdominal masses that are not confirmed and followed by imaging techniques; cystic lesions.

10.2 Specifications by methods of measurements

10.2.1 The same method of assessment and the same technique should be used to characterize each identified and reported treated lesion at baseline and during follow-up. Imaging based evaluation is preferred to

evaluation by clinical examination when both methods have been used to assess the anti-tumor effect of a treatment.

10.2.2 Study patients will have disease limited to liver. Serial measurements will be obtained from abdominal CT scans. MRI or ultrasound may be substituted for CT scan under special circumstances but whatever modality is used for baseline measurements should be consistently used in follow-up evaluations. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 4 mm contiguous reconstruction algorithm. As equipment becomes available volume estimates from 3 dimension reconstructions will be used.

10.2.3 Screening for the appearance of metastatic disease will be by abdominal CT and chest X-ray.

10.2.4 Unexplained elevations of serial AFP will trigger a more extensive search for metastatic disease.

10.2.5 AFP Tumor marker

Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response except when corroborative transaminase and viral levels convincingly demonstrate that the elevation is secondary to a hepatitis flare.

10.2.6 Assessment of overall tumor burden and measurable disease

To assess objective response, it is necessary to estimate the overall tumor burden at baseline and use this as a comparator for subsequent measurements. Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint. Measurable disease is defined by the presence of at least one measurable lesion (as defined in section 10.1). If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

10.2.7 Baseline documentation of “Target” and “Non-Target” lesions

All treated measurable lesions up to a maximum of 10 lesions representative of all involved organs should be identified as ***target lesions*** and will be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repetitive measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other treated lesions should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements are not

required and these lesions should be followed as “present” or “absent”.

Additionally, all lesions will be evaluated according to the following criteria: Presence of arterial enhancement: Yes or No.

10.3 Response Criteria

At each evaluation (see Study Calendar in Section 9.0), all lesions will be evaluated according to the following criteria: Presence of arterial enhancement: Yes or No and response will be classified as :

10.3.1 Evaluation of target lesions

Complete Response (CR)	Absence of enhancing tumor areas, reflecting complete tissue necrosis.
Partial Response (PR)	A decrease > 50% of enhanced areas, reflecting partial tissue necrosis.
Progression (PD)	An increase > 25% in the size of $\geq$ 1 measurable lesion(s) or the appearance of new lesions in the treated area (ie, specific segment or lobe of liver targeted by the intra-arterial infusion)
Stable Disease (SD)	A tumor response between PR and PD.

10.3.2 Evaluation of non target lesions

Complete Response (CR)	Absence of enhancing tumor areas, reflecting complete tissue necrosis
Non-Complete Response (non-CR) / Non–Progression (non-PD)	persistence of one or more non-target lesion or/and maintenance of tumor marker level above the normal limits.
Progression (PD)	appearance of one or more new lesions in the treated area. Unequivocal progression of existing non-target lesions in the treated area.

10.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). In general the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria (see section 10.3.4 below).

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note:

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as “symptomatic deterioration”. Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends upon this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

In the case of SD, follow-up measurements must have met the SD criteria at least once after the start of treatment at a minimum interval of 28 days after start of treatment.

10.3.4 Confirmatory measurement

To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies. The protocol specified interval is 7 weeks.

Note: Repeat studies to confirm changes in tumor size may not always be feasible or may not be part of the standard practice in protocols. In such cases, patients will not have “confirmed response” and this will be made clear when reporting the outcome of such studies.

10.3.5 Biopsy determination of response

At the time of RFA a biopsy will be obtained. Histologic demonstration of ≤ 10% of tumor in an adequate specimen will be considered a response even if dimension criteria are not met. This is viewed as appropriate because after IA therapy there is frequently a residual non-viable mass.

10.3.6 Duration of overall response

The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive

disease the smallest measurements recorded since the treatment started).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

10.3.7 Duration of stable disease

Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

10.4 Endpoint Definitions

10.4.1 Overall Survival. Defined as the time from first day of treatment to time of death due to any cause. If a patient is still alive, survival time is censored at the time of last follow-up.

10.4.2 Progression-free survival. Defined as the time from first day of treatment to the first observation of disease progression or death due to any cause. If a patient has not progressed or died, progression-free survival is censored at the time of last follow-up.

10.4.3 Time to progression. Defined as the time from first day of treatment to the first observation of disease progression or death due to disease. If failure has not occurred, failure time is censored at the time of last follow-up.

11.0 Special Instructions

11.1 Experimental Methods and Procedures

11.1.1 **Polymorphism:** We will isolate genomic DNA from whole blood samples. An approximate 230bp region of the TS gene containing the polymorphic site will be PCR amplified using a P-33 end-labeled forward primer and an unlabeled reverse primer. PCR products will be separated on sequencing gels and autoradiographed, and products will be sized by comparison to known genotypes.

11.1.2 **Quantitation of mRNA levels:** The cDNA library created from each tumor as part of this technology (RT-PCR) contains a quantitative and qualitative record of all of the tumor's expressed genes, and mRNA quantitation as well as mutations screening of 30-40 expressed genes is possible with material obtained from an average tumor biopsy. We have successfully quantitated gene expression in specimens of less than 1 mg and fine needle biopsies only by changing the PCR conditions. Quantitative RT-PCR is carried out essentially as previously described in with some modifications (31-37). The choice of the internal standard is critical for obtaining meaningful results with PCR quantitation. This gene should be one that is expressed constitutively with a level of per cell expression that is constant among different tissues or at least in similar tissues from different individuals.

11.2 Handling of blood samples for Pharmacogenetics

Purple top and red top blood samples should be sent within 2 hours to Dr. Lenz Laboratory at room temperature: Questions should be referred to Dana Agafitei or Dr. Zhang.

Dr. Wu Zhang Norris 5th Floor: 5410, Tel 323 865 0572.

11.3 Handling of tumor tissues

Tumor samples should be submitted when available, which have been collected at the time of diagnosis or prior to entry into this protocol. Unstained Paraffin embedded tumor section 10x 10um thick containing tumor and normal tissue should be send by room temperature to Dana Agafitei. A tumor block (paraffin embedded tumor tissues) can be used instead of sending the unstained slides. Questions should be referred to Dana Agafitei.

Dana Agafitei
USC/Norris Comprehensive Cancer Center
CISO office 7th floor
1441 Eastlake Ave
Los Angeles, CA 90033
Tel: 323 865 0467

12.0 Statistical Considerations

This is a one-stage Phase II study conducted at USC to evaluate intra-arterial chemotherapy with cisplatin and mitomycin-C as a 1st line chemotherapy for patients who are diagnosed with unresectable hepatocellular carcinoma with evident disease limited to liver and have never received prior treatment for advanced disease.

12.1 Study Design

This study was originally a two stage study of IA chemotherapy with cisplatin and mitomycin-C, followed by radio-frequency ablation (RFA). At the interim analysis, it was found that although the response rate was promising, patients were not going on to receive RFA after IA chemotherapy. The trial, thus has been amended, and the primary objective changed to response rate to IA chemotherapy with cisplatin and mitomycin-C. Prior to the amendment 29 patients were accrued, these patients will be included in the analysis of the primary endpoint.

The primary endpoint in this study will be response rate. If at least 15 objective responses are observed in 60 evaluable patients, this regimen would be considered worthy of further testing in this disease. This design yields at least 96% power to detect a true objective response rate of 35%. It yields at least .97 probability of a negative result if the true objective response rate is no more than 15%.

12.2 Analysis of Results

12.2.1 Analysis of Clinical Endpoints

The outcome status (in terms of response rate (the primary endpoint), toxicity, time to progression, reason off study, and survival) of all eligible patients who are registered, will be reported. Patients who complete at least 1 cycle of chemotherapy and are followed at least 3 weeks after completion of the first cycle, or who

experience any unacceptable toxicity will be included in the analysis of toxicity. Patients with measurable tumor who complete at least one cycle of chemotherapy, or who terminate treatment for reasons of toxicity, or who progress prior to completion of one cycle of chemotherapy, will be included in analysis of tumor response and time to progression. All eligible patients who begin treatment will be included in the analysis of overall survival.

Toxicities observed will be summarized in terms of type (organ affected, laboratory determination), severity (by NCI Common Toxicity Criteria and nadir or maximum values for the laboratory measures), time of onset (i.e. course number), duration, and reversibility or outcome. Tables will be created to summarize these toxicities and side effects by course. Demographic and baseline clinical information (i.e., performance status or prognostic index) will be presented to describe the patients treated in this study.

Tumor response will be assessed using the RECIST criteria at week 7 of each treatment cycle (lasting 8 weeks) of the IA regimen of cisplatin and mitomycin-C followed by RFA. Patients who receive less than one full course due to unacceptable toxicity or progressive disease will still be evaluable for response. All responses will be reported. Response rates will be calculated as the percent of evaluable patients whose best response is a CR or PR, and exact binomial 95% confidence intervals will be calculated for this estimate. Contingency tables will be constructed to summarize the associations between the categorical factors (i.e., genomic polymorphisms) and tumor response. Fishers' exact tests will be used to formally test for associations.

Time to progression, progression-free survival, and overall survival will be summarized with Kaplan-Meier plots to describe the outcome of patients treated on this protocol. The median time and 95% confidence intervals (CIs) of median time to progression and survival will be calculated. In addition the probability of progression at 3, 6, 9 months and survival at 6, 12, 18 months and Greenwood's standard errors will be summarized. The log-rank test will be used to examine whether prognostic factors are associated with time to progression, progression-free survival, or overall survival. The relative risk and corresponding 95% CIs will be used to summarize the differences in time to event of interest by prognostic factors.

12.2.2 Description of definitive therapy

Patients will be categorized according to the post-induction therapy they receive following completion of this study. Patients are expected to go one to receive on of the following:

1) Transplant

2) Local regional therapy

    a) RFA

b) Cryo

3) More IA chemo off trial

4) No further treatment

a) Due to Progressive Disease

b) Due to Stable Disease

The proportion of patients going on to each of these treatments will be computed and time to progression, progression free survival and overall survival will be summarized for each group separately.

12.2.3 Analysis of the prognostic index (PI)

The PI is defined as a sum of 2 points for elevated LDH and 1 point each for abnormal ALP (>200 u/L), abnormal AST (>100 u/L), and presence of ascites on baseline imaging. The PI will be ranged from 0 to 5. Categorization will be considered if a small number of patients are in a subgroup of the PI. A contingency table and percentages will be constructed to summarize the association between the PI and tumor response. Fishers' exact test will be used to formally examine the association. Kaplan-Meier plots will be constructed to describe time to progression, progression-free survival, and overall survival across the categories of the PI. The log-rank test will be performed to test the association between the PI and time to progression, progression-free survival, and overall survival. The analysis of the PI in association with clinical outcome will examine whether the PI developed in patients receiving chemoemolization is applicable to unresectable HCC patients.

12.2.4 Analysis of molecular biomarkers

Gene expression levels of (1) genes associated with induction of apoptosis or cell cycle regulation (bcl-2, bcl-xL, bcl-xS, bax, and p21), (2) DNA repair (ERCC1, RR, gamma-GT) will be summarized overall and according to tumor response, using medians, quartiles and ranges – or if a transformation is found to render the data compatible with the normal assumptions, with means, standard deviations, and 95% confidence intervals. The association with progression-free survival or overall survival will be assessed by categorizing the measures of gene expression at median (or by previously established cut-points) and constructing Kaplan-Meier plots. The log-rank test will be used to examine whether gene expressions are associated with time to progression, progression-free survival, or overall survival.

Association of types of polymorphisms for genes involved in the DNA repair (p53, ERCC1, XRCC1, and XPD) will be summarized overall and by response, using contingency tables and percentages. The association with progression-free survival or overall survival will be assessed by constructing the Kaplan-Meier

curves according to the polymorphisms observed. The log-rank test will be used to examine whether genomic polymorphisms are associated with time to progression, progression-free survival, or overall survival.

The analyses of associations between the molecular biomarkers and clinical outcome will be conducted in the end of this study. These analyses of the molecular biomarkers will be descriptive and exploratory, in order to identify questions and patterns for future study; the numbers of patients expected (nearly 100% for the specimens obtained at baseline) will not permit formal comparisons of patients according to response, but will allow us to estimate the patient-to-patient variability in this well-defined group of patients. These analyses will be descriptive in nature.

Justification of Study Design

There is sparse information on the association between molecular biomarkers and tumor response, time to progression, or survival among this group of patients. With 60 patients, we will have at least 90% power to detect the difference of 1.0 standard deviation in gene expressions between responders and non-responders (we expect that there will be at least 15 responders if this regimen is promising).

With 60 patients, we will have 84% power to identify the differences (at least 30%, equivalent to a hazard ratio of 2.4) in proportions without progression at 8 months when the prevalence of a predictor with favorable outcome varies from 33% to 67% (Table 2). There is a power of at least 78% that we will observe the difference in proportions of survival at 15 months (Table 2).

Table 2. Power to Test Differences in Clinical Outcome (Time to Event of Interest) by Prevalence of Prognostic Index, Genomic Polymorphisms, or Genetic Profile with Poorer Outcome

% of Patients in the poorer prognosis group	Power to Detect a Difference of 0.30 Proportion without Progression at 8 months: 0.35 vs. 0.65*	Power to Detect a Difference of 0.30 Proportion Surviving at 15 months: 0.35 vs. 0.65*
75%	78%	70%
67%	84%	78%
50%	90%	85%
33%	87%	83%
25%	82%	77%

Notes: Power calculations using methods in Rubinstein, Gail, and Santner, as programmed by Buckley, are based on a two-sided .05-level logrank test with annual accrual rate of 30 patients per year, 18 months of additional follow-up for time without progression and 2 years of additional follow-up for survival, and at most 2% of patients lost to follow-up annually.

* If the time to progression follows the exponential distribution (at least approximately), the proportion of 35% and 65% without progression at 8 months is equivalent to about 5.3 and 12.9 months of median progression,

respectively. Similarly, the proportion of surviving of 35% and 65% at 15 months is equivalent to about 9.9 and 24.1 months of median survival, respectively.

12.3 Monitoring for Excessive Unacceptable Toxicity

Although we expect that this regimen to be well tolerated, the guidelines listed below will be used to raise a flag if the number of patients who experience unacceptable toxicity is large enough to strongly suggest that the true probability of unacceptable toxicity is > 20%. Unacceptable toxicity will be defined as

any Grade 3 non-hematologic toxicity not reversible to Grade 1 or less within 96 hours or

any Grade 4 non-hematologic toxicity or

any Grade 4 hematologic toxicity not resolving to Grade 1 or less within 5 days, despite supportive care or

Grade 4 neutropenia associated with fever or

Grade 4 thrombocytopenia

An unacceptable toxicity, regardless of attribution, observed during any course, will be used in the decision to suspend accrual.

To be evaluable for unacceptable toxicity a patient must complete 1 course of treatment or have experienced unacceptable toxicity. Patients who do not complete 1 course and who do not experience any unacceptable toxicity will not be used in the decision to continue or suspend accrual to the trials, for reasons for excessive unacceptable toxicity. Every time unacceptable toxicity is observed, the number of patients (X) who have experienced unacceptable toxicity will be compared to the number of patients (N) who are evaluable for unacceptable toxicity. If the number of patients, N, is greater than N_X, the number given in column 2 of the Table 3, below, then accrual will not be suspended. If N is less than or equal to N_X, then accrual will be suspended for review of the data.

Table 3: Criteria for Continuing Accrual

X = Total Number of Patients with Unacceptable Toxicity	N_X: Suspend the Trial if Number of Evaluable Patients Is Less Than or Equal to:
4	≤ 6
5	≤ 10
6	≤ 14
7	≤ 18
8	≤ 22
9	≤ 27
10	≤ 31
11	≤ 35
12	≤ 39
13	≤ 43
14	≤ 47
15	≤ 52

16	≤ 56
≥17	Suspend

Using this rule with 60 patients, the probability of correctly suspending this regimen for review toxicities is 0.93 if the true chance of unacceptable toxicity is 35% or greater. The probability of falsely suspending this regimen for review toxicities is 0.04, if the true chance of unacceptable toxicity is ≤ 15%. Estimation of the probabilities of suspending this regimen is based on 10,000 simulations

13.0 Registration Guidelines

All patients will have signed an informed consent for participation in research activities in accord with all institutional, NCI and Federal regulations, and will have been given a copy of the Experimental Subject's Bill of Rights.

Note: At the time of registration, two copies of a signed and dated patient Informed Consent form with Bill of Rights must be available (an original for patient's medical chart; one copy for the patient; and the other for the CISO office).

14.0 Minorities and Women Statement

This study will be open to patients undergoing treatment at USC/Norris Cancer Center, USC University Hospital and LAC+USC Medical Center.

ETHNIC AND GENDER DISTRIBUTION OF NEWLY DIAGNOSED CANCER PATIENTS[1]
IN LOS ANGELES COUNTY IN 1999[2]

Primary Site of Tumor	Total # of Patients	Males %	Females %	White %	Black %	Hispanic %	Asian/Other %
ALL INVASIVE TUMORS	**33,194**	**49**	**51**	**61**	**12**	**18**	**10**
ORAL CAVITY/PHARYNX	**749**	**66**	**34**	**64**	**13**	**12**	**11**
DIGESTIVE SYSTEM	**6,727**	**52**	**48**	**56**	**12**	**18**	**13**
Esophagus	294	72	28	61	13	17	10
Stomach	823	60	40	41	12	27	21
Colon	2,677	49	51	62	14	14	11
Rectum/Anus	547	49	51	63	10	17	10
Liver	479	66	34	34	10	29	27
Pancreas	718	50	50	63	11	18	9
RESPIRATORY SYSTEM	**4,341**	**56**	**44**	**64**	**15**	**11**	**10**
Lung and Bronchus	3,949	54	46	65	15	11	10
BONES AND JOINTS	**72**	**58**	**42**	**43**	**11**	**33**	**13**
SOFT TISSUE INCL. HEART	**223**	**54**	**46**	**49**	**14**	**27**	**9**
MELANOMAS OF THE SKIN	**1,101**	**56**	**44**	**91**	**1**	**7**	**1**
BREAST	**5,489**	**1**	**99**	**63**	**11**	**16**	**11**
FEMALE GENITAL SYSTEM	**2,334**	**0**	**100**	**54**	**9**	**26**	**11**
Cervix Uteri	573	0	100	35	10	45	10
Corpus Uteri	954	0	100	63	10	18	9
Ovary	684	0	100	56	8	23	13
MALE GENITAL SYSTEM	**5,404**	**100**	**0**	**59**	**16**	**18**	**7**
Testis	195	100	0	54	2	41	4
Prostate	5,176	100	0	59	17	17	7
URINARY SYSTEM	**1,549**	**65**	**35**	**65**	**11**	**18**	**7**
Invasive Bladder	656	73	27	73	8	12	8
Renal	830	59	41	58	13	23	7
EYE AND ORBIT	**59**	**63**	**37**	**66**	**7**	**24**	**3**
BRAIN /NERVOUS SYSTEM	**481**	**55**	**45**	**61**	**8**	**24**	**6**
ENDOCRINE/THYROID	**656**	**26**	**74**	**53**	**6**	**26**	**15**
HODGKIN'S DISEASE	**245**	**49**	**51**	**53**	**10**	**33**	**4**
NON-HODGKIN'S LYMPHOMA	**1,430**	**55**	**45**	**62**	**8**	**20**	**10**
MULTIPLE MYELOMA	**408**	**57**	**43**	**49**	**23**	**20**	**8**
LEUKEMIA	**841**	**61**	**39**	**56**	**8**	**26**	**9**
Lymphocytic Leukemia	330	60	40	55	10	31	5
Non-Lymphocytic Leukemia	511	61	39	57	8	23	12
***IN SITU* DISEASE**							
in situ Breast	1,063	1	99	67	10	12	11
in situ Bladder	648	77	23	78	6	8	8
in situ Melanoma	658	54	46	92	1	6	1

(1) Invasive cancer with the inclusion of specified *in situ* breast, melanoma and bladder cancer cases. Data provided by Los Angeles County Cancer Surveillance Program; Department of Preventive Medicine; University of Southern California; 1540 Alcazar St.; LA CA 90033.

The numbers above reflect the ethnic and gender distribution of cancer patients in the County of Los Angeles. Although distributions may vary by disease type, our recruitment procedures have been developed to enroll patients who are representative of the target population.

15.0 Ethical and Regulatory Considerations

All institutional, NCI, and Federal regulations concerning the Informed Consent form will be fulfilled.

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