

TNF-based isolated hepatic perfusion

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1. ABSTRACT

Unresectable primary and metastatic cancers confined to the liver often determine the prognosis for patients with primary hepatic cancers, colorectal cancer, ocular melanoma, and neuroendocrine tumors. Although many locoregional therapies have emerged as options for patients with unresectable liver malignancies, these treatments frequently have limited clinical benefit. Isolated hepatic perfusion (IHP) has emerged as a regional therapy effective in inducing tumor regression in isolated liver metastases from multiple histologies. Tumor necrosis factor alpha (TNF) is a biologic agent well suited to isolated therapy because of its single-dose efficacy, synergistic effect with hyperthermia, and effects on tumor neovasculature. When combined with chemotherapeutic agents in IHP, TNF may improve response rates in patients with hepatic metastases of some histologies. However, there are additional toxicities associated with the administration of TNF and further studies are needed to determine whether TNF confers a clinical advantage in IHP.

2. INTRODUCTION AND SCOPE OF THE PROBLEM

The development of effective treatment options for patients with unresectable primary and metastatic cancers confined to the liver is a high clinical research priority. In addition to primary hepatic tumors such as hepatocellular carcinoma (HCC) and cholangiocarcinoma, the liver is a common site of metastases for colorectal carcinoma (CRC), ocular melanoma (OM), and neuroendocrine tumors (NET) of the midgut and pancreas. Despite improvements in patient outcome with hepatic resection in the treatment of metastatic and primary disease (1), its application is still limited by the overall condition of the patient, anatomic constraints based on the number or size of tumors, and the need to preserve an adequate remnant of functioning liver parenchyma. Several types of regional or hepatic-directed therapies for unresectable hepatic metastases are in clinical use; however, none have sufficient efficacy to be considered standard of care. Isolated hepatic perfusion (IHP) is a treatment technique

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designed to deliver hepatic-directed in patients with unresectable liver metastases. Hyperthermia and melphalan or other chemotherapeutics have been the most commonly administered agents during IHP. Tumor necrosis factor alpha (TNF), a biological agent with potent effects on tumor vasculature, has been used in isolation perfusion of the limb or liver; however, its role in IHP has not been clearly defined.

The need for more effective liver-directed regional therapies is highlighted by the fact that current treatment standards for primary or metastatic lesions confined to the liver frequently have limited clinical benefit. HCC is one of the most common cancers worldwide; most patients present with unresectable disease at the time of diagnosis and have a 3-year survival of less than 10% in advanced disease if left untreated (2,3). Combination chemotherapy has not been shown to confer a survival benefit (4-7). Similarly, cholangiocarcinoma is often unresectable at the time of diagnosis (8) and these patients rarely live beyond a year (9). Chemotherapy and radiation therapy have not been shown to prolong survival in these patients.

Colorectal cancer is the second leading cause of cancer deaths in North America. It is estimated that 153,000 new cases were diagnosed in 2006 (10). Of these patients, approximately 25% have synchronous liver metastases and 20-25% will develop metachronous liver tumors (11). Within the last 10 years, chemotherapeutic agents have been developed such as irinotecan, oxaliplatin, capecitabine, cetuximab, and bevacizumab. When these agents are combined with 5-fluorouracil and leucovorin, median survival has improved to up to 20 months (12, 13, 14). Despite these advances, most patients will experience disease progression within one year of treatment.

Ocular melanoma (OM) and neuroendocrine tumors (NETs) commonly metastasize to the liver as the sole or clinically dominant site of disease progression. As many as 40% of patients with OM will be initially diagnosed with liver metastases (15). While the 5-year survival for OM overall is 50-70%, patients with liver metastases have a median survival of 2-7 months despite aggressive therapy (16). Due to the multifocal distribution of OM liver metastases, surgical resection is usually not feasible (17). No therapies have been shown to meaningfully alter the natural history of the disease (18, 19). NETs with metastases to the liver are characterized by an indolent course; however, patients may suffer from debilitating symptoms associated with the hormone production of pancreatic NETs or serotonin production from midgut carcinoid tumors. Medical management of symptomatic NETs with a somatostatin analogue successfully controls symptoms early in the course of the disease. However with time tumors will become resistant to anti-secretory therapy necessitating further intervention (20). Over 90% of liver metastases from NETs are multifocal, potentially precluding resection (21).

Together these facts highlight the need for more effective therapies for patients with primary or metastatic

cancers confined to liver. The use of IHP with tumor necrosis factor will be presented below.

3. REGIONAL TREATMENT OPTIONS FOR PATIENTS WITH UNRESECTABLE HEPATIC MALIGNANCIES

Locoregional therapies such as radiofrequency ablation (RFA) and transarterial chemoembolization (TACE) have emerged as options for patients with unresectable liver malignancies. However, these treatments are limited by tumor size and number or diminished hepatic reserve. The presence of micrometastases is also not addressed at the time of treatment. The efficacy of RFA in HCC and CRC is limited by tumor size and is best suited for singular small metastases (3, 22-25). Similarly, TACE is most effective in HCC in tumors without vascular invasion and in patients with preserved hepatic function (26). TACE or RFA in combination with surgery has been shown to increase the 5-year survival of metastatic NETs, however, patients with > 50% of liver involvement did not benefit (27).

Regional chemotherapies for hepatic metastases include both hepatic arterial infusion (HAI) and IHP. The benefit of infusion therapy is the ability to limit unnecessary systemic drug toxicity while intensifying drug delivery to the affected organ. Chemotherapy can be targeted to tumor cells in HAI because of the unique anatomy of the liver, in which the parenchyma is perfused mainly by the portal vein while the tumor is primarily perfused by the hepatic artery (28). When agents are used with high first-pass hepatic extractions, high concentrations of the drug can be achieved in the liver while maintaining low systemic levels. In drugs with a dose-response curve this can be especially beneficial by enabling the use of higher doses than those tolerated in systemic therapy (29).

4. RATIONALE FOR IHP

IHP was first used reported in 1960 by Ausman and Aust; they treated 5 patients with nitrogen mustard using this technique. Response in one patient with a functioning carcinoid was demonstrated by decreased levels of serotonin post-treatment, although the levels eventually returned to their preoperative range. Two patients were palliated for 6 months and one patient died of perioperative complications (30). Since that report there have been refinements in IHP using a variety of cytostatic agents that have resulted in acceptable mortality and morbidity rates when performed in specialized centers. Because IHP involves a major operative procedure, ideal perfusion agents should be effective with a single administration. TNF is one such agent, with demonstrated anti-tumor activity after a single dose in murine models (31).

The technique of IHP results in a complete separation of the blood flow through a cancer-burdened organ from the systemic circulation resulting in eliminated or significantly limited systemic toxicity from the perfusion agents. IHP employs an oxygenated extra-corporeal

perfusion circuit analogous to that used in cardiac procedures. The tolerance of the perfused normal tissues to the agents being administered becomes the dose-limiting factor in treatment. Isolation perfusion of the liver allows one to use chemotherapeutics or biological agents that do not necessarily have a high first-pass extraction because they are continuously perfused through the liver with a recirculating circuit (32). One of the main advantages of IHP is its use in patients with extensive hepatic metastases. Partial responses have been reported in patients with greater than 50% of hepatic replacement (33-35). IHP allows one to apply clinically relevant levels of hyperthermia, which has independent cytotoxic actions on tumor cells (36) and has been shown to enhance the effects of chemotherapy and biologic agents (37, 38).

5. RATIONALE FOR TNF IN ISOLATION PERFUSION

TNF is now recognized as a multifunctional cytokine involved in apoptosis, the transduction of cell survival signals, inflammation, and immunity. The anti-tumor activity of TNF may have had its origins as one of the mediators of Coley's toxins that were used for the treatment of patients with cancer in the late 1890s and early 20th century. At that time, an American surgeon, William Coley, and a German physician, F. Fehleisen, began to independently investigate the apparent link between tumor regression and concomitant bacterial infection. Coley used a mixture of *Streptococcus pyogenes* and *Serratia marcescens*, known as Coley's toxins, on patients with unresectable tumors. These toxins emerged as the only form of systemic cancer therapy in the early 1900's (39). Anti-tumor activity was documented sporadically and was associated with the development of severe systemic inflammatory manifestations such as fever, chills, and tachycardia all reminiscent of the systemic toxicities of recombinant TNF (36).

The investigation of the tumoricidal effects of bacteria continued and in 1943, when Shear *et al* reported the isolation of bacterial lipopolysaccharide (LPS) and subsequently demonstrated LPS-induced hemorrhagic necrosis of tumors in murine models (40, 41). O'Malley and colleagues identified an endogenous factor produced in the serum of LPS-treated animals that induced hemorrhagic tumor necrosis and this substance was further characterized and named tumor necrosis factor in 1975 by Carswell and Old (31, 42). They demonstrated that a circulating factor in serum from LPS treated mice could produce necrosis in transplanted tumors as effectively as LPS. When recombinant TNF became available in 1985 several studies demonstrated marked anti-tumor effects of TNF in murine models (31). However, subsequent phase I and phase II trials of systemic TNF in humans demonstrated only rare and transient partial responses (43). Humans proved to be exceedingly sensitive to the toxic effects of systemically administered TNF; the maximum tolerated doses of TNF were 10-15 times lower than those used to produce tumor necrosis in murine models (44-47).

In light of TNF's dose-limiting toxicity with systemic administration, its marked tumoricidal effects in animal studies, and its efficacy with single dose administration in animal models, it appeared to be particularly well suited for administration via isolation perfusion. The first report of TNF in isolation perfusion was by Lienard and Lejueune in 1992. Twenty-three patients with metastatic melanoma and unresectable sarcoma were treated with interferon-gamma, TNF, and melphalan via isolated limb perfusion (ILP) with a complete response rate of 89% (48). Subsequent multicenter clinical trials demonstrated the efficacy of TNF-based ILP in the treatment of unresectable extremity sarcoma with a limb salvage rate of 74-87% (49). However, in later studies, the addition of TNF to melphalan in ILP for metastatic melanoma did not appear to affect overall response rates (50).

Several observations related to TNF were made in various clinical studies of ILP. When used as a single agent, TNF does not appear to have any meaningful anti-tumor activity (51, 52). However, when used with chemotherapeutics, most commonly melphalan, TNF was associated with anti-tumor activity against a broad range of tumor histologies and its principal target appeared to be the tumor-associated neovasculature (Figure 1). It is now appreciated that TNF initially augments the permeability of tumor neovasculature which promotes the selective accumulation of cytotoxic agents in the tumor interstitium; subsequently it causes a coagulative obliteration of the same tissues (53). Laboratory data have demonstrated that TNF increases vascular permeability in tumors in animal models (54) and has been shown to alter vascular endothelial cell integrity (55-58). The effects of TNF on vascular permeability may account for the increased tumor concentrations of chemotherapeutics in TNF-based isolated perfusion (59). TNF causes coagulation and central necrosis of murine tumors (31) and has been shown to increase the expression of tissue factor by endothelial cells (60-62). The effects of TNF on coagulation and vascular permeability may be synergistic as evidenced by the augmentation of TNF induced endothelial cell permeability by tissue factor *in vitro* (63). The advantages and disadvantages of TNF-based isolated hepatic perfusion are summarized in Table 1.

6. TECHNIQUE OF IHP

Because TNF alone is not clinically effective in isolated perfusion, it has been most commonly used with melphalan in IHP. The dose limiting toxicity of TNF when used with melphalan in IHP is coagulopathy and the maximum tolerated dose has been determined in Phase I trials as 1 mg. The maximum safe tolerated dose of melphalan with TNF is 1.5mg/kg. Higher doses of melphalan result in severe hepatic veno-occlusive disease (36, 64).

Preparation for IHP includes assessment of patient eligibility, preoperative imaging, and adequate intra-operative monitoring. Patients undergoing IHP should have isolated unresectable hepatic metastases, an

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Table 1. Advantages and Disadvantages of TNF-Based Isolated Hepatic Perfusion

Treatment	Advantages	Disadvantages
TNF	Augments delivery and retention of chemotherapeutic agents Synergistic effect when used with hyperthermia Efficacy with single dose administration	Systemic release of inflammatory cytokines Transient hepatotoxicity Not clinically effective when used alone
Isolated Hepatic Perfusion	High-dose therapy limited by hepatic not systemic toxicity Avoids first pass extraction of liver – broadening range of potential perfusion drugs Clinically effective in cases of large tumor burden > 50% Potential to treat micrometastases Hyperthermic administration of perfusate	Morbidity of extensive surgical procedure Limited ability to repeat treatment



Figure 1. Photographs of a female patient with an eccrine gland adenocarcinoma of the heel and extensive in-transit metastases refractory to chemotherapy. She was treated with ILP using TNF and melphalan. Note the eschar formation over the tumor post-operatively, with sparing of adjacent normal skin, which is a characteristic TNF effect.

ECOG performance less than or equal to 2 and normal hepatic synthetic function. Cirrhosis and portal hypertension should be excluded preoperatively as well as cardiac and pulmonary disease that would preclude a patient from safely undergoing a major operation. The vascular anatomy of the liver is evaluated by MRA to plan for cannula placement in the event of aberrant anatomy. Patients who have undergone prior HAI treatments may require a formal angiogram to assess the arterial vascular anatomy.

The technique of IHP includes the following steps: abdominal exploration, mobilization of the liver, systemic anticoagulation, establishment of IVC to axillary veno-venous bypass, establishment of a hepatic extra-corporeal vascular perfusion circuit and, after treatment, hepatic parenchyma flush and decannulation with repair of vascular structures (Figure 2). A wide operative field is prepared to include the chest, axillae, abdomen, and upper thighs. Initially a limited subcostal incision is made; if extra-hepatic disease is found, particularly evidence of

peritoneal dissemination or extensive lymphadenopathy, the procedure should be aborted. Resectable portal lymphadenopathy is not a contraindication to proceed and is resected in the course of preparing the porta hepatis for IHP. The liver is also assessed at this time to determine whether it can be mobilized safely and sufficiently to ensure complete isolation. The incision is then extended to a bilateral subcostal incision with extension to the xyphoid if necessary.

The liver is mobilized by dividing its diaphragmatic attachments bilaterally. It is reflected medially to expose the retro-hepatic inferior vena cava from the renal veins to the diaphragm. In order to prevent leak of perfusate, all retroperitoneal venous tributaries including the right adrenal vein and large phrenic veins must be identified and ligated. A cholecystectomy is performed to prevent post-perfusion chemical cholecystitis. The peri-portal vessels are dissected and isolated for cannulation. The right gastric artery and small arterial branches of the hepatic artery are ligated and divided. The gastroduodenal artery (GDA) is prepared as the cannulation site for perfusate inflow. The portal vein and common bile duct are dissected of lymph node bearing tissue to ensure no leak of perfusate through the adjacent fibrofatty tissues. Then, the axillary vein and saphenous veins are isolated in preparation of veno-venous bypass. A bolus of heparin is given to attain systemic anticoagulation and allowed to circulate for approximately 3-5 minutes before cannulation. Heparin should be re-administered at regular time intervals to maintain systemic anticoagulation throughout the perfusion.

Veno-venous bypass is established first in the following manner. Cannulae are advanced in to the saphenous vein terminating in the infrarenal IVC and into the axillary vein terminating in the central chest. The cannulae are secured with Rumel tourniquets and are connected to a bypass circuit powered by a centrifugal pump. The suprarenal IVC below the liver is then clamped to shunt the infra-hepatic IVC venous flow. To establish the perfusion circuit, a venous outflow cannula is positioned in the retrohepatic IVC through a venotomy just below the liver with the tip positioned adjacent to the orifices of the hepatic veins but no higher. The GDA is then opened and the arterial inflow cannula is positioned with the tip remaining in the GDA near the orifice of the proper hepatic artery. The position of the IVC outflow cannula is important so that when the vascular occluding clamp on the supra-hepatic IVC is placed it will not include the catheter tip. The placement of the arterial cannula is important so that the catheter tip or pressure from inflow of the perfusate will not cause an injury to the intima of the proper hepatic artery.

Once the cannulae are positioned, the common hepatic artery is occluded proximal to the GDA, the portal vein is occluded, and a final vascular cross clamp is placed on the supra-hepatic IVC. In the past, portal venous blood flow was routinely shunted with the IVC blood flow; however, this has been abandoned to simplify the

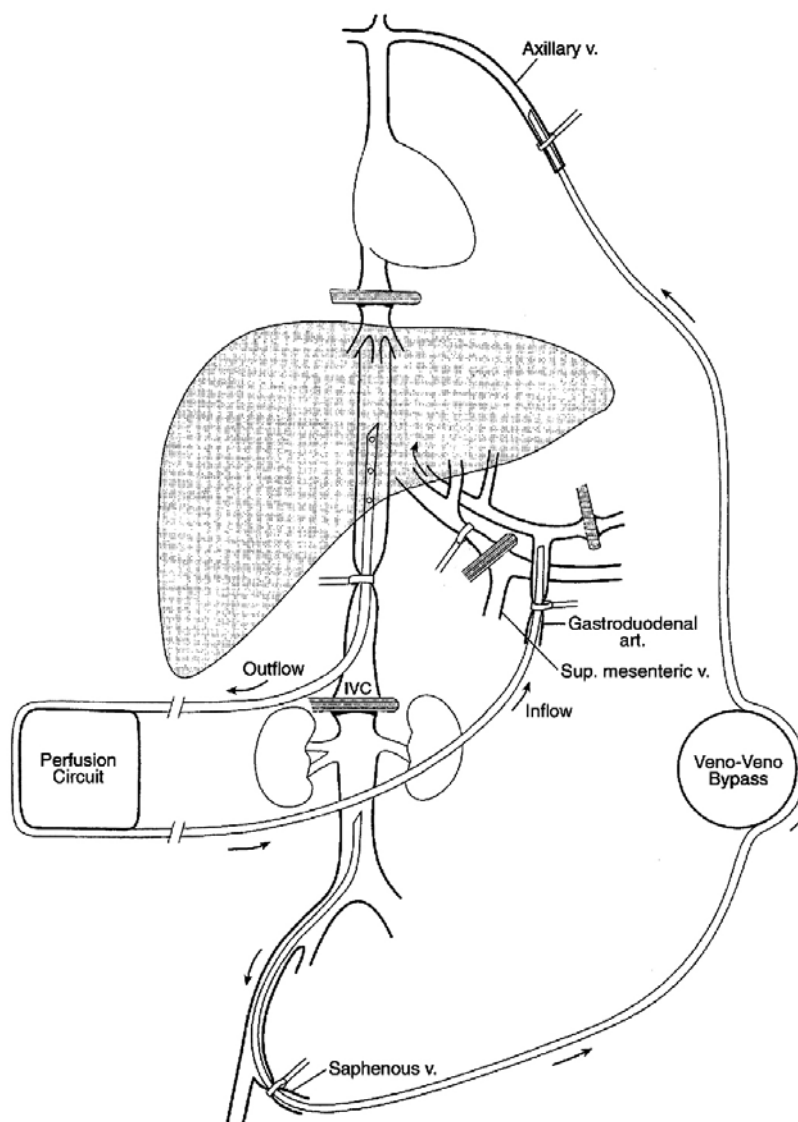


Figure 2. Schematic illustration of the IHP perfusion circuit. The arterial inflow is via the gastroduodenal artery and venous outflow is collected from a cannula positioned in an isolated segment of retro-hepatic vena cava. The inflow and outflow cannulae are connected to a perfusion circuit, shown on the patient's right. The veno-venous bypass circuit, depicted on the patient's left, shunts inferior vena cava blood flow back to the systemic circulation during therapy.

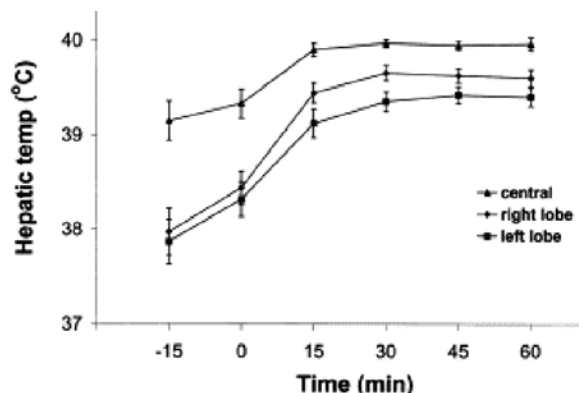
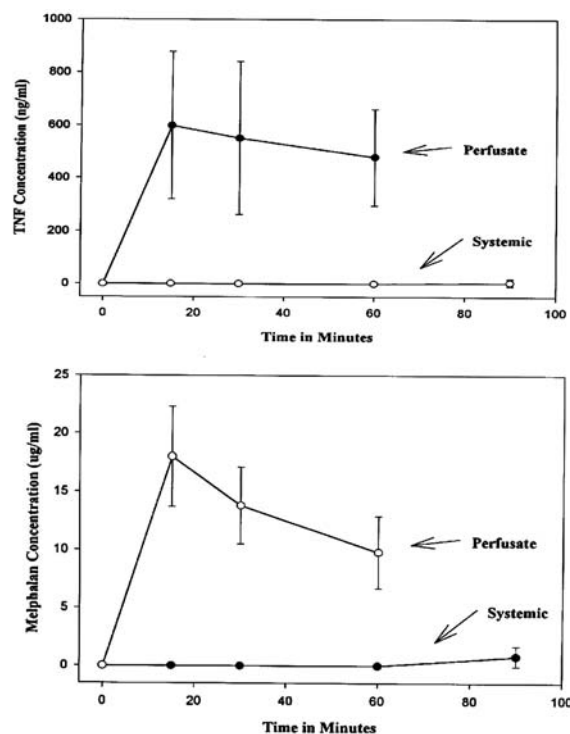
procedure and has not been associated with hemodynamic instability during the IHP.

If present, a replaced right hepatic artery may be cannulated and incorporated into the inflow line of the perfusion circuit via a Y-connector; an accessory left hepatic artery can be simply occluded during the procedure. The temperature of the liver parenchyma is monitored via perhepatically inserted probes in the left and right lobes of the liver. At this point, the vasculature of the liver is completely isolated and perfusion can begin; prompt and uniform heating of the liver parenchyma is usually observed and indicates adequate flow through both hepatic lobes (Figure 3).

The perfusion circuit is similar to that used in cardiac bypass procedures and consists of a roller pump, membrane oxygenator and heat exchanger. The 1 liter of perfusate is comprised of packed red blood cells and a balanced salt solution. The pH of the perfusate is monitored throughout the procedure and maintained above 7.2, with addition of sodium bicarbonate. Once stable perfusion parameters are achieved the liver is perfused for 60 minutes (Table 2). Intraoperative leak monitoring can be used as described; a gamma detection camera used to determine counts per minute (CPM) is placed over the centrifugal pump housing which holds a reservoir of systemic blood. Human serum albumin radio labeled with I-131 at a dose of 10-20 mCi is injected through a central vein and a baseline

Table 2. Typical Perfusion Parameters Used During IHP

Perfusion Parameter	Measure
Duration	1 hour
Hepatic tissue temperature	39.5-40° C
Flow rate	600-1200 ml/min
Arterial line pressure	110-200 mmHg
Veno-venous bypass	1.8-2.0 l/min
Flow perfusate volume	1 liter

**Figure 3.** Prompt tissue hyperthermia is routinely achieved during isolated hepatic perfusion. The temperatures measured peripherally in the right and left lobes are slightly lower than the central hepatic temperature, determined by a thermister probe positioned in the portal vein.**Figure 4.** Systemic and perfusate levels of TNF and melphalan during IHP. Measurements were obtained from samples taken at 0, 15, 30, 60, and 90 minutes after the start of perfusion. Peak perfusate concentrations of TNF and melphalan are observed within the first 15 minutes and levels are largely sustained during the 60 minutes of perfusion. Serum levels are undetectable for TNF and melphalan during perfusion.

CPM is determined. After a stable CPM is established, a 10-fold dose of the I-131 human serum albumin is injected into the perfusion circuit. Any increase in CPM represents a leak of perfusate into the systemic circulation and leak rates as small as 1% can be detected (65). Some centers have abandoned leak-monitoring for IHP after consistent experience with leak-free procedures (32). Pharmacokinetic data show that melphalan and TNF can be effectively confined to the IHP circuit (Figure 4). At the completion of perfusion, the liver is flushed through the GDA with crystalloid and then colloid. The cannulae are removed sequentially in order to reestablish flow first through the hepatic artery and the IVC.

7. TOXICITY OF TNF BASED IHP

IHP is an extensive operation as described above and usually lasts from 6-8 hours. Morbidity arises from the surgery as well as the agents used. Significant perioperative complications including hemorrhage, respiratory failure, and pleural effusion have been reported (66, 67). The mortality associated with the procedure has improved with experience and currently ranges from 0 to 6% (33, 65, 68-71). More recent studies have utilized TNF and melphalan or melphalan alone. The most common grade 3-4 toxicities associated with TNF in IHP are a transient rise in liver function tests and systemic hypotension. Liver enzymes characteristically return to preoperative levels approximately in 1-4 weeks after surgery and this transient elevation does not appear to have a significant effect on morbidity or mortality (33, 69, 70, 72). Elevated transaminases can also be seen with other agents. Weight gain, thrombocytopenia, tachycardia and fever have also been reported in the literature when TNF is used in hepatic perfusion without long term sequelae (33, 70).

Systemic toxicity associated with TNF can be observed after IHP when perfusate leak is controlled to the point where it is not measurable. One of the first studies focusing on the toxicities associated with TNF in IHP, independent of systemic exposure, came from the US National Cancer Institute in 2001. Lans *et al* compared hemodynamic parameters and cytokine release in patients with isolated liver metastases from colon cancer treated with IHP using melphalan alone (n=17) or melphalan and TNF (n=15). Complete vascular isolation was confirmed using the I-131 radio labeled albumin monitoring technique. Patients who received TNF exhibited significantly higher mean heart rate, lower systolic blood pressure, and higher peak mean pulmonary artery pressure compared to those patients treated with melphalan alone (Figure 5). The differences in hemodynamic parameters were gone after 48-60 hours. Hypotension was adequately treated with fluid resuscitation. Significantly elevated levels of IL-6 and IL-8 were observed in the TNF-treated patients and these cytokine levels returned to baseline within 24 hours (Table 3). The peak serum values of IL-6 and IL-8 occurred 4 to 6 hours after the procedure and were coincident with the maximum changes in systolic blood pressure observed in the patients receiving TNF. A transient elevation in serum bilirubin was observed in patients treated with TNF but not in those patients

Table 3. Systemic cytokine concentrations measured immediately after IHP with or without TNF. Time 0 is defined as the point when native blood flow to the liver had been re-established

Interleukin	Perfusate	0 hours after IHP	4-6 hours after IHP	24 hours after IHP
Mean serum IL-6 (pg/ml)	+ TNF	312 ± 43	23,543 ± 4,144	478 ± 95
Mean serum IL-6 (pg/ml)	- TNF	287 ± 31	841 ± 335	265 ± 77
Mean serum IL-8 (pg/ml)	+ TNF	250 ± 73	7,333 ± 1,113	53 ± 14
Mean serum IL-8 (pg/ml)	- TNF	65 ± 13	274 ± 42	52 ± 7

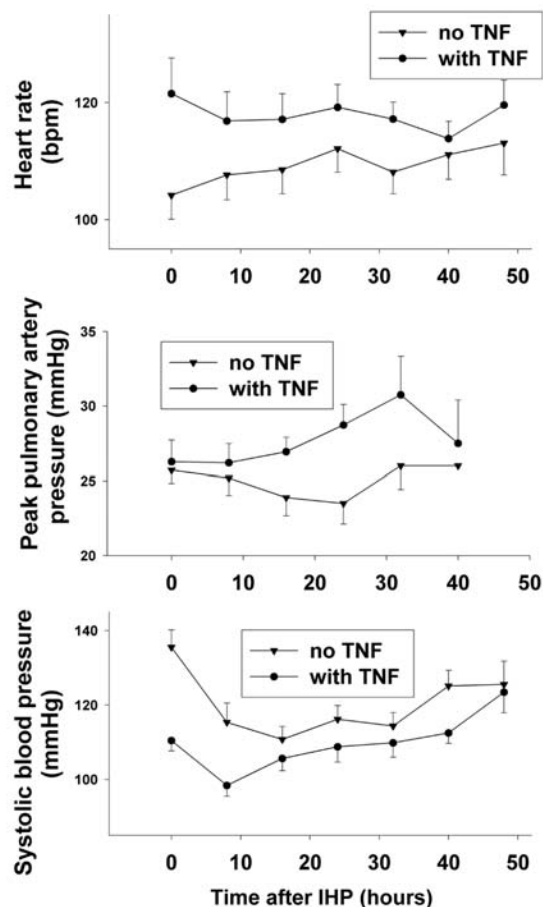


Figure 5. Mean heart rate, peak pulmonary artery pressure, and systolic blood pressure in patients after IHP with or without TNF. In patients receiving TNF, heart rate and peak mean pulmonary artery pressure were significantly higher and systolic blood pressure was significantly lower in patients receiving TNF compared with patients not treated with TNF.

receiving melphalan alone (Figure 6). In addition, patients treated with TNF exhibited significantly lower post-operative platelet counts. None of the patients required treatment for thrombocytopenia (73).

Whether the hemodynamic changes observed after TNF-based IHP are a direct result of elevated cytokines is unknown. Increased serum levels of IL-6 and

IL-8 have been linked to the hypotension in septic patients (74-76). Elevated serum levels of IL-6 in septic patients have also been associated with toxicities similar to those seen in IHP patients treated with TNF such as thrombocytopenia and changes in heart rate (77, 78). The liver is a metabolically active organ that releases cytokines in response to TNF which may account for the systemic toxicity observed in patients treated with TNF despite complete vascular isolation (79). However, the hemodynamic changes, cytokine release, and alterations in post-operative laboratory values in patients treated with TNF-based IHP are transient and do not appear to be of major clinical significance.

8. RESULTS OF TNF BASED IHP

IHP has been used with a variety of cytostatic agents on metastatic tumors of different origins. The treatment is often used in patients with tumors refractory to systemic therapy or in patients ineligible for other methods of localized therapy. Although response criteria have not been standardized, the majority of studies discussed here define partial response as > 50% reduction in tumor by imaging studies. The duration of response in IHP is variable. The outcomes of the most common histologies treated with TNF-based IHP are discussed below.

8.1. Colorectal cancer

Most of the experience with TNF-based IHP comes from treatment of unresectable CRC metastases. These trials with their results are summarized in Table 4. The largest studies of IHP come from the U.S. National Cancer Institute. The first large Phase II trial of TNF-based IHP was from Alexander *et al* in which 34 patients, 26 of whom had metastatic CRC, were treated with TNF and melphalan. This study demonstrated an overall response rate of 75% for a mean duration of 15 months (69). Bartlett and colleagues reported a follow-up study from that institution of IHP in 50 patients with CRC metastases in 2001. Thirty-two patients received IHP alone with 1 mg of TNF and 1.5 mg/kg of melphalan and 19 patients underwent hyperthermic IHP with melphalan alone followed by HAI with floxuridine and leucovorin. Twenty-six patients had received prior therapy that had failed to control their disease. There was an overall response rate of 76%, all partial, with a median duration of 10.5 months. Of the patients treated with IHP alone, 77% demonstrated partial response with a 16 month median survival. The patients treated with IHP followed by HAI had a 74% response rate and 27 month median survival (33). Currently TNF is no longer available in the United States for clinical trials in IHP and recent studies with melphalan alone demonstrate response rates of 40-60% and comparable median survival of 12-17 months (71, 80).

The investigators from the NCI have presented an updated but unpublished analysis of IHP in 120 patients with unresectable CRC liver metastases who underwent a 60 minute IHP with melphalan (n=69), TNF (n=10), or both (n=41). Wilcoxon rank sum and Fisher's exact tests were used to compare parameters by response category; survival and hepatic progression-free survival probabilities

Table 4. Results of IHP in patients with metastatic colorectal cancer

Reference	N	Treatment	Overall Response	Survival	Comments
67.	50 (26 CRC)	5-FU 29 pts. with additional HAI	50% (22% complete)	Median 14 mos. (18 mos. w/HAI)	8% post operative mortality
79.	6	Melphalan TNF	83% median 4.5. mos	Median 10 mos.	33% mortality infusion via HA and PV
69.	34 (26 CRC)	TNF Melphalan	75% mean 15 mos.	Not reported	3% post operative mortality
81.	12 (6 CRC)	Melphalan + TNF (6)	60%	Mean 1 month	
35.	11 (5 CRC)	Melphalan TNF	27% mean 6 mos	Median 16 mos.	No response for 3 CRC cases
33.	51	Melphalan +TNF (32) Melphalan + HAI (19)	76% all parital median 10.5. mos.	Median 16 mos (IHP alone) 27 mos. (+ RFA)	Response duration longer in HAI group (14.5. vs 8.5. mos.)
70.	7	Melphalan +TNF (5) TNF alone (2)	71% all partial median 10 mos.	Mean 19.7. mos.	2 patients with TNF only – no response
68.	73	Melphalan	59% median 7.7. mos.	Mean 28.8. mos.	6% mortality
80.	25	Melphalan	60% median 12 mos.	Median 12 mos. 28% 2-year survival	Preceeded by Irinotecan-based therapy
71.	30	Melphalan	43% median 11.5. mos.	Median 16.9. mos.	

were calculated by the Kaplan-Meier method, with log-rank (Mantel-Haenszel) tests used to determine the significance of the difference between pairs of Kaplan-Meier curves; parameters jointly associated with survival or hepatic progression-free survival were determined using Cox proportional hazards models. There were 69 responses in 114 evaluable patients for an overall radiographic response rate of 61 percent. Interestingly, there was no clinically meaningful anti-tumor activity in a small cohort of patients treated with TNF alone; there were 4 of 10 who experienced a partial response all of which were limited in duration. Age, pre-op CEA, prior chemotherapy, tumor burden, and post-IHP HAI therapy were not associated with response; however, total melphalan dose and combination melphalan and TNF were each associated with response. In other words, those patients who had TNF were more likely to have a partial or complete response compared to those treated with melphalan alone although this association was only marginally significant. Based on its presumed mechanism of action on tumor associated neovasculature and the fact that CRC liver metastases are hypovascular in nature may explain the relatively limited contribution of TNF in this clinical setting (Figure 7).

8.2. Ocular melanoma

Several studies have reported the use of combination TNF and melphalan IHP in OM in series of metastatic cancers of various histologies (69, 72, 81). However, these studies only consisted of 2-4 patients with liver metastases from OM. One of the earlier studies focusing on IHP in metastatic OM in 22 patients used melphalan alone in 11 patients and melphalan with TNF in 11 patients. The overall response rate reported was 62% with 2 patients (9.5%) exhibiting a complete response (Table 5). Although the median duration of response was 9 months, it is notable that the duration of response was significantly longer in the patients treated with TNF compared to melphalan alone (14 versus 6 months, respectively). The median overall survival was 11 months (82). The most durable responses were seen in patients treated with TNF and melphalan; one lasting for over 6 years (Figure 8).

8.3. Neuroendocrine tumors

Studies on isolated hepatic perfusion for neuroendocrine metastases are limited. Grover *et al* reported experience using melphalan, melphalan with TNF, and TNF alone in 13 patients. Most patients had > 25% of the liver involved with a median number of 40 tumors. A partial response was observed in 50% of patients with a marginal response in an additional 3 patients. Only 2 patients had functioning tumors, and therefore quality of life was not formally assessed. However, 1 patient reportedly had relief of symptoms for at least 3 years. The median actuarial survival was 48 months. There were no intraoperative deaths and there was one perioperative mortality from multi-system organ failure. The most common complication was a transient rise in liver function tests consistent with other experiences with IHP (83). In extensively metastatic NET to the liver, IHP is a viable option; however, response rates are generally very good in this patient population and it is not known if TNF contributes to the anti-tumor activity.

8.4. Primary hepatic tumors

There are few reports of IHP in primary liver cancer. Many patients with HCC present with evidence of cirrhosis or hypertension and therefore are not candidates for IHP. Feldman *et al.* reported the results of IHP in 9 patients with primary liver cancer (5 with HCC and 4 with cholangiocarcinoma). All treatments were performed under conditions of hyperthermia and 3 of the 9 patients received TNF with melphalan while 6 patients were treated with melphalan alone. There was a partial radiographic response in 67% of patients for a mean duration of 7.7. months. The mean survival reported was 15 months with a 27% 1-year survival. There were no perioperative deaths and the most common complications were fever, weight gain, and a transient rise in liver function tests (84). There have been other small studies of IHP for primary liver tumors without TNF. Lise *et al* reported 10 patients receiving hyperthermic IHP using melphalan with 2 perioperative deaths and a 63% overall response rate (85). Haefstrom *et al* demonstrated a partial response in 1 out of 4 patients treated with melphalan-based IHP without TNF (86).

Table 5. Response data after IHP with or without TNF for patients with ocular melanoma.

Variable	Total	Melphalan	Melphalan with TNF
N	22	11	11
Not assessable (n)	1	1	0
Total assessable (n)	21	10	11
CR, n (%)	2 (9.5%)	2 (0%)	2 (18%)
PR, n (%)	11 (52%)	7 (70%)	4 (36%)
Overall (CR + PR), n (%)	13 (62%)	7 (70%)	6 (54.5%)
Median duration of in-field response (mo)	9 (5-50)	6 (2-13)	14 (6-50)

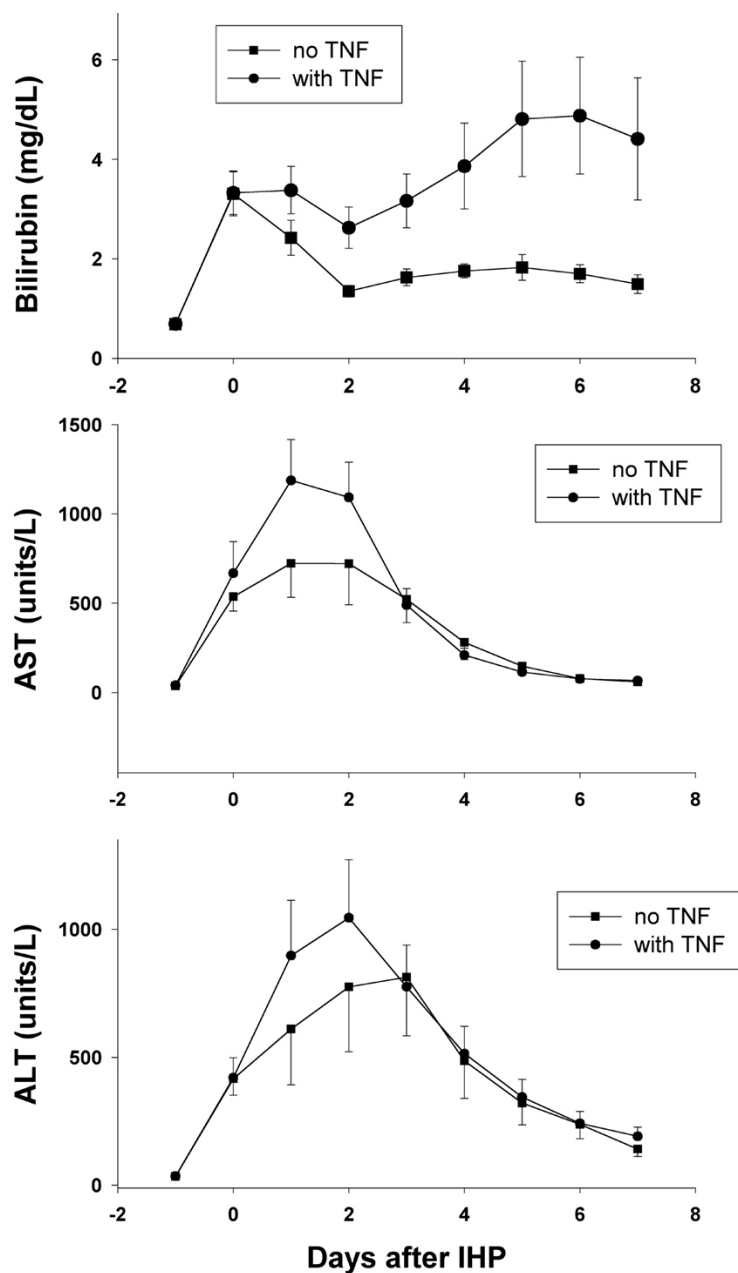


Figure 6. Hepatic bilirubin and transaminase levels in patients undergoing IHP with melphalan alone (n=17) and with melphalan and TNF (n=15).

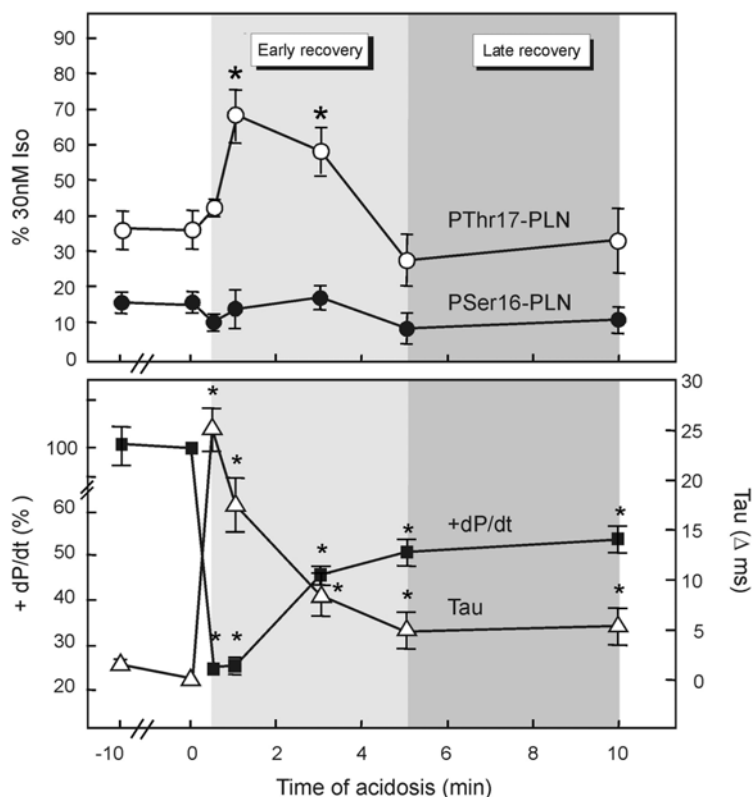


Figure 7. Gadolinium-enhanced $T1$ -weighted magnetic resonance imaging (MRI) scans through the liver of a patient with colon cancer metastases to the liver. Images are from a patient taken before and 10 months after IHP with melphalan and TNF. The patient had previously been treated with systemic 5-FU and leucovorin followed by intra-arterial floxuridine and leucovorin.

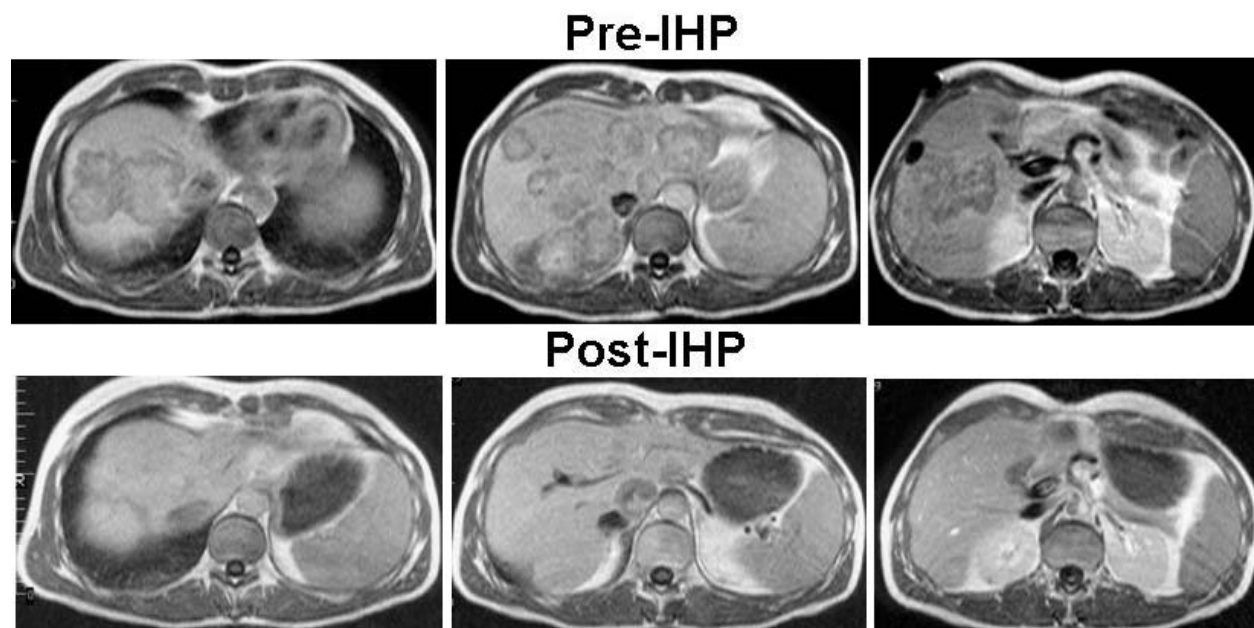


Figure 8. Gadolinium-enhanced $T1$ -weighted MRI of the liver before and 5 years after IHP with melphalan and TNF in a 38-year-old male with metastatic ocular melanoma. The patient was free of disease after resection of two perihepatic masses, presumed to be lymph nodes, 2 years after IHP and resection of a solitary brain metastasis 28 months after IHP.

9. CONCLUSIONS

The role of TNF in IHP has not been definitively established. Despite evidence that the addition of TNF may improve response rates in patients with CRC liver metastases and prolong response duration in ocular melanoma, it is unclear whether this translates to other histologies. There are several empirical advantages of using TNF in isolated perfusion as outlined in this chapter. However, given the additional toxicities associated with TNF in IHP, further studies are warranted to determine whether TNF confers a clinical advantage.

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Abbreviations: HCC: hepatocellular carcinoma, CRC: colorectal carcinoma, OM: ocular melanoma, NET: neuroendocrine tumor, IHP: isolated hepatic perfusion, TNF: tumor necrosis factor alpha, RFA: radiofrequency ablation, TACE: transarterial embolization, HAI: hepatic arterial infusion, LPS: lipopolysaccharide, ECOG: Eastern Cooperative Oncology Group, IVC: inferior vena cava, GDA: gastroduodenal artery, CPM: counts per minute, CEA: carcinoembryonic antigen

Key Words: Liver metastases, Isolation Perfusion, Regional Chemotherapy, Hyperthermia, Colorectal Carcinoma, Hepatocellular Carcinoma, Ocular Melanoma, Neuroendocrine Tumor, Review

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