30 Hyperthermic Isolated Limb Perfusion for Melanoma

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In 1956, the Department of Surgery at University of Tulane embarked on regional perfusion studies with a goal of increasing intratumoral chemotherapy drug concentrations in tumors located where the vascular supply and drainage could be completely isolated [1]. The use of a heart-lung machine to support isolated hyperthermic perfusion of the tumor was evaluated an approach to increase the dose of nitrogen mustard and at the same time avoid systemic toxic effects [2]. Cannulation of both the arterial inflow and the venous drainage for connection to an extracorporeal circuit maintained by a heart-lung machine for blood oxygenation represented an improvement over the technique previously described by Kopp and colleagues in which the chemotherapy was administered into the artery, with the venous drainage left unaltered, or clamped [2].

In 1957, a patient with a very high burden of melanoma metastases to the extremity presented to Charity Hospital 2 years following treatment of a melanoma on the ankle. Amputation was recommended, as the patient had over 80 satellite lesions, but the patient refused this recommendation. The team performed an isolated chemotherapy perfusion using melphalan, a chemotherapy agent that was new and under evaluation at the time for metastatic melanoma. The patient experienced a complete clinical response and remained melanoma-free until his death at age 92, some 16 years later.

The following year Creech presented the results of isolated perfusion in 24 patients—6 with melanoma and another 18 with other advanced cancers—before the American Surgical Association in New York [3]. For pelvic tumors, the aorta and IVC were occluded below their renal branches and cannulated just above the bifurcation. For perfusion of lung tumors, two circuits and caval occlusion were used to prevent mixing between the systemic and pulmonic circuits. And a motor pump was used to create negative pressure in the venous return circuit to minimize systemic mixing in cases in which tourniquets could not be applied (e.g., breast). Creech reported gross or microscopic responses in 18 of 19 cases followed long enough for changes to be evident. By 1962 they had treated a sufficiently large number of patients to report results of 303 patients, 123 with melanomas [4].

Many hospitals followed suit and began performing isolated limb perfusion. Unfortunately, an opportunity for progress was lost during this interval because hypotheses were not prospectively addressed and data were not collected in a scientific manner. Studies were generally single arm, absent appropriate control groups, and involved heterogenous patient populations including patients with completely resected

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tumors and unresectable tumors. Every study used a different types of patients and different doses of melphalan, perfusion duration, and temperature [5–13]. For example, in a report of 1139 perfusions performed over 35 years, the authors included patients with multiple indications: definitive treatment of in-transit metastases, unresectable recurrent or primary tumors, adjunctive therapy to surgical excision for regionally confined melanoma, conversion of advanced unresectable melanoma to resectable, and palliation in noncurable recurrent melanomas by maintaining a functional limb in the presence of systemic metastases [1]. Fortunately, clinical studies in the past two decades have been of significantly higher quality and with greater scientific rigor.

Equipment

The operation requires a standard heart-lung bypass device equipped with a roller pump, oxygenator with a gas source (95% oxygen 5% carbon dioxide), heater capable of reaching 42 °C, and venous reservoir (Fig. 30.1). Additional equipment necessary include an ultraviolet (black) light is used to evaluate for leakage of fluorescein from the extremity, access to a machine for activated clotting time measurements, a scintillation probe mounted over the chest (precordial) to monitor for I-131 or 99 m-Technetium labeled albumin or red cells as an indicator of leak from the circuit into the systemic circulation, a pulse volume recording machine to assess peripheral vasculature, and

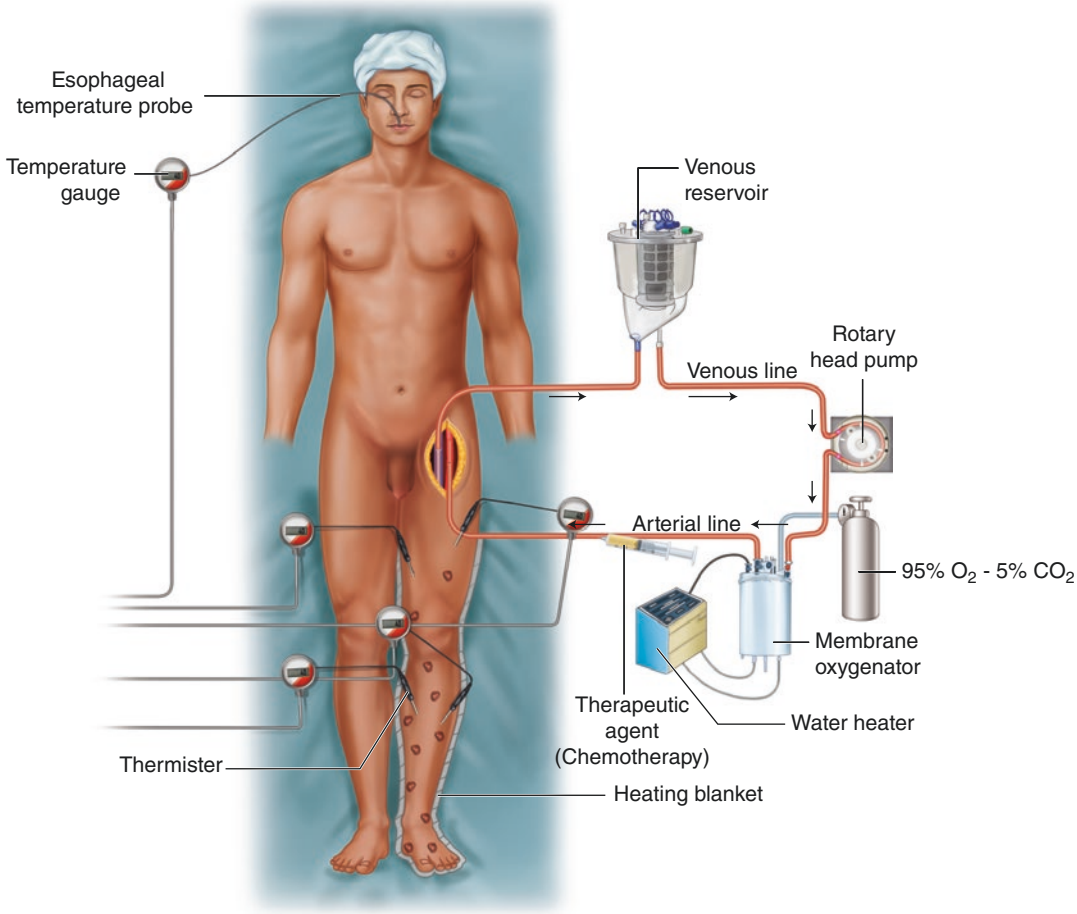

Fig. 30.1 Diagram showing typical set up for lower extremity hyperthermic isolated a limb perfusion

heating blankets for external warming of the extremity. Thermistors inserted under the skin are connected to digital temperature monitors to monitor temperature in different locations during the operation. A selection of different size arterial and venous cannulas should be on hand, as well as heparin-saline irrigation. A self-retaining retractor attached to the table is of significant help for approaching iliac vessels. Standard vascular instruments are used during the operation, as well as Rummel tourniquets, a hand drill for placement of Steinmann pins, and a Doppler probe.

Leak Monitoring

It is necessary during limb perfusion to assess for leakage from the circuit into the systemic circulation, or from the systemic circulation into the circuit. Leakage of melphalan into the systemic circulation can lead to acute nausea and delayed bone marrow suppression or hair loss. Leakage of even small amounts of tumor necrosis factor leads to proinflammatory cytokine storm responsible for sepsis-like side effects including intraoperative tachycardia, hypotension, and pulmonary edema [14].

A commonly used technique to measure leak involves mounting a shielded precordial scintillation detector over the precordium and injecting I-131 or Tm-99-labeled albumin or red cells into the perfusion circuit. A fraction of the total dose is administered into the systemic circulation to calibrate the system and allow for quantification of the leak, using the assumption that the volumes of the extracorporeal circuit and the systemic vasculature are in the proportion of 1:5. This technique allows quantification of the percent fractional leak over time.

A simpler but not quantitative approach involves administration of fluorescein into the circuit and then viewing different areas of the body with a Woods lamp. This technique reveals specific areas of skin outside the extremity that are receiving perfusate, thereby directing further dissection to identify and control specific collateral vessels. A disadvantage of this technique is that quantification is not possible, and once a significant systemic leak has occurred, it is not possible to confirm correction of the leak.

Another technique for leak detection that has been described but not used widely is administration of 3% desflurane into the bypass circuit using an anesthetic vaporizer. The expired breath is then monitored by standard gas analysis for desflurane as a sign of leakage [15].

Agents

Melphalan is the most widely used agent for HILP for melanoma. It is the agent that was used for the first patient treated with HILP and produced a clinical complete and durable response. Melphalan is a phenylalanine and a precursor for melanine biosynthesis and therefore taken up avidly by melanocytes and melanoma cells. The mechanism of action of melphalan is through its ability to interact directly with DNA and cause miscoding. A second mechanism by which alkylating agents cause DNA damage is by formation of cross-bridges in the DNA, thereby preventing strand replication or transcription.

Pharmacokinetic studies of melphalan in HILP demonstrate rapid uptake in tissue in the first 5—10 min, and continual reduction in drug concentration over 60 min to 10—20% of the starting concentration [16]. Dosing is calculated from limb volume or body weight, though notably, limb volume expressed as a percentage of total body weight results in as much as a twofold variation in the population for both lower and upper extremities. This could theoretically lead to double the amount of melphalan administered to the same volume of tissue in two different individuals when dosed by weight. When dosed by limb volume, optimal dosages of 10 mg/L limb volume in the leg and 13 mg/L limb volume in the arm have been determined as the highest dose with acceptable risk, and little variation in toxicity [11, 17, 18]. Melphalan is stable in sterile 0.9% sodium chloride for only 90 min at room temperature [19] and is therefore prepared immediately before administration. Melphalan is eliminated from plasma primarily by chemical

hydrolysis to inactive monohydroxymelphalan and dihydroxymelphalan. Renal excretion is extremely low. Identification of fluorescein in the urine from a leak test does not equate to a similar amount of melphalan in the urine. All discarded bodily fluids from an HILP case should be handled as chemotherapy biohazard waste. Side effects of melphalan administration as part of HILP are discussed below.

Tumor necrosis factor alpha TNFα gained considerable interest as an anti-cancer agent because it is a proinflammatory cytokine produced by multiple different immune cells and causes rapid and significant hemorrhagic necrosis of tumors. But humans are exquisitely sensitive to toxic effects of TNFα including a septic-like response with fevers, tachycardia, cardiovascular collapse, pulmonary edema, and shock. The maximum tolerated systemic dose has essentially no effect on tumors. With these observations in mind, TNFα is a logical choice of agent for isolated regional perfusion with a goal of achieving anti-tumor effects in the extremity without systemic side effects. TNFα alone has been used for isolated limb perfusion, with limited benefit observed [20]. Of six treated patients, partial response of less than 1 month duration was seen in two patients, and one patient had a complete response of only 7 months duration and then progressed. The observation that TNFα increases tumor neovascular permeability suggests that its best use is in combination with other agents. It has been combined most commonly with melphalan and interferon. Other agents used in the past for isolated limb perfusion either alone or in combination with other agents include cisplatin, dacarbazine, actinomycin D, and fotemusine [21].

Operative Technique

The operation involves the use of an extracorporeal circuit attached to a heart-lung machine (oxygenator and blood pump) to increase the oxygen tension and heat the circulating blood before delivery to the isolated limb and buffer with carbon dioxide. Anesthesia must be prepared for intraoperative fluid shifts between the vascular compartments of the limb and the remainder of the body, hypotension caused by low vascular tone, and sequela of ischemia reperfusion [22].

The operation typically lasts for 4–6 hours, depending on which vessels require isolation and whether a concomitant lymphadenectomy is indicated. Two large bore IVs are required, and anesthesia should be prepared for acute blood loss, particularly if surgical isolation of the vessels is anticipated to be difficult (e.g., iliac vessels and scarred vessels). Central venous pressure monitoring is not typically required. An arterial line is useful for repeated activated clotting time (ACT) measurements, and on occasion, close monitoring of blood pressure to enable manipulations necessary to manage leakage between the circuit and systemic circulation. A urinary catheter should be inserted. An epidural catheter for postoperative pain management is not typically used.

PVR is measured and saved for comparison after the operation. Similarly, peripheral pulses in the affected extremity are carefully assessed and recorded. Thermistors are placed in the proximal and distal extremity both medially and laterally (e.g., four thermistors) for real-time temperature monitoring during the operation. The extremity is wrapped in heating blankets, leaving the PVR cuff in place. It is necessary to place sterile surgical tubing (or Esmark bandage) around the root of the extremity for later use as a tourniquet.

A preoperative dose of antibiotic is administered. An incision is made over the vessels, with extension if needed for a lymphadenectomy. Axillary lymphadenectomy and iliac/hypogastric lymphadenectomy are performed as a matter of routine during isolated limb perfusion through the axillary or external iliac vessels, respectively. We do not perform superficial femoral lymphadenectomy at time of isolated limb perfusion unless there is clinical evidence of nodal metastases given that the incision used for this lymphadenectomy has high likelihood of infection or dehiscence, especially in a chemotherapy-treated field. Perfusion from an iliac approach does effectively perfuse lymph nodes in the femoral triangle [23]. The vessels are circumferentially

isolated, and small collateral vessels distal to the cannulation sites are tied off. A Steinmann pin is placed into the anterior superior iliac spine to serve as a cleat and prevent slippage of the tourniquet around the root of the extremity. Once the dissection is complete, 350 U/kg heparin is administered to achieve an ACT of over 450 s. The vessels are occluded proximally and distally with either vascular clamps or Rummel tourniquets. The vein and artery are cannulated through a transversely oriented incisions in the vessels, and each held in place with a Rummel tourniquet placed around the distal vessel and cannula, taking care to avoid fracturing any atherosclerotic plaque that is present. The tourniquet around the root of the limb is tightened to occlude superficial collateral vessels in the skin. After confirmation of a therapeutic ACT (longer than 450 s), the cannulas are connected to the extracorporeal circuit, and the roller pump is gradually brought up to the maximum flow rate at which the line pressure acceptable to avoid intimal injury and the reservoir volume does not diminish. Heparin resistance—defined by the inability to achieve therapeutic ACT with typical heparin doses—is typically successfully treated with additional heparin. However, antithrombin III deficiency should be suspected if this maneuver is unsuccessful, in which case use of argatroban instead, or transfusion of fresh frozen plasma or antithrombin is typically effective [24].

Once the extremity has reached the target temperature, melphalan is administered into the arterial side of the circuit based on the planned dose schedule. The heater for the heart-lung machine is adjusted based on the extremity temperatures registered by the thermistors. Isolated perfusion is conducted for the planned time, typically 60 or 90 min, during which time leak monitoring is employed to guide any necessary adjustments. Protocols for drug dosage, drug administration schedule, target temperature, and duration of perfusion differ among centers. After the perfusion is complete, the extremity is rinsed with crystalloid and/or colloid, with the drug-containing venous effluent discarded. The cannulas are removed, and the arteriotomy and venotomy are repaired with vascular sutures, meticulously avoiding narrowing of the vessels. PVR measurements in the distal extremity are obtained, and upon confirmation of a return to baseline, protamine is administered to reverse the effects of heparin. The wound is closed in multiple layers. A drain is left behind if a lymphadenectomy was performed.

In-transit metastases occur most commonly in the lower extremity, and therefore access for HILP is most commonly achieved via the iliac vessels or the femoral vessels. If in-transit metastases are located high in the extremity (e.g., within 6 inches of the inguinal crease), perfusion via the iliac vessels is required to achieve adequate perfusion of the proximal thigh. This operation involves an oblique incision in the lower abdominal wall, followed by incision of the external oblique fascia and splitting of the internal oblique musculature to reveal the transversalis fascia. This is incised, and the abdominal contents are retracted supero-medially to expose the iliac vessels. External iliac and obturator nodes are removed. Note is made of the quality and characteristics of the Doppler signals in the external iliac artery and vein. The hypogastric vein is ligated in situ or controlled with a bulldog vascular clip, and another bulldog clip is placed on the hypogastric artery. The external iliac vessels are followed under the inguinal ligament for as far as possible to allow for identification of small branches, which are clipped or tied off to prevent collateral flow. Removal of the clips on arterial branches at completion of the operation improves blood flow to portions of the healing wound. A drill is used to place a Steinmann pin in the anterior superior iliac spine to hold the tourniquet in place.

For approach to the axillary artery and vein, a generous incision is made in the axilla, and flaps are raised to allow a complete axillary lymphadenectomy. The pectoralis minor muscle is divided below its insertion onto the coracoid process. Level III axillary node are removed, which also provides additional exposure of the axillary vessels. Branches are tied off and divided. The brachial plexus trunks are carefully pushed aside to provide exposure to the artery with minimal disruption to the nerves. A

Table 30.1 Intraoprative leak identification and management

Precordial monitor (if used)	Circuit reservoir	Interpretation	Maneuver
Stable	Stable	Good isolation	None
Stable	Increasing	Systemic blood leaking into circuit	Tighten tourniquet, tilt table to place heart lower than limb (e.g., Trendelenberg position for leg perfusion), lower mean arterial pressure and venous pressure with nitroglycerin infusion, increase circuit flow rate
Increasing	Decreasing	Leakage from circuit into systemic circulation	Tighten tourniquet, tilt table to place heart higher than limb (e.g., reverse Trendelenberg position for leg perfusion), lower circuit flow rate, raise mean arterial pressure with pressor infusion, increase central venous pressure by infusing large amounts of intravenous fluid. If leakage persists, repeat flouresceine dye test to identify previously missed collateral vessels and guide dissection
Increasing	Stable	Two way leakage	Leakage in one direction is from venous collaterals, and leakage in the opposite direction is from arterial collaterals. First, stop leakage from circuit into systemic circulation, and once successful then stop leakage into the circuit. Tighten tourniquet and lower circuit flow rate. If this is unsuccessful, then raise mean arterial pressure with pressor infusion. If precordial monitor is still increasing, then lower mean arterial pressure back to baseline and instead increase central venous pressure by infusion large amounts of intravenous fluid. Once leakage from the circuit into the systemic circulation is stopped, the circuit reservoir should start increasing, in which case next lower the mean arterial pressure or the central venous pressure

Steinmann pin is placed to serve as a cleat for the tourniquet. An alternative approach is to use a retractor connected to the table to hold the tourniquet in place [25].

Specific maneuvers are employed to manage leakage between the circuit and systemic circulation during isolated limb perfusion (Table 30.1). Leakage from the circuit into the systemic circulation typically manifests as loss of volume in the venous reservoir. The route of leakage may be venous collaterals, arterial collaterals, or both. Leakage from the extracorporeal circuit that occurs after drug is administered results in systemic exposure to drug, and a lower concentration in the limb. The first step to manage leakage from the circuit to the systemic circulation is to lower the flow rate, which results in reduced pressure in collateral arteries and veins. The operating table can be tilted into reverse Trendelenberg position to lower the venous pressure in the leg relative to collateral veins. After infusion of fluorescein into the circuit, the skin should be examined with a Woods lamp to search for specific collateral vessels that were missed on initial dissection and can be tied off (e.g., inferior epigastric or circumflex iliac vessels). The systemic mean arterial pressure may be increased by infusion of pressor agents, and the central venous pressure may be increased by infusion of intravenous fluid.

Leakage from the systemic circulation into the isolated circuit manifests as an increase in reservoir volume over time. This results in unintended lowering of the drug concentration, as well as discarding more drug-contaminated blood at the end of the procedure. The first step is to increase the circuit flow rate, simultaneously monitoring outflow pressures to avoid intimal injury. The operating table can be tilted into Tredenlenberg position to raise the venous pressure in the lower limb relative to collateral veins. The central venous pressure and the systemic mean arterial pressure may be lowered by infusion of nitroglycerin.

A complex situation may arise whereby the precordial scintillation monitor suggests ongoing leak, yet the reservoir volume is stable or increasing. This set of observations indicates bi-directional leak, with blood movement into the limb via one set of collateral vessels (i.e., venous) and out of the limb via different collateral vessels (i.e., arterial). The approach to this condition involves simultaneous management of both types of leak (Table 30.1).

Hyperthermia

In Creech's original report, hyperthermia was not used in the isolated limb perfusion circuit, and rather, the treatment was with chemotherapy alone [3]. Subsequent work demonstrated that the combination of chemotherapy with mild hyperthermia produced higher response rates [26]. Hyperthermia during HILP affects cancer cells and non-cancer cell populations within tumors including neovasculature and stromal cells, and normal tissues in the extremity. The addition of hyperthermia clearly increases side effects (e.g., effects on normal tissues). In one study, factors associated with a greater toxicity were tissue temperatures 40 °C or higher, female gender, low pH in the circuit, and perfusion at a proximal level of isolation [27]. However, it is equally clear that tumor cells are more susceptible to adverse effects of hyperthermia compared to normal cells. Results of animal model studies of isolated limb perfusion with versus without hyperthermia suggest added cytotoxicity and increased efficacy with the addition of the hyperthermia [28]. These studies implicated a mechanism of enhanced cytotoxicity of l-phenylalanine mustard with hyperthermia rather than improved drug delivery and uptake. There are no prospective randomized clinical trial results comparing isolated limb perfusion with versus without hyperthermia.

Patient Selection

The most common indication for limb perfusion is *in-transit* metastases. HILP is used for patients with unresectable metastatic melanoma confined to an extremity without evidence of distant metastases. The definition of unresectable is subjective but integrates the frequency of *in-transit* metastases recurrences as well as the number and distribution of metastases. Rapid recurrence of multiple in tumor nodules soon after excision of in-transit metastases indicates that further surgical resection is not warranted, despite being technically achievable. Full staging including PET-CT and head MRI to exclude other metastases should be performed. Patients with peripheral vascular disease are not good candidates for HILP because of a significantly higher risk for toxicity and complications. The presence of peripheral vascular disease is typically evident on preoperative evaluation. Patients with declining performance status or who are unable to ambulate because of comorbidities are poor candidates for HILP.

Prior to effective molecularly targeted and immunotherapies, HILP was recognized as the most effective and appropriate treatment for patients with melanoma recurrences confined to an extremity. However, with advent of effective systemic therapies, most patients are treated with systemic therapy before resorting to HILP. BRAF V600 mutant melanomas are sensitive to targeted therapy using a BRAF inhibitor combined with a MEK inhibitor, with a response rate of 63% and acceptable toxicity [29, 30]. And for patients without BRAF V600 mutations in their melanoma, immune checkpoint inhibitor therapy to block CTLA-4, PD1, or PDL1 is commonly used. Response rates range from 11% with ipilimumab to 61% with ipilimumab and nivolumab [31]. Combined BRAF and MEK inhibitor therapy is typically first-line treatment for unresectable *in-transit* metastases that are BRAF mutant. And immune checkpoint inhibitor immunotherapy is typically first-line treatment for unresectable *in-transit* metastases that are BRAF wild type. HILP is typically considered for patients who progress on these therapies. And it is a good approach for patients who have a contraindication to immunotherapy, such as liver transplant, active colitis, and/or unmanageable and severe toxicity to immunotherapy.

Adjuvant HILP was historically used as adjuvant therapy after resection of high-risk primary

melanomas. A small, prospective randomized control trial conducted at the University of Cologne randomized to excision alone or excision with HILP and demonstrated a remarkable reduction in recurrences in the HILP arm [32]. But subsequently conducted randomize control trials that are of higher quality and larger patient number have convincingly demonstrated lack of benefit of adjuvant HILP. The clinical trial considered definitive in this area was conducted by a consortium of EORTC, WHO, and the North American Perfusion Group (NAPG-1) [23]. Over a period of 10 years, 852 patients were randomized to wide excision alone or wide excision and HILP. HILP-treated patients experienced no benefit in overall survival or time to distant metastasis, though HILP-treated patients benefited from a reduction in incidence of in-transit metastases as first site of recurrence (reduced from 6.6% to 3.3%), and of regional lymph node metastases, with a reduction from 16.7% to 12.6%. Adjuvant HILP was also examined as adjuvant to excision of *in-transit* metastases, and similar to other adjuvant trial results, improvement in regional disease control could be demonstrated but not improvement in overall survival [33]. In summary, HILP is not beneficial as an adjuvant therapy.

Results

The primary agent used by nearly all centers for HILP has been melphalan. Administration schedules differ among centers in drug dose, temperature, and duration of perfusion. Accordingly, it is difficult to reach definitive conclusions about which techniques and schedules are optimal in efficacy and have the least toxicity. The complete response rate for HILP with melphalan alone is in the range of 40–60%, and the overall response rate (e.g., including partial responses) ranges from approximately 60–90% (Table 30.2). For leg perfusions, the melphalan dose varies from 6 mg/L to 10 mg/L of leg volume, or when dose by body weight 0.8 mg/kg to 2 mg/kg of body weight. The dose used for arm perfusions is lower and ranges from 0.45 mg/kg to 0.8 mg/kg. Target limb temperatures vary range from 37° (normothermia) to 42°. Perfusion times vary from 50 to 120 min. Because of heterogeneity in the reports, it is not possible to draw a conclusion about the relationship between dose schedule and response rates. An approach utilizing sequential perfusions via the external iliac and common femoral vessels staged 6 weeks apart has also been used [13]. While the complete response rate with this approach jumped up to 77%, no benefit in overall

Table 30.2 Perfusion schedules and response rates with melphalan alone

Authors	*n*	Melphalan dose leg	Melphalan dose arm	Perfusion duration	Target limb temperature	Complete response rate	Partial response rate	Overall response
Rosin [5]	80	2 mg/kg	N/A	50 min	39–40 °C	21 (26%)	29 (36%)	50 (62%)
Di Filippo [6]	69	1.5 mg/kg	0.8 mg/kg	60 min	41.5 °C	27 (39%)	30 (43%)	57 (82%)
Skene [9]	67	2 mg/kg	N/A	60 min	39–40 °C	N/A	N/A	52 (74%)
Knorr [41]	87	10 mg/L	13 mg/L	90 min	38.5–40 °C	58 (66%)	21 (25%)	79 (91%)
Cornett [40]	58	10 mg/L	13 mg/L	90 min	38.5–40 °C	14 (25%)	22 (38%)	38 (64%)
Klaase [12]	120	10 mg/L	13 mg/L	60 min	37–40 °C	65 (54%)	30 (25%)	95 (79%)
Kroon [13]	43	First: 6 mg/L Second: 9 mg/L	13 mg/L	60 min	37–38 °C	33 (77%)	1 (2%)	34 (79%)

survival was observed relative to patients undergoing a single perfusion.

The heterogeneity among reports in key technical aspects of the procedure makes evaluation of the contribution of hyperthermia challenging. One retrospective analysis compared 218 patients treated with mild hyperthermia (39–40 °C) to 116 patients perfused under normothermic conditions (37–38 °C), in which no benefit in recurrence-free or overall survival was observed [34]. Interpretation of these data are complicated by the observation that other factors varied besides treatment temperature, including differences in number of perfusions. In this study, many of the patients receiving normothermic perfusion received a double perfusion, and double perfusions were associated with a higher response rate than single perfusions [12]. Other factors associated with a higher response rate in this study were negative regional lymph nodes and leg as the site of disease rather than the arm or foot. Another study of 216 patients reported that prognostic factors for survival in order of significance were stage of disease, gender, age, Breslow thickness, Clark level of infiltration of the primary melanoma, and the number of metastases [35]. In a study from Tulane University on 174 patients treated with limb perfusion between 1957 and 1982 including adjuvant treatment, the factors associated with decreased survival rates in patients that also underwent elective lymph node dissection were increasing age, presence of subcutaneous or both subcutaneous and dermal metastases, treatment at normothermic temperatures, or earlier date of treatment [36].

The addition of other agents to melphalan can enhance response rates. Tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ) appear to be associated with an increased rate of response. In one series, preoperative subcutaneous interferon was combined with a perfusate containing IFNγ 0.2 mg and TNFα 4 mg and melphalan 10 mg/L limb volume for lower extremities, or INFγ 0.2 mg and TNF 3 mg and melphalan 13 mg/L limb volume for upper extremities. The total perfusion treatment time was 90 min, with the melphalan added 30 min into the perfusion. In this phase II study, 90% of melanoma patients treated experienced a complete response, with time to best response achieved in one-third of the time compared to that typically observed with melphalan alone [37]. As is observed in animal models, the tumors liquefied quickly. Toxicity was significant and included cardiovascular instability and ARDS despite the use of prophylactic dopamine infusion. This regimen was evaluated again in a successor phase III trial designed to evaluate the contribution of IFNγ, but the results did not reproduce the extremely high response rates even in the IFNγ-TNFα-melphalan arm [38]. Response rates in absence of IFNγ were lower though this did not reach statistical significance. The addition of TNFα to melphalan appeared to provide superior response rates compared to melphalan alone as observed in historical controls.

A phase III randomized control trial was performed at the National Cancer Institute (NCI) and compared the Lienard triple-drug combination [37] to melphalan alone. An interim analysis revealed a complete response rate of 80% in the triple-drug regimen compared to 61% for the melphalan-alone arm. In another NCI trial, TNFα was dose escalated in combination with the standard melphalan and IFNγ doses [39], and the complete response rate in the 26 patients that received 4-mg TNFα was 76%, with an overall objective response rate of 92%. The complete response rate in the 12 patients that received 6 mg TNFα was 36% with an overall objective response rate of 100%. In the TNFα 6 mg group, regional toxicity was dose-limiting and greater in the group that received TNFα 4 mg, particularly skin blistering, painful myopathy, and neuropathy. The investigators concluded that HILP with TNFα at 4 mg combined with IFN and melphalan was highly effective but considerably less toxic than TNFα at 6 mg.

Subsequent reports of HILP with TNF in a three-drug regimen have been associated a range of complete response and survival rates. With this as a backdrop, the American College of Surgeons Oncology Group conducted an important clinical trial evaluating the effects of TNFα in a two-drug regimen. Patients with in-transit metastases were randomized to melanoma combined with TNFα

or melphalan alone [40]. HILP was completed in 124 patients of the 133 enrolled. Patients in the arm randomized to also receive TNFα experienced significantly greater toxicity. Grade 4 adverse events were observed in 3 of 64 (4%) patients in the melphalan-alone arm compared to 11 of 65 (16%) patients in the melphalan-plus-TNF-alpha arm ($p = .04$). The complete response rate at 3 months were similar: 25% in the melphalan-alone arm and 26% in the melphalan-TNFα arm. The complete response rate at 6 months was higher in patients treated with the TNFα-containing regimen (42%) compared to the melphalan-alone regimen (20%), although this difference did not reach statistical significance. These clinical trial results do not support addition of TNFα to melphalan for treatment of in-transit metastases.

Specific Toxicities and Management

Normal tissues are sensitive to the high concentrations of therapeutic agents, hyperthermia, and mild acidemia. HILP produces toxicities in the form of lymphedema, skin blistering, painful neuralgia, or painful myopathy. The latter two conditions are managed conservatively with gabapentin and analgesics. Leg edema is managed with elevation and compression wraps. Skin blistering is self-limiting and managed conservatively. Muscle injury and swelling is a grave sign because it can lead to compartment syndrome (see below).

Post-operative hypotension resulting from "cytokine storm" may be observed even in the absence of TNFα in the perfusate and requires pressor agents for management. This condition is typically self-limiting and resolves with time. Systemic exposure to melphalan occurs either through leakage during HILP, or after limb vascularization is restored following HILP and melphalan in tissues gains access to the systemic circulation. Systemic melphalan typically causes acute postoperative nausea and emesis, and these symptoms can be effectively managed with ondansetron. Systemic melphalan may also lead to marrow suppression, manifest by neutropenia or pancytopenia 7–14 days after HILP.

HILP-treated limbs temporarily have poor capacity to heal wounds. Surgical wounds and incidental abrasions on an extremity treated with HILP do not heal well for the first 3 months. It is therefore important for the patient to assiduously avoid cuts or skin abrasions in the first few months following HILP. And HILP procedures combined with superficial inguinal lymphadenectomy are at very high risk for wound breakdown. And when wounds do develop on the treated extremity, surgical debridement should be very conservative. Debridement down to healthy tissue is not typically rewarded with subsequent granulation tissue and, rather, most commonly results in simply a larger wound. Surgical debridement should be limited to unroofing areas of purulence.

Post-operative acute vascular compromise is typically a result of technical problems with the vessels following cannulation and de-cannulation. Even a short period of unrecognized post-operative ischemia that results from vascular inflow compromise potentiates the toxicity of the HILP. Thus, diligence in monitoring distal extremity pulses and perfusion is of paramount importance in the immediate post-operative period. For example, technical problems encountered not infrequently are an atherosclerotic plaque that is cracked during the operation or creation of an intimal flap; both may result in post-operative vascular compromise. Unilateral loss of pulses, cool extremity, or evidence of reduced perfusion should be investigated immediately with noninvasive studies (PVR, Doppler) and angiography or CT angiography. Immediate repair of compromised inflow should be the goal. And following restoration of blood flow, careful monitoring for compartment syndrome should be performed by pressure measurements. A two-incision, four-compartment fasciotomy is performed immediately for signs or symptoms of compartment syndrome. Evidence for rhabdomyolysis should be sought by monitoring muscle tenderness, serum CK, and urine myoglobin. If found, maneuvers commonly employed include administration of large volumes of intravenous fluids, sodium bicarbonate, and potentially mannitol.

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