Local and Recurrent Regional Metastases of Melanoma

Matthew C. Perez, Kenneth K. Tanabe, Charlotte E. Ariyan, John T. Miura, Dorotea Mutabdzic, Jeffrey M. Farma, and Jonathan S. Zager

Contents

M. C. Perez · J. T. Miura · J. S. Zager (✉)
Department of Cutaneous Oncology, Moffitt Cancer Center, Tampa, FL, USA
e-mail: mattperez22@gmail.com; john.miura@moffitt.org; jonathan.zager@moffitt.org

K. K. Tanabe
Division of Surgical Oncology, Department of Surgery, Massachusetts General Hospital Cancer Center, Boston, MA, USA
e-mail: ktanabe@partners.org

C. E. Ariyan
Memorial Sloan-Kettering Cancer Center, New York, NY, USA
e-mail: ariyanc@mskcc.org

D. Mutabdzic · J. M. Farma
Department of Surgical Oncology, Fox Chase Cancer Center, Philadelphia, PA, USA
e-mail: dorotea.mutabdzic@fccc.edu; jeffrey.farma@fccc.edu

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Abstract

Up to 10% of patients with cutaneous melanoma will develop recurrent locoregional disease. While surgical resection remains the mainstay of treatment for isolated recurrences, locoregional melanoma can often present as bulky, unresectable disease and can pose a significant therapeutic challenge. This chapter focuses on the natural history of local and regionally recurrent metastases and the multiple treatment modalities which exist for advanced locoregional melanoma, including regional perfusion procedures such as hyperthermic isolated limb perfusion and isolated limb infusion, intralesional therapies, and neo-adjuvant systemic therapy strategies for borderline resectable regional disease. Hyperthermic limb perfusion (HILP) and isolated limb infusion (ILI) are generally well-tolerated and have shown overall response rates between 44% and 90%. Intralesional therapies also appear to be well-tolerated as adverse events are usually limited to the site of injection and minor transient flu-like symptoms. Systemic targeted therapies have shown to have response rates up to 85% when used as neoadjuvant therapy in patients with borderline resectable disease. While combination immunotherapy in the neoadjuvant setting has also shown promising results, this data has not yet matured.

Introduction

The incidence of cutaneous melanoma continues to steadily increase and accounts for the majority of skin cancer-associated deaths (Siegel et al. 2017). Over 80% of melanoma patients present with clinically localized disease and can be managed with resection of the primary tumor with or without sentinel lymph node biopsy (Balch et al. 2009). However, up to

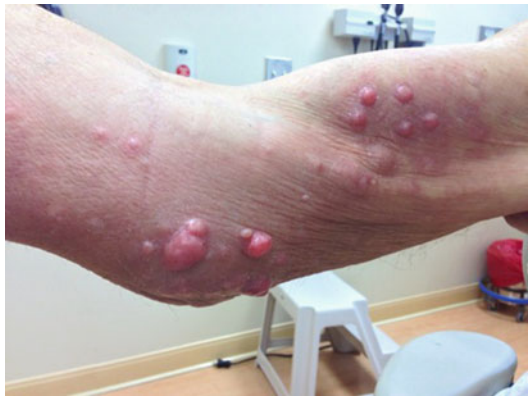

Image 1 In-transit melanoma of the upper extremity

10% of patients will develop recurrent locoregional disease (Borgstein et al. 1999). For isolated and resectable local and regional recurrences, complete excision of disease is the mainstay of therapy. However, patients often present with advanced, bulky, and unresectable disease either locally at the primary tumor site, in the regional nodal basin(s), or within the dermal lymphatic channels between the primary site and regional lymph nodes, and can pose a significant therapeutic challenge, as shown in Image 1 (Pawlik et al. 2005). Locoregionally advanced melanoma has also been shown to have significant adverse effects on patient's quality of life and emotional well-being (Weitman et al. 2018).

The last decade has seen a surge in the adoption of new immune and targeted therapies which have improved survival for patients with metastatic melanoma (Robert et al. 2015a, b; Chapman et al. 2011; Hodi et al. 2010). As a result of this prolonged survival, patients are living longer with advanced disease and therefore the importance of locoregional control has also increased. Fortunately, multiple management modalities exist for patients with advanced locoregional disease. This chapter focuses on the regional perfusion procedures, hyperthermic isolated limb perfusion (HILP) and isolated limb infusion (ILI), intralesional therapies, and neoadjuvant systemic therapy strategies for borderline resectable nodal metastases.

Local and Regional Recurrence of Melanoma

Local Recurrence

A local recurrence is a regrowth of melanoma in close proximity to the anatomic site from which the primary tumor was excised. Contemporary studies define this as regrowth within 2 cm of the surgical scar (Balch et al. 1993). This can be a result of either incomplete excision of the primary tumor or intralymphatic metastases and microscopic satellite lesions which are not contiguous but rather within the immediate area of the primary lesion. It is worth noting that although commonly used, a definition involving distance from the primary excision scar can lead to inconsistencies since primary resection margins vary between 1 and 2 cm, depending on the depth of the primary tumor.

Several features of the primary tumor and lymphatic metastases have been examined as potential prognostic factors that may be predictive of melanoma local recurrence. Long-term results of the Intergroup Melanoma Trial designed to evaluate 2 cm versus 4 cm surgical margins demonstrated a higher risk for local recurrence in patients with thicker primary melanomas (Karakousis et al. 1996; Balch et al. 2001). The risk of melanoma local recurrence increased with tumor thickness, despite the wider margins employed as tumor thickness increased (Table 1). The presence of ulceration in the primary melanoma is also an important risk factor for local recurrence, as evidenced by results of the Intergroup Melanoma Trial, as the presence or absence of ulceration had the greatest impact on risk for local recurrence (Balch et al. 2001). In the randomized group of patients with lesions on the trunk and proximal extremity, there was a sixfold increase in local recurrence rates (at any time) in patients with ulcerated primary melanomas, with local recurrence rates of 6.6% in patients with ulcerated primary melanomas compared with 1.1% in patients with nonulcerated primary lesions. In the nonrandomized group with lesions involving the distal extremity and head and neck sites, the local recurrence rate was 16.2% (at any time) in patients with ulcerated lesions compared to 2.1% in patients with

Table 1 Frequency of subgroups of thickness and incidence of local recurrence and other metastases

Thickness (mm)	No. of patients	Local recurrence (%)	In-transit metastases (%)	Regional nodal recurrence (%)	Distant recurrence (%)
1.00–2.0	445 (60%)	2.3	3.6	9.2	14.4
2.01–3.0	215 (29%)	4.2	8.4	15.8	27.9
3.01–4.0	77 (10%)	11.7 ($p = 0.001$)	16.9 ($p = 0.001$)	29.9 ($p = 0.001$)	44.2 ($p = 0.001$)
Total	737 (100%)	3.8	6.4	13.3	21.4

From Karakousis et al. (1996)

nonulcerated lesions ($p < 0.001$) (Balch et al. 2001). Resection margin size has not been shown to correlate with local recurrence as several prospective multi-institutional and large retrospective studies have shown that narrow margins for resection of thin melanomas result in similar rates of local recurrence. Furthermore, for tumors between 1 and 2 mm thick, a recent large retrospective study showed no significant difference in local recurrence or survival with 1 cm resection margins when compared to 2 cm margins (Doepker et al. 2016).

Surgery remains the mainstay of treatment for local recurrences of melanoma. In patients who are examined at regular intervals, local recurrences are may be detected at a stage at which they can be managed by surgical resection. Few data exist on appropriate surgical margins to employ during resection of a local recurrence. In the absence of significant data, most experts recommend excision using approximately 1 cm margins to ensure that a grossly clear margin is achieved. Clearly, radical surgery that makes use of extensive margins during surgical extirpation of a local recurrence is not justified. But even when more conservative margins are employed to excise a local recurrence, skin grafts are frequently required to close the resulting defect in an operative field that has already been compromised by a prior wide excision.

In-transit Recurrence

In-transit disease represents the clinical manifestation of small tumor emboli trapped within the dermal and subdermal lymphatics between the site of the primary tumor and regional lymph node drainage basin(s). Cascinelli et al. reported a 13% incidence of recurrent in-transit melanoma observed in a cohort of more than 1500 patients who had clinical stage I and II melanoma (Cascinelli et al. 1986). Dalal et al. reported a 4.8% incidence of in-transit metastases as the first site of recurrence in a cohort of 1046 patients with stage I or II disease at a median follow-up of 36 months (Dalal et al. 2007).

A number of factors may predispose patients to subsequent in-transit recurrence after resection of their primary tumors. In a review by Cascinelli et al., patients who had positive nodes had a higher incidence of recurrence than those who had negative nodes. Furthermore, in patients who had one positive node, the incidence of subsequent in-transit disease was 11%, whereas in those who had three or more positive nodes, the incidence of subsequent in-transit recurrence as the initial site of failure was 31% (Cascinelli et al. 1986). Calabro et al. reviewed 1001 patients who had positive nodes, observing metastatic in-transit melanoma as the initial site of recurrence in 99 of them (10%) (Calabro et al. 1990).

Gershenwald et al. reported a 3.3% incidence of in-transit metastases as the initial site of recurrence among 243 patients with stage I or II melanoma and a negative result on SLN biopsy that were followed for a median of 24 months (Gershenwald et al. 1998). Essner et al. reported a 3.7% incidence of in-transit metastases among 267 patients with clinical stage I to II melanoma who underwent SLN biopsy and were followed for a median of 45 months. For patients who had a negative result on SLN biopsy ($n = 225$), the incidence of in-transit metastases was 2.7% ($n = 6$), whereas for patients who had a positive result on SLN biopsy ($n = 42$), the incidence was 9.5% ($n = 4$) (Essner et al. 1999). Dalal et al. reported an increase in intransit

recurrences in SLN-positive patients compared with SLN-negative patients (30% versus 21%, respectively; p = NS). Factors predisposing to recurrence in this cohort of 1046 patients with stage I or II melanoma were a positive SLN, ulceration, thickness, and stage II disease (Dalal et al. 2007).

The median time to the appearance of in-transit metastatic melanoma is fairly consistent, ranging from 13 to 24 months (Dalal et al. 2007; Lee 1980; McCarthy et al. 1988; Wong et al. 1990). SLN-positive patients appear to have an earlier recurrence at a median of 13 months, compared with SLN-negative patients who have a recurrence at a median of 24 months (Dalal et al. 2007).

Regional Nodal Recurrence

The incidence of regional nodal recurrence after lymphadenectomy is variable and depends on a number of factors, primarily related to the tumor burden in the lymph node basin originally dissected. To a lesser extent, the likelihood of regional nodal recurrence depends on the extent of the procedure performed. Miller et al. found a 12% rate of nodal relapse at the site of 207 patients who underwent prior lymphadenectomy and noted relapse in a regional nodal basin after removal of negative nodes in 6.7% of patients. When one to three nodes were positive, 14% of patients subsequently suffered a relapse in the regional nodal basin; when more than four nodes were positive at the initial dissection, 53% of patients subsequently had a relapse in the regional nodal basin (Miller et al. 1992). Warso and Das Gupta found a 5.6% rate of nodal recurrence among 1030 patients undergoing prior lymphadenectomy (Warso and Das Gupta 1994). Regional nodal failure was often a harbinger of systemic relapse, because fewer than half of those patients had recurrences confined to the nodes alone. Calabro et al. reported 162 nodal relapses among 1001 patients undergoing lymphadenectomy with positive nodes (16%). Factors that predicted nodal basin relapse included the number of positive lymph nodes and the presence of extranodal tumor extension (Calabro et al. 1989). Of note, factors that did not predict nodal recurrence in that series included a history of prior lymph node biopsy, the clinical status of the nodes, or the site of regional lymphadenectomy. The highest reported rate of regional nodal recurrence after lymphadenectomy was from a series by Monsour et al. Of 48 patients undergoing therapeutic lymphadenectomy, 52% developed regional nodal recurrence as their initial site of failure. In that review, nodal relapse was age dependent as 31% in patients less than 50 years old and 66% in patients more than 50 years of age recurred. The authors did not comment on the factors that may have predisposed their patients to such a high rate of regional nodal recurrence (Monsour et al. 1993).

The widespread use of SLN biopsy has led to a new clinical scenario: nodal recurrence after both negative and positive SLN biopsy. Gershenwald et al. looked at 602 melanoma patients who underwent successful lymphatic mapping and SLN biopsy, followed for a median of 30 months. Of these patients, 105 (17%) had at least one histologically positive SLN, of whom 101 (96%) underwent completion lymphadenectomy. Of 36 patients who had recurrences, 10 had recurrences in the nodal basin at a median time of 14 months. The nodal basin was not the sole site of recurrence in any of these 10 patients. Reexamination of the sentinel node in patients who had nodal failure after a negative result on SLN biopsy revealed missed micrometastatic disease in eight (Gershenwald et al. 2000).

Nodal basin recurrence seems to be the result of aggressive disease biology and not surgical manipulation. Clary et al., at Memorial Sloan-Kettering Cancer Center, reported on the pattern of recurrence among 332 consecutive patients with localized primary cutaneous melanoma who underwent SLN biopsy. The overall incidence of recurrence was greater in the patients who had positive nodes (40% versus 14%), although the distribution between locoregional and systemic recurrences was not statistically different (Clary et al. 2001). Locoregional recurrences constituted 61% of all first-site recurrences, and distant recurrences accounted for 39%. As in Gershenwald's study, a re-examination of a sentinel node initially thought to be negative in 11 patients who had subsequent nodal

recurrence detected metastatic disease in seven (64%). In the MSLT-1 trial, 59 (6.3%) of the 944 patients with tumor-negative SLNs developed regional nodal recurrence at a median follow-up of 54 months (Morton et al. 2006). Of the 59 patients, 48 (81%) had recurrence in the SLN drainage basin and 11 had recurrence in a basin that was not sampled. More recently, Zogakis et al. reported on 773 patients with negative SLNs, of whom 8.9% ($n = 69$) had a recurrence. Distant metastases were seen in 4.8%: 1.8% were in-transit, 1.7% were nodal, and 1.2% were local recurrences (Zogakis et al. 2007).

For low-burden in-transit and recurrent regional nodal disease, complete resection of disease is often preferred when feasible. For unresectable and locoregionally advanced disease, multiple treatment modalities currently exist such as regional chemotherapy and intralesional therapies, as well as systemic immune and targeted therapies, and are discussed in more detail later in this chapter.

Hyperthermic Isolated Limb Perfusion

History and Early Clinical Studies

In 1956, the Department of Surgery at University of Tulane embarked on regional perfusion studies to increase the uptake of chemotherapy in tumors located in regions of the body whose vascular supply and drainage could be completely isolated (Krementz et al. 1994). Use of a heart-lung machine to support isolated hyperthermic perfusion of the tumor was evaluated in dogs as a strategy to avoid systemic toxic effects and at the same time increase the dose of nitrogen mustard (Ryan et al. 1958). The concept of cannulation of both the arterial inflow and the venous drainage for connection to an extracorporeal oxygenated circuit maintained by a heat-lung machine 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 (Ryan et al. 1958).

In June 1957, a patient with a very high burden of melanoma metastases to the extremity presented to Charity Hospital, 2 years after having been treated for a melanoma of the right ankle. Despite having over 80 satellite lesions, the patient refused the standard therapy at that time, namely, amputation. 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.

In 1958, Creech presented the results of isolated perfusion in six patients with melanoma and another 18 with other advanced cancers before the American Surgical Association in New York (Creech et al. 1958). For pelvic tumors, the aorta and IVC were occluded below their renal branches and cannulated just above the bifurcation. For perfusion of lung tumors, use of two circuits and caval occlusion were used to prevent mixing between the systemic and pulmonic circuits. And in cases, in which tourniquets could not be applied (e.g., breast), a motor pump was used to create negative pressure in the venous return circuit to minimize systemic mixing. They reported gross or microscopic responses in 18 of 19 cases followed long enough for changes to be evident. And by 1962, they had treated a sufficiently large number of patients to report results of 303 patients, 123 with melanomas (Krementz et al. 1962).

Over the ensuing four decades, many hospitals started performing isolated limb perfusion and reporting their results. Unfortunately, an opportunity for progress was lost during this interval because this experience lacked scientific rigor. The studies were generally single arm, absent appropriate control groups, and involved heterogenous patient populations including patients with completely resected tumors and unresectable tumors. Treatment schedules were varied in their dose of melphalan, perfusion duration, and perfusion temperature (Rosin and Westbury 1980; Di Filippo et al. 1989; Jonsson et al. 1983; Lejeune et al. 1983; Skene et al. 1990; Minor et al. 1985; Kroon 1988; Klaase et al. 1994a; Kroon et al.

1993). For example, in a report of 1139 perfusions performed over 35 years, the authors included patients in need of 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 (Krementz et al. 1994). However, clinical studies in the past two decades have been of significantly higher quality and with greater scientific rigor. Results of these studies are discussed below. See also chapter ▶ "Hyperthermic Regional Perfusion for Melanoma of the Limbs."

Patient Selection

Patients with metastatic melanoma confined to an extremity without evidence of distant metastases should be considered for hyperthermic isolated limb perfusion (HILP). The melanoma should be considered unresectable, although the definition of unresectable is subjective. The most common indication for limb perfusion is *in-transit* metastases, and the frequency and timing of *in-transit* metastases as well as the number and distribution of metastases are used to define when resection is appropriate. Rapid recurrence of multiple in tumor nodules soon after excision of in-transit metastases indicates that further surgical resection is not warranted. 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 (see below).

The role of HILP has shifted over the years. Prior to effective molecularly targeted immunotherapies, HILP was accepted as the most effective and appropriate treatment for patients with metastases or local recurrence 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 commonly sensitive to targeted therapy using a BRAF inhibitor combined with a MEK inhibitor, with a response rate of 63% and acceptable toxicity (Flaherty et al. 2012a, b). 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 (Wolchok et al. 2017). 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 wild type. HILP is 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 once accepted as appropriate adjuvant treatment for high-risk melanomas. A prospective randomized control trial was conducted at the University of Cologne randomized to excision alone or excision with HILP. This trial was stopped early because interim analysis showed a remarkable reduction in recurrences in the HILP arm (Ghussen et al. 1988). But subsequently conducted randomize control trials that are of higher quality and larger patient number have convincingly demonstrated lack of benefit of adjuvant HILP. Thus, the results of the Cologne study – positive and in favor of HILP – are generally discounted and considered an unreliable outlier based on its very small sample size (e.g., 34 patients treated with 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) (Koops et al. 1998). Over a period of 10 years, 852 patients were randomized to wide excision alone or wide excision and HILP. HILP-treated patients benefitted 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%. But importantly, HILP-treated patients experienced no benefit in overall survival or time to distant metastasis. 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 (Hafstrom et al. 1991). In summary, HILP is not beneficial as an adjuvant therapy.

Preoperative Evaluation

Preoperative evaluation requires careful staging to exclude metastases outside the limb, determination of preoperative ambulatory status, comorbidities, significant peripheral vascular disease, and patient motivation to handle side effects. Patients whose functional status has declined to a point where they are no longer ambulatory are poor candidates for HILP. Measurement of any preexisting leg edema to establish a baseline is appropriate. Careful evaluation for regional nodal metastases by CT or PET-CT establishes whether elective concomitant lymphadenectomy is required. In cases where the presence of significant peripheral vascular disease is detected on clinical examination, pulse volume recordings (PVR) are a useful noninvasive evaluation to determine the locations and severity of disease, and to establish a baseline. Patients with moderate or severe peripheral vascular disease are at high risk for complications from isolated limb perfusion and require alternative approaches for management of their melanoma. In situations where physical examination and PVR are insufficient to accurately assess the severity of peripheral vascular disease, preoperative angiography or CT angiography is indicated.

If drug dosing will be determined based on volume of the limb, several measurement techniques for limb volume are available. One involves physical measurements of the circumference of the leg at 2 cm intervals to calculate the cross-sectional area, and then calculation of the integral of this function. Another technique uses a water displacement. The leg or arm is immersed into a container filled completely to a brim, and the volume of displaced, overflowing water is the volume of the immersed extremity (Rabe et al. 2010). The last involves use of a CT or MRI scan of the entire extremity and use of 3D analytic software to measure leg volume (Brys et al. 2016).

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. It is helpful, though not required, to have a machine for activated clotting time measurements in the operating room. A scintillation probe mounted over the chest (precordial) is used 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. An ultraviolet (black) light is used to evaluate for leakage of fluorescein from the extremity. A pulse volume recording machine is used before, during and after the operation. Heating blankets warmed by a heated water circulator are used 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. 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. 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.

Operation

Isolated limb perfusion is a technically complex operation that requires closely integrated teamwork by a multidisciplinary team including anesthesiologists, perfusionists, nuclear radiologists, pharmacists, nurses, and surgeons. The quality and frequency of communication among these

team members affects outcomes. The procedure involves the use of an extracorporeal circuit attached to a heart-lung machine (oxygenator and blood pump) to heat the circulating blood, increase the oxygen tensions 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 sequel of ischemia-reperfusion (Ruschulte et al. 2013).

The operation is conducted under general anesthesia. Preparation for an operation of 4–6 h duration is appropriate, depending on which vessels require isolation and whether a concomitant lymphadenectomy is indicated. Anesthesia should be prepared for acute blood loss, particularly if surgical isolation of the vessels is anticipated to be difficult (e.g., iliac vessels, scarred vessels), and the operation should be conducted with two large-bore peripheral IVs. Central venous pressure monitoring is not typically required. An active type and crossmatch in the blood bank is mandatory. 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 bladder catheter should be inserted. An oral-gastric tube may be used during the operation. An epidural catheter for post-operative pain management is not typically used. A dose of prophylactic antibiotics is administered prior to the skin incision.

After induction of anesthesia, PVR is measured and saved for comparison after the operation. Similarly, peripheral pulses in the affected extremity are carefully assessed and recorded. It is useful to monitor temperatures of the extremity in several locations during the operation. Thermistors are placed in the proximal and distal extremity both medially and laterally (e.g., four thermistors) for real time temperature monitoring. The extremity is then wrapped in heating blankets, leaving the PVR cuff in place. The prep and drape should be wide. It is necessary to place sterile surgical tubing (or esmark bandage) around the root of the extremity for later use as a tourniquet.

The general approach is to use an incision 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. However, superficial femoral lymphadenectomy is performed at time of isolated limb perfusion only when clinical evidence of nodal metastases is present given that the incision used for this lymphadenectomy has high likelihood of infection or dehiscence, especially in a chemotherapy-treated field. Moreover, perfusion from an iliac approach does effectively treat nodes in the femoral triangle (Koops et al. 1998). 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 sec. The vessels are occluded proximally and distally with either vascular clamps or Rummel tourniquets. The vein and artery are cannulated through 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 maximally. After confirmation of a therapeutic ACT (typically >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 reservoir volume does not diminish. In rare patients, typically those that start with a very low hematocrit that also have very small limb volumes and consequently larger hemodilution from the priming volume, the hematocrit in the circuit is unacceptably low (e.g., below 18%). In these cases, a portion of a unit of packed red blood cells is transfused into the circuit. Heparin resistance – defined by the inability to achieve therapeutic ACT with typical heparin doses – is typically successfully treated with additional heparin. However, anti-thrombin III deficiency should be suspected if this maneuver

is unsuccessful. In these situations, options include changing to argatroban, or transfusion of fresh frozen plasma or antithrombin (Spiess 2008).

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 (see below). Protocols for drug dosage, drug administration schedule, target temperature, and duration of perfusion differ amongst 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 fine sutures. 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.

Because in-transit metastases occur most commonly in the lower extremity, access for HILP is most commonly achieved via the iliac vessels or the femoral vessels. If in-transit metastases are located within 6 inches of the inguinal crease, perfusion via the iliac vessels is required to achieve perfusion of the proximal thigh. The surgical approach to the iliac vessels starts with an oblique incision in the lower abdominal wall. The external oblique fascia is incised and the internal oblique musculature is separated to reveal the transversalis fascia. This is incised and the abdominal contents are retracted superomedially to expose the iliac vessels. The 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 (not necessary to divide) and a 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 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 inferior to its insertion onto the coracoid to allow removal of level III axillary nods and provide 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 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 (Stamatiou et al. 2017).

Leak Monitoring

During isolated limb perfusion it is necessary to assess for ongoing leakage from the circuit into the systemic circulation, or from the systemic circulation into the circuit. The former condition leads to systemic exposure to the drug, and the latter condition leads to lowering of the drug concentration in the perfusion circuit. One commonly used technique to measure leak involves mounting a shielded precordial scintillation detector over the precordium and injecting isotope labelled albumin or red cells into the perfusion circuit. I-131 and Tm-99 are used most commonly. 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 determination of the percent fractional leak as a function of time. An alternative approach involves administration of fluorescein into the circuit and then viewing different areas of the body and collected urine with a Woods lamp. This technique can reveal specific spots of skin outside the extremity

that are receiving perfusate, and lead to identification of specific collateral vessels to be tied off. Fluorescein in the urine collection chamber is easily identified with the black light, and increase in intensity over time provides a qualitative sense of the rate of leak. 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 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 (Stanley et al. 2000).

Leakage of significant amounts of melphalan into the systemic circulation can lead to acute nausea and delayed bone marrow suppression or hair loss. Leak from the circuit into the systemic circulation that occurs later during the perfusion is of less consequence, since most of the melphalan in the extremity will have been taken up by tissue. 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 (Laurenzi et al. 2004).

Specific maneuvers are employed to manage leakage between the circuit and systemic circulation (Table 1). Leakage from the circuit into the systemic circulation typically results in loss of volume in the venous reservoir. The leakage may be through 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 is to lower the flow rate, which results in reduced pressure in collateral arteries and veins. The operating table can be tilted into reverse Tredelenberg position to lower the venous pressure in the leg relative to the IVC. 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.

An increase in reservoir volume over time indicates a "steal" of systemic blood into the circuit, resulting in unintended lowering of the drug concentration, as well as discarding more drug-contaminated blood at the end of the procedure than intended. The first step is to increase the flow rate, though carefully monitoring outflow pressure to avoid intimal injury. The operating table can be tilted into Tredelenberg position to raise the venous pressure in the lower limb relative to the IVC. 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 some trial and error (Table 1).

Agents

Melphalan is the most widely used agent for HILP for melanoma. It is fortuitous that this was the agent selected for the first patient treated with HILP given the clinical complete and durable response it produced. Melphalan is a phenyl alanine mustard and taken up by melanoma cells (Luck 1956). Phenyl alanine itself is a precursor for melanine biosynthesis, and therefore taken up avidly by melanocytes. 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 following injection demonstrate that concentrations decline rapidly in a biexponential manner with distribution phase half-life of 10 min, and terminal elimination phase half-life of approximately

75 min. Pharmacokinetic studies 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 (Scott et al. 1992). Ideal dosing is calculated from limb volume rather than body weight. Limb volume expressed as a percentage of total body weight produced as much as a twofold variation in the population for both lower and upper extremities. This could 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 (Kroon 1988; Benckhuijsen et al. 1988; Wieberdink et al. 1982). Melphalan is stable in sterile 0.9% sodium chloride for only 90 min at room temperature (Desmaris et al. 2015). Therefore, for an HILP procedure it should be 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. Nonetheless, 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 is a pro-inflammatory cytokine produced by multiple different immune cells, and causes rapid and significant hemorrhagic necrosis of tumors. For these reasons there has been great interest in its potential as an anti-cancer agent. However, 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 no effect on tumors. TNFα therefore 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 (Posner et al. 1995). Of six treated patients, partial response of less than 1 month's 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-γ. 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 (Sanki et al. 2007).

Hyperthermia

In Creech's original report, isolated limb perfusion with chemotherapy was used without hyperthermia (Creech et al. 1958). Investigators subsequently observed that combined regional chemotherapy with mild hyperthermia produced higher response rates (Stehlin et al. 1975). There are no prospective randomized clinical trial results comparing isolated limb perfusion with versus without hyperthermia to inform this strategic decision. Hyperthermia during HILP affects tumor cells, other cell populations within the 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 (Klaase et al. 1994c). 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 (Abdel-Wahab et al. 2004). These studies implicated a mechanism of enhanced cytotoxicity of l-phenylalanine mustard with hyperthermia rather than improved drug delivery and uptake.

Results

HILP has been enthusiastically embraced over its greater than 60-year history, primarily because of a combination of the unique biology of melanoma in-transit metastases and the extraordinarily high response rate observed with this regional therapy. The primary agent used by nearly all centers has been melphalan, but most centers have developed protocols that differ from one another in drug administration schedule, temperature and duration of perfusion. Accordingly, it is difficult to reach conclusions about which techniques and schedules are optimal.

In general, the complete response rate for HILP with melphalan alone is in the range from 40% to 60% (Table 2). The overall response rate (e.g., including partial responses) generally ranges from 60% to 90%. For leg perfusions, the melphalan dose varies from 0.8 to 2 mg/kg of body weight, or when dosed per liter of extremity volume from 6 to 10 mg/L. The dose for arm perfusions is generally less and ranges from 0.45 to 0.8 mg/kg. Perfusion times vary from 50 to 120 min. Target limb temperatures vary range from 37 ° (normothermia) to 42 °. From this heterogeneous collection of reports it is not possible to draw a conclusion about the relationship between dose schedule and response rates. An interesting approach of sequential perfusions was evaluated and involved external iliac and common femoral approaches staged 6 weeks apart (Kroon et al. 1993). While the complete response rate with this approach jumped up to 77%, no benefit in overall survival was observed relative to patients undergoing a single perfusion.

Table 2 Wieberdink acute limb toxicity scale

Grade	Clinical characteristics
I	No subjective or objective evidence of reaction
II	Slight erythema or edema
III	Considerable erythema or edema with some blistering; slightly disturbed motility permissible
IV	Extensive epidermolysis or obvious damage to the deep tissues causing definite functional disturbances; threatened or manifest compartmental syndromes
V	Reaction that may necessitate amputation

The heterogeneity in procedures used also 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 (Klaase et al. 1995). However, interpretation of these data are complicated by the observation that treatment schedules varied in many ways beyond temperature, including differences in number of perfusions. Most of the patients receiving normothermic perfusion received a double perfusion, and double perfusions were associated with a higher response rate than single perfusions (Klaase et al. 1994a). 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. A separate study of 216 patients treated between 1978 and 1990 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 (Klaase et al. 1994b). In a similar analysis from Tulane University on 174 patients treated with limb perfusion between 1957 and 1982 – some in the adjuvant setting – 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 (Sutherland et al. 1987).

The addition of tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ) to melphalan appears to be associated with an increased rate of response. The combination of preoperative subcutaneous interferon 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 a 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 (Lienard et al. 1992). The tumors liquefied quickly, as has been observed with TNFα in animal models. Toxicity including shock and ARDS was observed despite use of prophylactic dopamine infusion. A successor phase III trial designed to evaluate the contribution of IFNγ did not reproduce the extremely high response rates even in the IFNγ-TNFα-melphalan arm (Lienard et al. 1999). There was a trend towards lower response rate in absence of IFNγ; however, this did not reach statistical significance. But 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 performed at the National Cancer Institute (NCI) comparing the triple drug combination as championed by Lienard (Lienard et al. 1992) to melphalan alone and an interim analysis revealed a complete response rate of 80% in the triple-drug regimen compared to 61% for the melphalan-alone arm. The difference was statistically significant, even though the response-rate observed with melphalan alone was higher than typically observed. At the same time as this trial, Fraker and colleagues at the NCI conducted a trial in which the TNFα dose was escalated in combination with the standard melphalan and IFNγ doses (Fraker et al. 1996). 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. Based on these data the investigators concluded that HILP with TNFα at 4 mg combined with IFN and melphalan was considerably less toxic than TNFα at 6 mg, yet can lead to complete local responses in the majority of patients.

Subsequent reports of HILP with TNFα in a three-drug regimen produced a range of observed complete response and survival rates. 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 (Cornett et al. 2006b). HILP was completed in 124 patients of the 133 enrolled. Greater toxicity was observed in the TNFα patients. 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 ($p = 0.04$). The complete response rate at 3 months was 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

Regional toxicities from HILP are caused by sensitivity of normal tissue to the high concentrations of toxic agents, hyperthermia, and mild acidemia. These may be 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).

Postoperative hypotension resulting from "cytokine storm" may be observed even in the absence of TNFα in the perfusate and requires pressor agents for management. Melphalan left in the tissues of the extremity at the completion of perfusion and wash enters the systemic

circulation once limb vascularization is restored. This may cause acute postoperative nausea and emesis, which can be effectively managed with odansetron. Systemic melphalan may also lead to marrow suppression, manifest by neutropenia or pancytopenia 7–14 days after HILP.

Wounds and 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 3 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.

Toxicity can also result from acute vascular compromise. Any period of unrecognized postoperative ischemia that results from vascular inflow compromise potentiates the toxicity of the HILP treatment. Diligence in monitoring distal extremity pulses and perfusion is of paramount importance in the immediate postoperative period for early detection of vascular compromise. An atherosclerotic plaque that is cracked during the operation or creation of an intimal flap may result in vascular compromise post-operatively. 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 is indicated. 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 if indicated. 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.

Isolated Limb Infusion

Background

Although effective, HILP is an invasive and costly procedure. Additionally, it is difficult to repeat the procedure secondary to the development of scar tissue from the initial procedure and as a result the overall complication rate increases from 28% to 51% for initial and repeat procedures, respectively (Cornett et al. 2006a). In the 1990s, at the now Melanoma Institute of Australia (MIA), Thompson et al. introduced the minimally-invasive and repeatable alternative to HILP known as isolated limb infusion (ILI) (Thompson et al. 1998). See also chapter ▶ "Isolated Limb Infusion for Melanoma." ILI uses the same principal of high-dose chemotherapy infusion into an isolated limb, however vascular access is gained by percutaneously placed arterial and venous catheters and cytotoxic drugs are instilled at a low-flow under hypoxic conditions (Thompson et al. 1998). Presently, ILI is used throughout the world and has shown favorable response rates for locally-advanced and in-transit melanoma (Kroon et al. 2014, 2016; Muilenburg et al. 2015; Li et al. 2018).

Patient Selection and Indications

Indications for ILI are primarily patients with unresectable locally-advanced or in-transit melanoma of the upper or lower extremity and no evidence of distant metastases. Patients need to be cleared for general anesthesia prior to the procedure, but it is usually well-tolerated in most patients. ILI may be repeated after partial responses, or recurrences and progression following an initial response (Chai et al. 2012). ILI may also be performed in patients who also have distant metastatic disease in a palliative effort to control symptomatic locoregional disease. Kroon et al. reported a limb salvage rate of 86% in a series of 37 patients with symptomatic limb disease and documented distant metastases at the time of ILI (Kroon et al. 2009a). While

predominately performed for cutaneous melanoma, ILI has also been used to treat locally-advanced soft tissue sarcoma and non-melanoma cutaneous malignancies including Merkel cell carcinoma, and squamous cell carcinoma (Mullinax et al. 2017; Turaga et al. 2011; O'Donoghue et al. 2017).

Technique

Usually performed in the radiology department on the morning of the procedure, arterial and venous catheters are percutaneously placed under fluoroscopic guidance and advanced into the affected limb with the catheter tips positioned distal to the level of the tourniquet to ensure adequate isolation of the extremity. Limb temperatures are maintained greater than 37 °C and usually closer to 40 °C by a combination of liquid warming blankets on the affected extremity and overhead heaters which are institution-specific. The infusion portion of the procedure is then performed in the operating room under general anesthesia. Prior to inflating the tourniquet, patients are fully heparinized to achieve an activated clotting time (ACT) greater than 350 or 400 sec, depending on the institution. The tourniquet is inflated once adequate circulation is achieved by manually drawing blood from the venous catheter and reinjecting it into the arterial catheter with a 20 cc syringe. After tourniquet inflation, papaverine is routinely administered through the arterial catheter to maximize vasodilation. Isolation of the limb is checked by confirming cessation of flow in pedal or radial arteries by a Doppler probe. Limb volume measurements are used to determine the dose of chemotherapeutic agents, usually melphalan with or without actinomycin-D, and melphalan dosing is corrected for ideal body weight. Infusion lasts for typically 30 minutes and the chemotherapeutic agent is manually circulated with a syringe connected in line to the closed circuit with a heating source. Following infusion of chemotherapy, the limb is washed out with Hartmann's or saline solution until the effluent is clear, and protamine is administered to reverse heparinization after the tourniquet is released.

Patients are usually admitted to a monitored care unit postoperatively and remain on bed rest for the first 24 h following the procedure. Extremity neurovascular checks are performed at least twice daily along with serum creatinine phosphokinase (CPK) levels. Normal saline and corticosteroids should be administered if the patient's CPK level exceeds 1000 IU/L. Patients are usually discharged from the hospital after CPK levels start to return towards baseline as long as other standard criteria for discharge have been met.

Response to Therapy

Multiple single-center and multi-institution studies throughout the world have been published in the last two decades on the efficacy of ILI. Kroon et al. published the largest single institution experience consisting of 185 melanoma patients undergoing ILI at the MIA and reported an ORR of 84% (Kroon et al. 2008). Duke University (DU) and Moffitt Cancer Center (MCC) subsequently reported their single-institution experiences of 61 procedures and 79 procedures, respectively. The ORR at DU was 44% with 30% of procedures achieving a CR, while MCC reported an ORR of 70% and a CR in 32% of procedures (Beasley et al. 2008; Wong et al. 2013). O'Donoghue et al. later extended the MCC experience to 145 ILI procedures for melanoma and reported an ORR of 59% with a CR in 26% of procedures (O'Donoghue et al. 2017). In an Australian study of five institutions, a 75% ORR (33% CR) was reported in 316 ILI procedures. (Kroon et al. 2016), while two multicenter studies in the United States reported an ORR of 64% (31% CR) and 57% (34% CR) in 128 patients and 160 patients, respectively (Beasley et al. 2009; Muilenburg et al. 2015). Kroon et al. also performed a systematic review of 576 patients around the world who underwent ILI and reported a 73% ORR with 33% of patients achieving a CR (Kroon et al. 2014).

Differences in response rates among varying institutions are likely related to multiple factors including heterogeneous patient populations, differences in technique, and inconsistencies in

response criteria used. The MIA series included patients with stage I-II disease, while the DU and MCC reports were comprised of stage III patients only. Drug-exposure times also differ between institutions and only certain institutions routinely use papaverine for vasodilation. For response criteria, DU and MCC determined response to ILI at 3 months using the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1, while the MIA determined best response using standard World Health Organization (WHO) criteria (Eisenhauer et al. 2009; Wold Health Organization 1979). However, the Australian multicenter analysis showed response rates to be significantly different on multivariate analysis among the included institutions which used the same response criteria (Kroon et al. 2016).

Survival After ILI

Response to therapy has shown a significant association with improved survival in patients who undergo ILI. O'Donoghue et al. reported both in-field progression-free survival (IPFS) and overall survival (OS) to be significantly higher in melanoma patients who responded to therapy when compared to those who did not respond to ILI (IPFS 14.1 vs. 3.2 months, $p < 0.0001$; OS 56.0 vs. 26.7 months, $p = 0.0004$) (O'Donoghue et al. 2017). Kroon et al. reported a median survival of 38 months for all patients who underwent ILI and showed a significantly higher OS in those patients who achieved a CR (53 months) compared to those who did not (25 months; $p = 0.005$) (Kroon et al. 2008). Furthermore, Wong et al. showed that resection of residual disease following ILI improves both disease-free and overall survival with rates similar to those who experienced a CR after ILI (Wong et al. 2014).

Burden of Disease

Multiple studies have shown burden of disease (BOD) to be associated with response to therapy. Patients with a lower BOD have been shown to have higher overall response rates (ORR) and complete response (CR) rates (Steinman et al. 2014; Muilenburg et al. 2015). Muilenburg et al. reported an ORR and CR rate of, respectively, 73% and 50% in 60 patients with low BOD (less than 10 lesions, none greater than 2 cm) and an ORR and CR rate, respectively, of 47% and 24% in those with a high BOD ($p = 0.002$) (Muilenburg et al. 2015). At the MIA, patients who underwent ILI for one lesion were found to have an improved OS when compared to those who had multiple lesions (Kroon et al. 2008). In the MIA single-center series, maximal depth of the primary tumor (Breslow depth), a well-known and strong indicator of overall survival in all patients with localized melanoma, was not shown to be a significant predictor of response to ILI (Kroon et al. 2008). However, in the multicenter analysis, a thinner primary melanoma was significantly associated with a higher response rate on multivariate analysis ($p = 0.04$) (Kroon et al. 2016).

Toxicity

ILI is generally a well-tolerated procedure with the majority of the toxicity limited to transient edema and hyperpigmentation of the limb postoperatively. The Wieberdink toxicity scale is routinely used to characterize toxicity related to ILI (Table 2) (Wieberdink et al. 1982). At the MIA, 56% of patients experienced grade II toxicity, 39% of patients experienced grade III toxicity, and 3% of patients experienced grade IV toxicity (Kroon et al. 2009b). The Australian multicenter study of 316 procedures reported a rate of 27% grade III toxicity, and 3% grade IV toxicity (Kroon et al. 2016). O'Donoghue et al. reported a grade III toxicity rate of 11.4% in a series of 201 ILI procedures at MCC with 145 of the procedures being performed for melanoma (O'Donoghue et al. 2017). Only one patient in this series developed a grade IV toxicity which resulted in a fasciotomy to treat compartment syndrome (O'Donoghue et al. 2017). DU used the National Cancer Institute Common Technology Criteria for Adverse Events (CTCAE) version 3 and has shown similar toxicity to MCC and the MIA, with grade III toxicities reported in 18%

of patients, excluding CPK elevation (Beasley et al. 2008).

Although toxicity score was significantly associated with an improved ORR on both univariate and multivariate analysis in the single-center MIA study, increased toxicity has consistently been shown to not improve rates of CR, in-field progression, or survival (Kroon et al. 2008, 2009b). Independent risk factors which have been shown to correlate with limb toxicity include postoperative CPK level, high peak and final melphalan concentration, and tourniquet time.

Systemic toxicities related to ILI are rare and mostly comprised of mild nausea and vomiting likely related to the general anesthesia used for the procedure. This is likely a result of the low rate of chemotherapy which leaks into the systemic circulation from the isolated limb. Systemic melphalan was only detected in 11 (6%) patients in the MIA single-center series, and 10 of these patients' systemic leakage was <1% of the infused melphalan (Kroon et al. 2008).

Intralesional Therapies for Cutaneous Melanoma

Introduction

The concept of intralesional injectional therapy dates back to 1890s when Dr. William B. Coley first began injecting a bacterial toxin termed "Coley's toxin" directly into inoperable tumors of patients resulting in tumor regression (Coley 1910). He developed the idea after seeing a patient who developed a staphylococcal cutaneous infection leading to complete regression of her sarcoma. However, there was skepticism in the medical community and ultimately the inability to standardize how the toxin was manufactured prevented this treatment from gaining much traction (Faries 2016).

Almost a century later, in the 1970s, Dr. Donald L. Morton was investigating immunological factors in malignant melanoma and noted that some patients treated with Bacille Calmette-Guerin (BCG) developed a rise in antimelanoma antibodies leading to a partial and at times complete response (Morton et al. 1970). He subsequently reported on a series of 36 patients with intradermal melanoma metastases treated with intralesional BCG and demonstrated a 91% regression rate of injected lesions, as well as, 17% regression rate of uninjected nontargeted lesions (Morton et al. 1974). This ability of intralesional therapies to stimulate immunologic response which affects uninjected lesions was initially referred to as a systemic effect and later termed the "bystander effect" (Thompson et al. 2008).

The discovery of these dramatic response rates along with a viable mechanism of action, in the context of a disease for which there were few effective systemic options at the time, prompted a flurry of investigation into intralesional therapies for malignant melanoma. Studies have been conducted using vaccines, cytokines, and viruses as intralesional therapies, but many have not proven to be as clinically applicable as was initially hoped. In 2015, the FDA approved intralesional injection of Talimogene laherparepvec (T-VEC) for advanced melanoma, and there have been promising results of other agents that are still under investigation in clinical trials.

In addition to the potential efficacy of intralesional therapies, there are additional benefits to this modality of treatment. First, it allows for injection of a smaller dose of medication and for a low systemic absorption and therefore lower rates of systemic adverse effects. This is particularly beneficial in patients who may have multiple comorbidities and may be unable to tolerate systemic therapies. Intralesional therapy is particularly applicable in localized in-transit or satellite lesions, as well as, lesions on the extremities, trunk or head and neck that are surgically unresectable. This section will review the existing evidence for intralesional therapies for cutaneous melanoma.

Bacille Calmette-Guerin (BCG)

BCG is a live, attenuated strain of *Mycobacterium bovis* which was initially developed as a vaccine for tuberculosis. Its effect on cancer was first demonstrated in animal models, where administration of BCG was shown to increase the resistance of

mice to transplanted tumors (Old et al. 1959). Subsequently, BCG has been investigated as an oncologic therapy in humans in multiple cancer types (Hersh et al. 1977). Morton et al. (1970) were the first to report its use in patients with melanoma and demonstrated that some patients treated with BCG developed tumor regression.

A larger case series was subsequently reported demonstrating that in patients with intradermal melanoma metastasis who were treated with intralesional BCG alone, 91% of injected lesions regressed and 17% of uninjected lesions regressed (Morton et al. 1974). Thirty-one percent of these patients remained disease-free 6–74 months after intralesional BCG therapy. Patients with subcutaneous or visceral metastasis had a worse overall response with only 31% of injected lesions regressing and no uninjected lesions completely regressing, some partial responses were seen. Multiple other case series showed similar response rates in patients with melanoma with an overall 58% regression rate in injected lesions and 14% regression rate in uninjected lesions (Bast et al. 1974).

However, BCG was also shown to be associated with significant complications including local reactions such as ulceration or abscess, as well as, systemic reactions including fever and malaise. More severe reactions such as erythema nodosum, hepatic dysfunction, hypersensitivity reactions, anaphylaxis, and disseminated intravascular coagulation have also been reported (Bast et al. 1974). In addition, the Eastern Cooperative Oncology Group randomized trial E1673, in which patients with stage I-–III melanoma were randomized to either intralesional BCG versus observation demonstrated no significant difference in disease-free or overall survival (Agarwala et al. 2004). Due to the concerns about serious adverse effects and the lack of benefit seen in a randomized trial, use of intralesional BCG has fallen out of favor.

Interleukin-2 (IL-2)

IL-2 was discovered in 1976 when it was found to be produced by lymphocytes and to lead to T lymphocyte growth (Morgan et al. 1976). IL-2 is a cytokine that stimulates IL-2 receptors resulting in boosting of the natural killer compartment, augmentation of the cytotoxicity of monocytes, induction of T-helper function, and increase in reactivity of cytotoxic T lymphocytes (Meloni et al. 1992). Intravenous infusion of IL-2 was shown to induce regression in 20–35% of patients with a variety of metastatic cancers including melanoma (Rosenberg et al. 1989). A systematic review of 270 patients from eight clinical trials conducted between 1985 and 1993 demonstrated an overall response rate of 16% in patients with metastatic melanoma with a complete response rate of 6% (Atkins et al. 1999). The US Food and Drug Administration approved use of systemic high-dose IL-2 for the treatment of advanced melanoma in 1998. However, there were considerable toxicities with this treatment leading to limited clinical use (Buchbinder and McDermott 2014).

However, intralesional IL-2 was shown in multiple small studies to lead to tumor regression. A systematic review of six phase II and III studies demonstrate a mean complete response rate *per lesion* of 78% (Byers et al. 2014). The mean complete response rate *per patient* was 49.6%. The reported adverse effects were generally mild, grade 1 and 2 events, with only two grade 3 events. The most common reactions were localized swelling and fever and flu-like symptoms including chills, night sweats, malaise, and fatigue (Byers et al. 2014). One of the studies included in the systematic review reported overall survival. The 5-year overall survival was 80% for patients with complete response and 33% for patients with partial response (Boyd et al. 2011). Mean time to relapse was 11 months for complete responders and 8 months for partial responders (Boyd et al. 2011).

Despite these excellent response rates, intralesional IL-2 has not been widely utilized in the United States. This may be due to an onerous injection schedule and a long duration of therapy as well as lack of demonstrable bystander effect (Sloot et al. 2016). The drug has most commonly been studied with two to three times per week injections for a duration of seven and up to 53 weeks (Byers et al. 2014).

Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF)

GM-CSF is a cytokine involved in the activation and recruitment of granulocytes and macrophages, by which it derived its name. However, it also plays a role in development and maturation of dendritic cells and proliferation and activation of T cells thereby linking the innate and acquired immune response (Kaufman et al. 2014). GM-CSF, used in the form of vaccines, was first shown to have anti-melanoma immune responses in mouse models (Kaufman et al. 2014).

However, data from clinical studies evaluating the use of subcutaneous injection of recombinant GM-CSF as adjuvant therapy in resected stage III and IV melanoma patients have been inconsistent. Initial smaller phase II studies showed promise (Elias et al. 2008; Spitler et al. 2009). The largest study, a randomized placebo-controlled trial involving 815 patients, demonstrated no difference in overall or recurrent-free survival in patients treated with adjuvant subcutaneous GM-CSF versus placebo (Lawson et al. 2015).

Similarly, intralesional GM-CSF demonstrated mixed results. Only small series of patients have been reported. One study showed three of 13 (23%) patients with partial response and none with complete response (Si et al. 1996). Another study involving intralesional injection of GM-CSF followed by perilesional IL-2 showed four of 14 (29%) patients with partial response, none with complete response, and seven of 14 (50%) with stable disease (Ridolfi and Ridolfi 2002). In addition, a new derivative of GM-CSF, talimogene laherparepvec (T-VEC), which is an oncolytic herpes simplex virus type 1 that has been genetically modified to drive expression of GM-CSF in infected cells, has shown much more promising results, and perhaps because of that interest in intralesional GM-CSF has waned.

Velimogene Aliplasmid (Allovectin)

Allovectin is a plasmid DNA formulated with a cationic lipid complex. The plasmid DNA encodes two proteins, a major histocompatibility complex (MHC) class I heavy chain (HLA-B7) and the light chain β2-microglobulin (β2M). As well, a mixture of a cationic lipid and a neutral lipid is formulated with the plasmid (Doukas and Rolland 2012). The product stimulates immune responses via induction of cytotoxic T-lymphocyte (CTL) responses directed against the allogenic target (HLA-B&) introduced on tumor cells, via induction of CTL responses directed against tumor antigens (following restoration of MHC class I expression), and via the induction of innate immune and inflammatory responses (Doukas and Rolland 2012).

A phase II trial was conducted with 133 patients. The overall response rate was 11.8% and the median duration of response was 13.8 months. In addition, 19% of patients with more than one lesion identified at baseline had a noninjected lesion response (Bedikian et al. 2010). However, two phase III trials failed to show a benefit of intralesional allovectin on either overall survival or progression-free survival leading to the discontinuation of this agent (Miura and Zager 2018).

Talimogene Laherparepvec (T-VEC)

Talimogene laherparepvec is an oncolytic herpes simplex virus type 1 in which the ICP34.5 gene has been deleted resulting in tumor-selective replication, the ICP47 gene has been deleted resulting in failure to block antigen presentation, and the GM-CSF gene has been added to enhance the immune response to tumor antigens released by virus replication (Liu et al. 2003).

Initial phase I data in patients with a variety of tumors including melanoma showed good tolerability and adverse events limited to low-grade events such as flu-like symptoms and local site reactions (Hu et al. 2006). A phase II trial in 50 patients demonstrated an overall response rate of 26%. Overall survival at 1 year was 58% and systemic responses in noninjected lesions were documented (Senzer et al. 2009).

A large phase III trial (OPTIM) including 436 patients randomized to receive either intralesional T-VEC or subcutaneous injections of GM-CSF and assessed for durable response rate (objective response lasting $\geq$6 months) (Andtbacka et al. 2015). The durable response rate was significantly

higher with T-VEC 16.3% than with GM-CSF 2.1%. Median overall survival was 23.3 months with T-VEC and 18.9 months with GM-CSF (hazard ratio, 0.79, 95% CI 0.62 to 1.00; $p = 0.051$). Adverse events were generally grade 1 and 2 as in the earlier studies including flu-like symptoms and local reactions. This led to the FDA approval of intralesional T-VEC for melanoma in 2015 (Miura and Zager 2018). There are ongoing clinical trials evaluating T-VEC used as a neoadjuvant tretament for recurrent resectable metastatic melanoma and other trials using T-VEC in combination with systemic immunotherapies, which will be addressed in a subsequent section.

Rose Bengal (PV-10)

PV-10 is a small molecule derivative of fluorescein which has been used by ophthalmologists for decades to stain damaged conjunctival and corneal cells. More recently, it was found to have anti-neoplastic properties in melanoma. PV-10 was found to cause necrotic cell death but also apoptotic cell death via accumulation in lysosomes, which trigger cell death by release of cathepsins into the cytosol (Mousavi et al. 2006).

A phase I study demonstrated good tolerability with only low-grade adverse events such as mild to moderate pain at injection sites, local inflammation, and pruritus (Thompson et al. 2008). One patient experienced a mild photosensitivity reaction in the treated limb after exposure to sunlight several days after treatment. The overall response rate of injected lesions was 48% and of non-injected lesions was 27%.

A subsequent phase II study including 80 patients with refractory cutaneous or subcutaneous metastatic melanoma was then conducted (Thompson et al. 2015). The overall response rate was 51% and complete response rate was 26%. Median duration of response was 4.0 months with 8% of patients having no evidence of disease after 52 weeks. Overall response rate in bystander lesions was 33%. Adverse events were predominantly mild to moderate with only 15% of patients with grade 3 or higher events. The most common reactions were local site reactions such as pain and swelling. Eight percent of patients experienced mild or moderate injection site photosensitivity and one experienced a severe generalized photosensitivity reaction. Phase III trials of intralesional PV-10 are currently underway.

Daromun (L19IL2 + L19TNF)

Daromun is the combination of two immunocytokines, L19IL2 and L19TNF. Immunocytokines are recombinant fusion proteins, consisting of a human cytokine (IL2 or TNF) linked to a monoclonal antibody or antibody fragment. By linking the cytokine to a tumor-specific antibody, the cytokine is inactive until it is internalized by tumor cells leading to the release of the parent drug to restore its cytotoxic activity (Schrama et al. 2006).

A phase II trial of intralesional L19IL2 alone in stage IIB and IIIC melanoma demonstrated an objective response rate of 53.9% with a complete response of all lesions achieved in 25% of patients and long-lasting in most patients (5 patients $\geq$24 months) (Weide et al. 2014). No serious adverse events were reported. Another phase II trial of intralesional L19IL2 and L19TNF in 22 patients with unresectable stage IIIC and IVM1a melanoma demonstrated an overall response rate of 55%. A complete response was seen in 28.3% of all lesions, 34.4% of which were noninjected lesions (Danielli et al. 2015). There is a phase III trial underway investigating intralesional L19IL2 and L19TNF in the neoadjuvant setting (Weide et al. 2017).

Coxackievirus A21

Coxackievirus A21 is a common cold virus that targets susceptible cells through specific viral capsid interactions with surface expressed virus receptors composed of intercellular adhesion molecule (ICAM-1) and decay-accelerating factor (DAF). DAF molecules are upregulated on the surface of malignant melanoma cells (Shafren et al. 2004).

A phase II (CALM) trial of intralesional Coxackievirus A21 was conducted including 57 patients with advanced melanoma demonstrating an overall response rate of 28.1% and a durable response rate lasting $\geq$6 months of 21.1% (Andtbacka et al. 2016). A summary of studies on intralesional therapies is shown in Table 3, and

Table 3 Summary of studies of intralesional therapies and their efficacy

Agent	Study	N	Administration	Overall response	Complete response	Median time to recurrence	Bystander effect
BCG	Bast et al. (1974) (review)	125 lesions		58% of lesions			14% of uninjected lesions
BCG	Morton et al. (1974)	754 lesions; 36 patients	Intralesional	91% of lesions	31% of patients		17% of patients
IL-2	Byers et al. (review) (2014)	2182 lesions; 140 patients	Intralesional 0.5–3 times/week; 1–53 weeks	81% of lesions	78% of lesions; 49.6% of patients		No
IL-2	Boyd et al. (2011)	629 lesions; 39 patients	Intralesional q2 weeks	76% of lesions	76% of lesions	77% of complete responders disease free at 5 years; 50% partial responders disease free at 5 months	No
GM-CSF	Si et al. (1996)	13 patients	Intralesional weekly	23% of patients	0% of patients		Yes, proportion unknown
GM-CSF/IL-2	Ridolfi and Ridolfi (2002)	14 patients	Intralesional GM-CSF ×1 followed by perilesional IL-2 1×/day × 5 days q 3 weeks	29% of patients	0% of patients		Yes, proportion unknown
Allovectin	Bedikian et al. (2010)	127 patients	Intralesional 1×/week	11.8% of patients	3.1% of patients	13.8 months	19% of patients

T-VEC	Andtbacka et al. (2015)	436 patients	Intralesional q2–3 weeks	26.4% of patients; durable (>6 months) response rate 16.3% of patients	10.8% of patients	Not estimable	22% of uninjected non-visceral lesions complete response; 9% uninjected visceral lesions complete response
PV-10	Thompson et al. (2015)	80 patients	Intralesional 1×	51% of patients; 58% of lesions	26% of patients; 53% of lesions	4 months	33% response in uninjected lesions
L19IL2	Weide et al. (2014)	24 patients (efficacy analysis)	Intralesional 1×/week for 4 weeks	50% of patients; 53.9% of lesions	25% of patients; 44.4% of lesions	93 days	
L19IL2 and L19TNF	Danielli et al. (2015)	20 patients (efficacy analysis)	Intralesional 1×/week up to 4 weeks	55% of patients	5% of patients; 28.3% of lesions		54% (7/13) of non-injected lesions
Coxackievirus A21	Andtbacka et al. (2016)	38 patients (efficacy analysis)	Intralesional 3×/week then 1× q3 weeks	24% of patients			
Electroporation + bleomycin or cisplatin	Mali et al. (2013) (review)	150 patients, 922 lesions	# pulses and duration of electroporation variable, 1× or 1× q4 weeks	80.6% of lesions	56.8% of lesions		

there are ongoing studies combining intralesional therapy with systemic immunotherapy agents.

Combination with Systemic Immune Therapies

Recently, the combination of intralesional therapies with systemic immune therapies has shown promising results. By combining different treatment modalities, particularly strategies with immunologic mechanisms, there appears to be a synergistic effect. A phase I trial of T-VEC combined with pembrolizumab in stage IIIB-IV melanoma demonstrated an objective response rate of 48% and complete response rate of 14% (Long et al. 2016). Grade 3 or 4 adverse events were reported in 33% of patients. A phase I trial of T-VEC combined with Ipilimumab established safety of the regimen and demonstrated an objective response rate of 50%. A $\geq$6 months durable response was seen in 44% of patients that responded (Puzanov et al. 2016).

A subsequent phase II randomized open-label study of T-VEC in combination with Ipilimumab versus Ipilimumab alone demonstrated an overall objective response rate of 39% in the combined arm versus 18% in the Ipilimumab alone arm (Chesney et al. 2018). Visceral lesion response rates were 53% in the combined arm versus 23% of the Ipilimumab alone arm. Incidence of $\geq$3 grade adverse events was 45% and 35%, respectively.

Electrochemotherapy (ECT)

Electrochemotherapy is the administration of a chemotherapeutic or cytotoxic drug that is normally not permeable to cell membranes, followed by local application of electrical currents which transiently permeabilize cell membranes and allow the cytotoxic drug to enter tumor cells. This therapeutic modality had initially shown promise in animal models of multiple tumor types (Mir et al. 1998).

In clinical studies in patients with melanoma the most commonly used cytotoxic drug has been bleomycin and cisplatin administered either intravenously or by intratumoral injection (Mali et al. 2013). In some studies, patients required either local or general anesthesia for the treatments but the treatment was well-tolerated with reported adverse effects including localized erythema, edema, and pain (Mir et al. 1998). In a systematic review and pooled analysis of 22 studies of electroporation used in patients with melanoma, 150 patients and 922 lesions were included. The objective response rate per lesion was 80.6% and the complete response rate was 56.8% (Mali et al. 2013). A phase II study including 19 patients randomized their lesions to intralesional bleomycin injection and electroporation versus intralesional bleomycin alone. In this study, the objective response rate was 78% for patients treated with electroporation compared to 32% with bleomycin alone ($x2 = 9.39$, 1df, $p = 0.002$) (Byrne et al. 2005). While these results are promising, larger randomized studies are needed to assess the efficacy of electrochemotherapy.

Conclusion

In summary, intralesional therapies have been investigated in melanoma since the beginning of the twentieth century. Over the years, some therapies such as BCG, GM-CSF, and Allovectin had shown promise in earlier reports but demonstrated not to be effective in larger randomized studies. Meanwhile other agents such as T-VEC have demonstrated efficacy in large randomized studies and gained FDA approval and wide-spread use. PV-10, Daromun, and Coxsackievirus A21, and electrochemotherapy are still under investigation but have shown promising results thus far. Finally, perhaps the most promising results of all have been with the combination of intralesional therapies and systemic immunotherapies in which a synergistic effect appears to be observed and could potentially be used both in the adjuvant and neoadjuvant setting. The recent flurry of research on intralesional therapies for melanoma have made this an exciting treatment modality for patients with advanced melanoma.

Neoadjuvant Therapy for Borderline Resectable Nodal Metastasis

Treatment of advanced melanoma now includes several options secondary to advances in systemic treatments with immunotherapy, or checkpoint blockade, and targeted therapy, or BRAF inhibition. While surgical resection remains a basic tenet of treatment, today there are multiple possibilities for the timing of surgery and use of systemic agents. This chapter will look at the role of neoadjuvant treatment in patients with melanoma. See also chapter ▶ "Neoadjuvant Systemic Therapy for High-Risk Melanoma Patients."

The advantage of neoadjuvant treatment in patients with borderline resectable disease is several. First of all, provides an opportunity to decrease the size of the tumor and make a surgical resection more feasible. The pathology of the tumor also provides a biological window into the mechanism of response and/or resistance to treatment which can also facilitate the development of biomarkers. Neoadjuvant treatment offers the opportunity to select out the patients most likely to respond to treatment, or better tumor biology. However, an alternative argument is that the patients who fail neoadjuvant therapy have lost the opportunity regional control through surgical resection. Therefore, it is important to understand the response rate and time to response prior to embarking on neoadjuvant treatment. Several issues in neoadjuvant treatment however, remain unclear. For example, the optimal time of neoadjuvant treatment prior to resection, whether the measurement of response based upon radiologic or pathologic response, and whether the response rate in the neoadjuvant setting supercedes therapy as an adjuvant.

Prior to the approval of checkpoint blockade, effective treatments in melanoma consisted of chemotherapy, temodar given as a single agent, or CVD (cisplatinum, vincristine, and dacarbazine), and immunotherapy consisting of IL-2 and interferon, which were also studied as neoadjuvant treatments in patients with Stage III melanoma. Amongst patients with measurable disease, an early trial of CVD given in 2–3 cycles followed by surgery and subsequent CVD if a response demonstrated a 48% response rate (Buzaid et al. 1994). This was followed by multiple trials which demonstrated efficacy of biochemotherapy, which includes CVD in combination with IL2 and interferon. Selected series demonstrated response rates of 39–50% (Buzaid et al. 1998; Gibbs et al. 2002). However, a phase II multicenter trial demonstrated a slightly lower response rate of 26% (22% partial and 4% complete) (Lewis et al. 2006). Overall, these response rates were not higher than the 48% response rate demonstrated in a Phase III trial of patients with metastatic melanoma, which unfortunately did not improve survival (Eton et al. 2002). Single agent temodar was also studied in a neoadjuvant trial of resectable Stage III, IVa disease. The overall response rate of 16% with two patients that had a complete response (CR), but this was not different than responses noted in Stage IV disease (Shah et al. 2010). Interferon-α2b (IFN) was also studied in a neoadjuvant/adjuvant fashion in patients with Stage III disease and palpable nodes. Patients underwent 4 weeks of treatment prior to surgery, followed by maintenance for a total of 1 year of treatment, with an impressive clinical response rate 55%, and three patients with a pathological CR (Moschos et al. 2006).

The studies above validated the feasibility of neoadjuvant trials, but unfortunately did not improve survival. However, subsequently the landscape of the treatment of melanoma changed with effective systemic therapies. The first drug to improve overall survival in patients with metastatic melanoma was anti-CTLA-4, or Ipilimumab. The response rate was 10% with a similar improvement in survival over standard therapy alone, vaccination with gp100 or dacarbazine (Hodi et al. 2010; Robert et al. 2011). Subsequently, anti-PD1 therapy has demonstrated a response rate of 30% and the combination of CTLA-4 and PD-1 blockade increases responses to 58% (Topalian et al. 2012; Wolchok et al. 2017). With active drug combinations, there was rationale to use these drugs in the neoadjuvant setting.

Tarhini et al. looked at the role of neoadjuvant Ipilimumab in patients with Stage IIIB/C melanoma. Patients had a radiologic assessment, pretreatment biopsy, and then received Ipilimumab for two doses, followed by repeat radiologic assessment and surgery. Of the 33 evaluable

patients, all had viable melanoma on the pathologic analysis. Investigation of the tumor demonstrated immune activation with CD4 and CD8 cells after treatment, and a low baseline CD20 cell number trended with a poor clinical response. On imaging, 9% had a partial response, 64% had stable disease, and 24% patients has progressive disease (Tarhini et al. 2014). Importantly, no patients lost the opportunity to have surgery, but the response rate was similar to that seen in Stage IV disease, and therefore subsequent trials have also employed anti-PD1 therapy. Early report of the Optimal Neoadjuvant Combination (OpACIN) trial of combination of Ipilimumab and nivolimab(IPI NIVO) demonstrated responses in 8 of 10 patients in the phase Ib portion of the trial. This trial is now being expanded to additional patients, but also noted many patients did not complete treatment because of toxicity (Rozeman et al. 2017). There are currently open trials of neoadjuvant therapy and the early results were pooled and presented in abstract form of the International Neoadjuvant Melanoma Consortium (INMC). Of the combined 21 patients receiving immunotherapy with IPI NIVO or NIVO alone, a pathologic complete response was found in 8 patients (38%), none of which have had a recurrence (Menzies et al. 2017). Further analysis of genomics of responding and non-responding patients is ongoing.

Targeted inhibition of BRAF mutated tumors has been the other area of major advance in melanoma. While single agent BRAF inhibition improved survival, resistance developed in many within a year (Chapman et al. 2011). Combination BRAF/MEK has been shown to improve survival in Stage IV patients beyond single agent and is now the preferred drug combination (Flaherty et al. 2012b). Studies utilizing a neoadjuvant approach with targeted therapy have also shown promise. A prospective trial randomized patients in a 2:1 fashion to neoadjuvant BRAF/MEK inhibition for 12 weeks followed by surgery and up to 44 weeks of postop treatment, for a total of 52 weeks. After 18 months of follow-up the trial was stopped because 10 of 14 patients in the treatment arm (71%) remained free of disease while none (0 of 7 patients) in the standard of care arm remained free of disease. The radiologic response rate in this cohort was 85%, and of the 12 patients that went on to have surgery, the pathological CR rate was 58% (Amaria et al. 2018). The toxicity was acceptable and similar to that seen in patients with Stage IV disease, the majority being fevers, chills and headache. Interestingly, a pretreatment biopsy demonstrating less pERK expression, and increased CD8 toxicity was associated with a CR. The trial was not powered to detect survival differences, and has remained open as a phase II single arm trial. However, the results are consistent with a larger experience of the international neoadjuvant melanoma consortium, which included patients from this trial, with a pathologic complete response rate of 55% (Menzies et al. 2017). All of these neoadjuvant targeted therapy trials administer targeted BRAF/MEK preoperatively and postoperatively for a year, and therefore do not answer the question of whether neoadjuvant therapy alone would be sufficient. However, the progression free survival (PFS) of targeted therapy in the adjuvant setting is 58% for combination therapy and 39% for placebo, so the benefit of the combination neoadjuvant plus adjuvant approach appear to be improved in these early studies.

While the results of neoadjuvant studies in the era of effective systemic therapies are still being finalized, the data to date demonstrates several important points. Responses to treatment occur within 6–12 weeks and no trial to date has reported the loss of control of a regional nodal basin by administering neoadjuvant therapy. Therefore, neoadjuvant therapy remains a safe option for patients with borderline resectable disease. The response rate to 12 weeks of targeted therapy with BRAF and MEK inhibition, 85%, with a CR rate of over 50% at surgery, is the highest noted to date, and better tolerated. The early reports of combination Ipilimumab and nivolimab are also encouraging, with responses from 80% after 6 weeks of treatment, and a CR rate at surgery of 38%. However, combination immunotherapy data is not as mature, and treatment is associated with more toxicity and this risk must be weighed on an individual basis. It will be interesting to see if the high response rates seen in

early combination trials persist in larger cohorts. Also, future studies will look at whether surgery can be delayed in responding patients to the point of maximal response. In general, for a patient with borderline resectable disease and a BRAF mutation, neoadjuvant therapy has the highest tolerability and chance of success. Hopefully the results of these trials will elucidate the patients who are most likely to benefit from the neoadjuvant approach in the future.

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