

Soft Tissue Sarcomas

Isolated Limb Perfusion with Melphalan and TNF- α in the Treatment of Extremity Sarcoma

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Opinion statement

Isolated limb perfusion (ILP) with chemotherapy alone has uniformly failed in the treatment of irresectable extremity soft tissue sarcomas. The addition of tumor necrosis factor-alpha (TNF- α) to this treatment approach contributed to a major step forward in the treatment of locally advanced extremity soft tissue sarcoma (STS). High response rates and limb salvage rates have been reported in multicenter trials, which combined ILP with TNF- α plus melphalan, which resulted in the approval of TNF- α for this indication in Europe in 1998. Subsequently a series of confirmatory single institution reports on the efficacy of the procedure have now been published. TNF- α has an early and a late effect; it enhances tumor-selective drug uptake during the perfusion and plays an essential role in the subsequent selective destruction of the tumor vasculature. These effects result in a high response rate in high-grade soft tissue sarcomas. This induction therapy thus allows for resection of tumor remnants some 3 months after ILP and thus avoidance of limb amputation. TNF- α -based ILP is a well-established treatment to avoid amputations. It represents an important example of tumor vasculature-modulating combination therapy and should be offered in large volume tertiary referral centers.

Introduction

Patients with a large, high-grade soft tissue sarcoma (STS) of the extremities have a high risk for local and systemic recurrences. The local situation in the limb

may require extensive and mutilating surgery followed by radiotherapy. Several studies suggested that amputation of the limb did not result in an improved

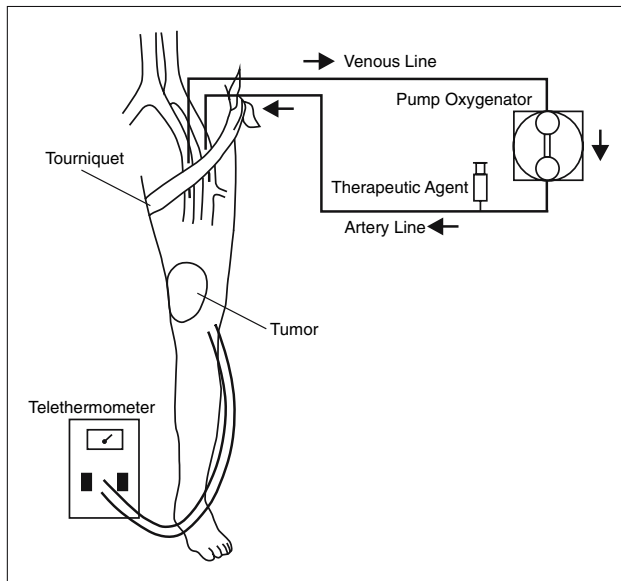


Figure 1. Isolated limb perfusion circuit

survival. The tendency to perform more limb-preserving surgery has led to the exploration of isolated limb perfusion (ILP) as a procedure enabling limb salvage surgery in cases of primarily STS, requiring mutilating surgery or even amputation of the limb. The technique of ILP achieves regional drug concentrations 15–25 times higher than systemic administration and is without systemic side effects [1,2]. Isolation of the limb is achieved by clamping and cannulation of the major artery and vein, connection to an oxygenated

extracorporeal circuit, ligation of collateral vessels, and application of a tourniquet (Fig. 1). Because of its efficacy and low regional toxicity profile, melphalan (L-phenyl-alaninemustard) is the standard drug and is most commonly used at a dose range of 10 mg/L (for the leg) to 13 mg/L (for the arm) [3]. ILP procedures are performed under mild hyperthermic conditions (38.5–40°C) as it has become clear that hyperthermia can increase the drug uptake in the cells, but that true hyperthermia (>41°C) is associated with increased local toxicity. Tissue temperatures are monitored and radiolabeled serum albumen or erythrocytes are injected into the perfusate to detect leakage into the systemic circulation by a precordial scintillation probe. Leakage monitoring is mandatory especially now that TNF- α is used, since leakage of TNF- α can theoretically lead to significant toxicity such as hypotension and septic shock-like syndrome. After 1–1.5 hours of perfusion, the limb is rinsed with an electrolyte solution, cannulas are removed, and the vessels are repaired. Acute tissue reactions after perfusion are classified in a 5-grade system according to Wieberdink *et al.* [4] (I) no reaction; (II) slight erythema and/or edema; (III) considerable erythema and/or edema with some blistering; (IV) extensive epidermolysis and/or obvious damage to the deep tissues causing definite functional disturbances; threatening or manifest compartmental syndrome; (V) reaction which may necessitate amputation. The advent of TNF- α has greatly expanded the successful application of ILP, and we will discuss these relatively recent developments.

Failure of ILP with chemotherapy alone

- Achieving limb salvage has become a key element in the management of locally advanced soft tissue sarcomas (STS) of the extremities, because there is ample evidence that amputations do not improve survival rates in patients with large (>5 cm) deep-seated high-grade sarcomas [5,6]. Locally advanced STS are often large at the time of diagnosis, and treatment options may consist of an amputation or a limb sparing extensive surgical procedure followed by radiation therapy which may cause severe disability of the limb. The insight that limb-preserving surgery is equal to amputation in terms of overall survival [7,8] greatly enhanced the interest in neoadjuvant treatment options that could reduce the size of the tumors and hence make function-preserving surgery possible. The possible role of induction chemotherapy was recently studied by the MD Anderson Cancer Center, but although a tumor response was observed in 43% of these patients, the therapy could only reduce the extent of operation in 13% and no scheduled amputation could be prevented [9]. Preoperative radiotherapy has not been studied as an induction therapy in patients with primary irresectable extremity STS. It has been shown to be as effective as postoperative radiotherapy in STS patients, but at the cost of higher wound complication rates [10]. Literature suggests that combined neoadjuvant chemoradiotherapy with limb salvage surgery

is an option for treating patients with deep STS of the extremity, but up to now, this treatment option remains investigational and results from large studies have to be awaited [11–13]. ILP has the ability to deliver the highest doses of (bio)chemotherapy to the affected limb and is thus a treatment modality that can be effective in the neoadjuvant setting. In contrast to the efficacy of melphalan-based ILP for small intransit melanoma metastases, however, results of ILP with melphalan, doxorubicin, or any other drug for large STS have been very disappointing. Presumably this is mainly due to impaired drug uptake in large tumors. For treating advanced STS, ILP with chemotherapy alone was abandoned after studies in the 1970s and 1980s [14–17]. These findings were reinforced by a study of ILP with doxorubicin, where Feig and coworkers reported a dismal 0% response rate and an amputation rate of 75% [18], in line with our experience.

Success of TNF- α plus chemotherapy in ILP

- The introduction of TNF- α in the ILP setting was pioneered by Lejeune *et al.* [19]. In 13 melanoma patients and four locally advanced STS cases impressive and very rapid responses were observed. This observation was followed by multicenter trials in patients with locally advanced STS [14,16,18,20] and melanoma [21–23]. Table 1 summarizes all literature reports on TNF- α -based ILP in STS patients. In the STS trials, TNF- α -based ILP was established as a highly effective new method of induction biochemotherapy for extremity STS with a 20%–30% complete remission (CR) rate and ~50% partial remission (PR) rate [20,41,26,28]. On the basis of our multicenter trials TNF- α was approved and registered in Europe for sarcoma treatment in 1998 [28]. In four studies over a 10-year-period, the European TNF/ILP assessment group evaluated 270 ILPs in 246 patients with irresectable STS. All cases were reviewed by an independent review committee and compared with a population based Scandinavian STS database of patients receiving conventional treatment (often an amputation). There were 246 patients with local and very advanced disease. Primary sarcomas occurred in 55% of patients, local recurrent sarcomas in 45%, multifocal primary or multiple local recurrences in 22%, and overt concurrent metastatic disease in 15%. Tumors >10 cm were found in 46% of patients, and tumors were a high grade with 95% showing grade II–III tumors. In 76% of cases a delayed marginal resection of the tumor remnant was usually performed 2–4 months after ILP. Major responses were seen in 56.5%–82.6% of patients, after which resection of the sarcoma became possible in most cases. Limb salvage was achieved in 74%–87% in these four studies. In 71% of the 196 patients who had been classified by the independent review committee, 87% were considered cases that could only have been managed by amputation and 13% by functionally debilitating resection plus radiotherapy. A matched control study with cases from the Scandinavian STS Databank showed that TNF- α had no negative effect on survival ($P = 0.96$). In this high-risk population overall survival was around 50% at 5 years [28]. It was concluded that ILP with TNF- α and melphalan represents a new and successful option in the management of irresectable locally advanced extremity STS [28]. The approval in Europe of TNF- α for its use in the ILP setting for locally advanced STS has led to training and activation of over 40 centers in Europe to provide one or more referral programs in each country.

Table 1. Study reports of TNF-based ILP for irresectable soft tissue sarcomas

Drugs	# Pts	CR (%)	PR (%)	Limb salvage (%)	Year	Author
TNF + Melphalan*	8	100 ^a	0	64	1993	Hill <i>et al.</i> [24]
TNF + IFN + Melphalan	55	18 ^a 36 ^b	64 ^a 51 ^b	84	1996	Eggermont <i>et al.</i> [20]
TNF + Melphalan	10	70 ^a	20 ^a	89	1996	Santinami <i>et al.</i> [25]
TNF ± IFN + Melphalan	186	18 ^a 29 ^b	57 ^a 53 ^b	82	1996	Eggermont <i>et al.</i> [26]
TNF ± IFN + Melphalan	35	37 ^b	54 ^b	85	1997	Gutman <i>et al.</i> [27]
TNF ± IFN + Melphalan	246	28 ^c	48 ^c	76	1999	Eggermont <i>et al.</i> [28]
	196	17 ^c	48 ^c	71 ^d		
TNF + Doxorubicin	20	26 ^e	64 ^e	85	1999	Rossi <i>et al.</i> [29]
TNF ± IFN + Melphalan	22	18 ^b	64 ^b	77	2000	Lejeune <i>et al.</i> [30]
TNF ± IFN + Melphalan	55	NS	NS	84	2001	Hohenberger <i>et al.</i> [31]
TNF ± IFN + Melphalan	49	8 ^b	55 ^b	58	2003	Noordap <i>et al.</i> [32]
TNF ± IFN + Melphalan ^h	48	38 ^b	31 ^b	85	2005	Grünhagen <i>et al.</i> [33]
TNF + Melphalan ^{g,h}	72	49	17	84	2005	Bonvalot <i>et al.</i> [34]
		35	22			
TNF + Doxorubicin ^h	21	5 ^a 55 ^e	57 ^a 35 ^e	71	2005	Rossi <i>et al.</i> [35]
TNF ± IFN + Melphalan	217	18 ^a 26 ^b	51 ^a 149 ^b	75	2006	Grünhagen <i>et al.</i> [36]
TNF + Doxorubicin/Melphalan ^h	51	41 ^a	55 ^a	82	2007	Pennacchioli <i>et al.</i> [37]
TNF ± IFN + Melphalan	73	25 ^a	69 ^a	61 ⁶	2007	van Ginkel <i>et al.</i> [38]
TNF + Melphalan	100	30	48	88	2007	Bedard <i>et al.</i> [39]
TNF + Melphalan	16	20	33	75	2007	Hayes [40]

#Pts—number of patients, CR—Complete response; PR—Partial response; Ref#—reference number, TNF—Tumour Necrosis Factor, IFN—Interferon gamma. *—low dose (<4 mg for leg-ILP, <3 mg for arm-LIP), NS—not stated

^aObjective Clinical Response Rate By WHO criteria

^bCR: clinical CR or 100% necrosis; PR: clinical PR or >50%–99% necrosis

^cCR only recognized by EMEA (European Medicine Evaluation Agency) when histopathology showed 100% necrosis

^dIndependent committee recognized 196 patients as pure amputation candidates

^eNo clinical response data; CR: >90% necrosis; PR: radiological and/or histopathological >50% necrosis.

^fOverall 10 years' limb salvage rate

^gDifferent scoring system: upper panel: CR/PR: loss of vasculature on Ultrasound/MRI; lower panel CR: >90% necrosis on histopathology

^hlow dose (<4 mg for leg-ILP, <3 mg for arm-ILP)

These programs are running well and experiences are rather uniform and in line with the initial trials as is indicated by the list of single center studies that have been published over the years (Table 1). Unfortunately TNF- α is not clinically available in North America because the patent and licensing rights of TNF- α are in the hands of a different company and thus the registration file has not been presented to the FDA.

Vasculotoxic mechanism of TNF- α plus chemotherapy

- As TNF- α targets the tumor vasculature, which is a common denominator in all these tumor types, the use of TNF- α is very attractive and it explains its efficacy in combination with chemotherapy across these numerous histologies. The selective destructive effects of TNF- α -based ILP on tumor-associated vessels are illustrated in the pre- and post-perfusion angiographies in Fig. 2. In magnetic resonance spectrometry studies, we have shown that shutdown of the tumor is virtually

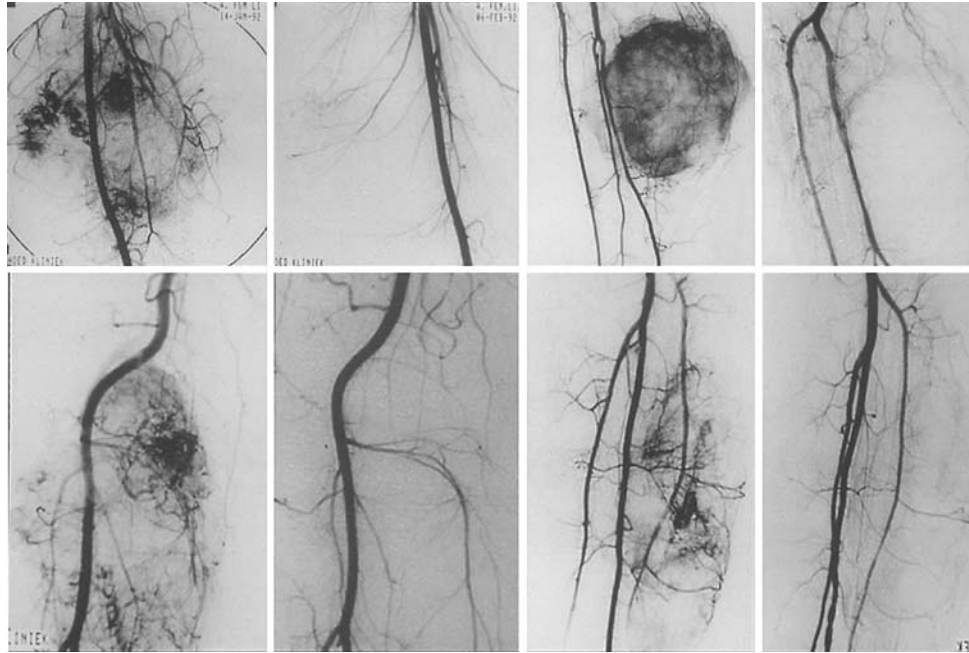


Figure 2. Selective eradication of tumor vasculature by TNF-based ILP (left pre-ILP; right post ILP)

complete within 16 hours after the perfusion, confirming the likelihood of TNF- α -mediated effects on the vasculature of the tumor [42]. At the histopathological level, it has been demonstrated that TNF- α induces microvascular damage through loss of endothelial cohesion [43]. This leads to intravascular effects, such as thrombocyte aggregation and erythrosthiasis, with hemorrhagic necrosis as the result. The vascular destruction is important in the early and late stages after ILP [44–46]. Moreover, besides the small direct cytotoxic effect of TNF- α and the far more important vasculotoxic mechanism, speculations exist on a third role, namely the ability of TNF- α to cause an immediate drop in interstitial pressure within the tumor [47].

TNF- α enhances selective drug uptake in tumors

- We have recently demonstrated that the addition of high-dose TNF- α to the perfusate results in a 4–6 fold increased uptake by the tumor of the cytostatic drugs melphalan and doxorubicin [48,49]. Importantly this uptake was tumor specific and no increased uptake was noted in the normal tissues, thus emphasizing the selective action of TNF- α on the tumor vasculature. The more vascular the tumor was, the better the synergistic effect was between TNF- α and the chemotherapeutic agent [50]. We also demonstrated that 10 μg of TNF- α (a 5-fold reduction of the 'standard dose of 50 μg ') was the minimum threshold dose for activity of TNF- α in our rat tumor extremity perfusion model. At 2 μg doses of TNF- α all effects were lost [51]. The results from our tumor models predict a minimum threshold activity for 1 mg TNF- α in the clinical situation and we advise not to go below that dose. We demonstrated that TNF- α and melphalan when combined with the effects of hyperthermia above 40°C leads to amputations because of normal tissue damage, and we advise against its use in the clinic [51]. Furthermore we showed that actinomycin D, commonly used in the

clinical setting, leads to idiosyncratic toxicity in our models of both tumor and normal tissues, resulting in limb amputation in all cases. We strongly advise not to use TNF- α in combination with actinomycin D in the clinical setting [52].

Reports on all STS patients

- Many single center studies of TNF- α plus melphalan have been reported over the past years that showed similar response and limb salvage rates (Table 1). Studies have reported overall experience in STS patients or of special categories (see below) of STS patients. The single center reports on overall STS populations showed that response rates varied from 53% to 91% and limb salvage rates from 58% to 89%. Two reports on the combination of TNF- α with doxorubicin show very similar response rates of 62%–90% and limb salvage rates of 71%–85%, with more regional toxicity after doxorubicin compared with melphalan [29,35]. We therefore consider melphalan to be the drug of choice.
- Another aspect that has been studied in overall populations is whether high doses of TNF- α (eg, 3–4 mg) are necessary or whether lower doses (eg, 1 mg) suffice. An early clinical report by Hill and coworkers suggested that low doses of TNF- α (i.e. up to 1 mg) were sufficient because in eight STS patients eight complete responses were observed [34]. The small study size, the concomitant use of high doses of corticosteroids, and the fact that a different type of TNF- α was used, however, meant that definitive conclusions were not possible. Definitive conclusions came from the Italian studies of 1 mg TNF- α in combination with doxorubicin [29,35], the French randomized phase II trial in 100 patients on four TNF- α doses (0.5, 1.0, 2.0, and 4.0 mg) [34], and our analysis of 240 ILPs where the TNF- α doses varied from 1.0, 2.0, 3.0, and 4.0 mg. All these studies seem to indicate that TNF- α doses of 1 mg (for the arm) and 1–2 mg (for the leg) are sufficient [33]. Recently, Bedard *et al.* [39] confirmed their previous findings [34] of the success of low-dose TNF- α in a prospective study of 100 patients with locally advanced STS treated by ILP and 1 mg TNF- α /melphalan. This is consistent with findings from our laboratory, which indicate that doses of 1 mg TNF- α were sufficient, but further dose reduction resulted in complete loss of activity [51].

Reports on special patient categories

Patients with overt metastatic disease

- Patients who have overt metastatic disease and a limb-threatening tumor are a special category whereby tumor control can be relatively easily achieved, and amputation avoided, by a palliative TNF- α -based ILP. In nine such cases, a 77% response rate and 89% limb salvage rate was reported [53], and we observed a 84% response rate and 97% limb salvage rate in 37 cases [54]. This demonstrates that TNF- α -based ILP is an extremely attractive option in these patients.

Patients with multiple tumors in the extremity

- TNF- α -based ILP is the ideal alternative to amputation in patients with multifocal primary tumors, who are difficult to treat, such as Kaposi sarcomas [55], multiple lymphangio-sarcomas (also known as

Steward Treves Syndrome) [56], or those with multiple primaries of various histologies or multiple recurrences after prior surgery [57]. Remarkably good results have been reported, and we observed a 87% response rate and a 80% limb salvage rate after 16 TNF- α -based ILPs were performed in 10 patients with Steward Treves Syndrome. In our experience, after 64 ILPs we observed a 77% response rate and 82% limb salvage rate in patients with multiple tumors. These results indicate that TNF- α -based ILP is very effective in this patient population.

Patients with recurrent tumors in an irradiated field

- We performed 29 ILPs in 26 patients with recurrent tumors in an irradiated field. In contrast to the belief that such tumors are unlikely to respond, we observed a 70% response rate and 65% limb salvage rate, indicating that in this very difficult patient category a TNF- α -based ILP approach is an attractive treatment option [58].

Patients with desmoids

- Patients with desmoid tumors and aggressive fibromatosis often present with recurrent disease, which is very difficult to treat surgically with or without radiotherapy. Lev-Lelouche *et al.* and our group report very similar results in 6 and 12 such cases, respectively. Response rates were 83% and 75%, respectively, and limb salvage rates were 100% in both reports, demonstrating the utility of this procedure [59,60].

Elderly patients >75 years old

- An important result was demonstrated by the report on the Rotterdam experience of patients older than 75 years with limb-threatening tumors treated with 50 TNF- α -based ILPs [61]. Results were very favorable in the 34 perfusions for limb-threatening sarcomas; a 38% CR and a 38% PR rate, achieving limb salvage in 76% of the patients, as well as in 16 perfusions for bulky melanoma intransit metastases resulting in a 75% CR and 25% PR rate, were observed. The procedure was proven safe in the elderly with limb salvage, which is of overriding importance in these patients as an amputation can abolish independency of the life [61]. In the present era of leakage-free ILPs, TNF- α -based ILPs are associated with very little toxicity problems. We have separately reported on the absence of toxicity in patients without leakage [33,62]. In the rare case of systemic leakage of TNF- α management is relatively easy and requests the use of ample hydration and non-steroid anti-inflammatory drugs, and toxicity can be minimal [63]. In our total experience of >450 ILP procedures, no toxic shock-like syndrome has occurred using this regimen. Moreover, TNF- α -based ILPs are not associated with a higher regional toxicity compared with melphalan-based ILPs, and have less regional toxicity than doxorubicin-based ILPs [64].

New vasoactive drugs

Histamine

- Because of the potential toxicity of TNF- α , alternative vasoactive drugs were investigated in our center. Histamine (Hi) is an inflammatory mediator that is formed and stored mainly in the granules of mast cells and basophils. It has also been identified in cells in regenerating or rapidly growing tissues. Its effect on fine vessels is to cause edema by increasing the flow of lump and lymph proteins into the extracellular space and also by promoting the formation of gaps between endothelial cells [65]. This effect could potentially be used to increase drug concentrations in tumor tissues. We used a sarcoma-bearing rat ILP model to assess the effects of Hi, melphalan, and the combination [66]. The antitumor effect of the combination Hi and melphalan was synergistic. The strong effect of Hi-based-ILP with melphalan was explained by three mechanisms: direct cytotoxicity to the tumor cells, direct cytotoxicity to the tumor-associated vasculature, and indirect effect through Hi-mediated increased melphalan concentration in the tumor. There are several theoretical advantages in using Hi instead of TNF- α . In brief; it has been hypothesized that ILP with Hi would likely enhance drug uptake more quickly and effectively than ILP with TNF- α , and Hi is a potentially safer drug than TNF- α in case of leakage into the systemic circulation during ILP [66].

Interleukin-2

- Interleukin-2 (IL-2) is another candidate to enhance tissue uptake of cytotoxic agents. IL-2 is a mediator of immune cell activation with some antitumor activity. IL-2 causes vascular leakage and edema and for this reason we examined the antitumor activity of a combined treatment with IL-2 and melphalan in an experimental ILP setting [67]. ILPs were performed in soft tissue sarcoma-bearing rats. ILP with either IL-2 or melphalan alone has no antitumor effect, but the combination of IL-2 and melphalan resulted in a strong synergistic tumor response, without any local or systemic toxicity. IL-2 significantly enhanced melphalan uptake in tumor tissue without signs of significant vascular damage to account for this observation. Observations indicated a potentially important role for macrophages as an explanation for the strong synergistic effect of IL-2. In systemic immunotherapy, the combination of IL-2 and Hi has been used for solid tumor treatment based on immunomodulatory effects. Also because of the success of IL-2 in ILP and the earlier-mentioned success of Hi in ILP model, we studied if the two drugs combined with melphalan could further improve response rates in an experimental ILP. In a sarcoma-bearing rat ILP model the effects were assessed [68]. A negative synergistic effect was found between IL-2 and Hi in a regional setting.

Conclusions and future directions

- ILP methodology provides us an excellent tool in the clinic to obtain local control and avoid amputations of limbs in patients with limb-threatening tumors. This has been largely achieved by the success of the antivascular TNF-based biochemotherapy in this setting. TNF- α ,

for the first time, has brought us an effective treatment against large, bulky tumors. TNF- α -based ILP is a very successful treatment option to achieve limb salvage in the management of advanced disease, multiple or drug-resistant extremity tumors. TNF- α -based ILPs are now performed in over 40 cancer centers in Europe with referral programs for limb salvage. Provision should be made to make TNF- α available in North America. TNF- α -based antivasular therapy of cancer is here to stay and its potential needs to be studied further, including its use in the systemic setting. In the field of isolated-perfusion newly discovered vasoactive drugs await evaluation in clinical trials [69].

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