TNF-α in Cancer Treatment: Molecular Insights, Antitumor Effects, and Clinical Utility

Remco van Horssen, Timo L. M. ten Hagen, Alexander M. M. Eggermont

Department of Surgical Oncology, Erasmus MC–Daniel den Hoed Cancer Center, Rotterdam, The Netherlands

Key Words. Cancer • TNF-α • TNFR-1 • Tumor vasculature • Isolated limb perfusion

Learning Objectives

After completing this course, the reader will be able to:

1. Discuss the role of TNF-α in cancer survival and apoptosis.
2. Describe the mechanism of chemotherapy potentiation by TNF-α.
3. Explain the selective targeting of tumor vasculature by TNF-α.
4. Discuss TNFR-1 and TNFR-2 signaling.

Abstract

Tumor necrosis factor alpha (TNF-α), isolated 30 years ago, is a multifunctional cytokine playing a key role in apoptosis and cell survival as well as in inflammation and immunity. Although named for its antitumor properties, TNF has been implicated in a wide spectrum of other diseases. The current use of TNF in cancer is in the regional treatment of locally advanced soft tissue sarcomas and metastatic melanomas and other irresectable tumors of any histology to avoid amputation of the limb. The interaction of TNF-α with TNF receptor 1 and receptor 2 (TNFR-1, TNFR-2) activates several signal transduction pathways, leading to the diverse functions of TNF-α. The signaling molecules of TNFR-1 have been elucidated quite well, but regulation of the signaling remains unclear. Besides these molecular insights, laboratory experiments in the past decade have shed light upon TNF-α action during tumor treatment. Besides extravasation of erythrocytes and lymphocytes, leading to hemorrhagic necrosis, TNF-α targets the tumor-associated vasculature (TAV) by inducing hyperpermeability and destruction of the vascular lining. This results in an immediate effect of selective accumulation of cytostatic drugs inside the tumor and a late effect of destruction of the tumor vasculature. In this review, covering TNF-α from the molecule to the clinic, we provide an overview of the use of TNF-α in cancer starting with molecular insights into TNFR-1 signaling and cellular mechanisms of the antitumor activities of TNF-α and ending with clinical response. In addition, possible factors modulating TNF-α actions are discussed. The Oncologist 2006;11:397–408

Introduction

Tumor necrosis factor alpha (TNF-α) is a multifunctional cytokine involved in apoptosis, cell survival, inflammation, and immunity acting via two receptors [1, 2]. Currently it...
is used in cancer treatment in the isolated limb perfusion (ILP) setting for soft tissue sarcoma (STS), irresectable tumors of various histological types, and melanoma in-transit metastases confined to the limb [3]. TNF-α was isolated in 1975 from the serum of mice treated with bacterial endotoxin as the active component of “Coley’s toxin” and was shown to induce hemorrhagic necrosis of mice tumors [4, 5]. It was almost a century ago that William Coley, a surgeon from New York, observed high fever and tumor necrosis in some cancer patients treated with his bacterial filtrate (“Coley’s mixed toxins”) [6]. A decade after its isolation, TNF-α was also characterized as “cachectin” and as T-lymphocyte differentiation factor [7, 8]. In 1984, the human TNF-α gene was cloned [9, 10], and a range of clinical experiments were set up, leading to a license from the European Agency for the Evaluation of Medicinal Products (EMEA) for the treatment of limb-threatening STS in an isolated perfusion setting [11].

**TNF-α and TNF Receptor 1 Signaling**

TNF-α is a 17-kDa protein consisting of 157 amino acids that is a homotrimer in solution. In humans, the gene is mapped to chromosome 6 [12]. Its bioactivity is mainly regulated by soluble TNF-α-binding receptors. TNF-α is mainly produced by activated macrophages, T lymphocytes, and natural killer (NK) cells. Lower expression is known for a variety of other cells, including fibroblasts, smooth muscle cells, and tumor cells. In cells, TNF-α is synthesized as pro-TNF (26 kDa), which is membrane-bound and is released upon cleavage of its pro domain by TNF-converting enzyme (TACE) [13].

As mentioned above, TNF-α acts via two distinct receptors [14]. Although the affinity for TNF receptor 2 (TNFR-2) is five times higher than that for TNFR-1 [15], the latter initiates the majority of the biological activities of TNF-α. TNF-1 (p60) is expressed on all cell types, while TNFR-2 (p80) expression is mainly confined to immune cells [16]. The major difference between the two receptors is the death domain (DD) of TNFR-1 that is absent in TNFR-2. For this reason, TNFR-1 is an important member of the death receptor family that shares the capability of inducing apoptotic cell death [17]. Besides this apoptotic signaling, TNFR-1 is widely studied because it is a dual role receptor: next to induction of apoptosis, it also has the ability to transduce cell survival signals. Although signaling pathways are well defined nowadays, the life-death signaling regulation is still poorly understood [18, 19]. The TNFR-1 signaling pathways are depicted in Figure 1. Upon binding of the homotrimer TNF-α, TNFR-1 trimerizes, and silencer of death domain (SODD) protein is released [20]. TNFR-associated death domain (TRADD) binds to the DD of TNFR-1 and recruits the adaptor proteins receptor interacting protein (RIP), TNFR-associated factor 2 (TRAF-2), and Fas-associated death domain (FADD) [21]. In turn, these adaptor proteins recruit key molecules that are responsible for further intracellular signaling. When TNFR-1 signals apoptosis, FADD binds pro-caspase-8, which is subsequently activated. This activation initiates a protease cascade leading to apoptosis, also involving the mitochondria and with caspases as key regulators [22]. The ultimate event in this apoptotic signaling is the activation of endonucleases, like EndoG, resulting in DNA fragmentation. Alternatively, when TNFR-1 signals survival, TRAF-2 is recruited to the complex, which inhibits apoptosis via cytoplasmic inhibitor of apoptosis protein (cIAP). The binding of TRAF-2 initiates a pathway of phosphorylation steps resulting in the activation of cFos/cJun transcription factors via mitogen-activated protein kinase (MAPK) and cJun N-terminal kinase (JNK) [23]. The major signaling event of TRAF-2 and RIP is the widely studied activation of nuclear factor kappa B (NF-κB) transcription factor via NF-κB–inducing kinase (NIK) and the inhibitor of κB kinase (IKK) complex [24]. Both the NF-κB and cFos/cJun transcription factors induce transcription of antiapoptotic, proliferative, immunomodulatory, and inflammatory genes. NF-κB is the major survival factor in preventing TNF-α–induced apoptosis, and inhibition of this transcription factor may improve the efficacy of apoptosis-inducing cancer therapies [25]. NF-κB activation in many human malignancies is aberrant or constitutive, and its role in the regulation of the apoptosis–proliferation balance in tumor cells indicates its role in oncogenesis [26, 27]. For further details on the dual signaling of TNFR-1, see Figure 1.

**Implications for Cellular Mechanisms Underlying TNF-α Effects During Solid Tumor Treatment**

It is widely known that TNF-α induces hemorrhagic necrosis in a certain set of tumor types. To investigate the underlying mechanisms of TNF-α action during ILP of solid tumors in humans, we set up perfusion models in rats and reported that hemorrhagic necrosis was much greater in tumors treated with TNF-α and chemotherapeutic drugs [28]. In addition, we showed a synergistic antitumor effect of the combination treatment with TNF-α and chemotherapeutic drugs [29]. In contrast, TNF-α alone induced only a mild central necrosis, and there was no objective tumor response observed. The same rat models also revealed that the addition of TNF-α improved the accumulation of chemotherapeutic drugs selectively in the tumor up to three- to sixfold. The augmented uptake of melphalan added to the molecular properties of this small molecule (distribution by gradient instead of convection) resulted in intratumoral concentra-
tions close to the 50% inhibitory concentration (IC50) in STS cells in vitro [30, 31]. These levels result in tumor cell kill in the ILP setting, and melphalan can distribute within the well-perfused parts of the tumor even though the intratumoral pressure is high. This selective uptake of melphalan by the tumor was also observed when other vasoactive drugs were used in the ILP setting (see below). It is important to note that the cell lines we used were not sensitive to TNF-α in vitro, which is in accordance with other reports describing a lack of effect of TNF-α and no synergism with cytotoxic drugs in cell lines [32, 33]. Next to these ILP data, studies in mice and rats showed that a systemic low dose of TNF-α augments the antitumor activity of pegylated liposomal doxorubicin [34, 35]. These observations are comprehensible clues that mechanisms underlying the TNF-α effect during solid tumor treatment cannot be caused by a direct cytotoxic or cytostatic effect of TNF-α toward the tumor cells. It was suggested that, rather than tumor cells themselves, cells of the tumor stroma may be responsible for the observed antitumor effect of TNF-α in patients. This hypothesis was confirmed by data from mice experiments revealing that TNF-α had a cytotoxic effect on tumor vasculature [36].

ANGIOGENESIS AND TUMOR-ASSOCIATED VASCULATURE

Angiogenesis, the formation of new blood vessels from pre-existing ones, has become a major field of research, mainly in cancer [37]. Angiogenesis is essential for a tumor to pro-
vide the tumor cells with oxygen and essential nutrients for growth and to metastasize hematogenically [38]. A growing tumor activates surrounding vessels by secreting angiogenic factors, thereby changing the dormant tumor phenotype toward an angiogenic one, the so-called “angiogenic switch” [39]. Activated endothelial cells have to migrate toward the tumor along a newly formed matrix, the components of which are synthesized by themselves, tumor cells, and other cells such as macrophages and fibroblasts [40]. Figure 2 shows schematically the process of tumor angiogenesis, which can be divided into four different stages. A small, dormant tumor (stage 1) can, depending on the nature of the tumor and its microenvironment, make the angiogenic switch to ensure exponential growth. The tumor secretes growth factors to activate endothelial cells of surrounding vessels (stage 2). Upon activation, these endothelial cells start to migrate and proliferate toward the tumor. Only one endothelial cell starts an angiogenic sprout and develops into an endothelial tip cell migrating along the extracellular matrix (ECM) and guiding the following so-called stalk endothelial cells (stage 3) [41]. Finally, the growing tumor is connected to the vasculature (stage 4). In addition to growth and proliferation, the tumor can metastasize. Malignant tumor cells, by invasion of the vessels, ECM degradation, attachment, and homing to target sites can form distal metastases [42]. The process of tumor angiogenesis results in a tumor-associated vasculature (TAV) that is rather chaotic, both in structure and function. In comparison with normal vessels, tumor vessels have a noncontinuous endothelium, an enlarged basal membrane, and an aberrant pericyte coverage [43]. Frequently in tumors, the vascular hierarchy of arterioles, capillaries, and venules is absent, resulting in loosely associated pericytes [44]. From animal experiments, it is known that pericytes are present in small tumors and more abundant in large tumors [45]. The contribution of pericytes to (anti)-angiogenic therapies is currently an attractive focus of research. On one hand, these characteristics impair tumor blood flow, delivery of oxygen, and therapeutics to the tumor cells and vessel functionality, but on the other hand, these differences may be used as a target. The solid tumors treated by ILP with TNF-α have a massive vascular structure consisting of vessels with a phenotype specific to tumor vessels, although detailed study needs to clarify the exact contribution of the TAV to the observed antitumor responses.

**Activity of TNF-α in Solid Tumors: Hypothetical Mechanism**

The vascular differences mentioned above are depicted in Figure 3A. These differences are responsible for a more leaky vasculature in the tumor, with average intraendothelial gaps of 400 nm, depending on the tumor type [46]. Blood cells such as lymphocytes and monocytes easily adhere and extravasate into the tumor. We speculate that the endothelial cells of the tumor vessels, compared with normal vessels, have an upregulation of TNFR-1 on their membranes, which may be dependent on TNFR-1–upregulating factors produced by vessel-surrounding cells like tumor cells and macrophages. This upregulation, along with the specific architecture of the

---

**Figure 2.** The sequential steps during tumor angiogenesis. The dormant tumor in stage 1 starts to secrete angiogenic growth factors (GF) after its “angiogenic switch”, which is accomplished by an imbalance in pro- and antiangiogenic factors. These GFs activate endothelial cells of surrounding vessels, and these cells start to migrate (stage 2) and proliferate toward the tumor. An endothelial tip cell (TC) is guiding this sprouting process (stage 3). In stage 4, the novel sprout has formed a lumen and the tumor is connected to the vasculature, thereby ensuring its growth and enabling it to metastasize hematogenically. Abbreviation: ECM, extracellular matrix.
endothelial lining, defines the tumor vessels as a specific target for TNF-α treatment (Fig. 3B). When TNF-α is administered via ILP to treat solid tumors, it binds soluble receptors, and because of the high dosage, TNFR-1 receptors on tumor endothelial cells become occupied. Healthy endothelium, in contrast, also binds TNF-α, however, because of a lower number of membrane-bound TNF-1 receptors (most TNF-1 is stored in the golgi apparatus [47]), there is no toxicity. We propose that this TNF-α to TNF-1 binding results in hyperpermeability of the tumor vessels, and erythrocytes and other blood cells extravasate. The strong extravasation of erythrocytes results in massive hemorrhagic necrosis of the tumor. As a result of the direct cytotoxicity of high-dose TNF-α to endothelial cells, some of these cells undergo apoptosis, and this process strongly enhances the induced hyperpermeability (Fig. 3B). Several studies have shown that a lower dose of TNF-α results in comparable responses [48, 49], suggesting that a lower dose still may induce these avascular effects. The healthy vessels, however, stay intact; no apoptosis and no extravasation occurs. The observed synergistic activity of TNF-α and chemotherapeutic drugs is a consequence of this double-induced hyperpermeability. This hyperpermeability throughout the tumor facilitates the augmented accumulation and distribution of the drug in the tumor, resulting in better exposure of the tumor cells to the cytostatic agent [30]. This double-induced hyperpermeability, along with the dual targeting—the TA V (by TNF-α) and the tumor cells (by the chemotherapy drug)—is one explanation for the observed synergistic response of tumors to TNF-α and chemotherapy that results in high response rates in patients.
CLINICAL EFFICACY OF TNF-α–BASED ISOLATED LIMB PERFUSION

The use of TNF-α in the ILP setting was pioneered by Lienard et al. [50]. In 19 melanoma patients and a few STS cases, impressive and very rapid responses were observed. This observation was followed by multicenter trials in patients with locally advanced STS and melanoma. In Table 1, we present an overview of the multicenter trials in Europe that led to the approval of TNF-α by the EMEA in 1998 for its application in ILP for the treatment of high-grade (2–3) STS. In these multicenter trials, an overall response rate of 76% and a median limb salvage rate of 82% were

Table 1. Principle studies on tumor necrosis factor (TNF)–based isolated limb perfusion (ILP) for irresectable soft tissue sarcomas

<table>
<thead>
<tr>
<th>ILP Studies</th>
<th>Pts</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>NC/PD (%)</th>
<th>Limb Salvage (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pivotal Multicenter Studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF + IFN + M</td>
<td>20</td>
<td>55 a</td>
<td>40 a</td>
<td>5 a</td>
<td>90</td>
<td>Eggermont et al. [51]</td>
</tr>
<tr>
<td>TNF + IFN + M</td>
<td>59</td>
<td>18 a</td>
<td>64 a</td>
<td>18 a</td>
<td>84</td>
<td>Eggermont et al. [52]</td>
</tr>
<tr>
<td></td>
<td>36 b</td>
<td>51 b</td>
<td>13 b</td>
<td></td>
<td></td>
<td>Eggermont et al. [53]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>195</td>
<td>18 a</td>
<td>57 a</td>
<td>25 a</td>
<td>82</td>
<td>Eggermont et al. [54]</td>
</tr>
<tr>
<td></td>
<td>29 b</td>
<td>53 b</td>
<td>18 b</td>
<td></td>
<td></td>
<td>Eggermont et al. [55]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>270</td>
<td>28 a</td>
<td>48 a</td>
<td>24 c</td>
<td>76</td>
<td>Eggermont et al. [56]</td>
</tr>
<tr>
<td></td>
<td>196</td>
<td>17 c</td>
<td>48 b</td>
<td>35 b</td>
<td>71 d</td>
<td>Eggermont et al. [57]</td>
</tr>
<tr>
<td><strong>Single-center studies (&gt;20 pts)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>35</td>
<td>37 b</td>
<td>54 b</td>
<td>9 b</td>
<td>85</td>
<td>Gutman et al. [58]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>34</td>
<td>35 b</td>
<td>59 b</td>
<td>6 b</td>
<td>85</td>
<td>Olieman et al. [59]</td>
</tr>
<tr>
<td>TNF + Dox</td>
<td>20</td>
<td>26 c</td>
<td>64 c</td>
<td>10 c</td>
<td>85</td>
<td>Rossi et al. [60]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>22</td>
<td>18 b</td>
<td>64 b</td>
<td>18 b</td>
<td>77</td>
<td>Lejeune et al. [61]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>55</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>84</td>
<td>Hohenberger et al. [62]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>49</td>
<td>5 b</td>
<td>55 b</td>
<td>37 b</td>
<td>58</td>
<td>Noorda et al. [63]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>29</td>
<td>38 b</td>
<td>38 b</td>
<td>18 b</td>
<td>76</td>
<td>van Etten et al. [64]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>64</td>
<td>42 b</td>
<td>45 b</td>
<td>13 b</td>
<td>82</td>
<td>Grünhagen et al. [65]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>29</td>
<td>20 b</td>
<td>50 b</td>
<td>30 b</td>
<td>65</td>
<td>Lans et al. [66]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>37</td>
<td>16 b</td>
<td>68 b</td>
<td>16 b</td>
<td>97</td>
<td>Grünhagen et al. [67]</td>
</tr>
<tr>
<td>TNF + Dox g</td>
<td>21</td>
<td>5 a</td>
<td>57 a</td>
<td>38 a</td>
<td>71</td>
<td>Rossi et al. [68]</td>
</tr>
<tr>
<td></td>
<td>55 b</td>
<td>35 b</td>
<td>10 b</td>
<td></td>
<td></td>
<td>Grünhagen et al. [69]</td>
</tr>
<tr>
<td>TNF ± IFN + M</td>
<td>217</td>
<td>18 a</td>
<td>51 a</td>
<td>31 a</td>
<td>87</td>
<td>Grünhagen et al. [70]</td>
</tr>
<tr>
<td></td>
<td>26 b</td>
<td>49 b</td>
<td>25 b</td>
<td></td>
<td></td>
<td>Grünhagen et al. [71]</td>
</tr>
<tr>
<td>TNF + M g</td>
<td>100</td>
<td>49 e</td>
<td>17 f</td>
<td>34 f</td>
<td>87</td>
<td>Bonvalot et al. [72]</td>
</tr>
<tr>
<td></td>
<td>35 f</td>
<td>22 f</td>
<td>43 f</td>
<td></td>
<td></td>
<td>Grünhagen et al. [73]</td>
</tr>
<tr>
<td>TNF ± IFN + M g</td>
<td>240</td>
<td>24 b</td>
<td>50 b</td>
<td>26 b</td>
<td>82</td>
<td>Grünhagen et al. [74]</td>
</tr>
</tbody>
</table>

a Objective clinical response rate by World Health Organization criteria.  
b CR, clinical CR or 100% necrosis; PR, clinical PR or >50%–90% necrosis.  
c CR only recognized by the European Medicines Agency when histopathology showed 100% necrosis.  
d Independent committee recognized 196 patients as pure amputation candidates.  
ε No clinical response data; CR >90%; PR, radiological and/or histopathological >50% necrosis.  
ζ CR/PR, loss of vasculature on ultrasound MRI; lower panel CR, >90% necrosis on histopathology.  
η Low-dose TNF of 1 mg or various doses of TNF-α (0.5–4 mg); Grünhagen et al. (1–4 mg), soft tissue sarcomas patients.  
υ Patients with metastatic disease.  
Ω Patients with multiple tumors in extremity.  
ρ Patients >75 years old.  
Ω Patients with recurrent sarcomas in 60–70 Gray irradiated fields.  
Abbreviations: CR, complete remission; IFN, interferon gamma; M, melphalan; NC, no change; PD, progressive disease; PR, partial remission; Pts, patients.
observed. Moreover, this table lists the largest single-center studies in STS that confirm the results of the multicenter experience [49, 51–67]. We observe strikingly consistent major response rates, with a median of 76% (range, 58%–91%), and with a median limb salvage rate of 84% (range, 58%–97%). TNF-α–based ILP now is performed in 35 cancer centers in Europe with national referral patterns for limb salvage. ILP with melphalan alone for melanoma in-transit metastases is reported in the literature to result in about a 50% complete response (CR) rate and an 80% overall response rate [68]. The introduction of TNF-α in this setting was reported to increase CR rates to 70%–90% and overall response rates to 95%–100%. These results are summarized in Table 2 [50, 69–78]. Early on, however, it was observed that ILP with TNF-α plus melphalan (TM-ILP) was especially effective against bulky tumors such as STSs, in which ILP with melphalan alone [79] or doxorubicin alone [79, 80] fails. It should also be noted, of course, that both drugs have no activity against melanoma in the systemic setting and that melphalan has no activity against STS in the systemic setting. TNF-α–based ILP with melphalan or doxorubicin results in similar tumor response rates, but because of less locoregional toxicity, melphalan is preferred over doxorubicin in the ILP setting [65–67]. In our own series of 50 ILPs in patients with bulky melanoma in-transit metastases, the CR rate was still 58% [73], identical to the CR rate that was seen in an interim analysis of a randomized trial by Fraker et al. [74], in which TNF-α–based ILP was shown to be of significant benefit in patients with a high tumor load, increasing the CR rate from 19% for M-ILP to 58% for TM-ILP. Apart from bulky melanoma, a further indication for TNF-α–based ILP is response failure to a prior ILP because excellent response rates have been reported in this situation [76, 77]. Similarly, high response rates have been reported for TNF-α–based ILP for nonmelanoma locally advanced skin cancers [78]. Because TNF-α acts primarily on the tumor vasculature, these observations make sense, and the propensity to respond to a TNF-α–based ILP is assumed to depend more on tumor vasculature than on the histologic type of the tumor.

Response of STS to TNF-α–based ILP is shown in Figure 4A. Magnetic resonance imaging of a patient with high-grade (6–7) leiomyosarcoma in the upper leg shows clear dark tumor masses with high gadolinium uptake before ILP. Five weeks after ILP, all tumor masses are gadolinium-negative. Along the distal femur, only small tumor remnants are visible, but at the proximal femur, a large but gadolinium-negative tumor mass without signs of regression is visible. All lesions were resected and found to be 100% necrotic. Thus the response was classified as a histopathologic CR.

Targeting by TNF-α of the tumor vasculature is revealed in patients by angiographies before and after ILP. The TAV is selectively destroyed by TNF-α–based ILP; the TAV is gone while normal vessels of the limbs are still intact after ILP (Fig. 4B). TNF-α targets the vasculature

### Table 2. Tumor necrosis factor (TNF)–based isolated limb perfusion (ILP) in melanoma and nonmelanoma skin cancer patients.

<table>
<thead>
<tr>
<th>ILP</th>
<th>Pts (n)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>Overall RR</th>
<th>TTLP Median (Mo)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>All melanoma patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>89</td>
<td>11</td>
<td>100^</td>
<td>8^</td>
<td>18</td>
<td>Lienard et al. [50]</td>
</tr>
<tr>
<td>44</td>
<td>90</td>
<td>10</td>
<td>100^</td>
<td>18^</td>
<td>26</td>
<td>Lejeune et al. [69]</td>
</tr>
<tr>
<td>58</td>
<td>88</td>
<td>12</td>
<td>100^</td>
<td>26</td>
<td></td>
<td>Eggermont et al. [70]</td>
</tr>
<tr>
<td>26</td>
<td>76</td>
<td>16</td>
<td>92^</td>
<td>ns</td>
<td></td>
<td>Fraker et al. [71]</td>
</tr>
<tr>
<td>32</td>
<td>78</td>
<td>22</td>
<td>100^</td>
<td>14</td>
<td></td>
<td>Lienard et al. [72]</td>
</tr>
<tr>
<td>32</td>
<td>69</td>
<td>22</td>
<td>91</td>
<td>ns</td>
<td>14</td>
<td>Lienard et al. [72]</td>
</tr>
<tr>
<td>100</td>
<td>69</td>
<td>26</td>
<td>95</td>
<td>16^</td>
<td></td>
<td>Grünhagen et al. [73]</td>
</tr>
<tr>
<td>Bulky melanoma only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>59</td>
<td>16</td>
<td>75</td>
<td>ns</td>
<td></td>
<td>Fraker et al. [74]</td>
</tr>
<tr>
<td>20</td>
<td>70</td>
<td>25</td>
<td>95</td>
<td>ns</td>
<td></td>
<td>Rossi et al. [75]</td>
</tr>
<tr>
<td>50</td>
<td>58</td>
<td>34</td>
<td>83</td>
<td>8</td>
<td></td>
<td>Grünhagen et al. [73]</td>
</tr>
<tr>
<td>Repeat ILP for melanoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>65</td>
<td>29</td>
<td>94</td>
<td>6</td>
<td></td>
<td>Bartlett et al. [76]</td>
</tr>
<tr>
<td>26</td>
<td>75</td>
<td>25</td>
<td>100</td>
<td>14</td>
<td></td>
<td>Grünhagen et al. [77]</td>
</tr>
<tr>
<td>Nonmelanoma skin cancers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>60</td>
<td>27</td>
<td>87</td>
<td>20^</td>
<td></td>
<td>Olieman et al. [78]</td>
</tr>
</tbody>
</table>

^TNF + IFN
Abbreviations: CR, complete remission; Overall RR, overall (CR + PR) response rate; PR, partial remission rate; Pts, patients; TTLP, time to local progression; ns, not specified.
Figure 4. Antitumor and antivascular effects of tumor necrosis factor alpha (TNF-α) upon isolated limb perfusion (ILP) treatment of sarcoma and melanoma patients. (A): Magnetic resonance imaging of a patient with a high-grade leiomyosarcoma in the upper leg showing the tumor mass before treatment on the left. Five weeks after ILP with TNF-α and melphalan, there is no gadolinium uptake in the tumor remnants (right). Tumor remnants were resected, and all were necrotic. (B): Angiographies of two patients with rapidly growing sarcomas in the leg before (left) and after (right) ILP with TNF-α and melphalan. Angiographies clearly show the well-developed tumor vasculature before ILP, which is selectively destroyed after treatment while the normal vessels are still present and intact. Both patients were classified as complete responders. (C): Endothelial lining of tumor vessels is destroyed. Endothelial staining (CD31) of vessels in normal skin (left) and melanoma (right) in biopsies of a melanoma patient taken after ILP. Vessels in the normal skin show a continuous endothelial lining (arrow), while in the melanoma-associated vessel, this lining is disrupted and the endothelial cells detach from the basement membrane (arrowheads). The elastic fibers (stars, stained aspecific) in the thickened basement membrane are also visible in the melanoma vessel. Scale bar, 50 µm.

of tumors with completely different histologies, but as the TAV is well developed in all these tumors, combination therapy in the ILP setting is very effective for the specific tumors treated. Synergistic and high response rates are achieved in sarcomas consisting of a broad range of subtypes, as well as in melanomas. At the histopathological level, massive hemorrhagic necrosis is observed inside melanomas treated with ILP [81], an effect likely caused by TNF-α–induced coagulation and extravasation of erythrocytes [82]. In accordance with the angiographies of STS, the vascular lining of melanoma tumor vessels is destroyed (Fig. 4C). Staining for endothelial cells reveals that, upon treatment in skin vessels, the endothelium is intact and continuous while this lining is heavily disrupted and the cells detach from the underlying basement membrane in melanoma-associated vessels. These antivasual TNF-α effects are achieved by the high concentration reached during ILP. At these high levels, TNF-α activity is antivascular and antiangiogenic, while at lower concentration TNF-α is known to promote angiogenesis [83]. In addition to direct TAV-mediated effects, TNF-α reduces blood flow in tumors in a dose-dependent fashion [84]. This set of antivascular TNF-α effects was recently confirmed by experiments revealing that tumor response to TNF-α correlates with the degree of tumor vascularity [85]. Along with this dual role in angiogenesis, TNF-α is also known for its dual role in cancer treatment, anti-TNF-α therapy is also used for several types of cancer. The neoplastic and tumor-promoting effects of TNF-α are discussed in a recent review [86].

**Approaches to Modulate TNF-α Action in Cancer Treatment**

High response rates in the ILP setting do not avoid the need to search for factors that modulate the TNF-α effect in solid tumors. In addition to the possible application of TNF-α in other settings (e.g., systemic treatment) and for other tumors types, nonresponding patients in the ILP setting may also benefit from TNF-α sensitizers. Some of these approaches are mentioned below. An obvious target is inhibition of the NF-κB survival pathway. Inactivation of NF-κB is known to sensitize several tumors to TNF-α [87]. NF-κB can be blocked in several ways: overexpression of its inhibitor IκB and selective NF-κB inhibitors have been shown to increase TNF-α–induced apoptosis of tumor cells [88, 89]. One such inhibitor, bortezomib, has entered the clinical arena as a combination therapy with chemotoxic drugs for prostate cancer and myeloma [90].

Nitric oxide (NO) is involved in survival of TNF-α–treated cells through NF-κB–induced expression of inducible NO synthase (iNOS) [91, 92]. We have previously shown that inhibition of NOS by the addition of L-NAME (N-nitro-L-arginine methyl ester) during TNF-α–based ILP resulted in an increased tumor response in rats bearing STSs [93]. These observations were confirmed by a recent study showing that NOS inhibition in endothelial cells reduces their sensitivity to TNF-α in vitro, leading to the hypothesis that tumor vessels exhibit a higher level of NOS, which might explain their higher TNF-α sensitivity [94]. These studies justify further evaluation of NOS inhibition in tumors of patients treated by ILP to stimulate the anti-TAV activities of TNF-α.

Apoptosis induced by TNF-α is also associated with the generation of reactive oxygen species (ROS). It has been shown that the key survival factor NF-κB induces ROS-neutralizing enzymes like superoxide dismutase [95]. Induction of ROS production or an inhibition of the
NF-κB pathway by cyclooxygenase-2 (COX-2) inhibitors is reported to be successful in sensitizing tumor cells to TNF-α–induced apoptosis [96].

Procedures with liposomal encapsulation of TNF-α to elongate its circulation time and to achieve effective TNF-α concentrations in the tumor have been shown to be effective in systemic treatment in combination with chemotherapeutic drugs in rats [97].

Another experimental approach is the development of TNF-α analogues like TNF-α mutants that selectively bind to TNFR-1 [98] and mutants affecting the pharmacokinetics of TNF-α to TNFR-1 interactions at the tumor vascular level [99]. In animal models, these approaches have shown promising results versus wild-type TNF-α, but this research is still too experimental, and no TNF-α analogues have reached clinical studies yet.

A more physiological and therefore potent way involves the role of other cytokines that serve as TNF-α sensitizers. Interferon gamma (IFN-γ) is one of the widely studied cytokines, and although no beneficial effects and some toxicity were observed in the ILP setting for melanomas and sarcomas, for other cancers, IFN-γ might be very suitable because of its reported actions on TNFR-1 and caspase-8, thereby regulating TNF-α–induced apoptosis [100]. Endothelial monocyte-activating polypeptide II (EMAP-II) is a cytokine produced by tumor cells, so local production of this cytokine is suggested to facilitating TNF-α antitumor activity. EMAP-II can sensitize reportedly TNF-α–resistant tumors to TNF-α, which was shown by the upregulation of EMAP-II resulting in an increased TNF-α effect [101]. Underlying mechanisms for this effect remain unclear, although it is postulated that an upregulation of TNFR-1 on endothelial cells might be one explanation [102]. Besides TNF-α–modulating effects, EMAP-II exhibits other antitumor effects including antiangiogenic and immunosuppressive activities [103, 104]. Combining these cytokines with TNF-α and investigating the expression patterns and local protein production might be very promising in modulating TNF-α activities in cancer treatment.

Besides modulating TNF-α actions, other TAV-manipulating agents are being tested in ILP as well. Recently, we showed that both histamine (via its own vascular activity) and interleukin 2 (via upregulation of TNF-α production) have strong synergistic antitumor effects when combined with melphalan. Importantly, with both these agents, an augmented accumulation of melphalan in the tumor specifically was observed, confirming TAV as an effective target in solid tumor treatment [105, 106].

**Conclusions**

The use of high-dose TNF-α locally administered in combination with melphalan for patients with metastatic in-transit melanoma and STS confined to the limb is a well-established treatment modality nowadays. Furthermore, the modulation of tumor pathophysiology by low-dose TNF-α indicates that, in combination with liposomal drugs, systemic therapy should be investigated in the clinic. Besides a greater understanding of the molecular events of the TNFR-1 signaling that takes place during tumor treatment, these studies likely will expand the use of TNF-α for other cancer types and for nonresponding ILP patients, as well. The multifunctional properties of TNF-α may well result in a more varied application of this cytokine.

**Disclosure of Potential Conflicts of Interest**

Dr. Eggermont has acted as a consultant for Boehringer-Ingelheim, GmbH.


