Systemic use of tumor necrosis factor alpha as an anticancer agent

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ABSTRACT:

Tumor necrosis factor-α (TNF-α) has been discussed as a potential anticancer agent for many years, however initial enthusiasm about its clinical use as a systemic agent was curbed due to significant toxicities and lack of efficacy. Combination of TNF-α with chemotherapy in the setting of hyperthermic isolated limb perfusion (ILP) has provided new insights into a potential therapeutic role of this agent. The therapeutic benefit from TNF-α in ILP is thought to be not only due to its direct anti-proliferative effect, but also due to its ability to increase penetration of the chemotherapeutic agents into the tumor tissue. New concepts for the use of TNF-α as a facilitator rather than as a direct actor are currently being explored with the goal to exploit the ability of this agent to increase drug delivery and to simultaneously reduce systemic toxicity.

This review article provides a comprehensive overview on the published previous experience with systemic TNF-α. Data from 18 phase I and 10 phase II single agent as well as 18 combination therapy studies illustrate previously used treatment and dose schedules, response data as well as the most prominently observed adverse effects. Also discussed, based on recent preclinical data, is a potential future role of systemic TNF-α in combination with liposomal chemotherapy to facilitate increased drug uptake into tumors.

INTRODUCTION

TNF-α was discovered in 1975 and subsequently cloned in 1984 [1, 2] and has been the focus of considerable interest as an anticancer agent. Initial enthusiasm for TNF-α as a systemic therapeutic agent stemmed from the observation that it could induce hemorrhagic necrosis in the tumors of bacillus Calmette-Guérin (BCG)-primed and endotoxin treated mice [1]. Recombinant human TNF-α (rhTNF-α) has been tested as a systemic treatment in several phase I and phase II clinical trials. These trials, conducted in the 1980s and 1990s, used TNF-α as both a single agent and in combination with other cytokines or chemotherapeutics. However, the initial enthusiasm for the development of TNF-α as a systemic treatment has waned in the face of significant toxicities and a lack of evidence for therapeutic benefit. Nevertheless, these studies have provided valuable data regarding the toxicity profile and pharmacological properties of systemically delivered TNF-α. More encouraging is the current clinical use of combination TNF-α with chemotherapeutic agents in the setting of hyperthermic isolated limb perfusion in limb-threatening soft tissue sarcomas and in-transit melanoma [3]. Here we review the phase I and phase II clinical trials of systemic use of TNF-α, the toxicities and responses observed, and highlight recent scientific advances that hint at reduced systemic toxicities and augmentation of the antitumor responses seen with this agent. Specifically, the recently identified vascular effects of TNF-α that lead to a targeted intra-tumoral enrichment of liposomes and macromolecules through an enhanced-enhanced permeability and retention effect (E2PR) [4-6].

BIOLOGY OF TNF-α

TNF-α is a 23 kilodalton (kDa) type II transmembrane protein arranged in stable homotrimers. A 51 kDa soluble homotrimeric cytokine is derived from
the transmembrane form via proteolytic cleavage by the metalloprotease TNF-α converting enzyme (TACE) [7]. TNF-α is primarily produced by macrophages, but also by a variety of other cells, including NK cells, T lymphocytes, smooth muscle cells, fibroblasts and others [8]. Release of TNF-α occurs in response to inflammatory stimuli and cytokines including peptidoglycan, lipopolysaccharide and other bacterial components [9]. Two receptors exist for TNF-α: 1) Tumor necrosis factor receptor 1 (TNFR1), which preferentially binds soluble TNF-α and is found almost ubiquitously on the surface of cells, and 2) Tumor necrosis factor receptor 2 (TNFR2), which is found on cells of the hematopoietic lineage and which has specificity for the transmembrane form of TNF-α [10]. The resulting biological effect of TNF-α binding to its receptor is depending on the type of receptor activated and the cellular state during activation. Stimulation of TNFR1 activates downstream inflammatory mediators through AP1, MAPK and NF-kB pathways [11]. The balance of activation of these pathways by TNF-α is critical in determining whether a cell undergoes apoptosis as a late stage event in TNF-α stimulation. For instance, in acute myeloid leukemia (AML), NF-kB dependent induction of HO-1 underlies resistance to TNF-α induced apoptosis [12]. Similarly, inhibition of NF-kB with concurrent TNF-α stimulation results in caspase activation and apoptosis [13]. Conversely, the biological role and downstream effects of TNFR2 stimulation are more poorly understood. TNFR2 can be up-regulated by cytokine stimulation and also mediate a variety of downstream inflammatory mediators [14].

**PRECLINICAL EVIDENCE FOR TNF-α AS AN ANTICANCER AGENT**

After the initial observation that TNF-α induced hemorrhagic necrosis of tumors in mice treated with BCG and endotoxin [1], the potential of TNF-α as a therapeutic agent was intensely studied in in vitro and in vivo studies. These studies highlighted the possible role of TNF-α as an anticancer agent and galvanized support for the numerous phase I and phase II studies that followed.

In vitro studies demonstrated that TNF-α had a growth inhibitory effect on SV40-tranformed human mammary epithelia cells and a cytotoxic effect on breast cancer cell lines. Interestingly, there was no effect on normal human mammary epithelial cells [15]. Similarly, TNF-α showed a cytostatic effect on hepatoma cells while having little effect on non-tumorigenic liver cells [16]. Intriguingly, Sugarman and colleagues showed that the cytostatic and cytotoxic effects of TNF-α were cell line specific, with only a proportion of tumor cell lines responding to TNF-α [17]. Comparison of the cytostatic and cytotoxic effect of TNF-α against a wide range of tumor types demonstrated that approximately a quarter of tumors (28%) are sensitive to the effects of TNF-α and that this sensitivity was greater in colorectal and lung cancers [18]. In vivo, TNF-α has shown activity against a wide variety of murine tumor types and human tumor xenografts [19-21]. Taken together, the in vitro and in vivo data were suggestive that TNF-α had the potential to be highly specific anti-cancer therapy, with activity against a number of tumor types.

Preclinical evidence also suggested a synergistic effect of TNF-α with a variety of chemotherapeutics in vitro and in vivo. TNF-α has been shown to enhance the cytotoxicity of DNA topoisomerase inhibitors actinomycin D, adriamycin, and etoposide against murine bladder tumor cell line (MBT-2) in in vitro and in vivo models [22]. The enhancing effect of TNF-α was not observed with other cytotoxic agents, such as: bleomycin, hydroxyurea, cisplatin, mitomycin C, vincristine and vinblastine. The timing of TNF-α treatment in relation to chemotherapy seems important with studies suggesting that the optimal time for TNF-α therapy is 48 hours prior to initiation of chemotherapy [23]. Interestingly, there are likely two mechanisms that underlie the importance of timing in regards to TNF-α treatment. Firstly, inhibitors of transcription, such as actinomycin D and flavopiridol, are used before or at the time of TNF-α treatment and block NF-kB pathway activation, sensitizing cells to the effects of TNF-α [24]. Secondly, inhibitors of topoisomerase II can be given at the time of, or after TNF-α and increase the sensitivity of TNF-α resistant cancer cell lines to TNF-α [25].

TNF-α also demonstrated enhanced antitumor effects in vitro when used in combination with other cytokines [26]. Induction of TNF-α receptors rather than an increased affinity of already present receptors explained the effect of IFN-γ on TNF-α binding [26, 27]. Similarly, in vitro and in vivo studies using the TNF-α resistant melanoma cell line B16BL6 demonstrated that IFN-γ sensitizes cancer cells to the effects of TNF-α, inducing necrosis and tumor response, which were previously absent [28]. Later, investigators showed that TNF-α induced synergistic growth inhibition against pancreatic cancer cell lines when combined with interferon alpha (IFN-α) and IFN-γ [29]. TNF-α and IFN-γ act against many other cancer cell lines as well. Orita et al. [30] tested TNF-α and IFN-γ on 23 cell lines in vitro and demonstrated that the combination acts synergistically, showing cytostatic and cytotoxic effects on cell lines previously resistant to TNF-α and IFN-γ individually.

Combinations of TNF-α with IFN-α and IL-2 also showed synergistic cytotoxic and cytostatic effects in vitro and in vivo. Concomitant TNF-α and IFN-α in a murine lung metastasis model significantly increased survival [31]. TNF-α and IL-2 in murine models with leukemia, mastocytoma, melanoma, lymphoma and sarcoma cell lines also demonstrate combinatorial effects and systemic immunological memory [32, 33].

The combination of TNF-α and radiotherapy has been
| Study                  | Total number of patients | Tumor Type         | Dose TNF-α<sup>a</sup> | Schedule                          | ORR<sup>b</sup> | MTD      | Dose Limiting Toxocities                                      |
|-----------------------|--------------------------|--------------------|-------------------------|-----------------------------------|-----------------|----------|-------------------------------------------------------------|
| Chapman 1987 [35]     | 13                       | Advanced cancer    | 1 - 200 µg/m²/day       | Twice weekly alternating SQ/IV rhTNF-α every week for 4 weeks | 8%              | NR       | Hypotension. Local tissue reaction. Nausea. Vomiting. Neurotoxicity. |
| Creaven 1987 [36]     | 29                       | Advanced cancer - solid tumors | 1 x 10³ - 48 x 10³ units/m² | Three doses 3 weeks apart | 0%              | 48 x 10⁶ units/m³ | Hypotension. |
| Kimura 1987 [37]      | 33                       | Advanced cancer - solid tumors | 1 x 10⁰ - 16 x 10³ units/m² | One dose | 0%              | 5 x 10⁵ units/m² | Hypotension. Thrombocytopenia. Hepatotoxicity. |
| Creagan 1988 [38]     | 27                       | Advanced cancer - solid tumors | 5 - 200 µg/m²/day       | Daily for 5 consecutive days every 2-3 weeks | 4%              | 150 µg/m² | Hypotension. Rigors. Phlebitis. |
| Feinberg 1988 [39]    | 39                       | Metastatic cancer  | 5 - 250 µg/m²/day       | Daily for five consecutive days every two weeks for 8 weeks, 30 minute vs. 4 hour infusion | 0%              | 200 µg/m²/day | Hypotension. Nausea. Vomiting. Myalgias. Fatigue. |
| Sherman 1988 [40]     | 19                       | Advanced cancer - solid tumors | 0.5 x 10⁴ - 3.0 x 10³ units/m²/day | 5-day continuous infusion every 4 weeks | 0%              | 3.0 x 10⁶ units/m³/day | Thrombocytopenia. Leukopenia. |
| Spriggs 1988 [41]     | 50                       | Advanced cancer    | 4.5 - 645 µg/m²         | Continuous infusion over 24 hours every 3 weeks | 2%              | 636 µg/m² | Hypotension. |
| Taguchi 1988 [42]     | 53<sup>a</sup>           | Malignant tumors   | 0.1 x 10⁴ - 5 x 10³units/dose (IV); 0.1 x 10⁴ - 2 x 10³units/dose (IT) | One dose for week 1, then three times a week for week 2-7 | 5%              | 1 x 10³ units/dose | Hypotension. |
| Creaven 1989 [43]     | 33                       | Advanced cancer    | 5 - 80 x 10³ units/m²/day | Daily for 5 consecutive days every 2 weeks | 6%              | 60 x 10³ units/m²/day | Hypotension. Hepatotoxicity. |
| Jakubowski 1989 [44]  | 19                       | Advanced cancer    | 5 - 200 µg/m²/day (IM)  | Daily for 5 consecutive days every 2 weeks | 0%              | 150 µg/m²/day | Hypotension. Local injection site reaction. Leukopenia. Thrombocytopenia. Hepatotoxicity. Neurotoxicity. |
| Wiedenmann 1989 [45]  | 15                       | Advanced cancer - adenocarcinoma | 40 - 400 µg/m² | Continuous infusion over 24 hours once or twice weekly for 8 weeks | 0%              | 200 µg/m² | Thrombocytopenia. Fever. Chills. Fatigue. Myalgia. |
| Gam 1991 [46]         | 62                       | Advanced cancer    | 2.5 - 200 µg/m²         | Twice daily for 5 consecutive days every 2 weeks for 8 weeks | 6%              | 150 µg/m²/dose | Hypotension. Hepatotoxicity. |
| Krigel 1991 [47]      | 27                       | Advanced cancer - solid tumors | 8.5 - 1000 µg/m²         | 100% dose on day 1, then 20% of initial dose on day 8 - day 12 repeated every 2 weeks | 0%              | 267 µg/m² (initial dose) and 160 µg/m² (subsequent daily dosing) | Hypotension. Hemorrhagic gastritis. |
| Logan 1991 [48]       | 24                       | Advanced cancer - solid tumors | 40 - 240 µg/m²          | 100% dose on day 1, then daily dosing on day 8 - day 12 repeated every 3 weeks | NR              | NR       | NR                                                        |
| Schiller 1991 [49]    | 53                       | Advanced cancer    | 5 - 275 µg/m²           | Three times a week for 4 weeks | 2%              | 225 µg/m² | Hypotension. Fatigue. Nausea. |
| Mittelman 1992 [50]   | 19                       | Advanced cancer - solid tumors | 40 - 200 µg/m²         | 24-hour infusion on day 1 followed by 120-hour infusion day 8 - day 12 repeated every 3 weeks | 0%              | 160 µg/m² | Hematologic toxicity. Neurotoxicity. |
| Furman 1993 [51]      | 27                       | Pediatric advanced cancer    | 100 - 350 µg/m²/day     | Daily for 5 consecutive days every two weeks | 4%              | 300 µg/m²/day | Cardiotoxicity. Hypotension. Hepatotoxicity. |
| Braczkowski 1998 [52] | 21                       | Advanced cancer - solid tumors | 75 - 150 µg/day        | Daily for 5 consecutive days every two weeks | 48%             | N/A      | NR                                                        |

<sup>a</sup> All Studies used intravenous infusion for delivery of TNF-α, unless otherwise indicated.  
<sup>b</sup> Objective response rate calculated using number of patients evaluable for response where available.  
<sup>c</sup> Intravenous (43 patients) and intratumoral (16 patients). IV dose only.  
<sup>d</sup> TNF-α - tumor necrosis factor alpha. ORR - objective response rate. MTD - maximum tolerated dose. IV - intravenous. IM - intramuscular. SQ - subcutaneous. IT - intratumoral.
less extensively studied. Investigation of the interaction of TNF-α and radiation in 14 human tumor cells lines demonstrated synergistic or additive cytotoxicity with the maximum effect when TNF-α was given 4-12 hours before irradiation [34]. The mechanism of this synergism is thought to be due to the induction of oxygen free radical species and resulting DNA damage.

**CLINICAL TRIALS OF SYSTEMIC RECOMBINANT HUMAN TNF-α**

**Systemic rhTNF-α as a single agent**

Numerous phase I and phase II studies have been conducted to ascertain the toxicity profile and efficacy of systemic TNF-α. Studies have encompassed a wide range of tumor types in both adult and pediatric patients. In the majority of phase I and phase II studies, TNF-α was administered as an intravenous bolus injection or infusion. However, a few phase I studies have evaluated TNF-α with subcutaneous or intramuscular administration.

Phase I studies conducted with TNF-α are detailed in Table 1 [35-52]. Eighteen phase I studies were conducted and published with rhTNF-α as a single agent systemic therapy, enrolling between 19 and 62 patients per study. Study design varied with single dose of rhTNF-α, multiple dosing (daily to every three weeks) and continuous infusion (one to five day duration) being tested. Overall, it appears that a systemic TNF-α dose of 150-200 µg/m², given as a 30 minute intravenous infusion was identified as MTD in several studies. Dose-limiting toxicities (DLT) as well as other side effects that were observed seemed to have been universal and in most cases reasonably well tolerated and reversible. Common DLTs included: hypotension, thrombocytopenia, leukopenia, neurotoxicity, fever, nausea/vomiting, as well as general symptoms of malaise and weakness (Table 2). Other pathological sequelae of a transient hypovolemic episode, including transient elevation of liver enzymes, were reported. Tumor responses however, when used as a single agent, even with more intense treatment schedules, were rare.

**Phase II studies using systemically administered rhTNF-α are detailed in Table 3 [53-62].** Studies typically investigated advanced/metastatic cases of: colorectal cancer, breast cancer, pancreatic cancer, malignant melanoma and renal cell carcinoma. The majority of studies involved a small number of cases (16-26), an exception being a phase II study of various malignancies that enrolled 147 patients [59]. Study design varied, with 150-200 µg/m² given as a 30 minute intravenous infusion daily for 3-5 days and repeated every 1-4 weeks being commonly employed. In all studies, tumor responses were rare and when they did occur, only partial responses were observed. In the largest study of 147 cancer patients treated with 150µg/m² for 5 days every other week, only 1 partial remission was noted while 13% of patients experienced a grade 4 or greater toxicity. The most serious toxicities included respiratory failure and coagulopathies. Other, less serious and more common side effects reported include: hypotension (31%), leukopenia (38%), thrombocytopenia (13%), fever / chills (69%), headache (25%), nausea / vomiting (69%) and hepatopathy (10%). However, compared to other phase II studies this regimen was fairly dose-dense which may have increased the significant toxicity observed.

**Systemic rhTNF-α in combination with chemotherapeutics**

Phase I and II studies that investigated the safety

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**Table 2: Side effects of single agent rhTNF-α**

| Side Effect               | Frequency  |
|---------------------------|------------|
| **Very Common**           |            |
| Hypotension               |            |
| Hepatotoxicity            |            |
| **Common**                |            |
| Nausea                    |            |
| Neurotoxicity             |            |
| Vomiting                  |            |
| Chills                    |            |
| Fatigue                   |            |
| Fever                     |            |
| Leukopenia                |            |
| Rigors                    |            |
| Thrombocytopenia          |            |
| Cardiotoxicity            |            |
| Gastrointestinal toxicity |            |
| Myalgia                   |            |
| Anemia                    |            |
| Dyspnea                   |            |
| Hematologic toxicity      |            |
| Local tissue reaction     |            |
| Pain                      |            |
| Pulmonary toxicity        |            |
| Anorexia                  |            |
| Arthropy                  |            |
| Coagulopathy              |            |
| Constituitive symptoms    |            |
| Diarrhea                  |            |
| Fever                     |            |
| Hematuria                 |            |
| Hemorrhagic gastritis     |            |
| Hyperglycemia             |            |
| Hypertension              |            |
| Intracranial hemorrhage   |            |
| Lethargy                  |            |
| Leukocytosis              |            |
| Lymphopenia               |            |
| Neuropathy                |            |
| Phlebitis                 |            |
| Renal toxicity            |            |
| Tachycardia               |            |
| Vascular thrombosis       |            |

Side effects to systemic rhTNF-α monotherapy observed as a dose-limiting toxicity or ≥ grade 3 toxicity in a phase I or phase II study. Very common side effect seen in > 10 studies. Common side effect seen in between 2 and 10 studies. Uncommon side effect seen in 1 study.
| Study             | Total number of patients | Tumor Type                      | Dose TNF-α | Schedule                              | Maximum Number of Cycles | ORRb | Major Reported Toxicitiesc |
|-------------------|--------------------------|---------------------------------|------------|---------------------------------------|--------------------------|------|----------------------------|
| Lenk 1989 [53]    | 22                       | Advanced cancer - solid tumors  | 683 - 956 μg/m² | Weekly                               | 6                        | NR   | Hypotension. Leukocytosis. Hepatotoxicity. Nausea. Vomiting. |
| Heim 1990 [54]    | 15                       | Advanced colorectal cancer      | 3 x 10⁵ U/m²/day | Daily for days 1-3 every 2 weeks     | 4                        | 9%   | Dyspnea. Fever. Leucopenia. |
| Kemeny 1990 [55]  | 16                       | Advanced colorectal cancer      | 100-150 μg/m²/day | 100 μg/m²/day BID on day one, 100 μg/m²/day BID on days 2-5. Repeat every other week | 4                        | NR   | Gastrointestinal toxicity. Neurotoxicity. Chills. Pain. Hypotension. Hypertension. Leukopenia. Hepatotoxicity. Vascular thrombosis. |
| Whitehead 1990 [56]| 25                       | Metastatic colorectal cancer    | 150 μg/m²/day | Daily for 5 days every 2 weeks       | 4                        | 0%   | Chills. Nausea. Vomiting. Anemia. Hepatotoxicity. |
| Brown 1991 [57]   | 26                       | Pancreatic adenocarcinoma       | 150 μg/m²/day | Daily for 5 days every 2 weeks       | 7                        | NR   | Hypotension. Hyperglycemia. Anemia. Dyspnea. Hepatotoxicity. Coagulopathy. Tachycardia. |
| Budd 1991 [58]    | 22                       | Metastatic breast cancer        | 150 μg/m²/day | Daily for 5 days every 2 weeks       | 4                        | 0%   | Hypotension. Diarrhea. Leukopenia. Hepatotoxicity. Intracranial hemorrhage. |
| Hersh 1991 [59]   | 147                      | Metastatic malignancies         | 150 μg/m²/day | Daily for 5 days every 2 weeks       | 4                        | 1%   | Hematological toxicity. Gastrointestinal toxicity. Renal toxicity. Hepatotoxicity. Cardiovascular toxicity. Chills/fever. Lethargy. Neurotoxicity. Pulmonary toxicity. Fever. Chills. Nausea. Vomiting. Hypotension. Hepatotoxicity. Constitutive symptoms. |
| Feldman 1992 [60]| 21                       | Malignant melanoma              | 150 μg/m²/day | Daily for 5 days every 2 weeks. For 4 cycles, then every three weeks | 4+                       | 5%   | Cardiovascular toxicity. Hypertension. Nausea. Vomiting. |
| Skillings 1992 [61]| 26                       | Metastatic renal cell carcinoma | 150 μg/m²/day | Daily for 5 days every other week for 4 weeks | 11                       | 9%   | Cardiovascular toxicity. Hypertension. Nausea. Vomiting. |
| Muc-Wierzgon 1996 [62]| 16                     | Advanced gastrointestinal cancers| 150 μg/m²/day | Daily for 5 days every 2 weeks       | 6                        | NR   | Fever. Rigor. Hypotension. Fatigue. Neuropathy. Myalgia. Arthopathy. Lymphopenia. |

*a* All Studies used intravenous infusion for delivery of TNF-α, unless otherwise indicated. *b* Objective response rate calculated using number of patients evaluable for response where available. *c* Grade 3 or greater toxicities. TNF-α - tumor necrosis factor alpha. ORR - objective response rate. NR - not reported in study.
Table 4: Studies of systemic TNF-α with chemotherapy

| Study | Total Number of Patients | Tumor Type | Study Design | Chemotherapy | Regimen | Dose of Chemotherapy/TNF-α | Maximum Number of Cycles | ORR | MTD | Major Reported Toxicities |
|-------|--------------------------|------------|-------------|--------------|---------|----------------------------|-------------------------|-----|-----|--------------------------|
| Jones 1992 [3] | 41 | Advanced melanoma | Phase II | BCNU | Daily for 5 days every 48 days | BCNU - 10.5% | 0% | 2 | N/A | Hepatotoxicity, Leukopenia, Hematological toxicity, Rigor |
| Seibel 1994 [63] | 33 | Pediatric cancer | Phase I | Actinomycin D | Daily for 5 days every 3 weeks | 87% | 200-220 µg/m² day x 5 | N/A | 33% | Leukopenia, Thrombocytopenia, Stomatitis, Hypotension, Pulmonary toxicity, Leukopenia, Thrombocytopenia, Stomatitis, Hypotension, Pulmonary toxicity, Leukopenia, Thrombocytopenia, Stomatitis, Hypotension, Pulmonary toxicity |
| Sella 1995 [64] | 21 | Metastatic melanoma | Phase I | Actinomycin D | Maximum 6 doses every 2 weeks | 4 | 10 | N/A | Leukopenia, Thrombocytopenia, Stomatitis, Hypotension, Pulmonary toxicity, Leukopenia, Thrombocytopenia, Stomatitis, Hypotension, Pulmonary toxicity |
| Yamamoto 2002 [65] | 10 | Recurrent malignant astrocytoma | Phase I | Carboplatin, Etoposide, TNF-α | Daily for 5 days every 4 weeks | 4 | 33% | Leukopenia, Thrombocytopenia, Stomatitis, Hypotension, Pulmonary toxicity, Leukopenia, Thrombocytopenia, Stomatitis, Hypotension, Pulmonary toxicity |
| Meany 2008 [66] | 21 | Recurrent or refractory Wilms tumor | Phase I | Dactinomycin, rTNF | Daily for 5 days every 3 weeks | 10 | 16% | 16% | Thrombocytopenia, Leukopenia, Thrombocytopenia, Leukopenia, Thrombocytopenia, Leukopenia, Thrombocytopenia, Leukopenia, Thrombocytopenia, Leukopenia |
| Gregorc 2009 [67] | 15 | Solid tumors | Phase I | Doxorubicin, NGR-hTNF | Every 3 weeks | 15 | 7% | N/A | Leukopenia, Thrombocytopenia, Leukopenia, Thrombocytopenia, Leukopenia, Thrombocytopenia, Leukopenia, Thrombocytopenia, Leukopenia, Thrombocytopenia, Leukopenia |

a All Studies used intravenous infusion for delivery of TNF-α. b Objective response rate calculated using number of patients evaluable for response who available. c Dose limiting toxicities for phase I studies and grade 3 or greater toxicities for phase II studies. TNF-α - tumor necrosis factor alpha. ORR - objective response rate. MTD - maximum tolerated dose. NR - not reported in study. N/A - not applicable for study design.
| Study          | Total Number of Patients | Tumor Type         | Study Design | Cytokine | Dose of Cytokine/TNF-α | Route | Bolus Infusion | Regimen | Cycles | ORR | MTD          | Major Reported Toxicities         |
|---------------|--------------------------|--------------------|--------------|----------|------------------------|-------|-----------------|---------|--------|-----|--------------|-------------------------------|
| Demetri 1989 [68] | 38 | Advanced cancer | Phase I | IFN-γ | IFN-γ (200 µg/m²/24hr); rhTNF-α (2-205 µg/m²/24hr) | IV   | Infusion | 24hr infusion of IFN-γ ; 24hr rhTNF-α infusion 12 hours after the start of IFN-γ | NR | 6% | 205 µg/m² of rhTNF-α | Hypotension. |
| Kurzrock 1989 [69] | 25 | Metastatic cancer | Phase I | IFN-γ | IFN-γ (5-75 µg/m²/24hr); rhTNF-α (5-75 µg/m²/24hr) | IM   | Bolus   | Daily for 5 days every 2 weeks | 2 | 0% | rTNF-α 75 µg and IFN-γ 50 µg | Dyspnea, Fatigue, Hyperthermia, Hypertensive encephalopathy - seizure, Thrombocytopenia. |
| Fiedler 1991 [70] | 16 | Colorectal cancer | Phase I/II | IFN-γ | TNF (50 µg/m² IV); IFN-γ (100 µg SC) | IV   | Infusion | Daily for 5 days every week | 4 | 0% | NA | Acute renal failure. Thrombocytopenia. |
| Smith 1991 [71] | 36 | Solid tumors | Phase I | IFN-γ | IFN-γ (10-100 µg/m²; rhTNF-α (10-100 µg/m²/24hr) | IM   | Bolus | IFN-γ followed 5 minutes later by rhTNF-α every other day | 0 | NR | 100 µg/m² of IFN-gamma plus 50 µg/m² of TNF-α | Fever. Thrombocytopenia. |
| Yang 1991 [72] | 16 | Non-small cell lung cancer | Phase I | IL-2 | IL-2 (6 x 10⁶ IU/m² IV); TNF-α (25-100 µg/m²/day IM) | IM | Infusion | Daily for 5 days every 3 weeks | 2 | 8% | 6 x 10⁶ IU/m² of L-2 plus TNF-α 50 µg/m²/day | Thrombocytopenia. |
| Schiller 1992 [73] | 24 | Advanced cancer | Phase I | IFN-γ | FN-γ (100 µg/day); TNF-α (25-100 µg/m²/day) | IV   | Infusion | Three times a week | 4 | NR | 50 µg/m² TNF-α and 100 µg/m² IFN-γ | Hypotension. |
| Krigel 1995 [74] | 15 | Metastatic cancer - solid tumors | Phase I | IL-2 | TNF-α (160 µg/m²); rIL-2 (6-18 x 10⁶ IU/m²/day IM) | IV | Infusion | TNF-alpha infusion for 5 days follow by rIL-2 for 5 or 7 days every 3-4 weeks | 2 | 14% | 160 µg/m² TNF-α and 18 x 10⁶ IU/m² TNF-α | Hypotension. Weight loss. Fatigue. |
| Schiller 1995 [75] | 8 | Non-small cell lung cancer | Plot Study | IL-2 | L-2 (6 x 10⁶ IU/m²/day IV); TNF-α (50 µg/m²/day IM) | IM | Bolus | IL-2 and TNF-α daily for 4 days every week for 3 wks | 2 | 0% | NA | Pulmonary toxicity. Cardiac toxicity. Renal toxicity. Neurotoxicity. |
| Schiller 1995 [75] | 7 | Non-small cell lung cancer | Phase I | IL-2 | L-2 (6 x 10⁶ IU/m²/day IV); TNF-α (50-150 µg/m²/day IM) | IM | Bolus | IL-2 and TNF-α daily for 5 days every 2 wks | 9 | 0% | < 6 x 10⁶ IU/m²/day of L-2 and 50 µg/m²/day of TNF-α | Pulmonary toxicity. Cardiac toxicity. Renal toxicity. Neurotoxicity. |
| Eskander 1997 [76] | 18 | Metastatic cancer | Phase I | IL-2 & IFN-α | FN-α (9 x 10⁶ IU/m²/24hr IM or SC); IL-2 (1-3 x 10⁶ IU/m²/24hr IV); TNF-α (40-120 µg/m² IV) | IV | Infusion | IFN-α w weekly on days 1, 3 and 5 for 3 wks. IL-2 weekly on days 1-5 for 3 wks. TNF-α on days 1-5 for w eek 1 | NR | 0% | 40-80 µg/m²/day as 2-hour infusion depending on regimen | Pulmonary toxicity. Cardiac toxicity. Gastrointestinal toxicity. Cytopenia. |

*Objective response rate calculated using number of patients evaluable for response where available. Dose limiting toxicities for phase I studies and grade 3 or greater toxicities for phase II studies. ORR - objective response rate. MTD - maximum tolerated dose. NR - not reported in study. N/A - not applicable for study design. IV - intravenous. IM - intramuscular. IFN-γ - interferon gamma. IL-2 - interleukin 2. IFN-α - interferon alpha. TNF-α - tumor necrosis factor alpha.*
and efficacy of systemic rhTNF-α combined with carmustine [3], actinomycin D [63, 64], carboplatin and etoposide [65], dactinomycin [66] and doxorubicin [67] have been reported and are detailed in Table 4. In all trials, intravenous rhTNF-α was given concurrently or sequentially to chemotherapeutics on multiple days and treatments being repeated for a number of cycles. Dose of intravenous rhTNF-α ranged from 88-200μg/m² and treatments being repeated for a number of cycles. Dose sequentially to chemotherapeutics on multiple days and trials, intravenous rhTNF-α was given concurrently or rhTNF-α to the treatment regimen improved outcome. chemotherapeutics have failed to prove that the addition of rhTNF-α. Together, trials of rhTNF-α combined with response to therapy was not conclusively due to the action in this study were previously treated with dactinomycin, in a 15.8% response rate [66]. However, while patients in this study were previously treated with dactinomycin, response to therapy was not conclusively due to the action of rhTNF-α. Together, trials of rhTNF-α combined with chemotherapeutics have failed to prove that the addition of rhTNF-α to the treatment regimen improved outcome.

**Systemic rhTNF-α in combination with cytokines**

Many studies have combined systemic administration of rhTNF-α with other cytokines such as: IFN-γ [68-72], IL-2 [73-76] and IFN-α [76], and these are summarized in Table 5. In general, phase I studies showed a reduction in the MTD of rhTNF-α when used in combination with other cytokines for patients with advanced solid tumors. This was largely due to the overlap in toxicities of these cytokines, that is: hypotension, fever, thrombocytopenia, acute renal failure, anemia, cardiac arrhythmias and pulmonary edema. Disappointingly, few objective responses were reported and none of the combinations were tested in larger randomized phase II studies; most likely because of the toxicity associated with combined therapy and a lack of efficacy seen in the initial studies.

**Systemic rhTNF-α in combination with radiotherapy**

Three studies that combined rhTNF-α with external beam radiation have also been reported [77-79] and they are detailed in Table 6. The most recent studies combined radiotherapy with both rhTNF-α and the chemotherapeutic ranimustine for the treatment of malignant astrocytoma [79]. In these studies DLTs were not observed and consequently, the maximum tolerated dose of this regimen was difficult to ascertain. In addition, no synergy in terms of objective response was noted.

**FUTURE DIRECTIONS OF SYSTEMIC TNF-α**

Translation of systemic TNF-α from research to clinic has been hampered by significant systemic toxicity and a lack of efficacy at MTD [43, 46]. Future directions for the development of TNF-α therapy rely on amelioration of the toxicity seen with systemic therapy and thereby increasing direct tumor response through higher TNF-α doses. Alternatively, the exploitation of novel mechanisms of action may increase efficacy and safety through indirect tumor effects.

Polyethylene glycol (PEG) conjugated proteins have shown increased retention and decreased immunogenicity in vivo [80]. Attempts to conjugate rhTNF-α with PEG has yielded a therapeutic with decreased toxicity and increased efficacy in murine preclinical models [81-84]. Thamm and colleagues conducted a phase I clinical trial of PEG-rhTNF-α in dogs with spontaneously occurring tumors [85]. Comparatively, Client-owned dogs provide an excellent model in which to develop novel anticancer agents. These dogs are genetically diverse, immunocompetent, share our environment and have similar types and size of tumors to people [86]. Interestingly, in this study the MTD of PEG-rhTNF-α

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### Table 6: Studies of systemic TNF-α with radiation +/- chemotherapy

| Study             | Total number of patients | Tumor Type                  | Chemotherapy | Study Design | Dose TNF-α | Regimen | Cycles | ORR | MTD | Major Reported Toxicities |
|-------------------|--------------------------|-----------------------------|--------------|--------------|------------|---------|--------|-----|----|--------------------------|
| Hallahan 1995     | 31                       | Advanced cancer             | NIA          | Phase I      | TNF-alpha 10-150 µg/m²; radiation: (150-300 µg/m²) total | TNF-alpha given 4 hours prior to radiotherapy | N/A | 40%  | 150 µg/m² | NR                         |
| Fukushima 1998    | 23                       | Malignant astrocytomas and glialblastomas | MCNU          | Pilot Study | TNF-SAM2 80 x 10⁶ U/m²; MCNU 100 mg/m² (IV); radiation (1.5Gy/day; 54-60Gy total) | 8 week cycle. MCNU day 1. TNF-SAM2 day 3. TNF-SAM2 given weekly for 5 doses | 4   | 12% | N/A | NR                         |
| Fukushima 2003    | 26                       | Malignant astrocytomas and glialblastomas | MCNU          | Pilot Study | TNF-SAM2 80 x 10⁶ U/m²; MCNU 100 mg/m² (IV); radiation (1.5Gy/day; 54-60Gy total) | 8-12 week cycle. MCNU day 1. TNF-SAM2 day 3. TNF-SAM2 given weekly for 5 doses | 4   | 17% | N/A | NR                         |

*All Studies used intravenous infusion for delivery of TNF-α. | Objective response rate calculated using number of patients evaluable for response where available. Grade 3 or greater toxicities. TNF-α - tumor necrosis factor alpha. ORR - objective response rate. NR - not reported in study. N/A - not applicable for study design. MCNU - ranimustine.
was found to be 26.7μg/kg (approximately 815μg/m²) and 4 of 15 dogs treated had a partial tumor response. DLT was similar to that observed with unconjugated TNF-α, with hypotension and coagulopathy being observed. This study suggests that PEG-rhTNF-α may limit some of the undesirable toxicity seen with unconjugated TNF-α and allow for greater antitumor responses.

Asparagine-glycine-arginine conjugated to the N-terminus of TNF-α (NGR-TNF-α) specifically binds the aminopeptidase N (CD13) of tumor vasculature [87]. CD13 is required for the pathological development of vasculature in the disease and presents an ideal target to modulate the effect of chemotherapeutics [88, 89]. Preclinical studies of NGR-TNF-α showed synergism with doxorubicin, cisplatin, plactitaxel and gemcitabine, increasing tumor penetration of cytotoxic compounds, anticancer efficacy and decreasing treatment associated toxicity [90]. Interestingly, increase in efficacy was seen in vivo but not in vitro with tumor cell lines, indicating that this synergism is due to an indirect effect of NGR-TNF-α on host vasculature [90]. A recent Phase Ib study of low-dose NGR-TNF-α with doxorubicin in advanced solid tumors demonstrated that this combination is well tolerated with no DLT observed [67]. A phase II dose of 0.8μg/m² of NGR-TNF-α and 75mg/m² of doxorubicin was recommended. The study provided hope for future development of TNF-α and doxorubicin combination therapy with 1 of 15 patients achieving a partial response and 10 of 15 patients with stable disease for a median duration of nearly 6 months.

An alternative concept for the use of TNF-α in the treatment of human cancers exists. Preclinical in vivo studies demonstrated that the uptake of radiolabeled liposomes in tumors was increased by approximately 6 fold in mice that were concomitantly treated with TNF-α [4]. The mechanism behind this enrichment is thought to be mediated through effects on the tumor vasculature and an enhanced-enhanced permeability and retention (EPR) effect. In vivo experiments using the combination of TNF-α and liposomal doxorubicin showed a significantly increased survival benefit in tumor-bearing mice treated with the combination in comparison to mice treated with either TNF-α or liposomal doxorubicin alone. Although single-agent liposomal doxorubicin alone delayed tumor growth and led to improved survival, the tumors eventually grew back, whereas the combination treatment with TNF-α and liposomal doxorubicin led to a long-term survival in 80% of the treated animals. These findings are in accordance with previously published data showing improved treatment outcomes in rat osteosarcoma and murine melanoma tumor models that were treated with low-dose TNF-α plus liposomal doxorubicin in comparison to TNF-α plus free doxorubicin [5, 91]. The development of low-dose TNF-α and liposomal doxorubicin may provide unique synergy to increase efficacy and decrease toxicity of combination therapy. Clinical studies are necessary to establish the safety and efficacy of this approach. These studies are worthwhile considering the novel mechanism of synergism between TNF-α and liposomal doxorubicin.

CONCLUSION

TNF-α has been proven an effective anticancer agent in vitro and in vivo preclinical studies. Sadly, the promise of systemic TNF-α has, as of yet, not translated to a patient therapy and enthusiasm has been curbed due to the toxicity profile and lack of efficacy at MTD. Combination with chemotherapy in the setting of hyperthermic isolated limb perfusion has proven quite successful, based not only on a direct anti-proliferative effect of TNF-α, but also due to its ability to increase drug penetration into tumor tissue. The future development of systemic TNF-α as an anticancer treatment will rely on exploring ways to reduced systemic toxicity and exploit novel mechanisms of action to deliver greater efficacy simultaneously with decreased toxicity. A number of avenues are currently being explored based on promising preclinical and early clinical data. The novel concept of using systemic TNF-α to facilitate increased tumor penetration of liposomal chemotherapy seems particularly promising and worth exploring clinically.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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