The immunocytokine L19-TNF eradicates Sarcomas in combination with Chemotherapy or with immune check-point inhibitors

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Abstract

Antibody-cytokine fusion proteins (also called “immunocytokines”) represent an emerging class of biopharmaceutical products, which are being considered for cancer immunotherapy. When used as single agents, pro-inflammatory immunocytokines are rarely capable of induce complete and durable cancer regression in mouse models and in patients. However, the combination treatment with conventional chemotherapy or with other immune-stimulatory agents typically increases the therapeutic efficacy of immunocytokines.

In this article, we describe combination treatments of a tumor-targeting antibody-cytokine fusion protein based on the L19 antibody (specific to a splice isoform of fibronectin) fused to murine tumor necrosis factor (TNF) with standard chemotherapy (dacarbazine, trabectedin or melphalan) or with an immune check-point inhibitor (anti-PD-1) in an immunocompetent murine model of sarcoma (WEHI-164).

All combination treatments led to improved tumor remission compared to monotherapy treatments, suggesting that these combination partners may be suitable for further clinical development in sarcoma patients.

Introduction

Sarcomas represent an heterogenous group of malignancies that can originate from soft tissue, cartilage, bone and other connective tissues. In particular, soft tissue sarcomas are tumors, which stem from non-epithelial extra skeletal tissue (e.g. muscle, fat, blood vessels and others). These cancer types are rare and they mainly affect young patients, however they represent 15% of all pediatric cancers [1–3].

For small and localized tumors (i.e. in the limbs) surgical resection (sometimes combined with radiation) represents a curative therapy, however postsurgical recurrence is observed in 50% of patients. This treatment option is often not applicable for patients with metastatic disease or with tumors with a different histology (e.g. head, neck or abdomen) and in these cases, chemotherapy is administered as first-line treatment [4]. Doxorubicin represents the standard-of-care for sarcomas with a response rate of 12-30% [5]. Doxorubicin may be combined with dacarbazine or other agents, however these combination treatments typically do not increase the survival benefit compared to the monotherapy [6]. Moreover, they are usually associated with substantial toxicities.

Already in the late 19th century, William B Coley observed a case of a sarcoma patient who experienced a spontaneous tumor regression as a consequence of an erysipelas infection. This finding was the basis for the development of the "Coley's toxin", a mixture of heat-inactivated bacteria, which was found to increase the overall survival of sarcoma patients, in spite of the development of high fever and chill [7,8]. It was later demonstrated that Coley's toxin treatment induced a dramatic increase of TNF levels [9].

TNF is a homo-trimeric pro-inflammatory cytokine, mainly produced by macrophages, NK and T cells [10,11]. TNF is involved in inflammatory processes and as indicated by its name, it may display a potent anticancer activity against some malignancies, by promoting hemorrhagic necrosis and by inducing direct apoptosis of tumor cells [12–17]. Moreover, TNF may activate the immune system as a result of the increased inflammation [18–20].
The systemic administration of recombinant human TNF (Beromun®) in the clinic is limited by substantial side effects and the maximal tolerated dose (MTD) was found to be 300µg/m^2 [21]. Beromun® obtained marketing authorization only for locoregional treatments (i.e., for isolated

limb perfusion procedure) in patients with soft-tissue sarcomas. The product is frequently administered in combination with melphalan [22].

The use of recombinant cytokines for cancer therapy may cause substantial toxicities already at low doses, therefore limiting their clinical use and preventing escalation to therapeutically active dose regimens [23–26]. To increase the therapeutic efficacy of cytokine payloads, the generation of fusion proteins with tumor-homing antibodies has been proposed. Antibody-cytokine fusion proteins (also termed "immunocytokines") are capable of increasing the therapeutic index of the corresponding cytokine payload, as results of a selective localization at the site of disease [24–28].

We have previously described the immunocytokine L19-mTNF, consisting of the fully human antibody fragment L19 (specific to the alternatively spliced EDB domain of fibronectin, a marker of angiogenesis) [29,30] fused to murine TNF. The fusion protein was able to induce tumor regression in different murine cancer types either alone or in combination with chemotherapy or other immunocytokines [31–35].

The fully human L19-TNF (featuring human TNF) has been investigated in cancer patients (phase I and II completed) either as systemic administration, intralesional injection or in isolated limb perfusion settings [36–38]. Moreover, L19-TNF is currently being investigated in Phase III clinical trials for the treatment of Stage III B,C melanoma (EudraCT number: 2015-002549-72 and NCT03567889) in combination with L19-IL2 and of metastatic soft-tissue sarcoma (EudraCT number: 2016-003239-38 and NCT03420014) in combination with doxorubicin.

Materials and Methods

Cell lines and reagents

CHO cells and WEHI-164 were obtained from the ATCC. Cell lines were received between 2017 and 2019, expanded, and stored as cryopreserved aliquots in liquid nitrogen. Cells were grown according the supplier's protocol and kept in culture for no longer than 14 passages. Authentication of the cell lines also including check of postfreeze viability, growth properties, and morphology, test for mycoplasma contamination, isoenzyme assay, and sterility test were performed by the cell bank before shipment.

The production and purification of L19-mTNF was performed as described before [31]. Commercially available dacarbazine (Dacin®, Lipomed AG), trabectedin (Yondelis®, PharmaMar AG), melphalan (Alkeran®, Aspen Pharma Schweiz GmbH) and the anti-PD-1 antibody (clone 29F.1A12, BioXCell, Catalog # BE0273) were purchased.

Therapy studies

Tumor cells were implanted subcutaneously in the flank of eight-week-old female BALB/c mice using 5 x 10^6 cells (WEHI-164). Mice were monitored daily and tumor volume was measured with a caliper (volume = length x $width^2$ x 0.5). When tumors reached a suitable volume (approx. 70-100 mm^3), pharmacological treatment was performed.

L19-mTNF was dissolved in phosphate buffered saline (PBS), also used as negative control, and administered at 0.1µg/g into the lateral tail vein three times every 48h.

Dacarbazine dissolved at 0.15 µg/g and Trabectedin dissolved at 0.15 µg/g were administered intraperitoneally once, four hours after the first L19-mTNF injection.

The anti-PD-1 antibody was administered at 10 µg/g into the lateral tail vein three times every 48h alone or in combination with L19-mTNF. Melphalan was administered once intravenously into the lateral vein once at 4.5 µg/g.

Experiments were performed under a project license (license number 04/2018) granted by the Veterinäramt des Kantons Zürich, Switzerland, in compliance with the Swiss Animal Protection Act (TSchG) and the Swiss Animal Protection Ordinance (TSchV).

Results and Discussion

In a first study, we compared the therapeutic activity of L19-mTNF, dacarbazine and trabectedin in immunocompetent BALB/c mice, bearing WEHI-164 sarcomas [**Figure 1**].
The treatment with saline and trabectedin alone, was not able to induce cancer regression. When used as single agents, L19-mTNF and trabectedin exhibited a mild tumor growth retardation compared to the saline group. Moreover, 25% of mice treated with L19-mTNF showed a complete response. By contrast, the combination therapy with these two agents led to an enhanced antitumor effect (75% of tumor eradication). Dacarbazine administered as a single agent showed the ability to cure 75% of the mice. This effect was further enhanced when administered in combination with L19-mTNF (100% complete response). [**Figure 1A, C**]. All treatments were well tolerated as evidenced by the comparison of body weight profiles.
A transient toxicity was observed upon the first pharmacological treatment with the two combination treatments (L19-mTNF with either dacarbazine or trabectedin). However, all treated mice recovered the body weight loss in few days [**Figure 1B**].

In a second experiment, we compared the therapeutic activity of L19-mTNF in combination with melphalan or with an immune check-point inhibitor (anti-PD-1) in the same murine sarcoma model (WEHI-164) [**Figure 2**]. The therapeutic activity of L19-mTNF was enhanced when used in combination with either melphalan or with an anti-PD-1 antibody, respectively, 60% and 80% of the treated mice showed a complete response [**Figure 2A, C**]. All the treatments were well tolerated as evidenced by the comparison of body weight profiles [**Figure 2B**].

Conclusions

In this work, we presented a pre-clinical evaluation of combination modalities for the treatment of sarcoma lesions. The immunocytokine L19-mTNF was able to synergize with chemotherapeutic agents, which are already being considered for the treatment of sarcoma patients. Moreover, L19-mTNF was able to potentiate the anti-cancer activity of an anti-PD-1 antibody. The fully-human analog of L19-mTNF (L19-TNF), which is currently being investigated in phase I clinical trials in combination with doxorubicin, may also be considered in combination with other chemotherapeutic agents or with immune check-point inhibitors for the treatment of sarcoma patients. This study may provide pre-clinical evidences for the upcoming phase I clinical trial of L19-TNF in pretreated Soft Tissue Sarcoma in combination with dacarbazine foreseen for 2020.

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Conflict of interest

Dario Neri is co-founder, shareholder and member of the board of Philogen, a company working on antibody therapeutics. The authors declare no additional conflict of interest.

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Figure 1

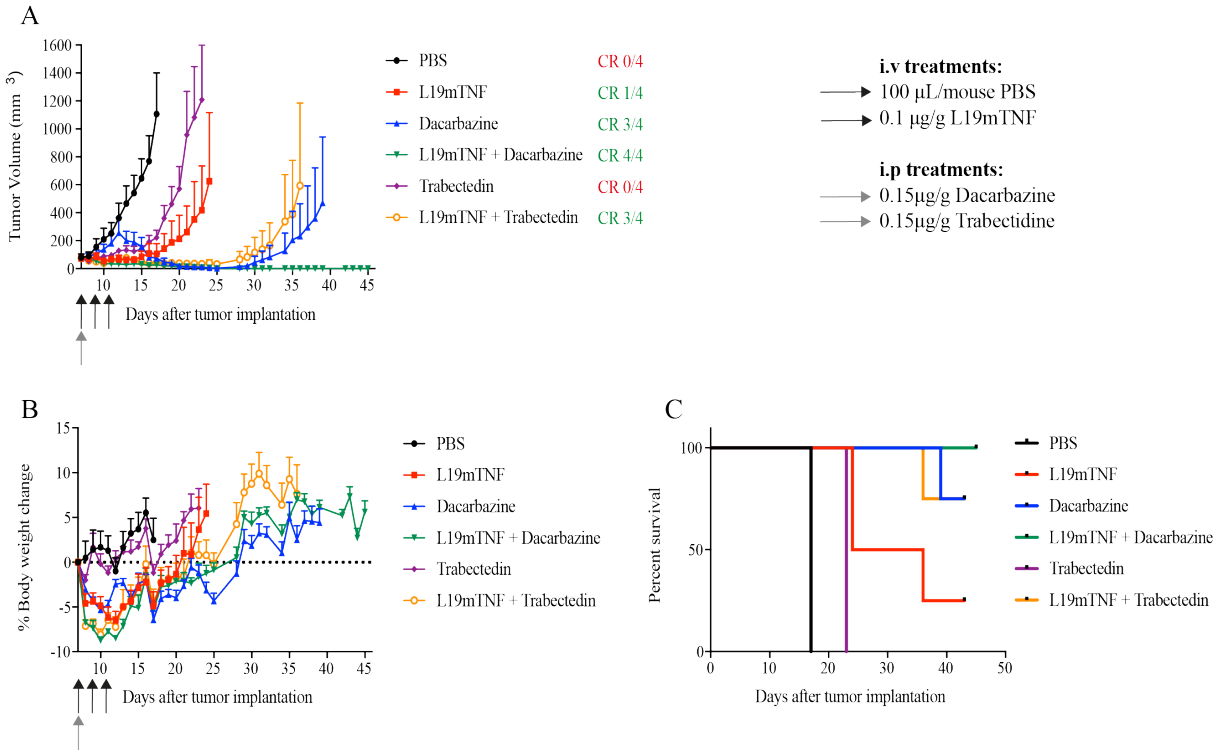

Figure 2

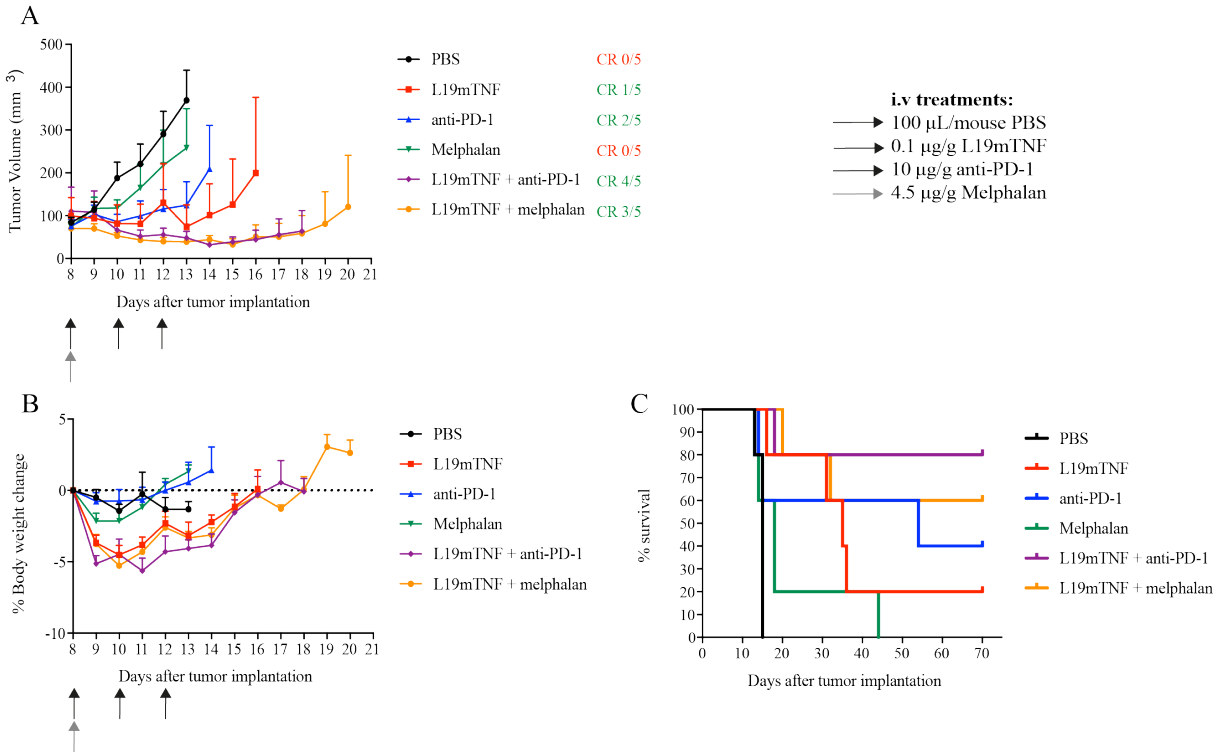

Figure captions

Figure 1 Therapeutic activity of L19-TNF in combination with chemotherapeutics (Dacarbazine and Trabectedin) in BALB/c mice bearing WEHI-164 tumors. A) Treatment started when tumors reached a volume between 70-100 mm^3, mice were injected three times intravenously (black arrows) every 48 hours with either PBS or L19-TNF. Dacarbazine and Trabectedin were administered intraperitoneally once (gray arrow), for hours after the first L19-TNF injection (combination group). Data represent mean tumor volume ± SEM, n = 4 mice per group, CR = complete response. B) Body weight monitoring. Weight change during the therapy of subcutaneous WEHI-164 tumors. Data represent mean % weight (± SEM), n = 4 mice per group. C) Survival curves.

Figure 2 Therapeutic activity of L19-TNF in combination with immune-checkpoint inhibitor (anti-PD1) or chemotherapeutics (Melphalan) in BALB/c mice bearing WEHI-164 tumors. A) Treatment started when tumors reached a volume between 70-100 mm^3, mice were injected three times intravenously (black arrows) every 48 hours with either PBS, L19-TNF, anti-PD-1 or the combination of L19-TNF plus anti-PD1. Melphalan were also administered intravenously once (gray arrow), both as a monotherapy and in combination with L19-TNF. Data represent mean tumor volume ± SEM, n = 5 mice per group, CR = complete response. B) Body weight monitoring. Weight change during the therapy of subcutaneous WEHI-164 tumors. Data represent mean % weight (± SEM), n = 5 mice per group. C) Survival curves.