

# The Anti-gp240 Immunocytokine scFvMEL/TNF Shows Potent Antitumor Activity

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## Abstract:

The recombinant fusion construct scFvMEL/TNF is composed of the human cytokine tumor necrosis factor- $\alpha$  (TNF) and a single-chain Fv recognizing gp240 present on 80 % of melanoma cell lines and fresh tumor samples. The fusion construct demonstrated specific binding to gp240 antigen-positive melanoma cells and retained biological activity of TNF. The fusion protein scFvMEL/TNF was more cytotoxic to antigen-positive A375 melanoma cells than TNF alone and, additionally, was active against AAB-527 melanoma cells completely resistant to TNF itself. Radiolabeled scFvMEL/TNF was administered to nude mice bearing human melanoma xenografts, and mice were sacrificed at 24, 48 and 72 h after administration. We found that kidney and spleen contained the highest tissue: blood ratio of all normal organs. At 24 h after administration, kidney, spleen and liver also contained the highest concentration of drug/gram tissue weight, although these concentrations declined over time. In contrast to normal organs, both the concentration of the label in tumor as well as the tissue: blood ratio of tumor tissue increased over time. Tumor was the highest site of accumulation by 72 hr after administration, followed by kidney, spleen and liver. Pharmacokinetic studies showed that the agent cleared from plasma triphasically with  $\alpha$ -,  $\beta$ - and  $\gamma$ -phase half-lives 0.38 h, 3.9 h and 17.6 h, respectively. We stably transfected A375 cells with enhanced green fluorescent protein (GFP) and further examined the antitumor effects of scFvMEL/TNF using A375GFP xenograft tumors growing subcutaneously. We monitored the efficacy of therapy using both caliper measurement and a Xenogen IVIS 200 Imaging System which allows intravital imaging of transfected cells. Treatment of scFvMEL/TNF at MTD doses demonstrated potent antitumor activity and tumor regression. These studies suggest that scFvMEL/TNF fusion construct may therefore have a significant potential for treatment of melanoma and some breast tumors.

## Background:

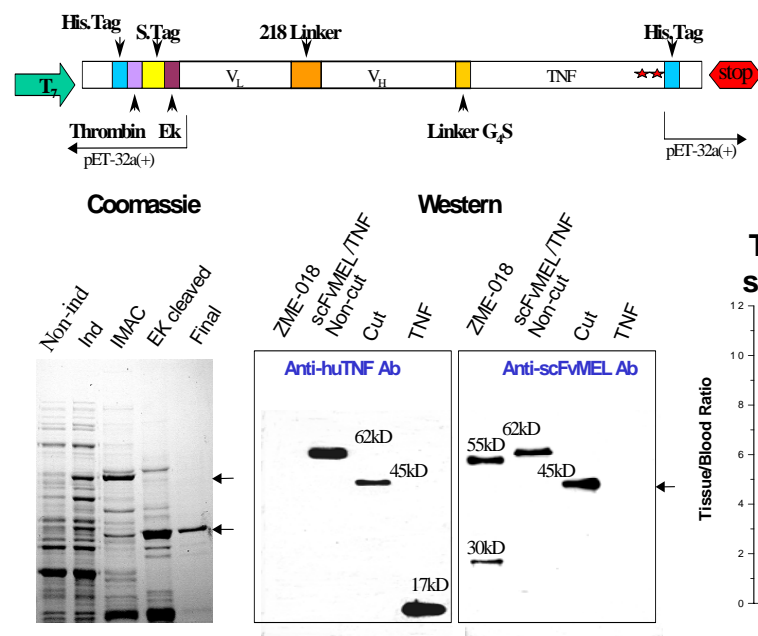
The anti-melanoma antibody scFvMEL is a single-chain recombinant construct which contains a variable light chain ( $V_L$ ) and a variable heavy chain ( $V_H$ ) linked by flexible peptide linker. This construct binds to the same epitope as the intact ZME-018 antibody. scFvMEL has improved tumor penetration and localizes well in tumor xenografts vs ZME-018.

TNF- $\alpha$  is an endogenous cytotoxic cytokine with cytotoxic effects against many tumors. Novel strategies have been suggested to target delivery of TNF to tumor cells.

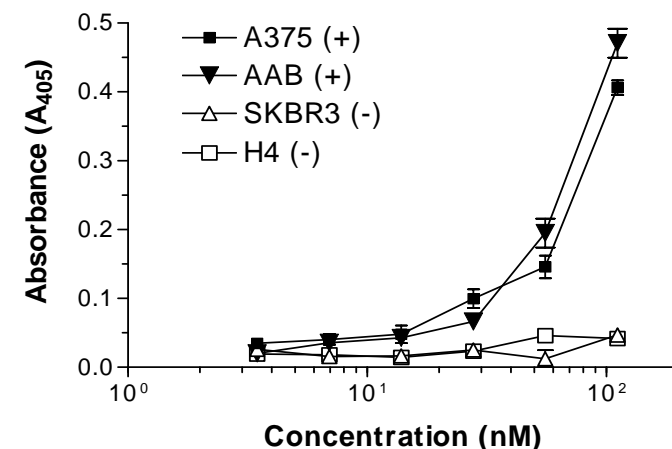
Our group initially utilized human recombinant TNF covalently linked to the murine anti-melanoma ZME-018. This chemical conjugate was extremely cytotoxic to target cells both sensitive and resistant to TNF. This suggests that targeted TNF might have a different biological effect compared to native TNF.

## Experimental Results:

### scFvMEL/TNF: Construction, Purification and Western Analysis



### scFvMEL/TNF Binds to gp240 Ag (+) Cells But not to gp240 (-) Cells.

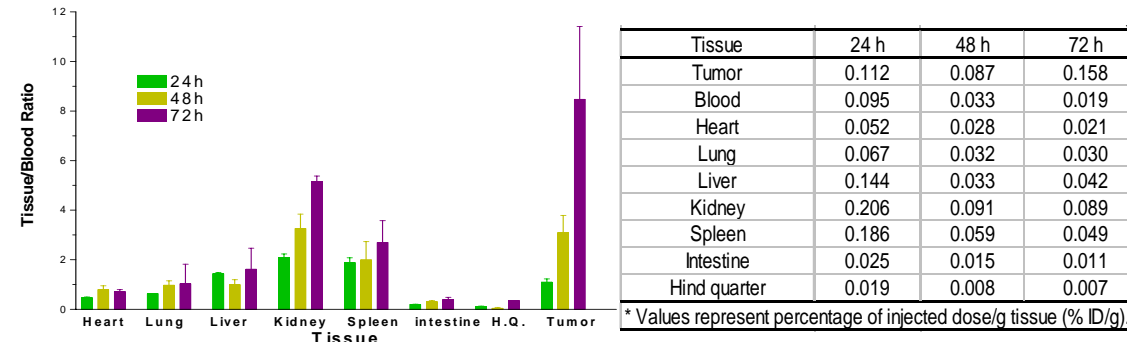


### ScFvMEL/TNF Is More Cytotoxic Than TNF to Human Melanoma Cells, Especially to Melanoma Cells Resistant to TNF Itself

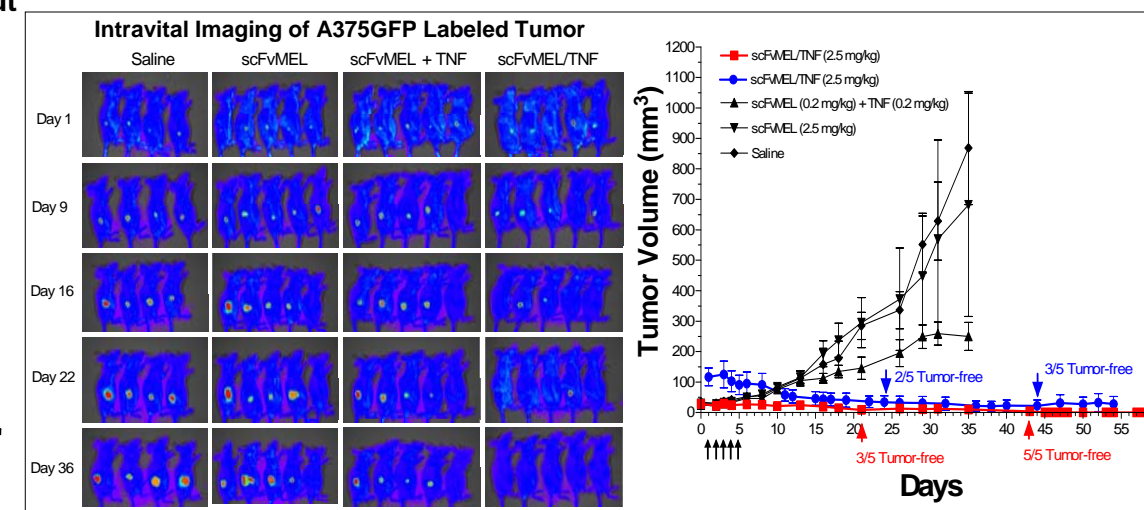
Cell lines	Cell features	I.C. <sub>50</sub> scFvMEL/TNF (nM)	I.C. <sub>50</sub> TNF (nM)	Targeting Index *	p value
Human melanoma AAB527	gp240 (+) TNF-resistant	20.62 ± 1.125	> 5000	>250	-
Human melanoma A375M	gp240 (+) TNF-sensitive	0.10 ± 0.013	1.37 ± 0.020	15	< 0.0001
Human breast cancer SKBR3-HP	gp240 (-) TNF-sensitive	2.55 ± 0.085	2.71 ± 0.014	1	> 0.05
Human neuroglioma H4	gp240 (-) TNF-resistant	> 100	> 5000	-	-

\*: Targeting Index = I.C.<sub>50</sub> of TNF / I.C.<sub>50</sub> of scFvMEL/TNF

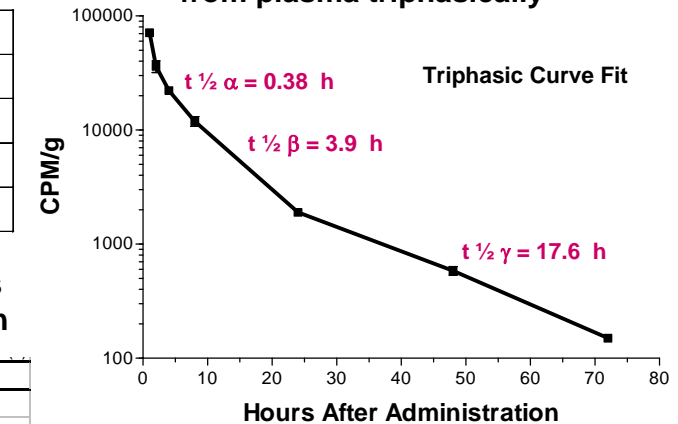
### Tissue Distribution of <sup>125</sup>I-scFvMEL/TNF in Mice Bearing A375 Xenografts scFvMEL/TNF Localizes in Human Melanoma Tumors after Administration



### scFvMEL/TNF Is Cytotoxic to A375GFP Tumor Xenografts (i. v. ↑)



### Pharmacokinetics: <sup>125</sup>I-scFvMEL/TNF cleared from plasma triphasically



## Conclusions:

- We have generated a fusion construct of the anti-melanoma single-chain antibody (scFvMEL) and the cytokine recombinant human TNF
- The immunocytokine is more cytotoxic than TNF to melanoma cells, especially to melanoma cells resistant to TNF itself
- Radiolabeled scFvMEL/TNF localized effectively in human melanoma xenografts in nude (nu/nu) mice at 72 h after administration (%ID/g, 0.158; T:B, 7.6 ± 2.2), followed by kidney (%ID/g, 0.089; T:B, 5.1 ± 0.2), spleen (%ID/g, 0.049; T:B, 2.6 ± 0.8) and liver (%ID/g, 0.042; T:B, 1.6 ± 0.8)
- The fusion construct cleared from plasma triphasically with 0.38 h, 3.9 h and 17.6 h of  $\alpha$ -,  $\beta$ - and  $\gamma$ -phase half-lives, respectively
- Treatment of scFvMEL/TNF at 2.5 mg/kg (total doses) demonstrated potent antitumor activity and tumor regression
- The scFvMEL/TNF fusion construct may therefore have a significant potential for treatment of melanoma and some breast tumors expressing gp240