

# NGR-hTNF, a selective vascular targeting agent (VTA), administered as single agent at low dose in patients with colorectal cancer refractory to standard regimens

A. Santoro<sup>1</sup>, L. Rimassa<sup>1</sup>, A. Sobrero<sup>2</sup>, V. Andretta<sup>2</sup>, V. Gregorc<sup>3</sup>, F. Scalfani<sup>1</sup>, F. Caprioni<sup>2</sup>, F. Caligaris-Cappio<sup>3</sup>, A. Lambiase<sup>4</sup>, C. Bordignon<sup>1</sup>

<sup>1</sup>Istituto Clinico Humanitas, Rozzano, Milan, Italy; <sup>2</sup>Ospedale S. Martino, Genova, Italy; <sup>3</sup>Istituto Scientifico San Raffaele, Milan, Italy; <sup>4</sup>Molmed, Milan, Italy

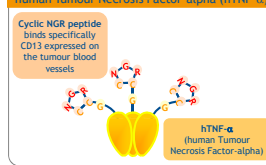
## ABSTRACT

**Background:** NGR-hTNF is a VTA exploiting a tumour-homing peptide (NGR) that selectively binds to aminopeptidase N (CD13) highly expressed on tumour blood vessels. NGR-hTNF combines activity on tumour vascular permeability and direct anticancer activity. In preclinical models, NGR-hTNF showed antitumour activity both at low doses and at high doses. **Methods:** Patients (pts) with CRC resistant or refractory to standard treatments, including biological agents, were enrolled to evaluate a low dose of NGR-hTNF given at 0.8 µg/m<sup>2</sup> as 1- hour intravenous infusion every 3 weeks (q3w). This phase II trial had a 2-stage design with 16 and 27 pts to be enrolled in stage 1 and 2, respectively. Progression-free survival (PFS) was the primary end point with tumour reassessment performed q6w. **Results:** From January to May 2007, 32 patients (median age 65 years, range 53-79; 16 M/16 F; PS 0/1 26/6) with documented progressive disease after last therapy were enrolled. Median number of prior regimens was 3 (range, 2-5), with eight pts (25%) pre-treated with ≥4 lines. Globally, 111 cycles (median, 2; range, 1-10) were administered and 13 pts (41%) received ≥4 doses. Neither grade 3-4 treatment-related adverse events nor toxicity-related deaths were observed. Most common grade 1-2 toxicities per patient were infusion-related chills (53%) and transient blood pressure increase (9%). Median duration of PFS was 2.3 months (range, 1.4-8.3). In an exploratory subset analysis, there were no significant differences in PFS between pts with CTC baseline value <3 and ≥3 cells and pts pre-treated with <3 and ≥3 regimens. One patient (3%) achieved a partial response lasting 5 months. An additional 12 pts (38%) had stable disease as best response with a median duration of 2.9 months (95% CI, 2.4-5.4). Median and 18-month overall survival was 13.1 months and 34%, respectively. **Conclusions:** Based on the favourable and manageable toxicity profile and preliminary evidence of activity in heavily pre-treated CRC patients, NGR-hTNF is currently developed both as single agent, exploring a weekly schedule, and in combination with standard chemotherapeutics.

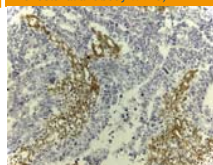
## Background

- A large number of preclinical studies have shown that tumour necrosis factor-α (TNF-α) has potent antitumour activity. However, its clinical use has been hampered by severe systemic toxicity, with MTD significantly lower than ED in humans<sup>1</sup>.
- NGR-hTNF is a selective vascular targeting agent (VTA) that has been genetically engineered by coupling the N-terminus of human TNF-α with the C-terminus of the tumour-homing peptide Cys-Asn-Gly-Arg-Cys (NGR) (Figure 1).
- The cell surface receptor for the NGR-containing peptide is a CD13/aminopeptidase N (APN) isoform selectively expressed by endothelial cells of newly formed human tumour vessels,<sup>2-4</sup> including colorectal cancer (Figure 2).

**Figure 1.** Recombinant fusion protein consisting of NGR peptide combined with human Tumour Necrosis Factor-α (hTNF-α)

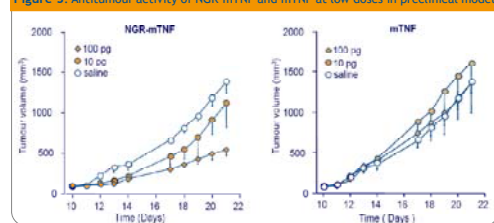


**Figure 2.** CD13 expression in colon cancer (staining with the anti-CD13 monoclonal antibody WM15)



- NGR-mTNF has shown antitumour activity in preclinical models<sup>5</sup> also when administered at doses in the picogram range (equivalent to a dose of 0.2 µg/m<sup>2</sup> in humans) (Figure 3).

**Figure 3.** Antitumour activity of NGR-mTNF and mTNF at low doses in preclinical model



## Phase I trials

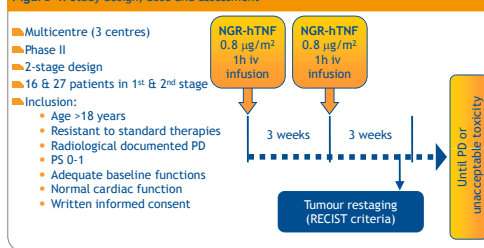
- A phase I study evaluating a dose-interval ranging from 0.2 to 60 µg/m<sup>2</sup> established the MTD of NGR-hTNF at 45 µg/m<sup>2</sup> when given as single agent once every 3 weeks<sup>5</sup>.
- Conversely, a further phase I trial exploring the low-dose range of NGR-hTNF from 0.2 to 1.6 µg/m<sup>2</sup> selected the dose of 0.8 µg/m<sup>2</sup> as the optimal biological dose (OBD), based on dynamic imaging changes and preliminary antitumour activity<sup>6</sup>.
- Notably, in this low-dose phase I trial a long-lasting (18 months) stabilization of disease was observed in a 43-year-old female patient with metastatic colon cancer refractory to three prior chemotherapy regimens administered in less than one year.

## Disease background

- CRC is the third most common cancer worldwide, with approximately 1 million new cases diagnosed yearly.
- Despite recent advances in the treatment of metastatic CRC, which include irinotecan- or oxaliplatin-based first-line regimens, and the increasing use of targeted monoclonal antibodies, most patients develop resistance to these therapies.
- Recently, two monoclonal antibodies have shown to be effective in disease refractory to fluorouracil, irinotecan and oxaliplatin<sup>7,8</sup>.
- A significant increase versus best supportive care of median PFS were reported for patients treated with cetuximab<sup>7</sup> (1.9 vs 1.8 months) and with panitumumab<sup>8</sup> (2.0 vs 1.8 months). Similar results were registered in terms of disease control rate (39% vs 11%, in the cetuximab study and 37% vs 10%, in the panitumumab study). However, there is a need for new treatment options in this setting.

## Methods

**Figure 4.** Study design, dose and assessment



## Results

- From January to May 2007, thirty-three colorectal cancer patients resistant or refractory to standard treatments, including biological agents, were enrolled in this phase II study. Median number of prior regimens was 3 (range, 2 to 5). Eight patients (25%) were pre-treated with ≥4 lines and 22 (67%) with biological agents. Baseline characteristics are summarized in Table 1.

**Table 1.** Baseline characteristics

Characteristics	n=33 (%)
Median age, years (range)	65 (53-79)
Gender	
Male	16 (48)
Female	17 (52)
ECOG performance status	
0	26 (79)
1	7 (21)
Primary diagnosis	
Colon cancer	23 (70)
Rectal cancer	10 (30)
Circulating tumour cells (CTC)	
< 3 cells/7.5 mL	17 (52)
≥ 3 cells/7.5 mL	12 (36)
≥ 3 cells/7.5 mL	4 (12)
Prior lines of systemic therapy	
2 lines	14 (42)
3 lines	10 (30)
4 lines	9 (28)
Best response to prior therapy	
Partial response	6 (18)
Stable disease	12 (36)
Progressive disease / Unknown	15 (45)

## Safety

- A total of 111 cycles of therapy were administered (mean, 3.4; median, 2; range, 1 to 10).
- Neither grade 3-4 treatment-related adverse events nor toxicity-related deaths were observed in the study population.
- The most common treatment-related adverse events were grade 1-2 chills (53%) and transient blood pressure increase (9%), generally occurring approximately 30 minutes after the start of the first infusions and lasting about 20 minutes. No cumulative toxicities were observed.

**Table 2.** Treatment-related adverse events occurring in > 5% of patients

Event	Grade 1	Grade 2	Grade 3	Grade 4
Chills	4 (12%)	13 (41%)	-	-
Blood pressure increase	3 (9%)	-	-	-
Fatigue	2 (6%)	-	-	-
Nausea	2 (6%)	-	-	-

## Efficacy

- In the first stage of the study (n=16), one partial response (6%) lasting 5 months and 9 stable disease (56%) were reported. Median and 3-month PFS were 2.9 months and 47%, respectively.
- Efficacy results after the completion of the enrollment into the second stage (n=33) are reported in Table 3 and 4. The actuarial progression-free survival curve is depicted in Figure 5 (2 patients censored).
- The subset of patients who achieved disease control (n=13) received a median of 4 cycles (range, 3 to 10 cycles). The 3-month and 4.5-month PFS rates in these patients were 67% and 49%, respectively.
- With a median duration of follow-up of 18.4 months (95% CI, 18.3 to 18.5 months), 11 patients (33%) were alive (Figure 6). Overall survival rates at 12 and 18 months among patients non-progressing at their first restaging (n=13) were 69% and 46%, respectively. The 6-month PFS rate in the prior-biological and biological-naïve cohorts was 5% and 20%, respectively, whereas 1-year OS rate was 41% and 72%, respectively.

**Table 3.** Best overall response

Variable	No. of patients	%
Partial Response (PR)	1	3
Stable disease (SD)	12	36
Disease control rate (DCR)	13	39
Progressive disease (PD)	17	51
Not assessed*	3	9

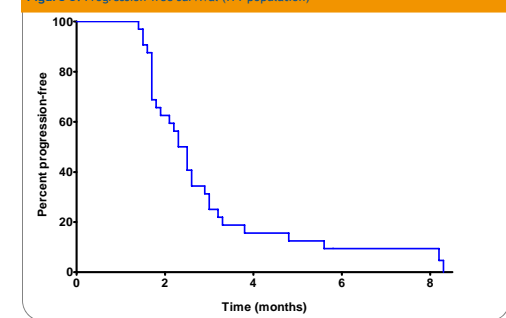
\*Two patients withdrew from the study before their first restaging scan for symptomatic deterioration and one patient before study treatment start.

**Table 4.** Time-related efficacy data

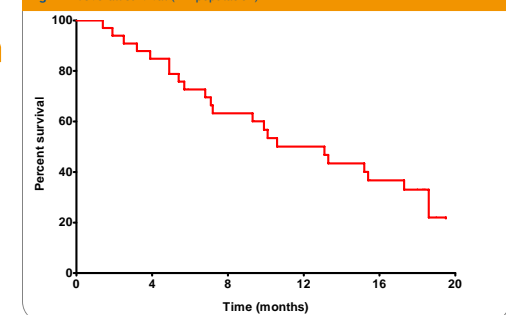
Variable	Estimate (months)	95% CI
Median PFS in ITT population (n=33)	2.5	2.2-2.8
Median PFS in patients with SD/PR (n=13)	3.8	1.4-6.1
Median OS in ITT population (n=33)	13.1	8.7-17.5
Median OS in patients with SD/PR (n=13)	15.4	11.5-19.2

PFS=progression-free survival; ITT= intent-to-treat; SD=stable disease; PR=partial response; OS= overall survival; CI=confidence interval

**Figure 5.** Progression-free survival (ITT population)



**Figure 6.** Overall survival (ITT population)



## Conclusions

- NGR-hTNF administered at low dose is safe and shows a favourable toxicity profile, with preliminary evidence of activity in heavily pretreated patients with advanced colorectal cancer
- Noteworthy, the toxicity profile is limited to reversible and easily manageable constitutional symptoms, such as chills, generally occurring during the administration of first infusions
- NGR-hTNF in colorectal cancer is currently developed both as single agent, exploring a weekly schedule of administration, and in combination with a standard capecitabine and oxaliplatin regimen

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