

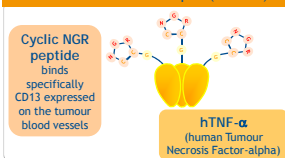
## ABSTRACT

**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:** Pts with CRC 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 pts with documented progressive disease after last therapy were enrolled. Pts characteristics were: 16 M/16 F; PS 0/1/2/6/6; median age: 65 years, range 53-79; circulating tumour cells baseline value <3/≥3 cells/17/12. Median number of prior regimens was 3 (range: 2-5), with 8 pts (25%) pre-treated with ≥4 lines. Globally, 111 cycles (median, 2, range, 1-10) were administered and 13 pts (41%) received ≥4 doses. A partial response lasting 5 months was achieved in one patient (3%). Twelve pts (38%) had stable disease as best response with a median duration of 2.9 months (95% CI, 2.4-5.4). Median PFS was 2.3 months (range, 1.4-8.3). In an exploratory subset analysis, no significant differences in PFS were detected between pts pre-treated with <3 and ≥3 regimens (HR=0.74, p=4). With a median follow-up time of 7.7 months (range, 1.4-15 months), 21 pts (66%) were still alive. Neither grade 3-4 treatment-related adverse events nor toxicity-related deaths were observed. Main grade 1-2 toxicities per patient were infusion-related chills (47%) and transient blood pressure increase (9%). **Conclusion:** Based on the favourable and manageable toxicity profile and preliminary evidence of activity in heavily pre-treated CRC patients, NGR-hTNF will be further developed both as single agent, exploring a weekly schedule, and in combination with standard chemotherapeutics.

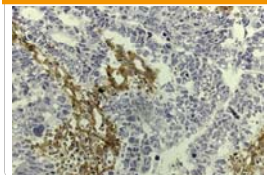
## Background

- A large body of preclinical evidences 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>
- The antivascular effects of TNF-α provided the rationale for developing a vascular targeting strategy aimed at increasing the local antitumour activity
- NGR-hTNF is a novel 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 and human Tumour Necrosis Factor-α (hTNF-α)

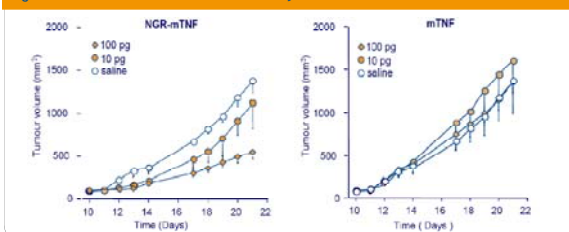


**Figure 2.** CD13 expression in colon cancer



- NGR-mTNF was found to have antitumour activity also at doses in the picogram range (equivalent to a dose of 0.2 µg/m<sup>2</sup> in humans) in preclinical model<sup>4</sup> (Figure 3)

**Figure 3.** Preclinical antitumour activity at low doses of NGR-mTNF and mTNF



## Phase I trials of NGR-hTNF

- In a phase I study evaluating a dose-interval ranging from 0.2 to 60 µg/m<sup>2</sup> the MTD of NGR-hTNF was established 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, based on dynamic imaging changes and preliminary antitumour activity<sup>6</sup>

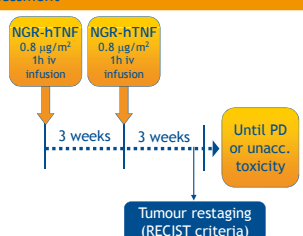
## Disease background

- Colorectal cancer (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>6,9</sup>
- A significant increase of median PFS durations versus BSC 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 first study and 37% vs 10%, in the second study). However, there is a need for new treatment options in this setting

## Methods

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

- Multicentre (3 centres)
- Phase II
- 2-stage design
- 16 & 27 patients in 1<sup>st</sup> & 2<sup>nd</sup> stage
- Inclusion:
  - Age >18 years
  - Resistant to standard therapies
  - Radiological documented PD
  - PS 0-1
  - Adequate baseline functions
  - Normal cardiac function
  - Written informed consent



## Results

- From January to May 2007, thirty-two colorectal cancer patients resistant or refractory to standard treatments, including biological agents, were enrolled in this phase II study
- Baseline characteristics are summarised in Table 1

**Table 1. Baseline characteristics**

Characteristics	n=32 (%)
Median age, years (range)	65 (53-79)
Gender	
Male	16 (50)
Female	16 (50)
ECOG performance status	
0	26 (81)
1	6 (19)
Primary diagnosis	
Colon cancer	22 (69)
Rectal cancer	10 (31)
Circulating tumour cells (CTC)	
< 3 cells/7.5 mL	17 (59)
≥ 3 cells/7.5 mL	12 (41)
Prior lines of systemic therapy	
2 lines	14 (44)
3 lines	10 (31)
≥ 4 lines	8 (25)
Best response to prior therapy	
Partial response	6 (18)
Stable disease	12 (38)
Progressive disease/Unknown	14 (44)

## Safety

- A total of 111 cycles of therapy were administered with a median of 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

**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 1<sup>st</sup> stage of the study (n=16), one patient achieved after 4 cycles a PR lasting 5 months and 9/16 pts (56%) had SD as best response. Median and 3-month PFS in this cohort of patients were 2.9 months and 47%, respectively
- Preliminary efficacy results after the 2<sup>nd</sup> stage (n=32) are reported in Tables 3-4
- The Kaplan-Meier plots for PFS and OS are depicted in Figures 4-5
- For patients with SD at their first restaging, the 3-month PFS rate was 67%
- With a median follow-up of 10.4 months (range, 1.4 to 19.5 months), the 12- and 18-month survival rates were 51% and 34%, respectively

**Table 3. Best overall response**

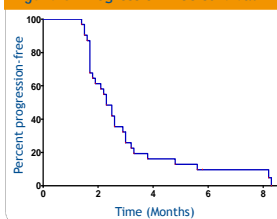
Variable	# pts	Estimate	95% CI
PR	1	3%	0-15
SD	12	38%	23-55
DCR	13	41%	26-58
PD	17	53%	36-69
Not assessed*	2	6%	2-20

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

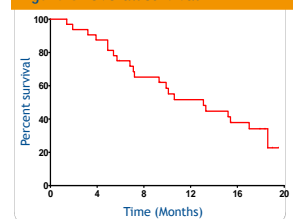
Variable	Median (months)	95% CI
PFS	2.3	1.9-2.7
PFS in pts with SD (n=13)	3.8	2.8-4.8
OS	13.1	7.6-18.6
OS in pts with SD (n=13)	15.4	11.5-19.2

PFS=progression-free survival; SD=stable disease; OS=overall survival; PR=partial response; DCR=disease control rate; PD=progressive disease; \*Two patients withdrew from the study before their first restaging scan for symptomatic deterioration

**Figure 5.** Progression-free survival



**Figure 6.** Overall survival



## Conclusions

- NGR-hTNF administered at low dose is safe and shows 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 is currently developed both as single agent, exploring a weekly schedule of administration, and in combination with a standard capecitabine and oxaliplatin regimen

## References

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