

A phase II study of NGR-hTNF, a novel vascular targeting agent (VTA), administered as single agent at low dose in patients with colorectal cancer refractory to standard regimens

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ABSTRACT

Background: NGR-hTNF is a VTA exploiting a tumor-homing peptide (NGR) that selectively binds to aminopeptidase N/CD13 highly expressed on tumor blood vessels. NGR-hTNF combines activity on tumour vascular permeability and direct anticancer activity. In preclinical models, NGR-hTNF showed antitumor activity at both low and 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/m² 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 and reassessment was performed q6w. **Results:** 32 pts (16 M/16 F; PS 0/1 26/6; median age: 65 years, range 53-79) received 111 cycles (median 2, range 1-10) and 13 pts (41%) were treated with ≥4 doses. The median number of prior regimens was 3 (range: 2-5), with 8 pts (25%) pre-treated with ≥4 lines. In the first stage, one pt (6%) achieved a confirmed partial response lasting 5 months and 9/16 pts (56%) had a stable disease (SD). The median and 3-month PFS were 2.9 months (95% CI 1.9-3.9) and 47% (95% CI 21-71%), respectively. After completion of the second stage, a total of 4 pts were progression-free at 4.5 months, with one pt still on treatment after 7 months. Neither grade 3-4 treatment-related adverse events nor toxicity-related death were observed. Main grade 1-2 toxicities per patient were infusion-related chills (41%) and transient blood pressure increase (9%). The study is currently recruiting patients into a subsequent cohort of 12 patients treated with a weekly schedule of administration. **Conclusions:** Based on the favourable and manageable toxicity profile and preliminary evidence of activity in heavily pretreated CRC patients, NGR-hTNF will be further developed both as single agent and in combination with standard chemotherapeutics.

Background

- A large number of preclinical studies have shown that tumor necrosis factor-α (TNF-α) has potent antitumor activity. However, its clinical use has been hampered by severe systemic toxicity, with MTD significantly lower than ED in humans¹.
- The antivascular effects of TNF-α provided the rationale for developing a vascular targeting strategy aimed at increasing the local antitumor activity and at enabling systemic administration of therapeutic doses.
- NGR-hTNF is a novel therapeutic vascular targeting agent (VTA) that has been genetically engineered by coupling the N-terminus of human TNF-α with the C-terminus of the tumor-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 tumor vessels,^{2,4} including colorectal cancer (Figure 2).
- Recently, in an APN-null mice model was shown that although aminopeptidase N activity is not essential for embryonic and fetal development including de novo blood vessel formation and normal adult function, it is critical for the pathological development of new blood vessels from existing blood vessels in disease.⁵

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

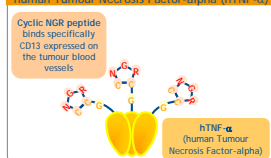
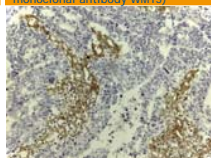
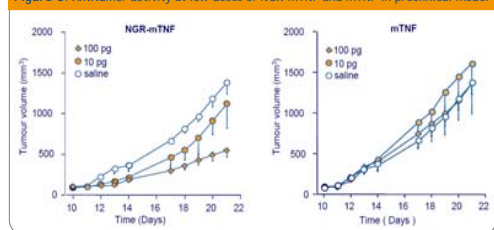


Figure 2. CD13 expression in colon cancer (staining with the anti-CD13 monoclonal antibody WM15)



- NGR-mTNF has shown antitumor activity in preclinical models⁶ also when administered at doses in the picogram range (equivalent to a dose of 0.2 µg/m² in humans) (Figure 3).

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



Phase I trials

- A phase I study evaluating a dose-interval ranging from 0.2 to 60 µg/m² established the MTD of NGR-hTNF at 45 µg/m² when given as single agent once every 3 weeks.⁶
- Conversely, a further phase I trial exploring the low-dose range of NGR-hTNF from 0.2 to 1.6 µg/m² selected the dose of 0.8 µg/m² as the optimal biological dose (OBD), based on dynamic imaging changes and preliminary antitumor activity.⁷
- 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

- Colorectal cancer is the third most common cancer worldwide, with approximately 1 million new cases diagnosed yearly.
- Although current use of the cytotoxic agents irinotecan, oxaliplatin, and fluoropyrimidines, as well as bevacizumab, the antibody against vascular endothelial growth factor, have increased the median survival of patients with advanced colorectal cancer, most patients develop resistance to these therapies.
- Recently, an additional two monoclonal antibodies targeting the epidermal growth factor receptor have shown to be effective in disease refractory to fluorouracil, irinotecan and oxaliplatin.^{8,9}
- A significant increase versus best supportive care (BSC) of median PFS durations were reported for patients treated with cetuximab⁸ (1.9 vs 1.8 months) and with panitumumab⁹ (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).
- The lack of an overall survival benefit in both studies was probably not surprising considering that the majority of patients in the BSC arms were allowed to cross over to the experimental treatments. However, there is a need for new treatment options in this setting.

Methods

- Multicenter, two-stage, phase II study with 16 and 27 patients to be enrolled after the first and second stage, respectively.
- NGR-hTNF given at 0.8 µg/m² as 1-hour intravenous infusion every 3 weeks until progressive disease or unacceptable toxicity.
- Primary endpoint: Progression-free survival with restaging performed every 6 weeks according to RECIST criteria. Secondary objectives: disease control rate, overall survival, safety and evaluation of circulating tumor cells (CTCs).
- Key inclusion criteria:
 - Patients >18 years old
 - Prior treatment with fluoropyrimidine, oxaliplatin and irinotecan based regimens (including combination with biological agents)
 - Patients with radiological documented progressive disease at study entry
 - ECOG Performance Status 0-1
 - Adequate baseline bone marrow, hepatic and renal function. Normal cardiac function and absence of uncontrolled hypertension
 - No clinical signs of CNS involvement
 - Written informed consent to participate in the study

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 summarized 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 tumor 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. 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 patient achieved a confirmed partial response (PR) lasting 5 months and 9/16 pts (56%) had a stable disease (SD) as best response.
- The median and 3-month rate of PFS in this first cohort of patients were 2.9 months (95% CI, 1.9 to 3.9 months) and 47% (95% CI, 21 to 71%), respectively.
- Preliminary efficacy results after the completion of the enrollment into the second stage (n=32) are reported in Table 4 and 5.
- The actuarial progression-free survival curve is depicted in Figure 4 (2 patients censored).
- For patients achieving SD/PR as best response (n=13), the 3-month and 6-month PFS rates were 67% and 25%, respectively.
- Median duration of follow up was 9.4 months (95% CI, 5.7 to 12.7 months) for all patients and 12.8 months (range, 5.9 to 15.8 months) for surviving patients.

Table 3. Best overall response

Variable	No. of patients	%	95% CI
Partial Response (PR)	1	3	0-15
Stable disease (SD)	12	38	23-55
Disease control rate (DCR)	13	41	26-58
Progressive disease (PD)	17	53	36-69
Not assessed*	2	6	2-20

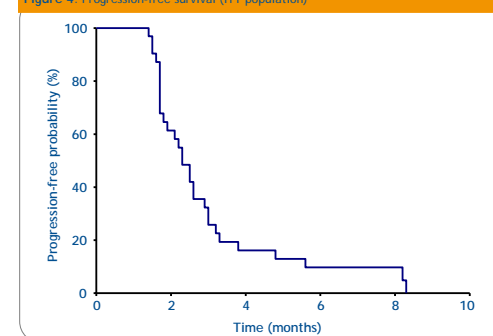
*Two patients withdrew from the study before their first restaging scan for symptomatic deterioration.

Table 4. Time-related efficacy data

Variable	Estimate	95% CI
Median PFS in ITT population (n=32), months	2.3	1.7-3.0
Median PFS in patients with SD/PR (n=13), months	3.8	2.6-6.0
Median OS (median follow up: 9.4 months)	NR	-
3-month OS rate, %	94	85-100
6-month OS rate, %	74	58-89

PFS=progression-free survival; ITT=Intent-to-treat; SD=stable disease; PR=partial response; OS=overall survival; CI=confidence interval; NR=not reached

Figure 4. Progression-free survival (ITT population)



Post-hoc analysis

- In an exploratory subset analysis, PFS rates were analyzed according to the prior number of regimens administered <3 (n=15) vs ≥3 lines (n=17) and the circulating tumor cells (CTC) baseline value <3 cells/7.5 mL (n=12) vs ≥3 cells/7.5 mL (n=12).
- No significant differences were detected between patients previously treated with <3 and ≥3 regimens (HR=0.74; 95% CI, 0.34-1.54; p=.40) and patients with CTC baseline values <3 and ≥3 cells (HR=0.68; 95% CI, 0.24-1.48; p=.28).

- Considering the favourable toxicity profile and the relatively short half-life, NGR-hTNF as single agent is currently evaluated by using a weekly schedule of administration in an additional cohort of 12 patients.
- Currently, eleven patients have been enrolled in this subsequent cohort.

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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