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Phase II study of NGR-hTNF, a selective vascular targeting agent, in patients with metastatic colorectal cancer after failure of standard therapy

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ABSTRACT

Background: NGR-hTNF consists of human tumour necrosis factor (hTNF) fused with the tumour-homing peptide Asp-Gly-Arg (NGR), which is able to selectively bind an aminopeptidase N overexpressed on tumour blood vessels. Preclinical antitumour activity was observed even at low doses. We evaluated the activity and safety of low-dose NGR-hTNF in colorectal cancer (CRC) patients failing standard therapies.

Patients and methods: Thirty-three patients with progressive disease at study entry received NGR-hTNF 0.8 μg/m^2 given intravenously every 3 weeks. The median number of prior treatment regimens was three (range, 2–5). One-quarter of patients had previously received four or more regimens and two-thirds targeted agents. Progression-free survival (PFS) was the primary study objective.

Results: NGR-hTNF was well tolerated. No treatment-related grade 3 to 4 toxicities were detected, most common grade 1 to 2 adverse events being short-lived, infusion-time related chills (50.0%). One partial response and 12 stable diseases were observed, yielding a disease control rate of 39.4% (95% CI, 22.9–57.8%). Median PFS and overall survival were 2.5 months (95% CI, 2.1–2.8) and 13.1 months (95% CI, 8.9–17.3), respectively; whereas in patients who achieved disease control the median PFS and overall survival were 3.8 and 15.4 months, respectively. In an additional cohort of 13 patients treated at same dose with a weekly schedule, there was no increased toxicity and 2 patients experienced PFS longer than 10 months.

Conclusion: Based on tolerability and preliminary evidence of disease control in heavily pretreated CRC patients, NGR-hTNF deserves further evaluation in combination with standard chemotherapy.

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1. Introduction

Tumour necrosis factor (TNF) was originally identified for its ability to induce powerful antivascular effects, mainly mediated by apoptosis of tumour endothelial cells, even though this cytokine binds unselectively to tumour vasculature.[1] Disappointingly, the systemic administration of recombinant human TNF was associated with severe toxicity and the maximum tolerated dose (MTD) resulted 10-fold lower than the estimated effective dose.[2–8] To mitigate this toxicity, the isolated limb perfusion was employed to deliver loco-regionally high doses of TNF combined with chemotherapy, with elevated response rates reported in melanoma and sarcoma patients.[9–12]

In order to selectively target TNF to tumour vasculature, NGR-hTNF was prepared by exploiting a ligand-directed approach, with recombinant human TNF fused with the tumour-homing peptide Asp-Gly-Arg (NGR). This peptide is able to specifically bind an aminopeptidase N/CD13 isoform overexpressed by endothelial cells of tumour vessels and its specific binding to the tumour neovasculature has been proven in several tumour types.[13–16] Recently, a CD13-null mice model confirmed that aminopeptidase N activity is crucial for the pathological development of newly formed blood vessels from pre-existing blood vessels.[17]

NGR-TNF induced antitumour activity at least 10-fold stronger than TNF in murine models and, interestingly, displayed antitumour effects even when delivered at very low doses in the nanogram range (0.005 μg/kg),[13,18] equivalent in humans to a dose of 0.2 μg/m^2, which was the selected starting dose of phase I trial.

The early-stage clinical development of NGR-hTNF was based on two dose-defining studies. A phase I study evaluating a wide-ranging dose interval from 0.2 to 60 μg/m^2 established the MTD of NGR-hTNF as 45 μg/m^2 once every 3 weeks.[19] In an additional trial aiming to further explore the low-dose range, 0.8 μg/m^2 every 3 weeks was selected as the optimal biological dose based on a combination of dynamic imaging changes, soluble TNF receptor kinetics, tolerability and preliminary activity.[20] In the latter study the antivascular effect of NGR-hTNF was confirmed by using dynamic contrast-enhanced magnetic resonance imaging. Interestingly, the decrease in tumour vascularity significantly correlated with radiologically assessed disease stabilisation, and a CRC patient previously refractory to three prior regimens experienced an 18-month PFS and then successfully underwent radical metastasectomy of a 30-cm large abdominal mass.[20] Considering the apparent similar activity and good tolerability, the low dose was chosen to be explored in phase II studies.

Despite the recent advances in the treatment of metastatic CRC, which include irinotecan- or oxaliplatin-based front-line regimens and increasing use of targeted agents, most patients develop resistance to these therapies.

Recently, two monoclonal antibodies targeting the epidermal growth factor receptor (EGFR) have shown in two large phase III trials to be more effective than supportive care alone in unselected chemorefractory CRC patients.[21,22] However, the benefits of these antibodies were almost entirely limited to patients whose tumours lacked KRAS mutation.[23] Moreover, chemorefractory metastatic CRC is a highly aggressive disease, as revealed by median survivals of 4–6 months reported in patients randomly assigned to receive supportive care only in the two aforementioned phase III trials.[21,22] Therefore, new treatment options are needed to continue the significant progress that has been made in this decade.

Here, we report a phase II study evaluating the activity and safety of NGR-hTNF given as single agent at 0.8 μg/m^2 in patients with metastatic CRC resistant or refractory to standard therapies, including biological agents.

2. Patients and methods

2.1. Eligibility criteria

Eligible patients had histologically proven CRC and received prior treatment with irinotecan, oxaliplatin and fluoropyrimidine, given in any combination or sequence in the adjuvant or advanced disease settings. Patients could also have received treatment with biological agents. Evidence of resistance to prior treatment was required at the study entry and was defined by radiologically documented progressive disease. Other inclusion criteria were the following: an age of 18 years or older; an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; an adequate bone marrow reserve (absolute neutrophil count $>1.5 \times 10^9$/L, platelets $>100 \times 10^9$/L, and haemoglobin >9 g/dL) and a serum creatinine <1.5 times the upper limit of normal.

Patients with significant cardiac, hepatic or renal dysfunction or known cerebral metastases were excluded, as well as patients completing radiation therapy or chemotherapy within 4 weeks or having surgery within 2 weeks of the study treatment start. All participants gave written informed consent before any study-related procedure was performed. The study was approved by the Ethical Committees of the respective participating Institutions.

2.2. Study design and dosing

This was an open-label, non-randomised, multicentre, phase II study of NGR-hTNF given as single agent. The primary study aim was the evaluation of progression-free survival (PFS). PFS was defined as the time from last tumour radiological assessment done at baseline (within 14 d before the first treatment administration) until progressive disease (PD) or death from any cause, dependent upon which occurred first or last follow-up the patient was known to be progression free and alive. Tumour restaging was repeated every other cycle (6 weeks) and the measurable target lesions were assessed for response using RECIST criteria.

Secondary objectives included safety, with toxicity graded according to the Common Terminology Criteria for Adverse Events, version 3.0; disease control rate (DCR), defined as the percentage of patients who had a best-response rating of complete response (CR), partial response (PR) or stable disease (SD); overall survival (OS), defined as the time from baseline until death or last contact the patient was known to be alive; and monitoring of circulating tumour cells (CTCs).

Peripheral blood was collected pre- and 24-h after each treatment cycle for CTCs isolation and enumeration. Patients were stratified into unfavourable and favourable prognostic groups based on CTC levels of 3 or more or less than 3 CTCs/7.5 mL, respectively.[24] For response to therapy at the first follow-up disease evaluation (after 2 cycles), the favourable group was defined as those with non-progressive disease (including SD/PR/CR categories) and the unfavourable group as those with PD or death.

NGR-hTNF was administered intravenously as 1-h infusion at the dose of 0.8 $\mu g/m^2$ every 3 weeks (triweekly schedule).

After study recruitment completion, in view of the lack of toxicity during inter-cycle period and in order to maintain a more constant antivascular effect, the protocol was amended to explore a more dense dosing schedule of NGR-hTNF given at same dose of 0.8 $\mu g/m^2$ on a weekly basis. Accordingly, a new cohort of 12 patients was planned to be enrolled. The weekly schedule was considered feasible and safe if two or fewer of 12 patients experienced grade 4 haematological or grade 3 to 4 non-haematological toxicity.

No NGR-hTNF dose reduction for toxicity was allowed. In the event of grade 3 or higher adverse event, treatment could be held or discontinued at investigator discretion, depending on the nature of toxicity.

Treatment duration was related to clinical outcome. In case of continued stable disease or objective response, study therapy was given until progressive disease, unacceptable toxicity or patient's refusal.

2.4. Statistical analysis

Primary study end-point was the 18-week progression-free rate according to the two-stage Simon's optimal trial design (P0 = 35%, P1 = 60%, α = 10%, β = 10%). The statistical parameters P0 and P1 were based on historical control.[25] The total planned sample size was 27 patients, with 16 patients to be enrolled in the first study stage. Study treatment could be considered worthy of additional testing if 7 (44%) and 13 patients (48%) were progression free at 18 weeks after the first and the second stages, respectively. All analyses were computed on an intent-to-treat (ITT) principle. Time-to-event variables were summarised with the use of Kaplan–Meier plots.[26] Median follow-up time was estimated with the inverse Kaplan–Meier method.[27] An exploratory Cox proportional hazards model[28] was fit to evaluate potential associations between a set of baseline characteristics and either PFS or OS. The variables assessed were age (as a continuous variable), gender, ECOG PS (0 *versus* 1), CTC baseline level (<3 *versus* $\geqslant$3 cells/7.5 mL), best overall response at any time on prior therapy (partial response or stable disease *versus* progressive disease) and number of prior regimens (<3 *versus* $\geqslant$3 lines). The Fisher's exact test was used to evaluate differences between progressors and non-progressors at first radiological assessment in the distribution of CTC levels and the log-rank test was used to evaluate the impact of variables on survival. All analyses were performed with GraphPad (GraphPad Software, Inc. San Diego, CA) and SPSS 13.0 (SPSS Inc., Chicago, IL). This trial is registered with ClinTrials.gov, number NCT00483080.

3. Results

3.1. Patients

Globally, 33 patients were enrolled. Patient demographics, baseline characteristics and prior treatments are listed in Table 1. All patients had radiologically documented PD at study entry and had received prior therapy with irinotecan, oxaliplatin and fluoropyrimidine. Twenty-two patients (66.7%) were also pretreated with at least one targeted agent, including cetuximab (n = 18), bevacizumab (n = 3), gefitinib (n = 1), while 11 (33.3%) had been pretreated with chemotherapy only. At baseline, two-thirds of patients had three or more sites involved by metastases and three-quarters presented with visceral metastases.

3.2. Safety

Thirty-two patients received at least one study-drug dose and resulted assessable for toxicity. One patient was withdrawn before treatment start for worsening of clinical condition. A total of 111 cycles (mean, 3.4; median, 2; range, 1–10) were delivered and 13 patients (40.6%) were treated with 4 or more courses. All patients received the planned dose on time with

Table 1 – Demographics, baseline characteristics and prior treatments.

Characteristics	Patients (n = 33)	%
Age (years)		
Median	65	–
Range	53–79	–
Gender		
Male	16	48.5
Female	17	51.5
ECOG performance status		
0	26	78.8
1	7	21.2
Primary tumour site		
Colon	23	69.7
Rectum	10	30.3
Sites of disease		
Median	3	–
Range	1–6	–
1 or 2	12	33.3
$\geqslant$3	21	63.7
Liver	25	75.8
Lung	24	72.7
Prior lines of systemic therapy		
Median	3	–
Range	2–5	–
Circulating tumour cells (CTC)		
<3 cells/7.5 mL	16	48.5
$\geqslant$3 cells/7.5 mL	13	39.4
Unknown	4	12.1
Best response to prior therapy		
Partial response	6	18.2
Stable disease	12	36.3
Progressive disease/unknown	15	45.5

the exception of three patients (9.4%) experiencing a 1–2-week delay in dosing as a result of toxicity. At the time of data analyses, no patient was on treatment. Globally, treatment discontinuation was the result of either symptomatic deterioration or radiologically documented PD in all patients.

In total, 237 study-emergent adverse events (AEs) were registered and the majority were mild to moderate in severity. Thirty grade 3 (12.7%) and three grade 4 (1.3%) AEs were reported. Most common AEs per patient are shown in Table 2. Five (15.6%) and 2 patients (6.2%) with metastatic liver disease experienced grade 3 to 4 hepatic toxicities, respectively, including hyperbilirubinaemia, increased γ-glutamyl transpeptidase and liver failure. Three patients (9.3%) with peritoneal carcinosis had grade 3 gastrointestinal obstruction. All of these grade 3 to 4 AEs were considered as likely related to the underlying disease.

Only 50 (21.1%) of the AEs were considered study-drug related and the most frequent were chills, experienced by 16 patients (50.0%) over 26 cycles (23.4%) and transient increase of blood pressure, reported in three patients (9.4%) during 11 cycles (9.9%). These events were short lived and presented a temporal relationship between their onset and the intravenous infusion, as they generally occurred 30–40 min after the start of administration and lasted approximately 20 min. Neither grades 3 to 4 treatment-related AEs nor toxicity-related deaths were observed in the study population.

3.3. *Efficacy*

After the first study stage ($n = 16$), one patient (6.2%) achieved a PR lasting 5 months and an additional 9 patients (56.3%) had SD, yielding an overall disease control rate of 62.5% (95% CI, 35.4–84.8%). Four patients (25.0%) were progression free at 18 weeks, whereas the median PFS was 2.9 months (95% CI, 2.0–3.8 months). Though this proportion was lower than the protocol-specified minimum requirement, the accrual was continued taking also into account the safety/tolerability profile of the experimental drug. According to an ITT analysis in a total of 33 patients enrolled after the second study stage, the median PFS was 2.5 months (95% CI, 2.1–2.8 months), and the Kaplan–Meier estimated that the 18-week PFS rate was 16.1% (Fig. 1A). The disease control rate by investigator assessment

Table 2 – Worst grade per patient of adverse events, irrespective of relationship to study drug, occurring in ⩾5% of patients (*n* = 32).

Adverse event	All grades *n* (%)	Grade 1 *n* (%)	Grade 2 *n* (%)	Grade 3 *n* (%)	Grade 4 *n* (%)
Pain	19 (60)	9 (28)	9 (28)	1 (3)	–
Chills	16 (50)	4 (13)	12 (37)	–	–
Asthenia	7 (22)	4 (13)	1 (3)	2 (6)	–
γ-Glutamyl transpeptidase increased	6 (19)	2 (6)	1(3)	3 (10)	–
Nausea	6 (19)	5 (16)	1 (3)	–	–
Vomiting	6 (19)	3 (10)	3 (10)	–	–
Hyperbilirubinaemia	5 (16)	1 (3)	–	3 (10)	1 (3)
Hyperglycaemia	5 (16)	3 (10)	1 (3)	1 (3)	–
Fever	5 (16)	5 (16)	–	–	–
Diarrhoea	4 (13)	3 (10)	1 (3)	–	–
Headache	4 (13)	1 (3)	3 (10)	–	–
Anorexia	3 (10)	2 (6)	–	1 (3)	–
Dyspnoea	3 (10)	1 (3)	1 (3)	1 (3)	–
Gastrointestinal obstruction	3 (10)	–	–	3 (10)	–
Hyperkalaemia	3 (10)	3 (10)	–	–	–
Hypertension	3 (10)	3 (10)	–	–	–
Partial thromboplastin time prolonged	2 (6)	–	–	2 (6)	–
Anaemia	2 (6)	1 (3)	–	1 (3)	–
Anxiety	2 (6)	2 (6)	–	–	–
Ascites	2 (6)	–	1 (3)	1 (3)	–
Aspartate aminotransferase increased	2 (6)	1 (3)	–	1 (3)	–
Blood creatinine increased	2 (6)	2 (6)	–	–	–
Constipation	2 (6)	–	2 (6)	–	–
Dizziness	2 (6)	1 (3)	1 (3)	–	–
Hypocalcaemia	2 (6)	2 (6)	–	–	–
Hypokalaemia	2 (6)	1 (3)	–	1 (3)	–
Hyponatraemia	2 (6)	1 (3)	–	1 (3)	–
Hypotension	2 (6)	2 (6)	–	–	–
Liver failure	2 (6)	–	–	–	2 (6)
Malaise	2 (6)	2 (6)	–	–	–
Oedema peripheral	2 (6)	1 (3)	1 (3)	–	–
Performance status decreased	2 (6)	–	1 (3)	1 (3)	–
Rectal tenesmus	2 (6)	2 (6)	–	–	–
Supraventricular extrasystoles	2 (6)	2 (6)	–	–	–
Tremor	2 (6)	2 (6)	–	–	–
Valve disease	2 (6)	2 (6)	–	–	–

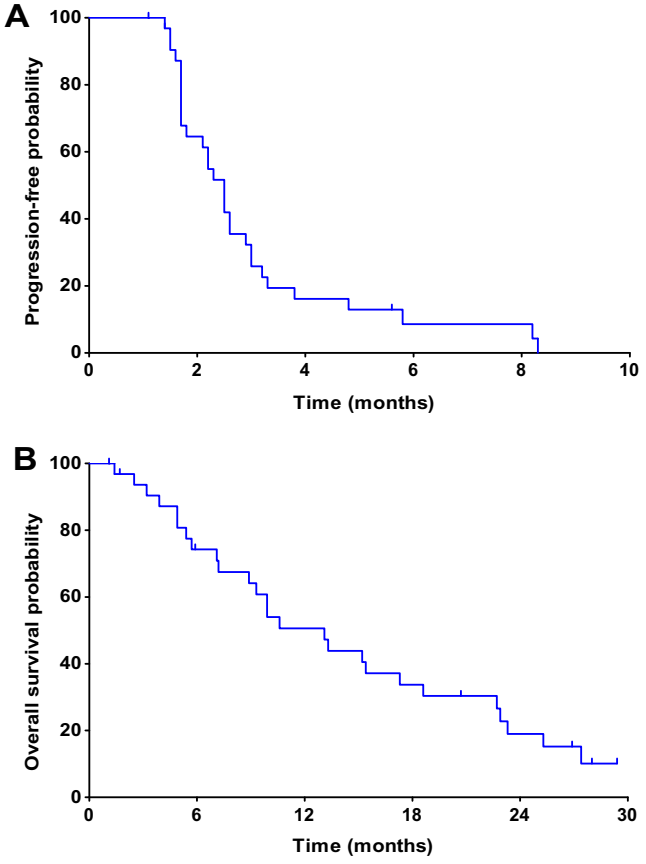

Fig. 1 – Kaplan–Meier curve of (A) progression-free survival and (B) overall survival for the intent-to-treat study population enrolled in the triweekly cohort (n = 33). Vertical ticks denote censored observations.

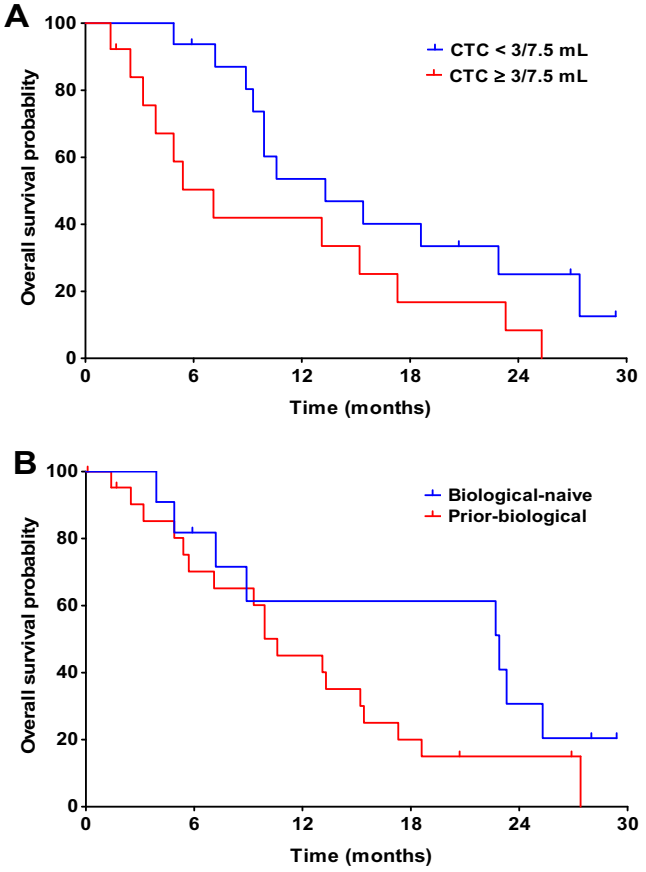

Fig. 2 – Kaplan–Meier curve of overall survival by (A) baseline CTC count and (B) prior treatment with biological agents for the study population enrolled in the triweekly cohort (n = 33). Vertical ticks denote censored observations.

was 39.4% (95% CI, 22.9–57.8%), including 1 PR (3.0%), 12 SD (36.4%) and 18 PD (54.5%). Two patients (6.1%) were withdrawn from the study before their first postbaseline tumour restaging for symptomatic deterioration. In patients who achieved disease control, the median number of treatment cycles was 4 (range, 3–10 cycles), the median PFS was 3.8 months (range, 1.4–8.3 months) and the 18-week PFS rate was 41.7%.

Paired serum samples (pre- and 24-h post first treatment cycle) for CTCs enumeration were available from 28 patients who had a subsequent follow-up imaging study performed after the second cycle. At baseline, unfavourable and favourable CTCs levels were detected in 13 patients (46.4%) and 15 patients (53.6%), respectively. These proportions did not significantly change after the first course (42.9% and 57.1%, respectively) and were not associated with outcome on first radiological tumour assessment (p = .27).

At a median follow-up time of 28.0 months, the median survival time was 13.1 months (95% CI, 8.9–17.3 months). The 1-year and 2-year survival rates were 50.6% and 19.0%, respectively (Fig. 1B). Patients who achieved disease control had a median survival of 15.4 months, while those who progressed or were unassessable had 9.3 months.

In univariate Cox analyses, no correlations were found between PFS and all variables assessed including age, sex, PS, baseline CTCs count, outcomes on prior therapy and number of prior treatment lines. A baseline level less than 3 CTCs showed a trend that correlated with OS (p = .059). All of the other variables failed to show any correlation with survival. In further post-hoc subset analyses, the median OS in patient cohorts with $\geqslant$3 or <3 CTCs were 7.1 months and 13.3 months (p = .052), respectively (Fig. 2A), whereas the median OS in patient cohorts previously treated or untreated with targeted agents were 10.6 months and 22.9 months (p = .125), respectively (Fig. 2B).

3.4. *Cohort of patients treated with a weekly schedule*

An additional 13 patients (9 women and 4 men) with a median age of 68 years (range, 56–78) were enrolled in a subsequent cohort exploring a more dose-dense schedule of NGR-hTNF given at 0.8 μg/m^2 on a weekly basis. Six patients (46.1%) had a PS of 0. Median number of prior regimens was two (range, 2–4) and most patients (n = 10; 76.9%) had been pretreated with targeted agents. A total of 138 (range, 3–42) weekly infusions were administered.

There was no worsening of the NGR-hTNF toxicity profile using the weekly dosing schedule. Most common drug-related toxicity was grade 1 to 2 chills experienced by 11 patients (84.6%) over 28 infusions (20.3%). One patient (7.7%) had grade 1 transient blood pressure increase and grade 2 fatigue.

Furthermore, neither drug-related grade 3 to 4 toxicities nor toxicity-related deaths were reported. Median PFS was 2.3 months (95% CI, 2.1–2.5 months). Two patients (15.4%) experienced PFS times of 10.5 and 11.0 months, which resulted in significantly longer PFS durations while on immediately prior therapy (3.8 months with panitumumab plus fluorouracil, leucovorin and irinotecan and 6.3 months with fluorouracil, leucovorin and irinotecan, respectively).

4. Discussion

The patient population enrolled in the current multicentre study had been heavily pretreated with a median of three prior chemotherapy lines. Additionally, the majority of patients had previously received also a targeted agent and half of patients presented at baseline three or more CTCs, which is an independent predictor of poor prognosis in metastatic CRC patients, regardless of therapy line.[24]

NGR-hTNF given as single agent at low dose was extremely well tolerated showing an easily manageable adverse event profile, characterised by the lack of drug-related grade 3 to 4 toxicity.

The disease control rate was 39.4%, whereas the median PFS and OS were 2.5 months and 13.1 months, respectively. Consistently with an advanced refractory disease,[21,22] most patients progressed within first postbaseline imaging assessments done after 6 and 12 weeks. Therefore, the 18-week PFS rate resulted lower than the challenging target of interest and the primary study end-point was not met. Moreover, as expected for a predominantly cytostatic agent, 1 patient only had an objective partial response. Nevertheless, about 40% of patients remained progression free for a median time of about 4 months and were alive for a median of 15 months.

Of note, in the patient cohort treated with a weekly schedule there was no increase of either incidence or severity of toxicity, whereas 2 patients with prolonged progression-free durations of more than 10 months were observed. In both cases, the PFS duration achieved in the current study was longer than that recorded on prior therapy. The rationale for exploring a weekly schedule was based on the hypothesis that a more frequent exposure to antivascular effect might prevent repair or regrowth of affected tumour vessels potentially arising during long treatment breaks.[29] However, given the apparent similar activity between the two schedules, the triweekly administration seems to be preferable for patient's convenience.

Taken together, these outcomes might be suggestive of potential tumour progression-delaying effect even for a subset of patients who did not achieve tumour shrinkage. Accordingly, previous studies have shown that disease stabilisation in chemorefractory CRC patients is clinically meaningful.[22,29] However, the interpretation of time-related outcomes from non-randomised studies is clearly complicated because the results might be biased by either patient selection or tumour-growth rate, rendering difficult to distinguish between treatment effect and natural history of disease.

Despite the inherent complexities associated with cross-study comparisons, the overall outcomes from the current study appear to be essentially in line with those reported in randomised phase 3 trials evaluating targeted agents in CRC patients failing standard therapies. Indeed, single-agent treatment with two anti-EGFR monoclonal antibodies resulted in median PFS of 1.9–2.0 months in unselected patients,[21,22] and 3.1–3.7 months in patients with wild-type KRAS tumours.[30,31] Similar results have been reported also in terms of disease control rate, with proportions ranging from 31% to 37% in unselected CRC patients.[21,22] Likewise, monotherapy with a vascular endothelial growth factor-binding monoclonal antibody resulted in median PFS of 2.7 months and disease control rate of 32% when administered as second-line therapy in CRC patients.[32] Moreover, the finding in the current study of a median survival of 1 year deserves interest when compared with median survivals of 4.6–7.3 months, regardless of KRAS status, reported in CRC patients who received supportive care only in the aforementioned trials with anti-EGFR monoclonal antibodies.[21,22,25,30]

In conclusion, the overall results from the current study showed a favourable toxicity profile of NGR-hTNF and hint of potential disease control in a subset of heavily pretreated CRC patients. Ultimately, the identification of markers of sensitivity or resistance either in tumour or in patient might help to select patients who eventually might benefit from treatment. Finally, although NGR-hTNF failed to show a meaningful single-agent activity, both the mode of action and the non-overlapping toxicity profile should facilitate its combination with standard chemotherapy for CRC patients. Consistently, significant preclinical synergism was displayed when low-dose NGR-hTNF was combined with several cytotoxic agents.[33] Currently, a phase II study is investigating NGR-hTNF given every 3 weeks in combination with a capecitabine plus oxaliplatin triweekly regimen in previously treated patients with metastatic CRC (clinicaltrials.gov/NCT00675012).

Conflict of interest statement

None declared.

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