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Two doses of NGR-hTNF in combination with capecitabine plus oxaliplatin in colorectal cancer patients failing standard therapies

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Background: asparagine-glycine-arginine-human tumour necrosis factor (NGR-hTNF), an agent selectively damaging the tumour vasculature, showed a biphasic dose–response curve in preclinical models. Previous phase I trials of NGR-hTNF indicated 0.8 and 45 μg/m^2 as optimal biological and maximum-tolerated dose, respectively.
Patients and methods: Two sequential cohorts of 12 colorectal cancer (CRC) patients who had failed standard therapies received NGR-hTNF 0.8 or 45 μg/m^2 in combination with capecitabine–oxaliplatin (XELOX).
Results: Median number of prior treatment lines was 3 in the low-dose and 2 in the high-dose cohort. Overall, 21 patients had been pretreated with oxaliplatin-based regimens. No grade 3–4 NGR-hTNF-related toxicities were observed. Grade 1–2 chills were reported in 43% and 40% of cycles in the low-dose and high-dose cohorts, respectively. In the low-dose cohort, one patient achieved a partial response and five had stable disease for a median of 4.6 months. In the high-dose cohort, six patients had stable disease for a median of 3.6 months. Three-month progression-free survival (PFS) rates were 50% and 33% in the low-dose and high-dose cohort, respectively. Three patients in low-dose cohort experienced PFS longer than PFS on last prior therapy.
Conclusions: Both NGR-hTNF doses were safely combined with XELOX in pretreated CRC patients. Hint of activity was apparent only with low-dose NGR-hTNF.
Key words: colorectal cancer, NGR-hTNF, vascular targeting agent

introduction

Over the past decade, major advances in the treatment of metastatic colorectal cancer (CRC) have occurred with the introduction of active chemotherapy combination (by adding either oxaliplatin or irinotecan to 5-fluoruracil) and targeted agents given either as first-line combination with chemotherapy (bevacizumab or cetuximab) or as single-agent salvage therapy (cetuximab or panitumumab). With these new drugs, there has been a paradigm shift with a doubling of median survival over 5-fluoruracil alone and a potential for long-term survival in certain patients [1]. Despite the growing number of options in first and subsequent lines, most patients develop resistance to these therapies [2]. Therefore, new treatment options are needed to continue the progress achieved in this decade.

NGR-hTNF is a vascular targeting agent consisting of human tumour necrosis factor-α (TNF) fused with the peptide asparagine–glycine–arginine (NGR), an aminopeptidase N (APN, CD13) ligand able to target tumour blood vessels [3–5]. The functional role of APN in promoting the angiogenic development of newly formed blood vessels from pre-existing blood vessels was recently confirmed in a CD13-null murine model [6]. Preclinically, NGR-hTNF induced stronger antitumour effects than TNF even with 30 times lower doses and displayed a biphasic dose–response curve showing anticancer activity when given either at very low or at high doses [2, 6].

In early-stage clinical development, a phase I study, testing doses from 0.2 to 60 μg/m^2, established the maximum-tolerated dose of NGR-hTNF at 45 μg/m^2 once every 3 weeks [7]. To further explore the low-dose range from 0.2 to 1.6 μg/m^2, a separate trial indicated 0.8 μg/m^2 as optimal biological dose mainly based on pharmacodynamic (PD) end points such as soluble TNF receptors (sTNF-Rs) kinetics and dynamic imaging assessment [8]. Across all dose levels, NGR-hTNF showed a favourable toxicity profile, most common toxicities being mild-to-moderate, short-lived, and infusion time-related chills. Recently, NGR-hTNF given as single agent at 0.8 μg/m^2 was evaluated in 33 metastatic CRC patients who had

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progressed through a median of three prior treatment lines. In this heavily pretreated patient population, a disease control rate of 39%, a median progression-free survival (PFS) of 2.5 months, and a median survival of 13.1 months were reported [9].

By damaging the integrity of cancer-associated endothelial cell barrier, TNF is also able to quickly reduce the interstitial fluid pressure, a process considered pivotal to increase the tumour-selective uptake of chemotherapy. Consistently, both significant preclinical synergism [10, 11] and safe clinical toxicity profile [12, 13] were observed by combining very low doses of NGR-hTNF with multiple chemotherapeutic agents.

The current study was designed to primarily assess the safety of low-dose (0.8 μg/m^2) and high-dose (45 μg/m^2) NGR-hTNF in combination with fixed doses of capecitabine plus oxaliplatin (XELOX) in CRC patients who had previously failed standard treatments. Secondary study aims included tolerability, pharmacokinetics (PKs), PDs, and preliminary activity.

patients and methods

eligibility criteria

Study population included patients >18 years who had metastatic CRC. They could have received no more than three prior treatment regimens, including irinotecan, oxaliplatin, fluoropyrimidine, and biological agents, given in any combination or sequence in the adjuvant or advanced disease settings. Additional eligibility requirements included Eastern Cooperative Oncology Group performance status (PS) of zero to one, absolute neutrophil count >1.5 × 10^9/l, platelet count >100 × 10^9/l, total bilirubin <1.5 × upper limit of normal (ULN), aspartate and alanine aminotransferase <2.5 × ULN in absence of liver metastasis or <5 × ULN in presence of liver metastasis, and serum creatinine <1.5 × ULN. Patients with significant cardiac, vascular or infectious diseases, uncontrolled hypertension, prolonged QTc interval, cerebral metastases, or symptomatic peripheral neuropathy of grade ≥1 were excluded as well as patients completing systemic therapy within 4 weeks or having surgery within 2 weeks before treatment start. All patients signed a written informed consent approved by the ethical committee of the participating institution.

Clinical trials.gov study identifier was NCT00675012.

study design, treatment plan, and safety

This was a single-centre, single-arm phase I study evaluating two doses of NGR-hTNF in combination with XELOX. Because study population consisted of patients generally pretreated with systemic therapy, chemotherapy doses slightly lower than standard were used for both chemotherapeutic agents.

The first cohort of patients ($n = 12$) received NGR-hTNF given i.v. in 1 h at 0.8 μg/m^2, followed by oxaliplatin 100 mg/m^2 delivered as a 2-h i.v. infusion on day 1 and oral capecitabine 825 mg/m^2 twice daily (equivalent to a total daily dose of 1650 mg/m^2) for 14 days, with the first dose taken the evening of day 1 and last dose the morning of day 15. Cycles were repeated on an every 3-week basis. The second cohort ($n = 12$) was treated with NGR-hTNF administered at 45 μg/m^2, followed by oxaliplatin and capecitabine administered as above.

The assessment of two sequential dose cohorts was considered more appropriate than a randomised study design to fully explore before the safety of the combination of chemotherapy with low-dose NGR-hTNF and then the association with high-dose NGR-hTNF. Either dose of NGR-hTNF evaluated in the two cohorts was considered safe in combination with XELOX regimen if no more than 2 of 12 patients experienced dose-limiting toxicity related to the experimental drug. The choice between the low dose and the high dose of NGR-hTNF to be selected for further testing in combination with XELOX was based on an overall assessment of safety, tolerability profile, and preliminary antitumour activity observed in the two cohorts.

Toxicity was graded according to the Common Terminology Criteria for Adverse Events, version 3.0. Dose modification for chemotherapy was applied according to standard clinical practice, while no NGR-hTNF dose reduction was allowed. On recycling, patients should have recovered from all treatment-related toxicities to grade 1 or less. If a patient was unable to meet retreatment criteria, all drugs were delayed for 1 week for up to 3 weeks. At investigator's discretion, in the presence of chills, prophylaxis with paracetamol was given for the next NGR-hTNF cycles.

Tumour restaging was done every other cycle (6 weeks) according to RECIST criteria. In case of stable disease or objective tumour response, both capecitabine and oxaliplatin were given for up to six cycles, whereas NGR-hTNF was continued until progressive disease, unacceptable toxicity, or patient's refusal.

PK and PD analysis

In selected consenting patients, PK blood sampling was performed on day 1 of the first three cycles with samples drawn at baseline and on-treatment at six time points after each infusion (20, 60, 90, 120, 180, 240, and 360 min).

NGR-hTNF and sTNF receptors 1 and 2 (sTNF-R1 and sTNF-R2) levels were determined by using a validated enzyme-linked immunosorbent assay. Maximum plasma concentration (C_{max}) and area under the plasma concentration–time curve (AUC) were estimated from plasma concentration–time data using standard non-compartmental methods.

The PD variables determined for sTNF-R1 and sTNF-R2 were E_{max} (maximum plasma concentration) and AUC. The levels of NGR-hTNF and sTNF-Rs were baseline normalised by subtracting the time zero value to all other time points values.

statistical methods

Descriptive statistics were provided using medians (with ranges) and means (with standard deviations) for continuous variables and proportions for categorical variables. All analyses were based on an intention-to-treat principle. Kaplan–Meier estimates were computed for PFS, defined as the time elapsed between first treatment and disease progression or death from any cause. A post hoc explorative analysis was conducted by using the growth modulation index (GMI), defined as the ratio of either uncensored or censored patient's PFS time on current study treatment relative to uncensored PFS time observed from the patient's most recent prior treatment, which served as the patient-specific historical control value [14, 15]. To compare differences in sTNF-Rs as a function of dose, the Mann–Whitney test was used. The degree of association between two continuous variables was quantified by Spearman rank correlation coefficient.

results

patients

From January to June 2008, 12 patients (7 men and 5 women) with a median age of 57 years (range 40–73 years) and a PS of zero ($n = 11$) or one ($n = 1$) were enrolled in the low-dose cohort. Between July 2008 and May 2009, an additional 12 patients (8 men and 4 women) with a median age of 56 years (range 43–65 years) and a PS of zero ($n = 8$) or one ($n = 4$) were recruited in the high-dose cohort. All patients presented with either clinical or radiologically documented progression at entry into the trial. All but two patients had received prior systemic therapy for metastatic disease with a median of 3

treatment lines (range 1–4) in the low-dose cohort and 2 (range 0–4) in the high-dose cohort. Twenty-one patients (87%) had been previously treated with an oxaliplatin-based regimen and 19 patients (79%) with at least one targeted agent.

safety

Globally, 44 cycles (median 3 and range 2–6) were delivered in the low-dose cohort and 35 courses (median 2 and range 1–6) in the high-dose cohort. In all the 24 enrolled patients, treatment discontinuation was a result of radiologically documented progressive disease either in target lesions ($n = 7$) or in nontarget lesions ($n = 12$), symptomatic deterioration ($n = 2$), declining of further treatment by the patient ($n = 2$), and death due to rapid progression ($n = 1$).

No grade 3–4 adverse events (AEs) clearly related to NGR-hTNF were observed in both study cohorts. Only 33 (17%) of 190 study-emergent AEs were considered related to NGR-hTNF. Most frequently, they were grade 1–2 chills, experienced by 10 patients during 19 cycles (43%) in the low-dose cohort and 6 patients during 14 courses (40%) in the high-dose cohort. These events were short lived and infusion related.

Most commonly reported AEs are listed in Table 1. The combination was well tolerated without apparent difference in either frequency or intensity of AEs by dose level. Three patients experienced five grade 4 AEs. Regarding chemotherapy-associated toxicity, a dose reduction was required in three patients for 11 cycles (25%) in the low-dose cohort and three patients for 8 cycles (23%) in the high-dose cohort. Chemotherapy was discontinued early for toxicity in one patient enrolled in the low-dose cohort.

PKs and PDs

Eight and two patients had first-cycle PKs and PDs studies completed in the low-dose and high-dose cohorts, respectively. Maximum plasma concentration and AUC of NGR-hTNF increased with dose ($P = 0.03$ for both). The mean C_{max} values of NGR-hTNF at doses of 0.8 and 45 μg/m^2 were 87 and 689 pg/ml, respectively, whereas the corresponding values of AUC were 5032 and 48 576 pg × min/ml, respectively. PK parameter estimates of NGR-hTNF in this study were generally within the ranges predicted from previous phase I trials testing NGR-hTNF alone either at high dose [7] or low dose [8] and in combination with chemotherapy at low dose [12, 13].

NGR-hTNF exposure significantly correlated with the AUC of both sTNF-R1 ($r = 0.79$, $P = 0.006$) and sTNF-R2 ($r = 0.81$, $P = 0.005$).

The concentration–time profiles of both receptors remained scattered around baseline value at 0.8 μg/m^2, indicating no significant induction of circulating receptors by NGR-hTNF at this dose level. Conversely, the mean AUC values of sTNF-Rs resulted higher at 45 μg/m^2 than at 0.8 μg/m^2 (sTNF-R1: 546 versus 141 pg × min/ml, respectively, $P = 0.11$ and sTNF-R2: 1805 versus 315 pg × min/ml, respectively, $P = 0.03$). Although the mean AUC values of sTNF-R2 were significantly higher than those of sTNF-R1 (613 versus 234 pg/ml, respectively, $P = 0.02$), plasma exposure of both sTNF-Rs tended to inversely correlate with patient's progression-free intervals ($r = -0.58$, $P = 0.08$ for both).

Table 1. Most frequent AE (worst grade per patient) during or shortly after treatment

	Any grade, *N*	Grade 1, *N*	Grade 2, *N*	Grade 3, *N*
AE/low-dose cohort				
Chills	10	1	9	–
Nausea	5	4	1	–
Hypersensitivity	5	1	1	3
Abdominal pain	5	1	4	–
Paraesthesia	3	1	1	1
Pyrexia	3	3	–	–
Fatigue	2	1	–	1
Anorexia	2	1	1	–
Pain	2	1	1	–
Vomiting	2	1	1	–
Duodenogastric reflux	2	1	1	–
AE/high-dose cohort				
Nausea	8	3	5	–
Chills	7	1	6	–
Asthenia	6	2	4	–
Vomiting	5	2	2	1
Pain	5	2	3	–
Abdominal pain	4	–	4	–
Paraesthesia	4	3	1	–
γ-glutamyltransferase increased	3	–	–	3
Diarrhoea	3	2	–	1
Constipation	3	1	2	–
Hyperuricaemia	2	–	–	1
Hyperglycaemia	2	–	1	1
Anorexia	2	1	–	1
Hypertension	2	1	1	–
Presyncope	2	1	1	–
Pyrexia	2	2	–	–
Hiccups	2	2	–	–
Weight loss	2	2	–	–
Anxiety	2	2	–	–
Oedema peripheral	2	2	–	–

antitumour activity

Twenty-three patients reached their first tumour restaging and were assessable for response (Table 2), while one patient came off study because of symptomatic deterioration just after the first cycle. A waterfall plot showing the maximal tumour change in target lesions for each patient by dose level is shown in Figure 1. In the low-dose cohort, one patient (8%) achieved a partial response and five patients (42%) had stable disease for a median duration of 4.6 months (range 4.3–8.0 months), whereas in the high-dose cohort, six patients (50%) maintained stable disease for a median time of 3.3 months (range 1.6–7.5). PFS rates at 3 months were 50% [95% confidence interval (CI) 22% to 78%) in the low-dose cohort and 33% (95% CI 7% to 59%) in the high-dose cohort. The GMI, defined by patient's PFS time on current study treatment relative to his/her PFS time on prior treatment, is shown in Figure 2. Three patients treated with low-dose NGR-hTNF had longer PFS than the PFS on prior therapy lines. There was no correlation between the durations of PFS on the last previous treatment and the current

Table 2. Patient demographics, prior treatments, and antitumour activity by dose levels

Patient no.	No. cycles	No. of prior regimens	Best response to last regimen/ PFS (months)	Best response to current regimen/PFS (months)
Low-dose cohort				
1	6	1	PR/13.1	SD/5.0
2	2	2	SD/4.2	PD
3	2	4	PD	PD
4	6	3	PD	SD/8.0
5	2	3	SD/10.4	PD
6	2	2	PD	PD
7	6	4	NA	SD/4.6
8	4	1	SD/5.7	PR/1.5
9	6	2	PR/9.4	SD/4.6
10	2	3	SD/8.5	PD
11	2	3	NA	PD
12	4	4	PD	SD/4.3
High-dose cohort				
1	2	2	SD/7.1	PD
2	2	3	PD	PD
3	4	2	PR/13.1	SD/7.5
4	2	2	PD	PD
5	2	2	PD	PD
6	4	4	SD/5.0	SD/3.3
7	4	3	SD/5.9	SD/2.7
8	2	2	PD	SD/1.6
9	2	0	–	PD
10	4	4	PD	SD/3.2
11	1	2	PD	PD
12	6	0	–	SD/5.7

Even though eligibility criteria required prior treatment with no more than three prior regimens, two patients (both in the high-dose cohort) who did not have previously received systemic therapy and five patients (three in the low-dose cohort and two in the high-dose cohort) who had previously received four regimens were, however, allowed to be enrolled and were included in the study analysis. Though measurable disease was not required by study protocol, all patients presented with at least one measurable target lesion according to RECIST criteria.

No. cycles, number of treatment cycles administered in the current study; PFS, progression-free survival; NA, not assessed; PD, progressive disease; SD, stable disease; PR, partial response.

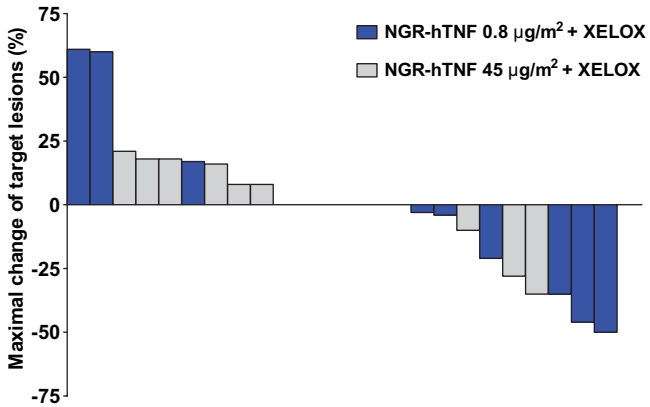

Figure 1. Waterfall diagram showing maximal change in target lesions per patient by dose level.

therapy ($r = -0.18$ in the low-dose cohort and $r = 0.46$ in the high-dose cohort).

discussion

This study has shown that both optimal biological and maximum-tolerated doses of NGR-hTNF can safely be added to XELOX with a well-tolerated and nonoverlapping toxicity profile in heavily pretreated CRC patients.

Most commonly reported toxicities were those expected from the chemotherapy regimen; however, the experimental drug also produced toxicity independently. The constitutional symptoms associated with the infusion of NGR-hTNF were mild-to-moderate in severity, short lived, and unrelated to dose. Notably, only 17% of all study-emergentAEs were considered clearly related to NGR-hTNF.

A further aim of this study was to evaluate the PD effect of the experimental drug on circulating TNF receptors. Both receptors for TNF can be proteolytically cleaved as soluble forms, with their shedding kinetics being linearly correlated with the TNF serum levels [16]. By competing for TNF with cell-surface receptors, these circulating receptors might block its bioavailability likely acting as physiological attenuators of TNF activity. Consistent with data from previous clinical trials [7, 8, 12, 13], an early induction of TNF receptor shedding was detected mainly following high-dose NGR-hTNF. Interestingly, the receptor levels tended to inversely correlate with progression-free times, thus supporting preclinical data suggesting the role of sTNF-R2 in regulating NGR-hTNF activity [10].

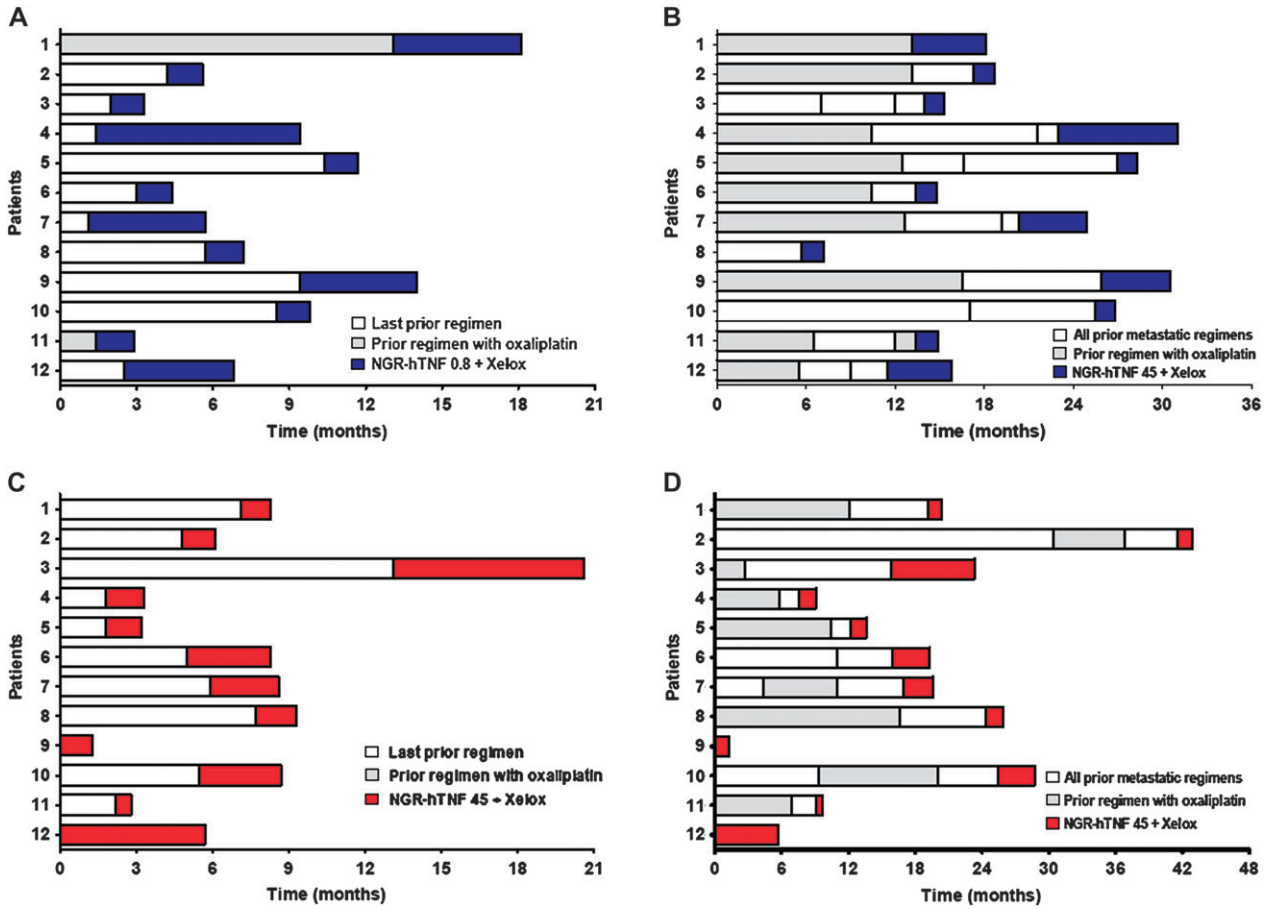

Figure 2. PFS duration for each patient enrolled in the low-dose cohort during the current study treatment versus (A) the last prior regimen and (B) all prior metastatic regimens. PFS duration for each patient enrolled in the high-dose cohort during the current study treatment versus (C) the last prior regimen and (D) all prior metastatic regimens. Each box in the horizontal bars indicates a treatment line administered for the metastatic disease. In panel A and C, the box on the left indicate the PFS time during the last prior regimen and the box on the right indicate the PFS time during the current study treatment. In panel B and D, all metastatic treatment lines are also shown. The patients 3, 7, 10, and 12 in the low-dose cohort and the patient 10 in the high-dose cohort received also adjuvant therapy (not shown in these panels). PFS, progression-free survival; XELOX, capecitabine + oxaliplatin.

Even though antitumour activity assessment was not the primary end point of this small-size, single-arm combination study, some comments about hint of activity are warranted (Table 2 and Figure 2). In the high-dose cohort, no responses were observed (Table 2). In addition, none of the stable diseases observed in this cohort lasted longer than the PFS reported from the previous treatment lines, suggesting very little if any activity (Figure 2).

However, among the 12 patients treated with XELOX plus low dose of NGR-hTNF, 6 had tumour growth control, 1 had partial response, and 5 had stable diseases (Table 2), and 3 of these had PFS longer than those reported for the previous treatment, indicating unequivocal antitumour activity. The patient number in this series is too low to conclude for a sufficient level of activity for phase III testing, but it is enough for a more formal and complete phase II trial, with a larger patient number. This is particularly true in the light of the lower than standard capecitabine and oxaliplatin doses used here and in consideration of the very favourable toxicity profile of NGR-hTNF. Current development plans include phase II testing of this combination in first line (with low dose of NGR-hTNF and regular dose of XELOX). Consideration is also given to the feasibility of more frequent NGR-hTNF administrating (weekly).

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disclosure

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