

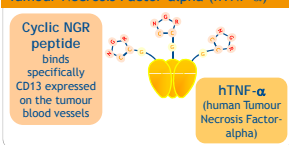
**ABSTRACT**

**Background:** NGR-hTNF is a VTA exploiting a tumour homing peptide (NGR) selectively binding angiogenic vessels in solid tumours, where NGR-hTNF specific binding relies on dynamic interactions with TNF-receptors and aminopeptidase N/CD13. NGR-hTNF combines activity on tumour vascular permeability and direct anticancer activity, both at low doses and at high doses. **Methods:** Pts with unresectable, recurrent or metastatic HCC were treated with 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 first and second stage, respectively. Progression-free survival (PFS) was the primary endpoint and tumour reassessment was performed q6w. **Results:** To date, 22 pts with progressive disease following prior loco-regional treatment (59%), systemic therapy (41%), or both (18%), have been recruited. Pts characteristics were: M/F 17/5; median age 67 years (range, 53 to 79); PS 0/1/17/5; Child-Pugh score A/B: 18/4. Globally, 63 cycles (median, 2; range, 1 to 8) were administered and 6 pts (27%) have received ≥4 doses. Neither grade 3-4 treatment-related adverse events nor toxicity-related death were observed. Main grade 1-2 toxicities per patient were infusion-related constitutional symptoms, including chills (59%), and transient blood pressure increase (14%). A confirmed partial response lasting 3.3+ months was observed in one patient with lung and node metastases. Stabilization of disease occurred in an additional 5 pts with a median duration of 4.3 months (range, 2.6 to 5.9 months). The median and 3-month PFS were 2.5 months (95% CI, 1.8 to 3.1 months) and 42% (95% CI, 18 to 63%), respectively. Currently, the study is completing recruitment into the second stage. **Conclusion:** NGR-hTNF given at 0.8 µg/m<sup>2</sup> q3w is well tolerated and appears to induce promising disease control in pre-treated patients with advanced HCC. The drug will be further developed as single agent exploring a weekly schedule of administration in this setting.

**Background**

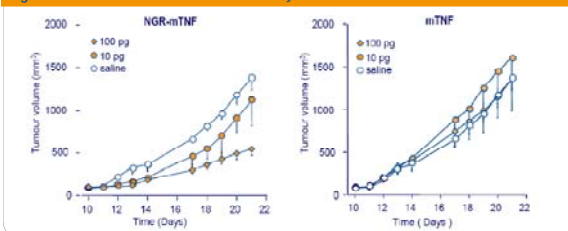
- A large body of preclinical evidences have shown that tumour necrosis factor-α (TNF-α) has potent antitumour activity. However, its clinical use has been hampered by severe systemic toxicity, with MTD significantly lower than ED in humans<sup>1</sup>
- The antivascular effects of TNF-α provided the 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 hepatocellular carcinoma

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



- In preclinical models<sup>4</sup>, NGR-mTNF was found to have antitumour activity also at doses in the picogram range (100 pg) (Figure 2), equivalent to a dose of 0.2 µg/m<sup>2</sup> in humans, the selected starting dose in phase I trial (Figure 3) NGR-mTNF

**Figure 2. Preclinical antitumour activity at low doses of NGR-mTNF and mTNF**



**Phase I trials of NGR-hTNF**

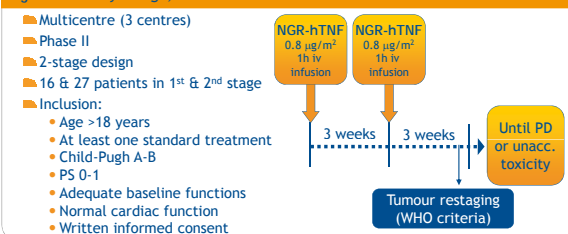
- In a phase I study evaluating a dose-interval ranging from 0.2 to 60 µg/m<sup>2</sup> the MTD of NGR-hTNF was established at 45 µg/m<sup>2</sup> when given as single agent once every 3 weeks<sup>5</sup>
- Conversely, a further phase I trial exploring the low-dose range of NGR-hTNF from 0.2 to 1.6 µg/m<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**

- Hepatocellular carcinoma (HCC) is the 3<sup>rd</sup> leading cause of cancer deaths worldwide and a disease that has increased in incidence in the western world over the past 20 years
- Until recently, there has been no agreed upon standard therapy for the significant majority of HCC patients whose tumours are not amenable to potentially curative therapy<sup>7</sup>
- In a recent phase III trial, Child-Pugh A patients treated with sorafenib experienced a significantly longer OS and TTP compared with patients receiving placebo. Additionally, a two percent partial response rate was reported in sorafenib-treated patients<sup>8</sup>
- Interestingly, HCC is a highly hypervascular tumour in which neovascularisation contributes to growth and metastasis<sup>9</sup>

**Methods**

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



**Results**

- From February 2007 to June 2008, twenty-seven patients with advanced hepatocellular cancer and previous treatment with loco-regional and/or systemic therapy were enrolled in this phase II study
- Baseline characteristics are summarised in Table 1

**Table 1. Baseline characteristics**

Characteristics	n=27 (%)
Median age, years (range)	67 (34-79)
Gender	
Male	21 (78)
Female	6 (22)
ECOG performance status	
0	18 (67)
1	9 (33)
Child-Pugh status	
A	21 (78)
B	6 (22)
AFP >400 ng/mL	
Yes	9 (33)
No	18 (67)
Prior treatments	
Resection/Transplantation	9 (33)
Ablation	5 (18)
TACE	16 (59)
Sorafenib/Chemotherapy/Hormonal therapy	15 (56)

**Safety**

- 82 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 (55%), and transient blood pressure increase (11%), generally occurring approximately 30 minutes after the start of the first infusions and lasting about 20 minutes

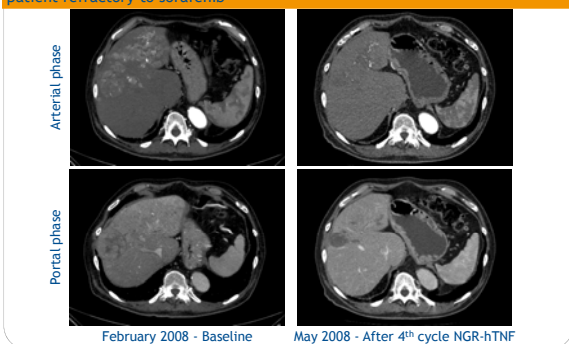
**Table 2. Treatment-related adverse events occurring in > 5% of patients**

Event	Grade 1	Grade 2	Grade 3	Grade 4
Chills	9 (33%)	6 (22%)	-	-
Blood pressure increase	3 (11%)	-	-	-
Fatigue	2 (7%)	-	-	-

**Efficacy**

- One complete response (CR) lasting 6.6+ months and one partial response (PR) lasting 4.4 months were observed
- An additional 6 patients had stable disease (SD) with a median duration of 3.8 months
- A 76-year-old sorafenib-refractory patient, with a large (6.5 x 5.5 cm) hepatic mass and extensive multifocal lesions, had dramatic tumour shrinkage starting from the 2nd cycle with complete necrosis of the primary lesion and absence of any foci of contrast-enhancement in the remaining parenchyma. The baseline and post 4th cycle CT scans are shown in Figure 4
- Preliminary efficacy results are reported in Table 3 and the actuarial progression-free survival curve is depicted in Figure 5 (3 patients censored)
- For patients achieving SD/PR/CR as best response, the 6-month PFS rate was 25%

**Figure 4. Complete necrosis in a hypervascular, infiltrating HCC after 4 cycles of NGR-hTNF, evaluated by contrast-enhanced CT scan in a 76-year-old patient refractory to sorafenib**

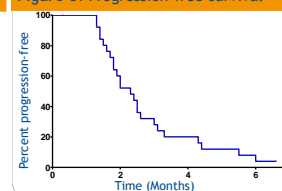


**Table 3. Time-related efficacy data**

Variable	Estimate (months)	Range
Median PFS	2.3	1.4-6.6
Median PFS in pts with SD/PR/CR	4.3	3.0-6.6
Median duration of SDs	3.8	2.6-5.9

PFS=progression-free survival; Pts=patients; SD=stable disease; PR=partial response; CR=complete response

**Figure 5. Progression-free survival**



**Conclusions**

- NGR-hTNF administered at low dose is safe and shows preliminary evidence of activity in pretreated patients with advanced HCC
- 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 will be further developed as single agent in pretreated patients, also exploiting a weekly schedule of administration
- The mechanism of action of NGR-hTNF, along with its safety profile, should also facilitate the combination with standard regimens

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