Abs #3540

Safety and anticancer activity of low dose regimen of NGR-hTNF, a new vascular targeting agent, in solid advanced malignancies (NGR002 phase I trial)

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Abstract

Background: NGR-hTNF is a vascular targeting agent (VTA) exploiting a tumour homing peptide (CNGRCG) 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. Consistently, mouse preclinical data indicate significant synergy between low dose NGR-hTNF and cytotoxic agents.

Methods: 4 dose levels of NGR-hTNF (0.2 up to 1.6 μg/m²) have been administered q3w in 16 patients. Main end-points included safety, anticancer activity and pharmacokinetic. Measurement of circulating tumor and endothelial cells (CTC and CEC), sTNFRI and sTNFRII, along with plasma cytochemokine profile have been performed.

Results: 16 patients were enrolled (6F/10M); median age 60, range 43-73). Toxicity was limited to constitutional symptoms, and chills were the most frequent event (40%). Over a median follow-up of 15 weeks, stable disease was achieved in 44% of patients, with long lasting disease control in 2 cases (27 and 75 weeks, with establishment of indication to radical surgery after 75 weeks, presently tumor free after removal of the residual tumor mass). In these 2 patients, VEGF, MMP-9, CA125, significantly decreased over time. DCE-MRI indicates that NGR-hTNF increases vascular permeability after first drug exposure, particularly at the dose of 0.4 μg/m², while following multiple infusions it exerts an antivascular effect, as demonstrated by the decrease of K^{trans} values. Moreover NGR-hTNF is able to elicit inflammatory and immune responses over time, as indicated by the modulation of expression of multiple cyto-chemokines. Finally, changes in CTC levels over time consistently matched the clinical outcome.

Conclusions: Low dose NGR-hTNF has an optimal safety profile along with anticancer activity acting on tumour vasculature and inducing relevant biological effects, thus rendering the agent suitable for a development both as monotherapy and in combination with chemotherapeutics. The phase II program is due to start in early 2007.

Background

- A large number of preclinical studies have shown that tumor necrosis factor-alpha (TNF-α) has potent antitumor activity. However, its clinical use is hampered by severe systemic toxicity, with MTD significantly lower than ED in humans[1] .
- More recently, isolated limb or hepatic perfusion with high doses of human TNF-α (hTNF-α) in combination with chemotherapeutic agent has produced high complete response rates in patients with melanoma or sarcoma of the extremities[2], as well as regression of bulky hepatic cancers confined to the liver[3].
- The antivascular effects of TNF 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 human TNF with the tumor-homing peptide Cys-Asn-Gly-Arg-Cys (NGR), a ligand of a CD13 (aminopeptidase N) isoform expressed by endothelial cells of newly formed human tumor vessels[4,5] (Figure 1).
- NGR-mTNF was found to have antitumor activity at doses in the picogram range (equivalent to a dose of 0.2 μg/m² in humans) in mice bearing RMA lymphoma[6] (Figure 2)
- The main objective of this phase I trial is to explore the safety profile of systemic administration of low doses of NGR-hTNF and to document its antivascular effects and preliminary antitumor activity.

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

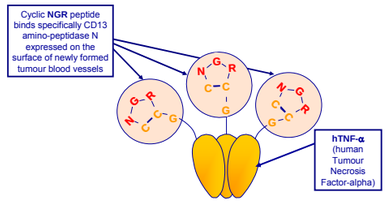
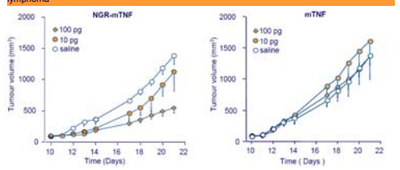


Figure 2. Antitumor activity at low doses of NGR-mTNF and mTNF in mice bearing RMA lymphoma



Methods

- Sixteen patients with advanced solid tumors refractory to standard treatments were enrolled at four escalating dose levels of NGR-hTNF (0.2-0.4-0.8-1.6 μg/m²) administered as 60-minute IV infusion every three weeks.
- These low dose levels have previously been tested and considered safe in an ongoing phase I trial aiming at exploring the entire dose range and defining the MTD of NGR-hTNF administered as single agent.
- Objective
  - To select the optimal biologic low dose of NGR-hTNF for further development in phase II trials by evaluating the:
    - safety profile (using NCI-CTC version 2.0)
    - changes on dynamic imaging (by DCE-MRI performed 48 hours before and 2 hours after the first and subsequent cycles)
    - PK and biomarkers analysis (with blood samples obtained just prior to and 6 time points up to 240 minutes after each infusion)
    - preliminary antitumor activity (according to RECIST criteria with re-assessment performed every 6 weeks)
- Key inclusion and exclusion criteria:
  - Patients >18 years old
  - ECOG performance status 0-2
  - Adequate baseline bone marrow, hepatic and renal function.
  - Absence of any conditions in which hypervoleamia or haemodilution could represent a risk for the patient
  - Normal cardiac function and absence of uncontrolled hypertension
  - No clinical signs of CNS involvement
  - Written informed consent to participate in the study

Results

Table 1. Baseline demographics, disease characteristics, and prior treatments

Characteristics	No. of patients (N=16)
Male/Female	10/6
Median age in years (range)	60 (43-73)
ECOG performance status	
0/1/2	5/10/1
Primary tumor type	
Colorectal	6
Renal	3
Liver/NSCLC	2
Carcinoid/Pancreatic/Gastric	1
Median number of prior chemo- or immuno-therapy lines (range)	4 (1-5)
1 line	16 (100%)
2 lines	12 (75%)
3 lines	10 (63%)
>3 lines	7 (44%)

Safety

- A total of 83 cycles of therapy were administered with a median of 2 (range:1-29).
- Neither grade 4 AE nor toxicity-related death were observed in the study population.
- The most frequent treatment-related adverse event was chills (n=11/16 patients), generally occuring approx 30-40 minutes after the start of the first infusion (n=7/11), and lasting about 20 minutes. This adverse event promptly resolved without (6/11) or with (5/11) appropriate treatment.
- Among all dose level cohorts (Table 2), only one patient treated at 1.6 μg/m² had grade 3 treatment-related adverse events (chills and dyspnea).
- Seven patients experienced grade 2 treatment-related AEs: chills (n=4), transient hypertension (n=2), fever (n=1).

Table 2. Treatment-related nonhematologic AEs by dose level and number of patients

Dose Levels (μg/m²)	No.pts with AE/ total pts per DL	Grade 1	Grade 2	Grade 3
0.2	1/4	chills, asthenia, tachycardia	fever	-
0.4	3/4 1/4	chills hypertension, vomiting	- -	- -
0.8	2/4 1/4	chills hypertension	- chills	- -
1.6	3/4 2/4 1/4	- hypertension -	chills hypertension -	- chills, dyspnea

Antitumor activity

- Out of eleven patients evaluable for response after at least two cycles of therapy, 6 had a stable disease with a median duration of 8 months (Table 3)

Tabe 3. List of patients with stable disease (SD)

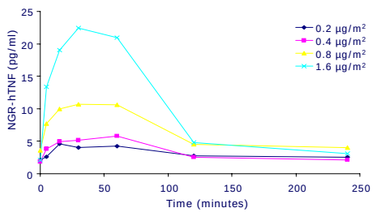
DL	Pt. No.	Gender/ Age (yrs)	Primary Tumor	Metastatic Sites	No.of prior Chemo-or Immuno-therapy Regimens	Dose (μg/m²)	Duration of SD (months)
1	1	F/61	Colon	Liver, peritoneal carcinosis	8	0.2	2
	3	F/43	Colon	Abdomen	4	0.2 (0.8)	18
2	9	F/43	Carcinoid	Liver	4	0.4	9
3	6	M/56	Renal	Nodes, bone	4	0.8	6
	11	M/70	Liver	Adrenal gland, nodes	6 (chemoem.)	0.8	11
4	14	F/70	NSCLC	Lung	2	1.6	7

- Notably, a very long-lasting SD was observed in a 43-year-old female patient with metastatic colon cancer refractory to three previous standard chemotherapy regimens administered in less than one year.
- She experienced a 18-month stable disease during treatment with NGR-hTNF given at 0.2 μg/m² for nine cycles, followed by twelve cycles administered at 0.8 μg/m².
- After twenty-one courses she successfully underwent radical metastasectomy of a 25-centimenter large abdominal (ovarian) mass.
- No significant cumulative toxicity during the treatment period was observed and the patient remains on study in a disease-free state following eight additional postoperative courses of NGR-hTNF delivered at 0.8 μg/m².
- In addition, it is worth noting that circulating tumor cells (CTCs) were undetectable before and after any NGR-hTNF post-operative course, and that circulating endothelial cells (CECs) progressively decreased over time, attaining values within the normal range from cycle 21 and onwards.

Pharmacokinetic analysis

- Concentration-time profiles for each patient were adjusted for baseline levels (pre-dose) prior to pharmacokinetic analysis, due to innate levels of TNF are generally present in patients with advanced solid tumors. Moreover, as mean plasma concentrations (adjusted for baseline) were similar between dosing cycles, pharmacokinetic data were summarized for all cycles at each dose level (Figure 3).
  - Following a 60-minute IV infusion of NGR-hTNF, plasma concentrations were detectable up to 4 hours post-dose.
  - Both maximum plasma concentration (C_{max}), reached after 15-60 minutes, and the area under the plasma concentration–time curve (AUC) were dose-related.

Figure 3. Plasma concentration-time profile for patients enrolled at each dose level

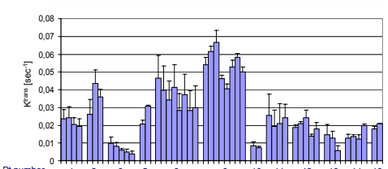


- Due to an extremely high variability of baseline levels of soluble TNF receptors (sTNF-RI and sTNF-RII), values obtained at different time points after each cycle were normalized against baseline levels, by subtracting the time 0 value to all other time points.
  - Overall, the data would suggest a treatment-induced release of soluble receptors. This effect was more evident in patients treated at 1.6 μg/m² and at this dose level, an increase two-fold higher for sTNF-RII than for sTNF-RI was observed.
  - However, no definitive conclusions can be drawn due to the limited number of patients evaluated and the very high interpatient variability at baseline.
- None of the 15 out of 16 patients evaluated for the detection of anti-NGR-hTNF showed circulating antibodies during treatment.

Dynamic Imaging

- The NGR-hTNF dose-antivascular effect relationship was evaluated in 12/16 patients with functional dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).
  - Overall, 8 out of 12 patients showed a reduction over time of the permeability surface area (K^{trans}) parameter. Of note, out of 6 patients achieving SD, 5 showed a decrease in K^{trans} values over time (Figure 4)
  - Interestingly, 3/4 patients enrolled at 0.8 μg/m² dose level had a relative decrease after the 1st cycle versus baseline value (Figure 5).
  - Figure 6 shows the progressive decline of K^{trans} values over time for the patient experiencing a 18-month stabilization of disease.

Figure 4. Average profile over time of K^{trans} (over all ROIs) for each patient



DCE-MRI performed 48h before the 1st administration and 2h after each cycles (columns)

Figure 5. Average relative difference of K^{trans} (over ROIs) after 1st cycle *vs* baseline

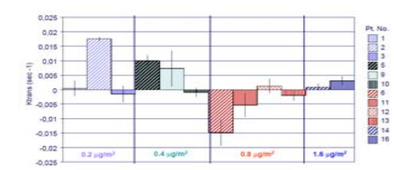
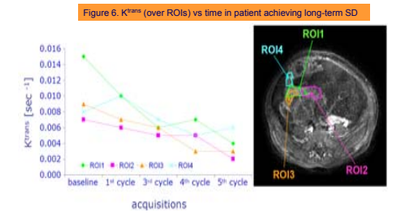


Figure 6. K^{trans} (over ROIs) vs time in patient achieving long-term SD



Biomarkers analysis

- In order to select biological markers potentially related to NGR-hTNF therapy, samples collected at 0-120-240 minutes after the treatment start from 15/16 patients were analysed for *Multi-Analyte Profile* testing (Rules Based Medicine Inc., Austin, TX), a screening analysis in which up to 90 markers are simultaneously evaluated on each sample.
- Screening analysis identified some analytes, including MIP-1β (Macrophage Inflammatory Protein-1 beta), for which a significant ($p<0.05$) increase was observed, and this increase was dose-related.

Conclusions

- NGR-hTNF administered at very low doses ranging between 0.2 and 1.6 μg/m² is safe and well tolerated in heavily pretreated patients with advanced solid tumors refractory to standard treatments.
- The proportion of patients achieving a stable disease in the present study is substantially similar to that reported in a recent meta-analysis on the preliminary antitumor activity registered in the phase I oncology setting[7], and is remarkable considering the extremely low doses administered and the easily manageable safety profile.
- Furthermore, long-lasting SDs (≥6 months) have been observed in five patients.
- Interestingly, an antivascular effect in terms of K^{trans} reduction, denoting changes in tumor vascular permeability or blood flow, was reported in 8 out of 12 assessed patients.
- Presently, NGR-hTNF is continuing the clinical phase I development aiming at establishing the maximum-tolerated dose in another trial, attaining 45 μg/m² dose level without evidence of DLTs/MTD.
- Based on the favourable toxicity profile, the preliminary antitumor activity, and the antivascular changes on dynamic imaging, the 0.8 μg/m² dose level has been selected for further phase II development both as single agent in selected tumor types and in combination with standard chemotherapeutics.

References

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