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MONOGRAPH

NGR-hTNF

Vascular Targeting Agent
Oncolytic

Human TNF fused with the NGR peptide that targets the tumor neovasculature by binding to CD13 (CNGRC peptide)
Asparagine-glycine-arginine (NGR) peptide-tumor necrosis factor alpha conjugate

EN: 400626

SUMMARY

NGR-hTNF is a vascular targeting agent that consists of a modified form of human TNF-α fused with an NGR peptide. The NGR peptide moiety of the compound acts by selectively targeting blood vessels, where TNF is released, resulting in the destruction of the tumor's blood supply and subsequent reduction in the size of the tumor. The NGR-mediated targeted delivery of TNF overcomes toxicity associated with systemic delivery of the agent. The compound has demonstrated good tolerability and promising antitumor activity in several phase I and II clinical studies in patients with a wide range of advanced/metastatic solid tumors, including malignant pleural mesothelioma, colorectal and hepatocellular carcinoma, as well as non-small cell lung cancer (NSCLC), administered either as monotherapy or in combination with chemotherapeutic regimens. NGR-hTNF is currently in phase III clinical development for the treatment of advanced malignant pleural mesothelioma.

PREPARATION*

NGR-TNF is obtained by recombinant DNA technology. In order to fuse the human TNF1-157 with the *C*-terminus of CNGRCG peptide, a polymerase chain reaction (PCR) is performed on plasmid pET11b/hTNF –containing human TNF cDNA– with specific primers encoding the CNGRCG sequence. The amplified fragment is then digested and cloned into plasmid pET-11b (NdeI/BamHI), which is transformed into *Escherichia coli* BL21 (DE3). The expression of NGR-TNF is induced with isopropyl-β-D-thiogalactoside. After fermentation, bacterial lysis is performed by sonication followed by centrifugation; the extract is then mixed with ammonium sulfate in order to precipitate proteins. The purification process continues with a hydrophobic interaction chromatography on a Phenyl-Sepharose 6 Fast Flow column, followed by ion-exchange chromatography on a DEAE-Sepharose Fast Flow column. Subsequently, trimers with the correct intrachain disulfide bridges are isolated by a four-step denaturation-refolding process using urea. Finally, the product is dialyzed and size-exclusion chromatography is performed on an HR Sephacryl S-300 column (1, 2).

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BACKGROUND

The effectiveness of chemotherapy in solid tumors depends largely on the ability of the drug to penetrate the neoplastic cells that are distant to the tumor vessels. Cyclic and linear peptides comprising the asparagine-glycine-arginine (NGR) motif identified by in vivo screening of phage display libraries have been shown to selectively recognize tumor vasculature and could potentially be exploited for the ligand-directed targeted delivery of anticancer agents to tumors or angiogenic tissues. The ability of such peptides to associate with the neovasculature relies on the formation of an interaction with a specific isoform of aminopeptidase N (CD13), which is expressed in angiogenic blood vessels but not in normal epithelium (3). Conjugation of an NGR-containing peptide (CNGRC) to the *N*-terminus of human TNF-α, a cytokine with potent antitumor effects that has the ability to alter the function of the endothelial barrier, reduces the interstitial pressure of tumors and consequently increases the penetration of chemotherapeutic molecules, led to the generation of the vascular targeting agent NGR-hTNF (4).

Systemic administration of TNF-α in preclinical studies has been associated with excessive toxicity and the maximum tolerated dose (MTD) has been determined to be 10-50 times lower than the estimated effective dose, thus resulting in compromised activity. Furthermore, the infusion of TNF in vivo or in humans has been linked to the induction of negative feedback mechanisms, such as the release of soluble p55 and p75 receptors that suppress the binding of TNF-α to receptors on the surface of cellular membranes. The intratumoral delivery of small amounts of TNF-α using NGR-hTNF was shown to improve the effects of chemotherapeutic compounds, such as melphalan or doxorubicin, without increasing the toxicity of these molecules, and did not correlate with the release of soluble TNF receptors into the circulation (5). Vascular targeting mediated by NGR-hTNF may therefore represent an efficient means to augment the effectiveness of chemotherapeutic modalities.

PRECLINICAL PHARMACOLOGY

In vitro pretreatment of murine T-cell lymphoma RMA cells with NGR-mTNF, a conjugate of NGR with the murine isoform of TNF-α, at 2 hours prior to the application of doxorubicin, paclitaxel, melphalan, cisplatin or gemcitabine for 48 hours had no impact on the cytotoxic effects of these agents. The cytotoxic activities of doxorubicin and melphalan in murine mammary adenocarcinoma TS/A and

murine fibrosarcoma WEHI-164 cells, respectively, were not affected by preincubation of these cell lines with NGR-mTNF. However, synergism between NGR-mTNF and chemotherapeutic agents was observed in vivo in mice bearing subcutaneous RMA, TS/A or WEHI-164 tumor xenografts, suggesting that the effects of NGR-mTNF may be mediated by the host cells rather than tumor cells. In mice bearing tumors resulting from the implantation of RMA cells, treatment with cisplatin alone (30 µg) demonstrated little or no antitumor activity, whereas prior administration of NGR-mTNF (0.1 ng) at 2 hours before cisplatin correlated with a significant antitumor response. Administration of cisplatin at 1 or 3 hours after NGR-mTNF resulted in a reduced antitumor response compared with the 2-hour interval. Similar effects were seen in RMA tumor-bearing animals treated with paclitaxel (60 or 80 µg), gemcitabine (1.8 mg) or melphalan (90 µg) following NGR-mTNF given at 2 but not at 1 or 3 hours prior to administration of the chemotherapeutic agent, implying a critical role in the timing of treatment administration. TNF is known to induce intravascular coagulation and vascular damage resulting in the occlusion of blood vessels, and therefore the effects of NGR-mTNF on tumor perfusion were assessed in C57BL/6 and BALB/c mice bearing RMA or WEHI-164 tumor xenografts. Mice received NGR-mTNF at doses of 0.1 or 10,000 ng (the latter dose is known to induce vascular damage and occlusion), and following a delay of 2 or 5 hours, the animals were given a dose of the nontoxic dye Patent Blue. Evaluation of the amount of Patent Blue present in tumors by spectrophotometric techniques at 5 minutes after administration of the dye revealed no impact for the low dose of NGR-mTNF, whereas the high dose was associated with almost complete inhibition of the perfusion of tumors. Repeated dosing of NGR-mTNF (0.1 ng given at 0 and 2 hours) in combination with cisplatin (40 µg administered at 2 and 4 hours) to mice bearing RMA tumor xenografts correlated with greater antitumor response compared to animals receiving a single 0.1-ng dose of NGR-mTNF at 0 hours prior to cisplatin. The findings indicated that the lack of synergism between NGR-mTNF and cisplatin when provided with a 4-hour delay may be due to the clearance of NGR-mTNF (6).

SAFETY

Evaluation of the dose-limiting toxicity (DLT) and establishment of the MTD of NGR-hTNF in patients with advanced solid tumors were the primary outcome measures of an open-label, interventional phase I trial. Secondary objectives included assessment of the clinical response by Response Evaluation Criteria in Solid Tumors (RECIST) and investigation of the mechanism of action of the agent by dynamic imaging (EORTC 16041) (7). Participants (N = 34) were treated with 20-minute i.v. infusions of NGR-hTNF at doses of 0.2, 0.4, 0.8, 1.3, 1.95, 2.6, 3.46, 4.6, 6.1 or 8.1 µg/m^2 given once every 3 weeks. Chills, fever, fatigue, bronchospasm, hypotension and nausea (n = 23, 12, 10, 1, 2 and 13, respectively) were the most frequent drug-related adverse events (AEs). Bronchospasm of grade 3 in severity was identified as the DLT in one patient treated with the dose of 1.3 µg/m^2 (8).

Treatment with NGR-hTNF showed no effects on DCE-MRI, which failed to identify the optimal biological dose of the molecule (9). Following additional dose escalation up to a dose of 60 µg/m^2, the MTD of NGR-hTNF was established at 45 µg/m^2. Pharmacokinetic (PK) profiling indicated dose-proportional increases in AUC and C_{max}. No anti-NGR-hTNF antibodies were detected (10). Additional dose escalation of NGR-hTNF (60, 80, 100 and 125 µg/m^2 given as a 2-hour infusion) was conducted in 12 patients with refractory solid tumors who were given a 1000-mg dose of acetaminophen prior to treatment with NGR-hTNF. The incidence of chills (grade 1 in severity) was reported as the most common drug-related AE seen in 58% of participants (11).

CLINICAL STUDIES

The safety and biological activity of NGR-hTNF were investigated in an open-label, nonrandomized, uncontrolled, single-group-assignment phase I study in patients with advanced solid tumors. The trial also aimed to determine the optimal biological dose of the agent (12). Sixteen volunteers received escalating doses of NGR-hTNF (0.2-1.6 µg/m^2) every 3 weeks. Stable disease was achieved by 44% of subjects over a median follow-up period of 15 weeks. DCE-MRI data showed increased vascular permeability following the first exposure to NGR-hTNF at a dose of 0.4 µg/m^2; however, multiple infusions with the agent correlated with an antivascular effect (13).

Verification of the safety of escalating doses of NGR-hTNF (0.2-1.6 µg/m^2) administered as a 1-hour infusion in combination with a fixed dose of cisplatin (80 mg/m^2) every 3 weeks in patients with advanced or metastatic solid tumors was the primary outcome measure of an open-label, nonrandomized, uncontrolled, single-group-assignment phase Ib study. Documentation of preliminary antitumor effects based on the rate of objective response by RECIST and assessment of the PK profile of the combination regimen were among the trial's secondary objectives (14). Tumor types included mesothelioma, sarcoma and melanoma, as well as lung and colorectal carcinoma (15). Participants had received a median number of three prior treatment regimens, including platinum- and oxaliplatin-based therapies (16). In 22 evaluable subjects, the combination of NGR-hTNF with cisplatin was described as well tolerated. PK analysis revealed no drug–drug interactions and indicated dose-proportional C_{max} and AUC values for NGR-hTNF (respective r^2 values of 0.91 and 0.67; P = 0.0001 and 0.001, respectively). No shedding of soluble TNF receptor 1 (TNF-R1) was observed following treatment with NGR-hTNF up to the dose of 0.8 µg/m^2 (17). No exacerbation of the toxicity of cisplatin was noted. The MTD was not attained and there were no DLTs at the dose range under investigation, except for a transient acute infusion reaction of grade 3 in severity observed in one patient in the 0.8 µg/m^2 cohort. The median progression-free survival (PFS) for the entire population of the study was estimated at 2.7 months. In patients receiving the 0.8 µg/m^2 dose (n = 12) and individuals pretreated with a platinum-based therapy (n = 9), the median PFS was 4.7 and 4.3 months, respectively (18).

An inverse association between the levels of intercellular adhesion molecule 1 (ICAM-1), matrix metalloproteinase-9 (MMP-9) and the clinical benefit of NGR-hTNF was identified in a phase I trial in 15 patients with refractory tumors. Participants were treated with NGR-hTNF (0.2-1.6 µg/m^2) administered every 3 weeks until disease progression. The levels of ICAM-1 correlated inversely with both the duration of treatment (r = –0.56; P = 0.03) and overall survival (r = –0.66; P = 0.01) of subjects at baseline and following treatment with the agent (r = –0.55 and P = 0.03 for the duration of treatment; r = –0.70 and P = 0.009 for overall survival). Following treatment

with NGR-hTNF, the levels of matrix metalloproteinase MMP-9 correlated inversely with overall survival, whereas a moderate inverse association was reported for the duration of treatment (r = –0.57 and –0.46, respectively; P = 0.03 and 0.08, respectively). Analysis by receiver operating characteristics (ROC) revealed that concentrations of ICAM-1 of ≤ 171 ng/mL at baseline and levels of MMP-9 of ≤ 47 ng/mL after treatment with NGR-hTNF could predict the duration of treatment with a sensitivity and specificity of 87% and 71%, respectively (19).

The antitumor activity of NGR-hTNF monotherapy was the primary outcome measure of an open-label, nonrandomized, single-group-assignment phase II trial performed in patients with advanced or metastatic malignant pleural mesothelioma (20). The agent was administered as a 1-hour i.v. infusion at a dose of 0.8 μg/m^2 once every 3 weeks to 43 subjects with progressive disease following treatment with pemetrexed/platinum-based regimens (21). The study was designed in a 2-stage protocol that involved the enrollment of 16 and 27 participants, respectively, in stages 1 and 2 (22). Safety assessments conducted in 43 individuals following 170 cycles of treatment revealed transient chills of grade 1-2 in severity developing during the initial infusions as the most frequent toxicity (71%). The median PFS was estimated at 2.8 months and the rate of disease control was established at 44%. A partial response (PR) lasting for 10 months was observed in 1 subject and 18 individuals exhibited stable disease (SD) with a median duration of 4.3 months. A separate cohort of 14 patients received a total of 242 infusions of NGR-hTNF (0.8 μg/m^2) administered on a once-weekly schedule. No exacerbated toxicity was reported in this treatment group. SD with a median duration of 8.1 months was reported in 50% of subjects receiving weekly doses of the treatment. The 6- and 12-month PFS rates were determined to be 36% and 19%, respectively. In the overall study population (N = 57) the rate of disease control was calculated at 46%, with a median duration of 4.7 months, and the median overall survival (OS) was 13.1 months (23).

Individuals with advanced or metastatic hepatocellular carcinoma (HCC) who had previously been treated with no more than one systemic anticancer therapy and/or locoregional treatment were included in an open-label, nonrandomized, single-group-assignment phase II study designed to investigate the antitumor effects of single-agent NGR-hTNF given as a 1-hour infusion (0.8 μg/m^2 i.v.) every 3 weeks (24). PFS was the primary endpoint of this trial. Tumor reassessments were performed every 6 weeks (25, 26). Infusion-related constitutional symptoms, including chills (55%) and transient increases in blood pressure (11%), were the most common toxicities of grade 1-2 in severity. There were no reports of grade 3-4 treatment-related AEs and no toxicity-induced deaths were observed (27). The median PFS was 2.3 months. A subject with sorafenib-refractory HCC exhibited a complete response lasting > 9 months and a PR was reported in 1 patient with Child-Pugh class B. Tumor shrinkage of 28% was seen in one of six subjects with SD (28). After a median follow-up period of 14 months, the median OS was 9.1 months, with survival rates at 12 and 18 months of 34% and 22%, respectively. In 12 subjects receiving treatment with the 0.8 μg/m^2 dose of NGR-hTNF on a once-weekly basis, no worsening of toxicity was reported. The rate of disease control in this cohort was estimated at 33% (29).

The combination of NGR-hTNF with doxorubicin for the treatment of patients with advanced or metastatic small cell lung cancer who had previously been treated with at least one chemotherapeutic agent was investigated in an open-label, nonrandomized, single-group-assignment phase II trial (30). A total of 16 subjects received doxorubicin (75 mg/m^2 i.v.) at 60 minutes after i.v. infusion of NGR-hTNF (0.8 μg/m^2) every 3 weeks. Administration of NGR-hTNF was continued until disease progression was observed, whereas doxorubicin was given until a cumulative lifetime dose of 550 mg/m^2 was reached. NGR-hTNF did not appear to alter the toxicity profile of doxorubicin. Brief, infusion time-related chills were the most frequent AEs classified as grade 1-2 in severity (56%). The median PFS was 3 months. The overall rate of disease control was estimated at 50% (including PR and SD; 25% each) (31).

Evaluation of the effects of NGR-hTNF monotherapy on PFS in subjects with advanced or metastatic colorectal cancer (CRC) who had received prior therapy with fluoropyrimidine-, oxaliplatin- and irinotecan-based regimens was the primary objective of an open-label, nonrandomized, single-group-assignment phase II trial. Assessments of the safety, PK and OS were among the study's secondary objectives (32). NGR-hTNF (0.8 μg/m^2) was administered as a 1-hour infusion every 3 weeks (33-35). In 33 evaluable patients who received a total of 111 cycles, no treatment-related AEs of grade 3-4 in severity or toxicity-related deaths were reported. The median PFS was estimated at 2.5 months (36). One patient exhibited PR and 12 individuals displayed SD, resulting in an overall disease control rate of 39%. In subjects with disease control, the median PFS was 3.8 months and the rates of PFS at 3 and 4.5 months were estimated to be 67% and 42%, respectively (37). At a median follow-up of 28 months the OS was established at 13.1 months (15.4 months in subjects who achieved disease control). An additional cohort of 13 patients was treated with the same dose of NGR-hTNF on a weekly basis. This dose-dense schedule did not correlate with worsening of the toxicity profile of NGR-hTNF. The median PFS in this group was determined to be 2.3 months, with 2 participants achieving PFS of 10.5 and 11 months, respectively (38).

Patients with metastatic CRC were enrolled in another open-label, nonrandomized, single-group-assignment phase II trial designed to determine the feasibility of coadministration of NGR-hTNF with a standard oxaliplatin-based regimen (primary outcome measure). The study also aimed to investigate the antitumor activity of the treatment in terms of the rate of objective response by RECIST and evaluate the PFS. The PK profile of NGR-hTNF was also assessed as a secondary objective (39). Two sequential cohorts of participants (n = 12/cohort) received treatment with NGR-hTNF at 0.8 μg/m^2 (low dose) and 45 μg/m^2 (high dose), respectively, as a 1-hour i.v. infusion every 3 weeks. Both cohorts were given oxaliplatin (100 mg/m^2 i.v. every 3 weeks) at 60 minutes after administration of NGR-hTNF plus capecitabine (825 mg/m^2 p.o. b.i.d. for 14 days). PR and SD (with a median duration of 5 months) were reported in 1 and 5 subjects, respectively, in the low-dose cohort. SD lasting for a median of 4.1 months was seen in four individuals treated with the high dose of NGR-hTNF. In the overall study population, the rates of PFS at 3 and 6 months were 48% and 38%, respectively (40). The combination therapy was described as well tolerated, with transient, infusion time-related chills being the most frequent toxicity of grade 1-2 in severity, seen in 58% and 54% of patients, respectively, in the low- and high-dose cohorts (41).

The efficacy of NGR-hTNF administered as second-line therapy in patients with advanced malignant pleural mesothelioma who had

received prior treatment with pemetrexed is under evaluation in an ongoing, randomized, double-blind, placebo-controlled, parallel-assignment phase III trial. Assessment of the OS is the primary outcome measure of the study, with PFS, rate and duration of disease control and safety being among the trial's secondary objectives. Participants will be given a 1-hour i.v. infusion of NGR-hTNF (0.8 μg/m^2) every week until confirmed evidence of disease progression is obtained or unacceptable toxicity is reported. Best supportive care (such as antibiotics, analgesics and antiemetics) will be provided and one chemotherapeutic agent (doxorubicin, gemcitabine or vinorelbine) may be administered in combination with NGR-hTNF based on the investigator's choice. The study is expected to be completed in February 2013 (42).

SOURCE

MolMed SpA (IT).

DISCLOSURES

The author states no conflicts of interest.

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