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Blockade of HLA Antibody-Triggered Classical Complement Activation in Sera From Subjects Dosed With the Anti-C1s Monoclonal Antibody TNT009—Results from a Randomized First-in-Human Phase 1 Trial

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Background. Complement may play a key role in antibody-mediated rejection. A promising therapeutic approach may be classical pathway (CP) inhibition at the level of early component C1. **Methods.** In this first-in-human, double-blind, randomized placebo-controlled phase 1 trial, we evaluated the safety and complement inhibitory effect of TNT009, a humanized monoclonal anti-C1s antibody. Sixty-four adult healthy volunteers received either single (n = 48; 7 consecutive cohorts, 0.3-100 mg/kg) or 4 weekly infusions (n = 16; 2 consecutive cohorts, 30 and 60 mg/kg per infusion) of TNT009 or placebo. To assess the effect of treatment on complement activity, sera from dosed subjects were analyzed in a CP activation assay evaluating C3d deposition on HLA-coated microbeads spiked with alloantibodies. **Results.** Single doses of TNT009 at 3 to 100 mg/kg uniformly and profoundly inhibited HLA antibody-mediated C3d deposition ($\geq 86\%$ after 60 minutes), whereby the duration of CP inhibition (2-14 days) was dose-dependent. Four weekly doses persistently blocked complement for 5 to 6 weeks. Ex vivo serum CP activity was profoundly inhibited when TNT009 concentrations exceeded 20 $\mu\text{g/mL}$. Infusions were well tolerated without serious or severe adverse events. **Conclusions.** Treatment with TNT009 was safe and potently inhibited CP activity. Future studies in patients are required to assess the potential of TNT009 for preventing or treating antibody-mediated rejection.

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Antibody-mediated rejection (AMR) is increasingly recognized as one of the cardinal causes of organ allograft dysfunction and loss.^{1,2} Even though donor-specific antibody (DSA) binding to the transplant endothelium may cause injury via direct signaling or Fc receptor-dependent mechanisms,^{3,4} there are several lines of evidence suggesting that

antibody-triggered complement activation by the classical pathway (CP) contributes to graft damage.^{5,6}

While clear-cut diagnostic criteria for AMR have been well defined,⁷ the clinical management of graft rejection has remained a major therapeutic challenge. There is still a need for new therapeutic paradigms to improve currently available treatment strategies. Indeed, even intense multimodal regimens have failed to completely prevent irreversible graft damage, as shown for kidney transplantation across HLA antibody barriers.⁸⁻¹⁰ One promising option may be the use

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of agents that specifically interfere with complement.^{11,12} Recent observational studies and case reports suggested that eculizumab, a monoclonal antibody against terminal component C5, may have efficacy in the prevention and treatment of acute AMR,¹³⁻¹⁶ but another study showed that complement inhibition was ineffective at preventing chronic AMR in patients with persistently elevated DSA, possibly due to upstream complement activation driving inflammation and subsequent tissue injury.¹⁵

An interesting alternative may be the use of agents that specifically target the CP at the level of complement component C1.¹² A potential advantage of this strategy over C5 inhibition is that in addition to preventing terminal pathway activation, inhibition at the level of C1 prevents the production of the potent C3a anaphylatoxin and C3b/iC3b opsonins. Recent intervention studies have provided the first evidence that C1 inhibition using a C1-esterase inhibitor (C1-INH) may have some therapeutic potential in transplant settings.¹⁷⁻¹⁹ However, C1-INH inhibits both lectin and CPs, and is also involved in other enzymatic pathways including the plasma kallikrein-kinin (contact) system. Another more selective approach may be the use of monoclonal antibodies that specifically target the C1 complex. Very recently, experimental studies have shown that TNT003, a mouse monoclonal antibody against the CP-specific serine protease C1s, effectively prevented cold agglutinin-mediated deposition of complement opsonins, release of anaphylatoxins, and hemolysis in vitro.²⁰ The same antibody potently inhibited HLA antibody-triggered complement split product deposition on HLA antigen-coated microbeads.²¹ These data suggested a therapeutic potential of C1s blockade in CP-driven complement-mediated disorders.

Here we report on the results of a first-in-human, double-blind, randomized, placebo-controlled phase 1 trial designed to assess the tolerability/safety (primary endpoint) and activity of the humanized anti-C1s monoclonal antibody TNT009 in healthy volunteers.²² TNT009-containing serum samples from healthy subjects dosed with the molecule were found to inhibit ex vivo HLA antibody-triggered CP activation. These data provide the basis for systematic studies evaluating the efficacy of TNT009 in transplant settings.

MATERIALS AND METHODS

Study design and Objectives

This first-in-human phase I trial was conducted as a single center, randomized, double-blind, placebo-controlled trial to evaluate the safety/tolerability profile and complement inhibitory potential of the humanized anti-C1s monoclonal antibody TNT009 (True North Therapeutics, Inc., South San Francisco, CA). The study was approved by the ethics committee of the Medical University Vienna and was performed in compliance with the Good Clinical Practice guidelines and the principles of the Declaration of Helsinki. The trial is registered at ClinicalTrials.gov (NCT 02502903) and EUDRACT (EUDRACT number: 2014-003881-26). This study used an integrated protocol design with a basket trial as described recently.²² Pharmacodynamic and pharmacokinetic results have been analyzed in a single and multiple ascending dose design. In the present analysis, we focus on the ex vivo effects of serum samples taken from healthy volunteers dosed with TNT009 on HLA antibody-triggered

CP activation. There were no deviations from the original protocol and its amendments or major changes of methods and trial outcomes after trial commencement.

Study Participants

After signed informed consent, 64 healthy adult (age, ≥ 18 years) male and female volunteers were included in the trial. Female volunteers were included only if they were postmenopausal, surgically sterilized, or using contraception throughout the study and for 30 days after the end of the trial. Major exclusion criteria were a significant medical history or ongoing chronic illness, clinically relevant infection within the preceding month, abnormal findings on physical or laboratory evaluation, or a history of infusion hypersensitivity, allergic, or anaphylactic reactions to other therapeutic proteins. If not previously vaccinated, participants underwent prophylactic vaccination against encapsulated bacterial pathogens (*Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*) at least 14 days before the first infusion.

Study participants were recruited between June and October 2015, and the 2 parts of the study were completed in December 2015. As previously described in detail, in compliance with a standard 3 + 3 phase 1 design, the trial included sample sizes of 4 or 8 subjects per dosing cohort.²² No classical sample size estimation was performed. For safety reasons, in each cohort, sentinel dosing groups comprising 1 active and 1 placebo-treated subject were used and observed for at least 24 hours before dosing of the remaining members of the cohort. All parts of the study were guided by an ongoing safety review process. Intercohort safety reviews were thereby conducted in consultation with an independent data safety monitoring board. The trial was carried out at the Department of Clinical Pharmacology (Medical University Vienna).

Intervention and Randomization

For drug administration study participants were admitted to the phase 1 study unit. TNT009 was infused over a period of 60 minutes. In the first part of the trial, 7 sequential cohorts of volunteers were given ascending single doses of TNT009 or placebo (3:1 randomization in each cohort; cohort 1 [n = 4; 0.3 mg/kg], cohort 2 [n = 4; 1 mg/kg], cohort 3 [n = 8; 3 mg/kg], cohort 4 [n = 8; 10 mg/kg], cohort 5 [n = 8; 30 mg/kg], cohort 6 [n = 8; 60 mg/kg], cohort 7 [n = 8; 100 mg/kg]). In the multiple ascending dose portion of the trial, 2 cohorts, each consisting of 8 subjects, were given 4 weekly intravenous doses of TNT009 or placebo (3:1 randomization within each cohort; cohort A [n = 8; 30 mg/kg], cohort B [n = 8; 60 mg/kg]). We applied computerized block randomization (restriction by block size; use of electronic case report forms). The allocation sequence was generated by an independent physician from the contract research organization. Medication/placebo was prepared by independent study pharmacologists, and study physicians were provided with blinded infusion bags. Subjects were enrolled and assigned to interventions by study nurses and physicians, respectively. Study subjects, staff, and researchers who assessed outcomes were blinded to treatment assignment (an emergency unblinding decode list was kept in an envelope securely stored on site).

Biological Material

After the sampling schedule of the single ascending dose part of the trial, serum was taken at eleven consecutive times and stored at -80°C until analysis, without repeated freezing and thawing (1 hour before and 0.5, 1, 4, 8, and 24 hours after infusion; days 2, 3, 4, 7, and 14 [end-of-trial visit]). For the multiple ascending dose part, 24 serial samples were drawn per subject (day 0, 1 hour before and 0.5, 1, 4, and 8 hours after the first infusion; days 1, 2, 3, and 4; day 7, 1 hour before and 4 hours after the second infusion; day 14, 1 hour before and 4 hours after the third infusion; day 21, 1 hour before and 0.5, 1, 4 and 8 hours after the fourth infusion; days 22, 23, 24, 25, 28, and 35 [end-of-trial visit]).

HLA Antibody and Complement Detection

For IgG type HLA antibody screening, sera were analyzed on bead arrays using a panel of 12 HLA class I and 5 HLA class II haplotype-coated screening beads (LABScreen Mixed assays) as described by the manufacturer (One Lambda Inc., Canoga Park, CA).

The C3d-fixing capability of HLA antibody-positive samples (preintervention sera of 7 sensitized healthy study participants or allosera obtained from 4 representative sensitized kidney transplant candidates; cytotoxicity panel reactivity: 54-70% to analyze the in vitro effect of increasing concentrations of TNT009 on CP activation) was assessed using a modification of the assay. Screening beads were incubated for 30 minutes with native test sera or heat-inactivated negative control serum obtained from a nonsensitized male volunteer, washed, and then stained with biotinylated anti-C3d antibody (30 minutes, Quidel, San Diego, CA, final concentration: 4 $\mu\text{g}/\text{mL}$). Beads were then washed and incubated for 30 minutes with phycoerythrin-conjugated streptavidin (eBioscience, San Diego, CA, final concentration: 1 $\mu\text{g}/\text{mL}$, 30 minutes) before analysis on a Luminex 200 flow analyzer (Luminex Corporation, Austin, TX).

The effect of C1s inhibition on HLA antibody-triggered CP activation in healthy volunteers dosed with TNT009 was assessed ex vivo using HLA-coated beads spiked with high level complement-activating HLA antibodies. LABScreen Mixed beads (One Lambda) were incubated for 30 minutes in a pool of 3 different heat-inactivated sera obtained from

highly sensitized end stage kidney disease patients immunized by prior kidney transplants (each serum contained $>99\%$ IgG virtual panel reactivity as assessed in single antigen bead assays). After washing, antibody-coated beads were incubated with test sera or heat-inactivated (negative control) serum, washed, and then stained with biotinylated anti-C3d antibody and phycoerythrin-conjugated streptavidin as described above. Results were expressed as a normalized C3d mean fluorescent intensity (MFI), whereby raw MFI obtained with the heat-inactivated serum were subtracted from test serum MFI (normalized MFI). For each test serum, we calculated the mean value of normalized C3d MFI recorded on the 12 HLA class I and 4 of the 5 HLA class II bead populations within the bead panel (1 HLA class II bead population [ID25] was excluded because of consistently negative C3d staining, $\text{MFI} < 100$).

Serum concentrations of C4 and 50% hemolytic complement (CH50) activity were measured using standard protocols. Alternative pathway complement activity was measured using Wieslab assays (Euro-Diagnostica, Malmö, Sweden) following the manufacturer's instructions. Serum levels of anti-nuclear antibodies and anti-dsDNA antibodies were assessed using standard technology. Circulating immune complexes were detected using C1q-binding tests (Quest Diagnostics; reference range: ≤ 25.1 mcg Eq/mL).

Statistical Methods

Continuous data are given as the median and the interquartile range (IQR) or mean and standard deviations as appropriate. Discrete data are presented as counts and percentages. The Fisher exact, Mann Whitney *U* or Pearson correlation tests were applied as appropriate. A 2-sided *P* value less than 0.05 was considered statistically significant. Half maximal inhibitory concentrations (IC_{50}) were computed from a 4-parameter logistic curve fit. Analyses were performed using GraphPad Prism 6.01 (GraphPad Software Inc., La Jolla, CA) and Predictive Analytics Software Statistics 18 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Overall, 64 healthy volunteers were randomized and dosed in this phase 1 trial. Demographic and immunological

TABLE 1.
Demographical and immunological data at baseline

Parameters	All study participants (n = 64)	TNT009 (n = 48)	Placebo (n = 16)	<i>P</i>
Baseline data				
Age, median (IQR)	30 (26-37)	30 (26-37)	30 (26-43)	0.54
Female sex, n (%)	26 (40.6)	22 (46)	4 (25)	0.12
Body mass index, median (IQR)	23.1 (20.7-24.9)	22.6 (21-24.7)	23.9 (20.6-25.5)	0.45
IgG type HLA sensitization ^a				
HLA class I and/or II, n (%)	7 (11)	6 (13)	1 (6)	0.67
Overall CP activity/levels ^b				
CH50 activity (%), median (IQR)	114 (102-126)	115 (103-127)	109 (100-123)	0.38
C4 (mg/dL), median (IQR)	17 (15-22)	17 (15-21)	18 (16-23)	0.28
HLA antibody-dependent CP activation ^c				
C3d MFI, median (IQR)	3494 (2794-4494)	3494 (2757-4361)	3470 (2830-4750)	0.69

^aSensitization was assessed on a Luminex platform using HLA antigen-coated screening beads as described in the methods section.

^bNormal ranges, 10-40 mg/dL (C4); 70-140% (CH50 activity).

^cCP activation was assessed on HLA antibody-spiked microbeads as detailed in the methods section. The assay read-out was the mean of normalized C3d MFI obtained on 15 different screening bead populations.

baseline data are provided in Table 1. Twenty-six (41%) volunteers were women. The median age was 30 years and the median body mass index 23.1 kg/m², respectively. All included participants had C4 levels (median, 17 mg/dL) and CH50 reactivity (median, 114%) within the normal range. In the HLA antibody-dependent CP assay, median C3d MFI levels of 3494 were detected (range, 1369-6312). As shown in Table 1, there were no significant differences between TNT009- and placebo-treated subjects regarding baseline data. Of note, HLA antibody prescreening revealed significant levels of HLA IgG class I and/or class II reactivity in 7 of the participants (Table 1). However, none of these subjects had complement (C3d)-fixing activity (data not shown).

Effect of TNT009 on HLA Antibody-Triggered CP Activation in Vitro

In a first step, we selected the preintervention sera obtained from 8 representative study participants (cohort 6, single ascending dose part of the trial) and, in parallel, sera obtained from 4 sensitized kidney transplant candidates to assess the effect of increasing concentrations of TNT009 on HLA antibody-dependent CP activation in vitro. As shown in Figure 1, TNT009 potently blocked C3d deposition in a concentration-dependent manner. Despite considerable interindividual variation in C3d-fixing capability at baseline, all tested samples showed a uniform and steep concentration-effect relationship (Figure 1). Individual levels of 50% inhibition (IC₅₀) were slightly higher in healthy volunteers (range, 37-58 µg/mL) than in sensitized patients (23-29 µg/mL).

Effect of Single Ascending Doses of TNT009 on HLA Antibody-Triggered CP Activation

In the single ascending dose part of the trial, 48 healthy volunteers were randomized to TNT009 (0.3-100 mg/kg) or placebo within 7 consecutive cohorts (Figure 2). HLA antibody-dependent CP activation (C3d deposition on HLA-coated screening beads preincubated with HLA antibodies) was induced by exposing the beads to serum samples taken from study participants at baseline (pre-TNT009 treatment) through the end of study (see Materials and Methods for time points). As shown in Figure 2, the extent and particularly the duration of CP blockade followed a dose-dependent pattern. While TNT009 at 0.3 mg/kg did not affect CP activity, infusion of the antibody at 1.0 mg/kg transiently inhibited complement (reduction of C3d deposition to 13-86% of pretreatment levels at 60 minutes). At higher doses (3-100 mg/kg), TNT009 inhibited complement immediately and profoundly (by ≥86% after 60 minutes). As anticipated from the extremely steep concentration-effect curve with a remarkable on-off pattern of inhibition, the dose-response relationship primarily manifested as an increase in the duration of complete CP inhibition, with at least 8 hours, 2, 7, or 14 days blockade at doses of 3, 10, 30 to 60, or 100 mg/kg, respectively. In placebo-treated volunteers, no significant change in CP activity was observed (Figure 2).

Effect of Multiple Doses of TNT009 on HLA Antibody-Triggered CP Activation

In the multiple ascending dose part of the trial, 16 healthy volunteers received 4 weekly doses of TNT009 (30 or 60 mg/kg per infusion; 6 subjects per cohort) or placebo (2 subjects per cohort) in 2 consecutive cohorts (Figure 3).

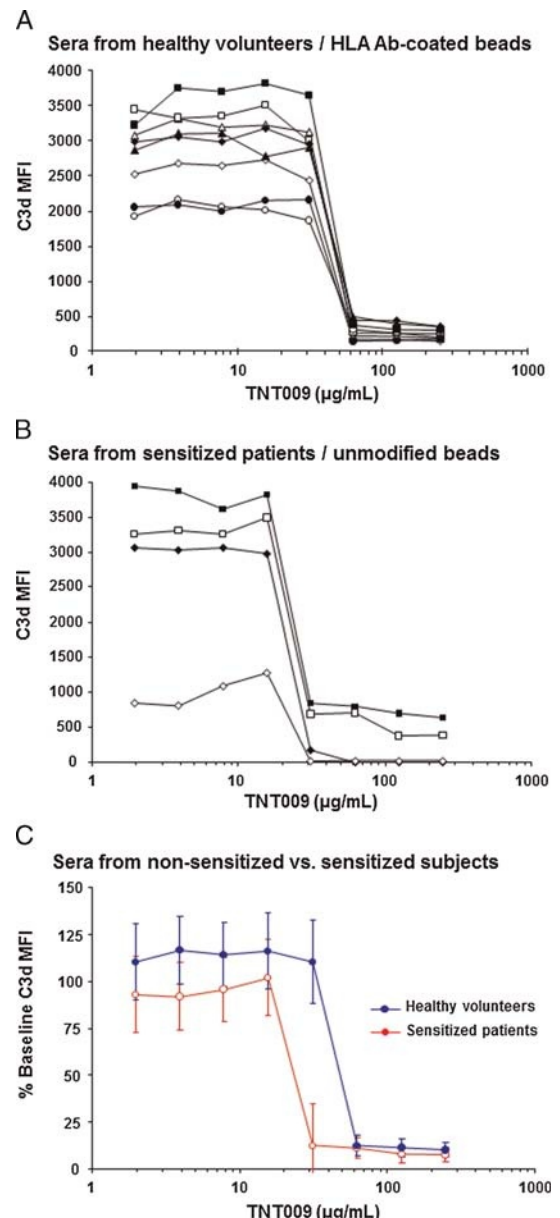


FIGURE 1. In vitro effect of TNT009 on HLA antibody-triggered CP activation in healthy volunteer and patient sera. To assess the effect of TNT009 on HLA antibody-dependent CP activation in vitro, serum samples were spiked with TNT009 in serial 1:2 dilution steps (concentrations between 250 and 1.95 µg/mL) and incubated with (A) HLA antibody-coated (samples obtained from 8 representative healthy volunteers) or (B) unmodified screening beads (samples obtained from 4 broadly sensitized patients). The assay read-out was the level of C3d MFI (healthy volunteers: mean of normalized MFI obtained on 15 different screening bead populations; sensitized patients: mean of normalized MFI on the 3 bead populations showing the highest levels of C3d fixation). In C, the mean (±SD) percentage of baseline MFI is shown for healthy volunteers and patient sera.

HLA antibody-dependent CP activation was monitored using samples from pre-TNT009 treatment out to 35 days (end-of-trial visit) after the first administration. As in the single ascending dose study, infusion of TNT009 immediately and potently inhibited CP activation. Complement inhibition was slightly less pronounced than in the corresponding 30 and 60 mg/kg single-dose cohorts. This was not due to differences in measured TNT009 serum concentrations, but

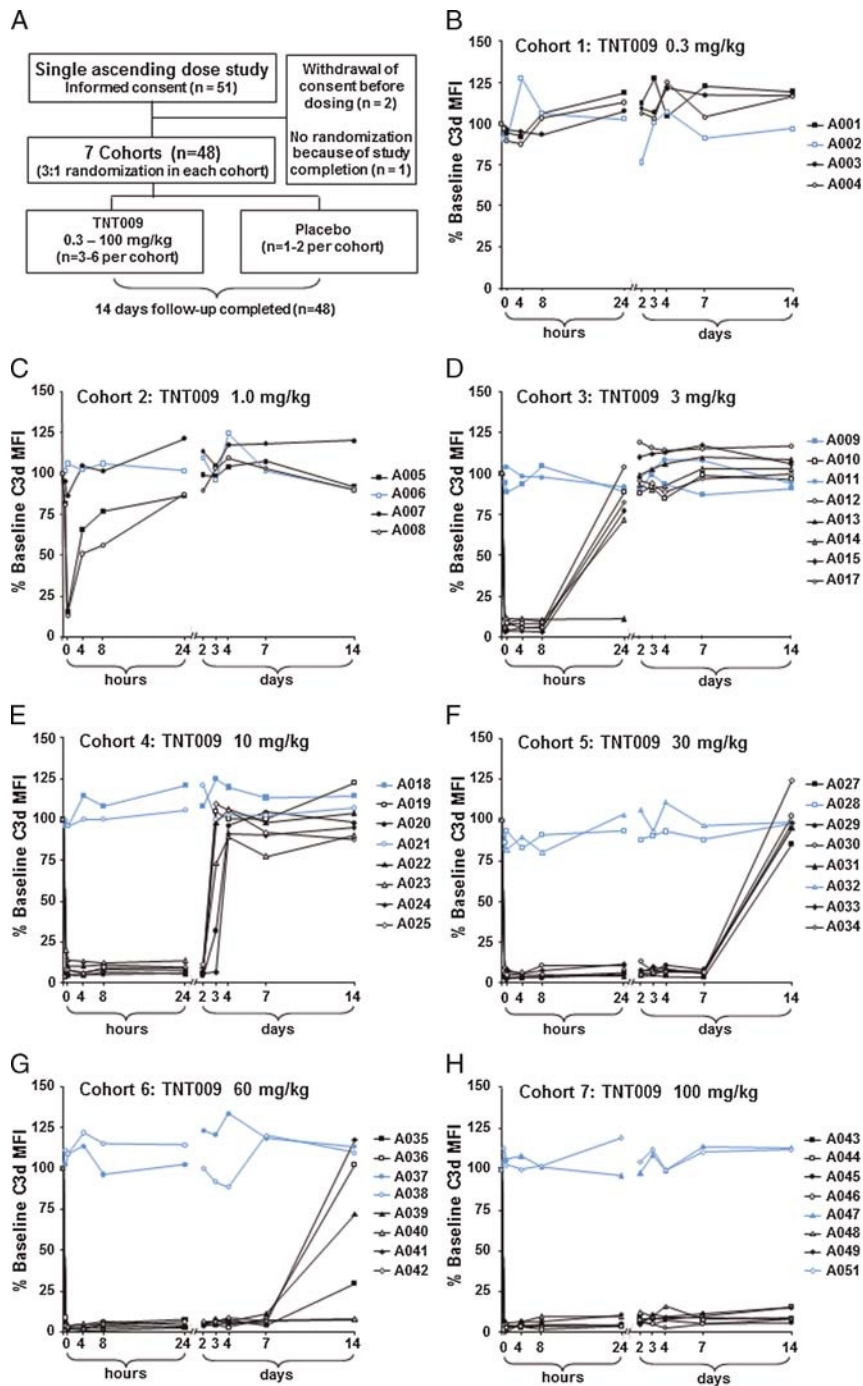


FIGURE 2. Effect of intravenous infusion of single doses (0.3-100 mg/kg) of TNT009 on HLA antibody-triggered CP activation. Forty-eight volunteers (study flow: A) were randomized in 7 cohorts (B-G) to receive ascending doses of TNT009 (black lines/symbols) or placebo (blue lines/symbols). Longitudinal serum samples obtained from study participants were incubated with presensitized HLA antibody-coated beads to assess the effect of TNT009 administration on ex vivo complement activation. The IDs of the participants are indicated. The effect of treatment on % preintervention C3d MFI (mean of normalized MFI obtained on 15 different screening bead populations) was recorded over a study period of 14 days (end-of-study visit).

presumably related to interassay differences in levels of baseline MFI (data not shown). Except for 1 volunteer (ID: B007) receiving 30 mg/kg (cohort A) who showed complete recovery of complement activity (to 92% of the pretreatment value) before the second, and partial recovery (to 55%) before the third infusion, all treated individuals showed a persistent and profound CP blockade for 4 weeks or longer. This was also observed in 1 volunteer

(ID: B015) who missed the second infusion of TNT009 (60 mg/kg, cohort B) due to diarrhea (and who was redosed uneventfully with the third dose). CP activity recovered, with some interindividual variation, between 1 and 2 weeks after the last infusion of 30 mg/kg, whereas CP blockade persisted until the end of study 2 weeks after the last infusion of 60 mg/kg. Placebo did not alter CP activity (Figure 3).

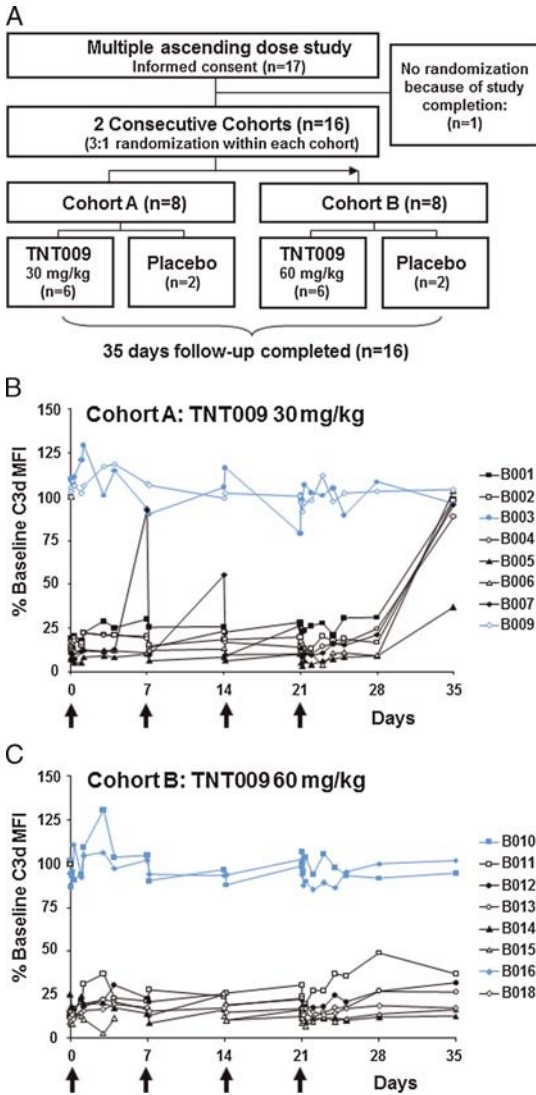


FIGURE 3. Effect of intravenous infusion of 4 weekly doses of TNT009 (30 or 60 mg/kg) on HLA antibody-triggered CP activation. Sixteen volunteers (study flow: A) were randomized in 2 cohorts (B, C) to receive 2 different repeated doses of TNT009 (black lines/symbols) or placebo (blue lines/symbols). Black arrows indicate the time points of infusion. The IDs of the participants are indicated. Longitudinal serum samples obtained from study participants were incubated with HLA antibody-coated beads to assess the effect of TNT009 administration on ex vivo complement activation. The effect of treatment on % preintervention C3d MFI (mean of normalized MFI obtained on 15 different screening bead populations) was recorded over a study period of 35 days (end-of-study visit).

Relationship Between TNT009 Plasma Concentration and CP Inhibition

TNT009 was not detected in any pretreatment samples and serial samples obtained from placebo-treated individuals. Concentrations measured after antibody infusion ranged from 0 to 2653 µg/mL. As shown in Figure 4, analysis of pretreatment and serial posttreatment samples of all the TNT009-treated individuals enrolled in the 2 study parts (n = 48) revealed a close relationship between TNT009 serum concentration and percent C3d or CH50 assay inhibition, with only a few outliers. At concentrations above 20 µg/mL, TNT009 profoundly inhibited HLA antibody-triggered C3d deposition

(median C3d MFI, 400 [IQR, 193-803] vs 3070 [IQR, 2442-3899] at concentrations below 20 µg/mL). For CH50 reactivity, which tightly correlated with levels of C3d deposition ($r = 0.858$; $P < 0.0001$), there was a comparable concentration dependency, even though its inhibition was less pronounced (median CH50 activity, 31% [38-36] at >20 µg/mL as compared with 114% [IQR, 102-132] at <20 µg/mL) (Figure 4). As shown in Figure 4, we found TNT009 serum levels did not inhibit alternative pathway activity.

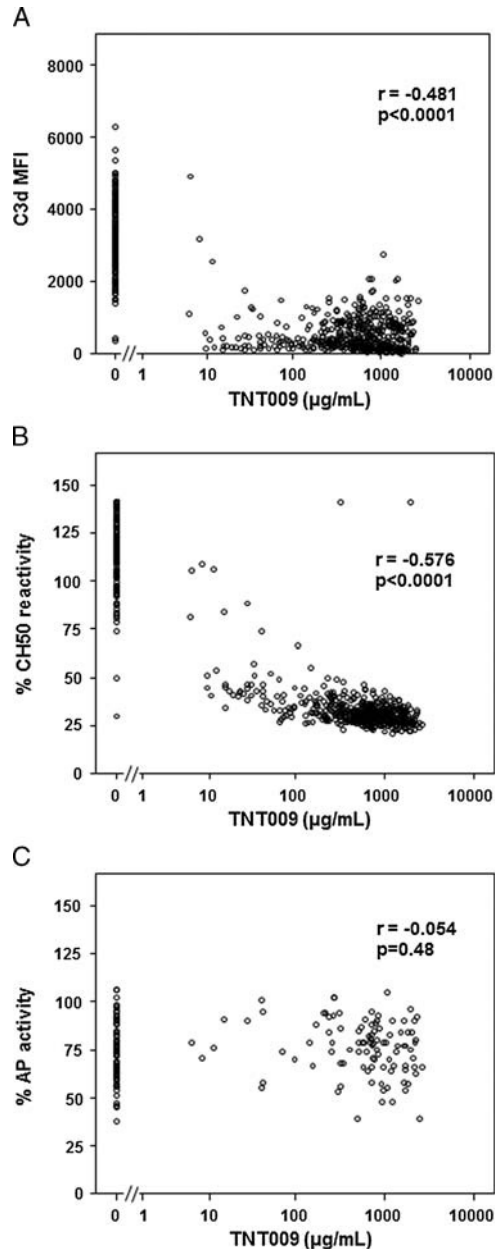


FIGURE 4. Relationship between TNT009 serum concentration and CP inhibition. Preintervention and postintervention sera obtained from 48 volunteers (single and multiple ascending dose parts of the study) allocated to verum treatment were evaluated for TNT009 serum concentration and, in parallel, C3d MFI (mean of normalized MFI obtained on 16 different screening bead populations) (A) CH50 activity (B), and (C) alternative pathway (AP) activity, respectively.

Safety

Single or multiple doses of TNT009 were well tolerated, and no serious adverse events (AE) were recorded during the study period. Table 2 provides a summary of mild or moderate AE occurring in single and multiple dose parts of the trial. Overall, 10 of 16 placebo-treated and 19 of 48 TNT009-treated subjects had 1 or more AE during study follow-up. TNT009 treatment was not associated with significantly more AE (Table 2). In 2 of the 62 included subjects, both allocated to TNT009 treatment, circulating immune complex levels were detected already before study initiation, without any change until the end of the study (A046 [TNT009 at 100 mg, single dose], 40 vs 46 mcg EQ/mL; B006 [TNT009 30 mg, multiple doses], 41 vs 39 µg EQ/mL). Another volunteer (A043, single dose of TNT009 at 100 mg) had detectable anti-dsDNA antibodies both at the time of screening and, at slightly lower levels, at the end-of-study visit (16 and 11 IU/mL, respectively). Finally, 2 subjects had low antinuclear antibody titers before and after the study (A023 [single dose of TNT009 at 10 mg], 1:160 vs 1:80; B010 [placebo arm]; 1:80 vs 1:80). There was only 1 volunteer (A046: TNT009, single dose of 100 mg/kg) who developed marginal de novo titers of antinuclear antibodies at the end-of study visit (1:80).

DISCUSSION

In this first-in-human phase 1 trial, we demonstrate that the humanized C1s monoclonal antibody TNT009 potently inhibited HLA antibody-triggered CP activation. This provides

a basis for future studies evaluating the ability of C1s inhibition to prevent or treat AMR.

TNT009 blocked C3d deposition over a wide range of doses (1-100 mg/kg). Duration of CP inhibition, which tightly correlated with changes in CH50 activity, was dose-dependent. A certain level of interindividual variation, as observed for a single dose of 60 mg/kg, was apparently not a result of varying susceptibility to complement inhibition. In vitro spiking of pretreatment sera obtained from these subjects revealed a uniform concentration dependency (Figure 1). This and a close relationship between TNT009 serum concentrations and C3d assay results suggest that differences between individuals are primarily due to variations in pharmacokinetics. Interpreting these results, however, it must be pointed out that the read-out of MFI in our ex vivo assays may be only an approximate measure of the extent of CP activation on the surface of HLA-coated beads. A remarkable result, which was also described for the mouse parental version of TNT009 (TNT003),^{20,21} was the distinct on-off pattern of CP modulation, which (both ex vivo and in vitro) manifested as a very steep concentration-effect curve between TNT009 levels and complement inhibition. A similar pattern was also observed in experiments using sera from HLA sensitized transplant candidates. A possible explanation may thereby be that due to its position at the top of the enzymatic cascade, only a few molecules of uncontrolled C1s may be sufficient to trigger complement amplification via C4 and C3. Once this chain reaction activates the amplification loop of complement (ie, the alternative pathway), this can lead to extensive C3 activation, especially in in vitro test situations which

TABLE 2.

Summary of AE

	Single ascending dose trial			Multiple ascending dose trial		
	Placebo	TNT009	<i>P</i>	Placebo	TNT009	<i>P</i>
No. subjects	12	36		4	12	
No. subjects with ≥ 1 AE (%)	6 (50)	11 (31)	0.30	4 (100)	8 (67)	0.52
No. subjects with mild/moderate AE (%)	3 (25)	3 (8)	0.16	1 (25)	3 (25)	>0.99
No. subjects with moderate AE (%)	5 (42)	6 (17)	0.11	3 (75)	5 (42)	0.57
No. subjects with SAE (%)	0 (0)	0 (0)		0 (0)	0 (0)	
AE, no. subjects (%)						
Headache	3 (25)	3 (8)	0.16	1 (25)	3 (25)	>0.99
Nausea	0 (0)	1 (3)	>0.99	0 (0)	0 (0)	—
Vertigo	0 (0)	2 (6)	>0.99	0 (0)	0 (0)	—
Flu-like symptoms	0 (0)	1 (3)	>0.99	0 (0)	2 (17)	>0.99
Itching	0 (0)	1 (3)	>0.99	0 (0)	1 (8)	>0.99
Urticaria	0 (0)	1 (3)	>0.99	0 (0)	0 (0)	—
Vaccination reaction	0 (0)	1 (3)	>0.99	0 (0)	0 (0)	—
Infections						
Common cold	1 (8)	3 (8)	>0.99	2 (50)	2 (17)	0.25
Upper respiratory tract infection	0 (0)	2 (6)	>0.99	0 (0)	3 (25)	0.53
Gastroenteritis	0 (0)	0 (0)		2 (50)	0 (0)	0.05
Urinary tract infection	0 (0)	1 (3)	>0.99	0 (0)	0 (0)	—
Vaginal yeast infection	0 (0)	1 (3)	>0.99	0 (0)	0 (0)	—
Gastrointestinal events						
Abdominal pain	1 (8)	0 (0)	0.25	0 (0)	1 (8)	>0.99
Diarrhea	0 (0)	0 (0)		0 (0)	1 (8)	>0.99
Other events	3 (25)	0 (0)	0.01	2 (50)	1 (8)	0.14

AE, adverse events; SAE, severe adverse events.

lack adequate control by complement-regulatory proteins. On the other hand, if all C1s molecules are controlled by bound TNT009, no activation signal is transmitted to C4 and no C3 activation (or C3 split product deposition) will occur.

Single doses of TNT009 were well tolerated, with no reported drug-related adverse events. Notably, there were no severe infectious complications despite complete CP inhibition for more than 4 weeks. As standard for complement blockade using eculizumab,^{23,24} our study participants were subjected to prophylactic vaccination against encapsulated bacterial pathogens. In this respect, a potential advantage over terminal complement blockade may be that C1s inhibition does not alter complement activation via the alternative pathway and thus may leave important components of innate defence intact.

It is well established that genetic deficiencies of C1 components or other early classical complement components, such as C1q and C4, associate with an excessive risk of autoimmune disease such as systemic lupus nephritis.^{25,26} However, pharmacological inhibition is not equivalent to congenital, life-long CP deficiency. To this point, TNT009-treated individuals did not develop any clinical signs of autoimmunity. Although there was no increase in circulating immune complexes or de novo formation of anti-dsDNA autoantibodies in any of the included subjects, only 1 of the 48 TNT009-treated volunteers developed a low titre of antinuclear antibodies.

There is emerging interest in C1 as a potential target of treatment in transplant settings.^{11,12,19} The early results reported for C1-INH^{17,18} may provide a basis for the development of more specific agents for selective and prolonged interference with this key component. The results of the present phase 1 trial may therefore provide a solid foundation for future studies evaluating the concept of C1 blockade in the context of AMR. Indeed, there are several lines of evidence for a role of complement as a trigger of injury in this specific context.⁵ For example, C4d staining in allografts, as a marker of antibody-triggered complement activation in the microcirculation, is associated with a more severe course of rejection.^{27,28} Moreover, DSA with high binding strength associated with the capability to fix complement components including C1q in bead array assays, associate with AMR occurrence and inferior transplant outcomes.²⁹⁻³¹ Finally, in direct support for a pathogenic role of complement as a trigger of injury, terminal complement blockade by eculizumab was shown to reduce acute AMR rates in crossmatch-positive living donor transplant recipients and reverse episodes of refractory AMR.¹³⁻¹⁶ Interestingly, use of eculizumab in sensitized recipients did not prevent chronic rejection processes.¹⁵ In this respect, it has to be pointed out that, maybe in contrast to early C1s blockade, terminal complement blockade may preserve important proximal steps of complement activation, such as C3 cleavage and C3a release, that maintain a continuous inflammatory process and tissue damage.

An important point is that the current trial is limited to analysis of complement activity in serum samples of healthy volunteers. It remains to be clarified whether TNT009 is also able to sufficiently block CP in the transplanted tissue. The ability of this antibody to counteract tissue injury triggered by the CP will have to be established in ongoing (uncontrolled study in kidney transplant recipients with late AMR, primarily designed to evaluate the safety and tolerability of C1s inhibition)²² and future clinical trials, for example,

evaluating its efficiency in the challenging population of transplant candidates with broad levels of complement-fixing HLA antibodies.

In conclusion, the results of this first-in-human trial suggest that infusion of a novel anti-C1s monoclonal antibody profoundly inhibited complement over a prolonged period. We demonstrate safety and tolerability of this treatment in healthy volunteers and report on potent ex vivo inhibition of HLA antibody-triggered CP activation over a wide range of doses and concentrations. In addition, this phase 1 trial may help build an in silico model of TNT009 pharmacokinetics/dynamics to use in future trials. Our results may provide a valuable basis for future studies evaluating the use of this agent in the prevention and treatment of AMR.

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