EXTENDED REPORT

First clinical trials of a new heteropolymer technology agent in normal healthy volunteers and patients with systemic lupus erythematosus: safety and proof of principle of the antigen-heteropolymer ETI-104

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Background: The heteropolymer technology was developed to remove pathogens from the circulation.

Objectives: To evaluate the safety and tolerability of a single administration and to establish proof of principle for ETI-104 in normal healthy volunteers (NHV) and patients with systemic lupus erythematosus (SLE)

Methods: The drug was given intravenously to 11 NHV and six patients with SLE. Over 28 days, vital signs were noted, a haematological and chemical analysis of blood and urine was carried out, and adverse events were recorded. CR1 receptor numbers, the ability of antigen based heteropolymers to bind to red blood cells (RBCs), and the clearance of high avidity and total anti-dsDNA antibodies were measured by Farr assays and FACS analysis.

Results: No safety measure differed significantly from normal in both groups; no drug related serious adverse events occurred. ETI-104 rapidly bound to RBCs in NHV and patients with SLE. Binding of the drug to RBCs of patients with SLE also caused a rapid reduction of circulating anti-dsDNA antibodies in the plasma 15 minutes after administration, with a maximum reduction of 55% (range 43–62). At 28 days statistically significant decreases were maintained in three patients, while in the other three the values had returned to baseline levels.

Conclusion: These clinical trials established the safety and the proof of principle of the new immunoconjugate ETI-104. This provides the basis for further development of this technology for numerous indications—for example, therapeutic options for autoimmune diseases or viral and bacterial infections.

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I mmune adherence (IA) is a physiological mechanism for the removal of immune complexes (ICs).[1][2] When a foreign substance, an antigen, enters the bloodstream, the immune system develops antibodies that bind to it specifically. The resulting antigen-antibody complex activates the complement system, resulting in formation of the complement activation product C3b, which binds to the antibody-antigen complex. The resulting IC binds to the complement receptor CR1 (C3b C4b receptor, CD35) on primate erythrocytes (red blood cells (RBCs)).[3][4] This binding allows the RBCs to act as an inner shuttle, delivering ICs to fixed tissue macrophages in the liver/spleen and away from vulnerable tissue. This is an important step for the safe clearance of circulating ICs and in preventing IC mediated diseases. After removal of the ICs, erythrocytes return to circulation unharmed but lacking some part of their CR1 content.[5][6]

We are using the physiological mechanism of IA and are developing a new heteropolymer (HP) system to allow more efficient removal of diverse pathogens from the bloodstream.[7] HPs consist of two crosslinked monoclonal antibodies, one of which is directed to the CR1 site and the other to a target pathogen/antigen. The HP provides "instant immunity"; it circumvents the need for an immune response against the pathogen, and allows faster clearance of the pathogen in a complement independent manner. Taylor *et al* demonstrated in a monkey model that an infused HP facilitates binding of a target (bacteriophage ΦX174) to RBCs and that the RBC bound ΦX174 is rapidly removed from the circulation with

the majority cleared to the liver, phagocytosed, and destroyed there within 24 hours.[7][8]

A subclass of HPs are the so-called "antigen based heteropolymers" (AHPs), which are designed to remove a pathogenic autoantibody with specificity to a single antigen. In this case, the anti-CR1 antibody is cross linked covalently to the antigen.[7][9][10]

The prototype of AHPs is ETI-104, containing double stranded (ds)DNA as the antigen linked to the anti-CR1 antibody (fig 1). We have shown that ETI-104 can be given safely (for example, no haemolysis caused by bound ETI-104) to monkeys, binds to primate RBCs in vivo, and clears anti-dsDNA antibodies infused into these animals[11] while failing to clear irrelevant antibodies, thus establishing the specificity of clearance.[9]

In humans the presence of anti-dsDNA antibodies is virtually diagnostic for systemic lupus erythematosus (SLE) and rarely occurs in other conditions.[12] SLE is a disease of unknown cause characterised by a number of immune abnormalities, including the formation of antibodies to nuclear and cellular antigens.[13–17] High avidity anti-dsDNA antibodies are seen mostly in patients with nephritis and may correspond with, or predict changes in, disease activity.[18–21]

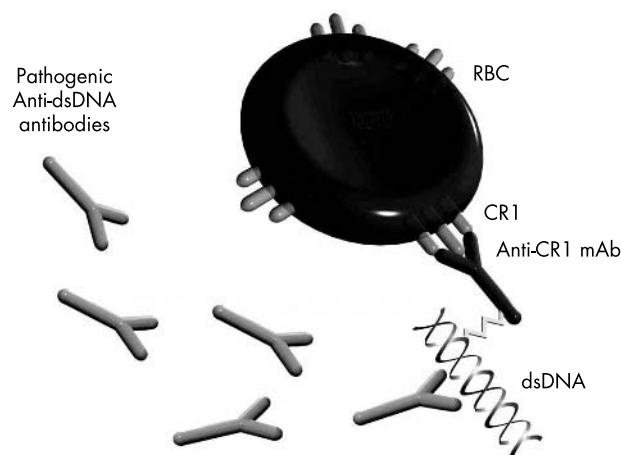...

Figure 1 Scheme of ETI-104 bound to an RBC.

These antibodies are believed to have a major role in the pathogenesis of SLE; a defective erythrocyte/IC clearing mechanism due to low CR1 levels on RBCs might lead to excessive IC deposit in vulnerable tissue such as the kidneys,[22–30] resulting in various degrees of cellular proliferation, inflammation, and fibrosis potentially leading to renal failure.[31 32] Numerous experimental and clinical studies have investigated anti-dsDNA antibodies and their relationship to the pathogen and flares in SLE nephritis.[27 28 33–39]

Our study aimed at establishing the safety and tolerability of a single dose of ETI-104 in normal healthy volunteers (NHV) and patients with SLE. Furthermore, we wished to establish for the first time the proof of principle for the new AHP technology in humans by demonstrating:

- Binding of ETI-104 to RBCs of NHV and patients with SLE
- Binding of the target (in this case anti-dsDNA antibody) to ETI-104 bound to RBCs of patients with SLE and
- Removal of the target from the blood.

PATIENTS AND METHODS
NHV and patients with SLE
Approval by the ethics committee of the University of Duesseldorf and informed consent was obtained from 11 NHV with an average age of 35 years (range 22–51), who were recruited by Focus Clinical Drug Development, Neuss, Germany. None of the NHV had significant diseases, a history of allergies, or took a regular drug.

Six patients with SLE (2 male, 4 female), diagnosed according to the ACR criteria for the classification of SLE,[40] with an average age of 36.6 years (range 26–55) were recruited by the Department of Rheumatology/University of Duesseldorf. Disease activity was measured before and after the study by the Systemic Lupus Activity Measurement (SLAM) score [41]. Patients were clinically inactive or had mild, stable disease activity and no actual major organ involvement for at least 6 months before entering the study. A SLAM score >1 in one organ system led to exclusion, unless the score was explained by stable values and was not caused by actual disease activity. All patients received immunosuppressive treatment, which was unchanged for 6 months before entering the study. Their steroid dose was <10 mg/day and had not been changed for at least 8 weeks. All patients had anti-dsDNA antibody titres >25 U/ml determined by the Farr assay, which had changed <10% over the past 3 months (''baseline'' Farr titre). Table 1 lists the demographic data and details of the patients with SLE .

Study description
The first eight healthy volunteers (NHV 1–8) were divided into two groups with four volunteers in each group; in the first group three subjects received 1 mg ETI-104 and the fourth a placebo solution. In the second group, 3 received 5 mg ETI-104, one placebo. Six patients with SLE (SLE 1–6) received 5 mg ETI-104, none of them placebo. Later on, in order to perform additional studies on the functional activity of the in vivo bound drug, three further volunteers (NHV 101–103) were given 5 mg ETI-104.

At day 0, the drug/placebo was given with a single infusion in a final 15 ml volume in saline over 15 minutes. Blood for pharmacokinetic assays and for determination of auto-dsDNA antibodies (patients) was drawn at 0, 15, 30, 60, 120, and 240 minutes, and at days 1, 2, 7, 14, and 28. Plasma was isolated and kept frozen at −70℃ until analysis.

Blood and urine for routine haematology and chemistry measurements (for example, serum proteins, enzymes, electrolytes, glucose, bilirubin, creatinine, blood cell counts or differentials, urinary constituents) was taken before the drug/placebo administration, at 4 hours, and at days 1, 7, and 28. Levels of complement factors C3/C4 were measured at 0 and 4 hours, and from day 1 to day 28. For safety reasons, NHV and patients stayed in hospital until 24 hours after administration and vital signs (including ECG) were measured continuously.

Study drug ETI-104
ETI-104 is a murine anti-CR1 monoclonal antibody (7G9) of IgG2a subclass conjugated to dsDNA derived from salmon testis.[11] The drug was manufactured in compliance with current Good Manufacturing Practices and was tested and characterised as described previously.[11]

Determination of human antimouse antibodies (HAMAs) to ETI-104
Production of HAMAs to the murine component of ETI-104 was measured in serum of NHV and patients before the study and at days 14 and 28 by a radiometric assay performed by Dr MB Khazaeli (University of Alabama, Birmingham Alabama).[42] A strong HAMA response in this assay has been defined as >250 ng/ml.[42] A positive HAMA response was defined as the mean on day 0+3 SD (>52 ng/ml for NHV and >22 ng/ml for patients with SLE), but at least twice the pre-dose level.

Pharmacokinetic assays
Blood samples from NHV or patients were collected into EDTA coated tubes (Becton Dickinson, Heidelberg, Germany) at the times described above by single venous puncture and subjected to FACS analysis.

The RBCs of NHV or patients were stained with Alexa-goat-antimouse IgG for detection of the murine component of ETI-104 and with PicoGreen for detection of the DNA component bound to the cells. For detection of anti-dsDNA antibodies bound to ETI-104, cells were stained with Alexa-goat-antihuman IgG and IgM according to the manufacturer's directions (all purchased from Molecular Probes, Leiden, The Netherlands). Complement receptor 1 (CR1) on RBCs was detected by staining cells with Alexa conjugated 7G9 antibody as described previously.[11] The number of CR1 on the RBCs was estimated by determining the amount of native 7G9 antibody required to block binding of the Alexa conjugate (prepared by Elusys and used at a 1:180 dilution).

Detection of ETI-104 and total human anti-dsDNA antibodies in plasma samples
To detect unbound ETI-104 in the plasma of NHV or patients, RBCs from a naïve donor (blood group O) were incubated

Table 1 Demographic data, drug treatment, medical history, anti-dsDNA antibodies, SLAM scores, C3/C4 levels, and CR1 levels of all six patients with SLE

Patient	Sex/age/onset of disease	Current drug for treatment of SLE	Clinical manifestation*	Anti-dsDNA† (Farr titre, U/ml) ANA titre ENA profile	SLAM Day 0/day 28	C3/C4 (g/l) t=0 h t=4 h t=24 h t=48 h	CR1 on RBC
1	Female/55/1995	7.5 mg Prednisone 400 mg Hydroxychloroquine	Arthritis Livedo reticularis Pleuritis	306 1/ 1280 Negative	6/8	0.98/0.24‡ 0.63/<0.06 0.67/0.11 0.82/0.11	372
2	Male/26/1984	5 mg Prednisone 250 mg Chloroquine 1000 mg Mycophenolatmofetil	Skin Arthritis Pleuritis Renal Liver/ascites Leucopenia	45 1/80 Negative	2/1	0.57/0.11 0.49/0.08 0.53/0.10 0.55/0.10	106
3	Female/28/1993	125 mg Chloroquine	Raynaud's syndrome Renal Skin vasculitis Leucopenia	42 1/320 U1RNP	9/7	1.08/0.16 0.95/0.17 0.1/0.15 1.04/0.17	253
4	Female/43/1980	5 mg Prednisone 50 mg Azathioprine	Arthralgia Myalgia Renal Raynaud's syndrome	62 1/ 640 SS-A, SS-B	12/9	0.85/0.12 0.76/0.11 0.78/0.11 0.82/0.11	106
5	Male/30/2000	2.5 mg Prednisone 400 mg Hydroxychloroquine Cyclophosphamide IV every 3 months 2000 mg Mesalazine	Renal Pleuritis Arthritis Endocarditis Leuco-lymphopenia Fever Colitis	484 1/640 Negative	7/9	0.82/0.11 0.62/0.06 0.78/0.09 0.77/0.10	27
6	Female/38/1999	10 mg Prednisone 250 mg Chloroquine	Raynaud's syndrome Alopecia Livedo reticularis Arthralgia Photosensitive rash Neuroradiculitis	27 1/640 Negative	9/6	0.83/0.14 0.77/0.13 0.77/0.13 0.82/0.15	54

*Clinical manifestation may be acute or in the medical history; †baseline levels of anti-dsDNA antibodies before entering the study; ‡levels at screening visit (2 weeks before) were 0.82/0.19.
ENA, extractable nuclear antigen.

with plasma samples and then stained as described above for in vivo RBC samples. Anti-dsDNA antibodies present in patient plasma were detected by incubating patient plasma with naïve RBCs opsonised with 250 ng AHP. The samples were stained with Alexa-goat-antihuman IgG and IgM for detection of total antibodies.

FACS analysis
Cells were analysed by FACS-Sort and CellQuest 4.1 software (Becton Dickinson). Ten thousand events were counted in an erythrocyte gate. All FACS assays were carried out in duplicate, and the results are expressed as geometric mean fluorescence.

Farr assay for detection of human anti-dsDNA antibodies
High avidity anti-dsDNA antibodies in serum were measured using an anti-dsDNA kit (Farr assay) from Trinity Biotech, Ireland, according to the manufacturer's instructions (normal limit <7 U/ml). All values were measured in quadruplicate.

Complement factors
C3/C4 were measured by nephelometry (Behring Werke AG, Marburg, Germany). Normal limits are: C3 0.6–0.9 g/l, C4 0.1–0.4 g/l.

Statistics
Differences between the groups and Farr titres at baseline, 15 minutes, and day 28 were calculated using a non-parametric signed rank Wilcoxon test, with $p < 0.05$ regarded as significant.

RESULTS
The goals of the studies in NHV and patients with SLE were to demonstrate, for the first time, that the new AHP ETI-104 can be safely given to man and, furthermore, to establish proof of principle for this platform technology.

Safety evaluation after ETI-104 administration
In NHV and patients with SLE the vital signs and cardiovascular measures gave no cause for concern about safety upon administration of ETI-104 at all follow up times up to 28 days. ETI-104 did not clinically significantly affect any chemistry, haematology, or urine analysis safety parameters in any of the subjects.

After ETI-104 administration, a slight and non-significant decrease in total haemoglobin was seen after 4 hours, which was not caused by haemolysis, as haptoglobin and lactate dehydrogenase stayed within normal limits and did not differ significantly between baseline and 4 hours. As patients were allowed to drink 1 hour after dosing, the haemoglobin decrease at 4 hours was probably caused by a dilution and/ or the blood sample taking (data not shown).

Table 2 Adverse events (AEs) recorded during the study in NHV and patients with SLE (subjects without any AE are not shown)

	Adverse event*	Intensity	Relation to ETI-104	Action taken
NHV No				
2†	Headache‡	Mild	Unrelated	None
6	Haematoma at IV line‡	Mild	Unrelated	None
	Skin reaction to tape dressing at IV line‡	Mild	Possibly related	None
7	Headache‡	Mild	Unrelated	None
	Headache‡	Moderate	Unrelated	500 mg Acetomephenone
	Headache	Mild	Unrelated	None
	Skin reaction to tape dressing at IV line‡	Mild	Possibly related	None
8	Skin reaction to tape dressing at IV line‡	Mild	Possibly related	None
101	Headache	Mild	Unrelated	500 mg Acetomephenone
	Headache	Mild	Unrelated	1000 mg Acetomephenone
Patient No				
2	Skin reaction to tape dressing at IV line‡	Mild	Possibly related	None
	Mild cold, sore throat, and rhinitis	Mild	Unrelated	None
	Diarrhoea, vomiting	Moderate	Unrelated	2 mg Loperamide
4	Headache	Mild	Unrelated	2000 mg Aspirin
	Meniscus lesion left knee	Moderate	Unrelated	Surgery§
	Headache	Moderate	Unrelated	500 mg Aspirin
5	Skin reaction to tape dressing at IV line‡	Mild	Possibly related	None
6	Skin reaction to tape dressing at IV line‡	Mild	Possibly related	None
	Increasing arthralgia, with reduction of steroids	Mild	Unrelated	None
	Diarrhoea	Mild	Unrelated	None
	Headache	Mild	Unrelated	500 mg Aspirin

*AEs lasted for up to 7 days, the six AEs considered as being possibly related to the drug administration lasted for up to 24 hours with a mean time period of 8 hours (range 2–24); †all subjects received 5 mg ETI-104 except NHV 2 who received 1 mg ETI-104; ‡AE was recorded within 24 hours after dosing, the rest days later; §fulfils criteria of a serious AE, but the meniscus lesion was diagnosed *before* entering the study.

As complement is an important factor in patients with SLE, levels for C3 and C4 were of specific interest. In four of six patients, C3 and C4 levels before administration were lower than the normal range and all patients showed a further decrease at 4 hours after administration. The mean decrease for C3 was 17% (range 7–36, p = 0.03) and 28% for C4 (range 7–75%, p = 0.063). The decreases were not clinically significant, were transient, and returned to baseline levels after 24 and 48 hours, respectively (except in patient No 1, see comment in table 1). For NHV C3/C4 levels did not decrease and were within normal ranges before and after administration.

Immunogenicity against ETI-104 was determined by drug-specific HAMA at days 0, 14, and 28 in both study groups. The mean day 28 HAMA level (98 ng/ml; SD 75) and the frequency of the response in patients with SLE (four of six patients) were not significantly different from the respective values in the six NHV receiving 5 mg ETI-104 (53 ng/ml, SD 33; four of six NHV, p = 0.22). All subjects stayed below 200 ng/ml at all times, a value regarded as a low level response.[42]

Overall, no drug related, serious adverse events occurred during the whole study. Ten non-serious events in NHV and 11 in patients with SLE were seen; and six of those were considered as being possibly related to the drug administration (table 2). In three NHV and three patients with SLE a skin reaction to the tape dressing at the intravenous site was seen. The reaction resolved without treatment within 2–24 hours. In three NHV and in two patients headache was seen; however, only in two NHV did the event occur during the first 24 hours after dosing. All other cases were recorded days later, so headache was not regarded as related to the drug administration.

All patients with SLE were clinically stable with essentially unchanged anti-dsDNA antibody values for 3 months before entering the study. Though the question of a clinical benefit of ETI-104 was not examined in this study, the SLAM score was taken to monitor patients' disease activity. It did not differ significantly between day 0 and day 28 (table 1).

In summary, these data show the safety of a single administration of 5 mg ETI-104 in NHV and patients with SLE.

Proof of principle
Binding of ETI-104 to RBCs of NHV in vitro and in vivo
Firstly, the binding of ETI-104 to each subject's RBCs was confirmed in vitro by FACS analysis (antimouse for detection of the murine CR1 antibody and PicoGreen for detection of the DNA component of ETI-104) (fig 2A). Its specificity for binding to CR1 was confirmed by its ability to compete with Alexa labelled anti-CR1 antibody (fig 2B) and the lack of binding of unconjugated DNA (not shown). In vivo, we also detected rapid binding of ETI-104 to RBCs in all NHV 15 minutes after administration—which was the first time this was measured (fig 2C). In NHV and patients with SLE receiving 5 mg ETI-105 and who had low CR1 numbers on RBCs some free drug was detected in plasma for up to 2 hours (data not shown).

Binding of ETI-104 and anti-dsDNA antibodies to RBCs of patients with SLE in vivo
Rapid binding of ETI-104 to RBCs and binding of the target pathogen, the anti-dsDNA antibodies, to bound ETI-104 could be demonstrated as early as 15 minutes after dosing (fig 3).

Removal of the pathogen (here anti-dsDNA antibodies) from plasma
Removal of anti-dsDNA antibodies from plasma was evaluated by measuring high avidity anti-dsDNA antibodies (by Farr assay) as well as total anti-dsDNA antibodies (by FACS analysis). At the first time (15 minutes) after administration, a substantial reduction of the Farr titre was achieved for all patients with SLE, with a mean reduction of 55% (range 43–62; fig 4A). Similarly, significant reductions were seen for total anti-dsDNA antibodies (mean 65%, range 40–79; fig 4B).

In five of the six patients, the maximum Farr titre reduction occurred at 15 minutes, in one patient (No 1) at 4 hours after ETI-104 administration. After 4 hours, the remaining five patients had Farr titre reductions of 15–62% of the initial values (fig 4A). For total anti-dsDNA antibodies, in five of six patients the maximum reduction also occurred at
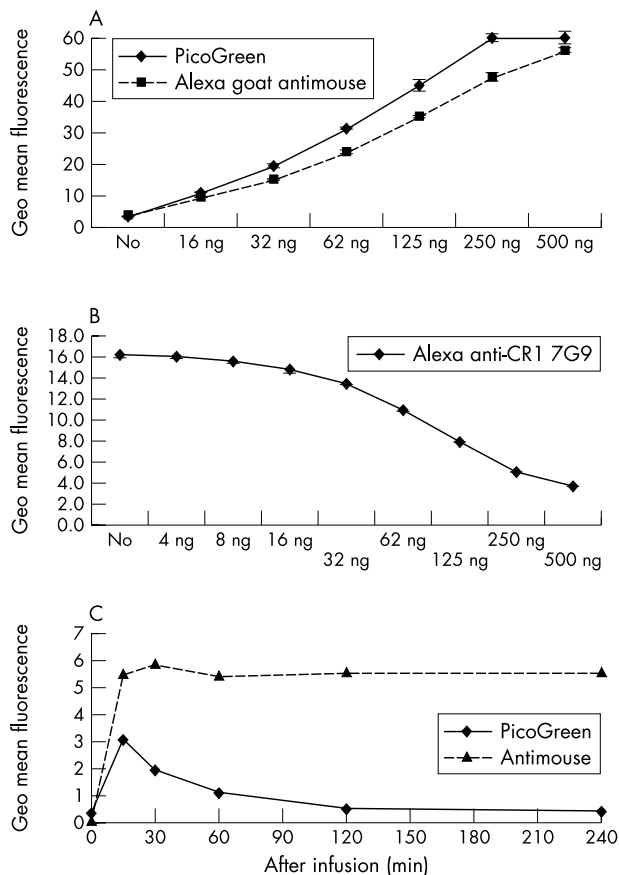
Figure 2 FACS analysis establishes binding of ETI-104 to RBCs of NHV. (A) Binding of ETI-104 to RBCs of each individual was confirmed by FACS analysis in vitro (goat antimouse for detection of the murine CR1 antibody and the PicoGreen DNA component of ETI-104). (B) RBCs from NHV 103 were bound in vitro to a saturating amount of Alexa-488 labelled anti-CR1 monoclonal antibody (mAb; 7G9) in the presence of varying concentrations of AHP. Increasing concentrations of AHP blocked 7G9 binding to the cells, demonstrating that AHP binds specifically to CR1. Thus, 250 ng AHP was not sufficient to block all available CR1. (C) RBCs from NHV 103 were stained after drug infusion to detect binding of the murine CR1 mAb (antimouse) and DNA (PicoGreen) components of ETI-104. The average of the geometric mean fluorescence intensity after subtracting non-specific fluorescence is given. The pattern of staining was similar in the other 10 NHV. All values were measured in duplicate.
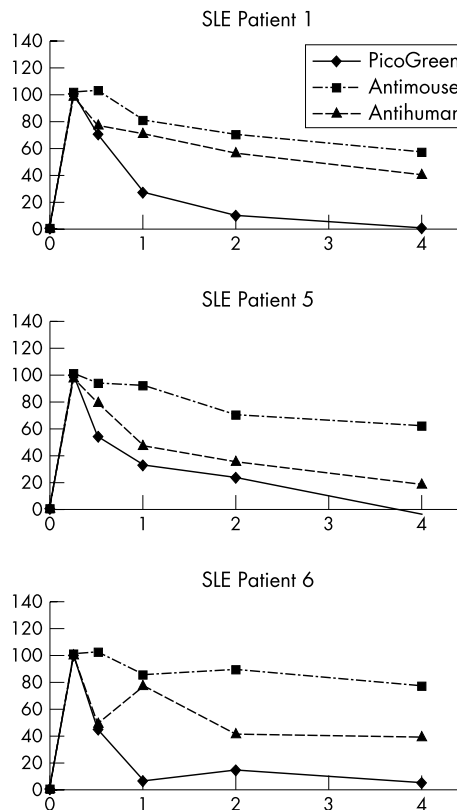
Figure 3 FACS analysis establishes binding of ETI-104 and target anti-dsDNA antibodies to RBCs in patients with SLE in vivo. RBCs from patients were stained after drug infusion to detect binding of the murine mAb (antimouse) and DNA (PicoGreen) components of ETI-104 and binding of the target anti-dsDNA antibody (antihuman). Average of the geometric mean fluorescence intensity of stained samples, after subtracting non-specific fluorescence, at each time was normalised to the 15 minute post-infusion value, which was set at 100%. The patients shown here represent the three patterns of association of anti-dsDNA antibodies with the RBCs; anti-dsDNA antibody tracked with the murine mAb component of ETI-104 (patient 1), with the DNA component of ETI-104 (patient 5), or non-parallel to either ETI-104 component (patient 6). Patients 2 and 3 showed essentially the same pattern as patient 1 and patient 4 as patient 5 (not shown). All values were measured in duplicate.

15 minutes, and in only one patient (No 4) at 2 hours. By 4 hours, total levels remained reduced by 11–71% (fig 4B).

On day 28 the Farr titres remained significantly reduced by 18% in patients Nos 1 and 2 and by 10% in patient No 6 (p<0.03). In the remaining patients the antibody values at day 28 were not significantly different from the starting values (data not shown).

Ability of ETI-104 to bind dsDNA antibodies in vivo

The ability of ETI-104 to reduce extremely high titres of anti-dsDNA antibodies was demonstrated for patients Nos 1 and 5 (table 3). If we assume the blood volume to be 5 litres, we observed removal of a total of 810 000 units (53% of 306 U/ml) in patient No 1 at 15 minutes, and a total of 1 403 000 units (58% of 484 U/ml) in patient No 5. The total anti-dsDNA antibody titres were reduced comparably (at 15 minutes by 66% in patient No 1 and by 68% in patient No 5). The similar amounts of antibody reduction in these two patients were achieved *despite* a significant difference in their CR1 number (372 *v* 27), resulting in differences in the amount of

ETI-104 bound (detected by different maximum PicoGreen and antimouse signals). Thus ETI-104 has the capacity to bind multiple molecules of anti-dsDNA antibodies. The amount of anti-dsDNA antibodies detected on the RBCs in patient No 5 *v* patient No 1 differed by only 50% despite the greater than 10-fold difference in the CR1 number on RBC.

Kinetics of drug components on RBC after initial binding

The rapid binding of ETI-104 to RBCs has been noted for both NHV and patients (figs 2B and 3). Whereas in both groups very little change of the murine component (measured by antimouse) was noted over the first 4 hours, the change of the DNA component of the drug (measured by PicoGreen) was much more rapid, with complete loss of the PicoGreen signal for all subjects by 4 hours. Detection of the anti-dsDNA antibodies (fig 3) on the RBCs showed different patterns for the patients. In three of six patients (for example, No 1) the antibody profile followed the murine component of ETI-104, in two patients (for example, No 5) the profile followed the DNA component, and in one patient (No 6) the pattern seemed intermediate between the murine and DNA component.
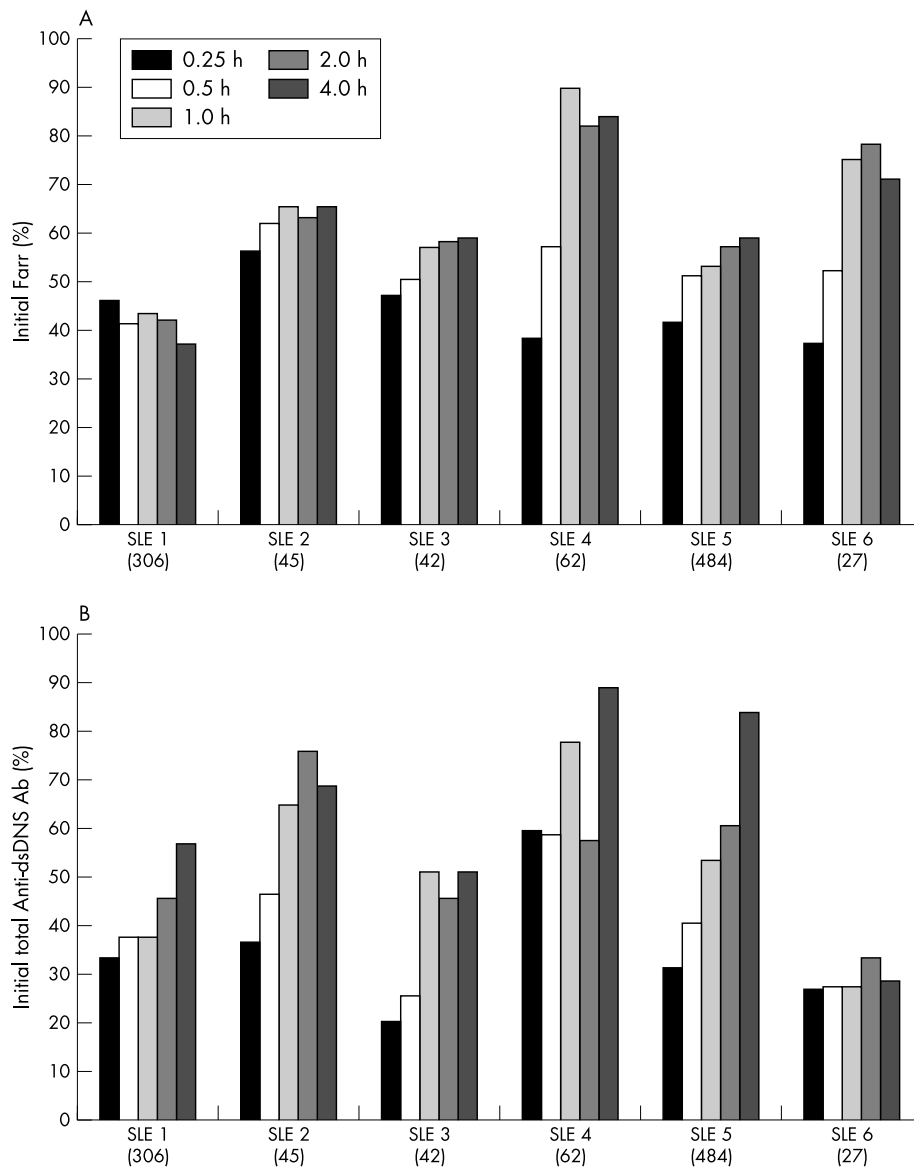
Figure 4 Administration of ETI-104 reduces the high avidity anti-dsDNA antibodies (Farr activity) and total dsDNA antibodies (FACS analysis) from the circulation of patients with SLE. (A) High avidity anti-dsDNA antibodies (Farr titre) reductions from 15 minutes to 4 hours after ETI-104 administration. The Farr titre was reduced from the initial values by 43–62% at 15 minutes and by 15–62% at 4 hours after ETI-104 administration; it was maximally reduced in patient No 1 at this time. The reduction was ~200 IU/ml in patients with starting titres of 306 and 484 IU/ml (starting titres of each patient are given in brackets). All Farr values were measured in quadruplicate. (B) Total anti-dsDNA antibody (FACS analysis) reductions from 15 minutes to 4 hours after ETI-104 administration. Patient plasma samples were incubated in vitro with naïve AHP opsonised RBCs (blood group O), washed, and stained with Alexa antihuman IgG and IgM antibody. Total dsDNA antibody levels were reduced from their initial value by 40–79% at 15 minutes and by 11–71% at 4 hours after ETI-104 administration. All FACS values were measured in duplicate.

Table 3 ETI-104 has a high binding capacity for anti-dsDNA antibodies in vivo

Patient with SLE	Calculated No of CR1 receptors on RBCs*	High avidity anti-dsDNA antibodies in plasma† (Farr titre)		Total anti-dsDNA antibodies in plasma‡ (by FACS analysis)		Anti-dsDNA antibodies on RBC‡ (by FACS analysis)	
		t=0 min	t=15 min	t=0 min	t=15 min	t=0 min	t=15 min
1	372	306	145	134.6	45.1	2.8	46.1
5	27	484	205	93.3	32.3	9.1	31.1

*Numbers were calculated using the fluorescence values from the CR1 FACS assay; †data are mean values from quadruplicate samples in U/ml; ‡data are given as geometric mean fluorescence intensity (mean of duplicate samples) from FACS analysis.

Kinetics of autoantibodies in plasma

In three of six patients the level of total anti-dsDNA antibody at 4 hours did not agree with the Farr titre. Two patients had a substantially greater reduction in Farr titre than in total anti-dsDNA antibody (patient No 1: 62% v 42% and patient No 5: 40% v 11%), one had a smaller reduction in the Farr titre than in the total anti-dsDNA antibody titre (patient No 6: 29% v 70%). In the remaining three patients, 4 hour levels of total anti-dsDNA antibody were in agreement with the Farr titre. The possible implications of this observation are discussed below.

DISCUSSION

The trials described here represent the first test in man of the HP technology platform. The principal purpose of the two trials on ETI-104 was to evaluate the safety of administration of a single dose of the drug using standard parameters for safety—physiological, cardiovascular, chemical, haematological, and urine analysis over a 28 day follow up period. Overall, there were no safety concerns and, particularly, no drug related serious adverse events. The only non-serious adverse event considered to be associated with the drug was a dermatological reaction in the area of the injection site and associated with the tape dressing. The reactions resolved spontaneously without treatment. The transient decrease of complement C3/C4 did not cause a haemolysis (no change in haptoglobin or lactate dehydrogenase) of RBCs nor did it affect the patients clinically.

The other purpose of these studies was to establish proof of principle for the new AHP platform. For the AHP technology to be successful in removing a target pathogen (in this case, autoantibodies to dsDNA), several steps must occur. AHP is a bispecific immunoconjugate, one part of the molecule binding to CR1 and the other to an antigen-specific antibody (see fig 1).

The proposed mechanism of action of the AHP technology is:

- The immunoconjugate binds to CR1 on RBCs (step 1)
- The pathogen binds to the immunoconjugate on RBCs (step 2)
- As a consequence, the pathogen levels in the plasma decrease (step 3) and
- The RBCs deliver the immunoconjugate/pathogen complexes to the reticulendothelial system in the liver/spleen for destruction. The RBCs then return functionally intact to the circulation with the loss of some expressed CR1 (step 4).

We decided to study the technology in patients with SLE; this disease is strongly associated with several defined autoantibodies, most notably anti-dsDNA antibodies. For this purpose ETI-104 was constructed, an AHP containing dsDNA in the immunoconjugate, which would bind the anti-dsDNA antibodies.

The studies presented here investigated steps 1–3 of the proposed mechanism in vivo. In both studies, in NHV as well as in patients with SLE, we could demonstrate (a) ETI-104 rapidly binds to CR1 on RBCs in vivo (step 1); (b) at the same time, the administration to patients with SLE leads to a rapid binding of the pathogen (here anti-dsDNA antibodies) to ETI-104 on RBCs (step 2); (c) leading to its reduction in the plasma (step 3). Thus, the proof of principle for the new AHP technology is established, in general, for the first time in man through these studies.

It was not possible to design a clinical trial to directly examine step 4, the in vivo removal of the immunoconjugate/pathogen complexes (ETI-104 and anti-DNA antibody) from the surface of the RBCs by the tissue macrophages, and to demonstrate that the clearance of the complex from the RBCs led to its destruction. As mentioned above, Taylor et al have demonstrated earlier this mechanism in a monkey model.[7] [8]

The data provided a proof of principle for the HP technology, in general. However, some specific factors have to be considered when dealing particularly with patients with SLE. The use of the AHP technology is dependent upon CR1 receptors being the binding site for the drug. Patients with SLE, however, have been found to have lower CR1 numbers on RBCs.[43] [44] We also observed a wide variation of CR1 levels (27–1381), with the patients having significantly less CR1 (mean (SD) 556 (365) for NHV and 153 (133) for patients; $p < 0.05$). The lower CR1 number and nature of the CR1 receptor in patients may compromise the ability to remove ICs through immune adherence. However, three features of the AHP technology that are pertinent to the discussion of CR1 in patients with SLE are (a) the removal mechanism is complement independent; (b) the CR1 expressed on RBCs of patients with SLE has been shown in this study to be capable of binding the HP; and (c) the binding of the CR1 receptor does NOT determine the capacity to bind autoantibodies; that is, the amplification provided by the dsDNA component of ETI-104 results in a huge capacity of binding anti-dsDNA antibodies (the patient with a CR1 number of 27 bound significant autoantibody titres).

One intention in the selection of a 5 mg dose of ETI-104 was to occupy only 20% of the available CR1 molecules based on reported values of 700 CR1/RBC in NHV and 400–500 CR1 in patients with SLE.[43] [44] With the wide variation mentioned above (27–1381 for both populations), the dose exceeded CR1 saturation, as evidenced by transient detection of the free drug in plasma of some NHV and patients. There were no safety problems connected with this oversaturation.

We observed a very fast and effective initial reduction of anti-dsDNA antibodies after 15 minutes in patients with SLE. Nevertheless, this decrease was transient, with some patients having an increase from the nadir of reduction during the first 4 hours after administration. We considered three possible explanations for the early rebound: (a) synthesis of new anti-dsDNA antibodies by memory B cells; (b) redistribution from sites of deposition within tissues/extravascular compartments; and (c) instability of the reagent. A new synthesis of autoantibodies by autoreactive plasma cells is unlikely because of the short timeframe of 15 minutes: stimulation of cells, synthesis, assembly, and transport of proteins cannot take place in such a short time. The second possibility is one we would like to pursue in future studies; although it confuses interpretation of the present data, it would be attractive to draw autoantibodies from other pools and clear them with multiple administrations at even lower doses of ETI-104. The clear shift in the nature of the anti-dsDNA antibody between high avidity (by Farr) and total anti-dsDNA antibodies (by FACS) during the early re-increase in three patients is consistent with a redistribution explanation. It is interesting to note that despite the rebound in the *early* phase, we saw a significantly reduced antibody level in three of the six patients after 28 days with only this single given dose. We did not observe a significant increase in total anti-dsDNA antibodies in any patient. The latter was seen in patients given other dsDNA antibody lowering treatments—for example, plasma exchange.

We explored the third possibility—instability of the molecule—because we noted a difference in the pattern of the PicoGreen staining of the DNA component of the molecule from the staining of the murine CR1 mAb component: when bound in vivo to RBCs of NHV and patients with SLE, PicoGreen decreases in fluorescence intensity over the first 4 hours, whereas the fluorescence intensity after

staining of the same samples for the CR1 component (Alexa-antimouse) remains essentially unchanged (fig 2C). Those data suggest that there might be a loss of the DNA component of ETI-104 in vivo, possibly due to a degradation of DNA through DNAse activity in the plasma of patients with SLE. However, binding of anti-dsDNA antibody to the RBCs does not always decrease in a manner similar to the PicoGreen signal (patient 1, see fig 3). In addition, we have examined the in vitro functionality of ETI-104 bound to RBCs of NHV in vivo. In these experiments we have sampled RBCs from NHV at various times after ETI-104 infusion and incubated these samples in vitro with a high titre SLE plasma. The level of binding of anti-dsDNA antibodies to the RBCs in vitro (antihuman) decreased with the sample time after administration, but it decreased at a rate less rapid than the loss of the PicoGreen signal (data not shown). These data suggest that the binding capacity of ETI-104 of anti-dsDNA antibodies is not necessarily linked to the PicoGreen signal.

As the role of anti-dsDNA antibodies in the pathogenesis of SLE is somewhat disputable, the question arises as to whether there is a need for an anti-dsDNA lowering treatment at all. Nevertheless, a number of treatments have been explored which aim at reducing the production of anti-dsDNA antibodies by autoreactive B cells (for example, by immunosuppressive treatment or by anti-CD20 antibodies) or removing antibodies from the circulation (for example, by plasma exchange and immunoadsorption).[33] HP technology may offer advantages over the physical methods of removal mentioned above. Plasma exchange and immunoadsorption using C1q or protein A are far less specific than the AHP; each of these removes a broader population of immunoglobulins, including beneficial antibodies as well as some complexes, and these methods have the potential for broader effects on immunoregulation. Such is not the case with the HP technology, which has been shown to be specific in its removal.

Controversial results were reported from the new agent LJP394. This molecule was shown to have high affinity for anti-dsDNA antibodies and decreased anti-dsDNA antibodies by ~30% in patients with SLE, and this was accompanied by a reduced risk for renal flares in the first phase 2/3 trials.[45 46] However, there was no significant difference from standard immunosuppressive treatment alone in a phase 3 trial.[47] Nevertheless, in both groups a significant reduction of the risk for renal and major flares was reported in patients with reduced anti-dsDNA antibodies, thus indicating the potential clinical benefit of an anti-dsDNA lowering treatment.[48]

The US Food and Drug Administration recently used its advisory committee meeting on 29–30 September 2003 to discuss with approximately 20 specialists the development of drugs to treat SLE. There was a broad consensus that anti-dsDNA antibodies are a biomarker for disease activity, although reliance on only antibody levels was not sufficient for establishment of clinical benefit. The Food and Drug Administration is also considering the measurement of anti-dsDNA antibodies as part of accelerated approval for SLE drugs. This opinion adds to our belief that further investigations of AHP as a potential treatment for SLE are warranted. We used a monoclonal mouse antibody in the early phase of the development to establish proof of principle. We know that the observed generation of a low level HAMA response against the murine component of ETI-104 precludes the further development of this compound because our data indicate that the administration of the immunoconjugate has to be repeated to achieve a continuous reduction in circulating anti-dsDNA antibodies. We have generated a humanised CR1 mAb which allowed the development of the AHP product ETI-201 that will facilitate investigations with multiple dosing regimens.

In summary, these clinical trials established that the immunoconjugate ETI-104, the first tested specimen from the new class of HPs, can safely remove very effectively high titres of pathogenic molecules (here, dsDNA antibodies). The data provide the basis for further development of this technology as a treatment option for numerous indications—for example, autoimmune diseases such as SLE, or viral and bacterial infections, including toxins.

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