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A clinical exploratory study with itolizumab, an anti-CD6 monoclonal antibody, in patients with rheumatoid arthritis

Pedro C. Rodriguez[a], Roberto Torres-Moya[b], Gil Reyes[c], Claudino Molinero[b], Dinorah Prada[b], Ana M. Lopez[b], Isabel M. Hernandez[b], Maria V. Hernandez[b], Jose P. Martinez[b], Xochel Hernandez[a], Angel Casaco[a], Mayra Ramos[a], Yisel Avila[d], Yinet Barrese[d], Enrique Montero[e], Patricia Hernandez[a],[*]

[a]Clinical Research Division, Center of Molecular Immunology, Havana, Cuba
[b]National Service for Rheumatology, Havana, Cuba
[c]Rheumatology Service, Center of Surgical and Clinical Research, Havana, Cuba
[d]National Coordinating Center of Clinical Trials, Havana, Cuba
[e]Experimental Immunotherapy Department, Center of Molecular Immunology, Havana, Cuba

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ABSTRACT

T cells are involved in the pathogenesis of rheumatoid arthritis (RA). CD6 is a co-stimulatory molecule, predominantly expressed on lymphocytes, that has been linked to autoreactive responses. The purpose of this study was to evaluate the safety, immunogenicity and preliminary efficacy of itolizumab, a humanized anti-CD6 monoclonal antibody, in patients with active rheumatoid arthritis. Fifteen patients were enrolled in a phase I, open-label, dose-finding study. Five cohorts of patients received a weekly antibody monotherapy with a dose-range from 0.1 to 0.8 mg/kg. Itolizumab showed a good safety profile, with no severe or serious adverse events reported so far. No signs or symptoms associated with immunosuppression were observed in the study. Objective clinical responses were achieved in more than 80% of patients after treatment completion, and these responses tend to be sustained afterwards. This clinical study constitutes the first evidence of the safety and positive clinical effect of a monotherapy using an anti-CD6 antibody in patients with rheumatoid arthritis.

1. Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease characterized by symmetric inflammation of synovial joints, leading to progressive erosion of cartilage and bone, restricted mobility, and reduced life expectancy [1,2].

The currently available disease-modifying anti-rheumatic drugs (DMARDs) used in conventional first-line therapy provide some benefit, but generally fail to control the disease in a significant number of patients and furthermore, their clinical effects are often limited by toxicity [3]. New and more effective DMARDs continue to emerge and in particular, biological agents that aim to inhibit cytokine activity, block T cell-mediated co-stimulation, or modify B cell biology [4,5]. In spite of some encouraging therapeutic results, it should be noted that none of the biological therapies tested in clinical trials has been able to induce ACR50 (approximately a 50% disease improvement) in at least half of the patients. In fact, the best drugs provide only 10–40% ACR70 [6]. In order to achieve additional significant gains in RA therapy, new therapeutic approaches need to be assayed.

The central role of T cells in the pathogenic immune response in RA has been described elsewhere [7]. T lymphocytes contribute to the initiation and perpetuation of RA immunopathology, leading to inflammation and, ultimately, joint destruction [8,9]. Activated T cells proliferate and recruit other immune cells such as monocytes, macrophages, and synovial fibroblasts, inducing them to produce proinflammatory cytokines (tumor necrosis factor-α, TNFα; interleukin-1, IL-1; interleukin-6, IL-6), prostaglandins, leukotrienes and oxygen free radicals [8,10], and to stimulate osteoclastogenesis and matrix metalloproteinase secretion [11]. One of the co-stimulatory pathways engaged in T cell activation involves the interaction between the activated leucocyte-cell adhesion molecule (ALCAM/CD166), found on antigen presenting cells, with the CD6 receptor on T cells [12,13].

CD6 is a highly glycosylated membrane protein predominantly expressed on lymphocytes. Its extracellular region is composed of three scavenger receptor cystein-rich (SRCR) domains [14]. The third, membrane proximal domain (SRCR3) contains the binding site for ALCAM
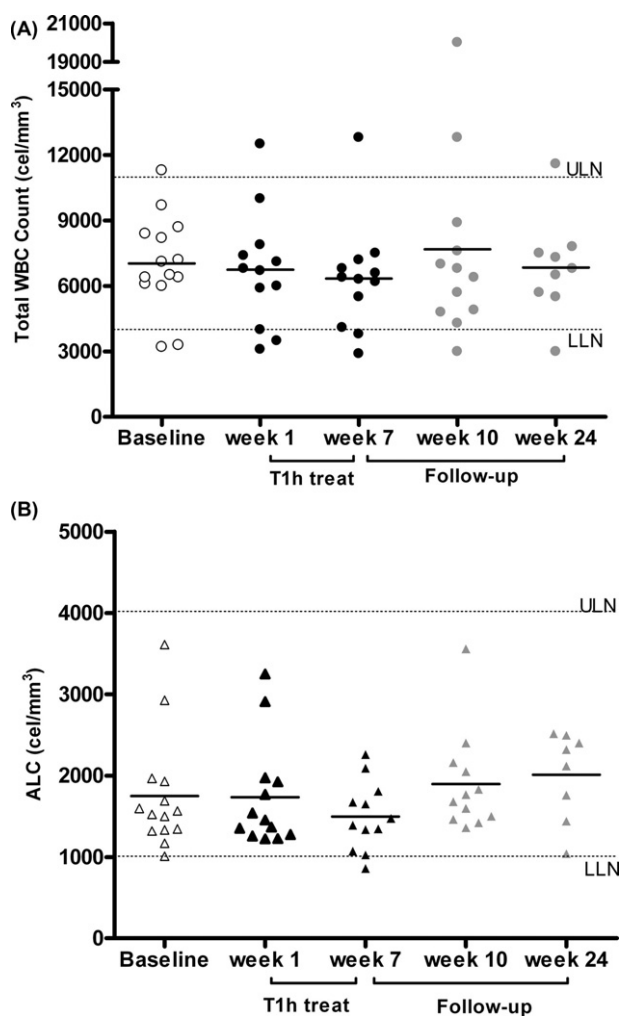
Fig. 1. Scatter of individual values for total white blood cells (WBC) (A) and absolute lymphocyte count (ALC) (B) for individual subjects of overall study cohort (safety population) over time. LLN: lower limit of normal; ULN: upper limit of normal. The graph also shows the normal laboratory reference ranges and the clinically significant range as per CTCAE v 3 Grade 1.

[15,16]. It has been argued that CD6 may play a role in cell proliferation, adhesion, differentiation and survival processes [17–19]. Recently, it was demonstrated that the CD6 co-stimulatory pathway contributes to the Th1 activation and differentiation of human T cells, promoting a preferentially proinflammatory response (TNFα, IL-6 and interferon-γ) [20]. Under particular conditions, such activation process may progress to an uncontrolled tissue inflammation, usually characterized by an autoimmune immunopathology.

The relevance of CD6 in an autoimmune scenario has been previously discussed [21–25,28–30]. Initial studies demonstrated that CD6 negative T cells show less alloreactivity than their CD6 positive counterparts, while anti-CD6 monoclonal antibodies (mAbs) prevent renal and bone marrow grafts rejection [26,27]. More recently, the finding of CD6 as a susceptibility gene in multiple sclerosis, a prototypic autoimmune disease [28–30], supports the role of CD6 in pathological autoimmunity leading to tissue inflammation and reinforces its relevance for targeted therapy.

It is worth noting that the therapeutic effectiveness of anti-human CD6 mAbs has been primarily associated with their ability to deplete CD6 cells by a complement-mediated mechanism [27] or the capacity of blocking the interaction between CD6 and its ligand ALCAM [31,32]. However, several evidences suggest that signals delivered

upon stimulation of different epitopes of the CD6 molecule may produce different effects on T cells [21,33].

Itolizumab (T1h) is a humanized monoclonal antibody [34] that recognizes the membrane-distal domain (SRCR1) of CD6 [35]. In *in vitro* experiments using a soluble construction of the ALCAM molecule, it was shown that T1h does not inhibit ALCAM binding to T-cells. Furthermore, it was shown that T1h does not produce T-cell depletion [35]. In spite of these properties, *in vitro* characterization showed that itolizumab inhibits the T-cell proliferation induced in the presence of ALCAM and excess IL-2, downregulates the phosphorylation of intracellular proteins implicated in the CD6-mediated activation pathways and reduces interferon-γ, IL-6 and TNF-α production [20]. Hence, targeting CD6 *in vivo* with itolizumab would modulate the immune response by reducing T-cell activation, proliferation and pro-inflammatory responses.

It is remarkable that these inmunomodulatory effects are produced without inhibiting ligand binding and inducing T cell depletion. In this regards, it has been reported that there are antibodies which instead of preventing ligand binding may cause receptor binding to be non-productive. These interactions can result in either an inhibition of new receptor formation, stimulation of the loss of existing receptors, or a blockage followed by internalization or downregulation of the receptors [36]. On the other hand, nondepleting mAbs have been used to establish persistent T-cell tolerance [37]. All together, these findings point to a potential new mechanism of action for itolizumab, as compared with other anti-human CD6 mAb previously used in clinical studies and other anti-CD6 antibodies assayed in preclinical studies [21,22,38].

The parent antibody of itolizumab, the murine mAb ior T1, was raised in BALB/c mice immunized with PBMCs from a patient with Sezary's syndrome [39,40]. Ior T1 mAb showed therapeutic effects in autoimmune diseases, such as psoriasis and RA [23,24,41–43]. However, due to its murine origin this antibody needed to be humanized aiming to eliminate most of the undesired properties of murine mAbs [34]. In this regard, the clinical use of humanized mAbs has revealed a striking absence of adverse reactions. Based on these evidences, together with our previous finding that itolizumab exhibits the same CD6 recognition profile and a similar affinity constant, but was less immunogenic in monkeys than its murine or chimeric counterpart [34,35], we expected itolizumab be less immunogenic and toxic than its predecessors, leading to additional benefits in RA patients.

The aim of the current study was to investigate the effect of a 6-week monotherapy with itolizumab in biologic-naïve patients with active moderate to severe RA despite the previous DMARD therapy. The primary intention of the study was to evaluate the safety and tolerability of different doses of itolizumab during 24 weeks. In addition, the study explores preliminary evidences of efficacy.

2. Methods

2.1. Study design and endpoints

The study was an open-label, non-controlled, dose-finding phase I trial, registered under number RPCEC00000007 at the Cuban Registry of Clinical Trials (www.registroclinico.sld.cu), and conducted at a single clinical center in Havana, Cuba. For trial recruitment active RA patients underwent an eligibility screening between July 2004 and October 2006.

After a washout period (at least 4 weeks for DMARDs and glucocorticoids, and 2 weeks for NSAIDs), patients were sequentially enrolled into cohorts of three patients, each receiving a different itolizumab dose (0.2, 04 or 0.8 mg/kg/day), once a week during 6 weeks. The dose range was selected based on *in vitro* experiments and from the preceding experiences on clinical trials were performed with the murine ior T1 mAb on RA patients [23,24,41–43].

Two patients were assigned to 0.1 and 0.6 mg/kg, in two additional

dose levels that were added during the course of the study. Subjects were followed up for a period of 18 weeks after the last antibody administration. The 24-weeks study period was divided into 2 stages: week 0–6 was considered the treatment period while from week 7 to week 24 was considered the post-treatment period (follow up). The study medication was administered intravenously, once a week during 6 weeks. The signs and symptoms were evaluated for 6 months, starting from the first dose administration. Clinical assessments were performed at baseline and at weeks 7, 10 and 24, according to the ACR core set of disease-activity measurements. Safety was monitored during the whole study (weeks 0–24). The restriction for the use of DMARDs, glucocorticoids and NSAIDs was extended from the washout period, including the administration phase and up to 4 weeks after the last itolizumab administration (follow-up, week 10), when these drugs could be administered if disease flares, according to the physician's criteria. Otherwise, only analgesics were permitted. Patients taking drugs for concomitant disease were required to have been on chronic stable doses prior to screening. Such stable dose had to be maintained throughout the study.

The primary endpoints were safety and tolerability of multiple doses of itolizumab as administered by intravenous infusion. The secondary endpoints were immunogenicity and prelimi-nary clinical activity evaluation of the administered mAb. Since this study was not designed for efficacy assessment a blinded parallel group using placebo was not included.

2.2. Ethics statement

This trial was conducted in full conformity with the principles expressed in the Declaration of Helsinki. The protocol and related documents were reviewed and approved by the institutional review board from the participating institution and approved by the Cuban National Regulatory Agency (State Center for Drug Quality Control). All patients were recruited within the National Service for Rheumatology in Havana and were given oral and written information about the trial. All patients provided written informed consent before any trial-specific procedure was performed. An institutional review board committee (IRB) safeguarded the rights, safety, and well-being of all trial subjects.

2.3. Study population

Eligible patients were aged 18–70 years, fulfilled the revised ACR criteria for RA [44], at least one year before the screening, and had active disease despite treatment with at least one DMARD. Active disease was defined by the presence of at least four swollen and four tender joints. Patients receiving a previous treatment with any DMARD, glucocorticoids or nonsteroidal anti-inflammatory drugs (NSAIDs) were eligible for participation after an appropriate washout period before enrolment. Laboratory values within normal reference range were required.

Patients were ineligible if they had history of, or current inflammatory joint disease, other than RA or other systemic autoimmune disorder or any overlap syndrome. All pa-tients had to be using a medically accepted form of contracep-tion at the time of enrolment and had to continue its use through the follow up period.

2.4. Immunogenicity evaluation

The study was primarily focused on the anti-idiotypic response after a prolonged exposure to the biological agent, when the IgG response is predominant. The IgG anti-idiotypic response against the variable region of the humanized itolizumab [34] was monitored weekly during 10 weeks after the first administration. Ninety-six well COSTAR® enzyme-linked immu-nosorbent assay plates (Corning Incorporated, Corning, NY, USA) were coated with ior T1 (the murine, parent antibody of itolizumab), at 5 μg/ml phosphate buffered saline

(PBS) and incubated overnight at 2–8 °C. The plates were washed with PBS containing 0.05% Tween 20 and blocked for 1 h at 37 °C with PBS containing 1% Bovine Serum Albumine (BSA). The plates were then washed again and 1:400 and 1:800 serial dilutions of test sera or positive control sera were added to the appropriate wells, followed by incubation for 1 h at 37 °C. A pool of sera from three Monkeys (*Cercopithecus aethiops*) immunized with a chimeric predecessor of T1h [34], having a known high reactivity in the assay, was used as control. The pool showed an average optical density (405 nm) of 2,148. Plates were washed and an alkaline phosphatase-conjugated goat anti–human IgG (Jackson Immunoresearch Laboratories, West Grove, USA) antibody was added. Following 1 h incubation at 37 °C, plates were washed again and 1 mg/ml paranitro-phenyl phosphate in diethanolamine buffer was added to each well. After 30 min at 25 °C in dark place, the reactions were stopped with 3 N NaOH, and the absorbance (405 nm) was recorded. A post-treatment OD/pre-treatment OD ratio = 2 was defined as cutoff value for positive responses.

2.5. Statistical analysis

Safety was evaluated in the population who received at least one dose of itolizumab, while clinical effect was evaluated in the evaluable population defined as patients who received at least six doses of the mAb. Patients who did not achieve an ACR20 were considered as non-responders. Patients who dropped out the study or did not attend physician evaluation at the time point to assess clinical effect were considered as not available.

The incidence of adverse events and the proportion of patients with a clinical benefit expressed in a 20% improvement of signs and symptoms (ACR20) or superior (ACR50 and ACR 70) were reported as counts and percentages.

The ACR core data set consists of seven components: swollen joint count (66 joints), tender joint count (68 joints), subject global assessment of pain (VAS 100 mm), subject global assessment of disease activity (VAS 100 mm), physician global assessment of disease activity (AS 100 mm), and subject assessment of physical function using HAQ and eritrosedimentation rate (ESR).

3. Results

3.1. Characteristics of the study population

A total of 15 patients were enrolled in the study. Three patients were included into the three dose levels groups previously defined (0.2 mg/kg, 0.4 mg/kg and 0.8 mg/kg). Two patients were additionally included in the 0.4 mg/kg group since two patients dropped-out the study before the clinical assessment was completed (week 7). A protocol amendment to include a 0.1 and 0.6 mg/kg dose cohorts was made after initiation of the trial, with two patients accrued in each one (Table 1).

Data on patient disposition, demographics and other characteristics at baseline are summarized in Tables 1 and 2. The patients were predominantly women (73%) with moderate disease activity (80%) and a median duration of the disease of 10 years across the five dose groups. Patients showed active disease at recruiting despite previous DMARD therapy, evidenced by more than four swollen and tender joints at baseline (data not shown). All patients had received two or more DMARDs before enrolment (Table 1). Since the washout period accounted for a high baseline disease activity, the clinical status immediately before the first itolizumab dose was considered as baseline (W0) (Table 3A).

Fourteen patients, out of 15 that participated in the study, received the scheduled six-infusions of itolizumab. Thirteen patients reached the first assessment point of the follow-up period (week 7); while nine patients completed all the scheduled follow-up visits. A
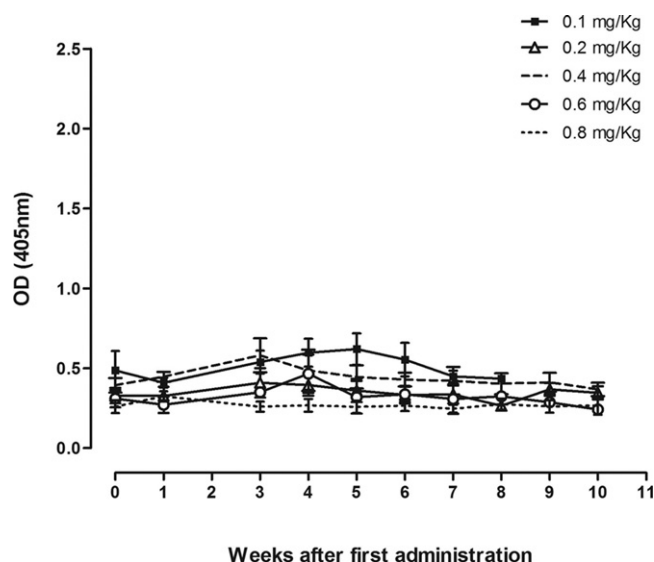
Fig. 2. Anti-idiotypic IgG response during T1h mAb therapy. The immunogenicity of the humanized T1h mAb was monitored prior to dosing and weekly until the week 10 after first administration. The IgG anti-idiotypic response of treated patients against the mouse variable region of the humanized mAb T1h was evaluated using an ELISA system coated with the anti- CD6 mAb iort1. Positive response was considered when the ratio post-treatment OD/ pre- treatment OD was >2 for each patient.

Table 1.
Demographic indicators and disease characteristics at screening of the RA patients in the intent-to-treat population enrolled in the trial, by treatment group. Data are number of patients (%) for categorical data and median (range) for continuous data. SJC = swollen joint count; TJC = tender joint count; DMARDs = disease-modifying antirheumatic drugs; NSAIDs = nonsteroidal antiinflammatory drugs; RF = rheumatoid factor; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; HAQ = Health Assessment Questionnaire.

Characteristics	T1h mAb dose levels					Combined T1h groups
	0.1 mg/kg (n = 2)	0.2 mg/kg (n = 3)	0.4 mg/kg (n = 5)	0.6 mg/kg (n = 2)	0.8 mg/kg (n = 3)	Total (n = 15)
Female, no. (%)	2 (100)	1 (33.3)	4 (80)	2 (100)	2 (66.6)	11 (73.3)
Age (years)	41.5 (31–52)	42 (41–49)	60 (41–62)	40	52 (39–61)	52 (31–69)
Weight (kg)	83 (82–84)	75 (63–90)	75 (51–96)	67.5 (65–70)	73 (65–82)	73 (47–96)
Disease duration (years)	6.5 (1–12)	18 (12–20)	3 (2–20)	11 (2–20)	6 (2–27)	10 (1–27)
Disease activity, no. (%)						
Moderate	0	3 (100)	5 (100)	2 (100)	2 (66.7)	12 (80)
Severe	2 (100)	0	0	0	1 (33.3)	3 (20)
Prior medications, no. (%)						
Methotrexate	2 (100)	2 (66.6)	3 (60)	2 (100)	2 (66.6)	11 (73.3)
Other DMARDs	2 (100)	3 (100)	1 (20)	2 (100)	3 (100)	11 (73.3)
Corticosteroids	1 (50)	3 (100)	5 (100)	1 (50)	3 (100)	13 (86.6)
NSAIDs	2 (100)	3 (100)	5 (100)	2 (100)	3 (100)	15 (100)
RF positive	1 (50)	2 (66.7)	4 (80)	1 (50)	2 (66.7)	10 (66.6)
CRP positive	2 (100)	2 (66.7)	4 (80)	2 (100)	3 (100)	14 (93.3)

total of six patients (40%) did not complete the study. Two (33.3%) out of six patients discontinued during the treatment period after using a restricted concomitant medication, one of them to treat ordinary symptoms of dyspepsia and the second one as rescue therapy because of lack of effectiveness. Three patients (50%) were discontinued because of loss of follow-up. One patient (16.6%) discontinued voluntarily (Table 2).

3.2. Primary endpoint

Safety analysis was based on all subjects who received at least one dose of itolizumab, which represented 100% of patients. None of the patients discontinued because of safety reasons.

No treatment-related serious adverse events (SAE) or severe infections were reported. All subjects experienced at least one adverse event during the 24-week study, but there was no evidence of a relationship between the dose and the intensity, duration or frequency of these adverse events. The majority of them were of mild (63.3%) or moderate (36.3%) severity. One subject from the 0.4 mg/kg dose

group experienced a severe adverse event (a headache) which was classified as not related to the study drug. No AEs resulted in either discontinuation or reduction of the dose of the study drug.

From the 225 AEs reported during overall study, 178 events (79%) were considered to be related to the study agent by the investigators. From these 178 EAs, 128 (72%) occurred during the treatment period while 50 (28%) took place during the follow-up period. The majority of them (77AEs, 43%) were suggestive of peri-infusional events (defined as adverse events occurring within the 24 h following the infusion) with a considerable decline in frequency observed after 3 weeks of treatment. The most commonly reported AEs included headache (27 AEs, 15%), fever (22 AEs, 12%) and chills (14 AEs, 7%). Only 43 AEs (24%) were considered to be likely or very likely related to the study agent.

Taking into account that CD6 is a lymphocyte marker that plays an important role in immune function, we determined whether itolizumab treatment has an effect on the white blood cells count (WBC) and in particular, the lymphocyte population (ALC) for all the 15 RA patients enrolled in the trial. Four patients showed laboratory

Table 2.
Most frequently reported adverse events ($n = 15$). All adverse events affecting more than 10% of patients in any one group are shown. Values are the number (%).

	Number (%) $n = 15$
Adverse events	
Death	0
Serious adverse events	0
Serious infections	0
Any adverse event	15 (100)
Most frequently adverse events	
Headache	11 (73)
Fever	9 (60)
Chills	9 (60)
Nausea	7 (46)
Anorexia	7 (46)
Asthenia	7 (46)
Local erythema	6 (40)
Pruritus	6 (40)
Vomiting	3 (20)
Diarrhea	2 (13)
Discontinuations (reasons)	6 (40)
Adverse event	0
Withdrawal of consent	1 (16)
Lost to follow-up	3 (50)
Use of restricted drugs	2 (33)
Death	0

values out of the normal reference ranges for WBC counts; two of them were under the lower limit (from the 0.4 mg/kg and 0.6 mg/kg groups), while the other two were over the upper limit (0.1 mg/kg and 0.4 mg/kg groups). In one of them (0.4 mg/kg group) the transient increase of WBC was associated with an increase in neutrophils and a severe RA. It has been reported that WBC elevation is primarily caused by the increase in neutrophils, and that those patients tend to have more active arthritis [45]. In three other patients these AEs came out before starting the treatment. All these AEs were graded as mild in intensity and were deemed not related to the study medication. In general, WBC and absolute lymphocyte counts were stable across the entire study, regardless of the dose levels. No abnormalities were observed in patients from the highest dose group (0.8 mg/kg) (Fig. 1 A and B).

The other hematological abnormalities frequently detected in the study were mild thrombocytosis and anemia. Such kinds of hematologic disorders are common in patients who suffer an active RA [46], therefore, these AEs were considered as primary disease progression or recurrence and not related to the study medication. Moreover, the hemoglobin values tended to increase throughout the study.

Three out of six patients with urinary symptoms (dysuria, polyuria and nicturia) were diagnosed of urinary tract infection (UTI) but only for one patient (arm 0.4 mg/kg) the symptom was considered as 'related' to the study drug. The other two patients (0.4 mg/kg arm and 0.6 mg/kg arm) showed low WBC counts before the study, and one of them had, in addition a history of type 2 Diabetes mellitus and recurrence of UTI. All patients with UTI were treated with oral antibiotics and recovered completely before the end of study.

Since concurrent treatment with any DMARDs, glucocorticoids or NSAIDs was allowed after week 10; four patients were consequently medicated because of disease flares. Three patients received low doses of oral corticosteroid and one patient used low doses of DMARD.

3.3. Secondary endpoints

3.3.1. Anti-idiotypic IgG antibody response

None of the serum samples from the 15 patients across the different dosage cohorts developed significant immunogenic responses after completion of week 10. The low measurable anti-idiotype antibody response was transient and independent of the amount of administered protein (Fig. 2). There were no evidences of any relationship between the anti-idiotype antibody response and the dose or clinical efficacy.

3.3.2. Efficacy evaluation

The clinical efficacy outcomes, assessed by the improvements in at least seven individual components of the ACR score and the rate of ACR 20, ACR 50 and ACR 70, were performed at weeks 7, 10 and 24 from the beginning of the study. The clinical assessment immediately before the first itolizumab dose was considered as baseline (W0). Taking advantage of the small number of patients included in the study and taking into account the safety aim of the study a preliminary efficacy analysis was performed by a full set analysis.

Already by the first assessment point of the follow-up period (week 7), the overall study cohort analysis showed improvements from baseline values in all ACR criteria components (Table 3A, W7). Most of the variables showed over 50% improvements. These results correlate with the high proportion of subjects achieving an ACR20 response rate (84%). The proportion of ACR50 and ACR70 responders was 76% and 23%, respectively (Table 3B, W7). At the subsequent assessment point (week 10, 4 weeks after the last itolizumab dose) the improvements tended to persist (Table 3 A and B, W10).

By week 24, there were significant improvements in all variables as compared with baseline (W0) and week 7. However, since the restriction for the use of DMARDs had previously already concluded, the clinical impact at this assessment point is limited. The analysis included three patients who received low-dose oral glucocorticoids but excluded one patient who was medicated with a DMARD (Table 3 A and B, W24).

Although six patients were missing at several assessment points, most of them dropped out during the follow-up (66.6%) and mostly after week 20 (50%). These patients showed ACR response in the last recorded visit (one ACR 50, two ACR 70 and one ACR 20).

A predominant effect was seen in some particular RA clinical markers such as SJC, PAP, HAQ and ESR. In these variables a trend toward the increase of improvement was sustained across the entire follow-up (Table 3A).

The clinical effect was also notable for hemoglobin values. The trend to decrease observed during the washout period was associated with RA exacerbation as a consequence of the restriction for the use of DMARDs. Once the treatment started a stabilization was observed and turned into increase until the end of treatment phase. The highest values were reached at week 24 (Fig. 3).

Among those who received different doses there were no differences between the proportions of patients who achieved ACR20 or higher clinical response, considering the small number of subjects in each group.

4. Discussion

Despite the evident success of several new biological therapies, concerns remain regarding their immunosuppressive effects and the associated increased risk of infection [47]. Therefore, the need for further advances and alternative therapies is clear.

A few agents are targeted to inhibit T-cell activation rather than block the consequences of activation—as most of current biological DMARDs do [48]. Evidences of clinical benefits of blocking T cell signalling in RA patients have been confirmed [49,50]. In this regard, anti-CD6 therapy is an emerging field to improve clinical benefit in active RA, with previous experiences obtained from studies developed in graft rejection and autoimmunity [51–54]. Such therapeutic intervention should modulate T lymphocyte activation, auto-recognition and traffic through the joints.

Table 3.
Efficacy of T1h at weeks 7, 10 and 24, by combined T1h groups. (A) Median (range) of ACR components. Median of percentage of improvement in ACR components (changes in the individual patient data from baseline (W0) to W7, W10 and W24): 100 × baseline value – day × value]/baseline value. SJC = swollen joint count; TJC = tender joint count; DMARDs = disease-modifying antirheumatic drugs; NSAIDs = nonsteroidal antiinflammatory drugs; RF = rheumatoid factor; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; HAQ = Health Assessment Questionnaire; PAP = Patient Assessment of Pain; Global Disease Assessment by Patient = GDAP; Global Disease Assessment by Observer = GDAO. (B) Proportion of patients with improvement in ACR criteria (ACR20, ACR50, ACR70).

RA assessment	Median (range)	Median (range)	Median change from baseline (range)	Median (range)	Median change from baseline (range)	Median (range)	Median change from baseline (range)
	W0, $n = 13$	W7, $n = 13$		W10, $n = 12$		W24[a], $n = 8$	
A							
SJC	21.0 (4.0–37.0)	2.0 (0.0–23.0)	81.0	3.5 (0.0–24.0)	76.3	1.0 (0.0–9.0)	95.0
TJC	26.0 (5.0–49.0)	7.0 (2.0–24.0)	60.0	5.0 (2.0–22.0)	67.5	2.0 (0.0–11.0)	85.0
PAP	8.0 (5.0–10.0)	4.0 (0.5–10.0)	50.0	2.5 (1.0 -8.0)	65.0	1.0 (0.0–8.0)	87.5
GDAP	8.0 (3.0–10.0)	3.0 (0.5–10.0)	66.7	3.0 (1.0–7.0)	62.8	1.0 (0.0–7.0)	81.9
GDAO	8.0 (4.0–10.0)	3.0 (0.0- 7.0)	66.7	3.0 (0.5–9.0)	62.8	1.0 (1.0- 6.0)	88.2
HAQ- DI	1.3 (0.3–3.0)	0.8 (0.0–2.0-)	55.4	0.6 (0.0 – 1.7)	60.5	0.1 (0.0–1.0)	97.3
ESR	42.0 (10.0–103.0)	40.0 (2.0–90.0)	22.1	35.0 (2.0- 94.0)	30.9	25.0 (7.0- 71.0)	44.9

B ACR responses	**Combined T1h groups**		
	W7, $n = 13$[b]	W10, $n = 12$[c]	W24, $n = 8$[d]
ACR ≥ 20	11 (84.6%)	11 (91.6)	8 (100%)
ACR ≥ 50	10 (76.9%)	8 (66.6%)	8 (100%)
ACR ≥ 70	3 (23.0%)	3 (25%)	2 (25%)

[a] One patient was excluded for the analysis because received DMARD.
[b] Two patients abandoned before first clinical assessment.
[c] One patient voluntary withdrawn.
[d] Two patients were lost to follow-up, one patient did not attend physician evaluation and one was additionally excluded for the analysis because received DMARD.
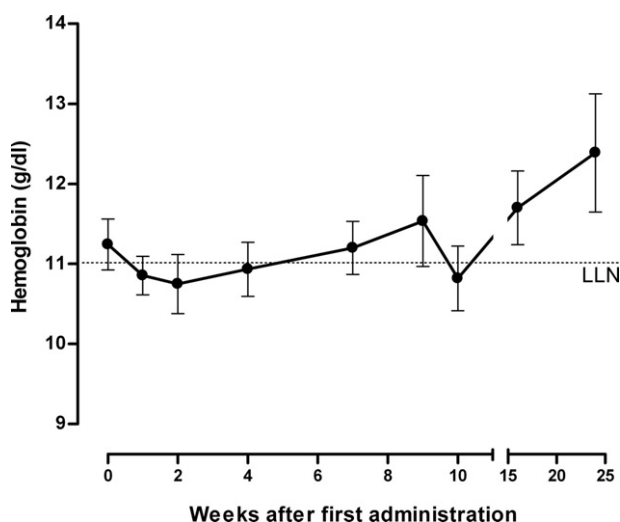

Fig. 3. Change in hemoglobin with T1h treatment during the study. LLN: lower limit of normal.

Itolizumab (T1h) is an anti-CD6 monoclonal antibody with clinical potential in the treatment of RA. A series of *in vitro* tests demonstrated that itolizumab inhibits CD6 mediated co-stimulation, reducing lymphocyte proliferation and pro-inflammatory cytokine production.

The study presented here was an exploratory, phase I, open label, dose range-finding trial involving biologically naïve patients with active RA. The primary endpoint of the study was to demonstrate and characterize the incidence of adverse events' rates of itolizumab monotherapy through dose escalation. Up to 6 weekly administrations of itolizumab at different doses (ranging from 0.1 to 0.8 mg/kg) were well tolerated and safe without any discontinuation because of safety reasons.

Immunogenicity is the main limitation for the use of mAbs. The treatment may result in the generation of antibodies against the therapeutic agent that, which interfere with the therapy. In particular, the generated antibodies may reduce the mAb half-life as consequence of increased clearance and produce undesired side effects which may limit the use of the drug. As we expected, based on a previous evidence of very low immunogenicity of itolizumab in monkeys, the study drug did not show significant immunogenicity in patients. There were no evidences of relationship between the low measurable anti-idiotype antibody response and the dose or clinical efficacy. The lack of anti-idiotypic response observed in RA patients correlated with a reduction in the type and intensity of AEs. Following an initial high incidence of mostly mild to moderate infusion-related AEs during the first week of treatment, itolizumab was well-tolerated.

Itolizumab monotherapy did not modify significantly the lymphocyte population during the course of the study. Likewise, there were not documented signs or symptoms which could be interpreted as immunosuppression induced by the mAb at any dose level during the therapy. These results suggest a different mechanism of action for itolizumab, not mediated by immune depletion, and provide a plausible explanation for the safety profile observed even at the highest dose level.

On the other hand, in previous clinical trials using ior T1, the mAb was administered intravenously once daily during 7 days, since the median half life time of this murine mAb was in the range 13.93–19.67 h [24,55]. In these studies most patients showed clinical benefits approximately up to 4 months after the first infusion. In our study, itolizumab was administered once weekly for 6 weeks and clinical benefits were observed at least up to 6 months after first infusion. Although we have not pharmacokinetics data at the time of this report, we hypothesize that the lack of anti-idiotypic response benefits the long term efficacy of itolizumab and permits a more comfortable schedule of administration.

The secondary endpoint evaluated preliminary evidences of therapeutic effect of itolizumab therapy in subjects without concomitant background DMARD therapy. In this scenario, itolizumab used as monotherapy achieved improvements in disease-related clinical markers. In the full set analysis, an objective clinical response was seen in most of the patients with ACR20, one week after the last dose administration. Itolizumab also showed effect at ACR50 and ACR70, which are more stringent measures of patient responses to treatment.

Significantly, the clinical response had a tendency to persist 4 weeks after the last itolizumab administration. Moreover, it is of note

that although the restriction for the use of DMARDs during the study was foreseen to extend just up to 4 weeks after the last itolizumab administration, most of the patients (53.3%) did not receive any DMARDs within the next 18 weeks from the last itolizumab dose (week 24) and nonetheless ACR 50 and ACR 70 were achieved. This sustained improvement along the follow-up period suggests that the treatment acts not only on the signs and symptoms, but also on the etiopathology of the disease. Moreover, it is important to mention that although there was a relatively high patient withdrawal rate (40%) in the study, the patients that abandoned the study did it in spite of the clinical benefits.

In patients with RA, anemia is the most common hematologic abnormality (prevalence ranges from 30% to 70%), which correlate with disease activity [56–59]. In our study we observed that itolizumab treatment leads to an increase in hemoglobin levels, which suggests a control of the disease despite no specific anti-rheumatic therapy but itolizumab monotherapy. The safe profile together with preliminary evidence of a sustainable efficacy response suggests that itolizumab is suitable for long-term treatment regimens.

Our study, nonetheless, has several limitations. This was the first-in-human dose escalation study conducted using itolizumab to define a safety dose range, which required an open-label design. This context and the consequent absence of a placebo-control arm limit our interpretation of efficacy. Moreover, the small sample size together with the relatively high number of non-compliances occurred in the trial reduces the power of the study, makes it difficult to define an optimal biological dose for treatment and to conduct a PK study, and leads to an underestimation of the safety and efficacy of the treatment. Finally, the 6-week treatment period was short. Since, proof-of-concept trials in RA require at least 3 months of treatment to allow sufficient time for improvements of the active disease to be demonstrated and to confirm that the benefit continues. This requirement has several important implications including the necessity for toxicology studies of sufficient duration to cover 12 weeks of dosing of a new agent in the clinic [60]. Third, the 24-week monitoring period is not enough for a long-term assessment to fully characterize the safety profile of itolizumab.

Nevertheless, this 6-week, ascending-dose trial involving 15 patients who had active moderate to severe rheumatoid arthritis despite non-biologic DMARD therapy, offers the first preliminary evidences of safety and clinical benefit using itolizumab monotherapy in RA patients. Our results point to a new potential mechanism for RA therapy, not mediated by immunosuppression, to achieve long lasting clinical benefits through T-cell response modulation. Although this study was not able to define a therapeutic dose based on efficacy results, it could be considered as the first valuable clinical application of mAb itolizumab. The encouraged safety profile shown by the antibody in this study prompted us to desing a long lasting schedule of monotherapy with itolizumab in larger cohorts of patient with active RA.

Acknowledgments

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