CSF1R inhibition with emactuzumab in locally advanced diffuse-type tenosynovial giant cell tumours of the soft tissue: a dose-escalation and dose-expansion phase 1 study

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Summary

Background Diffuse-type tenosynovial giant cell tumour (dt-GCT) of the soft tissue (alternatively known as pigmented villonodular synovitis), an orphan disease with unmet medical need, is characterised by an overexpression of colony-stimulating factor 1 (CSF1), and is usually caused by a chromosomal translocation involving CSF1. CSF1 receptor (CSF1R) activation leads to the recruitment of CSF1R-expressing cells of the mononuclear phagocyte lineage that constitute the tumor mass in dt-GCT. Emactuzumab (RG7155) is a novel monoclonal antibody that inhibits CSF1R activation. We have assessed the safety, tolerability and activity of emactuzumab in patients with Dt-GCT of the soft tissue.

Methods In this phase 1, first-in-human dose-escalation and dose-expansion study, eligible patients were aged 18 years or older with dt-GCT of the soft tissue with locally advanced disease or resectable tumours requiring extensive surgery, an Eastern Cooperative Oncology Group performance status of 1 or less, measurable disease according to Response Evaluation Criteria In Solid Tumors version 1.1, and adequate end-organ function. Patients with GCT of the bone were not eligible. Patients received intravenous emactuzumab at 900 mg, 1350 mg, or 2000 mg every 2 weeks in the dose-escalation phase and at the optimal biological dose in a dose-expansion phase. The primary objective was to evaluate the safety and tolerability of emactuzumab, and to determine the maximum tolerated dose or optimal biological dose. All treated patients were included in the analyses. Expansion cohorts are currently ongoing. This study is registered with ClinicalTrials.gov, number NCT01494688.

Findings Between July 26, 2012, and Oct 21, 2013, 12 patients were enrolled in the dose-escalation phase. No dose-limiting toxicities were noted in the dose-escalation cohort; on the basis of pharmacokinetic, pharmacodynamic, and safety information, we chose a dose of 1000 mg every 2 week for the dose-expansion cohort, into which 17 patients were enrolled. Owing to different cutoff dates for safety and efficacy readouts, the safety population comprised 25 patients. Common adverse events after emactuzumab treatment were facial oedema (16 [64%] of 25 patients), asthenia (14 [56%]), and pruritus (14 [56%]). Five serious adverse events (periorbital oedema, lupus erythematosus [occurring twice], erythema, and dermohypodermitis all experienced by one [4%] patient each) were reported in five patients. Three of the five serious adverse events—periorbital oedema (one [4%]), lupus erythematosus (one [4%]), and dermohypodermitis (one [4%])—were assessed as grade 3. Two other grade 3 events were reported: mucositis (one [4%]) and fatigue (one [4%]). 24 (86%) of 28 patients achieved an objective response; two (7%) patients achieved a complete response.

Interpretation Further study of dt-GCT is warranted and different possibilities, such as an international collaboration with cooperative groups to assure appropriate recruitment in this rare disease, are currently being assessed.

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Introduction

The molecular features of mesenchymal tumours have many similarities to those of haematological malignancies, including genetic aberrations creating deregulated kinases, overexpressed oncogenes, or fusion transcription factors. Such biologically disparate entities need subtype-specific treatments. Even when individual cases are rare, every effort should be made to validate the efficacy of new therapies with a treatment approach tailored to the underlying subtype-specific biology.[1]

Diffuse-type giant cell tumour (dt-GCT) of the soft tissue, alternatively known as pigmented villonodular synovitis, is a rare and destructive proliferation of synovial-like mononuclear cells, admixed with multinucleate giant cells, foam cells, siderophages, and inflammatory cells.[2] GCT can be localised or diffuse, and occurs more frequently in young adults, with no sex predilection in intra-articular disease, and a slight female predominance in extra-articular disease.[3] The knee is the most frequent (66–80%) intra-articular location for the development of these lesions, followed by the hip, ankle, elbow, and shoulder.[4] Total synovectomy is the standard of care for dt-GCT. However, recurrence is frequent (up to 55% of patients recur, depending on disease localisation) and

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Research in context

Evidence before this study

At the start of our study in 2012, the existing evidence on how to treat locally advanced and relapsed patients with diffuse-type tenosynovial giant cell tumors (dt-GCT) was inconclusive and the situation has not changed to date. We searched PubMed and ClinicalTrials.gov (without restriction in publication dates), and congress abstracts from the yearly meetings of the American Society of Clinical Oncology between 2007 and 2011 with search terms "pigmented villonodular synovitis", "diffuse type giant cell tumor", "tenosynovial giant cell tumor", "CSF1", "CSF1R", "c-fms", "imatinib", "nilotinib", "MCS110", "AMG820", and "PLX3397". No language restriction was used. We also held an international advisory board meeting to discuss this rare disease setting. We identified several case reports and patient series relevant to understanding the treatment strategy used for dt-GCT of the soft tissue. Probably because of the rarity of the disease, no randomised trials and only one larger prospective study was reported. Early clinical signs of antitumour activity were reported for imatinib; however, response rates were relatively low. It was postulated that the noted clinical activity was mediated by the CSF1 receptor targeting component of both tyrosine-kinase inhibitors. No systemic treatment was approved in this rare disease with high unmet need.

Added value of this study

Despite the non-life threatening character of dt-GCT, patients are often severely affected due to the long course of the disease, multiple surgeries (occasional amputation), perioperative morbidity, and secondary arthritis. Because dt-GCT is associated with characteristic cytogenetic abnormalities resulting in overexpression of CSF1, we designed a specific antibody to the CSF1 receptor. Emactuzumab (RG7155) showed clinical activity in locally advanced or relapsed dt-GCT and was associated with a manageable safety profile. Tumour responses were durable and most patients had profound symptomatic and functional improvement. Our results suggest that CSF1R targeting drugs, in particular specific monoclonal antibodies, could be very promising in this rare disease.

Implications of all the available evidence

At present, surgical resection remains the mainstay of treatment for dt-GCT; however, relapses are frequent and other treatment options are very limited. For advanced primary and recurring disease, specific therapeutic interference with the CSF1—CSF1R axis could represent a valuable alternative for this young patient population.

relapses are often multiple. Recurrence can severely compromise joint function and quality of life, and occasionally result in amputation.[2,5]

Dt-GCT is characterised by cytogenetic abnormalities, commonly involving a balanced translocation of 1p11 and 2q37 chromosomal loci.[6,7] This translocation involves *CSF1* and *COL6A3*.[8] *CSF1*, which encodes the ligand of the CSF1 receptor (CSF1R), is translocated in most cases of dt-GCT; however, only small numbers of cells in the tumour mass have been noted to carry the translocation resulting in overexpression of CSF1.[8] These cells recruit CSF1R-expressing macrophages and induce the formation of multinucleated giant cells. This results in the formation of a tumour landscape comprising non-neoplastic cells that respond to secreted CSF1, which eventually forms a locally destructive proliferation.[8]

The crucial role of aberrant CSF1 signalling in the development and progression of dt-GCT makes this pathway an ideal therapeutic target for patients with recurrent or unresectable disease, and potentially also for the first-line treatment of patients with diffuse disease to prevent perioperative morbidities arising from total synovectomy or total joint replacement. The potential therapeutic value of this approach was first shown by Blay and colleagues, who noted a complete response in one patient with advanced recurrent dt-GCT treated with imatinib.[9] The antitumour effect of imatinib is postulated to result from a blockade of CSF1R, however, studies assessing the efficacy of imatinib[10] or related drugs, such as nilotinib,[11] showed tumour shrinkage in only a few patients with dt-GCT.[12] So far, no systemic treatment is approved for dt-GCT.

Emactuzumab (RG7155) is a recombinant, humanised monoclonal antibody of IgG1 subclass directed against CSF1R expressed on macrophages. In a recent publication, we provided data supporting preclinical and pharmacodynamic activity of emactuzumab in in-vitro and animal models and in patients with dt-GCT.[13] We aimed to evaluate the safety and activity of emactuzumab in patients with locally advanced dt-GCT.

Methods

Study design and participants

This multicentre, open-label, phase 1 trial, included a traditional 3+3 dose-escalation phase followed by an expansion phase of the CSF1R inhibitor emactuzumab. This report only includes results for patients with dt-GCT; in addition to patients with dt-GCT, patients with all solid tumour types were eligible with the exception of hepatocellular carcinoma, non-small cell lung cancer, small cell lung cancer, gastric cancer, and malignant melanoma. Initial data about the clinical activity of emactuzumab in seven patients with dt-GCT have been reported recently.[13] Recruitment of patients with solid malignancies into this trial to be treated either with emactuzumab alone or with the combination of emactuzumab and paclitaxel is currently ongoing and will be reported elsewhere.

Eligible patients had locally advanced dt-GCT of the soft tissue or resectable tumours that required extensive

surgery. Diagnosis was reviewed for all cases by an expert pathologist in the French Sarcoma Group. Patients with GCT of the bone were not eligible. Patients were included if they were 18 years or older, had an Eastern Cooperative Oncology Group performance status of 1 or less, had measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) version 1.1, and had adequate end-organ function (serum total bilirubin ≤1·5×upper limit of normal [ULN] and aspartate aminotransferase or alanine aminotransferase ≤2·5×ULN; white blood cells ≥3×10⁹ cells per L, absolute neutrophil count ≥1·5×10⁹ cells per L, platelet count ≥100×10⁹ cells per L, haemoglobin ≥90 g/L; serum creatinine ≤1·5×ULN or creatinine clearance ≥50 mL per min; international normalised ratio ≤1·5×ULN, partial thromboplastin time ≤1·5×ULN). Patients were not eligible if they had received previous chemotherapy, radiotherapy, or any investigational drug or immunotherapy within 28 days of first infusion of emactuzumab. Patients with known autoimmune disease, acute infection, any poorly controlled comorbidities (eg, diabetes), and thromboembolic events (within 6 months of study entry) were also excluded from the study.

The protocol was approved by the institutional review board at each study centre. All patients provided written informed consent before study-related procedures were done. The study was done in accordance with the Declaration of Helsinki, current International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines, and all applicable regulatory and ethical requirements.

Procedures

Emactuzumab was given every 2 weeks (one cycle) as an intravenous infusion over 1·5 h. In both the escalation and expansion phases, treatment was continued until disease progression or unacceptable toxicity was recorded, or if the patient decided to withdraw from the study. In the event of grade 3 or worse adverse event believed to be related to study drug, the dose of study drug was held until adverse event fell to grade 2 or less within a maximum of 21 days. Patients would then resume treatment at a reduced dose of 50% for the remainder of the study. In the event of a haematological grade 4 adverse event believed to be related to study drug, the adverse event must revert to grade 2 or less in order for the patient to receive subsequent dose of study drug at a reduced dose of 500 mg.

In the traditional 3+3 dose-escalation phase, patients with dt-GCT were given one of three doses of emactuzumab (900 mg, 1350 mg, and 2000 mg). The protocol required a minimum of three evaluable patients per dose cohort; however, to avoid any delays, over-recruitment was allowed based on patient availability.

Based on pharmacokinetic, pharmacodynamic, and safety data from the dose-escalation phase, the optimal biological dose (OBD) of emactuzumab in patients with dt-GCT was estimated, and tested further in a separate cohort of patients with dt-GCT in the expansion phase.

Blood samples for safety and pharmacodynamic assessments were taken in both study phases at baseline, on day 1 of each 2-week cycle, at five additional timepoints during cycle one (days 2, 4, 8, 10, and 12), and one additional timepoint during cycle 2 (day 8).

Radiological assessments with MRI for all patients were done at baseline and after every three cycles of treatment using RECIST version 1.1. Assessments of metabolic response based on ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET imaging were done at baseline and after 4 weeks on treatment (two cycles of emactuzumab). ^{18}F-FDG PET imaging assessment was based on European Organisation for Research and Treatment of Cancer (EORTC) criteria[14] and both ^{18}F-FDG PET imaging and MRI were centrally reviewed by independent radiologists. Disease progression was defined as per RECIST version 1.1. Changes in joint function and other symptoms from baseline were documented based on the assessment of the treating physician. For pharmacodynamic assessment, tumour biopsies were taken at baseline and at 4 weeks on treatment. Samples were analysed for CD68, CD163, and CSF1R expression with immunohistochemical staining. Furthermore, we used fluorescence-activated cell sorting to monitor peripheral blood monocyte subsets over time. Specifically, we assessed the number of $CD45^{+}CD14^{+}$ and $CD14^{Dim}CD16^{High}$ monocytes; the latter express high levels of CSF1R and represent about 5–10% of all monocytes. The tissue biopsies were immediately subjected to fixation in 10% neutral-buffered formalin, and were transferred to tissue processing and paraffin embedding overnight incubation according to standard protocols. The following antibodies were obtained from Ventana Medical Systems (Tucson, AZ, USA): CD68 (clone KP-1) and CD163 (clone MRQ-26). An in-house-generated anti-human-CSF-1R monoclonal mouse antibody (clone 29; Roche Diagnostics GmbH, Germany) was used for immunohistochemical detection of CSF-1R. Sections were subjected to automated staining on a Benchmark XT instrument (Ventana Medical Systems).

Outcomes

The primary objective was to evaluate the safety and tolerability of emactuzumab and to determine the maximum tolerated dose (MTD) or OBD. The MTD was defined as the highest dose level at which no more than one of six patients in a dose-escalation cohort has a dose-limiting toxicity (DLT). Per protocol, DLTs were defined as any of the following toxicities occuring during the DLT assessment window: any non-hematological toxicity at grade 3 or worse (except for nausea, vomiting, fatigue, diarrhoea, skin disorders, oedema, hyperglycaemia, changes in serum electrolytes and laboratory parameters that might be increased because of reduced clearance in the liver if not associated with clinical signs and symptoms); haematological toxicities (febrile neutropenia

and/or documented infection with absolute neutrophil count $<1{\cdot}0\times10^9$ per L, thrombocytopenia grade 4, or bleeding necessitating a platelet transfusion); and any other study drug-related toxicity judged to be significant enough to be qualified as DLT in the opinion of the investigators after discussion with the sponsor). The OBD was defined based on the totality of all data obtained from the dose escalation phase of the study—ie, the dose level with acceptable safety, a favourable pharmacokinetic profile, observed clinical activity and/or effect on the disease course, modulation of biomarkers in skin, tumour, and whole blood, and pharmacodynamic imaging data.

Secondary objectives included pharmacokinetic and pharmacodynamic effects of emactuzumab (as measured by the number of CD68/CD163-positive macrophages and CSF1R-positive macrophages in the tumour tissue and the number of circulating monocytes in the peripheral blood including subsets), and identification of the recommended phase 2 dose (ie, the dose to be given for further study in patients with dt-GCT), and exploration of clinical activity of single agent emactuzumab.

Statistical analysis

The safety and efficacy cohorts included all patients who received at least one dose of emactuzumab before data cutoff. The safety data analysis cutoff date was June 30, 2014, whereas the efficacy data analysis cutoff date was Nov 5, 2014. Two different cutoff dates were used because of the availability of independent central review of all imaging data (FDG-PET and MRI) for response assessment. For logistical reasons, the latter was done batchwise and thus could not be coordinated at exactly the same time as the safety cutoff. Furthermore, for the efficacy analysis, we could only include patients who had undergone at least one on-treatment tumor assessment for them to be evaluable according to RECIST. The efficacy cohort included all patients who received emactuzumab between study start (July 26, 2012) and Nov 5, 2014, had measurable disease at baseline, and had at least one tumour evaluation during treatment (independent central review available). Immunohistochemical analyses included paired tumour specimens available on June 30, 2014.

Safety and tolerability were summarised for the type, incidence, severity, seriousness, and relatedness of adverse events; the type, incidence, and severity of laboratory abnormalities; and the incidence of dose-limiting toxic effects. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03.[15] Clinical activity as a pre-specified secondary objective is reported in terms of objective response (sum of complete and partial tumour responses observed, divided by the number of evaluable patients) and duration of clinically progression-free follow-up (from start of treatment to clinical detection of progressive disease). Changes in macrophage from paired tumour biopsy samples and monocyte levels from peripheral blood samples were were analysed with a Wilcoxon signed rank test done with R (version 3.1.1). All patients with both biopsy and blood samples providing evaluable samples were included in the analysis.

	Patients
Patients included at efficacy cutoff*	29
Patients evaluable for activity	28†
Age (years)	42 (18–82)
Sex	
Male	7 (24%)
Female	22 (76%)
Eastern Cooperative Oncology Group performance status	
0	26 (90%)
1	3 (10%)
Tumour location	
Knee	15 (52%)
Hip	4 (14%)
Foot or ankle	8 (28%)
Wrist	2 (7%)
Number of previous surgeries	1 (0–4)
Previous treatment with imatinib or nilotinib	8 (28%)

Data are n or n (%), or median (range). *Efficacy analysis included all patients who received emactuzumab between July 26, 2012, and Nov 5, 2014, had measurable disease at baseline, and had at least one tumour evaluation during treatment that was assessed by central review. †One patient withdrew consent from study after one cycle of treatment.

Table 1: **Patient characteristics**

	Grade 1	Grade 2	Grade 3	Grade 4
Adverse events*				
Facial oedema (including periorbital or eyelid oedema)	7 (28%)	9 (36%)	..	..
Asthenia	7 (28%)	7 (28%)	..	..
Pruritus	12 (48%)	2 (8%)	..	..
Rash	8 (32%)	2 (8%)	..	..
Peripheral oedema	5 (20%)	4 (16%)	..	..
Nausea	5 (20%)	2 (8%)	..	..
Dry skin	4 (16%)	..	..	..
Increased lacrimation	4 (16%)	..	..	..
Serious adverse events*				
Periorbital oedema	..	..	1 (4%)	..
Lupus erythematosus	..	1 (4%)	..	..
Skin erythema	..	1 (4%)	..	..
Subacute cutaneous lupus erythematosus	..	..	1 (4%)	..
Dermohypodermitis†	..	..	1 (4%)	..

Data are number of patients (%). *Multiple occurrences of the same adverse event in one patient are counted only once, with the highest grade reported. †Term commonly describes a bacterial infection of the dermis tissue, subcutaneous tissue, or both. Here, the term was used to describe the colocalisation of erythema and oedema on both lower legs.

Table 2: **Drug-related adverse events in more than three patients given emactuzumab and serious adverse events independent of incidence (safety population, n=25)**

This study is registered with ClinicalTrials.gov, number NCT01494688.

Role of the funding source

The funder of the study was involved in the study design, the data collection and analysis, the interpretation of the data, and the writing of the report. All authors had full access to all the data in the study, made the decision to submit these data for publication, were involved in writing the report, and agreed upon the final content of the report. The study funder provided funding for editorial assistance with report preparation. The corresponding author had final responsibility to submit for publication.

Results

Between July 26, 2012, and Sept 25, 2014, 29 patients were enrolled; 12 patients were enrolled in the dose-escalation phase; 17 patients were enrolled in the dose-expansion phase (cutoff for efficacy analysis was Nov 5, 2014). One patient withdrew consent after receiving one cycle of treatment; therefore the evaluable efficacy cohort consisted of 28 patients (table 1). At cutoff, the safety cohort consisted of 25 patients. Different cutoff dates for the safety and efficacy analyses were chosen because of availability of independent central review data for response assessment. The median age of patients was 42 years (range 18–82), and most patients were female. The median number of infusions per patient was six (range 1–14).

In the dose-escalation phase, three patients were enrolled in the 900 mg cohort, five in the 1350 mg cohort, and four in the 2000 mg cohort. No dose-limiting toxicities were observed. The MTD was not reached. We opted to proceed with the OBD of 1000 mg every 2 weeks in the dose-escalation cohort. This dose achieved the required 90% or more target saturation resulting in depletion of macrophages (both in the tumour and the skin [data not shown]) and was supported by further pharmacodynamic markers such as peripheral monocyte subsets allowing for determination of this optimum biological dose. 17 patients were enrolled in the dose-expansion cohort.

As of June 30, 2014, no deaths or life-threatening adverse events were reported in the safety cohort (n=25). No dose-limiting toxicities were recorded. The most frequent drug-related adverse events of any grade were facial oedema (including periorbital and eyelid oedema), asthenia, pruritus, and rash, all of grade 1 or 2 intensity (table 2). Five serious adverse events (periorbital oedema, lupus erythematosus [twice], erythema, and dermo-hypodermitis) were reported in five patients. Four of the five serious adverse events were reported at doses of 1350 mg and 2000 mg. Two patients who developed erythema and lupus erythematosus developed cutaneous and mucosal lesions consistent with subacute lupus erythematosus (no visceral involvement specific to systemic lupus disease was noted). The events were observed after six administrations of emactuzumab 2000 mg every 2 weeks and eight administrations of emactuzumab 1350 mg every 2 weeks, respectively. Emactuzumab was discontinued and both patients were treated with hydroxychloroquine associated with low-dose steroid, which led to rapid clinical improvement. Clinical signs and symptoms resolved without sequelae in both patients, lupus erythematosus-specific medication was discontinued, and clinical benefit for dt-GCT persisted. Three of five serious adverse events were assessed as grade 3. Two other grade 3 events were reported: mucositis (one [4%]) and fatigue (one [4%]). No grade 4 adverse events were documented. Doses of emactuzumab were not reduced in any patient. Five (20%) patients dropped out because of adverse events.

Pharmacokinetic analyses for patients with dt-GCT who received 900–2000 mg emactuzumab every 2 weeks showed linear drug pharmacokinetics at all doses (data not shown). At cutoff, independent central review imaging (MRI) analysis data, a prespecified secondary endpoint, were available for 28 patients and ^{18}F-FDG PET imaging data for 26 patients. Median follow-up at

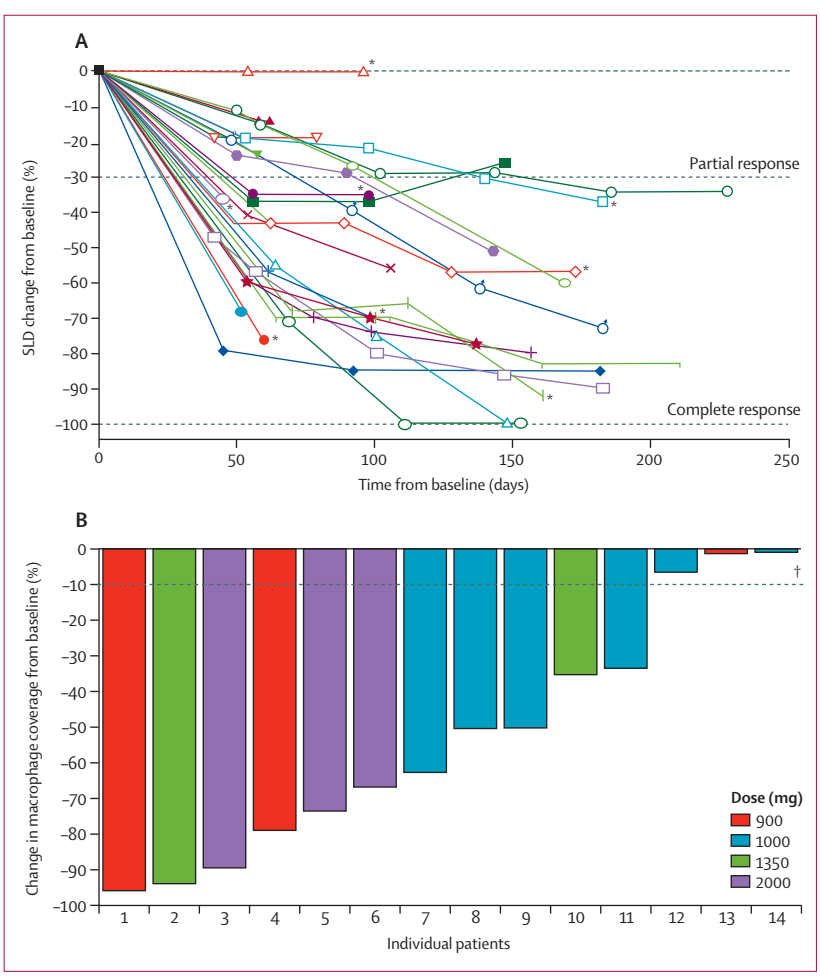

Figure: **Activity of emactuzumab in patients with dt-GCT**
(A) Change in sum of largest diameter (SLD) and (B) change in macrophage coverage (ie, area of the tumour specimen [biopsy] covered by macrophages) from baseline. Dt-GCT=diffuse-type tenosynovial giant cell tumours. *Patients who received previous nilotinib, imatinib, or both. †Greater than 10% change is regarded as clinically significant.

cutoff was 12 months (IQR 10–23 months). Using MRI, tumour shrinkage was detected in all but one patient, who had stable disease (figure A). According to the treating physician, this shrinkage of all 27 tumours was associated with early profound functional and symptomatic improvement. None of the patients showed an increase in tumour size.

24 (86%) of 28 patients achieved an objective response, and 19 (68%) showed a partial response as early as after 6 weeks (three cycles of emactuzumab) of treatment. Two (7%) patients achieved a complete objective response and 22 (79%) achieved a partial objective response. Repeated response assessments in our patient population suggest an early onset of tumour shrinkage; however, additional treatment cycles did not necessarily result in deepening of responses (figure A). We noted objective tumour responses in eight patients who had previously failed to respond to treatment with either imatinib or nilotinib or both (figure A). 24 (92%) of 26 patients achieved a metabolic response after 4 weeks of treatment (two cycles of emactuzumab). One (4%) patient achieved a complete metabolic response and 23 (88%) achieved a partial metabolic response. At cutoff, all but one patient remains clinically progression-free.

11 of 14 patients with evaluable paired tumour biopsy samples (taken at baseline and on treatment at 4 weeks after two cycles of emactuzumab) showed a clinically significant reduction in CD68/CD163-positive macrophages and CSF1R-positive macrophages of greater than 10% (p=0·0006; figure B).

All patients in the efficacy cohort had blood samples taken in both study phases (at baseline, on day 1 of each 2-week cycle, and at five additional timepoints during cycle 1 and one additional timepoint during cycle 2). In the peripheral blood, a near complete decrease in $CD14^{Dim}CD16^{High}$ monocytes, which express high levels of CSF1R, was noted (median reduction of 99% [IQR 97–99], p=0·004). Smaller decreases were detected for the total monocyte count and for the subsets of $CD14^{High}CD16^{Low}$ and CSF1R negative/low cells, respectively (median reductions of 43% [IQR 28–53], 68% [52–71], and 36% [25–49], respectively, p=0·004).

Discussion

We report the first clinical proof-of-concept study targeting CSF1R with the humanised IgG1 isotype monoclonal antibody emactuzumab (RG7155) in patients with dt-GCT, showing that overall, emactuzumab was well tolerated. The OBD was estimated at 1000 mg given every 2 weeks.

Objective responses were noted in 24 (86%) patients and were associated with early onset of symptomatic and functional improvement. Further, a reduction in metabolic activity of tumours was noted at 4 weeks (two cycles of emactuzumab), resulting in a metabolic responses in 24 (92%) patients by EORTC criteria. At cutoff, all patients but one remained clinically progression-free (median follow-up time of 12 months [IQR 10–23 months]). Paired tumour biopsy samples (at baseline and after 4 weeks on treatment, after two cycles of emactuzumab) showed a reduction in both CD68/CD163-positive cells and CSF1R-positive cells in the tumour tissue.

Eight patients who had previously failed to respond to, or discontinued treatment with, imatinib, nilotinib, or both, had an objective response when treated with emactuzumab. Imatinib is a tyrosine-kinase inhibitor with activity against BCR-ABL, c-Kit (CD117), PDGFR,[16] FLT3,[17] RET, and CSF1R.[18] Objective responses were observed in 19% of patients treated with imatinib in a retrospective case series of 29 patients with dt-GCT.[10] The results of a phase 2 clinical study with nilotinib in patients with dt-GCT have recently been reported.[11] The rationale for evaluating nilotinib was based on the assumption that it would exhibit similar antitumour activity to imatinib, albeit potentially with a better safety profile.[11,19] However, only three (6%) of 47 patients showed a partial response, and such responses were seen only during 24 weeks to 48 weeks after treatment initiation.[11] The striking difference in terms of activity noted between emactuzumab and either imatinib or nilotinib in patients with dt-GCT is likely attributable to the fact that imatinib and nilotinib are not particularly strong inhibitors of CSF1R. Indeed, in a preclinical xenograft model of dt-GCT, imatinib blocked the CSF1R pathway and monocyte or macrophage infiltration to a lesser extent than did the mouse antihuman CSF1 antibody 5H4.[19] Currently, novel tyrosine kinase inhibitors that are more selective for CSF1R are in clinical development. Initial clinical data for one of these drugs show improved activity in dt-GCT when compared with imatinib and nilotinib.[20]

The optimum imaging method to monitor tumour growth in dt-GCT of the soft tissue remains to be determined. MRI is commonly regarded as the method of choice; however, whether RECIST adequately captures antitumour responses for this diffuse form of GCT remains to be determined. Alternative response assessment criteria, such as the tumour volume score,[20] represent a feasible—albeit semiquantitative and unvalidated—method. Therefore, to use the potentially more stringent method and for comparability reasons with the only available prospective study in dt-GCT,[11] we decided to use RECIST in our study.

Overall, emactuzumab was well tolerated in patients with dt-GCT. Most adverse events were grade 1 or 2. Asthenia (56%), pruritus (56%), facial oedema, including periorbital and eyelid oedema (64%), and peripheral oedema (36%) were frequently reported adverse events. The precise pathophysiological cause of this oedema remains unknown; however, oedema has been reported in up to 70% of patients receiving imatinib for any indication, with periorbital oedema being the most common type.[21] The current hypothesis is that imatinib-induced inhibition of PDGFR results in an increase in the interstitial fluid pressure in the dermis, thereby

causing an increase in capillary permeability and extravasation of fluid.[22] Because emactuzumab specifically targets CSF1R, we postulate that the formation of all types of oedema with imatinib treatment could instead be induced by its CSF1R-targeting component. However, it cannot be ruled out that both PDGFR and CSF1R activate similar downstream signalling events resulting in increased vascular permeability. Histopathological analysis of imatinib-induced eyelid oedema showed loose dermis and mild acute and chronic inflammation.[23,24]

Besides oedema, emactuzumab was associated with the occurrence of adverse events of the skin (pruritus, 56%; rash, 40%). In this study, potential autoimmune events, with the skin being one of the target organs, were classified as adverse events of special interest. One patient with a medical history of psoriasis had worsening of this condition, and two patients with no medical history of skin diseases developed subacute cutaneous lupus-like disease. Clinically, adverse events including skin-related adverse events in both patients were rapidly reversed after stopping emactuzumab and with appropriate management. It has been observed that CSF1 as the only stimulus can polarise macrophages toward an immunosuppressive function.[25] Therefore, we speculate that in predisposed patients who have an increased likelihood of developing skin adverse events, the blockade of CSF1R can induce the development and migration of inflammatory macrophages, and result in the depletion of immunosuppressive macrophages. This could induce strong, potentially T-lymphocyte-mediated immune responses that are known to cause autoinflammatory skin disorders such as subacute cutaneous lupus erythematosus. Through this same mechanism, emactuzumab could prove effective as an immunomodulatory drug to induce a systemic anti-tumour immune response.[13] A study assessing the potential of emactuzumab in combination with other immunotherapies for solid tumours is currently underway (NCT02323191). However, we believe that the profound antitumour effect in Dt-GCT is mediated by direct interference with the CSF1–CSF1R axis rather than the induction of a systemic immune response. Recruitment of patients with solid malignancies to be treated with either emactuzumab alone or the combination of emactuzumab and paclitaxel are currently ongoing (NCT01494688).

Pre-clinical and clinical sudies[13,26,27] on CSF1R-targeted monoclonal antibodies have shown asymptomatic increases in short-lived enzymes, most likely caused by a decrease in physiologic clearance through partial depletion of sessile macrophages of the liver (CSF1R positive Kupffer cells). In this study, we noted an asymptomatic increase in aspartate aminotransferase up to grade 3 in three patients and grade 4 in one patient; however, no signs of impaired hepatocellular function or integrity were detected.

We noted a significant decrease from baseline in peripheral blood $CD14^{Dim}CD16^{High}$ monocytes, which express high levels of CSF1R, in patients treated with emactuzumab. This decrease has been described for other CSF1R targeting approaches and could serve as a specific pharmacodynamic marker of biological activity of emactuzumab rather than indicating a specific toxicity. Total monocyte count and the subsets of $CD14^{High}CD16^{Low}$ and CSF1R negative and $CSF1R^{low}$ cells were also reduced, but to a lesser degree. No specific adverse events (such as infectious manifestations) were associated.

20% of patients dropped out of the study due to adverse events, which compares favourably with imatinib;[10] however, our observations confirm that patients with dt-GCT represent a different patient group from one with life-threatening malignant disease. Our patients received a median of six infusions, suggesting that patients with dt-GCT might be less willing to cope with adverse event-related and study-related procedures (reinforced by the early onset of clinical benefit) than patients treated within phase 1 oncology trials for life-threatening malignancies. Nevertheless, it seems that a fairly short course of treatment with emactuzumab may be sufficient to induce durable clinical responses in patients with dt-GCT. We have recently amended our study protocol to add standardised follow-up assessments by MRI at 1 year and 2 year follow-up timepoints for correlation with clinical follow-up; however, data are not available yet. Further studies to identify a treatment regimen optimised for clinical activity, tolerability, and patient convenience are warranted and studies investigating alternative treatment schedules are ongoing.

Currently, one limitation of clinical studies of dt-GCT is the absence of specific and validated patient-reported outcome measures to document treatment-induced symptomatic and functional improvement. We have recently amended our study protocol to introduce the Western Ontario and McMasters Universities Osteoarthritis Index (WOMAC);[28] however, data are not yet available. Future studies of emactuzumab in dt-GCT will include patient-reported outcome instruments such as the WOMAC questionnaire to show whether tumour shrinkage correlates with clinical benefit for these patients.

There are few examples of the successful use of targeted inhibitors to disrupt the aberrant signalling pathways that mesenchymal tumours require for continued growth. These include imatinib-mediated inhibition of c-KIT in gastrointestinal stromal tumours,[29] inhibition of PDGFR β by imatinib in dermatofibrosarcoma protuberans,[30] mTor inhibition in perivascular epithelioid cell tumours,[31] and ALK inhibition in inflammatory myofibroblastic tumours.[32] Our study of emactuzumab confirms that CSF1R-specific targeting with a monoclonal antibody is highly effective in patients with dt-GCT. Even a short course of emactuzumab could offer durable benefit for patients for whom surgery is either not feasible or would result in major functional impairment. Further investigation of emactuzumab in dt-GCT is warranted and, in view of the rarity of the disease, different possibilities such as an international collaboration with cooperative groups are being assessed.

Contributors

PAC and AI and JB and DR contributed equally to this work. PAC, AI, CL, CAG-R, JD, JB, and DR contributed to the study design, data collection, analyses, and interpretation. PAC, AI, CAG, CL, MT, EB, JD, and JB served as clinical investigators at the study sites and conducted the clinical study. MAC, CR, AB, CM, AJ, AB, MD, KB, CF, GM, MB, RH, JR, KA, NG, KN, RDC, LU, and EB contributed to collection, analyses, and interpretation of the data. The primary data were made available to the investigators for independent central review and analyses. The first draft of the manuscript was written by AI, PAC, JB, and DR, with review and revision by the other coauthors. All authors had full access to all data in the study, made the decision to submit these data for publication, were involved in writing the report, and agreed upon final content of the paper.

Declaration of interests

MAC, CR, AB, CM, A-MJ, A-MB, MD, KB-F, CF, GM-L, MB, JR, RH, KA, NG, KN, RDC, LU, and DR are or have been employees of F Hoffmann-La Roche. DR, MAC, and CR have patents issued or pending in the use of emactuzumab (RG7155). PAC has received honoraria from Novartis, BluePrint Medicines, GlaxoSmithKline, PharmaMar, and Servier; and research support from Novartis and F Hoffmann-La Roche. AI has received honoraria from GlaxoSmithKline, Pfizer, PharmaMar, and Threshold; and research support from Novartis, Pfizer, PharmaMar, and F Hoffmann-La Roche. JB has received research support and honoraria from F Hoffmann-La Roche, Novartis, GlaxoSmithKline, Bayer, and PharmaMar. Other authors declare no competing interests.

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