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Efficacy and safety of emapalumab in macrophage activation syndrome

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ABSTRACT

Objectives Macrophage activation syndrome (MAS) is a severe, life-threatening complication of systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still's disease (AOSD). The objective of this study was to confirm the adequacy of an emapalumab dosing regimen in relation to interferon-γ (IFNγ) activity by assessing efficacy and safety. The efficacy outcome was MAS remission by week 8, based on clinical and laboratory criteria.

Methods We studied emapalumab, a human anti-IFNγ antibody, administered with background glucocorticoids, in a prospective single-arm trial involving patients who had MAS secondary to sJIA or AOSD and had previously failed high-dose glucocorticoids, with or without anakinra and/or ciclosporin. The study foresaw 4-week treatment that could be shortened or prolonged based on investigator's assessment of response. Patients entered a long-term (12 months) follow-up study.

Results Fourteen patients received emapalumab. All patients completed the trial, entered the long-term follow-up and were alive at the end of follow-up. The investigated dosing regimen, based on an initial loading dose followed by maintenance doses, was appropriate, as shown by rapid neutralisation of IFNγ activity, demonstrated by a prompt decrease in serum C-X-C motif chemokine ligand 9 (CXCL9) levels. By week 8, MAS remission was achieved in 13 of the 14 patients at a median time of 25 days. Viral infections and positive viral tests were observed.

Conclusions Neutralisation of IFNγ with emapalumab was efficacious in inducing remission of MAS secondary to sJIA or AOSD in patients who had failed high-dose glucocorticoids. Screening for viral infections should be performed, particularly for cytomegalovirus.

Trial registration number NCT02069899 and NCT03311854.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Macrophage activation syndrome (MAS) is a severe and potentially life-threatening complication of systemic juvenile idiopathic arthritis and adult-onset Still's disease.
⇒ There are no therapeutic options that have been prospectively investigated in MAS.
⇒ Data in animal models and ex vivo data from humans with MAS led to the hypothesis that interferon-γ (IFNγ) has a pathogenic role in MAS.

WHAT THIS STUDY ADDS

⇒ This open-label multicentre trial using emapalumab, an anti-IFNγ antibody, in patients who have failed to respond to high-dose glucocorticoids, demonstrates that IFNγ has a pathogenic role in MAS and that its neutralisation markedly improved MAS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results of this study show that neutralisation of IFNγ with emapalumab is a therapeutic option for patients with severe MAS who have failed standard of care with high-dose glucocorticoids.

INTRODUCTION

Macrophage activation syndrome (MAS) is a form of secondary haemophagocytic lymphohistiocytosis (HLH) occurring as a life-threatening complication of rheumatic diseases.[1] It is most frequent in systemic juvenile idiopathic arthritis (sJIA) and adult-onset Still's disease (AOSD), affecting about 10%–20% of patients.[1–4] sJIA and AOSD are considered the same disease named differently depending only on age at onset below or above 16 years, respectively.[2 3 5] The incidence and features of MAS are similar in the two age groups.[2 5] We use the term sJIA/AOSD to collectively identify these patients.

Similar to other forms of HLH, MAS is caused by excessive activation and expansion of T lymphocytes and macrophages resulting in hyperinflammation.[1 6 7] MAS is characterised by fever, hepatosplenomegaly, cytopenias, liver dysfunction, coagulation abnormalities and hyperferritinaemia, and may progress to multiple organ failure, with mortality rates of 10%–20%.[4]

MAS is treated with high-dose glucocorticoids with satisfactory response in two-thirds of the patients.[4 8] In patients unresponsive to glucocorticoids, ciclosporin is usually added. Treatment with cyclophosphamide, etoposide, intravenous immunoglobulin, etanercept, anakinra, tocilizumab, JAK inhibitors and plasmapheresis, have been described

in case reports or small series.[4 8–12] None of these regimens have been prospectively investigated.

Overproduction of interferon-γ (IFNγ) is present and pathogenic in animal models of MAS.[13–16] In sJIA/AOSD, high IFNγ activity demonstrated by high serum levels of C-X-C motif chemokine ligand 9 (CXCL9), a chemokine selectively induced by IFNγ,[17] is associated with MAS onset and severity.[18–22]

We studied emapalumab, a fully human anti-IFNγ antibody, in patients with MAS secondary to sJIA/AOSD who failed high-dose glucocorticoids.

METHODS

This phase II, open-label, single-arm trial (NI-0501-06; ClinicalTrials.gov Identifier NCT03311854), conducted at five sites in Italy, France, Spain, the UK and the USA, comprised screening, a treatment period of 28 days and a short-term 4-week follow-up. Data were collected up to 12 months in a long-term follow-up study (NI-0501-05; ClinicalTrials.gov Identifier NCT02069899) (online supplemental figure S1 in the online supplemental appendix). Results from both studies are reported.

FDB, AG, MB and CdM designed the study. The sponsor was also responsible for data collection, management and analysis. All authors vouch for the accuracy and completeness of the data and analyses, and for the fidelity of the study to the protocol.

Patient involvement

The study was presented and discussed at the 2016 and 2017 meetings of the sJIA Foundation in Washington, DC, USA. Information on the study for patients and families was published on the sJIA Foundation website. Dissemination of the data gathered from the study is planned to be discussed with the sJIA Foundation.

Patients

Patients had sJIA based on International League Against Rheumatism criteria,[23] or AOSD based on Yamaguchi criteria.[24] For patients presenting with MAS at sJIA onset, presumption of sJIA was based on Childhood Arthritis and Rheumatology Research Alliance criteria.[25] Patients had MAS, according to American College of Rheumatology/EULAR criteria,[26] and an inadequate response to high-dose intravenous glucocorticoids according to the treating physicians. High-dose glucocorticoids were defined as ≥2 mg/kg/day of prednisone equivalent in two divided doses, or at least 60 mg/day in patients weighing 30 kg or more, including but not limited to pulses up to 30 mg/kg/day for at least 3 consecutive days. Patients with active infections potentially favoured by IFNγ neutralisation (typical and atypical mycobacteria, *Histoplasma capsulatum*, *Shigella*, *Salmonella*, *Campylobacter* and *Leishmania*) were excluded (see online supplemental appendix 1). Eligibility criteria are listed in online supplemental table S1.

Treatment

Emapalumab infusions were administered on a background of glucocorticoids. The initial dose of emapalumab was 6 mg/kg on day 0, followed by 3 mg/kg every 3 days until day 15 and twice weekly until day 28. Treatment with emapalumab could be stopped on investigator's assessment of remission, but not before three emapalumab doses have been administered. Frequency between infusions could be shortened, dose could be increased or treatment prolonged on investigator's assessment of unsatisfactory response. Ciclosporin could be continued, if started at least 3 days before initiating emapalumab. Interleukin (IL)-1 and IL-6 inhibitors were not allowed. An amendment allowed continuation of anakinra, if started at least 3 days before initiating emapalumab, and its introduction during the study, at a maximum dose of 4 mg/kg/day to treat the underlying sJIA/AOSD. Tocilizumab and canakinumab were not allowed during the trial. Prophylaxis against herpes zoster was administered as per local standards. Glucocorticoid tapering could be initiated as soon as the patients' conditions allowed based on investigator's assessment.

Outcomes

The objective was to confirm the adequacy of the emapalumab dosing regimen in relation to IFNγ activity by assessing efficacy and safety. Serum levels of emapalumab, total (free and emapalumab-bound) IFNγ, CXCL9 and soluble IL-2 receptor were measured (online supplemental appendices 2 and 3).

The efficacy outcome was MAS remission by week 8, defined as resolution of clinical signs and symptoms (online supplemental table S2) according to the physician global assessment (visual analogue scale ≤1/10) and white blood cell and platelet count above lower limit of normal, lactate dehydrogenase (LDH), alanine aminotransferase and aspartate aminotransferase below 1.5 times upper limit of normal (ULN), fibrinogen >100 mg/dL and ferritin levels[27] decreased by at least 80% or below 2000 ng/mL, whichever was lower. Other efficacy evaluations included glucocorticoid dose (expressed as mg/kg/day of prednisone-equivalent) and survival. Adverse events (AEs) were assessed. During the long-term follow-up, evaluations included MAS episodes, AEs, pharmacokinetics and pharmacodynamics.

Statistical analysis

The analysis population included all patients. Categorical variables are presented with the number and percentage within each category. Continuous variables are reported as median (range). Laboratory parameters of MAS were measured locally.

RESULTS

Study population

Fourteen patients received emapalumab. All completed the study and entered long-term follow-up. Thirteen patients had sJIA onset before 16 years, and one AOSD with onset at 16 years and 9 months. Four patients had presumption of sJIA, which was later confirmed. Six patients had a total of 19 previous MAS episodes in their history, treated with high-dose glucocorticoids, with the addition of anakinra and/or ciclosporin in approximately half.

During the week preceding emapalumab, all patients were receiving high-dose intravenous glucocorticoids, as per protocol. In addition, eight were receiving ciclosporin and seven anakinra (table 1). Of the patients treated with anakinra, three were receiving standard doses for sJIA (≤4.0 mg/kg/day), and four high doses ranging from 7.5 to 15 mg/kg/day. Patients had severe MAS at baseline, shown by high physician assessment of MAS activity and marked abnormalities of laboratory parameters (table 2). Notably, worsening or no improvement of laboratory parameters was observed between screening and baseline, despite ongoing treatment.

Of the 14 patients, 6 received emapalumab up to day 28. In seven patients who had MAS remission per investigator's assessment, it was possible to discontinue emapalumab earlier. One patient continued emapalumab up to day 39. The median treatment duration was 27 days (range, 7–39) with the number of infusions ranging from 3 to 17 per patient.

Table 1 Baseline demographics and clinical characteristics of the patients

Demographic	(n=14)
Age, years, median (range)	11.0 (2–25)
Sex, female, n (%)	10 (71.4)
Weight, kg, median (range)	45.9 (12.0–68.8)
Age at diagnosis of sJIA/AOSD, years, median (range)	10.5 (1–17)
Previous MAS episodes	
Patients with previous MAS episodes, n (%)	6 (43%)
Total number of previous MAS episodes	19
Number of MAS episodes per patient*	3 (1–6)
Treatment of the current MAS episode prior to emapalumab	
High-dose intravenous glucocorticoids, n (%)	14 (100)
Average daily dose during week −1,† mg/kg prednisone-equivalent, median (range)	15.7 (0.8–36.4)
Ciclosporin,‡ n (%)	8 (57.1%)
Anakinra,‡ n, (%)	7§ (50)
Average daily dose during week −1,† mg/kg, median (range)	4.2 (1.6–13.9)
IVIG, n (%)	3 (21.4)

*Patients with no previous MAS episode(s) are excluded from the calculation of the median (range).
†Week prior to emapalumab administration.
‡Five patients received anakinra and ciclosporin, concomitantly.
§One additional patient received anakinra until 11 days prior to start of emapalumab administration.
AOSD, adult-onset Still's disease; IVIG, intravenous immunoglobulin; MAS, macrophage activation syndrome; sJIA, systemic juvenile idiopathic arthritis.

Pharmacokinetics and pharmacodynamics

All patients received an initial dose of emapalumab of 6 mg/kg followed by doses of 3 mg/kg. The initial dose allowed to rapidly reach serum concentrations of emapalumab close to the steady-state concentrations obtained with the doses of 3 mg/kg (figure 1A). The dosing interval was transiently shortened from 3 to 2 days in three patients based on clinical and laboratory parameters suggestive of incomplete response. In two of these patients the dosing interval was shortened for several infusions. In these two patients, measurement of emapalumab concentrations showed rapid drug clearance consistent with target-mediated drug disposition associated with high levels of total IFNγ (online supplemental figure S2).[28]

At baseline, CXCL9 concentrations were markedly elevated in all patients, indicating high IFNγ activity (figure 1B). Total IFNγ concentrations were variable across patients (figure 1C). Total IFNγ concentrations at day 3 (at equilibrium between free and emapalumab-bound IFNγ) reflect IFNγ production at baseline.[27 28] There was no correlation between total IFNγ at day 3 and CXCL9 levels at baseline (not shown). Soluble IL-2 receptor levels at baseline were markedly elevated in all patients (figure 1D).

Emapalumab administration led to a rapid decrease in serum CXCL9 levels, indicating neutralisation of IFNγ activity and therefore the appropriateness of the selected dosing regimen. Patients with elevated total IFNγ at day 3 also showed a decrease in total IFNγ concentrations over time indicating decreasing IFNγ production over time. Soluble IL-2 receptor concentrations also decreased markedly (figure 1D), indicating decreasing T cell activation. During long-term follow-up, in the absence of high IFNγ production, as shown by low levels of total IFNγ, emapalumab showed a slow linear terminal elimination phase with a half-life of 24 days, like that in healthy subjects (online supplemental figure S3).[28] Total IFNγ, CXCL9 and soluble IL-2 receptor levels were close to, or within, the normal range (online supplemental table S3).

Efficacy

During emapalumab administration, physician global assessment of MAS activity and MAS laboratory parameters rapidly improved in all patients while glucocorticoids were tapered.

By week 8, 13 patients (93%) achieved MAS remission at a median time of 25 days after emapalumab initiation, the earliest at day 9 (figure 2). At week 8, 2 of the 13 patients who had previously achieved MAS remission did not meet the criteria of MAS remission because of a single laboratory abnormality (one with LDH 1.7-fold above the ULN; one with white blood cells at 4.8×10^9/L with lower limit of normal at 5.5×10^9/L). One

Table 2 MAS activity, as defined by physician's assessment, and laboratory features of MAS before, during and after emapalumab treatment

	Screening (n=14)	Baseline (n=14)	Week 2 (n=14)	Week 4 (n=14)	Week 8 (n=14)
Physician assessment of MAS activity*, cm, median (range)	8.3 (2.0–10.0)	8.0 (2.0–10.0)	2.3 (0.0–9.5)	0.3 (0.0–6.5)	0 (0.0–1.0)
Ferritin, ng/mL, median (range)	19865 (367–192 584)	25 709 (716–192 584)	1410 (65–29 099)	180 (22–18 430)	55 (4–561)
Increased, n (%)†	13 (93)	14 (100)	9 (64)	2 (14)	0 (0)
WBCs, 10^9/L, median (range)	5.8 (1.0–25.7)	5.1 (0.9–25.7)	8.4 (0.9–32.6)	12.3 (2.4–34.7)	9.5 (4.8–22.6)
Decreased, n (%)‡	6 (43)	7 (50)	4 (29)	1 (7)	1 (7)
Platelets, 10^9/L, median (range)	152 (48–546)	112 (52–558)	284 (55–590)	336 (104–591)	383 (214–915)
Decreased, n (%)‡	6 (43)	9 (64)	4 (29)	2 (14)	0 (0)
Aspartate aminotransferase, U/L, median (range)	244 (26–3814)	190 (19–2039)	34 (5–146)	29 (16–100)	28 (15–52)
Increased, n (%)§	12 (86)	10 (71)	3 (21)	1 (7)	0 (0)
Alanine aminotransferase, U/L, median (range)	190 (139–3179)	302 (70–1492)	61 (18–327)	45 (23–195)	28 (7–59)
Increased, n (%)§	14 (100)	14 (100)	6 (43)	5 (35.7)	0 (0)
LDH, U/L, median (range)	1699 (859–12 734)	1502 (588–12 734)	518 (283–1572)	592 (277–810)	497 (306–741)
Increased, n (%)§	13 (100)	13 (93)	6 (43)	4 (29)	2 (14)
Fibrinogen, mg/dL, median (range)	230 (137–341)	174 (70–465)	244 (145–515)	279 (200–460)	337 (190–460)
Decreased, n (%)¶	0 (0)	1 (7)	0 (0)	0 (0)	0 (0)

*MAS activity was based on a 10 cm visual analogue scale, with scores ranging from 0 to 10 and higher score indicating higher activity.
†Increased ferritin was defined as ferritin >684 ng/mL as per 2016 Classification Criteria for MAS complicating sJIA.[23]
‡Decreased white blood cell and platelet were defined as counts below the lower limit of normal for age of local laboratory.
§Increased alanine aminotransferase, aspartate aminotransferase and LDH were defined as values above 1.5 the upper limit of normal range of local laboratory.
¶Decreased fibrinogen was defined as fibrinogen <100 mg/dL.
LDH, lactate dehydrogenase; MAS, macrophage activation syndrome; WBC, white blood cells.

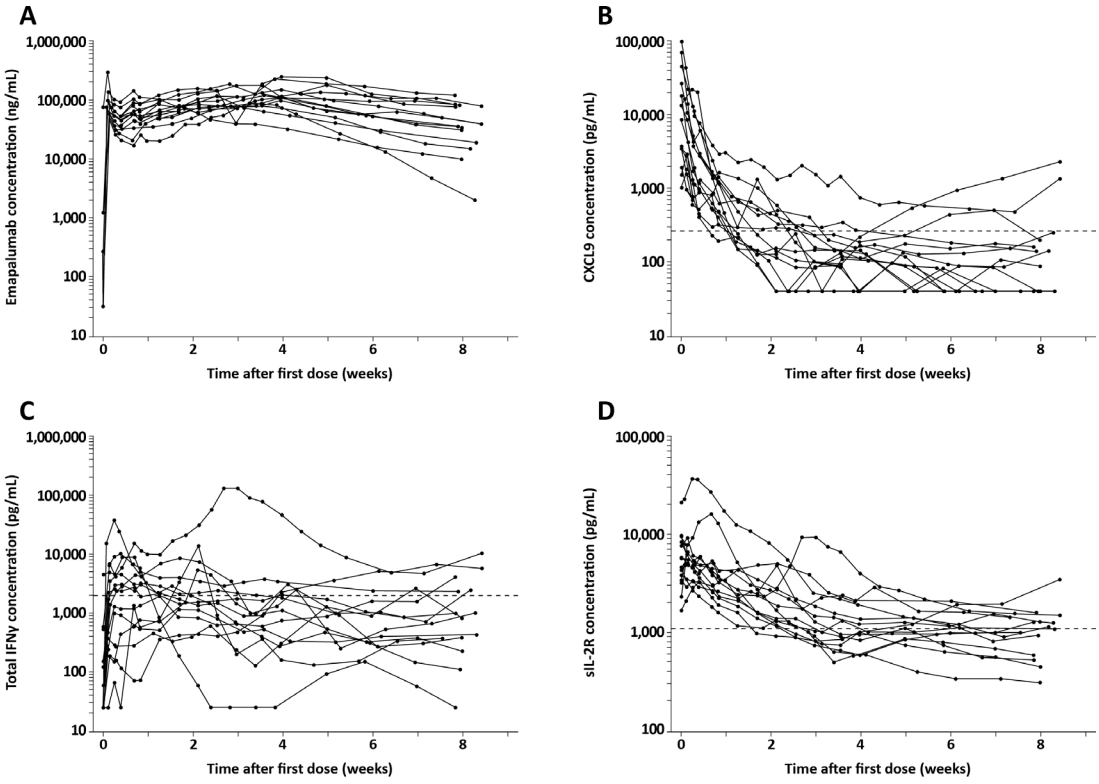

Figure 1 Pharmacokinetic (emapalumab* (A)) and pharmacodynamic (CXCL9† (B), total IFNγ‡ (C) and sIL-2R§ (D)) parameters in patients with MAS treated with emapalumab. *Serum emapalumab concentrations in patients treated with emapalumab (initial dose of 6 mg/kg, followed by intended maintenance dosing of 3 mg/kg every 3 days until day 15, and twice weekly until day 28). †Serum concentrations of CXCL9, a biomarker of IFNγ activity, being, at baseline, 4–362 times the 95th percentile of healthy volunteers (271 pg/mL),[24] indicated by the horizontal dotted line. ‡Total IFNγ (free and emapalumab-bound) that reflects IFNγ production, ranging, at day 3, from normal to 12 times the 95th percentile of healthy volunteers receiving emapalumab (2084 pg/mL) indicated by the horizontal dotted line. §Serum sIL-2R, a biomarker of T cell activation, being, at baseline, 1.6–20 times the 95th percentile of healthy volunteers (1071 pg/mL, unpublished data) indicated by the horizontal dotted line. CXCL9, C-X-C motif chemokine ligand 9; IFNγ, interferon-γ; MAS, macrophage activation syndrome; sIL-2R, soluble interleukin-2 receptor.

patient who stopped emapalumab after three doses on investigator's assessment of remission never met the criteria of MAS remission only because of LDH levels 1.5-fold above the ULN.

All laboratory parameters of MAS rapidly improved on initiation of emapalumab with evident improvement at week 1 and with all parameters being normal in the majority of patients by week 4 (figure 3, table 2 and online supplemental figure S4).

During long-term follow-up, 13 patients did not have MAS episodes. One patient had a MAS episode 11 months after stopping emapalumab, when emapalumab was undetectable in serum. At the end of the long-term follow-up, 10 patients met the MAS remission criteria. Four did not meet these criteria because of either mild abnormality in one laboratory value (n=1), MAS activity at 1.5 cm (n=1), missing data (n=1) or absence of data as the patient missed the 12-month visit (online supplemental table S4).

Tapering of glucocorticoids occurred rapidly after emapalumab initiation. During the week preceding emapalumab, the median average daily dose was 15.7 mg/kg/day prednisone-equivalent, 2.3 mg/kg during week 2 and 0.56 mg/kg during week 8 (figure 4). At the end of the long-term follow-up, five patients were not receiving glucocorticoids and six were receiving <0.3 mg/kg/day prednisone-equivalent. Two patients were receiving between 1 and 2 mg/kg: the above-mentioned patient with a MAS episode and one patient with lung disease associated with sJIA. Data on glucocorticoid dose for one patient at the last follow-up visit are not available, as the patient missed the visit.

Eight patients were receiving ciclosporin at baseline. Ciclosporin was discontinued in two patients early after emapalumab initiation (day 4 and 10) and in four additional patients during long-term follow-up.

During the trial, anakinra was continued in four patients at a dose of ≤4 mg/kg, the standard dose for sJIA/AOSD treatment and in one patient at a dose of 7.5 mg/kg. These patients did not present with flares of the underlying sJIA/AOSD. During the trial, while MAS was improving, six flares of sJIA/AOSD were observed: three in the three patients who discontinued anakinra before emapalumab initiation and three in three patients who had not previously received and were not receiving anakinra. During long-term follow-up, four sJIA/AOSD flares occurred in four patients. sJIA/AOSD flares and the background treatment at time of the flares are described in online supplemental table S5.

Safety

No deaths were reported during the trial and the long-term follow-up. During the trial, after initiation of emapalumab, 88 AEs were reported in 13 patients (table 3 and online supplemental table S6). All events were mild or moderate in intensity except two (one cardiopulmonary failure, and one neutropenia; neither related to emapalumab). The most frequently reported AEs were infections and positive tests for infectious agents in the absence of clinical symptoms. All infectious events were of viral origin. No bacterial or opportunistic infections were reported. Six viral events in three patients (two infections and four positive

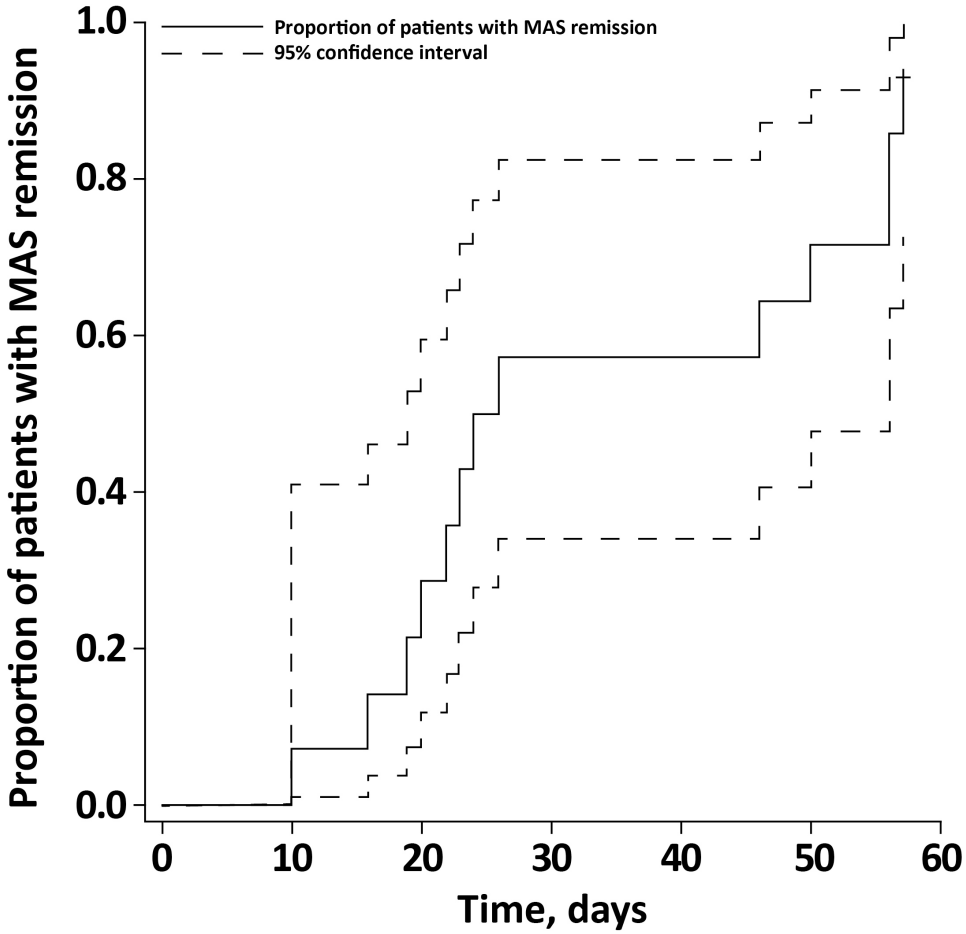

Figure 2 Time to MAS remission*. *Time to MAS remission defined as resolution of clinical signs and symptoms according to the investigator (visual analogue scale ≤1/10) (online supplemental table S2) and resolution of the abnormalities of MAS laboratory parameters: white blood cell and platelet count above lower limit of normal, LDH below 1.5 times ULN, alanine aminotransferase/aspartate aminotransferase below 1.5 times ULN, fibrinogen >100 mg/dL and ferritin levels decreased by at least 80% or below 2000 ng/mL, whichever was lower. The continuous line represents the proportion of patients with MAS remission, the dotted line represents the 95% CI. The cross indicates the censored patient. LDH, lactate dehydrogenase; MAS, macrophage activation syndrome; ULN, upper limit of normal.

tests) were reported as related to emapalumab. One cytomegalovirus (CMV) reactivation was reported as serious. In total, there were five CMV events (three reactivations, one infection and one positive test with no symptoms). All viral events resolved spontaneously or with standard treatment. Two infusion-related reactions (pruritic rash), not reported as severe, occurred during a total of 128 infusions. The rate of AEs and of infectious events was not increased during concomitant treatment with anakinra and emapalumab compared with treatment with emapalumab alone (table 4).

During long-term follow-up, 37 AEs were reported in 12 patients. Three were serious: one flare of sJIA (see online supplemental table S5), one oedema of the ankle and one MAS episode (that, as mentioned above, occurred 11 months after stopping emapalumab, when emapalumab was undetectable). Ten infectious events occurred in seven patients; four when emapalumab levels were measurable, and six after emapalumab levels became undetectable. All reported infections were viral, except for one cestode infection.

DISCUSSION

We show that neutralisation of IFNγ with emapalumab was efficacious in inducing MAS remission in patients with MAS secondary to sJIA/AOSD who failed standard of care including high-dose glucocorticoids with or without anakinra and/or ciclosporin.

The dosing regimen of emapalumab was chosen based on the data gathered in primary HLH patients on (a) the production rate of IFNγ (through the assessment of total IFNγ), (b) the rapid clearance of emapalumab consequent to target-mediated drug disposition in the presence of high IFNγ production and (c) the concentration of emapalumab required to neutralise high IFNγ levels. Through modelling and simulation, on the basis of the high levels of CXCL9 present in patients with MAS, it was possible to predict the dose of emapalumab required to achieve IFNγ neutralisation. A dosing regimen with an initial dose of 6 mg/kg, followed by 3 mg/kg every 3 days maintenance doses, was selected to achieve rapid efficacy as MAS usually has acute onset and may worsen rapidly, becoming life-threatening. In the majority of patients, the chosen regimen rapidly achieved IFNγ neutralisation, shown by prompt decrease in serum CXCL9 levels and prompt clinical and laboratory response. Shortening of the dosing interval might be considered in patients with unsatisfactory response. Indeed, in two patients with initial unsatisfactory response, shortening of the dosing interval led to achievement of response. In these patients, we found high IFNγ production and low emapalumab concentrations due

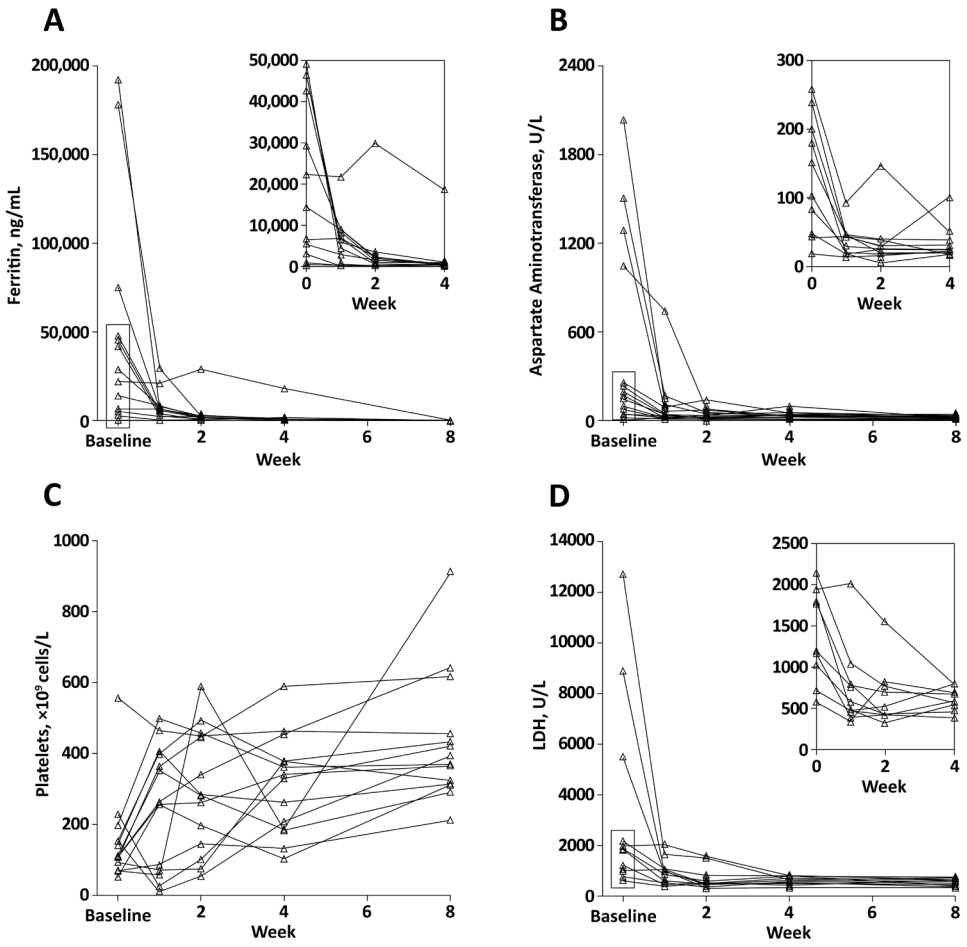

Figure 3 Changes in ferritin* (A), aspartate aminotransferase† (B), platelets (C) and LDH‡ (D) levels over time. *Panel A insert shows in detail changes from baseline to week 4 for patients with baseline levels of ferritin <50 000 ng/mL. †Panel B insert shows in detail changes from baseline to week 4 for patients with baseline levels of aspartate aminotransferase below 300 U/L. ‡Panel D insert shows in detail changes from baseline to week 4 for patients with baseline levels of LDH below 2500 U/L. LDH, lactate dehydrogenase.

to rapid drug clearance consistent with target-mediated drug disposition.

The patients recruited in this study had markedly elevated CXCL9 levels, reflecting high IFNγ activity. This finding is consistent with previous observations in patients with MAS.[16 18] In contrast with primary HLH patients treated with emapalumab,[27] we did not find a correlation of CXCL9 levels at baseline with total IFNγ levels at day 3, that, as mentioned, reflect IFNγ production. Therefore, in MAS, the pathogenic role of IFNγ may be due to increased IFNγ production and increased sensitivity to IFNγ. Indeed, monocytes from patients with MAS have increased responsiveness to IFNγ, possibly through increased expression of tripartite motif containing protein 8 (TRIM-8), which potentiates response to IFNγ.[29 30]

In this trial, the first prospectively investigating a treatment for MAS, we used a novel clinically meaningful efficacy measure, namely MAS remission, that combines resolution of clinical signs and symptoms and of the abnormalities in MAS laboratory parameters. Incidentally, the ferritin threshold (decrease by at least 80% or be below 2000 ng/mL, whichever was lower), previously used in the primary HLH trial with emapalumab,[27] is justified by the often-needed multiple transfusions and the frequent fluctuations of ferritin >1000 ng/mL in patients with HLH/MAS with good response to treatment. Notably, the highest value for ferritin at week 8 in our trial was 561 ng/mL.

After emapalumab initiation, MAS improved rapidly with median time to MAS remission of 25 days. This improvement occurred while background glucocorticoids were rapidly tapered: during the second week of emapalumab treatment, the median glucocorticoid dose was already 85% lower compared with that administered during the week preceding initiation of emapalumab. In general, patients seemed to benefit from emapalumab even when the MAS remission criteria were partially achieved. Indeed, in the few patients who did not reach MAS remission, the physician assessment of disease activity was ≤1 and MAS remission was not achieved because of minor abnormalities in only one of the laboratory parameters.

Elevated production of IFNγ is a feature of animal models of primary HLH and of secondary HLH, including infection-associated HLH and MAS.[13] In these models, when the effect of therapeutic IFNγ neutralisation was tested, benefit was always observed with prevention of death and/or disease improvement. The results of this trial, together with the efficacy of emapalumab demonstrated in primary HLH,[27] and the anecdotal cases of patients with different forms of secondary HLH successfully treated with emapalumab,[31–37] suggest that, in humans, IFNγ is an important driver of MAS/HLH, independently of the trigger or the underlying predisposing condition.

Notably, some inflammatory flares of the underlying sJIA/AOSD were observed, suggesting that, in the context of sJIA/

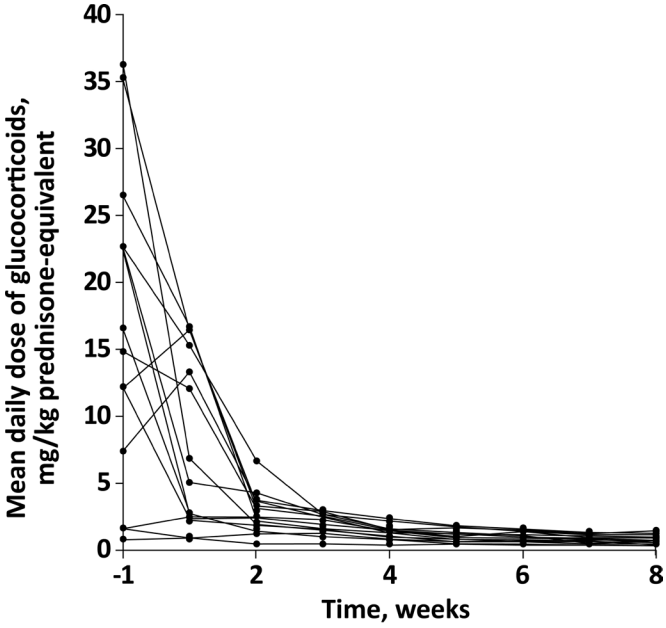

Figure 4 Changes in glucocorticoid dose* over time†. *The dose of glucocorticoids is shown as average daily dose in each week of the study, starting from the week preceding emapalumab treatment (week −1). The dose is shown in mg/kg of prednisone equivalent. †Weeks are defined starting form week −1 (from day −6 to day 0), followed by week 1 (form day 1 to day 7); week 2 (from day 8 to day 14); week 3 (from day 15 to day 21); up to week 8 (from day 50 to day 56).

AOSD, increased IFNγ activity is a feature of the hyperinflammation of MAS, but not of the autoinflammation that maintains sJIA. From a clinical perspective, we observed six flares of the underlying sJIA/AOSD in six out of the nine patients who were not receiving anakinra for the treatment of sJIA while receiving emapalumab. In contrast, no sJIA flares were observed in the five patients who continued anakinra after emapalumab initiation. Importantly, during the trial, no increase in AEs and/or infectious AEs was observed in patients concomitantly exposed to emapalumab and anakinra. Therefore, continuation of anakinra for the underlying sJIA/AOSD, at the doses conventionally used and allowed in this trial, should be considered.[38–40]

The contribution of the concomitant anakinra to the achievement of MAS remission with emapalumab is to be considered negligible. Only one patient continued anakinra at a dose >4 mg/kg/day, a so-called higher dose that has been reported as potentially efficacious in MAS.[10 14 41] Of note, several patients recruited to our study received anakinra before treatment with emapalumab and, in these patients, anakinra, even at doses >4 mg/kg/day, did not prevent or improve MAS. Furthermore, of the three patients in the trial who did not achieve MAS remission at week 8, two were receiving concomitant anakinra.

IFNγ neutralisation might increase predisposition to selected infections, as inferred based on data in humans with defective IFNγ activity, that is, subjects carrying autoantibodies to IFNγ or with IFNγ receptor deficiency.[42] While herpes zoster infections are common in individuals with defective IFNγ activity, no cases were reported in this trial. Notably, per-protocol, patients received prophylaxis with acyclovir. We did not observe any infections from typical or atypical mycobacteria, *H. capsulatum*, *Shigella*, *Salmonella*, *Campylobacter* or *Leishmania*, known to occur more frequently in subjects with defective IFNγ activity. Noteworthy, patients with infections from these agents were excluded from the trial. No bacterial or opportunistic

Table 3 AEs during the treatment with emapalumab and during the long-term follow-up

Event category		NI-0501-06 study (up to week 8) (n=14)	Long-term follow-up (up to week 52) (n=14)
AEs			
Events, n		88	37
Patients with at least one event, n (%)		13 (93)	12 (86)
		Emapalumab levels at or above LLOQ	**Emapalumab levels below LLOQ**
Serious AEs			
Events, n	9*	2	1
Patients with at least one event, n (%)	6 (43)	2 (14)	1 (7)
Cardiopulmonary failure†	1	0	0
sJIA flare‡	1	1	0
CMV infection reactivation§	1	0	0
Intracardiac thrombus	1	0	0
Pericarditis	1	0	0
Thrombocytopenia	1	0	0
Pneumatosis intestinalis	1	0	0
Juvenile myoclonic epilepsy	1	0	0
Rash	1	0	0
Oedema of the ankle region	0	1	0
MAS¶	0	0	1
Events related to study drug			
Events, n	9	0	1
Patients with at least one event, n (%)	4 (28)	0	1 (7)
CMV infection reactivation§	1	0	0
Viral infection	1	0	0
Viral URTI	0	0	1
Viral test positive	4	0	0
Infusion-related reaction	1	0	0
Rash pruritic	1	0	0
Diarrhoea	1	0	0
Infections			
Events, n	10	4	6
Patients with at least one event, n (%)	6 (43)	2 (14)	5 (35)
CMV infection reactivation	3	0	0
CMV infection	1	0	0
Epstein-Barr infection	0	0	1
Rhinovirus infection	0	1	0
Enterovirus infection	0	0	1
Viral infection	1	0	1
Viral URTI	0	3	2
Nasopharyngitis	1	0	0
Viral test positive**	4	0	0
Cestode infection	0	0	1
Systemic juvenile idiopathic arthritis flares			
Events, n	1	3	0
Patients with at least one event, n (%)	1 (7)	3 (21)	0
Infusion-related reactions to emapalumab			
Events, n	2	Not applicable	Not applicable
Patients with at least one event, n (%)	2 (14)	Not applicable	Not applicable

*Of the nine serious AEs reported during the trial, one was severe in intensity (cardiopulmonary failure) and the others were of moderate intensity. One of these events was reported as related to emapalumab. All nine events resolved.
†In the context of rapidly worsening MAS, the patient experienced worsening of pre-existing cardiocirculatory instability a few hours after the first emapalumab infusion. Due to this deterioration, the patient was transferred to the ICU and required intensification of inotropic support (dopamine dose increase). Of note, emapalumab dosing interval was shortened (see online supplemental figure S2) until MAS gradually improved and the patient recovered from the event.
‡Table 3 lists only the episodes of sJIA flare reported as a serious AE. Of note, sJIA flares were identified based on AE reporting as well as based on the rationale for change in medications or medication dose provided by the investigator and these are described in online supplemental table S5.
§This event is the same event reported in the Safety section of the Results and is reported in table 3 under serious AEs, under related AEs and is one of the three CMV reactivations reported under infections.
¶This event is the same event reported in the Efficacy section of the Results.
**One each for CMV, adenovirus, BK polyoma virus, respirovirus.
AE, adverse event; CMV, cytomegalovirus; ICU, intensive care unit; LLOQ, lower limit of quantification; MAS, macrophage activation syndrome; sJIA, systemic juvenile idiopathic arthritis; URTI, upper respiratory tract infection.

Table 4 Rate of AEs* and infectious AEs during the trial while exposed to emapalumab alone or emapalumab and anakinra

	Emapalumab	Emapalumab and anakinra
Exposure, days at risk	303	506
AEs		
Events, n	45	43
Rate per 100 patient-days	14.9	8.5
Infectious AEs		
Events, n	5	5
Rate of per 100 patient-days	1.7	1

*One event of flare of sJIA and two infusion-related reactions to emapalumab were excluded from this analysis.
AE, adverse event; sJIA, systemic juvenile idiopathic arthritis.

infections were reported. Viral infections or positive viral tests were reported in six patients during the trial and in two during follow-up while emapalumab was detectable. All events resolved spontaneously or with standard treatment. Of note, screening for Epstein-Barr virus, CMV and adenovirus every 2 weeks was required by protocol during the trial. Despite the fact that CMV is not reported at increased frequency in humans with defective IFNγ activity,[42] it should be noted that five events were related to CMV, with four reported as reactivations or infections and one as test positive. Whether concomitant prolonged immunosuppression with high-dose glucocorticoids in critically ill patients might have contributed to this observation remains to be established. Despite CMV infections not being reported in humans with defective IFNγ activity,[42] based on our data, patients with MAS receiving emapalumab should be screened for the presence of CMV. Although the entire age spectrum of AOSD is not covered, with only one patient above 20 years of age, it is highly unlikely that the infection risk changes with age.

The main limitation of this study is the single-arm nature of the design. However, a randomised controlled study in this patient population does not appear to be possible and, notably, ethical. This is based, first, on the absence of a standardised and/or validated treatment that has been prospectively investigated in patients with MAS who have failed high-dose glucocorticoids. Therefore, no drug is available as comparator for a head-to-head design. Second, the risk of death associated with this condition makes it unethical to continue, with no additional therapy, an ineffective treatment (ie, high-dose glucocorticoids) in patients who have already failed this treatment. Therefore, the option of continuing high-dose glucocorticoids plus placebo as a comparator arm is also not acceptable.

In conclusion, we demonstrate that IFNγ is an important driver of MAS secondary to sJIA/AOSD and that its neutralisation with emapalumab leads to remission of MAS in patients who failed high-dose glucocorticoids. Attention should be paid to viral infections, particularly to CMV; periodic screening for viral infections should be performed.

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the 'Methods' section for further details.

Patient consent for publication Not applicable.

Ethics approval Approval was obtained from institutional review boards or independent ethics committees at each participating centre. The initial approval was obtained from the Institutional Review Board at Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy (reference number 1245/2016). Participants gave written informed consent to participate in the study before taking part.

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Supplementary Information

Appendix 1: Methods for excluding infections potentially favoured by neutralization of interferon-γ (IFNγ)

Typical and atypical mycobacteria, *Histoplasma capsulatum, Shigella, Salmonella, Campylobacter* and *Leishmania* were excluded at screening by specific tests as outlined below:

- Searches for *Mycobacterium tuberculosis* was performed via IFNγ-release assay or purified protein derivative (PPD) test. In addition, a baseline via polymerase chain reaction (PCR) in a relevant specimen (e.g., urine or blood, if sputum is not easily obtained) had to be obtained. In the case of a patient having received Bacillus Calmette-Guérin (BCG) vaccination, a PPD test had to be performed and combined with an IFNγ-release assay.
- The presence of atypical mycobacteria was excluded at screening by assessing medical history, clinical examination including chest x-ray. In case of suspected clinically active atypical mycobacterial infection a PCR test and/or mycobacterial cultures of appropriate biological samples taken from the patient was performed.
- Searches for *Shigella*, *Salmonella* and *Campylobacter* were performed by stool and blood culture.
- A first screening for *Histoplasma capsulatum* could be performed using a galactomannan assay. However, in the presence of a positive test, confirmation had to be obtained using a *Histoplasma capsulatum*-specific test.
- The presence of *Leishmania* could be ascertained by direct bone marrow observation.

Appendix 2. Total interferon-γ, CXCL9, and soluble interleukin-2 receptor assays

Validated MesoScale Discovery (Rockville, MD, USA)-based immunoassays were used for the measurement of total interferon-γ (IFNγ), soluble interleukin-2 receptor and CXCL9. The respective ranges of quantification relative to the recombinant protein were: interferon-γ, 50 to 50,000 pg/mL; soluble interleukin-2 receptor (sIL-2R), 50 to 50,000 pg/mL; CXCL9, 80 to 50,000 pg/mL. Samples containing biomarker concentrations above these levels underwent dilution to achieve the working range of quantification, with the final result corrected for the sample dilution used. Runs failing to meet standard and/or quality controls (QCs) acceptance criteria were repeated.

Total IFNγ

Calibration standard (recombinant human IFNγ, R&D Systems, Minneapolis, MN, USA) ranging from 20 to 100,000 pg/mL was prepared in human pool serum (at least 10 individual human sera, Seralab/BioIVT, West Sussex, UK) and incubated 1h at 22°C in the presence of 50 ug/mL final of emapalumab to mimic clinical samples. Streptavidin Gold plates (MesoScale Discovery, Rockville, MD, USA) were pre-washed three times with phosphate-buffered saline-Tween 0.05% (wash buffer). Plates were coated with 25 µL of biotinylated mouse monoclonal antibody anti-human IFNγ clone 1-D1K (Mabtech, Nacka Strand, Sweden) at 0.50 µg/mL in phosphate-buffered saline-bovine serum albumin (PBS-BSA) 1%-Tween 0.05% and incubated 1h at 22°C with agitation at 600 rpm. Samples were tested neat or diluted 1:50 in pool serum.

Three QC samples were made of recombinant human IFNγ diluted in human pool serum respectively at 160, 2000, and 40,000 pg/mL with a final concentration of emapalumab of 50 ug/mL. Standard samples and QC samples were diluted at the minimum required dilution (1:3) in PBS-BSA 1%-Tween 0.05% with a final concentration of emapalumab of 50 ug/mL and incubated 1 h at 22°C. Streptavidin Gold plates were washed three times and 25 µL of standard, samples and QC samples were added per well, in duplicate. After 1h incubation at 22°C with agitation at 600 rpm, plates were washed three times and 25 µL of Sulfo-TAG® (MesoScale Discovery, Rockville, MD, USA)-labelled mouse monoclonal antibody anti-human IFNγ clone 1-D1K (Mabtech, Nacka Strand, Sweden) at 0.60 µg/mL in PBS-BSA 1%-Tween 0.05% were added per well. After 1 h incubation at 22°C with agitation at 600 rpm, plates were washed three times and 150 µL of MSD read buffer T 2X (MesoScale Discovery, Rockville, MD, USA) were added per well and the plates were read within 4 to 10 min on the MesoScale Discovery Meso Sector® S600. The raw data were analysed using the MesoScale Discovery Discovery Workbench 4.0 software.

Soluble interleukin-2 receptor

Streptavidin Gold plates (MesoScale Discovery, Rockville, MD USA) were pre-washed three times with PBS-Tween 0.05% (wash buffer). Plates were coated with 25 µL of biotinylated polyclonal goat immunoglobulin G anti-human CD25/interleukin-2 receptor α (R&D systems, Minneapolis, MN, USA) at 0.50 µg/mL in PBS-BSA 1% and incubated 1h at 22°C with agitation at 600 rpm. Calibration standard (recombinant human interleukin receptor 2α, Reprokine, St Petersburg, FL, USA) ranging from 20 to 100,000 pg/mL was prepared in foetal bovine serum (Sigma-Aldrich, Saint Louis, MO, USA). Samples were tested neat or diluted 1:2, 1:5, 1:10, 1:20 1:50 in foetal bovine serum. Three quality control samples were made of recombinant human sIL-2Rα spiked in foetal bovine serum at respectively 35,000 pg/mL, 1800 pg/mL and 150 pg/mL. Two MatrixQCs were made in human pool serum, one containing endogenous sIL-2Rα only, the other containing endogenous sIL-2Rα plus spiked recombinant sIL-2Rα. Standard, samples, QC samples and MatrixQCs were diluted at the minimum required dilution (1:10) in PBS-BSA 1%. Streptavidin Gold plates were washed three times and 25 µL of standard, samples, QC samples and MatrixQCs were added per well, in duplicate. After 1 h incubation at 22°C with agitation at 600 rpm, plates were washed three times and 25 µL of monoclonal mouse immunoglobulin G1 clone 24204 anti-human CD25/interleukin 2 receptor α (R&D systems, Minneapolis, MN, USA) labelled with Sulfo-TAG® (MesoScale Discovery, Rockville, MD, USA) at 0.80 or 1.20 µg/mL in PBS-BSA 1% were added per well. After 1 h incubation at 22°C with agitation at 600 rpm, plates were washed three times and 150 µL of MesoScale Discovery read buffer T 2X (MesoScale Discovery, Rockville, MD, USA) were added per well and the plates were read within 1 to 10 min on the MesoScale Discovery Meso Sector® S600. The raw data were analysed using the MesoScale Discovery Discovery Workbench 4.0 software.

CXCL9

Streptavidin Gold plates (MesoScale Discovery, Rockville, MD, USA) were pre-washed three times with PBS-Tween 0.05% (wash buffer). Plates were coated with 25 µL of mouse anti-human MIG biotinylated antibody (MesoScale Discovery, Rockville, MD, USA) at 0.25 µg/mL in PBS-BSA 1% and incubated 1 h at 22°C with agitation at 600 rpm. Calibration standard (recombinant human CXCL9, R&D systems, Minneapolis, MN, USA) ranging from 30 to 100,000 pg/mL was prepared in foetal bovine serum (Sigma-Aldrich, Saint Louis, MO, USA). Samples were tested neat or diluted 1:2, 1:5, 1:10, 1:20 and 1:50 in foetal

bovine serum. Three MatrixQCs were made of human pool serum containing endogenous CXCL9 only or containing endogenous plus spiked recombinant CXCL9. Standard, samples and MatrixQCs were diluted at the minimum required dilution (1:10) in PBS-BSA 1%. Streptavidin Gold plates were washed three times and 25 µL of standard, samples and QC samples were added per well, in duplicate. After 1 h incubation at 22°C with agitation at 600 rpm, plates were washed three times and 25 µL of goat anti-human MIG antibody labelled with Sulfo-TAG (MesoScale Discovery, Rockville, MD, USA) at 0.25X in PBS-BSA 1% were added per well. After 1 h incubation at 22°C with agitation at 600 rpm, plates were washed three times and reading was performed similarly as described above for sIL-2Rα.

De Benedetti F, *et al. Ann Rheum Dis* 2023;0:1–9. doi: 10.1136/ard-2022-223739

Appendix 3. Emapalumab assay

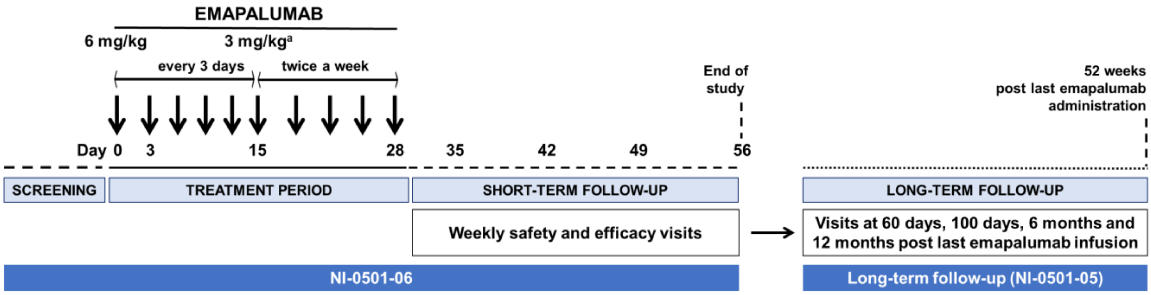
A validated Gyrolab (Gyros Protein Technologies AB, Uppsala, Sweden)-based immunoassay was used for the measurements of emapalumab in patient's serum. Quantification range was from 62.5 ng/mL to 8000 ng/mL. Samples containing emapalumab concentrations above these levels underwent dilution to achieve the working range of quantification, with the final result corrected for the sample dilution used. Runs failing to meet standard and/or quality control (QC) acceptance criteria were repeated.

Working calibration standards ranging from 31.25 to 16,000 ng/mL were freshly prepared in pooled human serum (at least 10 individuals, Seralab/BioIVT, West Sussex, UK) on the day of the assay. Two sets of QC samples thawed on the day of the assay were included on each compact disk (CD) and were comprised of pooled human serum, spiked with emapalumab at three concentration levels, 140 ng/mL (lower QC), 850 ng/mL (medium QC) and 6000 ng/mL (high QC). Two additional QC samples at 250,000 and 12,000 ng/mL were added to runs with clinical samples having a corresponding dilution to assess the validity of the 1:60 dilution. These QC samples were prepared by spiking reference standard into human pool serum and were diluted 1:60 in pooled human serum prior to dilution to the minimum required dilution.

Clinical samples were tested preferentially at the minimum required dilution or diluted 1:60 in addition to the minimum required dilution using pooled human serum, based on the amount of emapalumab expected to be present in the sample. QC samples and samples were diluted to a minimum required dilution of 1:3 in buffer Rexxip H (Gyros Protein Technologies AB, Uppsala, Sweden). Only one replicate of standards, QC samples and samples were prepared. The replicate was analysed twice by the Gyrolab Workstation on the CD to create the duplicate.

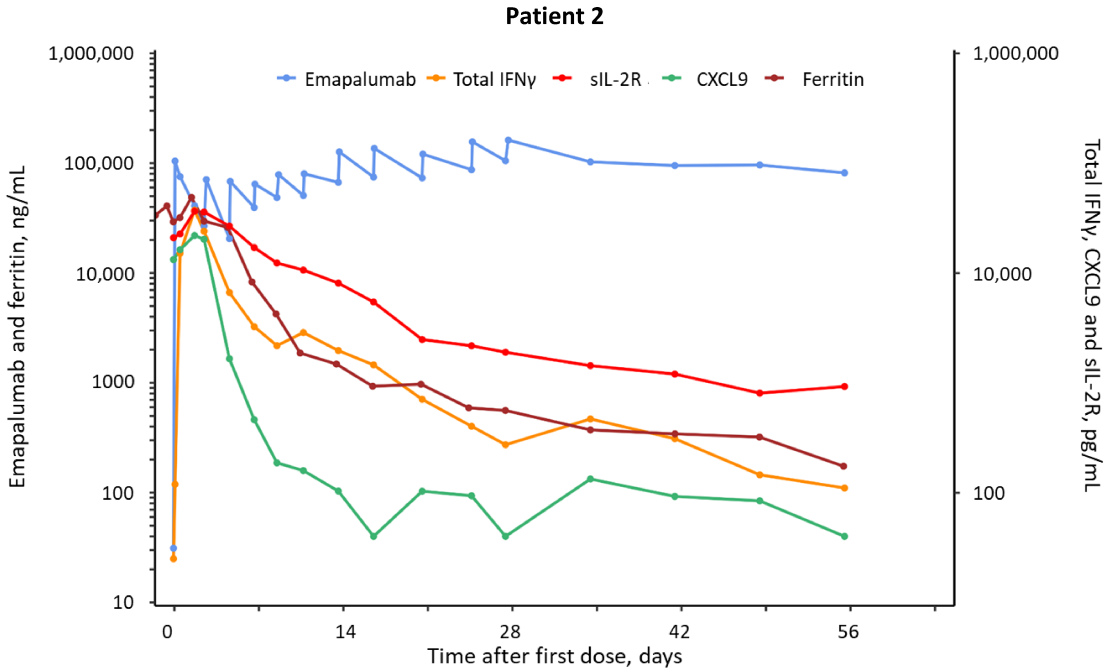
The CD contains micro-affinity columns which are nanostructures packed with streptavidin coated beads. The diluted samples, standard and QC samples as well as the reagents (capture and detection antibodies, wash solution) are prepared manually and loaded in a Gyrolab xP instrument via a polymerase chain reaction plate, which performs the pipetting, incubation, and wash steps of the immunoassay according to a set program where the plate contents are delivered to reservoirs in the CD and the spinning action of the CD causes them to flow through the column. A biotinylated monoclonal anti-idiotypic antibody specific to emapalumab diluted at 52.38 µg/mL with biotinylated bovine serum albumin at 22.73 µg/mL in phosphate-buffered saline-Tween 0.01% was added to the columns, allowing the biotin to bind to the streptavidin beads. The columns were washed, and samples, standards or controls added. After an additional wash the monoclonal anti-idiotypic detection antibody specific to emapalumab coupled with a fluorescent label diluted at 16.67 nM in Rexxip F (Gyros Protein Technologies AB, Uppsala, Sweden) was added. After a final wash step, the Gyrolab instrument measured the fluorescence in all of the columns, simultaneously. The raw data were analysed with Gyrolab Evaluator software.

Figure S1. Design of the NI-0501-06 study and of the NI-0501-05 long-term follow-up study

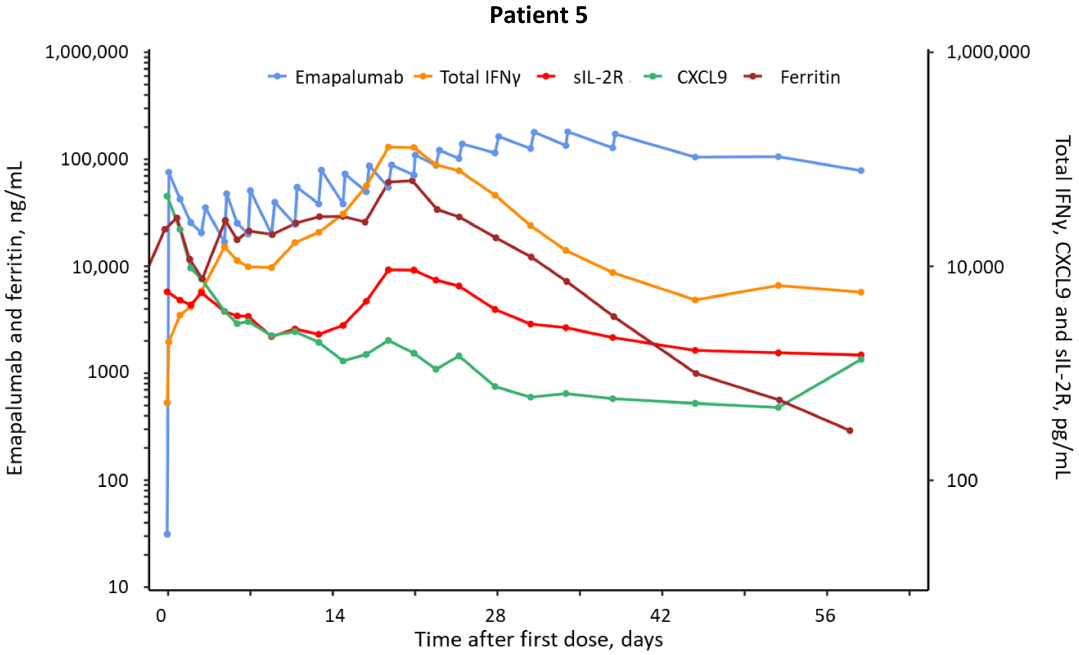


[a] Treatment with emapalumab could be shortened upon achievement of macrophage activation syndrome remission as per investigator's assessment, but not prior to the third dose; frequency between infusions could be shortened or dose could be increased or treatment prolonged in the absence of satisfactory response as per investigator's assessment.

Figure S2. Concentration-time profiles of emapalumab (blue, left log axis), ferritin (dark red, left log axis), total IFNγ (orange, right log axis), sIL-2R (red, right log axis) and CXCL9 (green, right log axis) in two patients showing initial rapid drug clearance with high levels of total IFNγ and incomplete neutralisation of IFNγ activity, as demonstrated by CXCL9 levels and insufficient initial response, as shown by ferritin levels



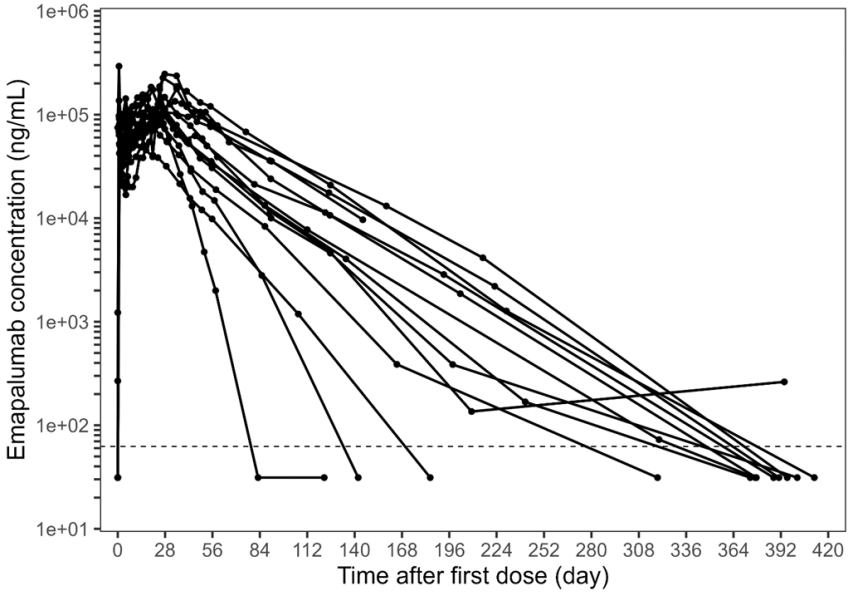
Patient 2 presented with rapidly worsening MAS leading to multiple organ failure and hypotensive shock not responding to multiple 1 g methylprednisolone pulses and required admission to intensive care unit. After start of emapalumab, CXCL9 levels, reflecting high IFNγ activity, and ferritin levels, indicating poor control of MAS, continued to increase. At the same time, the rapid decrease of emapalumab levels between infusions, together with high levels of total IFNγ (indicative of high IFNγ production) are consistent with the presence of target-mediated drug disposition. After shortening the dosing interval to 2 days (from day 5 to day 11), emapalumab levels started to increase and this was associated with neutralisation of IFNγ activity, as shown by decreased in CXCL9 levels and by a subsequent decrease in ferritin levels (and of all other MAS parameters [not shown]), reflecting progressive control of MAS activity.



Patient 5 presented with chronic relapsing MAS having failed multiple treatments in the previous 2 months, including multiple pulses of glucocorticoids, anakinra and tocilizumab. After an initial response to emapalumab, with an evident decrease in ferritin levels, an increase in ferritin is observed, occurring at the time of CMV reactivation. The concomitant rapid decrease of emapalumab levels between infusions, together with persistently high levels of total IFNγ (indicative of high IFNγ production) are consistent with the presence of target mediated drug disposition. Sustained increase in levels of total IFNγ (indicative of high IFNγ production), of sIL-2R (reflecting T cell activation) and of ferritin (reflecting poor control of MAS activity) subsided after shortening the dosing interval to 2 days (from day 3 to day 25). Progressive control of MAS activity was achieved.

Abbreviations:
CMV, cytomegalovirus; IFNγ, interferon gamma; MAS, macrophage activation syndrome; sIL-2R, soluble interleukin-2 receptor

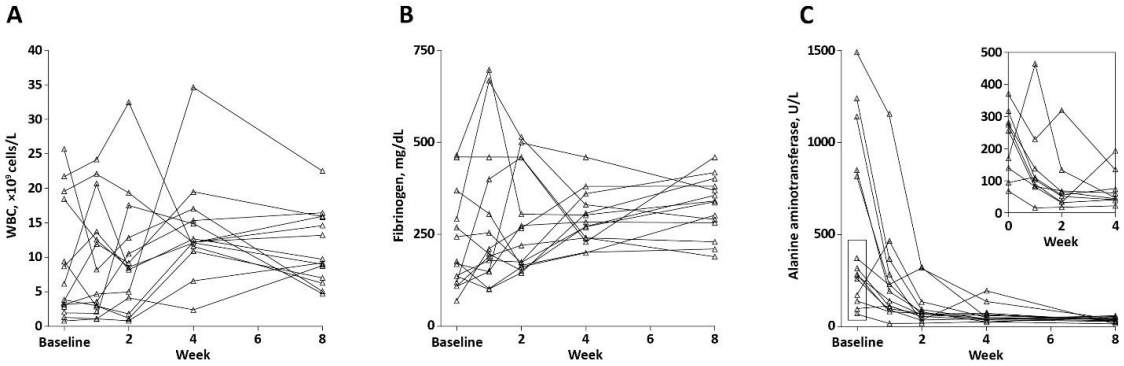
Figure S3. Serum emapalumab levels during the NI-0501-06 study and the long-term follow-up study



Median half-life during the long-term follow-up in the absence of interferon-γ production was 24 days, with a range from 6.1 to 32.4 days.

Dotted line represents the lower limit of quantification (62.5 ng/mL).

Figure S4. Laboratory parameters of MAS, in addition to those shown in Fig 3, of individual patients depicting WBCs (A), fibrinogen (B), alanine aminotransferase (C) before, during and after emapalumab treatment



The insert in panel C shows in detail the change from baseline to week 4 in alanine aminotransferase for patients with baseline levels of alanine aminotransferase below 500 U/L.

Abbreviations:

WBC, white blood cells

Table S1. Eligibility criteria (inclusion and exclusion) of the NI-0501-06 study

Inclusion criteria

- Patients of both genders.
- For patients with sJIA: Confirmed sJIA diagnosis. For patients presenting with MAS in the context of the onset of sJIA, high presumption of sJIA sufficed for eligibility. For AOSD patients: confirmed AOSD diagnosis as per Yamaguchi criteria.
- A diagnosis of active MAS confirmed by the treating rheumatologist, having ascertained the following:
  Febrile patient presenting with:
  - Ferritin >684 ng/mL

  and any 2 of:
  - Platelet count ≤181 x10^9 cells/L
  - Aspartate aminotransferase levels >48 U/L
  - Triglycerides >156 mg/dL
  - Fibrinogen levels ≤360 mg/dL.
- An inadequate response to high-dose intravenous glucocorticoid treatment administered for at least 3 days as per local standard of care (including but not limited to pulses of 30 mg/kg prednisone on 3 consecutive days).
- High-dose intravenous glucocorticoids should not be lower than 2 mg/kg/day of prednisone equivalent in two divided doses (or at least 60 mg/day in patients of 30 kg or more). In case of rapid worsening of the patient's condition and/or laboratory parameters, inclusion may occur within <3 days from starting high-dose intravenous glucocorticoids.
- Tocilizumab, tumour necrosis factor inhibitors, and canakinumab, if administered, had to be discontinued before emapalumab initiation.
- Informed consent provided by the patient (as required by local law) or by the patient's legally authorised representative(s) with the assent of patients who are legally capable of providing it as applicable.
- Having received guidance on contraception for both male and female patients sexually active and having reached puberty:
  - Females of child-bearing potential required the use of highly effective contraceptive measures (failure rate of <1% per year)[a] from screening until 6 months after receiving last dose of the investigational medicinal product.
  - Males with partners of child-bearing potential had to agree to take appropriate precautions (such as sexual abstinence, barrier contraception, or vasectomy) to avoid fathering a child from screening until 6 months after receiving last dose of the investigational medicinal product.

[a] Highly effective contraceptive measures included: sexual abstinence; hormonal contraceptives: combination or progesterone only; intrauterine methods (intrauterine devices or systems); bilateral tubal occlusion; vasectomised partner.

Exclusion criteria

- Diagnosis of suspected or confirmed primary haemophagocytic lymphohistiocytosis or haemophagocytic lymphohistiocytosis consequent to a neoplastic disease.
- Active mycobacteria (typical and atypical), *Histoplasma capsulatum*, *Shigella*, *Salmonella*, *Campylobacter*, and *Leishmania* infections.
- Clinical suspicion of latent tuberculosis.
- Positive serology for human immunodeficiency virus antibodies.
- Presence of malignancy.
- Patients who had another concomitant disease or malformation severely affecting cardiovascular, pulmonary, central nervous system, liver, or renal function that in the opinion of the investigator might have significantly affected likelihood to respond to treatment and/or assessment of emapalumab safety.
- History of hypersensitivity or allergy to any component of the study regimen.
- Receipt of bacillus Calmette-Guérin vaccine within 12 weeks prior to screening.
- Receipt of live or attenuated live vaccines within 6 weeks prior to screening.
- Pregnant or lactating female patients.

Abbreviations:

AOSD, adult-onset Still's disease; MAS, macrophage activation syndrome; sJIA, systemic juvenile idiopathic arthritis

Table S2. Signs and symptoms to be evaluated by the investigator while assessing the activity of MAS

- Fever (greater than 38.0°C [100.4°F]).
- Skin rash.
- Haemorrhagic manifestations:
  - Skin bleeding (petecchiae, ecchymosis, or purpura) (the investigator had to choose among “stable”, “worsened”, “improved”, or “resolved”).
  - Mucosal bleeding (gut, respiratory) (the investigator had to choose among “present” or “absent”).
- Evidence of CNS involvement:
  - Clinical (headache, irritability, seizures, confusion, lethargy, coma) (the investigator had to choose among “stable”, “worsened”, “improved”, or “resolved”).
  - Cerebrospinal fluid abnormalities (cell count) (if lumbar puncture performed based on clinical indication).
- Respiratory function:
  - Oxygen support (the investigator had to choose among “present” or “absent”; if present, the investigator had to indicate how many liters of oxygen were required to maintain oxygen saturation).
  - Mechanical ventilation (the investigator had to choose among “present” or “absent”).
- Cardiac:
  - Pericarditis (if echocardiography was performed, results were to be reported).
  - Inotropic support.
- Kidney:
  - Ultrafiltration/dialysis.

Abbreviations:

CNS, central nervous system; MAS, macrophage activation syndrome

Table S3. Total IFNγ, CXCL9, and sIL-2R levels during the study and the long-term follow-up study

	NI-0501-06 study						Long-term follow-up (NI-0501-05 study)		
	Day 0 (n=14)	Day 3 (n=14)	Day 7 (n=14)	Day 14 (n=14)	Day 28 (n=14)	Day 56 (n=14)	Day 100 (n=14)	6 months (n=10)[a]	12 months (n=9)[a]
Total IFNγ									
Median (range)	<50 (<50–4510)	2452 (66–37295)	2456 (73–9886)	1509 (59–20,744)	1200 (<50–46,186)	905 (<50–10,308)	1207 (105–24,290)	2177 (<50–12,675)	594 (<50–4010)
Normal, n (%)[b]	NA[c]	6 (43)	7 (50)	9 (64)	10 (71)	9 (64)	10 (71)	6 (60)	7 (78)
CXCL9									
Median (range)	10884 (1020–98,121)	1807 (598–21,963)	555 (192–3044)	217 (<80–1946)	136 (<80–750)	114 (<80–2296)	142 (<80–1043)	156 (<80–370)	211 (111–2323)
Normal, n (%)[b]	0 (0)	0 (0)	1 (7)	7 (50)	12 (86)	12 (86)	12 (86)	8 (80)	6 (67)
sIL-2R									
Median (range)	5077 (1664–20,954)	4669 (2646–36,481)	3319 (1579–17,059)	1756 (908–8090)	1105 (571–3945)	1098 (306–3432)	840 (344–3122)	853 (535–1803)	904 (501–4019)
Normal, n (%)[b]	0 (0)	0 (0)	0 (0)	1 (7)	7 (50)	7 (50)	9 (64)	6 (60)	5 (56)

[a] In the long-term follow-up, the protocol required pharmacokinetic/pharmacodynamic sampling until emapalumab was undetectable in blood. Two patients with undetectable levels at Day 100 were therefore not sampled at 6 and 12 months, and 1 patient with undetectable levels at 6 months was not sampled at 12 months. One patient was not sampled at 6 months. One patient missed the 6- and 12- month visits of the long-term follow-up because of Covid-19-related travel restriction.

[b] Below or equal to the 95th percentile of healthy controls: 2084 pg/mL for total IFNγ, 271 pg/mL for CXCL9[1] and 1071 pg/mL for sIL-2R.

[c] At baseline, in the absence of emapalumab, total IFNγ consists only of free IFNγ. Therefore, the above indicated 95th percentile for total IFNγ applies only to values obtained in the presence of emapalumab.

Abbreviations:

IFNγ, interferon-γ; sIL-2R, soluble interleukin-2 receptor

Reference: 1. Jacqmin P, Laveille C, Snoeck E, et al. Emapalumab in primary haemophagocytic lymphohistiocytosis and the pathogenic role of interferon gamma: A pharmacometric model-based approach. Br J Clin Pharmacol 2022;88(5):2128–2139.

Table S4. Assessment of MAS remission at the end of the long-term follow-up study (12 months)

Patients meeting MAS remission criteria: n=10
Patients not meeting MAS remission criteria: n=4 • One patient with MAS activity = 1.5 • One patient who stopped emapalumab after three administrations upon investigator's assessment of remission, continued to have mild abnormalities only in LDH levels • One patient had missing values for aspartate aminotransferase and LDH, with all other parameters of the criteria for MAS remission being met • One patient missed the 6- and 12-month visits of the long-term follow-up because of Covid-19–related travel restrictions

Abbreviations: LDH, lactate dehydrogenase; MAS, macrophage activation syndrome

Table S5. Inflammatory flares of underlying sJIA/AOSD and their treatment during the study and the long-term follow-up study

NI-0501-06 study		
Patient ID	**Flare and background treatment**	**Treatment of underlying sJIA/AOSD**
Patient 3	• Anakinra (2 mg/kg/day) discontinued on study day 0 • CRP increase on study day 3 while on 2.5 mg/kg/day prednisone equivalent and MAS improving (first MAS remission achieved on study day 25)	• Anakinra re-introduced at 2 mg/kg/day on study day 4
Patient 5	• Anakinra (13 mg/kg/day) discontinued 11 days prior to start of emapalumab • CRP increase on study day 14 while on 2.5 mg/kg/day prednisone equivalent and MAS improving (first MAS achieved on study day 45)	• Anakinra re-introduced at 4 mg/kg/day on study day 14
Patient 10	• Anakinra (9 mg/kg) discontinued on study day -1 • CRP increase on study day 2 while on 2.1 mg/kg/day prednisone equivalent and MAS improvement (first MAS remission achieved on study day 23)	• Glucocorticoid pulses at 35 mg/kg/day prednisone equivalent on study day 4, 5, and 6
Patient 13	• CRP increase on study day 21 while receiving 1.5 mg/kg/day prednisone equivalent (emapalumab last dose on study day 24) and in MAS remission (first achieved on study day 21)	• Anakinra introduced at 2 mg/kg/day on study day 25, increased to 4 mg/kg/day on study day 26
Patient 11	• Persistently increased CRP from study day 0 while receiving 25 mg/kg/day prednisone equivalent and MAS improvement (first MAS remission achieved on study day 15)	• 30 mg/kg/day prednisone equivalent on study days 4 and 5. • Anakinra introduced on study day 6 at 2 mg/kg/day
Patient 8	• CRP increase on study day 12 while on 0.4 mg/kg/day prednisone equivalent (last dose of emapalumab on study day 6) and MAS in resolution (only LDH > 1.5× ULN)	• Baricitinib introduced on study day 15

Long-term follow-up (NI-0501-05)		
Patient ID	**Flare and background treatment**	**Treatment of underlying sJIA/AOSD**
Patient 1	• Inflammatory flare of sJIA on study day 315 while on prednisone at 0.07 mg/kg/day and no MAS flare	• Glucocorticoid dose increased: methylprednisolone 0.9 mg/kg/day • Canakinumab introduced at 300 mg every 4 weeks
Patient 11	• Inflammatory flare of sJIA on study day 233 while on anakinra at 1.8 mg/kg/day and off glucocorticoids and no MAS flare	• Methyl prednisolone pulses (1 g/day) for 3 consecutive days, switched to oral prednisone (0.9 mg/kg/day) and then tapered • Anakinra continued at 1.8 mg/kg/day
Patient 12	• Inflammatory flare of sJIA on study day 189 while on anakinra at 14 mg/kg/day and off glucocorticoids	• Methylprednisolone pulses (1 g/day) for 2 days followed by prednisolone at 2.2 mg/kg/day • Subsequently tocilizumab (162 mg every 2 weeks)
Patient 14	• Inflammatory flare of sJIA with interstitial lung disease on study day 320 while on prednisolone at 1.5 mg/kg/day, anakinra at 4.2 mg/kg/day and methotrexate 7.5 mg/week	• Tofacitinib introduced at 2.5 mg/day

First MAS remission was the time of first fulfilment of MAS remission criteria

Flares of the underlying sJIA were identified based on adverse event reporting and on the rationale for change in medications or medication doses.

Abbreviations:

AOSD, adult-onset Still's disease; CRP, C-reactive protein; LDH, lactate dehydrogenase; MAS, macrophage activation syndrome; sJIA, systemic juvenile idiopathic arthritis; ULN, upper limit of normal

Table S6. TEAEs reported in ≥2 patients during the NI-0501-06 trial and the long-term follow-up study

Event category	**NI-0501-06 study (up to week 8)** (N=14)	**Long-term follow-up (up to week 52)[a]** (N=14)	
TEAEs reported in ≥2 patients			
		Emapalumab levels at or above LLOQ	Emapalumab levels below LLOQ
No. of events	23	9	5
Rash	4	–	–
CMV infection reactivation	3	–	–
Diarrhoea	4	–	–
Hyperglycaemia	3	–	–
Hypertension	3	–	–
Headache	4	1	1
Injection site reaction	2	–	–
Viral URTI	–	4	1
sJIA flare	–	3	–
Cholelithiasis	–	–	2
Dental caries	–	1	1

[a] The protocol mandated that only serious AEs were to be reported after the level of emapalumab was undetectable. However, five non-serious AEs were also reported during this time and are included in the table.

Abbreviations:

AE adverse event; CMV, cytomegalovirus; LLOQ, lower limit of quantification; sJIA, systemic juvenile idiopathic arthritis; TEAE, treatment-emergent adverse event; URTI, upper respiratory tract infection