Monoclonal antibody against macrophage colony-stimulating factor suppresses circulating monocytes and tissue macrophage function but does not alter cell infiltration/activation in cutaneous lesions or clinical outcomes in patients with cutaneous lupus erythematosus

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Summary

This study’s objective was to assess the effects of PD-0360324, a fully human immunoglobulin G2 monoclonal antibody against macrophage colony-stimulating factor in cutaneous lupus erythematosus (CLE). Patients with active subacute CLE or discoid lupus erythematosus were randomized to receive 100 or 150 mg PD-0360324 or placebo via intravenous infusion every 2 weeks for 3 months. Blood and urine samples were obtained pre- and post-treatment to analyse pharmacokinetics and pharmacodynamic changes in CD14+CD16+ monocytes, urinary N-terminal telopeptide (uNTX), alanine/aspartate aminotransferases (ALT/AST) and creatine kinase (CK); tissue biopsy samples were taken to evaluate macrophage populations and T cells using immunohistochemistry. Clinical efficacy assessments included the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI). Among 28 randomized/analysed patients, peak/trough plasma concentrations increased in a greater-than-dose-proportional manner with dose increases from 100 to 150 mg. Statistically significant differences were observed between active treatment and placebo groups in changes from baseline in CD14+CD16+ cells, uNTX, ALT, AST and CK levels at most time-points. The numbers, density and activation states of tissue macrophages and T cells did not change from baseline to treatment end. No between-group differences were seen in CLASI. Patients receiving PD-0360324 reported significantly more adverse events than those receiving placebo, but no serious adverse events. In patients with CLE, 100 and 150 mg PD-0360324 every 2 weeks for 3 months suppressed a subset of circulating monocytes and altered activity of some tissue macrophages without affecting cell populations in CLE skin lesions or improving clinical end-points.

Keywords: CD14/CD16 monocytes, cutaneous lupus erythematosus, immunohistochemistry, macrophage colony-stimulating factor, osteoclasts

Introduction

Cutaneous lupus erythematosus (CLE) is characterized by dermal inflammation that culminates in keratinocyte damage at the dermoepidermal junction [1]. CLE is categorized as acute, subacute or chronic, based on clinical morphology and lesional lifespan, and chronic CLE has several subtypes, including the most common subtype, discoid lupus erythematosus (DLE) [2,3]. The prevalence of CLE is comparable to or higher than that of systemic lupus erythematosus (SLE) [4,5]. Although CLE typically has a less severe course and better prognosis than SLE, it is associated none the less with substantial quality-of-life impairment and work disability because lesions frequently emerge on visible cutaneous areas, such as the face, neck, hands and arms [4,6]. Standard pharmacological treatment of CLE includes topical or systemic corticosteroids and anti-malarial agents, but these options may be of limited effectiveness and may result in burdensome local and systemic side effects [7]. Some patients with CLE or DLE resistant to standard therapies may respond to immunomodulators such as azathioprine [8], methotrexate [9] and mycophenolate mofetil [10], although the benefits of the latter agents must also be weighed against the potential for toxicity.
The pathogenic process that leads to CLE is incompletely understood. In genetically predisposed individuals, environmental factors, such as ultraviolet light, hormones, trauma or viruses, trigger abnormal immune responses. The latter responses stimulate activation of effector cells and influx of inflammatory cells, including T cells, macrophages and plasmacytoid dendritic cells (pDCs). pDCs and type I interferon (IFN) play a critical role in initiating and maintaining cutaneous lesions in patients with CLE [11–16]. pDCs have been shown to accumulate in affected skin in lupus erythematosus (but not in normal skin), where they perpetuate the inflammatory process by stimulating production of IFN-α/β [13,17]. IFN-α promotes cutaneous inflammation by inducing the production of chemokines that recruit autoreactive T cells from the peripheral blood into skin [17,18]. T cells have been found to be prominent in cutaneous lesions in DLE [19].

The macrophage colony-stimulating factor (M-CSF) is a growth factor that has also been implicated in the pathogenesis of lupus [20,21]. M-CSF supports the proliferation, differentiation and preservation of macrophages and monocytes, including CD14+ and CD16+ monocytes, which are precursors to proinflammatory macrophages and pDCs that infiltrate sites of inflammation. M-CSF levels are increased in serum of patients with SLE [22] and in urine of patients with lupus nephritis who experience renal flare [23]. In addition, elevations in serum or urine M-CSF levels in patients with SLE correlate with renal disease activity and are predictive of the onset and flare of lupus nephritis [21].

PD-0360324 is a fully human immunoglobulin G2 monoclonal antibody that binds M-CSF with high affinity and selectivity and inhibits the binding of M-CSF to its receptor. This Phase 1b study was conducted to assess the effects of this anti-M-CSF agent on circulating monocyte populations and tissue-based macrophages in patients with active CLE. The impact of PD-0360324 administration on pDC numbers and activation states, type I IFN activation and T cell recruitment was also evaluated, and the agent’s pharmacokinetics, clinical efficacy, safety and immunogenicity were explored.

Methods

Patients

Enrolled patients were between the ages of 21 and 70 years; had a clinical diagnosis of subacute CLE or DLE, with or without other features of SLE, confirmed via histological analysis of skin biopsy samples; had active disease, defined by a Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) disease activity score of 10 or greater; and were intolerant or had an inadequate response to anti-malarial therapy given for at least 3 months. Patients were permitted to receive stable doses of anti-malarials for ≥3 months before the study drug was first administered; topical corticosteroids of mild-medium potency for ≥2 weeks and oral prednisone or equivalent (≤20 mg/day) for ≥1 month; and other immunosuppressive/immunomodulatory medications for ≥2 months. Patients were excluded if they received treatment with an investigational biological agent, other than a B cell-depleting agent, within 1 year of baseline; an investigational non-biological agent within 1 month (or five half-lives); a B cell-depleting agent within 1 year; an antitumour necrosis factor (TNF) agent, belimumab or any other anti-B lymphocyte stimulator agent, intravenous (i.v.) cyclophosphamide or i.v. immunoglobulin within 6 months; or any bone active agent, e.g. calcitonin, calcitriol, an oestrogen receptor modulator or parathyroid hormone within 1 month. All patients provided written informed consent before undergoing screening procedures for the study.

Study design and treatment

This Phase 1b, randomized, double-blind, placebo-controlled, dose-escalation study was conducted between November 2011 and November 2013 at 17 centres in the United States, Canada and the Republic of Moldova (clinicaltrials.gov NCT01470313). After a 4-week screening, patients were assigned randomly in a 3 : 1 ratio (active treatment : placebo) to receive 100 or 150 mg PD-0360324 administered as an i.v. infusion every 2 weeks or placebo during the subsequent 12-week treatment period. A final follow-up visit was scheduled for all patients at week 16.

The study was conducted in compliance with the ethical principles of the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice guidelines. The study protocol and informed consent forms were approved by the institutional review boards and/or independent ethics committees at each participating study centre.

Study assessments

Pharmacokinetics. Blood samples were obtained before and after treatment administration at weeks 0, 2, 4, 6, 8 and 10 to measure serum concentrations of PD-0370324, using a validated enzyme-linked immunosorbent assay (ELISA). Other pharmacokinetic calculations were not performed because of limited pharmacokinetic data collection.

Pharmacodynamics. Whole blood and urine samples were obtained weekly from weeks 0–4 and at weeks 8, 12 and 16 for pharmacodynamic analyses, including analyses of changes from baseline in CD14+CD16+ monocytes, the bone remodelling biomarker urinary N-terminal telopeptide (uNTX; Osteomark® NTx Urine; Wampole Laboratories, Princeton, NJ, USA), alanine transaminase (ALT), aspartate transaminase (AST) and creatine kinase (CK).
CD14⁺ CD16⁺ monocyte fluorescence activated cell sorter (FACS) assay. Blood samples were incubated with human leucocyte antigen D-related (HLA-DR)-fluorescein isothiocyanate (FITC), CD14-phycocerythrin (PE) or PE-isotype control, CD3-peridinin chlorophyll (PerCP)-cyarin (Cy)5-5, CD19-PerCP-Cy5-5, CD16-AF647 or AF647-isotype control and CD45-allophycocyanin (APC)-H7 for 30 min at room temperature. The reaction was stopped by addition of 1 ml of 1X Pharm Lyse (BD Biosciences, San Jose, CA, USA), followed by a 15-min incubation period before analysis by FACS Canto II. The CD14⁺CD16⁺ assay was developed and validated for clinical use in this study.

Tissue immunohistochemistry. Skin tissue samples were collected to perform exploratory immunohistochemistry (IHC) histological analyses (data maintained at Pfizer). Skin punch biopsies (4 mm) were taken from non-lesional skin at week 0 and lesional skin at weeks 0 and 16 in 10 patients (placebo, n = 4; PD-0370324, n = 6). Formalin-fixed, paraffin-embedded lesional skin biopsies collected at baseline and post-treatment were evaluated for expression of the following markers by IHC: CD68 (clone: KP1, Mob 167-05; Diagnostic BioSystems, Pleasanton, CA, USA); CD163 (clone: 10D6, NCL-L-CD163; Novocastra/Leica Biosystems, Buffalo Grove, IL, USA); HLA-DR, -DP, -DQ (HLA-DR; clone: CR3/43 M0775; Dako North America, Inc., Carpinteria, CA, USA); CD3 (clone: SP7, RM-9107-R7; Thermo Scientific, Waltham, MA, USA); CXCR3/CD183 (clone: IC6, 557183; BD Biosciences, San Jose, CA, USA); and CD45, LCA (clone: RP2/18, 760-2505; Ventana Medical Systems, Tucson, AZ, USA). IHC assays were performed on a Discovery XT® automated stainer for CD68, CD3, CD45 and HLA-DR using UltraMap alkaline phosphatase red anti-rabbit or anti-mouse polymer detection kit (Ventana Medical Systems). CXCR3/CD183 and CD163 IHC assays were performed on BOND RX automated stainer (Leica Biosystems, Buffalo Grove, IL, USA) using the Bond Polymer Refine Red alkaline phosphatase detection kit. Slides were counterstained with haematoxylin and mounted with a synthetic mounting medium. The area of positive signal in epidermis and dermis in stained sections was quantified for each marker using Definiens Tissue Studio image analysis software (Definiens AG, Munich, Germany), and counts were normalized to the measured area of epidermis or dermis, respectively, on each slide.

Clinical efficacy and safety. Efficacy assessments included the routine laboratory safety tests and absolute change from baseline in CLASI activity and damage scores [24], the SLE Disease Activity Index-2K (SLEDAI-2K) score [25] and the Physician’s Global Assessment (PGA; 100-mm visual analogue scale) during the 12-week treatment period. Adverse events (AEs) were monitored at each visit.

Immunogenicity. Antibodies to PD-0370324 were determined in serum samples collected from all patients at weeks 1, 4, 8, 12 and 16, following a tiered approach using screening, confirmation and titre assays. The semi-quantitative ELISA included a positive control (M-CSF-A1 monoclonal antibody clone) and negative control (pooled normal human serum). Precision for the anti-drug antibody (ADA) assay, expressed as the between-day percentage coefficient of variation (CV%), was 19.6–23.1% for the positive control and 3.6–13.2% for the negative control. For the neutralizing ADA assay, CV% was 17.4 and 4.3 for the positive and negative controls, respectively.

Statistical analysis
The sample size was determined by clinical and practical criteria, not statistical criteria. Pharmacokinetic, pharmacodynamic, clinical efficacy and safety were analysed in patients who had been randomized to treatment and had taken at least one dose of study medication (defined as the full analysis population).

Changes from baseline in pharmacodynamic and clinical efficacy parameters were analysed using a linear mixed model (observed-case data) that included treatment and week as main effects, treatment by week as an interaction term and baseline score as a covariate. Overall rates of on-treatment adverse events were compared between treatments using Fisher’s exact test.

Results
Patients
Twenty-eight patients were randomized to treatment (placebo, n = 6; PD-0360324 100 mg, n = 12; PD-0360324 150 mg, n = 10), received treatment and were included in the full analysis population (Fig. 1). Baseline characteristics of patients in the three treatment groups are shown in Table 1. The mean age was lower in patients in the placebo group (37 years) than in those in the PD-0360324 groups (aged 47 and 50 years). Across all groups, most patients were female (75%) and white (75%). Patients in the PD-0360324 150-mg group had a longer mean duration since diagnosis of CLE (13 years) than those in the placebo (8 years) and 100-mg (9 years) groups.

Pharmacokinetics
Following i.v. infusion every 2 weeks, exposure (maximum) and trough plasma concentrations increased in a greater-than-dose-proportional fashion to increases in PD-0360324 dose from 100 to 150 mg (Fig. 2). This disproportionate exposure increase with increasing doses is consistent with the non-linear pharmacokinetics associated with
this agent’s target-mediated disposition and exposure data observed in previous studies (data on file, Pfizer).

Monocyte populations and other urinary/serum biomarkers

Circulating CD14$^+$ monocyte populations were within 20–40% of baseline values throughout the study period for all treatment groups (Fig. 3a). Therefore, neither the 100-mg nor the 150-mg dose altered the total populations of CD14$^+$ monocytes. Statistically significant differences were seen in the percentage change from baseline in CD14$^+$CD16$^+$ monocytes between the PD-0360324 100-mg and placebo groups at all time-points except weeks 2 and 8 and between the PD-0360324 150-mg and placebo groups at all weeks except week 2 ($P < 0.05$; Fig. 3b). Both

Table 1. Patient characteristics at baseline*

| Baseline characteristic                  | Placebo ($n = 6$) | PD-0360324 100 mg ($n = 12$) | PD-0360324 150 mg ($n = 10$) |
|------------------------------------------|-------------------|------------------------------|------------------------------|
| Age, years, mean (s.d.)                  | 37.0 (10.5)       | 46.8 (11.7)                  | 50.0 (11.6)                  |
| Female, $n$ (%)                          | 4 (67)            | 8 (67)                       | 9 (90)                       |
| Race, $n$ (%)                            |                   |                              |                              |
| Caucasian                                | 3 (50)            | 12 (100)                     | 6 (60)                       |
| Black                                    | 3 (50)            | 0                            | 4 (40)                       |
| Weight, kg, mean (s.d.)                  | 83.9 (23.4)       | 91.9 (31.0)                  | 79.7 (22.5)                  |
| Disease duration, years, median (range)  | 5.5 (0.4–21.2)    | 4.9 (0.3–28.5)               | 9.7 (1.8–40.3)               |
| CLASI, median (range)                    |                   |                              |                              |
| Activity, 0–70                           | 21.5 (9–40)       | 16.0 (10–40)                 | 18.0 (10–29)                 |
| Damage, 0–54                             | 24.5 (9–39)       | 6.0 (1–33)                   | 15.0 (3–25)                  |
| SLEDAI-2K, median (range)                | 12.5 (4–26)       | 6.0 (4–17)                   | 4.0 (2–27)                   |
| PGA, median (range)                      | 65.0 (24.0–85.0)  | 31.3 (17.0–99.0)             | 53.2 (17.2–86.3)             |
| Concomitant medications, $n$ (%)         |                   |                              |                              |
| Hydroxychloroquine                       | 3 (50)            | 6 (50)                       | 6 (60)                       |
| Mycophenolate mofetil                    | 1 (17)            | 0                            | 1 (10)                       |
| Methylprednisolone                       | 1 (17)            | 1 (8)                        | 0                            |
| Prednisone                               | 1 (17)            | 4 (33)                       | 4 (40)                       |
| Triamcinolone                            | 0                 | 2 (17)                       | 2 (20)                       |
| dsDNA autoantibodies, IU/ml, median (range) | 5.0 (0–149) | 2.0 (0–15)                 | 4.0 (0–130)                  |

*A total of 16 patients satisfied criteria of the American College of Rheumatology for SLE and all these patients had discoid lesions. CLASI = Cutaneous Lupus Erythematosus Disease Area and Severity Index; PGA = Physician’s Global Assessment; SLEDAI-2K = Systemic Lupus Erythematosus Disease Activity Index-2K.
active treatment groups showed statistically significant
decreases in the percentage change from baseline in the
osteoclast function marker uNTX compared with the pla-

cbo group at 3, 4, 8 and 12 weeks (P < 0.05; Fig. 3c).

In addition, consistent with previous studies, signifi-
cantly greater percentages of change from baseline in ALT,
AST and CK levels were observed in the active treatment
groups compared with the placebo group at most time-
points throughout the treatment period (Fig. 4). These
findings may have resulted from pharmacological inhibi-
tion of macrophage-derived cell populations (Küpffer cells)
in the liver and not direct muscle or liver toxicity [26].

Relationship between pharmacokinetics
and pharmacodynamics

The reductions in CD14⁺CD16⁻ monocytes, observed as
early as week 1, closely followed PD-0360324 trough con-
centration and remained suppressed throughout the dosing
period, whereas reduction in uNTx was more delayed.
Increases in AST and CK were sustained to week 12, with a
decline after dosing was discontinued at week 16, and were
related directly to PD-0360324 concentration (Fig. 5).

Exploratory tissue biomarkers

Numbers and activation states of macrophage populations
and T cells were evaluated by IHC in biopsies collected
from patients at baseline and after treatment. In total, four
complete sets of biopsies consisting of baseline lesional and
non-lesional samples and after-treatment lesional samples
were collected from patients receiving 100 mg, two sets
from patients receiving 150 mg and four sets from patients
receiving placebo. Despite suppression of CD14⁺CD16⁻ monocytes in circulation, no treatment-related effects were
observed on tissue macrophage density (CD68 and CD163)
or activation state (HLA-DR) in lesional biopsies collected
at the end of treatment compared with baseline samples. In
addition, no improvements were observed in the density
(CD3) or activation state (CXCR3) of T cell infiltrates or
overall leucocyte infiltrates (CD45) in baseline and post-
treatment samples, indicating that local IFN-driven inflam-

mation was not affected significantly by anti-M-CSF treat-
ment (Fig. 6).

Consistent with clinical scores and IHC end-points,
comparison of pre- and post-treatment biopsy histological
analyses by a board-certified dermatopathologist showed
no improvement in disease-associated end-points, includ-
ing epidermal thickness, basement membrane thickness
and inflammatory cell infiltrates (data not shown).

Clinical efficacy, safety and immunogenicity

No significant differences between the placebo and active
treatment groups were observed in the change from base-
line in CLASI or SLEDAI-2K scores at any time-point or in
the PGA at 8 or 12 weeks (data not shown). The frequency
of treatment-emergent AEs was generally higher in the
active treatment groups than in the placebo group (Table
2). Patients receiving PD-0360324 in the 150-mg group
had significantly more treatment-related AEs than those
receiving placebo (P < 0.01). AEs occurred with similar
frequency between patients in the PD-0360324 100-mg and
150-mg groups. No serious AEs occurred in the active
treatment groups.

Of 22 patients in the active treatment groups with eval-
uable samples, two patients developed ADAs over the 12-week
treatment period. One patient, who received the PD-
0360324 100-mg dose, was ADA-positive at weeks 4 and 12;
Fig. 3. Mean percentage of changes from baseline in CD14+ monocytes (a), CD14+ CD16+ monocytes (b) and urinary N-terminal telopeptide (uNTX) (c). *P < 0.05; †P < 0.01; ‡P < 0.001; §P < 0.0001, PD-0360324 versus placebo.
Fig. 4. Mean percentage of changes from baseline in alanine aminotransferase (ALT) (a), aspartate aminotransferase (AST) (b) and creatine kinase (CK) levels (c). *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$; §$P < 0.0001$, PD-0360324 versus placebo.
the second patient, who received the 150-mg dose, was ADA-positive at weeks 8 and 12. No neutralizing activity was detected in the serum samples of these patients. The ADAs did not have discernible effects on pharmacokinetics, pharmacodynamics, safety or clinical efficacy.

Discussion

In a 3-month study in patients with CLE, biweekly administration of either 100 or 150 mg of the anti-M-CSF monoclonal antibody PD-0360324 resulted in the suppression of a subset of circulating monocytes and altered activity of some tissue macrophages (osteoclasts and Küpffer cells). While circulating CD14<sup>+</sup>CD16<sup>+</sup> monocytes have been shown to be inflammatory [27–34] and probable precursors for tissue infiltration, the 40–50% suppression of these monocytes in the study did not alter macrophage numbers (CD68, CD163) or activation state (HLA-DR) of cells present in CLE lesions. In addition, M-CSF suppression did not alter T cell numbers (CD3) or activation state (HLA-DR, CXCR3) or the overall density or pattern of leucocyte (CD45) infiltration in post-treatment lesional skin biopsy samples. Consistent with these findings in tissue lesions, clinical data failed to demonstrate significant therapeutic activity based on CLASI, SLEDAI-2K or PGA clinical
Fig. 6. Immunohistochemistry results in tissue biopsy samples from completed patients. Representative images of CD68, CD163, human leucocyte antigen D-related (HLA-DR), CD3 and CXCR3 expression in baseline and post-treatment (d84) lesional biopsy samples from a patient receiving 100 mg PD-0360324 (a). Area of positive signal for each marker was determined by quantitative image analysis in epidermis or dermis (b). Counts were then normalized to measured tissue area (epidermis or dermis, respectively) and expressed as percentage area of positive signal. Results are presented for patients receiving placebo (grey bars) or 100 or 150 mg PD-0360324 (black bars).
A limitation of this study was the low sample size. Although the target sample size was 48 patients, enrollment of eligible patients was challenging and the study was terminated after enrollment of 28 patients due to negative interim data.

Inhibition of M-CSF in humans resulted in effects consistent with previous findings from studies in knock-out mice and other animal models [35]. Studies with PD-0360324 in non-human primates [26], in healthy volunteers [36] and in patients with rheumatoid arthritis [37] demonstrated similar dose- and time-dependent suppression in CD14⁺CD16⁺ monocyte subsets, osteoclasts and Kupffer cell activity. Data from this study of patients with CLE confirm this activity and establish the pharmacokinetics, pharmacodynamics and temporal relationship between antibody exposure and downstream effects. These human data show incomplete suppression of circulating monocytes under the conditions in this study, which may have contributed to the lack of efficacy observed in lesional sites. Regarding suppression of local M-CSF activity, there are three homodimeric isoforms of the M-CSF ligand, which have specific spatial and temporal expression resulting in unique functional activities [38]. Although the epitope for PD-0360324 is contained within the N-terminal 146 amino acid region of M-CSF, which is conserved in all isoforms (data not shown), we cannot rule out the potential for differences in tissue penetration or pharmacokinetic requirements for suppression of each isoform in vivo.

Although alterations in monocytes/macrophage functions have been proposed to contribute to the pathogenesis of SLE [39], their potential role in driving CLE is less clear. Previous research has shown that sunlight triggers CLE via a CSF-dependent and macrophage-mediated mechanism in mice [40]. The current study (data not shown) and others [41,42] have found that macrophage numbers are elevated in CLE lesions relative to non-lesional skin, but their functional role, if any, in these inflamed sites has not been demonstrated. In this study, macrophage numbers and activation state were not observably altered within skin lesions of patients administered anti-M-CSF, despite 40–50% suppression of CD14⁺CD16⁺ circulating monocytes. Several possibilities may explain this apparent discrepancy. CD14⁺CD16⁺ monocytes are considered to act as proinflammatory monocytes that infiltrate into inflamed tissue; however, the level and/or duration of suppression achieved in this study may have been insufficient to affect the inflammatory environment already established within lesions. Alternatively, the macrophage populations present within the CLE lesions may not derive wholly from CD14⁺CD16⁺ cells in circulation and/or may survive and develop

| Doses, n, median (range) | Placebo (n = 6) | PD-0360324 100 mg (n = 12) | PD-0360324 150 mg (n = 10) |
|-------------------------|----------------|-----------------------------|-----------------------------|
| Frequency of AEs        |                |                             |                             |
| No. of AEs              | 7              | 50                          | 60                          |
| Patients with AEs, n (%)| 3 (50)         | 11 (92)                     | 9 (90)                      |
| Patients with serious AEs, n (%) | 1 (17) | 0                           | 0                           |
| Patients with severe AEs, n (%) | 0            | 2 (17)                      | 2 (20)                      |
| Patients discontinued due to AE, n (%) | 1 (17) | 5 (42)                     | 4 (40)                      |
| Patients with dose reduction or temporary discontinuation due to AE, n (%) | 0 | 0                          | 0                           |

AEs by MEDRA system organ class

| Blood or lymphatic system disorders | 0 | 2 (17) | 0 |
| Ear and labyrinth disorders | 0 | 0 | 1 (10) |
| Eye disorders | 0 | 3 (2) | 5 (5) |
| Gastrointestinal disorders | 1 (17) | 3 (25) | 4 (40) |
| General disorders and administration site conditions | 0 | 5 (42) | 6 (60) |
| Infections and infestations | 1 (17) | 2 (17) | 3 (30) |
| Injury, poisoning and procedural complications | 0 | 0 | 1 (10) |
| Investigations (laboratory) | 1 (17) | 3 (25) | 3 (30) |
| Musculoskeletal and connective tissue disorders | 0 | 4 (33) | 3 (30) |
| Nervous system disorders | 1 (17) | 2 (17) | 3 (30) |
| Psychiatric disorders | 0 | 0 | 1 (10) |
| Renal and urinary disorders | 0 | 2 (17) | 0 |
| Respiratory, thoracic and mediastinal disorders | 0 | 1 (8) | 3 (30) |
| Skin and subcutaneous tissue disorders | 0 | 5 (42) | 4 (40) |
| Vascular disorders | 0 | 2 (17) | 1 (10) |

AE = adverse event; MEDRA = Medical Dictionary for Regulatory Activities.

Table 2. Summary of exposure and treatment-emergent AEs (all causalities)
Activity/numbers in the liver [26]. Similar elevations in serum, which may be attributed to reduced K effects were demonstrated by suppression of the osteoclast patients, the anti-M-CSF agent did alter the function of numbers or activation state at lesional skin sites in treated phage populations in non-inflamed skin could not be the anti-M-CSF monoclonal antibody on resident macro-
treatment non-lesional samples in this study, the effects of 
tially sensitive to M-CSF blockade. Due to lack of post-
inflammatory macrophage populations may be differen-
tially' sensitive to M-CSF blockade. Due to lack of post-
treatment non-lesional samples in this study, the effects of 
the anti-M-CSF monoclonal antibody on resident macro-
phage populations in non-inflamed skin could not be evaluated.

Despite having no observable effect on macrophage numbers or activation state at lesional skin sites in treated patients, the anti-M-CSF agent did alter the function of other monocyte-lineage cells in some tissues. The latter effects were demonstrated by suppression of the osteoclast activity marker uNTX and elevation of AST, ALT and CK in serum, which may be attributed to reduced K"upffer cell activity/numbers in the liver [26]. Similar elevations in serum kinases have been reported in the CSF1−/−/CSF1−/− mouse, which lacks functional M-CSF-1 receptors, and in monkeys treated with PD-0360324 [26], indicating that PD-0360324 can deplete resident macrophages in some tissues. Thus, resident and inflammatory macrophage populations may be differentially sensitive to M-CSF blockade. Due to lack of post-treatment non-lesional samples in this study, the effects of the anti-M-CSF monoclonal antibody on resident macrophage populations in non-inflamed skin could not be evaluated.

In addition to macrophages, anti-MCSF monoclonal antibody (mAb) administration also did not appear to affect numbers or activation state of T cells within the CLE skin samples collected in this study. Strong evidence indicates that type I IFN produced by infiltrating pDCs drive T cell recruitment and cytokine production in CLE lesions [18,41]. Consistent with previous reports [41,42,44], inflammatory infiltrates in baseline CLE lesions in this study contained infiltrating CD3+ T cells, including CXCR3+ cells, which did not appear to diminish with anti-
M-CSF treatment. The latter finding suggests that the type I IFN-driven inflammation loop was not affected. Given the proposed role of T cells, and CXCR3+ T cells in particular, in the local pathogenesis of CLE, the lack of clinical improvement in the skin lesions of these patients as measured by CLASI, SLEDAI-2K scores and PGA is not surprising. Together, these data indicate that the 40–50% suppression of CD14+CD16+ monocytes in circulation is not sufficient to alter macrophage populations in inflamed cutaneous sites in CLE over the time-frame examined in this study.

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Disclosure

All authors except L. A. C. are employees of Pfizer and owners of Pfizer stock. L. A. C. is an employee of Inventiv Health, contracted and paid by Pfizer to provide clinical support for the study and the development of this manuscript.

Author contributions

All authors contributed to the drafting and/or critical review/revision of the paper for important intellectual content; all authors also approved the final draft for submission and publication. Authors had full access to the data and take responsibility for data integrity and analysis accuracy. For study conception and design: K. M.-H., S. M., J. S., Y. Z., S. P. O. and D.B.; for data acquisition: K. M.-H., A. A., M. A., R. P. Q., J. B. C., J. S., Y. Z., S. P.-W., L. A. C. and D. B; for data analysis and interpretation: K. M.-H., A. A., S. M., E. P., R. P. Q., J. B. C., Y. Z., S. P. O., S. P.-W. and D. B.; and for manuscript preparation: K. M.-H., E. P., A. A., S. M., M. A., R. P. Q., J. B. C., J. S., Y. Z., S. P. O., S. P.-W., L. A. C. and D. B.

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