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RESEARCH ARTICLE

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SLN124, a GalNAc conjugated 19-mer siRNA targeting *tmprss6*, reduces plasma iron and increases hepcidin levels of healthy volunteers

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Abstract

SLN124, an N-acetylgalactosamine conjugated 19-mer short interfering RNA, is being developed to treat iron-loading anemias (including beta-thalassemia and myelodysplastic syndromes) and myeloproliferative neoplasms (polycythemia vera). Through hepatic targeting and silencing of the *TMPRSS6* gene, SLN124 increases endogenous hepcidin synthesis. This is the first clinical report of an siRNA targeting a component of iron homeostasis. This first-in-human, phase 1 study assessed the safety, tolerability, pharmacokinetics, and pharmacodynamics of single ascending doses of SLN124 (1.0, 3.0, and 4.5 mg/kg) in healthy volunteers. Twenty-four participants were randomized in three sequential cohorts of eight subjects, each to receive a single dose of either SLN124 or placebo (6:2 randomization), administered subcutaneously. There were no serious or severe adverse events, or discontinuations due to adverse events, and most treatment-emergent adverse events were mild, including transient mild injection site reactions, resolving without intervention. SLN124 was rapidly absorbed, with a median t_{max} of 4–5 h across all treatment groups, and largely eliminated from plasma by 48 h. Plasma concentrations increased in a greater than dose proportional fashion between treatment groups. In all SLN124 groups, a dose-related effect was observed across iron metabolism markers, and across erythroid markers, SLN124 resulted in increased plasma hepcidin levels, peaking around Day 29, and consequent dose-related sustained reductions in plasma iron and transferrin saturation with decreased reticulocyte production, MCHC, and MCV. Results suggest duration of action lasting up to 56 days after a single SLN124 dose, on hepcidin and hematological parameters of iron metabolism (serum iron and TSAT).

1 | INTRODUCTION

Iron is essential for multiple cellular processes,[1] including oxygen transport, nucleotide synthesis, mitochondrial respiration, and host defense.[2] The hormone hepcidin, synthesized predominantly in hepatocytes, is the central regulator of iron absorption and of distribution to tissues.[3] Dysregulation of hepcidin production leads to a variety of iron metabolism disorders[3,4] that are major sources of morbidity and mortality, including iron overload in hereditary hemochromatosis and iron-loading anemias.[2] Congenital or acquired iron-

loading anemias, such as alpha and beta-thalassemia and myelodysplastic syndromes (MDS), are characterized by ineffective erythropoiesis leading to decreased red blood cell (RBC) production, hepcidin suppression, excessive iron absorption and secondary iron overload.[2] Other acquired myeloproliferative diseases, such as polycythemia vera, are characterized by mutations in the JAK2 gene making red blood cell production erythropoietin independent resulting in high hematocrit, increased thrombotic risk and iron deficiency.[5]

Noncurative treatments for these iron-loading anemias have been mainly limited to RBC transfusions and iron chelators.[2] However, RBC transfusions exacerbate iron loading and lead to end-organ damage. Chelating agents can be inefficient at preventing or treating established iron overload-related complications[6] and may be associated with toxicity and tolerability issues.[2,7] Furthermore, chelation typically requires daily administration, which often leads to low adherence to treatment.

Novel drugs that target core pathophysiological mechanisms in thalassemia with greater efficacy and a better side-effect profile are needed to improve anemia. One approach is luspatercept, a recombinant fusion protein that traps transforming growth factor-β superfamily ligands.[8] Luspatercept reduces transfusion requirement in some transfusion-dependent thalassemias and increases hemoglobin (Hb) by at least 1 g/dL in over 70% of nontransfusion-dependent thalassemias.[9] Luspatercept has recently been approved for treating anemia resulting from MDS and transfusion dependent beta-thalassemias.

Another approach, which is effective in murine models of thalassemia,[9] is to improve ineffective erythropoiesis by restricting transferrin mediated iron delivery to the erythron by raising plasma hepcidin or inhibiting the hepcidin target ferroportin Potential agents include LJP-401 (subcutaneous injection of hepcidin mimetic),[10] rusfertide (PTG-300, subcutaneous injection of hepcidin mimetic),[11] vamifeport (VIT-2763, oral ferroportin antagonist),[12] IONIS-TMPRSS6-LRX (subcutaneous injection of antisense oligonucleotide targeting TMPRSS6),[13] and SLN124 (subcutaneous injection).[6,17] LJP-401,[10] rusfertide,[11] vamifeport (VIT-2763)[12] and IONIS-TMPRSS6[13] have all been shown to be well tolerated in healthy volunteer studies and to decrease serum iron parameters. Of these, rusfertide[14] and LJP-401,[15] were unsuccessfully used in clinical trials to treat thalassemia patients and led to their discontinuation in thalassemia patients. However, more recently, Rusfertide has shown utility in managing hematocrit and iron deficiency in polycythemia vera patients.[16]

SLN124, an N-acetylgalactosamine (GalNAc) conjugated 19-mer short interfering RNA (siRNA), is the first siRNA molecule being developed to treat a range of rare iron-loading anemias (including thalassemia and MDS) and rare myeloproliferative diseases (polycythemia vera). SiRNAs are known to have a long duration of action and potentially better safety profiles than other pharmaceutical modalities (for more information on mechanism see reference 17). SLN124 is optimized for the hepatic targeting and silencing of murine and human *TMPRSS6*, the gene that encodes matriptase 2, a membrane protease that attenuates the expression of hepcidin in the liver. By targeting TMPRSS6, SLN124 increases hepatic hepcidin synthesis and hence raises plasma hepcidin levels as demonstrated in mouse models for

and β-thalassemia[6,18–20] and hereditary hemochromatosis.[19,21] This is unlike previous efforts using lipid nanoparticles to target TMPRSS6 which although lacking the liver specificity still demonstrated the utility of siRNA targeting.[19,20] SLN124 lowered serum iron levels for at least 6 weeks after single administration in mice. It also normalized systemic iron levels and erythropoiesis in mouse models of hematochromatosis,[18] and improved RBC maturation and increased hemoglobin in mouse models of beta-thalassemia.[6]

The primary objective of this first-in-human (FIH) study was to evaluate the safety and tolerability of single ascending SC doses of SLN124 in healthy subjects. The secondary objective was to determine the pharmacokinetic (PK) parameters of SLN124. There were also two exploratory objectives: to assess the pharmacodynamic (PD) effect of SLN124 on biomarkers of iron metabolism and erythroid expansion.

2 | METHODS

2.1 | Study design

This was a FIH, randomized, double-blind, placebo-controlled, single ascending-dose study of SLN124 in 24 healthy adult volunteers. It was conducted between August 2020 and April 2021 by Hammersmith Medicines Research (HMR), London, in compliance with European Union (EU) Directives 2001/20/EC[22] and 2005/28/EC,[23] The Medicines for Human Use (Clinical Trials) Regulations 2004[24] and current amendments, the Declaration of Helsinki, Good Manufacturing Practice (GMP),[25] the SOPs issued by the Research Ethics Service for Research Ethics Committees in the UK,[26] and Good Clinical Practice (GCP). All participants gave written informed consent.

Subjects were randomized into three cohorts of eight subjects (six active; two placebo), to receive a single SC dose of SLN124 or placebo administered into their abdomen. SLN124 was presented as a solution for injection for SC use (100 mg/mL, presented as 0.5 mL extractable volume per vial). Placebo was sodium chloride 0.9% (weight/volume) solution for injection. As this was a FIH study, sentinel dosing was used, whereby the first two subjects in each cohort were randomized to receive either SLN124 or placebo on Day 1. Dosing of the remaining subjects at the same dose level was at least 23 h later, following a safety evaluation of the sentinel subjects by a Safety Review Group (SRG).

The three cohorts received SLN124 1.0, 3.0, and 4.5 mg/kg, respectively. Escalation to the next dose level was only after the SRG had deemed acceptable the safety, tolerability and PK data of preceding dose level(s). The maximum dose to be tested, which was based on the no observable adverse effect level (NOAEL) in mice, was 6.0 mg/kg.

2.2 | SLN124

SLN124 comprised a blunt ended double stranded oligonucleotide designed to bind to both mouse and human TMPRRS6 transcripts,

including all TMPRSS6 known isoforms. SLN124 drug substance was produced by chemical synthesis using standard solid phase technology for oligonucleotides. The antisense strand and the sense strand, which was conjugated to the GalNAc unit at the 5′end, were individually synthesized and purified by chromatographic and ultrafiltration steps. After hybridization of the single strands, the final double-stranded molecule was obtained as lyophilized powder. The manufacture was performed under GMP. The release and stability testing included appropriate tests for this kind of molecule and stage of development.

SLN124 drug product was manufactured by dissolving the drug substance in water for injection, followed by adjustment of required concentration, pH and osmolality, sterile filtration and finally aseptic filling into standard glass vials. The drug product manufacture was performed under GMP. The release and stability testing included appropriate tests for this kind of drug product and stage of development.

2.3 | Subject eligibility

Men or women aged 18–55 years with a body mass index (BMI) of 18–30 kg/m^2 were deemed healthy based on clinical history, physical examination, 12-lead electrocardiogram (ECG), vital signs, and laboratory tests of blood and urine. Subjects with serum ferritin outside the ranges 23.9–336.2 μg/L (men) or 11.0–306.8 μg/L (women), or TSAT < 20% (women) or <25% (men) were considered at risk of iron deficiency and excluded.

Eligible subjects were admitted to the ward on the day before dosing (Day −1) for a PCR test for COVID-19 and transferred to a separate ward only if it was negative. They remained on the ward until 48 h after dosing (Day 3), returning only for outpatient visits on Days 8 and 29 and a final follow-up visit on Day 57 (±3 days). They agreed to follow the MHRA requirements for contraception and gave fully informed consent.

2.4 | Endpoints

2.4.1 | Safety and tolerability

Safety and tolerability were assessed by vital signs, 12-lead ECG, physical examination, laboratory tests of blood (described in Supporting Information) and adverse events (AEs), including injection site reactions (ISR).

2.4.2 | Pharmacokinetics

SLN124 was detected using an anion exchange (AEX)-HPLC method with fluorescence detection that allows the specific detection of the analyte SLN124 from human plasma. The assay is based on the specific hybridization of the antisense strand of the analyte SLN124 to the complementary peptide-nucleic-acid (PNA)-probe. The PNA-probe is conjugated at both termini with Atto425-fluorescent dyes.

Blood for measurement of SLN124 was taken before and at 15 min, 0.5, 1, 2, 4, 6, 12, 24 (Day 2), 48 (Day 3), and 168 h (Day 8) after dosing for the following PK parameters: maximum observed plasma concentration (C_{max}), relative time of maximum observed plasma concentration (t_{max}), elimination half-life ($t_{\frac{1}{2}}$), elimination rate constant (λ_z), area under the concentration–time curve from dosing until time t, the last measured timepoint, and extrapolated to infinity (AUC$_{0–t}$, AUC$_{0–last}$ and AUC$_{0–\infty}$), and total clearance and apparent volume of distribution divided by the fraction absorbed (CL/F and V$_Z$/F) of SLN124.

2.4.3 | Pharmacodynamics

Blood was taken before and up to the last visit after dosing for the following PD biomarkers: iron metabolism (ferritin, plasma hepcidin, serum iron, total iron-binding capacity [TIBC], and TSAT) and biomarkers of erythroid expansion (hemoglobin, hematocrit, mean corpuscular volume [MCV], mean corpuscular hemoglobin concentration [MCHC], and reticulocyte count [absolute and percentage]). The assay to measure hepcidin-25 was WCX-TOF-MS as described by Kroot et al.[27]

2.5 | Statistical methods

A sample size of 24 was considered sufficient to evaluate the safety, tolerability and PK characteristics of SLN124 in a FIH study in healthy volunteers. Safety, tolerability, PK and PD data were analyzed by descriptive statistics. HMR conducted the analyses of safety, PK and PD data, using SAS version 9.4 and derived PK parameters using WinNonlin v8.3.

3 | RESULTS

3.1 | Subject characteristics

Twenty-four subjects (17 men and seven women) were enrolled and randomized into three cohorts of eight. A total of 18 subjects received SLN124 and six subjects received placebo. All subjects were included in the various analyses.

Demographic characteristics are shown in Table 1. Mean age was 31.1 years (range: 20–55 years), mean weight 72.8 kg (53.8–93.4 kg), and mean BMI 24.1 kg/m^2 (19.2–29.9 kg/m^2). Demographic characteristics were similar across the cohorts. All subjects who received SLN124 1.0 mg/kg were men.

3.2 | Safety and tolerability

SLN124 was well tolerated at all doses. There were no deaths, no nonfatal serious AEs (SAEs), and no other significant AEs or

TABLE 1 Summary of subject demographic details.

Variable		Placebo (N = 6)	1.0 mg/kg SLN124 (N = 6)	3.0 mg/kg SLN124 (N = 6)	4.5 mg/kg SLN124 (N = 6)	All subjects (N = 6)
Age (years)	Mean (SD)	31.5 (6.0)	32.5 (11.5)	27.5 (5.4)	33.8 (7.7)	31.2 (7.8)
	Range	23–38	22–55	20–33	24–44	20–55
Race n (%)	Asian	1 (16.7)	1 (16.7)	1 (16.7)	0	3 (12.5)
	Black or African American	1 (6.7)	0	0	1 (16.7)	2 (8.3)
	White	4 (66.7)	5 (83.3)	5 (83.3)	5 (83.3)	19 (79.2)
Ethnicity n (%)	Not Hispanic or Latino	6 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)	24 (100.0)
Gender n (%)	Female	3 (50.0)	0	2 (33.3)	2 (33.3)	7 (29.2)
	Male	3 (50.0)	6 (100.0)	4 (66.7)	4 (66.7)	17 (70.8)
Height (cm)	Mean (SD)	172.7 (3.9)	176.8 (5.4)	173.8 (7.9)	171.7 (13.4)	173.8 (8.1)
	Range	167–177	169–181	162–184	154–188	154–188
Weight (kg)	Mean (SD)	72.43 (7.2)	75.35 (10.7)	70.07 (8.4)	73.22 (15.4)	72.77 (10.3)
	Range	65.5–80.4	57.1–84.5	62.0–82.8	53.8–93.4	53.8–93.4
BMI (kg/m^2)	Mean (SD)	24.30 (2.1)	24.08 (3.2)	23.18 (2.2)	24.78 (3.7)	24.09 (2.8)
	Range	21.3–26.9	19.7–28.3	20.5–26.7	19.2–29.3	19.2–29.3
Cigarettes[a] (daily)	n	0	1	3	1	5
	Mean (SD)	-	-	4.0 (0.0)	-	3.6 (1.5)
	Range	-	5	4	1	1–5
Alcohol[a] 9 units/week)	n	4	5	4	3	16
	Mean (SD)	2.5 (1.3)	6.4 (4.2)	6.3 (3.3)	4.0 (2.0)	4.9 (3.3)
	Range	1–4	2–12	2–10	2–6	1–12

Abbreviations: BMI, body mass index; N, total number of subjects; n, number of applicable subjects.

[a]Includes only those subjects who smoke or drink alcohol.

treatment-emergent AEs (TEAEs) leading to subject withdrawal (Table 2). Of subjects who received SLN124, 61.1% (11/18) had at least one TEAE and 50.0% (9/18) had at least one potentially drug-related TEAE throughout the study, compared with 33.3% (2/6) and 16.7% (1/6), respectively, of subjects who received placebo. Overall, 34 TEAEs were reported, all of which were either mild (26 in total; 24 after SLN124 and 2 after placebo) or moderate (8 in total; 7 after SLN124 and 1 after placebo) in severity.

There was no clear relationship between SLN124 dose level and TEAE incidence. 50.0% of subjects who received SLN124 1.0 mg/kg had TEAEs (11 TEAEs in total), compared with 83.3% after 3.0 mg/kg (15 TEAEs) and 50.0% after 4.5 mg/kg (5 TEAEs). Potentially drug-related TEAEs were recorded in 33.3% (2/6) of subjects after 1.0 mg/kg (2 TEAEs), 83.3% (5/6) after 3.0 mg/kg (8 TEAEs), and 33.3% (2/6) after 4.5 mg/kg (4 TEAEs).

The most common TEAE was injection-site pain, which was reported by 38.9% (7/18) subjects across all SLN124 treatment groups. All were deemed study drug-related, mild in severity and transient in nature, and all resolved without medical intervention. There was no clear relationship with SLN124 dose. ALT increased (<3 × upper limit of normal) in one subject after SLN124 1.0 mg/kg and two subjects after SLN124 3.0 mg/kg, but none in the SLN124 4.5 mg/kg group. Thus, there was no evidence of a relationship with

SLN124 dose. The ALT increase in the subject receiving 1.0 mg/kg first increased on day 7 and lasted 18 days. The two subjects receiving SLN124 at 3.0 mg/kg who had ALT increases were first observed on day 32 for one case and day 34 for the other after dosing and lasted for 4.25 and 35 days respectively. All ALT increases resolved spontaneously. There were no other clinically significant findings.

3.3 | Pharmacokinetics

SLN124 was rapidly distributed from the SC injection site to the plasma. Absorption and elimination were largely independent of dose level, with a t_{max} of 2–6 h (median 4–5 h) across all dose groups. The arithmetic mean $t_{1/2}$ was approximately 4.0 h across all dosing groups (Table S1). SLN124 levels decreased rapidly from C_{max} in all groups, and was largely eliminated from plasma within 48 h after dosing (Figure 1 as seen in both linear and semilogarithmic plots), although remaining above the lower limit of quantification (LLQ) at 168 h after SLN124 3.0 and 4.5 mg/kg.

Plasma concentrations increased with SLN124 dose in a greater than dose-linear fashion: geometric mean C_{max} was 263, 995, and 1813 ng/mL, and geometric mean $AUC_{0-\infty}$ was 3230, 10 450, and 22 828 h·ng/mL, after SLN124 1.0, 3.0, and 4.5 mg/kg, respectively (Table S1).

TABLE 2 Summary of AEs and TEAEs.

	Placebo (N = 6) n (%) [number of TEAEs]	1.0 mg/kg SLN124 (N = 6)	3.0 mg/kg SLN124 (N = 6)	4.5 mg/kg SLN124 (N = 6)	Total active (N = 18)
Subjects with					
TEAEs	2 (33.3) [3]	3 (50.0) [15]	5 (83.3) [15]	3 (50.0) [5]	11 (61.1) [31]
Serious TEAEs	0	0	0	0	0
Drug-related TEAEs	1 (16.7) [1]	2 (33.3) [2]	5 (83.3) [8]	2 (33.3) [4]	9 (50.0) [14]
TEAEs leading to subject withdrawal	0	0	0	0	0
Mild TEAEs	1 (16.7) [2]	3 (50.0) [8]	5 (83.3) [12]	2 (33.3) [4]	10 (55.6) [24]
Moderate TEAEs	1 (16.7) [1]	3 (50.0) [3]	2 (33.3) [3]	1 (16.7) [1]	6 (33.3) [7]
Severe TEAEs	0	0	0	0	0
TEAEs by preferred term					
Injection site pain	-	2 (33.3) [2]	3 (50.0) [3]	2 (33.3) [3]	7 (38.9) [8]
Injection site erythema	-	-	2 (33.3) [2]	1 (16.7) [1]	3 (16.7) [3]
Catheter site pain	-	-	1 (16.7) [1]	-	1 (5.6) [1]
Headache	-	2 (33.3) [2]	1 (16.7) [1]	1 (16.6) [1]	4 (22.2) [4]
Dizziness	1 (16.7) [1]	1 (16.7) [1]	-	-	1 (5.6) [1]
Lethargy	-	1 (16.7) [1]	-	-	1 (5.6) [1]
Paraesthesia	-	-	1 (16.7) [1]	-	1 (5.6) [1]
Diarrhea	-	1 (16.7) [1]	1 (16.7) [1]	-	2 (11.1) [2]
Nausea	-	-	1 (16.7) [1]	-	1 (5.6) [1]
Alanine aminotransferase increased	-	1 (16.7) [1]	2 (33.3) [2]	-	3 (16.7) [3]
Epistaxis	-	1 (16.7) [2]	-	-	1 (5.6) [1]
Cough	1 (16.7) [1]	-	-	-	-
Wound infection	-	1 (16.7) [1]	-	-	1 (5.6) [1]
Procedural pain	-	-	1 (16.7) [1]	-	1 (5.6) [1]
Rash	-	-	1 (16.7) [1]	-	1 (5.6) [1]
Orthostatic hypotension	-	-	1 (16.7) [1]	-	1 (5.6) [1]
Dysmenorrhoea	1 (16.7) [1]	-	-	-	-

Note: Subjects with >TEAE are counted only once per SOC and preferred term.

Abbreviations: N, total number of subjects; n: number of subjects with a TEAE; SOC, system organ class; TEAE, treatment-emergent adverse event.

3.4 | Pharmacodynamics

3.4.1 | Iron metabolism

SLN124 had a dose-dependent effect on plasma hepcidin, serum iron, TSAT and ferritin. Compared with baseline, mean plasma hepcidin peaked at Day 8 after SLN124 1.0 mg/kg (+4.4 nM [+227%]) and 3.0 mg/kg (+5.8 nM [+379%]), and at Day 29 after 4.5 mg/kg (+7.8 nM [+536%]), whereas, after placebo, it changed little relative to baseline (Figure 2). On Day 57 (follow-up visit), mean hepcidin levels were still >150% above baseline in all SLN124 dose groups.

Consistent with the higher hepcidin levels, after SLN124 mean serum iron and TSAT were lower (Figures 3 and 4). Maximum reduction was at Day 29 for all SLN124 dose groups; the % change from baseline was: 43–48 for iron and 48–53 for TSAT (Figures 3 and 4). In contrast, mean iron and TSAT on Day 29 after placebo had changed little relative to baseline. In absolute terms, on Day 29, serum iron

levels were 6.8–10.1 μM after SLN124, compared with 20.0 μM after placebo; and TSAT levels were 10.3%–15.9%, compared with 32.8% after placebo. Iron and TSAT levels were still lower by 16%–34% relative to baseline at Day 57 across the SLN124 dose groups (Figures 3 and 4). In the 3.0 and 4.5 mg/kg dose groups, mean TSAT levels at Day 57 remained <17% lower, close to the limit that supports normal erythropoiesis (16%)[28] (Figure 4).

Compared with baseline, mean serum ferritin was higher by 17–32 μg/L from Day 29 across the SLN124 dose groups, in contrast to 15–18 μg/L lower in the placebo group (Table 3).

3.4.2 | Erythroid markers

There was a small, dose-related effect of SLN124 on mean levels of the erythroid markers hemoglobin, hematocrit, MCHC and MCV on Days 29 and 57 compared with placebo. On Day 57, hemoglobin
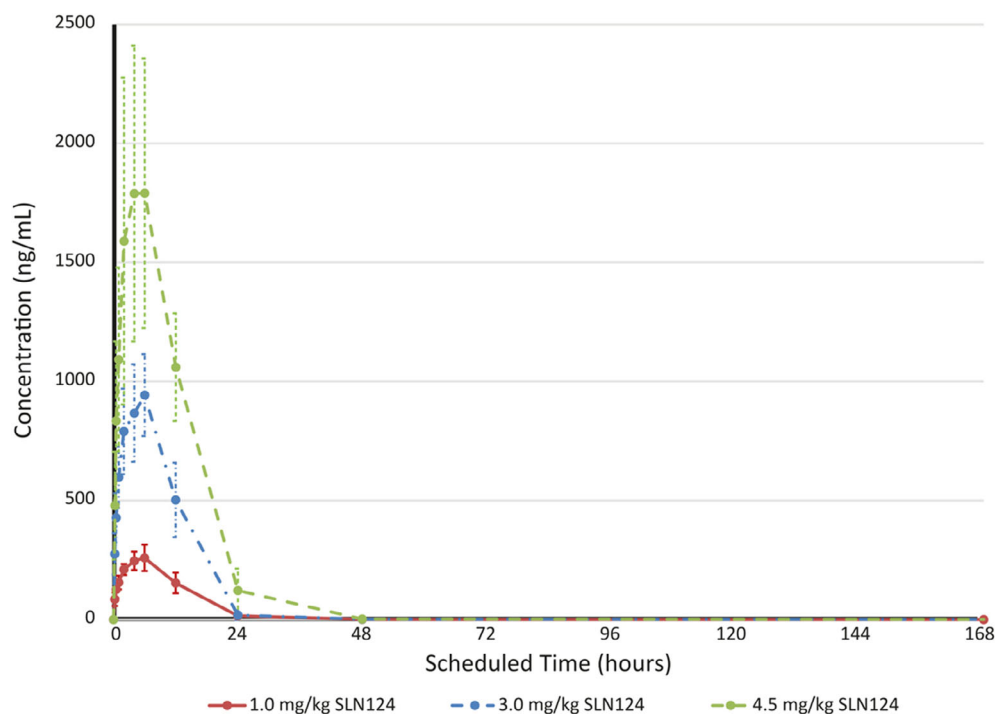
FIGURE 1 Mean plasma concentration-time plots (linear and semilogarithmic) of SLN124 up to 168 h after single 1–4.5 mg/kg subcutaneous doses in healthy subjects: PK concentration population; $N = 6$ per group; error bars represent 95% confidence intervals. [Color figure can be viewed at wileyonlinelibrary.com]
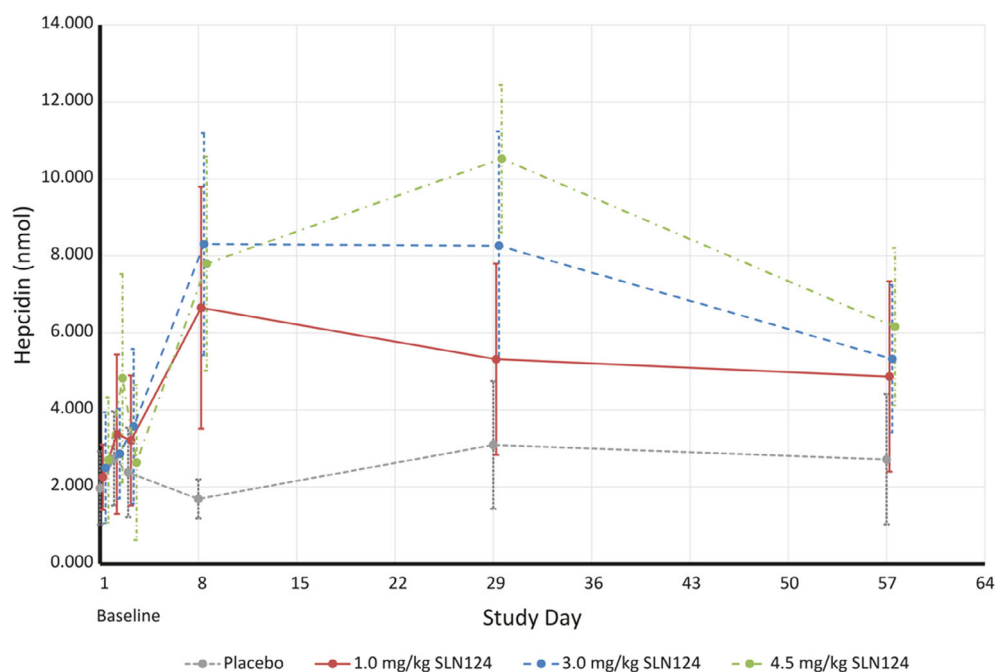
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FIGURE 2 Mean hepcidin level (nmol): PD population; $N = 6$ per group; error bars represent 95% confidence intervals. [Color figure can be viewed at wileyonlinelibrary.com]

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levels were lower relative to baseline by 3.5 g/L (2.2%), 4.3 g/L (3.3%), and 10.8 g/L (7.5%) after SLN124 1.0, 3.0, and 4.5 mg/kg, respectively, compared with a higher level of 7.5 g/L (5.6%) after placebo. Similarly, hematocrit levels on Day 57 were lower relative to baseline by 0.008 L/L (1.6%), 0.010 L/L (2.4%), and 0.020 L/L (4.8%) after 1.0, 3.0, and 4.5 mg/kg SLN124, respectively, compared with a higher level of 0.020 (4.9%) after placebo. Also, MCHC was lower at

Day 57 relative to baseline (−2.7 g/L [−0.8%], −3.2 g/L [−0.9%], and −10.5 g/L [−3.5%]; Table 4) and MCV (−1.7 fL [−1.8%], −2.3 fL [−2.7%], and − 2.3 fL [−2.6%]) after 1.0, 3.0, and 4.5 mg/kg SLP124, respectively, compared with changes after placebo of +2.2 g/L (+0.6%) for MCHC and −0.2 fL (−0.2%) for MCV (Table S2).

SLN124 3.0 mg/kg was associated with a lower mean absolute reticulocyte count at Days 8 and 29 (−18.1% and −14.0% relative to

FIGURE 3 Mean serum iron concentration (μmol): PD population; $N = 6$ per group; SD error bars represent 95% confidence intervals. [Color figure can be viewed at wileyonlinelibrary.com]

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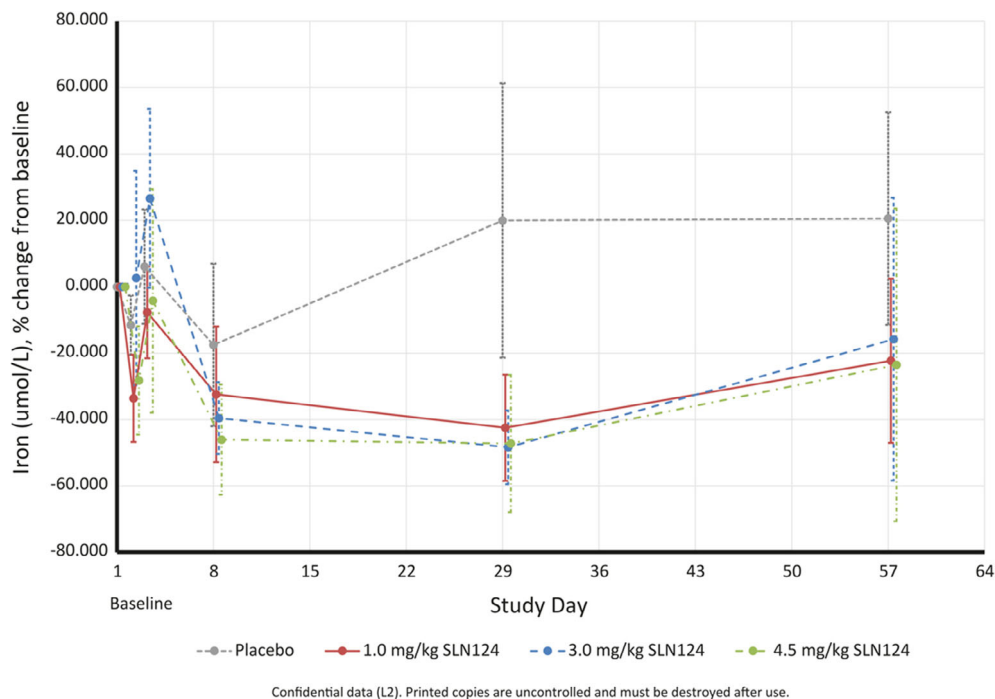
FIGURE 4 Mean TSAT (%): PD population; $N = 6$ per group; error bars represent 95% confidence intervals. [Color figure can be viewed at wileyonlinelibrary.com]

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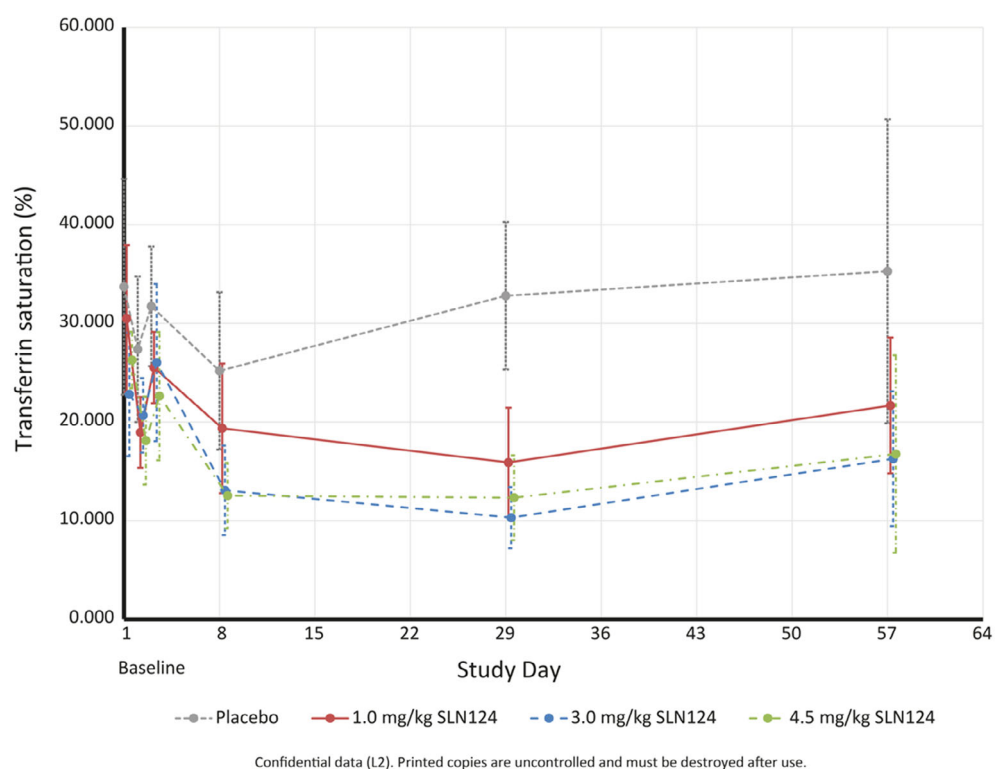
baseline, respectively, compared with +16.6% and −5.4% in the placebo group). Likewise, SLN124 4.5 mg/kg was associated with a lower mean absolute reticulocyte count at Day 29 (−29.0% relative to baseline compared with −5.4% in the placebo group) (Table 5). By Day 57, absolute reticulocyte count had largely returned to pre-dose values. Similar mean changes relative to baseline were observed for reticulocytes when expressed as a percentage of erythrocytes (Table S3).

4 | DISCUSSION

Single SC doses of SLN124 1.0, 3.0, and 4.5 mg/kg had an acceptable tolerability and safety profile. All subjects completed the study. There were no SAEs, and all TEAEs were mild or moderate in severity. Injection site pain was the most common TEAE; all were mild and resolved without medical intervention; and there was no clear relationship between incidence and SLN124 dose. TEAEs of injection site pain and

TABLE 3 Summary of ferritin levels.

Timepoint	Placebo (N = 6)	1.0 mg/kg SLN124 (N = 6)	3.0 mg/kg SLN124 (N = 6)	4.5 mg/kg SLN124 (N = 6)
Ferritin, pg/L; absolute mean (SD)				
Pre-dose (Day 1)	65.63 (54.9)	86.63 (43.0)	50.87 (33.1)	87.08 (90.7)
Day 2	69.48 (55.5)	101.70 (58.9)	58.10 (39.1)	116.7 (100.8)
Day 3	67.85 (51.8)	95.32 (56.8)	54.02 (34.6)	100.4 (94.6)
Day 8	56.98 (45.5)	106.40 (67.2)	49.08 (36.0)	92.77 (70.0)
Day 29	50.60 (27.8)	112.52 (67.3)	72.85 (35.5)	114.3 (56.5)
Follow-up (Day 57)	47.65 (35.7)	118.30 (78.0)	68.25 (44.1)	107.7 (64.8)
Ferritin, pg/L; mean change from baseline (SD)				
Day 2	3.85 (7.7)	15.07 (19.4)	7.23 (8.6)	29.57 (25.5)
Day 3	2.22 (7.3)	8.68 (17.9)	3.15 (9.5)	13.35 (14.1)
Day 8	−8.65 (11.6)	19.77 (27.6)	−1.78 (14.6)	5.68 (33.0)
Day 29	−15.03 (28.3)	25.88 (29.3)	21.98 (13.1)	27.18 (47.4)
Follow-up (Day 57)	−17.98 (22.2)	31.67 (42.1)	17.38 (18.1)	20.60 (41.4)
Ferritin, pg/L; percentage change from baseline (SD)				
Day 2	12.9 (22.1)	14.8 (11.9)	15.1 (12.2)	59.9 (56.3)
Day 3	13.2 (26.7)	6.5 (12.8)	8.2 (21.6)	29.2 (37.2)
Day 8	−5.4 (22.3)	19.3 (24.5)	3.1 (49.5)	41.4 (52.7)
Day 29	−4.4 (35.8)	29.6 (25.4)	70.9 (80.3)	117.7 (128.3)
Follow-up (Day 57)	−16.1 (29.0)	36.2 (38.2)	52.6 (70.7)	85.6 (92.2)

Abbreviation: *N*, total number of subjects.

TABLE 4 Summary of mean corpuscular hemoglobin concentration.

Timepoint	Placebo (N = 6)	1.0 mg/kg SLN124 (N = 6)	3.0 mg/kg SLN124 (N = 6)	4.5 mg/kg SLN124 (N = 6)
MCHC, g/L; absolute mean (SD)				
Pre-dose (Day 1)	343.7 (7.6)	346.5 (4.5)	341.7 (4.5)	344.2 (9.9)
Day 2	344.0 (6.7)	349.2 (4.2)	344.5 (8.9)	341.3 (5.9)
Day 3	345.0 (7.9)	348.3 (3.4)	342.0 (6.4)	342.5 (9.6)
Day 8	346.7 (8.6)	344.8 (3.1)	344.5 (9.0)	338.7 (7.0)
Day 29	344.8 (7.8)	345.8 (4.0)	339.5 (9.1)	337.5 (7.1)
Follow-up (Day 57)	345.8 (8.0)	343.8 (3.9)	338.5 (5.5)	333.7 (10.7)
MCHC, g/L; mean change from baseline (SD)				
Day 2	0.3 (3.3)	2.7 (3.2)	2.8 (5.6)	−2.8 (6.5)
Day 3	1.3 (2.9)	1.8 (4.0)	0.3 (3.3)	−1.7 (6.9)
Day 8	3.0 (2.7)	−1.7 (3.2)	2.8 (5.2)	−5.5 (5.0)
Day 29	1.2 (4.8)	−0.7 (2.8)	−2.2 (5.1)	−6.7 (4.2)
Follow-up (Day 57)	2.2 (5.0)	−2.7 (3.1)	−3.2 (2.5)	−10.5 (4.6)
MCHC, g/L; percentage change from baseline (SD)				
Day 2	0.1 (1.0)	0.8 (0.9)	0.8 (1.6)	−0.8 (1.9)
Day 3	0.4 (0.9)	0.5 (1.2)	0.1 (1.0)	−0.5 (2.0)
Day 8	0.9 (0.8)	−0.5 (0.9)	0.8 (1.5)	−1.6 (1.4)
Day 29	0.3 (1.4)	−0.2 (0.8)	−0.6 (1.5)	−1.9 (1.2)
Follow-up (Day 57)	0.6 (1.5)	−0.8 (0.9)	−0.9 (0.7)	−3.1 (1.3)

Abbreviation: *N*, total number of subjects.

TABLE 5 Reticulocyte counts.

Timepoint	Placebo (N = 6)	1.0 mg/kg SLN124 (N = 6)	3.0 mg/kg SLN124 (N = 6)	4.5 mg/kg SLN124 (N = 6)
Reticulocytes, 10^9/L; absolute mean (SD)				
Pre-dose (Day 1)	57.0 (25.5)	72.2 (12.1)	68.0 (13.1)	58.0 (20.8)
Day 2	60.5 (32.7)	78.3 (15.7)	74.2 (13.3)	62.7 (17.4)
Day 3	64.3 (34.8)	88.2 (20.0)	71.3 (16.9)	58.2 (16.4)
Day 8	63 8 (18.8)	76.3 (14.8)	56.8 (22.6)	59.1 (10.4)
Day 29	52.8 (24.6)	73.7 (19.2)	58.0 (10.1)	39.8 (14.3)
Follow-up (Day 57)	55.7 (19.4)	74.2 (5.5)	61.3 (9.3)	53.8 (15.6)
Reticulocytes, 10^9/L; mean change from baseline (SD)				
Day 2	3.5 (9.1)	6.2 (11.5)	6.2 (6.5)	4.7 (11.0)
Day 3	7.3 (11.1)	16.0 (16.6)	3.3 (16.3)	0.2 (6.8)
Day 8	6.8 (8.5)	4.2 (10.3)	−11.2 (15.8)	1.2 (15.5)
Day 29	−4.2 (12.4)	1.5 (15.3)	−10.0 (7.9)	−18.2 (14.4)
Follow-up (Day 57)	−1.3 (13.9)	2.0 (10.1)	−6.7 (9.4)	−4.2 (18.8)
Reticulocytes, 10^9/L; percentage change from baseline (SD)				
Day 2	4.3 (13.4)	9.2 (16.3)	9.7 (10.5)	12.5 (20.5)
Day 3	9.8 (17.2)	23.1 (23.9)	6.0 (22.6)	3.4 (13.7)
Day 8	16.6 (15.8)	6.0 (13.9)	−18.1 (26.4)	10.6 (32.4)
Day 29	−5.4 (21.8)	2.4 (19.5)	−14.0 (10.1)	−29.0 (16.7)
Follow-up (Day 57)	1.3 (25.3)	5.1 (18.4)	−8.6 (12.4)	−0.4 (34.9)

Abbreviation: *N*, total number of subjects.

other ISRs were not unexpected: mild, transient ISRs (including erythema, rash and pruritus) have been reported in a minority of recipients in studies involving other hepatocyte-specific oligonucleotide therapeutic products using similar platforms (i.e., GalNAc–siRNA conjugates).[29–31] Although three subjects had elevated ALT after SLN124, there was no evidence of a relationship to dose; no subject in the highest dose group (4.5 mg/kg) had elevated ALT. In all three subjects, ALT levels were <3 × ULN and returned to pre-dose levels without medical intervention. Sporadic, transient, asymptomatic elevations in ALT (≥3 × ULN) have been reported in other studies involving GalNAc–siRNA conjugates.[32,33]

PK in healthy subjects was consistent with modeling of preclinical PK data,[34] and similar to that of other GalNAc-siRNAs.[35–37] At all dose levels tested, SLN124 was rapidly distributed from the SC injection site(s) to the plasma (median t_{max} 4–5 h), and was largely eliminated from plasma within 48 h post-dose (Figure 1). The rapid elimination of SLN124 from plasma was expected: GalNAc-conjugated siRNAs are known to facilitate rapid hepatocyte targeting and uptake following SC administration and any siRNA remaining in plasma is quickly eliminated via the kidneys.[38]

The PD effects of SLN124 were longer-lasting than the PK effects, as expected from animal models of hereditary hemochromatosis[18] and beta-thalassemia.[6] There were dose-related increases in plasma hepcidin by Day 8, which were still evident at the final follow-up visit on Day 57, indicative of robust target engagement and *TMPRSS6* gene

knockdown throughout the period of observation (Figure 2). The increased plasma hepcidin levels coincided with sustained reductions in serum iron and TSAT (Figure 3), consistent with restriction of iron supply to the plasma from red cells catabolized in macrophages. A small but discernible increase in serum ferritin was observed from Day 29, which is also consistent with iron sequestration in macrophages via the same mechanism (Table 3). The reductions in serum iron and TSAT were highest after SLN124 3 mg/kg, suggesting a ceiling effect. TSAT below 16%, in conjunction with serum iron <9 μM, is considered insufficient to support normal erythropoiesis in healthy people.[28] The reduction in TSAT is consistent with the concomitant small decrease in reticulocyte count, MCV, and MCHC. MCV and MCHC decreased up to Day 57, the last time-point, while reticulocyte counts were lowest on Day 29. That is consistent with the short life of reticulocytes, which exist for only 2–3 days before becoming mature red cells with a lifespan of ~120 days. The sustained PD effects in this study support an infrequent dosing regimen for SLN124.

Hepcidin, serum iron and TSAT varied considerably among subjects at early timepoints (Day 1, 2 and 3). Hepcidin is subject to diurnal variation and varies with age and sex.[39] Serum iron[40,41] and TSAT[42] also vary over time within subjects, more so with respect to TSAT than ferritin.[43]

The limitations of the study are the low number of subjects, the short follow-up period, and the parallel group design. A crossover design is less variable than a parallel design, but a crossover study was

not feasible because of the long duration of activity of SLN124 and the need for an additional washout period between doses.

In summary, this is the first report of an siRNA targeting iron homeostasis in healthy volunteers. SLN124 had an acceptable tolerability and safety profile. It increased plasma hepcidin and reduced TSAT, both in a dose-responsive manner and with prolonged duration of action, which supports an infrequent dosing regimen. Two studies are in progress using SLN124, one is in patients with thalassemia (NCT04718844) and the second is in patients with polycythemia vera (NCT05499013). In these studies, it will be of interest to compare the dose response effect seen in healthy volunteers to that in patients with different disease pathologies.

CONFLICT OF INTEREST STATEMENT

JBP, RW, and LJ declare fees from consulting. MUM declares research funding. AS, AM, and GVC are employees of Silence Therapeutics PLC and MA, US are employees of Silence Therapeutics GmbH.

DATA AVAILABILITY STATEMENT

Reasonable requests for clinical data presented in this paper can be made to Silence Therapeutics by a qualified researcher.

PATIENT CONSENT

All subjects gave written consent to participate in this trial. Before giving consent, subjects read the information about the trial, discussed it with the investigator, and were given the opportunity to ask questions. All study-specific materials provided to volunteers were approved by the REC. Each subject was free to withdraw from the trial at any time, without giving a reason.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

All content is original to Silence Therapeutics PLC.

CLINICAL TRIAL REGISTRATION

EudraCT no 2020-002544-23; ClinicalTrials.gov Identifier: NCT04559971.

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Additional supporting information can be found online in the Supporting Information section at the end of this article.