Supplementary Materials

Immunophenotyping methodology

For ORACLE-MS, the T, B, NK assay (also called the basic panel) was a 7 colour flow cytometry assay run on CantoII using the FACSCantoTM software version 6.1. For the flow cytometry samples, selected sites close to the central laboratories were instructed to send the tubes at ambient temperature on the day of blood draw to the central laboratories for analyses to comply with the central laboratories laboratory manuals. The good comparability of data between studies support the quality of the analysis.

The samples were stained with specific ready to use reagents (labelled CD45, CD3, CD4, CD8, CD19 and CD16$^+$56 beads) provided by Becton and Dickinson. Absolute counts and % of CD3$^+$ (from total CD45$^+$ cells) and CD4$^+$, CD8$^+$, CD19$^+$, CD16$^+$56$^+$ cells (from CD3$^+$ lymphocytes) were measured. CD4$^+$ absolute counts and % were also measured for naïve (CD45RA$^+$) & memory (CD45RO$^+$) CD4$^+$ or CD8$^+$ T-cells (from CD45$^+$CD3$^+$ cells).

The extended T-cell panel used additional surface markers (including chemokine receptors) represented in two panels which were validated and specified as Research Use Only. Absolute counts and % were measured for central and effector memory CD4$^+$ T-cell types cells (from CD4$^+$ cells) and naïve and memory CD4$^+$ Treg cells (from CD4$^+$ cells). Cell population ratios were also provided for CD4$^+$/CD8$^+$ cells.

Supplementary Table 1. Lymphocyte surface markers evaluated in the ORACLE-MS study

Cell type	FACS marker staining	Label
Central Memory TH Cells (Chemo R1)	CD3$^+$CD4$^+$CD45RO(DIHI)$^+$CCR7(DIHI)$^+$	CD4$^+$RO$^+$CCR7$^+$ Central memory CD4$^+$ cells
Effector Memory TH Cells (Chemo R1)	CD3$^+$CD4$^+$CD45RO(DIHI)$^+$CCR7$^-$	CD4$^+$RO$^+$CCR7$^-$ Effector memory CD4$^+$ cells
Th-1 Type TH Cells	CD3$^+$CD4$^+$CXCR3$^+$	CD4$^+$CXCR3$^+$ TH1 type CD4$^+$ cells
Nat. Ocurr. Treg	CD3$^+$CD4$^+$CD25(DIHI)$^+$CD127(LO/-)	CD4$^+$CD25$^+$CD127$^-$ Nat. occurring CD4$^+$ TREG
Naïve-Like Nat. Ocurr. Treg	CD3$^+$CD4$^+$CD25(IDIHI)$^+$CD127(LO/-)CD45RA(HI)$^+$	CD4$^+$CD25$^+$CD127$^-$RA(HI)$^+$ Naïve-like Nat. occurring CD4$^+$ TREG
Memory-Like Nat. Ocurr. Treg	CD3$^+$CD4$^+$CD25(DIHI)$^+$CD127(LO/-)CD45RA(LO/-)	CD4$^+$CD25$^+$CD127$^-$RA
T-Helper (Th) cells	CD3$^+$CD4$^+$CD45$^+$CD8$^-$	CD4$^+$
T cytotoxic (TC)/Suppressive	CD3$^+$CD8$^+$CD45$^+$CD4$^-$	CD8$^+$
B-cells	CD19$^+$CD45$^+$CD3$^-$CD(16+56)$^-$	CD19$^+$
NK-cells	CD19$^+$CD45$^+$CD16$^+$56$^+$CD3$^-$	CD16$^+$/56$^+$
Naïve Th cells	CD3$^+$CD4$^+$CD45RA(BRIGHT)$^+$CD45RO$^-$	CD4$^+$/CD45RA$^+$
Memory Th cells	CD3$^+$CD4$^+$CD45RO(DIM+BRIGHT)$^+$CD45RA$^-$	CD4$^+$/CD45RO$^+$
Naïve T-cytotoxic cells	CD3$^+$CD8$^+$CD45RA(BRIGHT)$^+$CD45RO$^-$	CD8$^+$/CD45RA$^+$
Memory T-cytotoxic cells	CD3$^+$CD8$^+$CD45RO(DIM+BRIGHT)$^+$CD45RA$^-$	CD8$^+$/CD45RO$^+$

Shading indicates markers that were also assessed in the CLARITY and CLARITY Extension studies. FACS, fluorescence-activated cell sorting; Nat. Ocurr. Treg, naturally occurring T-regulatory lymphocytes

Supplementary Table 2 A and B

International reference ranges as reported by central laboratories: Lymphocyte subpopulations

A CLARITY and CLARITY Extension

Lymphocyte subpopulation	CLARITY		CLARITY Extension	
	Low	High	Low	High
NK cells CD16/56+ % (cells/µL)	5 (90)	27 (590)	5 (90)	27 (590)
			6 (65)*	20 (730)*
B cells CD19+ % (cells/µL)	3 (0)	24.2 (539)	3 (0)	24.2 (539)
			8 (190)*	19 (380)*
T cells CD3+ % (cells/µL)	58.9 (379)	89.3 (2287)	-	-
			58 (700)*	76 (1900)*
T helper cells CD4+ % (cells/µL)	31.9 (210)	65.9 (1546)	31.9 (210)	65.9 (1546)
			36 (400)*	55 (1100)*
CD4/CD8 Ratio	0.9	4.5	-	-
			1.6*	2*
Naïve T helper cellsCD4+/CD45RA+ % (cells/µL)	-	-	0 (0)	-
			20 (272)*	40 (1123)*
Memory T helper cells CD4+/CD45RO+ % (cells/µL)	-	-	0 (0)	-
			5 (68)*	25 (702)*
Cytotoxic T cells CD8 % (cells/µL)	7.5 (0)	37.5 (824)	7.5 (0)	37.5 (824)
			17 (300)*	37 (700)*

			0 (0)	-
Naïve cytotoxic T cells CD8+/CD45RA+ % (cells/µL)	-	-	0 (0)*	0 (0)*

			0 (0)	-
Memory cytotoxic T cellsCD8+/CD45RO+ % (cells/µL)	-	-	0 (0)*	0 (0)*

\- means no value given

*Duplicate samples processed by Unimed. All other samples were processed by Quintiles.

B ORACLE-MS

Lymphocyte subpopulation	ORACLE-MS	
	Low	High
B cells CD19+ % (cells/µL)	5 (80)	22 (616)
CD4/CD8 Ratio	0.91	3.68
NK Cells CD16+56+ % (cells/µL)	5 (84)	26 (724)
Cytotoxic T cells CD8+ % (cells/µL)	13 (220)	39 (1129)
T helper cells CD4+ % (cells/µL)	33 (404)	58 (1612)
T lymphocytes CD3+CD45+, %	56 (723)	86 (2737)

All samples were processed by Covance.

Calculation of cell count changes from baseline

For each subject in the 3.5 mg/kg group, the change from baseline to Week X (5, 13, 24, 48) was calculated as follows:

$$num\_WeekX - num\_baseline$$

This is the difference between the Week X number of cells/µL and the baseline number of cells / µL. The median of these changes is reported in the line denoted "Change vs baseline" in Table 1.

For each subject in the 3.5 mg/kg group, the % change vs baseline to Week X (5, 13, 24, 48) was also calculated, as follows:

$$(numWeekX - num\_baseline) / num\_baseline * 100$$

This is the difference between the Week X number of cells/μL and the baseline number of cells/μL divided by the baseline number of cells/μL and multiplied by 100. The median of these % changes is reported in the line denoted "% change vs baseline" in Table 1.

Figures

Supplementary Figure 1. Median counts over time of A) neutrophils and B) monocytes in patients randomised to placebo or CT 3.5 mg/kg during the first year of the ORACLE-MS study.
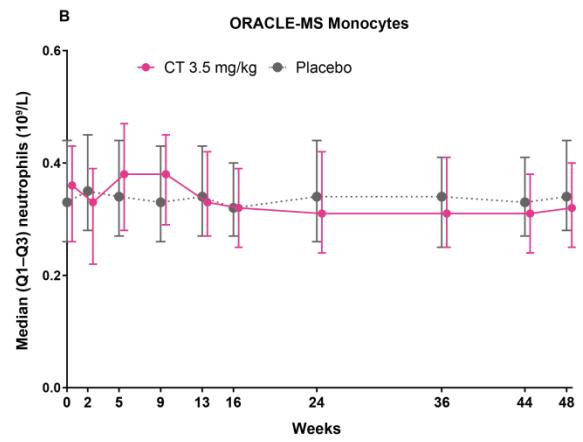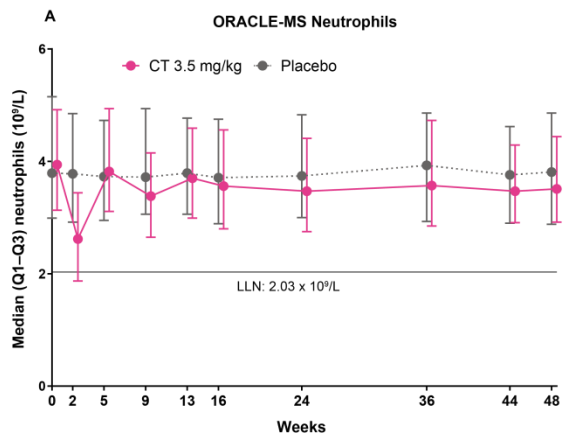
Supplementary Figure 2 Proportions of patients with neutrophil counts stratified according to CTCAE grade (Grade 0 – 1; Grade 2; and Grade 3) by visit during ORACLE-MS in patients randomised to cladribine tablets 3.5 mg/kg.
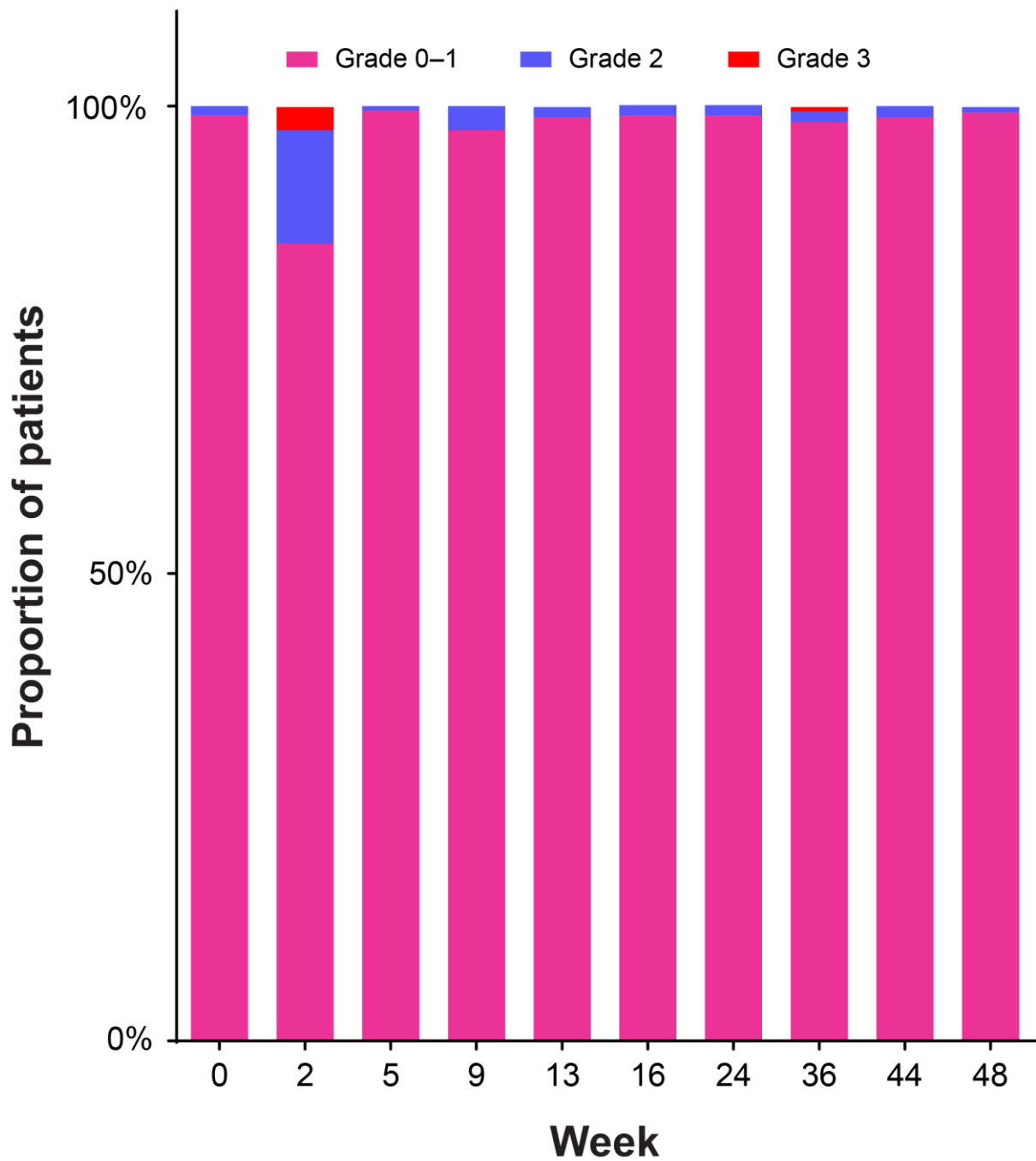
Numbers within columns indicate the percentage of patients treated with CT 3.5 mg/kg who had Grade 0–1 neutrophil counts. Numbers above columns indicate the percentage of patients treated with CT 3.5 mg/kg who had Grade 3 neutrophil counts. There were no cases of Grade 4 neutrophil counts during Weeks 0 to 48.