Lymphocytes and Fingolimod

Temporal pattern and relationship with Infections

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Disclosures

- **This study was funded by Novartis Pharma AG Basel, Switzerland**
  - G Francis and D Tang are employees of Novartis Pharmaceuticals Corporation; W Collins, L Zhang-Auberson L, A de Vera and M Looby are employees of Novartis Pharma AG; and F. Mercier is an employee of Novartis Institutes for BioMedical Research, Cambridge, MA, USA
  - Prof. L Kappos, is the Principal Investigator for FREEDOMS study and has received research support from Actelion, Advancell, Allozyne, BaroFold, Bayer Health Care Pharmaceuticals, Bayer Schering Pharma, Bayhill, Biogen Idec, BioMarin, CLC Behring, Elan, Genmab, Genmark, GeNeuro SA, GlaxoSmithKline, Lilly, Merck Sereno, Medicinova, Novartis, Novo Nordisk, Peptimmune, Sanofi-Aventis, Santhera, Roche, Teva, UCB and Wyeth
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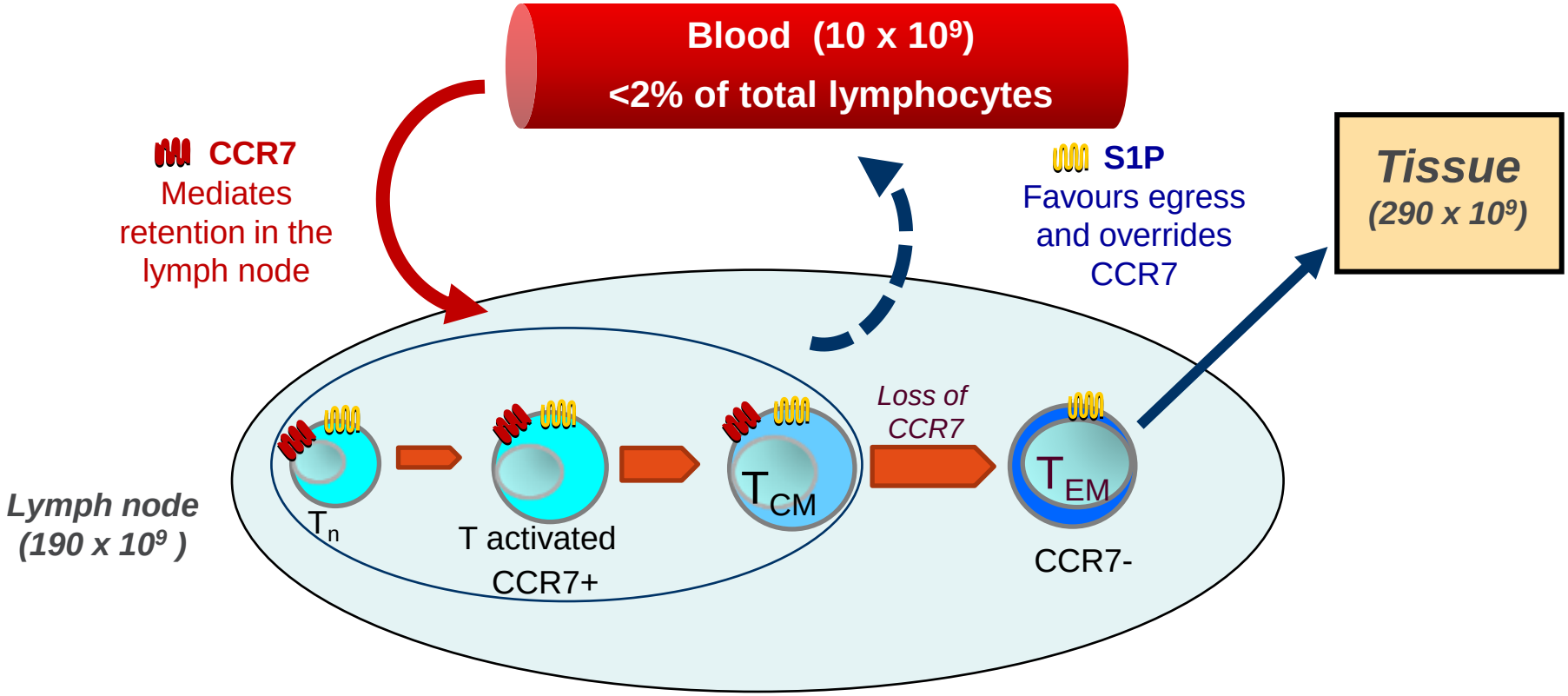
Methods

- **Lymphocyte count data**
  - Pharmacology studies in healthy volunteers and renal transplant patients to illustrate time of onset and offset of effect and impact on subsets
  - Phase 3 MS placebo-controlled study (FREEDOMS) to evaluate timing and persistence of effect on WBC
  - All MS studies pooled (Phase 2 and Phase 3 including extension phases)
    - to evaluate long-term effect on lymphocytes
    - to evaluate return to circulation after drug discontinuation
- **Infection data**
  - Phase 3 MS placebo-controlled study (FREEDOMS) to evaluate overall infection rate, rate by exposure time and relationship to lymphocyte counts
    - Incidence rate determined by both mean & nadir lymphocyte counts
    - Incidence of infection adjusted for time of exposure to study drug

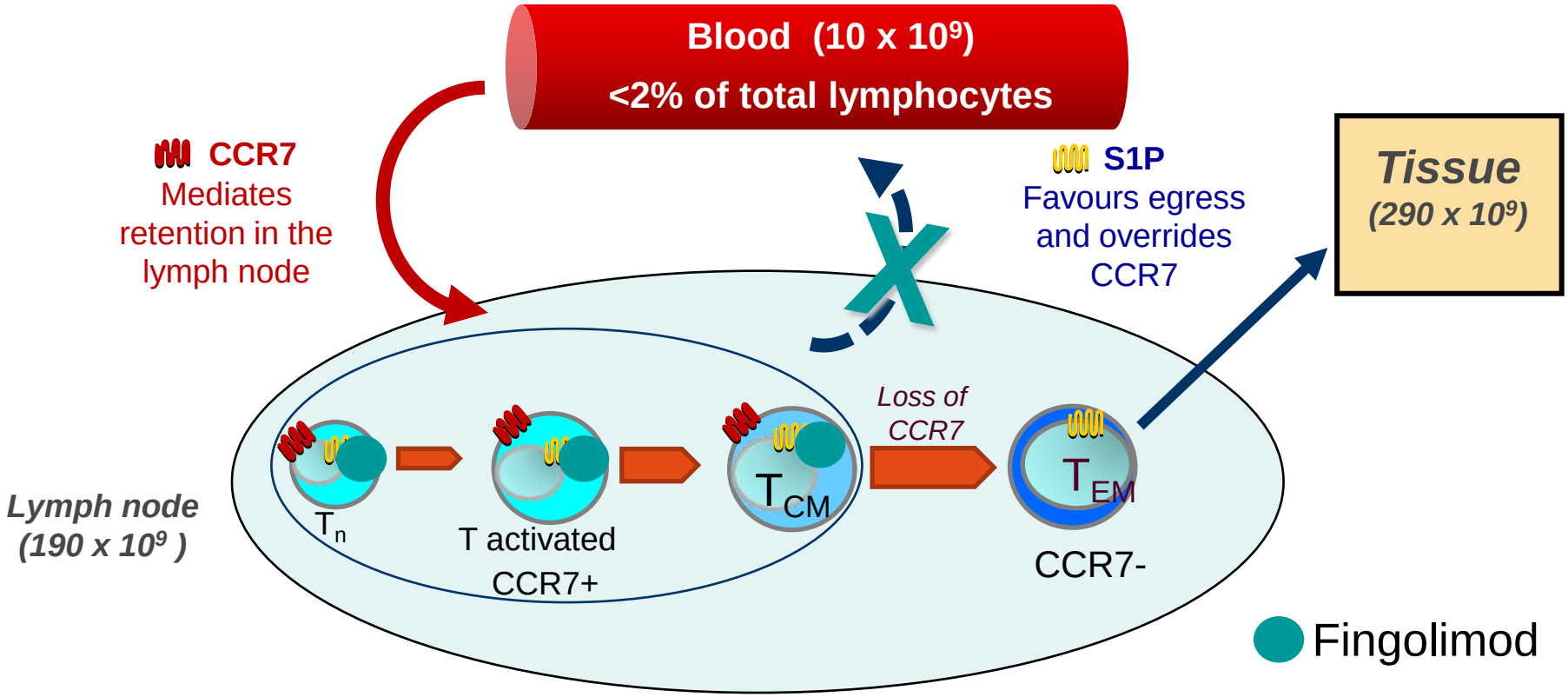
T cell circulation through lymph nodes: CCR7-mediated retention vs. $S1P_1$-mediated egress



- **$S1P/S1P_1$ signaling promotes lymphocyte egress by overriding CCR7 mediated retention**
- **T_{EM} lack the homing and retention-promoting receptor CCR7, they do not regularly re-circulate between blood and lymphoid tissues and are not retained in the lymphoid tissue like naïve and T_{CM} cells**

T n, naive T cells; TCM, central memory T cell; TEM, effector memory T cell. Sallusto F *et al. Nature* 1999; Mackay CR. *Nature* 1999; Sallusto F *et al. Annu Rev Immunol* 2004; Lanzavecchia A & Sallusto F *Science* 2000; Appay V *et al. Cytometry A* 2008; Westermann J & Pabst R. *Clin Investig* 1992; Pham TH *et al. Immunity* 2008; Brinkmann V *et al*. *Nat Rev Drug Discov* 2010

Fingolimod selectively retains circulating lymphocytes in lymphoid organs

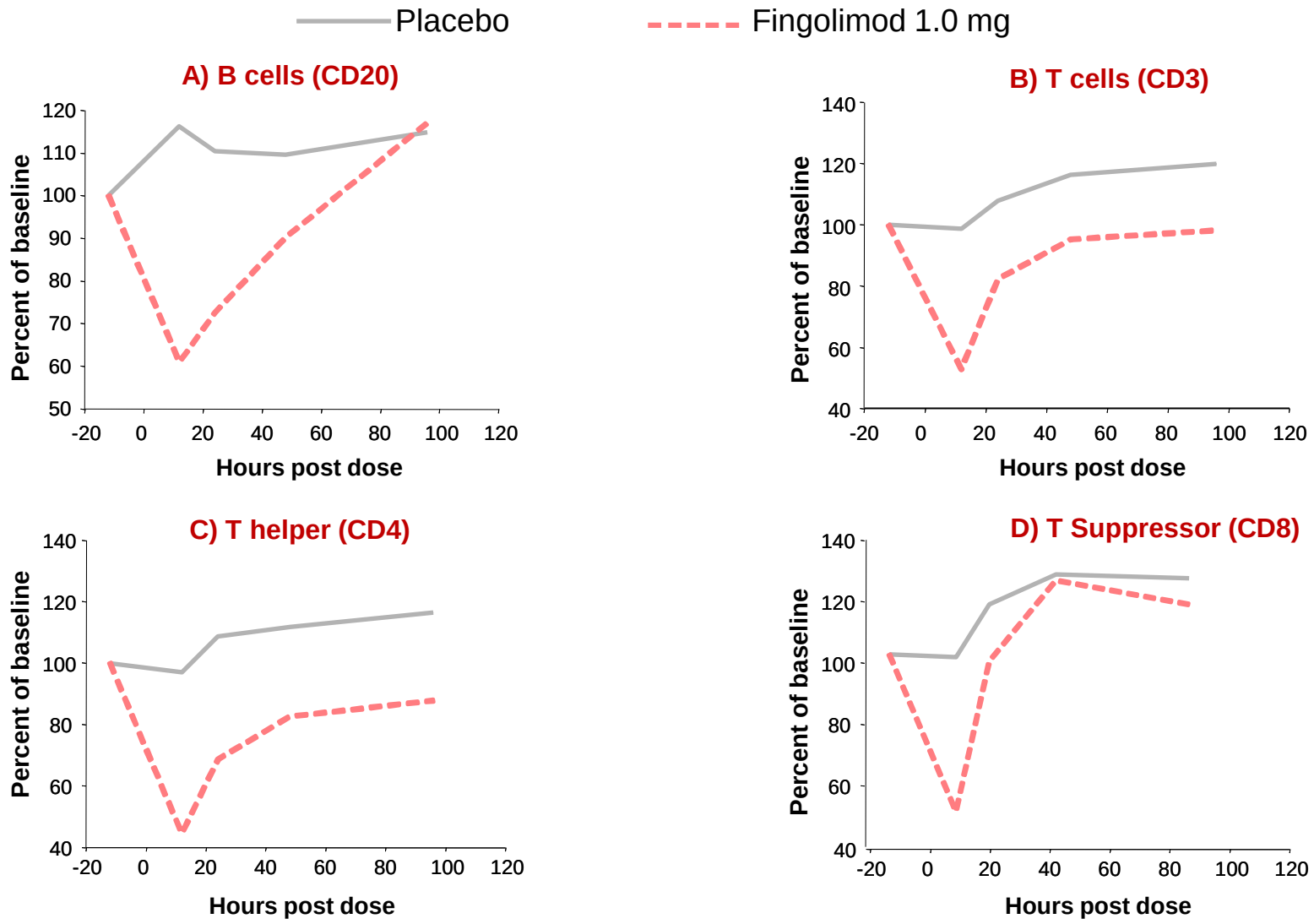


- **Fingolimod induces internalisation of S1P thereby favouring selective retention of CCR7+ T_n and T_{CM} (including Th17) cells involved in MS pathology**
- **T_{EM}, which are important for immune surveillance and maintenance of protective immunity, lack the homing and retention-promoting receptor CCR7, and are therefore largely spared by fingolimod**

T n, naive T cells; TCM, central memory T cell; TEM, effector memory T cell. Sallusto F *et al. Nature* 1999; Mackay CR. *Nature* 1999; Sallusto F *et al. Annu Rev Immunol* 2004; Lanzavecchia A & Sallusto F *Science* 2000; Appay V *et al. Cytometry A* 2008; Westermann J & Pabst R. *Clin Investig* 1992; Pham TH *et al. Immunity* 2008; Brinkmann V *et al*. *Nat Rev Drug Discov* 2010

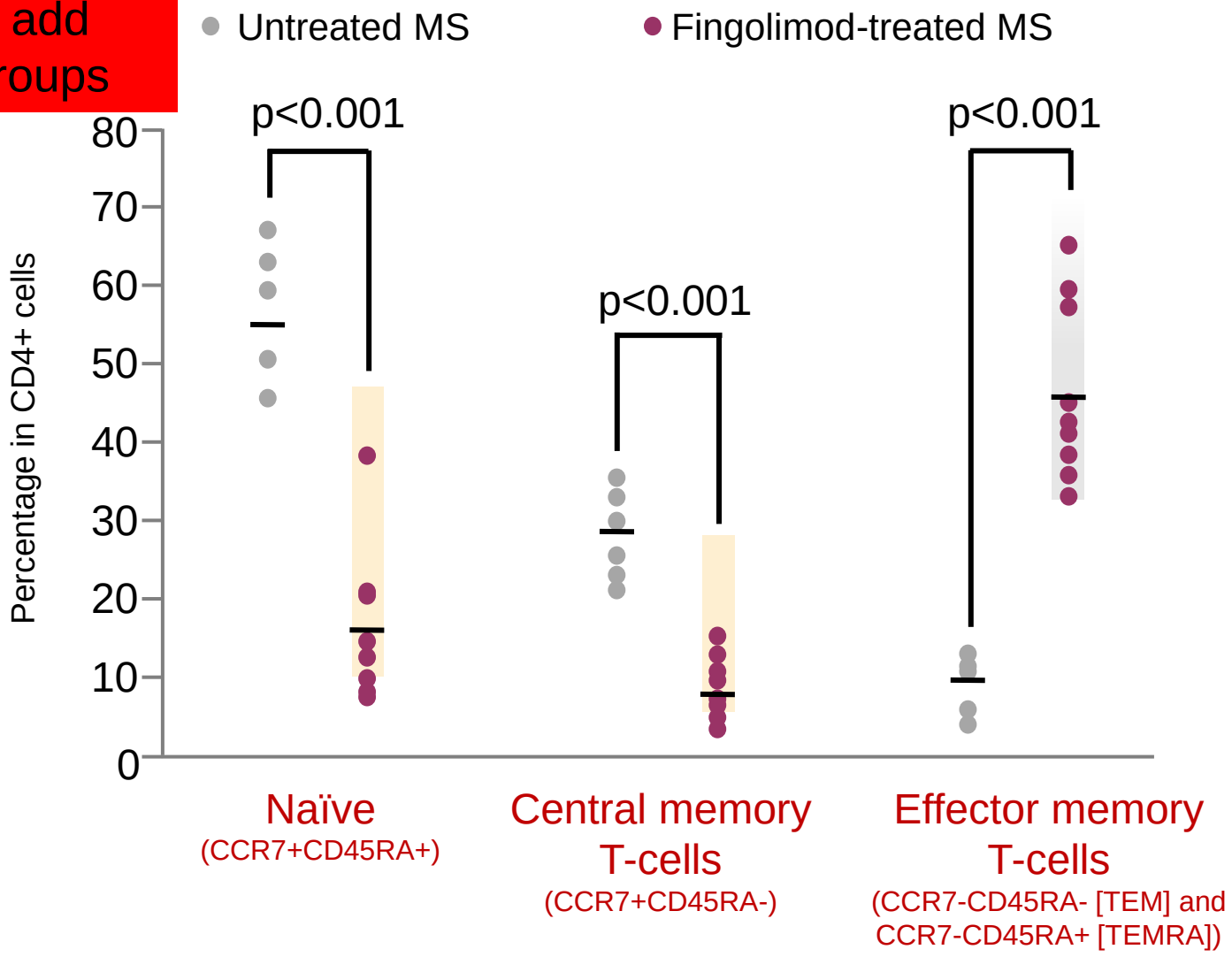
Fingolimod Effect on Lymphocyte Subsets

Percent change from baseline in mean counts of leukocyte subsets after a single dose of placebo or fingolimod 1.0 mg



Oral fingolimod blocks naïve and central memory T-cell egress but spares effector memory T-cells[1]

need to add n's to groups

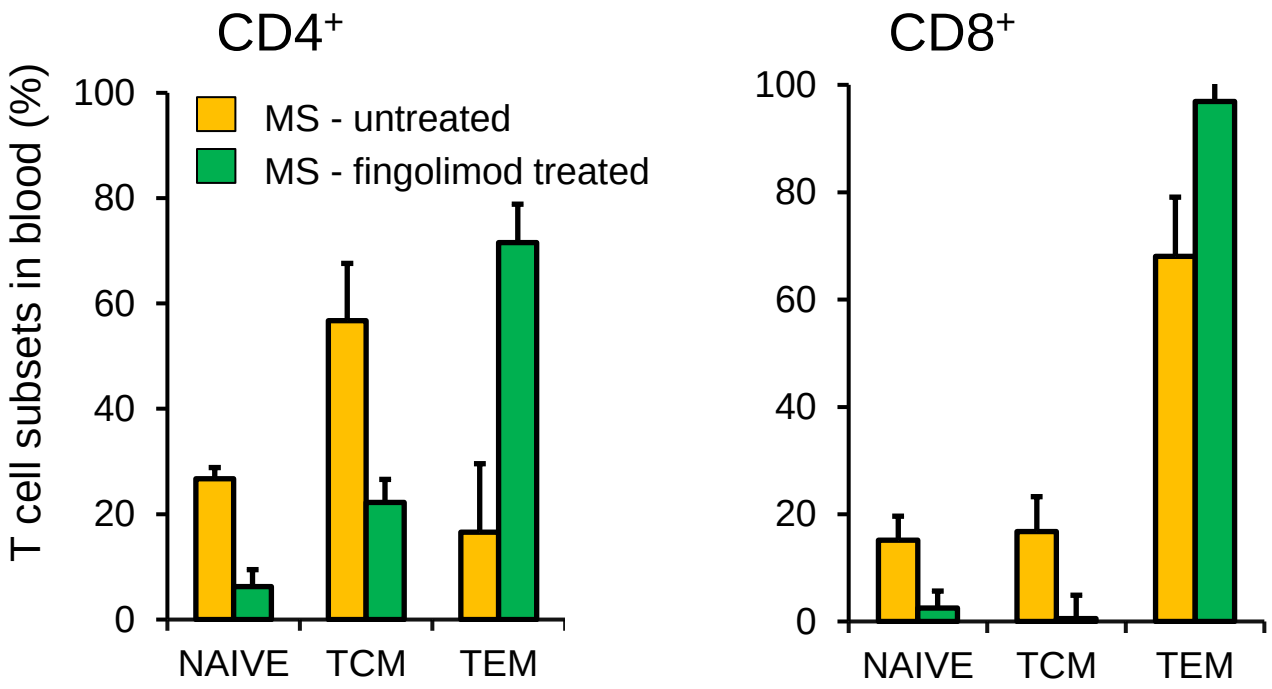


CCR7 and CD45RA expression on blood T-cells of patients with MS (FACS)
MS, Multiple Sclerosis

1. Mehling M et al. *Neurology* 2008.

Fingolimod retains CCR7^{+} naive and central memory but not CCR7^{-} effector memory T cells in lymph nodes

need to add n's to groups



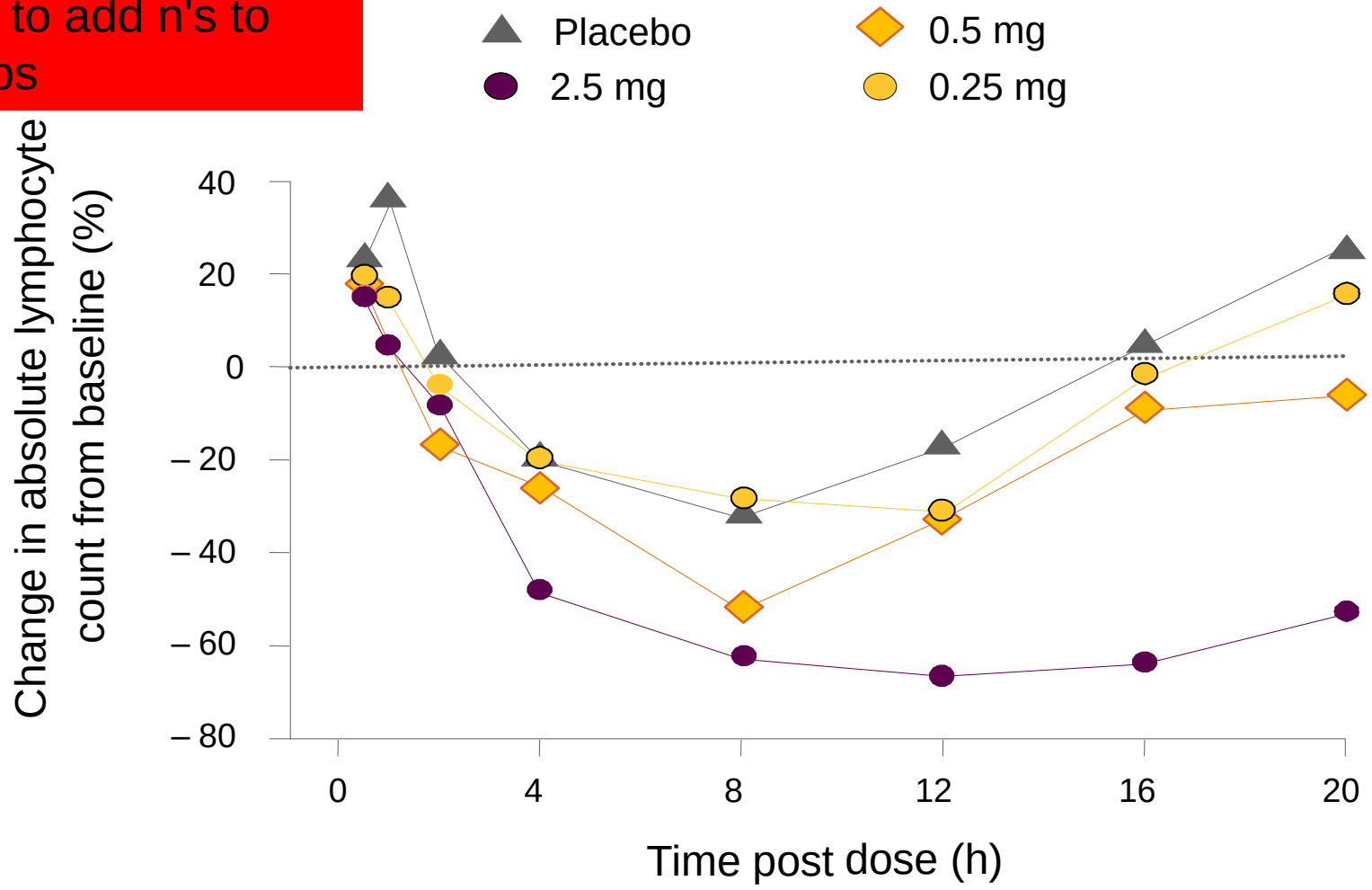
- Fingolimod internalizes S1P1, thereby favoring CCR7-mediated retention
- Fingolimod preferentially retains CCR7^{+} naive and TCM cells in LNs, but largely spares CCR7^{-} TEM cells

Mehling & Brinkmann et al., *Neurology* 2008; Metzler et al., *Int Immunol* 2008

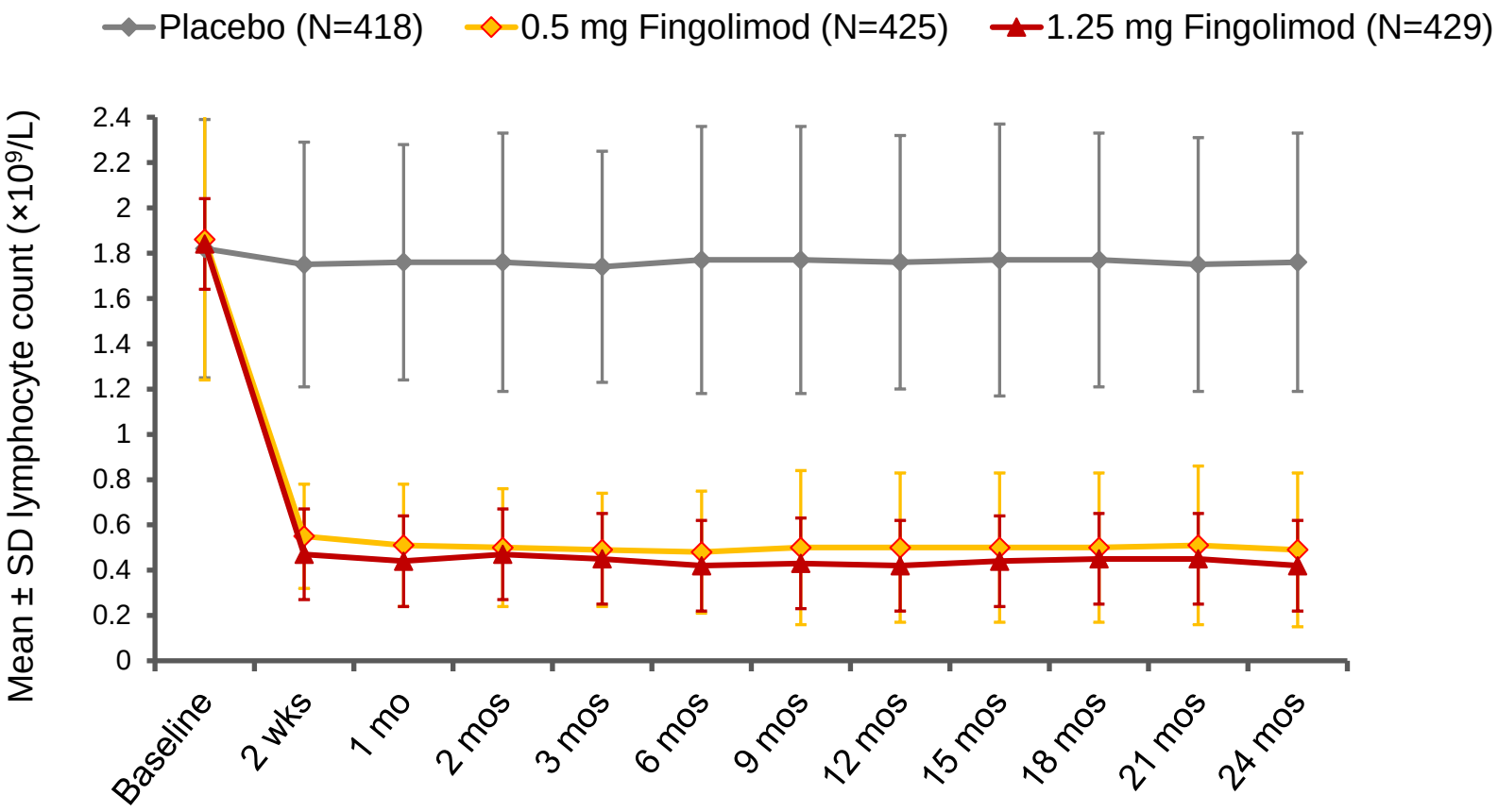
Chronology of Fingolimod Effect on Lymphocytes

Percent change from baseline with first dose in mean absolute lymphocyte count: renal transplant patients

need to add n's to groups

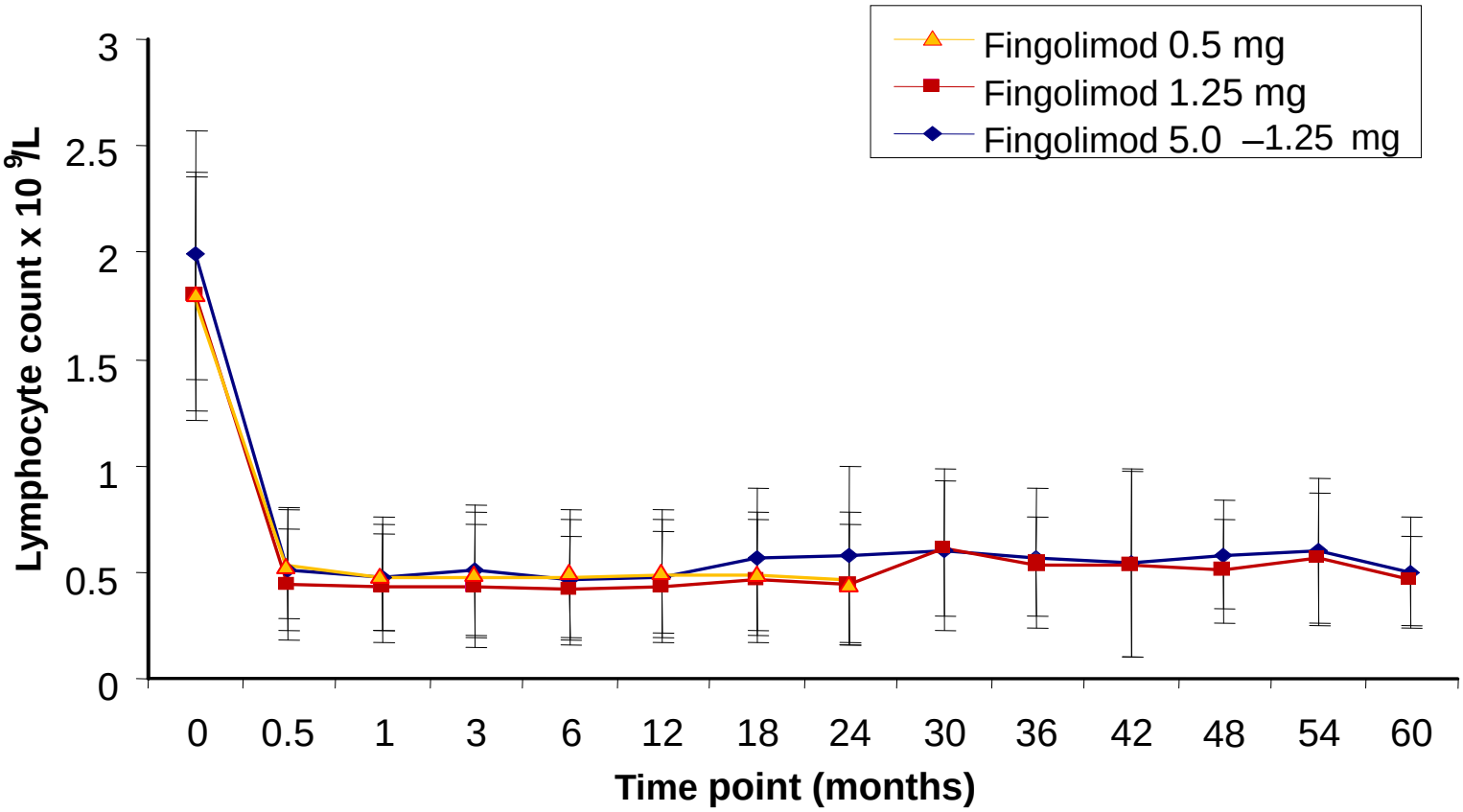


Lymphocyte count during treatment with fingolimod: FREEDOMS



Values represent the mean; error bars are the standard deviation.

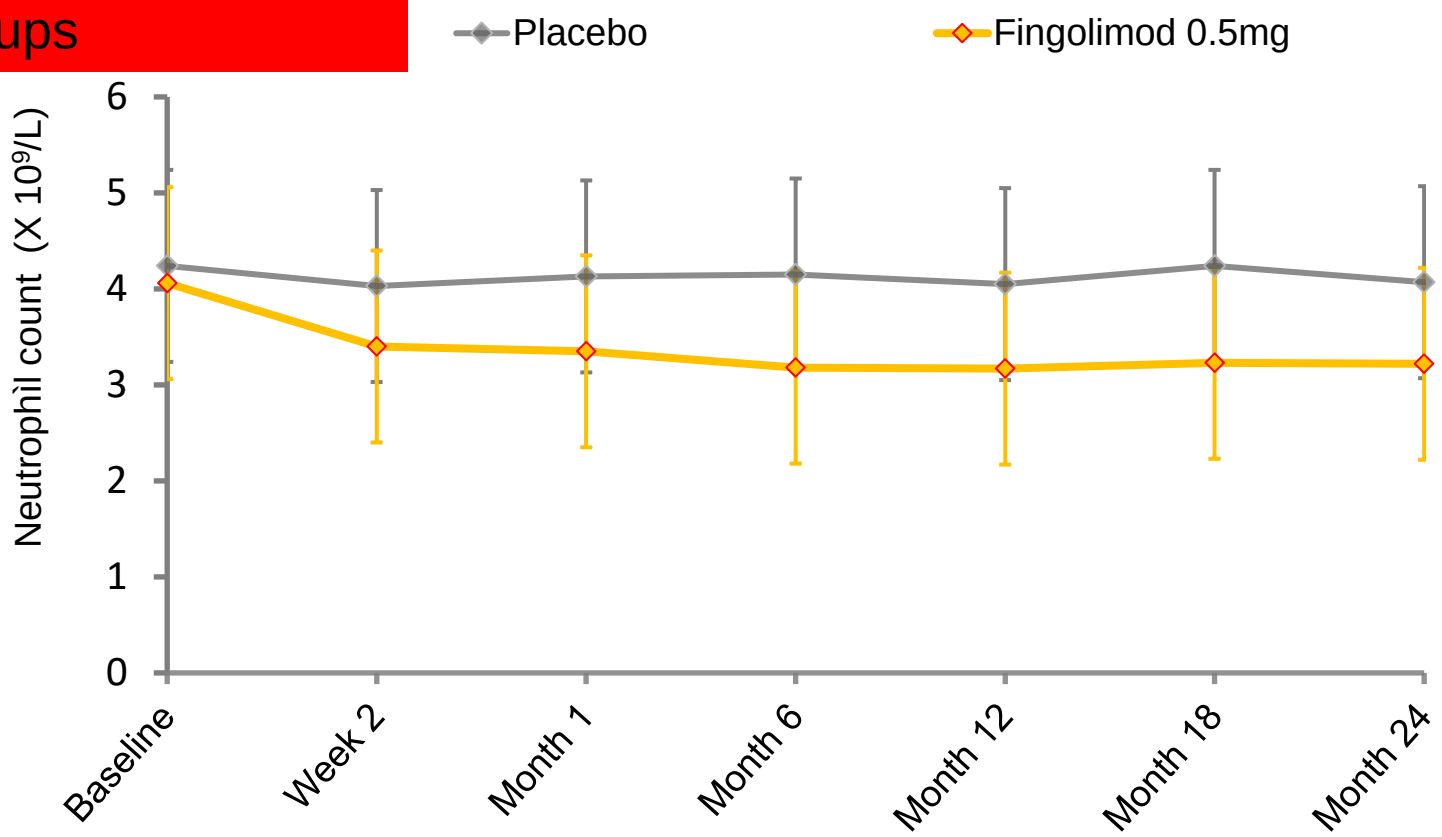
Mean lymphocyte count over time for up to 60 months (All Studies group; n=2315)



Fingolimod 0.5 mg, n	1015	941	944	957	928	803	674	452						
Fingolimod 1.25 mg, n	1148	1026	1036	1027	992	862	699	497	86	79	68	72	69	47
Fingolimod 5.0 –1.25 mg, n	137	93	93	99	121	103	92	93	91	79	74	71	61	39

Neutrophil count during treatment with fingolimod: FREEDOMS

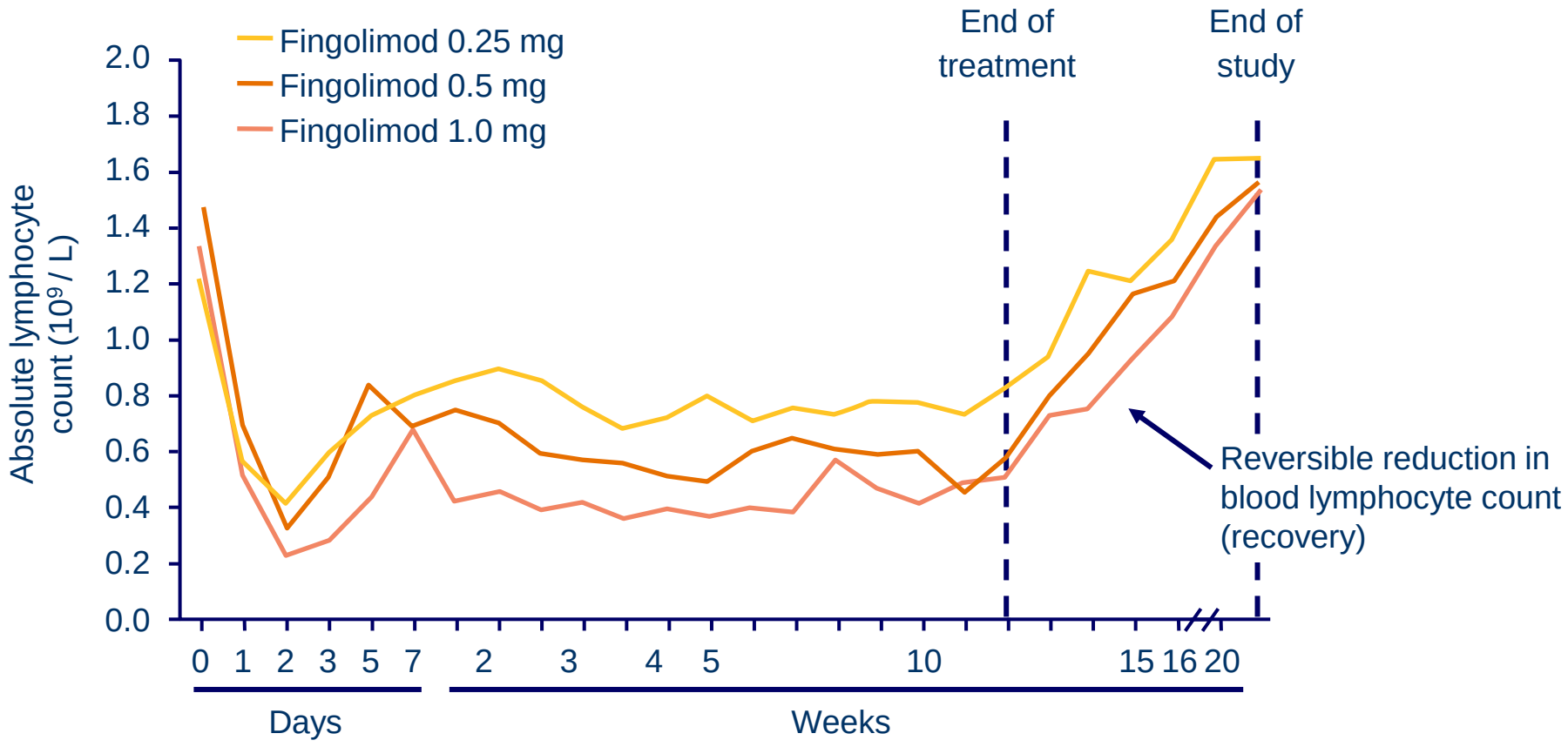
need to add n's to groups



Values represent the mean; error bars are the standard deviation.

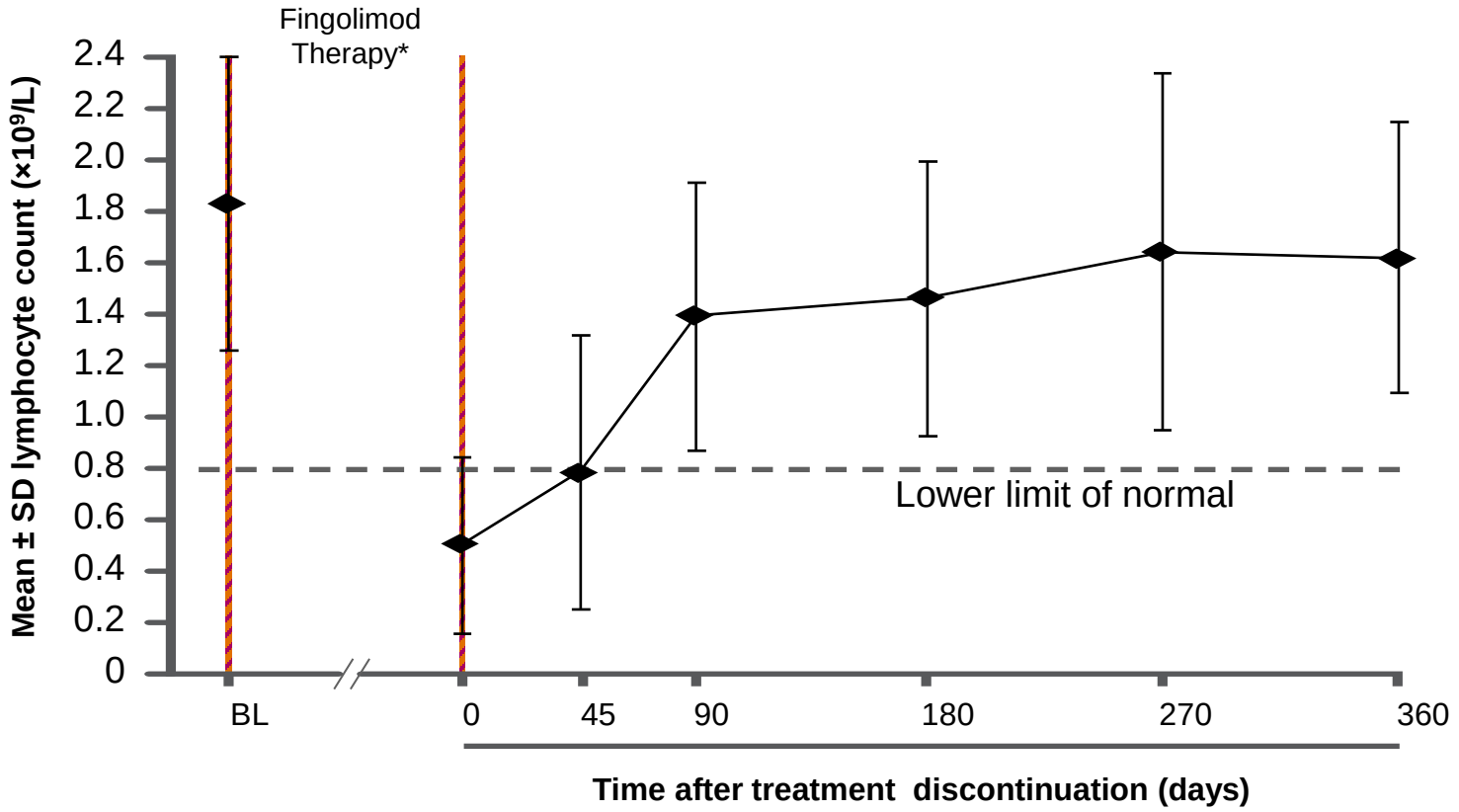
Lymphocyte recovery after stopping fingolimod Renal Transplant[1]

need to add n's to groups



1. Tedesco-Silva et al. *Transplantation* 2004;77:1826-33; 2. Schmouder R et al. Poster presented at ECTRIMS 2006

Lymphocyte count recovery after stopping fingolimod MS patients



- When therapy is stopped, mean lymphocyte counts return to above the lower limit of normal range (> 0.8 × 10^9/L) within 6 weeks

*All doses of fingolimod combined across all phase 2/3 studies, including patients who discontinued fingolimod therapy and had lymphocyte count data. Number of patients with evaluable data: baseline (n = 538), end of treatment (n = 518), after treatment discontinuation, days 0–45 (n = 294), days 46–90 (n = 358), days 91–180 (n = 130), days 181–270 (n = 72), and days 271–360 (n = 55). BL, baseline.

Clinical Impact of Low Lymphocyte Counts

Malignancies

	Phase III placebo-controlled (D2301)			All studies	
	Placebo	Fingolimod		Fingolimod	
		0.5 mg	1.25 mg	0.5 mg	1.25 mg
	N=418	N=425	N=429	N=1176	N=1302
Basal cell carcinoma	3 (0.7)	4 (0.9)	1 (0.2)	9 (0.8)	5 (0.4)
Squamous cell ca. skin	-	-	1 (0.2)	1 (0.1)	1 (0.1)
Malignant melanoma	1 (0.2)	-	1 (0.2)	3 (0.3)	5 (0.4)
Breast cancer	3 (0.7)	-	1 (0.2)	3 (0.3)	3 (0.3)
Cervix carcinoma	1 (0.2)	-	-	-	-
Endometrial carcinoma	1 (0.2)	-	-	-	-
Prostate cancer	1 (0.2)	-	-	-	-
Ovarian epithelial cancer	-	-	-	1 (0.1)	-
Total	**10 (2.4)**	**4 (0.9)**	**4 (0.9)**	**17 (1.4)**	**14 (1.1)**

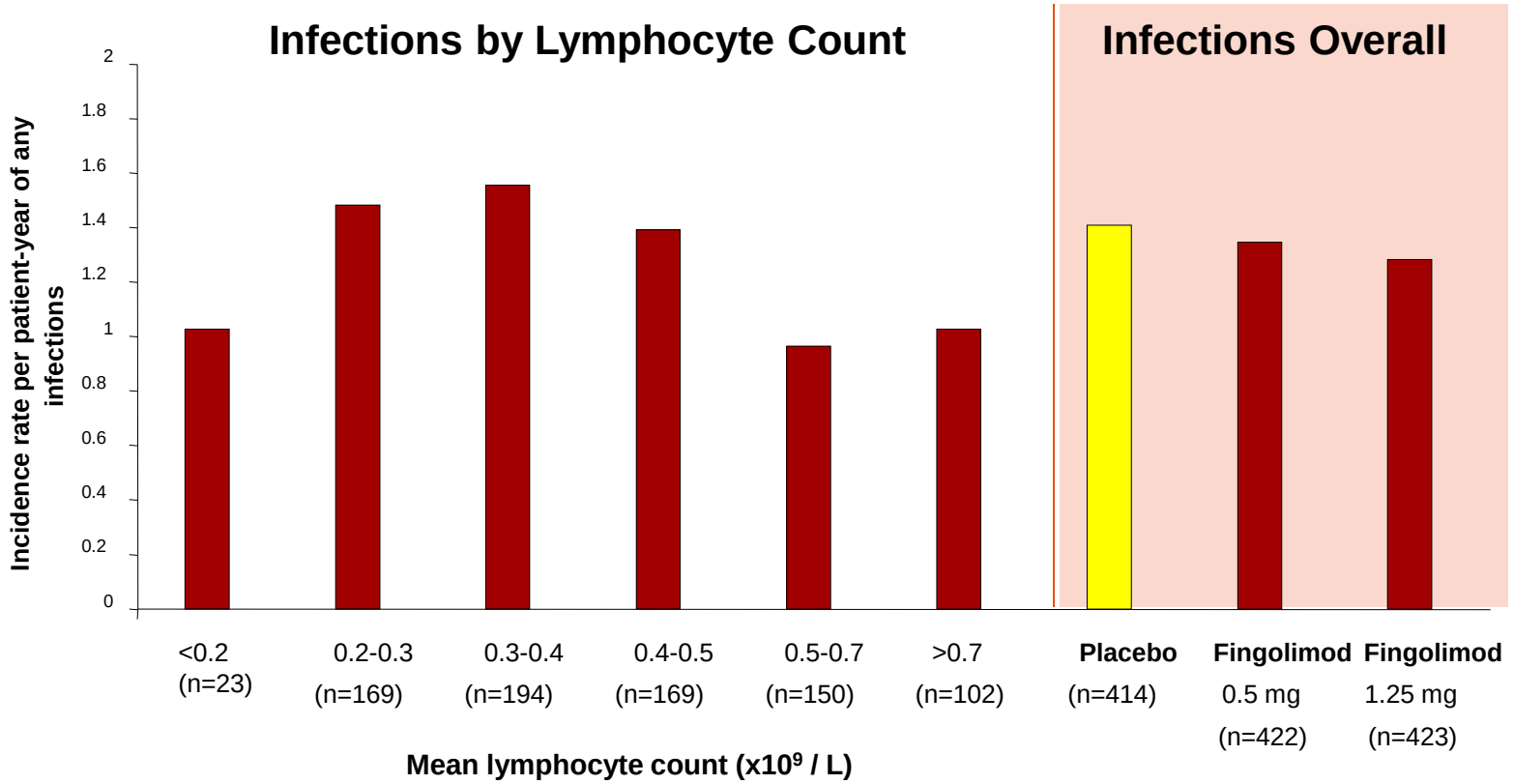
All cancer in situ are included in their respective terms

Infections

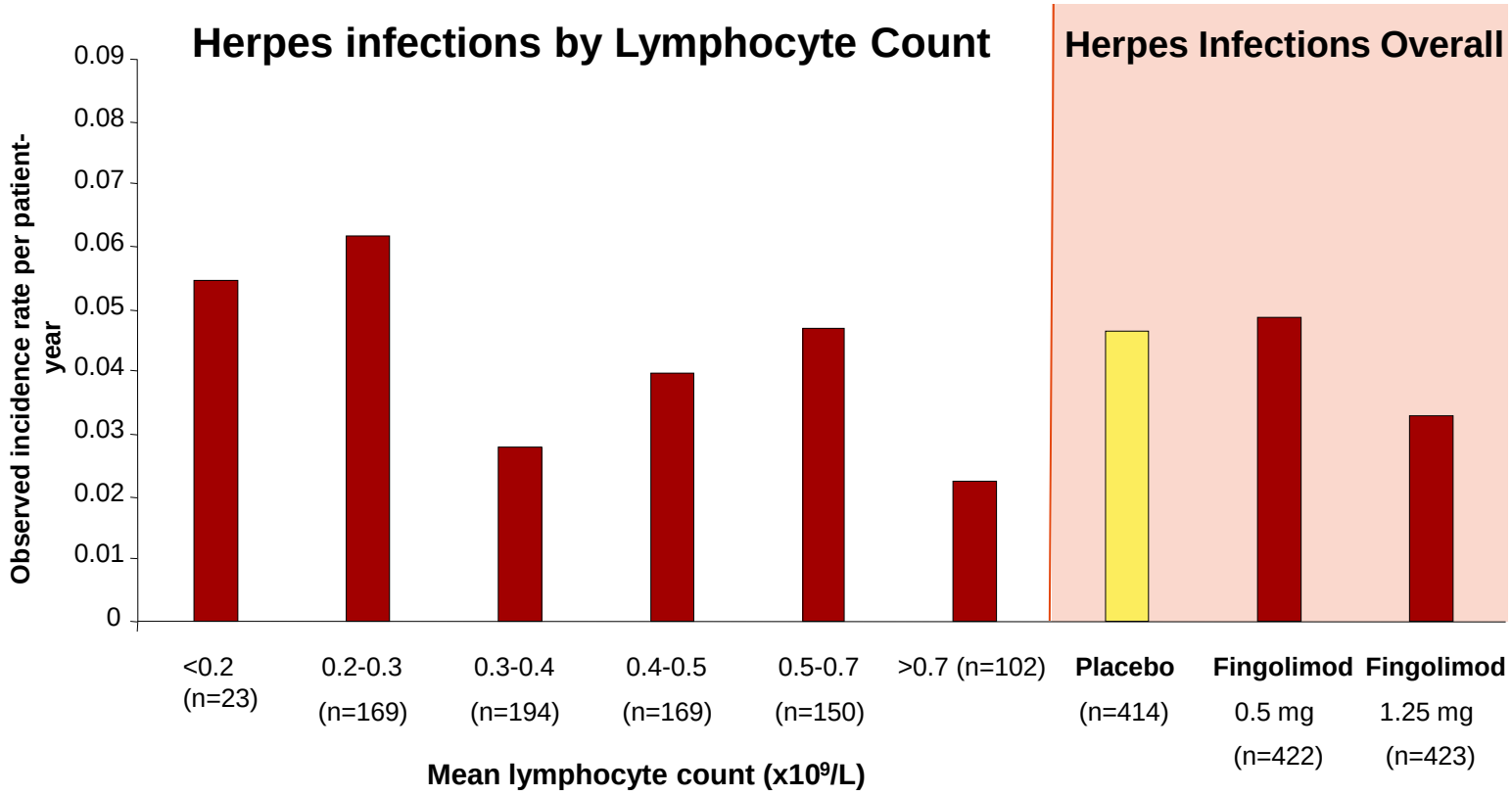
	Phase III placebo-controlled (D2301)			All studies	
	Placebo	Fingolimod		Fingolimod	
		0.5 mg	1.25 mg	0.5 mg	1.25 mg
	N=418	N=425	N=429	N=1176	N=1302
At least one infection, n(%)	301 (72.0)	304 (71.5)	294 (68.5)	751 (63.9)	847 (65.1)
Infections (events per 100 patient-years)	128.1	126.9	123.0	120.7	121.9
Any severe infection, n (%)	13 (3.1)	12 (2.8)	15 (3.5)	29 (2.5)	44 (3.4)
Any serious infection, n (%)	8 (1.9)	7 (1.6)	11 (2.6)	18 (1.5)	33 (2.5)
Infections of interest:					
LRTI and lung infections*	25 (6.0)	41 (9.6)	49 (11.4)	100 (8.5)	130 (10.0)
Any Herpes infection	33 (7.9)	37 (8.7)	25 (5.8)	95 (8.1)	122 (9.4)
Varicella-zoster	4 (1.0)	7 (1.6)	3 (0.7)	19 (1.9)	30 (2.6)
Herpes Infection SAEs	-	1 (0.2)	1 (0.2)	3 (0.3)	8 (0.6)

*LTRI = Lower respiratory tract infections. Only type of infection with higher incidence (>1% difference vs placebo) in the 1.25 mg group in Phase III. Bronchitis was the most frequently reported LRTI.

Incidence of overall infections/p-y categorized by mean lymphocyte counts: FREEDOMS core study group

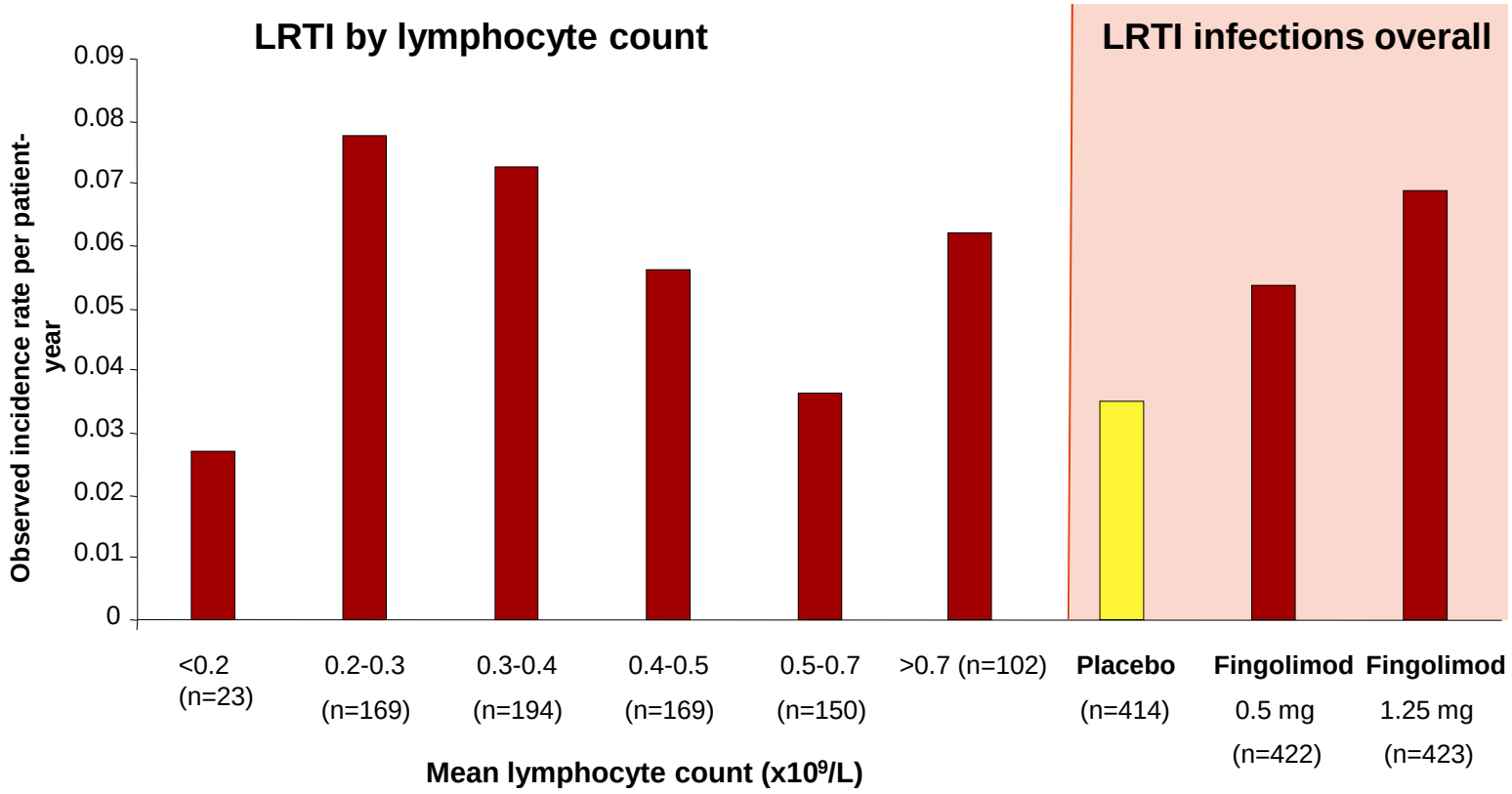


Incidence of herpes infections/p-y overall and categorized by mean lymphocyte counts: FREEDOMS core study group



2 fatal cases of herpes infections – Lymphocyte counts > 0.3x10^9/L

Incidence of LRTI /p-y overall and categorized by mean lymphocyte counts: FREEDOMS core study group



Conclusions

- Fingolimod leads to rapid reduction in peripheral lymphocyte counts whilst preserving key immune functions (no cytotoxicity) and which persists in stable fashion while treatment continues
  - ~70% decrease from baseline at target 0.5mg dose
- Most lymphocyte subsets are affected
  - CCR7+ T_n and T_{CM} affected while CCR7- T_{EM} cells are relatively unaffected
  - Effect on CD4 > CD8 leading to inverted CD4:CD8 ratio *in circulation*
- Recovery to within normal range occurs within 1-2 months and follows drug concentration ($T^{1/2}$ = 6-9 days)
- Neutrophil counts reduced by about 20%
- Infections, including serious ones are not increased on fingolimod
  - No clear relationship between lymphocyte count and infections
  - Lower infection incidence, compared to control, in patients treated with fingolimod who retain higher lymphocyte counts is unexplained