

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: The CAMMS223 Trial Investigators. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N Engl J Med* 2008;359:1786-801.

## **Supplementary Appendix: statistical analysis and additional safety data.**

*Submitted by Dr AJ Coles on behalf of the writing committee of the CAMMS223 Trial Investigators*

### **Statistical Analysis**

The estimate of sample size was based on prior open-label experience at Addenbrooke's Hospital (Cambridge, UK)<sup>14</sup> and the Prevention of Relapses and Disability by Interferon-beta-1a Subcutaneously in Multiple Sclerosis study<sup>15;16</sup>. With the use of two-sided tests, an estimated 16% drop-out rate, a Bonferroni correction for the two alemtuzumab treatment arms, and the allocation of 0.04 alpha to the sustained accumulation of disability comparisons and 0.01 to the relapse rate comparisons, the initial planned sample size of 285 provided the study with 75% power to detect a treatment effect on sustained accumulation of disability when the 36-month sustained accumulation of disability rate was 12% for the alemtuzumab treatment arms and 30% for the IFN $\beta$ 1a treatment arm. At the time that 285 patients had been randomized (April 2004), more than 75 patients had recently entered the screening process. The sponsor allowed these patients, if eligible, to remain in the study and 49 were ultimately randomized.

Pre-planned interim analyses were performed when approximately all patients had completed at least one year (results issued September 2005) and two years of follow-up (results issued September 2006). A Lan-DeMets error spending function with an O'Brien-Fleming boundary was used to pre-specify the amount of alpha spent at each analysis based on the approximate amount of statistical information available. The overall alpha spent was split further to account for multiple comparisons on dose group and co-primary endpoints. For the first interim analysis, alpha was allocated between the sustained accumulation of disability and relapse endpoints with a 1:4 ratio and, for the second and final analyses, alpha was allocated between the sustained accumulation of disability and relapse endpoints with a 4:1 ratio. For each treatment arm the p-values to

declare statistical significance for the sustained accumulation of disability endpoint at the 1<sup>st</sup>, 2<sup>nd</sup> and final analyses were: 0.00015, 0.01194 and 0.01646. Likewise, for the relapse rate endpoint the p-values to declare statistical significance at the 1<sup>st</sup>, 2<sup>nd</sup> and final analyses were: 0.00267, 0.00328 and 0.00396. There was no plan to stop the trial for efficacy based on the interim analyses.

Efficacy was analyzed on an intention-to-treat principle and included 334 randomised patients excluding one individual discussed in the Study Populations section who did not have multiple sclerosis. The effect of treatment on the time to sustained accumulation of disability co-primary endpoint was assessed using a Cox proportional hazard model with treatment group (indicator variables for the 12 mg/day treatment group and the 24 mg/day treatment group), country, and baseline EDSS score (grouped by 0 to 1.5 and 2.0 to 3.0) as factors. Using the same covariates, the comparison of relapse rates was conducted using an Andersen-Gill multiplicative intensity model with robust variance estimation<sup>17</sup>.

The proportion of patients relapse-free at 36 months was assessed using logistic regression with treatment group, EDSS group and country as covariates, and with patients censored prior to month 36 classified as relapse-free. The estimated percentage of patients experiencing sustained accumulation of disability or relapse in specific time intervals was generated via the Kaplan-Meier method. Annualised relapse rate was estimated via Poisson regression. The number needed to treat is equal to the inverse of the absolute risk reduction and, in the context of this manuscript, interpreted as the number of patients who would need to be treated with alemtuzumab instead of IFNB-1a to avoid one sustained accumulation of disability event. The numbers needed to treat were calculated using the proportion of patients free of sustained accumulation of disability at month 36 and, separately, using the proportion of patients free of relapse at month 36<sup>18</sup>. EDSS comparisons were based on a repeated measures analysis of covariance (ANCOVA) with study visit, EDSS at baseline, country, treatment group and study visit by treatment group interaction. In addition, the distribution of patients who improved, stayed the same or deteriorated in the EDSS at the last available observation compared to baseline was summarized and a proportional odds model used to estimate

the odds of improving or staying the same versus worsening, or (equivalently in the proportional odds model setting) the odds of improving versus staying the same or worsening. The model included the same covariates as the time-to-sustained accumulation of disability proportional hazards model, and patients without at least one post-treatment EDSS assessment were not included in this analysis. MRI percent change from baseline to specific time points was analyzed using the Wilcoxon-Mann-Whitney test and, over the 36 month time period, using the multivariate Wei-Lachin test<sup>19,20</sup>. Fisher’s exact test and Poisson regression were used for safety event incidence and event rate. The reported p-values are 2-sided and not adjusted for multiple testing.

Study conduct was monitored by an independent Data and Safety Monitoring Board. The statistical analysis was performed by Genzyme employees and ratified by independent statisticians.

***Online only Appendix Table: all other serious adverse events not listed in Table 3 of printed manuscript***

	<b>Interferon</b>	<i>12 mg/day</i>	<i>24 mg/day</i>	<b>Pooled</b>
<b><u>Other serious adverse events</u></b>	<b>beta-1a</b>	<i>Alemtuzumab</i>	<i>Alemtuzumab</i>	<b>Alemtuzumab</b>
<b>General condition – no. patients (%)</b>				
Adverse drug reaction	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Chest discomfort	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Drug toxicity	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Hypokalemia	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Pyrexia	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>

Dehydration	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
Fatigue	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
Dental necrosis	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
<b>Cardiovascular condition – no. patients (%)</b>				
Cardiovascular disorder	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Myocardial infarction	<b>1 (0.9)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Arrhythmia	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
<b>Neurological symptom – no. patients (%)</b>				
Neurological event	<b>14 (13.1)</b>	<i>8 (7.4)</i>	<i>6 (5.6)</i>	<b>14 (6.5)</b>
Urinary incontinence	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>2 (1.9)</i>	<b>2 (0.9)</b>
Bladder spasm	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Cerebral hemorrhage	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Cerebrovascular accident	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Extraocular muscle disorder	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Headache	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Optic atrophy	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Syncope	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Hearing impaired	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
Hypoesthesia oral	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
Intervertebral disc protrusion	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>

Paraesthesia oral	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
Urinary retention	<b>2 (1.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
<b>Gastrointestinal condition – no. patients (%)</b>				
Abdominal hernia	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Dyspepsia	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Gastritis	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Gastroduodenitis	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Ileus	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Nausea	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Esophagitis	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Peritonitis	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Salivary gland calculus	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Vomiting	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Enterocutaneous fistula	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
<b>Musculoskeletal condition – no. patients (%)</b>				
Arthritis	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Back pain	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Musculoskeletal discomfort	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Pain in extremity	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
<b>Psychiatric condition – no. patients (%)</b>				

Bipolar disorder	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Depression	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Anxiety	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
<b>Pulmonary symptom – no. patients (%)</b>				
Asthma	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>2 (1.9)</i>	<b>2 (0.9)</b>
Pulmonary embolism	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Wheezing	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
<b>Renal and urinary disorder – no. patients (%)</b>				
Dysuria	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Nephrolithiasis	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
<b>Reproductive disorder – no. patients (%)</b>				
Spontaneous abortion	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Threatened abortion	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Miscarriage of partner	<b>0 (0.0)</b>	<i>0 (0.0)</i>	<i>1 (0.9)</i>	<b>1 (0.5)</b>
Ovarian cyst	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Ovarian cyst ruptured	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
Uterine leiomyoma	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Endometriosis	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
Pelvic pain	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
<b>Menstrual disorder – no. patients (%)  </b>	<b>1 (1.4)</b>	<i>0 (0.0)</i>	<i>2 (2.9)</i>	<b>2 (1.4)</b>

<b>Surgical and medical procedures – no. patients (%)</b>				
Jaw operation	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Carpal tunnel decompression	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
<b>Injury, poisoning and procedural complications</b>				
Alcohol poisoning	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>
Limb traumatic amputation	<b>0 (0.0)</b>	<i>1 (0.9)</i>	<i>0 (0.0)</i>	<b>1 (0.5)</b>
Fracture	<b>1 (0.9)</b>	<i>0 (0.0)</i>	<i>0 (0.0)</i>	<b>0 (0.0)</b>