Detecting anti-drug-antibodies in alemtuzumab therapy

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Introduction

Alemtuzumab was the ***first humanised monoclonal antibody*** produced, designed to reduce the immunogenicity of the parent rat anti-CD52 immunoglobulin. It is licensed for the treatment of relapsing multiple sclerosis (MS)[1,2]. It acts by depleting peripheral T and B lymphocytes. Despite humanisation, alemtuzumab induces the ***highest frequency of binding and neutralizing anti-drug antibodies (ADA)*** of all antibodies used in MS therapy (Table 1A). We show that in some individuals, ADA may limit the therapeutic efficacy of the drug.

Background

Alemtuzumab-specific ADA have been repeatedly cited as being clinically insignificant[1-3]. However, attempts to develop strategies to inhibit the occurrence of alemtuzumab-specific ADA, would suggest otherwise[4]. We found unpublished data, indicating that alemtuzumab induces binding ADA (BAbs) in about 85% of people with MS within 24 months (n=811) and 92% of those people develop neutralising ADA (NAbs)[5,6].The clinical dosing schedule of alemtuzumab[2] was perhaps formulated in order to minimise the impact of ADA (Table 1B), which are probably a product of the depletion/repletion kinetics of immune cells, the tissue-expression of CD52 and the biology of alemtuzumab (Figure 1).

Table 1. ADA as a major driver of the dosing schedule of alemtuzumab

A

Antibody	Frequency ADA
Rituximab[1,2] (Chimeric CD20. 1000mg Q26W)	BAbs 15-37%
Ocrelizumab[3] (Humanised CD20) (600mg Q26W)	BAbs 0.4% NAbs <0.1%
Natalizumab[4] (Humanised CD49d. 300mg Q4W)	Babs 5-9%
Alemtuzumab[5] Humanised CD52	Babs 85% NAbs78%

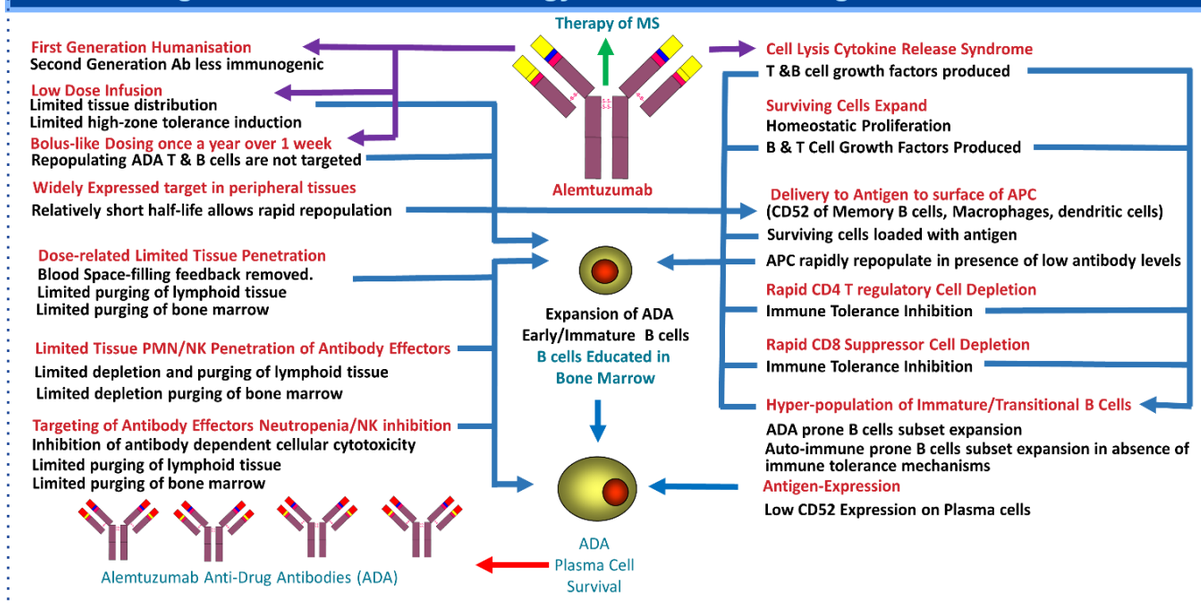
B

Dosing Schedule	Observed Effects (CARE-MS trials)[5]
First Infusion cycle Five daily 12mg alemtuzumab 6h infusions	ADA develop in most pwMS (62% BAbs, 54% NAbs in 1 month)[5] Primary antibody Responses take at least 6 days to generate
Second Infusion cycle Three daily 12mg alemtuzumab 6h infusions	ADA develop in most pwMS (83% BAbs, 79% NAbs in 1 month) [5] Secondary Antibody Responses take 3-4 days to generate
Repeat infusion cycle at minimum 12 month Intervals Retreatment after disease activity, 12 months from last dose Original Schedule 2013-2017 Two cycles New Schedule. 2017 up to 4 cycles (EU), 2018 up to 3 cycle (UK)	Antibodies levels subside slowly Pre-dose 1 Binding ADA = 0.9% Neutralizing ADA = 0% [5] Pre-dose 2. Binding ADA = 29% Neutralizing ADA = 0.6%[5] Pre dose 3. Binding ADA = 75% Neutralizing ADA = 31%[5]
Prophylactic Anaphylactoid Treatment Anti-histamines, paracetamol, steroids	Reduction of Infusion reactions/cytokine release syndrome Masks anaphylactoid responses which occur rarely[6]

1.Hauser et al. N Eng J Med 2008 358:676, 2.Dunn et al. Mult scler 2018 24:1224, 3. Hauser et al. N Eng J Med 2017 376:221, 4. Calibresi et al. Neurol 2007 69:1391, 5. Li et al. Clin Exp Immunol 2018 194:295, 6. Cohen et al. Lancet 2012 380:1819

Each infusion cycle ends before primary and secondary antibody responses are generated. In addition, there must be at least 12 months between each treatment cycle, rather than treating following disease breakthrough, to allow ADA to subside (Table 1B). These ADA become more prominent and potentially problematic after two treatment cycles of alemtuzumab[5,6].

Figure 1. Alemtuzumab biology contributes to the generation of ADA



We hypothesised that ADA may become clinically relevant for some individuals after repeated treatment, notably three or more cycles of alemtuzumab.

Aims

1) **To detect binding and neutralising ADA in sera samples from people with relapsing MS receiving 3 or more cycles of alemtuzumab.**
2) **To examine the relationship between lymphocyte depletion and ADA titre.**

Methods

Binding ADA assay

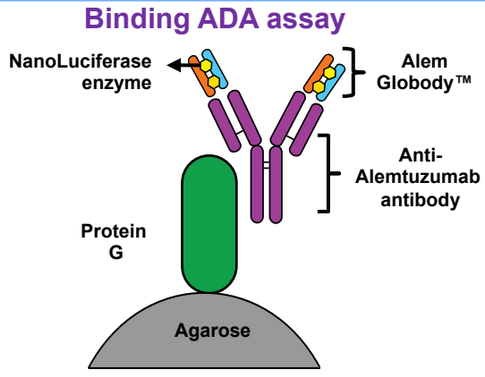


Figure 2. Binding ADA assay

Alemtuzumab (Alem) Globody™ was engineered. This molecule consists of the variable domains of alemtuzumab bridged via a dual nanoluciferase containing linker. ADA-Alem GloBody complexes were captured on Protein G agarose and the retained luciferase activity determined.

We developed light-based alemtuzumab-specific antibody binding (Figure 2) and neutralising assays (Figure 3). These were used to retrospectively screen blinded, banked, serum samples from 32 individuals received three (n=24), four (n=2), five (n=4) or six (n=1) cycles of alemtuzumab. These were obtained with informed consent from the Welsh Neuroscience Research Tissue Bank. Research Ethics Committee approval reference 19/WA/0058.

Neutralising ADA assay

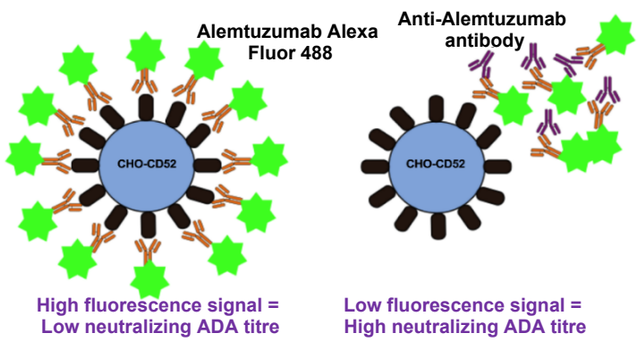


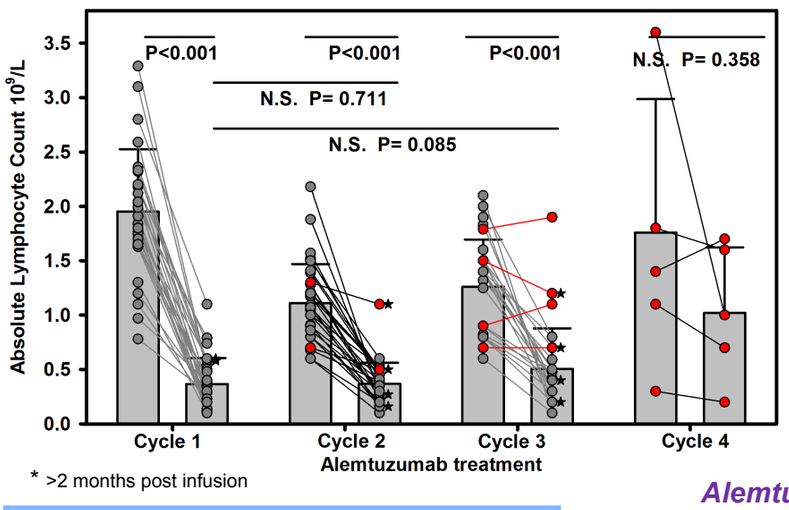
Figure 3. Neutralizing ADA assay

A plastic-adherent human CD52-expressing Chinese hamster ovary cell line was made and used to enable detection of neutralising ADA in a competitive binding assay using serial serum dilutions and an alemtuzumab Alexa-Fluor 488 conjugate, detection agent.

Results

Figure 4. Alemtuzumab depletion failure occurs in MS

Lymphocyte depletion in people with MS receiving 3 or more cycles of alemtuzumab



* >2 months post infusion

At the population level there was comparable depletion after cycle 1 to 3 (Figure 4), but an increasing number of individuals failed to deplete below the lower limit of normal (1.0 x 10^9 cells/L) or depleted-poorly (red) with an increasing number of treatment cycles.

Loss of lymphocyte depletion was associated with subsequent treatment failure.

Figure 5. Binding ADA developed in most people treated with alemtuzumab compared to baseline

Baseline sera samples were available in some people (n=17). ADA titre in baseline sera samples was significantly (P<0.001) less than the ADA titre recorded from 50µg/mL of a monoclonal alemtuzumab ADA standard (n=32). The results show the highest titre for each individual (n=32) in any of 4-6 samples/ individual banked during treatment. The mean ± standard deviation group scores are shown.

29/32 (90.6%) of the individuals had detectable binding ADA (above 2x standard deviation of baseline samples) following infusions of alemtuzumab.

Alemtuzumab-specific binding ADA in individual samples

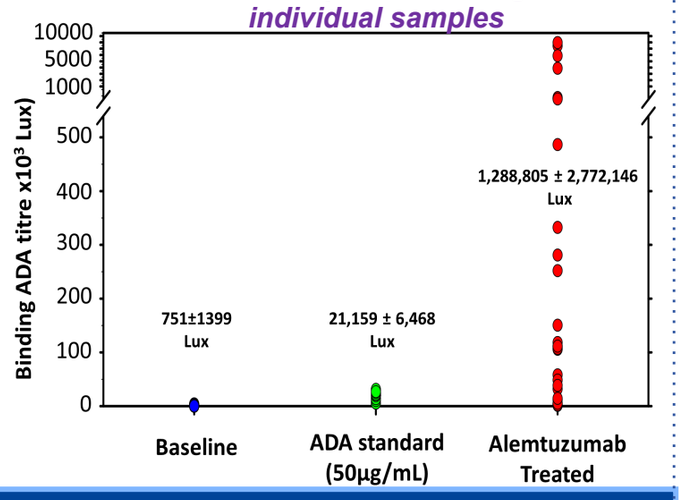
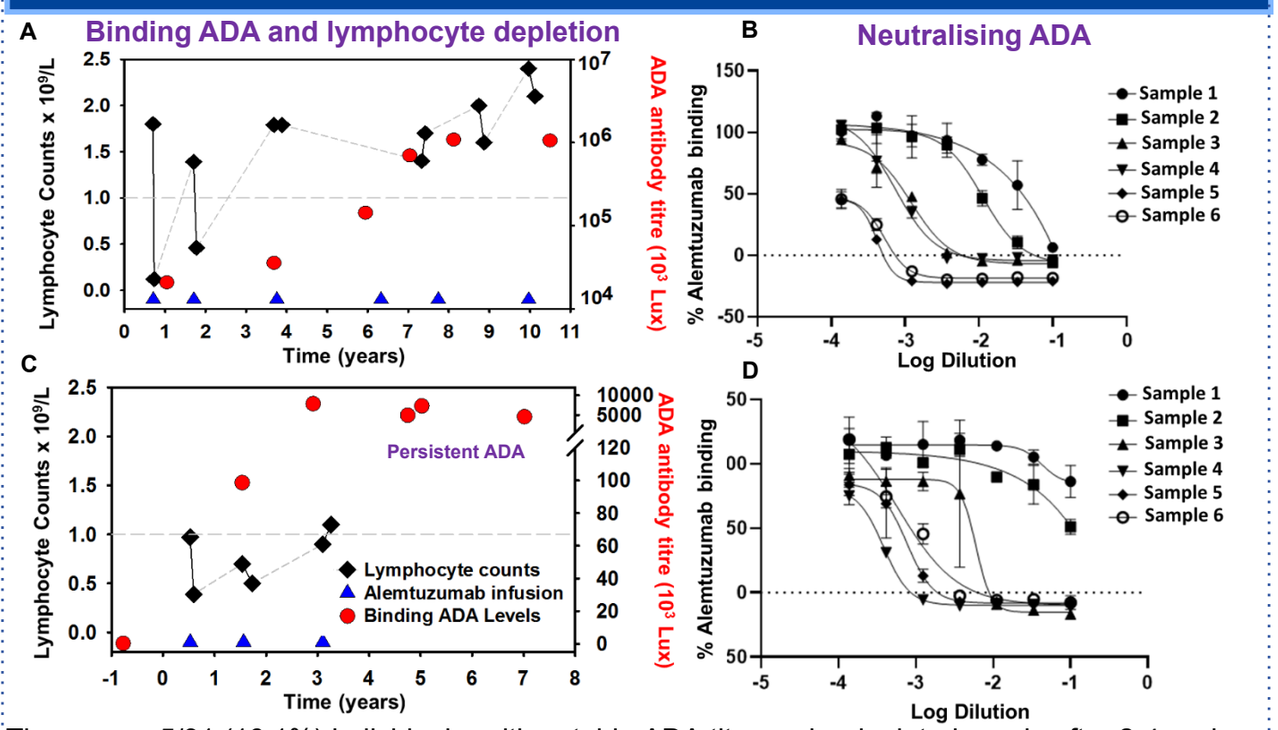


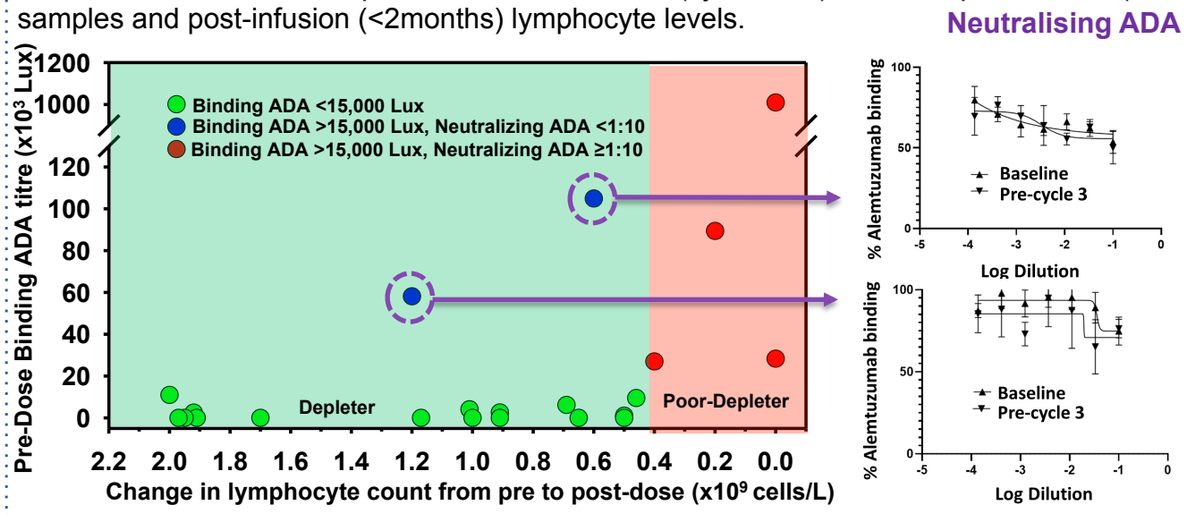
Figure 6. Development of ADA can be associated with inhibition of lymphocyte depletion in some individuals treated with alemtuzumab.



There were 5/31 (16.1%) individuals, with notable ADA titres, who depleted poorly after 2-4 cycles. Once this occurred, lymphocyte depletion failure was maintained (Figure 6: A&B = 1 individual who received 6 alemtuzumab infusion cycles, C&D = 1 individual who received 3 infusion cycles)

Figure 7. Pre-dose ADA titres can be associated with post-dose lymphocyte depletion

There were 22 serum samples from 19 individuals (cycles 1-4) who had pre-infusion (<2months) samples and post-infusion (<2months) lymphocyte levels.



Stratification according to binding and neutralizing ADA titre appears to identify poor-lymphocyte depleters. These individuals may be more likely to fail treatment.

Conclusions

- Alemtuzumab-specific ADA seem to be clinically relevant for some individuals.
- Lack of lymphocyte deletion may be a prognostic feature for future disease activity and treatment failure.
- Monitoring of ***pre-dose ADA titre*** and ***post-dose lymphocyte depletion*** may help inform whether to re-treat or switch to another disease modifying treatment. This may provide risk:benefit improvements and help in the cost-effective treatment of MS.

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Disclosures GS, has nothing to declare. ASK has filled patents on the assay technology. SG, GG, DB, NPR and ET have received consultancy, speaker honoraria or research funds from Sanofi-Genzyme within the past 3 years. Others (multiple) are considered irrelevant.

References: 1. Coles et al. N Eng J Med 2008 359:1786, 2. Cohen et al. Lancet 2012 350:1819. 3. Jacobs et al. Mult Scler 2018 24(S2): 302. 4. Somerfield et al. J Immunol 2010; 185:763. Somerfield et al. J Immunol 2010; 185:763. 5. Baker et al. JAMA Neurol 2017;74:961. 6. Dubuisson et al. Immunol 2018; 154:253.