



Randomized Placebo Controlled Trial of IVIG in autoimmune LGI1/CASPR2 epilepsy

Running Head: LGI1/CASPR2-antibody associated epilepsies

Authors:

Divyanshu Dubey MD^{1,2,3}, Jeffrey Britton MD^{1,3}, Andrew McKeon MD^{1,2,3}, Avi Gadoth MD^{1,3}, Anastasia Zekeridou MD^{1,2,3}, Sebastian A Lopez Chiriboga MD^{1,3}, Michelle Devine MD^{1,3}, Jane H. Cerhan, PhD¹, Katie Dunlay BS^{1,3}, Jessica Sagen MA^{1,3}, Melanie Ramberger PhD⁴, Patrick Waters PhD⁴, Sarosh R Irani MD⁴, Sean J. Pittock MD^{1,2,3}

¹Department of Neurology and Immunology, Mayo Clinic, Rochester MN

²Department of Laboratory Medicine and Pathology Mayo Clinic, Rochester MN

³Center MS and Autoimmune Neurology, Mayo Clinic, Rochester MN

⁴Oxford Autoimmune Neurology Group, Nuffield Department of Clinical Neurosciences, University of

Oxford, Oxford, UK

Corresponding Author Information:

Name: Dr. Sean Pittock

Address: Mayo Clinic, Department of Neurology, 200 First Street SW, Rochester, MN 55905

Email: Pittock.Sean@mayo.edu; Phone: 507-284-2511

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Abstract

Objective: Drug-resistant seizures are common in patients with Leucine-rich, glioma-inactivated-1 (LGI1) and contactin-associated-protein-like-2 (CASPR2)-IgG associated encephalitis. We performed the first randomized double-blind placebo-controlled trial to evaluate efficacy of intravenous immunoglobulin (IVIG) in reducing seizure frequency.

Methods: Our enrollment goal was 30 LGI1/CASPR2-IgG-seropositive adult patients with ≥ 2 seizures per-week. Patients were randomized to receive IVIG (0.5g/kg day-1, 1g/kg day-2, 0.6g/kg week 3 and 5) or volume-matched intravenous normal saline. Following the blinded-phase, the non-responders in the placebo group received IVIG. The primary clinical outcome was 50% reduction in seizure frequency from baseline to 5 weeks.

Results: After enrollment of 17 patients (LGI1-IgG, 14; CASPR2-IgG, 3) over 34 months the study was terminated due to slow enrollment. Six of eight patients in the IVIG group were responders compared to two of nine in the placebo group ($p=0.044$, OR 10.5, 95% CI 1.1 to 98.9). For the LGI1-IgG seropositive subgroup: 6 of 8 patients in the IVIG group were responders, compared to zero of 6 in the placebo group. Two LGI1-IgG seropositive patients receiving IVIG, but none receiving placebo, were seizure free at the end of the blinded-phase. Four of the six patients entering the open-label IVIG arm reported $\geq 50\%$ reduction in seizure frequency. There were no correlations with LGI1/CASPR2-IgG1-4 subclasses.

Interpretation: Superiority of IVIG to placebo reached statistical significance for the primary end-point for all patients and the subset with LGI1-IgG. These results have to be interpreted with the caveat that the study did not reach its originally selected sample size.

Introduction

Seizures are a common manifestation of autoimmune encephalitis and paraneoplastic disorders.¹ A considerable minority of patients with focal epilepsy of unknown etiology have neural specific antibodies,^{2,3} with voltage-gated potassium channel complex (VGKC) antibodies as a common serological biomarker among these reported cases.⁴⁻⁶

VGKC IgG were initially reported in association with neurological autoimmunity in 2004.⁷ However, discovery of autoantibodies against the extracellular domains of Leucine-rich, glioma inactivated-1 (LGI-1)⁸ and contactin-associated protein-like 2 (CASPR2)⁹ facilitated a change in our understanding of clinical implications of VGKC antibodies.^{10,11} LGI-1 IgG is typically associated with seizures and/or memory deficits among older adults whereas CASPR2 IgG seropositive cases predominantly have peripheral nervous system involvement (neuromyotonia, myokymia or dysautonomia).^{12,13} However, some CASPR2 IgG seropositive patients present with epilepsy and/or encephalopathy as the primary neurological manifestation.^{9,12} Both conditions are male predominant and affect patients in later life. Currently, the presence of VGKC IgG in the absence of LGI1 and/or CASPR2 IgG seropositivity is not considered to be a specific biomarker of neurological autoimmunity, and such patients are typically not responsive to immunotherapy and do not carry the strikingly robust HLA associations seen in patients with LGI1 or CASPR2-IgG.^{10-12,14,15}

Management of autoimmune epilepsy currently centers on immunotherapies.¹⁶ There are clear data supporting a variety of immunotherapies over AEDs.¹⁶⁻¹⁸ However, the current immunotherapy evidence base is limited to retrospective case-series with largely retrospective data collection and to expert opinions.^{7,13,18-23} To date, there have been no randomized control trials evaluating the efficacy of immunotherapy in autoimmune epilepsy.

Designing a clinical trial in autoimmune epilepsy is fraught with many specific and generic challenges. Firstly, the heterogeneity of clinical presentation makes a unifying outcome measure

difficult. Secondly, outcome measures such as brain magnetic resonance imaging (MRI),^{19, 24, 25} brain positron emission tomography (PET), formal neuropsychological assessment, and seizure diaries have not been validated. Thirdly, there remain difficulties in establishing the diagnosis due to limited clinician recognition and difficult logistics, costs and limited scalability of serologic testing. Fourthly, study size is limited by the rarity of the condition^{23, 26} and the potential rates of drop out in the event of complete recovery or suspected adverse events. Furthermore, due to increased recognition of the importance of early immunotherapy among neurologists,^{7, 16} most physicians will treat the patients acutely rather than delaying treatment to allow enrollment in a randomized control clinical trial.

Despite these limitations, there is a clear need for a randomized clinical trial evaluating efficacy of immunotherapy in the setting of LGI1 or CASPR2 IgG associated autoimmune epilepsy. The existing data is biased due to lack of a comparator arm and no reported placebo-treated outcomes. In the absence of class I or class II evidence, most patients or physicians have difficulty in getting approval for insurance coverage of immunotherapy costs especially intravenous immunoglobulin (IVIG). Thirdly, some studies have suggested a self-limiting nature, albeit with more limited recovery, of some of the LGI1-IgG associated epilepsies, further necessitating a placebo-controlled study assessing efficacy of immunotherapy in the clinical outcomes of these patients.²⁷

We designed a randomized placebo controlled clinical trial evaluating efficacy of IVIG in LGI1 and/or CASPR2 IgG associated autoimmune epilepsy. We hypothesized that IVIG would be more efficacious than placebo in achieving 50% reduction in seizure frequency.

Methods

Participants

Placebo was the chosen comparator and a maximum possible duration of 5 weeks on placebo was determined acceptable following ethical consideration and recognition that no approved

therapies nor controlled trials had been performed to establish the risk/benefit profile of off-label medications in LGI1/CASPR2 IgG associated autoimmune epilepsy. The trial protocol (ClinicalTrials.gov registration number NCT02697292) and supporting documentation were approved by the Mayo Clinic institutional review board (IRB# 15-005649).

Between February 19, 2016, and December 6, 2018, patients were identified through the Mayo Clinic Neuroimmunology Laboratory service line testing and directly recruited into a randomized double-blind placebo-controlled trial at Mayo Clinics in Rochester, MN, following consent of the patient and managing physician. Inclusion and exclusion criteria are summarized in Supplementary Table 1. Patients provided written informed consent at enrollment.

Antibody testing

Radioimmunoprecipitation assays were performed to detect VGKC IgG. All positive sera and cerebrospinal fluid (≥ 0.03 nmol/l)¹³ were tested for LGI1-IgG and CASPR2-IgG specificities using a transfected cell based immunofluorescence assay (CBA; EUROIMMUN, Lübeck, Germany). CSF was tested undiluted and serum at 1:10 dilution. Other neural specific antibodies which are a part of the Mayo Neuroimmunology Laboratory's comprehensive neural autoantibody evaluation were also tested as previously described.²⁸

LGI1 and CASPR2 IgG subclass quantification

Human embryonic kidney (HEK) 293 cells were transiently transfected with membrane-tethered LGI1 or CASPR2 C-terminally fused to EGFP.^{12, 29} After 24-48 hours, cells were detached and mixed with untransfected HEK293T cells in a ~1:1 ratio. Next, cells were incubated with serum/CSF dilutions for 30 minutes at room temperature. After two washing steps, a single mouse anti-human secondary antibody (IgG1-3: AF647, IgG4: PE, Southern Biotech [IgG1 Hinge-Alexa Fluor® 647 (9052-31); IgG2 Fc-Alexa Fluor® 647 (9070-31); IgG3 Hinge-Alexa Fluor® 647 (9210-31); IgG4 Fc-PE (9200-09)]) was incubated for 30 minutes at room temperature. Subsequently, cells were washed once with buffer containing DAPI (4',6-

diamidino-2-phenylindole), once without DAPI, and analysed on an Attune NxT flow cytometer. The serum dilutions used for LGI1 IgG1-3 was 1:50 and LGI1 IgG4 was 1:200 (normalised to 1:50). The serum dilutions used for CASPR2 IgG1-3 was 1:100; and CASPR2 IgG4 was 1:400-1:8000 (normalised to 1:100). The CSF dilution used for all samples was 1:2.

The antibody levels were then calculated by subtracting the median IgG fluorescence of the GFP negative from GFP positive cells (to generate delta medians of the fluorescence intensity HEK/single cells/viable/GFP positive – HEK/single cells/viable/GFP negative) and normalized to calibration beads (Quantum™ Simply Cellular® microspheres, Bangs Laboratories) resulting in Antibody Binding Capacities (ABC) of the cells. The cut-off was calculated for each subclass individually using 9-10 healthy control serum samples (mean+3SD).

Human leukocyte antigen (HLA) analysis

All patients (except patient #6) were genotyped for HLA-DRB, using PCR-sequence specific primers (SSP).¹⁴

Procedures

Once consent was obtained and it was determined that the participant met all inclusion criteria, the participants were enrolled into the study. Patients were randomized into two groups (A vs B) in a 1 to 1 ratio using a simple randomization method by utilizing JMP Pro 13 software (M.M.). The details of randomization table were unknown to all researcher who enrolled the participants (J.S.) and the clinicians who evaluated the patients. The randomization code (A was active arm and B was placebo arm) was kept sealed until the completion of the blinded phase of the study. Patients in the active arm received IVIG and patients in control arm, received placebo for 5 weeks. During the period between enrollment and unblinding, no changes were made to their anti seizure medication (ASMs) regimen. All patients were instructed to keep a seizure diary documenting the seizure frequency and seizure semiologies, spouses and family members were also asked to assist. Following completion of 5 weeks of treatment with either IVIG or placebo participants returned to Mayo Clinic for evaluation.

Sample size of 30 (i.e. 15 per group) was calculated based on preliminary observations of 70% efficacy in the experimental group vs 10% anticipated as the placebo effect (Power 89% and significance level alpha of 5% [Nquery advisor software version 7.0]). We proceeded with the target sample of 30 due to budgetary restrictions, limited IVIG availability for the research study and feasibility of patient enrollment for a rare disease within 2 years (target study duration at the time of initiation).

Blinded phase of the trial (all infusions given at Mayo Clinic infusion center)

Active Comparator: Intravenous Immunoglobulin (IVIG) (Gamunex-C ®) Group

The IVIG dose was determined based on ideal weight with a dose not to exceed 80 grams. Patients received IVIG (0.5g/kg) on day 1 [week 1 day 1], then received IVIG (1g/kg not exceeding 80 gram) on day 2 [week 1 day 2]. Patients also received 500 ml normal saline before and after the higher dose infusion of 1g/kg. Then once every 2 weeks patients received 0.6g/kg IVIG [week 3 and week 5] for 2 more infusions. After completion of all 4 infusions, all patients were again evaluated at Mayo Clinic and unblinded.

Placebo Comparator: Placebo/Normal Saline Group

Patients received volume matched placebo on day 1 [week 1 day 1], then they received placebo on day 2 [week 1 day 2]. Then once every 2 weeks patients received placebo [week 3 and week 5] for 2 more infusions. After completion of all 4 infusions, all patients were again evaluated at Mayo Clinic and unblinded.

Open label of arm of the trial

The patients in the placebo group with persistent symptoms received IVIG in an open label fashion in their homes through Option Care home infusion services. Patients received IVIG (0.5g/kg) on day 1, and then they received IVIG (1g/kg not exceeding 80 gram) on day 2. Patients also received 500 ml normal saline before and after the higher dose infusion of 1g/kg.

Then once every 2 weeks patients received 0.6g/kg IVIG for 2 more infusions in their homes through Option Care. Patients were evaluated at Mayo Clinic following completion of all 4 infusions.

Evaluations

During the clinic visit (Baseline and 5 weeks), complete neurological examination and formal cognitive assessment was performed for each patient. Electroencephalography data were obtained during initial visit (Supplementary Table 2). Estimation of seizure frequency prior to enrollment was based on history provided by the patient and family at the initial visit. All patients and family members were instructed to maintain seizure dairies. Data in the seizure dairies were corroborated with history taken at the time of the clinical visits. Where a discrepancy was identified, the frequency reported at the time of clinical visits was used. Maximum seizure frequency over the last 3 days prior to clinic evaluation was established utilizing patient report and/or documentation in seizure dairy. Cognitive status was measured using the Repeatable Battery for the Assessment of Neuropsychological Status [RBANS].³⁰ The RBANS is a well-standardized battery which includes twelve subtests with widely-used task formats tapping five major cognitive domains. It includes alternate forms to minimize practice effect. For patients who entered into the open label IVIG arm, the above testing was performed following completion of all 4 infusions. The study coordinators completed adverse event questionnaires as appropriate (J.S. and K.D).

Clinical outcome

The primary clinical outcome was reduction in seizure frequency by $\geq 50\%$ during the follow up visit following completion of the blinded phase at 5 weeks. Secondary outcome measures included RBANS.³⁰ The proportion of patients with improvement in seizure frequency or seizure freedom were also calculated. Withdrawal from the blinded phase of the study due to worsening of seizure frequency and cognitive status was considered as an intervention failure.

Statistical analysis

Descriptive summaries are reported as median (range) for continuous variables and frequencies (%) for categorical variables. Comparisons of categorical and continuous outcomes of interest were done with Fisher's exact test and Mann-Whitney U tests, respectively. One-sided statistical test was utilized to demonstrate improved efficacy of IVIG compared to placebo. Graphical analysis illustrated comparison of responder rate and seizure freedom between IVIG and placebo arms. All analyses were done in SPSS (IBM, version 23) and JMP® Pro 13. Odds ratio estimates are not reported when cells with 0 are observed.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Patient disposition

Between February 19, 2016, and December 6, 2018, the physicians of 594 LGI1/CASPR2-IgG positive patients were contacted by telephone regarding possible enrollment. Among these, the majority of patients (570) either did not meet the criteria (Supplementary Table 1) or were unable to travel to Mayo Clinic, Rochester, MN for evaluation (Figure 1). Twenty four patients were evaluated at Mayo Clinic for possible enrollment but seven failed the screening process. Since the planned duration of the study was 2 years and a decline in patient enrollment occurred in 2018 [none between July to December 2018]), the investigators terminated the study before the statistically based sample size target of 30 was reached.

Baseline demographic and clinical characteristics

At the time of study termination, seventeen patients (12 men; 5 women) had undergone randomization to receive either placebo or IVIG. None of the patients enrolled in the study were on any immunosuppressive medications at the time of enrollment and only one patient had received any immunotherapy prior to enrollment: a 3 day course of IVIG >8 months ago. As expected, at baseline 11 of 17 reported more than 10 seizures daily and 6 of 17 experienced over 60 seizures per day. The majority (n=14, 82%) were seropositive for LGI1-IgG, and three patients had CASPR2-IgG. None were positive for both antibodies. The demographic and clinical characteristics of all 17 patients are outlined in Supplementary Table 2. Baseline characteristics are summarized separately for each treatment arm in Table 1. There was no significant difference in demographic and clinical characteristics, including duration of symptom onset to time of enrollment between the two study arms. One LGI1-IgG seropositive patient (patient #9) required hospitalization due to significant deterioration of her neurological conditions with increasing seizures (≥ 3 seizures per hour) and worsening cognitive status. She was unblinded at 3 weeks and found to be in the placebo arm.

All LGI1-IgG seropositive patients tested carried one copy of HLA-DRB1*07:01 and all CASPR2-IgG seropositive patients carried one copy of HLA-DRB1*11:01. In addition, one patient with LGI1-antibodies also carried DRB1*11:01 (patient #4).

Efficacy

Primary outcome: A higher proportion of patients in the IVIG arm experienced $\geq 50\%$ reduction in seizure frequency compared to the placebo arm at the completion of the blinded phase (75% [6 of 8 patients] vs 22% [2 of 9 patients], $p=0.044$, Odd's ratio [OR] 10.5, 95% Confidence Interval [CI] 1.1 to 98.9, Table 3, Figure 2A and 2B). Among the LGI1-IgG seropositive subgroup who received IVIG, the 50% responder rate was 75% [6 of 8 patients], compared to 0 [0 of 6 patients] in those in the placebo arm. Two LGI1 IgG seropositive patients receiving IVIG, but none in the placebo arm, were seizure free at the end of the blinded phase. All CASPR2 IgG patients ($n=3$) were randomized to receive placebo: one reported $\geq 50\%$ reduction in seizure frequency and another reported seizure freedom at 5 week visit.

Secondary Outcome: The median change in RBANS total-score among LGI1-IgG seropositive patients showed a trend towards improvement for the IVIG group compared to placebo (3 [0 to 13] vs -1 [-12 to 6], $p=0.077$, 95% CI 0.84 - ∞) (Table 3). Eight of eight LGI-1 IgG seropositive patients in the IVIG group showed zero change or increase in RBANS total-score percentiles, compared with 2 of 5 in the placebo group (Figure 3). There were no significant differences in the RBANS index scores for immediate memory, visuospatial/constructional, language, attention, or delayed memory (Table 3). Although there was no statistical difference between IVIG and placebo in the combined cohort, a trend towards a favorable cognitive outcome was observed in the IVIG arm (100% [8 out of 8] vs 40% [2 out of 5], $p=0.100$, 95% CI 0.45 - ∞).

Open Label Arm:

Seven of the nine patients who initially received placebo were subsequently administered open label IVIG.

Two patients did not enter the open label arm of the study: one LGI1 IgG patient who was unblinded prior to completion of the 5 week regimen and another CASPR2 IgG patient had near complete resolution of symptoms on placebo. One CASPR2 IgG patient, who was seizure free at week 6 follow up, received IVIG for management of ataxia attributed to his condition.

Six patients with persistent seizures who were non-responders at week 5 on placebo during the blinded phase of the study entered the open label IVIG arm of the study. Among these, four patients reported $\geq 50\%$ reduction in seizure frequency during the week 11 evaluation. However, none achieved seizure freedom. Furthermore, five of the seven patients (including the CASPR2-IgG seropositive patient with ataxia but no seizure, Supplementary Table 2) who received IVIG in the open label part of the study showed stabilization or improvement of RBANS percentiles.

LGI1 and CASPR2 IgG subclasses

All LGI1 and CASPR2 autoimmune epilepsy cases had IgG4 as the predominant subclass (serum). Both the LGI1 IgG seropositive patients who achieved seizure freedom during the blinded phase of the study had undetectable LGI1-IgG1 antibodies. However, there was no significant difference in % IgG1-4 subclasses among the two LGI1 patients who did not have a favorable response to IVIG compared to others (Supplementary Table 2). Proportion of LGI1 IgG1-4 subclass did not differ significantly based on seizure semiology. Among eight patients with fasciobrachial dystonic seizures (FBDS), four had undetectable or barely detectable (0.2%) LGI1-IgG1 antibody subclass.

Treatment Tolerance and Toxicity

One LGI1-IgG (patient #9) seropositive patient was withdrawn from the study due to progressive decline in her neurological status. Three weeks into the study period, she became stuporous and non-responsive to questions or commands with frequent witnessed seizures. She was taken to the local emergency department. MRI brain obtained demonstrated bilateral limbic encephalitis. Due to significant clinical deterioration, she was unblinded and found to be in the placebo arm.

Two falls without serious injuries were reported during the blinded phase of the study; one in a CASPR2-IgG seropositive patient receiving placebo and another in a LGI1-IgG seropositive patient receiving IVIG, likely secondary to FBDS. No other drug-related adverse effects were reported during the blinded phase of the study.

During the open label phase one patient developed a mild to moderate headaches attributed to IVIG infusion, and another patient developed generalized body rash. The skin rash persisted even after completion of IVIG regimen, and was later attributed to levetiracetam.

Immunotherapy post blinded/open label IVIG

All patients were recommended to continue immunotherapy following completion of the trial period (Supplementary Table 2). Neurology assessment follow up details were available on fifteen cases. Among these, three patients had achieved seizure freedom prior to clinical trial completion. Nine of the remaining twelve patients received high dose intravenous corticosteroids along with additional immunotherapies (Mycophenolate mofetil [n=2], plasmapheresis [n=1], oral prednisone [n=5], IVIG every 2 weeks [n=2]) and AEDs. Seizure freedom was achieved in 56% cases (n=5/9, Supplementary Table 2). The median duration of intravenous corticosteroids infusion to seizure freedom was 1 month (range 0.5 to 4 months). One patient who received IVMP (1g daily for 5 days) followed by oral prednisone 60 mg daily developed acute psychosis and hyperglycemia (measured serum glucose 600 mg/dl). He was admitted to a hospital and corticosteroids were held. Subsequently, he was treated with rituximab (1000 mg, 2 infusion 2 weeks apart), IVIG (0.4g/kg every week for 12 weeks), plasmapheresis (5 sessions) and azathioprine (2 mg/kg/day) leading to seizure freedom. Two patients achieved seizure freedom following treatment with IVIG (1g/kg every 2 weeks for 12 weeks) and mycophenolate mofetil (1000 mg BID), and did not receive corticosteroids. The patient in the placebo arm of the study who was unblinded at 3 weeks due to clinical deterioration, received rituximab (1000 mg) and IVIG (2g/kg over 5 days) with partial clinical improvement (more conscious and communicative).

Discussion:

This study represents the first double-blind randomized placebo controlled clinical trial of any immunotherapy in antibody-mediated autoimmune epilepsy. The data support the use of IVIG and present the first placebo data in this condition. Due to limited enrollment and early termination, the study did not reach its statistically based sample size and was underpowered. Nevertheless, among the randomized patients, administration of IVIG was associated with favorable responder rate, especially among patients with LGI1-IgG, compared to placebo. Furthermore, LGI1 IgG seropositive patients who received IVIG also had a significant association with stabilization/improvement of RBANS cognitive scores. The efficacy of IVIG was further supported by the open label arm of the study in which the majority cases demonstrated $\geq 50\%$ reduction in seizure frequency following 6 weeks of IVIG. Additionally, in the setting of autoimmune epilepsy, IVIG administration showed a promising safety profile which compares with the frequent side effects seen with AEDs in these patients.^{14, 17}

This study also highlights the morbidity associated with autoimmune epilepsies, with the majority of patients (11/17) reporting more than 10 seizures daily. High seizure frequency and co-existing cognitive deficits limited precise seizure counting in some patients.^{31, 32} However, corroborative history during clinical visits by the non-responders and their family members denoting similar or increased seizure frequency estimates during follow up clinic evaluation helped with determination of efficacy.

A potential limitation of the study is that the median duration of symptom onset to enrollment was 3 months longer for placebo group compared to the IVIG group (Table 1), however this time difference was not found to be statistically significant.

Despite the significant improvement in seizure frequency associated with IVIG, only two patients achieved seizure freedom at the end of the blinded phase of the study. Additionally, 63% (5/8) of the patients continued to have frequent (≥ 5) daily seizures. High dose intravenous

corticosteroid infusion was initiated for 9 of 12 patients following completion of blinded or open label IVIG infusion, with the majority attaining seizure freedom (Supplementary Table 2). This modest effect appears to contrast with more dramatic effect in patients treated with steroids as a consistent medication, often alongside PLEX / IVIG.^{17, 29}

Prior studies have reported limited efficacy of IVIG in Musk IgG myasthenia³³ and Neurofascin 155 IgG associated CIDP, both IgG4-dominant diseases.³⁴ Indeed, patients with LGI1- and CASPR2-IgG both show a IgG4 predominance,^{29, 35} and the ratio of IgG1:4 has been reported to correlate with clinical outcomes in LGI1-antibody associated diseases.^{13, 29} Therefore, we evaluated the proportion of IgG1-4 subclasses (Supplementary Table 2). All the LGI1 or CASPR2 IgG seropositive cases were IgG4 subclass predominant, including the two LGI1 IgG seropositive patients who did not have a favorable response to IVIG during the blinded phase. Interestingly, two LGI1-IgG seropositive patients receiving IVIG who achieved seizure freedom during the blinded phase of the study had undetectable LGI1-IgG1 antibodies. Therefore, at least among patients with LGI1/CASPR2 autoimmune epilepsy, the IgG4 predominance doesn't appear to limit IVIG efficacy.

Consistent with their autoantibody profiles and phenotypes,¹⁴ patients carried the established HLA associations with LGI1-IgG and CASPR2-IgG seropositivity. LGI1-IgG or CASPR2-IgG patients with variable responses to IVIG and different seizure semiologies did not have variability in HLA-DRB genotypes. Suggesting that HLA association do not distinguish between outcomes or sub-phenotypes.

This study is especially important for patients in whom corticosteroids are relatively contraindicated, or for those refractory to corticosteroid therapy. Additionally, for management of some cases with high neurological morbidity, co-administration of both IVIG and IVMP, or addition of plasma exchange, may be considered.

Accepted Article

In this randomized control trial we limited our assessment to patients with epilepsy associated with LGI1/CASPR2 autoimmunity. Patients with other clinical presentations LGI1/CASPR2 IgG associated autoimmunity such as cognitive impairment and/or peripheral hyper-excitability syndromes without co-existing seizures were excluded. Therefore, efficacy of IVIG among LGI1 or CASPR2 IgG seropositive patients with inclusion of broader clinical spectrum may need to be analyzed in the future. Nearly all patients in our study had not received any other immunotherapy prior to enrollment in the clinical trial. Future studies should also compare safety profile and efficacy of IVIG to other first-line immunotherapies such as high dose corticosteroids or plasma exchange. Its efficacy as an add-on therapy to corticosteroids or second line agents should also be evaluated. Furthermore, utility of scheduled or monthly IVIG to prevent disease relapse should be assessed in a long-term study.

These data support consideration of IVIG as a therapeutic option in the acute management of autoimmune epilepsy due to LGI1 IgG, and provide preliminary data supportive of the development of larger, multicenter randomized controlled studies to evaluate this rare treatable condition.

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Author Contributions

SJP, JB and AM contributed to concept and design of the study.

All authors contributed to acquisition and analysis of the data.

DD, MD and SJP contributed to drafting the text and preparing the figures.

All authors contributed to critical revision of the manuscript.

Potential Conflicts of Interest

AG, AZ, SLC, JC, KD, JS, MR, PW have no conflicts to report.

DD has received research support from Grifols (the company involved manufactures a drug used in the study).

JB received research support from Grifols.

AM has consulted for Grifols.

SRI is a coapplicant and receives royalties on patent application WO/2010/046716 (U.K. patent no., PCT/GB2009/051441) entitled 'Neurological Autoimmune Disorders'. The patent has been licensed to Euroimmun AG for the development of assays for LGI1 and other VGKC-complex antibodies.

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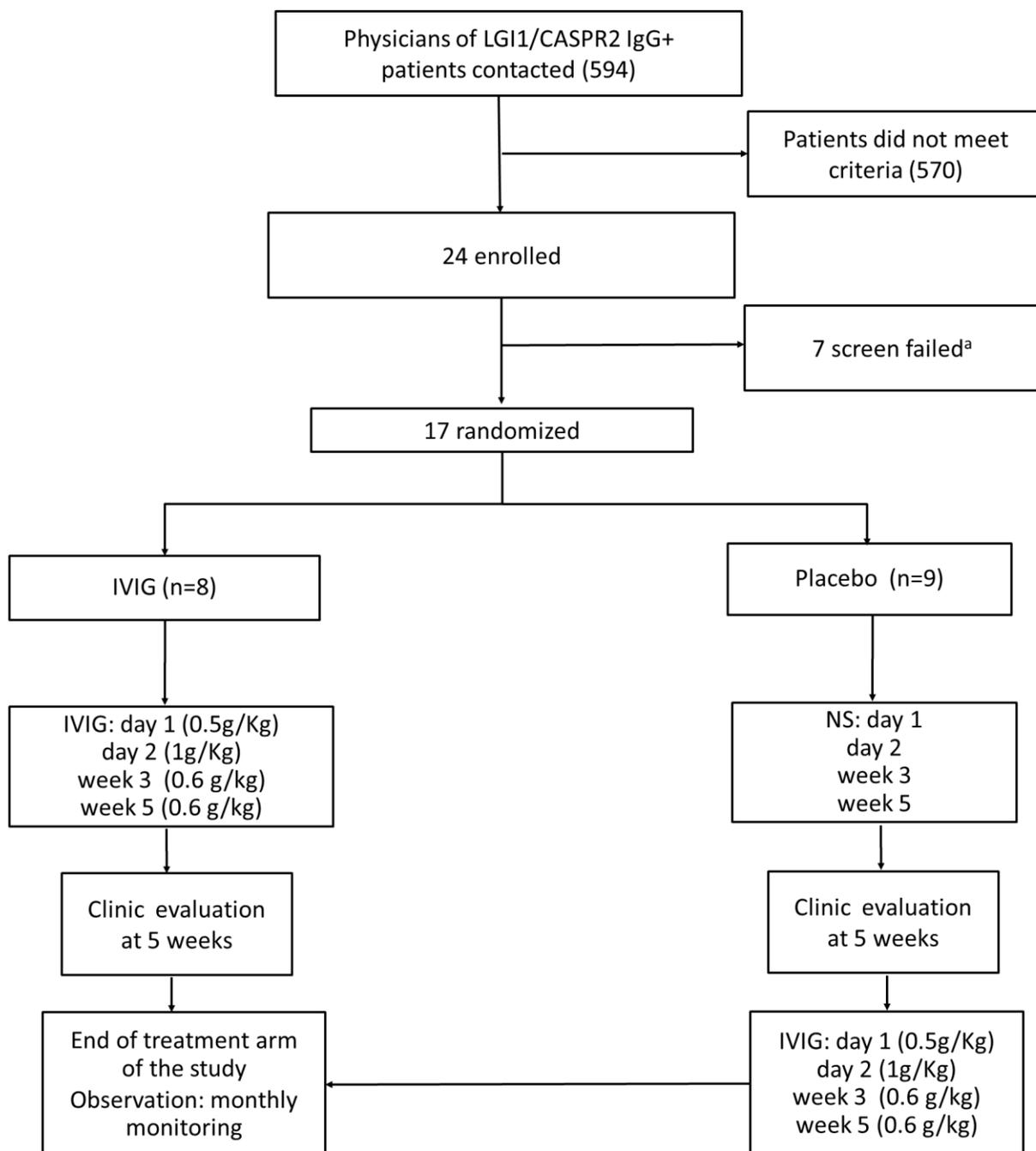
Figure 1: Patient enrollment summary and trial design.

Key: IVIG intravenous immune globulin, NS normal saline.

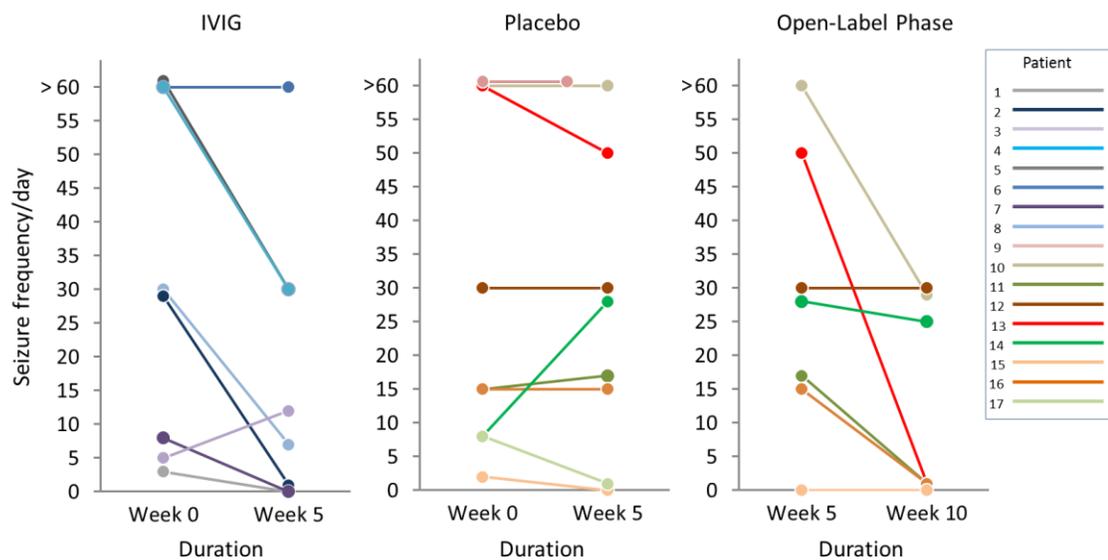
Figure 2 (A, B): Clinical outcomes. Change in seizure frequency following administration of placebo or IVIG in the blinded and open label phase of the study (panel A). Proportion of the patients achieving $\geq 50\%$ reduction in seizure frequency, seizure frequency improvement, seizure freedom and stabilization or improvement in cognitive measure in the two study arms during the blinded phase of the study (panel B).

Key: IVIG intravenous immune globulin, RBANS Repeatable Battery for the Assessment of Neuropsychological Status, bar representing significant difference ($p < 0.05$), ^aReason for failed screening (Seven patients: patient refused due to concern of receiving placebo, $n=2$; patient refused to participate in the study due to inability to return for required follow up visits, $n=1$; change in regimen of anti-epileptic drugs < 1 week prior to enrollment, $n=1$; seizure frequency < 2 per week, $n=1$; patient refused to keep a seizure diary, $n=1$; IgA deficiency, $n=1$)

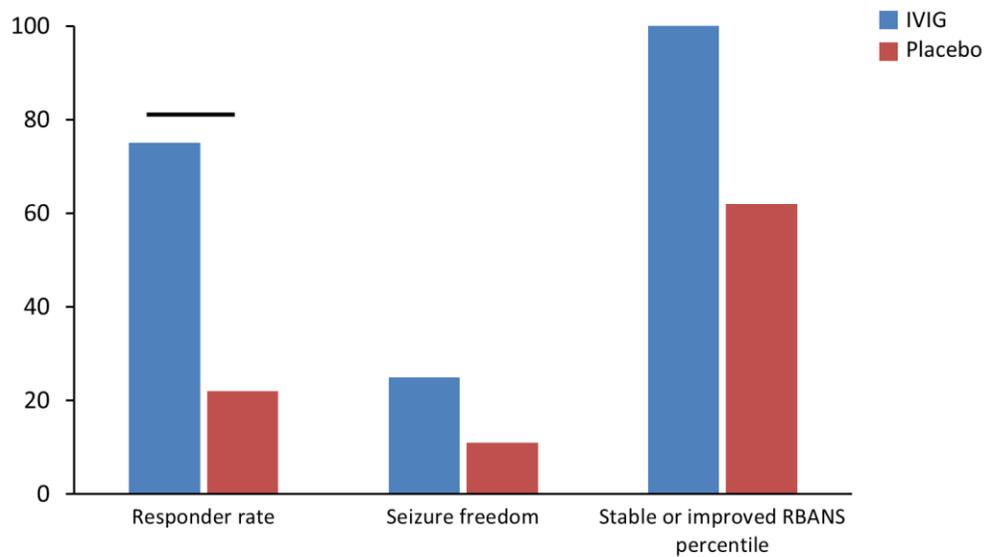
Figure 3. Cognitive outcomes among LGI1 IgG seropositive cases. Change in RBANS percentiles among LG1 IgG seropositive cases following administration of placebo or IVIG in the blinded and open label phase of the study. Key: IVIG intravenous immune globulin, LGI1 leucine rich glioma inactivated 1, RBANS Repeatable Battery for the Assessment of Neuropsychological Status



A



B



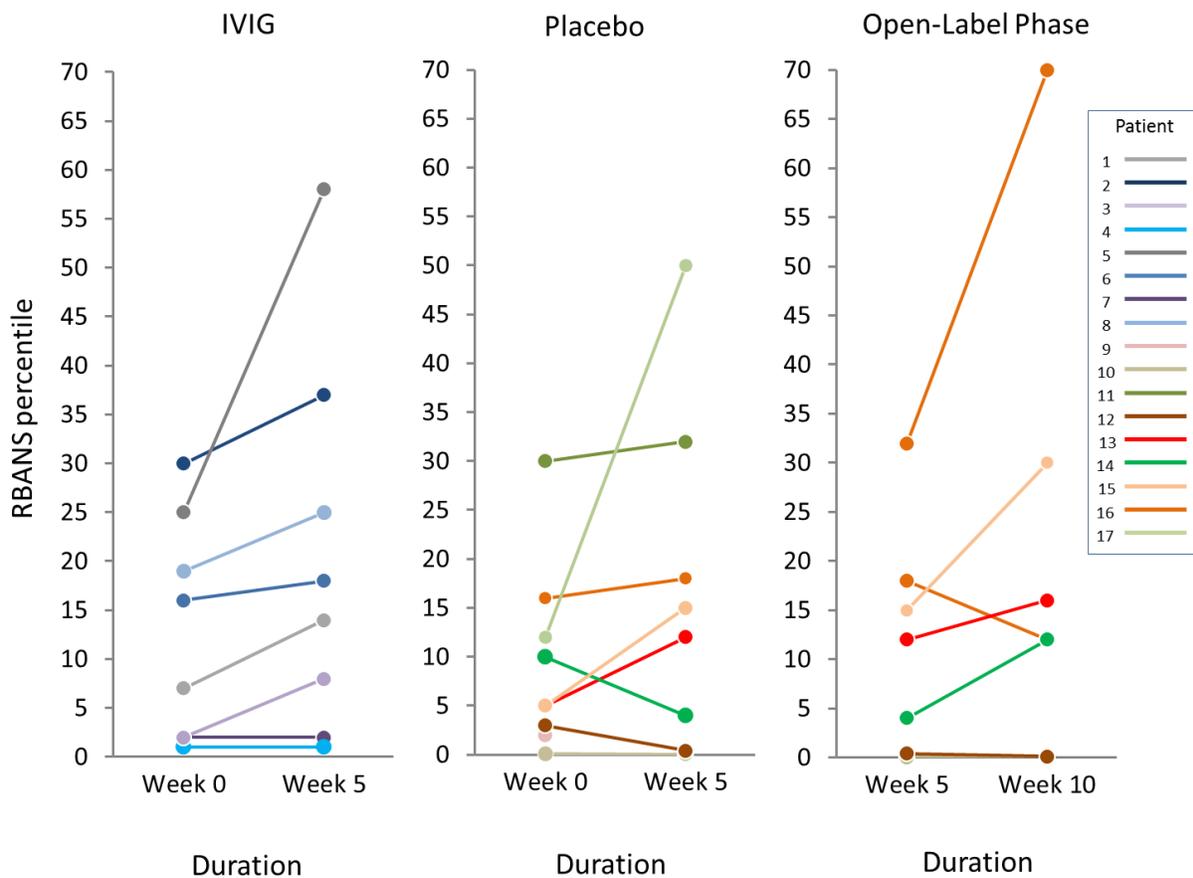


Table 1: Clinical and demographic characteristics and the two groups.

	IVIG (n=8)	Placebo (n=9)	P-value
Median age (range)	70 (66-77)	70 (59-77)	0.773
Male (%)	6 (75)	6 (67)	0.707
LGI1 IgG (%)	8 (57)	6 (43)	0.206
CASPR2 IgG (%)	0	3 (100)	0.206
Median duration of symptom onset to enrollment, months (range)	5 (1-12)	8 (1-13)	0.359
≥5 seizures every day (%)	4 (50)	5 (55)	1.000
Facio-brachial dystonic seizures (%)	4 (50)	5 (66)	1.000
Secondarily generalized seizures (%)	1 (13)	3 (33)	0.576
Cognitive dysfunction (%)	8 (100)	8 (89)	0.331
Mesial temporal hyperintensity on MRI (%)	1 (13)	4 (44)	0.294
ASMs (%)	3 (38)	5 (56)	0.457
Levetiracetam (%)	0	4 (44)	0.637
Sodium channel blocking ASMs : ZNS, OXC, LMT, LCM (%)	3 (38)	2 (22)	0.620

Key: ASM antiseizure medication, CASPR2 contactin-associated protein-like 2, CBZ carbamazepine, FBDS faciobrachial dystonic seizures, LGI1 leucine rich glioma inactivated 1, LMT Lamictal, LCM lacosamidem, MRI magnetic resonance imaging, OXC oxcarbazepine, ZNS zonisamide

Table 2: Comparison of clinical outcomes and adverse effects between IVIG and placebo arms.

	LGI1 and Caspr2 IgG		
	IVIG	Placebo	P-values (OR, 95% CI)
50% seizure rate reduction, week 5 (%)	6/8 (75)	2/9 (22)	0.044 (10.5, 1.1– 98.9)
Improvement in Seizure frequency, week 5 (%)	6/8 (75)	2/9 (22)	0.044 (10.5, 1.1– 98.9)
Seizure freedom, week 5 (%)	2/8 (25)	1/9 (13)	0.453 (2.7, 0.11 – 176.6)
Improvement in FBDS frequency, week 5 (%)	2/4 (50)	0/5	0.167 (NR, 0.3- ∞)
Change in seizure semiology (%)	1/7 (14)	1/6 (17)	0.731 (0.8, 0.01, 78.4)
Stable or improved RBANS percentile, week 5 (%)	8/8 (100)	5/8 (62)	0.100 (NR, 0.45- ∞)
Adverse effects (%)	1/8 (13)	2/9 (22)	0.547 (0.5, 0.01 – 12.3)
Open label IVIG phase			
50% seizure rate reduction, week 11 (%)	-	4/6 (67)	-
Improvement in Seizure frequency, week 11 (%)	-	5/6 (83)	-
Seizure freedom, week 11(%)	-	0/6	-
Stable or improved RBANS percentile, week 11 (%)	-	4/7 (57)	-

Key: CASPR2 contactin-associated protein-like 2, FBDS faciobrachial dystonic seizures, IVIG intravenous immune globulin, LGI1 leucine rich glioma inactivated 1, NS not significant, NR not reported (Odds ratio estimates are not reported when cells with 0 are observed), RBANS Repeatable Battery for the Assessment of Neuropsychological Status, CI confidence interval

Table 3: Comparison of clinical outcomes and adverse effects between intravenous immune globulin and placebo arms of the study in the LGI1-IgG subgroup.

	LGI1 IgG		
	IVIG	Placebo	P-values (95% CI)
50% seizure rate reduction, week 5 (%)	6/8 (75)	0/6	0.009 (NR, 1.51 - ∞)
Improvement in Seizure frequency, week 5 (%)	6/8 (75)	0/6	0.009 (NR, 1.51 - ∞)
Seizure freedom, week 5 (%)	2/8 (25)	0/6	0.308 (NR, 0.14 - ∞)
Improvement in FBDS frequency, week 5 (%)	2/4 (50)	0/5	0.167 (NR, 0.254 - ∞)
Change in seizure semiology (%)	1/7 (14)	0/5	0.583 (NR, 0.02 - ∞)
Stable or improved RBANS percentile, week 5 (%)	8/8 (100)	2/5 (40)	0.035 (NR, 0.84 - ∞)
Median change immediate memory scores (range)	6.5 (-4 to 14)	-4 (-4 to 9)	0.174 (-13.4 - 2.2)
Median change in visuospatial memory score (range)	-1.5 (-9 to -11)	-9 (-27 to 19)	0.378 (-26.7 - 15.6)
Median change in language score (range)	-1 (-7 to 7)	0 (-3 to 11)	0.505 (-4.5 - 9.7)
Median change in attention score (range)	0 (-11 to 9)	0 (-12 to 6)	0.412 (-12.2 - 5.6)
Median change in delayed memory score (range)	2.5 (-9 to 35)	-8 (-47 to 13)	0.188 (-14.3 - 0.01)
Median change in total RBANS score (range)	3 (0 to 13)	-1 (-12 to 6)	0.077 (-15.9 -

			1.6)
Adverse effects (%)	1/8 (13)	1/6 (17)	0.692 (0.75, 0.03-14.56
Open label IVIG phase			
50% seizure rate reduction, week 12 (%)	-	3/5 (60)	-
Improved cognitive score, week 12(%)	-	3/5 (60)	-

Key: CASPR2 contactin-associated protein-like 2, FBDS faciobrachial dystonic seizures, IVIG intravenous immune globulin, LGI1 leucine rich glioma inactivated 1, NS not significant, NR not reported (Odds ratio estimates are not reported when cells with 0 are observed), RBANS Repeatable Battery for the Assessment of Neuropsychological Status