Cyclophosphamide Pulses Therapy after Natalizumab Discontinuation for Multiple Sclerosis: A Multicentre Study

Marco Capobianco1*, Marianna Lo Re1, Francesca Sangalli1, Lucia Moiola1, Paola Perini1, Paolo Gallo1, Maura Danni2, Leandro Provinciali3, Annamaria Repice4, Luca Massacesi5, Silvia Messina5, Francesco Patti5, Alice Laroni6, Gian Luigi Mancardi5, Eugenio Pucci5, Massimiliano Calabrese6 and Antonio Bertolotto1

1Regional Multiple Sclerosis Centre, AOU San Luigi Gonzaga Orbassano
2Department of Experimental Medicine and Clinical Neuroscience, University of Palermo
3Institute of Experimental Neurology, University Vita e Salute San Raffaele, Milano
4Multiple Sclerosis Centre, University of Padova
5Multiple Sclerosis Centre University of Ancona
6Neurofarba Department, University of Firenze
7Multiple Sclerosis Centre, University of Catania
8Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genova
9Neurology section, Department of Neurological and Movement Sciences, University Verona

Abstract

**Importance:** Natalizumab discontinuation induces the recurrence of Multiple Sclerosis (MS) disease activity: Currently no therapeutic approach has been found able to abolish disease reactivation.

**Objective:** To collect data from patients with MS switching from natalizumab to cyclophosphamide.

**Design:** Retrospective multicentre study.

**Setting:** Nine Multiple Sclerosis Centers in Italy.

**Participants:** A total of 47 patients with clinically definite RR-MS switched to cyclophosphamide after natalizumab discontinuation. Two patients were excluded from the analysis because received less than 12 natalizumab infusions. The remaining 45 patients were subdivided into two main groups: Early Treatment (period of washout between natalizumab and cyclophosphamide 1 to 3 months), Late Treatment (washout between natalizumab and cyclophosphamide higher than 3 months).

**Intervention:** Cyclophosphamide intravenous pulses after natalizumab discontinuation.

**Main outcome measure:** Number of relapses, Expanded Disability Status Scale scores, number of new T2/fluid-attenuated inversion recovery lesions and contrast-enhancing lesions on brain magnetic resonance imaging, rebound effect, adverse events.

**Results:** In the Early Treatment group, only 3/23 patients (13%) experienced a clinical relapse and only 2 out of 13 (15%) patients showed brain Magnetic Resonance Imaging (MRI) activity at 3 months, while none developed MRI activity at 6 months after cyclophosphamide introduction. In the Late Treatment Group 12/22 patients (63%) had relapses during the washout period and 4/22 (40%) after the introduction of cyclophosphamide; MRI disease activity was shown in 5/9 (56%) at 3 months and in 5/14 (36%) at 6 months after cyclophosphamide introduction.

**Conclusions and relevance:** These data show that cyclophosphamide could be able to reduce disease reactivation after natalizumab, in particular with a short washout period after natalizumab discontinuation. It can be suggested that a short period (3-6 months) of cyclophosphamide monthly pulses could be used as “re-induction” treatment in patients discontinuing natalizumab.

**Keywords:** Multiple sclerosis; Natalizumab discontinuation; Disease reactivation; Cyclophosphamide; Rebound

**Introduction**

Natalizumab is a very efficient monoclonal antibody used in Relapsing Remitting Multiple Sclerosis (RRMS) [1]. However, despite its high efficacy, natalizumab is associated with Progressive Multifocal Leukoencephalopathy (PML), a potentially fatal infection caused by the John Cunningham virus (JCV) [2].

According to the current risk stratification scheme, JCV antibody positivity, natalizumab treatment >2 years, and immunosuppressive treatment before natalizumab increase PML risk. Particularly JCV antibody-positive patients with at least one additional risk factor are at a high PML risk [3-5]. As a consequence these patients and/or neurologists often choose to discontinue natalizumab therapy. Following natalizumab discontinuation, in the majority of patients, relapses and Magnetic Resonance Imaging (MRI) activity return to pre-treatment levels, which peaks 4–7 months after the last infusion. [2,6]

The risk of return of disease activity appears to be greater in patients with high disease activity pre-natalizumab than in those with low disease activity [2].

In addition, although a unique definition does not exist, rebound phenomena have been described in some patients during natalizumab discontinuation. [7-8]

**Keywords:** Multiple sclerosis; Natalizumab discontinuation; Disease reactivation; Cyclophosphamide; Rebound

**Introduction**

Natalizumab is a very efficient monoclonal antibody used in Relapsing Remitting Multiple Sclerosis (RRMS) [1]. However, despite its high efficacy, natalizumab is associated with Progressive Multifocal Leukoencephalopathy (PML), a potentially fatal infection caused by the John Cunningham virus (JCV) [2].

According to the current risk stratification scheme, JCV antibody positivity, natalizumab treatment >2 years, and immunosuppressive treatment before natalizumab increase PML risk. Particularly JCV antibody-positive patients with at least one additional risk factor are at a high PML risk [3-5]. As a consequence these patients and/or neurologists often choose to discontinue natalizumab therapy. Following natalizumab discontinuation, in the majority of patients, relapses and Magnetic Resonance Imaging (MRI) activity return to pre-treatment levels, which peaks 4–7 months after the last infusion. [2,6]

The risk of return of disease activity appears to be greater in patients with high disease activity pre-natalizumab than in those with low disease activity [2].

In addition, although a unique definition does not exist, rebound phenomena have been described in some patients during natalizumab discontinuation. [7-8]

**Keywords:** Multiple sclerosis; Natalizumab discontinuation; Disease reactivation; Cyclophosphamide; Rebound

**Introduction**

Natalizumab is a very efficient monoclonal antibody used in Relapsing Remitting Multiple Sclerosis (RRMS) [1]. However, despite its high efficacy, natalizumab is associated with Progressive Multifocal Leukoencephalopathy (PML), a potentially fatal infection caused by the John Cunningham virus (JCV) [2].

According to the current risk stratification scheme, JCV antibody positivity, natalizumab treatment >2 years, and immunosuppressive treatment before natalizumab increase PML risk. Particularly JCV antibody-positive patients with at least one additional risk factor are at a high PML risk [3-5]. As a consequence these patients and/or neurologists often choose to discontinue natalizumab therapy. Following natalizumab discontinuation, in the majority of patients, relapses and Magnetic Resonance Imaging (MRI) activity return to pre-treatment levels, which peaks 4–7 months after the last infusion. [2,6]

The risk of return of disease activity appears to be greater in patients with high disease activity pre-natalizumab than in those with low disease activity [2].

In addition, although a unique definition does not exist, rebound phenomena have been described in some patients during natalizumab discontinuation. [7-8]

**Keywords:** Multiple sclerosis; Natalizumab discontinuation; Disease reactivation; Cyclophosphamide; Rebound

**Introduction**

Natalizumab is a very efficient monoclonal antibody used in Relapsing Remitting Multiple Sclerosis (RRMS) [1]. However, despite its high efficacy, natalizumab is associated with Progressive Multifocal Leukoencephalopathy (PML), a potentially fatal infection caused by the John Cunningham virus (JCV) [2].

According to the current risk stratification scheme, JCV antibody positivity, natalizumab treatment >2 years, and immunosuppressive treatment before natalizumab increase PML risk. Particularly JCV antibody-positive patients with at least one additional risk factor are at a high PML risk [3-5]. As a consequence these patients and/or neurologists often choose to discontinue natalizumab therapy. Following natalizumab discontinuation, in the majority of patients, relapses and Magnetic Resonance Imaging (MRI) activity return to pre-treatment levels, which peaks 4–7 months after the last infusion. [2,6]

The risk of return of disease activity appears to be greater in patients with high disease activity pre-natalizumab than in those with low disease activity [2].

In addition, although a unique definition does not exist, rebound phenomena have been described in some patients during natalizumab discontinuation. [7-8]
interruption. There are no established guidelines for the timing and choice of treatment in patients who discontinue natalizumab. Disease control has been incomplete when patients switched to interferon beta (IFNβ) or Glatiramer Acetate (GA), or were treated with high-dose corticosteroids [7,8]. There are controversial opinions in the literature on the use of Fingolimod (FTY) after natalizumab discontinuation. [7,9-15] The aim of our study is to evaluate the efficacy and safety of cyclophosphamide (CTX) to reduce disease reactivation after natalizumab discontinuation.

**Method**

Data were collected from nine different Italian MS Centres and retrospectively evaluated from clinical records. 47 patients with clinically definite RR-MS, who switched to CTX after natalizumab discontinuation in the period between May 2011 and September 2014, were enrolled.

Two patients were excluded from the analysis because received less than 12 natalizumab infusions. The remaining 45 patients were subdivided into two main groups according to the period of washout between natalizumab and CTX: 23/45 patients had a period of washout between 1 to 3 months (Early Treatment) while 22/45 had CTX after at least 3 months of washout (Late Treatment) [Figure 1].

Clinical MS stability was defined as the absence of documented relapses and the absence of Expanded Disability Status Scale (EDSS) progression during the period of examination. MRI activity was defined as the appearance of new T2+/FLAIR lesions and/or Gd-enhancing lesions. Rebound effect has been evaluated as the recurrence of clinical and/or MRI disease activity at a higher level than before natalizumab introduction. High dose intravenous methylprednisolone has been used in case of clinical relapse according to the normal daily clinical activity of each Centre.

Descriptive statistics were used to summarize baseline characteristics of patients in each of the two groups: Continuous data were expressed as median with interquartile range as measure of variability. Mann-Whitney and Chi square test were performed to assess differences at baseline between groups.

The frequency of relapses and MRI activity (presence/absence of lesions) over the follow-up period were reported along with 95% confidence intervals computed using Bayesian methods with Jeffreys prior [16]. Bootstrap resampling was used to compute 95% confidence intervals of EDSS.

Since our study is a retrospective analysis, the CTX-based therapeutic protocol was different among Italian MS Centres. The protocol applied was constituted by monthly intravenous pulses for 3 or 6 months after natalizumab discontinuation, but with a different dose of CTX infused: the more used protocol in our samples was monthly pulses of 800-1000 mg/m<sup>2</sup> adjusted for lymphocytes count at the nadir.

In our analysis we report the cumulative dose of CTX expressed as milligrams for body surface (mg/m<sup>2</sup>).

In Italy the CTX is an approved drug for autoimmune diseases of the Nervous System (Note 4 AIFA) and informed consent was obtained from the patients before starting therapy. No specific ethics committee approval was needed according to local regulations.

**Results**

Demographic and baseline characteristics. The demographic and clinical features of the patients are summarized in [Table 1]. No statistical differences were observed between the two groups.

The main reason for natalizumab discontinuation was the risk of PML (respectively 78% and 73%): no data on anti-JCV antibodies Index were available at the time of the study. Developments of neutralising antibodies, adverse events or lack of efficacy were minor reasons and they were equal distributed between the 2 groups.

**Relapse and EDSS after CTX**

In the Early Treatment group, only 3/23 patients (13%) experienced a clinical relapse: one patient after 2 monthly pulses of CTX and two patients after 3 monthly pulses of CTX. Interestingly, one patient experienced 2 relapses after CTX introduction and he was the only with a washout period of 3 months. The other two patients that experienced 1 relapse each after CTX, had respectively 1 and 2 months of washout from natalizumab. In addition, in the washout period between natalizumab and CTX, two patients present a clinical relapse, one of which had experienced a clinical relapse also after CTX treatment, indicating a very aggressive disease. Median EDSS improved at the end of follow-up (median EDSS 3,82 at the end of follow-up, median EDSS 4,0 at natalizumab withdrawal). No patients had rebound. Mean follow-up was 9,3 months (range 1-16).

In the Late Treatment Group 12/22 patients (54%) had relapses.
Citation: Capobianco M, Re ML, Sangalli F, Moiola L, Perini P, et al. (2015) Cyclophosphamide Pulses Therapy after Natalizumab Discontinuation for Multiple Sclerosis: A Multicentre Study. J Mult Scler (Foster City) 2:151. doi:10.4172/2376-0389.1000151

during the washout period (mean 10, range 4-42 months) and 4/22 (18%) after the introduction of CTX. In 9 patients (41%) a rebound effect was described. A median increase of 1-point EDSS has been seen in this group of patients (median EDSS 4,07 at the end of follow-up, median EDSS 3,0 at natalizumab withdrawal).

### MRI disease activity

A total of 61 brain MRI scans had been performed in 45 patients after CTX introduction (31/61 in Early Treatment and 30/61 in Late Treatment). In the Early Treatment group only 2 out of 13 (15%) patients showed brain MRI activity at 3 months while none developed MRI activity at 6 months. On the contrary in the Late Treatment group MRI disease activity was shown in 5/9 (56%) at 3 months and in 5/14 (36%) at 6 months after CTX introduction [Table 2].

### Adverse events

The CTX was well tolerated and no patient discontinued treatment for adverse events. We report adverse events in five patients: pneumonia, urinary infection, alopecia, amenorrhoea and asymptomatic neutropenia.

| Relapses during wash-out | % (N/T) | 95% CI | % (N/T) | 95% CI | OR | P-value |
|--------------------------|---------|--------|---------|--------|----|---------|
| Early Treatment          |         |        | Late Treatment |       |     |         |
| Active                   | 13% (3/23) | 3.95; 32.09 | 54% (12/22) | 40.87; 81.76 | 10.86 | 0.002 |
| Inactive                 | 0% (0/23) | 0; 10.24 | 9% (2/22) | 3.37; 5.02 | 1.23 | 0.688 |

| MRI Pre Natalizumab     |         |        |         |        |     |         |
|-------------------------|---------|--------|---------|--------|----|---------|
| Active                  | 100% (23/23) | 89.76; 100 | 91% (20/22) | 73.91; 98.06 | 0.174 | 0.200 |
| Inactive                | 0% (0/23) | 0; 10.24 | 9% (2/22) | 1.94; 26.09 |     |         |

| MRI Pre CTX             |         |        |         |        |     |         |
|-------------------------|---------|--------|---------|--------|----|---------|
| Active                  | 30% (7/23) | 14.77; 50.68 | 58% (11/19) | 35.9; 77.68 | 3.14 | 0.078 |
| Inactive                | 70% (16/23) | 49.32; 85.23 | 42% (8/19) | 22.32; 64.1 |     |         |

| MRI 3-months            |         |        |         |        |     |         |
|-------------------------|---------|--------|---------|--------|----|---------|
| Active                  | 15% (2/13) | 3.34; 40.9 | 56% (5/9) | 25.41; 82.7 | 6.87 | 0.059 |
| Inactive                | 85% (11/13) | 59.1; 96.66 | 44% (4/9) | 17.30; 74.59 |     |         |

| MRI 6-months            |         |        |         |        |     |         |
|-------------------------|---------|--------|---------|--------|----|---------|
| Active                  | 0% (0/15) | 0; 15.82 | 36% (5/14) | 15.15; 61.55 | 17.94 | 0.012 |
| Inactive                | 100% (15/15) | 84.81; 100 | 64% (9/14) | 38.45; 84.85 |     |         |

| MRI 12-months           |         |        |         |        |     |         |
|-------------------------|---------|--------|---------|--------|----|---------|
| Active                  | 0% (0/3) | 0; 53.56 | 57% (4/7) | 23.45; 86.11 | 9.00 | 0.128 |
| Inactive                | 100% (3/3) | 46.44; 100 | 43% (3/7) | 13.88; 76.55 |     |         |

Abbreviations: EDSS=Expanded Disability Status Scale, MRI=Magnetic Resonance Imaging, PML=Progressive Multifocal Leukoencephalopathy, CTX=Cyclophosphamide.

### Table 1: Shows demographic and baseline characteristics of patients.

### Table 2: MRI disease activity: Shows brain MRI scans before natalizumab, before CTX and after CTX introduction. Before natalizumab introduction MRI was active in the majority of patients in both groups. Before CTX introduction MRI was active in the most part of the Late Treatment group. After CTX introduction in the Early Treatment group only 2 out of 13 patients showed brain MRI activity at 3 months while none developed MRI activity at 6 months. In the Late Treatment group MRI disease activity was shown in 5/9 at 3 months and in 5/14 at 6 months after CTX introduction.
Discussion

As far as we know, this is the first study on CTX after discontinuation of natalizumab. Our results showed an important efficacy of CTX after natalizumab discontinuation: only 3 patients (13%) experienced a clinical relapse when CTX treatment is started early (1-3 months).

CTX could be able to reduce disease reactivation after natalizumab discontinuation for marked immunosuppression and an anti-inflammatory immune effect.

CTX is an alkylating agent used to halt rapidly progressive forms of multiple sclerosis, high doses of CTX produce marked immunosuppression and an anti-inflammatory immune deviation. CTX effectively reduces the number of circulating T and B cells in addition to creating a more favourable cytokine balance, shifting away from autoimmune Th1 responses and reductions in interleukin 12 (IL-12) and interferon gamma (IFN-γ). This is the rationale for its use in the treatment of autoimmune diseases, including Multiple Sclerosis. CTX and its metabolites are also capable of penetrating the blood–brain barrier and can exert direct intrathecal immunologic effects. These beneficial biologic effects are potentially favourable for disease control after natalizumab discontinuation [17].

As we have enrolled patients in the period between May 2011 and September 2014 not many therapeutic options were available at that time: In particular neither BG12 nor alemtuzumab nor ocrelizumab were available. Off-label use of rituximab should also be an option but it is also at risk of PML development limiting its use in these kinds of patients.

In the literature there are controversial opinions on the treatment strategies for patients who discontinue natalizumab therapy. There are a large number of studies analysing the use of FTY: some have reported increased relapse rates and severe relapses in patients switching to FTY after natalizumab discontinuation [12,13,17,18]. Other studies reported that FTY have a potential role to reduce disease reactivation between 15% and 30% [7,9,11,19,20]. Nevertheless, due to the fact that FTY steady state kinetics is achieved only two months after initiation, it could be a longer time to achieve a complete control of disease reactivation [21]. On the contrary CTX has intrathecal immunologic rapid effects that could play a crucial role to arrest MS disease reactivation, which peaks 4–7 months after the last infusion of natalizumab.

For these reasons cyclophosphamide was preferred in our population characterised by a very aggressive disease course. Our study confirms, moreover, that washout between natalizumab and other Disease Modifying Treatment (DMTs) should be shorter than 3 months, as other have described [7,9,14].

In the Late Treatment group we observed a higher number of relapses after CTX introduction; moreover the only patient with 3 months of washout in the Early Treatment group experienced 2 clinical relapses after the introduction of CTX. In addition, the risk of relapse during the washout is correlated with MS disease activity before natalizumab initiation and with the duration of the washout. The risk increased significantly for patients with a washout of 3 months or longer. The use of methylprednisolone or an immunomodulatory drug did not mitigate the risk of relapse. Conversely, patients who had a short washout (<3 months) had a lower risk of relapse. In fact, in our study in the Late Treatment Group 12 (63%) patients had more relapses than in the Early Treatment group during the washout period between natalizumab and CTX. Our data support choosing a short switch period (2 months or less) between natalizumab and other DMT.

CTX has an acceptable safety profile; it is important to monitor the patients during and after CTX therapy for toxicity, dose-related adverse events, and the risk for long-term effects due to immunosuppression, as the risk of opportunistic infections like PML. The main cause of natalizumab discontinuation is the risk of PML, no data has been reported in the literature that quantify the PML risk after CTX therapy, but it must be taken into consideration the possibility of cumulative risk. This potential risk has to be considered as much as the high risk of disease activity after natalizumab discontinuation.

The retrospective nature of this study is an important limitation as the different therapeutic CTX protocol among Italian MS Centres. Despite these limitations, this report represents a first study of using CTX after natalizumab discontinuation. Of course, we are aware that a larger sample size, longer follow-up and unique infusion protocol are needed to confirm our preliminary results.

According to our results, we propose to standardize the protocol to monthly intravenous pulses, using a dose of 1000 mg/m² per pulse, with a short natalizumab-CTX washout (<3 months). Due to the potential cumulative risk of infections, a short course (3-6 months) of CTX therapy should be recommended and it can precede the initiation of other DMTs, as BG12 or Fingolimod, almost in selected patients with an aggressive pre-natalizumab MS.

References

1. Polman CH, O’Connor PW, Havrdova E, Hutchinson M, Kappos L, et al. (2006) A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med 354: 899-910.
2. O’Connor PW, Goodman A, Kappos L, Lublin FD, Miller DH, et al. (2011) Disease activity returns during natalizumab treatment interruption in patients with multiple sclerosis. Neurology 76: 1858-1865.
3. Gorelik L, Lerner M, Bixler S, Crossman M, Schlaun B, et al. (2010) Anti-JC virus antibodies: implications for PML risk stratification. Ann Neurol 68: 295-303.
4. Plavina T, Subramanym M, Bloomgren G, Richman, Pace A, et al. (2014) Anti–JC Virus Antibody Levels in Serum or Plasma Further Define Risk of or Plasma Further Define Risk of Multifocal Leukoencephalopathy. Ann Neurol 76: 802–812.
5. Serensen PS, Bertolotto A, Edan G, Giovannoni G, Gold R, et al. (2012) Risk stratification for progressive multifocal leukoencephalopathy in patients treated with natalizumab. Mult Scler 18: 143-152.
6. Fox RJ, Cree BA, De Séze J, Gold R, Hartung HP, et al. (2014) MS disease activity in RESTORE: A randomized 24-week natalizumab treatment interruption study. Neurology 82: 1491-1498.
7. Cohen M, Maillart E, Tourbah A, De Séze J, Vukusic S, et al. (2014) Switching from natalizumab to fingolimod in multiple sclerosis: A French prospective study. JAMA Neurol 71: 436-441.
8. Magrainer MJ, Corel F, Navarré A, Boscá I, Simó M, et al. (2011) Pulsed steroids followed by glatiramer acetate to prevent inflammatory activity after cessation of natalizumab therapy: a prospective, 6-month observational study. J Neurol 258: 1805-1811.
9. Jokubaitis VG, Li V, Kalincik T, Izzieqo G, Hodgkinson S, et al. (2014) Fingolimod after natalizumab and the risk of short-term relapse. Neurology 82: 1204-1211.
10. Comi G, Gold R, Dahlke F, Sinha A, von Rosenstiel P, et al. (2015) Relapses in patients treated with fingolimod after previous exposure to natalizumab. Mult Scler 21: 786-790.
11. Laroni A, Brogi D, Miliesi V, Abate L, Uccelli A, et al. (2013) Early switch to fingolimod may decrease the risk of disease recurrence after natalizumab interruption. Mult Scler 19: 1236-1237.
12. Emspe RC, Martin-Medina P, Berenguer-Ruiz L, Pérez-Campona N, Sanchez-Perez R, et al. (2013) Switching from natalizumab to fingolimod: An observational study. Acta Neurol Scand 128: e6-e10.
13. Centonze D, Rossi S, Rinoldi F, Gallo P (2012) Severe relapses under fingolimod treatment prescribed after natalizumab. Neurology 79: 2004-2005.
14. Rinaldi F, Seppi D, Calabrese M, Perini P, Gallo P (2012) Switching therapy from natalizumab to fingolimod in relapsing–remitting multiple sclerosis: Clinical and magnetic resonance imaging findings. Multiple sclerosis Journal 18: 1640-1643.

15. Capobianco M, di Sapio A, Malentacchi M, Malucchi S, Malta M, et al. (2015) No impact of current therapeutic strategies on disease reactivation after natalizumab discontinuation: A comparative analysis of different approaches during the first year of natalizumab discontinuation. Eur J Neurol 22: 585-587.

16. Garthwaite PH, Crawford JR (2011) Confidence intervals for a binomial proportion in the presence of ties. Journal of Applied Statistic 38: 1915-34.

17. Weiner HL, Cohen JA (2002) Treatment of multiple sclerosis with cyclophosphamide: critical review of clinical and immunologic effects. Mult Scler 8: 142-154.

18. Jander S, Turowski B, Kieseier BC, Hartung HP (2012) Emerging tumefactive multiple sclerosis after switching therapy from natalizumab to fingolimod. Mult Scler 18: 1650-1652.

19. Daelman L, Maitrot A, Maarouf A, Chaunu MP, Papeix C, et al. (2012) Severe multiple sclerosis reactivation under fingolimod 3 months after natalizumab withdrawal. Mult Scler 18: 1647-1649.

20. Havla J, Tackenberg B, Helweg K, Meiri I, Krumbholz M, et al. (2013) Fingolimod reduces recurrence of disease activity after natalizumab withdrawal in multiple sclerosis. J Neurol 260: 1382-1387.

21. David OJ, Kovarik JM, Schmouder RL (2012) Clinical pharmacokinetics of fingolimod. Clin Pharmacokinet 51: 15-28.