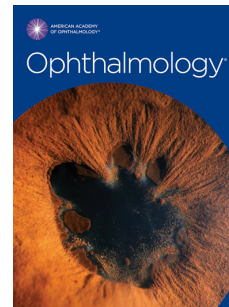


# Journal Pre-proof



Macular Atrophy Incidence and Progression in Eyes with Neovascular Age-Related Macular Degeneration Treated with VEGF Inhibitors Using a Treat-and-Extend or a *Pro-Re-Nata* Regimen. Four Year Results of the MANEX Study.

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1 **Macular Atrophy Incidence and Progression in Eyes with Neovascular Age-Related**  
2 **Macular Degeneration Treated with VEGF Inhibitors Using a Treat-and-Extend or a *Pro-Re-***  
3 ***Nata* Regimen. Four Year Results of the MANEX Study.**

4  
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17

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36 **Running head:** 4-year results of MANEX study. Macular Atrophy Incidence and Progression in nAMD.

37

38

39 **ABSTRACT**

40 **Purpose:** To compare the incidence and progression of macular atrophy (MA) in eyes with  
41 neovascular age-related macular degeneration (nAMD) treated with anti-vascular endothelial  
42 growth factor (VEGF) agents using either a treat-and-extend (T&E) or a *pro-re-nata* (PRN) regimen  
43 over 4-years in a real-life setting.

44 **Design:** 4-year, multicenter, retrospective comparative study

45 **Participants:** 264 patients with treatment-naïve nAMD.

46 **Methods:** Consecutive patients with nAMD received anti-VEGF therapy according to a T&E (n=163)  
47 or PRN (n=101) regimen. Eyes were included if they had received anti-VEGF injections for a period of  
48 at least 4-years and had annual fundus autofluorescence (FAF) and optical coherence tomography  
49 (OCT) imaging using Heidelberg Spectralis. Two masked graders independently delineated areas of  
50 MA from serial FAF images using Heidelberg region finder software, and growth rates were  
51 calculated. Incident MA was assessed using proportional hazard ratios.

52 **Main Outcomes Measures:** MA incidence and progression over 4-years, association between  
53 treatment strategy, and number of injections.

54 **Results:** At baseline, MA was present in 24% and 20% of study eyes in T&E and PRN groups,  
55 respectively ( $p=0.32$ ). At year-4, 27% (34/124) and 25% (20/81) eyes without baseline MA had  
56 detectable MA, in the T&E and PRN groups respectively. In those with MA at baseline, the mean  
57 square root area of MA progressed by a rate of  $0.4\pm 0.2$  and  $0.4\pm 0.1$ mm/year in the T&E and PRN  
58 groups, respectively ( $p=0.23$ ). Multivariate analysis for baseline predictors of MA growth  
59 demonstrated older age, poorer baseline VA and presence of RAP, had a higher risk of greater MA  
60 progression ( $P=0.03$ ). Regression analysis demonstrated no association between T&E and PRN  
61 treatment strategies with the risk of developing new MA during the four years of follow-up or the  
62 progression of pre-existing MA at year-4 ( $p=0.692$ ).

63 **Conclusion:** Over four years, neither incidence nor progression of macular atrophy in eyes with  
64 nAMD treated with anti-VEGF injections was influenced by the treatment regimen and injection  
65 frequency. Eyes treated with a T&E regimen received more injections and had better visual  
66 outcomes compared to those treated with a PRN approach.

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67 **INTRODUCTION**

68 Neovascular age-related macular degeneration (nAMD) is a progressive retinal disease that may  
69 cause significant vision loss if untreated<sup>1</sup>. Defects in the retinal pigment epithelium (RPE) layer  
70 associated with aberrant choroidal vessel growth cause leak and fluid accrual leading to fast visual  
71 decline due to impairment of the overlying retina.<sup>2</sup> Vascular endothelial growth factor (VEGF)-A  
72 overexpression is a crucial feature in the pathogenesis of choroidal neovascularisation (CNV).<sup>3,4</sup> Anti-  
73 VEGF drugs prevent the binding of several active types of VEGF-A to their receptors and have  
74 become the first line treatment for nAMD.<sup>5-8</sup> These agents reduce leak and fluid, and lead to  
75 inactivation of choroidal new vessels.

76 Despite anti-VEGF treatment effectiveness on the neovascular component of the disease, patients  
77 with nAMD can develop progressive visual loss due to macular atrophy (MA), a condition  
78 characterized by RPE, choriocapillaris and photoreceptors loss.<sup>9,10</sup> Recently there has been some  
79 question as to whether RPE atrophy development and progression could be accelerated by more  
80 intensive anti-VEGF therapy.<sup>11-13</sup> In fact, VEGF appears to also have an effect on non-vascular tissues  
81 and to play a critical role in the survival and maintenance of the RPE and choriocapillaris integrity. Its  
82 suppression could therefore induce the development or progression of MA.<sup>14</sup>

83 The relationship between number of injections and incidence of MA appears to be inconsistent in  
84 different studies. The Comparison of AMD Treatment Trials (CATT) found an association between the  
85 intensive anti-VEGF therapy and macular atrophy.<sup>15,16</sup> In this study, 18.3% of patients developed  
86 MA within 2 years of starting anti-VEGF therapy. At 5-years, eyes on a monthly dosing regimen  
87 exhibited a higher risk of developing MA than those on *pro re nata* (PRN) regimen.<sup>16</sup> MA developed  
88 in almost all eyes (98%) in the SEVEN-UP study which included eyes treated for nAMD over seven  
89 years.<sup>17</sup> In one study, there was an association of MA growth with ocular factors in the study and  
90 fellow eyes but not with the number of injections or drug.<sup>18</sup> However, in another study by the same  
91 authors, there was an inverse relationship between the number of injections and incidence of MA.<sup>19</sup>  
92 A study by Munk et al, demonstrated number of injections were not associated with MA size.<sup>20</sup>

93 Previous studies have not demonstrated an effect of monthly versus treat and extend dosing on the  
94 development of new MA.<sup>21</sup>

95 Fundus autofluorescence (FAF) is a non-invasive imaging modality used to evaluate the condition of  
96 the RPE and the overlying neurosensory retina<sup>22,23</sup>, and has become the gold standard by which  
97 atrophy is detected and observed.<sup>24</sup> Areas where RPE atrophy is present appear  
98 hypoautofluorescent, whereas areas with higher distribution of lipofuscin will appear hyper-  
99 autofluorescent.<sup>25</sup> Various limitations have to be considered for FAF imaging. Media opacities may  
100 result in FAF images that cannot be analyzed adequately, and FAF changes do not always correlate  
101 with RPE changes, such as in cases of soft and hard drusen, haemorrhages and pigmented  
102 plaques.<sup>26</sup> However, the FAF images are usually analysed considering and assessing other image  
103 modalities such as OCT, to exclude other causes of FAF hypoautofluorescence such as blockage due  
104 to hemorrhages or fibrosis.

105 Our study aimed to examine whether there was a different rate in incidence and/or progression of  
106 MA in eyes receiving treatment for nAMD, using a treat and extend (T&E) regimen compared to a  
107 PRN regimen. When using a T&E regimen, the aim is to keep eyes free of fluid or 'dry' whereas with  
108 PRN approach, eyes are only treated when fluid is present. We hypothesised that there may be a  
109 difference in the rate of incidence and progression of MA. In fact, while eyes in the T&E group may  
110 develop atrophy at a greater rate due to the more sustained VEGF suppression, greater fluid  
111 fluctuations and recurrence of CNV activity may lead to greater RPE injury with resultant atrophy in  
112 eyes treated using a PRN regimen.

**113 METHODS****114 Protocol/Inclusion and Exclusion Criteria and treatment**

115 In this retrospective, multi-center study, consecutive patients undergoing anti-VEGF therapy for  
116 neovascular AMD from two retinal clinics in Sydney, Australia and Milan, Italy were included if they  
117 fulfilled the following criteria: (1) angiographically confirmed choroidal neovascular membrane  
118 (CNV) in the context of nAMD; (2) recurrent and continuous administration of anti-VEGF therapy for  
119 the treatment of nAMD to one eye for a minimum of 4-years; (3) the study eye was treatment-naïve  
120 at the initiation of treatment; and (4) fundus autofluorescence imaging available at least yearly  
121 during the 4 years of follow-up.

122  
123 Patients who initiated treatment between January 2009 and January 2014, with a minimum follow-  
124 up of 4-years were eligible for inclusion. Patients with concurrent intraocular condition that may  
125 reduce the potential for visual improvement or impede clinical outcomes, specifically, those with an  
126 active diabetic retinopathy, or inflammatory disease such as uveitis, retinal dystrophies, severe  
127 media opacities, and RPE rip/tears were excluded.

128  
129 The study was approved by the local institutional ethics committee (The University of Sydney), and  
130 was conducted in accordance with the Declaration of Helsinki.

**132 Data acquisition**

133 Medical records were reviewed for demographic data, visual acuity (VA) converted to an Early  
134 Treatment Diabetic Retinopathy Study (ETDRS) letter score,<sup>27</sup> number of intravitreal injections  
135 administered, and anti-VEGF therapy administered. The formula to convert Snellen visual acuity  
136 measurements to approximate ETDRS letter scores is  $85 + 50 \times \log(\text{Snellen fraction})$ , which may be  
137 rounded to the nearest letter.<sup>27</sup> All patients initially received three monthly intravitreal injections,  
138 followed by either a PRN or T&E protocol. This study was a retrospective study, and as such strict

139 criteria for follow-up and retreatment were not pre-established. However, each site followed their  
140 own internal guidelines for the management of patients with nAMD. All procedures including follow-  
141 up visits took place at the Eye Clinic, Department of Biomedical and Clinical Sciences, Luigi Sacco  
142 Hospital, University of Milan and Sydney Retina Clinic, Sydney, Australia. These clinics were chosen  
143 as the retinal specialists consistently treated their patients using the one protocol as standard  
144 clinical practice already in place in the respective clinics. T&E regimen group consisted of patients  
145 from the clinic in Sydney, and PRN group consisted of patients from Milan.

146

147 For the first group (PRN), the usual protocol of the treating doctor, was 3 loading doses of anti-VEGF  
148 injections, with subsequent injections only given if there was a drop in visual acuity, new  
149 haemorrhage or exudation on OCT. After treatment by 3 monthly intravitreal injections of anti-VEGF  
150 therapy during the period from January 2008 to January 2014, subsequent single injections were  
151 given as needed according to changes in the patient's visual acuity and/or signs of exudation on  
152 optical coherence tomography (OCT) or fluorescein angiography (FA). In the absence of retreatment  
153 criteria, no further injections were administered, and patients were asked to follow-up again in 4 to  
154 8 weeks.

155

156 For the second group (T&E group), the usual protocol of the treating doctor, was 3 monthly loading  
157 doses of anti-VEGF treatment. If there was no new haemorrhage or signs of exudation on OCT, the  
158 interval between injections was extended a further 2 weeks, up to a maximum of 12 weeks. If new  
159 haemorrhage or exudation were present, then the interval was decreased by 2 weeks to a minimum  
160 of 4 weeks. The aim of this regimen was to keep the macula dry.

161

162 Baseline fundus fluorescein angiographic (FA) and OCT (Spectralis OCT; Heidelberg Engineering,  
163 Heidelberg, Germany) images were graded by 2 independent graders, blinded to site, for active CNV  
164 lesion type (type 1, type 2, type 3/retinal angiomatous proliferation (RAP) or polypoidal choroidal



165 vasculopathy (PCV),<sup>28</sup> and for the presence of atrophy. Fundus autofluorescence imaging (FAF) were  
166 obtained on Heidelberg Spectralis using a laser with an excitation wavelength of 488nm and barrier  
167 filter of 495nm. Macular atrophy was defined as sharp, delineated hypoautofluorescence with  
168 corresponding attenuation of the RPE band and loss of overlying ellipsoid zone and external limiting  
169 membrane with thinning of the outer nuclear layer, together with enhanced signal transmission into  
170 the choroid as evidenced on OCT.

171 The quantification of macular atrophy using FAF was performed by two graders, blinded to all  
172 patient details, using the Heidelberg region finder software (version 2.5.8.0) (**Figure 1**), which is able  
173 to semi automatically quantify atrophic areas. Once atrophic areas and constraints had been defined  
174 for the baseline image, they could then be copied to the subsequent visit images.<sup>29</sup> The minimal  
175 lesion size was defined as an atrophic area measuring 0.02 mm<sup>2</sup>, quantified using region finder  
176 software.<sup>30</sup>

177 Various limitations have to be considered for FAF imaging. Media opacities may result in FAF images  
178 that cannot be analyzed adequately, and FAF changes do not always correlate with RPE changes,  
179 such as in cases of soft and hard drusen, haemorrhages and pigmented plaques.<sup>26</sup> However, the FAF  
180 images are usually analysed considering and assessing other image modalities such as OCT, to  
181 exclude other causes of FAF hypoautofluorescence such as blockage due to hemorrhages or fibrosis.

182 Image quality of FAF were analyzed by two graders, in cases of poor FAF image, multimodal imaging  
183 was assessed by both graders for unanimity. In cases of hemorrhage, FAF imaging available within 3  
184 months of target visit was used. In cases where there was a difference greater than 20% between  
185 measurements obtained by the two observers, arbitration through open adjudication was  
186 performed. In the few cases in which agreement was not achieved, a resolution was established by a  
187 third expert grader who evaluated the images (SFB). An average of the measurements of the two  
188 observers was used for statistical analysis. Areas of peripapillary atrophy were not classified as MA  
189 and accordingly were not included in MA measurements. In images that showed two or more

190 distinct MA areas each measuring  $0.02\text{mm}^2$  or greater, each distinct area was measured and  
191 summed to generate the total MA area.

192 Incident MA was defined as a well-demarcated region or regions of marked hypo-autofluorescence  
193 from an absence of the RPE measuring at least  $0.02\text{mm}^2$ . The progression of MA was classified as the  
194 expansion of pre-existing areas of MA equated to baseline. The variance in the entire area of MA at  
195 each annual visit and baseline were determined and the degree of progression was calculated by  
196 dividing the change in MA size by the time points.<sup>31,32</sup> As MA progresses at a non-linear rate, MA  
197 size was also calculated as a square root transformation<sup>31</sup> of lesion area to reduce the reliance on  
198 baseline lesion size for test-retest variability and the growth rates.<sup>33</sup> All MA results are presented as  
199 the square root transformation value. MA that was confluent with peripapillary atrophy were  
200 excluded.

201 The measurement of central subfield retinal thickness (CSRT), defined as the distance from the inner  
202 retinal surface to Bruch's membrane within the central 1mm of the ETDRS grid.. All measurements  
203 were performed using the Heidelberg Eye Explorer software (version 1.9.10.1; Heidelberg  
204 Engineering, Heidelberg, Germany). The results from two independent masked graders were  
205 compared. If the difference in quantitative results between graders was less than 20%, the individual  
206 grader results were averaged. If the difference was  $\geq 20\%$ , a third examiner adjudicated a consensus  
207 among graders.

208 Intra-observer reliability was evaluated by the intraclass correlation, which was calculated from the  
209 measurements of the two graders

210

### 211 **Expected patient numbers and power calculations**

212 We estimated that there may be a 15% difference in incidence and progression of MA in eyes  
213 treated with the T&E regimen compared to PRN. The prevalence of MA at onset of neovascular AMD  
214 varies in the literature of between 6% and 40.9%,<sup>16,21,34</sup> so we assumed a baseline prevalence of MA

215 of 20% in each group. A total of 236 participants would be required to find a difference between the  
216 2 groups with a power of 80% and false positive rate of 5%.

217

## 218 **Statistical Analyses**

219 Statistical analysis was performed using SPSS software (version 24.0, SPSS Inc., Chicago, IL, USA).

220 Results were presented as means and standard deviation. Mann-Whitney's nonparametric test was  
221 used to compare statistical distributions. Inter-observer agreement was assessed using the interclass  
222 correlation coefficient (ICC). The statistically significant difference between the two treatment  
223 groups was also proven by a more robust procedure, Welch test.

224 Univariate and multivariate analyses with logistic regression were used to determine factors  
225 associated with atrophy at baseline and proportion of patients with new MA at each annual visit. In  
226 respect to macular atrophy, predictive factors of visual acuity, number of injections, CSRT, and  
227 atrophy size were assessed with linear regression. The generalised estimating equation (GEE) was  
228 used to account for the inclusion of bilateral eyes from the same patient. A sensitivity analysis using  
229 mixed model after data imputation following the LOCF method for mean change in VA was  
230 consistent with the secondary analysis. A 95% confidence interval with 5% level of significance was  
231 adopted; thus,  $P$  values of  $<0.05$  were considered to be statistically significant. Missing data were  
232 imputed using the last observation carried forward method. Treatment exposure and follow-up  
233 frequency were only analysed in patients concluding the entire 4-years of the study.

234

235 **RESULTS**236 ***Study patients***

237 Of 2,041 eyes identified with beginning anti-VEGF treatment between 2009 and 2014, 264 eyes met  
238 the inclusion and exclusion criteria. All eyes commenced treatment with intravitreal injections of  
239 anti-VEGF injections between 2009-2014, 206 eyes were initiated on ranibizumab treatment, 45 on  
240 aflibercept, and 13 on bevacizumab. A total of 163 eyes were treated according to a treat-and-  
241 extend regimen (T&E), and 101 eyes were treated according to *pro re nata* (PRN) regimen. Follow-up  
242 data were available at least annually for 4-years post initiation of anti-VEGF therapy. Bilateral eyes  
243 were included in 24 cases. During the 4-year follow-up period, 130 eyes (49%) changed anti-VEGF  
244 therapy at least once (62 eyes in the PRN group and 68 eyes from the T&E group).

245

246 ***Baseline characteristics***

247 The groups were well balanced at baseline for visual acuity ( $P=0.45$ ) and CSRT ( $P=0.67$ ). The other  
248 demographic and ocular parameters of the two treatment groups are presented in **Table 1**.

249 The PRN group included 66 women and 35 men, aged from 52 to 91 years (mean,  $74.3\pm 8.1$  years).  
250 This group included 61 eyes with type 1 CNV (60%), 17 eyes (17%) with type 2 CNV, 14 eyes (14%)  
251 with type 3 or retinal angiomatous proliferation (RAP), and 9 eyes (9%) with polypoidal choroidal  
252 vasculopathy lesions (PCV). Initial visual acuity ranged from 20 to 85 letters with a mean of  
253  $65.5\pm 14.7$  (Snellen equivalent: 20/50) at baseline.

254 The T&E group included 82 women and 81 men aged 55 to 95 years (mean,  $77.8\pm 8.5$  years). There  
255 were 68 right eyes and 85 left eyes. This group included 95 eyes (58%) with type 1 CNV, 31 eyes  
256 (19%) with type 2 CNV, 21 eyes (13%) with Type 3 (RAP) lesion and 16 polypoidal cases (10%). Initial  
257 visual acuity ranges from 20 to 90 letters with a mean of  $66.9\pm 14.4$  (Snellen equivalent: 20/50).

258 A predetermined sub analysis assessed the incidence, and progression of macular atrophy. The inter-  
259 grader reliability was excellent for both ( $k=0.91$ ).

260

### 261 ***Pre-existing Macular Atrophy***

262 At baseline, MA was present in 39 eyes (24%) in T&E group and 20 eyes (20%) in the PRN group  
263 ( $p=0.45$ ). Mean baseline MA area was greater in the T&E group than the PRN group ( $1.1\pm 0.7$ mm and  
264  $0.8\pm 0.4$ mm in the T&E and PRN groups, respectively using the square root transformation,  $P=0.06$ ).

265 At year 4, mean MA area increased to  $2.2\pm 0.9$ mm in the T&E group, and  $1.7\pm 0.6$ mm in the PRN  
266 group ( $p=0.06$ ) (**Figure 2**), showing continuous growth over the course of the study regardless of the  
267 treatment regimen.

268 Continuous progression of MA was seen in all eyes with MA at baseline based on FAF and OCT  
269 images at each annual visit. The MA progression rate over 4 years for eyes with pre-existing MA was  
270  $0.4\pm 0.2$ mm/year in the T&E group,  $0.4\pm 0.1$ mm/year in the PRN group ( $P= 0.23$ ) (**Figure 2**). All eyes  
271 demonstrated increased area with MA, with 93% expanding by 1-disc area or more by 4 years  
272 follow-up.

273 The progression rates by CNV type were significantly higher in the eyes with Type 3 lesions (RAP)  
274 ( $p=0.04$ ), in both groups with a progression rate of  $0.9\pm 0.8$ mm/year and  $1.0\pm 0.7$ mm/year in the T&E  
275 and PRN groups respectively ( $P=0.62$ ). The progression rate of MA was smallest in those with type 1  
276 CNV:  $0.5\pm 0.2$ mm/year and  $0.3\pm 0.1$ mm/year in the T&E and PRN groups, respectively ( $P=0.45$ ).

277 Of all the eyes with MA at baseline, 31% was unifocal and in 69% it was multifocal. The proportion of  
278 eyes with unifocal and multifocal MA was similar in each group ( $p=0.09$  and  $0.08$ , respectively).

279 There was no difference in the rate of progression of MA in those with unifocal compared to  
280 multifocal MA ( $0.2\pm 0.1$ mm/year and  $0.3\pm 0.2$ mm/year, respectively [ $p=0.68$ ]). In those with MA at  
281 baseline, it included the fovea in 58% and was extrafoveal in 42%. There was no difference in the

282 proportion of foveal involving and extrafoveal MA between the 2 groups ( $p=0.82$  and  $0.81$ ,  
283 respectively). There was no difference in mean progression rate of MA in foveal involving MA  
284 compared to extrafoveal MA ( $0.5\pm 0.2\text{mm/year}$  vs.  $0.6\pm 0.2\text{mm/year}$ ,  $p=0.22$ ). As expected foveal  
285 centred MA had lower baseline VA ( $46.7\pm 15.2$  letters vs.  $68.4\pm 12.9$  letters,  $p<0.001$ ).

286 The main outcome, the correlation coefficient among treatment regimen and progression of GA in  
287 SQRT, was Pearson's  $r=0.3$ ,  $P=0.29$ . As predicted, the correlation was positive for progression  
288 expressed in mm/year,  $r=0.7$ ,  $P<0.001$  (FIGURE 2). On univariate analysis, increasing age ( $p<0.001$ ),  
289 poorer baseline VA ( $p=0.02$ ), foveal location of MA ( $p=0.01$ ), and presence of RAP ( $p=0.04$ ) and  
290 presence of intraretinal fluid ( $p=0.05$ ), were all associated with increased progression of MA.

291 Multivariate analysis for baseline predictors of MA growth demonstrated older age, poorer baseline  
292 VA and presence of RAP, had a higher risk of greater MA progression ( $P=0.03$ ). Other variables, such  
293 as sex, presence of RSD, drusen, CSRT, lens status and treatment group were not significant.

294

### 295 ***Incidence of New Macular Atrophy***

296 Incident MA developed in 34 eyes (27% of eyes without MA at baseline) and 20 (25%) eyes in the  
297 T&E and PRN groups respectively during 4-years of anti-VEGF therapy ( $P=0.70$ ) (Figure 4). The mean  
298 size of MA on first presentation was  $1.2\pm 0.9\text{mm}^2$  and  $1.1\pm 1.6\text{mm}^2$  in the T&E and PRN groups,  
299 respectively ( $P=0.88$ ). A total of 13 (10%) T&E eyes and 9 (11%) PRN eyes developed atrophy within  
300 the first year of initiating anti-VEGF therapy. The incidence in the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> years was, 5 eyes  
301 (4%), 9 eyes (7%) and 7 eyes (6%) in the T&E group; and 6 eyes (7%), 3 eyes (4%), and 2 eyes (2%) in  
302 the PRN group.

303 Incidence of MA varied across eyes with different CNV types. particularly those with type 3 (RAP)  
304 CNV, that demonstrated greater increase in MA size ( $P=0.04$ ). Subset analysis of incidence of MA for

305 each CNV subtype, found a higher incidence of MA among those with type 1 CNV (51%), followed by  
306 RAP lesions (17%), PCV (15%) and type 2 (7%).

307

308 Unifocal lesions accounted for 48% of eyes, and multifocal in 52% of eyes. Multifocal MA had a  
309 significantly greater progression rate compared to unifocal lesions ( $0.4\pm 0.2\text{mm/year}$  vs.  
310  $0.2\pm 0.1\text{mm/year}$ ,  $p<0.001$ ). Foveal MA was present in 44% of eyes at baseline, and 56% were  
311 classified as extrafoveal. Extrafoveal MA demonstrated a greater mean progression rate compared  
312 to foveal MA ( $0.4\pm 0.2\text{mm/year}$  vs.  $0.3\pm 0.1\text{mm/year}$ ,  $p=0.05$ ). Again, as predicted foveal centered MA  
313 significantly lost vision by year-4 ( $-12.9\pm 13.1$  letters) compared to those eyes with extrafoveal MA  
314 who gained  $6.3\pm 9.2$  letters, at the end of follow-up,  $p<0.001$ ).

315

316 Overall, there was no significant difference in the risk of developing MA between the two treatment  
317 groups during the four years of the study,  $P=0.69$ . Injection frequency in the T&E group did not  
318 appear to be associated with MA presence at year-4 (**Figure 5**). There was no meaningful difference  
319 in new MA rates by injection frequency.

320 Time was a significant predictor of development of new MA by year-4, ( $P<0.001$ ), each year there  
321 was a 38% higher risk in developing MA. The presence of PED, reticular pseudodrusen, and number  
322 of injections were found to be not significant in predicting the development of new MA.

323 No significant difference was found regarding gender, pseudophakic status or type of anti-VEGF  
324 therapy.

325 The mean progression rate of MA that developed over the course of the study was  $0.8\pm 0.6\text{mm/year}$   
326 and  $0.8\pm 0.9\text{mm/year}$  in the T&E and PRN groups respectively ( $P=0.89$ ) (**Figure 6**).

327 Linear regression analysis demonstrated a positive association between treatment regimen, with a  
328 gain in VA at year-4 in the T&E group ( $P=0.006$ ) compared to baseline. Multiple regression analysis

329 adjusted for VA, age at diagnosis, OCT findings at baseline demonstrated no association between the  
330 T&E and PRN treatment strategies with the progression of preexisting MA at year-4 ( $P=0.09$ ).  
331 Multivariate analysis indicated that higher baseline CSRT, type of CNV lesion and presence of  
332 intraretinal fluid at year 1 was associated with a higher progression rate of atrophy ( $P<0.001$ ,  $P=0.03$ ,  
333 and  $P<0.001$  respectively). Eyes in the T&E cohort received a significantly higher number of anti-  
334 VEGF injections during the follow-up period ( $29.3\pm 10.8$  versus  $15.7\pm 8.8$ ,  $P<0.01$ ). Interestingly,  
335 number of injections was not statistically significant as a risk factor for MA progression ( $P=0.06$ ).

336

### 337 ***Vision and CSRT Outcomes***

338 Baseline CSRT was similar between the treatment groups:  $410.2\pm 105.5\mu\text{m}$  versus  $416.6\pm 136.5\mu\text{m}$  in  
339 the T&E and PRN groups respectively ( $P=0.67$ ). There was no significant difference for the first 2  
340 years, but by year 3 there was a significant difference ( $P=0.01$ ) among the T&E and PRN groups  
341 (**Figure 7**).

342 VA was evaluated in eyes in the absence versus presence of MA. Eyes with and without MA at  
343 baseline had mean VA gains from baseline to Year 1 of  $0.3\pm 8.2$  and  $2.7\pm 10.9$  letters in the T&E  
344 group, respectively ( $P=0.10$ ); and  $0.5\pm 13.8$  and  $4.4\pm 12.9$  letters, respectively in the PRN group  
345 ( $P=0.25$ ). However, these gains were lost by Year-4 in eyes with and without MA at baseline: -  
346  $0.5\pm 13.6$  and  $+0.9\pm 13.9$  in the T&E group respectively, ( $P=0.57$ ); and  $-4.2\pm 17.2$  and  $-2.6\pm 23.7$  in the  
347 PRN group respectively, ( $P=0.74$ ) (**Figure 8a**). VA was also evaluated at baseline, year -1, -2, -3 and -4  
348 with and without concurrent MA, that is, eyes with detectable atrophy at baseline at each time point  
349 (**Figure 8b**).

350 Reassuringly, 72 eyes (44%) and 40 eyes (40%) from the T&E and PRN groups respectively, never  
351 developed MA during the 4-years of observation ( $P=0.65$ ). Those eyes that developed new MA had a  
352 greater decline in vision in contrast to those eyes that never developed atrophy, most evident after  
353 4-years of treatment.



354 **DISCUSSION**

355 VEGF inhibitors have revolutionised the outcomes of eyes with nAMD and decreased the rate of  
356 blindness amongst those affected. Despite this, the impact of atrophy on nAMD patient's visual  
357 function and quality of life is significant and a correlation between anti-VEGF treatment and a higher  
358 MA development/progression has been proposed.<sup>12</sup>

359 Different treatment regimens for nAMD are used in real life settings in order to control the disease  
360 and prevent overtreatment, the commonest being T&E and PRN. Both these approaches may have  
361 an impact on MA development/progression. In fact, while eyes in the T&E group may develop  
362 atrophy at a greater rate due to the more sustained VEGF suppression, greater fluid fluctuations and  
363 recurrence of CNV activity may lead to greater RPE injury with consequent atrophy in eyes treated  
364 using a PRN regimen.<sup>12,35,36</sup>

365 In the MANEX study we compared two group of eyes treated with anti-VEGF following a T&E and a  
366 PRN regimen respectively and we compared the incidence and progression of MA between these 2  
367 groups. We found that the incidence of MA and the mean square root area of MA increase was  
368 similar in the two groups, regardless of the treatment regimen. Eyes treated with T&E however  
369 received significantly more injection than those on a PRN regimen and had significantly better visual  
370 outcomes at 4 years.

371 Natural history studies have shown that MA occurs in eyes with nAMD without anti-VEGF treatment,  
372 so susceptibility to macular atrophy is part of the disease process. However, growth rate of MA in  
373 untreated eyes is between 1.5 and 2.2mm<sup>2</sup>/year over 4 years,<sup>37,38</sup> significantly more to results seen  
374 in the present study. This suggests a possible increase in the risk to develop new MA following anti-  
375 VEGF treatment regardless of the re-injection strategy, and the possible protective effects of CNV  
376 lesions.<sup>39</sup> Despite this, VA in eyes treated with anti-VEGF injections is significantly higher than that of  
377 untreated eyes thus the benefit deriving from the neovascular component control overcomes the  
378 negative effect of new GA development on VA outcomes, justifying the treatment.

379 Previously reported incidence of MA in eyes treated with anti-VEGF injections is heterogenous in the  
380 literature. In the present study, MA was present in 24% of the T&E group, and 20% in the PRN group  
381 at baseline, which is similar to the 24% observed in newly diagnosed treatment naïve nAMD eyes in  
382 a study by Sikorav et al<sup>28</sup> whom had a similar baseline mean atrophy size ( $1.2\pm 1.8\text{mm}^2$ ). The  
383 incidence of new atrophy developed in 11% and 10% in the PRN and T&E groups, respectively at  
384 year-1, a percentage comparable to month-12 findings of the RIVAL study,<sup>34</sup> and in a retrospective  
385 study by Kuroda et al,<sup>40</sup> in which newly diagnosed eyes were treated with aflibercept for 12-months.  
386 At 4 years 27% of eyes in the T&E group and 25% of eyes in the PRN group developed new MA.  
387 These figures are comparable to results seen in the HARBOR study (29%)<sup>41</sup> and slightly below the 24-  
388 month results seen in the RIVAL study (27% and 32% in the ranibizumab and aflibercept arms,  
389 respectively).<sup>34</sup>

390 The enlargement of atrophic lesions corresponds to loss of increasingly larger areas of the visual  
391 field and almost invariably occurs in eyes affected by MA. A higher rate of progression of these areas  
392 could mean a faster loss of VA. For this reason, determining whether a different anti-VEGF injections  
393 regimen could affect the MA progression is of extreme relevance. In our study, the change in square  
394 root area of MA at 4-years was similar between the two arms. We found a MA growth rate similar to  
395 that seen at 24-months of the RIVAL study ( $0.36$  and  $0.28\text{mm}^2/\text{year}$  in the ranibizumab and  
396 aflibercept arms respectively) and less than that that seen in the CATT study ( $0.7\text{mm}^2/\text{year}$ ). This  
397 suggest absence of correlation between the treatment regimen. The macular atrophy area  
398 demonstrated a positive correlation with larger baseline areas progressing faster, as previously  
399 reported.<sup>33</sup> The LOESS regression analysis did not differ from the linear regression, after square root  
400 transformation, comparable to results seen in a study by Mones et al.<sup>42</sup>

401 Using adjusted linear regression analysis, we found no relationship between treatment regimen and  
402 progression of existing MA and incidence of new MA over 4-years. We found no significant  
403 association with the total number of injections with the apparent growth of MA. Although it is

404 possible that there were not have enough eyes to power this statistical finding. There was a  
405 significant difference in injection rates between the 2 groups, yet the number of injections of anti-  
406 VEGF or treatment strategy had no association with the incidence and progression of MA. The  
407 injection rates seen in the T&E group were similar to both groups in the RIVAL study, where the  
408 mean number administered in the first 12 months was 9.7, and 8.9 in the final 12 months,<sup>34</sup> which is  
409 higher than that observed in other observational real-world studies. Although the number of  
410 injections in the PRN group in the present study, may explain the poorer visual outcomes compared  
411 to T&E group.

412 Although the CATT trial demonstrated that eyes receiving monthly treatment had a higher incidence  
413 of atrophy compared to those being treated with PRN,<sup>16</sup> the IVAN and RIVAL studies found no  
414 statistical difference in incidence of atrophy among differing anti-VEGF therapies.<sup>34,43</sup> Injections of  
415 intravitreal anti-VEGF has been shown to have no association with RPE damage in animal models.<sup>44-46</sup>  
416 A possible explanation for the inconsistent results reported in the literature is that MA could depend  
417 more on specific features of the single neovascular lesions included in the studies or the underlying  
418 MA phenotype rather than to the treatment itself. Furthermore, the subtype of neovascularisation is  
419 believed to influence the risk of atrophy progression. It has been proposed that type 3 (retinal  
420 angiomatous proliferation- RAP) lesions may confer a greater risk in the development and  
421 progression of atrophy, whilst type 1 are associated with a lower risk of MA progression.<sup>39,47</sup> Our  
422 study confirmed this association and it is possible that an uneven distribution of type 3 (RAP) lesion  
423 in the arms of the above-mentioned trials affected the post-hoc analysis on MA.

424 This study has several limitations that must be considered when interpreting the findings. Being a  
425 retrospective study, our cohort represents only a subgroup of treated patients, that is those with 4-  
426 years of follow-up and adequate imaging, thus selection bias cannot be excluded. It is possible that  
427 patients were not always compliant with the recommended follow-up. Furthermore, a limitation of  
428 the study common to longer term studies was the eyes excluded due to insufficient data or lost to

429 follow-up of patients. It is possible that patients who responded extremely well or especially poor  
430 were more likely to cease treatment or be lost to follow-up, thus limiting generalizability of the  
431 findings. This would affect the aggregate data, notwithstanding these limitations, these would not  
432 change the main conclusions of the study, which are centred on comprehensive examinations made  
433 in each patient over-time. Finally, the Heidelberg Region Finder Software, to the best of our  
434 knowledge, is so far, the only validated method to assess atrophy in CNV, however, we are aware it  
435 has limitations such as masking due to fibrosis and disease activity.<sup>28,30,34,48-50</sup> These changes  
436 sometimes lead to irregular, not clearly demarcated hypoFAF lesions. This can impact the  
437 assessment using the region finder.

438 Our study was not powered to compare difference between anti-VEGF agents. However, previous  
439 studies including the RIVAL study and the CATT did not find a difference in the development or  
440 incidence of MA in eyes treated with ranibizumab vs aflibercept or ranibizumab vs bevacizumab.<sup>16,34</sup>  
441 Finally, a proportion of patients (49%) within our study switched agents during the 4-year follow-up  
442 period. However, the large number of patients and long follow-up make this data set an extremely  
443 valuable addition to the literature.

444 In conclusion, the MANEX observational study found no significant difference in the incidence or  
445 progression of MA in eyes with nAMD treated with anti-VEGF intravitreal injections using a T&E or  
446 PRN regimen over 4-years. Eyes treated using a T&E regimen had significantly better visual outcomes  
447 and received more injections. Since visual outcomes are better with a T&E protocol and the present  
448 study demonstrated that MA is not influenced by the frequency of treatment, T&E may be the  
449 preferred treatment regimen as it allows for better functional outcomes with no increased risk for  
450 MA.

451

452 **Figure Captions**

453 Figure 1: Example of measurement of atrophy area by two masked graders using Heidelberg Region  
454 Finder semi-automatic progression tool at (A) baseline; (B) Year 1; (C) Year 2; (D) Year 3; and (E) Year  
455 4.

456 Figure 2: Mean progression of macular atrophy (MA) area over 4-years of follow-up in eyes with pre-  
457 existing atrophy (n=59).

458 Figure 3: Mean change in macular atrophy (MA) area by square root (SQRT) transformation over 4-  
459 years of follow-up in eyes with pre-existing atrophy (n=59).

460 Figure 4: Incidence of New Atrophy over 4-years

461 Figure 5: Total number of injections received (n=264).

462 Figure 6: Mean change in macular atrophy (MA) area over 4-years of follow-up in eyes with new  
463 incident atrophy.

464 Figure 7: Mean change in central macular thickness (CSRT) over 4-years of follow-up.

465 Figure 8: Visual Acuity (VA) in the presence and absence of detected macular atrophy (MA). **A**, VA  
466 change from baseline over time among study eyes with and without MA detected at baseline. **B**, VA  
467 at baseline, year 1, year 2, year 3, and year 4 with and without MA detected at each time point.

468 Error bars in both **A** and **B**, 95% confidence intervals (CIs) are shown. ETDRS= Early Treatment  
469 Diabetic Retinopathy Study.

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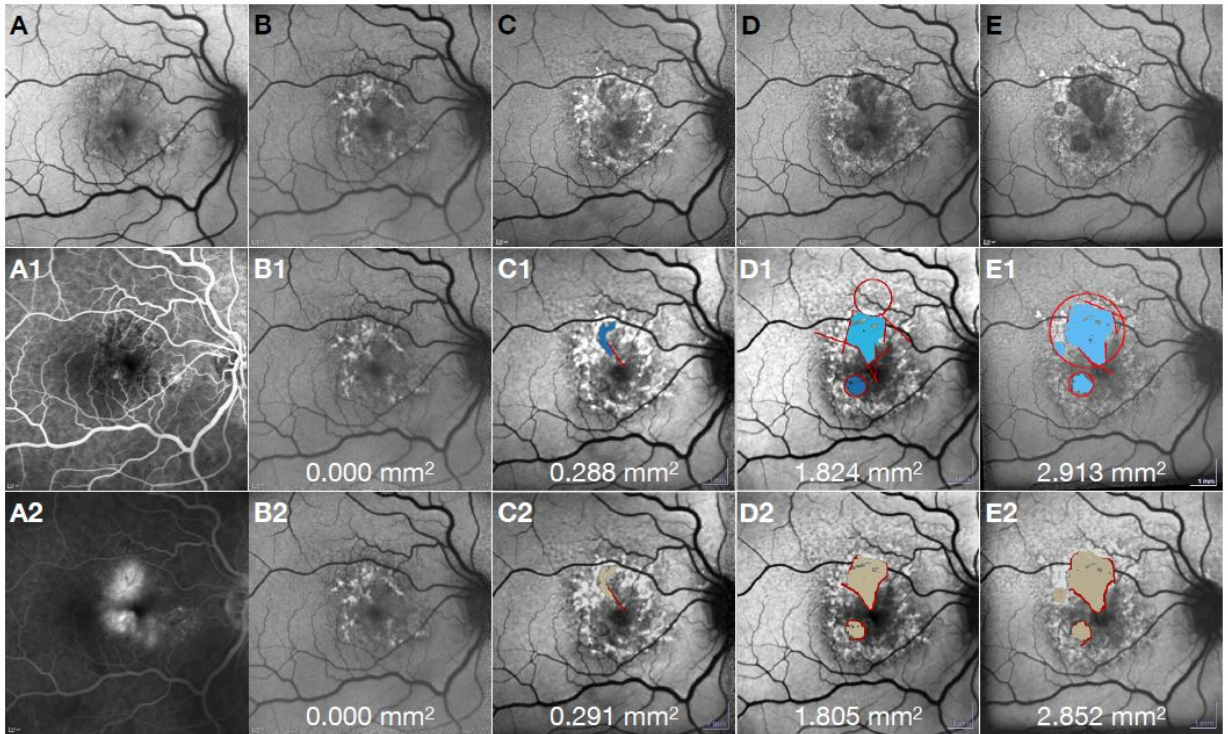
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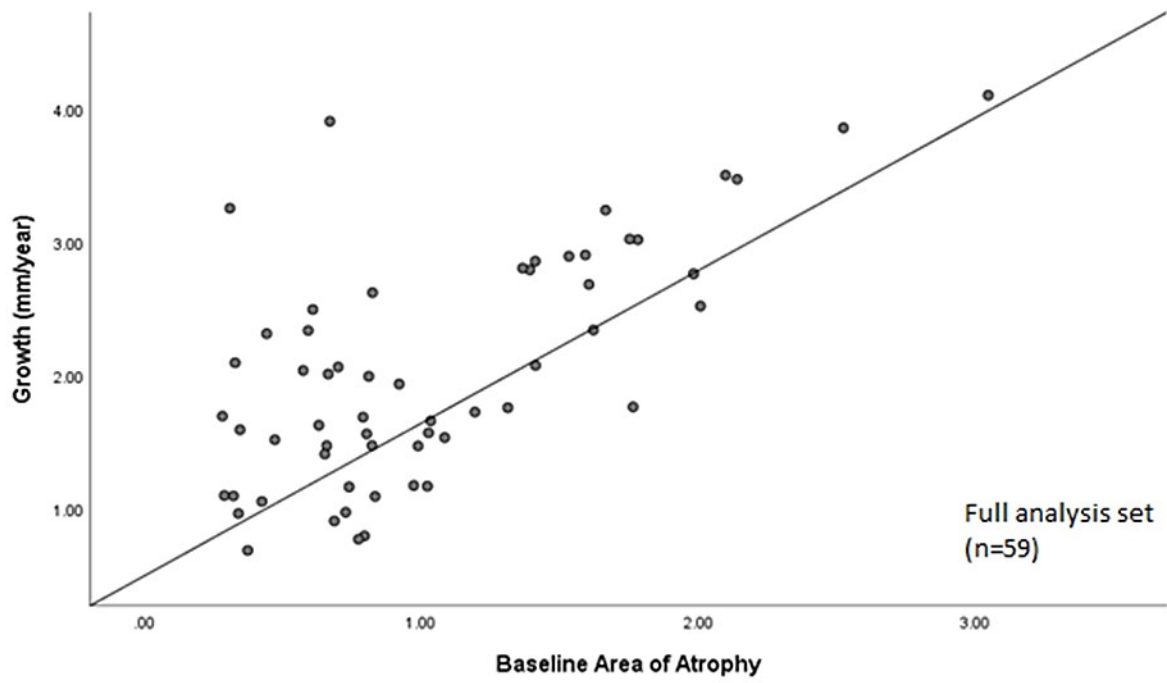
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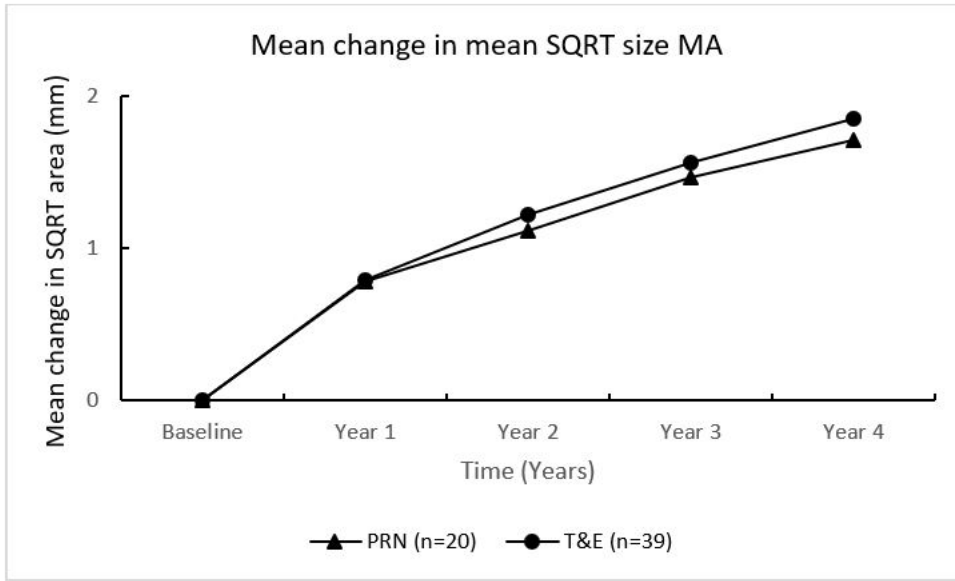
**Precis:** This comparative study of 264 eyes with nAMD treated with VEGF inhibitors demonstrated no difference in incidence or progression of macular atrophy using a treat-and-extend versus Pro re nata regimen over 4-years.

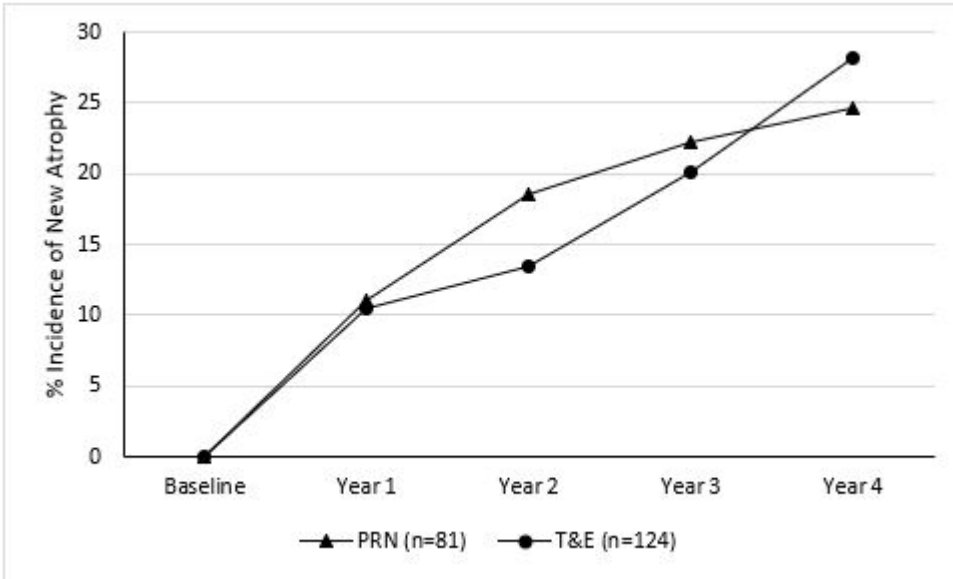
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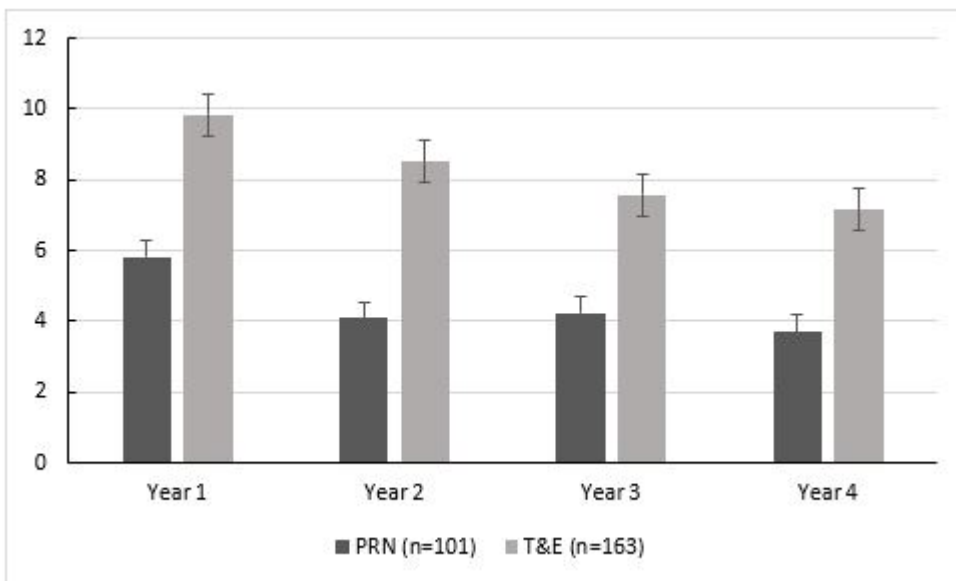


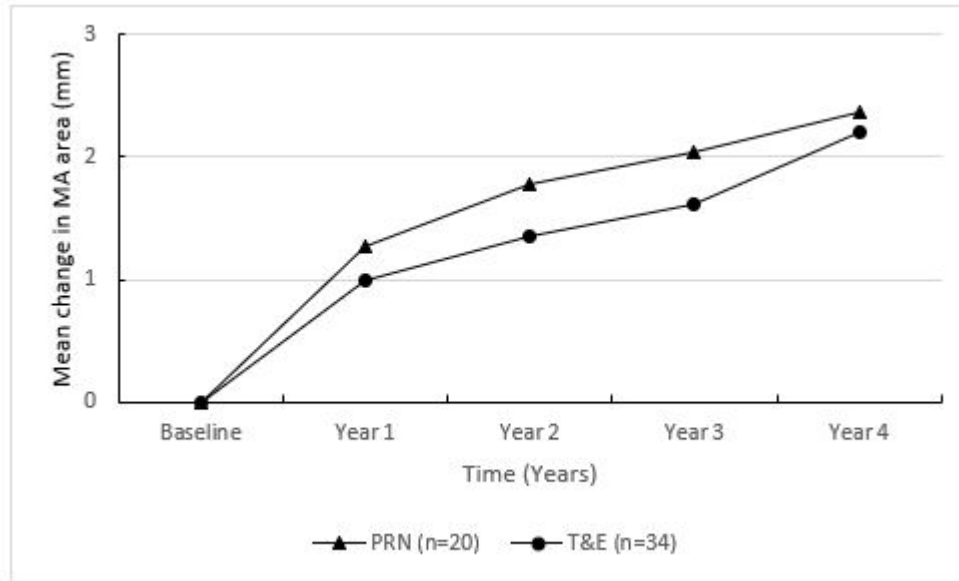


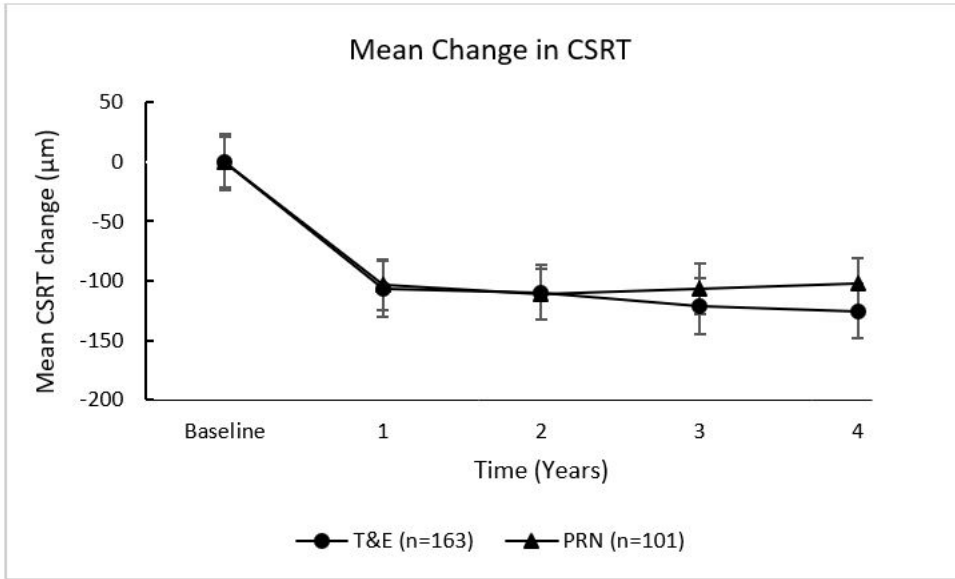
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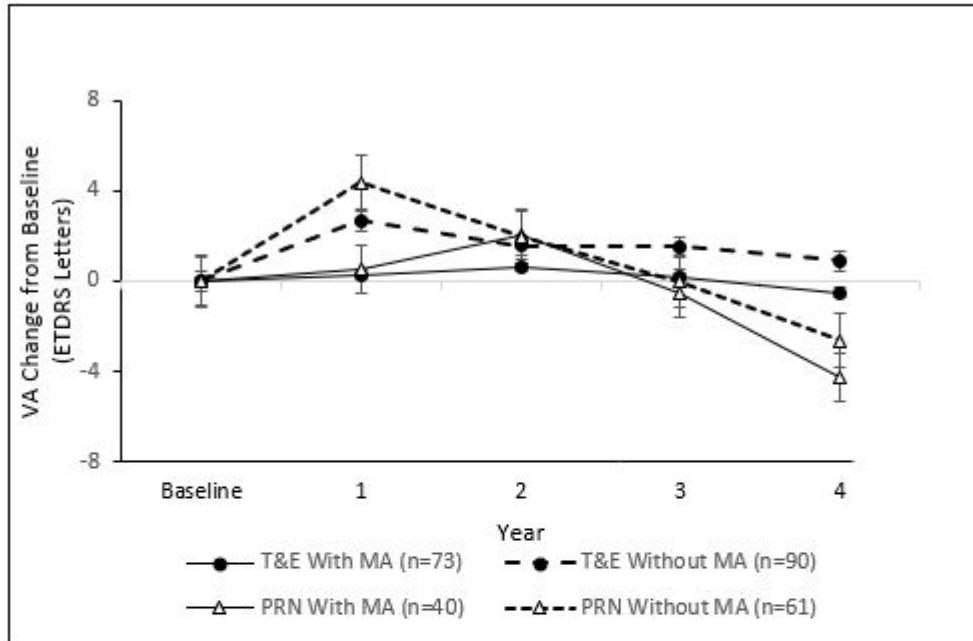




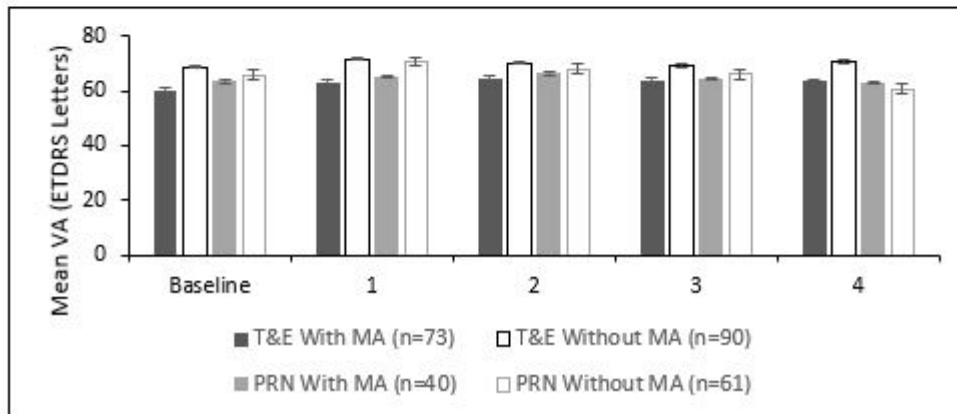




A.



B.



**Table 1. Baseline Characteristics of Included Patients**

	PRN (n=101)	T&E (n=163)	P Value
Age, years	74.3 ± 8.4	76.9 ± 8.8	0.33
VA (ETDRS letters)	65.5 ± 14.7	66.9 ± 14.4	0.44
CMT (µm)	416.6 ± 136.5	410.2 ± 105.5	0.67
MA at diagnosis	20 (20%)	39 (24%)	0.45
Unifocal	7 (35%)	11 (28%)	0.09
Multifocal	13 (65%)	28 (72%)	0.08
Foveal	12 (60%)	22 (56%)	0.82
Extrafoveal	8 (40%)	17 (44%)	0.81
MA lesion size (mm <sup>2</sup> )	0.8 ± 0.76	1.9 ± 1.9	0.014
Reticular Pseudodrusen	15 (15%)	19 (12%)	0.18
Drusen	57 (56%)	113 (69%)	0.051
Subretinal fibrosis	4 (4%)	6 (4%)	0.91
Gender			0.019
Male	35 (35%)	81 (50%)	
Female	66 (66%)	82 (50%)	
Laterality			0.269
Right	43 (43%)	78 (48%)	
Left	58 (57%)	85 (52%)	
CNV lesion type			0.43
Type 1	61 (60%)	86 (53%)	
Type 2	17 (17%)	30 (18%)	
Type 3 (RAP)	14 (14%)	22 (13%)	
PCV	9 (9%)	15 (9%)	
Lens Status			0.06
Phakic	72(71%)	83 (51%)	
Pseudophakic	29 (29%)	80 (49%)	
History of PDT/Laser	8 (8%)	8 (5%)	0.53

*CMT, central macular thickness; CNV, choroidal neovascular membrane; ETDRS, early treatment of diabetic retinopathy score; MA, macular atrophy; PCV, polypoidal choroidal vasculopathy; PDT, photodynamic therapy; RAP retinal angiomatous proliferation; VA, visual acuity*