



HAL
open science

Predicting Progression to Advanced Age-Related Macular Degeneration from Clinical, Genetic, and Lifestyle Factors Using Machine Learning

Soufiane Ajana, Audrey Cougnard-Grégoire, Johanna Colijn, Bénédicte M.J. Merle, Timo Verzijden, Paulus T.V. M. de Jong, Albert Hofman, Johannes Vingerling, Boris Hejblum, Jean-François Korobelnik, et al.

► **To cite this version:**

Soufiane Ajana, Audrey Cougnard-Grégoire, Johanna Colijn, Bénédicte M.J. Merle, Timo Verzijden, et al.. Predicting Progression to Advanced Age-Related Macular Degeneration from Clinical, Genetic, and Lifestyle Factors Using Machine Learning. *Ophthalmology: Journal of The American Academy of Ophthalmology*, 2021, 128 (4), pp.587-597. 10.1016/j.opthta.2020.08.031 . hal-03100320

HAL Id: hal-03100320

<https://inria.hal.science/hal-03100320>

Submitted on 7 Jan 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Predicting progression to advanced age-related macular degeneration from clinical, genetic and lifestyle factors using machine learning

Authors: Soufiane Ajana PhD,¹ Audrey Cougnard-Grégoire PhD,^{1*} Johanna M Colijn M.D., MSc,^{2,3*} Bénédicte MJ Merle PhD,^{1*} Timo Verzijden MSc,^{2,3} Paulus TVM de Jong M.D., PhD,^{4,5} Albert Hofman PhD,^{3,6} Johannes R Vingerling M.D., PhD,² Boris P Hejblum PhD,^{1,7} Jean-François Korobelnik M.D.,^{1,8} Magda A Meester-Smoor PhD,^{2,3} Marius Ueffing⁹, Hélène Jacqmin-Gadda PhD,^{1**} Caroline CW Klaver M.D., PhD,^{2,3,10, 11**} and Cécile Delcourt PhD^{1**} for the EYE-RISK Consortium[§]

¹ Univ. Bordeaux, Inserm, Bordeaux Population Health Research Center, UMR 1219, Bordeaux, France.

² Department of Ophthalmology, Erasmus University Medical Center, Rotterdam, The Netherlands.

³ Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands.

⁴ Department of Retinal signal processing, Netherlands Institute of Neurosciences, KNAW; Department of Ophthalmology, Amsterdam University MC, Amsterdam, The Netherlands.

⁵ Department of Ophthalmology, Leiden University MC, Leiden, The Netherlands.

⁶ Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA.

⁷ Inria, SISTM, Bordeaux Sud-Ouest, Bordeaux, France.

⁸ Department of Ophthalmology, Bordeaux University Hospital, Bordeaux, France.

⁹ Department of Ophthalmology, Institute for Ophthalmic Research, University of Tübingen, Tübingen, Germany

¹⁰ Department of Ophthalmology, Radboud University Medical Center, Nijmegen, The Netherlands.

¹¹ Institute of Molecular and Clinical Ophthalmology Basel, Basel, Switzerland.

- 1 *Audrey Cougnard-Grégoire, Johanna M Colijn and Bénédicte Merle contributed
- 2 equally to this work and are considered co-second authors.
- 3 **Hélène Jacqmin-Gadda, Caroline CW Klaver and Cécile Delcourt contributed
- 4 equally to this work and are considered co-last authors.

§ Members of the EYE-RISK consortium are listed in annex.

5 Corresponding author: Cécile Delcourt, “lifelong exposure health and aging” team,
6 Research Center Inserm U1219, Université de Bordeaux – ISPED, 146, rue Léo
7 Saignat, CS61292, 33076 Bordeaux Cedex. Email: cecile.delcourt@u-bordeaux.fr

8

9 This article contains additional online-only material. The following should appear
10 online-only: Figures S1, S2 and S3 and Table S1.

11 **Financial support:** The EYE-RISK project was supported by the European Union’s
12 Horizon 2020 Research and Innovation Programme (grant no.: 634479). The
13 Rotterdam Study is funded by Erasmus Medical Center and Erasmus University,
14 Rotterdam, The Netherlands; the Organization for the Health Research and
15 Development (ZonMw); the Research Institute for Diseases in the Elderly (RIDE); the
16 Ministry of Education, Culture and Science; the Ministry for Health, Welfare and
17 Sports; the European Commission (DG XII), and the Municipality of Rotterdam,
18 Rotterdam, The Netherlands. The funders had no role in study design, data collection
19 and analysis, decision to publish, or preparation of the manuscript.

20 Additionally, the ophthalmic research within the Rotterdam Study was supported by
21 the following foundations: Oogfonds; Bartiméus Sonneheerdt Vereniging; Landelijke
22 Stichting voor Blinden en Slechtienden; Algemene Nederlandse Vereniging Ter
23 Voorkoming Van Blindheid; Novartis Foundation; and MaculaFonds, which
24 contributed through UitZicht (grant nos.: 2015-36 and 2016-19). The Antioxydants,

25 Lipides Essentiels, Nutrition et Maladies Oculaires (ALIENOR) Study was funded by
26 Laboratoires Théa; Fondation Voir et Entendre; Retina France; Agence Nationale de
27 la Recherche (ANR 2010-PRSP-011 VISA); Programme Hospitalier de Recherche
28 Clinique (PHRC) (ECLAIR project-2012) ; CFSR Recherche, the French Speaking
29 Retina Specialists' Club and Caisse Nationale pour la Solidarité et l'Autonomie.
30 The funders had no role in study design, data collection and analysis, decision to
31 publish, or preparation of the manuscript. Laboratoires Théa participated in the
32 design of the Antioxydants, Lipides Essentiels, Nutrition et Maladies Oculaires
33 (ALIENOR) Study, but none of the sponsors of this study participated in the
34 collection, management, statistical analysis, or interpretation of the data, or in the
35 preparation, review, or approval of the present manuscript.

36

Running head: Predicting progression to advanced age-related macular degeneration

37

38 **Abstract:**

39 **Objective:** Current prediction models for advanced age-related macular
40 degeneration (AMD) are based on a restrictive set of risk factors. The objective of
41 this study was to develop a comprehensive prediction model, applying a machine
42 learning algorithm allowing selection of the most predictive risk factors automatically.

43 **Design:** Two population-based cohort studies

44 **Participants:** The Rotterdam Study I (RS-I, training set) included 3838 participants
45 aged 55 years or more, with a median follow-up period of 10.8 years and 108
46 incident cases of advanced AMD. The ALIENOR study (test set) included 362
47 participants aged 73 years or more, with a median follow-up period of 6.5 years and
48 33 incident cases of advanced AMD.

49 **Methods:** The prediction model used the bootstrap lasso for survival analysis to
50 select the best predictors of incident advanced AMD in the training set. Predictive
51 performance of the model was assessed using the area under the receiver operating
52 characteristic curve (AUC).

53 **Main outcome measures:** incident advanced AMD (atrophic and/or neovascular),
54 based on standardized interpretation of retinal photographs.

55 **Results:** The prediction model retained i) age, ii) a combination of phenotypic
56 predictors (based on the presence of intermediate drusen, hyper-pigmentation in one
57 or both eyes and age-related eye disease study (AREDS) simplified score), iii) a
58 summary genetic risk score based on 49 single nucleotide polymorphisms, iv)
59 smoking, v) diet quality, vi) education, and vii) pulse pressure. The cross-validated
60 AUC estimation in RS-I was 0.92 [0.88-0.97] at 5 years, 0.92 [0.90-0.95] at 10 years
61 and 0.91 [0.88-0.94] at 15 years. In ALIENOR, the AUC reached 0.92 at 5 years

62 [0.87-0.98]. In terms of calibration, the model tended to underestimate the cumulative
63 incidence of advanced AMD for the high-risk groups, especially in ALIENOR.

64 **Conclusions:** This prediction model reached high discrimination abilities, paving the
65 way towards making precision medicine for AMD patients a reality in the near future.

66

67 Age related macular degeneration (AMD) is a leading cause of blindness, worldwide.¹
68 It is a multifactorial degenerative eye disease affecting the central part of the retina,
69 named macula.² Its prevalence is rising alongside the general ageing of the human
70 population³, with a projected number of individuals affected by AMD worldwide of 196
71 million in 2020 and up to 288 million in 2040.⁴ AMD typically progresses through
72 different stages from an early to an advanced form, subdivided into atrophic and
73 neovascular subtypes. Advanced AMD results in permanent severe visual
74 impairment or blindness, with major consequences on quality of life and functional
75 independence.² Major therapeutic progress has been made for the neovascular form
76 of the disease in the last 15 years, with the intravitreal injection of anti-angiogenic
77 agents.² However, these treatments are not curative, represent a major cost for
78 society (for instance 2.3 billion of dollars annually for Medicare beneficiaries) and do
79 not always successfully stave off vision loss.⁵ Moreover, there still is no treatment
80 available for the atrophic form of the disease (about 50% of advanced cases).

81 Prediction models are increasingly being used by clinicians in order to stratify
82 patients according to their risk and adapt therapies, recommendations and follow-up
83 frequency. Such models can also be used for patient selection in clinical trials testing
84 new therapies or interventions. For instance, several prediction models are now
85 widely used in clinical practice for cardiovascular disease risk assessment.⁶

86 Both genetic and modifiable risk factors (in particular smoking, nutrition and vascular
87 risk factors) for advanced AMD have been previously identified⁷, and several
88 prediction models have been proposed.⁸⁻¹⁴ However, these prediction models did not
89 include dietary factors, relied only on a limited set of lifestyle factors and incorporated
90 only a restricted set of genetic variants.

91 Furthermore, stepwise selection methods are still widely used to develop such
92 models despite their lack of stability^{15, 16} and the availability of more principled
93 alternatives. In particular, penalized regression methods can handle overfitting and
94 multicollinearities among the predictors and have been proven to improve stability of
95 variable selection as well as prediction performance.^{17, 18} In addition, in elderly
96 cohorts, death is a competing event for the onset of advanced AMD. But due to
97 interval censoring (AMD is assessed only at follow-up visits in the cohort), AMD
98 status at death is unknown for subjects without advanced AMD at their last visit.
99 Standard survival analyses (e.g. Cox model) thus underestimate the incidence of
100 advanced AMD because they ignore advanced AMD cases developing between the
101 last visit and death. More importantly, regression parameters from Cox model may
102 be biased if the excess risk of death among AMD patient is large and predictors of
103 AMD are also associated with death risk.¹⁹

104 The aim of this article is to develop a prediction model for advanced AMD from a
105 wider set of potential predictors. Thus, our prediction model was developed from a
106 population-based cohort using a machine learning (ML) approach performing both
107 parameter estimation and automatic variable selection simultaneously. In addition,
108 this prediction model was validated on an independent population-based cohort to
109 better assess its generalizability.

110

111 **Methods**

112 **Study design and participants**

113 The training data set included participants enrolled in the Rotterdam Study I (RS-I).

114 The validation dataset included participants enrolled in the Antioxydants, Llipids

Code de champ modifié
Code de champ modifié
Code de champ modifié

115 Essentiels, Nutrition et maladies OculaiRes (ALIENOR) study. The complete
116 methodologies of these two population-based cohorts have been detailed elsewhere.
117 ^{20, 21} Data from both studies were harmonized and included in the database of the
118 EYE-RISK consortium (www.eyerisk.eu) which aims at proposing new clinical
119 guidelines and recommendations for prevention, and at providing a better
120 understanding of the pathogenetic causes of AMD.³ All participants gave written
121 informed consent for participation in accordance with the declaration of Helsinki and
122 ethics approval was obtained in both studies.

123 RS-I included participants aged 55 and over. Baseline examinations took place from
124 1990 to 1993 and 4 follow-up examinations were performed in 1993-1995, 1997-
125 1999, 2002-2004 and 2009-2011.

126 ALIENOR included participants aged 73 years or more at first eye examination.
127 Baseline eye examinations took place from 2006 to 2008 and 4 follow-up
128 examinations were performed in 2009-2010, 2011-2012, 2013-2015 and 2015-2017.
129

130 **Main outcome**

131 Classification of advanced AMD (neovascular or atrophic AMD) was based on retinal
132 photographs, interpreted by trained graders according to a modification of the
133 Wisconsin Age-Related Maculopathy Grading System²² for RS-I and according to the
134 International Classification²³ for ALIENOR. These classifications were identical
135 regarding for advanced AMD, but showed some differences regarding features of
136 early AMD (such as drusen area or location, for instance). Only features using
137 identical definitions in both classification were used in the analyses.

138 Incidence of advanced AMD was defined as participants without advanced AMD at
139 baseline progressing to advanced AMD at any point during the study period. All
140 advanced AMD cases were adjudicated by retina specialists of the corresponding
141 study and harmonized within the EYE-RISK consortium.³

142

143

144 **Statistical analysis**

145 We compared excluded participants with those included in the final set, both in RS-I
146 and ALIENOR using the t-test for continuous variables and the chi-square test or the
147 Fisher exact test for categorical variables accordingly. All the p-values were adjusted
148 for multiple testing using the Holm correction method (controlling the Family Wise
149 Error Rate).²⁴

150 The training dataset comprised a set of 33 variables which were common to RS-I and
151 ALIENOR: 7 variables describing retinal alterations (including the AREDS simplified
152 scale,²⁵ the Beckman classification²⁶ and the Rotterdam classification²⁷), 25 variables
153 for demographic, lifestyle (including the Mediterranean diet score²⁸) and
154 comorbidities, and a genetic risk score based on 49 previously identified single
155 nucleotide polymorphisms (SNPs). Based on the 52 single nucleotide polymorphisms
156 (SNPs) proposed by Fritsche et al.²⁹, the genetic risk score was calculated only for
157 participants without missing data for the following 5 major risk alleles:
158 CFH_rs10922109, CFH_rs570618, C2_rs11603772 (merged with: C2_rs429608),
159 C3_rs2230199 and ARMS2_rs3750846. In addition, 3 minor alleles with high
160 missingness were excluded from the analyses: TRPM3_rs71507014,
161 CNN2_rs67538026 and MMP9_rs142450006. Thus, the final genetic risk score was

162 calculated as a linear combination of 49 SNPs using the beta coefficients of Fritsche
163 et al. study.²⁹

164 We performed variable selection using the lasso method in a survival framework.³⁰
165 However, since the lasso may not be consistent in terms of variable selection when
166 there is a strong correlation between the relevant and the irrelevant predictors^{30, 31, 31,}
167 ³² we insured the stability of the selected predictors using the bootstrap as
168 recommended by several authors.^{33,34} As shown in Figure 1, we started by performing
169 a resampling with replacement from the entire Rotterdam Study I (RS-I) cohort in
170 order to generate 100 bootstrap samples. Of note, we stratified the individuals in
171 each bootstrap sample according to the incidence rate of advanced AMD. The
172 objective was to maintain the same ratio of events in each generated bootstrap
173 sample since there is a low number of events in RS-I (2.8%). We then estimated a
174 lasso in a survival framework³⁰ in each bootstrap sample in order to perform variable
175 selection while handling the multicollinearity of some predictors. The penalization
176 parameter of the lasso was estimated by minimizing the partial likelihood deviance
177 using a 5-fold cross-validation stratified on the incidence rate of advanced AMD. The
178 final set of selected variables was composed of the most frequently selected
179 variables on all the bootstrap samples using the elbow criterion.³⁵

180 The risk score was then estimated by including the final set of selected variables in a
181 semi-parametric Illness-Death Model (IDM) (i.e., the baseline transition intensities
182 were left unspecified and approximated by M-splines) to account for the probability of
183 developing advanced AMD between the last visit without the disease and death.³⁶
184 The IDM was estimated with the R package *smoothHazard* v1.4.1.³⁷ The parameters
185 of the IDM were estimated by maximization of the penalized likelihood. The
186 penalization parameters, which control the trade-off between the data fit and the

187 smoothness of the intensity functions, were estimated by leave-one-out cross-
188 validation. We selected an equidistant sequence of 5 knots between the minimal and
189 maximal event times to fit the splines for each transition intensity. Finally, we
190 included the variables selected by bootstrap lasso as inputs for the regression model
191 of the transition intensity to advanced AMD and we added gender (in addition to the
192 variables selected by bootstrap lasso) as input for the regression models of the two
193 transition intensities to death.

194 The risk score for advanced AMD was defined as the linear predictor for the
195 transition intensity to advanced AMD estimated from this IDM. We then defined 2
196 cutpoints at 5 years in RS-I, in order to classify participants in 3 risk categories (low,
197 intermediate and high). The first threshold was chosen to be highly sensitive
198 (sensitivity closest to 95%) and the second to be highly specific (specificity closest to
199 95 %) similarly to Delcourt *et al.*³⁸

200 Finally, prediction performance was evaluated both internally in RS-I (using cross-
201 validation) as well as externally in ALIENOR. Discrimination performance was
202 assessed by calculating the area under the receiver operating curve (AUC) for
203 incident advanced AMD while accounting for the competing risk of death.³⁹ All AUCs
204 are reported with 95% confidence intervals, based on the quantiles of the standard
205 normal distribution.³⁹ Calibration was evaluated by comparing the observed
206 cumulative incidence to the model-estimated cumulative incidence of advanced AMD
207 in each risk category. Of note, the predicted CIF of advanced AMD was estimated
208 using a parametric IDM where the baseline transition intensities were modelled by
209 Weibull distributions. The observed CIF was estimated using a semi-parametric IDM
210 where the baseline transition intensities were left unspecified and approximated by
211 M-splines.³⁶

212 In a sensitivity analysis, other missing data for comorbidities, phenotypic and
213 environmental variables were imputed using multiple imputation by chained
214 equations.⁴⁰ In RS-I, participants for whom either all genetic data (n= 572) or all
215 nutritional data (n= 768) was missing were excluded from the training set, because it
216 became impossible to build a reliable imputation model in such cases. We generated
217 5 imputed datasets and the imputation procedure was iterated 20 times. We
218 performed all the analyses using R, version 3.3.4 (R Foundation). We used two-sided
219 P values with an $\alpha = .05$ threshold for statistical significance.

Code de champ modifié

220

221 **Results**

222 **Study participants**

223 As shown in Figure 2, among the 5,214 RS-I participants without advanced AMD at
224 baseline and with at least one follow-up examination, 572 had no genetic data,
225 mostly because of lack of blood samples, 144 participants had incomplete phenotypic
226 data and 690 had incomplete lifestyle data (including comorbidities, nutritional and
227 environmental data), leading to a final sample size of 3,838 subjects and 108 cases
228 of incident advanced AMD. Among the 617 participants of the ALIENOR study with
229 incidence data and without advanced AMD at baseline, 112 had no genetic data, 107
230 had incomplete phenotypic data and 36 had incomplete lifestyle data, leading to a
231 final sample size of 362 participants and 33 cases of incident advanced AMD.

232 The description of the included and excluded participants of both cohorts is provided
233 in Table S1 (available at <http://www.aaojournal.org>). In both RS-I and ALIENOR
234 study, included participants tended to be younger than those excluded. Additionally,
235 in RS-I, the included participants tended to have less frequently early AMD, to have

236 more frequently secondary education and to take less frequently anti-diabetic drugs.
237 Furthermore, included participants of RS-I were 10 years younger on average than
238 those of ALIENOR — they were less frequently affected by early AMD and less
239 frequently used anti-diabetic and lipid lowering drugs. They were also more often
240 smokers, and adhered less to the Mediterranean diet. Interestingly, the incidence of
241 advanced AMD was higher in ALIENOR compared to RS-I, especially in the first 2
242 years of follow-up (see Figure S1, available at <http://www.aaojournal.org>).

243

244 **Prediction model**

245 The 33 variables in the analysis and the results of the variable selection obtained by
246 the bootstrap lasso technique are presented in Figure 3. Using the elbow criterion,
247 we selected the first 9 variables as being the most predictive of incident advanced
248 AMD. Particularly, 4 variables (presence of intermediate drusen, genetic risk score,
249 AREDS simplified scale and age) were selected in 100% of the 100 bootstrap
250 samples. In addition, smoking, pulse pressure (the difference between systolic and
251 diastolic blood pressure), presence of hyperpigmentation, education and the
252 Mediterranean diet score were selected in more than 80% of the bootstrap samples,
253 while other variables were selected less frequently. Interestingly, these 9 variables
254 were also the most frequently selected in the 5 imputed datasets.

255 Our prediction model reached very high discrimination performances (Figure S2,
256 available at <http://www.aaojournal.org>). In RS-I, the cross-validated AUCs were 0.92
257 (95% CI, 0.88 to 0.97), 0.92 (95% CI, 0.90 to 0.95) and 0.91 (95% CI, 0.88 to 0.94) at
258 5, 10 and 15 years of follow-up, respectively. In ALIENOR, the AUC was 0.92 (95%
259 CI, 0.87 to 0.98) at 5 years of follow-up.

260 As genetic testing is not currently available in routine ophthalmological practice, we
261 tested the prediction performance of an IDM model with 8 variables (after exclusion
262 of the genetic risk score). In the Rotterdam study, the cross-validated AUCs were
263 0.91 (95% CI, 0.86-0.95), 0.90 (95%CI, 0.87-0.93) and 0.88 (95%CI, 0.84-0.92) at 5,
264 10 and 15 years of follow-up, respectively. In ALIENOR, the AUC was 0.93 (95%CI,
265 0.89-0.97).

266 **Risk categories characteristics**

267 We described the distribution of the risk score calculated in RS-I and validated in
268 ALIENOR for incident advanced AMD and non-incident advanced AMD participants
269 (Figure S3, available at <http://www.aaajournal.org>). As expected, we observe a
270 bimodal distribution in both studies, although some overlap persisted.

271 To further investigate the prediction ability of the final prediction model, we classified
272 the participants in 3 risk categories (low, intermediate and high). The definition of the
273 cut-points was performed in RS-I. The first cut-point was set at 1.49, corresponding
274 to a sensitivity of 93.8%, and the second cut-point was set at 2.73, corresponding to
275 a specificity of 95.0%.

276 We estimated the cumulative incidence of advanced AMD in each of the risk
277 categories for RS-I and ALIENOR using a semi-parametric IDM (Figure 4). In both
278 samples, the high-risk group was characterized by a high cumulative incidence
279 increasing steeply from baseline and reaching 21% at 8 years and 44% at 15 years
280 in RS-I and 32% at 8 years in ALIENOR. In both samples, the intermediate-risk and
281 the low-risk categories showed much lower incidence rates than the higher risk
282 category, at all-time points. Particularly, the low-risk category showed a cumulative
283 incidence close to 0 during the whole follow-up in both cohorts. In RS-I, the incidence
284 in the intermediate-risk and low-risk groups were distinguishable only in the long term

285 (after 5 years). In ALIENOR, both incidences remained very close until 8 years of
286 follow-up. This is probably due to the very low number of events in both of these
287 categories (see Table 1). It is however to be noted that in RS-I, the incidence of
288 advanced AMD in the intermediate group was only after 7-10 years clearly higher
289 than that in the low risk category.

290

291 As shown in Table 1, in RS-I, 3,146 (81.9%) participants were classified as low-risk,
292 405 (10.6%) as intermediate-risk and 287 (7.5%) as high-risk. Overall, 49% of the
293 incident cases were observed in the high-risk category versus 24% in the
294 intermediate-risk category and 27% in the low-risk category (due to the larger size of
295 this last group). The AMD genetic risk score ranging from -3.36 to 4.54 in the whole
296 sample had a mean of 0.2, 1.2 and 1.6 in the low-risk, intermediate-risk and high-risk
297 categories respectively. Regarding phenotypic characteristics, 93.3% of participants
298 in the low-risk category were classified as free of AMD in the AREDS simplified scale
299 (score 0). Interestingly, this was also the case for 73.1% and 26.1% of the
300 participants classified in the intermediate-risk and high-risk groups, respectively. The
301 high-risk participants with an AREDS score of 0 were characterized by a high
302 frequency of intermediate drusen at baseline (84%), a very high genetic risk score
303 (mean of 2.06) and a high cumulative incidence of advanced AMD (9 incident cases
304 out of 75, 12 %). Finally, regarding lifestyle and co-morbidities, the high-risk category
305 showed a higher frequency of primary education, a lower adherence to the
306 Mediterranean diet score and a higher mean pulse pressure compared to the
307 intermediate-risk and low-risk groups.

308

309 In ALIENOR, 52.5% of the participants were classified as low-risk, 22.3% as
310 intermediate-risk and 25.2% as high-risk. Overall, 81.8% of the incident cases were
311 observed in the high-risk category, 6.1% in the intermediate-risk category and 12.1 %
312 in the low-risk category. Again, 15.4% of the participants classified as high-risk had a
313 score of 0 on the AREDS simplified scale. Regarding lifestyle and co-morbidities, we
314 observed the same trends as in RS-I.
315 Finally, we assessed the calibration of the prediction model (i.e., the ability of the
316 model to correctly predict the incidence of advanced AMD in each risk category). As
317 displayed in Figure 5, the model tends to underestimate the cumulative incidence of
318 advanced AMD for the high-risk groups, especially in ALIENOR.

319

320 **Discussion**

321 We developed a prediction model for advanced AMD based on phenotypic, genotypic
322 and lifestyle factors. This model reached high discrimination performances with an
323 AUC equal to 92% at 5 years in the external validation set. In addition, the proposed
324 model showed a good stratification of participants into low-risk and high-risk groups
325 of incident advanced AMD both in RS-I and ALIENOR.

326 Buitendijk et al. developed a prediction model for incident advanced AMD based on
327 RS-I and validated it in two independent population-based cohorts.¹⁰ Other prediction
328 models for incident advanced AMD trained on the Age-Related Eye Disease Study
329 (AREDS) clinical trial were proposed.^{8, 9, 12, 14, 41} These models were similarly based
330 on genetic, phenotypic and lifestyle variables and also reached high discrimination
331 performances externally (AUC of 0.85 to 0.90), although somewhat lower than the
332 model presented here. The main differences with the present model lie in the

a supprimé: by Seddon et al

334 inclusion of a wider set of lifestyle and comorbidities factors as well as a genetic
335 score based on 49 AMD-related SNPs. To our knowledge, the present study is the
336 first to use an automatic variable selection approach to select the most predictive
337 variables from a wide set of predictors.³³ Indeed, this is a principled way to identify
338 new predictors compared to less rigorous strategies used in the aforementioned
339 prediction models where only a limited set of variables were selected *a priori*. Of
340 particular interest, among the predictors selected by the bootstrap lasso approach
341 here, pulse pressure as well as the Mediterranean diet score were never included in
342 the previously reported AMD prediction models. These predictors have previously
343 been reported as associated with AMD and are modifiable factors, which could lead
344 to preventive interventions.⁴²⁻⁴⁴ Interestingly, the bootstrap lasso also selected
345 education, which we believe is more likely to be a surrogate for other unmeasured
346 lifestyle risk factors rather than a direct risk factor of advanced AMD. We also
347 considered the competing risk of death during both the training of the model³⁶ and
348 when assessing its discrimination performance³⁹, which was not done in previous
349 studies.

350 Furthermore, in order to insure comparability with the previously developed prediction
351 models, we ran a standard Cox model (instead of an IDM) on the variables selected
352 by bootstrap lasso, considering subjects who died without AMD as censored at their
353 last visit before death. This “naïve” model reached slightly better predictive
354 discrimination performances than the IDM with a cross-validated AUC in RS-I equal
355 to 0.94 (95% CI, 0.89 to 0.99) at 5 years, 0.93 (95% CI, 0.90 to 0.96) at 10 years and
356 0.92 (95% CI, 0.89 to 0.95) at 15 years. In ALIENOR, the AUC remained at 0.92 at 5
357 years (95% CI, 0.87 to 0.97). The differences in AUC between the Cox and IDM
358 models were small, most probably because the risk of death was not much higher

359 among AMD patients and only a few of the selected predictors for AMD were
360 predictors of death.¹⁹
361 Since genetic testing is not currently available in routine ophthalmological practice,
362 we also ran an IDM model without the genetic risk score. Although the genetic risk
363 score was selected in 100% of the bootstrap lasso samples, the AUCs of this model
364 were similar to those obtained with the complete model both in the Rotterdam (91%,
365 90% and 88% at 5, 10 and 15 years, respectively) and Alienor studies (93% at 5
366 years). However, in a minority of cases, genetic information might help identify high
367 risk subjects. In particular, a small number of subjects with a simplified AREDS score
368 0 were classified as high risk. They had a high genetic score (average 2.06) and
369 exhibited an incidence of late AMD of 12%.

a supprimé: at 5 years

370 The main limitations of this study were the low number of events making it impossible
371 to investigate AMD subtypes. In addition, our model tended to underestimate the
372 incidence of advanced AMD for the high-risk groups, particularly in the validation set,
373 which is most probably due to the higher incidence rates in ALIENOR compared to
374 RS-I. As discussed previously, these differences in incidence rates are partially due
375 to age differences among the cohorts⁴², but may also be due to differences in design,
376 classification of disease and potentially differences in AMD incidence among the
377 Netherlands and France. Further validation in other cohorts, in particular in
378 populations with a different genetic make-up (such as Asian populations), would
379 enhance the generalizability of this model.

a supprimé: , while the performance at long term appeared somewhat lower (88% versus 91% at 15 years, in the Rotterdam study).

380 Further enhancements of this prediction model could still be achieved by integrating
381 multiple data sources as color fundus photographs (CFPs) and Optical Coherence
382 Tomography (OCT) examinations. Indeed, in the present model, description of
383 phenotype is based on human interpretation of a few standard retinal alterations on

388 CFPs (large drusen and pigmentary abnormalities composing the AREDS simplified
389 score, together with intermediate drusen and hyperpigmentation). Automated grading
390 of retinal images using deep learning methods can improve the prediction accuracy
391 of the model compared to manual grading which is subjective and time-consuming.⁴⁵⁻
392 ⁴⁹ Moreover, previous studies have suggested that SD-OCT parameters, such as
393 drusen volume, drusen reflectivity, presence of hyperreflective foci or retinal
394 thickness, may improve prediction of progression to advanced AMD.^{46, 50-52}

395 In conclusion, this prediction model for advanced AMD based on a wide set of risk
396 factors achieved high discrimination performances. It will be made available to
397 ophthalmologists and patients through the website www.macutest.net. The approval
398 by the U.S. Food and Drug Administration of devices based on artificial intelligence
399 highlights the potential of combining such prediction models – based on ML
400 techniques – with ophthalmologists' expertise to suggest personalized
401 recommendations and to lower the risk of AMD. Our prediction model paves the way
402 towards making precision medicine for AMD patients a reality in the near future.

403

404

405 **References**

- 406 1. Bourne RR, Stevens GA, White RA, et al. Causes of vision loss worldwide, 1990-2010: a
407 systematic analysis. *Lancet Glob Health* 2013;1:e339-349.
- 408 2. Mitchell P, Liew G, Gopinath B, Wong TY. Age-related macular degeneration. *Lancet*
409 2018;392:1147-1159.
- 410 3. Colijn JM, Buitendijk GHS, Prokofyeva E, et al. Prevalence of Age-Related Macular
411 Degeneration in Europe: The Past and the Future. *Ophthalmology* 2017;124:1753-1763.
- 412 4. Wong WL, Su X, Li X, et al. Global prevalence of age-related macular degeneration and
413 disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob*
414 *Health* 2014;2:e106-116.
- 415 5. Erie JC, Barkmeier AJ, Hodge DO, Mahr MA. High Variation of Intravitreal Injection Rates and
416 Medicare Anti-Vascular Endothelial Growth Factor Payments per Injection in the United States.
417 *Ophthalmology* 2016;123:1257-1262.

- 418 6. Pylpchuk R, Wells S, Kerr A, et al. Cardiovascular disease risk prediction equations in 400 000
419 primary care patients in New Zealand: a derivation and validation study. *Lancet* 2018;391:1897-1907.
- 420 7. Sobrin L, Seddon JM. Nature and nurture- genes and environment- predict onset and
421 progression of macular degeneration. *Prog Retin Eye Res* 2014;40C:1-15.
- 422 8. Seddon JM, Reynolds R, Maller J, et al. Prediction model for prevalence and incidence of
423 advanced age-related macular degeneration based on genetic, demographic, and environmental
424 variables. *Invest Ophthalmol Vis Sci* 2009;50:2044-2053.
- 425 9. Seddon JM, Reynolds R, Yu Y, et al. Risk models for progression to advanced age-related
426 macular degeneration using demographic, environmental, genetic, and ocular factors.
427 *Ophthalmology* 2011;118:2203-2211.
- 428 10. Buitendijk GH, Rohtchina E, Myers C, et al. Prediction of age-related macular degeneration
429 in the general population: the Three Continent AMD Consortium. *Ophthalmology* 2013;120:2644-
430 2655.
- 431 11. Chiu CJ, Mitchell P, Klein R, et al. A risk score for the prediction of advanced age-related
432 macular degeneration: development and validation in 2 prospective cohorts. *Ophthalmology*
433 2014;121:1421-1427.
- 434 12. Seddon JM, Rosner B. Validated Prediction Models for Macular Degeneration Progression
435 and Predictors of Visual Acuity Loss Identify High-Risk Individuals. *Am J Ophthalmol* 2019;198:223-
436 261.
- 437 13. Chen Y, Zeng J, Zhao C, et al. Assessing susceptibility to age-related macular degeneration
438 with genetic markers and environmental factors. *Arch Ophthalmol* 2011;129:344-351.
- 439 14. Klein ML, Francis PJ, Ferris FL, 3rd, et al. Risk assessment model for development of advanced
440 age-related macular degeneration. *Arch Ophthalmol* 2011;129:1543-1550.
- 441 15. Breiman L. Heuristics of Instability and Stabilization in Model Selection. *The Annals of*
442 *Statistics* 1996;24:2350-2383.
- 443 16. Heinze G, Wallisch C, Dunkler D. Variable selection – A review and recommendations for the
444 practicing statistician. *Biometrical Journal* 2018;60:431-449.
- 445 17. Mansiaux Y, Carrat F. Detection of independent associations in a large epidemiologic dataset:
446 a comparison of random forests, boosted regression trees, conventional and penalized logistic
447 regression for identifying independent factors associated with H1N1pdm influenza infections. *BMC*
448 *Med Res Methodol* 2014;14:99.
- 449 18. Obermeyer Z, Emanuel EJ. Predicting the Future - Big Data, Machine Learning, and Clinical
450 Medicine. *N Engl J Med* 2016;375:1216-1219.
- 451 19. Leffondre K, Touraine C, Helmer C, Joly P. Interval-censored time-to-event and competing risk
452 with death: is the illness-death model more accurate than the Cox model? *Int J Epidemiol*
453 2013;42:1177-1186.
- 454 20. Ikram MA, Brusselle GGO, Murad SD, et al. The Rotterdam Study: 2018 update on objectives,
455 design and main results. *Eur J Epidemiol* 2017;32:807-850.
- 456 21. Delcourt C, Korobelnik JF, Barberger-Gateau P, et al. Nutrition and Age-Related Eye Diseases:
457 The ALIENOR (Antioxydants, Lipides Essentiels, Nutrition et Maladies Oculaires) Study. *J Nutr Health*
458 *Aging* 2010;14:854-861.
- 459 22. Klein R, Davis MD, Magli YL, et al. The Wisconsin age-related maculopathy grading system.
460 *Ophthalmology* 1991;98:1128-1134.
- 461 23. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for
462 age-related maculopathy and age-related macular degeneration. The International ARM
463 Epidemiological Study Group. *Surv Ophthalmol* 1995;39:367-374.
- 464 24. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scandinavian Journal of*
465 *Statistics* 1979;6:65-70.
- 466 25. Ferris FL, Davis MD, Clemons TE, et al. A simplified severity scale for age-related macular
467 degeneration: AREDS Report No. 18. *Arch Ophthalmol* 2005;123:1570-1574.
- 468 26. Ferris FL, 3rd, Wilkinson CP, Bird A, et al. Clinical classification of age-related macular
469 degeneration. *Ophthalmology* 2013;120:844-851.

470 27. Van Leeuwen R, Klaver CC, Vingerling JR, et al. The risk and natural course of age-related
471 maculopathy: follow-up at 6 1/2 years in the Rotterdam study. *Arch Ophthalmol* 2003;121:519-526.

472 28. Sofi F, Macchi C, Abbate R, et al. Mediterranean diet and health status: an updated meta-
473 analysis and a proposal for a literature-based adherence score. *Public Health Nutr* 2014;17:2769-
474 2782.

475 29. Fritsche LG, Igl W, Bailey JN, et al. A large genome-wide association study of age-related
476 macular degeneration highlights contributions of rare and common variants. *Nat Genet* 2016;48:134-
477 143.

478 30. Tibshirani R. The lasso method for variable selection in the Cox model. *Stat Med*
479 1997;16:385-395.

480 31. Zou H, Hastie T. Regularization and variable selection via the elastic net. *Journal of the Royal*
481 *Statistical Society: Series B (Statistical Methodology)* 2005;67:301-320.

482 32. Zou H. The Adaptive Lasso and Its Oracle Properties. *Journal of the American Statistical*
483 *Association* 2006;101:1418-1429.

484 33. Bach FR. Bolasso: model consistent Lasso estimation through the bootstrap. Proceedings of
485 the 25th international conference on Machine learning. Helsinki, Finland: ACM, 2008.

486 34. Breiman L. Bagging Predictors. *Machine Learning* 1996;24:123-140.

487 35. Cattell RB. The Scree Test For The Number Of Factors. *Multivariate Behav Res* 1966;1:245-
488 276.

489 36. Touraine C, Helmer C, Joly P. Predictions in an illness-death model. *Stat Methods Med Res*
490 2016;25:1452-1470.

491 37. Touraine C, Gerds TA, Joly P. SmoothHazard: An R Package for Fitting Regression Models to
492 Interval-Censored Observations of Illness-Death Models. *Journal of Statistical Software* 2017;79:1-22.

493 38. Delcourt C, Souied E, Sanchez A, Bandello F. Development and Validation of a Risk Score for
494 Age-Related Macular Degeneration: The STARS Questionnaire. *Invest Ophthalmol Vis Sci*
495 2017;58:6399-6407.

496 39. Blanche P, Dartigues JF, Jacqmin-Gadda H. Estimating and comparing time-dependent areas
497 under receiver operating characteristic curves for censored event times with competing risks. *Stat*
498 *Med* 2013;32:5381-5397.

499 40. van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations
500 in R. *Journal of Statistical Software; Vol 1, Issue 3 (2011)* 2011.

501 41. Seddon JM, Silver RE, Kwong M, Rosner B. Risk Prediction for Progression of Macular
502 Degeneration: 10 Common and Rare Genetic Variants, Demographic, Environmental, and Macular
503 Covariates. *Invest Ophthalmol Vis Sci* 2015;56:2192-2202.

504 42. Merle BMJ, Colijn JM, Cougnard-Gregoire A, et al. Mediterranean Diet and Incidence of
505 Advanced Age-Related Macular Degeneration: The EYE-RISK Consortium. *Ophthalmology*
506 2019;126:381-390.

507 43. Cougnard-Gregoire A, Delyfer MN, Korobelnik JF, et al. Long-Term Blood Pressure and Age-
508 Related Macular Degeneration: The ALIENOR Study. *Invest Ophthalmol Vis Sci* 2013;54:1905-1912.

509 44. Areds. Risk factors associated with age-related macular degeneration. A case-control study in
510 the age-related eye disease study: age-related eye disease study report number 3. Age-Related Eye
511 Disease Study Research Group. *Ophthalmology* 2000;107:2224-2232.

512 45. Peng Y, Dharssi S, Chen Q, et al. DeepSeeNet: A Deep Learning Model for Automated
513 Classification of Patient-based Age-related Macular Degeneration Severity from Color Fundus
514 Photographs. *Ophthalmology* 2019;126:565-575.

515 46. Schmidt-Erfurth U, Waldstein SM, Klmscha S, et al. Prediction of Individual Disease
516 Conversion in Early AMD Using Artificial Intelligence. *Invest Ophthalmol Vis Sci* 2018;59:3199-3208.

517 47. De Fauw J, Ledsam JR, Romera-Paredes B, et al. Clinically applicable deep learning for
518 diagnosis and referral in retinal disease. *Nature medicine* 2018;24:1342-1350.

519 48. Burlina PM, Joshi N, Pekala M, et al. Automated Grading of Age-Related Macular
520 Degeneration From Color Fundus Images Using Deep Convolutional Neural Networks. *JAMA*
521 *Ophthalmol* 2017;135:1170-1176.

522 49. Burlina PM, Joshi N, Pacheco KD, et al. Use of Deep Learning for Detailed Severity
523 Characterization and Estimation of 5-Year Risk Among Patients With Age-Related Macular
524 Degeneration. *JAMA Ophthalmol* 2018;136:1359-1366.
525 50. Hallak JA, de Sisternes L, Osborne A, et al. Imaging, Genetic, and Demographic Factors
526 Associated With Conversion to Neovascular Age-Related Macular Degeneration: Secondary Analysis
527 of a Randomized Clinical Trial. *JAMA Ophthalmol* 2019;137:738-744.
528 51. Ferrara D, Silver RE, Louzada RN, et al. Optical Coherence Tomography Features Preceding
529 the Onset of Advanced Age-Related Macular Degeneration. *Invest Ophthalmol Vis Sci* 2017;58:3519-
530 3529.
531 52. Waldstein SM, Vogl WD, Bogunovic H, et al. Characterization of Drusen and Hyperreflective
532 Foci as Biomarkers for Disease Progression in Age-Related Macular Degeneration Using Artificial
533 Intelligence in Optical Coherence Tomography. *JAMA Ophthalmol* 2020.

534

535

536

537 **Figures legends:**

538 Figure 1. The bootstrap lasso and IDM procedures for developing the prediction model
539 of advanced AMD.

540

541 Figure 2. Flowchart of Rotterdam Study I (RS-I) and Antioxydants, Llipids Essentiels,
542 Nutrition et maladies OculaiRes (ALIENOR) study.

543

544 Figure 3. Selection frequency of variables in the Rotterdam Study I (RS-I) using the
545 Bolasso method.

546 The vertical dashed line represent the threshold corresponding to a selection
547 frequency equals to 80%.

548

549 Figure 4. Cumulative incidence of advanced age-related macular degeneration
550 (AMD) according to risk categories.

551 A, Rotterdam Study I (RS-I). B, Antioxydants, LIpids Essentiels, Nutrition et maladies
552 OculaiRes (ALIENOR) study.

553

554 Figure 5. Calibration of prediction risk scores per risk group.

555 A,B,C. Comparison of the cross-validated predicted and observed cumulative
556 incidence in the Rotterdam Study I (RS-I). D. Comparison of the predicted and
557 observed cumulative incidence in the Antioxydants, LIpids Essentiels, Nutrition et
558 maladies OculaiRes (ALIENOR) study.

559 The vertical bars represent the 95% confidence intervals of the observed cumulative
560 incidences.

561

562

563 **Annex:**

564 Members of the Eye-Risk consortium: Erkin I. Acar, MSc (Department of
565 Ophthalmology, Radboud University Medical Center, Nijmegen, Netherlands), Blanca
566 Arango-Gonzalez, M.D. (Centre for Ophthalmology, Institute for Ophthalmic
567 Research, Eberhard Karls University Tübingen, University Clinic Tübingen, Tübingen,
568 Germany); Angela Armento, PhD (Centre for Ophthalmology, Institute for Ophthalmic
569 Research, Eberhard Karls University Tübingen, University Clinic Tübingen, Tübingen,
570 Germany); Franz Badura (PRO RETINA Deutschland e.V.); Vaibhav Bhatia, PhD
571 (Department of Regeneration and Cell Therapy, Andalusian Molecular Biology and

572 Regenerative Medicine Centre (CABIMER), Seville, Spain); Shomi S. Bhattacharya,
573 PhD (Department of Regeneration and Cell Therapy, Andalusian Molecular Biology
574 and Regenerative Medicine Centre (CABIMER), Seville, Spain); Marc Biarnés, PhD
575 (Barcelona Macula Foundation, Barcelona, Spain); Anna Borrell, MSc (Barcelona
576 Macula Foundation, Barcelona, Spain); Sofia M. Calado, PhD (Department of
577 Regeneration and Cell Therapy, Andalusian Molecular Biology and Regenerative
578 Medicine Centre (CABIMER), Seville, Spain); Sascha Dammeier, PhD (Centre for
579 Ophthalmology, Institute for Ophthalmic Research, Eberhard Karls University
580 Tübingen, University Clinic Tübingen, Tübingen, Germany); Anita de Breuk, M.D.
581 (Department of Ophthalmology, Radboud University Medical Center, Nijmegen,
582 Netherlands); Berta De la Cerda, PhD (Department of Regeneration and Cell
583 Therapy, Andalusian Molecular Biology and Regenerative Medicine Centre
584 (CABIMER), Seville, Spain); Anneke I. den Hollander, PhD (Department of
585 Ophthalmology, Radboud University Medical Center, Nijmegen, Netherlands,
586 Department of Human Genetics, Radboud University Medical Center, Nijmegen,
587 Netherlands); Francisco J. Diaz- Corrales, M.D., PhD (Department of Regeneration
588 and Cell Therapy, Andalusian Molecular Biology and Regenerative Medicine Centre
589 (CABIMER), Seville, Spain); Sigrid Diether, PhD (Centre for Ophthalmology, Institute
590 for Ophthalmic Research, Eberhard Karls University Tübingen, University Clinic
591 Tübingen, Tübingen, Germany); Eszter Emri, PhD (Centre for Experimental
592 Medicine, Queen's University Belfast, Belfast, United Kingdom); Tanja Endermann,
593 PhD (Assay Development, AYOXXA Biosystems GmbH, Cologne, Germany); Lucia
594 L. Ferraro, M.D. (Barcelona Macula Foundation, Barcelona, Spain); Míriam Garcia,
595 OD, MSc (Barcelona Macula Foundation, Barcelona, Spain); Thomas J.
596 Heesterbeek, M.D. (Department of Ophthalmology, Radboud University Medical

597 Center, Nijmegen, Netherlands); Sabina Honisch, PhD (Centre for Ophthalmology,
598 Institute for Ophthalmic Research, Eberhard Karls University Tübingen, University
599 Clinic Tübingen, Tübingen, Germany); Carel B. Hoyng, M.D. (Department of
600 Ophthalmology, Radboud University Medical Center, Nijmegen, Netherlands); Ellen
601 Kilger, PhD (Centre for Ophthalmology, Institute for Ophthalmic Research, Eberhard
602 Karls University Tübingen, University Clinic Tübingen, Tübingen, Germany); Elod
603 Kortvely, PhD (Roche Innovation Center Basel); Claire Lastrucci, PhD (Centre for
604 Genomic Regulation, Barcelona, Spain); Hanno Langen, PhD (Roche Innovation
605 Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland); Imre Lengyel, PhD
606 (Centre for Experimental Medicine, Queen's University Belfast, Belfast, United
607 Kingdom); Phil Luthert, PhD (Institute of Ophthalmology, University College London,
608 London, United Kingdom); Jordi Monés, MD, PhD (Barcelona Macula Foundation,
609 Barcelona, Spain); Everson Nogoceke, PhD (Roche Innovation Center Basel, F.
610 Hoffmann-La Roche Ltd, Basel, Switzerland); Tunde Peto, M.D., PhD (Centre for
611 Public Health, Queen's University Belfast, Belfast, United Kingdom); Frances M.
612 Pool, PhD (Ocular Biology, UCL Institute of Ophthalmology, London, United Kingdom);
613 Eduardo Rodriguez-Bocanegra, MSc (Barcelona Macula Foundation, Barcelona,
614 Spain); Luis Serrano, PhD (Centre for Genomic Regulation, Barcelona, Spain); Jose
615 Sousa, PhD (Advanced Informatics CTU, Queen's University Belfast, Belfast, United
616 Kingdom); Eric Thee, M.D. (Department of Epidemiology, Erasmus Medical Center,
617 Rotterdam, Netherlands, Department of Ophthalmology, Erasmus Medical Center,
618 Rotterdam, Netherlands); Marius Ueffing, PhD (Centre for Ophthalmology, Institute
619 for Ophthalmic Research, Eberhard Karls University Tübingen, University Clinic
620 Tübingen, Tübingen, Germany, Department of Ophthalmology, University
621 MedicalCentre Tübingen, Tübingen, Germany); Karl U. Ulrich Bartz-Schmidt, M.D.

622 (Centre for Ophthalmology, Institute for Ophthalmic Research, Eberhard Karls
623 University Tübingen, University Clinic Tübingen, Tübingen, Germany, Department of
624 Ophthalmology, University MedicalCentre Tübingen, Tübingen, Germany); Markus
625 Zumbansen, PhD (Research and Development, AYOXXA Biosystems GmbH,
626 Cologne, Germany).

627

628