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Pimasertib-associated ophthalmological adverse events

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7 1 Pimasertib-associated ophthalmological adverse events

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7 **Abstract**

8 Purpose: To analyse ophthalmological adverse events associated with mitogen-activated protein

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10 kinase kinase (MEK) inhibition with pimasertib treatment for metastatic cutaneous melanoma (CM).

11 Methods: In this prospective observational, cohort-based, cross-sectional study, 8 patients treated with
12 the MEK inhibitor pimasertib received a complete ophthalmic examination. This included Early
13 Treatment of Diabetic Retinopathy Study best-corrected visual acuity, visual field testing, color vision
14 testing, slit-lamp examination, applanation tonometry, indirect ophthalmoscopy, digital color fundus
15 photography, and optical coherence tomography (OCT). In selected cases fluorescein angiography
16 was performed.

17 Results: Serous subretinal fluid (SRF) developed in all patients, within a time frame of ~~92-207~~ days
18 after the start of treatment. The fovea was involved in 6/8 patients (75%). None of the patients with
19 foveal SRF (excluding a patient who developed a bilateral retinal vein occlusion (RVO)) experienced
20 visual symptoms. SRF decreased or resolved in all patients, despite continuation of study medication
21 in 6/8 patients (75%). Complaints in the CM patient (13%) consisted of ~~experiencing a dark fleck in~~
22 ~~the inferior part of the visual field of the right eye~~~~unilateral vision loss~~ 1 week after the start of
23 treatment, due to an RVO. Subsequent intravitreal bevacizumab treatment resulted in functional and
24 anatomical improvement.

25 Conclusions: Patients with metastatic CM who are treated with the MEK inhibitor pimasertib are at
26 high risk of development of ocular adverse events including serous retinopathy and possibly RVO,
27 stressing the need of adequate ophthalmological follow-up including OCT during ~~the~~ administration
28 of pimasertib, despite the fact that SRF generally does not lead to ophthalmological complaints.
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45 **Key words**

46 MEK inhibitor; ophthalmological adverse events; pimasertib; retinal vein occlusion; serous
47 retinopathy
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52 Introduction

53 Spectral-domain optical coherence tomography (OCT) is a sensitive means to detect serous
54 retinopathy, which is defined as retinal lesions on ophthalmoscopy corresponding to a localized
55 separation of the neuroretina and retinal pigment epithelium (RPE).(van Dijk et al. 2015) ~~Serous~~
56 ~~retinopathy may be classified according to the underlying cause, which is either rhegmatogenous,~~
57 ~~tractional, or exudative.(Ghazi & Green 2002)~~It may occur as an independent entity, but may also be
58 associated with a broad spectrum of other diseases or may follow the use of certain drugs.(Ghazi &
59 Green 2002) We and others recently described that serous retinopathy is seen in patients with
60 metastatic melanoma during the treatment with mitogen-activated protein kinase kinase (MEK)
61 inhibitors such as binimetinib,(van Dijk et al. 2015; Weber et al. 2016) cobimetinib,(McCannel et al.
62 2014) trametinib,(Infante et al. 2012) and RO5126766.(Martinez-Garcia et al. 2012) ~~For many drugs,~~
63 ~~it is unknown how often this complication occurs.~~Luckily, in many cases vision is not affected by the
64 treatment, but serious problems including retinal vein occlusion (RVO) may occur.(LoRusso et al.
65 2010; Leijten et al. 2012)

66 As treatment options for metastatic malignant cutaneous melanoma (CM) are scarce, much
67 effort is being put into the development of possible new treatments for this patient group. One of the
68 target pathways for treatment is the mitogen-activated protein kinase (MAPK) signalling (Ras - Raf -
69 MEK - extracellular signal-regulated kinase) pathway. A broad spectrum of cellular processes is
70 coordinated by this pathway, ~~which can be activated by various stimuli, such as hormones, growth~~
71 ~~factors, stress, and by ischemic and inflammatory injuries.~~ Activation of the MAPK pathway
72 influences processes such as cell differentiation and metabolism, cell migration, and cell
73 death.(Krishna & Narang 2008) Although visual symptoms may be absent or relatively mild in
74 patients to whom MEK inhibition is prescribed, OCT imaging has shown that some of the MEK
75 inhibitors induce retinal changes in a high percentage of patients.(Flaherty et al. 2012; Ascierto et al.
76 2013; McCannel et al. 2014; Urner-Bloch et al. 2014; van Dijk et al. 2015)

77 ~~Preliminary results of p~~Preclinical studies have shown that the new MEK inhibitor pimasertib
78 displays activity against ~~NRAS~~ and ~~BRAF~~ mutated cell lines.(Kim et al. 2010; Yoon et al. 2011)

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7 79 Moreover, induction of apoptosis in *BRAF* mutated human malignant melanoma cell lines has been
8 80 described after the administration of a combination of pimasertib and the *BRAF* inhibitor PLX4032,
9 81 whereas either drug alone did not (Park et al. 2013) In the first human trial IMP28062, activity against
11 82 *NRAS* mutated tumors could be detected after prescription of pimasertib. In a recently published
13 83 report of a patient with metastatic ovarian cancer bilateral multifocal retinal detachments were
15 84 described 2 days after the start of pimasertib. (AlAli et al. 2016) This patient developed complaints of
17 85 blurred vision, and treatment was discontinued. Three days after stopping pimasertib treatment, ocular
18 86 complaints and lesions had disappeared. (AlAli et al. 2016) The occurrence of serous retinal
20 87 detachments in patients, to whom pimasertib was prescribed, was not further specified in another
22 88 studies. (Houede et al. 2011; Macarulla et al. 2015) In 1 of these studies, the occurrence of an RVO
24 89 in a patient was also not discussed in detail. (Houede et al. 2011) No other ocular adverse events
26 90 during the prescription of pimasertib have been reported so far.

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28 91 ~~As we are very interested in the mechanism behind the development of serous retinopathy~~
29 92 ~~during MEK inhibition, w~~We hypothesized that this drug may also lead to subretinal fluid (SRF) and
31 93 wondered whether SRF would occur in only in a sensitive subset of patients, or that it might be a
32 94 general phenomenon. As patients are in quite a good general condition when using this treatment, we
34 95 were able to perform a prospective study of patients using the new MEK inhibitor pimasertib, and
36 96 included high resolution OCT imaging to examine the macular area in detail.

98 **Materials and methods**

99 *Patient characteristics*

100 Eight patients from an academic medical center (Erasmus University Medical Center, Rotterdam, the
101 Netherlands) were included in this study. All patients were diagnosed with a measurable,
102 histologically or cytologically confirmed, locally advanced or metastatic *NRAS* mutated malignant
103 CM. Patients with a medical history of retinal degenerative disease, uveitis, or RVO were excluded.
104 Local ethics committee approved the study. Each patient gave written informed consent ~~after~~
105 ~~explanation of the nature and possible consequences of the study~~, and the study was performed in
106 accordance with the Declaration of Helsinki. The clinical trial was registered in the Clinical Trial

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7 107 Register with number NCT01693068. Patients were included in this trial from September 2013 to
8 108 June 2014.
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12 110 *Treatment*

13 111 All patients received ~~the MEK inhibitor~~ pimasertib in a randomized phase II trial, in which the
14 112 comparator arm was chemotherapy with dacarbazine (intravenous administration at dose of 1000 mg
15 113 per square meter of body surface area, every 3 weeks). Patients received 60 mg of pimasertib orally
16 114 twice a day, continuously for 21 days (defined as a 1 treatment cycle, for scheduling purposes).
17 115 Within this trial 7 patients were primarily randomized to pimasertib, whereas 1 patient made a cross-
18 116 over to treatment with pimasertib after having developed progressive disease upon chemotherapy with
19 117 dacarbazine. ~~In all patients study treatment was continued until either disease progression or~~
20 118 ~~unacceptable toxicity occurred.~~
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30 120 *Ophthalmic examinations*

31 121 All patients received complete ophthalmic examination, including Early Treatment of Diabetic
32 122 Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) testing, visual field testing, color
33 123 vision testing using the Desaturated Panel D-15 test, slit-lamp examination, intraocular pressure
34 124 measurement, indirect ophthalmoscopy, digital color fundus photography (Topcon Corporation,
35 125 Tokyo, Japan), and OCT using the spectral-domain OCT (Spectralis HRA+OCT (Heidelberg
36 126 Engineering, Heidelberg, Germany)), before ~~the~~ start of the study. ~~Part of these examinations was~~
37 127 ~~performed after dilation of pupils by topical administration of 1% tropicamide and 5% phenylephrine~~
38 128 ~~drops. Moreover, study protocol instructed the BCVA measurement OCT scanning was at every~~
39 129 ~~evaluation visit: performed~~ at the beginning of ~~treatment~~ cycle 2, and at the beginning of every
40 130 subsequent odd ~~treatment~~ cycle. ~~At these evaluation visits, Pupils were dilated by topical~~
41 131 ~~administration of 1% tropicamide and 5% phenylephrine drops slit-lamp examination and assessment~~
42 132 ~~of the posterior pole using indirect ophthalmoscopy were also prescribed to be executed, together with~~
43 133 ~~OCT scanning.~~ When ETDRS BCVA testing was not available, Snellen BCVA was determined and a
44 134 previously-established conversion method was used to achieve ETDRS values. (Gregori et al. 2010)

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7 135 In patients with visual disturbances or abnormal retinal findings on ophthalmological
8 136 assessments, fluorescein angiography was performed with the spectral-domain OCT. Treatment had to
9 137 be interrupted when a serous retinal detachment with a decrease in ETDRS BCVA of ≥ 15 letters
10 138 occurred. A serous retinal detachment was defined as a localized separation of the neuroretina and
11 139 RPE on OCT, with an accumulation of SRF between these layers. Restart of treatment could only be
12 140 scheduled after full resolution of the detachment and full recovery of vision within 2 weeks, after
13 141 weekly follow-up visits. In case of the occurrence of an RVO the study medication had to be stopped,
14 142 unless the event resolved within 2 weeks.
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24 144 *Safety and efficacy monitoring*

25 145 ~~During every visit to the outpatient clinic of the Department of Oncology, a report was completed.~~
26 146 After clinical assessment at the evaluation visits at the Department of Oncology, the dose of study
27 147 medication was reduced or medication was discontinued, when either unacceptable signs of toxicity
28 148 or disease progression had developed. Subjects with documented tumor progression on the
29 149 dacarbazine arm could choose to switch to receiving pimasertib. ~~Every 2 treatment cycles, a CT scan~~
30 150 ~~was made for evaluation of disease.~~
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36 152 *Statistical analysis*

38 153 Both ETDRS BCVA at the time point of most pronounced SRF on OCT and at final follow-up were
39 154 compared to ETDRS BCVA at initial screening, using an independent t-test in IBM SPSS Statistics,
40 155 version 23.0 (IBM Corp., Armonk, NY, United States).
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45 157 **Results**

47 158 The 8 CM patients (4 male, 4 female) had a mean age of 62.9 years (median: 64 years; range, 56-67
48 159 years). The clinical patient characteristics ~~of the CM patients~~ are summarized in Table 1.
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51 160 During this study, 1 patient (13%) developed visual complaints, consisting of experiencing a
52 161 dark fleck in the inferior part of the visual field of the right eye. These complaints started 1 week after
53 162 the restart of pimasertib treatment, after a previous discontinuation because of a reversible decrease in
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7 163 left ventricular ejection fraction. In the right eye, ETDRS BCVA had dropped from 95 to 67 letters,
8 164 while ETDRS BCVA of the left eye had not changed at the time of visual complaints ~~in the right eye~~.
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10 165 Indirect ophthalmoscopy and fluorescein angiography led to the diagnosis of a hemi-RVO of the
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12 166 superior temporal vein of the right eye and an asymptomatic mild hemi-RVO of the inferior temporal
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14 167 retinal vein of the left eye (Fig. 1A-L). Because of cystoid macular edema, a single intravitreal
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16 168 injection of bevacizumab in the right eye was given to this patient, after which ETDRS BCVA
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18 169 recovered to 89 letters at follow-up visit 8 days later (Fig. 1M-P). Because of the need for palliative
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20 170 care, which this patient preferred to receive in another hospital, and because of the good functional
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22 171 and anatomical result of this single injection, no additional follow-up was scheduled.

23 172 OCT indicated that SRF developed in 16/16 eyes (100%). None of the patients (excluding the
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25 173 patient with RVO) experienced visual symptoms ~~during the onset or presence of SRF~~. Moreover, no
26
27 174 measurable significant influence on visual acuity could be detected, as (converted) median BCVA
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29 175 ETDRS was 88 letters (range, 77-93 letters) at screening, 87 letters (range, 66-98 letters) at the
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31 176 moment of most prominent SRF on OCT, and 89 letters (range, 75-98 letters) at the final follow-up
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33 177 visit. Differences in ETDRS BCVA were not statistically significant ($p=0.58$ (screening versus most
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35 178 prominent SRF), $p=0.31$ (most prominent SRF versus final follow-up visit), and $p=0.51$ (screening
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37 179 versus final follow-up visit)).

38 180 Ophthalmoscopy revealed transparent to yellowish vitelliform lesions at the time that SRF
39
40 181 was observed on OCT (Fig. 1D, 1K-L, 2A), whereas either no abnormalities or mild RPE changes
41
42 182 were observed after disappearance of SRF. The SRF, ~~detected on OCT in all CM patients~~, was
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44 183 bilateral and fairly symmetrical (Fig. 2K-T). These lesions were seen in all patients at the time of their
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46 184 first OCT after the start of the study medication, which was performed after ~~92-207~~ days (median: ~~44~~
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48 185 ~~20~~ days). Foveal SRF accumulation was present in 6/8 patients (75%), ~~and this occurred bilaterally in~~
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50 186 ~~all patients~~. The center of these lesions was hyperreflective on infrared reflectance (~~IRR~~) imaging, and
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52 187 the lesions were surrounded by a hyporefective zone. On OCT, SRF was detected extrafoveally in all
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54 188 8 patients (Fig. 1K-L, 2D, ~~2I-J~~). ~~In 2 of these patients (25%), only extrafoveal SRF could be detected~~
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56 189 ~~(Fig. 2I-J)~~. The median total number of extrafoveal lesions in the posterior pole, based on a 30°
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58 190 infrared reflectance photograph, was 6 (mean: 5, range, 3-13 lesions) in the 16 eyes.

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7 191 At last ophthalmological follow-up at 14-126 days (median: 81 days) after the first
8 192 appearance of SRF, the SRF had disappeared in both eyes in 5/8 patients (63%). In the patient who
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10 193 received intravitreal bevacizumab ~~for cystoid macular edema associated with hemi-RVO~~, resolution
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12 194 of both cystoid macular edema and SRF had occurred. Administration of pimasertib had been
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14 195 discontinued in this patient. In the other 4 patients, SRF disappeared spontaneously while pimasertib
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16 196 was either still administered according to the original dose ~~to (2 of these patients) who showed~~
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18 197 ~~spontaneous resolution, 1 patient had received pimasertib or~~ dose of pimasertib was reduced (1
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20 198 patient) or, and or in 1 patient pimasertib was discontinued because of a decrease in left ventricular
21
22 199 ejection fraction (1 patient). Three patients had persistent SRF, during treatment with 60, 60, and 30
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24 200 mg of pimasertib twice a day, respectively. ~~These patients were seen for follow up after 56, 41, and 34~~
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26 201 ~~days after the (re)start of pimasertib, respectively.~~ However, during follow-up the amount of SRF had
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28 202 decreased in all 3 patients. Color vision testing and visual field testing revealed no significant
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30 203 changes, despite macular SRF accumulation during pimasertib treatment. For all patients, information
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32 204 on the occurrence and evolution of SRF, and the possible relationship with orally administered dose
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34 205 of pimasertib has been depicted in Fig. 3.

35 206 During the period of the administration of ~~the~~ study medication, in 1 patient who had
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37 207 previously been diagnosed with bilateral diabetic macular edema, (Fig. ~~43~~A-D) edema had increased
38
39 208 unilaterally at ophthalmological follow-up, 9 days after the start of study medication (Fig. ~~34~~E-F).
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41 209 This patient also developed bilateral foveal SRF, ~~but did not report visual complaints.~~ No signs of
42
43 210 other ophthalmological diseases were detected during follow-up. Eleven days later, SRF and edema
44
45 211 had decreased spontaneously (Fig. ~~34~~G-H). At final follow-up 106 days later, only minimal unilateral
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47 212 foveal SRF remained (Fig. ~~34~~I-J). Ophthalmic characteristics of all patients are summarized in Table
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50 214 **Discussion**

51 216 Spectral-domain OCT is a sensitive means to detect a typical serous retinopathy in patients treated
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53 217 with MEK inhibitors such as pimasertib. Despite these striking MEK inhibitor-associated retinal
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55 218 abnormalities in all 8 metastatic CM patients, all but 1 patient remained asymptomatic. This patient

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7 219 experienced central vision loss due to bilateral RVO, which responded to intravitreal bevacizumab
8 220 treatment. Lesions occurred both foveally and extrafoveally in most of the 8 patients, indicating a
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10 221 more extensive dysfunction of the retina and/or RPE that extended beyond the fovea. Serous
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12 222 retinopathy had either disappeared or decreased at the last ophthalmological follow-up in all patients.
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14 223 The exact pathogenesis of both serous retinopathy and RVO associated with MEK inhibitor
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16 224 treatment is as yet unclear. A time-dependent and reversible serous retinopathy, resembling the
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18 225 clinical picture we present in the current study, was previously described in association with several
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20 226 other MEK inhibitors such as cobimetinib,(McCannel et al. 2014) trametinib,(Infante et al. 2012) and
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22 227 RO5126766.(Martinez-Garcia et al. 2012) We described this specific phenotype by a mildly
23
24 228 symptomatic, time-dependent, and reversible accumulation of both foveal and extrafoveal serous
25
26 229 SRF, with abnormalities on electro-oculography but without any evidence of choroidal
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28 230 abnormalities.(van Dijk et al. 2015) This serous retinopathy is most probably related to a class effect
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30 231 of this type of drugs, and thereby an on-target side effect of treatment,(Duncan et al. 2015; Kurbel et
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32 232 al. 2015) although pharmacological differences between several MEK inhibitors exist.(Hatzivassiliou
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34 233 et al. 2010) We have previously described prolonged abnormalities on electro-oculography in patients
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36 234 with transient binimetinib-associated serous retinopathy, indicating panretinal RPE dysfunction and
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38 235 dysfunction of the RPE pump.(McCannel et al. 2014; van Dijk et al. 2015) In addition, anti-RPE and
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40 236 anti-retinal autoantibodies may play a role ~~in the pathogenesis of MEK inhibitor associated serous~~
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42 237 ~~retinopathy.~~ (van Dijk et al. 2015) ~~Both direct RPE toxicity and the presence of autoantibodies could~~
43
44 238 ~~contribute to both these abnormalities and to the occurrence of SRF, which can occur within a few~~
45
46 239 ~~days after the start of MEK inhibition treatment.(McCannel et al. 2014; Urner Bloch et al. 2014; van~~
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48 240 ~~Dijk et al. 2015)~~In a cell model of RPE and neuroretina, binimetinib administration resulted in
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50 241 inactivation of the MAPK pathway, and discontinuation of administration of the MEK inhibitor
51
52 242 binimetinib led to reactivation, mimicking the mild and reversible retinopathy.(van Dijk et al. 2016)
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54 243 The occurrence of an RVO, during the prescription of MEK inhibition has also been described
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56 244 previously in up to 5% of patients.(LoRusso et al. 2010; Houede et al. 2011; Leijen et al. 2012) MEK
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58 245 inhibitors could influence the vasculature of the retina, leading to both this serous retinopathy and
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60 246 RVO.(van der Noll et al. 2013; van Dijk et al. 2015) The RVO that occurred in 1 of our patients could

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7 247 | have been associated with the previous reversible decrease in left ventricular ejection fraction in this
8 248 | case, which had been detected 3 weeks before the diagnosis of RVO had been established. Moreover,
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10 249 | ~~After oral administration of the MEK inhibitor PD0325901 to rats, retinal gene expression suggested~~
11
12 250 | an increase in inflammatory and oxidative stress response, endothelial and blood-retinal barrier
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14 251 | damage, and effects on blood coagulation, possibly characteristic for RVO.(Huang et al. 2009) Effects
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16 252 | of oxidative stress and endothelial cell inflammation could lead to vascular hyperpermeability and
17
18 253 | damage to the blood-retinal barrier.(Huang et al. 2009) Moreover, an imbalance between thrombosis
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20 254 | and fibrinolysis has previously been described to play an active role in the development of RVO.(Lip
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22 255 | et al. 1998; Rehak & Rehak 2008) ~~However, the administration of PD0325901 to rats did not result in~~
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24 256 | ~~the occurrence of clinical effects of RVO similar to the effects as observed in treated patients. This~~
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26 257 | ~~was in contrast with intravitreal injection of a MEK inhibitor in rabbits, which did lead to effects of~~
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28 258 | ~~RVO.(Huang et al. 2009)~~

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30 259 | MEK-associated serous retinopathy has to be discerned from several other retinal diseases. In
31
32 260 | contrast to central serous chorioretinopathy, in which lesions usually start unilaterally or bilateral
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34 261 | asymmetrically, the MEK-associated serous retinopathy is not associated with RPE detachments on
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36 262 | OCT, and there are no “hot spots” of trans-RPE subretinal leakage on fluorescein
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38 263 | angiography.(McCannel et al. 2014; Uner-Bloch et al. 2014) In cancer-associated retinopathy,
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40 264 | melanoma-associated retinopathy, or non-neoplastic autoimmune retinopathy, symptoms may include
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42 265 | a relatively rapid-onset photopsia, night blindness, scotomas, and progressive visual field
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44 266 | loss.(Heckenlively & Ferreyra 2008) Interestingly, this spectrum of autoimmune retinopathy is
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46 267 | associated with anti-retinal and/or anti-RPE antibodies, which we have also described in association
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48 268 | with MEK inhibitor-associated serous retinopathy.(van Dijk et al. 2015) Hereditary retinal dystrophies
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50 269 | such as autosomal dominant inherited Best vitelliform macular dystrophy and autosomal recessive
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52 270 | bestrophinopathy usually have an earlier onset in combination with typical fundus lesions and a
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54 271 | markedly abnormal electro-oculogram.(Boon et al. 2009)

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56 272 | In conclusion, we show that serous retinopathy associated with MEK inhibition treatment
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58 273 | such as pimasertib is very common. This ~~MEK inhibitor associated~~ serous retinopathy generally does
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60 274 | not lead to marked ophthalmological complaints,(van Dijk et al. 2015) and lesions may resolve

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7 275 | despite continuous treatment. Therefore, discontinuation of ~~MEK inhibition~~ treatment in most patients
8 276 | with metastatic cancer is not necessary. As ophthalmological complaints ~~due to serous retinopathy~~
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10 277 | may occur in a noteworthy number of patients,(van Dijk et al. 2015) and as survival may increase in
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12 278 | these patients because of the use of this new type of treatment for metastatic melanoma, performing
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14 279 | OCT scanning before, during, and after MEK inhibition treatment may be advisable for monitoring
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16 280 | and follow-up of fundus lesions ~~in relation to possible visual complaints~~. Another ophthalmological
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18 281 | side effect of MEK inhibition is RVO, which can cause visual complaints that requires therapeutic
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20 282 | intervention. Especially OCT follow-up ~~was is~~ able to precisely monitor such abnormalities even in
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22 283 | patients that remain asymptomatic. It is currently unclear if MEK inhibitor-associated serous
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24 284 | retinopathy correlates with other clinical parameters of MEK inhibition treatment such as treatment
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26 285 | response of metastases.(van Dijk et al. 2015) Further studies are needed to unravel the exact
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28 286 | pathogenesis and clinical consequences of MEK inhibitor-associated ophthalmological adverse
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380 **Figure legends**381 **Figure 1. Bilateral retinal vein occlusion associated with pimasertib treatment**

382 A-B, Optical coherence tomography (OCT) scan of the right (A) and left eye (B) of a 60-year-old
383 male patient ~~with metastatic cutaneous melanoma~~ revealed an ~~entirely~~ normal macular structure at ~~the~~
384 baseline examination, ~~before the first administration of pimasertib~~. C-D, Fundus photography of the
385 right (C) and left eye (D), 41 days after the start of pimasertib treatment, revealed bilateral foveal and
386 extrafoveal yellowish lesions. E-F, Foveal OCT scanning of both the right (E) and left eye (F) showed
387 a serous neuroretinal detachment ~~at that time~~. ~~Pimasertib~~ ~~treatment~~ was discontinued 22 days later,
388 ~~because of a decrease in left ventricular ejection fraction~~. Nine days after discontinuation, treatment
389 with 45 mg pimasertib twice daily could be restarted and patient attended a follow-up visit 12 days
390 later. At that time, ~~the patient~~ ~~experienced~~ ~~had visual complaints of experiencing~~ a dark fleck in the
391 inferior part of the visual field of the right eye. ~~Visual acuity had dropped from 95 to 67 Early~~
392 ~~Treatment of Diabetic Retinopathy Study (ETDRS) letters in that eye, whereas visual acuity in the left~~
393 ~~eye was stable~~. G-J, Fluorescein angiography and fundus photography revealed a hemi-retinal vein
394 occlusion (RVO) ~~of the superior temporal vein~~ in the right eye (G, I), and ~~a hemi-RVO of the inferior~~
395 ~~temporal retinal vein in the~~ left eye (H, J). K, OCT of the right eye showed cystoid macular edema,
396 ~~and a single intravitreal injection of bevacizumab was given to this patient. On OCT, and~~ both foveal
397 and extrafoveal neuroretinal detachments were also present. L, OCT scanning of the left eye revealed
398 both foveal and extrafoveal neuroretinal detachments, ~~but no cystoid macular edema~~. At this time,
399 administration of pimasertib was discontinued. M-N, At follow-up 8 days ~~after a single intravitreal~~
400 ~~injection of bevacizumab~~ later, fundus photography of the right (M) and ~~the~~ left eye (N) revealed
401 persistent cotton-wool spots and haemorrhages associated with the RVO in the right eye, and an
402 increase of haemorrhages and cotton-wool spots in the left eye. ~~At that time, in the right eye ETDRS~~
403 ~~BCVA had recovered to 89 letters, whereas visual acuity was still stable in the left eye~~. O-P, On an
404 OCT scan during this ~~follow-up~~ visit, both cystoid macular edema and SRF had resolved in the right
405 eye (O), and resolution of the neuroretinal detachment had occurred in the left eye (P). ~~No additional~~
406 ~~follow-up was available because of a further palliative care strategy in this patient~~.

407

408 **Figure 2. Spectrum of ophthalmological findings in pimasertib-associated serous retinopathy**

409 A, Fundus photography of the right eye of a 67-year-old male patient ~~with metastatic cutaneous~~
410 ~~melanoma (CM)~~ who developed foveal and extrafoveal yellowish lesions, at 13 days after the start of
411 ~~the mitogen-activated protein kinase kinase inhibitor~~ pimasertib treatment. B, Infrared reflectance
412 (IRR) imaging showed ~~a~~ hyperreflective foveal and extrafoveal lesions, which were surrounded by a
413 hyporeflective zone, ~~corresponding to the lesion on fundus photography. Lesions with similar~~
414 ~~reflectance characteristics could be detected throughout the posterior pole.~~ C, Optical coherence
415 tomography (OCT) had showed a normal macular structure at ~~the baseline examination, before the~~
416 ~~administration of pimasertib.~~ D, Thirteen days after the start of study treatment, both foveal and
417 extrafoveal serous neuroretinal detachments on OCT had developed, ~~but the patient experienced no~~
418 ~~visual complaints.~~ E, At final follow-up, 63 days after the start of the prescription ~~of pimasertib,~~
419 resolution of ~~both the foveal and extrafoveal~~ lesions had occurred. ~~At this final examination,~~
420 ~~pimasertib was still administered to the patient in a dosage of 30 mg twice daily.~~

421 F, Fundus photography of the right eye of a 63-year-old female ~~CM~~ patient showed subtle extrafoveal
422 transparent to yellowish lesions, 27 days after the start of pimasertib treatment. G, IRR imaging
423 showed corresponding ~~subtle~~ hyperreflective lesions. H, Before ~~treatment~~ ~~the administration of~~
424 ~~pimasertib,~~ OCT had showed a normal macular structure. I, An extrafoveal neuroretinal detachment
425 could be detected on OCT, 27 days after the beginning of pimasertib. ~~At that moment, the patient had~~
426 ~~no visual complaints.~~ J, This serous retinopathy was still present at final follow-up 14 days later, ~~when~~
427 ~~the patient still received 60 mg pimasertib twice daily.~~

428 K-L, In a 66-year-old ~~CM~~ patient, showing ~~bilateral and fairly symmetrical~~ pimasertib-associated
429 serous retinopathy, fundus photography revealed both foveal and extrafoveal yellowish lesions, 20
430 days after the start of pimasertib. ~~M-NP-Q,~~ These lesions corresponded to hyperreflective ~~foveal and~~
431 ~~extrafoveal~~ lesions, surrounded by a hyporeflective zone on IRR imaging. ~~OPM-N,~~ At baseline
432 examination, ~~before the start of pimasertib treatment~~ no abnormalities had been found ~~were seen~~ on

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7 433 OCT. ~~QO and~~-R, Twenty days after the start of ~~pimasertib~~-treatment, an OCT scan showed a bilateral
8 434 foveal neuroretinal detachment, ~~which was not accompanied by any visual complaints~~. S-T, ~~Bilateral~~
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10 435 ~~R~~esolution of this ~~serous neuroretinal~~ detachment was detected ~~on OCT~~, 62 days after
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12 436 discontinuation of ~~pimasertib~~ treatment.
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17 438 **Figure 3. Occurrence and evolution of subretinal fluid over time, and possible relationship with**
18
19 439 **orally administered dose of pimasertib**

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21 440 ~~For all 8 patients included in this study, the prescribed dose of pimasertib and the occurrence of~~
22
23 441 ~~subretinal fluid (SRF) on optical coherence tomography (OCT) over time have been depicted. Colors~~
24
25 442 ~~in the ‘pimasertib dose’ rows correspond to the following doses: black = 60 mg twice daily, dark grey~~
26
27 443 ~~= 45 mg twice daily, light grey = 30 mg twice daily, white = no treatment. ‘STOP’ corresponds to end~~
28
29 444 ~~of treatment. ‘Y’ in the ‘SRF on OCT’ rows corresponds to presence of SRF on OCT at evaluation,~~
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31 445 ~~with the maximum amount of SRF over time in bold. ‘N’ corresponds to the absence of SRF on OCT.~~
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35 447 **Figure 43. Worsening of diabetic macular edema and occurrence of serous retinopathy**
36
37 448 **associated with pimasertib treatment**

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39 449 A-B, Fluorescein angiography in a 67-year-old male patient with metastatic cutaneous melanoma and
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41 450 diabetes mellitus type 2 ~~at baseline examination~~ before the start of pimasertib treatment, showed
42
43 451 bilateral microaneurysms and mild fluorescein leakage. C-D, Optical coherence tomography (OCT) at
44
45 452 baseline examination showed very mild diabetic macular edema (DME) ~~without other abnormalities~~
46
47 453 ~~an otherwise normal macular structure in the right eye~~ (C), and mild DME in the left eye (D). E, OCT
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49 454 scanning at 9 days after the start of pimasertib treatment showed a foveal serous neuroretinal
50
51 455 detachment ~~and roughly stable DME~~ in the right eye. F, At this moment, the left eye showed both a
52
53 456 foveal serous neuroretinal detachment and worsening of the DME ~~edema~~ on OCT. ~~The patient did not~~
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55 457 ~~report any ocular complaints during follow-up.~~ G-H, Eleven days later the amount of serous subretinal

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458 fluid had decreased spontaneously in the right (G) and the left eye (H). ~~H. The left eye also showed a~~
459 ~~spontaneous decrease in the amount of subretinal fluid, as well as in DME.~~ I-J, Despite the fact that
460 this patient still used pimasertib in a dosage of 60 mg twice daily, the neuroretinal detachment had
461 almost resolved in both eyes, together with the DME in the left eye, at final ophthalmological follow-
462 up ~~at 126 days after the start of pimasertib treatment.~~

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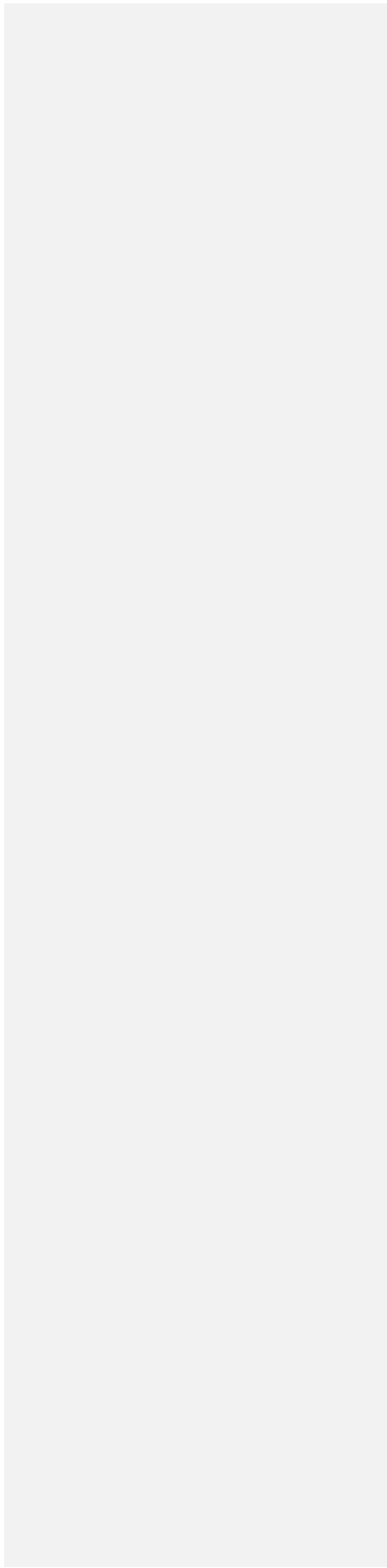
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464 **Tables**

465 **Table 1. Characteristics of the cutaneous melanoma patients.**

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For Peer Review



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3 1 Pimasertib-associated ophthalmological adverse events
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3 **Abstract**

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5 Purpose: To analyse ophthalmological adverse events associated with mitogen-activated protein
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7 kinase kinase (MEK) inhibition with pimasertib treatment for metastatic cutaneous melanoma (CM).

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9 Methods: In this prospective observational, cohort-based, cross-sectional study, 8 patients treated with
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11 the MEK inhibitor pimasertib received a complete ophthalmic examination. This included Early
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13 Treatment of Diabetic Retinopathy Study best-corrected visual acuity, visual field testing, color vision
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15 testing, slit-lamp examination, applanation tonometry, indirect ophthalmoscopy, digital color fundus
16
17 photography, and optical coherence tomography (OCT). In selected cases fluorescein angiography
18
19 was performed.

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21 Results: Serous subretinal fluid (SRF) developed in all patients, within a time frame of 9-27 days after
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23 the start of treatment. The fovea was involved in 6/8 patients (75%). None of the patients with foveal
24
25 SRF (excluding a patient who developed a bilateral retinal vein occlusion (RVO)) experienced visual
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27 symptoms. SRF decreased or resolved in all patients, despite continuation of study medication in 6/8
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29 patients (75%). Complaints in the CM patient (13%) consisted of experiencing a dark fleck in the
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31 inferior part of the visual field of the right eye 1 week after the start of treatment, due to an RVO.

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33 Subsequent intravitreal bevacizumab treatment resulted in functional and anatomical improvement.

34
35 Conclusions: Patients with metastatic CM who are treated with the MEK inhibitor pimasertib are at
36
37 high risk of development of ocular adverse events including serous retinopathy and possibly RVO,
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39 stressing the need of adequate ophthalmological follow-up including OCT during administration of
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41 pimasertib, despite the fact that SRF generally does not lead to ophthalmological complaints.
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46 **Key words**

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48 MEK inhibitor; ophthalmological adverse events; pimasertib; retinal vein occlusion; serous
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50 retinopathy

51 Introduction

52 Spectral-domain optical coherence tomography (OCT) is a sensitive means to detect serous
53 retinopathy, which is defined as retinal lesions on ophthalmoscopy corresponding to a localized
54 separation of the neuroretina and retinal pigment epithelium (RPE).(van Dijk et al. 2015) It may occur
55 as an independent entity, but may also be associated with a broad spectrum of other diseases or may
56 follow the use of certain drugs.(Ghazi & Green 2002) We and others recently described that serous
57 retinopathy is seen in patients with metastatic melanoma during the treatment with mitogen-activated
58 protein kinase kinase (MEK) inhibitors such as binimetinib,(van Dijk et al. 2015; Weber et al. 2016)
59 cobimetinib,(McCannel et al. 2014) trametinib,(Infante et al. 2012) and RO5126766.(Martinez-Garcia
60 et al. 2012) Luckily, in many cases vision is not affected by the treatment, but serious problems
61 including retinal vein occlusion (RVO) may occur.(LoRusso et al. 2010; Leijen et al. 2012)

62 As treatment options for metastatic malignant cutaneous melanoma (CM) are scarce, much
63 effort is being put into the development of possible new treatments for this patient group. One of the
64 target pathways for treatment is the mitogen-activated protein kinase (MAPK) signalling (Ras - Raf -
65 MEK - extracellular signal-regulated kinase) pathway. A broad spectrum of cellular processes is
66 coordinated by this pathway. Activation of the MAPK pathway influences processes such as cell
67 differentiation and metabolism, cell migration, and cell death.(Krishna & Narang 2008) Although
68 visual symptoms may be absent or relatively mild in patients to whom MEK inhibition is prescribed,
69 OCT imaging has shown that some of the MEK inhibitors induce retinal changes in a high percentage
70 of patients.(Flaherty et al. 2012; Ascierto et al. 2013; McCannel et al. 2014; Uner-Bloch et al. 2014;
71 van Dijk et al. 2015)

72 Preclinical studies have shown that the new MEK inhibitor pimasertib displays activity
73 against *RAS* and *BRAF* mutated cell lines.(Kim et al. 2010; Yoon et al. 2011) Moreover, induction of
74 apoptosis in *BRAF* mutated human malignant melanoma cell lines has been described after the
75 administration of a combination of pimasertib and the BRAF inhibitor PLX4032, whereas either drug
76 alone did not.(Park et al. 2013) In the first human trial IMP28062, activity against *NRAS* mutated
77 tumors could be detected after prescription of pimasertib. In a recently published report of a patient

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3 78 with metastatic ovarian cancer bilateral multifocal retinal detachments were described 2 days after the
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5 79 start of pimasertib.(AlAli et al. 2016) This patient developed complaints of blurred vision, and
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7 80 treatment was discontinued. Three days after stopping pimasertib treatment, ocular complaints and
8
9 81 lesions had disappeared.(AlAli et al. 2016) The occurrence of serous retinal detachments in patients,
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11 82 to whom pimasertib was prescribed, was not further specified in other studies.(Houede et al. 2011;
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13 83 Macarulla et al. 2015) In 1 of these studies, the occurrence of an RVO in a patient was also not
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15 84 discussed in detail.(Houede et al. 2011) No other ocular adverse events during the prescription of
16
17 85 pimasertib have been reported so far.

18
19 86 We hypothesized that this drug may also lead to subretinal fluid (SRF) and wondered whether
20
21 87 SRF would occur in only in a sensitive subset of patients, or that it might be a general phenomenon.
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23 88 As patients are in quite a good general condition when using this treatment, we were able to perform a
24
25 89 prospective study of patients using the new MEK inhibitor pimasertib, and included high resolution
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27 90 OCT imaging to examine the macular area in detail.
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31 92 **Materials and methods**

32 93 *Patient characteristics*

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34 94 Eight patients from an academic medical center (Erasmus University Medical Center, Rotterdam, the
35
36 95 Netherlands) were included in this study. All patients were diagnosed with a measurable,
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38 96 histologically or cytologically confirmed, locally advanced or metastatic *NRAS* mutated malignant
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40 97 CM. Patients with a medical history of retinal degenerative disease, uveitis, or RVO were excluded.
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42 98 Local ethics committee approved the study. Each patient gave written informed consent, and the study
43
44 99 was performed in accordance with the Declaration of Helsinki. The clinical trial was registered in the
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46 100 Clinical Trial Register with number NCT01693068. Patients were included in this trial from
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48 101 September 2013 to June 2014.
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52 103 *Treatment*

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54 104 All patients received pimasertib in a randomized phase II trial, in which the comparator arm was
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56 105 chemotherapy with dacarbazine (intravenous administration at dose of 1000 mg per square meter of
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3 106 body surface area, every 3 weeks). Patients received 60 mg of pimasetib orally twice a day,
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5 107 continuously for 21 days (defined as a 1 treatment cycle, for scheduling purposes). Within this trial 7
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7 108 patients were primarily randomized to pimasetib, whereas 1 patient made a cross-over to treatment
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9 109 with pimasetib after having developed progressive disease upon chemotherapy with dacarbazine.

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13 111 *Ophthalmic examinations*

14
15 112 All patients received complete ophthalmic examination, including Early Treatment of Diabetic
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17 113 Retinopathy Study (ETDRS) best-corrected visual acuity (BCVA) testing, visual field testing, color
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19 114 vision testing using the Desaturated Panel D-15 test, slit-lamp examination, intraocular pressure
20
21 115 measurement, indirect ophthalmoscopy, digital color fundus photography (Topcon Corporation,
22
23 116 Tokyo, Japan), and OCT using the spectral-domain OCT (Spectralis HRA+OCT (Heidelberg
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25 117 Engineering, Heidelberg, Germany)), before start of the study. Part of these examinations was
26
27 118 performed after dilation of pupils by topical administration of 1% tropicamide and 5% phenylephrine
28
29 119 drops. Moreover, study protocol instructed the BCVA measurement at every evaluation visit: at the
30
31 120 beginning of treatment cycle 2 and at the beginning of every subsequent odd treatment cycle. At these
32
33 121 evaluation visits, slit-lamp examination and assessment of the posterior pole using indirect
34
35 122 ophthalmoscopy were also prescribed to be executed, together with OCT scanning. When ETDRS
36
37 123 BCVA testing was not available, Snellen BCVA was determined and a previously-established
38
39 124 conversion method was used to achieve ETDRS values.(Gregori et al. 2010)

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41 125 In patients with visual disturbances or abnormal retinal findings on ophthalmological
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43 126 assessments, fluorescein angiography was performed with the spectral-domain OCT. Treatment had to
44
45 127 be interrupted when a serous retinal detachment with a decrease in ETDRS BCVA of ≥ 15 letters
46
47 128 occurred. A serous retinal detachment was defined as a localized separation of the neuroretina and
48
49 129 RPE on OCT, with an accumulation of SRF between these layers. Restart of treatment could only be
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51 130 scheduled after full resolution of the detachment and full recovery of vision within 2 weeks, after
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53 131 weekly follow-up visits. In case of the occurrence of an RVO the study medication had to be stopped,
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55 132 unless the event resolved within 2 weeks.

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3 134 *Safety and efficacy monitoring*
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5 135 After clinical assessment at the evaluation visits at the Department of Oncology, the dose of study
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7 136 medication was reduced or medication was discontinued, when either unacceptable signs of toxicity
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9 137 or disease progression had developed. Subjects with documented tumor progression on the
10
11 138 dacarbazine arm could choose to switch to receiving pimasertib.
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15 140 *Statistical analysis*
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17 141 Both ETDRS BCVA at the time point of most pronounced SRF on OCT and at final follow-up were
18
19 142 compared to ETDRS BCVA at initial screening, using an independent t-test in IBM SPSS Statistics,
20
21 143 version 23.0 (IBM Corp., Armonk, NY, United States).
22

23 144

24
25 145 **Results**
26

27 146 The 8 CM patients (4 male, 4 female) had a mean age of 62.9 years (median: 64 years; range, 56-67
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29 147 years). The clinical patient characteristics are summarized in Table 1.
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31 148 During this study, 1 patient (13%) developed visual complaints, consisting of experiencing a
32
33 149 dark fleck in the inferior part of the visual field of the right eye. These complaints started 1 week after
34
35 150 the restart of pimasertib treatment, after a previous discontinuation because of a reversible decrease in
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37 151 left ventricular ejection fraction. In the right eye, ETDRS BCVA had dropped from 95 to 67 letters,
38
39 152 while ETDRS BCVA of the left eye had not changed at the time of visual complaints. Indirect
40
41 153 ophthalmoscopy and fluorescein angiography led to the diagnosis of a hemi-RVO of the superior
42
43 154 temporal vein of the right eye and an asymptomatic mild hemi-RVO of the inferior temporal retinal
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45 155 vein of the left eye (Fig. 1A-L). Because of cystoid macular edema, a single intravitreal injection of
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47 156 bevacizumab in the right eye was given to this patient, after which ETDRS BCVA recovered to 89
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49 157 letters at follow-up visit 8 days later (Fig. 1M-P). Because of the need for palliative care, which this
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51 158 patient preferred to receive in another hospital, and because of the good functional and anatomical
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53 159 result of this single injection, no additional follow-up was scheduled.
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56 160 OCT indicated that SRF developed in 16/16 eyes (100%). None of the patients (excluding the
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58 161 patient with RVO) experienced visual symptoms. Moreover, no measurable significant influence on
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3 162 visual acuity could be detected, as (converted) median BCVA ETDRS was 88 letters (range, 77-93
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5 163 letters) at screening, 87 letters (range, 66-98 letters) at the moment of most prominent SRF on OCT,
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7 164 and 89 letters (range, 75-98 letters) at the final follow-up visit. Differences in ETDRS BCVA were
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9 165 not statistically significant ($p=0.58$ (screening versus most prominent SRF), $p=0.31$ (most prominent
10
11 166 SRF versus final follow-up visit), and $p=0.51$ (screening versus final follow-up visit)).

12
13 167 Ophthalmoscopy revealed transparent to yellowish vitelliform lesions at the time that SRF
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15 168 was observed on OCT (Fig. 1D, 1K-L, 2A), whereas either no abnormalities or mild RPE changes
16
17 169 were observed after disappearance of SRF. The SRF was bilateral and fairly symmetrical (Fig. 2K-T).
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19 170 These lesions were seen in all patients at the time of their first OCT after the start of the study
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21 171 medication, which was performed after 9-27 days (median: 20 days). Foveal SRF accumulation was
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23 172 present in 6/8 patients (75%). The center of these lesions was hyperreflective on infrared reflectance
24
25 173 imaging, and the lesions were surrounded by a hyporeflective zone. On OCT, SRF was detected
26
27 174 extrafoveally in all 8 patients (Fig. 1K-L, 2D, 2I-J). The median total number of extrafoveal lesions in
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29 175 the posterior pole, based on a 30° infrared reflectance photograph, was 6 (mean: 5, range, 3-13
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31 176 lesions) in the 16 eyes.

32
33 177 At last ophthalmological follow-up at 14-126 days (median: 81 days) after the first
34
35 178 appearance of SRF, the SRF had disappeared in both eyes in 5/8 patients (63%). In the patient who
36
37 179 received intravitreal bevacizumab, resolution of both cystoid macular edema and SRF had occurred.
38
39 180 Administration of pimasertib had been discontinued in this patient. In the other 4 patients, SRF
40
41 181 disappeared spontaneously while pimasertib was either still administered according to the original
42
43 182 dose (2 patients) or dose of pimasertib was reduced (1 patient), or pimasertib was discontinued
44
45 183 because of a decrease in left ventricular ejection fraction (1 patient). Three patients had persistent
46
47 184 SRF, during treatment with 60, 60, and 30 mg of pimasertib twice a day, respectively. However,
48
49 185 during follow-up the amount of SRF had decreased in all 3 patients. Color vision testing and visual
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51 186 field testing revealed no significant changes, despite macular SRF accumulation during pimasertib
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53 187 treatment. For all patients, information on the occurrence and evolution of SRF, and the possible
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55 188 relationship with orally administered dose of pimasertib has been depicted in Fig. 3.
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3 189 During the period of the administration of study medication in 1 patient who had previously
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5 190 been diagnosed with bilateral diabetic macular edema, (Fig. 4A-D) edema had increased unilaterally
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7 191 at ophthalmological follow-up, 9 days after the start of study medication (Fig. 4E-F). This patient also
8
9 192 developed bilateral foveal SRF. No signs of other ophthalmological diseases were detected during
10
11 193 follow-up. Eleven days later, SRF and edema had decreased spontaneously (Fig. 4G-H). At final
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13 194 follow-up 106 days later, only minimal unilateral foveal SRF remained (Fig. 4I-J). Ophthalmic
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15 195 characteristics of all patients are summarized in Table 1.
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17 196

19 197 **Discussion**

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21 198 Spectral-domain OCT is a sensitive means to detect a typical serous retinopathy in patients treated
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23 199 with MEK inhibitors such as pimasertib. Despite these striking MEK inhibitor-associated retinal
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25 200 abnormalities in all 8 metastatic CM patients, all but 1 patient remained asymptomatic. This patient
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27 201 experienced central vision loss due to bilateral RVO, which responded to intravitreal bevacizumab
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29 202 treatment. Lesions occurred both foveally and extrafoveally in most of the 8 patients, indicating a
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31 203 more extensive dysfunction of the retina and/or RPE that extended beyond the fovea. Serous
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33 204 retinopathy had either disappeared or decreased at the last ophthalmological follow-up in all patients.
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35 205 The exact pathogenesis of both serous retinopathy and RVO associated with MEK inhibitor
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37 206 treatment is as yet unclear. A time-dependent and reversible serous retinopathy, resembling the
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39 207 clinical picture we present in the current study, was previously described in association with several
40
41 208 other MEK inhibitors such as cobimetinib,(McCannel et al. 2014) trametinib,(Infante et al. 2012) and
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43 209 RO5126766.(Martinez-Garcia et al. 2012) We described this specific phenotype by a mildly
44
45 210 symptomatic, time-dependent, and reversible accumulation of both foveal and extrafoveal serous
46
47 211 SRF, with abnormalities on electro-oculography but without any evidence of choroidal
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49 212 abnormalities.(van Dijk et al. 2015) This serous retinopathy is most probably related to a class effect
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51 213 of this type of drugs, and thereby an on-target side effect of treatment,(Duncan et al. 2015; Kurbel et
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53 214 al. 2015) although pharmacological differences between several MEK inhibitors exist.(Hatzivassiliou
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55 215 et al. 2010) We have previously described prolonged abnormalities on electro-oculography in patients
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57 216 with transient binimetinib-associated serous retinopathy, indicating panretinal RPE dysfunction and
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3 217 dysfunction of the RPE pump.(McCannel et al. 2014; van Dijk et al. 2015) In addition, anti-RPE and
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5 218 anti-retinal autoantibodies may play a role.(van Dijk et al. 2015) In a cell model of RPE and
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7 219 neuroretina, binimetinib administration resulted in inactivation of the MAPK pathway, and
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9 220 discontinuation of administration of the MEK inhibitor binimetinib led to reactivation, mimicking the
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11 221 mild and reversible retinopathy.(van Dijk et al. 2016) The occurrence of an RVO, during the
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13 222 prescription of MEK inhibition has also been described previously in up to 5% of patients.(LoRusso
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15 223 et al. 2010; Houede et al. 2011; Leijen et al. 2012) MEK inhibitors could influence the vasculature of
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17 224 the retina, leading to both this serous retinopathy and RVO.(van der Noll et al. 2013; van Dijk et al.
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19 225 2015) The RVO that occurred in 1 of our patients could have been associated with the previous
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21 226 reversible decrease in left ventricular ejection fraction in this case, which had been detected 3 weeks
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23 227 before the diagnosis of RVO had been established. Moreover, after oral administration of the MEK
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25 228 inhibitor PD0325901 to rats, retinal gene expression suggested an increase in inflammatory and
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27 229 oxidative stress response, endothelial and blood-retinal barrier damage, and effects on blood
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29 230 coagulation, possibly characteristic for RVO.(Huang et al. 2009) Effects of oxidative stress and
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31 231 endothelial cell inflammation could lead to vascular hyperpermeability and damage to the blood-
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33 232 retinal barrier.(Huang et al. 2009) Moreover, an imbalance between thrombosis and fibrinolysis has
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35 233 previously been described to play an active role in the development of RVO.(Lip et al. 1998; Rehak &
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37 234 Rehak 2008)

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39 235 MEK-associated serous retinopathy has to be discerned from several other retinal diseases. In
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41 236 contrast to central serous chorioretinopathy, in which lesions usually start unilaterally or bilateral
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43 237 asymmetrically, the MEK-associated serous retinopathy is not associated with RPE detachments on
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45 238 OCT, and there are no “hot spots” of trans-RPE subretinal leakage on fluorescein
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47 239 angiography.(McCannel et al. 2014; Urner-Bloch et al. 2014) In cancer-associated retinopathy,
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49 240 melanoma-associated retinopathy, or non-neoplastic autoimmune retinopathy, symptoms may include
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51 241 a relatively rapid-onset photopsia, night blindness, scotomas, and progressive visual field
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53 242 loss.(Heckenlively & Ferreyra 2008) Interestingly, this spectrum of autoimmune retinopathy is
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55 243 associated with anti-retinal and/or anti-RPE antibodies, which we have also described in association
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57 244 with MEK inhibitor-associated serous retinopathy.(van Dijk et al. 2015) Hereditary retinal dystrophies
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3 245 such as autosomal dominant inherited Best vitelliform macular dystrophy and autosomal recessive
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5 246 bestrophinopathy usually have an earlier onset in combination with typical fundus lesions and a
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7 247 markedly abnormal electro-oculogram.(Boon et al. 2009)
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9 248 In conclusion, we show that serous retinopathy associated with MEK inhibition treatment
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11 249 such as pimasertib is very common. This serous retinopathy generally does not lead to marked
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13 250 ophthalmological complaints,(van Dijk et al. 2015) and lesions may resolve despite continuous
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15 251 treatment. Therefore, discontinuation of treatment in most patients with metastatic cancer is not
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17 252 necessary. As ophthalmological complaints may occur in a noteworthy number of patients,(van Dijk
18
19 253 et al. 2015) and as survival may increase in these patients because of the use of this new type of
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21 254 treatment for metastatic melanoma, performing OCT scanning before, during, and after MEK
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23 255 inhibition treatment may be advisable for monitoring and follow-up of fundus lesions. Another
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25 256 ophthalmological side effect of MEK inhibition is RVO, which can cause visual complaints that
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27 257 requires therapeutic intervention. Especially OCT follow-up is able to precisely monitor such
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29 258 abnormalities even in patients that remain asymptomatic. It is currently unclear if MEK inhibitor-
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31 259 associated serous retinopathy correlates with other clinical parameters of MEK inhibition treatment
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33 260 such as treatment response of metastases.(van Dijk et al. 2015) Further studies are needed to unravel
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35 261 the exact pathogenesis and clinical consequences of MEK inhibitor-associated ophthalmological
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37 262 adverse events.
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3 355 **Figure legends**

4
5 356 **Figure 1. Bilateral retinal vein occlusion associated with pimasertib treatment**

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8 357 A-B, Optical coherence tomography (OCT) scan of the right (A) and left eye (B) of a 60-year-old
9
10 358 male patient revealed a normal macular structure at baseline examination. C-D, Fundus photography
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12 359 of the right (C) and left eye (D), 41 days after the start of pimasertib treatment, revealed bilateral
13
14 360 foveal and extrafoveal yellowish lesions. E-F, Foveal OCT scanning of both the right (E) and left eye
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16 361 (F) showed a serous neuroretinal detachment. Treatment was discontinued 22 days later. Nine days
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18 362 after discontinuation, treatment with 45 mg pimasertib twice daily could be restarted and patient
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20 363 attended a follow-up visit 12 days later. At that time, patient experienced a dark fleck in the inferior
21
22 364 part of the visual field of the right eye. . G-J, Fluorescein angiography and fundus photography
23
24 365 revealed a hemi-retinal vein occlusion (RVO) in the right (G, I), and left eye (H, J). K, OCT of the
25
26 366 right eye showed cystoid macular edema and both foveal and extrafoveal neuroretinal detachments
27
28 367 were also present. L, OCT scanning of the left eye revealed both foveal and extrafoveal neuroretinal
29
30 368 detachments. At this time, administration of pimasertib was discontinued. M-N, At follow-up 8 days
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32 369 after a single intravitreal injection of bevacizumab, fundus photography of the right (M) and left eye
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34 370 (N) revealed persistent cotton-wool spots and haemorrhages associated with the RVO in the right eye,
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36 371 and an increase of haemorrhages and cotton-wool spots in the left eye. O-P, On an OCT scan during
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38 372 this visit, both cystoid macular edema and SRF had resolved in the right eye (O), and resolution of the
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40 373 neuroretinal detachment had occurred in the left eye (P).

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44 375 **Figure 2. Spectrum of ophthalmological findings in pimasertib-associated serous retinopathy**

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47 376 A, Fundus photography of the right eye of a 67-year-old male patient who developed foveal and
48
49 377 extrafoveal yellowish lesions, at 13 days after the start of pimasertib treatment. B, Infrared reflectance
50
51 378 (IRR) imaging showed hyperreflective foveal and extrafoveal lesions, which were surrounded by a
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53 379 hyporefective zone. C, Optical coherence tomography (OCT) had shown a normal macular structure
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55 380 at baseline. D, Thirteen days after the start of study treatment, both foveal and extrafoveal serous
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3 381 neuroretinal detachments on OCT had developed. E, At final follow-up, 63 days after the start of the
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5 382 prescription, resolution of lesions had occurred.
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8 383 F, Fundus photography of the right eye of a 63-year-old female patient showed subtle extrafoveal
9
10 384 transparent to yellowish lesions, 27 days after the start of pimasertib treatment. G, IRR imaging
11
12 385 showed corresponding hyperreflective lesions. H, Before treatment, OCT had shown a normal
13
14 386 macular structure. I, An extrafoveal neuroretinal detachment could be detected on OCT, 27 days after
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16 387 the beginning of pimasertib. J, This serous retinopathy was still present at final follow-up 14 days
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18 388 later.
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21 389 K-L, In a 66-year-old patient, showing pimasertib-associated serous retinopathy, fundus photography
22
23 390 revealed both foveal and extrafoveal yellowish lesions, 20 days after the start of pimasertib. P-Q,
24
25 391 These lesions corresponded to hyperreflective lesions, surrounded by a hyporefective zone on IRR
26
27 392 imaging. M-N, At baseline examination, no abnormalities had been found on OCT. O and R, Twenty
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29 393 days after the start of treatment, an OCT scan showed a bilateral foveal neuroretinal detachment. S-T,
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31 394 Resolution of this detachment was detected, 62 days after discontinuation of treatment.
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37 396 **Figure 3. Occurrence and evolution of subretinal fluid over time, and possible relationship with**
38
39 397 **orally administered dose of pimasertib**

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42 398 For all 8 patients included in this study, the prescribed dose of pimasertib and the occurrence of
43
44 399 subretinal fluid (SRF) on optical coherence tomography (OCT) over time have been depicted. Colors
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46 400 in the 'pimasertib dose' rows correspond to the following doses: black = 60 mg twice daily, dark grey
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48 401 = 45 mg twice daily, light grey = 30 mg twice daily, white = no treatment. 'STOP' corresponds to end
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50 402 of treatment. 'Y' in the 'SRF on OCT' rows corresponds to presence of SRF on OCT at evaluation,
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52 403 with the maximum amount of SRF over time in bold. 'N' corresponds to the absence of SRF on OCT.
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3 405 **Figure 4. Worsening of diabetic macular edema and occurrence of serous retinopathy associated**
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5 406 **with pimasertib treatment**
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8 407 A-B, Fluorescein angiography in a 67-year-old male patient with metastatic cutaneous melanoma and
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10 408 diabetes mellitus type 2 before the start of pimasertib treatment, showed bilateral microaneurysms and
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12 409 mild fluorescein leakage. C-D, Optical coherence tomography (OCT) at baseline examination showed
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14 410 very mild diabetic macular edema (DME) without other abnormalities (C), and mild DME in the left
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16 411 eye (D). E, OCT scanning at 9 days after the start of pimasertib treatment showed a foveal serous
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18 412 neuroretinal detachment in the right eye. F, At this moment, the left eye showed both a foveal serous
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20 413 neuroretinal detachment and worsening of the DME on OCT. G-H, Eleven days later the amount of
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22 414 serous subretinal fluid had decreased spontaneously in the right (G) and the left eye (H). I-J, Despite
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24 415 the fact that this patient still used pimasertib in a dosage of 60 mg twice daily, the neuroretinal
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26 416 detachment had almost resolved in both eyes, together with the DME in the left eye, at final
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28 417 ophthalmological follow-up.
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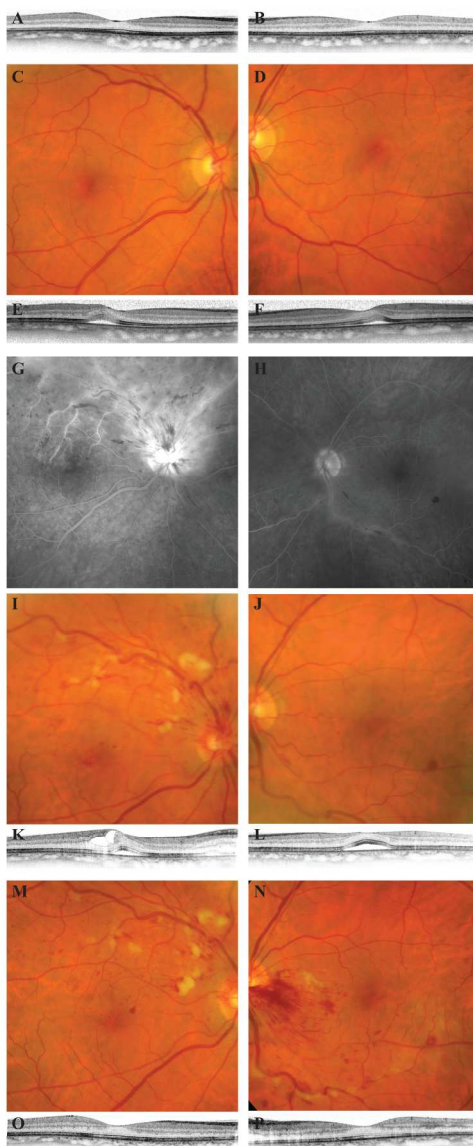
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419 **Tables**

420 **Table 1. Characteristics of the cutaneous melanoma patients.**

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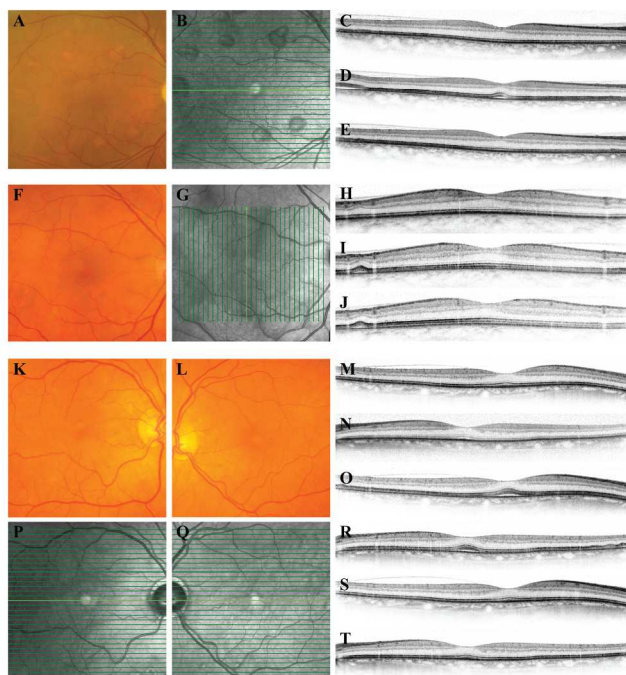


A-B, Optical coherence tomography (OCT) scan of the right (A) and left eye (B) of a 60-year-old male patient revealed a normal macular structure at baseline examination. C-D, Fundus photography of the right (C) and left eye (D), 41 days after the start of pimasertib treatment, revealed bilateral foveal and extrafoveal yellowish lesions. E-F, Foveal OCT scanning of both the right (E) and left eye (F) showed a serous neuroretinal detachment. Treatment was discontinued 22 days later. Nine days after discontinuation, treatment with 45 mg pimasertib twice daily could be restarted and patient attended a follow-up visit 12 days later. At that time, patient experienced a dark fleck in the inferior part of the visual field of the right eye. G-J, Fluorescein angiography and fundus photography revealed a hemi-retinal vein occlusion (RVO) in the right (G, I), and left eye (H, J). K, OCT of the right eye showed cystoid macular edema and both foveal and extrafoveal neuroretinal detachments were also present. L, OCT scanning of the left eye revealed both foveal and extrafoveal neuroretinal detachments. At this time, administration of pimasertib was discontinued. M-N, At follow-up 8 days after a single intravitreal injection of bevacizumab, fundus photography of the right (M) and left eye (N) revealed persistent cotton-wool spots and haemorrhages

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3 associated with the RVO in the right eye, and an increase of haemorrhages and cotton-wool spots in the left
4 eye. O-P, On an OCT scan during this visit, both cystoid macular edema and SRF had resolved in the right
5 eye (O), and resolution of the neuroretinal detachment had occurred in the left eye (P).
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A, Fundus photography of the right eye of a 67-year-old male patient who developed foveal and extrafoveal yellowish lesions, at 13 days after the start of pimasertib treatment. B, Infrared reflectance (IRR) imaging showed hyperreflective foveal and extrafoveal lesions, which were surrounded by a hyporefective zone. C, Optical coherence tomography (OCT) had shown a normal macular structure at baseline. D, Thirteen days after the start of study treatment, both foveal and extrafoveal serous neuroretinal detachments on OCT had developed. E, At final follow-up, 63 days after the start of the prescription, resolution of lesions had occurred.

F, Fundus photography of the right eye of a 63-year-old female patient showed subtle extrafoveal transparent to yellowish lesions, 27 days after the start of pimasertib treatment. G, IRR imaging showed corresponding hyperreflective lesions. H, Before treatment, OCT had shown a normal macular structure. I, An extrafoveal neuroretinal detachment could be detected on OCT, 27 days after the beginning of pimasertib. J, This serous retinopathy was still present at final follow-up 14 days later.

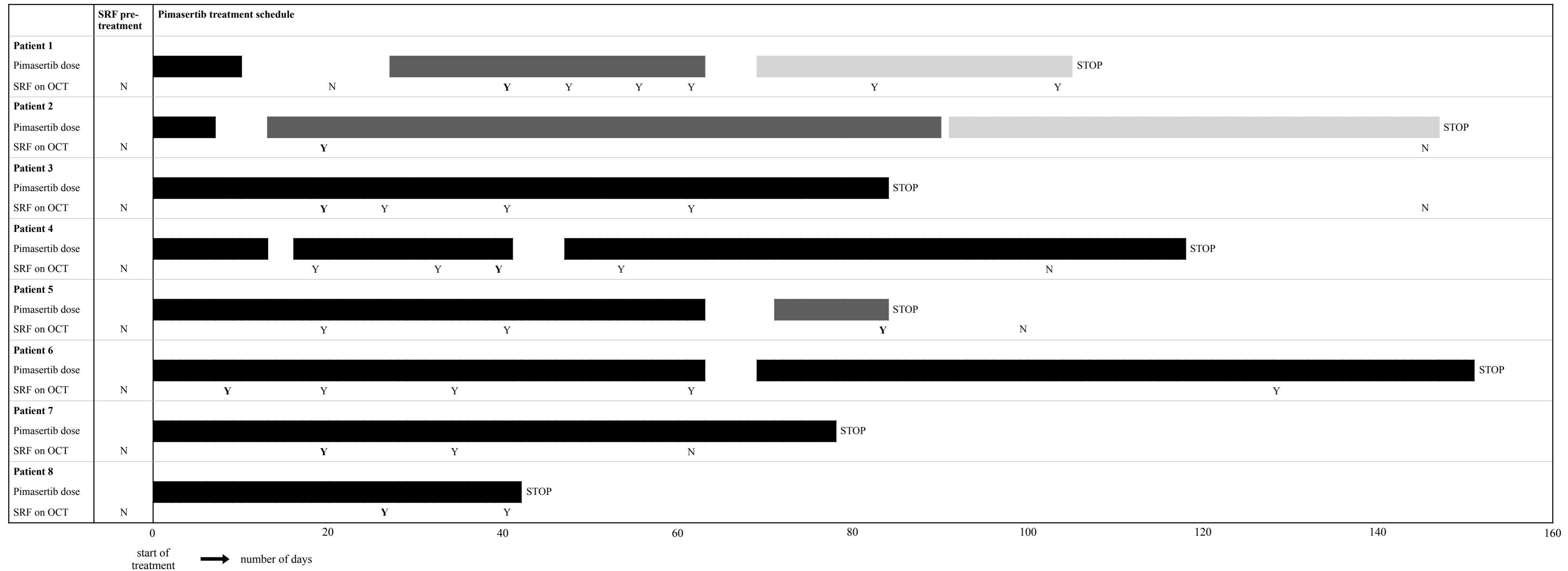
K-L, In a 66-year-old patient, showing pimasertib-associated serous retinopathy, fundus photography

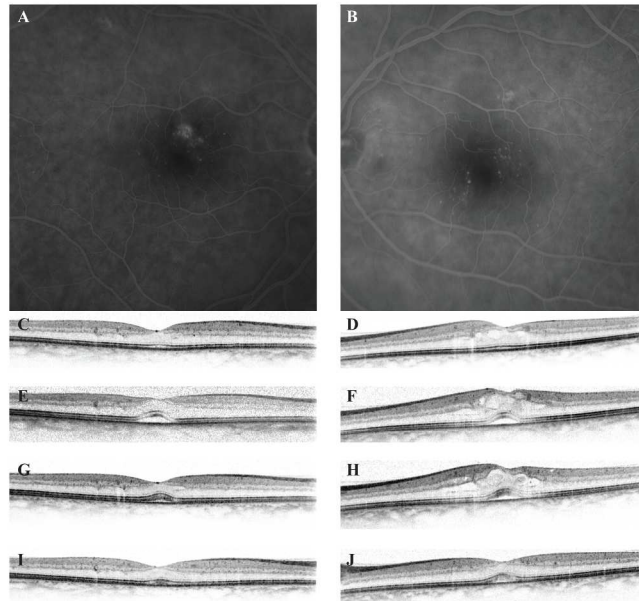
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3 revealed both foveal and extrafoveal yellowish lesions, 20 days after the start of pimasertib. P-Q, These
4 lesions corresponded to hyperreflective lesions, surrounded by a hyporefective zone on IRR imaging. M-N,
5 At baseline examination, no abnormalities had been found on OCT. O and R, Twenty days after the start of
6 treatment, an OCT scan showed a bilateral foveal neuroretinal detachment. S-T, Resolution of this
7 detachment was detected, 62 days after discontinuation of treatment.

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A-B, Fluorescein angiography in a 67-year-old male patient with metastatic cutaneous melanoma and diabetes mellitus type 2 before the start of pimasertib treatment, showed bilateral microaneurysms and mild fluorescein leakage. C-D, Optical coherence tomography (OCT) at baseline examination showed very mild diabetic macular edema (DME) without other abnormalities (C), and mild DME in the left eye (D). E, OCT scanning at 9 days after the start of pimasertib treatment showed a foveal serous neuroretinal detachment in the right eye. F, At this moment, the left eye showed both a foveal serous neuroretinal detachment and worsening of the DME on OCT. G-H, Eleven days later the amount of serous subretinal fluid had decreased spontaneously in the right (G) and the left eye (H). I-J, Despite the fact that this patient still used pimasertib in a dosage of 60 mg twice daily, the neuroretinal detachment had almost resolved in both eyes, together with the DME in the left eye, at final ophthalmological follow-up.

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Table 1. Characteristics of the cutaneous melanoma patients.

Clinical characteristics	
Number of patients	8
Number of males	4/8 (50%)
Number of females	4/8 (50%)
Mean age (years)	62.9
Median age (years)	64
Ophthalmic characteristics	
Number of patients developing visual complaints	1/8 (13%)
<i>Ophthalmoscopy images available during ophthalmological follow-up (patients)</i>	8
Transparent to yellowish fundus lesions (eyes)	16/16 (100%)
Single foveal lesion (eyes)	0
Multifocal lesions (eyes)	16/16 (100%)
<i>OCT imaging available during ophthalmological follow-up (patients)</i>	8
SRF on OCT (eyes)	16/16 (100%)
<u>Foveal SRF</u> (eyes)	12/16 (75%)
- Only foveal SRF	0/12
- Foveal and extrafoveal SRF	12/12
<u>Extrafoveal SRF</u> (eyes)	16/16 (100%)
- Only extrafoveal SRF	4/16
- Foveal and extrafoveal SRF	12/16
<i>Infrared reflectance imaging available during ophthalmological follow-up (patients)</i>	8
Extrafoveal lesions (eyes)	16/16 (100%)
Mean number of extrafoveal lesions	5
Median number of extrafoveal lesions	6

Abbreviations: OCT, optical coherence tomography; SRF, subretinal fluid