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## Comparative analysis of predictive values of the kinetics of 11 circulating miRNAs and of CA125 in ovarian cancer during first line treatment (a GINECO study)

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1 **Comparative analysis of predictive values of the kinetics of 11 circulating miRNAs and**  
2 **of CA125 in ovarian cancer during first line treatment (a GINECO study).**

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52 **Running title:** Kinetics of miRNAs and of CA125 in ovarian cancer

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66 Abstract

67 **Objective:** MicroRNAs (miRNAs) are promising biomarkers in ovarian cancer. Their kinetics  
68 during treatment might be useful for monitoring disease burden, and guiding treatments in  
69 patients treated with peri-operative chemotherapy and interval debulking surgery (IDS).

70 **Methods:** Serial blood samples of patients enrolled in the randomized phase II CHIVA trial,  
71 comparing first line carboplatin-paclitaxel +/- nintedanib (NCT01583322) and IDS, were  
72 investigated to assess the kinetics of 11 relevant miRNAs. Their prognostic/predictive values  
73 regarding the likelihood of complete IDS, and the patient survival, were assessed and  
74 compared to those of CA125 kinetics. The selection of the miRNAs (miR-15b-5p, miR-16-5p,  
75 miR-20a-5p, miR-21-5p, miR-93-5p, miR-122-5p, miR-150-5p, miR-195-5p, miR-200b-3p,  
76 miR-148b-5p and miR-34a-5p) was based on the expression levels found with a large  
77 explorative panel, and on the literature data.

78 **Results:** 756 serial blood samples from 119 patients were analyzed for a total of 8172 miRNA  
79 assays, and 1299 CA125 values. The longitudinal kinetics of the miRNA expressions were  
80 highly inconsistent, and were not related to CA125 dynamics. The miRNA changes during  
81 neoadjuvant treatment were not found associated with RECIST tumor response or IDS  
82 outcomes. Decreases of miR-34a-5p and miR-93-5p were associated with PFS benefit  
83 ( $p=0.009$ ) and OS benefits ( $p<0.001$ ), respectively, using univariate tests.

84 **Conclusions:** The longitudinal kinetics of miRNA expressions during neo-adjuvant treatment  
85 in ovarian cancer patients were inconsistent, and were not found to be associated with tumor  
86 burden changes. Although some prognostic value could be discussed, no predictive value  
87 regarding tumor responses or IDS quality could be identified.

88 Introduction

89 Ovarian cancer ranks fifth for mortality linked to female cancer in western countries [1]. In  
90 2011, the Gynecologic Cancer Intergroup (GCIG) defined CA125 response as a  $\geq 50\%$   
91 reduction in CA125 levels from baseline, on a minimum 28 day period [2]. However, the  
92 relevance of this specific criterion, has recently been questioned, since it was not found to  
93 exhibit prognostic or predictive values regarding progression free survival (PFS) and overall  
94 survival (OS) in several studies [3–6].

95 In first line setting, stage III or IV ovarian cancer patients with diseases considered to be not  
96 amenable to primary cytoreductive surgery are usually treated with neoadjuvant  
97 chemotherapy and interval debulking surgery (IDS) [7]. One of the main prognostic factors in  
98 patients treated with such a strategy is the completeness of the cytoreductive surgery  
99 (complete CC0 versus incomplete CC1-CC2) [8]. In this context, there are no validated  
100 biomarkers prone to help predict the likelihood of complete IDS, or patient survival.

101 MicroRNAs (miRNAs) are small non-coding RNA molecules that bind specifically to their  
102 target mRNAs for inhibiting the expression of genes after transcription [9]. Several studies  
103 reported promising outcomes about the diagnostic [10–14], prognostic [10,12,13,15] or  
104 predictive values [16–18] of miRNAs in ovarian cancer patients. Some data suggested that the  
105 tumor burden changes observed during treatment correlated with the kinetics of some  
106 circulating miRNAs in ovarian cancers patients [17,18].

107 We hypothesized that the kinetics of some miRNAs of interest may be more helpful than  
108 CA125 for monitoring disease burden and guiding clinicians in decision making for ovarian  
109 cancers patients. The aim of the present study was designed to assess the kinetics of 11  
110 relevant miRNAs and of CA125 in first line treatment, as a way of comparing their prognostic  
111 and predictive values regarding the likelihood of complete IDS.

112

113 Materials and methods

114 Patient population

115 Clinical and biological data were derived from the randomized phase II trial CHIVA  
116 (NCT01583322). In this trial, patients with International Federation of Gynecology and  
117 Obstetrics (FIGO) stage IIIC or IV ovarian carcinomas planned to be treated with peri-  
118 operative chemotherapy and IDS, were randomly allocated to 1) the standard arm carboplatin  
119 AUC 5 or 6 and paclitaxel 175mg/m<sup>2</sup> every 3 weeks with placebo, or 2) the experimental arm  
120 with the same regimen combined to nintedanib at 200mg twice daily. The enrolled patients  
121 received 3 to 4 cycles before cytoreductive surgery, and then 3 to 4 cycles after surgery,  
122 followed by a maintenance treatment with nintedanib/placebo for up to 2 years [19].

123 Nintedanib (BIBF 1120, VARGATEF<sup>TM</sup>) is a receptor tyrosine kinase (RTK) inhibitor with  
124 potential antiangiogenic and antineoplastic activities. The multitargeted tyrosine kinase  
125 inhibitor BIBF 1120 selectively binds to and inhibits vascular endothelial growth factor  
126 receptor (VEGFR), fibroblast growth factor receptor (FGFR) and platelet-derived growth  
127 factor receptor (PDGFR) tyrosine kinases, which may result in the induction of endothelial  
128 cell apoptosis; a reduction in tumor vasculature; and the inhibition of tumor cell proliferation  
129 and migration.

130 Blood samples for miRNA, along with CA125 concentrations, were assayed at the 8  
131 following times during the study: at screening, cycles 2, and 3; before IDS; the three cycles  
132 after IDS; and at progression, if any. Moreover, the CA125 values were assayed every 3  
133 months after the end of chemotherapy for follow-up. The following parameters were collected  
134 for the present study: age, weight, height, pathology type, grade, FIGO stage, treatment arm,  
135 post-operative disease residuals as judged by the surgeon (no macroscopic residual, tumor

136 residuals < 0.25cm, tumor residuals 0.25 to 2.5 cm, tumor residuals > 2.5 cm), and the  
137 radiological tumor objective response to treatment according to RECIST V1.1 criteria  
138 (complete response (CR), partial response (PR), stable disease (SD) or progressive disease  
139 (PD)).

140 The protocol was approved by an ethic committee and health authorities in 2012. Written  
141 informed consent was obtained from all patients before study entry.

142 Serum miRNAs quantification and analysis

143 The plasma samples were centrifuged, and stored at -80°C. MiRNAs were extracted with the  
144 Qiagen miRNeasy microkit. The targets miRNAs were quantified by qRT-PCR using the  
145 Qiagen kit custom miScript miRNA PCR assay. After performing the Qiazol lysis step, *C.  
146 elegans* miR-39 miRNA (cel-miR-39) was added as an external control for extraction  
147 efficiency and normalization.

148 Analysis were performed using the  $2^{-\Delta\Delta Ct}$  method [20]. The  $\Delta Ct$  is the difference of expression  
149 (expressed as cycle threshold (Ct)) between the target miRNA (miRNA being tested) and a  
150 housekeeping miRNA (external control, miRNA cel-miR-39) for each sample. The difference  
151 between the  $\Delta Ct$  of the target sample and the  $\Delta Ct$  of the reference sample (calibrator sample:  
152 first sample of the first patient) is the  $\Delta\Delta Ct$ . By calculating the  $2^{-\Delta\Delta Ct}$ , the relative  
153 quantification (RQ) of the target gene expression sample was compared to the reference  
154 sample. RQs greater or less than 1 were considered as over-expression or under-expressions,  
155 respectively. Samples with expressions lower than the limit of quantification (Ct result > 35  
156 cycles) were excluded from the analyses and the graphics, because they were considered as  
157 non-significant expressions of miRNAs.

158 MiRNAs selection



159 More than 1000 cancer miRNAs have been reported in the literature [21]. Eleven miRNAs  
160 were selected for the present study. This number resulted from a selection performed in 2  
161 steps. First, a large panel of 84 human circulating miRNAs (MIHS-106ZA, QIAGEN) were  
162 tested in 8 randomly selected patients in order to identify the 8 miRNAs which were the most  
163 relevant for our kinetic analysis with the following criteria: sufficient expression for  
164 replication (at least 75% of Ct lower than 35 cycles), and kinetics considered to be consistent  
165 during treatment (in the sense that the miRNA level should increase or decrease during  
166 treatment periods). Moreover, the selection was guided by the literature analysis with the  
167 requirement that these miRNAs had to be involved in the ovarian carcinogenesis, and they  
168 were shown to exhibit differential expressions in between ovarian cancer patients and control  
169 groups. The eight miRNAs selected from this step were: hsa-miR-15b-5p, hsa-miR-16-5p,  
170 hsa-miR-20a-5p, hsa-miR-21-5p, hsa-miR-93-5p, hsa-miR-122-5p, hsa-miR-150-5p and hsa-  
171 miR-195-5p (Supplementary Figure S1). In addition, three additional miRNAs were selected  
172 based on publications reporting relevant kinetics of miRNAs during chemotherapy: hsa-miR-  
173 200b-3p, hsa-miR-148b-5p, and hsa-miR-34a-5p [17,18,22].

174 The eleven selected miRNAs were quantified in the remaining 111 patients.

175 Kinetic analyses of longitudinal miRNAs and CA125 changes during CHIVA trial

176 The time changes of miRNA titers and of CA125 were assessed on the following time  
177 windows: 1) screening to pre-surgery timepoint; 2) pre-surgery timepoint to first cycle after  
178 surgery (cycle 4); and 3) third cycle after surgery to progression, if any. The differences in  
179 between plasma miRNA RQs and CA125 concentrations on these time periods were assessed  
180 using Wilcoxon signed-rank tests. The longitudinal changes in miRNAs RQ and in CA125  
181 levels were graphically assessed for each patient, and for the global population.

182 Prognostic and predictive values of miRNA and of CA125 longitudinal time changes during  
183 neo-adjuvant chemotherapy

184 As previously done for CA125 [2] and other serum biomarkers [23], the time changes of  
185 miRNA RQs and of CA125 concentrations during neoadjuvant chemotherapy were calculated  
186 using their percentage decreases from the baseline timepoint (screening value) to the pre-  
187 operative timepoint (before IDS).

188 Receiver operating characteristic (ROC) curves were used to evaluate the discriminative  
189 predictive ability of miRNA & CA125 percentage decreases regarding the likelihood of  
190 complete CC0 IDS, and of radiological tumor response (assessed using RECIST V1.1  
191 criteria).

192 PFS and OS were calculated as the time intervals from treatment start to the date of  
193 progression or death, whichever occurred first, for PFS; and to the death only for OS.

194 The 5 following thresholds were arbitrarily chosen: the cutoffs maximizing 1) the prediction  
195 ability of CC0 IDS (CC0 vs CC1-CC2); 2) the prediction ability of tumor response (complete  
196 response and partial response (CR & PR) versus stable disease and progressive disease (SD &  
197 PD), using the Youden index statistic criterion with ROC curves; along with 3) the 1<sup>st</sup>  
198 quartiles of the biomarker percentage decrease distributions; 4) the cutoffs associated with a  
199 decline of miRNA or CA125 by 25%; 5) the cutoffs associated with a decline of miRNA or  
200 CA125 by 50%.

201 The predictive values of every miRNA and CA125 percentage variations, dichotomized by  
202 the above thresholds, regarding PFS and OS were first investigated using c-index tests [24].  
203 The prognostic values of CA125 and miRNA percentage variations found to be the most  
204 significant with the c-index analyses, were subsequently assessed using Kaplan-Meier method  
205 and log-rank tests.

206 All tests were two-sided with 5% alfa risks. All statistical analyses were performed using R  
207 3.3.2 software with “survival”, "ROC" and "pec" packages.

208

209 Results

210 Patients characteristics

211 188 patients were included in the CHIVA trial from May 2013 to July 2015. The data from  
212 119 of them (63%) could be analyzed for the present study, comprising 8 patients chosen for  
213 the selection of 8 miRNAs from the large panel, and 111 remaining patients for the kinetic  
214 analyses of 11 miRNAs (Supplementary Figure S2). The characteristics of the included  
215 patients are presented in Table 1 and were similar for the 2 groups.

216 Total 8172 miRNAs concentrations were assayed. The concentrations were above the limits  
217 of quantification for 6674 samples (82%), ranging from 23% to 98% depending of the  
218 miRNA. All the 1299 CA125 measured values were above the limits of quantification (Table  
219 2).

220 Kinetic analysis of miRNAs and CA125 longitudinal time changes in CHIVA trial

221 The kinetics of miRNAs were poorly assessable. No statistically significant changes of the  
222 miRNAs expressions during treatment were found using Wilcoxon signed-rank tests.  
223 However, statistically significant increases of miRNAs expressions were identified at disease  
224 progressions for 6 mi-RNA (Table 2).

225 The comparative analyses of miRNA and CA125 kinetics during the disease managements  
226 showed that the time-changes of CA125 were more homogeneous and rational, than those of  
227 miRNAs (Table 2, Figure 1 and Supplementary Figures S3 to S7). Declines of CA125 by  
228 50% or more during chemotherapy were found in 98% of patients (GCIG CA125 response

229 criterion), while such analyses were not assessable for the miRNAs, due to their hectic and  
230 irrational kinetics, with high inter-individual variability.

231 Predictive value of miRNAs regarding the tumor response rate or interval debulking  
232 cytoreductive surgery score

233 The predictive abilities of every miRNA expression percentage variation during neoadjuvant  
234 chemotherapy regarding the likelihood of complete IDS (yes vs no) using ROC area under the  
235 curve (AUC) are presented in Table 3. The ROC AUCs ranged from 0.51 to 0.88; none of  
236 them being statistically significant, except for the hsa-miR-200b-3p. The same lack of  
237 significant predictive value was found for the likelihood of tumor response (CR-PR vs SD-  
238 PD) with ROC AUCs ranging from 0.50 to 0.86; all of them being non-significant, except for  
239 hsa-miR-200b-3p (Table 3).

240 The same analyses done with CA125 percentage decrease showed a ROC AUC at 0.58 (95%  
241 CI 0.45-0.71) for prediction of CC0 IDS, and at 0.64 (95% CI 0.51-0.76) for prediction of  
242 tumor response (Table 3).

243 The values of the different thresholds maximizing the likelihood of complete surgery and of  
244 tumor response for miRNA and CA125 percentage decreases are presented in Table 3.

245 Predictive value of miRNA kinetics for survivals

246 The predictive values of miRNA and CA125 variations, dichotomized by the different cutoffs,  
247 regarding PFS and OS are shown in Table 4. The c-index values of miRNA time changes  
248 ranged from 0.47 to 0.67 for PFS; and from 0.44 to 0.81 for OS. The highest significant  
249 predictive values regarding PFS were observed with the hsa-miR-15b-5p (categorized by the  
250 threshold optimizing the tumor response likelihood) at 0.63 (95% CI 0.57-0.69), and with the  
251 hsa-miR-34a-5p (categorized by the cutoff associated with a decline by 25%) at 0.64 (95% CI  
252 0.52-0.76).

253 For OS, the highest significant predictive values were obtained with the has-miR-15b-5p  
254 (categorized by the threshold optimizing the tumor response likelihood) at 0.63 (95% CI 0.53-  
255 0.73), and with the hsa-miR-93-5p (categorized by the threshold optimizing the prediction of  
256 complete IDS) at 0.66 (95% CI 0.56-0.76). The similar analyses done with the CA125  
257 kinetics showed c-index values ranging from 0.50 to 0.54 for PFS, and 0.50 to 0.56 for OS.

258 The outcomes regarding the prognostic values of the most significant miRNAs using Kaplan-  
259 Meier survival curves and log-rank tests are presented in Figure 2. Hsa-miR-15b-5p and hsa-  
260 miR-34a-5p were associated with better PFS (median PFS = 13.2 vs 20.8 months and 13.7 vs  
261 24.8 months, respectively,  $p < 0.01$ ), whilst hsa-miR-15b-5p and hsa-miR-93-5p were  
262 associated with better OS (median OS = 34.2 vs 52.0 and 34.3 vs NR months, respectively,  
263  $p < 0.01$ ). CA125 kinetics assessed with the similar methodology were not significant,  
264 including with the 50% decrease cutoff recognized as the GCIG CA125 response criterion.

265

## 266 Discussion

267 This present study represents the first analysis on the actual predictive and prognostic values  
268 of miRNA longitudinal kinetics in cancer patients managed with medical-surgical treatments.  
269 MiRNAs have been recently reported as major actors in the tumorigenesis [25]. The miRNA  
270 expression time changes were expected to correlate with the tumor burden changes, thereby  
271 representing promising predictive biomarkers [17,18,26,27]. The ovarian cancer population  
272 was particularly adequate, since there is a need for reliable serum biomarkers to overcome the  
273 limitation of imaging, and also because miRNA kinetic value could be assessed with respect  
274 to the validated serum marker CA125 [2,28].

275 Several authors reported the prognostic or predictive values of miRNA expressions (e.g. miR-  
276 34a-5p level before treatment associated with CC0 cytoreductive surgery in 56 patients, ROC

277 AUC = 0.78) [22], or miRNA expression changes during treatments (e.g. miR-200b change  
278 from baseline to post-operative period regarding PFS in 33 patients) [17].

279 The innovative design of the randomized phase II trial CHIVA, in which serial blood samples  
280 were regularly performed during disease management, enabled us to assess the kinetics of 11  
281 miRNAs measured in a properly conducted prospective trial. This miRNA selection was  
282 based on initial explorative analyses with a large panel of miRNAs in 8 patients, and based on  
283 the literature data. Our selection appeared adequate since the miRNAs were highly expressed  
284 in 82% of samples.

285 The kinetic analysis of miRNA demonstrates that no rational longitudinal profiles could be  
286 identified for any of them throughout the treatment. The large intra-individual and inter-  
287 individual variability of miRNA time-changes made any longitudinal assessment impossible  
288 with mathematical modeling. As a consequence, it was not adequate to search for  
289 relationships between tumor burden alterations and miRNA kinetics using mathematical  
290 modeling, contrarily to what was done with CA125 [3,29–32].

291 Our outcomes in favor a lack of predictive value of miRNA are not consistent with the data  
292 reported in the literature earlier [17,18,22,26,27,33]. This discrepancy could be understood by  
293 the methodology used by the other teams so far. In those studies, the kinetic analyses were  
294 performed on maximum two or three time points (contrarily to our approach based on 8  
295 timepoints per patient), and it is possible that the limited numbers of time points would have  
296 induced biases in the outcomes given the high inter-individual variability of miRNA  
297 expressions. Moreover, the large inconsistency in the types of miRNAs reported in the  
298 literature assumed to exhibit prognostic or predictive values highlights the difficulty to  
299 identify miRNAs with reproducible prognostic/predictive values.

300 The present study present limitations prone to reduce the relevance of data. First, it was not  
301 possible to assess all miRNAs, and we had to select an affordable panel. We selected 11  
302 miRNAs that appeared rational, based on an explorative analysis including a large panel of 84  
303 miRNAs, and based on the literature. Our conclusions about the lack of assessable  
304 longitudinal prognostic or predictive kinetic profiles may not necessarily be transposable to  
305 other miRNAs. The selection of the best cut-offs for analyzing miRNAs kinetics in a way that  
306 would make the analyses helpful for clinical purpose was another difficulty. We chose to  
307 implement common statistical approaches, such as ROC curve tests and quartiles, but the  
308 relevance is questionable. None of the cutoffs that we assessed appeared superior. Moreover  
309 the expressions of the assessed miRNAs were below the lower limit of quantification in 18%  
310 of measurements, thereby making the kinetics analysis even more complicated, especially for  
311 miR-200b-3p. Furthermore, the impact of the tumor histological subtypes or BRCA status on  
312 miRNAs kinetics could not be assessed. Of note, recent data on the kinetics of CA125  
313 assessed using mathematical modeling suggest that the modeled kinetic parameter KELIM  
314 could be a promising predictor of complete IDS likelihood, or of survival, in ovarian cancer  
315 patients [34–36]. However this parameter was not assessed here, because we thought that  
316 miRNA and CA125 kinetics had to be investigated with the same methodologies.

317 The present study is the largest work including serial assessment of a high numbers of  
318 miRNA, in cancer patients managed with multi-modal treatment in a properly conducted  
319 phase II trial. The outcomes indicate miRNA may not be adequate biomarkers for longitudinal  
320 kinetic analyses during treatment. Consistently with the inconsistent data of the literature on  
321 the prognostic or the predictive values of miRNA titers or time changes, we could not identify  
322 any miRNA prone to be reproducible predictors of IDS score, tumor response, or survivals in  
323 ovarian cancer patients.

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328 Conflict of interest statement

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- 466

467 **Figures Legends**

468 **Figure 1. Longitudinal time changes of the miR-195 expression and of CA125**  
469 **concentrations.** (A) Individual patient profiles of miR-195 and CA125 kinetics according to  
470 completeness of cytoreduction (green line: complete cytoreductive surgery; red: incomplete  
471 cytoreductive surgery); (B) Changes in populations values of blood miR-195 expressions and  
472 CA125 concentrations during management steps and at progression

473 Abbreviations: S: Screening; C: Pre-surgery; P: Progression.

474

475 **Figure 2. Prognostic values of the expression changes of the most significant miRNAs**  
476 **and of CA125 regarding PFS and OS using Kaplan-Meier survival curves of patients.**

477 (A) PFS with miR-15b categorized by the threshold optimizing the tumor response likelihood  
478 and miR-34a categorized by the 25% decline threshold; (B) OS with miR-15b categorized by  
479 the threshold optimizing the tumor response and miR-93 categorized by the threshold  
480 optimizing the complete IDS likelihood; (C) PFS with CA125 categorized by the first quartile  
481 of the RV and OS with CA125 categorized the threshold maximizing the complete IDS  
482 likelihood.

483 IDS: interval debulking surgery; PFS: progression free survival; OS: overall survival.

484

485 **Tables**

486 **Table 1. Patient characteristics at inclusion**

487

488 **Table 2. Expressions of the 11 miRNA and of CA125. Statistical significances of the**  
489 **comparisons of expressions on 3 different time windows using Wilcoxon signed-rank**  
490 **tests: 1) from screening (baseline) to IDS; 2) from IDS to cycle 4; 3) from cycle 6 to**  
491 **disease progression.**

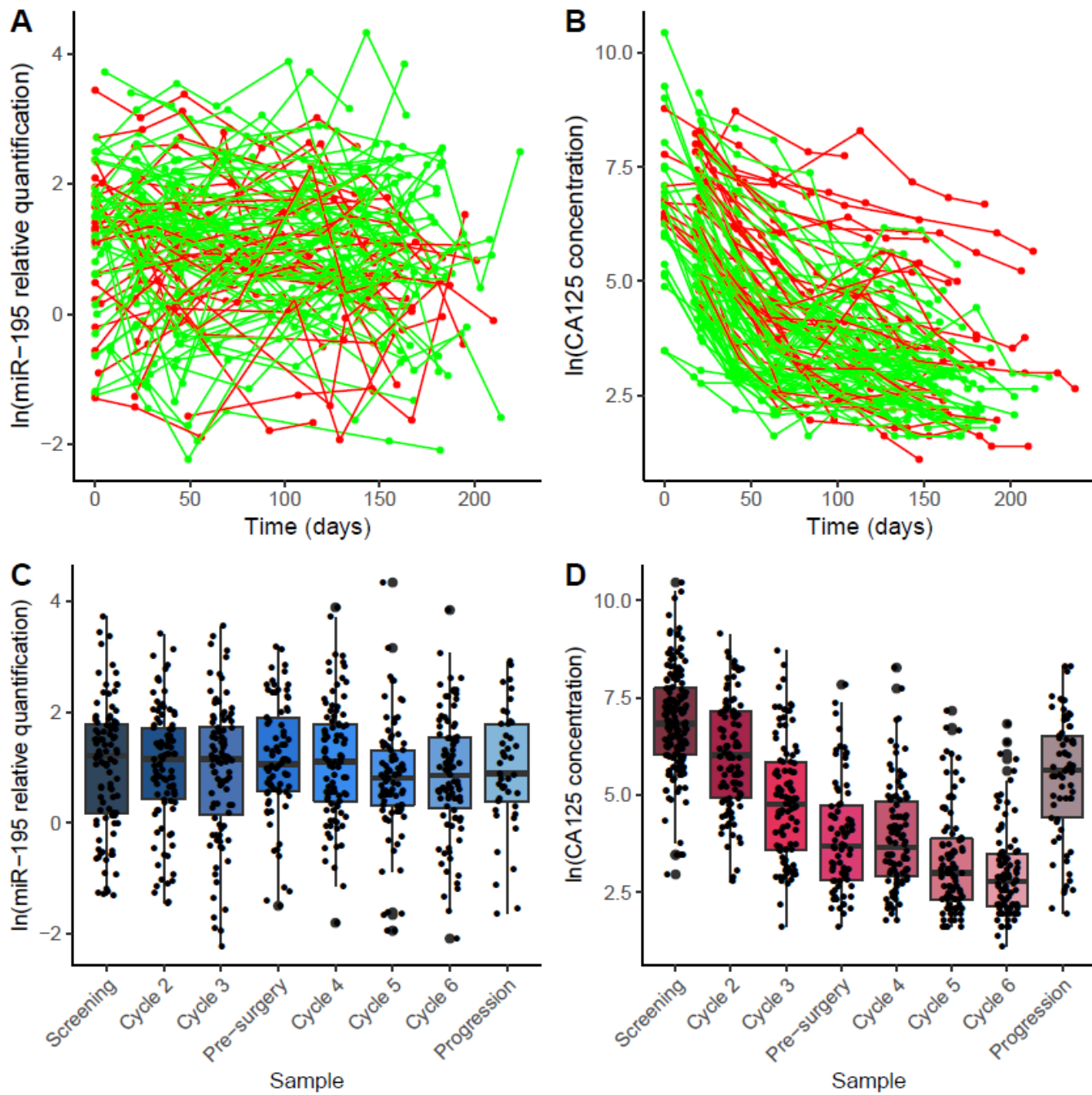
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493 **Table 3. Outcomes of the ROC curve AUC analyses on 1) Predictive ability of miRNA**  
494 **and CA125 percentage decreases during neoadjuvant chemotherapy regarding the**  
495 **likelihood of complete cytoreductive surgery (complete cytoreductive surgery with no**  
496 **macroscopic residuals vs incomplete debulking with residuals), or of tumor response (CR +**  
497 **PR vs SD + PD); 2) Identification of the optimal cutoff for predictions of the likelihood of**  
498 **complete cytoreductive surgery, or of tumor response using Youden criterion.**

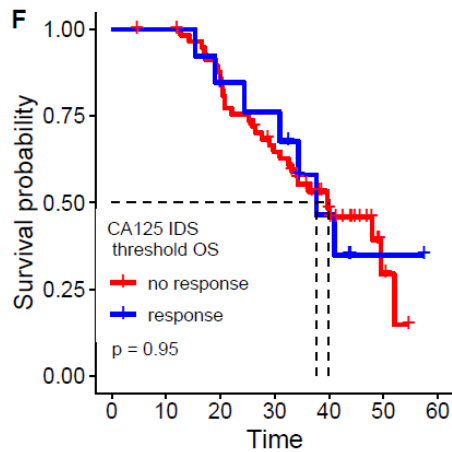
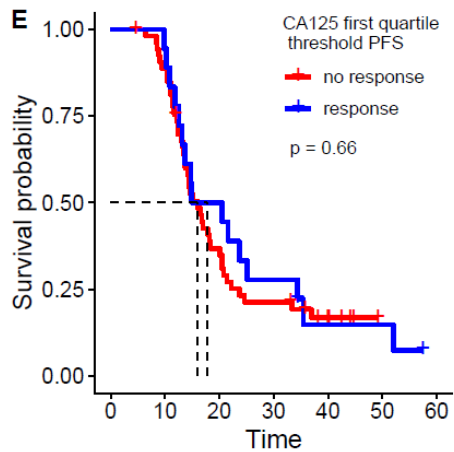
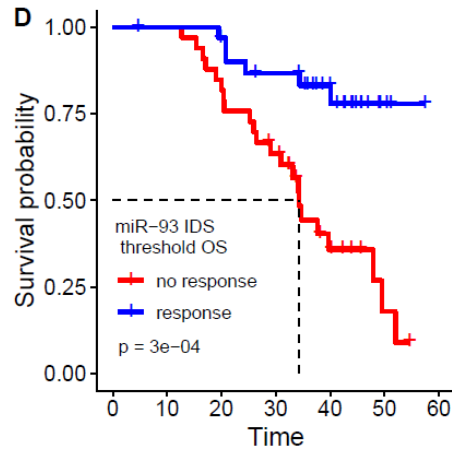
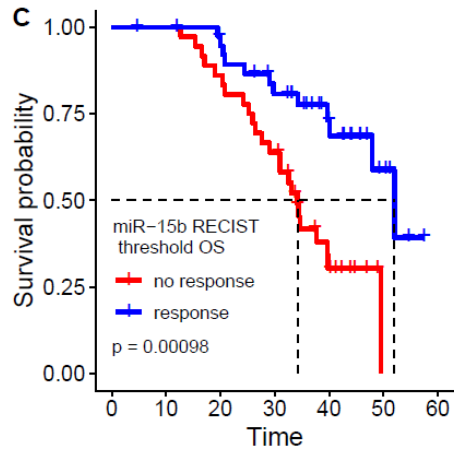
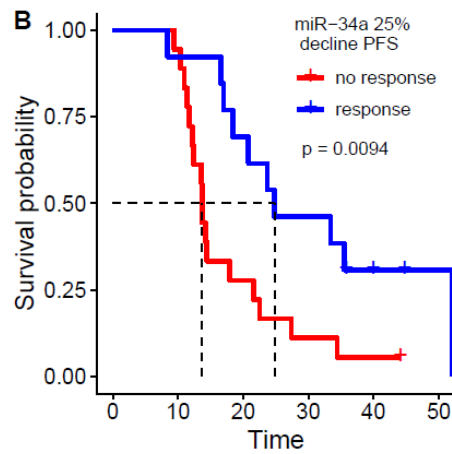
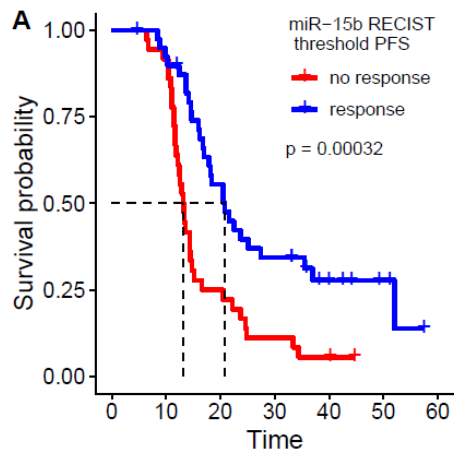
499

500 **Table 4. C-index values regarding the predictive values of miRNA and CA125**  
501 **percentage decreases, categorized by the 5 different cutoffs for PFS and OS.**

502



**Figure 1.**



**Figure 2.**

**Table 1. Patient characteristics at inclusion**

Characteristics	MiRNA selection	
	patients	Test patients
Patients number, <i>n</i>	8	111
Age, years, median (range)	65 (31-71)	64 (31-79)
ECOG PS, <i>n</i> (%)		
0-1	7 (88%)	98 (88%)
2	1 (12%)	12 (11%)
NA	0 (0%)	1 (1%)
FIGO stage, <i>n</i> (%)		
IIIc	7 (88%)	85 (77%)
IV	1 (12%)	26 (23%)
Histological type, <i>n</i> (%)		
Serous/papillary	8 (100%)	98 (88%)
Endometrioid	0 (0%)	1 (1%)
Mucinous	0 (0%)	1 (1%)
Clear cells	0 (0%)	1 (1%)
Undifferentiated	0 (0%)	3 (3%)
NA	0 (0%)	7 (6%)
Histological grade, <i>n</i> (%)		
Grade 1	0 (0%)	3 (3%)
Grade 2	1 (12%)	10 (9%)
Grade 3	5 (63%)	79 (71%)
NA	2 (25%)	19 (17%)
CA125 at inclusion (xULN), median (range)	24.4 (2.6-250.4)	26.9 (0.5-784.3)
IDS performed, <i>n</i> (%)	8 (100%)	86 (77%)
Completeness of cytoreduction, <i>n</i> (%)		
No macroscopic residuals	5 (63%)	70 (81%)
Tumoral residuals < 0,25 cm	0 (0%)	6 (7%)
Tumoral residuals 0,25 < R < 2.5 cm	3 (37%)	7 (8%)
Tumoral residuals > 2.5 cm	0 (0%)	2 (2%)
NA	0 (0%)	1 (1%)
Treatment with nintedanib, <i>n</i> (%)	5 (63%)	68 (61%)
Response to neo-adjuvant chemotherapy, <i>n</i> (%)		
CR	1 (12%)	2 (2%)
PR	3 (37%)	45 (41%)
SD	4 (50%)	57 (51%)
PD	0 (0%)	2 (2%)
NA	0 (0%)	5 (5%)
Progression free survival in months, median (95% CI)	15.6 (11.8-NR)	16.2 (14.4-18.4)
Overall survival in months, median (95% CI)	NR (38.6-NR)	40.1 (36.2-NR)
Death, <i>n</i> (%)	3 (37%)	55 (50%)

ECOG PS: ECOG Performance Status; IDS: Interval debulking surgery; NA: Non-available;

ULN: Upper limit of normal; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease CI: Confident interval; NR: Not reached.



**Table 2. Expressions of the 11 miRNA and of CA125. Statistical significances of the comparisons of expressions on 3 different time windows using Wilcoxon signed-rank tests: 1) from screening (baseline) to IDS; 2) from IDS to cycle 4; 3) from cycle 6 to disease progression.**

Serum biomarkers	% of samples with RQ greater than LoQ	Significances of expression differences on 3 time windows		
		1) Screening to IDS <i>p</i> value	2) IDS to Cycle 4 <i>p</i> value	3) Cycle 6 to Progression <i>p</i> value
hsa-miR-15b-5p	97%	0.986	0.184	0.001
hsa-miR-16-5p	98%	0.397	0.454	0.001
hsa-miR-20a-5p	92%	0.886	0.441	0.013
hsa-miR-21-5p	98%	0.405	0.920	0.005
hsa-miR-93-5p	90%	0.974	0.441	0.026
hsa-miR-122-5p	96%	0.296	0.309	0.437
hsa-miR-150-5p	90%	0.182	0.012	0.081
hsa-miR-195-5p	96%	0.660	0.186	0.007
hsa-miR-34a-5p	57%	0.915	0.080	0.117
hsa-miR-148b-5p	54%	0.379	0.624	0.181
hsa-miR-200b-3p	23%	0.375	0.461	0.500
CA125	100%	<0.001	0.109	<0.001

IDS: Interval debulking surgery; RQ: Relative quantification; LoQ: limit of quantification;

**Table 3. Outcomes of the ROC curve AUC analyses on 1) Predictive ability of miRNA and CA125 percentage decreases during neoadjuvant chemotherapy regarding the likelihood of complete cytoreductive surgery (complete cytoreductive surgery with no macroscopic residuals vs incomplete debulking with residuals), or of tumor response (CR + PR vs SD + PD); 2) Identification of the optimal cutoff for predictions of the likelihood of complete cytoreductive surgery, or of tumor response using Youden criterion.**

Serum marker	Predictive value of miRNA and CA125 kinetics using ROC curve AUCs		Optimal threshold of the percentage decline maximizing the prediction ability of	
	Prediction of complete cytoreductive surgery (95% CI)	Prediction of tumor response (95% CI)	Complete cytoreductive surgery	Tumor response
hsa-miR-15b-5p	0.55 (0.41-0.70)	0.50 (0.36-0.63)	-6%	+27%
hsa-miR-16-5p	0.55 (0.40-0.69)	0.64 (0.50-0.76)	-23%	-11%
hsa-miR-20a-5p	0.53 (0.38-0.68)	0.57 (0.43-0.72)	+11%	+29%
hsa-miR-21-5p	0.52 (0.37-0.66)	0.53 (0.40-0.66)	-32%	+9%
hsa-miR-93-5p	0.52 (0.37-0.67)	0.67 (0.54-0.80)	+15%	+111%
hsa-miR-122-5p	0.58 (0.43-0.73)	0.58 (0.45-0.72)	+8%	+18%
hsa-miR-150-5p	0.56 (0.41-0.72)	0.51 (0.37-0.65)	-32%	+5%
hsa-miR-195-5p	0.52 (0.37-0.67)	0.60 (0.46-0.73)	-13%	-33%
hsa-miR-34a-5p	0.54 (0.27-0.80)	0.53 (0.32-0.74)	-41%	+124%
hsa-miR-148b-5p	0.63 (0.40-0.86)	0.58 (0.37-0.79)	-30%	+168%
hsa-miR-200b-3p	0.88 (0.65-1.00)	0.86 (0.60-1.00)	-10%	-3%
CA125	0.57 (0.43-0.70)	0.63 (0.49-0.76)	-99%	-97%

ROC: Receiver operating characteristic; AUC: Area under the curve; IDS: Interval debulking surgery; CI: Confident interval.

**Table 4. C-index values regarding the predictive values of miRNA and CA125 percentage decreases, categorized by the 5 different cutoffs for PFS and OS.**

Serum marker	Optimal threshold for predicting complete cytoreductive surgery (SD)	Optimal threshold for predicting tumor response (SD)	1 <sup>st</sup> Quartile of percentage decrease distribution (SD)	Percentage decrease of 25% (SD)	Percentage decrease of 50% (SD)
<b>Analyses for PFS</b>					
hsa-miR-15b-5p	0.61 (0.04)	0.63 (0.03)	0.60 (0.03)	0.60 (0.03)	0.58 (0.03)
hsa-miR-16-5p	0.55 (0.04)	0.57 (0.04)	0.55 (0.03)	0.53 (0.03)	0.53 (0.03)
hsa-miR-20a-5p	0.57 (0.04)	0.51 (0.04)	0.50 (0.03)	0.52 (0.04)	0.51 (0.04)
hsa-miR-21-5p	0.59 (0.03)	0.60 (0.03)	0.58 (0.03)	0.61 (0.04)	0.60 (0.03)
hsa-miR-93-5p	0.60 (0.04)	0.57 (0.03)	0.58 (0.04)	0.58 (0.04)	0.57 (0.03)
hsa-miR-122-5p	0.54 (0.04)	0.56 (0.03)	0.55 (0.03)	0.57 (0.04)	0.56 (0.03)
hsa-miR-150-5p	0.59 (0.04)	0.57 (0.04)	0.59 (0.04)	0.58 (0.04)	0.56 (0.03)
hsa-miR-195-5p	0.60 (0.04)	0.56 (0.03)	0.57 (0.03)	0.58 (0.03)	0.53 (0.03)
hsa-miR-34a-5p	0.60 (0.05)	0.56 (0.04)	0.57 (0.05)	0.64 (0.06)	0.55 (0.05)
hsa-miR-148b-5p	0.49 (0.06)	0.54 (0.04)	0.47 (0.05)	0.55 (0.05)	0.51 (0.05)
hsa-miR-200b-3p	0.58 (0.11)	0.67 (0.11)	0.64 (0.11)	0.64 (0.11)	0.59 (0.08)
CA125	0.51 (0.03)	0.51 (0.04)	0.52 (0.03)	0.50 (0)	0.50 (0.02)
<b>Analyses for OS</b>					
hsa-miR-15b-5p	0.60 (0.05)	0.63 (0.05)	0.61 (0.04)	0.61 (0.04)	0.62 (0.04)
hsa-miR-16-5p	0.57 (0.05)	0.58 (0.05)	0.56 (0.04)	0.55 (0.04)	0.55 (0.04)
hsa-miR-20a-5p	0.57 (0.05)	0.53 (0.05)	0.55 (0.04)	0.55 (0.05)	0.48 (0.05)
hsa-miR-21-5p	0.59 (0.04)	0.61 (0.04)	0.59 (0.04)	0.60 (0.05)	0.60 (0.04)
hsa-miR-93-5p	0.66 (0.05)	0.60 (0.05)	0.59 (0.05)	0.64 (0.05)	0.57 (0.04)
hsa-miR-122-5p	0.54 (0.05)	0.56 (0.04)	0.54 (0.04)	0.59 (0.05)	0.55 (0.04)
hsa-miR-150-5p	0.58 (0.04)	0.55 (0.05)	0.58 (0.04)	0.58 (0.04)	0.59 (0.04)
hsa-miR-195-5p	0.63 (0.05)	0.58 (0.04)	0.59 (0.04)	0.60 (0.04)	0.55 (0.04)
hsa-miR-34a-5p	0.53 (0.07)	0.52 (0.06)	0.49 (0.07)	0.51 (0.07)	0.51 (0.06)
hsa-miR-148b-5p	0.53 (0.07)	0.52 (0.05)	0.54 (0.06)	0.55 (0.07)	0.50 (0.06)
hsa-miR-200b-3p	0.81 (0.18)	0.75 (0.19)	0.44 (0.18)	0.44 (0.18)	0.58 (0.12)
CA125	0.50 (0.03)	0.54 (0.04)	0.51 (0.04)	0.50 (0)	0.52 (0.02)

Abbreviations: SD: Standard deviation; IDS: Interval debulking surgery; PFS: Progression

free survival, OS: Overall survival, CI: Confident interval; NR: Not Reached.