SUPPLEMENTARY INFORMATION TO:

Development and validation of a microRNA-based signature (MiROvaR) to predict early relapse or progression of epithelial ovarian cancer: a cohort study.

Marina Bagnoli PhD[§], Silvana Canevari PhD[§], Daniela Califano PhD, Simona Losito MD, Prof. Massimo Di Maio MD, Francesco Raspagliesi MD, Maria Luisa Carcangiu MD, Giuseppe Toffoli MD, Erika Cecchin PhD, Roberto Sorio MD, Vincenzo Canzonieri MD, Daniela Russo PhD, Giosué Scognamiglio PhD, Gennaro Chiappetta PhD, Gustavo Baldassarre MD, Domenica Lorusso MD, Prof. Giovanni Scambia MD, Prof. Gian Franco Zannoni MD, Antonella Savarese MD, Mariantonia Carosi MD, Paolo Scollo MD, Enrico Breda MD, Viviana Murgia MD, Francesco Perrone MD, Sandro Pignata MD[#], Loris De Cecco PhD[#], Delia Mezzanzanica PhD [#$]. Multicentre Italian Trials in Ovarian cancer (MITO) translational group.

[§] Equally contributing first authors

[#] Equally contributing senior authors

[$] Corresponding author:

Delia Mezzanzanica - Unit of Molecular Therapies, Department of Experimental Oncology and Molecular Medicine - Fondazione IRCCS Istituto Nazionale dei Tumori, Via Amadeo, 42; 20133 Milan Italy. delia.mezzanzanica@istitutotumori.mi.it. Phone: +390223902569

Other collaborators
We are grateful to: Renato Franco, Carmela Pisano, Gaetano Facchini, Giovanni Salvatore Bruni, Lucia Cannella, Davide Leopardo, Stefano Greggi, Francesco Iodice (deceased), Gennaro Casella Maria Carmela Piccirillo, Gennaro Daniele, Jane Bryce, (Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G.Pascale", IRCCS, Napoli). Vanda Salutari, Antonia Testa, Rosa De Vincenzo, Alessia di Legge, Claudia Masi, Valeria Masciullo, Mirella Di Stefano (Policlinico Universitario Gemelli, Università Cattolica del Sacro Cuore, Roma); Francesco Cognetti, Gianluigi Ferretti, Enrico Vizza, Emanuela Mancini (Istituto Nazionale Tumori Regina Elena, Roma); Simona Scalone, Giorgio Giorda, Elio Campagnutta (Centro di Riferimento Oncologico, Aviano [PN]); Elena Cicerone, Antonella Mecozzi, Emanuela Proietti, Loredana Rossi, Angelo Fedele Scinto (Ospedale S. Giovanni Calibita Fatebenefratelli, Roma); Alessandra Vernaglia Lombardi, Carmine Malzoni, Mario Malzoni, Giuseppina Farnetano (Casa di Cura Malzoni Villa dei Platani, Avellino); Antonio Febbraro, Claudia Corbo, Ilaria Spagnoletti (Ospedale Fatebenefratelli, Benevento); Giuseppe Scibilia, Gabriella D'Agate (Ospedale Cannizzaro**,** Catania); Mattia Barbareschi, Enzo Galligioni, Antonella Ferro, Viviana Murgia, (Ospedale S. Chiara, Trento); Stefano Tamberi, Laura Amaducci, Carmelo Bucolo, (Ospedale Civile, Faenza); Donato Natale, Bruna Fornarini, Dante Orlando (Osp. S. Massimo**,** Penne [PE]); Fabrizio Artioli, Laura Scaltriti, Lorenzo Aguzzoli (Ospedale Ramazzini, Carpi [MO]); Cesare Gridelli, Filomena Del Gaizo (Ospedale S.Giuseppe Moscati, Avellino); Rossella Lauria, Valeria Forestieri (Università Federico II, Napoli); Ilaria Franceschetti, Rocco De Vivo, (Ospedale S. Bortolo ULSS 6, Vicenza); Giovanni Lo Re (Ospedale S.Maria degli Angeli, Pordenone); Vito Lorusso (Ospedale Vito Fazzi, Lecce).

miRNA expression profiling from formalin-fixed paraffin embedded (FFPE) and fresh frozen samples and quality controls

RNA from the OC179 FFPE case material, derived from MITO2 trial [1], was extracted from three 20-µm-thick FFPE sections using the miRNeasy FFPE kit (Qiagen, Valencia, CA, USA). RNA concentration was determined using the ND-1000 spectrophotometer (Agilent Technologies, Palo Alto, CA USA). A quality check was carried out using RT-qPCR and Agilent 2100 Bioanalyzer Small RNA assay (Agilent Technologies). Briefly, RNA quality was evaluated by RT-qPCR amplification of two amplicons of different length for three housekeeping genes (GAPDH, RPL13a, ACTB) according to Ravo et al. [2]. Samples with short/long amplicon ratio lower than 5 were considered of acceptable quality for microarray analysis. In addition, 3 miRNAs (hsa-miR-16, hsa-miR-21, hsa-miR-451) expected to be highly expressed by literature data, were detected by RT-qPCR and samples with Ct values lower than 28 cycles were considered of acceptable quality for microarray analysis. miRNA expression profiling was performed using custom Agilent Sureprint G3 8×60K microarrays designed on miRBase 17.0 and identifying 1520 miRNAs. 100 ng of total RNA were labelled and hybridised following the recommended Agilent's protocols. Microarray slides were then washed and scanned with a DNA microarray scanner (Agilent Technologies). Raw data were generated using the Feature Extraction Software v10.7.3.1 (Agilent Technologies) and data normalisation and filtering procedures were performed by means of the AgiMicroRna R package [3]. The robust multiarray average algorithm (RMA) was used to summarise the results. Data were log2-transformed and normalised, using the quantile algorithm; eventually, miRNAs flagged as absent were removed.

Fresh frozen tumour specimens from the OC133 case material included in the OC263 series were mechanically disrupted and homogenised in the presence of QIAzol Lysis reagent (Qiagen) using a Retsch MM200 dismembrator (Sigma-Aldrich, St Louis, MO, USA). RNA was extracted using the miRNeasy Mini kit (Qiagen) according to the manufacturer's instructions. RNA concentration and quality were assessed using the NanoDrop ND-100 Spectrophotometer and the Agilent 2100 Bioanalyzer using the RNA 6000 Nano kit (Agilent Technologies), respectively. Samples included in the study had a RIN (RNA Integrity Number) score >6. miRNA expression profiling was performed using the Illumina Human_v2 MicroRNA expression profiling kit, based on the DASL (cDNA-mediated Annealing, Selection, Extension, and Ligation) assay, according to the manufacturer's instructions (Illumina Inc., San Diego, CA, USA). Briefly, 300 ng/sample total RNA was converted to cDNA followed by annealing of a miRNA-specific oligonucleotide pool consisting of: (i) a universal PCR priming site at the 5′ end; (ii) an address sequence complementary to a capture sequence on the BeadArray; and (iii) a miRNA-specific sequence at the 3′ end. After PCR amplification and fluorescent labelling, probes were hybridised on Illumina miRNA BeadChips, washed, and fluorescent signals were detected by the Illumina BeadArrayTM Reader. Data were collected using BeadStudio V3.0 software and raw data were quantile normalised using GenomeStudio v2011.1 and Illumina Expression module v19.0 (Illumina).

Concerning miRNA profiling, the TCGA used only frozen tissue while we used both frozen as well as FFPE samples. However, as previously reported in Bagnoli et al. [4] for the dataset OC130 (included in validation set 1 OC263), data obtained on frozen samples could be highly reproduced in FFPE samples and vice versa.

Our previous work [5] clearly demonstrated the inter-platform reproducibility of Agilent and Illumina platform. Since the microarrays are designed on different miRBase versions, instead of developing multiple signature (one for each dataset) containing different list of miRNAs that cannot be confirmed because of their absence in the validation sets, our strategy was to build a single model that could be validated. The number of miRNAs shared in all platforms that entered the process of model development was 385.The complete list is reported in Supplementary Table 2.

Assessment of miROvaR performance.

MiROvaR performance was evaluated by ROC curves, following the guidelines established by Steyerberget al. [6] Prediction accuracy was evaluated at maximum time point of follow-up using time dependent ROC curves, computed using SurvJamda R package [7] in training and validation sets. A non-parametric estimator for censored survival data, based on nearest neighbor bivariate distribution developed by Heagerty et al. [8] was applied to estimate time-dependent sensitivity and 1-specificity. The accuracy of the prediction was plotted as the mean and standard deviation following a 10-time cross-validation procedure (see Supplementary Figure 3A) We then decided to analyze the performance of our predictor in the sub-set of high-grade serous cases present in the OC263 validation set for a more direct comparison with the second validation set OC452 derived from TCGA data. The analysis of Type II sub-set was done taking into consideration the new proposed classification of ovarian cancer that, besides HGSOC, includes in this sub-set also endometroid high grade, undifferentiated ovarian cancer and Malignant mixed mullerian tumor. The performance of MiROvaR in separating patients included in these two sub-groups of OC263 is reported in Supplementary Figure 3B and is definitely higher that the performance on TCGA data-set.

Definition of clinical end-points and statistical analysis.

PFS was chosen as primary end-point. The identification of molecular classifiers like MiROvaR is based on an a priori choice of the outcome of interest. In our case, since the main goal of our predictor was to identify early relapsing patients, we reasoned that PFS would be the more appropriated end point. PFS is widely accepted as a reasonable end-point in ovarian cancer [9], both clinically and in terms of new drug development, particularly in the first-line of treatment

due to the fact that post-progression survival may be quite long and affected also by different and heterogeneous second-line treatments diluting the differences eventually seen in PFS. Since the training set OC179 derived from the MITO2 clinical trial (see supplementary Figure 1), we necessary used in this analysis the same definitions of PFS used in the MITO2 clinical trial [1]. Therefore, PFS is defined starting from the date of randomization and this is an out-of-discussion rule in randomized trials and within the intention-to-treat analysis. Date of surgery in OC179, could not be used as a starting point for several reasons: for many patients there would be a window time between surgery and informed consent; for some patients surgery might have been diagnostic only and certainty and quality of data might not be warranted because pertaining to procedures out of the study. As regard with median follow-up and data maturity, they are very high in the MITO2 trial, from which OC179 training set has been derived, and this is typical of prospective clinical trials. Therefore, we don't believe that further updates are possible within this data set, also because increasing the follow-up there will be a growing effect of competitive non-cancer-related death. The same applies for the two validations sets (OC263 and OC452). Since no other public datasets with fully annotated clinical data are available, to further validate MiROvaR we are already working on subsequent MITO trials where tumor collection has become mandatory to possibly avoid attrition bias (MITO 16 program NCT01706120 and NCT01802749).

We used multivariable analysis by Cox regression model to evaluate the independent prognostic impact of MiROvaR. Among the variables with known prognostic value we selected those considered as the stronger ones in terms of PFS prediction, i.e. FIGO stage and residual disease after primary surgery and the impact of the latter was independent of the categories use: SOD vs. OD (see Table 3) or NED vs. mRD vs. GRD (Supplementary Table 3).

MiROvaR confirmed its ability in classifying high vs. low risk patients both in training and in the two validation sets also when overall survival was considered as an alternative endpoint (see Supplementary Figure 4 and Supplementary Table 4)

Distribution of MiROvaR high- and low-risk patients according to clinical and pathological characteristics is reported in Supplementary Table 5.

Supplementary Table 1
Characteristics of the 179 MITO2 patients evaluable for miRNA expression compared with the whole MITO2 population

	OC179		Overall population	
	N° (179)	**%**	**N° (820)**	**%**
Age				
Median (range)	59	(28-78)	57	(21-77)
Histology				
Serous	124	69	530	65
Endometrioid	24	13	98	12
Clear cell	6	3	27	3
Mucinous	0	0	25	3
Undifferentiated	10	6	60	7
Mixed or other	13	7	44	5
Missing information	2	1	36	4
Stage (FIGO)				
Ic	17	10	74	9
II	15	8	79	10
III	123	69	493	60
IV	24	13	174	21
Grade				
G1	5	3	32	4
G2	27	15	137	17
G3	126	70	453	55
Undifferentiated	10	6	60	7
Missing information	11	6	138	17
Amount of residual disease				
None	73	41	298	36
< 1 cm	42	23	149	18
> 1 cm	53	30	227	28
Not operated	11	6	146	18
Treatment assigned				
Carboplatin – paclitaxel	78	44	410	50
Carboplatin – Caelyx	101	56	410	50

Supplementary Table 2: List of miRNA shared by the three platforms used for profiling the three case materials

Mature miRNA name	Accession	Mature miRNA name	Accession	Mature miRNA name	Accession	Mature miRNA name	Accession	Mature miRNA name	Accession
hsa-let-7a-3p	MIMAT0004481	hsa-miR-151a-3p	MIMAT0000757	hsa-miR-223-5p	MIMAT0004570	hsa-miR-365a-3p	MIMAT0000710	hsa-miR-542-3p	MIMAT0003389
hsa-let-7a-5p	MIMAT0000062	hsa-miR-151a-5p	MIMAT0004697	hsa-miR-22-3p	MIMAT0000077	hsa-miR-369-3p	MIMAT0000721	hsa-miR-542-5p	MIMAT0003340
hsa-let-7b-5p	MIMAT0000063	hsa-miR-152-3p	MIMAT0000438	hsa-miR-224-5p	MIMAT0000281	hsa-miR-369-5p	MIMAT0001621	hsa-miR-543	MIMAT0004954
hsa-let-7c-5p	MIMAT0000064	hsa-miR-154-3p	MIMAT0000453	hsa-miR-22-5p	MIMAT0004495	hsa-miR-370-3p	MIMAT0000722	hsa-miR-548c-5p	MIMAT0004806
hsa-let-7d-3p	MIMAT0004484	hsa-miR-154-5p	MIMAT0000452	hsa-miR-23a-3p	MIMAT0000078	hsa-miR-371a-5p	MIMAT0004687	hsa-miR-548d-5p	MIMAT0004812
hsa-let-7d-5p	MIMAT0000065	hsa-miR-155-5p	MIMAT0000646	hsa-miR-23a-5p	MIMAT0004496	hsa-miR-374a-5p	MIMAT0000727	hsa-miR-550a-3p	MIMAT0003257
hsa-let-7e-5p	MIMAT0000066	hsa-miR-15a-5p	MIMAT0000068	hsa-miR-23b-3p	MIMAT0000418	hsa-miR-374b-5p	MIMAT0004955	hsa-miR-564	MIMAT0003228
hsa-let-7f-1-3p	MIMAT0004486	hsa-miR-15b-3p	MIMAT0004586	hsa-miR-23b-5p	MIMAT0004587	hsa-miR-375	MIMAT0000728	hsa-miR-566	MIMAT0003230
hsa-let-7f-5p	MIMAT0000067	hsa-miR-15b-5p	MIMAT0000417	hsa-miR-24-1-5p	MIMAT0000079	hsa-miR-376a-3p	MIMAT0000729	hsa-miR-570-3p	MIMAT0003235
hsa-let-7g-5p	MIMAT0000414	hsa-miR-16-2-3p	MIMAT0004518	hsa-miR-24-2-5p	MIMAT0004497	hsa-miR-376a-5p	MIMAT0003386	hsa-miR-571	MIMAT0003236
hsa-let-7i-3p	MIMAT0004585	hsa-miR-16-5p	MIMAT0000069	hsa-miR-24-3p	MIMAT0000080	hsa-miR-376b-3p	MIMAT0002172	hsa-miR-574-3p	MIMAT0003239
hsa-let-7i-5p	MIMAT0000415	hsa-miR-17-3p	MIMAT0000071	hsa-miR-25-3p	MIMAT0000081	hsa-miR-376c-3p	MIMAT0000720	hsa-miR-574-5p	MIMAT0004795
hsa-miR-100-3p	MIMAT0004512	hsa-miR-181a-2-3p	MIMAT0004558	hsa-miR-26a-5p	MIMAT0000082	hsa-miR-377-3p	MIMAT0000730	hsa-miR-582-5p	MIMAT0003247
hsa-miR-100-5p	MIMAT0000098	hsa-miR-181a-3p	MIMAT0000270	hsa-miR-26b-3p	MIMAT0004500	hsa-miR-378a-5p	MIMAT0000731	hsa-miR-583	MIMAT0003248
hsa-miR-101-3p	MIMAT0000099	hsa-miR-181a-5p	MIMAT0000256	hsa-miR-26b-5p	MIMAT0000083	hsa-miR-379-5p	MIMAT0000733	hsa-miR-584-5p	MIMAT0003249
hsa-miR-103a-3p	MIMAT0000101	hsa-miR-181c-3p	MIMAT0004559	hsa-miR-27a-3p	MIMAT0000084	hsa-miR-381-3p	MIMAT0000736	hsa-miR-585-3p	MIMAT0003250
hsa-miR-105-5p	MIMAT0000102	hsa-miR-181c-5p	MIMAT0000258	hsa-miR-27b-3p	MIMAT0000419	hsa-miR-382-5p	MIMAT0000737	hsa-miR-592	MIMAT0003260
hsa-miR-106b-3p	MIMAT0004672	hsa-miR-181d-5p	MIMAT0002821	hsa-miR-27b-5p	MIMAT0004588	hsa-miR-409-3p	MIMAT0001639	hsa-miR-596	MIMAT0003264
hsa-miR-106b-5p	MIMAT0000680	hsa-miR-182-3p	MIMAT0000260	hsa-miR-28-3p	MIMAT0004502	hsa-miR-409-5p	MIMAT0001638	hsa-miR-598-3p	MIMAT0003266
hsa-miR-107	MIMAT0000104	hsa-miR-183-3p	MIMAT0004560	hsa-miR-28-5p	MIMAT0000085	hsa-miR-410-3p	MIMAT0002171	hsa-miR-610	MIMAT0003278
hsa-miR-10a-3p	MIMAT0004555	hsa-miR-183-5p	MIMAT0000261	hsa-miR-296-3p	MIMAT0004679	hsa-miR-411-3p	MIMAT0004813	hsa-miR-614	MIMAT0003282
hsa-miR-10a-5p	MIMAT0000253	hsa-miR-185-5p	MIMAT0000455	hsa-miR-296-5p	MIMAT0000690	hsa-miR-411-5p	MIMAT0003329	hsa-miR-615-3p	MIMAT0003283
hsa-miR-10b-3p	MIMAT0004556	hsa-miR-186-3p	MIMAT0004612	hsa-miR-298	MIMAT0004901	hsa-miR-421	MIMAT0003339	hsa-miR-622	MIMAT0003291
hsa-miR-10b-5p	MIMAT0000254	hsa-miR-186-5p	MIMAT0000456	hsa-miR-299-3p	MIMAT0000687	hsa-miR-423-3p	MIMAT0001340	hsa-miR-624-5p	MIMAT0003293
hsa-miR-1225-3p	MIMAT0005573	hsa-miR-188-5p	MIMAT0000457	hsa-miR-299-5p	MIMAT0002890	hsa-miR-423-5p	MIMAT0004748	hsa-miR-625-3p	MIMAT0004808
hsa-miR-1225-5p	MIMAT0005572	hsa-miR-18a-3p	MIMAT0002891	hsa-miR-29a-3p	MIMAT0000086	hsa-miR-424-3p	MIMAT0004749	hsa-miR-625-5p	MIMAT0003294
hsa-miR-122-5p	MIMAT0000421	hsa-miR-18a-5p	MIMAT0000072	hsa-miR-29a-5p	MIMAT0004503	hsa-miR-424-5p	MIMAT0001341	hsa-miR-627-5p	MIMAT0003296
hsa-miR-1226-5p	MIMAT0005576	hsa-miR-18b-5p	MIMAT0001412	hsa-miR-29b-1-5p	MIMAT0004514	hsa-miR-425-3p	MIMAT0001343	hsa-miR-628-3p	MIMAT0003297
hsa-miR-1228-3p	MIMAT0005583	hsa-miR-190a-5p	MIMAT0000458	hsa-miR-29b-3p	MIMAT0000100	hsa-miR-429	MIMAT0001536	hsa-miR-628-5p	MIMAT0004809
hsa-miR-1228-5p	MIMAT0005582	hsa-miR-191-3p	MIMAT0001618	hsa-miR-29c-3p	MIMAT0000681	hsa-miR-431-5p	MIMAT0001625	hsa-miR-629-3p	MIMAT0003298
hsa-miR-1229-3p	MIMAT0005584	hsa-miR-192-5p	MIMAT0000222	hsa-miR-29c-5p	MIMAT0004673	hsa-miR-432-5p	MIMAT0002814	hsa-miR-632	MIMAT0003302
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hsa-miR-129-1-3p	MIMAT0004548	hsa-miR-198	MIMAT0000228	hsa-miR-30d-5p	MIMAT0000245	hsa-miR-485-3p	MIMAT0002176	hsa-miR-708-5p	MIMAT0004926
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hsa-miR-139-3p	MIMAT0004552	hsa-miR-206	MIMAT0000462	hsa-miR-335-3p	MIMAT0004703	hsa-miR-503-5p	MIMAT0002874	hsa-miR-887-3p	MIMAT0004951
hsa-miR-139-5p	MIMAT0000250	hsa-miR-20a-3p	MIMAT0004493	hsa-miR-335-5p	MIMAT0000765	hsa-miR-505-3p	MIMAT0002876	hsa-miR-889-3p	MIMAT0004921
hsa-miR-1-3p	MIMAT0000416	hsa-miR-20a-5p	MIMAT0000075	hsa-miR-337-3p	MIMAT0000754	hsa-miR-505-5p	MIMAT0004776	hsa-miR-890	MIMAT0004912
hsa-miR-140-3p	MIMAT0004597	hsa-miR-20b-5p	MIMAT0001413	hsa-miR-338-3p	MIMAT0000763	hsa-miR-506-3p	MIMAT0002878	hsa-miR-92a-1-5p	MIMAT0004507
hsa-miR-140-5p	MIMAT0000431	hsa-miR-210-3p	MIMAT0000267	hsa-miR-339-3p	MIMAT0004702	hsa-miR-507	MIMAT0002879	hsa-miR-92a-3p	MIMAT0000092
hsa-miR-141-3p	MIMAT0000432	hsa-miR-211-5p	MIMAT0000268	hsa-miR-33a-5p	MIMAT0000091	hsa-miR-508-3p	MIMAT0002880	hsa-miR-92b-3p	MIMAT0003218
hsa-miR-142-3p	MIMAT0000434	hsa-miR-212-3p	MIMAT0000269	hsa-miR-340-3p	MIMAT0000750	hsa-miR-509-3p	MIMAT0002881	hsa-miR-92b-5p	MIMAT0004792
hsa-miR-142-5p	MIMAT0000433	hsa-miR-21-3p	MIMAT0004494	hsa-miR-342-3p	MIMAT0000753	hsa-miR-509-5p	MIMAT0004779	hsa-miR-93-3p	MIMAT0004509
hsa-miR-143-3p	MIMAT0000435	hsa-miR-214-3p	MIMAT0000271	hsa-miR-342-5p	MIMAT0004694	hsa-miR-513a-5p	MIMAT0002877	hsa-miR-93-5p	MIMAT0000093
hsa-miR-143-5p	MIMAT0004599	hsa-miR-214-5p	MIMAT0004564	hsa-miR-345-5p	MIMAT0000772	hsa-miR-513b-5p	MIMAT0005788	hsa-miR-939-5p	MIMAT0004982
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hsa-miR-148b-3p	MIMAT0000759	hsa-miR-221-3p	MIMAT0000278	hsa-miR-361-5p	MIMAT0000703	hsa-miR-525-5p	MIMAT0002838	hsa-miR-99a-3p	MIMAT0004511
hsa-miR-149-5p	MIMAT0000450	hsa-miR-221-5p	MIMAT0004568	hsa-miR-362-3p	MIMAT0004683	hsa-miR-532-3p	MIMAT0004780	hsa-miR-99a-5p	MIMAT0000097
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hsa-miR-150-5p	MIMAT0000451	hsa-miR-223-3p	MIMAT0000280	hsa-miR-363-3p	MIMAT0000707	hsa-miR-539-5p	MIMAT0003163	hsa-miR-99b-5p	MIMAT0000689

Supplementary Table 3. Multivariable analysis (Cox regression) of progression-free survival for clinical and biological variables in the test set (OC179) and validation sets (OC263 and OC452): three categorizations for residual disease.

Datasets	Variables	HR	95% CI	P value
OC179 (n=179, events=124)	***Stage***			
	III–IV *vs* I–II	2·60	1·23–5·49	*0·012*
	Surgical debulking			
	mRD *vs* NED	2·35	1·43–3·87	*<0·0001*
	GRD vs NED	2·19	1·37-3·49	*0·0011*
	miRNA predictor			
	High *vs* Low risk	1·85	1·29–2·64	***<0·0001***
OC263 (n=262, events=194)	***Stage***			
	III–IV *vs* I–II	1·76	0·95–3·28	*0·072*
	Surgical debulking			
	mRD *vs* NED	1·57	1·03–2·40	*0·035*
	GRD vs NED	2·06	1·35-3·13	*0.00079*
	miRNA predictor			
	High *vs* Low risk	2·96	2·14–4·10	***<0·0001***
OC452 (n=409, events=300)	***Stage***			
	III–IV *vs* I–II	1·50	0·86–2·61	*0·14*
	Surgical debulking			
	mRD *vs* NED	1·70	1·23–2·35	*0·0012*
	GRD vs NED	1·87	1·31-2·67	*<0·00051*
	miRNA predictor			
	High *vs* Low risk	1·32	1·04–1·69	***0·022***

HR=hazard ratio; CI=confidence interval; mRD=minimal residual disease; NED= no evident residual disease; GRD= gross residual disease.

Supplementary Table 4
Univariate analysis (Cox regression) of overall survival for MiROvaR in the three dataset

		Univariate analysis		
		HR	**95% CI**	**P value**
OC179 **(n=179, events= 77)**	***miRNA predictor***			
	High *vs* Low risk	1·79	1·12–2·79	*0·015*
OC263 **(n=263, events= 105)**	***miRNA predictor***			
	High *vs* Low risk	3·24	2·13–4·93	*<0·0001*
TCGA **(n=452, events= 223)**	***miRNA predictor***			
	High *vs* Low risk	1·33	1·01–1·76	*0·046*

HR=hazard ratio; CI=confidence interval;

Supplementary Table 5
Test for interaction miRNA-predictor-treatment in OC179 cohort

		HR	**95% CI**	**P value**
OC179 **(n=179, events=124)**	***miRNA predictor***			
	High *vs* Low risk	1.66	0.97-2.86	*0.06*
	treatment			
	Platinum-Caelyx vs Platinum-Taxane	0.91	0.52-1.58	*0.73*
	miRNA H : Platinum-Caelyx	1.2	*0.58-2.48*	*0.62*

Supplementary Table 6
Distribution of MiROvaR high- and low-risk patients in relation to clinical and pathological variables

	OC179 (N 179)					*OC263 (N 263)*					*TCGA (N 452)*				
	Low risk		**High risk**		**P value**	**Low risk**		**High risk**		**P value**	**Low risk**		**High risk**		**P value**
	N	**%**	**N**	**%**		**N**	**%**	**N**	**%**		**N**	**%**	**N**	**%**	
Stage (FIGO)															
I/II	23	72	9	28		15	60	10	40		12	44	15	56	0·54
III/IV	67	46	80	54	**0·013**	107	45	131	55	0·19	157	37	267	63	
Missing information											0	0	1	100	
Amount of residual disease															
OD	64	56	51	44		90	56	71	44		122	39	188	61	0·32
SOD	26	41	38	59	0·055	32	32	69	68	**0·0005**	34	34	66	66	
Missing information						0	0	1	100		13	31	29	69	
Histology															
Serous	63	51	61	49		82	43	108	57		169	37	283	63	
Undifferentiated	5	50	5	50		10	43	13	57						
Clear cells	2	33	4	67	0·39	4	58	3	42	0·28					na
Endometroid	15	62	9	38		15	58	11	42						
Mucinous						0	0	1	100						
Others + Mixed	4	31	9	69		10	62	5	38						
Missing information	1	50	1	50		1	100	0	0						
Grade															
Borderline						2	67	1	33		1	100	0	0	
1: well differentiated	3	60	2	40		4	57	3	43		2	40	3	60	
2: moderately differentiated	15	56	12	44	0·93	30	59	21	41	0·25	25	46	30	54	0·46
3: poorly differentiated	62	49	64	51		75	42	102	58		138	36	244	64	
Undifferentiated	5	50	5	50		10	43	13	57						
GX											3	37	5	63	
Missing information	5	45	6	55							0	0	1	100	

N= number; na=not applicable; OD= optimal debulking; SOD =sub-optimal debulking.

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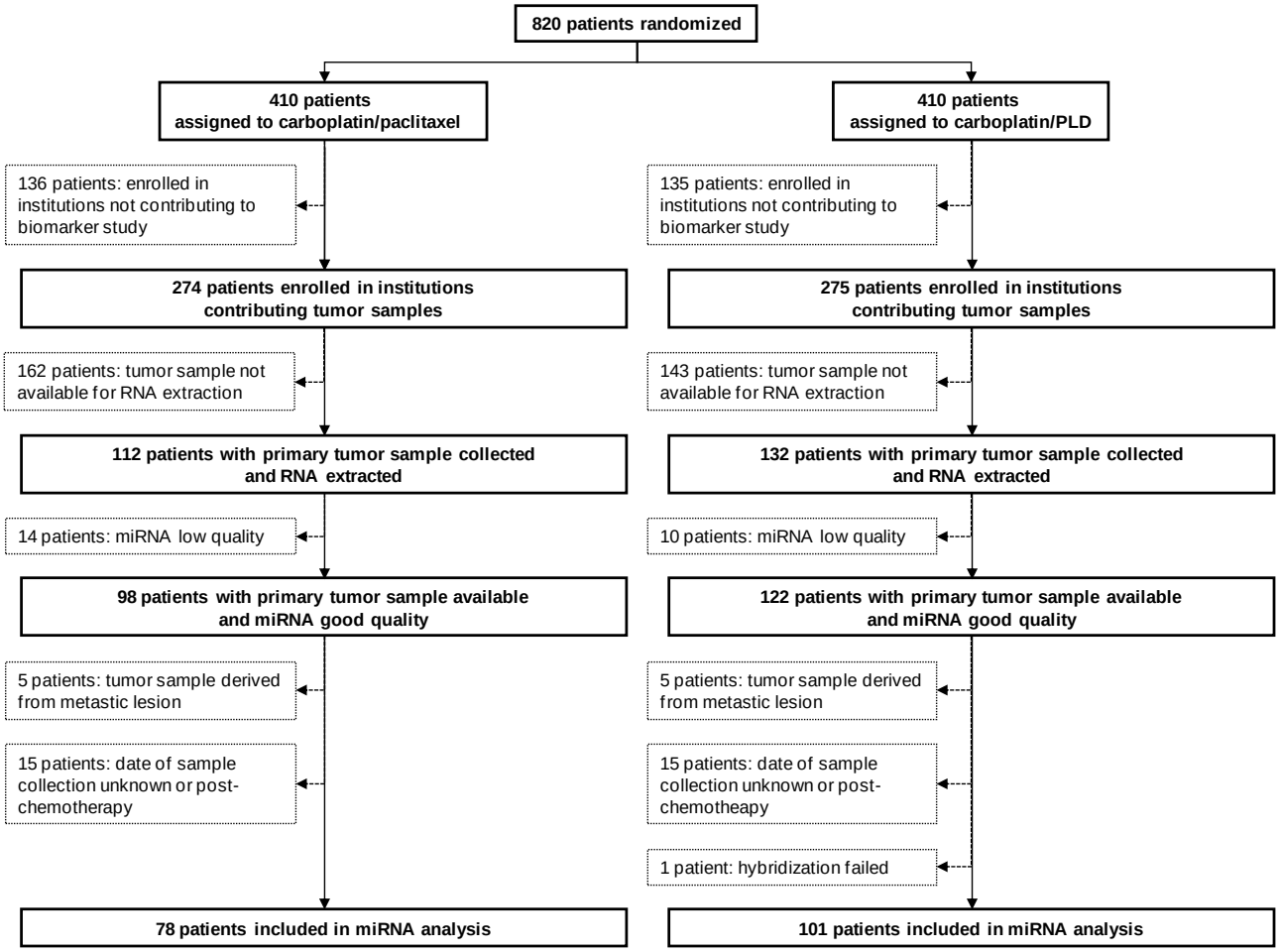
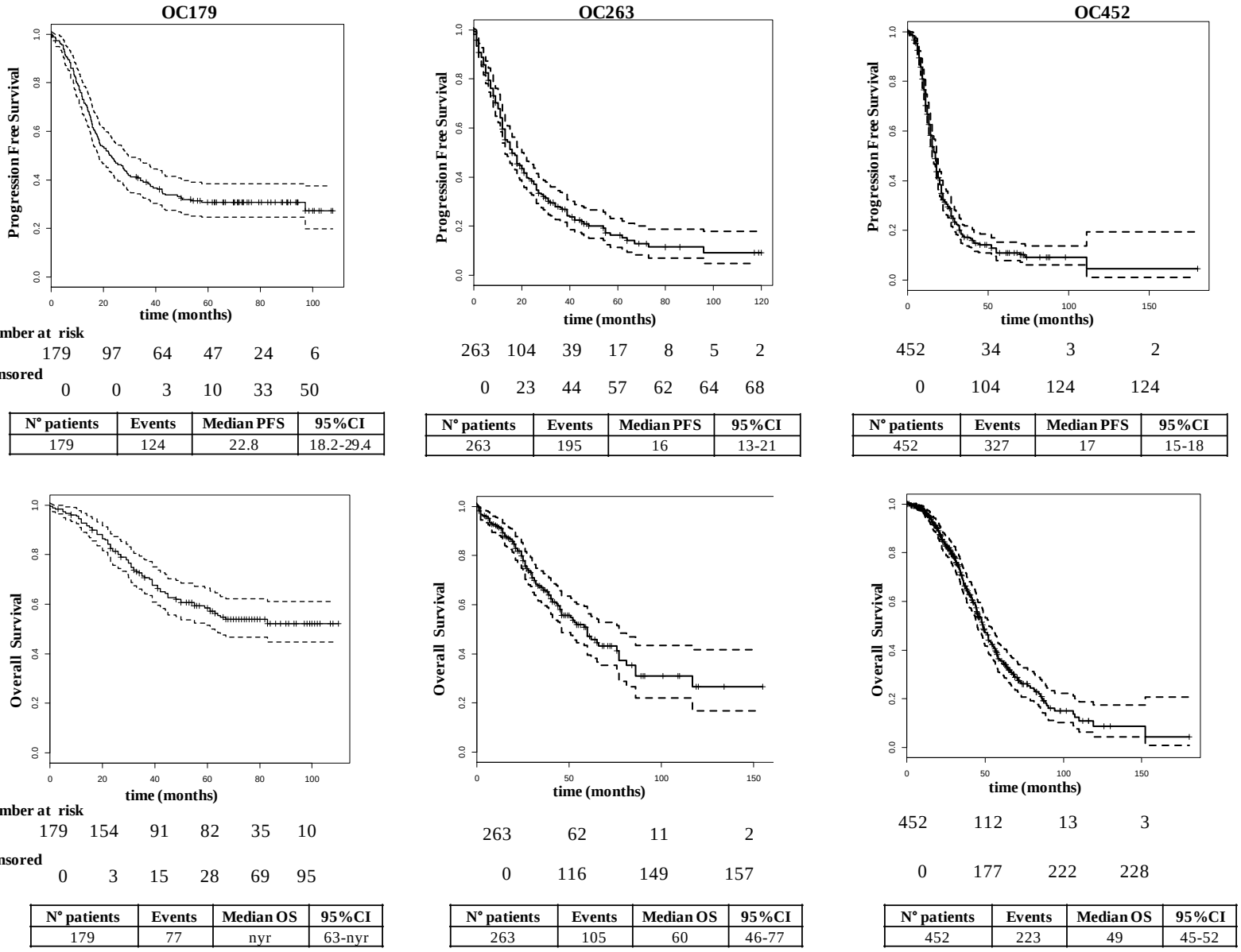


Supplementary Figure 1. Consort diagram for inclusion criteria of OC179 case material used as a training set and derived from samples collected for translational research purposes from the MITO2 clinical trial [1].



N° patients	Events	Median PFS	95%CI
179	124	22.8	18.2-29.4

N° patients	Events	Median PFS	95%CI
263	195	16	13-21

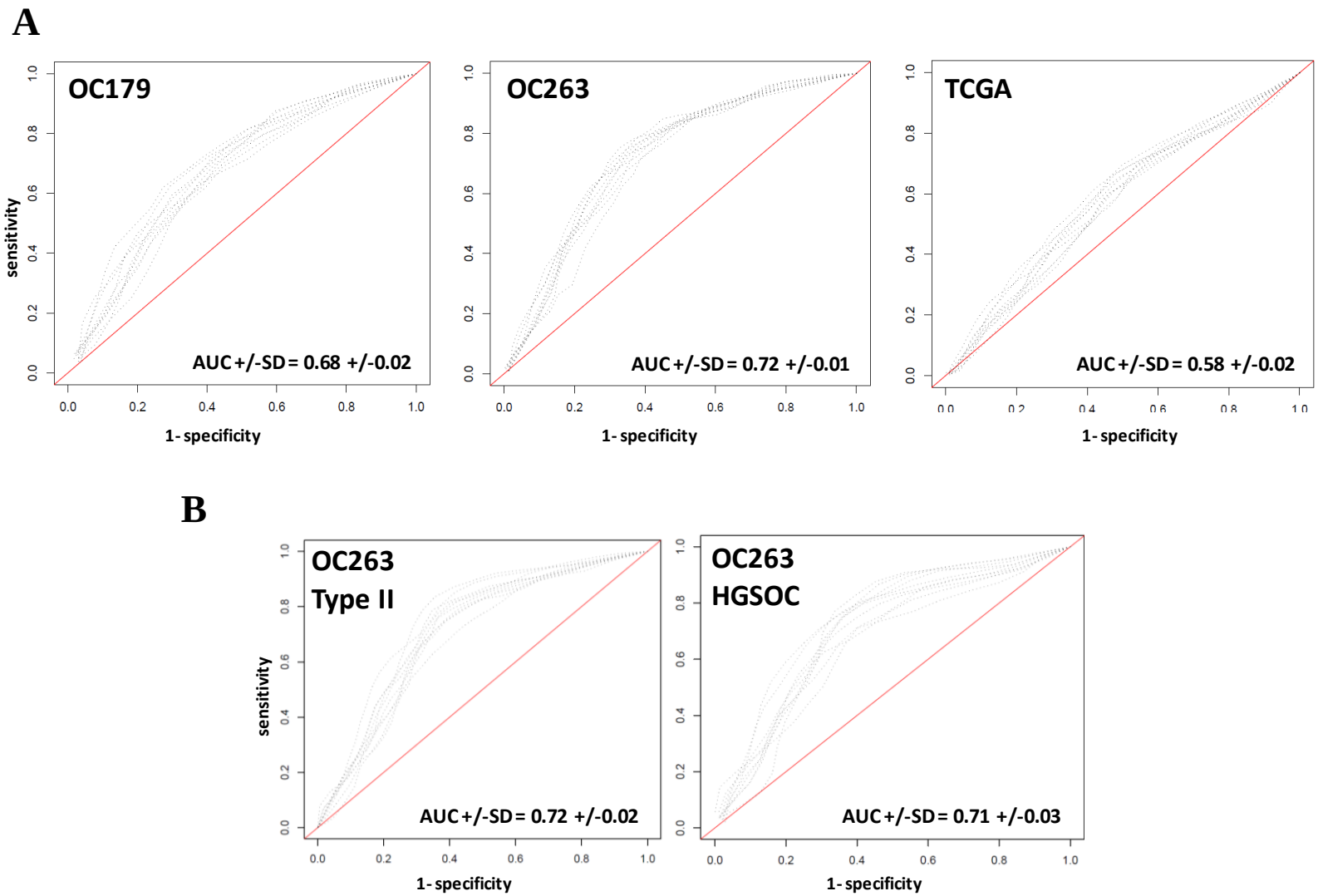
N° patients	Events	Median PFS	95%CI
452	327	17	15-18

N° patients	Events	Median OS	95%CI
179	77	nyr	63-nyr

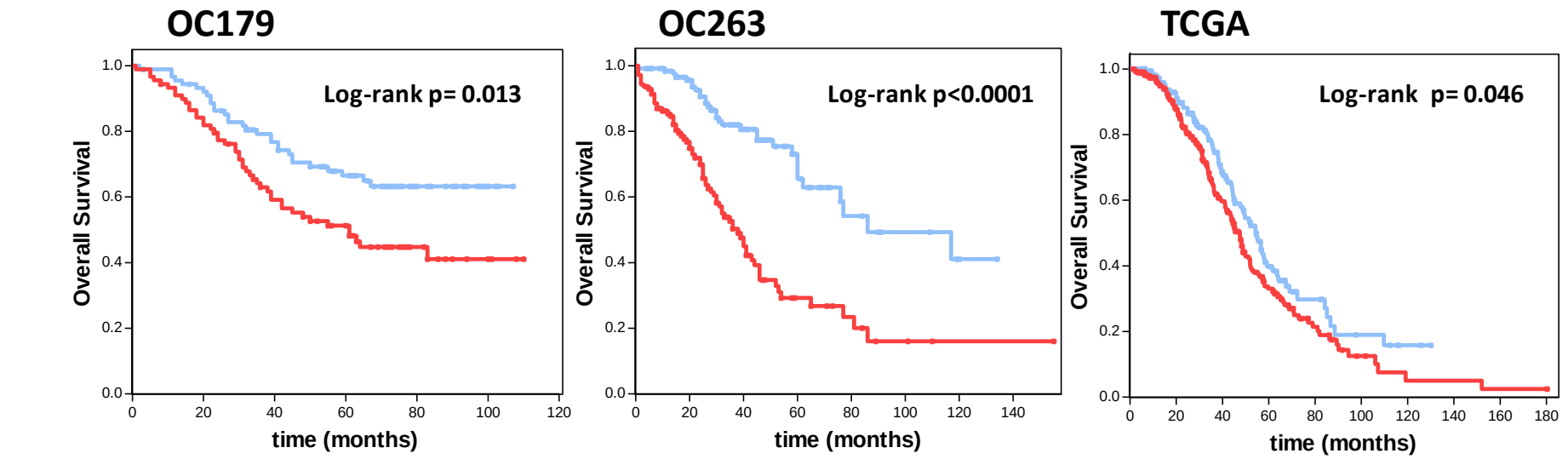
N° patients	Events	Median OS	95%CI
263	105	60	46-77

N° patients	Events	Median OS	95%CI
452	223	49	45-52

Supplementary Figure 2. Progression Free Survival (upper panels) and overall survival (lower panels) curves for the three case materials included in the study: OC179, training set (left panels); OC263, validation set 1 (middle panels); OC452, validation set 2 (right panels). Solid lines=survival curves; dotted lines=95% CI.



Supplementary Figure 3. A: Performance of miROvaR to detect high risk patients in all populations. ROC curves from training set (OC179), validation set1 (OC263) and validation set2 (TCGA) are reported. **B:** Performance of miROvaR in Type II and High Grade Serous (HGSOC) subgroup of OC263 validation set1 population. Accuracy of prediction was tested in 230 Type II and 185 HGSOC cases from OC263 validation set. The accuracy of the prediction (AUC) was plotted as the mean and standard deviation (SD) following a 10-time cross validation procedure.



Risk prediction	N° patients	Events	Median OS	95%CI
Low	90	31	nyr	nyr-nyr
High	89	46	39	39-nyr

Risk prediction	N° patients	Events	Median OS	95%CI
Low	122	32	86	66-nyr
High	141	73	38	30-46

Risk prediction	N° patients	Events	Median OS	95%CI
Low	169	73	54.3	48-62
High	283	150	47.4	42-51

Supplementary Figure 4. Overall survival curves of patients stratified according to the miRNA predictor in the three dataset included in the study: OC179, training set (left panels); OC263, validation set 1 (middle panels); OC452, validation set 2 (right panels). High-risk (red line) and low-risk (blue line) curves were compared using a log-rank test. Nyr = not yet reached