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A validated microRNA profile with predictive potential in glioblastoma patients treated with bevacizumab

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Abstract

Purpose: We investigated whether microRNA expression data from glioblastoma could be used to produce a profile that defines a bevacizumab responsive group of patients.

Patients and Methods: TCGA microRNA expression data from tumors resected at first diagnosis of glioblastoma in patients treated with bevacizumab at any time during the course of their disease were randomly separated into training (*n*=50) and test (*n*=37) groups for model generation. MicroRNA-seq data for 51 patients whose treatment included bevacizumab in the BELOB trial were used as an independent validation cohort.

Results: Using penalized regression we identified 8 microRNAs as potential predictors of overall survival in the training set. We dichotomized the response score based on the most prognostic minimum of a density plot of the response scores (log-rank HR=0.16, $p=1.2e^{-5}$) and validated the profile in the test cohort (one-sided log-rank HR=0.34, p=0.026). Analysis of the profile using all samples in the TCGA glioblastoma dataset, regardless of treatment received, (*n*=473) showed that the prediction of patient benefit was not significant (HR=0.84, p=0.083) suggesting the profile is specific to bevacizumab. Further independent validation of our microRNA profile in RNA-seq data from patients treated with bevacizumab (alone or in combination with CCNU) at glioblastoma recurrence in the BELOB trial confirmed that our microRNA profile predicted patient benefit from bevacizumab (HR=0.59, p=0.043).

Conclusion: We have identified and validated an 8-microRNA profile that predicts overall survival in patients with glioblastoma treated with

bevacizumab. This may be useful for identifying patients who are likely to benefit from this agent.

Keywords: microRNA, glioblastoma, bevacizumab, glioma, prediction

1. Introduction

Bevacizumab (BEV) is an anti-angiogenic monoclonal antibody that acts by slowing the growth of new blood vessels in tumors through inhibition of VEGFA (vascular endothelial growth factor A) (Glade-Bender et al., 2003). In glioblastoma, two prospective, randomized, placebo controlled clinical trials, AVAglio and RTOG 0825 have been performed to assess whether BEV improves survival in patients with newly diagnosed glioblastoma (Chinot et al., 2014; Gilbert et al., 2014). Both studies reported improved progression-free survival (PFS) but no overall survival (OS) benefit. Despite these results there is anecdotal evidence, and some evidence from observation of tails of Kaplan Meier survival curves, that certain patients may benefit from BEV treatment. Identification of these patients is an unmet need (Field et al., 2014; Prados et al., 2015).

Prediction of benefit from BEV in glioblastoma patients has been attempted previously. Colman *et al* developed a prognostic signature for glioblastoma (all treatments) which was based on expression of genes associated with mesenchymal differentiation and angiogenesis (Colman et al., 2010). This was then assessed using tumor samples from the RTOG 0825 patients[3]. The results did not show the expected association between worse OS and PFS with the mesenchymal subtype.

A smaller phase II trial (the BELOB trial) in the Netherlands assessed BEV or CCNU mono-or combination therapy in recurrent glioblastoma, with a primary endpoint of OS (Taal et al., 2014). However, survival benefit in glioblastoma could not be confirmed in the phase III EORTC 26101 trial (Wick et al., 2015). When patients from the BELOB trial were assigned to molecular subtypes

results showed that the EGFR amplified, classical glioblastoma subtype responded well to the combination therapy and the mesenchymal subtype showed a poor response to combination therapy. It should be noted that these data included only 28 patients in the mesenchymal single agent BEV group, and they are not yet fully published (Eraslan et al., 2014). Overall, these, and other data may suggest that anti-angiogenic therapy resistance is associated with the mesenchymal transition, and that tumors with more infiltrative phenotypes are more resistant to these drugs (Piao et al., 2013; 2012). Retrospective analysis of the AVAglio trial showed patients with IDH wild-type proneural tumors had improved OS when treated with BEV first-line, and these are the most encouraging data linking tumor sub-type to outcome thus far (Omuro et al., 2014).

Further analysis of translational data from the AVAglio trial suggested that neither VEGFA or VEGFR2 (vascular endothelial growth factor receptor 2) are predictive or prognostic biomarkers in the context of BEV treatment, although a VEGFA SNP rs2010963 is associated with vascular toxicity (Di Stefano et al., 2014; Field et al., 2014). Plasma levels of MMP2 (matrix metalloproteinase 2) have been shown to be associated with response and survival in BEV-treated patients (single agent therapy) in a study by the Chinot lab (Tabouret et al., 2014).

MicroRNAs have not been studied as predictive indicators for BEV response to date. Their stability in clinical samples and role in glioma biology suggest they represent prime candidates for use in predictive signatures/profiles (Hall et al., 2012; Hayes et al., 2014).

In this study, we have attempted to identify a prognostic microRNA profile in BEV treated patients using OS as an endpoint. Our results show that an 8-microRNA profile can define patients treated with BEV who have a better prognosis.

2. Materials and methods

2.1. TCGA clinical information and expression data

Level 3 Agilent microRNA 8x15k microarray expression data plus clinical and treatment information for 563 glioblastoma samples, 90 of which were from patients treated with BEV used either as an adjuvant with first-line treatment, at progression or recurrence, were downloaded from TCGA (Cancer Genome Atlas Research Network, 2008). Patients had been treated using varying numbers of 2-3 week cycles of BEV therefore treatment time was determined as the date from the start of treatment to the date of the end of treatment. Samples were taken at diagnosis and OS was measured from the date of diagnosis, regardless of timing of BEV treatment (however timing of treatment was analyzed as a variable in a separate multivariable analysis). Three patients were removed due to lack of start date information, resulting in a total of 87 patients. These were randomly split into test and training set groups of 50 and 37 patients respectively (Table 1). These numbers were chosen to maximize power in generation of the model, whilst allowing a sufficient validation cohort for testing of the model.

2.2. Generation of a risk algorithm for OS in bevacizumab-treated glioblastoma patients using microRNAs

The training set samples were assessed using LASSO penalized regression (Tibshirani, 1996) with leave-one-out cross-validation using R software (v2.15.1) and the Penalized package (Goeman, 2010). This produced 8 microRNAs with non-zero coefficients.

A response score was generated using the sum of microRNA expression values weighted by the coefficients from the LASSO regression.

This was: E_miR-n = expression of microRNA n.

Response score = 0.055E_miR-124a + 0.309E_miR-202 + -0.184E_miR-204 + 0.170E_miR-222 + -0.194E_miR-363 + -0.025E_miR-630 + -0.322E_miR-663 + 0.161E_miR-7

The response score was applied to all samples in the training set. The most prognostic cut-off was chosen based on log-rank tests at each minimum value on a density plot. The rationale behind this is that biologically it is assumed that patients either show some benefit to the treatment or not. The training set samples were then separated into responders and non-responders using this cut-off. A Cox regression model incorporating age and the log-rank test were used to assess OS of the two groups in the training set. 200 permutations of a 50-patient training set from the 87 original patients were used as input to LASSO to determine model differences with different patients. The response score was also assessed as a predictor of PFS. A statistical significance threshold of p=0.05 was used throughout, with two-tailed log rank tests for the training set and one-tailed tests for all validations. The length of treatment time was tested for correlation with the survival time in both responder and

non-responder groups. Fisher's exact test was also performed on the responder groups for the molecular subtype, treatment regimen and histological features.

2.3. Validation of the response score in the test set

The response score was calculated with the above algorithm using the microRNA expression values for the 37 test set samples. The defined cut-off from the training set of a response score of 0 was used to separate the test set into two groups of responders and non-responders. A Cox regression model incorporating age and the log-rank test were used to assess OS of the responder groups. The length of treatment time was assessed for correlation with survival time in both responder and non-responder groups. Multivariate analysis of other prognostic indicators assessed in the trial was performed to determine whether the responder groups are independent prognosis predictors.

2.4. Testing of the algorithm across all treatment types

The response score was applied to all 473 patients in the TCGA (treated with various treatment regimens not including BEV) (Table S1). This cohort was split into two responder groups based on the response score cut-off of 0 and the two groups were assessed by Cox regression and log-rank test.

2.5. Validation of the profile using BELOB trial data

Patients were eligible for the BELOB trial if they were ≥18 years and had a first recurrence of glioblastoma after temozolomide and radiotherapy

treatment. Details of the study have been described previously (Taal et al., 2014). Total RNA extraction, purification, and quantification from formalin-fixed and formalin-fixed paraffin-embedded (FFPE) material were reported previously (Gravendeel et al., 2011). 500 ng RNA was used for sequencing on an Illumina TruSeq and ~35-40 million 40 base paired end-reads were generated per sample. RNA-seq (*n*=96) was run by Expression Analysis (Durham, NC). Gene expression levels (Ref-seq genes) were extracted from the RNA-seq data using featureCounts (Liao et al., 2014), after alignment on hg19 with Tophat2 (Trapnell et al., 2009) of clipped/trimmed reads as provided by the manufacturer. The response score was calculated using read per million counts and the cut-off value was defined by using the minimum value of a density plot of the response scores.

3. Results

3.1. An 8-microRNA profile generated from the training set predicts prognosis in bevacizumab treated patients

Using the LASSO method, 8 microRNAs were identified with non-zero regression coefficients in our training dataset of 50 glioblastomas. A response score was created using the algorithm stated in methods. The response score was then plotted as a density plot (Fig 1). The response score itself showed a normal distribution (Fig S1) and therefore the minimum values of a density plot at a bandwidth of 0.009 were used as a guide for determining a cut-off. The response score value at each minimum of the density plot was determined and a log-rank test was performed with this value as a cut-off to determine an optimal cut-off for dichotomization. The minimum density that

occurred around a response score of 0 showed the highest significance with the best hazard ratio, and so a response score of 0 was chosen for the cut-off (Fig 2A, Table S2). This cut-off is justified because the expression data are quantile normalized, and therefore a cut-off of 0 represents the expression of each of the microRNAs in the signature at median survival of all patients. The median survival time of the responder group defined in this way was 22 months and the median of the non-responder group was 12 months. A log rank test performed on 200 permutations of the 50-patient training set was significant for predicting altered survival in 100% of tests with $p<0.05$ and in 96.5% of tests with $p<0.005$.

Spearman's correlation of duration of BEV treatment with survival time showed that the responders showed a correlation (correlation coefficient=0.48, p=0.01) whereas the non-responders did not (correlation coefficient=0.36, p=0.12). Multivariable Cox regression of the responder group and age showed the responder group to be an independent predictor of survival irrespective of age (group HR=0.11, CI=0.04-0.29, $p=5.4e^{-6}$, age HR=1.03, CI=1.00-1.06 $p=3.3e^{-2}$).

3.2. Assessment of the profile in the test group of 37 patients

Response scores were calculated for the 37 test set patients using a cut-off of 0 to separate the patients into responder and non-responder groups. This produced a group of 18 responders, with a median survival 21 months and a group of 19 non-responders with a median survival of 15 months. A one-sided log rank test showed that the responders survived significantly longer than the non-responders (HR=0.34, CI=0.11-1.01, p=0.026, Fig. 2B). Multivariable

Cox regression with age confirmed that the responder group was a prognostic factor (HR=0.33, CI=0.11-0.99, p=0.049) independent of patient age.

3.3. Testing of the profile across all the glioblastoma patients in the TCGA database, independent of treatment

To test whether our profile is predictive of patient outcome in general or specific to BEV, we calculated the response score for all 473 glioblastoma patients in the TCGA database. These were treated with various drugs and regimes not including those treated with BEV (Table S1). This identified 256 patients in the responder group (median survival 9.25 months) and 217 in the non-responder group (median survival 7.55 months). A two-sided log rank test between the responder groups showed this profile is not prognostic for OS (HR=0.84, CI=0.68-1.02, p=0.083, Fig 3). This indicates the profile is predicting benefit from BEV specifically.

3.4. Characterization of the responder groups defined by the profile

We determined the proportions of each molecular glioblastoma subtype in the responder and non-responder groups of the combined test and training sets. Analysis using Fisher's exact test showed that there were significantly fewer mesenchymal type tumors in the responder group (p=0.041). The other subtypes did not show any significant difference between the responder and non-responder groups (classical glioblastoma p-value=0.15, neural glioblastoma p-value=0.56, proneural G-CIMP glioblastoma p-value=1.00, proneural non-G-CIMP glioblastoma p=1.00). Multivariable Cox regression using the microRNA profile and subtype (as the only variables in the model)

showed that the microRNA profile was independent of subtype using a two-tailed test in the training set (HR= 0.13, 95% CI=0.05-0.37, $p=1.1e^{-4}$) and using a one-tailed test in the test set (HR= 0.28, 95% CI=0.07-1.15, p=0.04). Previous data have suggested that molecular subtype may be predictive of benefit from BEV (Eraslan et al., 2014); however, the microRNA-based BEV response profile we have identified here has more predictive power than the molecular glioblastoma subtypes when directly tested.

The cohort of BEV treated patients includes different treatment start points during the course of a patient's disease. There was no significant difference in any treatment regime between the responder groups when tested with Fisher's exact test (adjuvant p-value=0.79, progression p-value= 1.00, recurrence p-value=0.45). Multivariable Cox regression using the microRNA profile and subtype showed that the microRNA profile was independent of treatment time using a two-tailed test in the training set (HR=0.12, 95% CI=0.04-0.32, $p=2.4e^{-5}$). A similar trend was observed in the test set (HR= 0.44, 95% CI=0.14-1.31, p=0.07) (although treatment time data were available for only 70% of patients in the test set cohort).

3.5. Ability of the profile to predict progression free survival

We tested whether the 8-microRNA profile predicts PFS using Cox regression in both the test and training sets. As the cut-off for PFS may be different than that for OS, the score was also assessed for ability to predict survival. The training set showed that decreasing response score predicts PFS (HR=0.37, 95% CI=1.42-5.03, p=0.0024) and the dichotomized responder groups have significantly different PFS by log-rank test (HR=0.48, 95% CI=0.25-0.93,

p=0.029). In the test set, decreasing response score predicted PFS (HR=0.44, 95% CI=1.02-5.04, p=0.045) but the dichotomized responder groups did not (HR=0.58, 95% CI=0.29-1.13, p=0.11). This may be because of the small size of the test set.

3.6. Validation of the profile using data from the BELOB trial

To independently validate the profile we calculated response scores for each of the patients in the BELOB trial using the microRNA algorithm. This was done using aligned reads from RNA-seq data and was performed blind, with no knowledge of clinical data. An identical cut-off to that in the training set was not possible, because this used microRNA reads from an RNA-seq dataset and could not be normalized in the same way as the microarray data from the TCGA. The response score was plotted on a density plot and the minimum value of this density plot used as a cut-off to generate two response groups (Fig 4A-B). One-tailed log-rank tests in all arms of the trial, and in just the arms that included bevacizumab as a treatment (either monotherapy or in combination with CCNU) are shown in Fig 4 and patient OS data from the BELOB trial are shown in Fig S2. In the BEV treated arms, the responder groups were significantly associated with survival (HR 0.59, 95% CI 0.32-1.09, p= 0.043) validating that the profile delineates patient groups with differing benefit from BEV. Log rank test of the predicted responders from the BEV arms with the predicted non-responders from the CCNU showed no significance (HR 1.12, 95% CI 0.51-2.49, p=0.36, Fig S3), which may suggest that the profile specifically defines a group of patients who do not benefit when treated with BEV. These results indicate that even the patient responder

group treated with BEV show no more improvement in survival than those from the non-responder group treated with CCNU.

Clinico-pathological markers including age, gender, MGMT methylation status, IDH1 mutation status, were assessed by univariate and multivariate analysis in the whole trial and this showed that the responder groups defined by the profile were independently associated with survival (Table 2).

4. Discussion

This study has identified an 8-microRNA profile that is associated with prognosis in BEV-treated glioma patients. The profile, with further validation, could be used as an indicator at diagnosis to determine whether a patient will benefit from BEV, at any time during their disease. This profile is preferable to previously published signatures/profiles because it uses only 8 predictors, which can easily be assayed in a clinical setting, using stable genetic markers (microRNAs) and has better prediction power than other factors such as MGMT promoter methylation status, IDH mutation and molecular subtype. Sandmann *et al* reported that proneural IDH- wild-type tumors might respond to first line BEV treatment (Sandmann et al., 2015). We did not find that there were more proneural non-G-CIMP patients in the responder group. This may be because this group is not highly represented in our data. There were fewer patients with tumors of mesenchymal molecular glioblastoma subtype in the responder category although 38% of mesenchymal tumors still stratified to the responder group, which indicates that the profile is not simply predicting a mesenchymal subtype.

If the microRNAs in the profile are associated with BEV response it is assumed they have a role in angiogenesis. The microRNAs that have a positive weight in the profile (miR-7, miR-124a, miR-202 and miR-222), and therefore are lower in responders, are likely to be anti-angiogenic. This is because responders should have more angiogenic tumors than non-responders. The converse is also likely to be true for the negatively weighted microRNAs (miR-204, miR-663, miR-630 and miR-363).

Of the eight microRNAs identified in the profile, seven are reported in the literature to be involved in angiogenesis. Of the positively-weighted microRNAs, miR-124a has been shown to transcriptionally decrease VEGF through RAS signaling (Shi et al., 2014) and miR-222 is considered one of three most important anti-angiogenic microRNAs in coronary artery disease (Zhang et al., 2011). Overexpression of miR-7 in a neuroblastoma mouse model significantly reduced angiogenesis and in endothelial cell lines miR-7 overexpression decreased tube formation and sprouting (Babae et al., 2014). Of the microRNAs that were negatively weighted, miR-363 and miR-663 are reported to improve endothelial cell angiogenesis and endothelial interaction with hematopoietic precursors(Costa et al., 2013) and miR-663 indirectly increases VEGF and promotes angiogenesis (Afonyushkin et al., 2012). The reports on these microRNAs are concordant with their effect in the profile. However, miR-204 and miR-630 show anti-angiogenic properties and are negatively weighted microRNAs; miR-204 directly decreases VEGF and also targets angiopoietin-1 (Kather et al., 2014; Zhao et al., 2014), and miR-630 has been shown to be induced by the anti-angiogenic protein angiopoietin-like protein 1 (Kuo et al., 2013). These unexplained functions of certain

microRNAs in the signature are likely reflective of the complex biology involved in the response of a patient and their tumor to BEV.

In summary, we have defined a promising approach to predicting response to BEV in GBM patients and further studies are warranted to test this profile further, in larger cohorts using clinically relevant assays.

Figures and tables

Table 1. Summary of data from the test and training set cohorts. Samples from TCGA were randomly split into training and test sets of 50 and 37 patients respectively. The test set has a marginally poorer prognosis and KPS and, on average, 28.5 days shorter treatment time for BEV. Additionally, 22% of test set patients were treated with BEV as an adjuvant treatment, whereas only 16% of patients in the training set were treated as an adjuvant treatment. Days to death are recorded where possible, and where the patient was living at the end of the data collection, days to last follow-up were used.

Figure 1. Density plot of the response scores to determine a cut-off for dichotomization. The response score, calculated according to the microRNA profile algorithm was illustrated as a density plot (bandwidth 0.009). This shows the fraction of patients with scores at each value on the X-axis and was used to determine whether a natural cut-off of the response score could be ascertained for dichotomization of the score into two groups of responders and non-responders. As multiple minima were identified from the density plot, the optimal minimum was determined by assessing prognostic ability. Each minimum value was used as a cut-off to define two ‘response’ groups. These

two groups were then assessed for differences in survival using the log rank test. The hazard ratio and p-value at each minimum are shown on the plot. The most significant association with survival occurred when a cut-off score of 0 was used which corresponds to the baseline hazard determined by the microRNAs.

Figure 2. MicroRNA expression and patient survival in the responder and non-responder groups in the test and training sets. The responder and non-responder groups were calculated using the microRNA profile and split into two responder groups using a cut-off score of 0. (A) A heat map and survival curve of microRNA expression of the 8 profile microRNAs and survival of the responder and non-responder groups in the training set. Negative microRNAs are those that are higher in samples from patients with poorer survival and conversely, positive microRNAs are those that are lower in patients with poorer survival. The accompanying survival curve confirms that patients who stratified to the responder group using the profile had a better outcome than those stratified to the non-responder group. (B) A heat map and survival curve of microRNA expression of the 8 profile microRNAs and survival of the responder and non-responder groups in the test set. A one-tailed log-rank test showed that patients stratified to the responder group in the test set had a better outcome than those in the non-responder group, as shown in the accompanying survival curve.

Figure 3. The survival of the profile-defined responder and non-responder groups when calculated for all glioblastoma patients in the

TCGA. In order to determine whether the profile-based definitions of responder and non-responder groups are specific to patients treated with BEV, the response score was calculated and a cut-off score of 0 was used to test all the patients in the TCGA, regardless of their treatment (not including those patients treated with BEV). The profile-defined responder groups were not associated with prognosis (HR BEV-treated training set = 0.16, HR bev-treated test set 0.34, HR all treatments = 0.84). This suggests that the profile predicts prognosis more strongly in BEV treated glioblastoma.

Figure 4. Survival curves for the profile-defined responder and non-responder groups in different arms of the BELOB trial. In order to validate the profile, the microRNA profile response score was calculated for patients in the BELOB trial who were treated with BEV (either as monotherapy or in combination with CCNU). (A) A density plot at bandwidth 0.009 of the response scores showed one minimum at a response score of 1.71 and this was used as a cut-off to dichotomize. The calculation was performed blind, with the investigator having no knowledge of the clinical details of the patients. (B) MicroRNA expression of the response groups in the validation group. Negative microRNAs are those that are higher in samples from patients with poorer survival and conversely, positive microRNAs are those that are lower in patients with poorer survival. MiR-202 sequences were not detected in this group but microRNA reads are of low abundance in RNA-seq data and miR-202 may therefore be expressed in these samples. (C) Survival of responder and non-responder groups in all treatment arms from the BELOB trial (*n*=73). (D) Survival of responder and non-responder groups in the arms that included

BEV as a treatment (monotherapy or in combination with CCNU, *n*=51). The responder groups from other arms in the trial have been included for clarity.

Table 2. Univariate and multivariate analysis of parameters from the validation group. Using univariate analysis, the microRNA profile, Karnofsky performance score (KPS), MGMT methylation (MGMT) were also associated with survival in the trial cohort. Four different multivariate analyses, using survival and one other variable stated in the table, showed that the microRNA profile and KPS were independently associated with survival (although only KPS was significant after multiple testing correction (p=0.042)). IDH1 refers to IDH1 mutation status.

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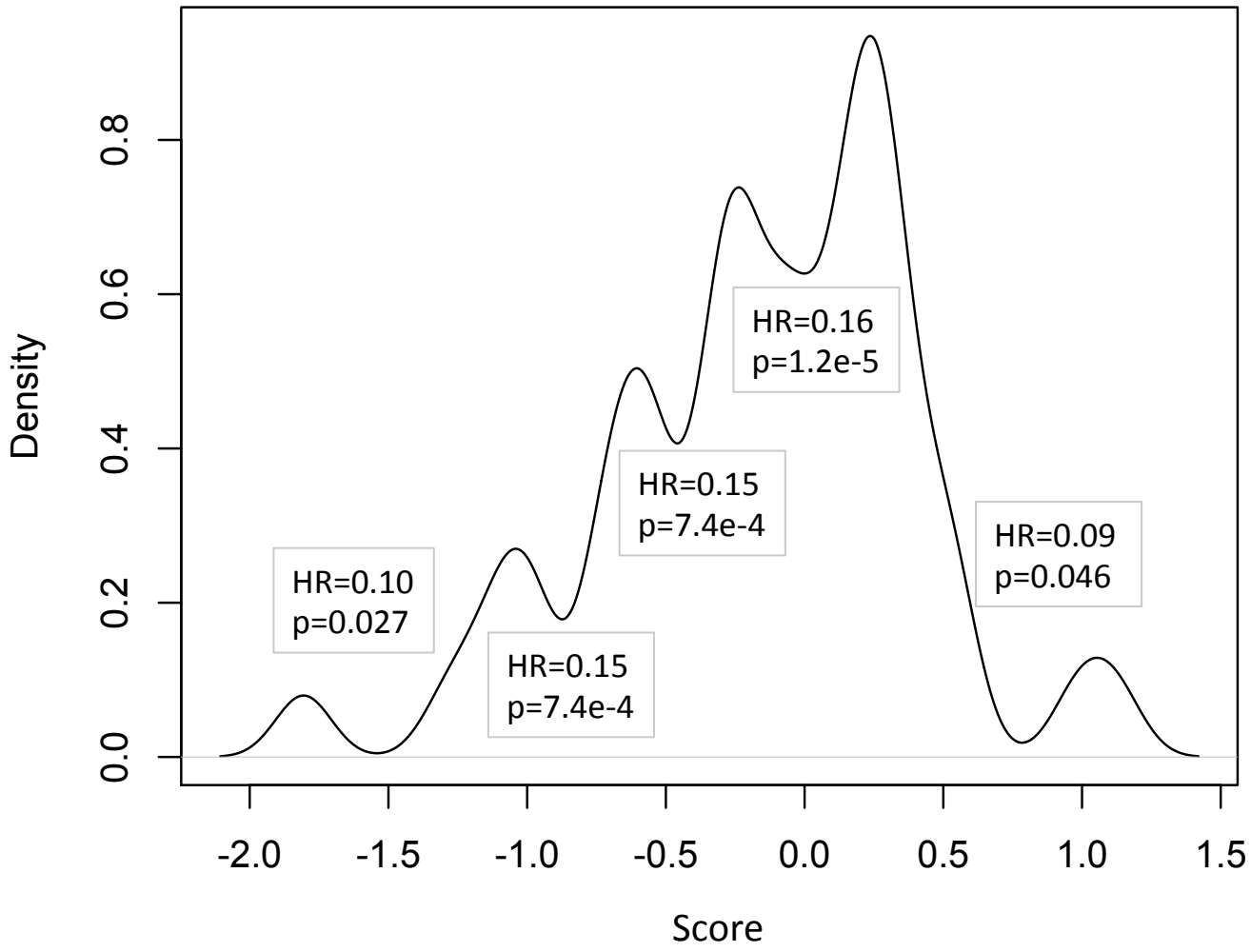
	Training set (*n*=50)	Test set (*n*=37)
Age	median 54.5 years	median 56 years
<60 years	33	27
≥60 years	17	10
Gender		
Male	27	23
Female	23	14
Karnofsky performance score		
≤70	21	18
>70	29	19
Days to death/last follow-up		
<450 days	25	23
≥450 days	25	14
<30 days	0	0
Treatment regimen		
Adjuvant	8	8
Progression	29	16
Recurrence	5	2
Not available	8	11
Mean treatment length	205.9	177.4

Univariate analysis

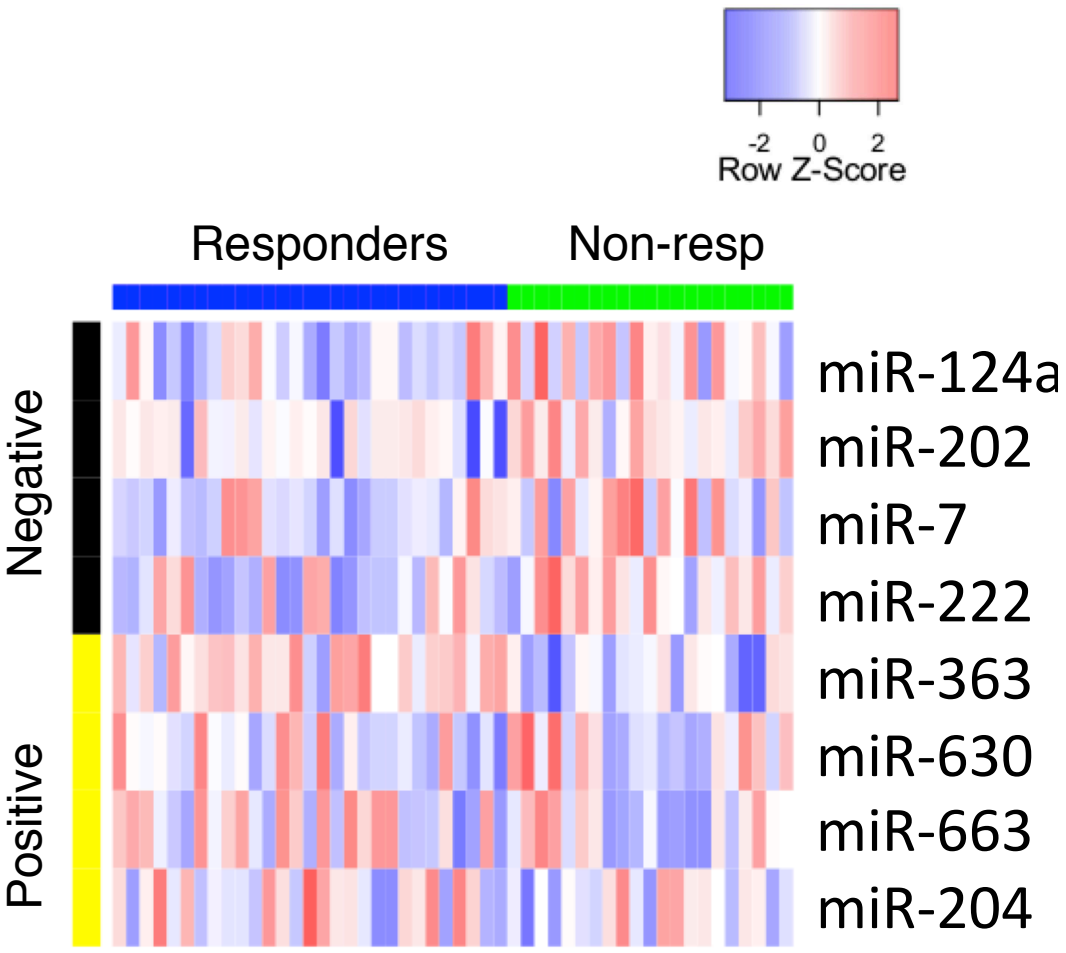
Variable	Number of subjects	HR	95% CI	p-value
MGMT	71	0.59	0.36-0.96	0.034
IDH1	70	0.55	0.23-1.29	0.169
KPS	73	1.82	1.17-2.82	0.007
Age	73	1.01	0.99-1.03	0.546
Gender	73	0.87	0.54-1.41	0.579
miR profile	73	0.58	0.35-0.95	0.031

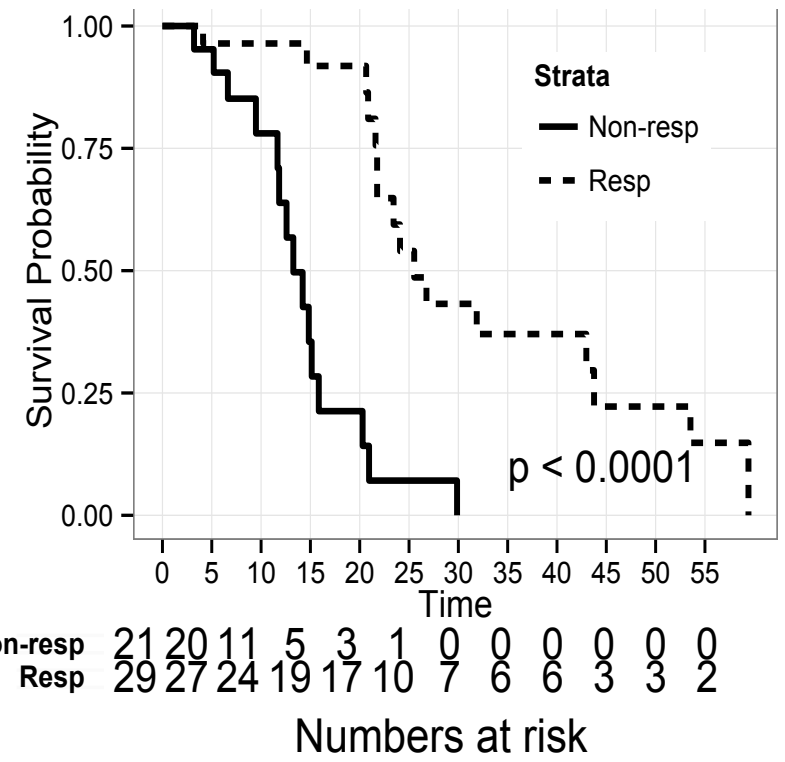
Multivariate analysis (68 subjects)

Variable	HR	95% CI	p-value
MGMT	0.71	0.43-1.18	0.185
KPS	2.02	1.31-3.12	0.001
IDH	0.74	0.31-1.77	0.493
miR profile	0.59	0.35-0.99	0.046

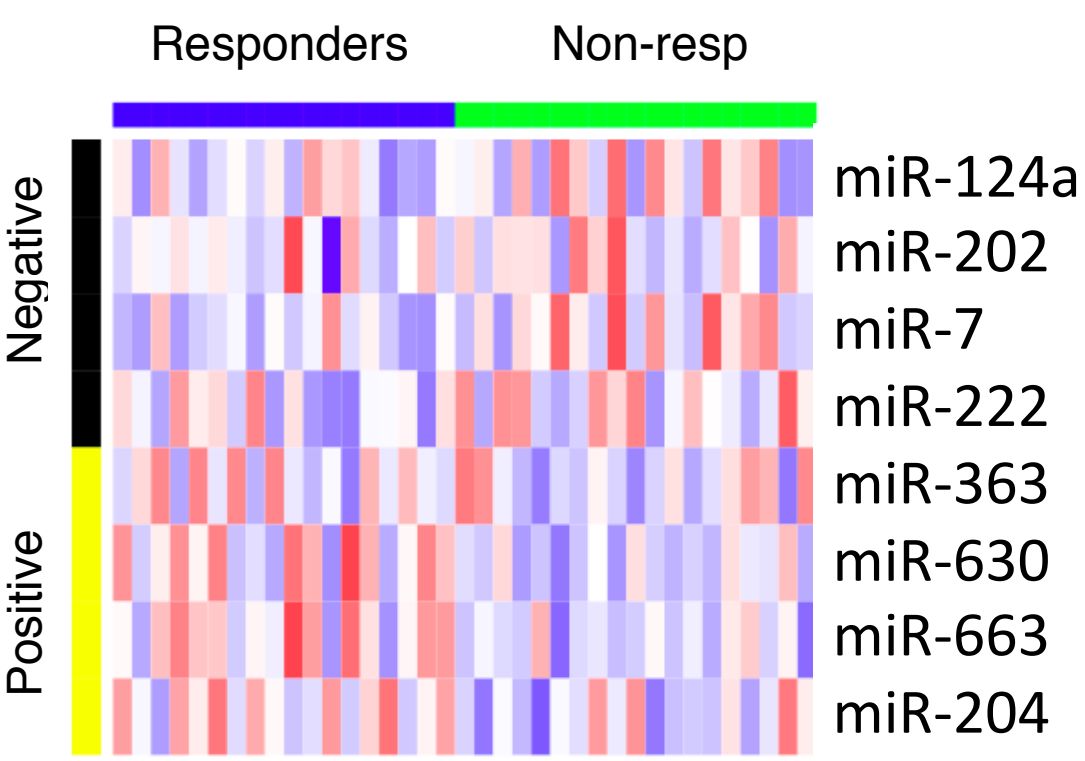

Density
0.0
0.2
0.4
0.6
0.8
HR=0.10
p=0.027
HR=0.15
p=7.4e-4
HR=0.15
p=7.4e-4
HR=0.16
p=1.2e-5
HR=0.09
p=0.046
-2.0
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
Score

A


-2
0
2
Row Z-Score
Responders
Non-resp
Negative
Positive
miR-124a
miR-202
miR-7
miR-222
miR-363
miR-630
miR-663
miR-204


Survival Probability
1.00
0.75
0.50
0.25
0.00
Strata
Non-resp
Resp
p < 0.0001
0 5 10 15 20 25 30 35 40 45 50 55
Time
Non-resp 21 20 11 5 3 1 0 0 0 0 0 0
Resp 29 27 24 19 17 10 7 6 6 3 3 2
Numbers at risk

B


Responders
Non-resp
Negative
Positive
miR-124a
miR-202
miR-7
miR-222
miR-363
miR-630
miR-663
miR-204

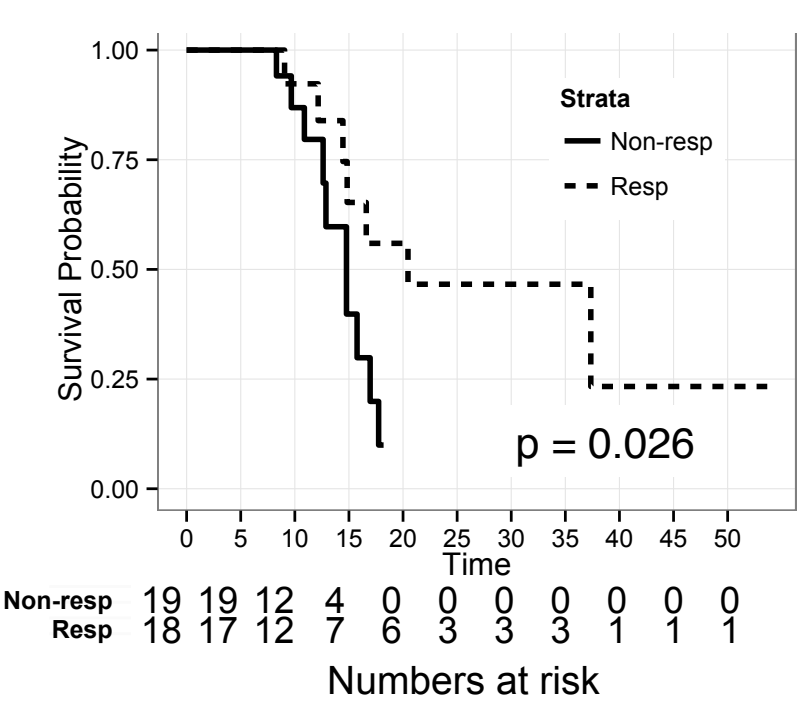
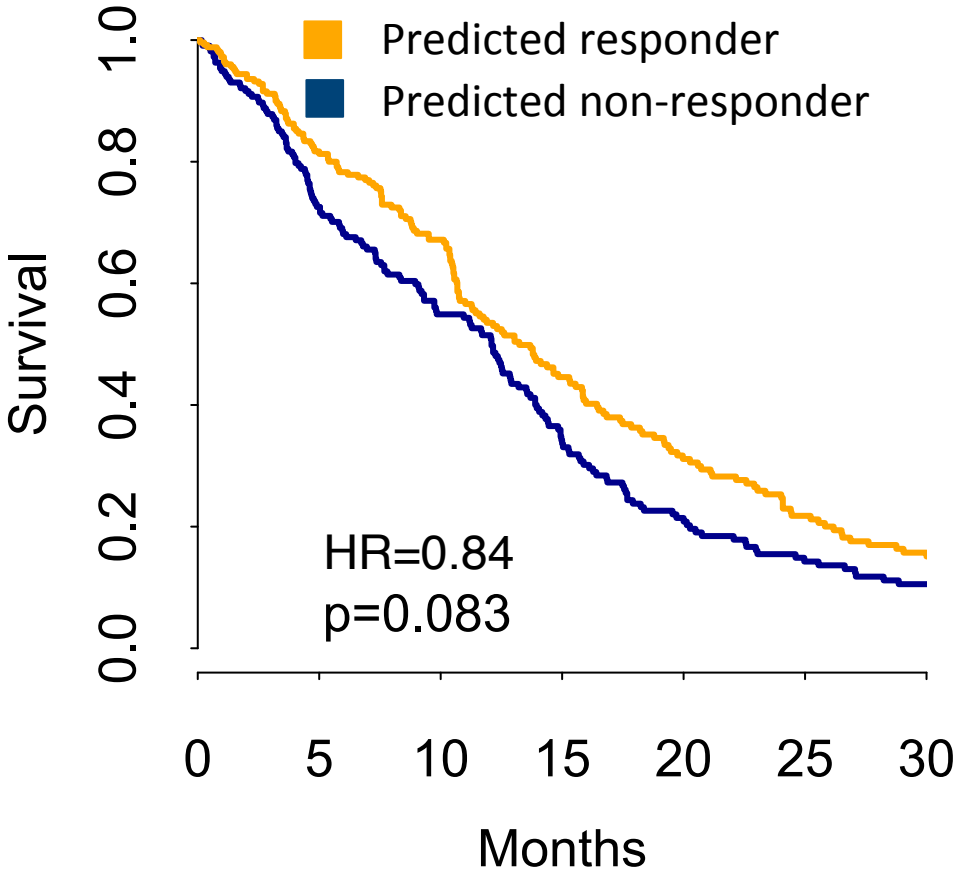
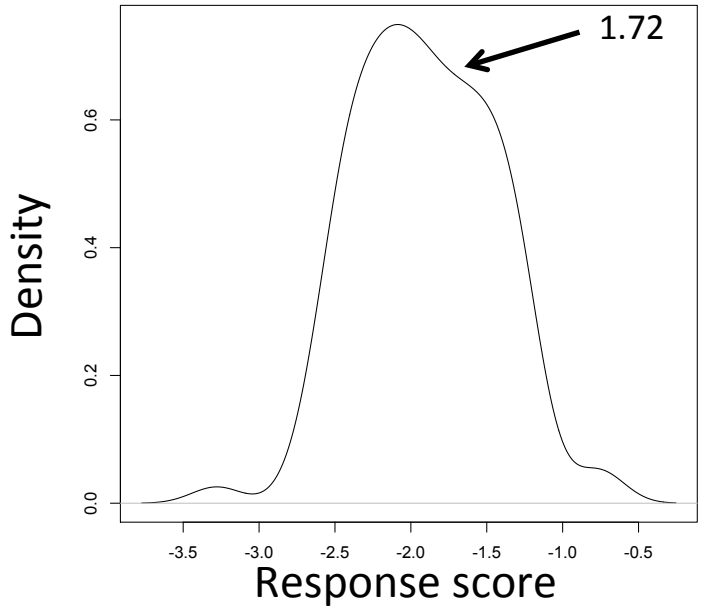

Survival Probability
1.00
0.75
0.50
0.25
0.00
Strata
Non-resp
Resp
p = 0.026
0 5 10 15 20 25 30 35 40 45 50
Time
Non-resp 19 19 12 4 0 0 0 0 0 0 0
Resp 18 17 12 7 6 3 3 3 1 1 1
Numbers at risk

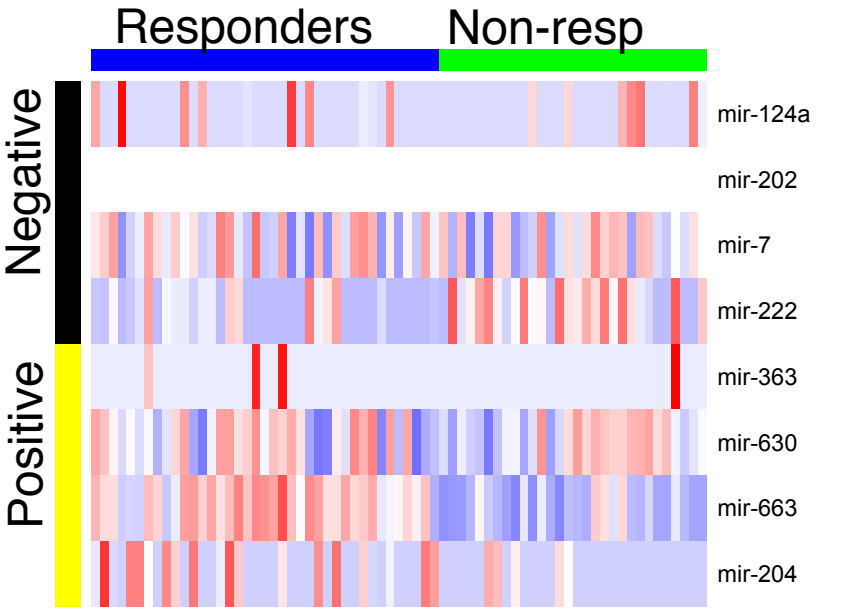
Figure 2


Predicted responder
Predicted non-responder
Survival
0.0
0.2
0.4
0.6
0.8
1.0
HR=0.84
p=0.083
0
5
10
15
20
25
30
Months

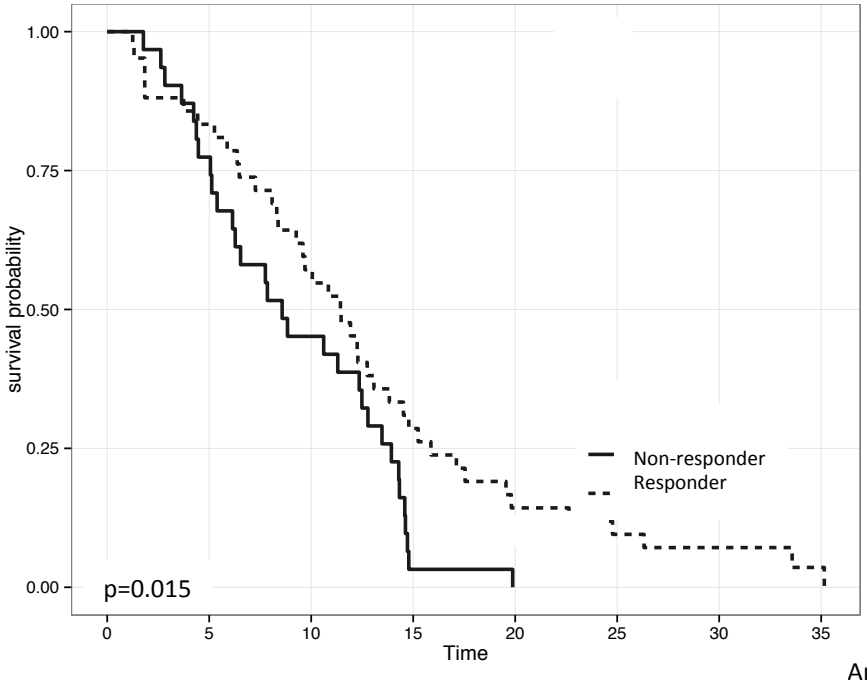
A



B

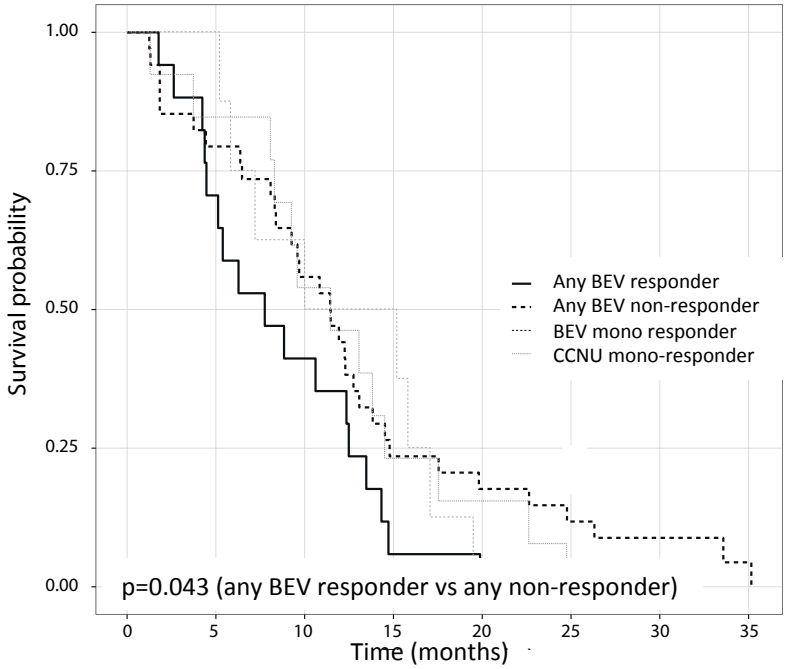


C



Responder	42	35	24	12	6	4	2	1
Non-responder	31	24	14	1	0	0	0	0

D



Any BEV responder	34	27	19	8	6	4	2	1
Any BEV non-responder	17	12	7	1	0	0	0	0
BEV mono responder	8	8	5	4	0	0	0	0
CCNU mono-responder	13	11	7	3	2	0	0	0