Les mégadonnées en médecine : Un exemple vécu

1082 – Biological factors predicting response to chemotherapy in advanced NSCLC: a prospective study

Background and objectives

- Lung cancer frequenty diagnosed at an advanced stage
- Cisplatin based doublet chemotherapy is the standard for treatment
- No reliable biological signature for predicting chemosensitivity (objective response 20%-40%) for most patients
- Protocol 1082 was designed to find biological signatures to predict **response** to chemotherapy (CDDP-VNR) as well as overall survival
- Derivation and validation sets planned in the protocol; 50 patients in the derivation

Main eligibility criteria

- Histologically confirmed NSCLC
- Bronchial biopsy available for analysis of biomarkers
- Candidate for first-line chemotherapy (no prior chemotherapy)
- Signed informed consent

- First chemotherapy regimen studied : CDDP-VNR (CDDP 60 mg/m^2 d1 and VNR 25 mg/m^2 d1-d8)

Primary endpoint:

To identify a predictive molecular signature for **response to chemotherapy**, according to WHO criteria by studying

- the transcriptome (**mRNAs** and **miRNAs**) and
- the single nucleotide polymorphism (**SNPs**) by using high throughput techniques.

Prospective validation obtained in a similar group of patients and with similar high throughput biological tests failed to confirm signatures for prediction of response to chemotherapy and survival in advanced NSCLC: a prospective study from the European Lung Cancer Working Party

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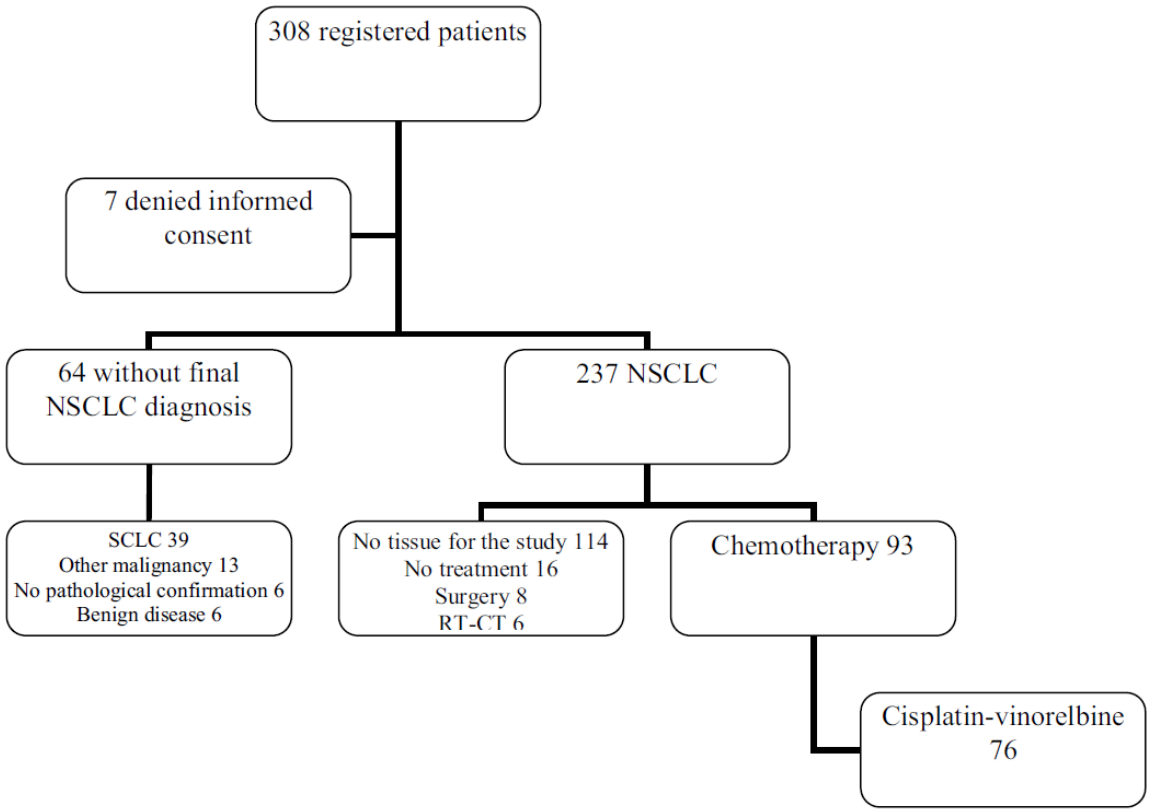


FIGURE 1 | Flow chart of registered patients. NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; RT-CT, radiochemotherapy.

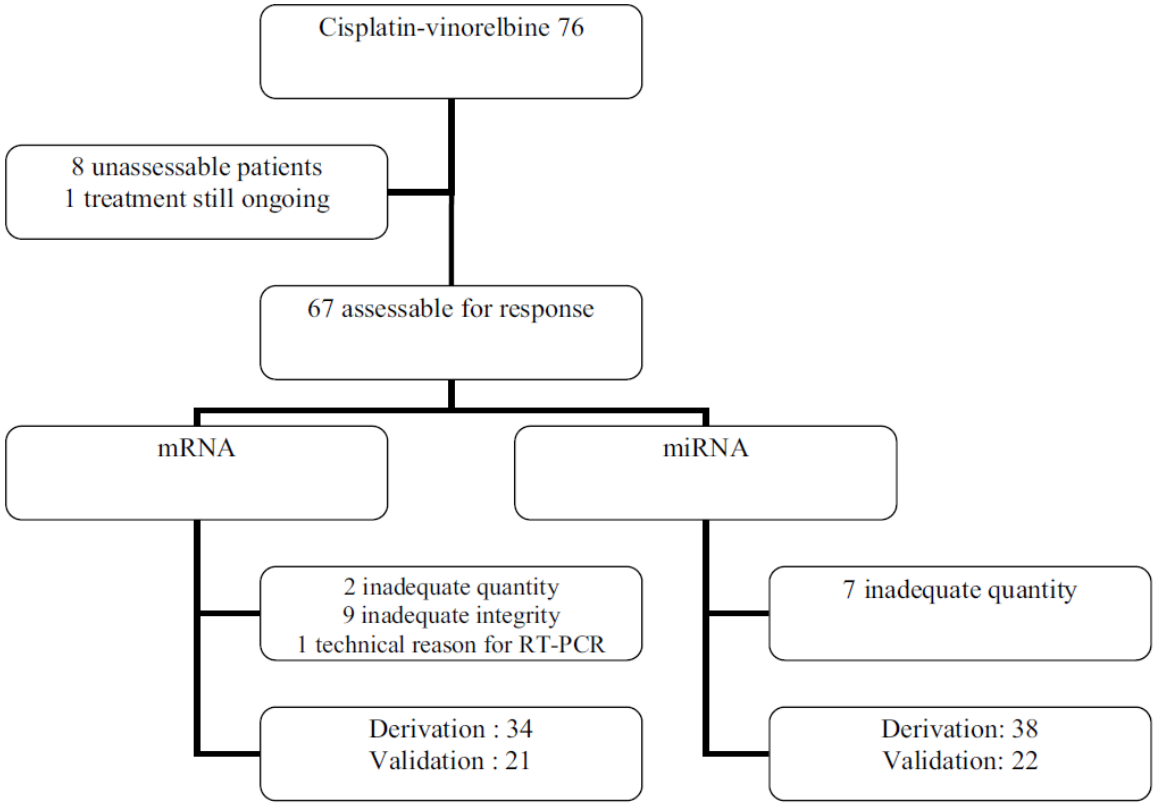


FIGURE 2 | Flow chart of the patients treated with cisplatin–vinorelbine for transcriptomic analysis.

	Derivation group ($N = 38$)		Validation group ($N = 22$)		*P*-value
Gender					
Male	27	(71%)	17	(77%)	0.60
female	11	(29%)	5	(23%)	
Age					
Mean ± STD	59 ± 11		62 ± 10		0.27
Median (min–max)	60 (33–78)		62 (45–76)		
Karnofsky PS					
50	–	–	1	(5%)	
60	2	(5%)	1	(5%)	
70	9	(24%)	1	(5%)	
80	6	(16%)	6	(30%)	
90	17	(45%)	9	(45%)	
100	4	(11%)	2	(10%)	
Missing data			2		
Median (min–max)	90 (60–100)		90 (50–100)		0.83
Metastases					
No	8	(21%)	7	(32%)	0.36
Yes	30	(79%)	15	(68%)	
Histology					
Adenocarcinoma	20	(53%)	8	(36%)	0.44
Squamous	15	(39%)	12	(55%)	
NSCLC NOS	3	(8%)	2	(9%)	
Response to chemotherapy					
No response	21	(57%)	13	(59%)	0.86
Response	16	(43%)	9	(41%)	
Unassessable	1				
Median OS (months) (95% CI)	25 (19–40)		29 (15–40)		0.63

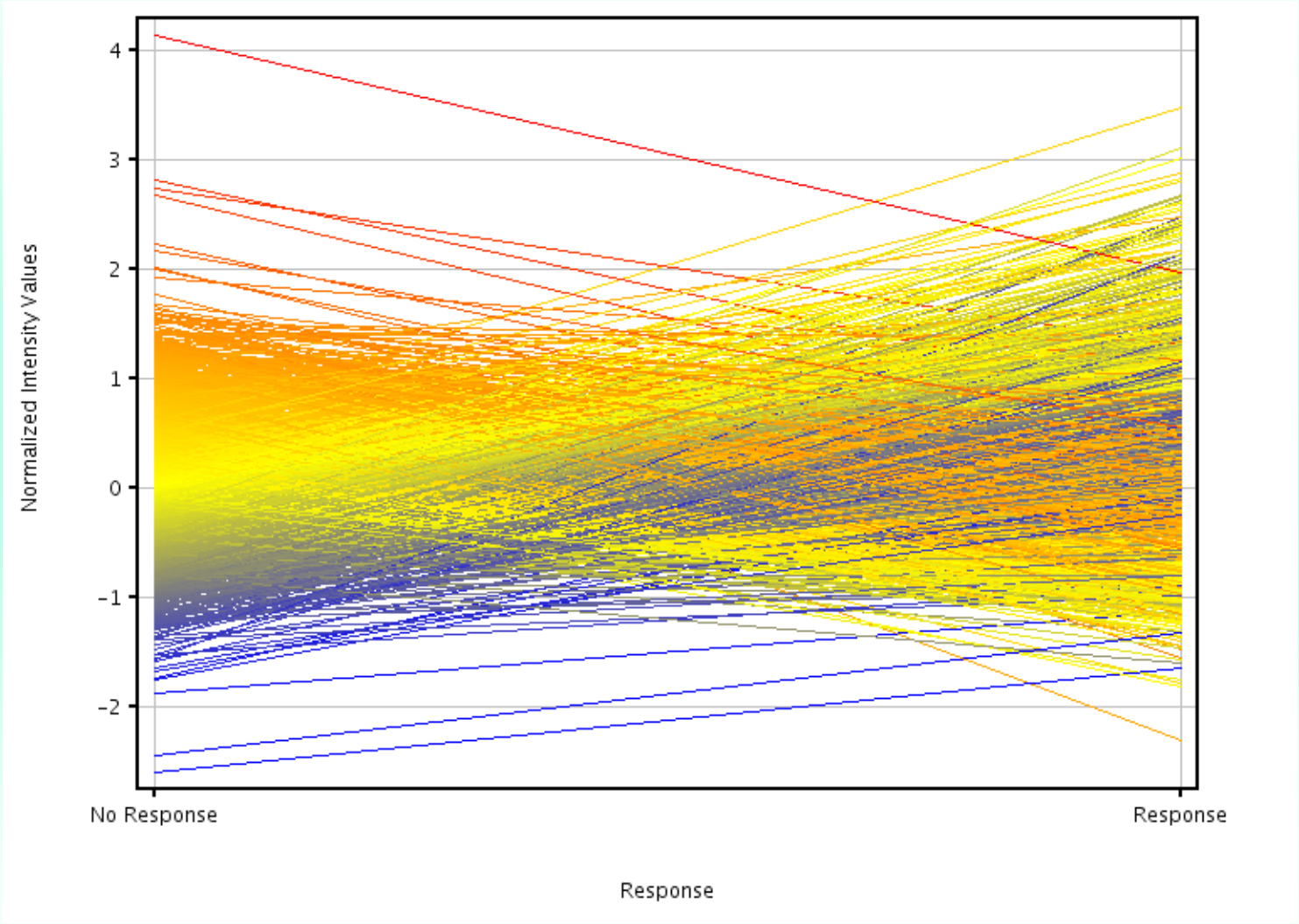
Microarray data

- 41093 genes analyzed
- Big Data ?
- Microarrays of 36 patients performed.
  - For one patient, two microarrays didn't succeed. No DNA left to do further microarray.
- 35 microarrays available
  - One patient with response inevaluable
- 34 patients with response and microarray available
  - 14 response (41%)
  - 20 no response (59%)

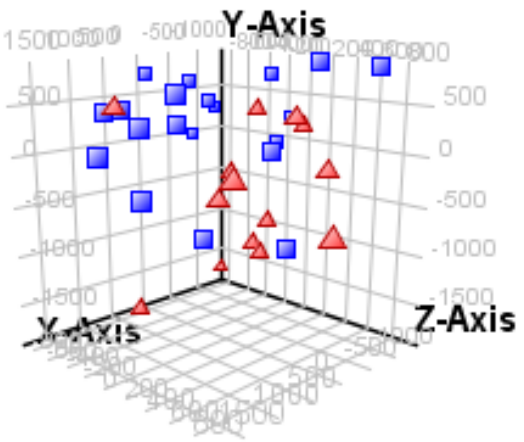
Preprocessing

- Data imported in Genespring as Agilent two-color data
- Data was already preprocessed by Feature Extraction (FE) software. Agilent FE pre-processing software produces columns that indicates the reliability of the intensity value for each feature:
  - Whether intensity is above saturation level
  - Whether feature is population outlier
  - Whether feature is uniform spot
- Agilent FE performs lowess normalization
- Baseline transformation: baseline to median of all samples. For each probe, the median of the log summarized values from all the samples is calculated and subtracted from each of the sample.

Profile plot of all 41093 genes



Quality control



X-Axis Component 1 (35.01%) Y-Axis Component 2 (28.29%) Z-Axis Component 3 (19.22%)

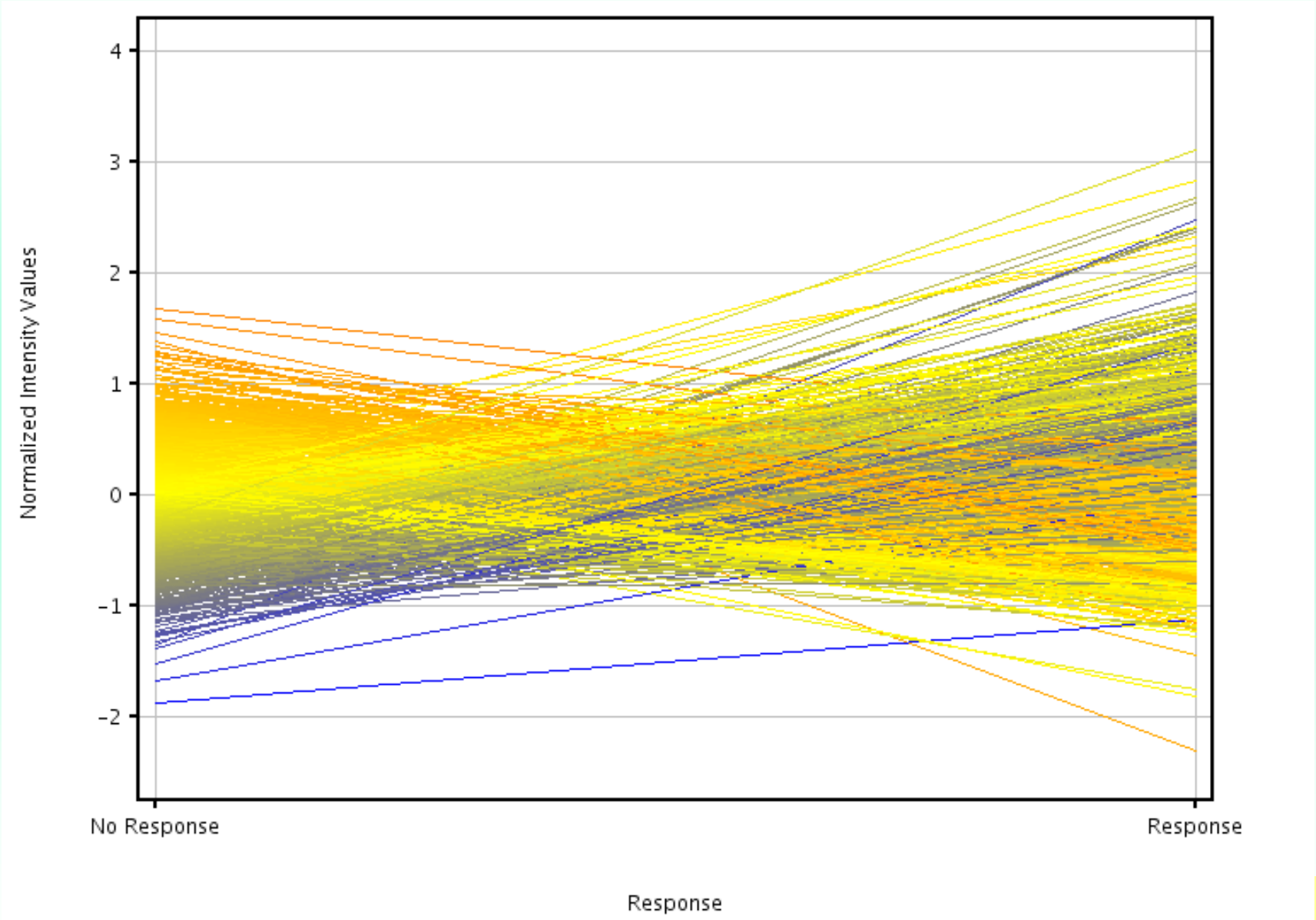
No outliers.

Filter probesets

- Before proceeding with analysis, we removed genes not expressed in any of the samples

- Of the 41093 probes, 26570 pass the filter.

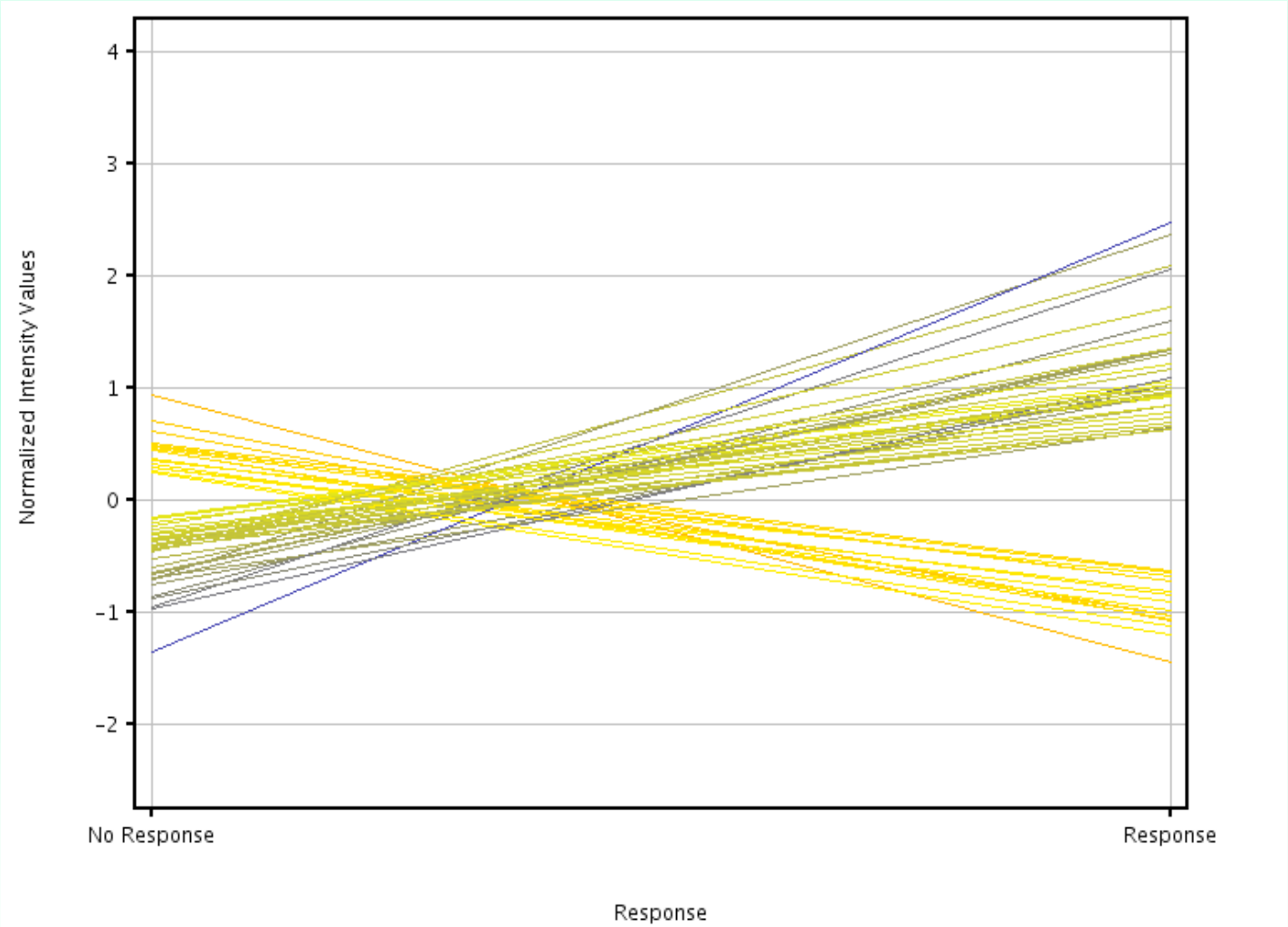
Profile plot of the 26570 genes



Significance analyses, correcting for multiple testing with False Discovery Rate , FDR (B-H method)

- Apply t-test, adjust for multiplicity by FDR<0.05
- 115 genes differentially expressed between response and non response
  - 50 genes have a fold change >2 between response and non response
  - 19 genes have a fold change >3

- We restrict to genes with FC >2 (N=50)
  - 16 **down**regulated
  - 34 **up**regulated



Creating signature

Stepwise variable selection

Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-2.2753	1.0157	5.0179	0.0251
a_23_p157879	1	-2.2393	1.0798	4.3010	0.0381
a_24_p567454	1	5.5365	2.4143	5.2590	0.0218

ProbeName	Gene-Symbol	Area under ROC curve	Description
A_23_P157879	FCN1	0.86	Homo sapiens ficolin (collagen/fibrinogen domain containing) 1 (FCN1), mRNA [NM_002003]
A_24_P567454	RNF168	0.94	E3 ubiquitin-protein ligase RNF168 (EC 6.3.2.-)(RING finger protein 168) [Source:UniProtKB/Swiss-Prot;Acc:Q8IYW5] [ENST00000318037]

Area under the ROC curve when including both FCN1 and RNF168 is **0.97**.

Signature: **-2*FCN1 + 5*RNF168**

Signature

-2xFCN1 + 5xRNF168 > 2.3 → response

-2xFCN1 + 5xRNF168 < 2.3 → no response

- Sensitivity 13/14 = 93%
- Specificity 20/20 = 100%
- PPV 13/13 = 100%
- NPV 20/21 = 95%

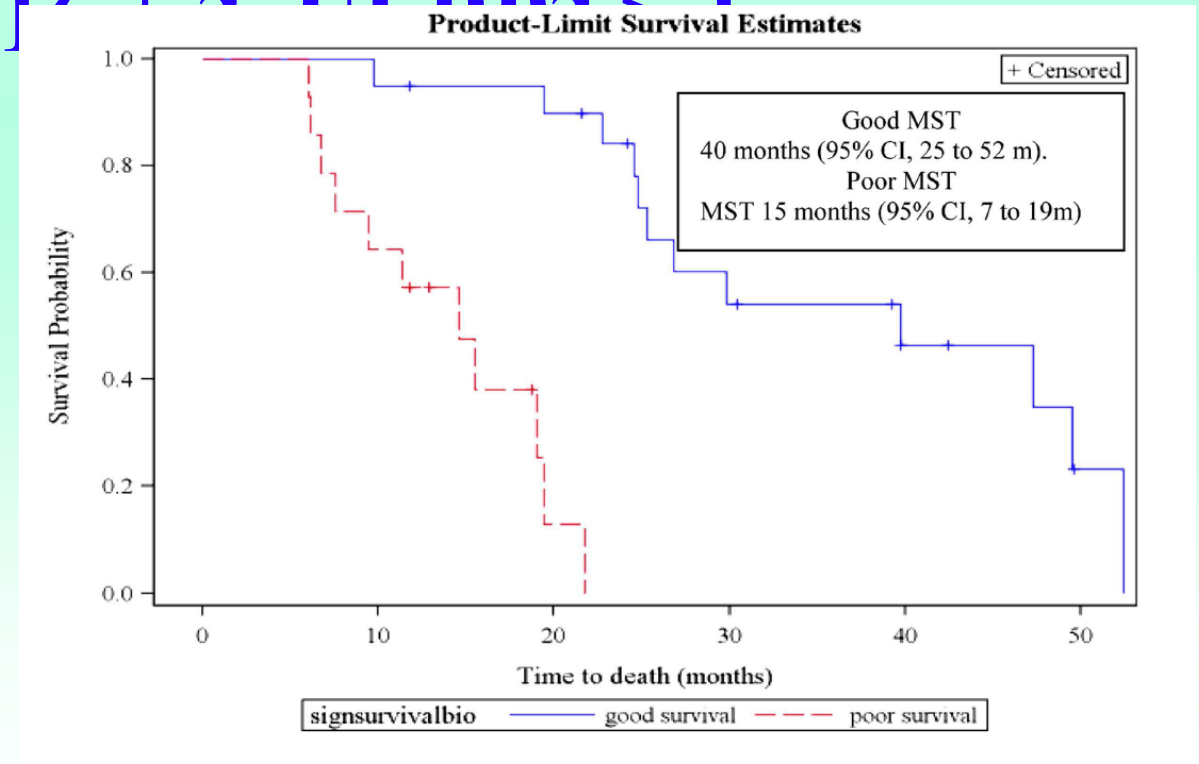
Signature

Another signature was found restricting the analysis to the 19 genes with FC > 3 on the basis of KRT16 and SEMA3D

Signature for overall survival

-3xKRT16 + 2xULBP2 < 1 → better survival

-3xKRT1 r survival



miRNAs data

- 39 patients
  - 1 response not evaluable
  - 1 not enough RNA left for assessing the miRs on plaque B
- 756 miRs
  - **396 had a CT>32 (no expression or low) in all patients** and can therefore be excluded from the analysis.

- 37 patients and 360 miRs
  - 16 (43%) Response
  - 21 (57%) Non response

Significance analyses, correcting for multiple testing with False Discovery Rate, FDR

- Apply Wilcoxon-test, adjust for multiplicity by FDR<0.05
- No miRNAs retained.
- Go on with uncorrected p-value<0.05

Creating signature

Stepwise variable selection

Analysis of Maximum Likelihood Estimates					
Parameter	DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	1	2.0263	2.7622	0.5381	0.4632
hsa_miR_149_4395366	1	-1.1225	0.4198	7.1493	0.0075
hsa_miR_375_4373027	1	0.7300	0.3542	4.2471	0.0393

Area under the ROC curve 0.90.

Signature

-4*hsa-miR-149 + 3*hsa-miR-375 > -6 → response

-4*hsa-miR-149 + 3*hsa-miR-375< -6 → no response

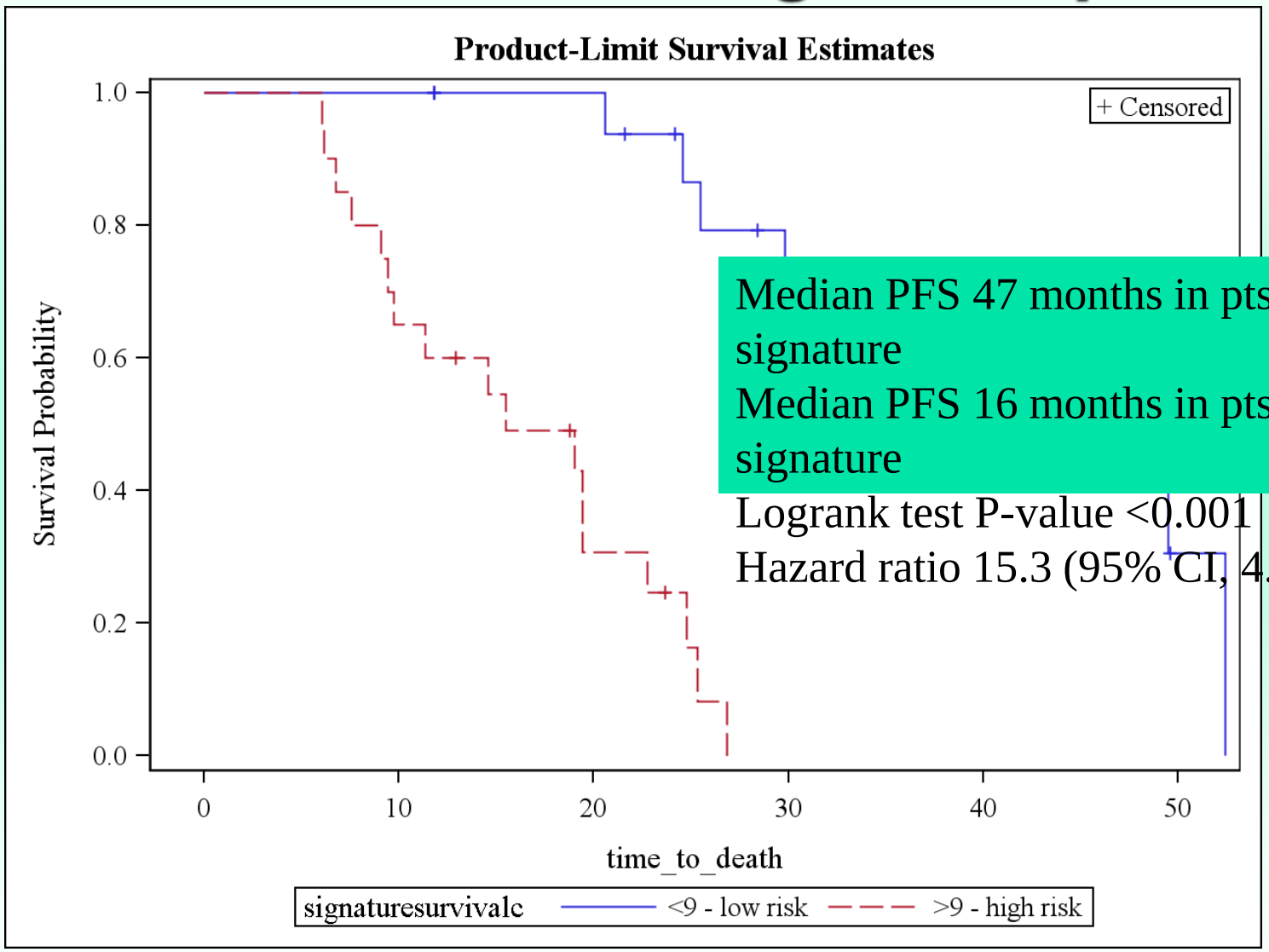
- Sensitivity 14/16 = 88%
- Specificity 17/21 = 81%
- PPV 14/18 = 78%
- NPV 17/19 = 89%

Signature for overall survival

- Again, no miR fulfills the FDR restriction.
- If we build a signature based on the above 19 miRs with uncorrected P-value (PH model) <0.05, we obtain 7 miRs (stepwise selection):

Analysis of Maximum Likelihood Estimates								
Parameter	DF	Parameter Estimate	Standard Error	Chi-Square	Pr > ChiSq	Hazard Ratio	95% Hazard Ratio Confidence Limits	
hsa_miR_200c_4395411	1	1.10044	0.32075	11.7709	0.0006	3.005	1.603	5.636
hsa_miR_29c_4395171	1	-1.21471	0.40228	9.1180	0.0025	0.297	0.135	0.653
hsa_miR_663B_002857	1	-0.33797	0.12471	7.3437	0.0067	0.713	0.559	0.911
hsa_miR_424__002309	1	0.40827	0.12501	10.6657	0.0011	1.504	1.177	1.922
hsa_miR_219_5p_4373080	1	1.09097	0.39032	7.8127	0.0052	2.977	1.385	6.398
hsa_miR_124_4373295	1	-0.29936	0.12546	5.6934	0.0170	0.741	0.580	0.948
hsa_miR_1274A_002883	1	-0.55726	0.27989	3.9641	0.0465	0.573	0.331	0.991

How well does the signature predict OS?



Median PFS 47 months in pts with low risk signature

Median PFS 16 months in pts with high risk signature

Logrank test P-value <0.001

Hazard ratio 15.3 (95% CI, 4.2 to 55.5)

Validation

- 5 genes assessed by RT-qPCR (FNC1, RNF168, KRT16, SEMA3D, ULBP2)
- One patient excluded due to technical problems
- With or without correction for the reference genes (HPRT and actin), none of the 5 genes was differentially expressed between responders and non responders
- But difference in the reference genes was significant
- No predictive value for the signatures (response and overall survival) : validation failed (mRNAs)

Further analyses

- No gene out of 25 693 could be retained when adjustment for multiplicity is applied in the validation set
- Analyses without correction for multiplicity of genes differentially expressed in both sets
- Genes in common : 3994 (derivation) and 4597 genes (validation), 402 in common, 153 regulated in the same direction, 10 with $p<0.01$ in both sets and 1 with FC >2

Conclusions

- Validation of the signatures failed – No clinical usefulness in routine although the validation set was likely very close to the derivation set and the techniques are assumed to be the same and both sets came from a prospective study
- Not the only failure in the literature
- Many signatures published, few have genes in common
- Is the methodology wrong ?
- Is there a technical failure ?
- Is the sample size really too small ? Overfitting despite the adjustment for multiplicity ?