A New Cross-Platform Multi-Signature Classifier Approach To Predict Neuroblastoma Patients Outcome

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Background

- Neuroblastoma is the most common pediatric solid tumor of the sympathetic nervous system

- High variability in clinical behavior

- Reliable patients outcome predictions are often difficult to assess

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Development of new predictive tools to assist established Neuroblastoma risk factors is mandatory

Background

Previous Work

Generation of a Multi-Signature Ensemble classifier for NeuroBlastoma patients outcome prediction (NB-MuSE-classifier)

➢ Ability to take into account the biological and prognostic information derived from a-priori knowledge (gene expression signatures).

➢ Possibility to combine different machine learning algorithms prediction power.

- 182 Neuroblastoma patients: U133Plus2 Gene Expression Profiles
- 35 Neuroblastoma related gene signatures from literature
- 22 Machine Learning algorithms tested

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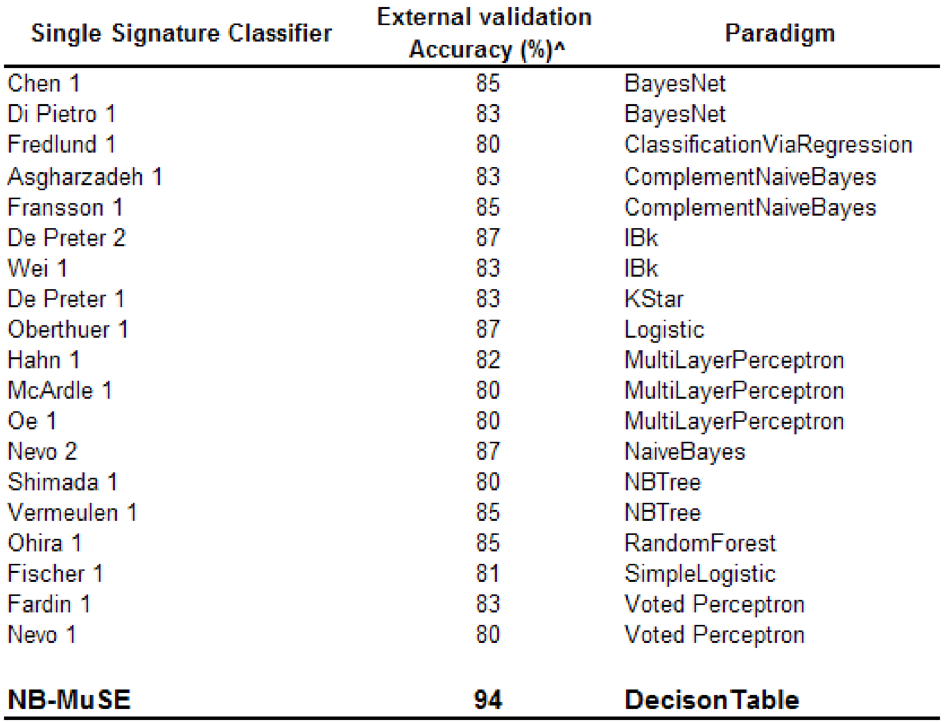
NB-MuSE-classifier

Background

Previous Work Results



Single Signature Classifier	External validation Accuracy (%)^	Paradigm
Chen 1	85	BayesNet
Di Pietro 1	83	BayesNet
Fredlund 1	80	ClassificationViaRegression
Asgharzadeh 1	83	ComplementNaiveBayes
Fransson 1	85	ComplementNaiveBayes
De Preter 2	87	IBk
Wei 1	83	IBk
De Preter 1	83	KStar
Oberthuer 1	87	Logistic
Hahn 1	82	MultiLayerPerceptron
McArdle 1	80	MultiLayerPerceptron
Oe 1	80	MultiLayerPerceptron
Nevo 2	87	NaiveBayes
Shimada 1	80	NBTree
Vermeulen 1	85	NBTree
Ohira 1	85	RandomForest
Fischer 1	81	SimpleLogistic
Fardin 1	83	Voted Perceptron
Nevo 1	80	Voted Perceptron
NB-MuSE	**94**	**DecisonTable**

Promising preliminary results

but

Evaluation Limited by relatively small test dataset

Background

The availability of samples is one important limiting factor in developing reliable prognostic classifiers, especially for rare tumors such as neuroblastoma.

Neuroblastoma repositories are often characterized by heterogeneous high-througput datasets

A multi-signature classification framework which can use different array datasets (different gene expression platforms, arrayCGH, etc.) to:

- improve biological and prognostic a-priori information
- extend the sample size used for validation

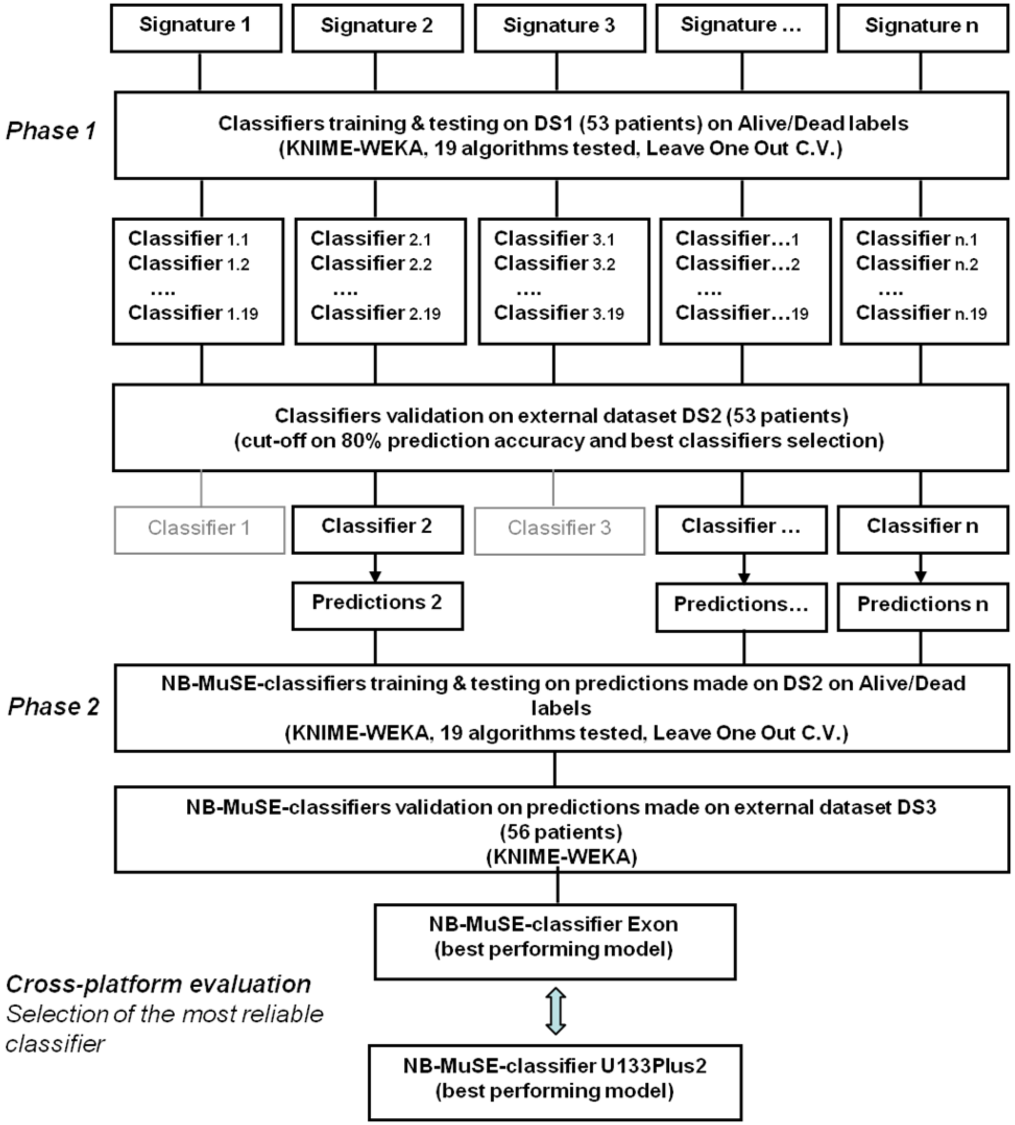
Generation of a cross-platform NB-MuSE-classifier
Exon 1.0 ST ⟺ U133Plus2 Data

- Ability to take into account the biological and prognostic information derived from a-priori knowledge (gene expression signatures).

- Possibility to combine different machine learning algorithms prediction power.

- Ability to be trained and tested on different type of high-throughput datasets (cross-platform feature), such as different gene expression arrays.
This feature permits the integration of heterogeneous datasets and the extension of sample size used for validation.

Workflow for cross-platform NB-MuSE-classifier construction
Exon 1.0 ST ⟺ U133Plus2 Data



Signature 1 | Signature 2 | Signature 3 | Signature … | Signature n

Phase 1

Classifiers training & testing on DS1 (53 patients) on Alive/Dead labels
(KNIME-WEKA, 19 algorithms tested, Leave One Out C.V.)

Classifier 1.1, Classifier 1.2, …., Classifier 1.19 | Classifier 2.1, Classifier 2.2, …., Classifier 2.19 | Classifier 3.1, Classifier 3.2, …., Classifier 3.19 | Classifier…1, Classifier…2, …., Classifier…19 | Classifier n.1, Classifier n.2, …., Classifier n.19

Classifiers validation on external dataset DS2 (53 patients)
(cut-off on 80% prediction accuracy and best classifiers selection)

Classifier 1 | Classifier 2 | Classifier 3 | Classifier … | Classifier n

Predictions 2 | Predictions… | Predictions n

Phase 2

NB-MuSE-classifiers training & testing on predictions made on DS2 on Alive/Dead labels
(KNIME-WEKA, 19 algorithms tested, Leave One Out C.V.)

NB-MuSE-classifiers validation on predictions made on external dataset DS3
(56 patients)
(KNIME-WEKA)

NB-MuSE-classifier Exon
(best performing model)

NB-MuSE-classifier U133Plus2
(best performing model)

Cross-platform evaluation
Selection of the most reliable classifier

- 162 new neuroblastoma patients: Affymetrix Exon 1.0 ST Array (gene level).

Clinical Characteristics	DS1	DS2	DS3	Global Dataset
NB stage	%	%	%	%
st4s	7.55	9.43	12.50	9.88
st4	35.85	47.17	50.00	44.44
st3	20.75	20.75	12.50	17.90
st2	13.21	11.32	10.71	11.73
st1	22.64	11.32	14.29	16.05
age at diagnosis				
<=1 y.o.a.	43.40	28.30	42.86	38.27
>1 y.o.a	56.60	71.70	57.14	61.73
mycn amplification				
yes	21.15	25.00	20.00	22.01
no	78.85	75.00	80.00	77.99
overall survival				
alive	71.70	83.02	80.36	78.40
dead	28.30	16.98	19.64	21.60
number of patients	53	53	56	112

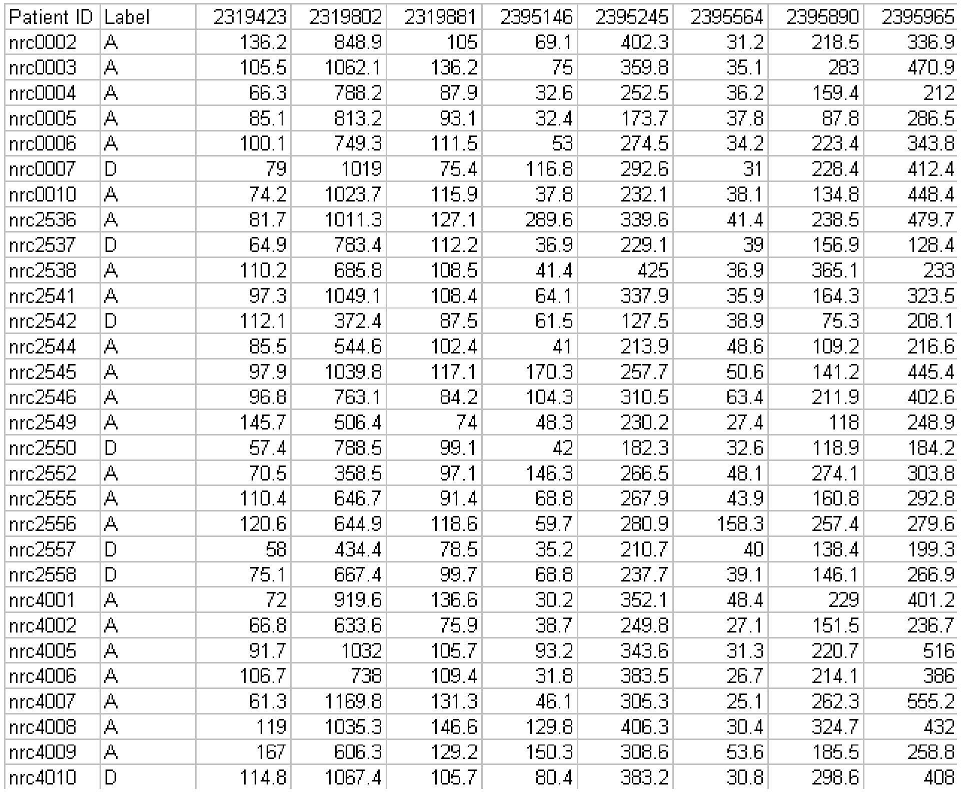
- 20 neuroblastoma related gene signatures

- 19 Machine Learning algorithms

Machine learning algorithm	Cathegory
Bayes Logistic regression	Bayesan
BayesNet	Bayesan
Complement Naive Bayes	Bayesan
Naive Bayes	Bayesan
Logistic	Functions
Multi Layer Perceptron	Functions
Simple Logistic	Functions
Voted perceptron	Functions
IB1	Lazy
IBK	Lazy
Kstar	Lazy
Bagging	Meta-learner
Classification via regression	Meta-learner
Decision Table	rules
Zero R	rules
J48	tree
NBTree	tree
Random Forest	tree
Random Tree	tree

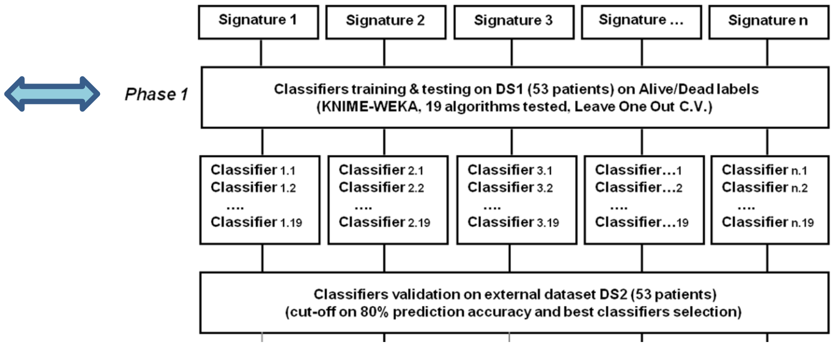
DS1 trainingdataset (53 patients): one for each signature

Expression dataset used to train the single signature classifiers to predict patients overall survival (Alive/Dead labels)



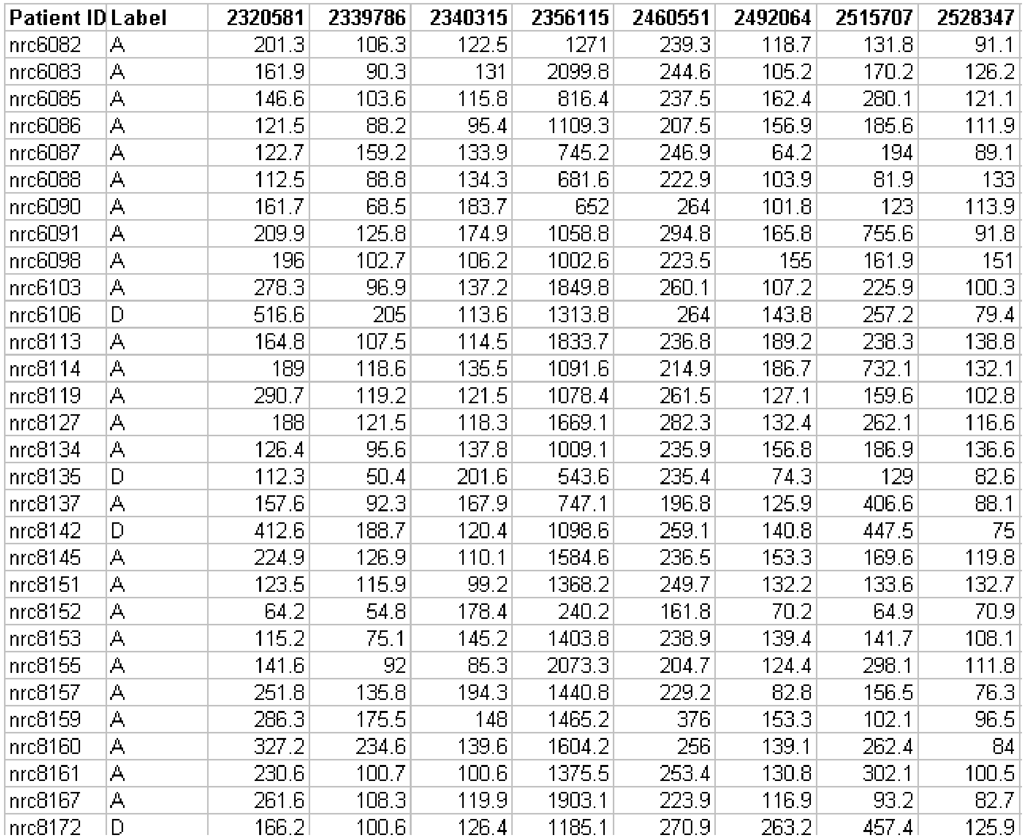
Patient ID	Label	2319423	2319802	2319881	2395146	2395245	2395564	2395890	2395965
nrc0002	A	136.2	848.9	105	69.1	402.3	31.2	218.5	336.9
nrc0003	A	105.5	1062.1	136.2	75	359.8	35.1	283	470.9
nrc0004	A	66.3	788.2	87.9	32.6	252.5	36.2	159.4	212
nrc0005	A	85.1	813.2	93.1	32.4	173.7	37.8	87.8	286.5
nrc0006	A	100.1	749.3	111.5	53	274.5	34.2	223.4	343.8
nrc0007	D	79	1019	75.4	116.8	292.6	31	228.4	412.4
nrc0010	A	74.2	1023.7	115.9	37.8	232.1	38.1	134.8	448.4
nrc2536	A	81.7	1011.3	127.1	289.6	339.6	41.4	238.5	479.7
nrc2537	D	64.9	783.4	112.2	36.9	229.1	39	156.9	128.4
nrc2538	A	110.2	685.8	108.5	41.4	425	36.9	365.1	233
nrc2541	A	97.3	1049.1	108.4	64.1	337.9	35.9	164.3	323.5
nrc2542	D	112.1	372.4	87.5	61.5	127.5	38.9	75.3	208.1
nrc2544	A	85.5	544.6	102.4	41	213.9	48.6	109.2	216.6
nrc2545	A	97.9	1039.8	117.1	170.3	257.7	50.6	141.2	445.4
nrc2546	A	96.8	763.1	84.2	104.3	310.5	63.4	211.9	402.6
nrc2549	A	145.7	506.4	74	48.3	230.2	27.4	118	248.9
nrc2550	D	57.4	788.5	99.1	42	182.3	32.6	118.9	184.2
nrc2552	A	70.5	358.5	97.1	146.3	266.5	48.1	274.1	303.8
nrc2555	A	110.4	646.7	91.4	68.8	267.9	43.9	160.8	292.8
nrc2556	A	120.6	644.9	118.6	59.7	280.9	158.3	257.4	279.6
nrc2557	D	58	434.4	78.5	35.2	210.7	40	138.4	199.3
nrc2558	D	75.1	667.4	99.7	68.8	237.7	39.1	146.1	266.9
nrc4001	A	72	919.6	136.6	30.2	352.1	48.4	229	401.2
nrc4002	A	66.8	633.6	75.9	38.7	249.8	27.1	151.5	236.7
nrc4005	A	91.7	1032	105.7	93.2	343.6	31.3	220.7	516
nrc4006	A	106.7	738	109.4	31.8	383.5	26.7	214.1	386
nrc4007	A	61.3	1169.8	131.3	46.1	305.3	25.1	262.3	555.2
nrc4008	A	119	1035.3	146.6	129.8	406.3	30.4	324.7	432
nrc4009	A	167	606.3	129.2	150.3	308.6	53.6	185.5	258.8
nrc4010	D	114.8	1067.4	105.7	80.4	383.2	30.8	298.6	408

…. Gene IDs



DS2 external validation dataset (53 patients): one for each signature

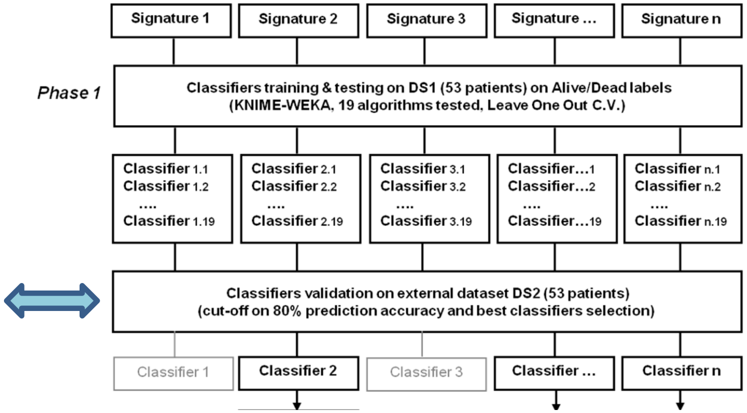
Expression dataset used to validate the single signature classifiers trained on DS1



Patient ID	Label	2320581	2339786	2340315	2356115	2460551	2492064	2515707	2528347
nrc6082	A	201.3	106.3	122.5	1271	239.3	118.7	131.8	91.1
nrc6083	A	161.9	90.3	131	2099.8	244.6	105.2	170.2	126.2
nrc6085	A	146.6	103.6	115.8	816.4	237.5	162.4	280.1	121.1
nrc6086	A	121.5	88.2	95.4	1109.3	207.5	156.9	185.6	111.9
nrc6087	A	122.7	159.2	133.9	745.2	246.9	64.2	194	89.1
nrc6088	A	112.5	88.8	134.3	681.6	222.9	103.9	81.9	133
nrc6090	A	161.7	68.5	183.7	652	264	101.8	123	113.9
nrc6091	A	209.9	125.8	174.9	1058.8	294.8	165.8	755.6	91.8
nrc6098	A	196	102.7	106.2	1002.6	223.5	155	161.9	151
nrc6103	A	278.3	96.9	137.2	1849.8	260.1	107.2	225.9	100.3
nrc6106	D	516.6	205	113.6	1313.8	264	143.8	257.2	79.4
nrc8113	A	164.8	107.5	114.5	1833.7	236.8	189.2	238.3	138.8
nrc8114	A	189	118.6	135.5	1091.6	214.9	186.7	732.1	132.1
nrc8119	A	290.7	119.2	121.5	1078.4	261.5	127.1	159.6	102.8
nrc8127	A	188	121.5	118.3	1669.1	282.3	132.4	262.1	116.6
nrc8134	A	126.4	95.6	137.8	1009.1	235.9	156.8	186.9	136.6
nrc8135	D	112.3	50.4	201.6	543.6	235.4	74.3	129	82.6
nrc8137	A	157.6	92.3	167.9	747.1	196.8	125.9	406.6	88.1
nrc8142	D	412.6	188.7	120.4	1098.6	259.1	140.8	447.5	75
nrc8145	A	224.9	126.9	110.1	1584.6	236.5	153.3	169.6	119.8
nrc8151	A	123.5	115.9	99.2	1368.2	249.7	132.2	133.6	132.7
nrc8152	A	64.2	54.8	178.4	240.2	161.8	70.2	64.9	70.9
nrc8153	A	115.2	75.1	145.2	1403.8	238.9	139.4	141.7	108.1
nrc8155	A	141.6	92	85.3	2073.3	204.7	124.4	298.1	111.8
nrc8157	A	251.8	135.8	194.3	1440.8	229.2	82.8	156.5	76.3
nrc8159	A	286.3	175.5	148	1465.2	376	153.3	102.1	96.5
nrc8160	A	327.2	234.6	139.6	1604.2	256	139.1	262.4	84
nrc8161	A	230.6	100.7	100.6	1375.5	253.4	130.8	302.1	100.5
nrc8167	A	261.6	108.3	119.9	1903.1	223.9	116.9	93.2	82.7
nrc8172	D	166.2	100.6	126.4	1185.1	270.9	263.2	457.4	125.9

…. Gene IDs

⋮



Signature 1
Signature 2
Signature 3
Signature …
Signature n

Phase 1

Classifiers training & testing on DS1 (53 patients) on Alive/Dead labels
(KNIME-WEKA, 19 algorithms tested, Leave One Out C.V.)

Classifier 1.1
Classifier 1.2
….
Classifier 1.19

Classifier 2.1
Classifier 2.2
….
Classifier 2.19

Classifier 3.1
Classifier 3.2
….
Classifier 3.19

Classifier…1
Classifier…2
….
Classifier…19

Classifier n.1
Classifier n.2
….
Classifier n.19

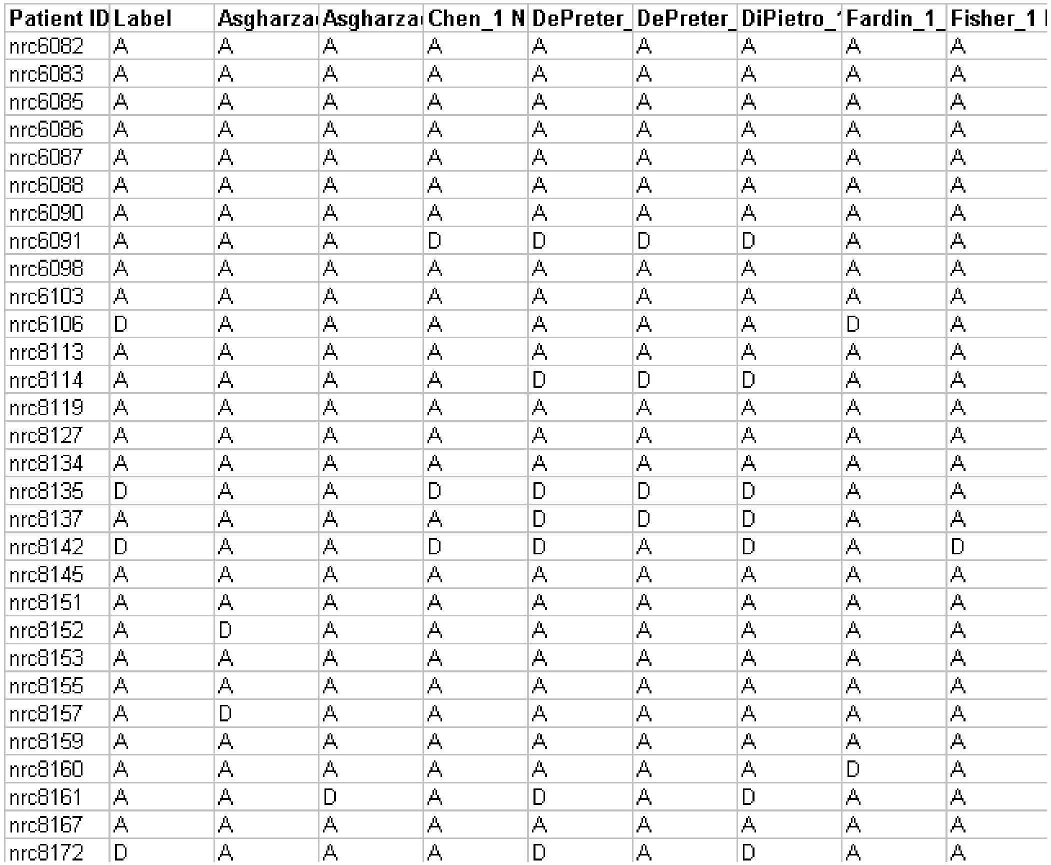
Classifiers validation on external dataset DS2 (53 patients)
(cut-off on 80% prediction accuracy and best classifiers selection)

Classifier 1
Classifier 2
Classifier 3
Classifier …
Classifier n

- Selection of the best single signature classifiers: evaluation prediction accuracy (>80%), sensitivity, specificity and recall.

DS2 transformed in Prediction Matrix (NB-MusE-classifier training set, 53 patients)

Prediction matrix assembled from the predictions performed on DS2 by the best single-signature classifiers selected during the first phase



Patient ID	Label	Asgharza	Asgharza	Chen_1 N	DePreter_	DePreter_	DiPietro_	Fardin_1_	Fisher_1
nrc6082	A	A	A	A	A	A	A	A	A
nrc6083	A	A	A	A	A	A	A	A	A
nrc6085	A	A	A	A	A	A	A	A	A
nrc6086	A	A	A	A	A	A	A	A	A
nrc6087	A	A	A	A	A	A	A	A	A
nrc6088	A	A	A	A	A	A	A	A	A
nrc6090	A	A	A	A	A	A	A	A	A
nrc6091	A	A	A	D	D	D	D	A	A
nrc6098	A	A	A	A	A	A	A	A	A
nrc6103	A	A	A	A	A	A	A	A	A
nrc6106	D	A	A	A	A	A	A	D	A
nrc8113	A	A	A	A	A	A	A	A	A
nrc8114	A	A	A	A	D	D	D	A	A
nrc8119	A	A	A	A	A	A	A	A	A
nrc8127	A	A	A	A	A	A	A	A	A
nrc8134	A	A	A	A	A	A	A	A	A
nrc8135	D	A	A	D	D	D	D	A	A
nrc8137	A	A	A	A	D	D	D	A	A
nrc8142	D	A	A	D	D	A	D	A	D
nrc8145	A	A	A	A	A	A	A	A	A
nrc8151	A	A	A	A	A	A	A	A	A
nrc8152	A	D	A	A	A	A	A	A	A
nrc8153	A	A	A	A	A	A	A	A	A
nrc8155	A	A	A	A	A	A	A	A	A
nrc8157	A	D	A	A	A	A	A	A	A
nrc8159	A	A	A	A	A	A	A	A	A
nrc8160	A	A	A	A	A	A	A	D	A
nrc8161	A	A	D	A	D	A	D	A	A
nrc8167	A	A	A	A	A	A	A	A	A
nrc8172	D	A	A	A	D	A	D	A	A

…. **Signature IDs**

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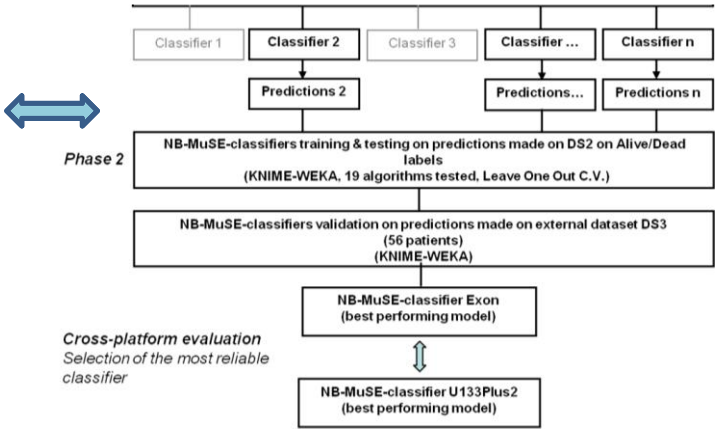
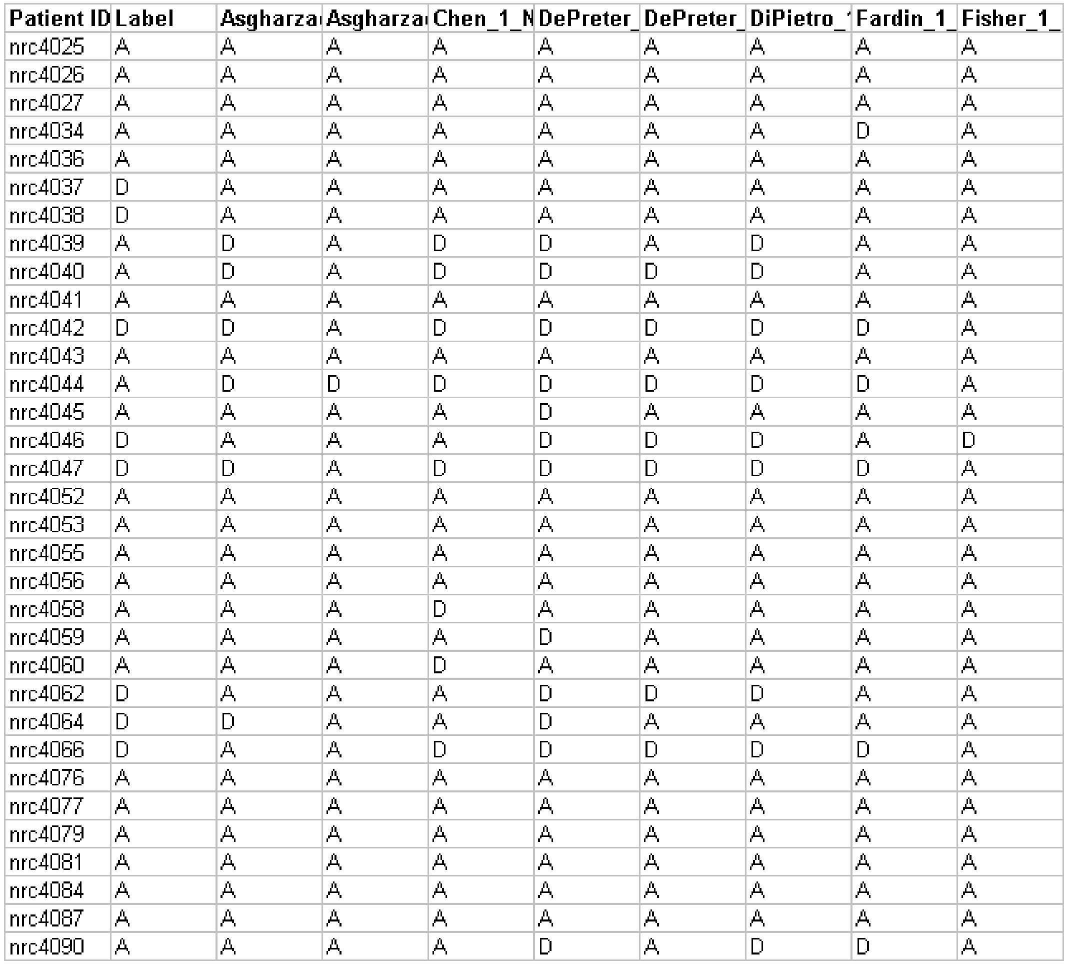

Classifier 1
Classifier 2
Classifier 3
Classifier …
Classifier n
Predictions 2
Predictions…
Predictions n
Phase 2
NB-MuSE-classifiers training & testing on predictions made on DS2 on Alive/Dead labels
(KNIME-WEKA, 19 algorithms tested, Leave One Out C.V.)
NB-MuSE-classifiers validation on predictions made on external dataset DS3
(56 patients)
(KNIME-WEKA)
NB-MuSE-classifier Exon
(best performing model)
Cross-platform evaluation
Selection of the most reliable classifier
NB-MuSE-classifier U133Plus2
(best performing model)

DS3 transformed in Prediction Matrix (NB-MusE-classifier validation set, 56 patients)

Prediction matrix assembled from the predictions performed on DS3 by the best single-signature classifiers selected during the first phase



Patient ID	Label	Asgharza	Asgharza	Chen_1_N	DePreter_	DePreter_	DiPietro_	Fardin_1_	Fisher_1_
nrc4025	A	A	A	A	A	A	A	A	A
nrc4026	A	A	A	A	A	A	A	A	A
nrc4027	A	A	A	A	A	A	A	A	A
nrc4034	A	A	A	A	A	A	A	D	A
nrc4036	A	A	A	A	A	A	A	A	A
nrc4037	D	A	A	A	A	A	A	A	A
nrc4038	D	A	A	A	A	A	A	A	A
nrc4039	A	D	A	D	D	A	D	A	A
nrc4040	A	D	A	D	D	D	D	A	A
nrc4041	A	A	A	A	A	A	A	A	A
nrc4042	D	D	A	D	D	D	D	D	A
nrc4043	A	A	A	A	A	A	A	A	A
nrc4044	A	D	D	D	D	D	D	D	A
nrc4045	A	A	A	A	D	A	A	A	A
nrc4046	D	A	A	A	D	D	D	A	D
nrc4047	D	D	A	D	D	D	D	D	A
nrc4052	A	A	A	A	A	A	A	A	A
nrc4053	A	A	A	A	A	A	A	A	A
nrc4055	A	A	A	A	A	A	A	A	A
nrc4056	A	A	A	A	A	A	A	A	A
nrc4058	A	A	A	D	A	A	A	A	A
nrc4059	A	A	A	A	D	A	A	A	A
nrc4060	A	A	A	D	A	A	A	A	A
nrc4062	D	A	A	A	D	D	D	A	A
nrc4064	D	D	A	A	D	A	A	A	A
nrc4066	D	A	A	D	D	D	D	D	A
nrc4076	A	A	A	A	A	A	A	A	A
nrc4077	A	A	A	A	A	A	A	A	A
nrc4079	A	A	A	A	A	A	A	A	A
nrc4081	A	A	A	A	A	A	A	A	A
nrc4084	A	A	A	A	A	A	A	A	A
nrc4087	A	A	A	A	A	A	A	A	A
nrc4090	A	A	A	A	D	A	D	D	A

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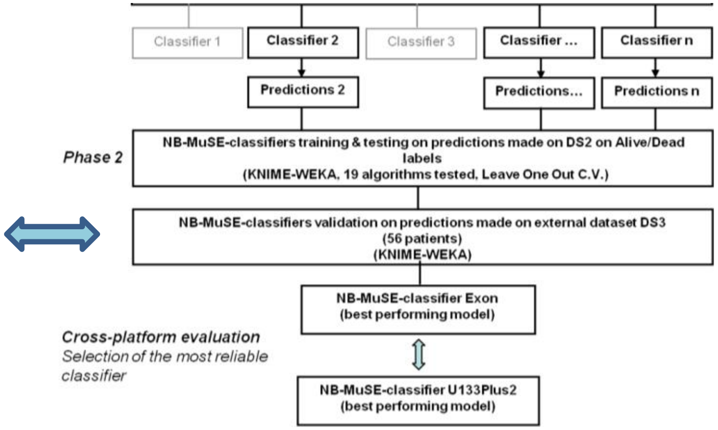
…. **Signature IDs**



Phase 2

NB-MuSE-classifiers training & testing on predictions made on DS2 on Alive/Dead labels
(KNIME-WEKA, 19 algorithms tested, Leave One Out C.V.)

NB-MuSE-classifiers validation on predictions made on external dataset DS3
(56 patients)
(KNIME-WEKA)

NB-MuSE-classifier Exon
(best performing model)

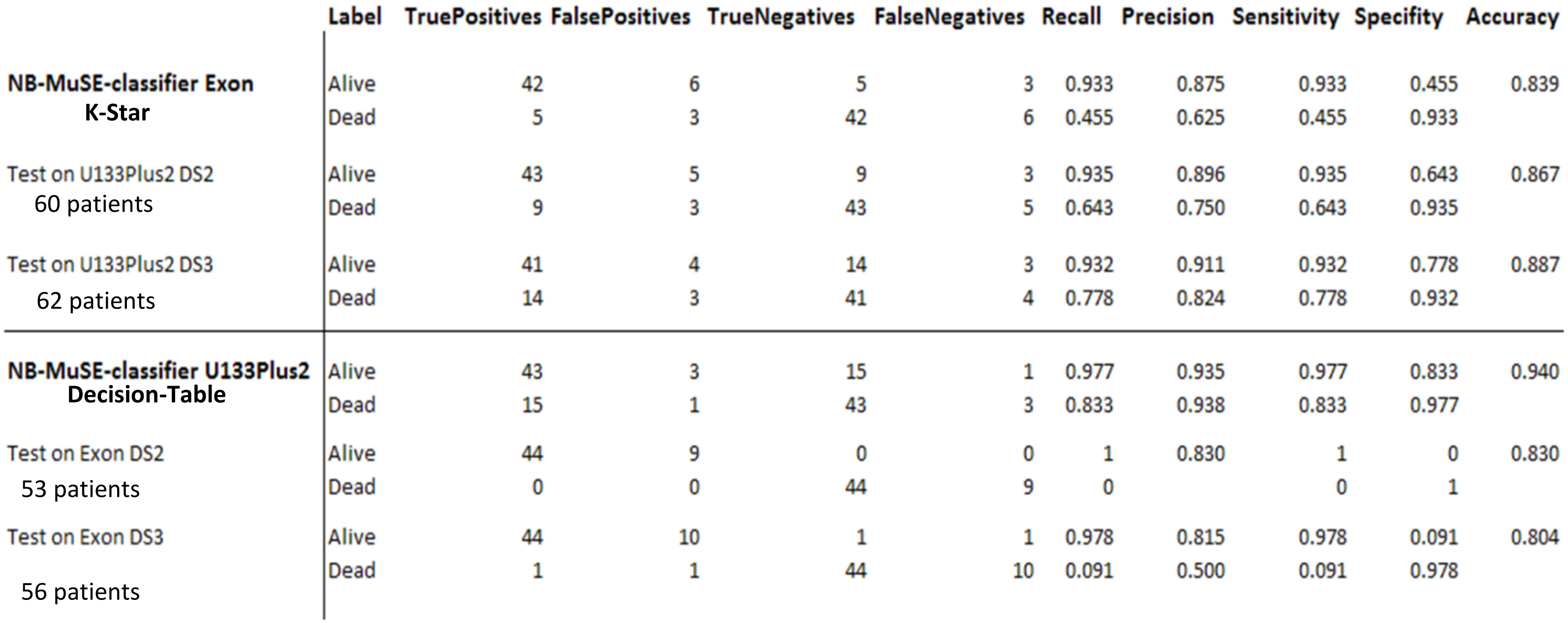
NB-MuSE-classifier U133Plus2
(best performing model)

Cross-platform evaluation
Selection of the most reliable classifier

DS2 and DS3 transformation steps are the core of the Cross Platform feature

Preliminary Results

Cross-platform evaluation of Multi-Signature Classifiers performance.
The resulting multi-signature classifiers have been cross-tested on the external datasets and the relative performance has been evaluated. The Exon based NB-MuSE-classifier showed higher stability and reliability across the test datasets.



	Label	TruePositives	FalsePositives	TrueNegatives	FalseNegatives	Recall	Precision	Sensitivity	Specifity	Accuracy
NB-MuSE-classifier Exon **K-Star**	Alive	42	6	5	3	0.933	0.875	0.933	0.455	0.839
	Dead	5	3	42	6	0.455	0.625	0.455	0.933	
Test on U133Plus2 DS2 60 patients	Alive	43	5	9	3	0.935	0.896	0.935	0.643	0.867
	Dead	9	3	43	5	0.643	0.750	0.643	0.935	
Test on U133Plus2 DS3 62 patients	Alive	41	4	14	3	0.932	0.911	0.932	0.778	0.887
	Dead	14	3	41	4	0.778	0.824	0.778	0.932	
NB-MuSE-classifier U133Plus2 **Decision-Table**	Alive	43	3	15	1	0.977	0.935	0.977	0.833	0.940
	Dead	15	1	43	3	0.833	0.938	0.833	0.977	
Test on Exon DS2 53 patients	Alive	44	9	0	0	1	0.830	1	0	0.830
	Dead	0	0	44	9	0		0	1	
Test on Exon DS3 56 patients	Alive	44	10	1	1	0.978	0.815	0.978	0.091	0.804
	Dead	1	1	44	10	0.091	0.500	0.091	0.978	

Conclusions

- We developed a new classification model based on Exon expression data testable on the prediction matrices previously assembled from U133Plus2 data

- We successfully tested the cross-platform feature of NB-MuSE-classifier

- We have been able to evaluate and compare the two classifiers performance on respectively 109 and 122 (DS2+DS3) new neuroblastoma patients.

Future Directions

- Optimization of classifiers learning parameters and cross-validation set-ups
- Optimization of a-priori information selection (NB-related signatures)
- Test on randomized datasets
- Integration of arrayCGH and miRNA datasets