

Molecular Classification of Cutaneous Malignant Melanoma by Gene Expression Profiling

Supplement II - Statistical Analysis of Clinical and Culture Characteristics of Melanoma Clusters

Contact:

Michael Radmacher and R. Simon
Biometric Research Branch
National Cancer Institute
Bethesda, Maryland 20892
(Correspondence: mdradmac@helix.nih.gov)

SUMMARY REPORT:

Thirty-one tissue specimens were clustered using the Bioclust clustering algorithm (see text), resulting in one tight cluster of 19 specimens (Group A) and 12 specimens that showed no specific clustering pattern (Group B). We performed statistical tests to determine whether any clinical or tumor cell characteristics were specifically associated with cluster group. For categorical variables we created a contingency table and used Fisher's exact test to compute a p-value (the Chi-square test was not used because each table had at least one expected cell frequency less than 5). For continuous and ordered variables, we used the Wilcoxon two-sample (rank-sum) test, a non-parametric alternative to the two-sample *t* test. Tests were performed in S-plus 4.5 and StatXact 3.1.

The two groups consisted of the following patient IDs:

Group A				Group B		
M93-007	M91-054	UACC091	UACC502	HA-A	UACC827	UACC1529
UACC1256	UACC1273	UACC2534	M92-001	UACC647	UACC930	M93-047
UACC457	UACC383	UACC3093	A-375	UACC2837	TC-F027	WM1791C
UACC1022	TD1376-3	TD1683	TD1720	UACC1012	UACC1097	UACC903
TD1384	TD1730	TC1376-3				

As noted in the text, two pairs of specimens in Group A were derived from the same patient. The two pairs are M93-007 & M92-

001 and TD1376-3 & TC1376-3. In our analyses, we only considered the data for each of these patients once or, as specifically noted, entirely removed the specimens for these patients from the analysis.

We first performed an analysis that included all specimen types (tissues and cell lines). We tested for associations between group and the following variables: sex, age, mutation status, biopsy site*, pigment, Breslow thickness, Clark level, and specimen type. There was no variable tested, which was shown to be associated with cluster group (at the 0.05 significance level).

Although there was not a statistically significant association between group and specimen type ($p=0.106$) it was noteworthy that all 5 tissue specimens were located in Group A. We therefore performed another analysis in which we only considered data from cell lines. In the analysis of cell lines, no variables were associated with cluster group at the 0.05 significance level, although “age” did have a marginal association ($p=0.0812$). Passage number was also tested in this analysis and had no association with group ($p=0.8570$).

Next, we investigated for differences in survival between the two cluster groups. We used a measure of survival that indicated survival time from the date of biopsy. Four cases (including the previous two) had a biopsy date falling in 1998 and a known status (alive or dead) for which a specific date of death or last follow-up was unknown. In order to use these cases in the survival analysis, the survival/follow-up time in these cases was arbitrarily set to 1 year if the biopsy date occurred prior to 7/1/98 or 0.5 years if the biopsy date occurred on or after 7/1/98.

The data used in the survival analysis are shown in Figure 1. A total of 15 cases were included in the analysis, 10 from Group A and 5 from Group B. Survival/follow-up times were rounded to the nearest quarter year. A Kaplan-Meier survival plot was created and log-rank test performed. No statistically significant association between group and survival was found ($p=0.135$).

* Biopsy site was broken down into the following three categories: skin/external (including ankle, abdomen/chest, shoulder, breast, neck/forehead and back), internal (including chest wall, distal ileum, paraspinal, thyroid lobe, small bowel, rectus muscle and intra-abdominal), and lymph nodes (including axillary, cervical and thigh femoral).

The analyses performed resulted in no significant association with cluster group. However, this does not necessarily mean associations do not exist between the groups and the clinical and tumor characteristics tested. The power of the tests we performed is limited by the amount of data available for each variable. For example, only 6 specimens in Group A and 3 in Group B have information on Breslow thickness. Finding significant associations with so few data is unlikely. The power of the tests would increase with more complete data on the existing specimens and by the addition of new specimens to the data set. Such studies are underway in our laboratory.

ANALYSIS OF ALL SPECIMENS:

Group A = specimens that cluster; Group B = others.

Two pairs of specimens in Group A (M93-007/M92-001 & TD1376-3/TC1376-3) were derived from the same patient. The clinical and tumor characteristics for each of these patients are only considered once in the below analyses.

SEX - no statistically significant association with group

Contingency table with Fisher's exact test

	A	B	
F	4	4	p-value = 0.6754
M	12	7	alternative hypothesis: two-sided

AGE - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.1397

data: x: age w/group = A , and y: age w/group = B

Mann-Whitney Statistic: $W = 102.0$, $n=15$, $m=10$

alternative hypothesis: two-sided

MUTATION STATUS - no statistically significant association with group

Contingency table with Fisher's exact test

	A	B	
mutated	2	4	p-value = 0.1713
deleted	6	1	alternative hypothesis: two-sided
WT	4	2	

Contingency table with Fisher's exact test

Combined mutated and deleted into one category.

	A	B	
mut./del.	8	5	p-value = 1
WT	4	2	alternative hypothesis: two-sided

BIOPSY SITE - no statistically significant association with group

Contingency table with Fisher's exact test

	A	B	
skin/external	3	3	p-value = 0.8763
internal	4	3	alt. hypothesis: two-sided
LN	7	4	

PIGMENT - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.2631

Pigment Type: light=1, med=2, dark=3

(amelanotic = light; tan = med; pigmented = dark.)

data: x: pig. type w/group = A , and y: pig. type w/group = B

Mann-Whitney Statistic: $W = 76.5$, $n=13$, $m=9$

alternative hypothesis: two-sided

BRESLOW THICKNESS - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.2619

data: x: thickness w/group = A , and y: thickness w/group = B

Mann-Whitney Statistic: $W = 14.0$, $n=6$, $m=3$

alternative hypothesis: two-sided

CLARK LEVEL - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.4481

Clark level: II=2, III=3, IV=4

data: x: Clark level w/group = A , and y: Clark level w/group = B

Mann-Whitney Statistic: $W = 19.5$, $n=6$, $m=5$

alternative hypothesis: two-sided

For the below analysis, the two pairs of specimens in Group A derived from the same patient (M93-007/M92-001 & TD1376-3/TC1376-3) were removed.

SPECIMEN TYPE - no statistically significant association with group

Contingency table with Fisher's exact test

	A	B	
cell line	11	12	p-value = 0.106
tissue4	0		alternative hypothesis: two-sided

ANALYSIS OF CELL CULTURES:

Group A = specimens that cluster; Group B = others.
A pair of cell lines in Group A (M93-007/M92-001) was derived from the same patient. The clinical and tumor characteristic for this patient is only considered once in the below analyses.

SEX - no statistically significant association with group

Contingency table with Fisher's exact test

	A	B	
F	4	4	p-value = 1
M	8	7	alternative hypothesis: two-sided

AGE - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.0812

data: x: age w/group = A , and y: age w/group = B

Mann-Whitney Statistic: $W = 80.0$, $n=11$, $m=10$

alternative hypothesis: two-sided

MUTATION STATUS - no statistically significant association with group

Contingency table with Fisher's exact test

	A	B	
mutated	2	4	p-value = 0.1713
deleted	6	1	alternative hypothesis: two-sided
WT	4	2	

Contingency table with Fisher's exact test

Combined mutated and deleted into one category.

	A	B	
mut./del.	8	5	p-value = 1
WT	4	2	alternative hypothesis: two-sided

BIOPSY SITE - no statistically significant association with group

Contingency table with Fisher's exact test

	A	B	
skin/external	2	3	p-value = 0.7272
internal	2	3	alt. hypothesis: two-sided
LN	6	4	

PIGMENT - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.4212

Pigment Type: light=1, med=2, dark=3

amelanotic = light; tan = med; pigmented = dark.

data: x: pig. type w/group = A , and y: pig. type w/group = B

Mann-Whitney Statistic: $W = 50.5$, $n=9$, $m=9$

alternative hypothesis: two-sided

BRESLOW THICKNESS - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.2000

data: x: thickness w/group = A , and y: thickness w/group = B

Mann-Whitney Statistic: $W = 8.0$, $n=3$, $m=3$

alternative hypothesis: two-sided

CLARK LEVEL - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.6349

Clark level: II=2, III=3, IV=4

data: x: Clark level w/group = A , and y: Clark level w/group = B

Mann-Whitney Statistic: $W = 13.0$, $n=4$, $m=5$

alternative hypothesis: two-sided

For the below analysis, the pair of specimens derived from the same patient in Group A (M93-007/M92-001) was removed.

PASSAGE NUMBER - no statistically significant association with group

Wilcoxon rank-sum test: p-value = 0.8570

Passage #'s for established cell lines were set equal to 21.

data: x: passage # w/group = A , and y: passage # w/group = B

Mann-Whitney Statistic: $W = 34.0$, $n=8$, $m=8$

alternative hypothesis: two-sided

Contingency table with Fisher's exact test

	A	B	
1-5	3	4	p-value = 0.8695
6-10	4	2	alternative hypothesis: two-sided
11-20	4	5	
>20	1	1	

SURVIVAL ANALYSIS:

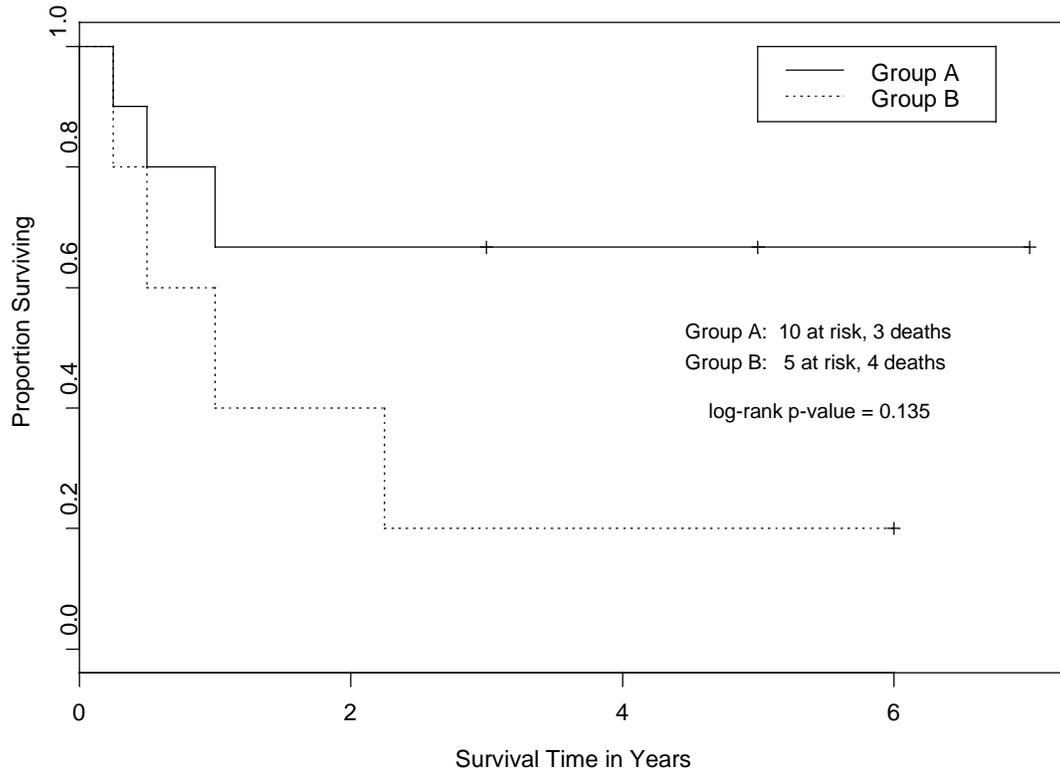
Data used in the survival analysis:

<u>Pt.ID</u>	<u>Group</u>	<u>Status</u>	<u>Time</u>
M93-007	A	0	7
M91-054	A	0	7
UACC091	A	0	7
UACC502	A	1	0.5
UACC2534	A	1	0.25
TD1683	A	1	1
TD1720	A	0	0.5
TD1348	A	0	5
TD1730	A	0	0.5
TC1376-3	A	0	3
UACC827	B	1	0.5
UACC930	B	1	2.25
M93-047	B	0	6
TC-F027	B	1	1
UACC903	B	1	0.25

Status: 0 = alive, 1 = dead

Time is in years.

Kaplan-Meier Survival Plot - All Specimens



There is not a statistically significant association between group and survival (p-value=0.135).