Can melanoma treatment be guided by a panel of predictive and prognostic microRNA Biomarkers?

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Survival is stage dependant at time of diagnosis

Initial Project Background

- No effective biomarkers that were sensitive or specific enough to be beneficial for early diagnosis

- Melanoma progression markers are infrequently used due to their lack of adequate precision to detect disease relapse.

- Tumours have been shown to release microRNA (miRNA) into the blood, which is indicative of tumour progression.

- miRNAs exhibit ‘tissue-specific’ expression.

- In serum/plasma and FFPE tissues - miRNAs are “resistant” to degradation

  ✓ Excellent biomarker potential
Origin of circulating miRNA

Melanoma-miRNA Discovery

Objectives

- Profile all currently known miRNAs (miRBase v18 at study design)
  - melanoma cell lines (n=55)
  - other solid malignancies (breast, ovarian, prostate, colorectal, etc) (n=34)

- Discover melanoma ‘enriched’ miRNA (Aim 1)
- Serum expression analysis (biomarker identification) (Aim 2)
- Correlate with progression (Aim 3)
55 Melanoma cell lines Vs. 34 ‘other’ solid malignancies
- Mann-Whitney (unpaired)
- \(P\) value \(\leq 0.05\); Fold Change \(\geq 2\); Benjamini-Hochberg (FDR)

233 miRNAs (\(P\) value \(\leq 0.05\); Fold Change \(\geq 2\))
Hierarchical clustering allows for the Discovery of melanoma-enriched miRNAs

Selected Gene Tree: Normalised data (All Samples)_1-Way ANOVA_MELvsOthersvsNORM_p.0.05_287genes
Selected Condition Tree: Normalised data (All Samples)1-Way ANOVA_MELvsOthersvsNORM_p.0.05_287genes
Branch color parameter: Tissue Type
Gene List: Selected (287)

miRNA genes

Red = Up-regulated
Green = Down-regulated

Stark et al. (2015) Oncotarget
Serum Cohorts Analysed - Multi-Centre Study

‘Controls’

- no history of melanoma (n=102)
- previous melanoma history (n=16)
- high nevus count (n=12)

‘Melanoma’

- stage I/II (n=86)
- stage III (n=50)
- stage IV (n=119)
High Throughput and Sensitive Detection
Heatmap of miRNA expression in serum

Stage IV melanoma patients

Healthy Controls with no melanoma

Marker Controls

v. high

high

medium

low

v. low

zero

Spike-in Control

miRNAs
Significant differences comparing ‘Melanomas’ vs. ‘Controls’

hsa-miR-211-5p

Median Normalised CT value

<table>
<thead>
<tr>
<th>Stage</th>
<th>Controls</th>
<th>Stage I/II</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

- Controls: ns, p=0.025
- Stage I/II: p<0.0001

Stark et al. (2015) EbioMedicine
MELmiR-7 panel members can classify Stage I/II vs Stage IV

AUC=0.99

Stark et al. (2015) EbioMedicine
MELmiR-7 panel members can classify Stage III vs Stage IV

AUC=0.99

Stark et al. (2015) EbioMedicine
Potential for predicting stage IV survival

Stark et al. (2015) EbioMedicine

Stage at Blood Draw
≤ Stage III
p<0.001

≤ Stage I/II
100% (n=86)

> Stage III
miR-211
≥ Ct 24.01 (low)

≤ Ct 24.0 (high)
36% (n=44)

4.8 months

100% (n=50)

2.7 years

63% (n=75)
Potential for monitoring tumour burden

Stark et al. (2015) EbioMedicine
Research Paper

The Prognostic and Predictive Values of Melanoma-related MicroRNAs Using Tissue and Serum: A MicroRNA Expression Analysis

Mitchell S. Stark a,b,*, Kerenhaftali Klein c,d, Benjamin Weide e, Lauren E. Haydu f,g, Annette Pflugfelder c,h, Yue Hang Tang i, Jane M. Palmer a, David C. Whiteman j, Richard A. Scolyer f,g, Graham J. Mann f,g, John F. Thompson f,g, Georgina V. Long f,g, Andrew P. Barbour i, H. Peter Soyer h, Claus Garbe e, Adrian Herington b, Pamela M. Pollock b, Nicholas K. Hayward a
Can melanoma treatment be guided by a panel of predictive and prognostic microRNA Biomarkers?
Potential for monitoring disease activity

Stark et al. (2016) Unpublished

Fold Change Relative to Initial Blood draw

Complete Responder (CR)

Blood Sampling Dates

- PR noted via PET *
- SD noted via PET **
- New scalp lesion noted on PET ***
- CR noted via PET ****

Dabrafenib + Trametinib
Potential for monitoring disease activity

Stark et al. (2016) *Unpublished*

**Partial Responder (PR)**

* PR noted via PET

Fold Change Relative to Initial Blood draw

Blood Sampling Dates

- Ipilimumab
- Pembrolizumab
Potential for monitoring disease activity

**Stark et al. (2016)**
*Unpublished*

Progressive Disease (PD)

- Brain Met *
- PD noted via CT **
- Cyberknife treatment ***
- PD noted via CT ****
- Death from Melanoma ☠

Blood Sampling Dates

1/04/2014 7/05/2014 29/07/2014 20/08/2014

Fold Change Relative to Initial Blood draw
What’s next?

• Alignment with a melanoma clinical trial using targeted therapies in stage IIIc and IV (n=50-100) and Stage IIIa/b (n=50)
  • Centres – Brisbane and Perth
  • Serial collection of bloods every 3 weeks up to 12mths (IIIc/IV)
  • Serial collection monthly for 12mths (IIIa/b)

• Expected Results:
  • Steady increase in relative miRNA expression = progressive disease (i.e. Treatment resistance)
  • Steady decrease in relative miRNA expression = reduction in tumour burden (i.e. effective treatment)
Acknowledgments and Funding

Prof. H. Peter Soyer
A/Prof Helmut Schaider
A/Prof Andrew Barbour

Prof. Claus Garbe
Dr Benjamin Weide
Dr Annette Pflugfelder

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Dr Benjamin Weide
Dr Annette Pflugfelder

Dr Elin Gray
Prof. Mel Ziman

Prof. Nick Hayward
Dr Kere Klein
Prof. David Whiteman
Jane Palmer

Prof. Richard Scolyer
Prof. Graham Mann
Prof. Georgina Long
Dr. Lauren Haydu
Dr. James Wilmott

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A/Prof. Pam Pollock
Prof. Adrian Herington

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Prof. Michael Millward
A/Prof. Adnan Khattak

Funding

Merchant Charitable Foundation

Dr Victoria Atkinson
Dr W. Phillip Law

Dr Victoria Atkinson
Dr W. Phillip Law

Australian Government
National Health and Medical Research Council

Australian Government
National Health and Medical Research Council
SAVE THE DATE!

9th WORLD CONGRESS OF MELANOMA
A JOINT MEETING WITH THE SOCIETY FOR MELANOMA RESEARCH

www.worldmelanoma2017.com