Comprehensive Molecular Characterization of Regional Metastatic Melanoma

Ian Watson
On behalf of Melanoma AWG and TCGA

Tuesday May 13th, 2014
TCGA SKCM AWG Contributors

Co-Chairs: Lynda Chin, Jeffrey Gershenwald and Ian Watson
Analysis Coordinator: Terrence Wu
Data Coordinator: Lihua Zou
Manuscript Coordinator: Ian Watson
NIH/NCI: Ina Felau, Margi Sheth, Kenna Shaw, Jean Claude Zenklusen
Broad Firehose Team: Dan Dicara, Lihua Zou, Mike Noble, Gad Getz

Clinical data: Jeffrey Gershenwald, Richard Scolyer, William Burns, Genevieve Boland, Stergios Moschos
Graham Mann, John Thompson
Pathology: Richard Scolyer, Alex Lazar, Ken Tsai, Carolyn Shaiu, James Wilmott, Lauren Haydu, Jeff Gershenwald Victor Prieto
Whole exome sequencing analysis: Lihua Zou, Gad Getz, Mike Lawrence, Eran Hodis, Carrie Sougnez, Terrence Wu, Ian Watson
Copy number: Andrew Cherniack, Terrence Wu, Ian Watson
Methylation: Leslie Cope, Ludmila Danilova, James Herman
miRNAseq: Gordon Robertson, Da Yang, Andy Chu, Denise Brooks, Reanne Bowlby, Payal Sipahimalani, Andy Mungall, Yussanne Ma, Steven Jones, Marco Marra, Anders Jacobsen
RNAseq: Norman Sharpless, William Jeck, Katherine Hoadley, Stergios Moschos
LowP WGS: Raju Kucherlapati, Peter Park, Per Wu, Lixing Yang, Terrence Wu, Angela Hadjipanayis, Semin Lee, Xingzhi Song, Seth Sahil, Jianhua Zhang, Alexei Protopopov
RPPA: Gordon Mills, Scott Woodman, Jason Roszik, Mike Davies, Terrence Wu
Pathway Analysis: Chris Sander, Niki Schultz, Giovanni Ciriello, Anil Korkut, Ronglai Shen
Integrative Analysis: Sheila Reynolds

...And the rest of the TCGA Community ... the patients
Primary Focus: Untreated Regional Metastases

- Paradigm shift from nearly all other TCGA efforts (80% of samples analyzes are metastatic origin)
- Originating from non-glabrous skin primaries (exclude - palm of hand and soles of feet) – reduce heterogeneity
- No prior systemic treatment (exception – IFN >90 days ok)

- **Rationale**
  - Scarcity of frozen primary tumor tissue in sufficient quantity
  - If discovered early melanoma highly curable, however, 5-year survival rate drops to 62% for regional disease (ACS: Cancer Facts and Figures 2012)
  - Most common first site of metastasis in melanoma (lymph node and regional subcutaneous cutaneous sites)
TCGA Subtypes

Note: only 2 cases with matched primary and metastatic samples

<table>
<thead>
<tr>
<th>Metastatic Subtypes</th>
<th>nSamples</th>
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<tbody>
<tr>
<td>Regional_Lymph_Node</td>
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<tr>
<td>Regional_Skin_or_Soft_Tissue</td>
<td>52</td>
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<tr>
<td>Distant_Metastases</td>
<td>35</td>
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</tbody>
</table>

Subtypes

- All Primaries: 67 samples
- Regional Lymph Nodes: 160 samples
- Regional Skin and Soft Tissue: 52 samples
- All Distant: 35 samples

MDACC
William Burns
Genevieve Boland
Jeffrey Gershenwald
Manuscript Data Freeze (2014_02_23) (Batches 180, 198, 206, 240, 262, 277, 291)
Melanoma has the Highest Mutation Rate of Cancers Sequenced to Date

Somatic mutation frequencies observed in exomes from 3,083 tumour–normal pairs.
A breakdown of mutation rates per category discovered for this individual set.

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<th>category</th>
<th>n</th>
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<th>relative_rate</th>
<th>exp_ns_s_ratio</th>
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<td>105743</td>
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<td>2.5</td>
<td>1.6</td>
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<tr>
<td>(A/G)p*G-&gt;T</td>
<td>11812</td>
<td>2126234554</td>
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<td>5.6</td>
<td>0.33</td>
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<td>1.2e-06</td>
<td>1.2</td>
<td>0.073</td>
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<td>17</td>
<td>1</td>
<td>3.5</td>
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<table>
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<th>count</th>
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<tr>
<td>Translation_Start_Site</td>
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</table>

| Total             | 228987  |
Significantly Mutated Genes by MutSig

Mutational heterogeneity in cancer and the search for new cancer-associated genes


-Identified 42 significantly mutated genes (Q <0.1)
Significantly Mutated Genes by InVEx

InVEx:
 permutes called mutations within a gene’s exons and introns (the covered region sequenced) and asks whether in the real data we observe more ‘non-silent’ mutations than in the permutations (which approximate what we might expect by chance).

A Landscape of Driver Mutations in Melanoma

Mapping the Hallmarks of Lung Adenocarcinoma with Massively Parallel Sequencing

Broad Institute
Eran Hodis
Landscape of Somatic Mutations
(InVEx, P. Bonferroni <0.05, Q <0.1)
(MutSig, Q <0.1, 13/42)

Fisher's exact test:
- BRAF hotspot– NRAS: p-value = 6.461e-16
- NRAS – NF1: NS
- BRAF hotspot- NF1: p-value = 1.888e-06
NF1 Mutated in 14% of Samples:

<table>
<thead>
<tr>
<th>Mutation type</th>
<th>n_Total</th>
<th>% of mutation total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsense</td>
<td>22</td>
<td>36%</td>
</tr>
<tr>
<td>Loss of function</td>
<td>6</td>
<td>10%</td>
</tr>
<tr>
<td>Splice site</td>
<td>6</td>
<td>10%</td>
</tr>
<tr>
<td>Frame Shift Deletions</td>
<td>3</td>
<td>5%</td>
</tr>
<tr>
<td>Missense</td>
<td>21</td>
<td>34%</td>
</tr>
<tr>
<td>Silent</td>
<td>9</td>
<td>15%</td>
</tr>
<tr>
<td>n_Loss of Function</td>
<td>31</td>
<td>51%</td>
</tr>
<tr>
<td>n_Total:</td>
<td>61</td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing mutation types and locations]

*MDACC*  
Ian Watson  
Terrence Wu  
Lynda Chin  
Broad Institute  
Lihua Zou
Co-occurring *NF1* and *BRAF* Mutations:

Exon 11 mutations

- P.G466E
- P.S467L
- P.G468R

- P.N581S
- P.D594N
- P.V600E
- P.V600K

ATP (By similarity).

Missense_Mutation

MDACC
Ian Watson
Terrence Wu
Lynda Chin

The Cancer Genome Atlas
Landscape of Somatic Mutations
(InVEx, P. Bonferroni <0.05, Q <0.1)
(MutSig, Q <0.1, 13/42)

Subgroups:

1) **BRAF**
   - V600E/R/K
   - K601E

2) **RAS**:
   - Q61R/K/L/H
   - G12R/D/A
   - G13R/D

3) **NF1**:
   - All NF1 mutants

4) **Triple wild-type**
SCNAs in Each Genetic Subtype

**BRAF hotspot**
- \( n = 122 \)

**NRAS hotspot**
- \( N = 70 \)

**NF1 mutant**
- \( N = 38 \)

**Triple wild-type**
- \( N = 39 \)

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**Broad Institute**
- Andy Cherniack

**MDACC**
- Ian Watson
- Terrence Wu
- Lynda Chin

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The Cancer Genome Atlas
Landscape of Copy Number Gains and Cosmic Mutations in Melanoma

Triple wild-type

MDACC
Ian Watson
Terrence Wu
Negative Enriched Copy Number Gains in BRAF/NRAS/NF1 Mutant Hotspot Samples

% of sub group (BRAF/NRAS/NF1/wild-type) harboring an amplification in the indicated gene

*Fisher's test (BRAF comparison)
Segmented value above 0.5 (no consideration for focality)
Fusion Analysis Flowchart

Harvard Medical School/Brigham & Women’s Hospital/MD Anderson Cancer Center
Alexei Protopopov, Netty Santoso, Semin Lee, Michael Parfenov, Jianhua Zhang, Harshad S. Mahadeshwar, Jiabin Tang, Xiaojia Ren, Sahil Seth, Lixing Yang, Andrew W. Xu, Xingzhi Song, Angeliki Pantazi, Christopher A. Bristow, Ruibin Xi, Angela Hadjipanayis, Jonathan Seidman, Lynda Chin, Peter J. Park, Raju Kucherlapati

RNA-Seq:
1. 338 samples
2. Three callers (Mapsplice; ChimeraScan; PRADA)

DNA Deep Seq:
1. 38 samples
2. One caller (MeerKat)

Low Pass DNA Seq:
1. 119 samples
2. One callers (BreakDancer; MeerKat)

Filters:
1. 1 junction read
2. 2 discordant reads
3. Homologous filter

Filters for detection of drivers:
1. Fusion centrality
2. Germline fusions
3. Fusions with function domains

Investigate potential fusion detection rates using intragenic copy number
Integration of all potential fusions Identified by all callers
784 were detected by at least two callers

221 potential fusion drivers

Poster #63
<table>
<thead>
<tr>
<th>Gene1</th>
<th>Gene2</th>
<th>Samples</th>
<th>Mutation Subtype N of Callers</th>
<th>Domain_kept</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATG7</td>
<td>BRAF</td>
<td>TCGA-BF-A5EP-01</td>
<td>Unknown</td>
<td>2</td>
</tr>
<tr>
<td>TAX1BP1</td>
<td>BRAF</td>
<td>TCGA-D3-A2JC-06A</td>
<td>Triple Wild-type</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: 1. PF07714 is **Protein kinase_Tyr domain**

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**HMS/MDACC GCC**
Per Wu, Chris Bristow, Lixing Yang, Terrence Wu, Angela Hadjipanayis, Semin Lee, Peter Park, Lynda Chin, Raju Kucherlapati

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**Poster #63**
Differential MAPK Signaling in Genetic Subtypes
(190 antibodies on 201 cases)

MDACC
Terrence Wu
Scott Woodman
Jason Roszik
Mike Davies
Gordon Mills

MSKCC
Debyani Chakravarty

**MAPK1/3**

**MEK1 S217/221**

**ERK1/2 T202/Y204**
Recurrent Mutations in *IDH1* in Melanoma (~5%) • R132C caused by C>T transition (UVB)
Pattern of High DNA Methylation at CpG Islands is Associated with *IDH1* Mutations

Johns Hopkins University
Leslie Cope
Ludmila Danilova
James Herman
Examples for Clinical Analysis

- **Days to Death/Last Follow Up**
  - From Time of Initial Diagnosis = OS
  - From Time sample procurement = “TCGA survival”
  - Difficulty is that this may be influenced by unaccounted for factors

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**Overall Survival**

- Initial Dx
- Sample Procurement
- Death or Last Follow-UP

**Time**

“TCGA Survival”

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MDACC
Terrence Wu
William Burns
Genevieve Boland
Jeffrey Gershenwald
Hierarchical clustering identifies melanomas subgroups with elevated epithelioid, lymphocytic and MITF expression signatures not associated with mutation status.
Lymphocyte Score by Tissue Source Sites

- Primary Tumor (N=46)
- Regional Skin or Soft Tissue (N=52)
- Distant Skin or Soft Tissue (N=21)
- Regional Lymph Node (N=161)
- Distant Lymph Node (N=6)

Pathology Team
- Richard Scolyer (Sydney)
- Alex Lazar (MDACC)
- Ken Tsai (MDACC)
- Carolyn Shaiu (Vancouver)
- James Wilmott (Sydney)
- Lauren Haydu (stats, Sydney/MDACC)
- Jeff Gershenwald (MDACC)
- Victor Prieto (MDACC)

Tumors from Lymph nodes have higher Lymphocyte Infiltration
Lymphocyte Infiltration vs TCGA Survival

All_Regional_Meta : TCGA Survival
Lymphocyte Score

Logrank P=0.00778, HazardRatio=1.7, C-index=0.628

Regional Lymph Node

Logrank P=0.0155, HazardRatio=1.8, C-index=0.635

↑ Lymphocyte Infiltration
=> Longer Survival
TERT Promoter Mutation in SKCM TCGA Data

Highly Recurrent TERT Promoter Mutations in Human Melanoma

- 106 TCGA melanoma tumors
  - C228T mutation in 25 tumors (23.6%)
  - C250T mutation in 41 tumors (38.7%), mutually exclusive with C228T
  - Wild-type TERT promoter in 40 tumors (37.7%)

TERT Promoter Mutations in Familial and Sporadic Melanoma

HMS/BWH/MDACC GCC
Angela Hadjipanayis, Terrence Wu, Franklin Huang, Lynda Chin, Raju Kucherlapati
TERT Promoter Mutation in SKCM TCGA Data (PCR and Sanger sequencing)

Rank-sum test: C228T >> WT, P=0.001

HMS/BWH/MDACC GCC
Terrence Wu, Angela Hadjipanayis, Franklin Huang, Lynda Chin, Raju Kucherlapati
Pathway Analysis: Melanoma Subtypes

**MSKCC**
Anil Korkut, Giovanni Ciriello, Niki Schultz, Chris Sander
Propose 4 genetically distinct subgroups of melanoma:

- **BRAF** mutant (hotspot)
- **RAS** mutant (hotspot)
- **NF1** LoF mutations (**BRAF/NRAS WT**) with UV signature
- **Triple wild-type** lacking UV signature driven by copy number alterations of known oncogenes

Integration of other data platforms identifies differential MAPK signaling pathways altering in genetic subtypes

Methylation clustering analysis identified a CIMP subtype enriched for IDH1 R132 mutations
Ongoing Analysis

- Incorporation of Oncosign analysis (Memorial Sloan-Kettering Cancer Center)
- miRNA clustering analysis (The Genome Sciences Centre, BC Cancer Agency)
- Primary vs Metastatic comparative analyses
- Genetic determinants of lymphocytic infiltration
# Manuscript Writing Committee

**MDACC**  
Lynda Chin (Co-Chair)  
Jeff Gershenwald (Co-Chair)  
Ian Watson (Co-Chair)  
Terrence Wu  
Genevieve Boland  
William Burns  
Alex Lazar  
Ken Tsai  
Scott Woodman

**Johns Hopkins University**  
Leslie Cope  
Ludmila Danilova

**Emory University**  
Keith Delman

**Melanoma Institute Australia**  
Richard Scolyer  
Graham Mann

**Memorial Sloan-Kettering Cancer Center**  
Anil Korkut  
Chris Sander

**Translational Genomics Research Institute**  
Jeffrey Trent

**University of North Carolina**  
Stergios Moschos

**The Weizmann Institute of Science**  
Yardena Samuels

** Firestore**  
Gordon Robertson

**Broad Institute**  
Lihua Zou  
Andrew Cherniack

**The Genome Sciences Centre, BC Cancer Agency**  
Gordon Robertson

**Institute for Systems Biology**  
Sheila Reynolds
## Acknowledgements

**Broad Institute**
- Douglas Voet
- Daniel DiCara
- Gordon Saksena
- Hailei Zhang
- David Heiman
- Juok Cho
- William Mallard
- Harindra Arachchi
- Michael Lawrence
- Petar Stojanov
- Lihua Zou
- Chip Stewart
- Scott Frazer
- Pei Lin
- Kristian Cibulskis
- Jaegil Kim
- Lee Lichtenstein
- Aaron McKenna
- Andrey Sivachenko
- Carrie Sougnez
- Lee Lichtenstein
- Steven Schumacher
- Raktim Sinha

**Belfer/DFCI/MDACC**
- Juinhua Zhang
- Spring Liu
- Sachet Shukla
- Terrence Wu

**IGV & GenePattern teams @ Broad**
- Jill Mesirov
- Michael Reich
- Peter Carr
- Marc-Danie Nazaire
- Jim Robinson
- Helga Thorvaldsdottir

**Broad Institute Leadership:** Todd Golub, Eric Lander

**Harvard Medical School**
- Matthew Meyerson
- Andrew Cherniack
- Juliann Chmielecki
- Rameen Beroukhim
- Scott Carter

- Peter Park
- Nils Gehlenborg
- Semin Lee
- Richard Park

**The Cancer Genome Atlas**
Acknowledgements Melanoma AWG
(all participants 04-01-2014)


The Cancer Genome Atlas
miRNA Analysis: Primary vs Metastatic Comparison

MDACC  
Da Yang
