Molecular Genetics of Sezary Syndrome

Pedro Horna, M.D.
Mayo Clinic
Rochester, MN
Chromosomal alterations on CTCL
High resolution CNV analysis


- Variably recurrent genomic gains and losses involving genes relevant to cancer.
- **MF:** loss of cell cycle inhibitors, gains of anti-apoptotic proteins.
- **SS:** activation of C-MYC, inhibition of p53, activation of cytokine signaling.
Copy number chromosomal alterations in Sezary Syndrome


- 16 patients with Sezary syndrome
- SNP array, inner track.
- WES read depth, outer track.
- Activation of MYC
- Inhibition of P53
- Activation of cytokine signaling.
Mycosis Fungoides

- Gains in antiapoptotic proteins
- Loss of cell cycle inhibitors
- Increased cell survival.

Sezary Syndrome

- Activation of C-MYC
- Inhibition of P53
- Activation of cytokine pathways
- Increased cell proliferation

Model adapted from U Leiden (Netherlands):
Gene mutations in CTCL by NGS

Complex and heterogeneous mutational landscape.

No single pathognomonic mutation or mutated gene.

Mutations occur in commonly affected pathways:
- Control of gene expression
- Cytokine signaling
- T-cell receptor signaling

(*) Genes mutated in WGS study of 5 t-MF
Whole exome sequencing in CTCL


- 25 SS and 8 MF.
- Again, mutations on genes involved in epigenetic regulation, cytokine signaling and TCR signaling.
- Mutations in P53.
Mutated driver genes in Sezary Syndrome


WES on 10 Sezary patients, followed by targeted sequencing of 549 genes on 101 patients with Sezary syndrome.

Sequence mutations (gold), in addition to amplification and deletions (green and red).

Frequent mutations in genes involved in DNA repair (including telomere maintenance), epigenetic regulators, and cell death.

In addition, mutations in genes involved in cytokine signaling (JAK1/3, STAT3, STAT5A/B), and TCR signaling (PLCG1, PRKCQ, NFKB1, NFATC2, CARD11)

No clear association between genetic alterations and survival.
Whole exome sequencing of Sezary syndrome at Moffitt

**Protocol**

- Cases where tumor cells >50% of MNC by FC.
- TCC protocol, logistics and biorepository.
- Sequencing at Hudson Alpha Genomic Lab (AL)
  - GATK WES pipeline.
  - 100x mean coverage
  - >90% 30x coverage
- Non-tumor controls: Flow sorted CD3-negative mononuclear cells.

**Progress Report**

- WES finalized on 8 unique patients (pending analysis of normal controls).
Preliminary analysis of 8 patients with Sezary syndrome

Variant selection

DP > 30x   VAF ≥ 10%   VAD ≥ 10   FS < 60
Excluded common variants
AF≥1% in 1000g, ESP, ExAC
Gene coding, non-synonymous
N = 2528

2447 Variants on non-cancer genes
81 (3.20%) variants on cancer genes

PolyPhen-2
19 (23.46%) Potentially Damaging
43 variants on 38 genes

COSMIC Cancer Gene Census (11/2015)
(572 genes)
Cancer gene mutations in Sezary syndrome

- No recurrent mutations but some recurrently mutated genes: DNMT3A (3), PRDM16, ESR1, and MECOM (2 each).

- DNMT3A mutations in 3 of 8 cases (38%), all novel
  - Splice donor variant, intron 19: XM_005264175.1:c.2322+1G>A
    Previously reported in 1 case of PTCL-NOS and 1 case of AML (COSM144495)
  - Exon 15 variant: c.1750T>G_p.Tyr584Asp, no COSMIC.
  - Exon 19 variant: c.2227C>T_p.Pro743Ser, no COSMIC.

- DNMT3A mutated cases had less mutational burden on other cancer genes (mean = 2.3) compared to non-DNMT3A mutated cases (mean = 6.6, p=0.0397)
### Cancer gene mutations by gene function in Sezary syndrome

- Mutations on genes related to:
  - Transcriptional regulation
  - Cytokine/growth factor signaling and, less commonly
  - T-cell receptor signaling.

- DNMT3A mutations and cytokine/growth factor signaling mutations appear to be exclusive.

<table>
<thead>
<tr>
<th>Function</th>
<th>Genes</th>
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<tbody>
<tr>
<td>Transcriptional regulation</td>
<td>DNMT3A, PRDM16, EZH2,</td>
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<tr>
<td></td>
<td>BCOR, TRIM24, MECOM</td>
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<tr>
<td>Transcription factors</td>
<td>ESR1, TAL1, TRIP11,</td>
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<tr>
<td></td>
<td>HNF1A, IKZF1, ETV5,</td>
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<td>CUX1</td>
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<td>T-cell receptor signaling</td>
<td>PLCG1, ITK</td>
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<tr>
<td>Cytokine/growth factor signaling</td>
<td>JAK2, LIFR, STAT5B,</td>
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<td>ROS1, IL21R, EPS15</td>
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<tr>
<td>Apoptosis</td>
<td>FAS, NDRG1</td>
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<tr>
<td>DNA repair</td>
<td>WRN, ERCC2, BRCA1</td>
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<tr>
<td>Mitosis</td>
<td>PCM1, NUMA1, EML4</td>
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<td>Cell adhesion/motility</td>
<td>MYH9, MLLT4, LPP</td>
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<tr>
<td>Others</td>
<td>CLTCL1, GOLGA5, GRIN2A,</td>
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<tr>
<td></td>
<td>PICALM, RANBP2, COL1A1</td>
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</tbody>
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- TCR signaling
- Epigenetic regulators
- Cytokine signaling
- Cell cycle
- DNA repair