Molecular Pathology of Cutaneous Melanoma

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Houston, Texas

Disclosure Information

• Nothing to disclose

Lecture Outline

• Melanoma diagnosis
• Mutations in melanoma
• Melanoma targeted therapy

Introduction

• Melanoma is the most deadly skin cancer
• Lifetime risk of developing melanoma 1:50
• American Cancer Society projection for 2013 will be 76,690 new diagnosis and 9,480 deaths

Photo provided by Dr. Kim
Distinction Among Melanocytic Nevi and Melanoma

- Morphologic features
- Immunohistochemical characterization
  - HMB45
  - Mart-1/Ki-67
- Molecular features
  - Comparative genomic hybridization (CGH)
  - Fluorescence in situ hybridization (FISH)

Morphologic Features of Melanocytic Nevi vs. Melanoma

- Architecture
  - asymmetric
- Border
  - poor circumscription
- Cytology
  - severe atypia
- Epidermal melanocytes
  - confluent proliferation
  - prominent Pagetoid extension
  - single cell pattern
- Dermal melanocytes
  - absence of maturation pattern
  - presence of host response
  - increase mitosis

Invasive Melanoma

Immunohistochemical Features of Melanocytic Nevi vs. Melanoma

- HMB45
  - Glycoprotein 100 in stage I and II melanosomes
  - Marker of melanocytic differentiation
- Mart-1/Ki-67
  - Mart-1 detects melanocytes
  - Ki-67 labels proliferating cells

HMB45 and Mart-1
Immunohistochemistry: HMB-45

Diffuse

Patchy

Immunohistochemistry: Mart-1/Ki-67

Nevus

Melanoma


Low in nevi

High in melanoma

Comparative Genomic Hybridization (CGH) in Melanocytic Nevi vs. Melanoma

- Analyzed 186 melanocytic tumors by CGH

96% of melanomas carry some chromosomal copy aberration: Gains or losses in discrete fragments of chromosomes


Comparative Genomic Hybridization (CGH)

Melanoma cell

"Normal" cell

Identify regions of chromosomal gains/amplifications versus losses/deletions

Limitations of CGH as a Diagnostic Test

- Expensive, labor intensive and technical expertise.
- Requires large amount of pure tumor cell population.
Fluorescence in situ hybridization (FISH)

• Allows evaluation of copy number at particular locus to distinguish nevi from melanoma
• Four FISH probe set commercially available:
  – RREB1 (6p25)
  – CEN6
  – MYB (6q23)
  – CCND1 (11q13)

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<th>Cut-Off</th>
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<td>RREB1 &gt; 2</td>
<td>&gt;16%</td>
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<tr>
<td>RREB1 &gt; CEN6</td>
<td>&gt;53%</td>
</tr>
<tr>
<td>MYB &lt; CEN6</td>
<td>&gt;42%</td>
</tr>
<tr>
<td>CCND1 &gt;2</td>
<td>&gt;9%</td>
</tr>
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FISH

• Overall sensitivity approximately 80% in unambiguous melanocytic lesions
  – 20% of melanomas may have negative FISH results
• FISH for ambiguous melanocytic lesions
  – Sensitivity as low as 50-40%
  – Awareness of polyploidy in Spitz tumors
  – Gain in entire chromosome (6p25, 11q13, and 6q23)
  – Misinterpreted as positive for melanoma

Second Generation FISH

• Four FISH probe set commercially available:
  – RREB1 (6p25)
  – CDKN2A (9p21)
  – MYC (8q24)
  – CCND1 (11q13)

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</tr>
<tr>
<td>CDKN2A</td>
<td>&gt;10% &gt;29%*</td>
</tr>
<tr>
<td>MYC &gt;2</td>
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</tr>
<tr>
<td>CCND1 &gt;2</td>
<td>&gt;19% &gt;29%*</td>
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Probe Set Sensitivity Specificity
First Generation 75% 96%
Second Generation 94%* 98%*

Diagnosis of Melanoma

• Involves integration of
  – Morphology
  – Immunohistochemistry
  – CGH or FISH
• Aware of polyploidy in Spitz nevi
  – Reduce false positive rate of FISH
  – Exclude tetraploid cells as abnormal cells (abnormal/total)

Lecture Outline

• Melanoma diagnosis
• Mutations in melanoma
• Melanoma targeted therapy
**Genetics of Melanoma**

- Recent insights in melanoma genetics
- Rapid, evolving molecular testing platforms from single gene analysis to whole exome sequencing
- Provided an exciting era of targeted therapy

**Somatic Mutations Cutaneous Melanoma**

- **G-protein**
- **KIT**
- **GNAQ/GNA11**
- **NRAS**
- **BRAF**
- **PI3K/AKT**
- **PTEN**
- **CDKN2A/p16**
- **BAP1**

**Cell Pathway**

- Inhibition of apoptosis, cell cycle progression, differentiation
- Inhibition of apoptosis, proliferation

**Mutations in Cutaneous Melanoma**

- **Somatic: Sporadic melanomas**
  - KIT
  - NRAS
  - BRAF
  - GNAQ/GNA11
  - BAP1
- **Germline: Hereditary melanoma**
  - CDKN2A/p16
  - BAP1

**Germline and Somatic Melanoma Mutations**

- **Hereditary (~6-12%)**
- **Sporadic (~90%)**
**KIT Mutation**

- **KIT** is a tyrosine kinase growth factor receptor
- **KIT** mutation promotes ligand-independent KIT dimerization and constitutive activation of MAPK and PI3K-AKT pathway to promote proliferative and survival advantage for melanoma cells
- **KIT** mutations and or increased copy numbers are seen in
  - acral lentiginous/mucosal melanomas
  - melanomas in skin with chronic sun damage (CSD)

**Approximate Frequency of Somatic Mutations in Cutaneous Melanoma**

<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>BRAF (%)</th>
<th>KIT (%)</th>
<th>NRAS (%)</th>
<th>GNAQ/ GNA11 (%)</th>
<th>BAP1 (%)</th>
</tr>
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<tr>
<td>Superficial spreading</td>
<td>50-60</td>
<td>1</td>
<td>20</td>
<td>not detected</td>
<td>&lt;10</td>
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<tr>
<td>Nodular</td>
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<tr>
<td>Lentigo maligna</td>
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</tr>
<tr>
<td>Acral lentiginous</td>
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<tr>
<td>Melanoma with blue nevus like features</td>
<td>not detected</td>
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<tr>
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<td>25-75% (APS)</td>
<td>not reported</td>
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- **Germline: Hereditary melanoma**
  - CDKN2A/p16
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**RAS**

- The RAS oncogene family includes NRAS, HRAS, and KRAS
  - Neuroblastoma (NRAS) chr. 1p13
  - Harvey rat sarcoma (HRAS) chr.11p15
  - Kirsten rat sarcoma (KRAS) chr. 12p12
  - Share 90% identity with HRAS and KRAS
  - Activated RAS recruits RAF which activates MEK and ERK and promotes cell proliferation, differentiation and survival
  - Mutated NRAS demonstrates impaired GTP hydrolysis and constitutively activated
### NRAS

- 6 exons spanning 12,438 bp
- Oncogenic mutations in NRAS are commonly activating mutations
- Occur in ~15-25% of cutaneous melanoma
- Mutations frequently involve exon 2 and 3

### KRAS

- KRAS and HRAS mutations are infrequent in cutaneous melanoma
- HRAS isoform is more commonly mutated in Spitz nevi
  - nearly 30%
  - increase copy number of chr. 11p in 20%
- Congenital melanocytic nevi demonstrate the most prevalent NRAS mutations
  - approximately 65% of these mutations occur at codon 61 of exon 3

### NRAS Protein

- The most frequent mutations involve
  - codon 12 exon 2
    - amino acid substitution of glycine (G) with glutamic acid (D) or serine (S) at position 12
      - G12D
      - G12S
  - codon 61 exon 3
    - amino acid substitution of glutamine (Q) with lysine (K) or arginine (R) at position 61
      - Q61K
      - Q61R

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### Mutations in Cutaneous Melanoma

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  - BAP1
- Germline: Hereditary melanoma
  - CDKN2A/p16
  - BAP1
RAF Kinase Family

- RAF is a serine-threonine protein kinase in the MAPK pathway involved in cell proliferation, differentiation, and transformation.
- Three isoforms
  - ARAF, chromosome Xp11
  - BRAF, chromosome 7q34
  - CRAF (RAF1), chromosome 3q34
- Mutation in ARAF and CRAF genes are rare events in human cancer
- BRAF mutations are more frequent in cancer
  - higher basal kinase activity
  - preferred mutation target

BRAF Gene

- 18 exons spanning 190,284 bp
- Oncogenic mutations in BRAF are commonly activating mutations
- Occur in ~50-60% of cutaneous melanoma
- Mutations frequently involve exon 15

BRAF Mutation

- BRAF V600E seen in over 90% of the cases
  - single point mutation with a DNA base substitution from T (thymine) to A (adenine) or T to A
  - valine to glutamic acid at the 600 position of the amino acid (BRAF V600E)

- BRAF V600K is second most frequent mutations, 5-10%
  - two DNA base pair change from G (guanine) and T (thymine) to A (adenine) and A (adenine) or GT to AA
  - valine to lysine at the 600 position of the amino acid (BRAF V600K)
  - V600K more associated with chronic sun exposure than V600E
- Others combine for less than 1% BRAF

BRAF Protein

Melanoma

- Clinical
  - <55 years old
  - Non-sun damage site
  - Acquired nevi
  - Nodal nevi
- Histologic
  - Pagetoid: greater
  - Size of intraepithelial nests: larger
  - Pigment: heavy
  - Circumscription: greater
  - Solar elastosis: less

- Clinical
  - Older patients
  - Sun damage sites
  - Congenital nevi
  - May demonstrate NRAS and KIT mutations
- Histologic
  - Pagetoid: less
  - Size of intraepithelial nests: smaller
  - Pigment: light
  - Circumscription: less
  - Solar elastosis: greater

Broekaert et al. Pigment cell Melanoma Res. 2010
### Mutations in Cutaneous Melanoma

**Somatic:** Sporadic melanomas
- KIT
- NRAS
- BRAF
- GNAQ/GNA11
- BAP1

**Germline:** Hereditary melanoma
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### GNAQ/GNA11
- **GNAQ** (guanine nucleotide binding protein (G protein), alpha, q polypeptide) on chr. 9q21
- **GNA11** (guanine nucleotide binding protein (G protein), alpha 11) on chr. 19p13
- Encode for the α-subunit of G-protein-coupled receptor
- GNAQ and GNA11 share 90% homology
- Mutations in GNAQ/GNA11 in melanocytic lesions
  - Subsequent amino acid changes of glutamine to leucine or proline (Q209L or Q209P)
  - GTP hydrolysis is prevented in mutant forms of GNAQ, thus resulting in a constitutive activation

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**BAP1**

Germline mutations in BAP1 predispose to melanocytic tumors

- **BAP1 (BRCA1 associated protein 1)** on chr. 3p21
- Tumor suppressor functions
  - Ubiquitin properties
  - Interactions with BRCA1 and BARD1
- Two families with recurring deletions involving BAP1
- Autosomal dominant inheritance
- Each family had one individual with uveal melanoma
- One family had three members with cutaneous melanoma

- Affected individuals with multiple (5-50)
  skin colored, red to brown dome shaped papules
- Beginning in the second decade
- Clinically, reminiscent of Spitz nevus
  - differ molecular profile

- Histologically, dermal tumors
- Composed of epithelioid shaped melanocytes
- Large nuclei, prominent nucleoli
- Cytologically cells appear Spitzoid
  - Absence of hyperplasia, Kamino bodies, clefting
- Atypical features included
  - Increase cellularity
  - Nuclear pleomorphism
  - Chromosomal abnormalities seen in melanoma (loss of 1, 3, 6, 9, and 22)

- Sporadic melanocytic lesions evaluated for BAP1 mutation:
  - Common nevi
  - Spitz nevi
  - Atypical spitz tumors 2/18 (11%)
  - Acral lentiginous 1/15 (7%)
  - Mucosal
  - CSD
  - Non-CSD 2/15
  - Uveal 13/33 (40%)

- Examination of 32 sporadic atypical spitz tumors (AST)
  - 28% AST with loss of BAP1
  - 89% concomitant loss of BAP1 and with BRAF V600E mutation
  - Three category of spitzoid tumors
    - 1) HRAS mutant
    - 2) BRAFV600E/BAP1 mutant
    - 3) Unknown genetics

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Sequencing Platforms in Mutation Analysis of Melanoma

- Early sequencing technology
  - Sanger Sequencing
  - Pyrosequencing
- Cobas 4800 BRAF
- Next generation sequencing technology

BRAFV600E Mutation Detected

Limitations of Molecular Test

- Amplification of DNA by PCR remains central in evaluating for genomic mutations in melanoma
- Melanin pigment is a known inhibitor of PCR reaction
- May yield no results due to non-amplifiable DNA

Limitations of Molecular Test

- This may become an issue with melanomas that are heavily pigmented
- Repeat mutational analysis on tumor specimens from different blocks and/or different tissue sources from a patient if material is available

Molecular Analysis of Primary vs. Metastatic Melanoma

- High consistency of mutation status in primary melanomas and metastasis
  - Lymph node
  - Visceral organs
- Consistency of mutation status appears to decrease when metastasis include
  - Skin
  - Brain

Frequency of BRAF/NRAS Mutations in Paired Melanoma Samples

- 99 patients with paired primary and metastatic melanoma
- 84/99 (85%) had same BRAF or NRAS mutation pattern in paired primary and metastatic samples
- Brain and skin metastasis demonstrated lower mutation pattern of 80% and 75%

Columbino J Clin Oncol 2012
Discrepant Mutation Patterns in Paired Tissue Samples

- 15/99 (15%) paired primary and metastatic lesions were discrepant in BRAF or NRAS mutation status
- 8 mutated primary and wild type metastatic lesions (7 BRAF and 1 NRAS)
- In different metastatic lesions, discrepant WT and BRAF/NRAS mutations observed (7 BRAF and 2 NRAS)
- Subclones of tumors generated in some patients

Immunohistochemical Detection of BRAFV600E Mutation

- Anti-BRAF V600E (VE1)
- 37/38 known BRAFV600E mutant melanomas by genetic analysis positive with BRAFV600E immunostain
- All 11 BRAF mutant variants negative for BRAFV600E stain
- 47/48 BRAF wild type by genetic analysis were negative with BRAF antibody
- Sensitivity 97%, Specificity 98%

Lecture Outline

- Melanoma diagnosis
- Mutations in melanoma
- Melanoma targeted therapy
Targeted Therapy

- Small molecule inhibitors target genomic abnormality of cancer cell and function
- Monoclonal antibodies (MABs)
  - Target extracellular receptors
  - Prevent growth, incite death
- Small molecule inhibitors (NIBs)
  - Target cellular signaling pathways
  - Prevent growth, tumor cell survival

Melanoma Targeted Therapy

- KIT inhibitor
- Tyrosine kinase inhibitor (TKI)
  - Sorafenib
- MEK inhibitor
  - Selumetinib, Trametinib
- AKT inhibitor
- PI3K inhibitor
- RAF inhibitor
  - Vemurafenib

Imatinib, Phase II Study

- 42% patients had regression of tumor mass after initiation of therapy
- 6 month progression free survival of 36.6%

Dabrafenib, Phase I Trial

- Inhibitor of BRAF
- Targets mutant V600E and non-V600E (e.g., V600K)
- Penetrates blood-brain barrier
- Cutaneous toxicities with SCC and KA

V600-Selective BRAF Inhibitors: Vemurafenib

- 48% with confirmed objective response
- Improved overall survival
  - 84% vs. 65%

Toxicities of Cancer Therapy

- Dermatologic toxicities (DT) is ten most common adverse reaction to adjuvant therapy
- Examination of DT will become increasingly important component of our daily clinical practice
- Era of “oncodermatology and oncodermatopathology”
Cutaneous Toxicities

- Keratinocytic proliferations
- Sorafenib therapy
  - Invasive SCC (SCC with KA features)
    - 64% of lesions
  - AK
    - 2% of lesions

- AKT and PI3K Inhibitors Skin Toxicities
  - Perivascular lymphocytic infiltrate = dermal hypersensitivity reaction (DHR)
  - Responsive to anti-histamines and/or topical steroids

- Tyrosine Kinase Inhibitors
  - MEK Inhibitor Selumetinib and Trametinib

- Suppurative Folliculitis

- Vemurafenib Associated Toxicities
  - Most common adverse events
    - Arthralgia
    - Fatigue
    - Cutaneous events
      - All cutaneous epithelial proliferations
        - 54%
      - 18% developed either a SCC or KA or both

References:
- Desar et al. Acta Oncologica. 2010
### Dermatologic Toxicities with Vemurafenib

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Vemurafenib (N=336) N (%)</th>
<th>Dacarbazine (N=282) N (%)</th>
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</thead>
<tbody>
<tr>
<td>Rash</td>
<td>165 (49)</td>
<td>9 (3)</td>
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<tr>
<td>Pruritus</td>
<td>74 (22)</td>
<td>4 (1)</td>
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<tr>
<td>Xerosis</td>
<td>16 (54)</td>
<td>3 (1)</td>
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<tr>
<td>Erythema</td>
<td>38 (11)</td>
<td>4 (1)</td>
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<td>HFSR</td>
<td>22 (7)</td>
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<td>Photosensitivity</td>
<td>101 (30)</td>
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<td>Sunburn</td>
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<td>Squamous carcinoma</td>
<td>40 (12)</td>
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### Clinical and Histologic Features

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<th>Drug</th>
<th>Clinical</th>
<th>Histology</th>
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<td>KITRi</td>
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<td>Maculopapular rash</td>
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<tr>
<td>MEKi</td>
<td>Selumetinib, Trametinib</td>
<td>Papulopustular rash</td>
<td>Suppurative folliculitis</td>
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### Dermatologic Toxicities with Selective RAF Inhibitor Vemurafenib

**MDACC Experience**

**Evaluation of 141 DTs During Vemurafenib Therapy**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of Patients (%)</th>
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<tbody>
<tr>
<td>Sex</td>
<td>Male: 13 (29)</td>
</tr>
<tr>
<td></td>
<td>Female: 20 (44)</td>
</tr>
<tr>
<td>Age (in years) at initiation of VT</td>
<td>Median: 56</td>
</tr>
<tr>
<td></td>
<td>Range: 27-84</td>
</tr>
<tr>
<td>Tumor type treated</td>
<td>Melanoma: 29 (88)</td>
</tr>
<tr>
<td></td>
<td>Papillary thyroid cancer: 4 (12)</td>
</tr>
<tr>
<td>Onset (in months) of first DT(s)</td>
<td>Median: 3.0</td>
</tr>
<tr>
<td></td>
<td>Range: 0.3-36</td>
</tr>
<tr>
<td>No. of DT(s)/patient</td>
<td>1-5: 26 (79)</td>
</tr>
<tr>
<td></td>
<td>6-10: 2 (6)</td>
</tr>
<tr>
<td></td>
<td>&gt;10: 5 (&lt;15)</td>
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</tbody>
</table>

### Clinically Distinct BRAFi Induced Cutaneous Epithelial Proliferations

- Clinical distinct lesions
  - Papules/nodules with keratosis
  - Size range: 0.3-1.0 cm
  - Peripheral rim of erythema

### Diagnostic Category of Cutaneous Epithelial Proliferations

<table>
<thead>
<tr>
<th>Category of DTs</th>
<th>No. of Lesions (%)</th>
<th>Diagnosis</th>
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</thead>
<tbody>
<tr>
<td>CEP</td>
<td>48 (a,c) (40)</td>
<td>VV</td>
</tr>
<tr>
<td></td>
<td>29 (24)</td>
<td>SCC, invasive</td>
</tr>
<tr>
<td></td>
<td>25 (a, b) (21)</td>
<td>AK</td>
</tr>
<tr>
<td></td>
<td>6 (5)</td>
<td>SCIS</td>
</tr>
<tr>
<td></td>
<td>4 (3)</td>
<td>FAD</td>
</tr>
<tr>
<td></td>
<td>4 (3)</td>
<td>KA</td>
</tr>
<tr>
<td></td>
<td>1 (2)</td>
<td>KP</td>
</tr>
<tr>
<td></td>
<td>1 (&lt;1)</td>
<td>BLK</td>
</tr>
<tr>
<td></td>
<td>1 (&lt;1)</td>
<td>FC</td>
</tr>
<tr>
<td></td>
<td>1 (&lt;1)</td>
<td>IFK</td>
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<tr>
<td>Total</td>
<td>120</td>
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</table>
Summary of Histologic Features with BRAFi

• Papillomatosis
• Hyperkeratosis with parakeratosis
• Hypergranulosis, hemorrhage in stratum corneum
• Changes of verruca
• Associated with abnormal follicle/folliculcentric
• Endophytic/exophytic
• In situ component not prominent
• Collaret of epidermis without significant atypia

BRAFi Induced Cutaneous Epithelial Proliferations

• Histologically reproducible architectural appearance on scanning magnification
  – Crateriform proliferation with histologic changes of verruca
  – Associated with abnormal follicle
• Range of diagnosis
  – Keratosis to SCC

Mechanisms of Vemurafenib Induced Squamous Cell Carcinoma

Vemurafenib Associated Melanocytic Lesions

<table>
<thead>
<tr>
<th>Melanocytic lesions</th>
<th>4 (36)</th>
<th>DN</th>
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<tbody>
<tr>
<td></td>
<td>3 (27)</td>
<td>CMN</td>
</tr>
<tr>
<td></td>
<td>1 (9)</td>
<td>AMP</td>
</tr>
<tr>
<td></td>
<td>1 (9)</td>
<td>IDN</td>
</tr>
<tr>
<td></td>
<td>1 (9)</td>
<td>Melanoma, metastatic</td>
</tr>
<tr>
<td></td>
<td>1 (9)</td>
<td>MIS+nevus</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
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Morphologic Changes

• Involution-regressed features
• Alteration in color and size-increase pigment and size
• Development of new nevi
• Development of melanoma

Melanoma with Associated Nevus
Conclusion

- Examined diagnosis of melanoma
  - Incorporation of IHC and FISH
- Reviewed frequent mutations in cutaneous melanoma
- Described the range of cutaneous toxicities from melanoma targeted therapy

Thank You

- Acknowledgements
  - Section of Dermatopathology
    - Dr. Prieto
    - Dr. Torres-Cabala
    - Dr. Tetzlaff
    - Dr. Ivan
  - Division of Dermatology
    - Dr. Duoc
    - Dr. Tsai
  - Department of Melanoma Medical Oncology
    - Dr. Kim