1 **"Biomarkers in Ocular Melanoma"**

Patricia Chévez-Barrios, MD
Pathology and Genomic Medicine, Houston Methodist Hospital
Professor of Pathology and Laboratory Medicine and Ophthalmology, Weill Cornell Medical College

2 **Skin Melanoma**

- Accounts for 75% skin-related deaths worldwide.
- Diagnosis is challenging due to large diversity of morphological patterns.
- Highest source of litigation in surgical pathology.
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3 **Biomarkers**

4 **Diagnosis**
Melanoma

5 **Immunohistochemical Markers**
Identification Biomarkers

Challenges :

- Melanin pigment (DAB chromogen is brown)
- Tumor associated lymphocytes, stromal cells (may mark with proliferation markers)

Options:

- Bleach prior to immunestaining (may alter results)
- Use of different chromogen (red, blue)
- Double staining with melanocytic marker (cytoplasmic) + proliferation marker (nuclear)

6 **Immunohistochemical Markers**
Identification Biomarkers

- S100: sensitive melanocytic marker but not specific. Excellent marker, for desmoplastic melanoma. Usually not positive or faintly positive in uveal melanoma

- HMB-45: Helps differentiating benign nevi (decreased positivity with lesion depth/maturation) vs melanoma (consistent positivity in deeper part), not definitive in nevoid variants of melanomas.

7 **Immunohistochemical Markers**
Identification Biomarkers

- MITF-1 & SOX-10: nuclear stains, preferred in continuous lentiginous proliferations and pagetoid spread in difficult in-situ melanocytic lesions. Can stain intraepithelial dendritic cells .
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- SOX-10: High sensitivity for melanocytic differentiation. Used in desmoplastic melanoma along with S100 (recently reported in scar). *Caviate*: metastatic vs. breast, salivary gland carcinomas, neural creast tumors and clear cell sarcomas.

- Ki-67 and pHH3: Estimates proliferation rate
- (inflammatory cells). Co-staining with a melanocytic marker such as HMB-45 may improve accuracy of the test.

8 **Clinical Approach to Patient with Melanoma**

9 **Timeline of Advances in Melanoma Diagnosis &Prognosis, Treatment- 1**

10 **Timeline of Advances in Melanoma Diagnosis &Prognosis, Treatment- 2**

11 **Timeline of Advances in Melanoma Diagnosis &Prognosis, Treatment- 3**

12 **BIOMARKERS IN OCULAR MELANOMAS**

13 **Clinical Approach to Patient with Uveal Melanoma**

14 **Most Common Malignant Intraocular Tumors**

15 **Choroidal Melanoma**

- Predisposing factors
  - melanosis oculi and Nevus of Ota
  - BAP1 associated tumor predisposition syndrome
- 6 cases per million in USA (60-70 years of age)
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- 50% uveal melanoma patients will develop metastasis
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16 **Melanoma of the Uvea**

- Location = symptoms specific to the location will develop and facilitate early or late diagnosis
- Ciliary body melanoma presents later because of location (hiding behind the iris/lens)
- Most frequent in whites and lightly pigmented individuals

17 **Melanoma**

- Iris = nonaggressive
- 3.3 – 16.6% are from iris (~10 years earlier dx than choroidal)

18 **Clinical Diagnosis**

- Standard of care
- Patients treated based on clinical/imaging features

19 **Uveal Melanoma**

- Metastasis 80% to liver (90% in liver prior to death)
- 1/3 of metastatic MM solely to the liver
- No difference in survival: local treatment with radiation plaque vs. enucleation (COMS prospective trial)
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20 **High Risk Prognostic Factors**

21 **Malignant Melanoma – Size, location**

22 **Callender Classification**

23 **Vasculogenic Mimicry**

24 **Uveal Melanoma**

Genetic features associated with metastasis include:

- monosomy 3 and gain of chromosome 8q

25 **Molecular Heterogeneity**

- FISH analysis on paraffin sections showed that heterogeneity of monosomy of chromosome 3 is a frequent phenomenon in uveal melanoma.

26 **Choroidal melanoma**

- Monosomy 3 in uveal melanomas associated with approximately 50% of all uveal melanomas.
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- Follow-up showed that 57% developed metastases within 3 years, in contrast to patients with tumors that retained both copies of chromosome 3, who only rarely developed metastases.

27 **Molecular genetics in Uveal melanoma**

- Somatic mutations in the heterotrimeric G protein alpha-subunit, GNAQ
- Blue nevi (83%) and ocular melanoma of the uvea (46%).
- The mutations occur exclusively in codon 209 in the Ras-like domain and result in constitutive activation, turning GNAQ into a dominant acting oncogene.
- An alternative route to MAP kinase activation in melanocytic neoplasia

28 **Molecular genetics in Uveal melanoma**

- BAP1 (BRCA1-associated protein 1) is a tumor suppressor gene .
- BAP1 gene locus is on chromosome 3 (3p21.1).
- Deletions of the 3p21 region were commonly seen in small cell and nonsmall cell lung cancer cell lines, and breast cancers
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29 **BAP1 – Epithelioid phenotype**

30 **BAP1**

- Inactivating mutations in BAP1 are seen in 47% (27/57) uveal melanomas.
- Metastasis developed in the vast majority (26/27) of these tumors, many of which also showed monosomy of chromosome 3.
- These findings support the thesis that BAP1 is a classical tumor suppressor gene (Knudson's two-hit model)
  - One allele of BAP1 being lost via monosomy of chromosome 3 and the second allele being lost by inactivating BAP1 mutation(s).

31 **Molecular genetics in Uveal melanoma**

- BAP1 mutations recently reported to increase susceptibility for the development of uveal melanoma, cutaneous atypical and epithelioid melanocytic lesions, clear cell renal cell carcinoma, mesotheliomas and other tumors.
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32 **BAP1 testing**

- Direct (Sanger) sequencing of the BAP1 gene using blood or salivary DNA from the individual(s) of interest (suspect of BAP1 related predisposition syndrome).
- BAP1 testing in tumors by RT-PCR

- BAP1 IHC in paraffin embedded tumor tissue (BAP1 loss of staining by IHC)
  - Biallelic BAP1 genomic loss correlates with positive and negative predictive values of 100% and 98.6%, respectively
- Equivocal IHC results may undergo subsequent confirmatory sequencing.

33 **Uveal Melanoma**

- Gene-expression signatures (15 genes) have also been identified that accurately distinguish tumors at low metastatic risk (class 1 signature) and high metastatic risk (class 2 signature)
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35 **GEP – Uveal Melanoma**

- Based upon the clinical outcomes from the prospective, 5-year multi-center Collaborative Ocular Oncology Group (COOG) study, the test reports Class 1A, Class 1B and Class 2 phenotype:
- Class 1A: Very low risk, with a 2% chance of the eye cancer spreading over the next five years;
- Class 1B: Low risk, with a 21% chance of metastasis over five years;
- Class 2: High risk, with 72% odds of metastasis within five years.

36 **Choroidal Melanoma**

37 **Requirement of Tissue for Molecular Testing**

- Adequacy of sampling
  - GEP does not discriminate between normal, benign or malignant melanocytic lesion
  - Blood elements = Class 1
  - Some metastatic carcinomas = Class 2
- Confirmation of diagnosis
- FNABx before radioactive plaque
- Tissue retreival at time of enucleation
- FFPE tissue acceptable

38 **Cytology : Sampling prior to plaque**

- Diagnosis and molecular prognosis of uveal melanoma

39 **Cytology : Sampling prior to plaque**

- Diagnosis and molecular prognosis of uveal melanoma

40 **Rapid on site evaluation (ROSE) in uveal melanomas for diagnostic adequacy and optimal triaging for molecular testing: A series analysis of 67 cases**

41 **UVEAL MELANOMA BIOMARKERS**

- Uveal melanoma does not have effective treatment for metastatic phase
- GEP or other prognostic molecular testing shows no detrimental psycological effect in most patients
- Early treatment of localized metastasis and possible enrollment in clinical trials

42 **Examples of Current Trials**

1 MD Anderson Cancer Center
Elizabeth Grimm, MD ; Sapna Patel, MD

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- Not stratifying uveal melanomas based on any biomarkers
- Usually test for GNAQ and GNA11 and often do a post-hoc look at responses in these groups.
- To date, targeted therapy trials do not show a difference in the GNAQ/11 wild-type or mutated populations.
- Other melanomas, next generation panel (BRAF, NRAS, CKIT, GNAQ and GNA11) .
- Standard of care and clinical trial options based on mutation status.
- PD-L1 status on a metastatic specimen to drive treatment decisions regarding single-agent versus combination checkpoint blockade (selective cases)
- Trial using iNOS as a biomarker for entry onto an anti-inflammatory/NF-KB inhibitor study in combination with checkpoint blockade for melanoma (Elizabeth Grimm, MD).
- Engineered T cell trials based on HLA-A02:01 status, and MART-1 expression in the tumor tissues.

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3 Massachusetts General Hospital Cancer Center
Keith T. Flaherty, M.D.

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- Uveal - Developing clinical trial to combine intralesional oncolytic virus with PD-1/CTLA-4 therapy, given the relative lack of efficacy for immune checkpoint antibody therapy in this population to date.
- Cutaneous, mucosal, and acral melanomas large clinical trial portfolio aiming to
- (1) expand the benefits of immune checkpoint antibody therapy by targeting novel immune targets,
- (2) extending the benefits of BRAF inhibitor-based therapy by targeting additional vulnerabilities outside of the MAP kinase pathway with triplet drug regimens
- (3) developing molecular targeted therapy strategies pertinent to BRAF wild type melanomas that build on the modest efficacy of single agent MEK inhibition

43 **Conclusions**
- Oncogenes in melanoma are relevant for prognosis and therapeutic biomarkers
- Multimarker assays are needed
- Immunohistochemistry as surrogate for mutations (BRAF, BAP1)
- Noninvasive biomarkers needed (blood circulating tc)
- Immunoprofiling is a valid form of biomarker
- Need for predicting response of immuno-based therapies
- Epigenetic alteration are an expanding group of potential biomarkers
- Special type melanomas have different tumorogenesis
- Development of combination therapies appear to be important in melanoma