Multiplatform biomarker analysis of non-sun-exposed mucosal melanoma

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Abstract #9042

Background: Mucosal melanoma is a rare malignancy, notoriously resistant to conventional chemotherapy, with few treatment options. Because of their origin, they do not receive screening and, hence, are detected in advanced stages where prognosis and curative rates are poor. The purpose of this study is to identify novel, potential targets and therapeutic options for this disease, utilizing a multiplatform approach.
Methods: In total, 93 mucosal melanoma specimens were tested via a multiplatform profiling service (Caris Life Sciences, Phoenix, AZ) consisting of gene sequencing (Sanger or next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]) and/or gene amplification (CISH or FISH). Conjunctival melanoma was excluded.
Results: Notable protein overexpression rates included co-expression of PD-1 and PD-L1 (71.4%, 5/7) and cKIT (41.9%, 13/31). Percent agreement between c-KIT by IHC and sequencing was 62% (16/26). Overall, sequencing revealed the highest mutation rates in TP53 (17%, 4/23), KIT (18.2%, 14/77), BRAF (12.0%, 10/83), and NRAS (10.0%, 4/40). A sub-analysis of BRAF, KIT, and NRAS (BRAF/KIT/NRAS) based on the anatomic location of the melanoma revealed the following: sinonasal (5.3%, 0.0%, 27.0%), vulvovaginal (21.2%, 27.3%, 7.1%), and anorectal (5.0%, 18.0%, 0.0%).
Details on the TP53-mutated specimens (n=4) are shown below:

Specimen Primary Site	TP53 Mutation(s)
Sinonasal	H179R (c.536A>G)
Nasopharyngeal	V272L (c.814G>T), R248W (c.742C>T)
Vaginal	R337C (c.1009C>T), R273C (c.817C>T)
Rectal	N268fs

Conclusion: Multiplatform tumor profiling identified multiple, potentially actionable targets. Given the high rate of PD-1 and PD-L1 co-expression, new immunotherapies should be strongly considered in advanced stages of this disease. In addition, mutations detected may provide further guidance in this rare malignancy. The highest rates of NRAS mutations occurred in the sinonasal melanomas. Meanwhile, KIT mutations were highest in vulvovaginal and anorectal mucosal melanomas. This variability in mutation rates of BRAF, KIT, and NRAS based on the primary site's location should be further elucidated in larger studies for potential diagnostic and theranostic purposes.

Background

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Methods

In total, 93 mucosal melanoma specimens were tested via a multiplatform profiling service (Caris Life Sciences, Phoenix, AZ) consisting of gene sequencing (Sanger or next generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]) and/or gene amplification (CISH or FISH). NGS sequencing was performed to a depth of 1500x. As it was considered sun-exposed, conjunctival melanoma was excluded from this analysis.

Results

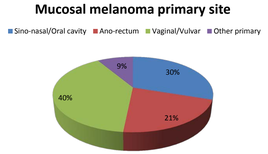


Figure 1. Distribution of mucosal melanoma series. The pie chart on the left shows percentage of the melanomas used in this analysis. Conjunctival melanoma was not included as it was identified as a sun-exposed form of mucosal melanoma. Most specimens analyzed were from the primary site (67.7%, 63/93) .

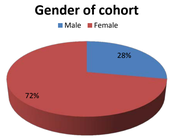
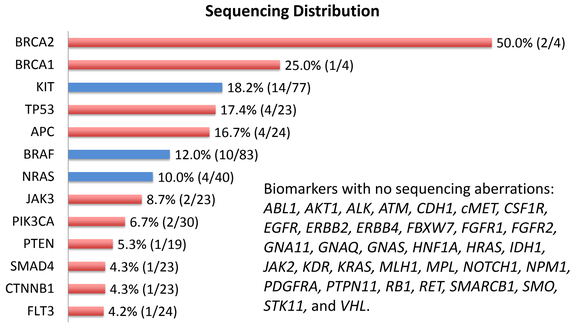


Figure 2. Gender distribution. More women (n=67) than men (n=26) is expected in this cohort given the number of vulvovaginal mucosal melanomas.

Biomarker	Number Amplified	Number Analyzed	Percent Amplified
EGFR	2	6	33.3%
MET	1	25	4.0%
ERBB2 (HER2)	0	27	0.0%
TOP2A	0	6	0.0%

Figure 3. ISH (amplification) distribution. Amplification was detected in EGFR and MET. Newer EGFR-targeted therapy may be considered in those with increased EGFR gene copy number (GCN), perhaps in combination with approved therapies. Also, amplified MET may respond to agents like crizotinib. ERBB2 and TOP2A, both on the same chromosome, were not found.

Results (cont.)



Biomarkers with no sequencing aberrations: *ABL1, AKT1, ALK, ATM, CDH1, cMET, CSF1R, EGFR, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, JAK2, KDR, KRAS, MLH1, MPL, NOTCH1, NPM1, PDGFRA, PTPN11, RB1, RET, SMARCB1, SMO, STK11,* and *VHL*.

Figure 4. Sequencing distribution. The graph above shows distribution sorted from highest to lowest. The variability seen in the number of tests performed is secondary to what test(s) had been requested by the ordering physician. *BRAF*, *KIT (c-KIT)*, and *NRAS* – mutations associated with mucosal melanoma - have been highlighted in blue. Mutations were highest in *TP53* and, although small (n=4), *BRCA1/2*. Of the four specimens with *TP53* mutations, two were found to have two *TP53* mutations each. The other variants/mutations shown may be actionable and/or serve as a baseline for understanding the underlying tumor biology.

Head and Neck Mucosal Melanoma (i.e. sinonasal, pharyngeal and oral cavity melanoma)

Biomarker	# Mutated	Total	% Mutated
BRAF	1	22	5.0%
KIT	1	20	5.0%
NRAS	3	13	23.0%

A

Vulvovaginal Mucosal Melanoma

Biomarker	# Mutated	Total	% Mutated
BRAF	7	33	21.2%
KIT	9	33	27.3%
NRAS	1	14	7.1%

B

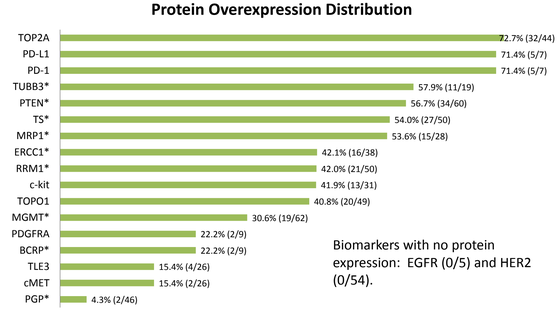
Anorectal Mucosal Melanoma

Biomarker	# Mutated	Total	% Mutated
BRAF	1	20	5.0%
KIT	3	17	18.0%
NRAS	0	9	0.0%

C

Figure 5. *BRAF, KIT, NRAS* (by sequencing) comparison based on primary anatomical location. The figures (A, B, C) on the left show mutation rates of *BRAF*, *KIT*, and *NRAS* in three distinct, anatomic locations. For reference, head and neck mucosal melanomas include those melanomas arising from the sinonasal and oral cavity. Also, note that specimens arising from other sites (e.g. small intestine) were excluded from this analysis due to small numbers (n). *BRAF* mutation rates were highest in vulvovaginal melanomas (21.2%). *KIT* mutations were highest in vulvovaginal and anorectal mucosal melanomas (27.3% and 18.0%, respectively). *NRAS* mutation rates were highest in head and neck mucosal melanomas (23.0%). In a sub-analysis of the head and neck melanoma specimens, most NRAS mutations were from the sinonasal cavity (27.0%, 3/11).

Results (cont.)



Biomarkers with no protein expression: EGFR (0/5) and HER2 (0/54).

Figure 6. Immunohistochemistry (IHC) distribution. The graph above shows distribution of potentially prognostic, predictive biomarkers sorted from highest to lowest. TOP2A, a biomarker associated with high cellular proliferation and (in specific lineages) potential benefit to anthracycline therapy, showed the highest protein expression. Although low (n=7), the percentage of patients with overexpression along the PD-1/PD-L1 axis is worth mentioning (71.4%) as novel immunotherapeutics are now available.

Conclusions

- Multiplatform tumor profiling can identify multiple potentially actionable targets for therapy in mucosal melanoma.
- The tumor heterogeneity of mucosal melanoma imparts a potential benefit of using multiplatform tumor profiling.
- *NRAS* mutation rates where highest in sinonasal melanomas and *KIT* mutations were highest in vulvovaginal and anorectal mucosal melanomas.
- Future studies involving *BRAF*, *KIT*, and *NRAS* should verify the variable mutation rates based on the anatomic location of the primary site. Such information could potentially be utilized for diagnostic and theranostic purposes.
- The high rate of PD-1 and PD-L1 co-expression in advanced mucosal melanoma warrants further exploration in clinical trials involving novel immunotherapeutics (e.g. pembrolizumab, nivolumab, MPDL3280A).

Conflicts of Interest

Drs. Arguello, Gatalica, Millis, and Reddy are employees of Caris Life Sciences . Dr. Cherington and Bryce have no disclosures. Dr. Gonzalez has consulting /advisory roles with Amgen, Bristol-Meyers-Squibb, and Roche/Genentech.

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