

Anal Melanoma: A Comparative Genomic Profiling Study

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ABSTRACT

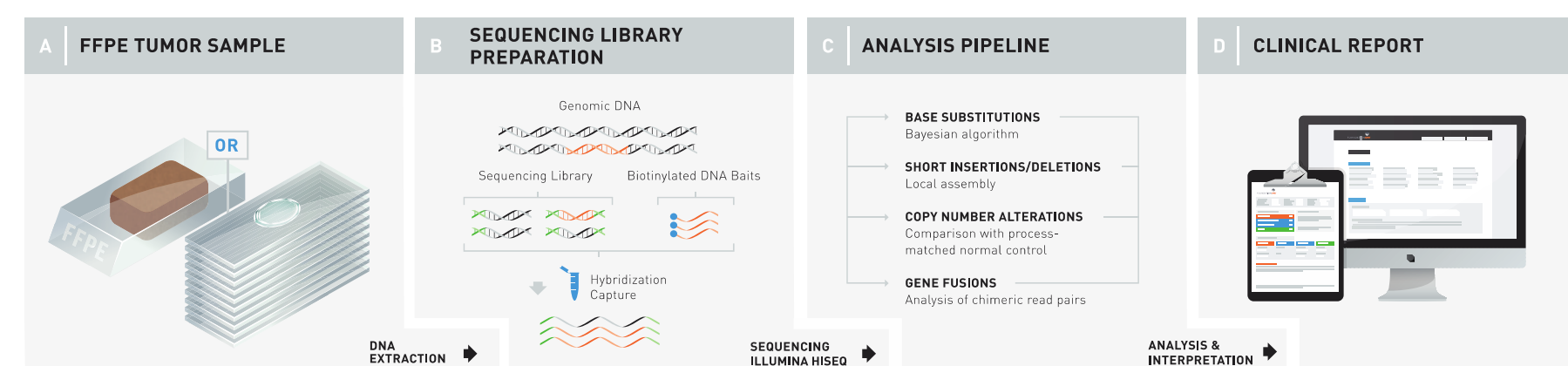
Background: We performed a comprehensive genomic profiling (CGP) study of anal melanoma (AM) and cutaneous melanoma (CM) to learn of potential genomic alterations (GA) linked to targeted and immune checkpoint inhibitor (ICPI) therapies.

Methods: 90 AM and 1,804 CM formalin-fixed paraffin-embedded (FFPE) tissues from late-stage disease underwent hybrid-capture based CGP to evaluate all classes of genomic alterations (GA). Tumor mutational burden (TMB) was determined on 0.83-1.14 Mbp of sequenced DNA and microsatellite instability (MSI) was determined on 114 loci. PD-L1 expression was determined by IHC.

Results: AM and CM had a similar age, but AM were a majority female (61%) while CM were a majority male (64%) ($P < 0.0001$). GA/tumor was significantly higher in CM (UV light exposure) as were the median TMB and frequency of TMB ≥ 10 and 20 mutations/Mb ($P < 0.0001$ for all comparisons). PD-L1 expression was higher in AM than CM ($P = 0.0023$). AM and CM were all MS-stable. The contrast in *SFB1* mutations in AM and *TERT* GA in CM were significant ($P < 0.0001$). Of potentially targetable GA, AM featured significantly more *KIT* GA than CM ($P < 0.0001$), whereas CM featured significantly more *BRAF* GA ($P < 0.0001$). Only 11% of AM *BRAF* GA were V600E whereas 74% of CM *BRAF* GA were V600E ($P < 0.0001$). mTOR pathway GA were common in both tumor types. Additional potentially targetable alterations in *PDGFRA* and *ERBB2* kinases were seen in AM but not in CM.

Conclusions: CM is distinct from AM, featuring higher GA/tumor, higher TMB and frequent *BRAF* V600E GA that predict benefit from ICPI and anti-*BRAF* therapies. Although both AM and CM feature mTOR pathway targets, AM does have higher PD-L1 expression than CM and is characterized by an array of potentially targetable kinase genes including *KIT*, *PDGFRA*, *ERBB2* and to a lesser extent than CM, *BRAF*.

MATERIALS AND METHODS



- ≥ 50 ng DNA extracted from 40 μ m of FFPE sections
- Sequencing performed for up to 315 cancer-related genes and introns from 28 genes commonly rearranged in cancer
- Hybrid capture-based sequencing using adaptor ligation-based libraries
- Mean coverage depth $>600X$
- Base substitutions, insertions and deletions (short variants; SV), rearrangements, and copy number changes were assessed
- Tumor mutational burden (TMB) calculated from 0.83-1.14 Mb sequenced DNA
- PD-L1 expression was determined by IHC (Dako 22C3)

RESULTS

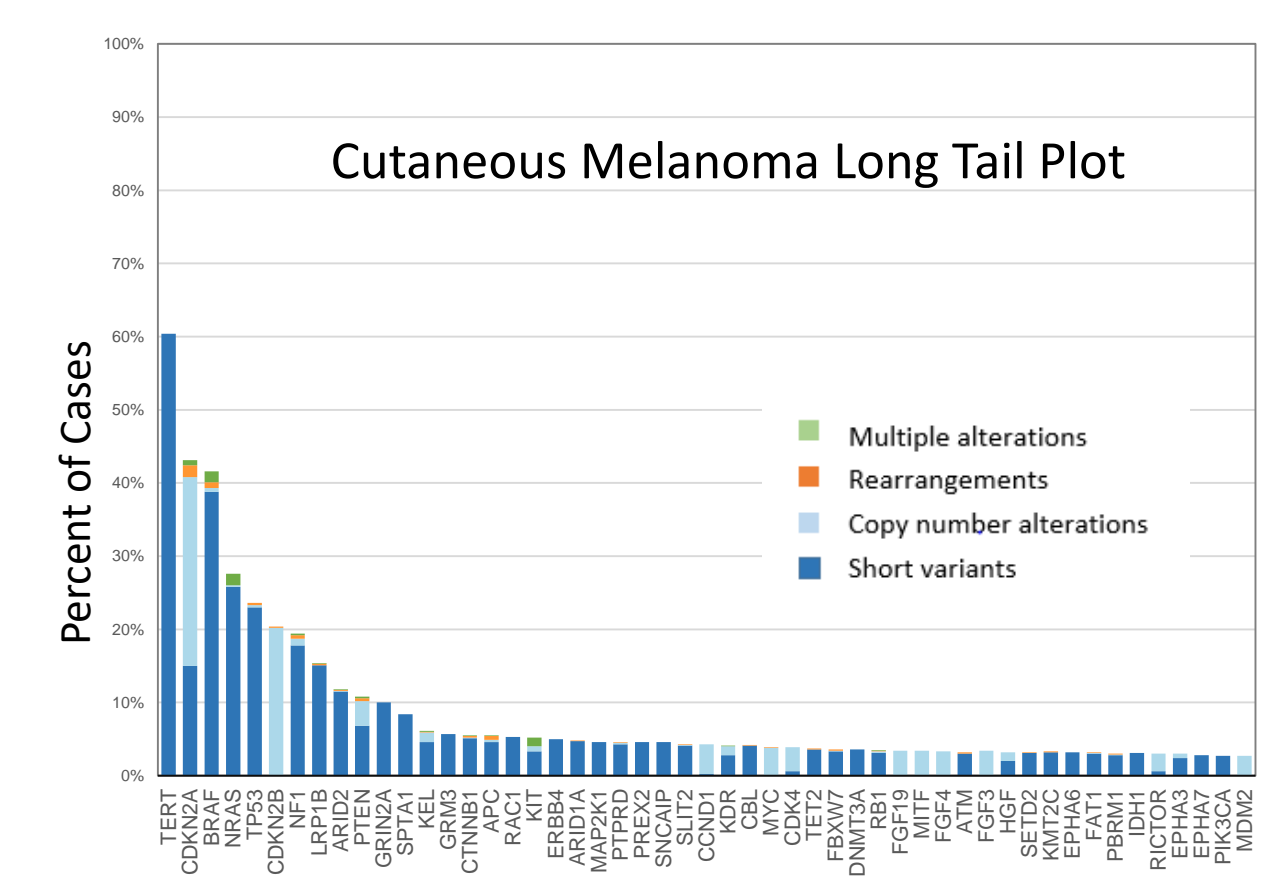
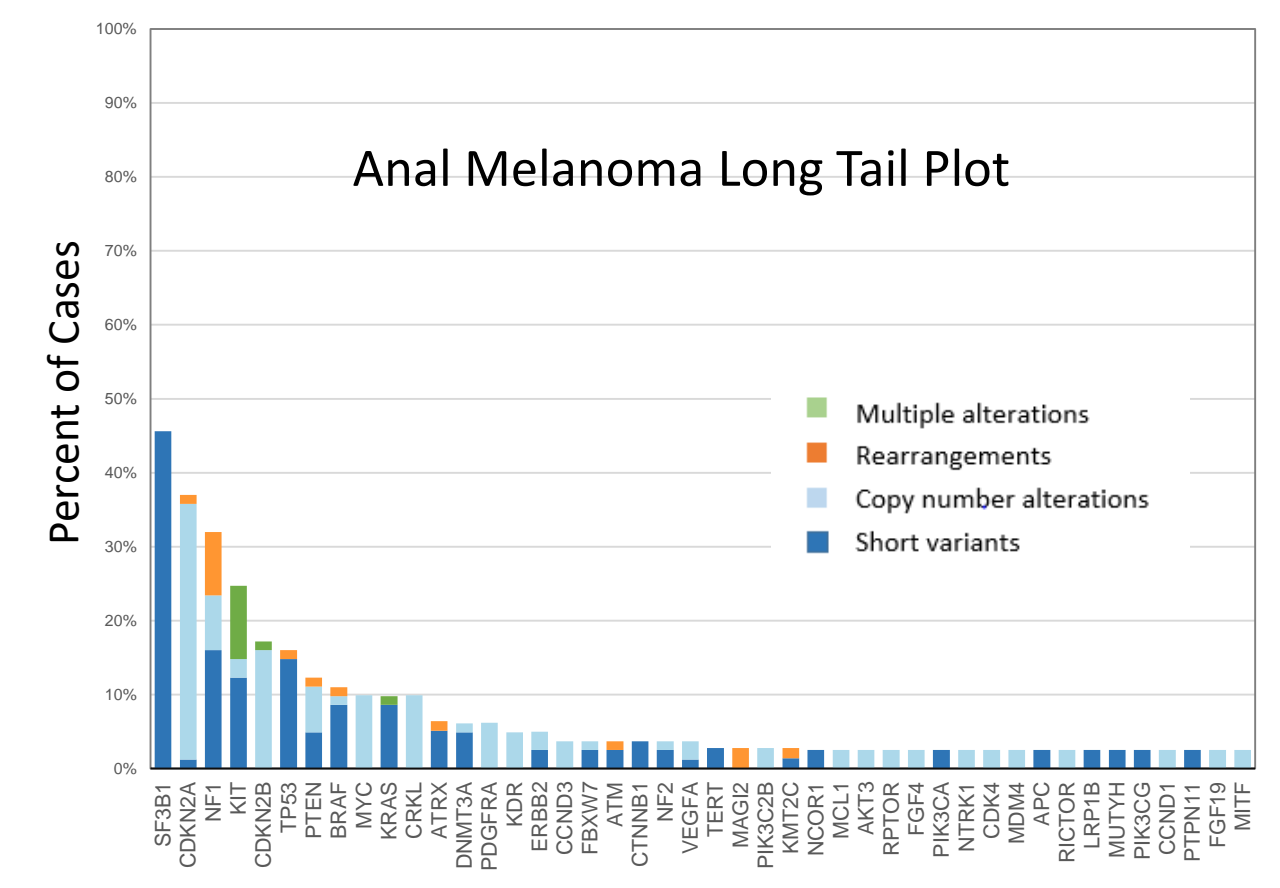
- AM and CM had a similar age
- AM were a majority female (61%) while CM were a majority male (64%) ($P < 0.0001$)
- Given the UV light exposure in the CM, the GA/tumor was significantly higher than AM as were the median TMB and frequency of TMB ≥ 10 and 20 mutations/Mb ($P < 0.0001$ for all comparisons)
- All AM and CM were MS-stable
- The contrast in *SFB1* mutations in AM and *TERT* GA in CM were significant ($P < 0.0001$)
- AM featured significantly more *KIT* GA than CM ($P < 0.0001$), whereas CM featured significantly more *BRAF* GA ($P < 0.0001$)
- Only 11% of AM *BRAF* GA were V600E whereas 74% of CM *BRAF* GA were V600E ($P < 0.0001$)
- mTOR pathway targets including *NF1* and *PTEN* were commonly altered in both tumor types
- Additional potentially targetable alteration in *PDGFRA* and *ERBB2* kinases were seen in AM but not in CM

	AM	CM
Number of Cases	90	1,723
Males/Females	35/55	1,110/613
Median age (range)	66 (32-89)	62 (8-88)
GA/tumor	4.2	6.6
Top Un-targetable GA	<i>SF3B1</i> (42%) <i>CDKN2A</i> (36%) <i>CDKN2B</i> (17%) <i>TP53</i> (16%) <i>MYC</i> (10%) <i>KRAS</i> (9%)	<i>TERT</i> (60%) <i>CDKN2A</i> (43%) <i>NRAS</i> (28%) <i>TP53</i> (24%) <i>CDKN2B</i> (20%) <i>LRP1B</i> (15%)
Top Targetable CRGA	<i>NF1</i> (33%) <i>KIT</i> (23%) <i>PTEN</i> (12%) <i>BRAF</i> (11%) <i>PDGFRA</i> (6%) <i>ERBB2</i> (4%)	<i>BRAF</i> (42%) <i>NF1</i> (20%) <i>PTEN</i> (11%) <i>KIT</i> (5%)
MSI-High	0%	0%
Median TMB	2.6	14.4
TMB > 10 mut/Mb	1%	64%
TMB > 20 mut/Mb	0%	40%
PD-L1 Expression	35%	21%

CASES

Stage IV Anal melanoma in a 64 year old Caucasian man. PD-L1 IHC was 0% staining. On CGP, this tumor featured both *KIT* amplification and a L576P activating short variant mutation. Other alterations included amplification of *PDGFRA*, *CCND3*, *NTRK1*, and *VEGFA*. *CDKN2A* loss and *KMT2C* (*MLL3*) deletion were also present. The tumor was MS-Stable and had a low TMB at 4 Mutations/Mb. *KIT* encodes a cell surface tyrosine kinase receptor that, upon ligand binding and dimerization, leads to activation of the PI3K-AKT and RAS-MAPK signaling pathways. *KIT* mutation or amplification has been reported in from 16 to up to 53% mucosal melanomas. *KIT* mutations have been reported to occur predominantly in vulvar melanoma. The majority of *KIT* mutations in mucosal melanomas occurred in exon 11, with the L576P mutation being the most common. The prognostic significance of *KIT* mutation in mucosal melanomas is not established. *KIT* activating alterations are associated with sensitivity to *KIT* tyrosine kinase inhibitors including imatinib, sunitinib, sorafenib, dasatinib, nilotinib, pazopanib, regorafenib, and ponatinib. In addition, the use of mTOR inhibitors has demonstrated some success in *KIT*-activated melanoma, suggesting mTOR inhibitors are effective in targeting kinase inhibitor-resistant tumors.

Anal melanoma in a 77 year old Caucasian female that advanced to Stage IV disease. CGP revealed major amplification of *ERBB2* at 185 copies. Short variant mutations were also seen in *ERBB3* and *SF3B1*. The tumor was MS stable and had a low TMB at 3 mutations/Mb. The PD-L1 IHC stain (shown) was negative. In the TCGA datasets, *ERBB2* amplification has been reported in fewer than 1% of cutaneous melanoma samples analyzed, and not in any of 80 uveal melanoma cases (cBioPortal, Aug 2017). IHC-based studies have shown that *HER2* over-expression is an extremely rare event in anal melanoma. As widely demonstrated, *ERBB2* amplification may predict sensitivity to therapies targeting *HER2*, including antibodies such as trastuzumab, pertuzumab, ado-trastuzumab emtansine (T-DM1) and dual EGFR/*HER2* kinase inhibitors such as lapatinib, afatinib, neratinib and dacomitinib. In patients with breast cancer, concurrent *PIK3CA* or *PTEN* alterations that activate the PI3K pathway have been associated with resistance to therapies that target *HER2*.



CONCLUSIONS

- UV light exposure drives high GA/tumor and TMB in CM, both associated with immune checkpoint inhibitor (ICPI) responsiveness, whereas AM lacks high TMB and therefore is far less likely to benefit from ICPI treatments
- CM is classically associated with opportunities for anti-*BRAF* therapies and AM with an array of kinase targets including *KIT*, *BRAF*, *PDGFRA* and *ERBB2*
- Finally, mTOR pathway targets are common to both tumor types
- The *SF3B1* genomic alterations found in this anal melanoma study have been linked to adverse prognosis in other non-cutaneous melanomas