Triple wild type melanoma profiling in the Caris Molecular Intelligence™ registry

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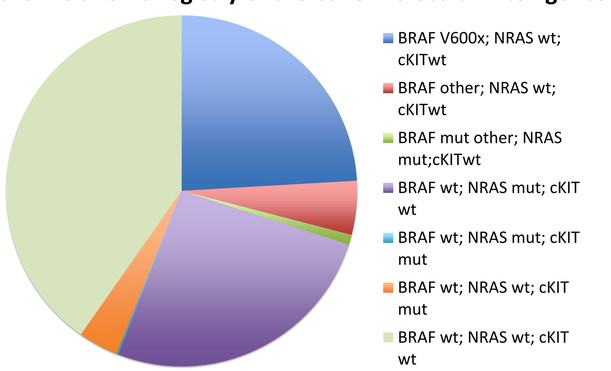
Background

Malignant melanoma is a genetically diverse disease. The most frequent mutation is BRAF (60%), followed by NRAS (30%) and cKIT (5%) mutations (1). While BRAF, NRAS and cKIT mutations represent the largest fraction of patients, for whom targeted therapies can be proposed using BRAF, MEK or cKIT inhibitors respectively (2), there is also an important group of patients lacking all of these three mutations, referred to as the triple wild type population (3xWT). These patients cannot currently benefit from the wealth of targeted therapies, except immunotherapies, and hence are in dire need for potential actionable targets. In order to better describe this important group of patients, we made use of the database of Caris Molecular Intelligence™. In this platform the tumors of patients were analyzed by next generation sequencing (NGS) of select hotspot mutations and also protein expression of selected protein markers by immunohistochemistry (IHC) including expression of PD-L1.

Methods

We used the entire available database of 541 melanoma patients. Patient samples were anonymized. Immunohistochemistry (IHC) and next generation sequencing (NGS) data was performed using standard technologies (3). qPCR was also available for BRAF V600E mutations. Age, sex and location of tumor was available as clinical variable while prior or later therapies were not specified. Out of the 541 samples 89 samples also had PD-L1 expression data available. Samples were grouped into 4 subtypes: BRAF^{mut}(n=169), NRAS^{mut} (n=151), cKIT^{mut} (n=25) and 3xWT (n=197) and further analyzed. Data was analyzed using Excel and R.

Figure 1. The distribution of mutation subtypes of melanoma in the melanoma registry of the Caris Molecular Intelligence™



Mutations	% of total samples
BRAF V600x; NRAS wt; cKITwt	24
BRAF other; NRAS wt; cKITwt	5
BRAF mut other; NRAS mut;cKITwt	0.9
BRAF wt; NRAS mut; cKIT wt	26
BRAF wt; NRAS mut; cKIT mut	0.2
BRAF wt; NRAS wt; cKIT mut	3.7
BRAF wt; NRAS wt; cKIT wt (3pl wt)	40.3
All samples	**100% (541 pts)**

Results

As shown in **Figure 1.,** the melanoma registry of the Caris Molecular Intelligence™ contrasts with the previously described mutation distribution (1). 3xWT melanoma are enriched in the database. Although unexpected, this observation can be explained by an increased likelihood of BRAF, NRAS and cKIT wild type patients to be referred for identification of targets by NGS analyses. Age and sex distribution was similar in all groups with cKIT mutant patients being slightly older and male patients more significantly referred than female patients (p=0.0014).

Figure 2. Demographics (age, sex) of subtypes of melanoma

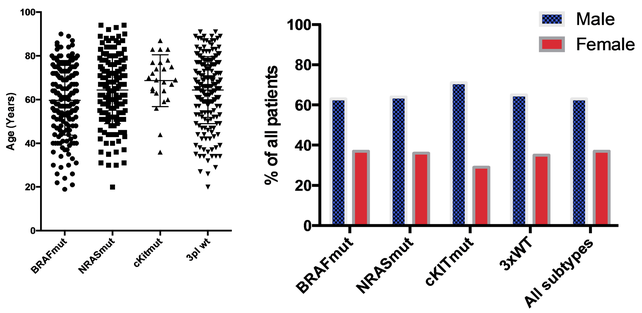


Figure 3. Frequency of a, protein expression (IHC 3+) and b, hot spot mutations (NGS) in mutation subtypes of melanoma

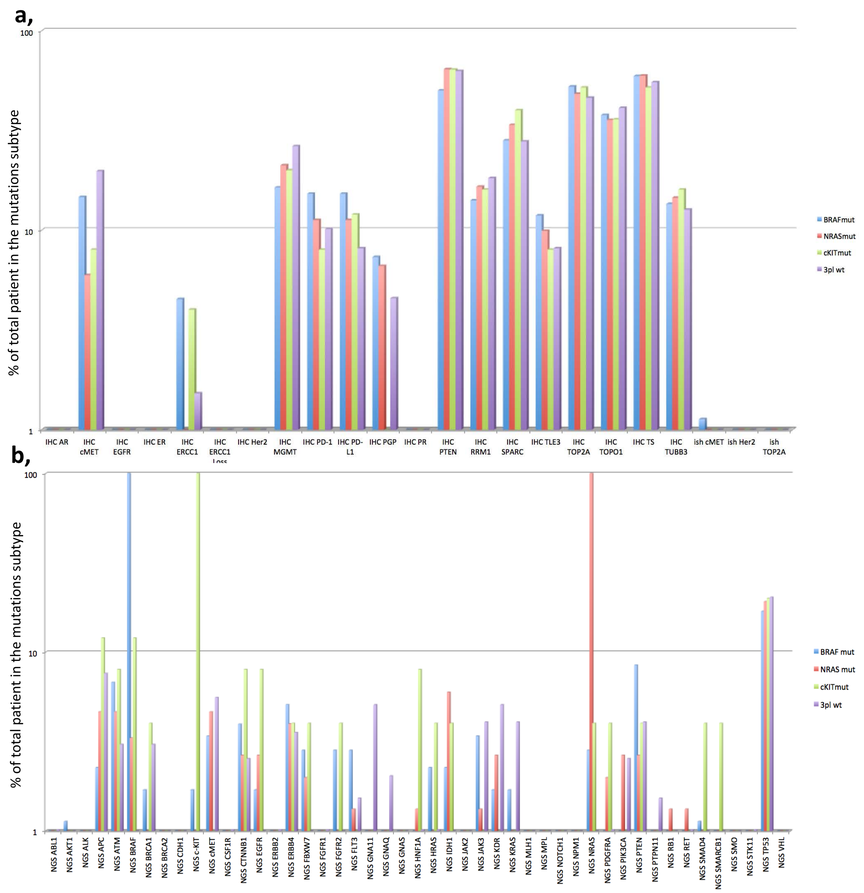


Figure 4. Frequency of hot spot mutations in the 3xWT melanoma is below 10% except for TP53. However overall targetable mutations can be identified in up to 40% of this patient population.

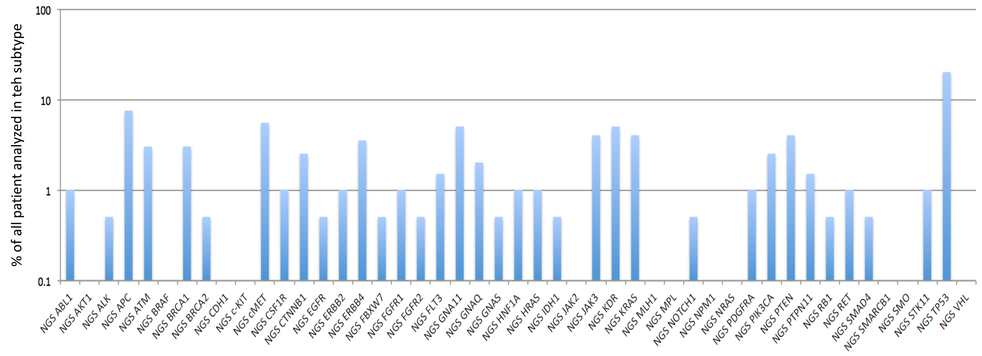


Figure 5. PD-L1 expression is uniform amongst all the mutation subtypes with a non significant higher portion in BRAF mutant patients

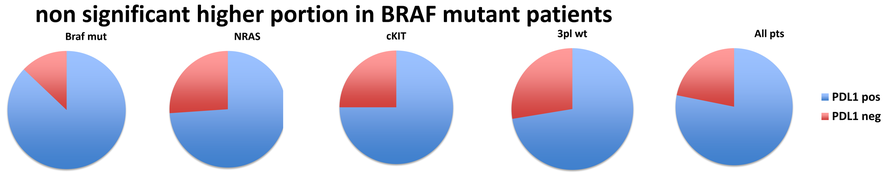
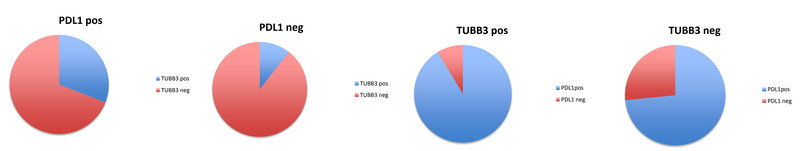


Figure 6. The putative taxan resistance marker, TUBB3 shows an increased expression in PD-L1 positive patients



Conclusions

1. 3xWT tumors have a different spectrum of mutations compared to the other three mutated subtypes
2. These mutations individually represent less than 10% but more than 1% frequency.
3. Actionable mutations are KRAS, JAK3, cMET, GNA11, GNAQ, APC, KDR, BRCA1, ERBB4 and their cumulative incidence is as high as 40% of patients
4. None of the 541 tumors had mutation in Akt, BRCA2, IDH1, CSF1R, GNAS, Notch1, Smo, STK11, VHL, MLH1, MPL, MPM1
5. All PD-L1 positive patients were also PD-1 positive and, conversely, all PD-L1 negative patients were PD-1 negative as well
PDL1 positive patients were 2.5 fold more likely to have TUBB3 overexpression a marker of taxol resistance
6. While more than 75% of patient had PD-L1 positive disease, there were more BRAF mutant patients positive for PD-L1 than with any other mutation subgroups
7. 3xWT and NRAS mutant tumors were more frequently PD-L1 negative
8. TP53 mutation did not seem to have any increase in PD-L1 positivity

References

1. E. Hodis et al., Cell. 2012 Jul 20; 150(2): 251-263
2. KT. Flaherty., N Engl J Med. 2012 November; 367(18): 1694–1703
3. JT. Miura., Cancer Biol Ther. 2015 May 4;16(5):764-9.