Real-world experience and clinical utility of a non-invasive gene expression rule-out test for melanoma and additional validation against high risk driver mutations in BRAF, NRAS and the TERT promoter

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SYNOPSIS

About 3 million surgical biopsies are performed in the US annually to diagnose fewer than 200,000 new cases of in situ and invasive melanomas using the current care standard of visual assessment and histopathology. Tools that reduce the number of surgical biopsies performed on benign skin lesions have the potential to improve patient care and reduce cost. The non-invasive pigmented lesion assay (PLA) is such a tool. It helps rule out melanoma and the need for surgical biopsies of pigmented skin lesions clinically suspicious of melanoma with a negative predictive value of over 99% by assessing LINC and PRAME gene expression.[1-5] Analyses of over 20,000 PLA samples in real-world routine-use settings in over 600 US dermatology offices demonstrate that only 12% of assessed lesions are PLA(+) (gene expression consistent with melanoma; LINC and/or PRAME detected). An ongoing real-world utility study of now over 500 cases demonstrates that clinicians follow the guidance of the PLA by surgically biopsying all (100%) of PLA(+) cases (n=61) while monitoring the vast majority (99%) of PLA(-) cases thereby reducing surgical biopsies by almost 90% while missing fewer melanomas. To date, 461 PLA(-) cases have been followed for 6 months and 272 cases have been followed for 1 year since the initial PLA(-) test result was obtained.

Efforts to validate the PLA beyond correlating it with histopathology demonstrated that somatic hotspot mutations in three genes known to be drivers of early melanoma development (BRAF other than V600E, NRAS and the TERT promoter) could be detected in non-invasively collected PLA samples in 2 patient cohorts with skin lesions clinically suspicious of melanoma. In cohort 1 samples (n=103, archival, histopathology available), at least one hotspot driver mutation was present in 77% of melanoma samples compared to only 14% in non-melanoma samples (p=0.0001). TERT promoter mutations were the most prevalent mutation type in PLA(+) melanomas. Eighty-two percent of PLA(-) lesions had no mutations and 97% of histopathologically confirmed melanomas were PLA and/or mutation (+). Mutation frequencies were similar (p=ns) in cohort 2 samples (n=519, prospectively collected real-world PLA samples, histopathology not available) in which 88% of PLA(-) samples had no detectable hotspot mutations.

Combining PLA gene expression analyses for LINC and PRAME and mutation analyses for BRAF other than V600E, RNAS and the TERT promoter enhances the ability to non-invasively detect early melanoma.

OBJECTIVES

- To assess the real-world utility of the PLA and determine, if physicians follow the guidance of the test
- To determine if BRAF, RNAS and TERT promoter mutations can be used as additional validation of PLA gene expression and if combining gene expression and mutation analyses further improves test performance

METHODS

All studies were IRB approved. Gene expression analyses were performed as previously described. Mutation analyses were performed by Sanger sequencing of adhesive patch and FFPE tissue block samples.

RESULTS

We demonstrate in an ongoing real-world utility study of now over 500 representative cases that clinicians follow the guidance of the PLA by surgically biopsying all (100%) of PLA(+) cases (n=61) while monitoring the vast majority (99%) of PLA(-) cases thereby reducing surgical biopsies by almost 90% while missing fewer melanomas. To date, 461 PLA(-) cases have been followed for 6 months and 272 cases have been followed for 1 year since the initial PLA(-) test result was obtained. Ninety three percent that tested double positive for gene expression of both LINC and PRAME were histopathologically classified as invasive melanoma or melanoma in situ. PRAME only and LINC only lesions were assessed as melanomas histopathologically in 50% and 7%, respectively. Assuming PLA(-) results without a follow up biopsy are true negatives, a sensitivity of 95% and a specificity of 91% with a negative predictive value of >99% was calculated. An additional also ongoing survey of 594 PLA routine-use cases in which clinicians self report actions taken further corroborates the PLA's utility - 99.4% of PLA(-) tests were not biopsied and monitored, 98.2% of PLA(+) cases were biopsied (only a single LINC only case without mutations was monitored).

Recent optimizations enabled the reliable extraction of both DNA and RNA from adhesive patch skin samples which made it possible to non-invasively analyze pigmented lesion cases suspicious for melanoma not only for gene expression via PLA, but also for the presence of somatic mutations. Mutation analyses in cohort 1 samples (n=103, archival, histopathology available) showed hotspot driver mutations (BRAF other than V600E, NRAS or TERT promoter mutations) in 77% of the 30 melanoma cases but only in 14% of the 73 non-melanoma cases. The frequency of the assessed early hotspot driver mutations in histopathologically confirmed melanomas is statistically higher than the frequency in non-melanoma cases p<0.0001). Ninety-seven percent of cases with a histopathologic consensus diagnosis of melanoma were either PLA gene expression or mutation positive, and 48% of non-melanomas were negative for expression of LINC, PRAME and driver mutations, highlighting the allure of an approach that looks at both RNA and DNA risk factors in a single non-invasively obtained sample. TERT promoter mutations were the most prevalent mutation type in PLA positive melanomas (79%). BRAF V600E mutations were present at similar frequencies in melanoma and non-melanoma samples (in 10% and 8% respectively). Conversely, BRAF V600K mutations (6%) and NRAS G61R and K5E (10%) mutations were found in melanomas only. Thirty-eight percent of invasive and 17% of in situ melanomas harbored multiple hotspot mutations. Figure 1 summarizes correlations of mutation, PLA and histopathologic analyses.

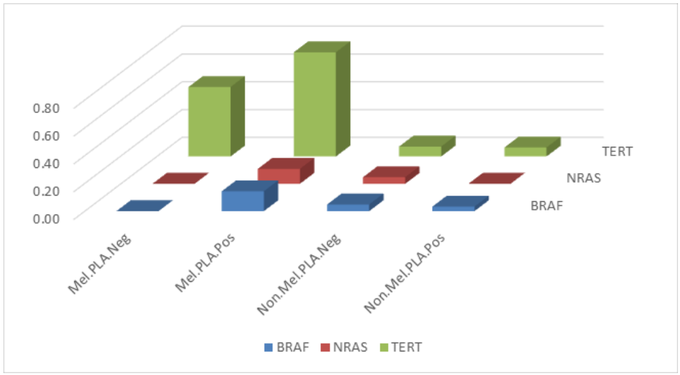

Figure 1: Correlation of mutation analyses (at least one BRAF non-V600E, NRAS or TERT promoter hotspot mutation detected), PLA gene expression analyses (LINC and/or PRAME detected) and histopathologic analyses (consensus diagnosis) in cohort 1 samples (n=103). Differences between melanoma and non-melanoma groups were highly statistically significant (p<0.0001). Mel.PLA.Neg.: PLA negative samples of histopathologically confirmed melanomas, Mel.PLA.Pos.: PLA positive samples of histopathologically confirmed melanomas, Non.Mel.PLA.Neg.: PLA negative samples of histopathologically confirmed non-melanomas, Non.Mel.PLA.Pos.: PLA positive samples of histopathologically confirmed non-melanomas.

To further corroborate the described hotspot mutation results in epidermal skin samples of pigmented skin lesions suspicious for melanoma, we compared findings from adhesive patch samples to findings in formalin-fixed paraffin embedded (FFPE) tissue blocks of surgical biopsies from the same lesions. Ninety-three percent of mutations detected in adhesive patch samples correlate with mutations in FFPE tissue blocks of the same lesions (n=41).

Subsequently, 519 prospectively collected real-world PLA samples from cohort 2, 387 PLA(-) and 132 PLA (+) cases were analyzed for these same mutations and a similar difference in the frequency of hotspot driver mutations was found. Eighty eight percent of real-world PLA(-) samples were also negative for any of these melanoma related mutations, similar to the 82% in cohort 1. Ten percent of PLA negative cases harbored mutations in the TERT promoter region. NRAS mutations (Q61K and G60L) were found in 1% of PLA (-) cases while none of these cases harbored G12 or G13 mutations. All BRAF mutations in PLA (-) cases were V600E mutations (4%). PLA(+) cases in real-world cohort 2 samples had mutation frequencies similar to the cohort 1 validation set. The two groups were not statistically different. Figure 2 depicts the comparison of cohort 1 and 2 for the absence of mutations in PLA (-) samples irrespective of histopathology (available only for cohort 1).

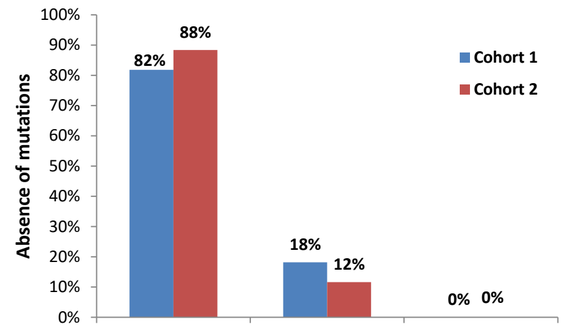

Figure 2: Comparison of hotspot driver mutations in PLA(-) cases of cohort 1 and cohort 2. PLA(-) cases were assessed for the absence of BRAF (non-V600E), NRAS and TERT promoter hotspot mutations. There were no statistically significant differences between cohort 1 and cohort 2 (p=ns).

CONCLUSIONS

- The PLA reduces surgical biopsies by 88% while missing fewer melanomas
- A PLA NPV >99% correlates with a less than 1% probability of a negative test missing a melanoma compared to an NPV of 83% for histopathology (17% probability of missing melanoma).
- Physicians follow the guidance of the test and surgically biopsy PLA(+) lesions while PLA(-) lesions are monitored.
- Hotspot driver mutation analyses further validate gene expression results – combining gene expression and mutation analyses further improves test performance.

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Conflict of interest disclosures: LF, MS, GP and CC are advisors and/or investigators, BJ, ZY and JR are employees of DermTech

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