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

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ABSTRACT

Tools that reduce the number of surgical biopsies performed on benign skin lesions have the potential to improve patient care. The non-invasive pigmented lesion assay (PLA) gene expression test is such a tool. It helps rule out melanoma and the need for surgical biopsies of atypical pigmented skin lesions with a NPV >99%. Analyses of ~15,000 PLA samples in the real-world routine use setting of over 600 US dermatology offices demonstrated that ~88% were PLA(-). A real-world utility study in 381 cases demonstrated that 99% of PLA(-) cases were monitored, while all PLA(+) cases were surgically biopsied demonstrating that clinicians follow the guidance of the test. With efforts to validate the PLA beyond histopathology, we found that we can identify somatic mutations in three genes known to be drivers of melanoma development (BRAF, NRAS and the TERT promoter) in PLA samples. Mutations in adhesive patch PLA samples were concordant with mutations in biopsies. The frequency of mutations in melanoma samples was 77% and statistically higher than the 14% found in non-melanoma samples (p=0.0001). TERT promoter mutations were the most prevalent mutation type in PLA(+) melanomas (79%). Eighty-six percent of non-melanomas had no mutations and 97% of histopathologically confirmed melanomas were PLA and/or mutation positive (n=103). Mutation frequencies were similar in 519 additional prospectively collected real-world PLA samples, with 88% of PLA(-) samples having no mutations. Combining gene expression and mutation analyses 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 described. (Ref. 1-5) Mutation analyses were performed by Sanger sequencing of adhesive patch and FFPE tissue block samples.

RESULTS

A total of 381 real-world use cases were analyzed, 330 in the PLA(-) cohort and 51 in the PLA(+) cohort. Table 1 shows the summary of clinical management for these 2 cohorts.

	Melanoma	Non-Melanoma	Clinical Management
PLA(+)	19 (12 MIS, 7 Invasive)	32	100% Biopsied
PLA(-)	1**	329* (3 follow-up biopsies, all non-melanoma)	99% Surveillance

*Assumes for this analysis that tests negative for biopsy (biopsies not performed during 3-6 month follow-up visits) are non-melanomas. An additional study that observes PLA negative lesions for up to 2 years and re-assesses the gene expression status during this prolonged period has been initiated recently. **Chart reviews of 330 cases for biopsies 3-6 months after PLA testing identified one case where a surgical biopsy was performed on the same day PLA testing was performed.

Table1: Assessment of real-world PLA results from four US dermatology sites. 330 PLA(-) and 51 PLA(+) real-world cohorts and their clinical management were studied. Pathology reports of PLA(+) tests and follow-up procedures for PLA(-) tests at 3-6 months after PLA testing were reviewed.

Ninety-nine percent (99%) of the PLA(-) results were managed with follow up surveillance per standard of care. Three (<1%) PLA(-) lesions were subject to follow up biopsy at 3-6 months and histopathologic diagnoses for these three lesions were non-melanoma. Of the 51 PLA(+) lesions, all (100%) were subject to surgical biopsies (65% tangential / scoop procedures often describes as shaves, 33% excisional and 2% punch biopsies). Histopathologically, 47 (92%) of these 51 PLA(+) lesions were melanocytic in nature and 4 (8%) were non-melanocytic with a diagnosis of seborrheic or actinic keratosis. Nineteen (37%) were histopathologically diagnosed as melanoma (12 MIS and 7 invasive, Stage 1) resulting in a a number needed to biopsy (NNB) to detect one melanoma of 2.7 and a biopsy ratio of 1.7 benign lesions for each melanoma detected. The number of excisions performed per melanoma found was 1.6. Moderately to severely atypical nevi constituted 22% of cases while 27% of cases were nevi with mild atypia. Six percent of nevus cases showed no atypia. Thirteen (13) of 14 lesions (93%) that tested double positive for gene expression of both LINC518 and PRAME were histopathologically classified as invasive melanoma or MIS with the remaining case being characterized histopathologically as atypical melanocytic proliferation. PRAME only and LINC00518 only lesions were 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. The prevalence of melanoma in this study was 5% (19 of 381 cases). For comparative purposes, the sensitivity of VAH from histopathology review was determined to be 84%, with 16% of 108 melanomas (MIS/Stage 1) having a consensus read discordant to the primary read.

Using pigmented lesion tissue collected non-invasively via adhesive patches, we identified somatic mutations in three genes known to be drivers of melanoma development (BRAF, NRAS and the TERT promoter). The mutation frequency in BRAF, NRAS, and TERT genes was characterized in 103 Cohort 1 adhesive patch platform PLA samples in histopathologically confirmed melanomas (n=30) and non-melanoma cases (n=73). Figure 1 summarizes these findings.

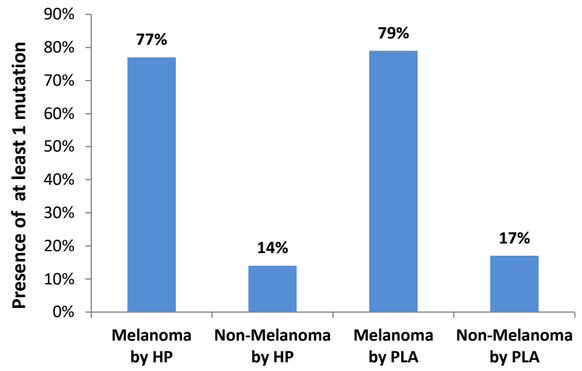

Figure 1: Samples with at least one BRAF (non-V600E), NRAS and TERT promoter hotspot mutation in fully annotated pigmented lesion samples with histopathologic consensus diagnoses (Cohort 1, n=103). Differences between melanoma and non-melanoma groups were highly statistically significant (p<0.0001). TERT promoter mutations were the most prevalent mutations observed in 79% of PLA positive cases.

BRAF V600E mutations were present at similar frequencies in melanoma and non-melanoma samples (in 10% and 8% of cases, respectively). Conversely, BRAF V600K mutations (6%) and NRAS G61R and K5E (10% of cases) mutations were found only in melanoma and often co-existed with TERT promoter mutations. Among melanomas in Cohort 1 with TERT mutations, 11 harbored -124G>A mutations and -146G>A mutations were also found in 11 cases. Furthermore, -126G>A, -132G>A and -138G>A were observed in cases harboring multiple TERT promoter mutations. Of the 30 cases with a histopathologic consensus diagnosis of melanoma, 6 were melanoma in situ / lentigo maligna cases and 24 were invasive melanomas with a median tumor thickness of 0.58 mm. Of the 73 non-melanoma cases, 61 were nevi (44 atypical and 17 conventional nevi) and 12 were non-melanocytic lesions.

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 (n=132) in real-world Cohort 2 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 (which was available only for Cohort 1).

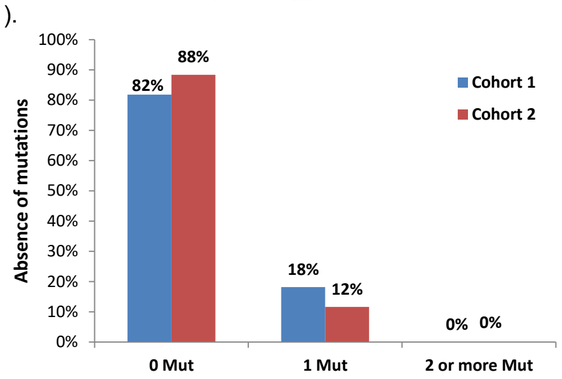

Figure 2: Comparison of hotspot driver mutations in PLA(-) cases of Cohort 1 and Cohort 2. PLA(-) cases were assessed for the absence of BRAF (non-V600E), NRAS and TERT promoter hotspot mutations. There were no statistically significant differences between Cohort 1 and Cohort 2 (p=ns).

CONCLUSIONS

- The PLA reduces surgical biopsies by 88% while missing fewer melanomas
- A PLA NPV >99% is linked to 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, RM, PG and CC are DermTech Scientific Advisory Board Members. BJ and ZY are employees of DermTech

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