# A minimally-invasive collection technique for the assessment of molecular signatures in cutaneous melanocytic lesions: a pilot study

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**Background:** 'Precision dermatology' requires the identification and validation of biomarkers in relation to histologic diagnosis and prognosis. In patients with melanocytic lesions, such molecular signatures would help stratify patients according to their risk for skin melanoma. Most of the excised suspicious lesions are benign, thus being able to excise only those who are truly malignant is of relevance to avoid unnecessary stress and scarring for the patients. Recently, a microbiopsy device (MB) has been developed that enables the anesthetic and scar-free collection of tiny pieces of skin (Figure 1a & Figure 1b).

**Aims**: To evaluate in samples obtained from microbiopsies: (a) the quantity of nucleic acids obtained upon extraction from two different types of MBs, and (b) the accuracy and sensitivity of the BRAF c600 mutation screening results.

**Figure 1a**. Conventional (3 mm) vs. Micro biopsies

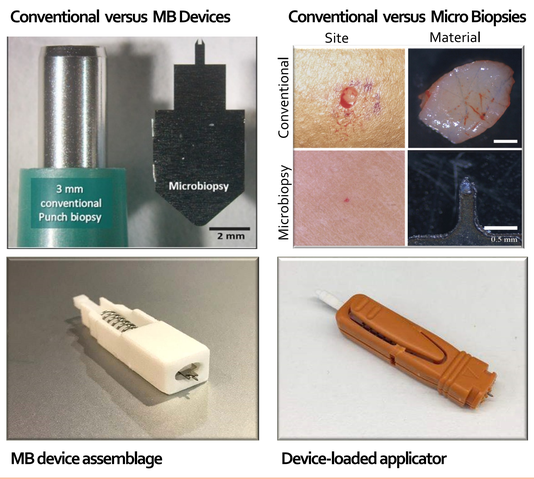
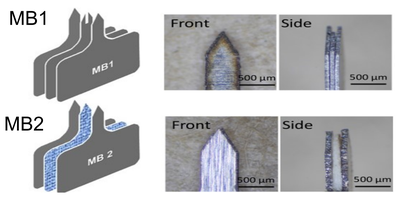
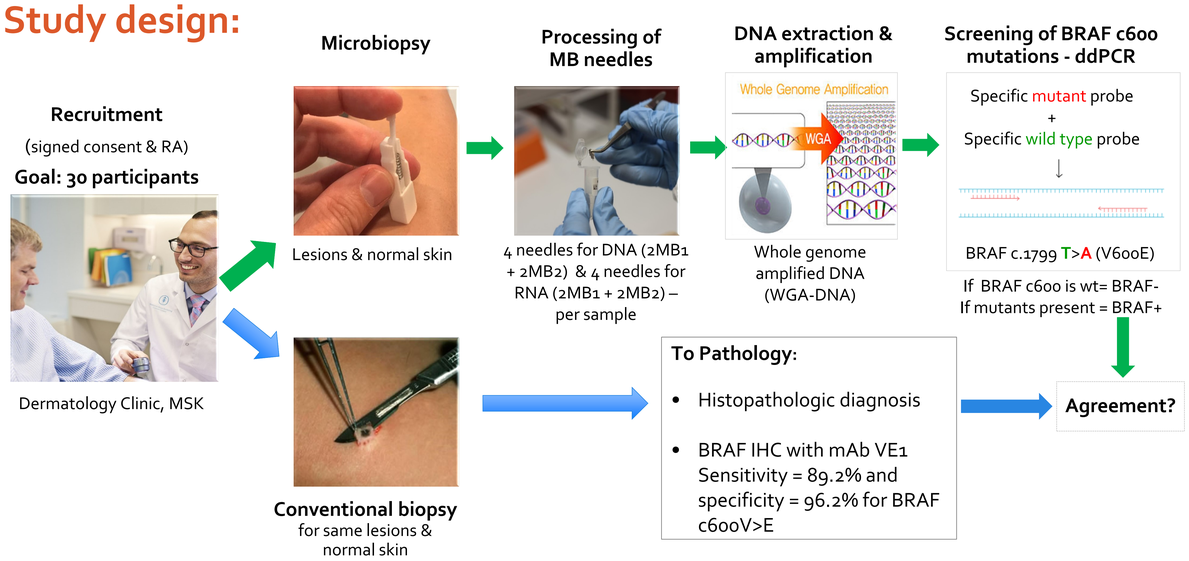



**Figure 1b**. Two types of Microdevices




Note that MB2 (bottom device) includes an absorbent Whatman paper (shown in blue)

## Study design:




**Results:** To date we tested 19 samples obtained with MB1 and MB2 from 6 banal nevi 4 normal skin with MB1 of 6 participants (Table 1).

- DNA yield**:** The purified WGA-DNAs' mean, median, and ranges for samples from melanocytic lesions were:
  - From undetectable to 24600 ng; ave 5103 ng; med 29 ng for MB1.
  - From undetectable to 26000 ng; ave 10224 ng; med 222 ng for MB2.
- Screening of BRAF V600E mutations by ddPCR (Figure 2):
  - Mutations were detected in 5 of 6 banal nevi (BRAF+).
  - Normal skin was BRAF+ in 1 of 4 NL skin samples.
  - The percentage of detected mutants was approximately the same between the two devices.
- Histopathology diagnosis: One specimen turned to be a neuroma instead of a banal nevus.
- BRAF-IHC: All the specimens tested to date, with the exception of the neuroma, were BRAF+ (Figure 3; Table 1).

**Figure 2**. ddPCR outcome

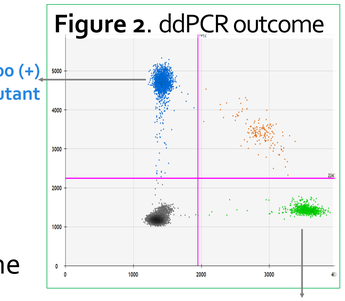
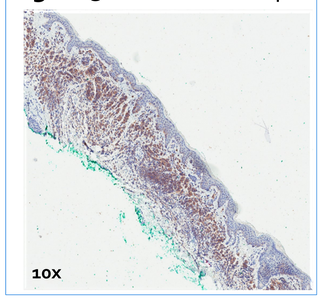



**Table 1. ddPCR and IHC results**

| | Type of sample | # Mutant droplets | # WT Droplets | Proportion of mut+ by ddPCR | BRAFmut by IHC |
|---|---|---|---|---|---|
| 1 | Banal nevus | 46 | 497 | 8.5 % | + |
| 2 | Banal nevus | 52 | 175 | 22.9 % | + |
| 3 | Banal nevus | 0 | 44 | 0 | - |
| | NL skin | 0 | 168 | 0 | - |
| 4 | Banal nevus | 213 | 316 | 40.3 % | + |
| | NL skin | 2 | 196 | 1% | - |
| 5 | Banal nevus | 1316 | 2476 | 34.7 % | pending |
| | NL skin | 0 | 758 | 0 | pending |
| 6 | Banal nevus | 825 | 3157 | 20.7% | pending |
| | NL skin | 0 | 4073 | 0 | pending |

**Figure 3**. IHC BRAF+ sample




## Discussion and future steps:

- In this ongoing IRB approved study, preliminary data from 19 specimens show full agreement between ddPCR and conventional BRAF-IHC results.
- As of now, the device 2 (MB2) seems to collect a greater amount of tissue.
- ddPCR detected BRAF+ in a normal skin indicating that this method might be more sensitive than IHC.
- Next: We will continue testing additional samples; we will evaluate RNA quantity and gene expression from the already collected frozen specimens.
- Future plans: We would like to compare our minimally invasive technique and data to results obtained with a commercially available adhesive patch.

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