Ultra-late melanoma recurrence is infrequent, poorly understood and, in most cases, difficult to unambiguously distinguish from a new primary melanoma. We identified a patient with a second melanoma diagnosed after a 30-year disease-free interval, and sought to determine if this new lesion was a recurrence of the original melanoma. Here we report the genomic sequence analysis of the exomes of 2 melanoma lesions isolated from the same individual in 1985 and 2015, and their comparison to each other and to the germline DNA of the patient. Identification of many shared somatic mutations between these lesions proves a lineal relationship spanning 30 years. Unlike prior reports of ultra-late melanoma recurrence, the availability of the original tumor and the use of comprehensive genomic analysis allowed us to confirm that the second lesion is truly a recurrence. We demonstrate the acquisition of numerous additional mutations during the 30 decade asymptomatic period. These data highlight the low but very long-lasting risk of recurrence in this patient population.

KEYWORDS
exome sequencing, melanoma, recurrence, tumor evolution

1 | INTRODUCTION

The incidence of ultra-late recurrence (beyond 10-15 years) of cutaneous melanoma has been estimated to be 2.0% to 6.9% from large series, with occasional reports of apparently recurrent melanoma after 40 years and 41 years. Two major factors are potentially confounding. Firstly, the risks of second primary melanoma (5%) and of melanoma of unknown primary (2.7%) create uncertainty about whether at least some of these late-onset tumors might be independent lesions. Secondly, the original pathology specimen is often unavailable for comparative analysis.

Next-generation sequencing can unambiguously resolve the relationship, if any, between metachronous lesions by comparing somatic mutations. Here we have used exome sequencing to confirm the lineal relationship between 2 melanomas resected from a patient at our institution in 1985 and 2015. This is, to our knowledge, the longest disease-free interval that has been so rigorously confirmed in melanoma or any other solid tumor type.

2 | CASE REPORT

A Caucasian male in his 70’s with a previous history of melanoma presented to the Dermatology Clinic on referral for evaluation of a new onset subcutaneous nodule of the left thorax unresponsive to antibiotics given by his primary care provider. The patient also had a concurrent prostate cancer diagnosis. In 1985, the patient had a superficial spreading malignant melanoma, Clark Level 4, Breslow depth of 2 mm biopsied from his left flank. He underwent a wide local excision with left axillary lymphadenectomy. The pathology report showed no residual melanoma and no lymph node involvement. Subsequent skin checks over 30 years showed no suspicious lesions and no clinical evidence of recurrence.

The skin exam revealed 2 well-healed surgical scars of his left infra-axillary/thorax area. The posterior scar corresponded to the...
excision of the primary melanoma. The anterior scar corresponded to the lymphadenectomy excision. At the midpoint between the scars was a somewhat mobile 2 cm subcutaneous nodule with no overlying pigmentation. No palpable masses or pigmentation were associated with the surgical scars. No clinical adenopathy was detected. Fine needle aspiration biopsy of the nodule was interpreted as showing malignant melanoma. In light of the clinical history and anatomical location, the subsequent excision was histopathologically interpreted as consistent with metastatic melanoma as there was neither a junctional component nor deep dermal scar indicative of prior excision present.

Given this history, we considered the possibility that this lesion was a recurrence of the earlier melanoma. The 1985 specimen was retrieved from our institution’s Cancer Biobank. Genomic DNA was isolated by macrodissection of pathologist-verified neoplastic cells from 10 micron sections of both tumors (Figure 1) and from whole blood. Exome libraries targeting 21,522 genes were sequenced to an average depth of 64×, 73× and 96× on the germline, 1985 and 2015 samples, respectively.

Using Varscan7 to compare both tumor samples with the patient’s germline DNA sequence, 157 shared somatic variants were identified in both melanoma specimens (Figure 2). A representative sample of somatic mutations is listed in Table 1, ordered by their variant allele frequency in the 1985 sample. Both samples shared identical alterations in *BRAF* (V600E) and *EGFR* (S768I), genes commonly altered in melanoma8 and likely driver genes in this case. The majority of the other alterations listed are likely passenger mutations. The first 11 alterations listed, detected in both tumors, confirm the common origin of both lesions. The remaining 9 alterations were only found in the 2015 sample, providing possible evidence of tumor evolution during the process of recurrence.

3 | DISCUSSION

Although likely ultra-late melanoma recurrences have previously been noted, this is the first case in which analysis of somatic mutations was used to unambiguously exclude the possibility that the second tumor is an independent lesion. Hypotheses advanced to explain the long clinically tumor-free periods observed in these patients include dormancy mediated by interaction with the immune system, and due to limitations in angiogenic factors, as well as cellular dormancy in which quiescence is induced by intrinsic or microenvironmental factors.9 In each case, melanoma cells remain as individual cells or small foci, the expansion of which is constrained for a prolonged period of time.

Once established, escape from dormancy may require several changes in the tumor cells and/or their microenvironmental niche. As recently reviewed,10 this may involve modulation of multiple factors in the neoplastic cells (including FAK, SRC and β1-integrin), their microenvironment (eg, TGFβ), reduced immune cell function or systemic instigation.11 This recurrence acquired several additional mutations, perhaps arguing against a model solely involving release from a microenvironmental checkpoint. Notably, prostate cancer is associated with increased risk of primary melanoma.12 If the systemic changes resulting from prostate tumor growth can support the outgrowth of primary melanoma, it is tempting to speculate that similar mechanisms facilitated expansion of this secondary melanoma after 3 dormant decades in this patient.

---

**FIGURE 1** Histology of melanoma specimens. A, Hematoxylin and eosin stained section of the 1985 melanoma specimen. B, Hematoxylin and eosin stained section of the 2015 melanoma specimen. The main images were captured at 4×. Insets, showing the neoplastic cells, were captured at 10×.

**FIGURE 2** Circos plot of somatic mutations shared between 1985 and 2015 melanoma specimens. Somatic mutations (as called by the Varscan 2 somatic algorithm) which were shared between both samples are depicted on the Circos plot. The outer red track shows somatic mutations in the 1985 sample. The inner green track shows somatic mutations in the 2015 sample. Each bar length is proportional to the variant allele frequency of that mutation.
MATERIALS AND METHODS

4.1 Ethical approval of studies and informed consent

This study was approved by our Institutional Review Board, and the patient consented to the publication of this report.

4.2 DNA isolation

Slides were stained using hematoxylin & eosin and regions of malignant cells were identified by a pathologist. Corresponding regions were scraped into a small volume of ethanol from 10 micron slides, transferred to a microcentrifuge tube, dried and extracted in xylene. Tumor DNA was purified using the QIAamp DNA FFPE kit (Qiagen; Germantown, MD, USA). Germline DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen; Germantown, MD, USA). Double-stranded DNA was quantified using Quant-IT Picogreen (Invitrogen; Carlsbad, CA, USA) and a Synergy 2 plate reader (Biotek; Winooski, VT, USA).

4.3 Exome sequencing

Samples were transferred to BGI Tech Solutions (Hong Kong) for exome sequencing. Exome libraries were prepared to target a total of 50 megabases from 357,999 exons of 21,522 genes using the SureSelect All Exon V5 kit (Agilent; Santa Clara, CA, USA). Sequencing was performed by BGI on an Illumina (San Diego, CA, USA) HiSeq 4000 using 150 bp paired-end reads.

4.4 Data analysis

Sequencing reads meeting QC thresholds were mapped to the human genome (hg19) using BWA.13,14 Local alignment around indels was performed using GATK15,16 and duplicate reads were removed using Picard. SNVs and indels were called using HaplotypeCaller of GATK. Variants were annotated using wANNOVAR.17 SNVs were also independently called using Partek Genomics Suite and Varscan 2.7 The Circos plot was generated using J-Circos.18 The primary goal of the analysis was to identify somatic variants with high confidence, and determine which variants were in common or different in the 2 tumor samples. Accordingly, an arbitrary minimum threshold of 22× sequence depth at each variant was required in order to be included in Table 1.

ACKNOWLEDGEMENTS

This study was supported by research funds from the Gundersen Medical Foundation. PK holds the Dr. Jon & Betty Kabara Endowed Chair in Precision Oncology.

REFERENCES