



SKIN CANCER (OTHER THAN MELANOMA)

## IMPLEMENTATION OF A NEXT-GENERATION SEQUENCING PANEL TO IDENTIFY MOLECULAR ALTERATIONS IN BASAL CELL CARCINOMA SUBTYPES.

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**Background:** Basal cell carcinoma (BCC) is the most common cutaneous malignant neoplasia in fair-skinned individuals with increasing incidence rates worldwide. Aberrant activation of the Hedgehog pathway (HH) is the molecular driver in BCC pathogenesis, with the majority of BCCs carrying mutations in PTCH1 or SMO genes. However, the great variability in morphology, aggressiveness and response to treatment of BCC remains unexplained and highlights the hypothesis that additional genes might contribute to tumorigenesis of different BCC subtypes.

**Objective:** We evaluated the feasibility of a next-generation target sequencing (NGS) approach to identify additional molecular alterations beyond HH signaling, in the pathogenesis of BCC. For this purpose, we developed a custom-designed BCC-mutation panel for NGS, using the Ion Torrent™ Technology.

**Materials and methods:** 54 fresh-frozen BCCs and matched normal tissues were analysed by targeted NGS using a custom-designed panel of 12 genes (CSMD1, CSMD2, DPH3 promoter, PTCH1, SMO, GLI1, NOTCH1, NOTCH2, TP53, ITIH2, DPP10, STEAP4). The BCC-mutation panel has been designed with Ion Ampliseq technology and used on Ion PGM System (ThermoFisher). Somatic mutations in TERT promoter were analysed by Sanger sequencing.

**Results:** Our study showed a good performance of the BCC mutation panel, including high coverage of target regions (94.4%) and high sequence coverage uniformity (> 85%). Variants were identified in 10/12 (83.3%) genes analysed. In details, PTCH1 and TP53 genes were mutated in 72% and 65% of BCCs respectively and a high mutation rate was observed in CSMD2 (80%), NOTCH1 (80%), CSMD1 (60%), and NOTCH2 (40%) genes.





Additionally, we identified TERT promoter mutations in 59% of cases, being -146C>T the most frequent.

Conclusions: These findings demonstrated the feasibility of our custom-designed NGS approach for the study of molecular variability of BCCs and confirmed a complex network of genetic alterations beyond HH signalling, in the pathogenesis of BCC.

