Circulating tumour DNA (ctDNA) based detection of genetic immune escape in advanced prostate cancer (aPCa)

Aslı D. Munzur[1], Jack V.W. Bacon[1], Gillian Vandekerkhove[1], Gráinne Donnellan[1], Cecily Q. Bernales[1], Jenny N. Tran[2], Franz Fenninger[2], Karen R. Sherwood[2], Alexander W. Wyatt[1,3], Nicolette M. Fonseca[4,5]

[1]Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, Canada; [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada; [3]Michael Smith Genome Sciences Centre, BC Cancer, Vancouver, British Columbia, Canada; [4]Department of Biochemistry and Microbiology, University of Victoria, Canada; [5]Department of Basic and Translational Research, Deeley Research Centre, BC Cancer, Victoria, Canada

Background: T-cell recognition of neoantigens presented by the Major Histocompatibility Complex Class I molecules (MHC Class I) is essential for anti-tumour immunity. Loss of MHC Class I expression therefore represents a mechanism of immune evasion in aPCa, with implications for the efficacy of T-cell based immunotherapies. However, the genomic events contributing to MHC Class I loss, particularly loss of heterozygosity (LOH) of the polymorphic *HLA-A*, *HLA-B* and *HLA-C* genes, remain poorly defined. Furthermore, ctDNA has not been investigated as an analyte for tracking tumour-specific HLA alterations, as current approaches rely on whole genome or exome sequencing of tissue.

Methods: We developed a targeted DNA sequencing panel (ImmGen) to capture 6597 unique *HLA-A*, *HLA-B* and *HLA-C* alleles. Probe design was optimised using machine learning applied to the IMGT-HLA database (v3.10). HLA genotyping and HLA-LOH detection was performed using published tools and validated in select cases with targeted NGS-based clinical grade HLA genotyping (Holotype HLA, v2 Omixon).

Results: We assembled a cohort of 40 aPC patients with cell-free DNA (n=28), tumour tissue (n=17) and matched germline DNA (n=40). ImmGen achieved 90% HLA allele concordance (216/240) across 3 HLA genotyping tools, versus 79% (90/114) via exome sequencing (where available). In 19 patients with available Holotype HLA results, ImmGen HLA types were 100% concordant. Germline HLA homozygosity was detected in 39% of patients (16/40). HLA-LOH was detected in ctDNA (>30% purity) and tissue from 33% (8/24) and 35% (6/17) of patients respectively.

Conclusions: Our results demonstrate that HLA-LOH can be detected non-invasively and in real-time using cost-effective targeted sequencing of ctDNA.

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