Comprehensive molecular profiling of single Circulating Tumor Cells (CTCs) reveals intra-patient heterogeneity

Claudio Forcato¹, Valentina del Monaco¹, Alberto Ferrari¹, Mario Terracciano¹, Massimiliano Pellicano¹, Paola Tonini¹, Marianna Garonzi¹, Francesca Fontana¹, Genny Buson¹, Rui P. Neves², Nikolas H. Stoecklein³, Johann De Bono³, Penny Flohr³, Maryou Lambros³, Karim Riham³, Andrea Ardizzoni³ and Nicolò Manaresi³

¹Menarini Silicon Biosystems S.p.A., Bologna, Italy; ²Oncologia Medica, Policlinico Sant’Orsola-Malpighi, Bologna, Italy; ³The Institute of Cancer Research and Royal Marsden NHS Foundation Trust, London, United Kingdom; ⁴University Hospital of the Heinrich-Heine-University Düsseldorf, Department of General, Visceral and Pediatric Surgery, Düsseldorf

Introduction Cell-based liquid biopsy offers an unprecedented opportunity to identify novel cancer biomarkers, study tumor heterogeneity and monitor cancer progression, without the need for invasive procedures. Here we present a complete and comprehensive workflow* to detect hotspot mutations, focal copy-number (CN) amplifications and genome-wide CN alterations in single cells from clinical samples. The whole process includes a LM-PCR Whole Genome Amplification (WGA) followed by library preparation with a WGA-tailored NGS targeted panel and a low-pass WGS protocol.

Methods Blood samples from patients affected by prostate (n=3) and lung cancer (n=3) were enriched with CellSearch® and 60 single cells were isolated using DEPArray™ Nxt technology. Single cell DNA was then whole-genome amplified using Ampli1™ WGA kit (Menarini Silicon Biosystems). Starting from the same Ampli1™ WGA products, Illumina-compatible libraries were generated using the Ampli1™ OncoSeek Panel and the Ampli1™ LowPass (Menarini Silicon Biosystems) kits. After sequencing on MiSeq/HiSeq platforms, raw data were analysed using assay-specific applications on the MSBiosuite platform (Menarini Silicon Biosystems).

Results Targeted sequencing revealed the homozygous missense TP53:p.P278A and KRAS:p.G12C mutations, found in most CTCs of two different lung patients, respectively (Fig.2). Similarly, a prostate patient showed a homozygous frameshift mutation (APC:p.T1556Nfs*) shared among all sequenced CTCs, while the heterozygous missense TP53:p.R248W mutation was detected only in 25% (2/8) of CTCs, implying intra-patient heterogeneity (Fig.3). Inter- and intra-patient heterogeneity was also confirmed for CN amplifications on AR, MYC and KIT genes. Additional information was offered by genome-wide CN profiles which highlighted, on lung and prostate patients, patterns of sub-chromosomal losses – and then Loss-of-Heterozygosity – and gains on genome regions including known oncogenes as MYC (Fig.3).

Conclusions Here we presented a solution for a comprehensive and accurate molecular characterization of single CTCs from clinical samples, which revealed precious information on inter- and intra-patient tumor heterogeneity.

*The CELLSEARCH® - DEPArray - Ampli1 workflow is for Research Use Only. Not for use in diagnostic procedures.

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