ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

NEW MODELS AND TECHNOLOGIES FOR PERSONALISED MEDICINE

Annals of Oncology 28 (Supplement 7): vii2–vii11, 2017
doi:10.1093/annonc/mdx508

5 Genome-wide identification of actionable copy number alterations from targeted sequencing panels with Excavator2

L. Mazzarella[1], R. D'aurizio[2], G. Frige[1], A. Guida[1], E. Belloni[1], E. Marino[1], L. Bernard[1], P. Pelicci[1], A. Magi[3]

[1]European Institute of Oncology, Milan, Italy, [2]Institute for Informatics and Telematics, CNR, Pisa, Italy, [3]University of Florence, Florence, Italy

Background: Alterations of clinical interest, ("actionable") include Copy Number Alterations (CNA), but most sequencing panels and bioinformatic pipelines are optimised for detection of Single Nucleotide Variants, not informative for CNA-driven tumours. Furthermore, identifying CNA boundaries becomes clinically relevant when genes with opposing effects on drug sensitivity are involved on single CNAs. With hybridization-based enrichment methods, a sizeable fraction of reads align off-target. Our Excavator2 algorithm exploits off-target reads to call CNAs and outperforms standard, on-target read-based CNA-calling algorithms on Whole Exome Sequencing (NAR 2016 Nov 16;44(20):e154). We tested whether Excavator2 calls genome-wide CNAs using sequencing data from small, hybridization-based targeted enrichment panels.

Methods: We compared CNA callers (EXCAVATOR2, CopywriteR and CNVkit) on simulated cancer genomes containing CNAs of varying size (100-5000 kb), on-target and off-target (both = class1, on-target only= class 2 in Fig. 1) with different tumour cellularity (10-90%), generated with Xome-Blender (Semeraro et al., Submitted).We then compared genome-wide CNA calls from targeted sequencing using the Agilent ClearSeq cancer panel (coding sequences of 46 genes) vs high-density SNP-array on 5 HER2-amplified breast cancer samples. Finally, we called CNAs involving 58 genes identified as actionable from literature search in a separate set of 7 lung tumours, ClearSeq-sequenced.

Results: Precision/recall for Excavator2 was superior across the whole range of simulations (Fig. 1). Excavator2 and CopywriteR both showed comparable resolution with SNP array for on-target (True Positive Rate (TPR)=0.75 and 0.66 and False Positive Rate (FPR)=0 and 0.09 respectively) and, more interestingly, on off-target genes genome-wide (TPR=0.9 and 0.9 and FPR=0.21 and 0.1 respectively). As expected, precision/recall increased with higher tumour cellularity and CNA size for both algorithms (Fig. 2, with representative sample). On lung tumours, Excavator2 identified multiple actionable alterations on both on-target and off-target genes (Fig. 3).

Conclusions: Excavator2 calls actionable CNAs, otherwise unidentifiable with targeted sequencing panels, with comparable resolution to gold standard, significantly enlarging analytical capabilities and clinical usefulness of routine clinical sequencing. Additional validation with other sequencing panels is ongoing.

Legal entity responsible for the study: IEO
Funding: Italian Ministry of Health GR-2011-02352026
Disclosure: All authors have declared no conflicts of interest.

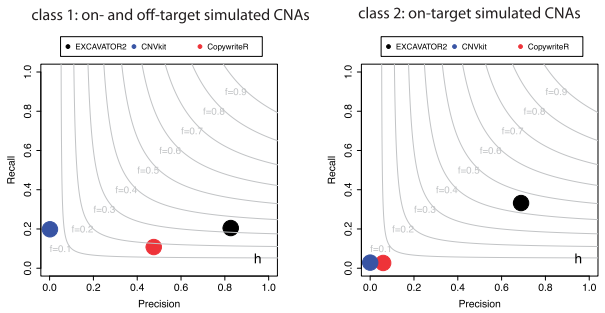

Figure 1: Abstract 5

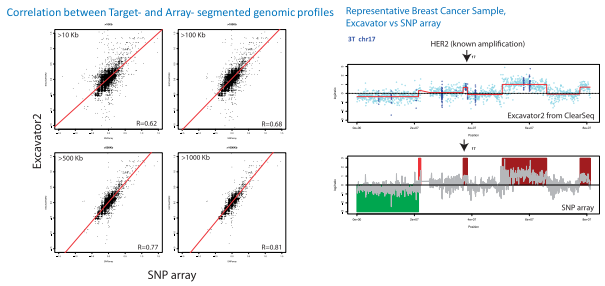

Figure 2: Abstract 5

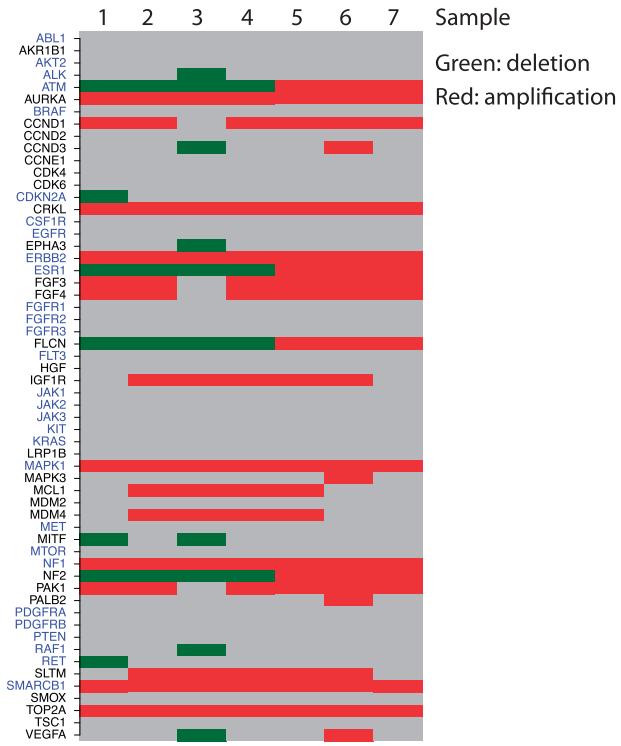

Figure 3: Abstract 5