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Comparison of data from two commercially available tissue-based comprehensive genomic profiling (CGP) solutions using AMP/ASCO/CAP guidelines and ESMO ESCAT

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Summary

The AVENIO CGP and TSO-500 assays, two commercially available CGP solutions with variant reporting analysis software, had differences in the detection of TMB and ESCAT biomarkers, including CNAs. These differences are important considerations for any CGP solution.

Introduction

- CGP, a next-generation sequencing approach, enables the detection of novel and known variants of all four classes of genomic alterations in known cancer-related genes and genomic signatures, such as TMB, MSI and gLOH.[1]
- Increased adoption of personalised medicine has brought CGP solutions to the fore; however, while testing solutions can broaden access to genomic profiling, assays, and especially bioinformatics and variant reporting analysis software, differ.
- The study compared differences in data generated by the AVENIO Tumor Tissue CGP RUO Kit (AVENIO CGP RUO; Roche, Branchburg, NJ, USA and Foundation Medicine GmbH, Penzberg, Germany) paired with navify® Mutation Profiler RUO software (Roche Sequencing Solutions, Inc, Pleasanton, CA, USA), and the TruSight Oncology 500 assay (TSO-500; at Signature Diagnostics GmbH) paired with PierianDx Clinical Genomics Workspace v6.21.0 software (PierianDx, Inc., Creve Coeur, MO, USA).

Methods

- Sequencing was performed using the AVENIO CGP (RUO) and TSO-500 (RUO) assays per the manufacturer instructions. Aliquots of DNA (and RNA for the TSO-500 assay) were analysed from 145 formalin-fixed, paraffin-embedded solid tumour tissue specimens of various origins (prostate: n=28; breast: n=27; colon: n=26; lung: n=25 [others: miscellaneous]).
- AVENIO CGP sequence data were analysed using a proprietary software system developed by Foundation Medicine, Inc. (FoundationOne® Analysis Platform) (bioinformatics analysis). AVENIO CGP has been shown to have a high degree of alignment with the FoundationOne® CDx assay in a previous study.[2]
- TSO-500 sequence data were analysed using manufacturer-provided software (bioinformatics analysis).
- Key variant annotation (variant reporting analysis) data outputs were variant tier classification[3,4] and ESCAT guideline inclusion per tumour type.[5]
- AMP/ASCO/CAP variant tiers were obtained with navify® Mutation Profiler software for AVENIO CGP or PierianDx software for TSO-500. ESCAT inclusion was determined manually.
- All Roche materials used are for RUO and not for use in clinical diagnostic procedures in the USA or EU, except the navify® Mutation Profiler, which is CE-IVD.

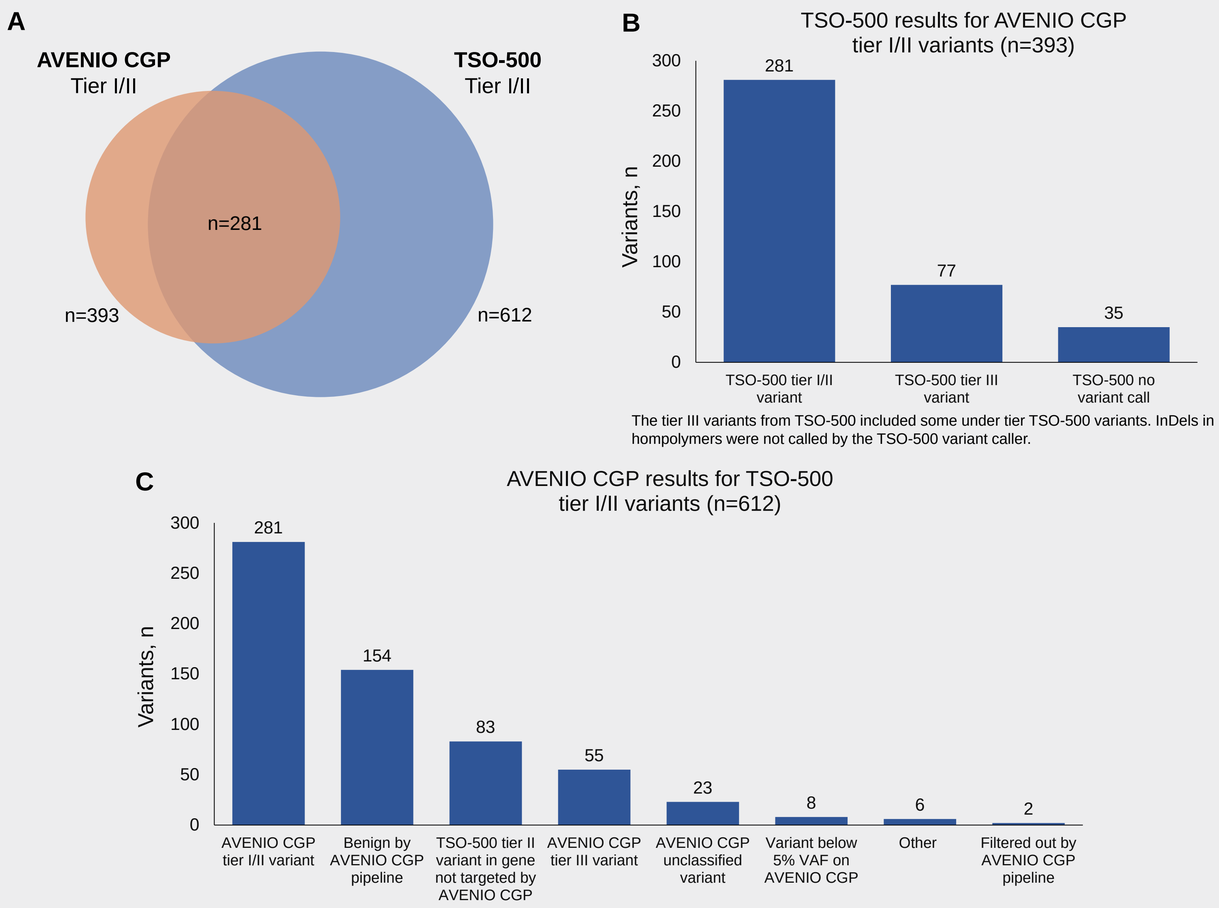
Results

- **This study identified 34 ESCAT variants; AVENIO CGP detected all 34, while TSO-500 missed 5/11 CNAs in *ERBB2* (*HER2*).**
- **TMB with TSO-500 was significantly higher than with AVENIO CGP, leading to the potential for some samples to be mis-classified based on a TMB cut-off of 10 mut/Mb.**
- **The AVENIO bioinformatic pipeline normalises for tumour ploidy and purity when calculating copy number and also filters putative benign variants.**

Variant detection and ESCAT categorisation

- Variants detected using the AVENIO CGP and TSO-500 assays and categorised by AMP/ASCO/CAP guideline inclusion are shown in Figure 1.
- Tier I/II variants detected by both assays were: 724 short variants (SVs; single nucleotide and insertion/deletion variants), 408 copy number alterations (CNAs) and 54 gene fusions.

Figure 1. Tier I/II variants (tiered by the AMP/ASCO/CAP guidelines) and top identified genes. A) Venn diagram showing the proportion of tier I/II variants in each assay. Assay results for tier I/II variants for the B) TSO-500 assay (versus the AVENIO CGP assay tier I/II variants) and C) AVENIO CGP assay (versus the TSO-500 tier I/II variants).



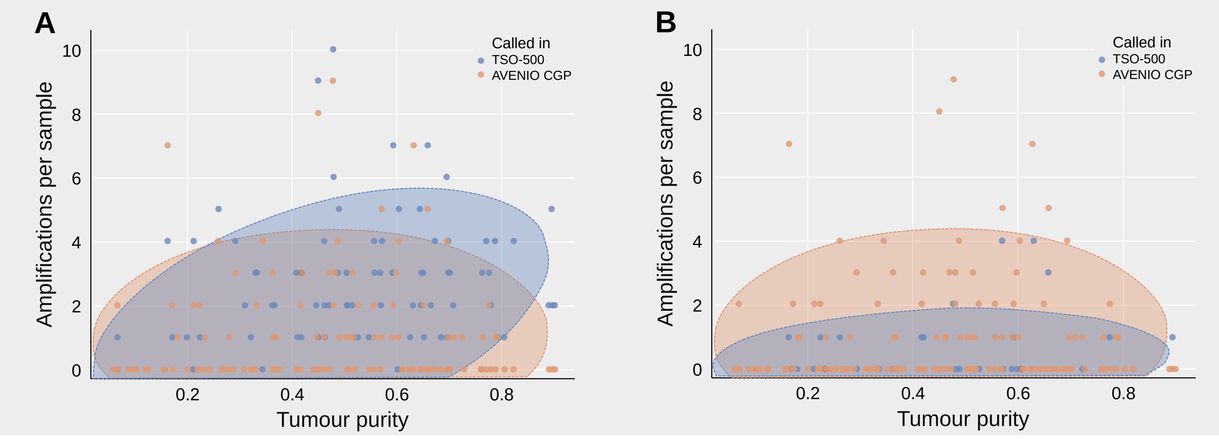
The tier III variants from TSO-500 included some under tier TSO-500 variants. InDels in hompolymers were not called by the TSO-500 variant caller.

- For ESCAT tier I/II variants identified by either AVENIO CGP or TSO-500 (n=34), all were identified by AVENIO CGP whereas TSO-500 missed 5/11 CNAs (all *ERBB2* amplifications; see Figure 2).
- Fewer variants were identified by the AVENIO CGP assay, mostly due to the assay design and filtering of putative benign variants.
- Only a single variant (*KRAS* G12F mutation) characterised as tier I by TSO-500 (using the AMP/ASCO/CAP guidelines) was not identified by the AVENIO CGP assay due to being slightly below the 5% VAF cutoff.

Copy number differences

- The AVENIO CGP assay models tumour purity to accurately call CNAs, whereas CNA calls are dependent on purity (Spearman correlation coefficient: 0.53; p<0.0001) (Figure 2).
- Average positive agreement for CNAs was 28.5% and average negative agreement was 98.0%.

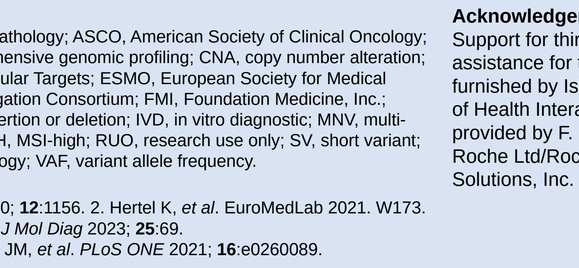
Figure 2. CNAs per sample (y-axis) vs tumour purity (from FMI; x-axis) for the TSO-500 (blue) and AVENIO CGP (orange) assays. Variants were filtered for tier I/II and only genes where both assays called amplifications. TSO-500 fold change thresholds were set at A) 1.5 and B) 3.19.[6] No thresholds were required for AVENIO CGP.



TMB differences

- TMB with TSO-500 was significantly higher versus AVENIO CGP (average 3.4 mut/Mb; p<0.001).
- TSO-500 called n=41/145 samples TMB >10 mut/Mb, vs 31/145 samples for AVENIO CGP (Figure 3).
- Differences in bioinformatic approaches could account for TMB overestimation by TSO-500, given the high degree of alignment demonstrated between AVENIO CGP and the FoundationOne® CDx assay in a prior study.[2]

Figure 3. Comparison of TMB scores between the two assays – TSO-500 (x-axis; log scale) and pairwise difference (y-axis). Reasons for differences between assays are shown in the table.



Reasons for differences between the TSO-500 and AVENIO CGP assays

Assay	Reason	n
AVENIO GCP	AF below threshold	54
	Close to 5% AF threshold	31
	Gene not targeted	80
	In TMB file, but excluded from score	3
	MNVs are excluded	11
	Other	49
	TSO-500 filter: GermlineFilterProxi	64
	TSO-500 filter: No CodingVariant	6
TSO-500	AF below threshold	100
	ExAC allele count above threshold (>2)	52
	Gene not targeted	967
	Known/likely/ambiguous variant	266
	No potential germline call	42
	Other	43
	Potential germline variant	89

Other biomarkers and variants

- Overall agreement for MSI was good between assays (MSI-H by AVENIO CGP: n=5; 4/5 also called MSI-H by TSO-500).
- A comparison of gLOH could not be performed since the TSO-500 assay did not offer a comparable biomarker; however, 1/4 patients with an 'eligible' ovarian cancer disease ontology presented with loss of heterozygosity based on the AVENIO CGP assay.
- The data set had limited value for comparing fusion-calling functionality and the only relevant fusions previously described in the context of the investigated diseases were *TMPRSS2-ERG*, where there was good agreement.

Conclusions

- With growing knowledge of molecular drivers, future clinical CGP solutions must accurately detect guideline-relevant alterations. Two commercially available CGP solutions with variant reporting analysis software had differences in TMB scoring and ESCAT variant detection, specifically for CNAs. Differences in variant and biomarker detection can be explained by different bioinformatic approaches to variant calling, filtering, tiering and normalisation. These are important considerations for any future clinical CGP solution and may result in different results across CGP tests if not properly controlled.

https://ter.li/gxqgrn

Please contact the lead author at *twieland@foundationmedicine.com* for any questions.

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Abbreviations
AF, allele frequency; AMP, Association for Molecular Pathology; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; CGP, comprehensive genomic profiling; CNA, copy number alteration; ESCAT, ESMO Scale for Clinical Actionability of molecular Targets; ESMO, European Society for Medical Oncology; EU, European Union; ExAC, Exome Aggregation Consortium; FMI, Foundation Medicine, Inc.; gLOH, genome-wide loss of heterozygosity; InDel, insertion or deletion; IVD, in vitro diagnostic; MNV, multi-nucleotide variant; MSI, microsatellite instability; MSI-H, MSI-high; RUO, research use only; SV, short variant; TMB, tumour mutational burden; TSO, TruSight Oncology; VAF, variant allele frequency.

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