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Benchmarking whole exome sequencing in the German network for personalized medicine

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ABSTRACT

Introduction: Whole Exome Sequencing (WES) has emerged as an efficient tool in clinical cancer diagnostics to broaden the scope from panel-based diagnostics to screening of all genes and enabling robust determination of complex biomarkers in a single analysis.
Methods: To assess concordance, six formalin-fixed paraffin-embedded (FFPE) tissue specimens and four commercial reference standards were analyzed by WES as matched tumor-normal DNA at 21 NGS centers in Germany, each employing local wet-lab and bioinformatics. Somatic and germline variants, copy-number alterations (CNAs), and complex biomarkers were investigated. Somatic variant calling was performed in 494 diagnostically relevant cancer genes. The raw data were collected and re-analyzed with a central bioinformatic pipeline to separate wet- and dry-lab variability.
Results: The mean positive percentage agreement (PPA) of somatic variant calling was 76 % while the positive predictive value (PPV) was 89 % in relation to a consensus list of variants found by at least five centers. Variant filtering was identified as the main cause for divergent variant calls. Adjusting filter criteria and re-analysis increased the PPA to 88 % for all and 97 % for the clinically relevant variants. CNA calls were concordant for 82 % of genomic regions. Homologous recombination deficiency (HRD), tumor mutational burden (TMB), and microsatellite instability (MSI) status were concordant for 94 %, 93 %, and 93 % of calls, respectively. Variability of CNAs and complex biomarkers did not decrease considerably after harmonization of the bioinformatic processing and was hence attributed mainly to wet-lab differences.
Conclusion: Continuous optimization of bioinformatic workflows and participating in round robin tests are recommended.

1. Introduction

Currently, the implementation of clinical Whole Exome Sequencing (WES) in predictive molecular cancer diagnostics is expedited by decreasing sequencing costs and government reimbursement schemes in Germany. WES offers several substantial advantages [1] over sequencing panels, such as eliminating the risk to miss targetable alterations in the coding sequences of the genome and enabling *post-hoc* retrospective research in an unrestricted manner once new putative targets emerge. Further, using WES provides a more robust measurement of complex biomarkers [2,3].

To evaluate reproducibility between different pipelines and uncover opportunities for improvement of workflows, we compared an unprecedented number of diagnostic centers regarding their initial implementation of WES analysis. Previous WES implementation studies with clinical focus were either single center [4–6], or focused on limited metrics, such as single complex biomarkers or only variant calling [7–17]. An earlier study performed in Germany included a smaller number of laboratories investigated WES of fresh frozen tissue samples [1]. WES for cancer patients from FFPE samples has not been compared between a large number of centers before.

Six clinical FFPE tissue specimens and four commercial reference samples of matched tumor and normal DNA samples were analyzed in the German National Initiative for Personalized Medicine (DNPM) at 21 participating centers, using locally established wet-lab workflows and dry-lab bioinformatics pipelines. Somatic and germline mutations in diagnostically relevant genes, complex biomarkers, and copy-number alterations (CNAs) were reported and evaluated for concordance. Discrepancies were cooperatively assessed to identify relevant factors for optimization and harmonization of WES in clinical cancer diagnostics. Additionally, raw sequencing data sets were re-analyzed using a central bioinformatic pipeline to separate wet- and dry-lab variability. This study presents the results of a national comprehensive FFPE tissue specimen based evaluation for WES analysis in a clinical routine diagnostic setting.

2. Material and methods

Six FFPE tissue specimens were selected to represent various cancer types and biological features (Table 1). Broad consent was given by the patients and all analysis were performed in line with the Declaration of Helsinki. Further, four reference samples were included with two cases of low and high tumor mutational burden (TMB), as well as two cases of low and high Homologous Recombination Deficiency (HRD) score. Matched tumor-normal DNA from FFPE tissue specimens was extracted centrally. DNA from the FFPE specimen and the reference samples were shipped to the 21 participants for wet- and dry-lab analysis.

Each center used their wet and dry-lab protocols to analyze a defined set of parameters, including somatic and germline variants, CNA, and complex biomarkers. Next, the results were gathered and compared for concordance and against the known values of the reference samples. Raw data was collected as unfiltered variant calls and unaligned reads. Those were re-analyzed using the pipeline of Center-5

Table 1
Samples used in the pilot study with previously determined characteristics from TSO500 (samples 3 - 16) and reference material (samples 17 – 20). Complex biomarker were were not determined (N.D.) for all samples. *CytoSNP; $^{+}$confirmed by Marker panel; ‡determined by Seraseq.

Sample ID	Entity / Reference	Pathological tumor purity (%)	HRD	TMB	MSI (%)
3	Undifferentiated pleomorphic sarcoma of the lateral femur	90	70 *	3.6	1.23
4	SMARCA4 deficient undifferentiated uterine sarcoma (SDUS)	80	N.D.	39.4	56.3^{+}
9	Breast cancer of no special type (NST)	80	N.D.	3.2	1.65
13	Endometrioid carcinoma grade 2	80	N.D.	77.3	58.5^{+}
14	Moderately differentiated adenocarcinoma of the colon	50	2	44.7	20.34^{+}
16	Moderately differentiated adenocarcinoma of the colon	80	11	48.6	62.6^{+}
17	Seraseq TMB Mix Score 7	66	N.D.	7‡	N.D.
18	Seraseq TMB Mix Score 13	66	N.D.	13‡	N.D.
19	Seraseq gDNA HRD Low-Positive	66	58‡	N.D.	N.D.
20	Seraseq gDNA HRD Negative	66	33‡	N.D.	N.D.

to separate effects of wet and dry-lab. Detailed information can be found in the Supplementary Methods.

3. Results

WES was performed using local wet-lab and bioinformatic pipelines (Supplementary Table 1). Most centers successfully sequenced all of the samples, while Center-1 and Center-11 excluded samples 3 and 16 because of sequencing quality issues.

Across 18 centers and the sequenced tumor samples, the median mean target coverage was 268x (lower quartile 218, upper quartile 354). A total of 154 (88 %) samples were covered with > 100x for at least 80 % of the target region (Supplementary Figure 1). Of the remaining three centers, two (Center-10 and Center-12) had a lower mean coverage (87x and 73x), while Center-19 had a considerably higher coverage (1374x).

3.1. Somatic variants

Somatic variants in the predefined list of 454 diagnostically relevant cancer genes (Supplementary Table 2) were compared by chromosomal position and base alteration. Based on a total of 1014 unique non-synonymous somatic variants detected by 5799 variant calls, we defined a consensus list of 321 variants that were called by at least five centers (Fig. 1a). The distribution of missed variant calls shows that more variant calls (60 %) where missed by few (1–5 centers), while 40 % of calls where missed by more centers (Supplementary Figure 2a). Across centers, a positive percentage agreement of 76 % (5000 variant calls) was reached with respect to this list. By contrast, a total of 1590 (24 %) variant calls were missed by one or more centers which were examined in an in-depth analysis to uncover the causes for the discrepancies (Fig. 1a). Of the missed variants, 750 (47 %) could be found by reviewing the unfiltered variant calls and were reported to the respective centers for review. Variant filters, including PASS filter (18.3 %) and low variant allele fraction (24.4 %) were reported as predominant causes for not reporting (Fig. 1b). Several local filter rules that were only used in a single or in few centers were observed (Supplementary Figu. 2b). Further, several missed calls were due to incorrect filter rules and reported as erroneous.

For 31 % of the reported variant calls the VAF was below 5 %. In the consensus list only four variants (1 %) were below this threshold as many centers filtered variant calls with a fixed VAF threshold. We evaluated the influence of a central VAF cut-off on PPV and PPA. For a 5 % VAF cut-off the mean PPV increased from 89 % to 91 %, due to the removal of possible false-positives, the mean PPA increased from 74 % to 75 %. For a 10 % VAF cut-off the mean PPV increased further to 95 %, while the mean PPA increased from 74 % to 80 % as more consensus variants were found between 5–10 % VAF (55 Variants, 17 %).

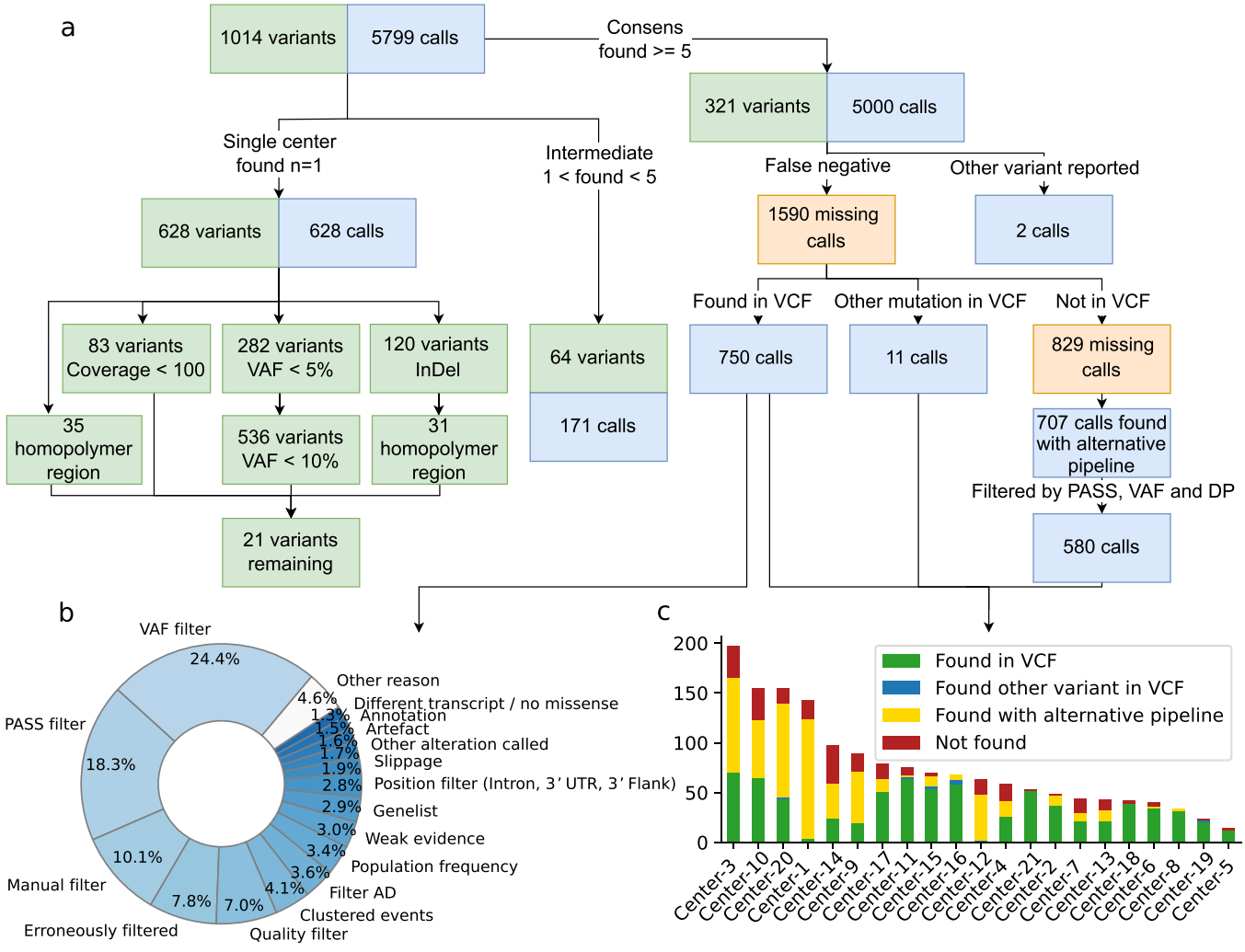

Fig. 1. Somatic variant calls. a: Variants separated by occurrence. b: Reasons reported by centers for missed variants. c: Fractions of missed variants found by another method or not found separated by center.

Of the remaining 829 missed calls, 580 (70 %) could be identified by the re-analysis of raw-data with the central bioinformatics pipeline. An additional 127 variants were found but filtered out by either depth, variant allele fraction (VAF) or PASS filter. Overall, only 122 (1.9 %) of all expected somatic variant calls were not identified by either initial analysis, raw variant calls or re-analysis. Those variants showed a low mean VAF of 2.4 %. The fractions of found to miss ratio varied among the centers (Fig. 1c). The set of missed variant calls showed a significant enrichment of InDels (Supplementary Figure 3a). The missed InDels were often located at homopolymer sites (120 of 144 = 83 %). Further, a negative correlation of PPA with the sample sequencing depth was observed (slope=−0.011, p = 0.007, Supplementary Fig. 3b).

A total of 628 variants were only reported by single centers (Fig. 1a) of which all but 21 variants could be classified as either low VAF (< 5 % or < 10 %), low depth (< 100 reads), InDels or variants at homopolymer sites. Most of these variants were reported by centers 11, 13, 16, 20 and especially 19, which reported the most variants which was in line with a much higher sequencing coverage.

In the absence of a gold-standard for somatic variants in the analyzed cases different consensus lists were selected based on the number of missed calls. For each of the lists, PPA and positive predictive value (PPV) were determined. The median PPA for the five-center consensus list (16 missed calls allowed) was 75 %, while a median PPV of 89 % was reached (Fig. 2a,b). Considerably lower PPVs were observed for the five centers that submitted the highest numbers of variants (listed above).

Based on the five-center consensus list, the unfiltered variant calls of each center were searched for the missed calls. Including these variant calls led to a considerable increase of PPA for previously lower performing centers (Fig. 2c, middle). Re-analysis of raw data using the same bioinformatics pipeline further increased the PPA to a mean of 88 %. This correction especially improved the results of the worst performing centers (Fig. 2c, right). Clustering of centers by detected somatic variants showed no relation to wet- or dry-lab procedures (Supplementary Fig 4). No correlation between self-reported experience with WES analysis and variant calling performance (PPA, PPV) was observed (p values between 0.3 - 0.6).

The five center consensus list was screened for druggable targets using OncoKB without considering cancer type [18]. Altogether, there were 31 druggable variants, of which on average 80 % were identified by the centers (523 calls). An additional 17 % (111 calls) could be found either in the list of raw variant calls or with the re-analysis of raw-data as described previously, resulting in total of 97 % of the druggable variants that were identified (Fig. 3).

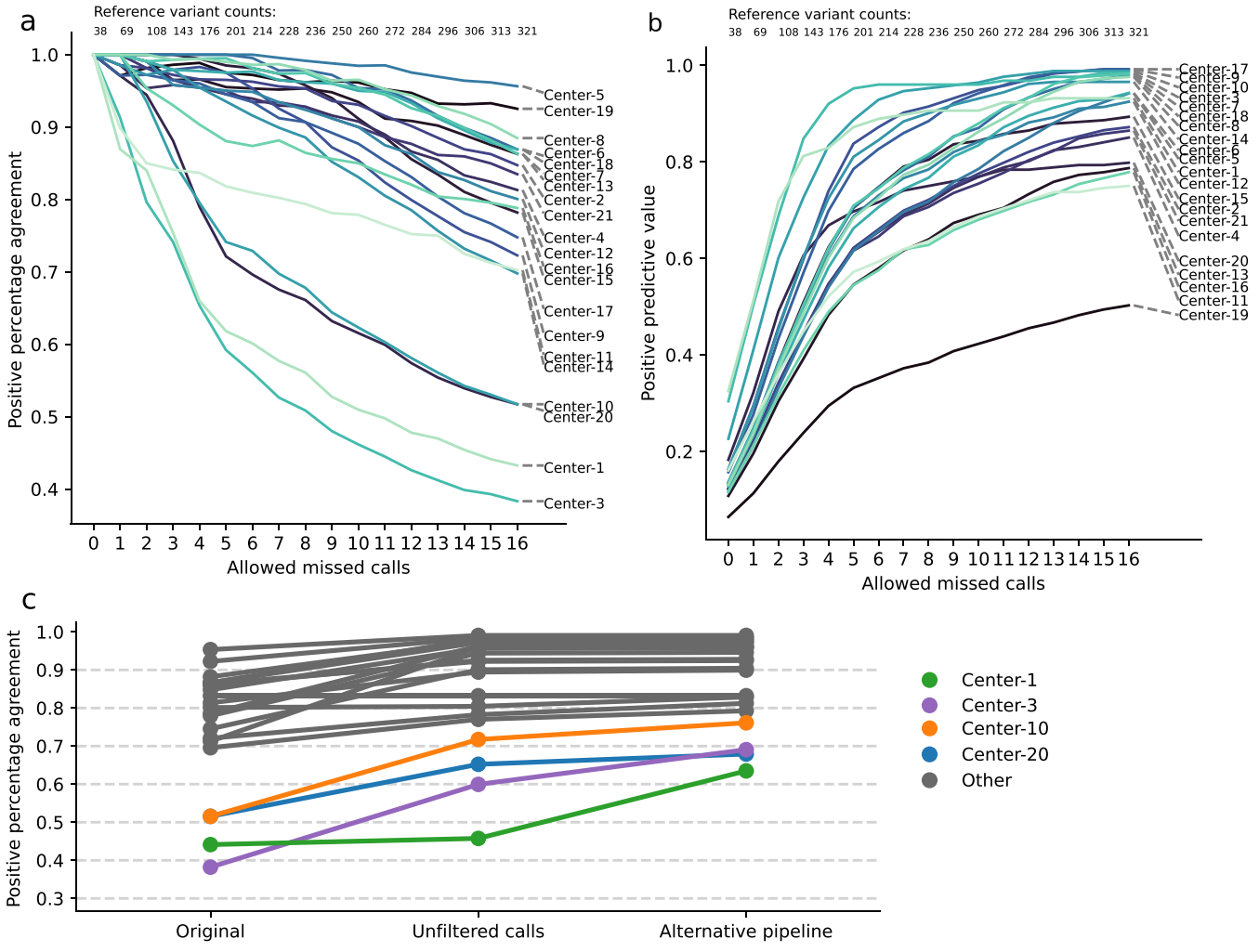

Fig. 2. Positive percentage agreement (PPA) and positive predictive values (PPV) of variant calls. a: PPA in relation to allowed missed calls and inversely to the consensus counts. For zero missed calls a consensus of all 21 centers is found, for 16 allowed misses a consensus of five centers is found. The consensus counts were created by selecting the variants missed by at most the number of centers annotated at the bottom. The top shows the number of variants for each of the consensus counts. b: PPV in relation to decreasing consensus counts. c: Change in PPA for unfiltered calls (middle), or the single re-analysis with basic filters (PASS filter, VAF >= 3.5 %, depth >= 100) in relation to the five center consensus.

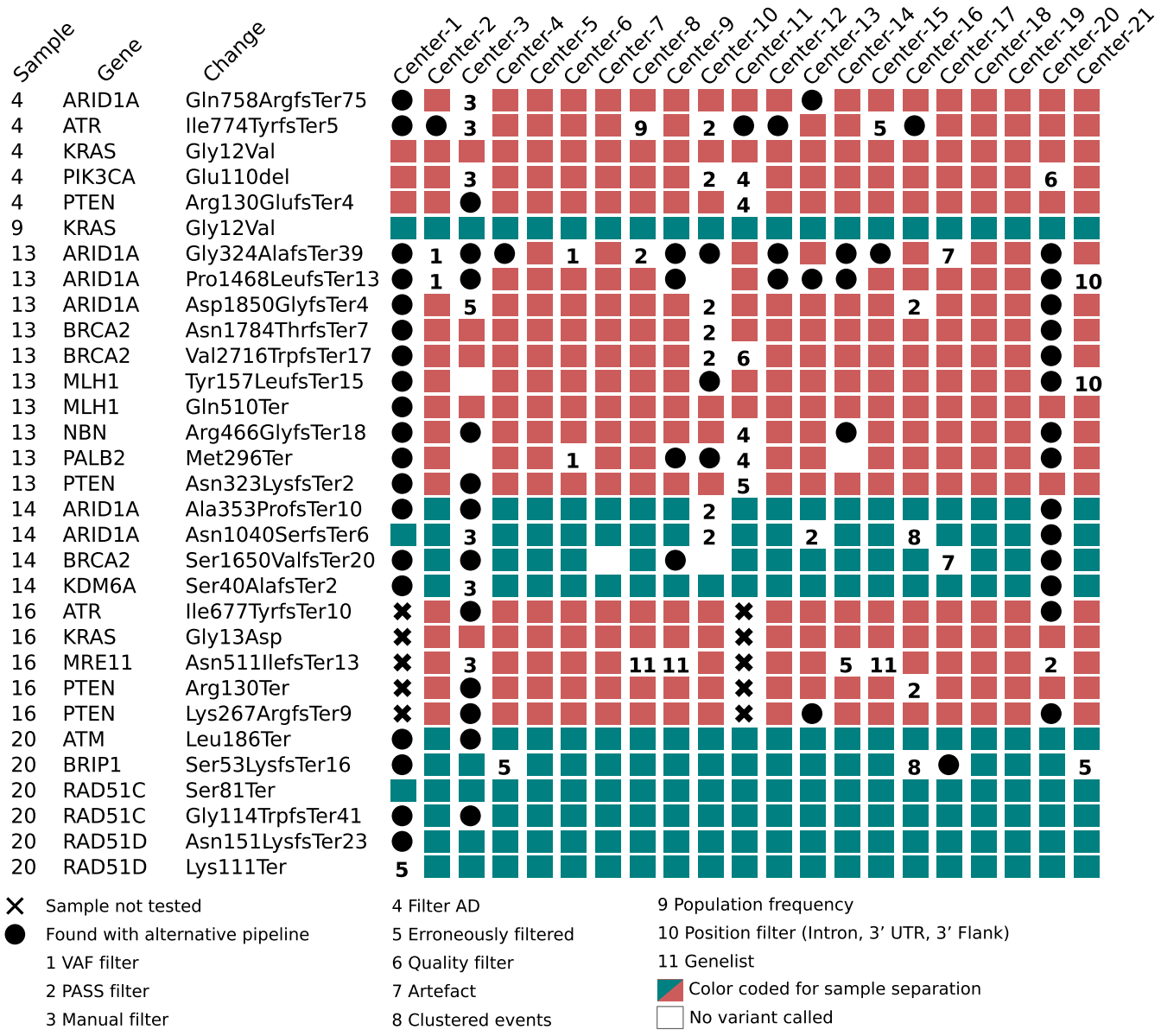

Fig. 3. Variants filtered for possible therapeutic targets. Each box shows a reported calls, they are colored for better samples separation. 51 missed variant calls were found in the raw calls and were annotated with the reason for the miss as reported by the respective center. Further 60 variants were found using the central bioinformatic pipeline. White fields indicate that neither local nor central could find the variant call.

3.2. Germline variants

In total, 10 centers reported pathogenic and likely pathogenic germline variants and their classification. The consensus results, i.e., variants identified by at least five centers, included two (likely) pathogenic variants in *PMS2* (p.E504X) and *BRCA1* (p.Q1756Pfs*74) and two variants classified as (likely) benign or of unknown significance (VUS) in *RET* (p.Y791F) and *TP53* (p.R283C), reported as likely pathogenic by one center (Supplementary Figure 5a). The *BRCA1* variant was identified by all centers. The *PMS2* variant was not reported by two centers due to a pseudogene filter. The variants in *RET* and *TP53* were each identified by all but one center.

All of the centers classified the germline variants according to ACMG criteria. Classification of the *BRCA1* variant was concordant and reported as pathogenic by nine centers and as likely pathogenic by the remaining center. The classification of the *PMS2* variant was more heterogeneous, with a consensus classification as likely pathogenic (pathogenic: 2 centers, likely pathogenic: 6 centers, quality filtered: 2 centers). The consensus classification for the variants in *RET* and *TP53* was benign (7 out of 10 centers in each case), while being reported as either VUS or likely pathogenic by two centers. ACMG criteria for the pathogenic variants in *BRCA1* and *PMS2* showed high similarity (Supplementary Figure 5b), with differences leading to differential classification of the *PMS2* variant as pathogenic or likely pathogenic. In line with the detected (likely) pathogenic variants in *BRCA1* and *PMS2*, high HRD and MSI scores were observed for the corresponding tumors, respectively.

3.3. Somatic copy number alterations

Genome-wide allele-specific CNA segments with absolute CN were submitted by 18 centers. Pairwise comparison of genomic regions across the whole genome lead to an agreement of 61 % of bases with 11 % of bases matching when accounting for genome duplications, 28 % of bases showed divergent values (Fig. 4a). Re-analysis of raw data with a single pipeline improved the concordance to 72 % match and while

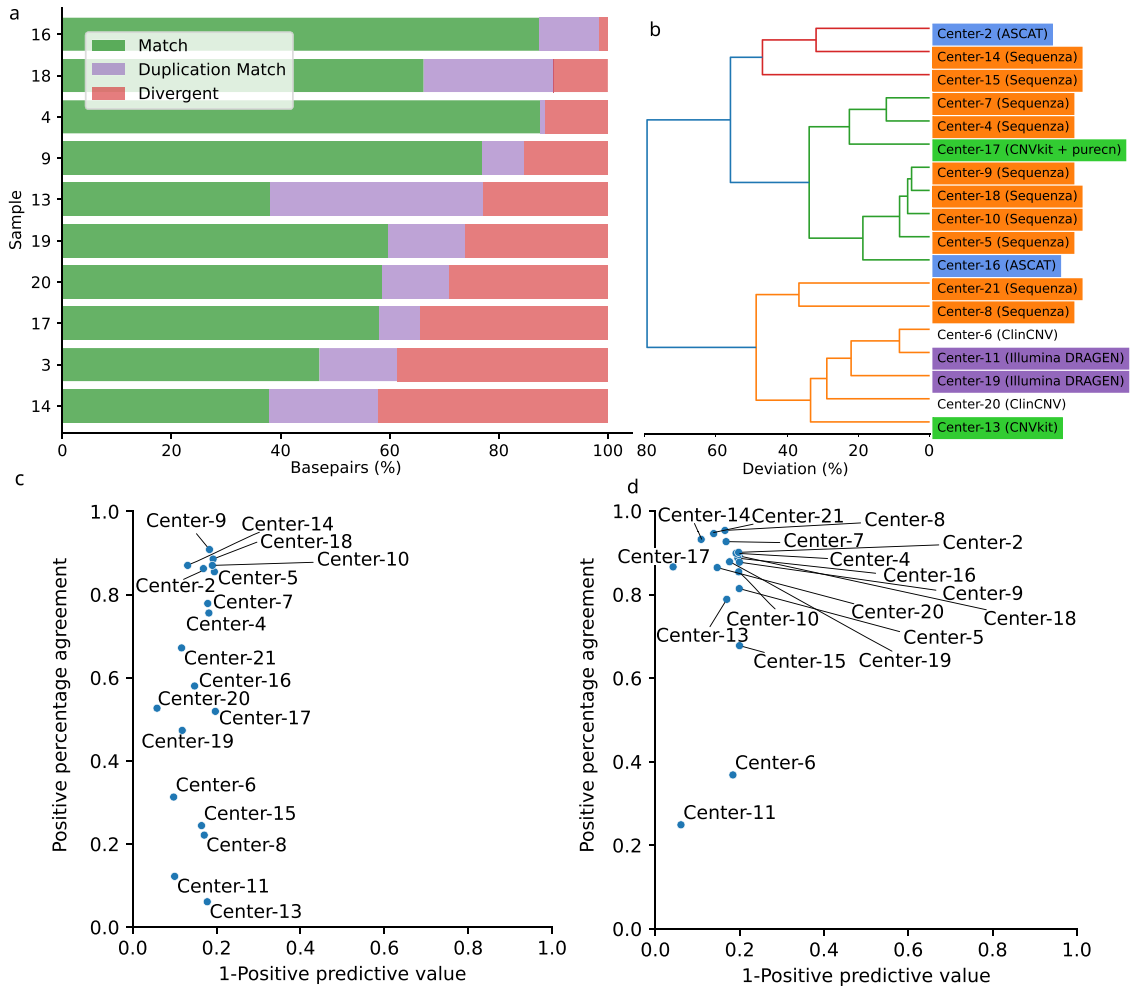

Fig. 4. Comparison of CNA calls. a: Pairwise comparison of CN profiles by sample and separated into segments with matching (green), not matching (red), and matching when normalized for genome duplications (purple). b: Hierarchical clustering of CN profiles annotated with bioinformatic segmentation tool. c: Gene amplification calls by center in relation to the five center consensus. d: Gene deep deletion or LOH calls in relation to the five center consensus.

duplication match decreased to 7 % (Supplementary Figure 6a). Hierarchical clustering of CNA revealed three main clusters, which can be attributed to differences by bioinformatics tools (Fig. 4b), re-analysis of raw data with a central pipeline led to a clustering by panel for most centers (Supplementary Figure 6b).

Concordance of gene amplifications, deep deletion and LOH calls was calculated in reference to alterations found by at least five centers in the list of 454 diagnostically relevant genes (Supplementary Table 2). While the mean PPA for the detection of amplifications was moderate (59 %), a mean PPV of 77 % was achieved (Fig. 4 c). Concordance of deep and LOH calls were observed to be higher with a mean PPV of 81 % and mean PPV of 82 % (Fig. 4d). Re-analysis of raw data with a single pipeline improved the PPA for detection of amplifications only by 10 %, while the PPV did not change. Similarly, PPA for the detection of deletions was improved by only 1 %, PPV remained unchanged (Supplementary Figure 6c,d).

Gene specific copy number alterations were investigated for genes with level 1–4 of OncoKb [18] and found two elevated CN for *MDM2* and *MET*, as well as two high-level amplifications: sample 3 *FGFR1* with a median of 17 copies found by 16 of 18 centers and *ERBB2* in sample 20 with a median of 19 copies found by 16 of 18 centers (Supplementary Fig. 7a). A deep deletion of *CDKN2A* was found by 6 centers in sample 3 as well as a varying counts of losses for *CDKN2A* in sample 17 and *TP53* in sample 20 (Supplementary Figure 7b).

3.4. Complex biomarkers

HRD scores were determined using eight different bioinformatics segmentation tools (Fig. 5a) and three different methods to count genomic scars. Fourteen centers reported results using the commonly used cut-off of 42 inferred from breast and ovarian carcinoma [19], one center used a cut-off of 65, while the six remaining centers did not perform HRD classification (Supplementary Table 1). Unanimous status calls were observed for four of 10 samples. Overall, 134 (93 %) of the status calls were consistent across centers. Identical status calls were reported for the reference samples (Fig. 5a). Correlations of HRD scores between centers showed a mean of 0.88 ± 0.18 with a 75 % percentile above 0.98, with only Center-1 showing correlations below 0.69, which applied a different cut-off and used a different bioinformatics tool (Supplementary Figure 8).

Overall, 163 TMB status calls (93 %) showed agreement, based on the cut-off 10 Mut/Mb for TMB-high vs. low (Fig. 5b). Most centers were in agreement of the status calls, but some center-specific discordance were observed: Center-11 showed overall lower TMB values, this center also had the most missed somatic variant calls. Center-20 showed considerably higher TMB values. Other deviations from the consensus were close to the cut-off point. For the two reference samples, 33 status calls (86 %) were concordant over all centers. Correlation of TMB values showed a mean of 0.89 ± 0.2 with 99 % of calls in the 75 % percentile.

Four different bioinformatics tools were utilized for the calculation of MSI scores in 20 centers, one center did not submit MSI values. MSI

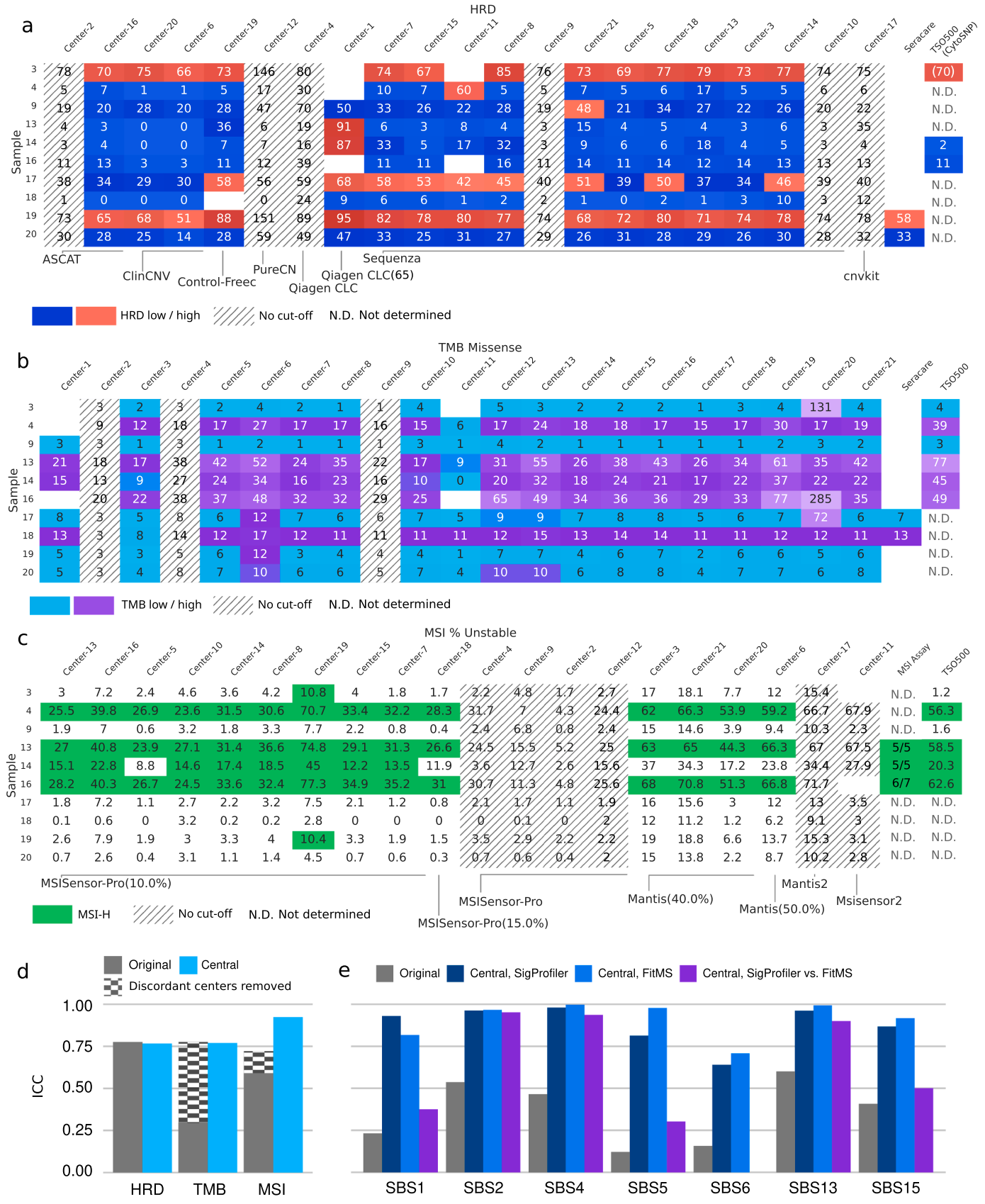

Fig. 5. Reported values for complex biomarkers. a: HRD scores ordered by segmentation tool, cut-off 42 was used in all but Center-1. b: Missense TMB values colored by TMB status with 10 Mut/MB cut-off. c: MSI percentage unstable sites ordered by bioinformatic tool with the cut-off value. The rightmost column shows the fraction of instable sites by MSI assay. d: Interclass correlation (ICC) for the three biomarkers from both bioinformatic pipelines. e: ICC for the main mutational signatures from the original data, the two algorithms from the central pipeline, and between the two algorithms.

status was determined with different cut-off values. Six centers did not submit a cut-off value. In seven samples full concordance was observed, while overall 132 (94 %) status calls were concordant (Fig. 5c). The majority status calls were consistent with assay-based (qPCR or gold standard fragment length analysis) and TSO500 status calls. MSI values showed the highest mean correlation (0.96 ± 0.07) with only Center-9 displaying a correlation coefficient below 0.9.

Re-analysis of biomarkers from the raw data with the central pipeline improved of status calls concordance for MSI (+2 %pt), while no improvement for TMB (+0 %pt) and decrease for HRD status calls (−3 %pt) were observed (Supplementary Figure 9). Comparative Interclass correlation (ICC) between original and central bioinformatics showed varying results for the original data (TMB: 0.30, MSI: 0.59, HRD: 0.77). The largest impact on variance was found to be center-specific as values for TMB and MSI were improved distinctly by removing two highly variant centers regarding TMB (Center-11 and Center-20, increased ICC from 0.30 to 0.77) and five centers applying different cut-offs in the MSI analysis (increased from 0.59 to 0.72) (Fig. 5d). Changes in ICC between original and central bioinformatics were comparable to the improvements of status calls discussed before with better concordance for MSI (0.59/0.72 to 0.92) and slight changes in TMB (0.30/0.77 to 0.77) and HRD (0.77 to 0.77) (Fig. 5d).

A total of 67 different single base substitution (SBS) signatures were submitted by 10 centers using three different bioinformatics tools showing a mean ICC of 0.36 (Fig. 5e, original). Re-analysis of all raw data with two bioinformatics tools substantially improved the mean ICC to 0.73 and 0.88 (Fig. 5e, central). ICC comparing both central methods shows high correlation for the signatures SBS2, SBS4 and SBS13. Lower ICC was observed for the more prevalent signatures SBS1 and SBS5. The signature SBS6 was called, yet, in different samples (Fig. 5e, central).

HLA class I status was determined by 10 centers using six different bioinformatic tools. Overall, 576 of 660 (87 %) HLA calls were concordant. Hierarchical clustering indicated a strong correlation of HLA predictions for all but one tool (Supplementary Figure 10).

4. Discussion

As data on inter-center comparability of diagnostic WES using FFPE material are scarce [1], we initiated a national WES benchmark study involving 21 major cancer centers in Germany. DNA of six paired tumor and normal specimen and four reference samples was analyzed with local wet-lab workflows and bioinformatics pipelines, as well as a central bioinformatics pipeline to allow separation between wet-lab and dry-lab variability.

Somatic variant calls showed an average PPA of 76 % compared to the five-center consensus list. Deviations could largely be explained by different variant filter rules. Re-analysis revealed that in principle an average PPA of 98 % was achievable from the raw-data. Therapeutically relevant variants reached an average PPA of 80 %, which potentially could be improved to 97 %. An influence of using FFPE instead of fresh-frozen samples on concordances was not observed [1].

Based on unfiltered somatic variant calls and re-analysis we were able to determine the main factors for the four centers with overall lower concordance: Center-1 used different bioinformatics tools (Supplementary Table 1) and most variant calls could be found in the re-analysis (Fig. 1c). Center-10 missed most variant calls due to lower coverage (87x) as variants were found, but often not labeled as PASS (Supplementary Figure 2a). Center-3 implied a strict manual filter accountable for about two-thirds of missed calls while most others were filtered due to a misconfigured pipeline (Supplementary Figure 2b). Center-20 implied strict variant filtering and variants could often be found in the unfiltered calls (Fig. 2c).

Four germline variants in cancer risk genes were identified and classified with high concordance between centers, with some notable exceptions. Two centers did not report the variant in *PMS2*, which lies in a region in exon 11 which is homologous to a correponding region of the pseudogene *PMS2CL* and was therefore removed by the center's quality filters. While almost all centers agreed that the variants in *RET* and *TP53* are (likely) benign, one center classified these variants as likely pathogenic. At the time of the round robin test the two variants were annotated as likely pathogenic in at least one of the three databases OnkoKB, CKB and LOVD used by this. In the meantime, the *RET* variant class was reduced to class 2–3 in all three databases, while the *TP53* variant remained a class 4 only in CKB, showing that pathogenicity classification is highly dependent on utilized databases.

CNA concordance was observed for 72 % of the genomic regions, which was very similar to 76 % of matching regions observed in the earlier study in fresh frozen tissue specimens [1].

Deviations between bioinformatic tools were also observed, a results that ties well with a systematic evaluation of wet-lab influences and bioinformatics evaluation on CNV calling [20]. The differences between centers were not resolved by using the same bioinformatics tools as seen for SNVs. Therefore, the underlying cause appears to be wet-lab driven. In line with this notion, a recent study showed that WES and FFPE processing had a large impact on CNV concordance, especially on losses [20].

Status calls for HRD, TMB, and MSI agreed for 93 %, 93 %, and 94 % across samples and centers, respectively, even though different bioinformatics tools were used. The results align with previous results in fresh frozen tissue specimens (HRD: 96 %, TMB: 99 %, MSI: 100 %). Re-analysis in the central pipeline did not increase the concordances beyond well-aligned wet- and dry-lab procedures. The estimation of HRD scores is strongly influenced by the estimation of tumor purity and ploidy [21]. For the artificial reference sample 17 different ploidy solutions between 1.8 and 4.4 were chosen, which presumably lead to deviant results of the HRD score estimation while results were around the cut-off value of 42 further increasing the discrepancies. The re-analysis showed a higher concordance, but the scores still scattered around the cut-off (Supplementary Figure 9). The influence of the segmentation tool, as shown in Fig. 2a, seems to be minor, except for Center-1, as neither a systematic deviation is apparent between the tools, nor did the re-analysis improve HRD scores. Re-analysis of MSI still showed large deviations in status for sample 14, where also a high variance in bioinformatically estimated tumor purity between 10–100 % were observed, emphasizing the difficulties in evaluating this sample (Supplementary Figure 9).

Aside from the center-specific deviations described before, increasing sequencing depth correlated with less missed variant calls (Supplementary Figure 3). However, no clustering by sequencing depth was observed (Supplementary Figure 4), indicating that low sequencing depth lead to less variant identification, yet, beyond a certain depth, it does not increase concordance. No significant correlation between sequencing depth and PPV or PPA of CNA were found (p values between 0.2 - 0.6).

Findings in this study are limited by the absence of a gold standard for somatic mutations, complex biomarkers, and CNA for clinical samples. Missed somatic variants could be recovered in unfiltered VCFs, however, this approach needs to be balanced with false positive detection. Furthermore, it should be noted that the central bioinformatics pipeline used in the re-analysis of raw data only represents a single possible approach. Other bioinformatics tools could improve the concordance further.

Previous studies have laid the foundation for clinical WES [22] and highlighted the benefits of moving from gene panels to WES, which allows for rapid and flexible expansion of the reportable gene list and precise measurement of complex biomarkers while reducing the burden of assay revalidation [23]. Our multicentric benchmark study, which is to our best knowledge the largest of its kind, closes a significant gap in the field, supports the implementation of decentralized WES in clinical diagnostics for cancer patients and demonstrates its fundamental feasibility. The results also highlight processes in the dry laboratory that require further standardization and harmonization. Finally, our study

provides a basis and blueprint for the design of standardized EQA schemes for clinical WES.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: MMT reports speaker and travel Expenses from Twist. JS reports speaker honoraria from DLS, Molecular Health, AstraZeneca and Biocartis, outside the submitted work. UL reports speaker fees from AstraZeneca, GSK, Novartis, Menarini, advisory board from AstraZeneca and Novartis. DH reports speaker honorary AstraZeneca, adboard BMS, WD speaker honoraries BMS & Novartis. SMB reports speaker honoraria, advisory board fees and research grants from AstraZeneca, Daiichi, Menarini, Novartis, Roche, BMS, Pfizer, Bayer, MSD, Merck, Amgen, Molecular Health, Targos, DLS, Janssen, GSK, QuIP, outside the submitted work. SL reports research grant from BMS, advisory board/speaker invitation from AstraZeneca, Eli Lilly, Roche and Takeda outside of this work. NTG reports research support from Janssen-Cilag and Advisory Boards from Janssen-Cilag, AstraZeneca, Daiichi-Sankyo and BMS outside the submitted work. WW reports research grants from Roche, MSD, BMS and AstraZeneca. Advisory board, lectures and speaker bureau fees from Roche, MSD, BMS, AstraZeneca, Pfizer, Merck,

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Data Availability

The datasets generated during the current study are available from the corresponding authors on reasonable request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2024.114306.

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