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Introduction

The genomic characterization of soft tissue sarcomas is increasingly applied in patient care to better understand the pathogenesis of these diseases. However, most pan-cancer comprehensive genomic profiling (CGP) tests cover few gene fusions of clinical relevance in sarcomas. Hence, the contribution of all classes of genomic alterations to the pathogenesis of sarcomas is often subjected to methodological bias.

Objective: This study investigates all actionable genomic variants present in soft tissue sarcoma patients using a sarcoma-targeted CGP approach that also interrogates most sarcoma-relevant gene fusions

CGP RNA Fusion Genes List

ABL1, AKT3, ALK, AR**, AXL, BCL2, BRAF, BRCA1, BRCA2, CDK4, CSF1R, EGFR**, EML4, ERBB2, ERG, ESR1, ETS1, ETV1, ETV4, ETV5, EWSR1, FGFR1, FGFR2, FGFR3, FGFR4, FLI1, FLT1, FLT3, JAK2, KDR, KIF5B, KIT, KMT2A, KMT2AT3, MET**, MSH2, MYC, NOTCH1, NOTCH2, NOTCH3, NRG1, NTRK1, NTRK2, NTRK3, PAX3, PAX7, PDGFRA, PDGFRB, PIK3CA, PPARG, RAF1, RET, ROS1, RPS6KB1, TMPPRS2

** Denotes genes with splice variants including AR-V7, EGFRvIII, and MET exon 14 skipping.

The assay detects single-nucleotide variants (SNVs), insertions/deletions (InDels), copy number variants (CNVs), and RNA fusions and splice variants in a total of 517 genes (517 genes analyzed by DNA, 55 genes by RNA), plus microsatellite instability (MSI*) and tumor mutational burden (TMB).

Sarcoma Targeted Panel Genes List

A next-generation sequencing panel that detects translocations and fusions with known and novel fusion partners of these genes:

ACTB, AHRR, ALK, ASPSCR1, ATF1, ATIC, BCOR, BRAF, C11orf95, CAMTA1, CARS1, CCNB3, CDH11, CIC, CLTC, CNBP, COL1A1, COL1A2, CREB1, CREB3L1, CREB3L2, CSF1, CTNNA1, DDIT3, DUX4, EML4, EPC1, ERG, ETV1, ETV4, ETV6, EWSR1, FEV, FLI1, FOXO1, FRK, FUS, GLI1, HAS2, HEY1, HMGA2, IL2RB, ITK, JAZF1, LMNA, LPP, MEAF6, MRTFB, MYH9, MYLK, NAB2, NCOA1, NCOA2, NFATC2, NFIB, NR4A3, NTRK1, NTRK2, NTRK3, NUTM2A, NUTM2B, OMD, PAX3, PATZ1, PAX7, PBX1, PDGFB, PDGFRB, PHF1, PLAG1, POU5F1, RAD51B, RANBP2, ROS1, SEC31A, SRF, SS18, SSB1, SSB2, SSB4, STAT6, SUZ12, SYK, TAF15, TCF12, TEAD1, TFE3, TFG, THRAP3, TPM3, TPM4, USP6, WT1, WWTR1, YAP1, YWHAE and ZNF444.

Methodology

We screened 20,660 test orders for cancer patients with available diagnosis tested by either of two complementary tests: a pan-cancer CGP panel and a sarcoma fusions panel, both routinely performed in our clinical laboratory. The first detects SNV/Indels in 517 genes, CNA in 59 genes, MSI and TMB, and common solid tumor RNA fusions on 55 genes. The second analyzes additional known and novel RNA fusions in 97 genes with high diagnostic, prognostic, and therapeutic value in sarcomas, utilizing fusion-enrichment-based RNA-seq. Of 12,885 CGP-tested patients, 346 had sarcoma, while 1,406 sarcoma patients were tested with the sarcoma fusion panel. Of those, 62 patients were characterized using both tests.

Comprehensive Genomic Profiling (n=346)

Variant Type	#	Prevalence (%)
SNV/Indel (pathogenic/likely pathogenic)	246	71.09%
CNV	85	24.56%
Fusion	22	6.35%
No alterations	2	0.57%

Table 1: Sarcoma Variant Characterization (n=346). Breakdown of pathogenic/likely pathogenic variants by type (SNVs/Indels, CNVs, and fusions) and the frequency of samples without alterations. Prevalence is calculated individually for each variant class.

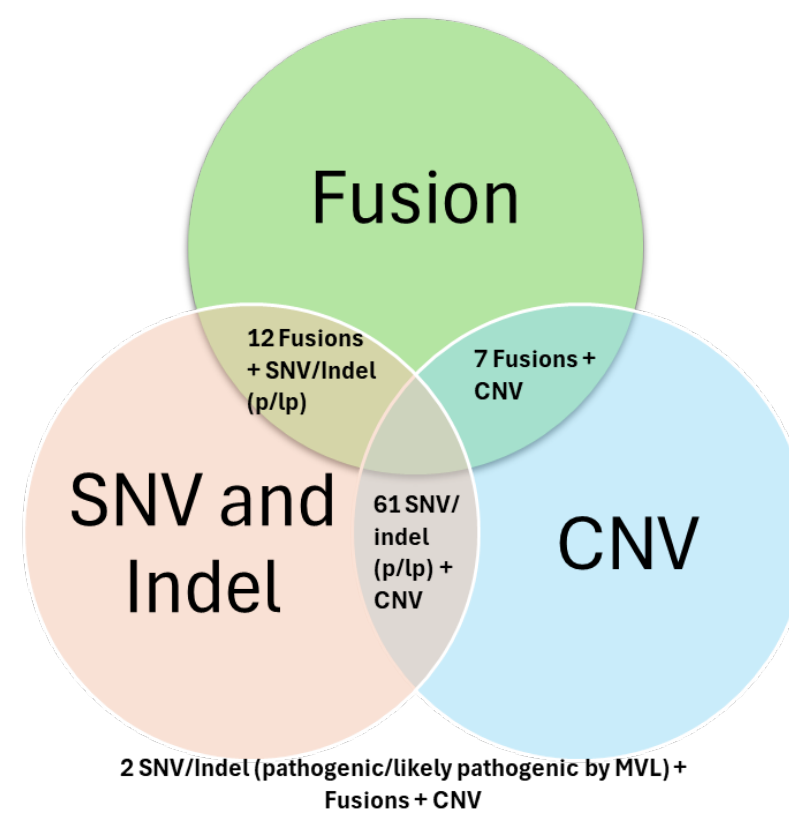


Figure 1: Distribution and Intersection of Variant Types. CGP results for the 346-sample sarcoma cohort, showing the prevalence of individual variant types and the degree of genomic overlap between them.

Solid Fusion- Sarcoma Targeted Panel (n=1406)

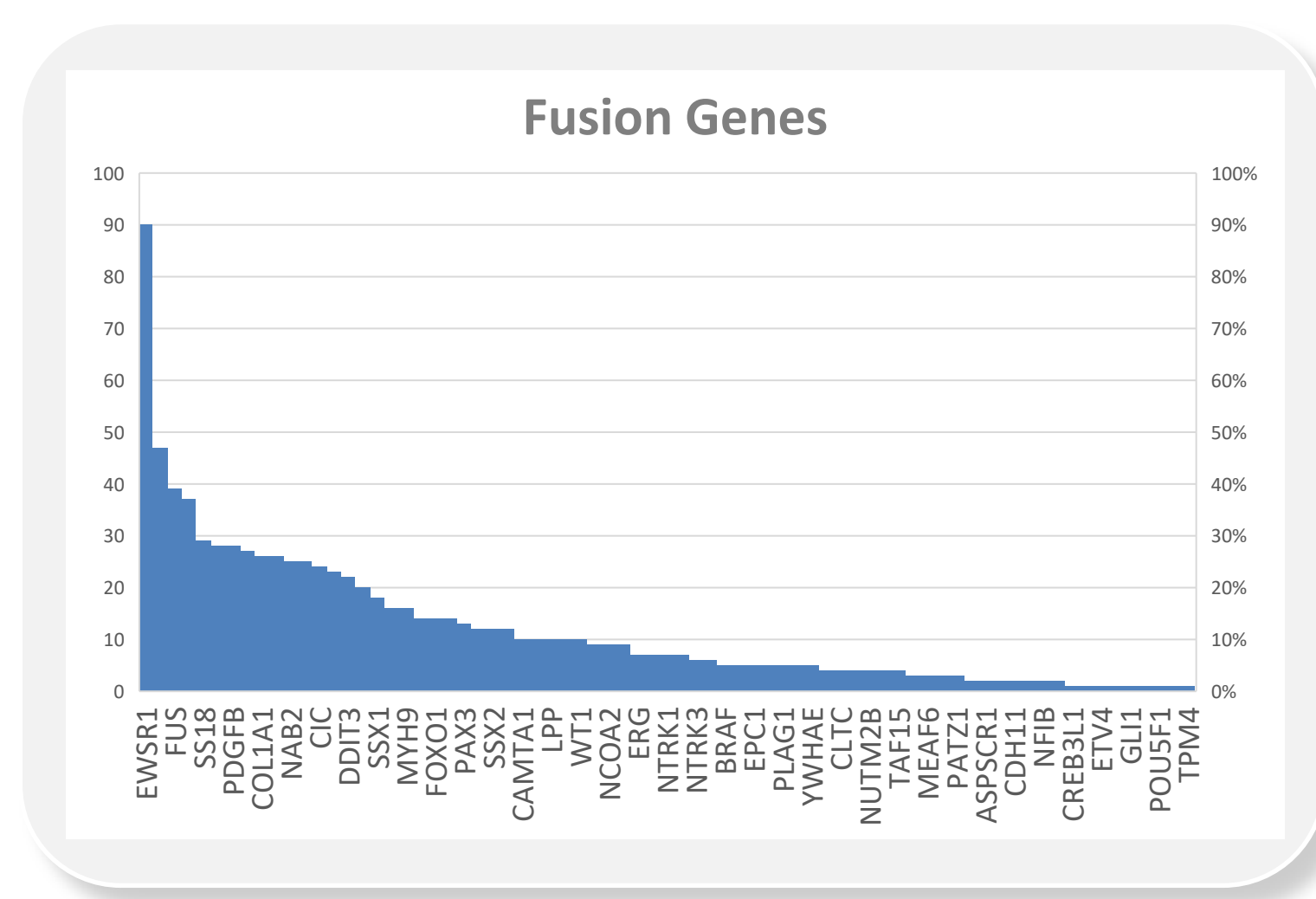


Figure 2: Rank of relevant detected fusions. Rearrangements in EWSR1, HMGA2, FUS, FLI1, and SS18 represent the most frequent alterations identified across the cohort (n=1406).

Overlap between Sarcoma Fusion Panel & CGP

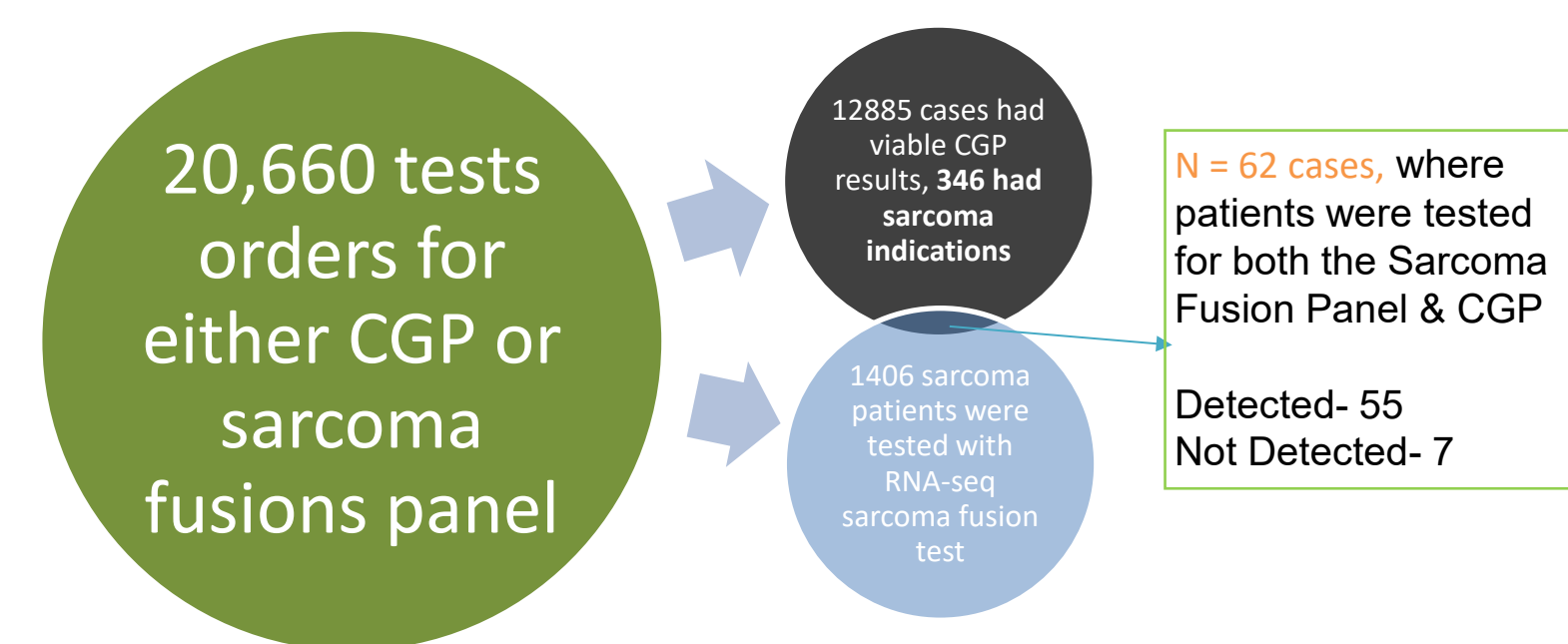


Figure 3: Comparative detection results of concurrent Sarcoma Fusion Panel and CGP testing (n = 62). Molecular alterations were identified in 55 patients, while 7 showed none.

Results

From the 346 sarcoma patients tested with the pan-cancer CGP test, 268 patients had pathogenic DNA alterations, and 22 patients had RNA fusions. Conversely, among 1,406 sarcoma patients tested with the sarcoma fusion panel, fusions were detected in 399 patients, with 207 distinct fusions identified. The top 5 genes rearranged were EWSR1, HMGA2, FUS, FLI1, and SS18. We next analyzed the data from the 62 sarcoma patients tested with both assays and found that all pathogenic alterations (DNA/RNA) were detected in 56 cases. DNA alterations were found in 54 of them. SNV/indels were present in 53 patients with 86 genes harboring pathogenic mutations, most frequently in TP53, TERT, LRP1B, NF1, TET, and CDKN2A. CNAs were identified in 15 patients, of whom one had only CNAs (KRAS, MDM2, PDGFRA, and KIT). The most frequent CNAs were on MDM2 (n=5), followed by CCND1, CDK4, EGFR, KRAS, and MYC (n=3). Gene fusions were detected in 10 patients. Three (3) patients had fusions detected by both panels, 2 had fusions detected by the CGP panel but not covered by the sarcoma panel, and 5 patients had a fusion only detected by the Sarcoma fusion panel.

Solid Fusion (5)		Both (3)		CGP (2)	
6752262	COL1A1:PDGFB	5902207	AKT3::FCF1P7; SDCCAG8::AKT3	7209144	RPS6KB1::VMP1
6981315	CEP170::RAD51B	6673607	SQSTM1::ALK		
7222025	RPL11:PAX7 (Strong Fusion) NT5C3A:HMGA2 (Strong Fusion)				
7164061	HMGA2::RP11-81H14.2; CCT2::ESR1; RPRD1B::RAB22A; CPSF6::GLS/RP11-61102.6	7297978	EWSR1::FLI1	7255643	FGFR1::KDR
5917804	LPP::TP63; MYOCD::ARHGAP44				

Table 2: Comparative detection of gene fusions via CGP and Sarcoma Fusion Panel (n=10). Distribution of findings across 10 positive patients, highlighting concordant results (n=3), CGP-specific findings (n=2), and Sarcoma Fusion Panel-specific detections (n=5).

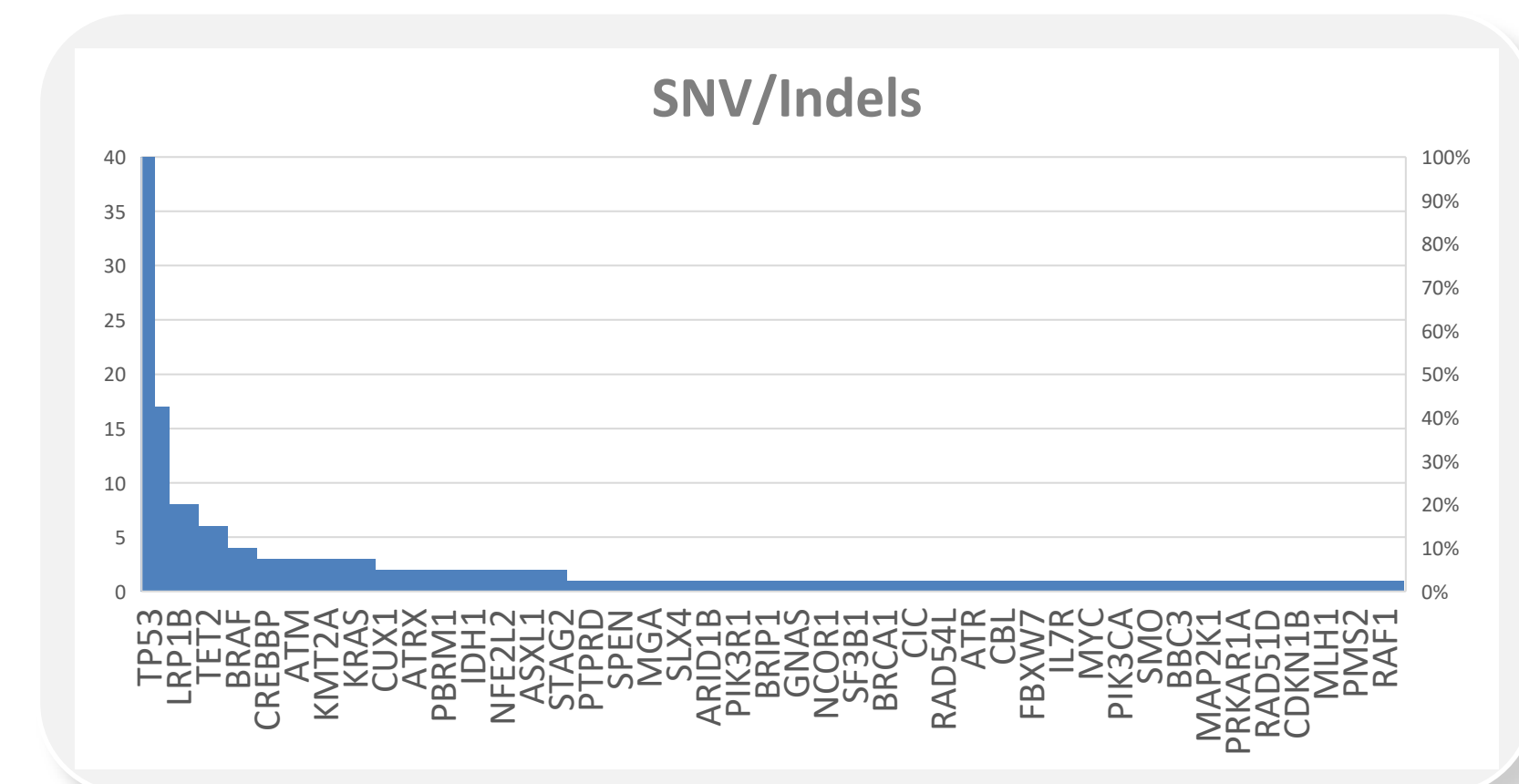


Figure 4: Frequency and distribution of SNV/Indels in dual-tested sarcoma patients (n = 62). Pathogenic DNA alterations were identified in 54 patients with 53 harboring mutations across 86 distinct genes. These genes, in descending order of frequency, are led by TP53, TERT, LRP1B, NF1, TET, and CDKN2A.

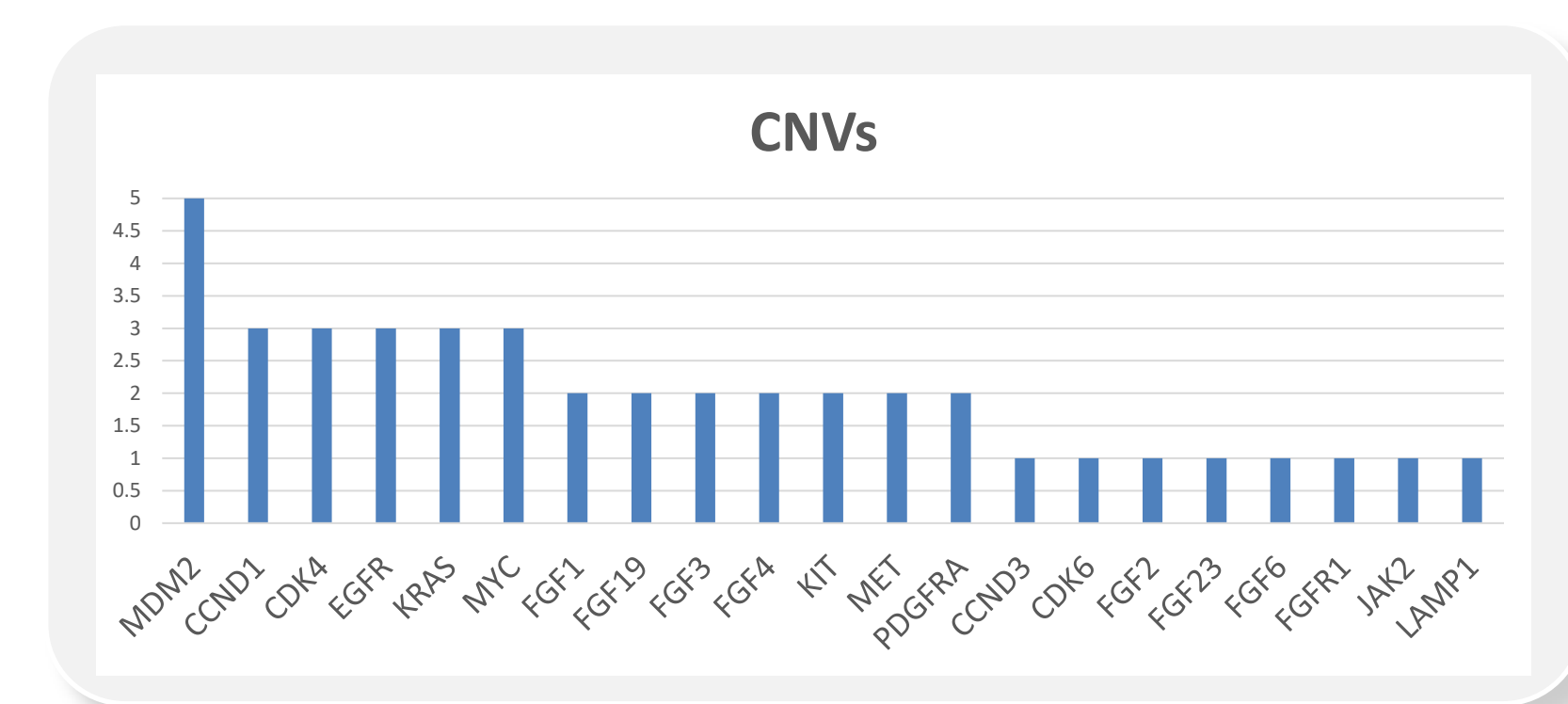


Figure 5: Characterization of Recurrent CNAs. Summary of copy number changes across 15 patients. The overall cohort was dominated by MDM2 alterations, followed by other key oncogenic drivers ranked by frequency.

Key Takeaways

- Complementary Coverage:** While pan-cancer CGP maps the broad tumor molecular landscape, the sarcoma-targeted panel captures critical and specialized fusions.
- Synergistic Molecular Profiling:** Combining both assays provides a more complete genomic evaluation, maximizing the detection of pathogenic drivers.
- Enhanced Precision in Patient Care:** Dual-testing ensures a more accurate diagnosis and expands the potential for personalized, fusion-directed therapies.