Genomic Medicine Sweden

GMS560: a broad targeted NGS gene panel for comprehensive genomic profiling of solid tumors

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Design, validation and implementation

The GMS560 gene panel consists of a genomic DNA (gDNA) target capture panel module and an RNA (cDNA) junction capture panel module for comprehensive genomic profiling of solid tumors in clinical practice as well as for research applications. The modules can be used separately. The design, optimization and validation is the result of a nationwide collaborative effort within the Genomic Medicine Sweden (GMS) Solid Tumor work package. This synopsis describes the intended use, range and type of genetic aberrations that can be analyzed by the panel as well as basic technical specifications of GMS560. The national validation, which included >200 clinical samples with known genetic aberrations and multiple commercial reference standards, is completed and forms the basis for the summary below. This effort has involved pathologists, molecular biologists, medical geneticists and bioinformaticians at the seven Swedish Genomic Medicine Centers (GMC). Implementation at regional GMC/Clinical Genomics nodes is ongoing. More details will be released shortly (manuscript in preparation). Please contact your local GMS representative for more information about use and clinical implementation.

Intended use

The GMS560 gene panel is a next generation sequencing (NGS) based assay for the detection of base substitutions (SNVs), insertions and deletions (INDELs), copy number variations (CNVs) and gene rearrangements (fusions) as well as the genomic signatures microsatellite instability (MSI) and tumor-mutational burden (TMB). For clinical application, the assay is intended for comprehensive genomic profiling of formalin-fixed paraffin-embedded (FFPE) tumor samples and cytological specimens. The panel is designed to cover treatment predictive genetic aberrations, diagnostic biomarkers for tumor subtyping, and to support the detection of exploratory targets for patient inclusion in clinical trials. Moreover, in research settings, the assay can be used to assess a broad range of cancer-associated biomarkers of relevance for tumor biology, regardless of established clinical significance. The gene panel is intended to harmonize broad cancer testing on a national level and to allow for the addition of novel cancer biomarkers in future iterations.

Panel design and analytical performance

The gene content of GMS560 is described in **Figure 1**. The detection of SNVs, INDELs and CNVs together with biomarker signatures is based on extracted gDNA and analyzed with the gDNA target capture panel. Gene rearrangements can be detected by the gDNA panel with expected technical limitations common to all gDNA designs. The detection of rearrangements is

supplemented by a panel module for targeted sequencing of total RNA which subsequently is converted into cDNA. Kapa Biosystems reagents, in combination with Twist Bioscience probes and reagents are used for library preparation and enrichment, respectively, following the manufacturers' protocols with minor modifications. The validation sequencing was performed on Illumina NextSeq and NovaSeq sequencers.
In order to confidently define all types of genomic aberrations in patient samples, the following sample criteria have been evaluated and are recommended for analysis:
DNA input amount = 100 ng, Tumor cell content ≥ 20 %, RNA input amount = 50 ng.
Samples with lower input amounts can be analyzed but need to be interpreted with care as sensitivity and specificity might be lower especially in regions outside clinical hotspot positions.

SNVs and INDELs: The exons of 544 genes are targeted by DNA capture (top panel, **Figure 1**). GMS560 is validated for the detection of small variants (SNVs and INDELS) in DNA extracted from FFPE and cytology samples with a tumor cell content of at least 10%. Mutations can be detected at variant allele frequency (VAF) ≥2% in validated clinical hotspot positions, and at VAF ≥5% across the rest of the panel content in samples with a total read coverage of ≥50M and a median target coverage of ≥500x. At lower coverage the criteria for a positive mutation call are VAF ≥5% and at least 20 variant reads.
CNVs: The main CNV backbone, spanning the entire genome, has probes placed with an average distance of 1 Mb. Amplifications (high-copy number gains, above 6x) are reported for 21 genes (**Figure 1**, Copy number analysis, Amplifications). Additionally, there is a reinforced CNV backbone extending 3 Mb on each side of 20 genes where copy number losses may be clinically relevant (**Figure 1**, Specific copy number analysis, Deletions).
TMB: GMS560 covers approximately 1.6 Mb of coding sequence and is thus suitable for TMB-score calculation. TMB-values can be scored with a high concordance to current reference methods.
MSI: A backbone of microsatellite loci in non-complex genomic regions known to be informative for the classification of MSI-H cases are included in the design. MSI scores as computed from GMS560 are highly sensitive and specific for determining MSI-status in colorectal cancer samples.
Fusion gene detection by targeted RNA-sequencing: Capture probes for the cDNA panel were designed by the "junction capture method" with a dense probe tiling around the 5´and 3´exon junctions in 44 genes. Treatment-predictive fusion genes were given the highest priority; the design includes all exons in all genes for which there are currently available approved targeted treatments in any solid tumors (bottom panel, **Figure 1**).

Bioinformatics

The GMS560 data has been analyzed using the Twist Solid Pipeline and reports SNVs, Indels, CNVs, fusions and biomarkers (TMB and MSI) as well as visualization of CNVs and QC. The pipeline, which is a collaboration project between multiple GMC/CG nodes, is constructed using Hydra-Genetics modules. These modules are based on Snakemake, a workflow language that ensures flexibility and scalability. All tools within the pipeline are containerized, to ensure portability and reproducibility. To maintain good quality standards, the pipeline undergoes peer-review by multiple experienced bioinformaticians. Furthermore, a comprehensive suite of automatic tests, including unit tests, integration tests and code style checks are executed with each code and configuration update to guarantee reliability.
The source code is publicly available on GitHub and documentation is accessible via ReadTheDocs.

Ongoing validation efforts

Current efforts to supplement the clinical validation of additional diagnostic modules include: Fusion gene detection on DNA-level, homologous recombination deficiency (HRD) and calling of a larger range of copy number variations (including larger structural deletions and loss of heterozygosity).

DNA (mutation- and copy number analysis) (544 genes)										
Mutation analysis										
ABL1	*BRAF*	*CTCF*	*ERCC3*	*GLI1*	*JAK2*	*MGMT*	*PALB2*	*PTMA*	*SESN2*	*TEK*
ABRAXAS1	*BRCA1*	*CTLA4*	*ERCC4*	*GNA11*	*JAK3*	*MITF*	*PARP1*	*PTPN11*	*SETBP1*	*TENT5C*
ACVR1	*BRCA2*	*CTNNB1*	*ERCC5*	*GNA13*	*JUN*	*MLH1*	*PARP2*	*PTPN14*	*SETD2*	*TERT*
ACVR1B	*BRD4*	*CTNND1*	*ERG*	*GNAQ*	*KANSL1*	*MLH3*	*PAX5*	*PTPRC*	*SF1*	*TET1*
ACVR2A	*BRD7*	*CUL1*	*ERRFI1*	*GNAS*	*KAT2B*	*MN1*	*PBRM1*	*PTPRD*	*SF3B1*	*TET2*
ADGRG6	*BRIP1*	*CUL3*	*ESR1*	*GPS2*	*KDM5A*	*MRE11*	*PCBP1*	*PTPRS*	*SFRP1*	*TGFBR1*
AJUBA	*BTG2*	*CYLD*	*ETV1*	*GREM1*	*KDM5C*	*MSH2*	*PDCD1*	*PTPRT*	*SH2B3*	*TGFBR2*
AKT1	*CACNA1A*	*CYSLTR2*	*ETV6*	*GRIN2D*	*KDM6A*	*MSH3*	*PDCD1LG2*	*RAB35*	*SIN3A*	*THRAP3*
AKT2	*CARD11*	*DACH1*	*EWSR1*	*GSK3B*	*KDR*	*MSH6*	*PDGFRA*	*RAC1*	*SLX4*	*TLE1*
AKT3	*CASP8*	*DAXX*	*EZH2*	*GTF2I*	*KEAP1*	*MST1*	*PDGFRB*	*RAD21*	*SMAD2*	*TLR4*
ALB	*CASR*	*DAZAP1*	*FANCA*	*H1-2*	*KEL*	*MTOR*	*PDPK1*	*RAD50*	*SMAD3*	*TMEM127*
ALK	*CBFB*	*DCHS1*	*FANCB*	*H1-4*	*KIF1B*	*MUC6*	*PDS5B*	*RAD51*	*SMAD4*	*TMPRSS2*
ALOX12B	*CBL*	*DCHS2*	*FANCC*	*H2AX*	*KIT*	*MUTYH*	*PGR*	*RAD51B*	*SMARCA1*	*TNFRSF14*
AMER1	*CCND1*	*DCUN1D1*	*FANCD2*	*H3-3A*	*KLF4*	*MXI1*	*PHOX2B*	*RAD51C*	*SMARCA2*	*TOP1*
ANKRD11	*CCND2*	*DDR2*	*FANCE*	*H3-3B*	*KLF5*	*MYB*	*PIK3CA*	*RAD51D*	*SMARCA4*	*TP53*
APC	*CCND3*	*DDX3X*	*FANCF*	*H3-5*	*KLF6*	*MYBL1*	*PIK3CB*	*RAD52*	*SMARCB1*	*TP63*
APOB	*CCNE1*	*DIAPH2*	*FANCG*	*H3C10*	*KMT2A*	*MYC*	*PIK3CD*	*RAD54L*	*SMARCD1*	*TRAF3*
APOBEC3B	*CCNQ*	*DICER1*	*FANCI*	*H3C2*	*KMT2B*	*MYCL*	*PIK3CG*	*RAF1*	*SMARCD2*	*TRAF7*
AR	*CD274*	*DIS3*	*FANCL*	*H3C3*	*KMT2C*	*MYCN*	*PIK3R1*	*RARA*	*SMARCE1*	*TSC1*
ARAF	*CD70*	*DKK1*	*FANCM*	*HDAC1*	*KMT2D*	*MYD88*	*PIK3R2*	*RASA1*	*SMC1A*	*TSC2*
ARHGAP35	*CD79B*	*DKK4*	*FAT1*	*HDAC2*	*KNSTRN*	*MYH9*	*PIM1*	*RB1*	*SMC3*	*U2AF1*
ARID1A	*CDC27*	*DMD*	*FAT2*	*HES2*	*KRAS*	*MYOD1*	*PLCB4*	*RBM10*	*SMO*	*USP9X*
ARID1B	*CDC73*	*DNAJB1*	*FAT3*	*HES3*	*LATS1*	*NBN*	*PLCG1*	*RECQL*	*SOCS1*	*VEGFA*
ARID2	*CDH1*	*DNMT1*	*FAT4*	*HES4*	*LATS2*	*NCOA2*	*PLCG2*	*RECQL4*	*SOS1*	*VHL*
ARID5B	*CDK12*	*DNMT3A*	*FBXW7*	*HES5*	*LDB1*	*NCOA3*	*PLK2*	*RET*	*SOX17*	*WIF1*
ARRDC1	*CDK4*	*DNMT3B*	*FGF19*	*HEY1*	*LYN*	*NCOR1*	*PLXNB2*	*RHEB*	*SOX2*	*WRN*
ASXL1	*CDK6*	*DOT1L*	*FGF3*	*HGF*	*LZTR1*	*NCOR2*	*PMS1*	*RHOA*	*SOX9*	*WT1*
ASXL2	*CDK8*	*DPYD*	*FGF4*	*HIF1A*	*MACF1*	*NF1*	*PMS2*	*RHOB*	*SPEN*	*WWC1*
ATF7IP	*CDKN1A*	*E2F1*	*FGFR1*	*HLA-A*	*MAD2L2*	*NF2*	*POLD1*	*RICTOR*	*SPOP*	*XPO1*
ATM	*CDKN1B*	*E2F3*	*FGFR2*	*HLA-B*	*MAML3*	*NFE2L2*	*POLE*	*RIT1*	*SPRED1*	*XRCC1*
ATR	*CDKN2A*	*EED*	*FGFR3*	*HLA-C*	*MAP2K1*	*NFKBIA*	*POLQ*	*RNF111*	*SPTA1*	*XRCC2*
ATRX	*CDKN2B*	*EEF1A1*	*FGFR4*	*HNF1A*	*MAP2K2*	*NFKBIE*	*POLR2A*	*RNF43*	*SPTAN1*	*YAP1*
AURKA	*CDKN2C*	*EGFR*	*FH*	*HRAS*	*MAP2K4*	*NIPBL*	*POT1*	*ROS1*	*SRC*	*YES1*
AURKB	*CEBPA*	*EGR3*	*FLCN*	*HUWE1*	*MAP3K1*	*NKX2-1*	*PPARG*	*RPL22*	*SRSF2*	*YY1*
AURKC	*CENPA*	*EIF1AX*	*FLT1*	*ID3*	*MAP3K13*	*NKX3-1*	*PPM1D*	*RPL5*	*STAG2*	*ZBTB7B*
AXIN1	*CHD1*	*EIF4A2*	*FLT3*	*IDH1*	*MAP3K4*	*NOTCH1*	*PPP2R1A*	*RPS6KA4*	*STAT3*	*ZC3H12A*
AXIN2	*CHD3*	*ELF3*	*FLT4*	*IDH2*	*MAPK1*	*NOTCH2*	*PPP2R2A*	*RPS6KB2*	*STAT5A*	*ZFHX3*
AXL	*CHD4*	*ELOC*	*FOXA1*	*IFNGR1*	*MAPK3*	*NOTCH3*	*PPP4R2*	*RPTOR*	*STAT5B*	*ZFP36L1*
B2M	*CHD8*	*EMSY*	*FOXA2*	*IGF1R*	*MAX*	*NOTCH4*	*PPP6C*	*RRAS2*	*STAT6*	*ZFP36L2*
BAP1	*CHEK1*	*EP300*	*FOXL2*	*IKBKE*	*MCL1*	*NPM1*	*PRDM1*	*RUNX1*	*STK11*	*ZMYM2*
BARD1	*CHEK2*	*EPAS1*	*FOXO1*	*IKZF1*	*MCPH1*	*NRAS*	*PRDM14*	*RXRA*	*STK19*	*ZMYM3*
BCL10	*CIC*	*EPCAM*	*FOXO3*	*IL6ST*	*MDC1*	*NSD1*	*PREX2*	*SAV1*	*SUFU*	*ZNF750*
BCL2	*CNTN6*	*EPHA2*	*FOXP1*	*IL7R*	*MDM2*	*NSD2*	*PRKAR1A*	*SCAF4*	*SUZ12*	*ZNRF3*
BCL2L1	*COL5A1*	*EPHA3*	*FUBP1*	*INPP4B*	*MDM4*	*NTRK1*	*PRKCI*	*SDHA*	*TAF1*	*ZRSR2*
BCL2L11	*CRB1*	*EPHA7*	*FYN*	*INPPL1*	*MECOM*	*NTRK2*	*PRKD1*	*SDHAF2*	*TAOK1*	
BCL6	*CREBBP*	*EPHB1*	*GAB1*	*IRF2*	*MED12*	*NTRK3*	*PRSS1*	*SDHB*	*TBL1XR1*	
BCOR	*CRKL*	*ERBB2*	*GAB2*	*IRF4*	*MEF2B*	*NUF2*	*PSEN2*	*SDHC*	*TBX3*	
BCORL1	*CRLF2*	*ERBB3*	*GABRA6*	*IRF6*	*MEN1*	*NUP93*	*PSIP1*	*SDHD*	*TCF12*	
BLM	*CSDE1*	*ERBB4*	*GATA2*	*IRS2*	*MET*	*PAK1*	*PTCH1*	*SERPINB3*	*TCF3*	
BMPR1A	*CSF1R*	*ERCC2*	*GATA3*	*JAK1*	*MGA*	*PAK5*	*PTEN*	*SERPINB4*	*TCF7L2*	
Copy number analysis										
Amplifications:										
*ALK**	*BRAF**	*CDK6**	*ERBB2**	*FGFR2**	*FGFR4**	*KRAS**	*MTOR**	*MYCN**	*NTRK2**	*PDGFRA**
*AR**	*CDK4**	*EGFR**	*FGFR1**	*FGFR3**	*KIT**	*MET**	*MYC**	*NTRK1**	*NTRK3**	
Deletions:										
ATM	*BRCA1*	*CDH1*	*CDKN2B*	*FANCA*	*MCPH1*	*PALB2*	*RAD51C*	*SPRED1*	*TSC1*	
BAP1	*BRCA2*	*CDKN2A**	*CHD1*	*FAT1*	*NF1*	*PTEN**	*RB1*	*TP53*	*TSC2*	
** reported genes*										
Mutation signatures										
*TMB**	*MSI**	*HRD*								
** reported signatures*										
RNA (fusion gene analysis) (44 genes)										
AKT3	*EGFR*	*ESR1*	*EWSR1*	*FGFR4*	*MET*	*NCOA1*	*NTRK3*	*PPARG*	*PRKD3*	*ROS1*
ALK	*ERBB2*	*ETV1*	*FGFR1*	*FUS*	*MGMT*	*NRG1*	*PDGFRA*	*PRKCA*	*RAF1*	*SS18*
AR	*ERBB4*	*ETV4*	*FGFR2*	*HMGA2*	*MYB*	*NTRK1*	*PIK3CA*	*PRKD1*	*RELA*	*TMPRSS2*
BRAF	*ERG*	*ETV5*	*FGFR3*	*MAML2*	*MYBL1*	*NTRK2*	*PLAG1*	*PRKD2*	*RET*	*YAP1*

Figure 1. GMS560 content.