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A clinical decision support software to integrate next-generation sequencing and cytogenetics assays for myeloid cancers

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INTRODUCTION

Sanger sequencing and karyotyping are traditionally used to detect molecular alterations in myeloid cancer that inform diagnosis and treatment. Recently, new myeloid biomarkers have expanded the scope of molecular testing. To address the need for broader profiling, we developed a next-generation sequencing (NGS) assay to research alterations in 58 myeloid cancer genes and a cytogenetics assay to detect chromosomal alterations. Furthermore, we developed a decision support software that integrates results from both platforms into a single report.

MATERIALS AND METHODS

NGS assay was developed on Ion AmpliSeq™ targeted sequencing technology to detect DNA and RNA variants from 30ng of input. The cytogenetics assay was developed on an Affymetrix platform with enriched coverage for cancer genes. Evidence from drug labels (FDA, EMA), guidelines (NCCN, ESMO), and global clinical trials were identified using automated text searches and manual curation. In addition, all curated evidence was associated with appropriate AMP/ASCO/CAP tiers. A web application was developed to generate biomarker specific custom reports in 11 languages.

RESULTS

Figure 1: Myeloid NGS Assay was developed using Ion AmpliSeq™ targeted sequencing technology* to provide detection of DNA and RNA variants from 30ng of input nucleic acid per pool. The assay contains a total of 40 DNA genes and 29 RNA driver genes that are prognostic, diagnostic, or therapeutic markers associated with open clinical trials, clinical guidelines from ESMO and NCCN, and therapies approved by European EMA and US FDA authorities. Targeted hotspot variants in 23 genes include activating, sensitizing or resistant mutations. Full coding sequence coverage for 17 tumor suppressor genes enables the detection of de novo deleterious mutations. The assay detects single-nucleotide variants, insertion/deletions, and fusions in 58 genes with an average read depth of >2000 reads per amplicon and average uniformity of >95%. Variant calling algorithms were optimized and combined into a single Ion Reporter workflow. A custom algorithm for *FLT3* internal tandem duplications (*FLT3-ITD*) was developed to increase sensitivity of detection.

Hotspot genes (23)		
ABL1	*IDH1*	*NRAS*
BRAF	*IDH2*	*PTPN11*
CBL	*JAK2*	*SETBP1*
CSF3R	*KIT*	*SF3B1*
DNMT3A	*KRAS*	*SRSF2*
FLT3	*MPL*	*U2AF1*
GATA2	*MYD88*	*WT1*
HRAS	*NPM1*	

Full gene (17)		
ASXL1	*IKZF1*	*SH2B3*
BCOR	*NF1*	*STAG2*
CALR	*PHF6*	*TET2*
CEBPA	*PRPF8*	*TP53*
ETV6	*RB1*	*ZRSR2*
EZH2	*RUNX1*	

Fusion driver genes (29)			
ABL1	*FGFR1*	*MLLT10*	*RARA*
ALK	*FGFR2*	*MLLT3*	*RBM15*
BCL2	*FUS*	*MYBL1*	*RUNX1*
BRAF	*HMGA2*	*MYH11*	*TCF3*
CCND1	*JAK2*	*NTRK3*	*TFE3*
CREBBP	*KMT2A (MLL)*	*NUP214*	
EGFR	*MECOM*	*PDGFRA*	
ETV6	*MET*	*PDGFRB*	

Figure 2: Whole Genome Cytogenetics Assay contains over 2.6 million markers for copy number including 750,000 SNPs with >99% genotype accuracy. The assay allows the detection of whole-genome copy number gains/losses, LOH, cnLOH, ploidy, mosaicism, and clonal heterogeneity.

- **All probes empirically selected for optimal performance**
  - Empirical Probes selection based on actual performance on 1,000s of clinical samples.
  - Backbone (non-gene) coverage of 1 marker per 1,737 bases
- **Whole-genome platform with enriched coverage for cancer genes enabling high functional resolution across the genome**
  - 98% RefSeq genes cover with 1 marker per 880 bases
  - 98% DDD/DECIPHER (1,309 genes)
  - 100% ClinGen (3,484 genes)
  - 100% Sanger cancer gene coverage (1 marker / 553 bases)
  - 100% OMIM genes (1 marker / 659 bases)
  - 100% X chromosome OMIM Morbid genes (177 genes, 1 marker / 486 bases)

Figure 3: Oncomine Knowledgebase is a curated database of biomarker based information from drug labels (USA and Europe), guidelines (USA and Europe), global clinical trials, and peer-reviewed literature.

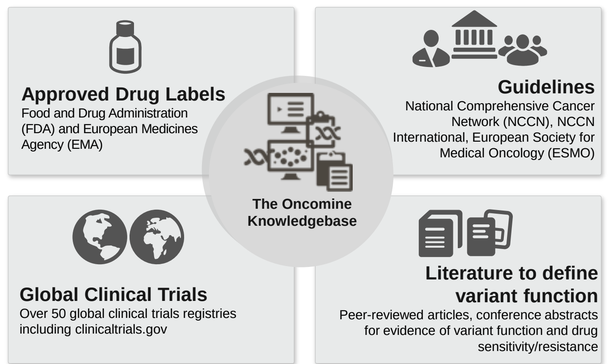
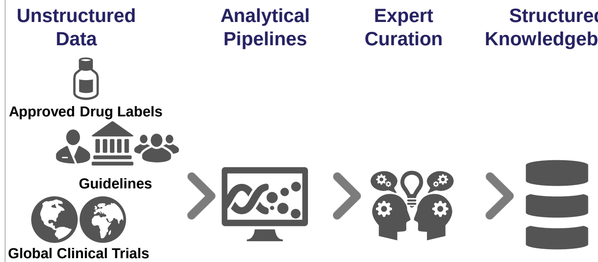


Figure 4: Oncomine Knowledgebase Workflow. Unstructured data is evaluated for candidate evidence using proximity search algorithms. All candidate evidence is manually analyzed by curation experts for context and standardization. Structured data supports the reporting in the Ion Torrent™ Oncomine™ Reporter and is updated quarterly



Structured Data Metrics: Oncomine Reporter 4.0 (September 2018)

- Approved Drugs: 15 genes, 1 cytogenetic abnormality, 65 labels
- Guidelines: 44 genes, 23 cytogenetic abnormalities, 29 guidelines
- Clinical Trials: 204 genes, 22 cytogenetic abnormalities, >1,200 trials
- >250 literature citations with evidence for NGS biomarkers

Figure 5: AMP/ASCO/CAP Tier IA genes and cytogenetic markers associated with § =approved labels (FDA and/or EMA) and/or * =clinical guidelines (NCCN and/or ESMO evidence for therapeutic, prognostic, or diagnostic implication.

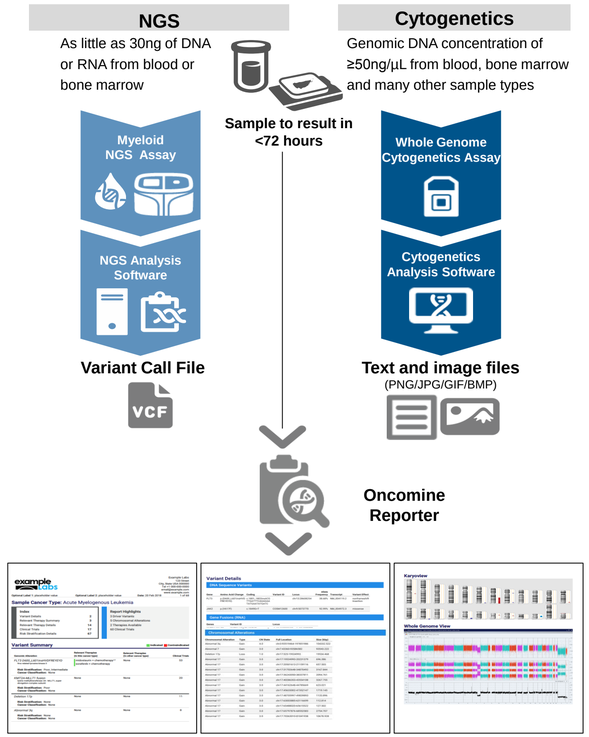
AMP/ASCO/CAP Tier IA Solid Tumor Genes

- **Breast** — ERRB2 § *
- **Colorectal** — KRAS § *, NRAS § *, BRAF*
- **Gastric/GEJ** — ERRB2 § *
- **GIST** — NF1*, KIT*, PDGFRA*
- **Head and Neck** — AR*, ERBB2*
- **Melanoma** — BRAF § *, KIT*
- **Non-Small Cell Lung** — ALK § *, BRAF § *, EGFR § *, ROS1 § *, KRAS*, MET*, RET*
- **Ovarian** — BRCA1 § *, BRCA2 § *
- **Soft Tissue Sarcoma** — ALK*
- **Thyroid** — BRAF § *

AMP/ASCO/CAP Tier IA Myeloid Cancer Genes

- **Acute Myeloid Leukemia** — FLT3 § *, IDH2 § *, ABL1*, ASXL1*, CEBPA*, CREBBP*, JAK2*, KIT*, KMT2A*, MECOM*, MLLT3*, MYH11*, NPM1*, NUP214*, RUNX1*, TP53* 5qDel*, inv(16)*, 7qDel*, Inv(3)*, -5*, -7*
- **APL** — RARA § *
- **CML** — ABL1 § *, Abn3q26*
- **CMML** — ASXL1*
- **Myelodysplastic Syndrome** — ASXL1*, BCOR*, CREBBP*, ETV6*, IDH2*, KMT2A*, MECOM*, NRAS*, NUP214*, RUNX1*, SF3B1*, SRSF2*, STAG2*, TP53*, U2AF1*, ZRSR2* 5qDel § *, Del3q*, Del7q*, Del9q*, Del11q*, Del12p*, Del13*, Del17p*, Del20q*, -Y, inv(3), Iso17q, -5*, -7*, Trisomy 19*, Trisomy 8*
- **ET** — CALR*, JAK2*, MPL*
- **MDS/MPN** — PDGFRA § *, PDGFRB § *
- **PV** — JAK2*
- **Primary Myelofibrosis** — ASXL1*, CALR*, EZH2*, IDH1*, IDH2*, JAK2*, KMT2A*, MECOM*, MPL*, SRSF2*, TET2*, TP53* 5qDel*, 12pDel*, 7qDel*, inv(3)*, Iso17q*, -5*, -7*, Trisomy 8*

Figure 6: Sample-to-result workflow of Myeloid NGS assay and Whole Genome Cytogenetics Assay using Oncomine Reporter. Oncomine Reporter is a software that generates integrated custom reports with clinically relevant annotations for the molecular biomarkers identified using the NGS and Cytogenetics assays. A typical workflow for a Myeloid cancer sample is that somatic alterations (SNVs, indels, fusions) can be assessed using a Myeloid NGS Assay* while large chromosomal and copy number alterations can be detected using the Whole Genome Cytogenetics Assay. Variant analysis for the NGS Assay is performed with NGS Analysis Software, which generates a Variant Call Format (VCF) file. Copy number analyses for the Whole Genome Cytogenetics Assay are performed using the Cytogenetics Analysis Software. The user can then combine the VCF, TXT, and image files in the Oncomine Reporter to generate a single integrated report.



CONCLUSIONS

We developed two complementary molecular profiling platforms to detect relevant mutations and chromosomal alterations in myeloid cancer. Combined with an integrative decision support software, we demonstrate a streamlined and robust sample-to-result workflow.