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Comprehensive analysis of cell-free DNA with whole-exome sequencing and its application to minimal residual disease (MRD) monitoring

Amy Xiaohong Wang, Zhixin Zhao, Chao Dai, Kemin Zhou, Shidong Jia, Pan Du, Shujun Luo. Predicine Inc, Hayward, CA 94545, USA

Predicine

INTRODUCTION

Liquid biopsy has been increasingly used in cancer diagnosis, monitoring of therapy response and minimal residual disease (MRD). In this study, we report the development of a novel PredicineALERT liquid biopsy solution for personalized and generalized MRD detection, regardless of tumor tissue status.

If baseline sample (tissue or liquid biopsy such as blood, urine, CSF) is available, a personalized PredicineALERT MRD approach is recommended where the PredicineWES assay will be used for genome-wide coverage of coding regions and the 600-gene PredicineATLAS™ NGS panel for in-depth molecular profiling. Variants detected in baseline samples will be used for personalized MRD detection with assay sensitivity down to 0.005%.

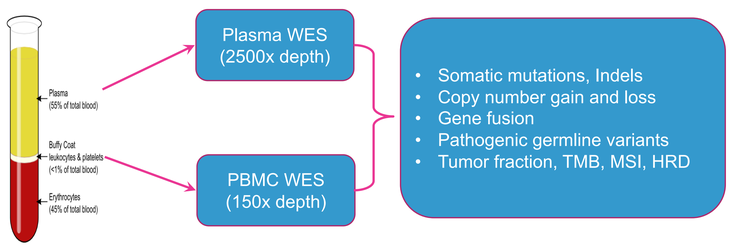
If baseline sample is not available, generalized MRD approach will leverage the multidimensional detection of genomic and epigenomic variants in liquid biopsy.

Important cancer-related actionable genes are included in the PredicineALERT MRD platform to provide actionable insights for MRD-positive patients.

METHODS

PredicineWES:

Fig. 1: Workflow for plasma PredicineWES

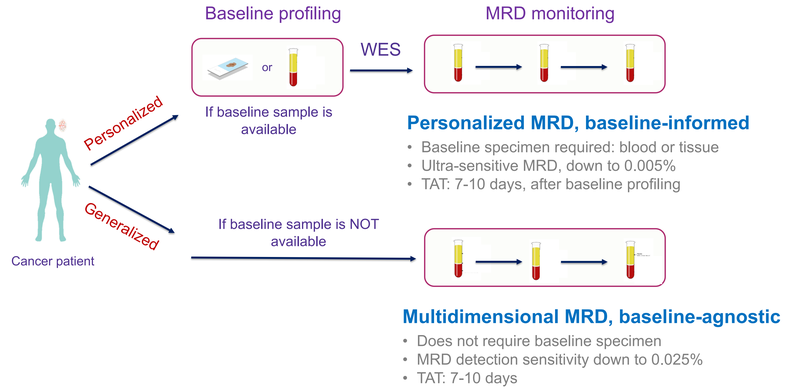


PredicineWES assay features:

- Incorporating 20,000-gene WES panel and 600-gene PredicineATLAS panel
- Deep sequencing (20,000x, 0.25% LOD) on 600-cancer genes and important DNA fusions
- Whole exome coverage at 2,500x (1% LOD) enables genomic profiling beyond cancer genes
- Exome-wide SNP skeleton enhances LOH and CNV detection
- Precise estimation of tumor fraction, HRD score, tumor mutation burden (TMB) and microsatellite instability (MSI)
- Applies to tissue and liquid biopsy samples, including plasma and urine

PredicineALERT MRD assay:

Fig. 2: Workflow for PredicineALERT MRD assay, regardless of baseline sample availability



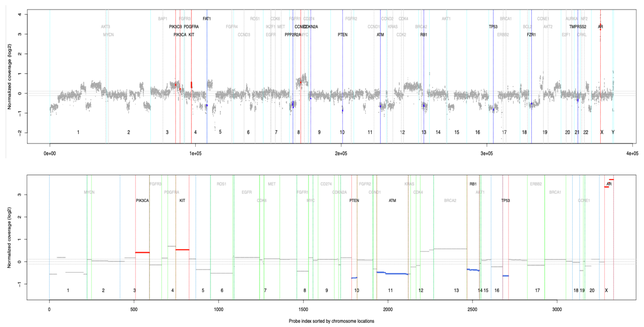
RESULTS

PredicineWES:

Table. 1: PredicineWES detects more variants than PredicineCARE panel in plasma

	PredicineWES						PredicineCARE
	Somatic	Synonymous	Variant (AF < 1%)	Variant (AF >= 1%)	Max MAF	Tumor Fraction	Variant (AF>0.1%)
Plasma 1	284	80	10	274	28.1	26.7	22
Plasma 2	106	27	11	95	70.6	49.5	6
Plasma 3	95	16	38	57	84.6	49.0	10
Plasma 4	85	23	11	74	48.9	48.9	5
Plasma 5	49	19	2	47	93.3	46.7	2
Plasma 6	5	1	0	6	1.74	1.74	1

Fig. 3: PredicineWES confirms the CNVs detected by PredicineCARE in plasma



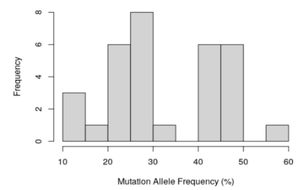
PredicineWES detects and confirms all copy number variations (CNV) detected in patient plasma samples using the 152-gene PredicineCARE CLIA-certified NGS assay

PredicineALERT - Case study of baseline informed, personalized MRD assay:

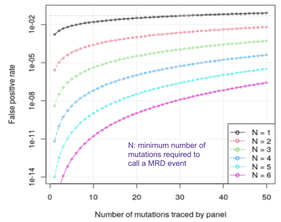
- mCRPC plasma samples profiled by PredicineWES, somatic mutations are selected to design PredicineALERT personalized MRD panel.
- Patient plasma cfDNA is diluted in normal cfDNA background at five titration levels: 0.1, 0.05, 0.025, 0.01 and 0.005%.

Fig. 4: Personalized MRD detection in plasma samples

A. AF distribution of PredicineWES-based mutations selected for personalized MRD monitoring



B. MRD assay specificity study



C.

Number of mutations used by Personalized panel		0.005% (TF: 0.0025%)	0.01% (TF: 0.005%)	0.02% (TF: 0.01%)	0.05% (TF: 0.025%)	0.1% (TF: 0.05%)
32 mutations	Avg # of detected mutations	1.5	3.75	5.75	12.5	20
	Sensitivity	50%	100%	100%	100%	100%
16 mutations	Avg # of detected mutations	0.75	1.88	2.88	6.25	10
	Sensitivity	0	50%	100%	100%	100%

A. 32 mutations were selected from baseline PredicineWES profiling and used for longitudinal MRD tracking. B. MRD specificity study with different numbers of mutations used in tracking. C. MRD detection sensitivity at different titration levels (tumor fraction is roughly half of titration %): 0.005% for 32 mutations.

	PredicineWES	PredicineALERT	
Feature	Enhanced whole exome sequencing	Baseline informed, personalized MRD detection	Baseline agnostic, indication specific MRD detection
Genes	~20,000 (WES + PredicineATLAS)	• MRD variant detection • Actionable core genes	• MRD variant detection • Actionable core genes • Chromosomal abnormalities, methylation
Sample Type			
Limit of Detection	• cfDNA: 0.25-1% • Tissue: 5%	cfDNA: 0.005%	cfDNA: 0.025%
Indication	Personalized MRD assay, pan-cancer		Generalized MRD assay, indication-specific

CONCLUSIONS

We have developed a proprietary PredicineALERT MRD liquid biopsy assay that can detect cancer variants down to 0.005% with actionable variants detected simultaneously. PredicineWES assay is used to generate genome-wide and in-depth baseline profiling, enabling personalized and ultra-sensitive monitoring of therapy response and disease recurrence.