Analytical and clinical validation of liquid biopsy-based copy number loss in cancer

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Introduction

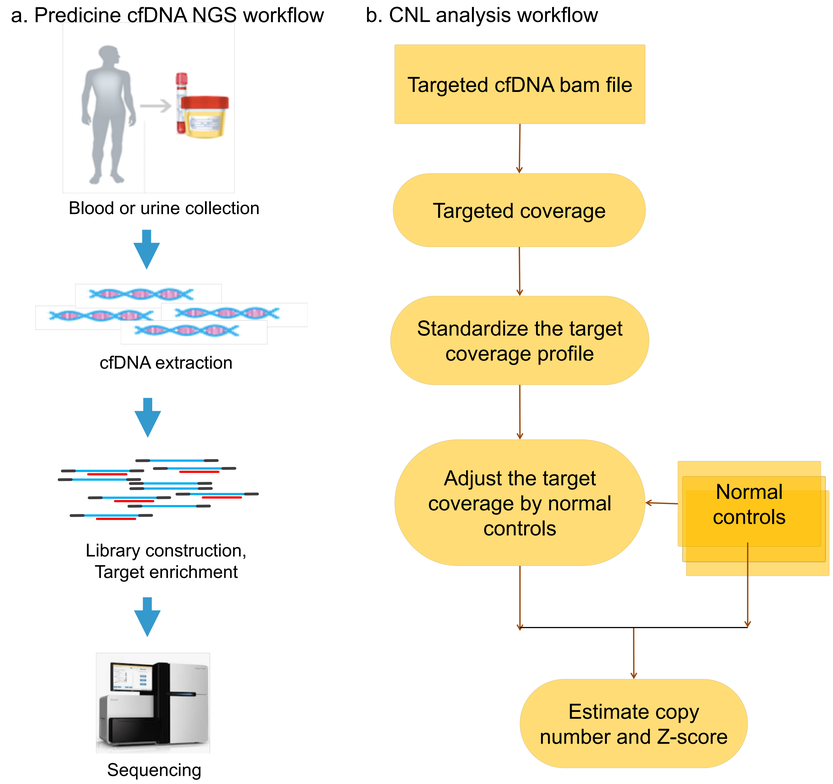
Copy number loss (CNL) of cancer-related genes, like *PTEN*, *BRCA1, BRCA2, ATM, RB1 and CDKN2A*, is a common genomic event in cancer. Detection of CNL is important for cancer diagnosis and treatment selection. Multiple methods based on whole genome sequencing (WGS) have been developed to detect long range copy number loss in tissue and blood. However, the development of a highly sensitive liquid biopsy assay for CNL is under high demand in clinic.

Here we report the development and validation of Predicine cfDNA assay, a liquid-biopsy based NGS test that detects CNL, in addition to SNV, Indel, rearrangement and copy number gain, in circulation. Unique double-stranded fragment coverage was estimated using in-house proprietary NGS analysis pipeline. Predicine cfDNA assay has provided a useful tool to fully capture the genomic landscape of cancer using biofluid samples such as blood or urine.

Predicine Pipeline

Cell-free DNA (cfDNA) was extracted from biofluid samples and used for the detection of genomic alternations including copy number variations. Proprietary pipeline was developed for accurate detection of CNL in circulation.

Fig. 1: Workflow for Predicine's cfDNA CNL measurement



Results

Fig. 2: Predicine cfDNA assay detects copy number loss to expected values in a titration study

a. Cell line titration study

b. Low pass WGS confirmation study

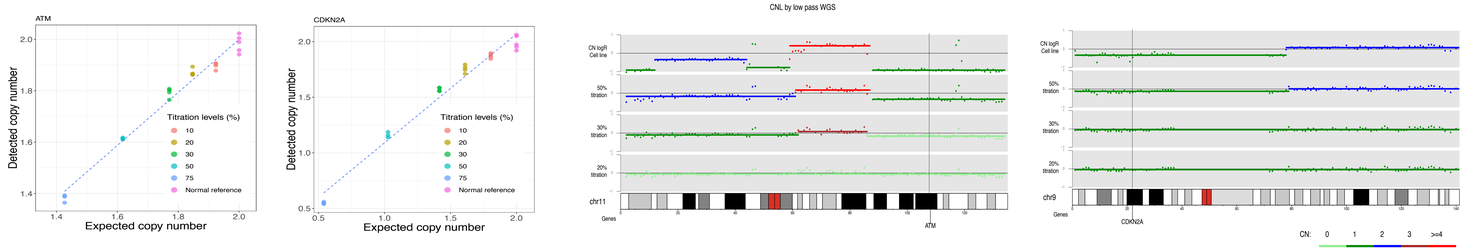


Fig. 2: Predicine's cfDNA CNL assay detects CNL in cell line reference materials with known CNL status in a titration study. The CNL detection at different tumor fractions was assessed to the expected copy number (a) and was further confirmed by orthogonal platform Low-Pass Whole-Genome Sequencing (LP-WGS) (b).

Fig 3. High concordance observed between Predicine cfDNA CNL assay in plasma and tWES in matched core needle biopsy from breast cancer patients

Sample	CNV Loss Calling (for ATM, BRCA1, BRCA2, CDKN2A, RB1)	
	cfDNA (Predicine)	tWES (Independent CAP Lab)
S1	ATM, CDKN2A, RB1	ATM, CDKN2A, RB1
S2	CDKN2A	CDKN2A
S3		
S4	ATM, CDKN2A	CDKN2A
S5	ATM, BRCA1, BRCA2, RB1	ATM, BRCA1, BRCA2, RB1
S6	CDKN2A	ATM
S7		
S8		
S9		RB1
S10		

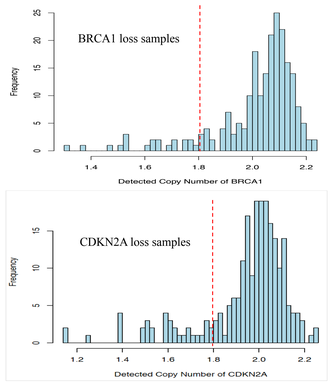


Fig 3. Matched core needle biopsy and plasma samples from breast cancer patients were used for CNL detection using tissue-based WES (tWES) and plasma-based Predicine cfDNA assay. A highly comparable result was achieved between two platforms, with positive predictive value 81.8%.

Fig 4. Predicine cfDNA-based CNL detection is associated with shorter overall survival in mCRPC patients.

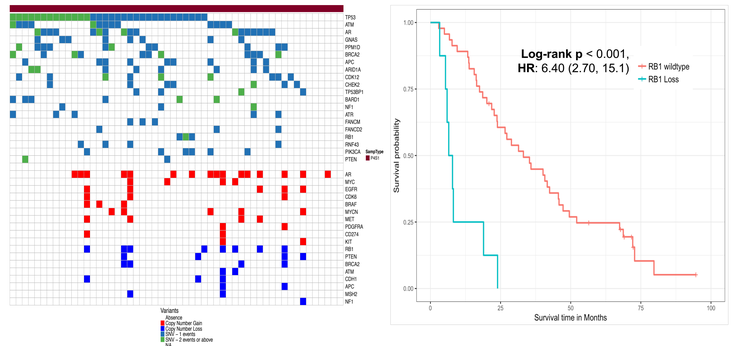


Fig. 4: Predicine cfDNA assay detects cancer variants in clinical progressed mCRPC plasma samples. Data shown are the distribution of top SNV and CNVs in mCRPC patients (a) and the correlation between RB1 (CNL) and overall survival based on Kaplan-Meier analysis (b).

Predicine cfDNA Panel - Metrics and CNV genes

Table 1. Assay Metrics / Sample Requirement

Regions Analyzed	180 genes
Panel Size	576kb
Sequencing and Bioinformatics	Illumina NGS / in-house pipeline
Report Range	SNV/Indels/DNA fusions/CNA/CNL
Assay sensitivity for CNL	≤ 1.8 copies
Target Sequencing Coverage	>20,000X for biofluid, >2,000X for tissue
Sample Requirement	Blood or plasma, urine, FFPE tissue

Table 2. Copy number variations coverage

AKT2	ALK	AR	AREG	ATM	AURKA
BAP1	BCL2	BRAF	BRCA1	BRCA2	CCND1
CCND2	CCND3	CCNE1	CD274	CDK4	CDK6
CDKN2A	CDKN2B	CRKL	EGFR	ERBB2	ERBB3
EREG	FGF19	FGF3	FGF4	FGFR1	FGFR2
FGFR3	FGFR4	HGF	IGF1R	IGF2	IKZF1
KIT	KRAS	MDM2	MET	MYC	MYCN
NF2	PDGFRA	PIK3CA	PPP2R2A	PTEN	RAF1
RB1	ROS1	TOP2A	VEGFA		

Conclusions

- We report the development of Predicine cfDNA assay, which is capable of detecting copy number loss in biofluid samples such as blood and urine.
- The detection of CNL by Predicine cfDNA assay is confirmed by orthogonal LP-WGS in cell titration study and further validated by tissue WES where up to 81.8% of CNL in breast cancer tissues was detected in matched plasma samples.
- The status of cfDNA-based copy number loss of RB1 tumor suppressor gene associates with shorter overall survival in mCRPC patients.

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