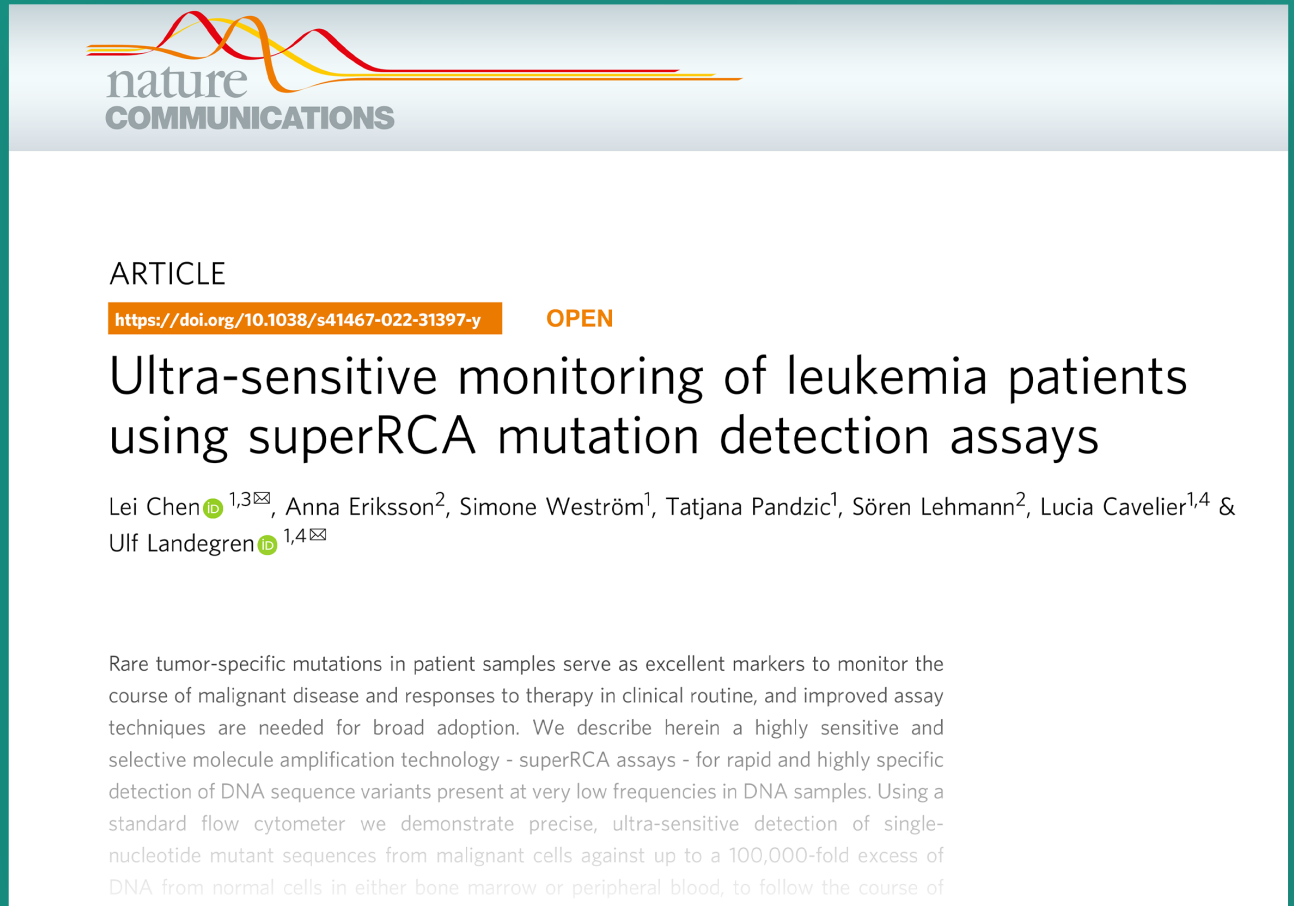


Reference:



Ultra-Sensitive Multiplex Mutation Monitoring in Cancer Patients

SuperRCA - A Flow Cytometer based Multiplex assay for gDNA and ctDNA



UPPSALA
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*Equal contribution to the study

Assay Performance

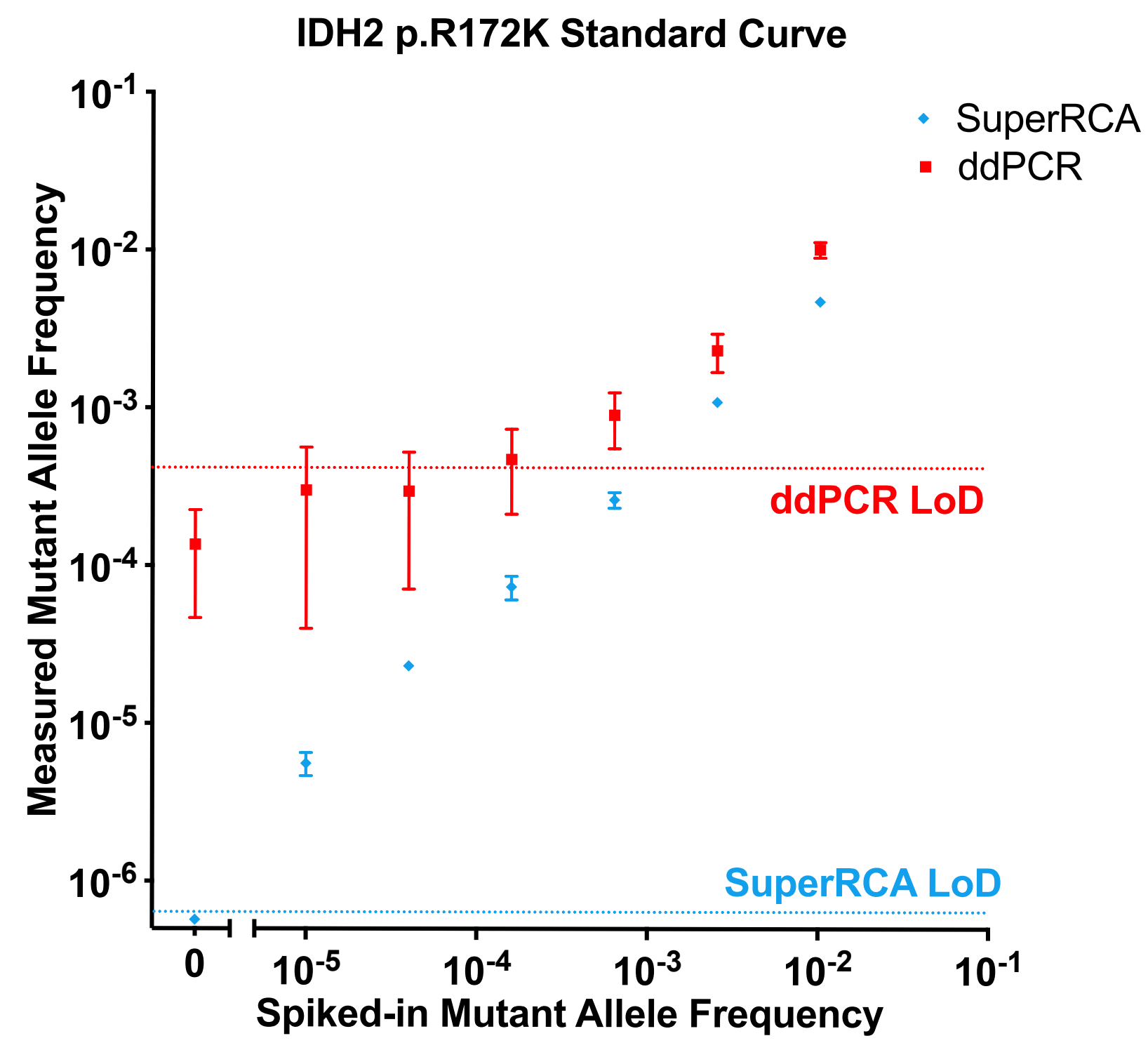


Figure 1: Analysis of IDH2 p.R172K dilution series. With SuperRCA and ddPCR. 330 ng DNA were analyzed per sample for the SuperRCA assay, while 66 ng were used for ddPCR.

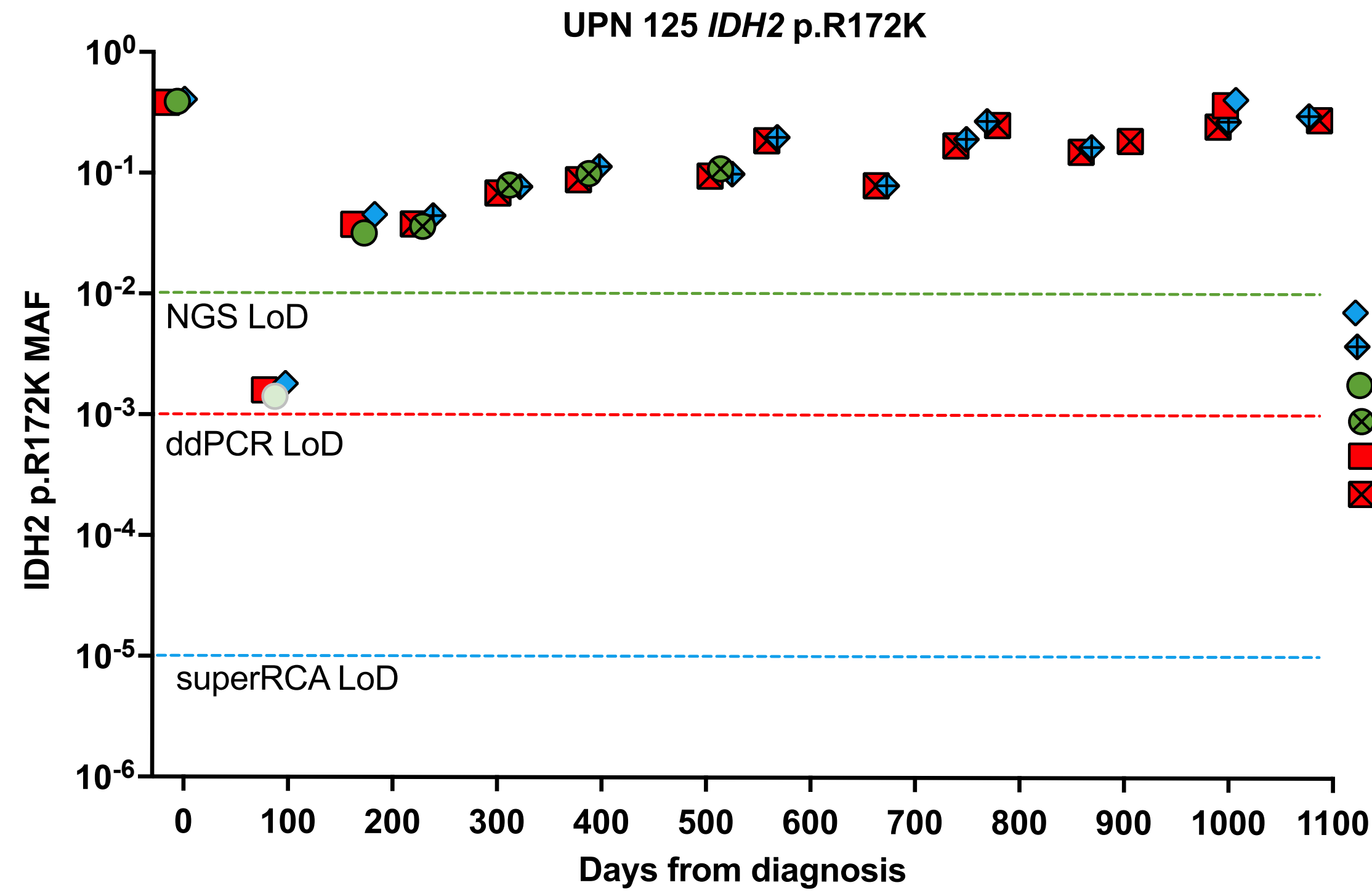


Figure 2: Patient UPN125 was followed by IDH2 p.R172K mutation. NGS of a BM sample (day 0) revealed high levels of IDH2 p.R172K, mutation as well as subsequent superRCA and ddPCR analyses of the same sample. After 3 months of therapy, subsequent analysis by superRCA and ddPCR assays revealed significant levels of this mutation but not NGS. All the following samples have been positive by all three detection techniques.

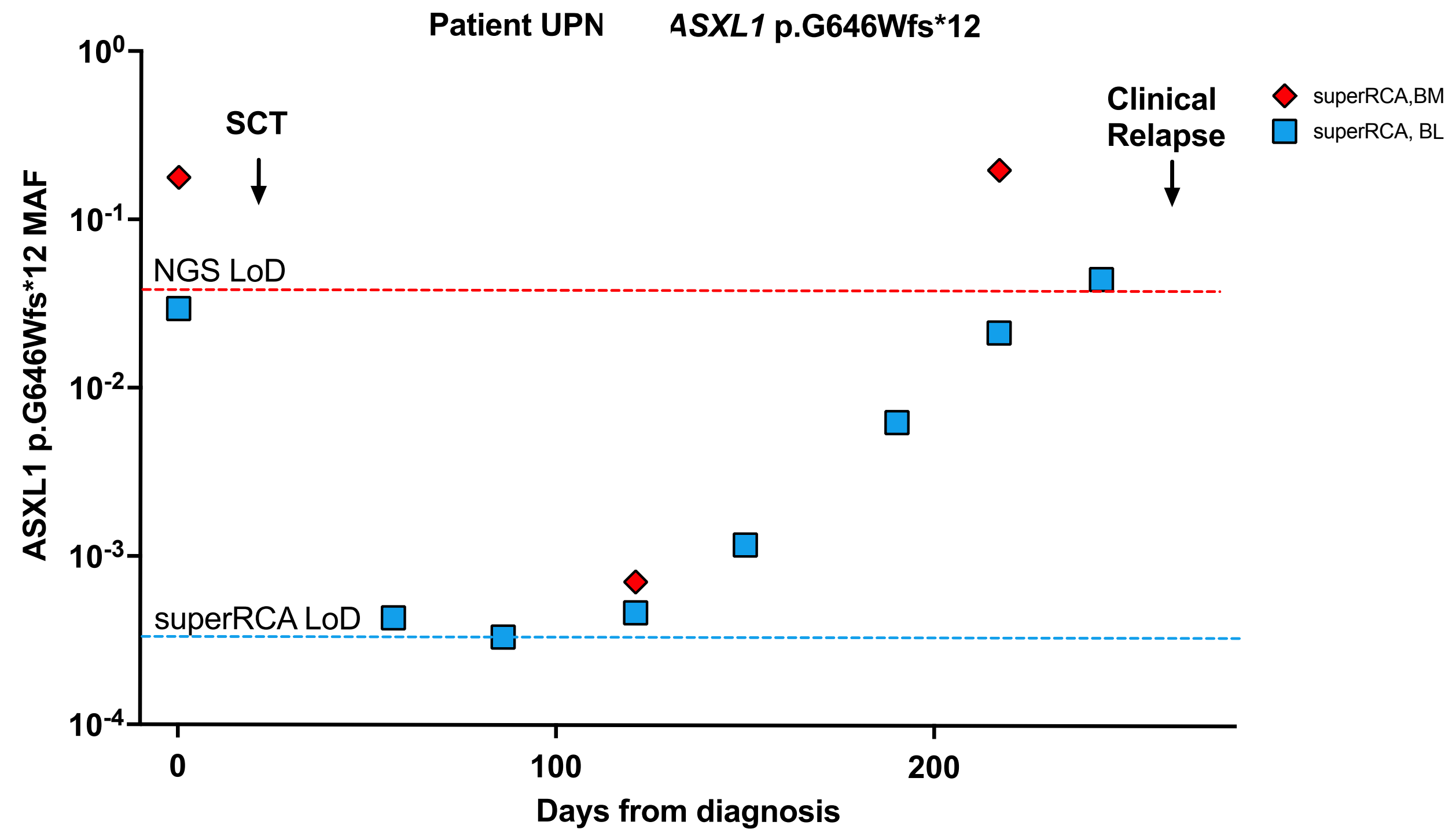


Figure 3: Patient UPN was followed by ASXL1 p.G646Wfs*12, **85% GC Target** (+/- 10bp) GGAGGGGGGGG[-/C]TGGCCCGGGTG, with a 5-10% limited sensitivity using NGS, and impossible to detect with ddPCR. SuperRCA can clearly detect the molecular relapse of this patient with ASXL1 p.G646Wfs*12 mutation three months earlier than clinical relapse. ***unpublished data**

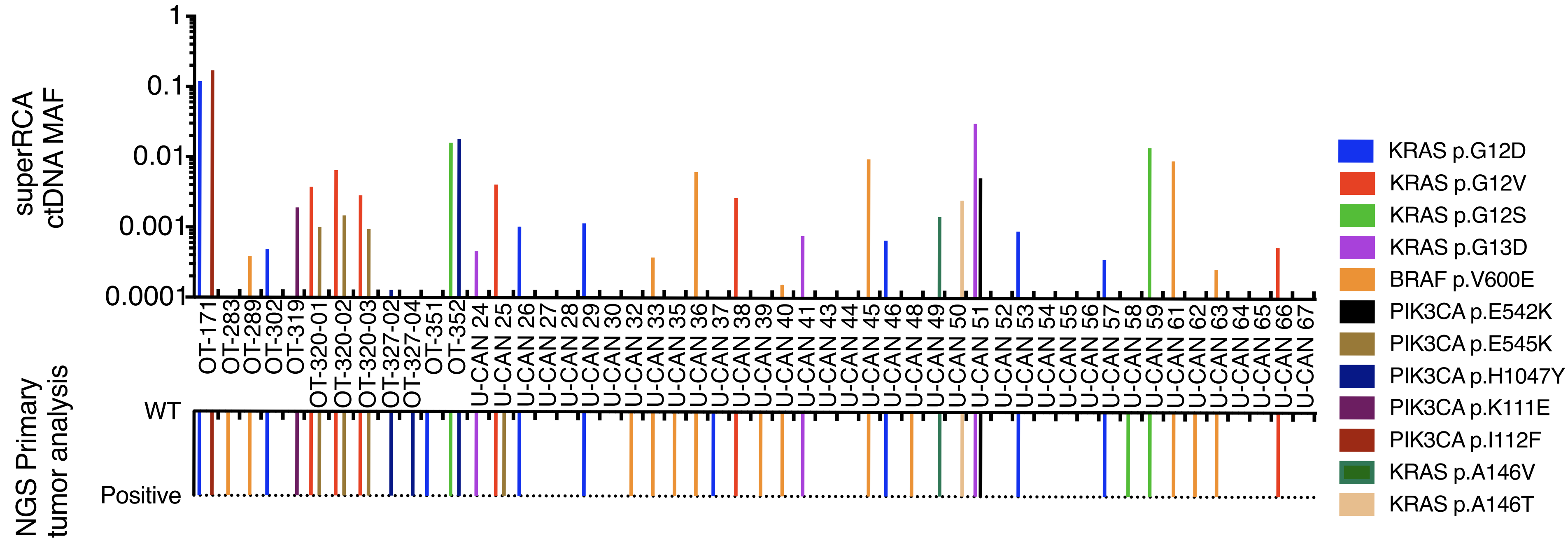
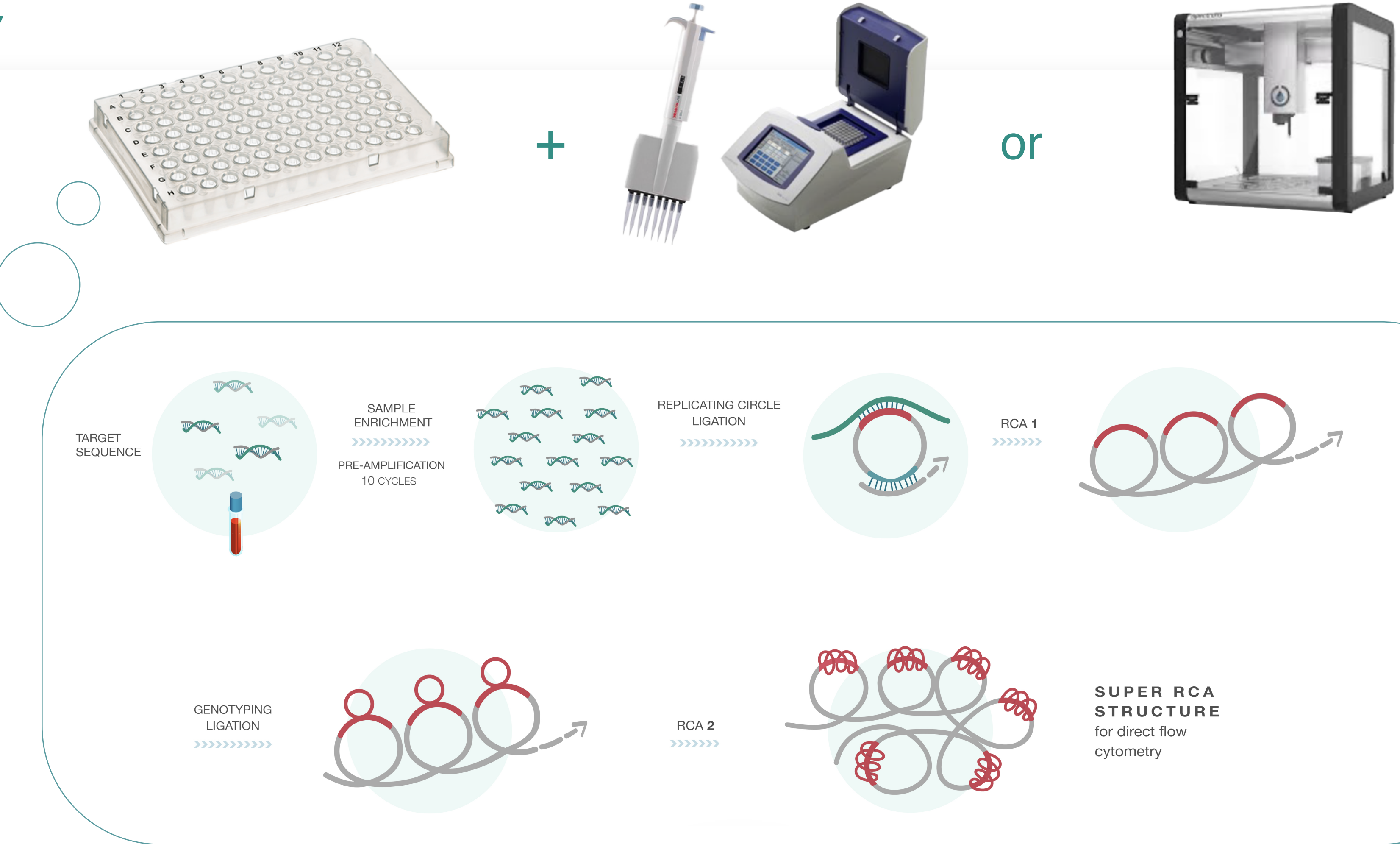


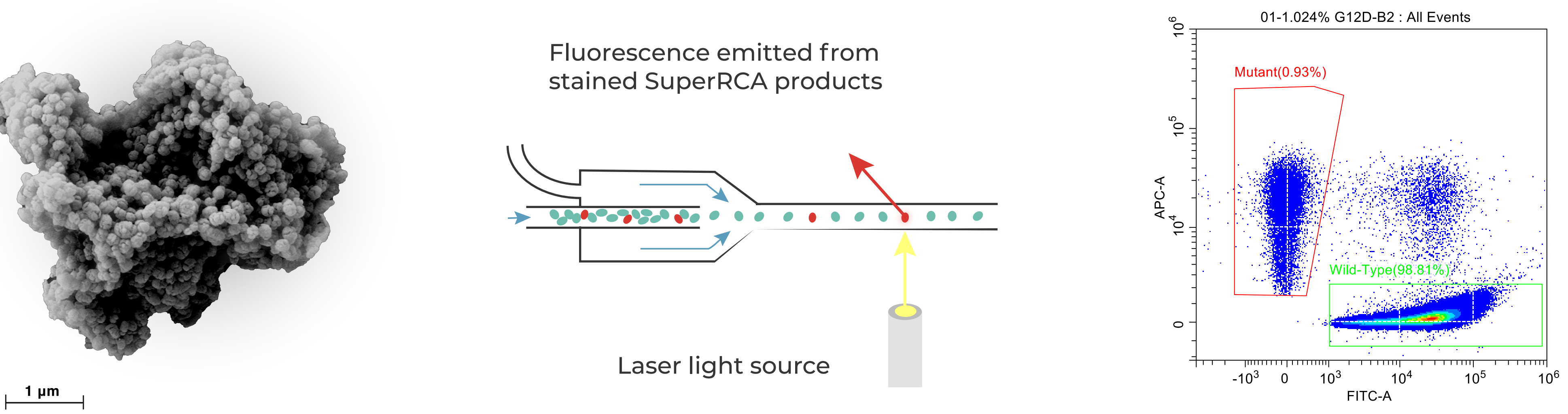
Figure 4: Multiplexed Analysis of recurring mutations in 51 plasma from colorectal cancer patients with a subset of the 42-plex colon cancerSuperRCA panel. The bars in the bottom panel identify the mutations found in the patients' primary tumors with NGS, while the top panel reflects the identity and frequency of mutant in cell-free plasma DNA from the patients collected on the same day of operation. ***unpublished data**

SuperRCA Workflow

Assay



Readout



The superRCA technology is a proprietary technology developed by Dr. Lei Chen commercialized through Rarity Bioscience, Sweden. Using superRCA technology, sequences of interest are developed into giant DNA particles with existing lab equipment. During such giant DNA particle generation process, a majority voting based sequence genotyping step is embedded, and the fluorescent signal of each DNA particle reflects the genotyping result. It is possible to detect up to 50 different mutations present in the same DNA sample at a sensitivity of 1 in 100,000 readout by standard flow cytometer.

Assay Panels

superRCA CRC panel						superRCA Lung Cancer Panel			
Gene	Mutation	Gene	Mutation	Gene	Mutation	Gene	Mutation	Gene	Mutation
KRAS	G12C (34G>T)	KRAS	K117N (351A>T)	NRAS	Q61H (183A>C)	KRAS	G12C (34G>T)		T790M (2369C>T)
	G12R (34G>C)		A146T(436G>A)		Q61H (183A>T)		G12R (34G>C)		C797S (2389T>A)
	G12S (34G>A)		A146V (437C>T)		I112F (334A>T)		G12S (34G>A)		C797S (2390G>A)
	G12A (35G>C)		K147Q (439A>C)	PIK3CA	K111E (331A>G)		G12A (35G>C)		L858R (2573 T>G)
	G12D (35G>A)	NRAS	G12C (34G>T)		E542K (1624G>A)		G12D (35G>A)	EGFR	L861Q (2582 T>A)
	G12V (35G>T)		G12R (34G>C)		E545K (1633G>A)		G12V (35G>T)		E746_A750del (2235_2249del)
	G13S (37G>A)		G12S (34G>A)		E545G (1634A>G)		G13S (37G>A)		E746_A750del (2235_2250del)
	G13C (37G>T)		G12A (35G>C)	BRAF	Q546K (1636C>A)		G13C (37G>T)	BRAF	L747_T751del (2240_2254del)
	G13R (37G>C)		G12D (35G>A)		M1043I (3129G>T)	AKT1	G13R (37G>C)		L747_A750>P (2235_2248delinsC)
	G13D(38G>A)		G12V (35G>T)		H1047Y (3139C>T)		G13D(38G>A)		L747_A750>P (2235_2248delinsC)
	A59T (175G>A)		G13R (37G>C)		H1047R (3140A>G)		A59T (175G>A)		L747_T751del (2240_2254del)
	Q61K (181C>A)		G13D(38G>A)		H1047L (3140A>T)		Q61K (181C>A)		V600E (1799T>A)
	Q61L (182A>G)		G13V (38G>T)		V600K (1798_1799 GT>AA)		Q61L (182A>G)		V600K (1798_1799 GT>AA)
	Q61H (183A>C)		Q61K (181C>A)		E17K (49G>A)		Q61H (183A>T)		
	K117N (351A>C)		Q61L (182A>G)				Q61H (183A>C)		

