

HER2 Expression and Amplification in CTCs of Metastatic Breast Cancer patients

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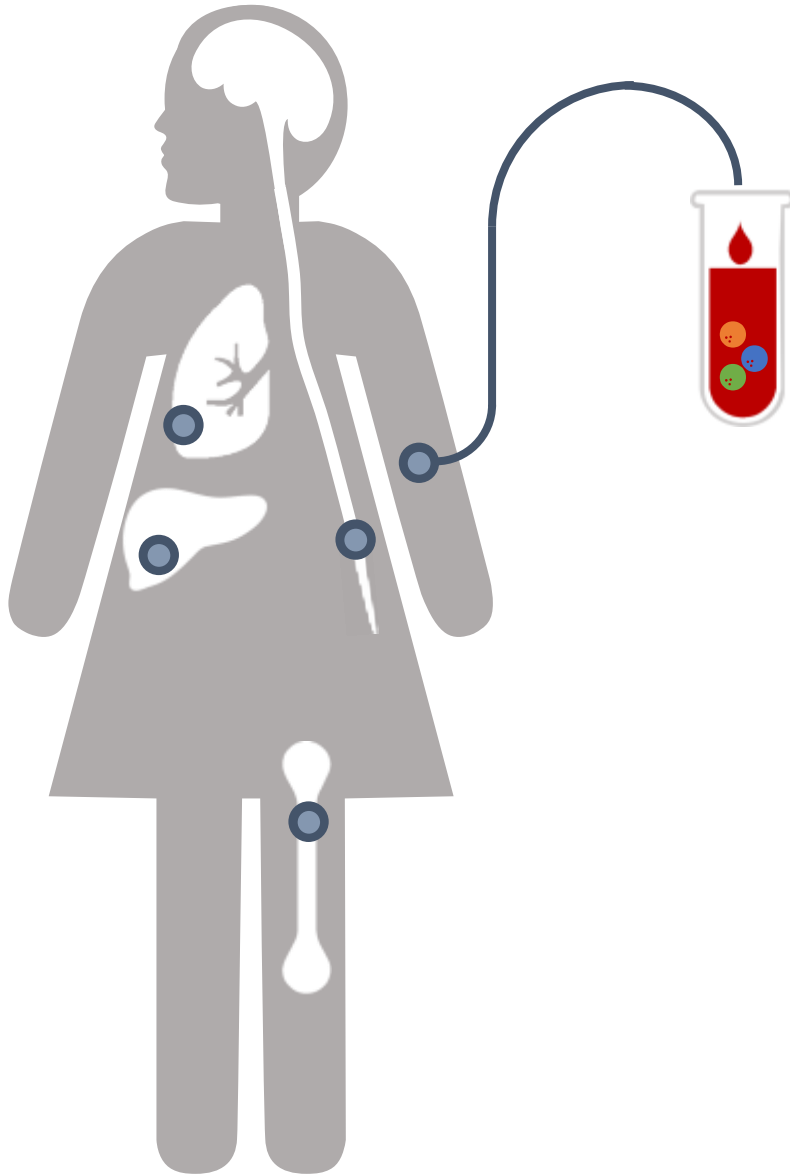
Translational Cancer Research Unit

TCRU

Sint-Augustinus
GZA . Ziekenhuizen

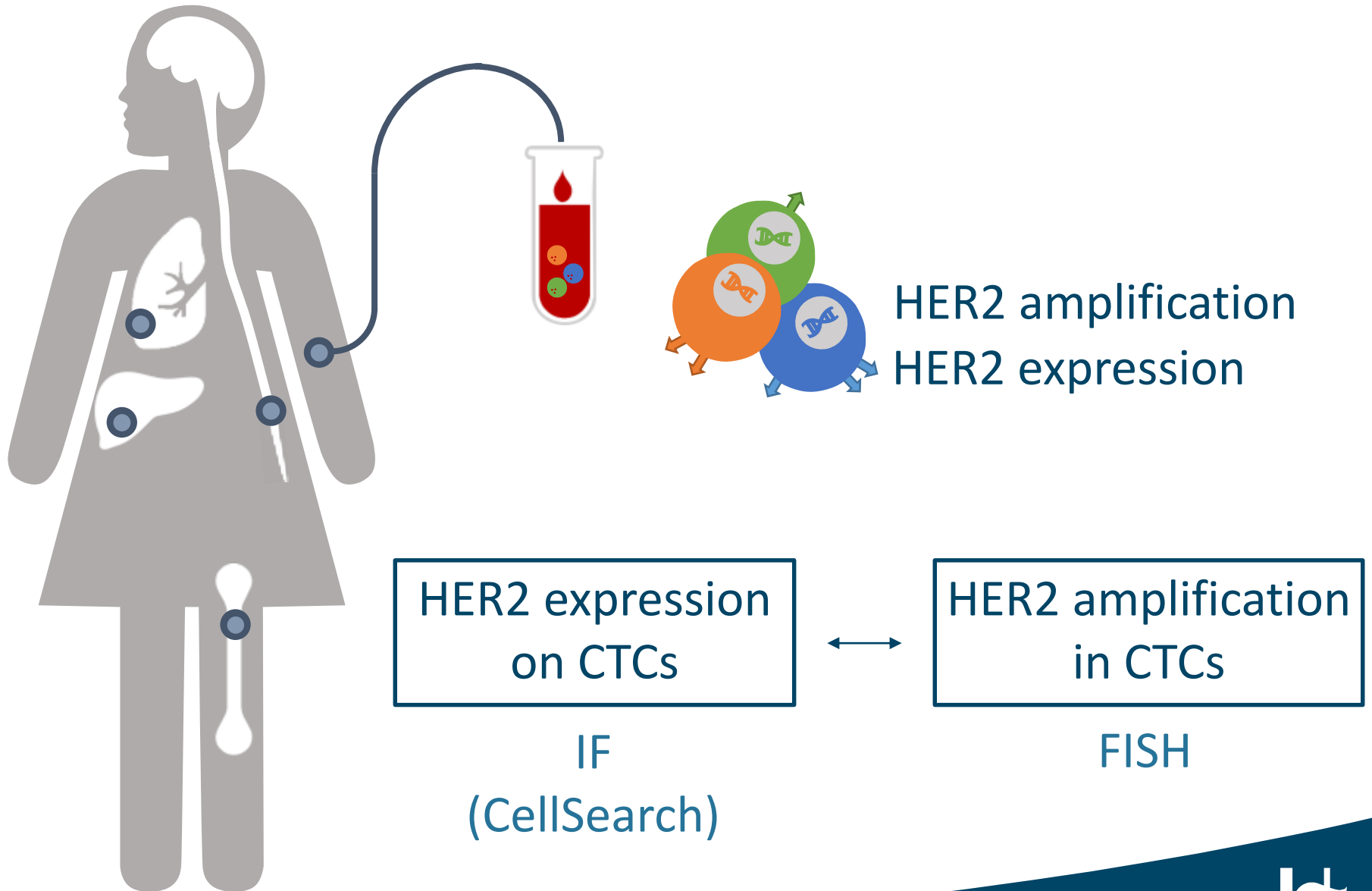


Circulating Tumor Cells



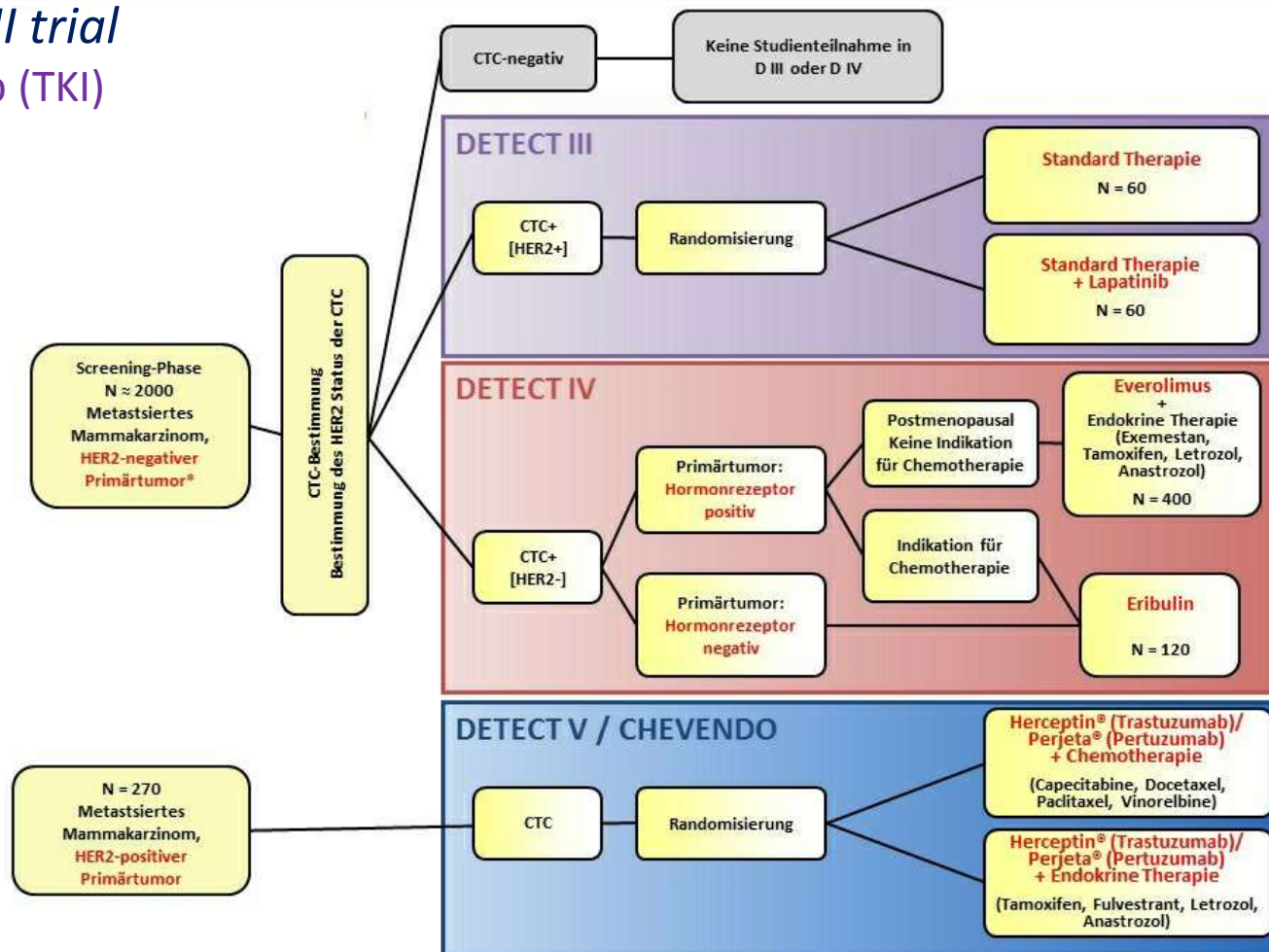
- In metastatic disease **multiple clones** of tumor cells **evolve** in time and under therapy pressure.
- CTCs are known to **represent** metastatic disease and are an easy source for **repeated tumor analysis**.

Circulating Tumor Cells



DETECT III

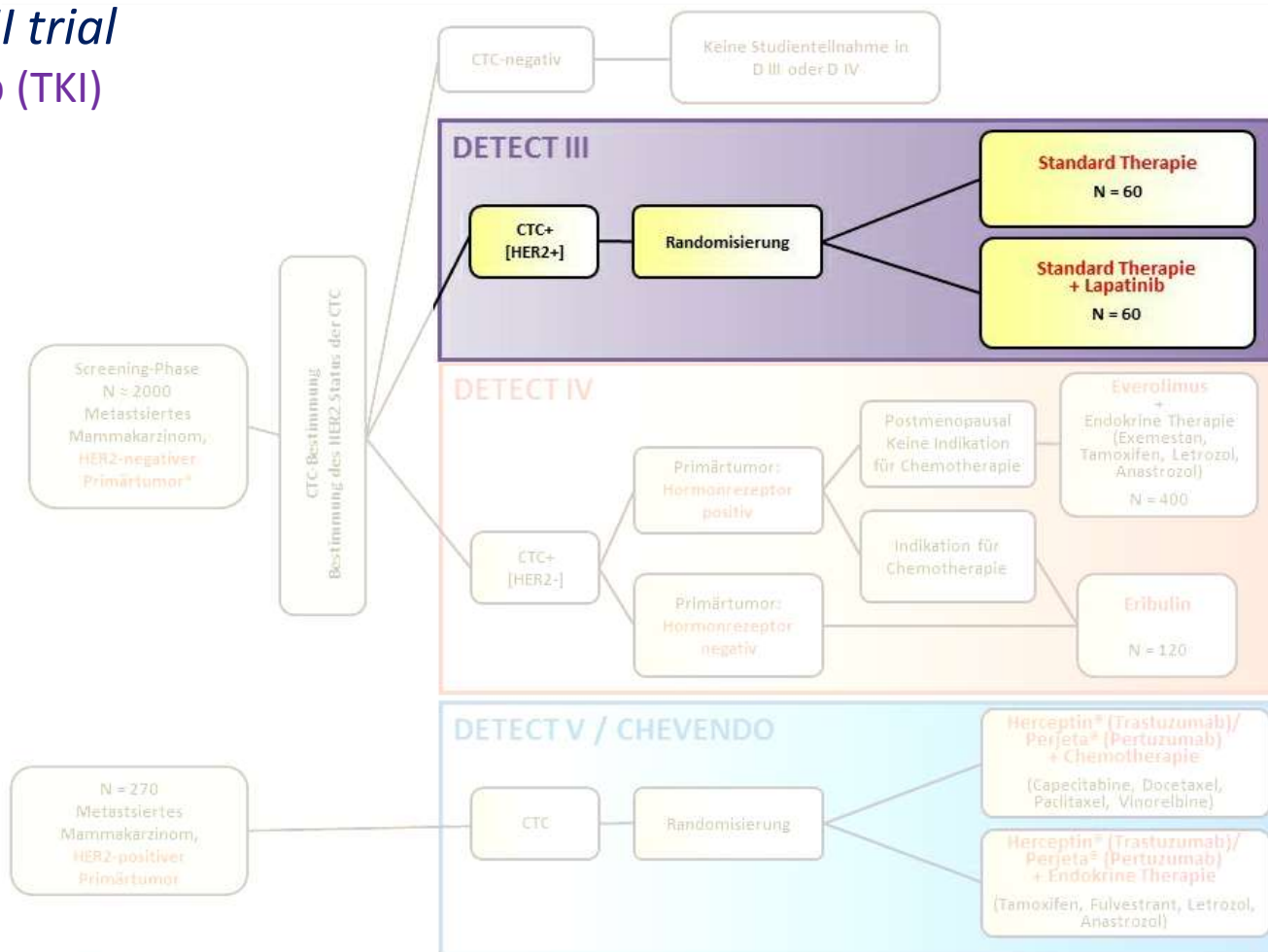
Phase III trial Lapatinib (TKI)



* einschließlich HER2-negativer metastatische Läsionen – sofern eine Biopsie durchgeführt wurde

DETECT III - CTC scoring with HER2 IF

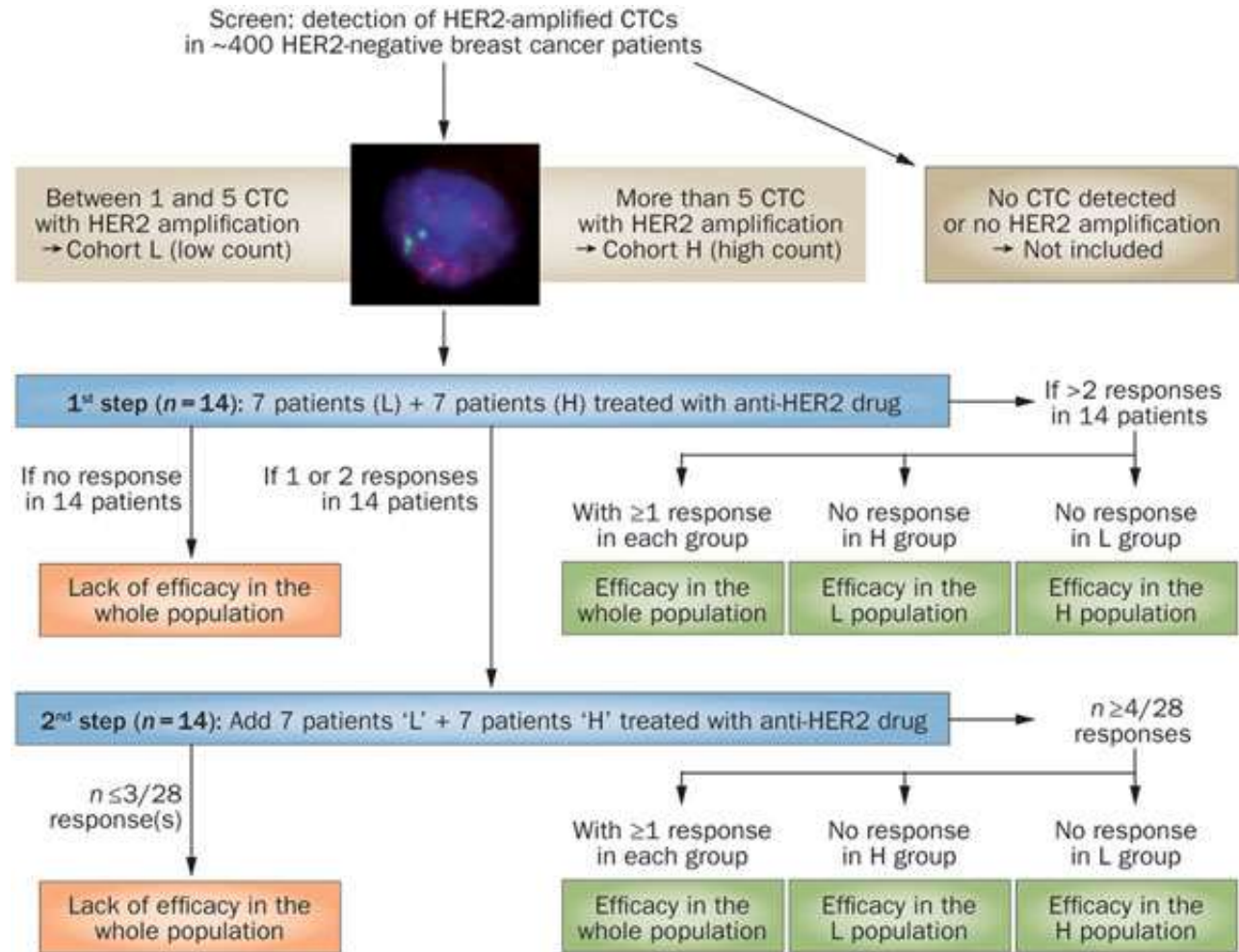
Phase III trial
Lapatinib (TKI)



* einschließt HER2-negative metastatische Läsionen – sofern eine Biopsie durchgeführt wurde

CirCe T-DM1 - CTC scoring with HER2 FISH

Phase II trial T-DM1



Methods

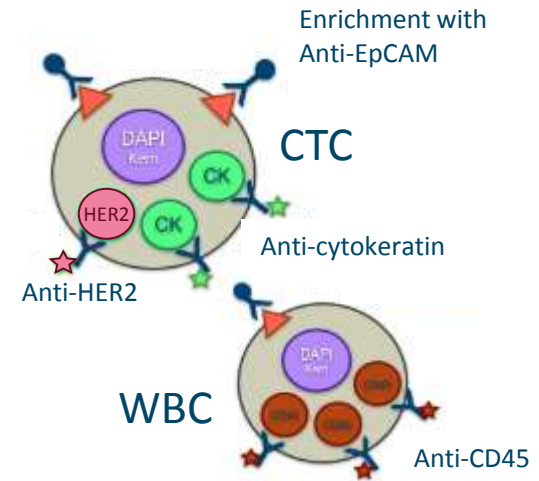
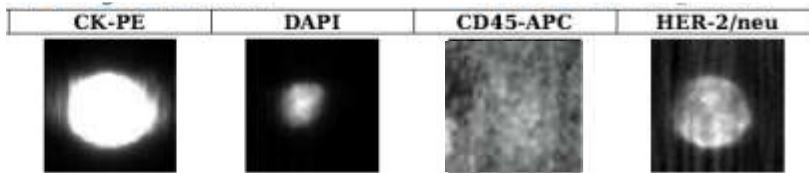
HER2 expression by IF

HER2 amplification by FISH

Methods

HER2 expression by IF

- CellSearch (FDA approved)

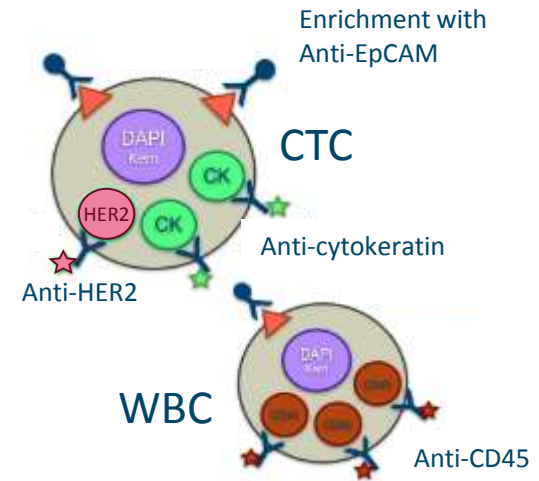
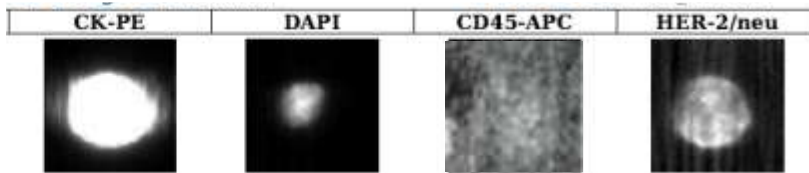


HER2 amplification by FISH

Methods

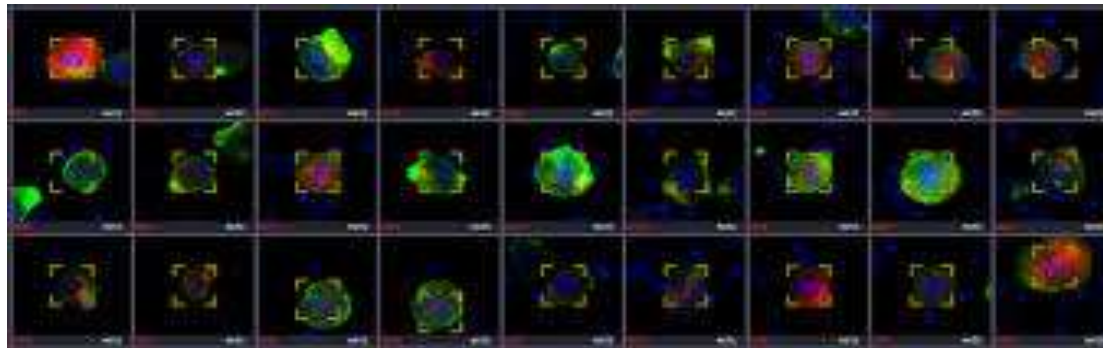
HER2 expression by IF

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HER2 amplification by FISH

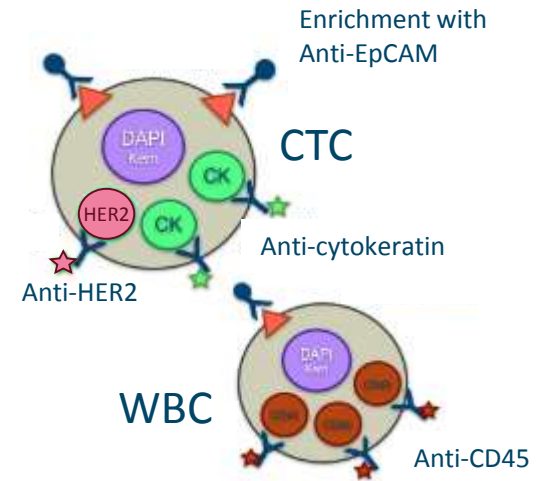
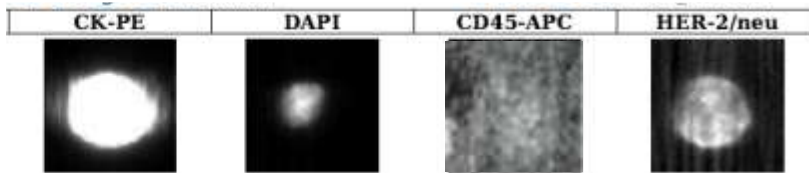
- BioView



Methods

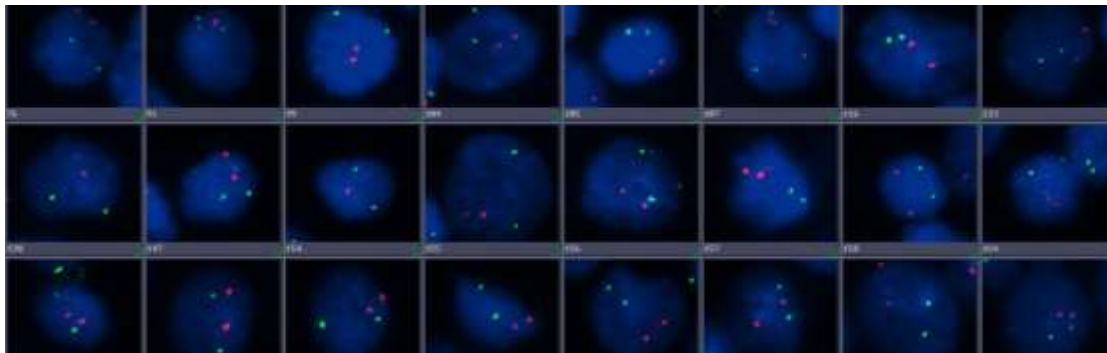
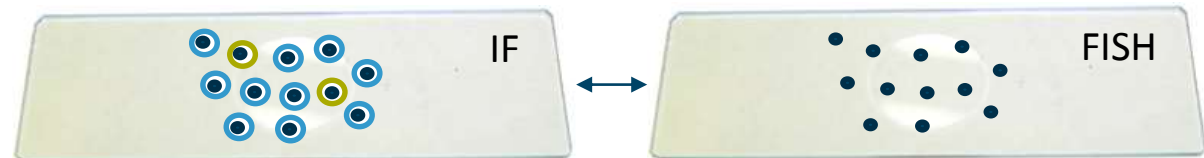
HER2 expression by IF

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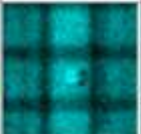

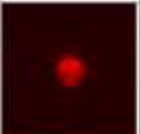
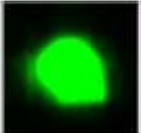


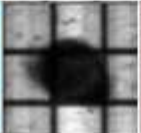

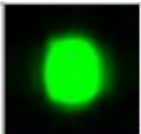

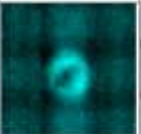
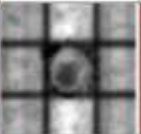

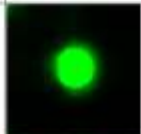

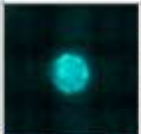
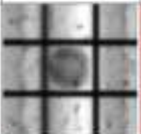

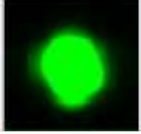

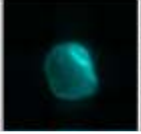

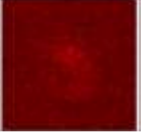


HER2 amplification by FISH

- BioView

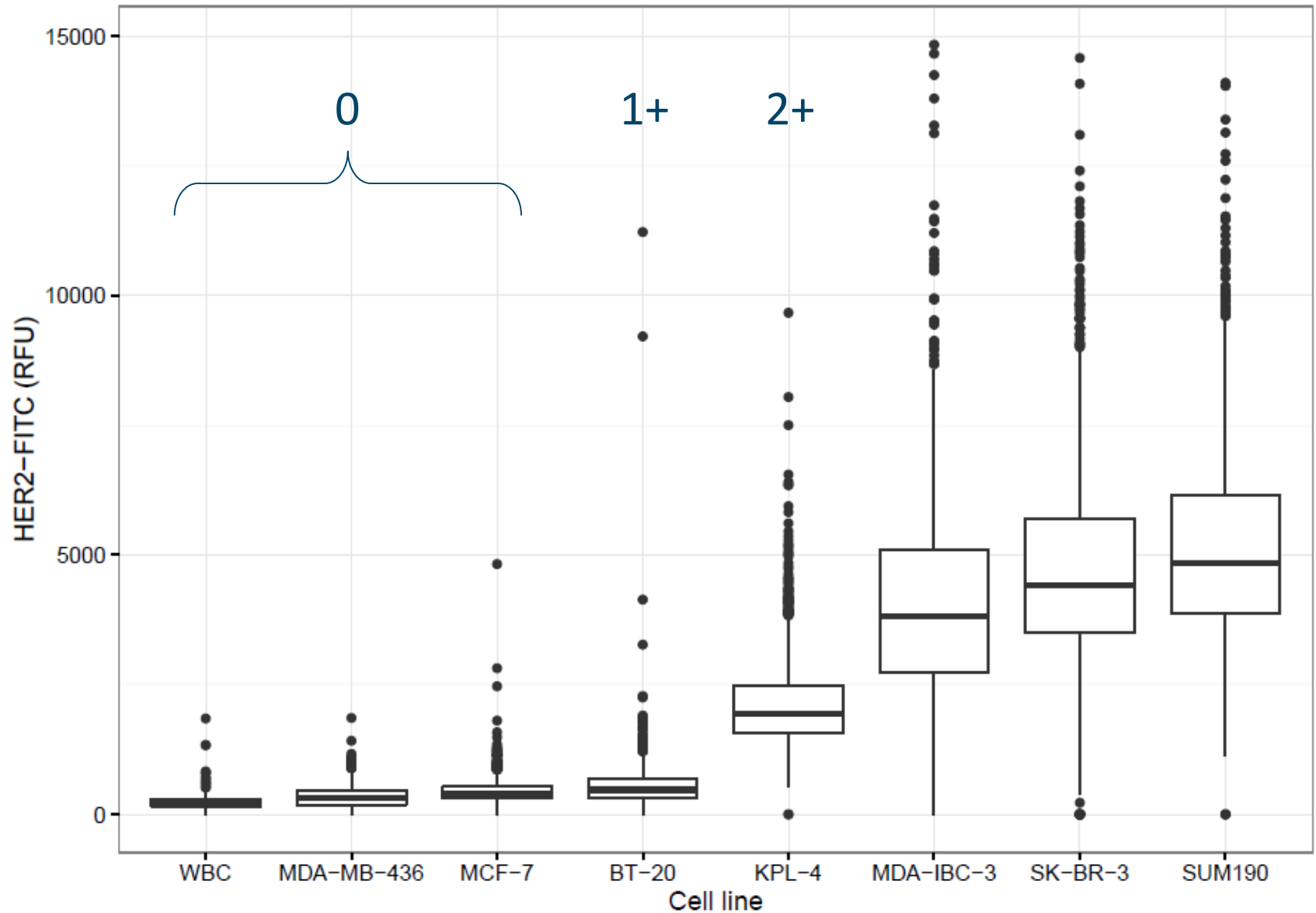


Cell line model

Group	CK	DAPI	HER2	Bright Field	CD45	
WBC						Leukocytes
CTC						MCF-7 MDA-MB-436 (0+)
CTC						BT-20 (1+)
CTC						KPL-4 (2+)
CTC						SUM190 IBC-3 (3+) SKBR-3

HER2 intensity per Cell line

3+



Automatic HER2 scoring on IF images

Patient samples:

- GZA St Augustinus: **40.000 CTCs** in 128 pt samples
- Erasmus MC (NL): **10.000 CTCs** in 38 pt samples

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Manual scoring

HER2



0 - no cell recognizable



1+ - background darker than cell
- pixelated edge
- pixelated staining



2+ - darker background
- relative sharp edge
- fluent staining

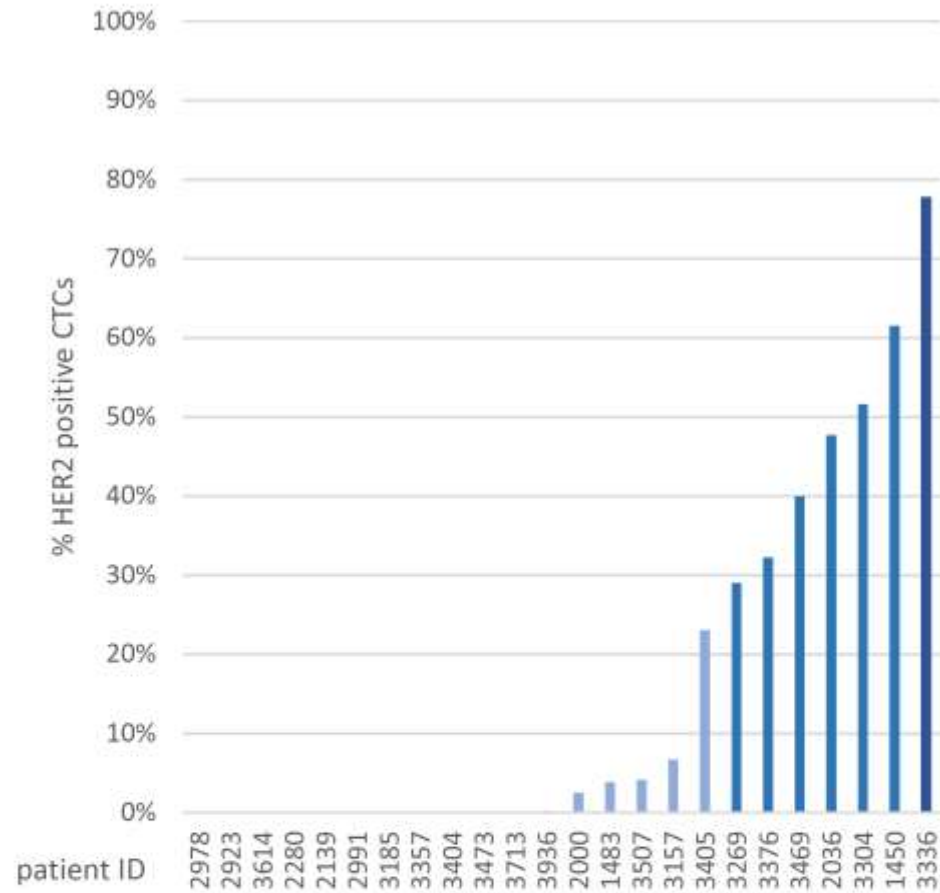


3+ - very dark background
- sharp edge
- fluent staining

HER2 scores on CTCs of 58 HER2- patient



HER2 scores on CTCs of 24 HER2+ patient

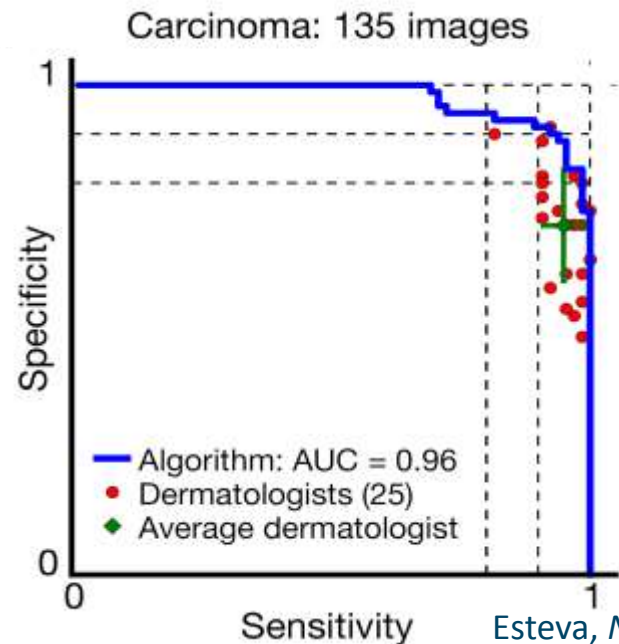
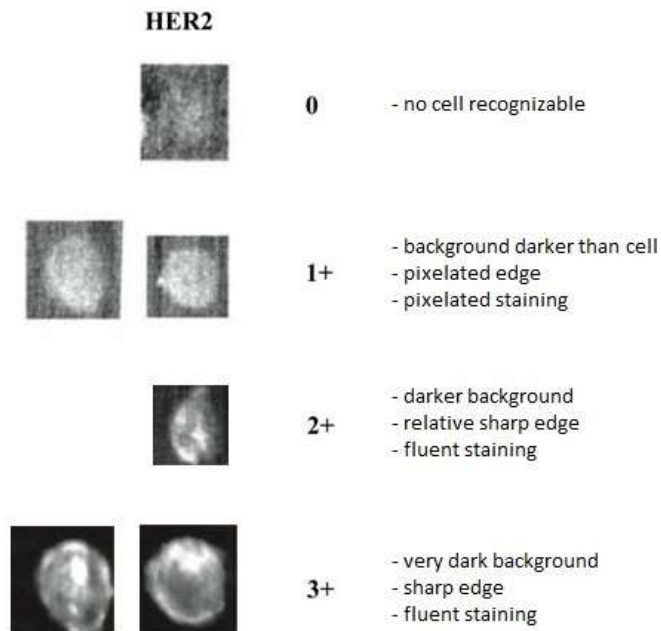


Automatic HER2 scoring on IF images

Patient samples:

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Manual scoring vs. Machine learning



Performance of Image machine learning

ImageJ Patients		Manual Scoring			
		0	1+	2+	3+
Prediction	0	201	45	1	4
	1+	508	1608	83	0
	2+	7	101	170	8
	3+	2	2	13	135

	Sens	Spec
0	28,0	97,7
1+	91,6	47,8
2+	63,7	95,6
3+	91,8	99,4

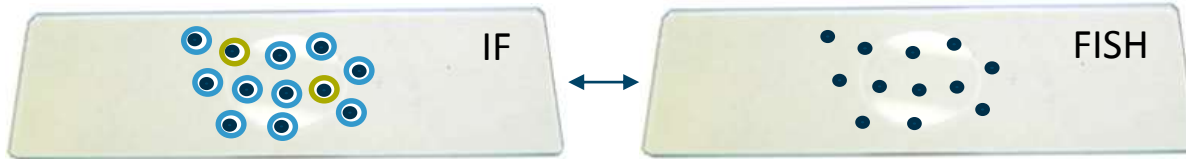
Accuracy: 73,2%

Machine Learning Patients		Manual Scoring			
		0	1+	2+	3+
Prediction	0	1239	169	3	0
	1+	562	4440	138	0
	2+	0	195	538	20
	3+	1	4	16	394

	Sens	Spec
0	68,8	97,1
1+	92,3	76,0
2+	77,4	96,9
3+	95,2	99,7

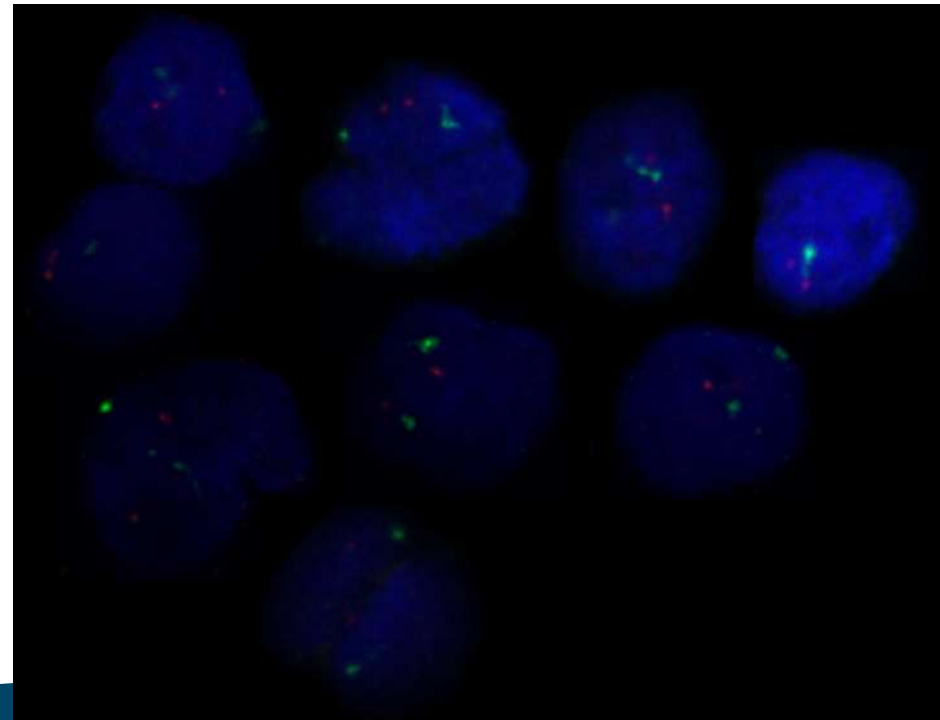
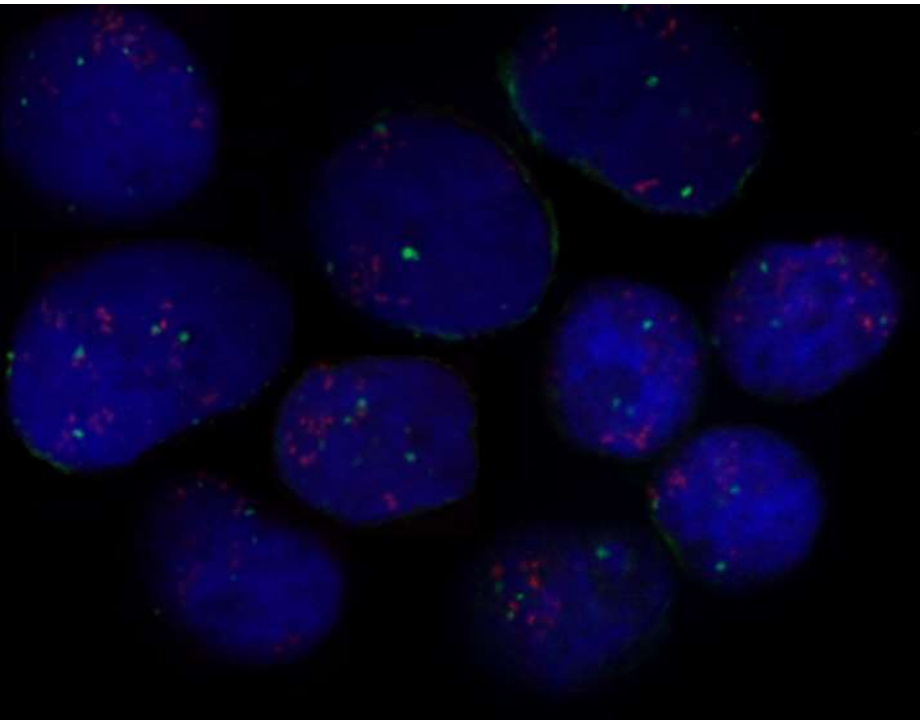
Accuracy: 85,6%

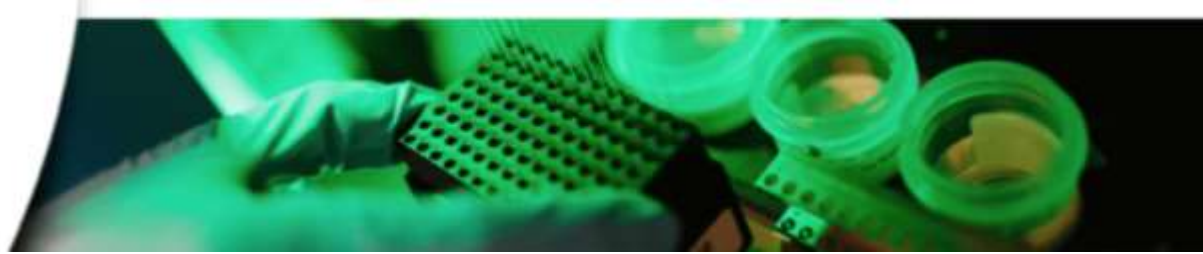
BioView after DAKO-IQ FISH



SKBR-3

WBC





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CORE

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