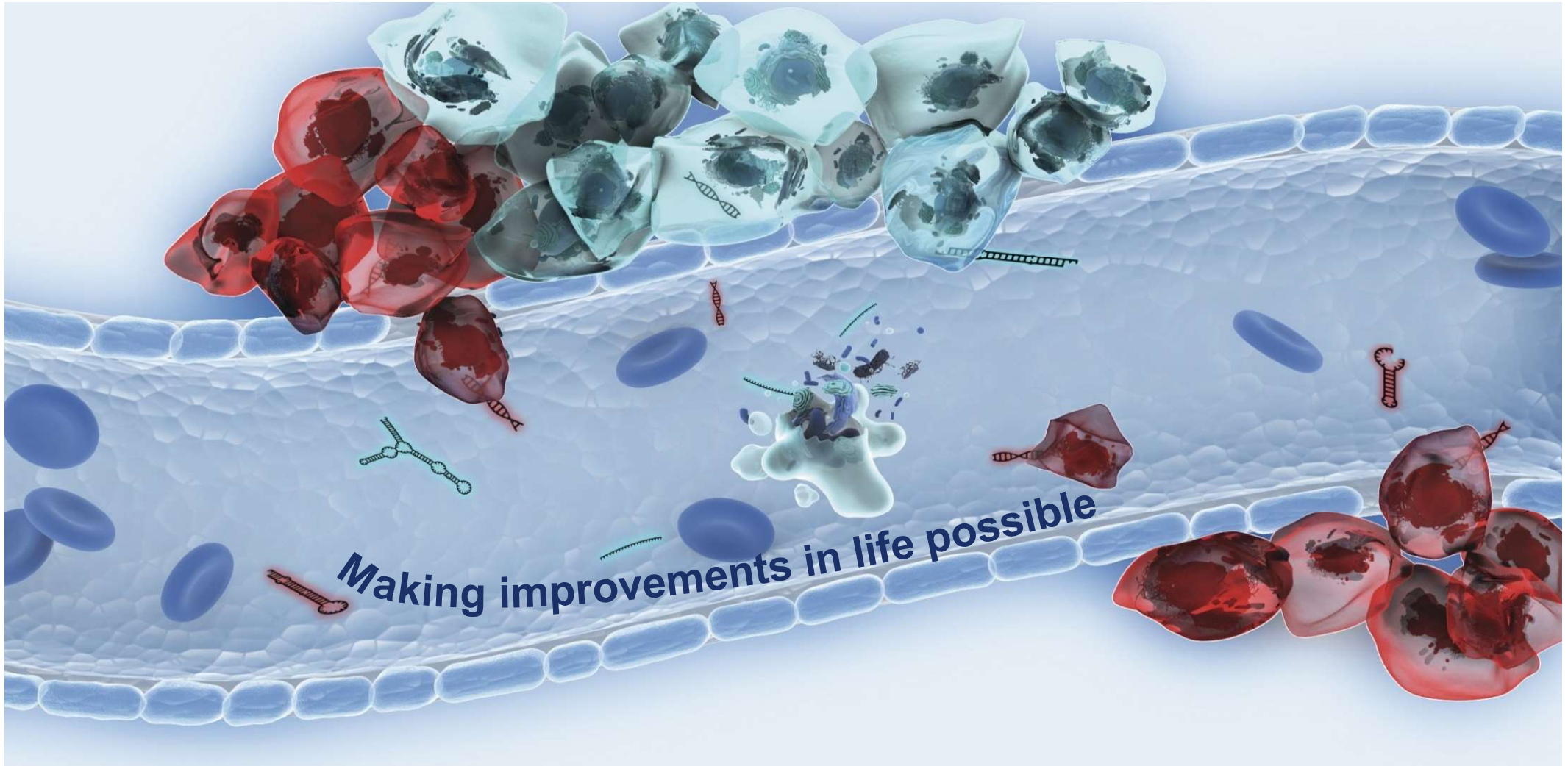


CTC Detection and Molecular Characterization – Challenges and Solutions

Dr. Siegfried Hauch

Did circulating tumor cells tell us all they could..... ?

Maria Grazia Daidone,
Istituto Tumori, Milano 2015

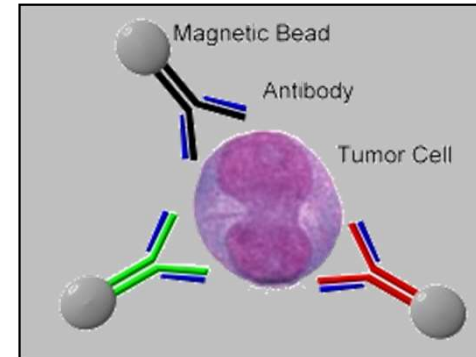


The AdnaTest Principle – about how to open a treasure

The COCP „combination of combination“ Principle

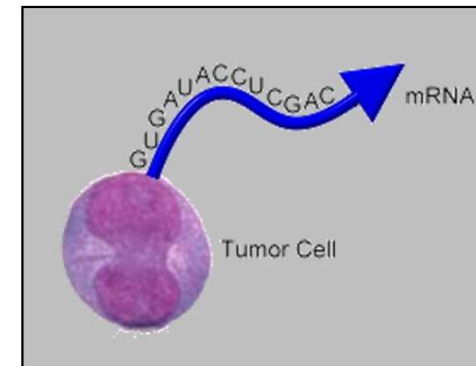
(1) Immunomagnetic cell enrichment

Tumor cell enrichment using magnetic particles conjugated with an antibody mixture to ensure CTC capturing in case of EpCAM loss .



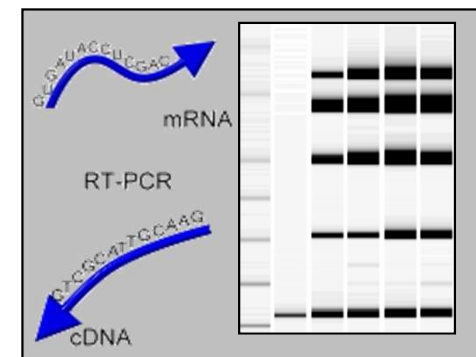
(2) mRNA isolation and RT

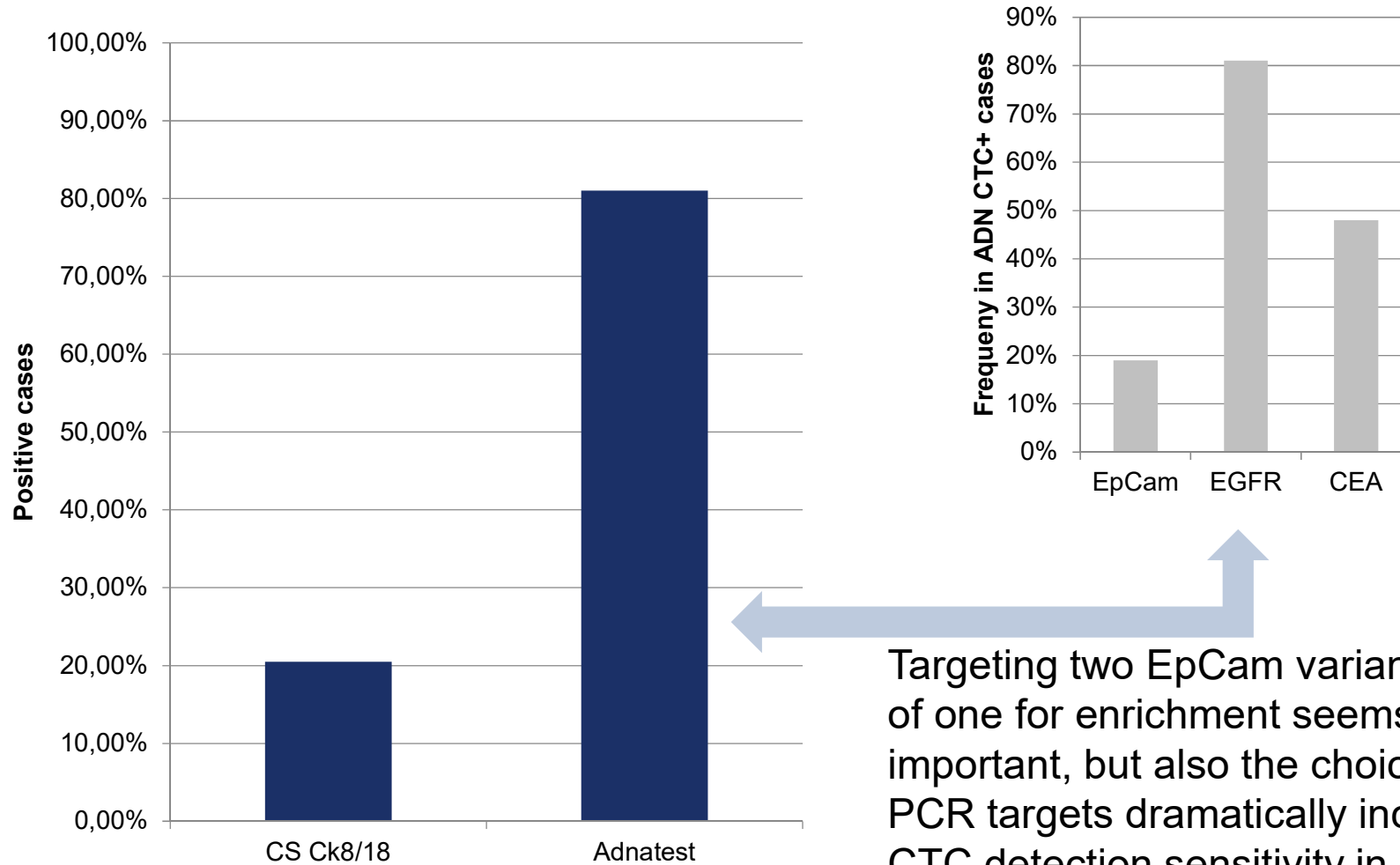
mRNA stabilization, mRNA isolation with oligo(dT) magnetic beads and cDNA synthesis by reverse transcription.



(3) Multiplex-PCR

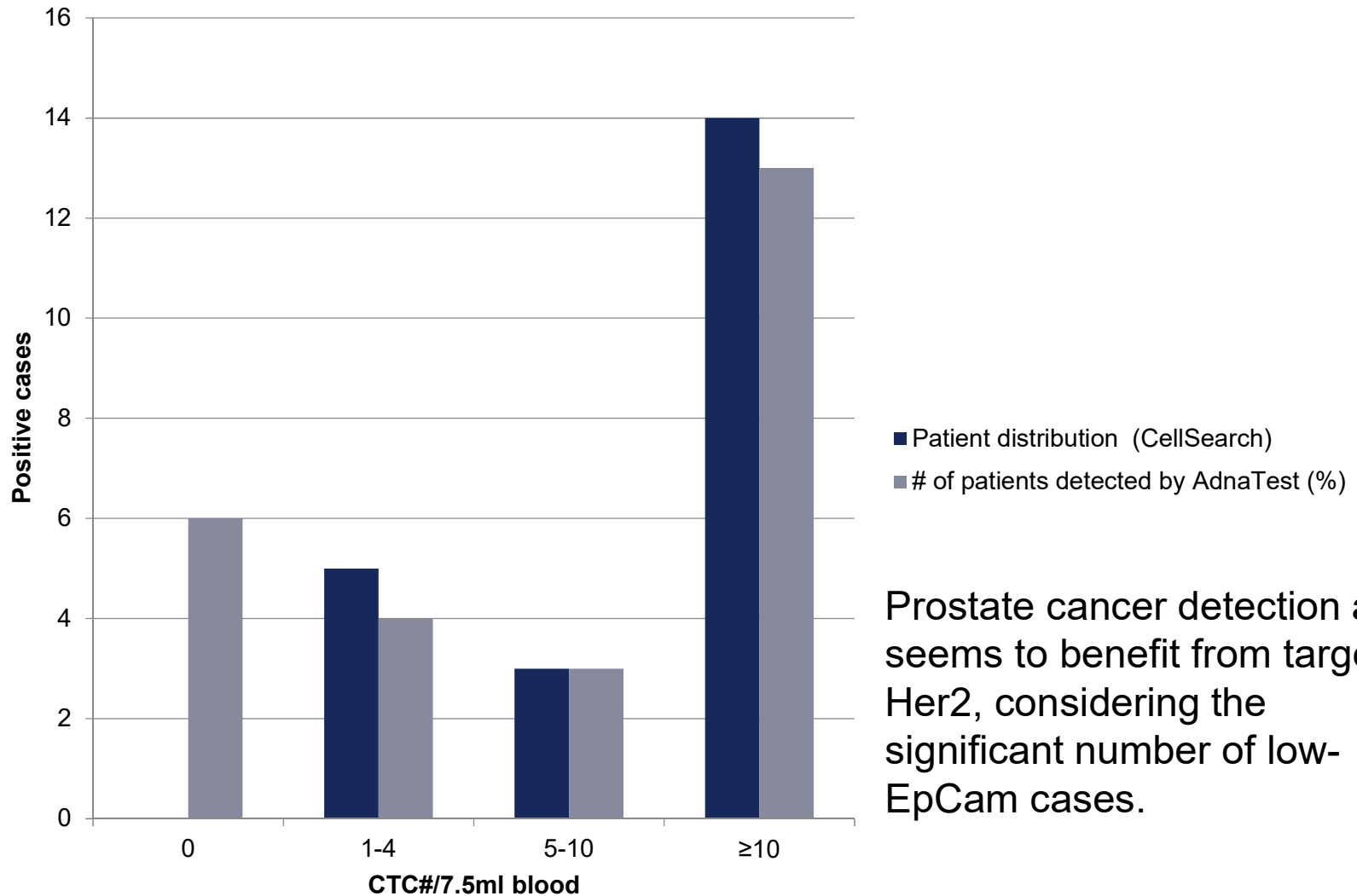
Expression analysis of tumor associated markers. The sample is positive for CTCs if one of the markers is detected





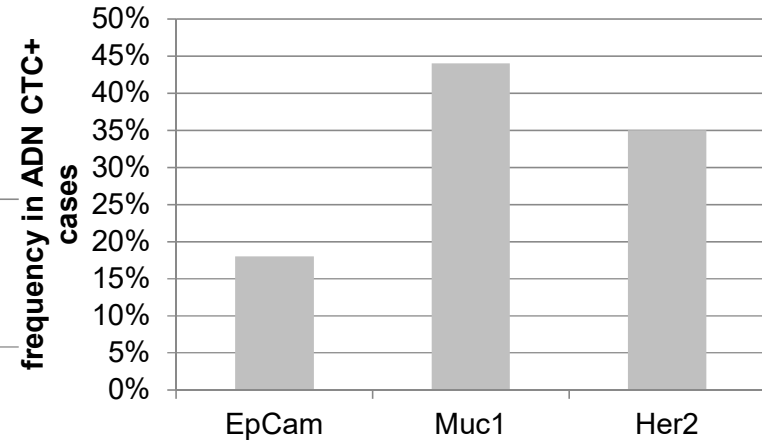
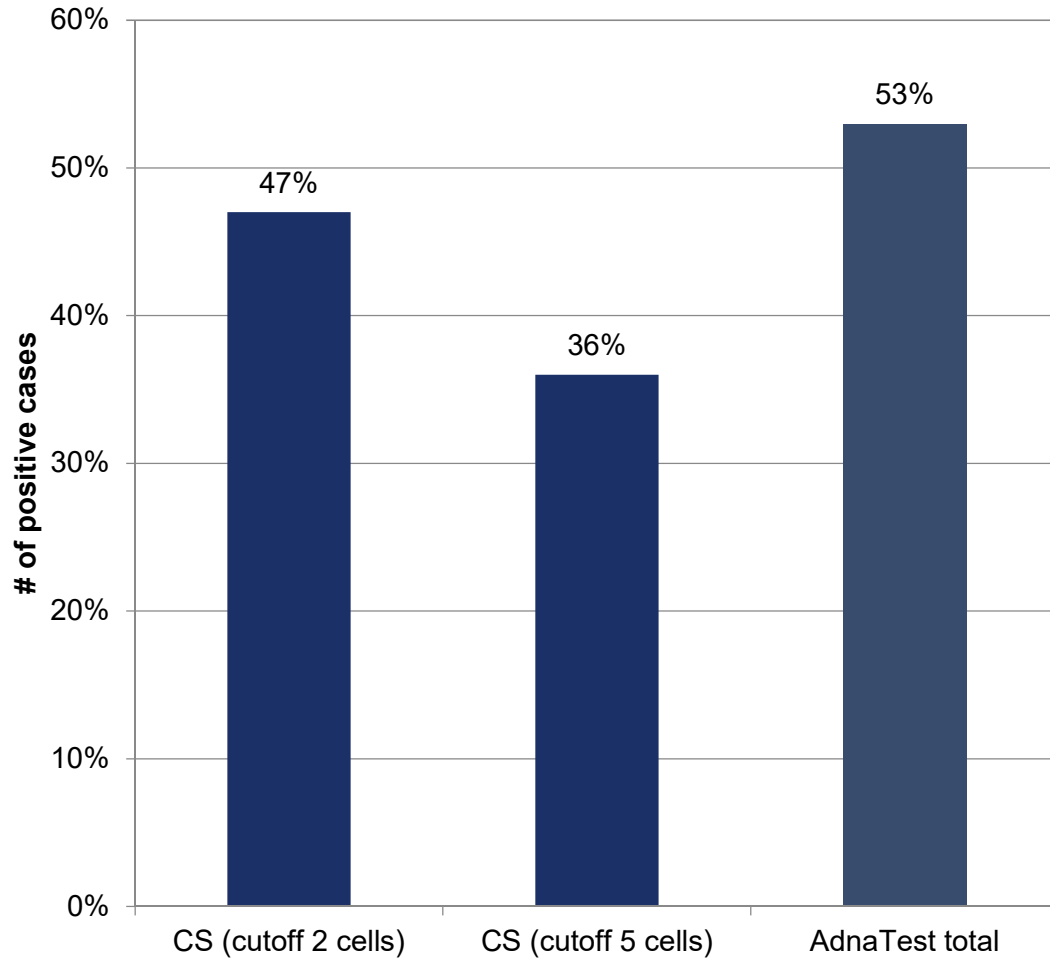
Targeting two EpCam variants instead of one for enrichment seems to be important, but also the choice of the PCR targets dramatically increases CTC detection sensitivity in colorectal cancer.

Raimondi et al. Cancer Biol Ther. 2014 May;15(5):496-503



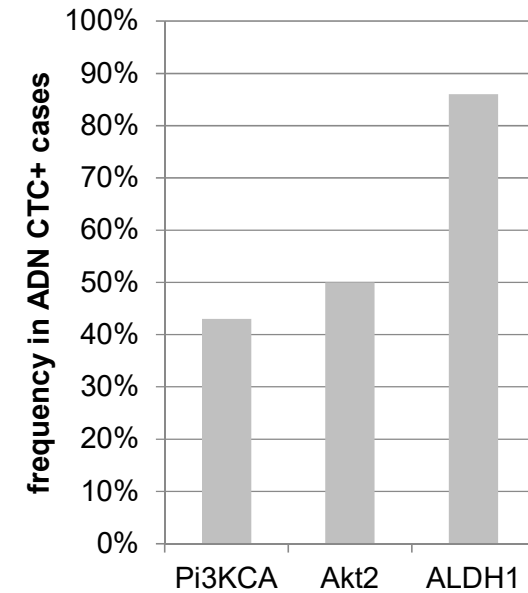
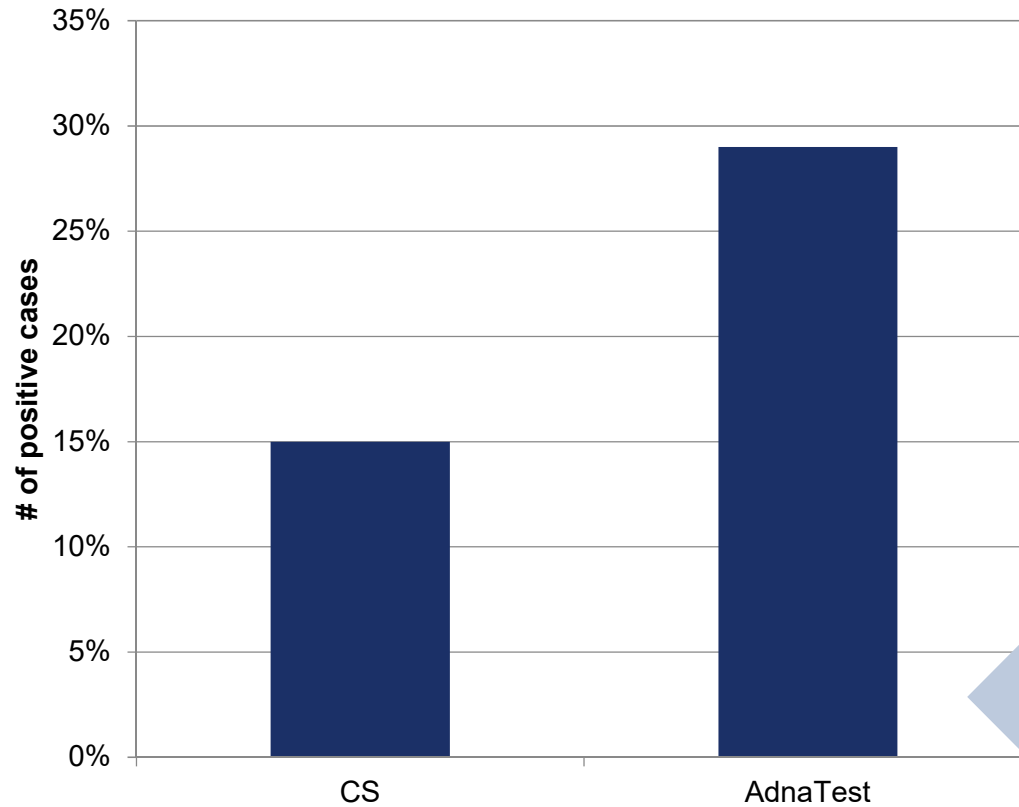
Prostate cancer detection also seems to benefit from targeting Her2, considering the significant number of low-EpCam cases.

Samoila et al. 2012
 Poster at http://www.adnagen.com/m089v01_upload/Samoila_poster_2012.pdf



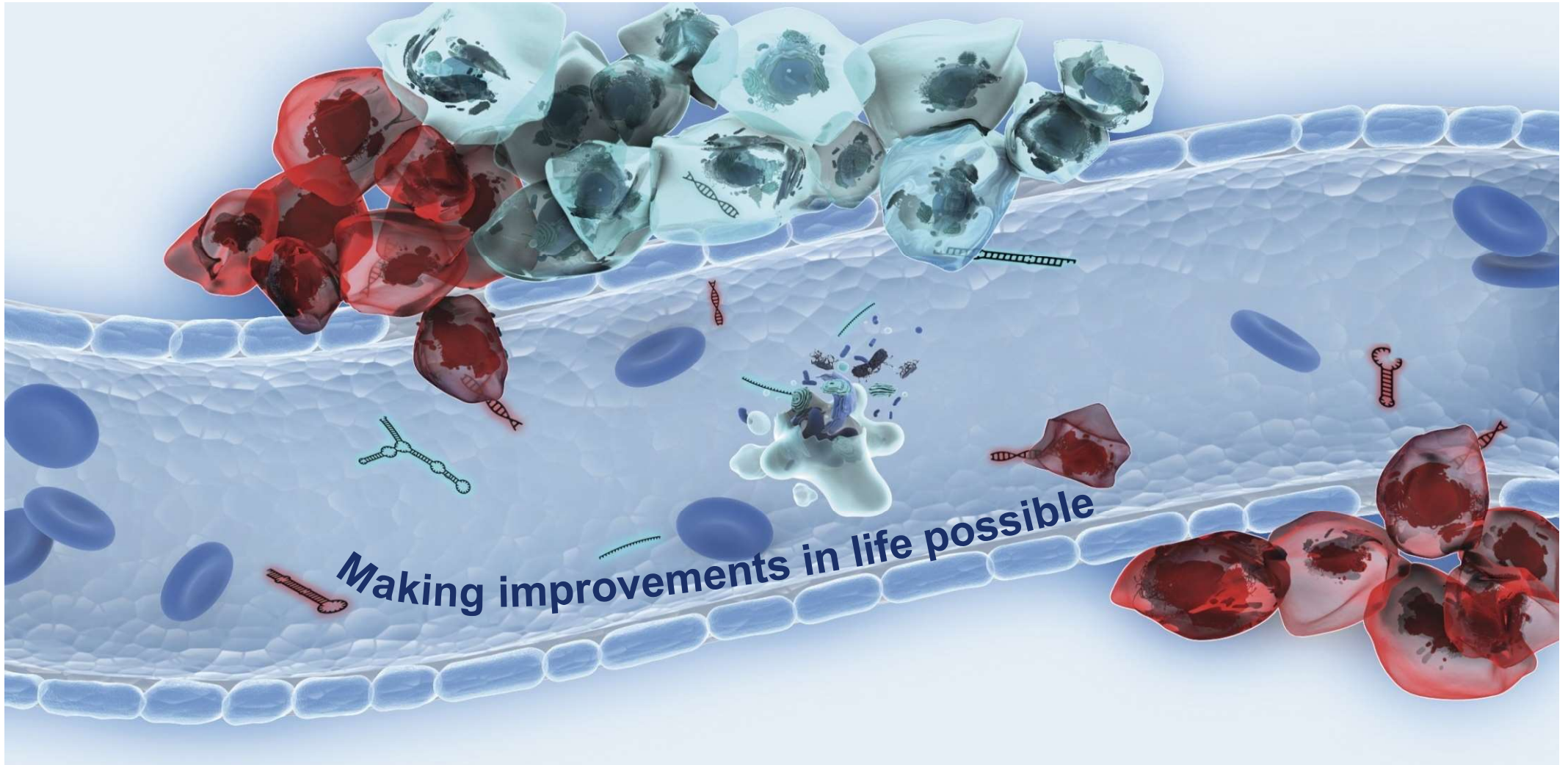
Using EpCam as well as high- and low-glycosylated MUC1, target sensitivity increases 17% over the gold standard as per definition.

Andropoulou et al. ; Int J Cancer. 2012 Apr 1;130(7):1590-7

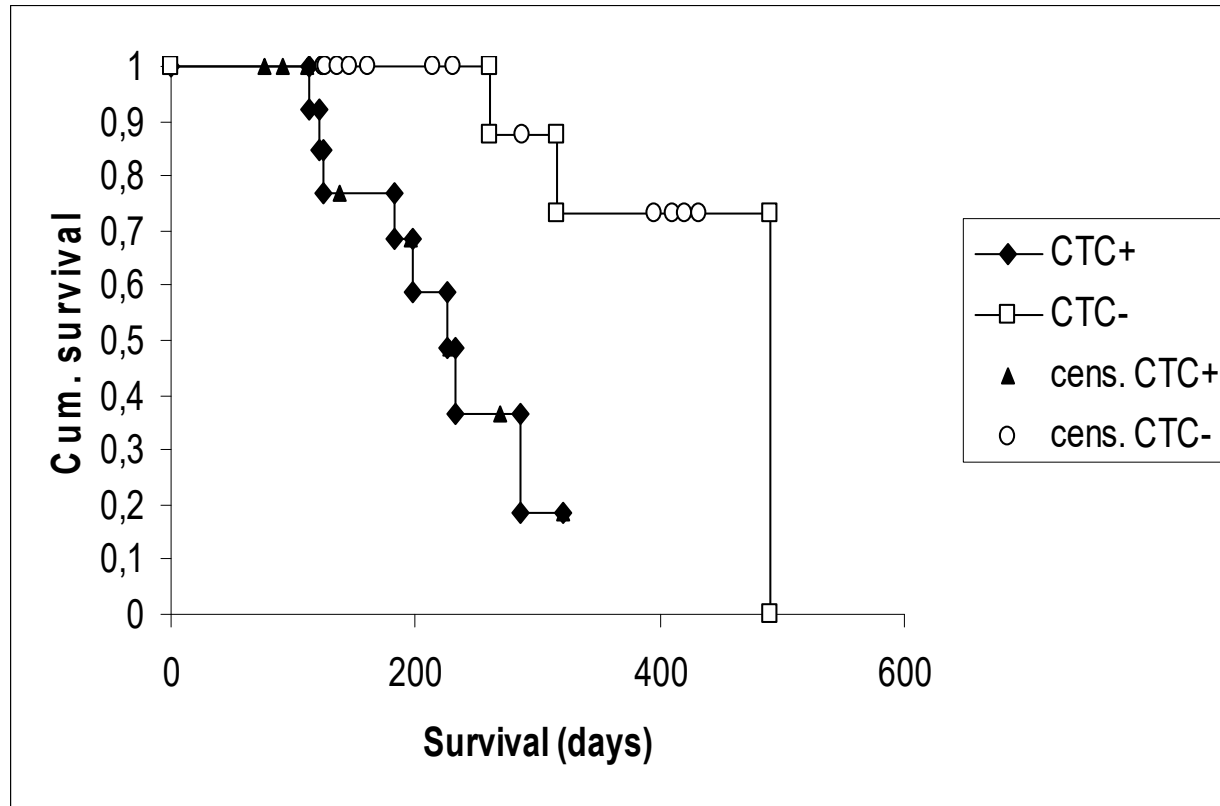


In lung cancer, the AdnaTest EMT2 provides additional antibodies against Her2 and EGFR. This doubles performance and choosing the right detection markers increases even more.

Hanssen et al. ; Nature Sci Rep. 2016 Jun 15;6:28010.



CTCs in Breast Cancer



Tewes et al., Breast Cancer Res Treat, 115(3) p581-90, 2009

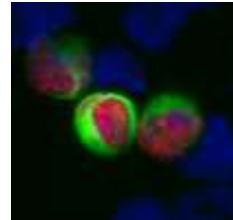
QIAGEN AdnaTest

Metastatic Tissue



=

CTC

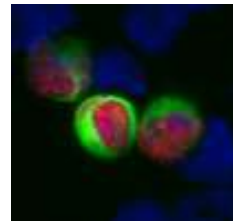


P=0,04

CellSearch

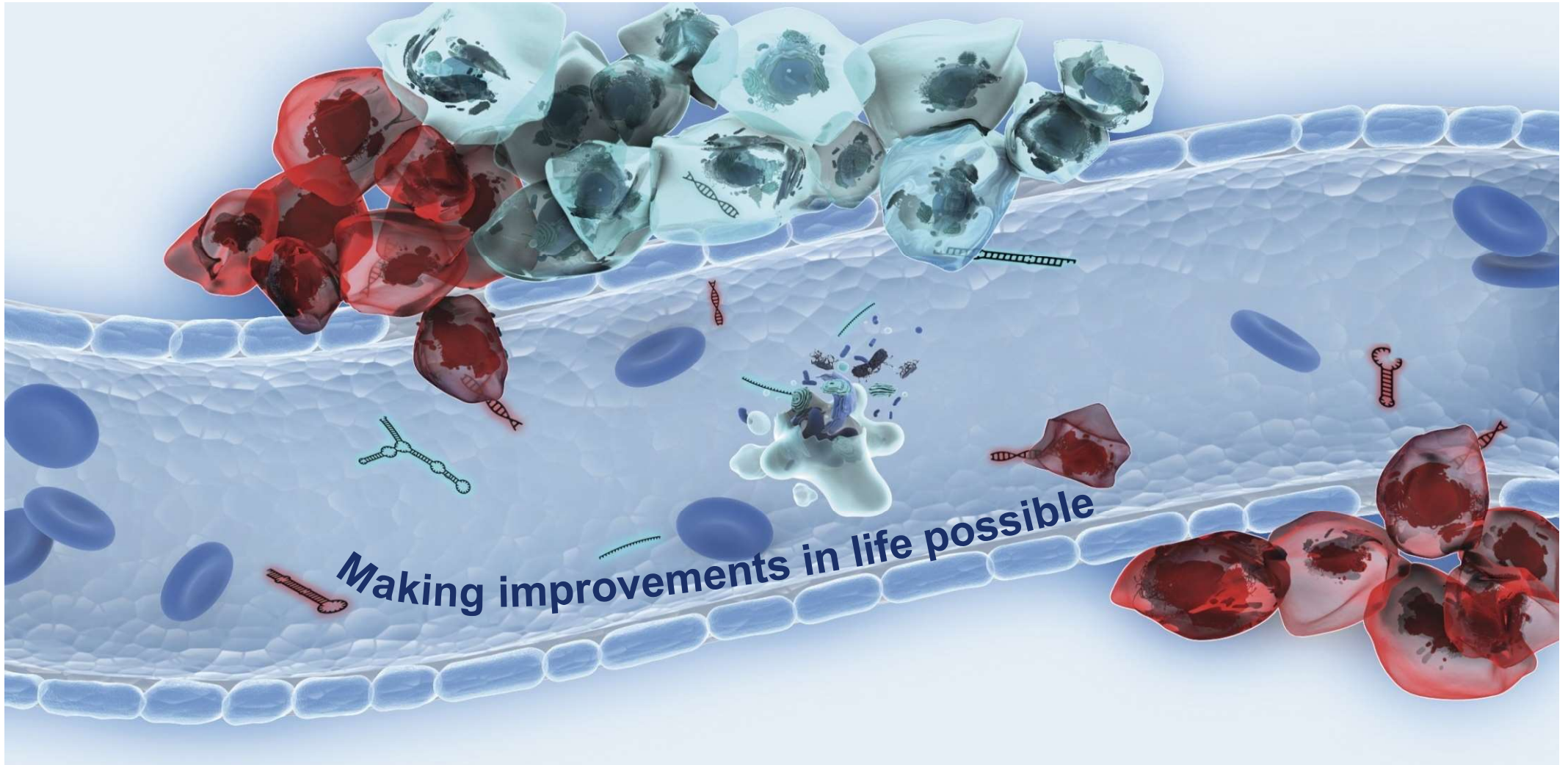


≠



P=0,9

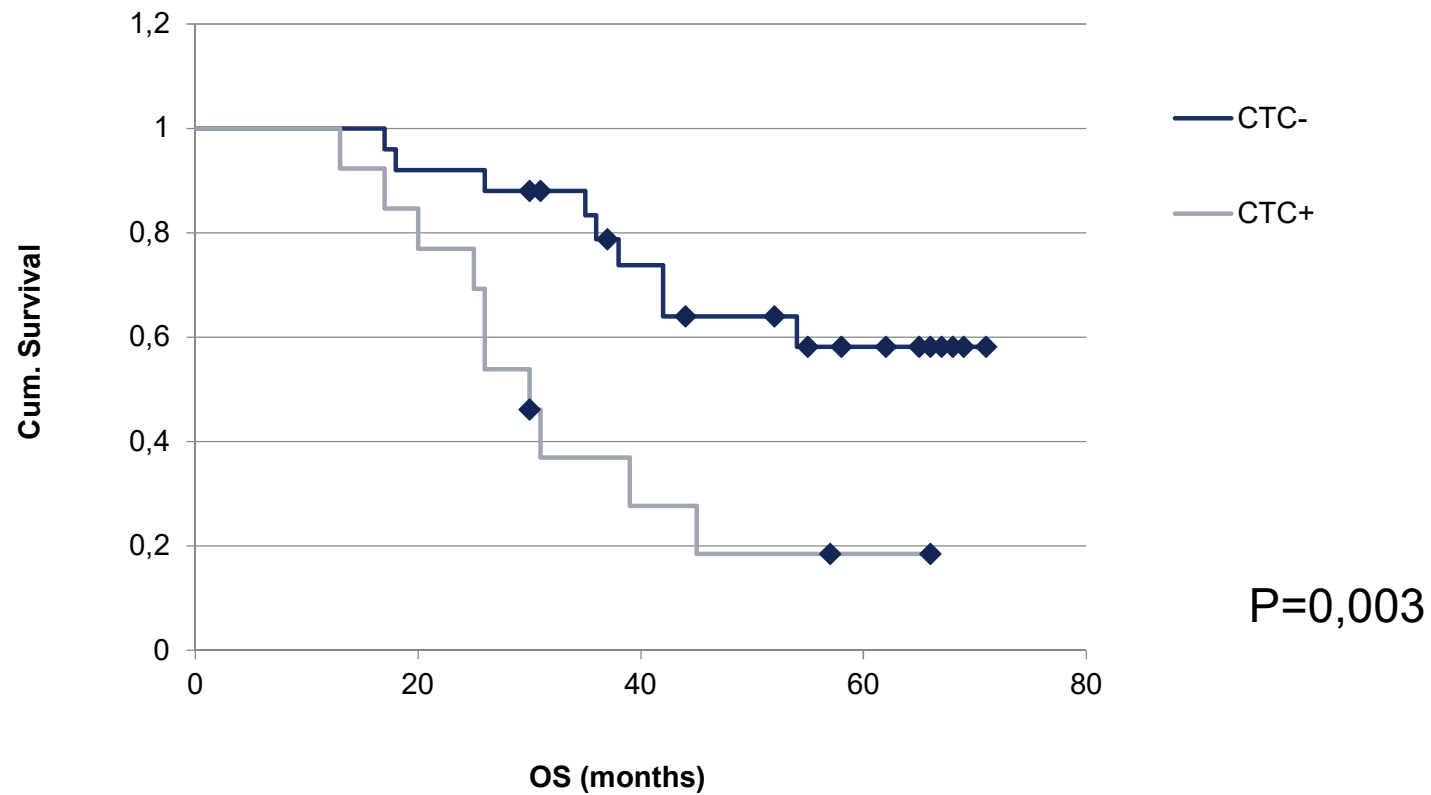
Aktas et al., BMC Cancer 2016



CTCs in Ovarian Cancer

Prognostic impact for overall survival

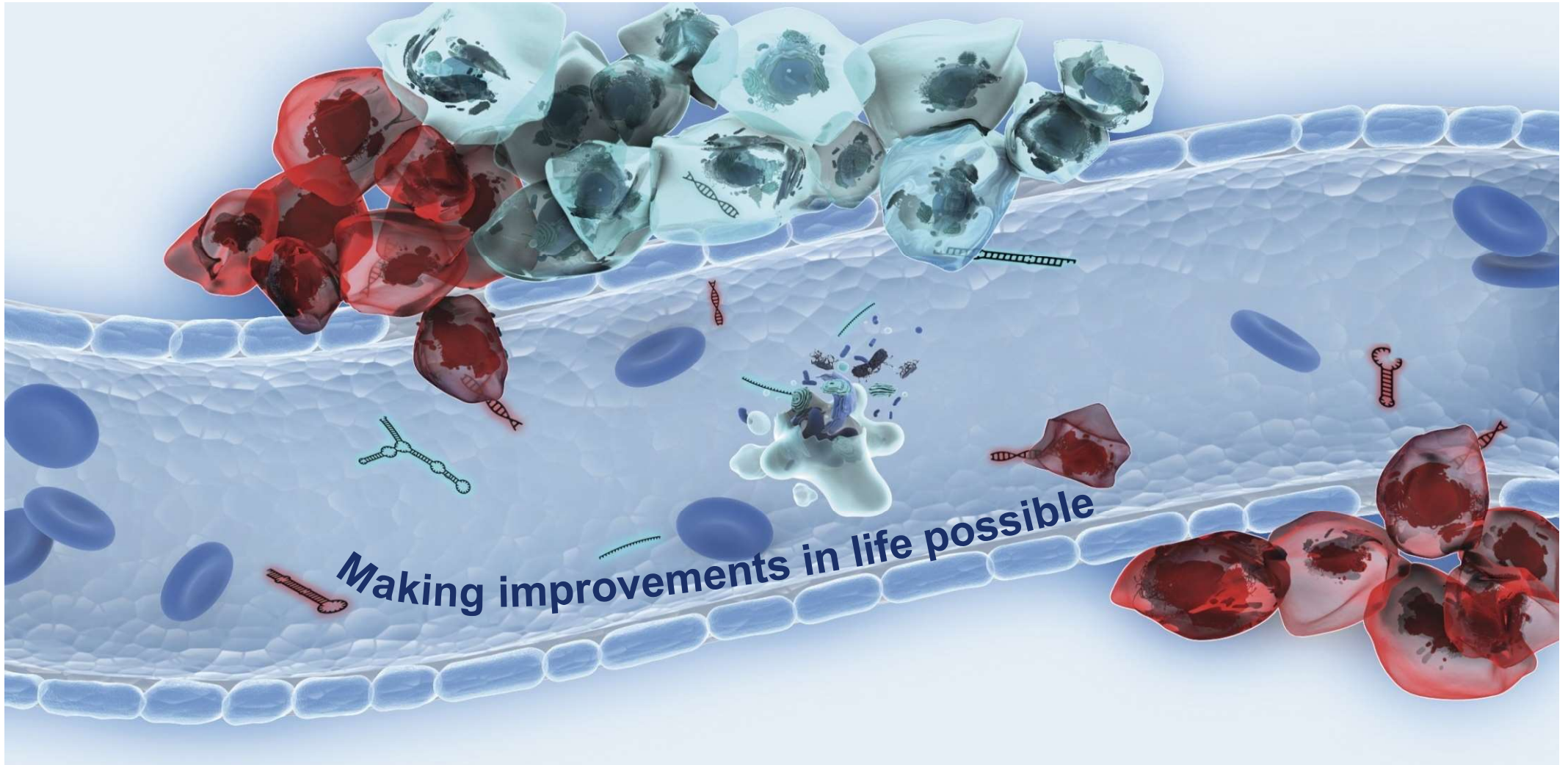
CTC in Ovarian Cancer before or after surgery



Kuhlmann et al., Clin. Chem., 2014

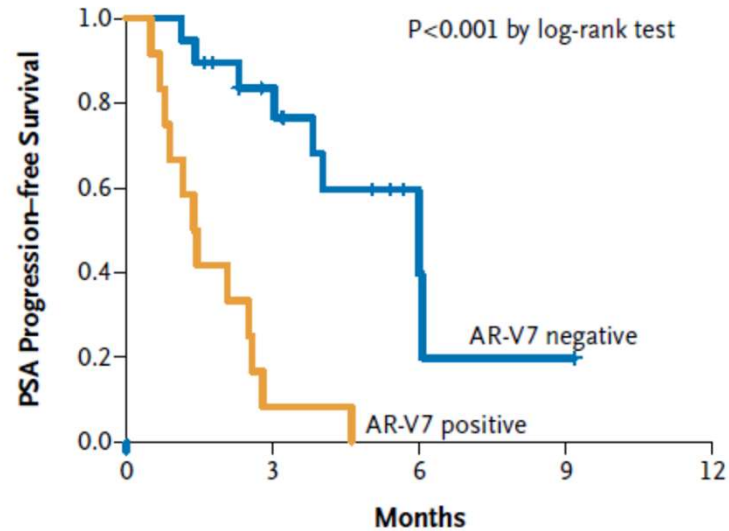
Cox Regression	Cox-Regression: DFS			log. Regression Platinum resistance		
	Independent	HR	95%-CI	Independent	HR	95%-CI
pT	<0,0005	11	3,7-30,1	*	*	*
pN	*	*	*	*	*	*
M	<0,0005	4	1,9-7,9	0.001	9.3	2,6-33,5
G	<0,0005	0	0,15-0,57	0.006	0.2	0,07-0,63
RTB	*	*	*	*	*	*
CTC (ERCC1+)	0.007	4	1,4-9,1	0.012	8.7	1,6-46,5

Kuhlmann et al., Clin. Chem., 2014

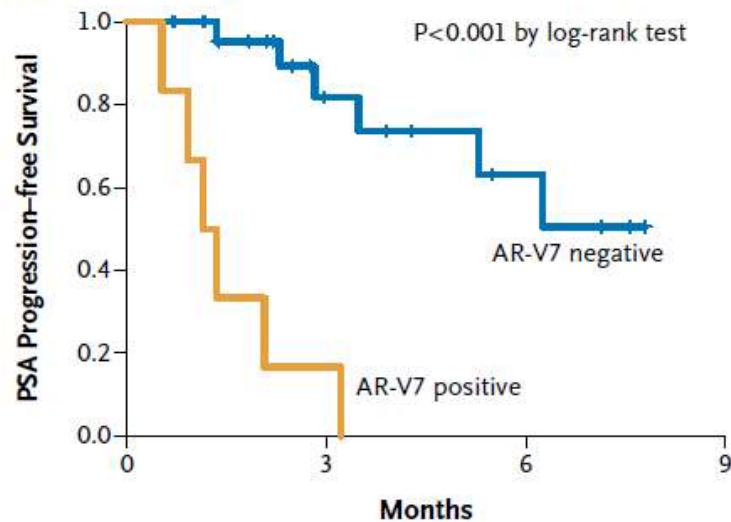


CTCs in Prostate Cancer

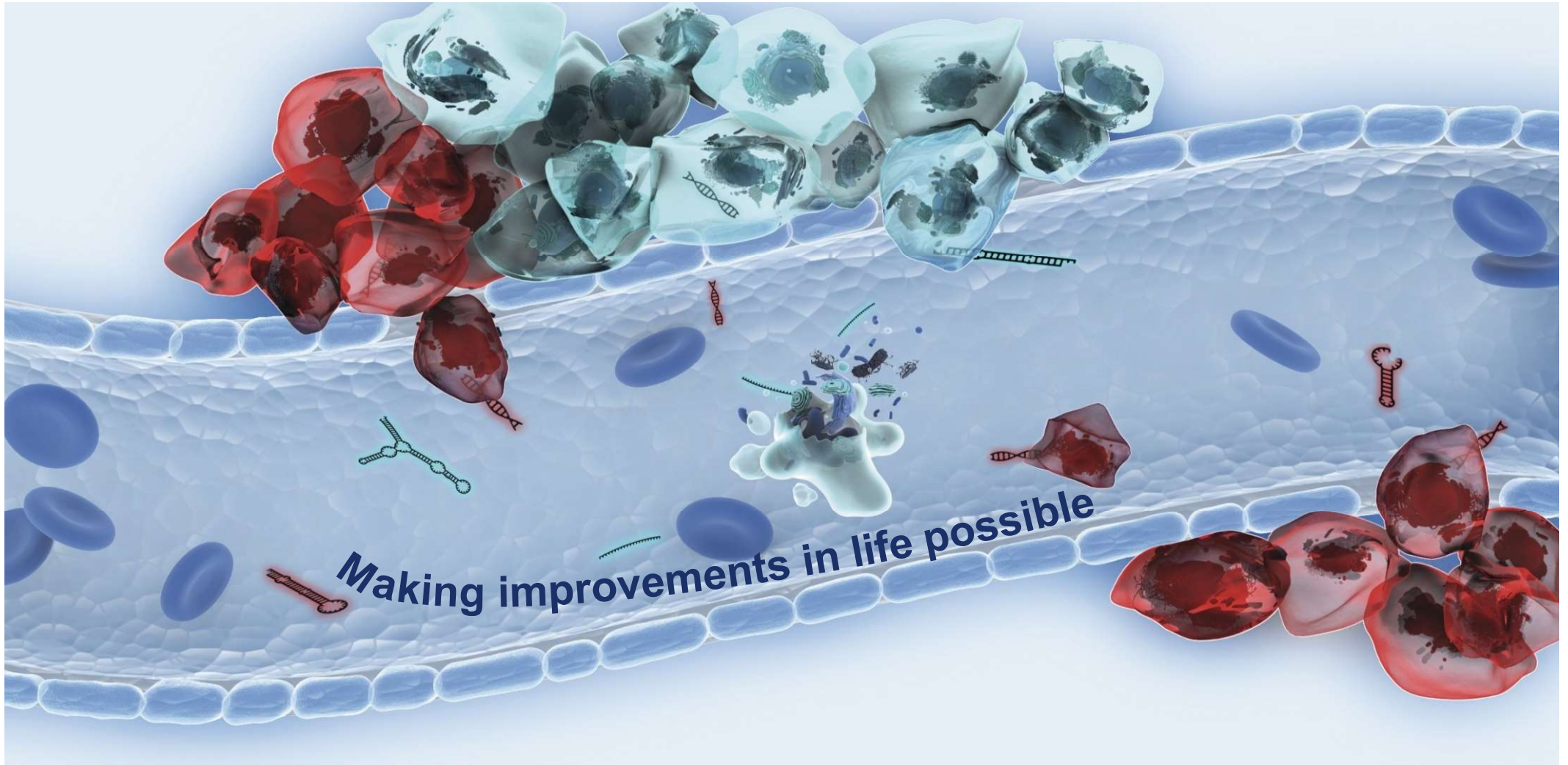
A Enzalutamide-Treated Patients



B Abiraterone-Treated Patients

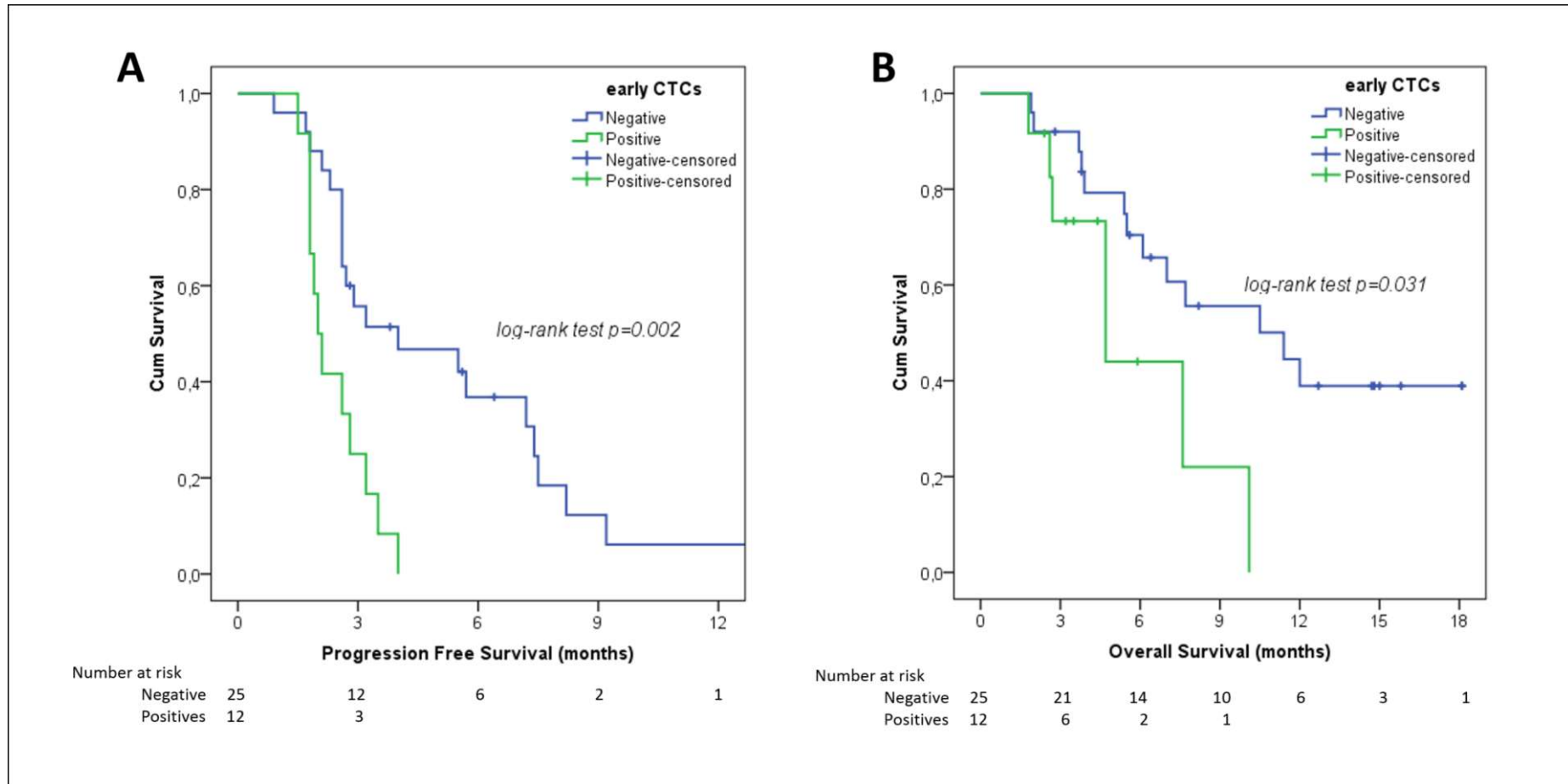


Antonarakis et al.; NEJM 2014, 371(11), 1028ff



CTCs in Colon Cancer

CTCs correlate with therapy failure for Cetuximab or Panitumumab in wt RAS/RAF patients

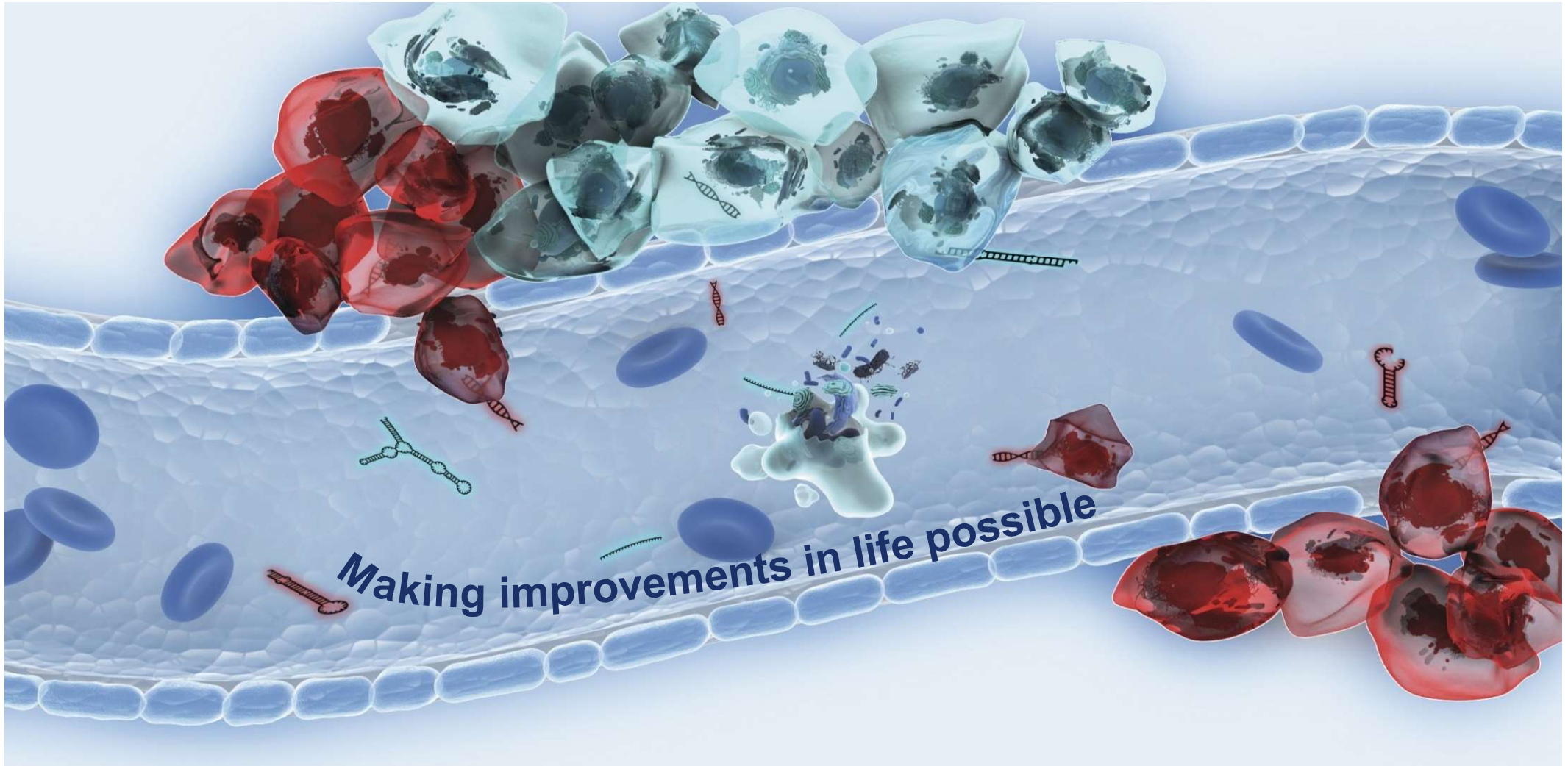


Musella et al.; Int J. Cancer
2015, 137(6), 1467ff

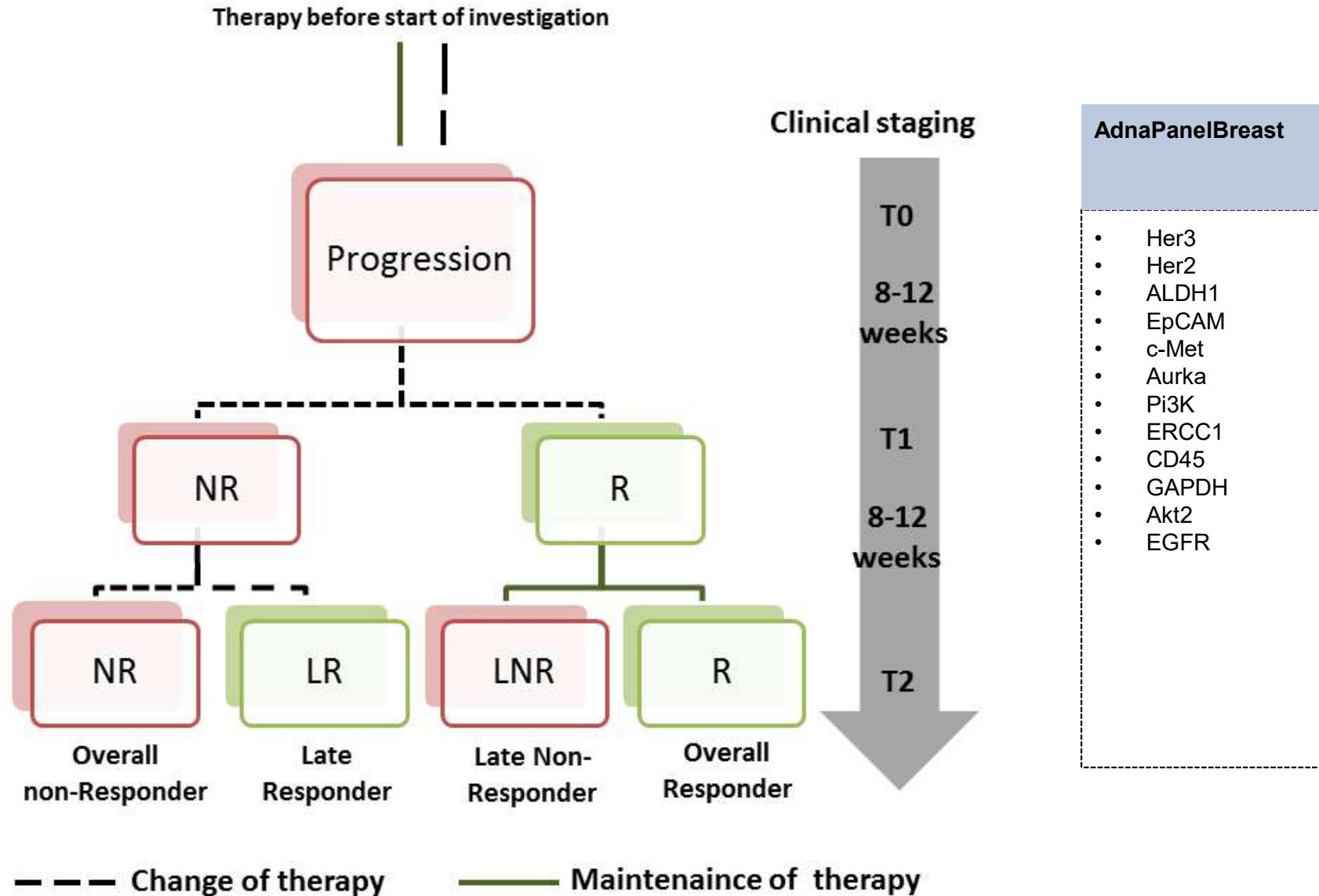
KRAS overexpression vs. Mutation (codon12)

	Kras+	Kras-
Tissue wt	8	8
Tissue mut	0	12
		P=0,0005

Raimondi et al.;Cancer biol.
Ther. 2013, 85(4), 164ff

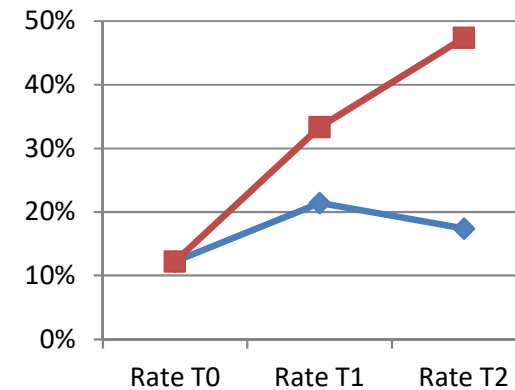
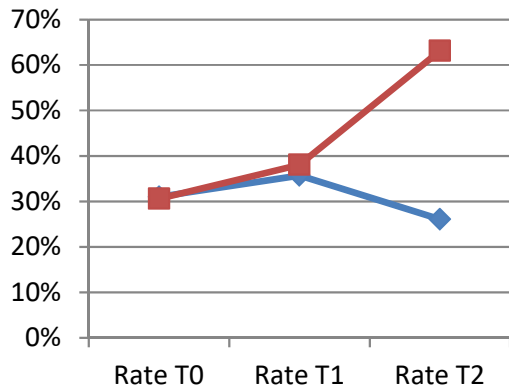
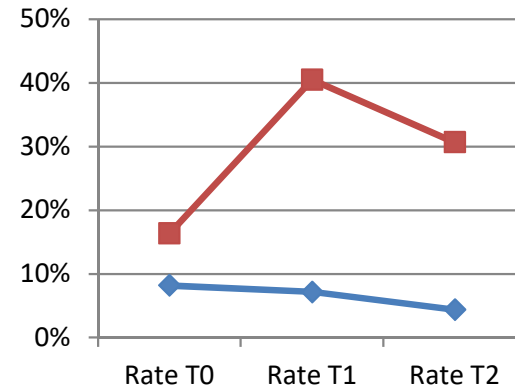
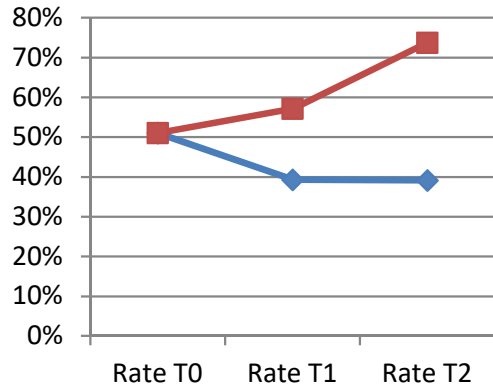


The AdnaPanel Breast



Bredemeier et al.;
Oncotarget 2016

Overexpression frequencies over all time points T0-T2



Bredemeier et al.;
Oncotarget 2016

- Molecular detection of CTC using the AdnaTest proved to be a highly sensitive and specific method for the detection of CTC
- Using the AdnaTest therapy efficacy can be determined early after the beginning of the treatment.
- Molecular characterisation of CTC shows EMT and Tumor Stem Cell related phenotype of CTC and their relation to resistance to conventional therapy
- The AdnaTest is an open platform that enables an intense investigation of CTC characteristics and molecular signal transduction pathways in all major tumor entities