New Diagnostics in personalized Cancer Medicine

FEAM Spring Conference
Bern, May 20th 2016

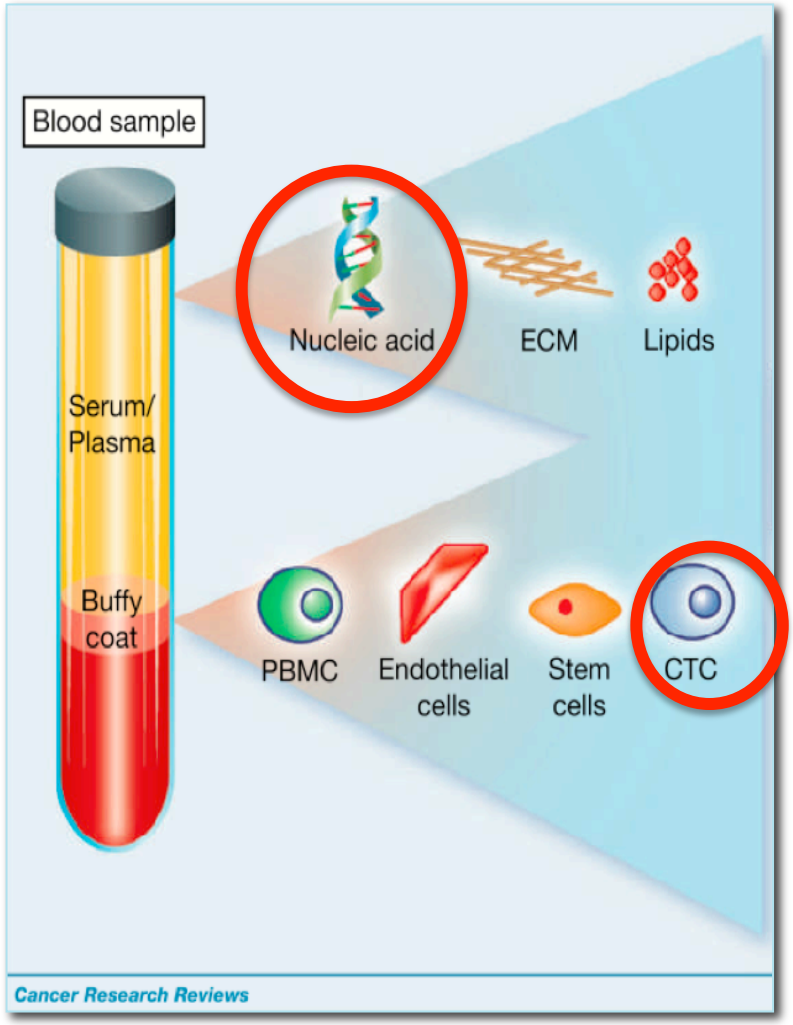
Michael Neumaier
Institute for Clinical Chemistry
Medical Faculty Mannheim
Universität Heidelberg

Present & Future of Tumour Diagnostics: Implications for personalized Medicine

- **Past & Present**
  - **hereditary/familial Tumours**
    - Predisposition by Human Geneticist
  - **primary Tumour Tissue**
    - Histopathology/molecular Pathology
  - **metastasized Disease and Follow-Up**
    - Imaging
    - **Lab Medicine (Serum Tumor Markers)**
    - **Detection of Tissue-specific Expression**
  - **paraneoplastic Phenomena**

    „non-specific“ Role for detecting Complications:
    e.g. Anemia, Clotting Abnormalities, Hormones .....

- **Future**
  - **primary Diagnosis & Follow-Up with liquid Profiling**
    - **CTC and DTC**
    - **fcDNA (ctDNA,** exosomes, µ-particles**)**
    - **digital PCR**
    - **MPS (CAPP; *iDES*-CAPP)**



Screening & Diagnosis

Companion Diagnostics

Response Prediction

Prognosis

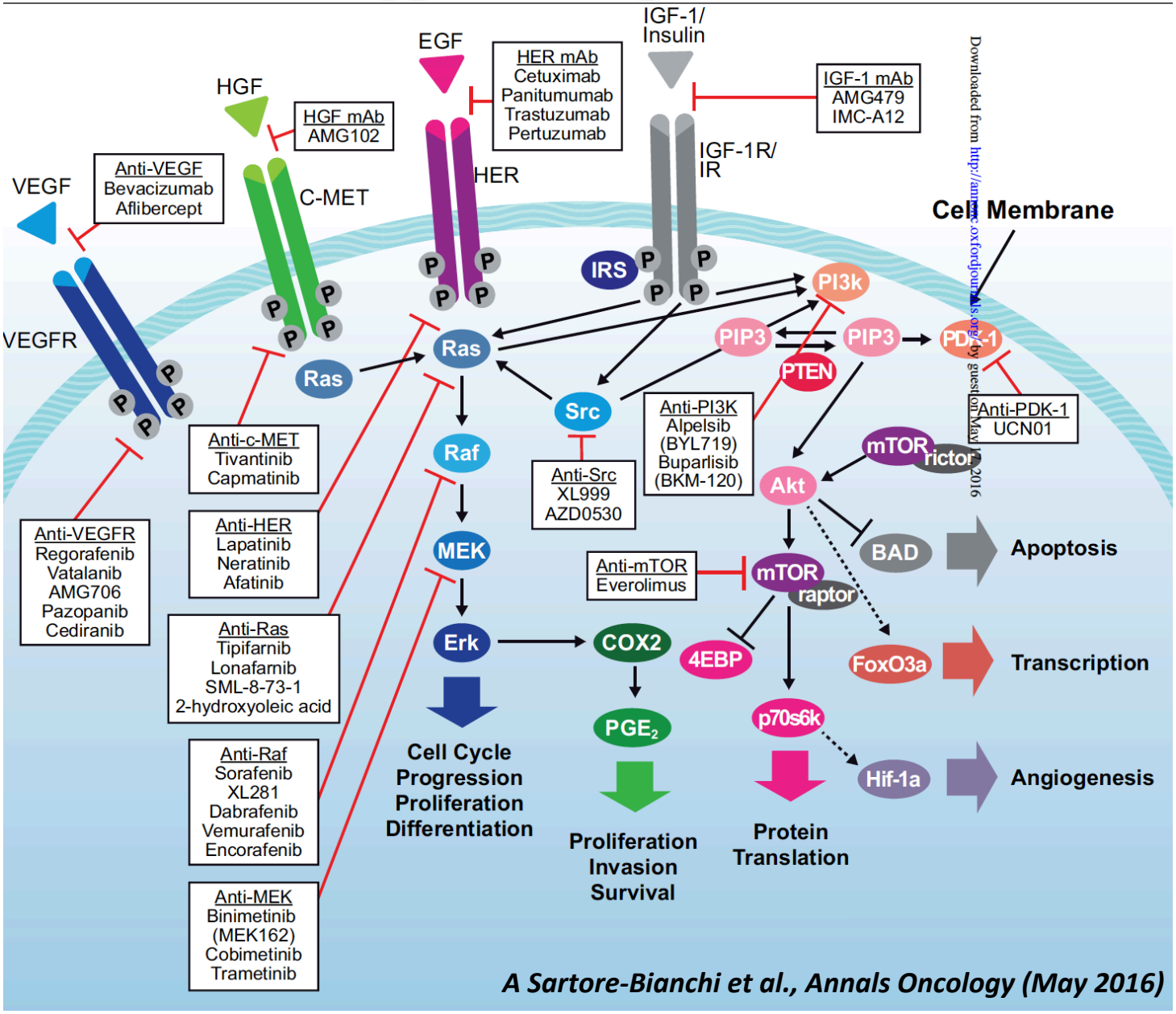
Monitoring Resistence/ Recurrence (MRD)

FDA/EMA-approved Drugs associated with Eligibility testing* (selection)

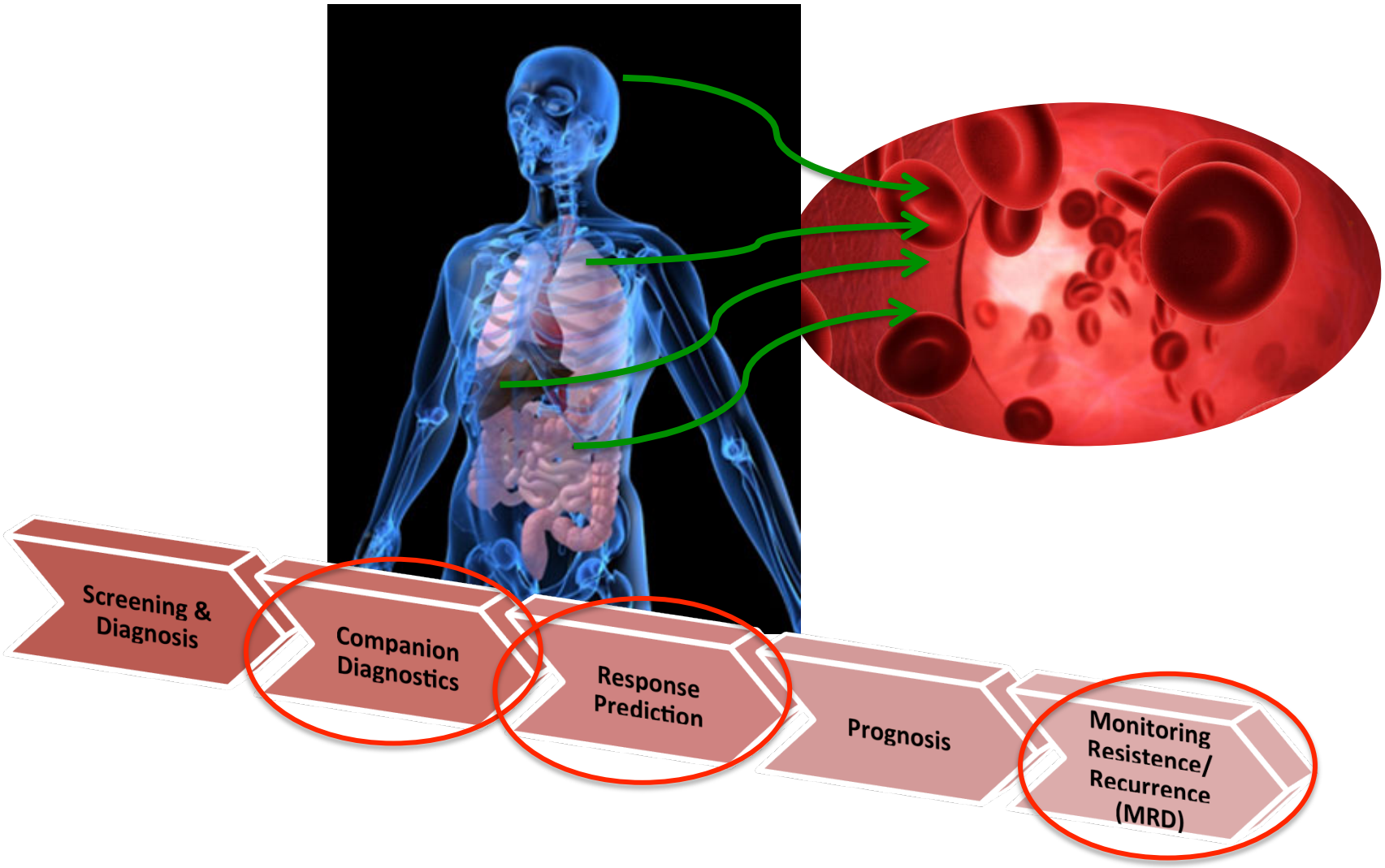
- Trastuzumab/Lapatinib → metastatic breast cancer, overexpression/amplification of ***HER-2***
- Tamoxifen+/- chemo → ER+/HER2 - breast cancer, mutation pattern - multigene assays
- Cetuximab → metastatic colorectal cancer, overexpressing **EGFR/wild-type *KRAS***
- Panitumumab → colorectal cancer with **wild-type *KRAS*** (mutation excluded)
- Nimotuzumab → metastatic colorectal cancer (still experimental)
- Gefitinib → non-small cell lung cancer with **mutated *EGFR***
- Erlotinib → non-small cell lung cancer with **mutated *EGFR***
- Crizotinib → non-small cell lung cancer with **mutated EML4-ALK**
- Vemurafenib (PX4032) → malignant melanoma with **mutated B-RAF**
- Olaparib (Lynparza) → ovarial cancer (Platin-sens., high grade) with **mutated BRCA1/BRCA2**
- Gemtuzumab-Ozogamicin → AML with **CD33** (> 60 yrs.), mal. melanoma
- Imatinib → CML, ***bcr/abl*–positive** (activated PK),
- Imatinib → GIST with activated **c-kit receptor tyrosine kinase/CD117, exon 9 mut**
- Rituximab (+ CHOP), Y90-Ibritumomab, I131-Tositumomab → NH Lymphoma with **CD20**

*Strongly suggested by FDA's Drug-Diagnostic Co-Development Initiative

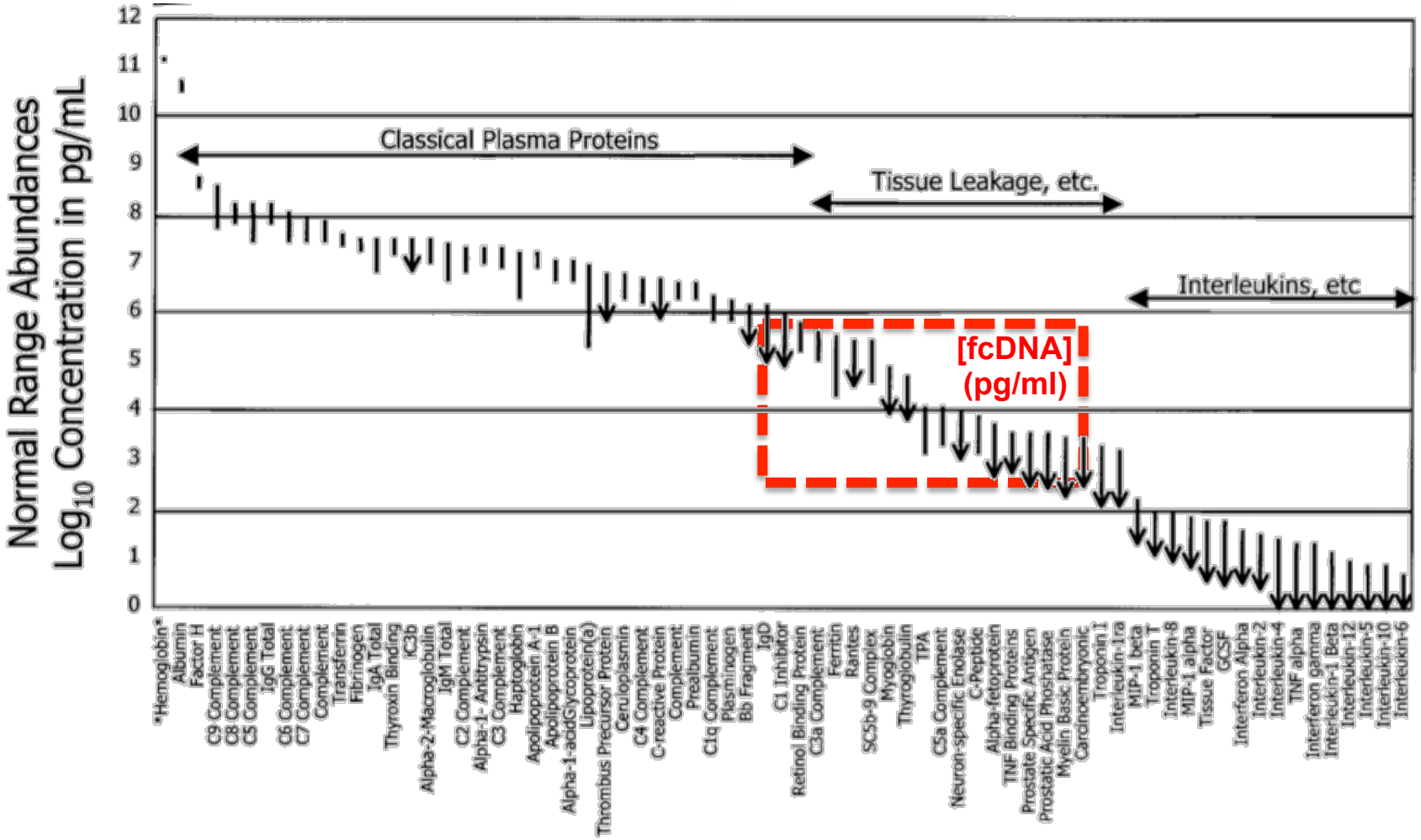
Selection of druggable Targets in metastatic CRC



finally „actionable Health Information“ through Dx



Concentration of Biomolecules in human Plasma: Proteome, fcDNA and ctDNA



mod. acc: N.L. Anderson & N.G. Anderson Mol Cell Proteomics 2002

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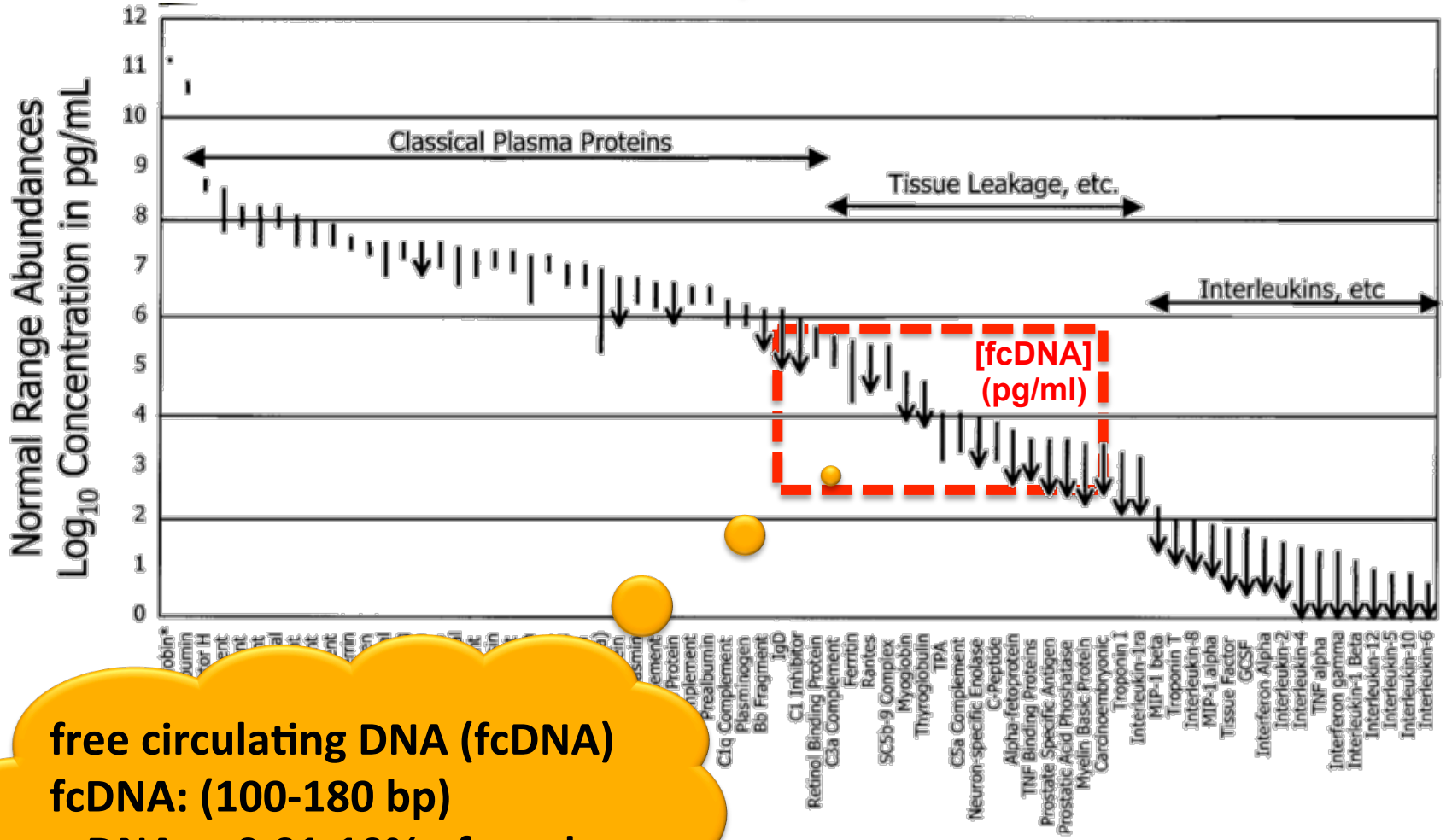

free circulating DNA (fcDNA)
fcDNA: (100-180 bp)
ctDNA: ~ 0.01-10% of total

mod. acc: N.L. Anderson & N.G. Anderson Mol Cell Proteomics 2002


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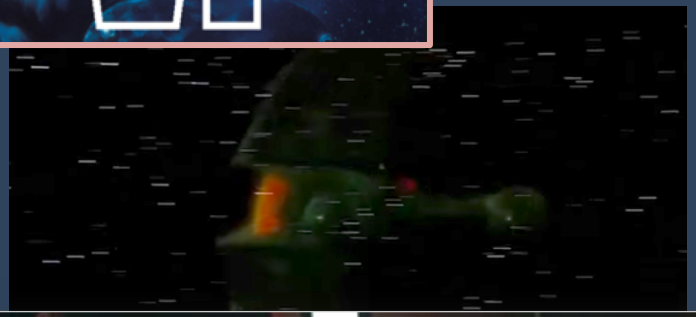
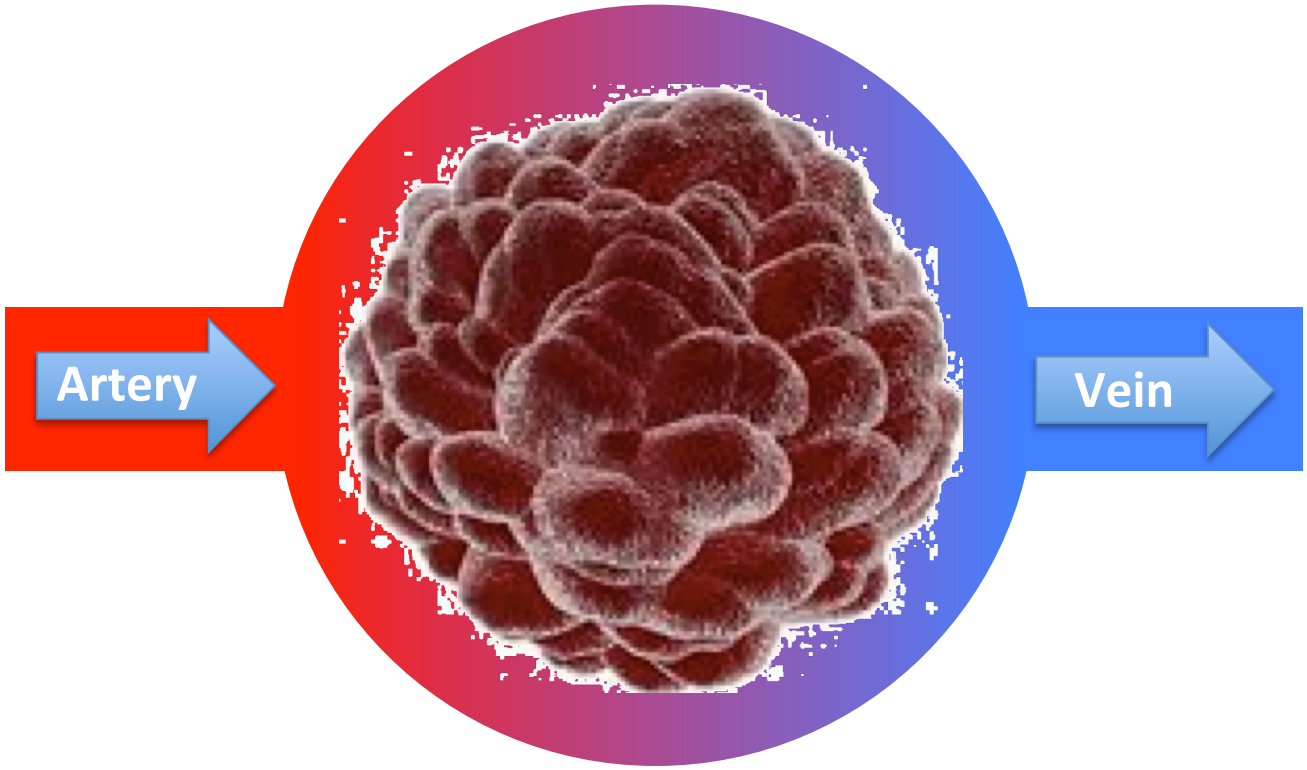
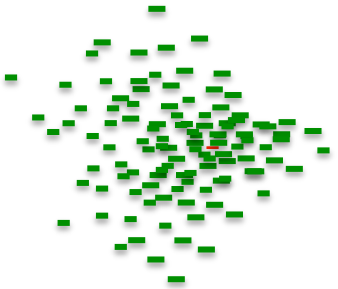
DAS UNENTDECKTE LAND



the Vein is the Tumour´s „Exhaust Pipe“





ctDNA [ng/ml]

and other molecules

the seminal Techniques: digital PCR & Emulsion PCR

DETECTION OF *APC* MUTATIONS IN FECAL DNA FROM PATIENTS WITH COLORECTAL TUMORS

GIOVANNI TRAVERSO, B.A., ANTHONY SHUBER, M.S., BERNARD LEVIN, M.D., CONSTANCE JOHNSON, R.N., M.S., LOUISE OLSSON, M.D., DAVID J. SCHOETZ, JR., M.D., STANLEY R. HAMILTON, M.D., KEVIN BOYNTON, B.S., KENNETH W. KINZLER, PH.D., AND BERT VOGELSTEIN, M.D.

NEJM (2002)

limiting Dilution of Preamplicons

Emulsions-PCR (dPCR)

high Sensitivity ASO (BEAMing)

NGS

Genome sequencing in microfabricated high-density picolitre reactors

Marcel Margulies[1]*, Michael Egholm[1]*, William E. Altman[1], Said Attiya[1], Joel S. Bader[1], Lisa A. Bemben[1], Jan Berka[1], Michael S. Braverman[1], Yi-Ju Chen[1], Zhoutao Chen[1], Scott B. Dewell[1], Lei Du[1], Joseph M. Fierro[1], Xavier V. Gomes[1], Brian C. Godwin[1], Wen He[1], Scott Helgesen[1], Chun He Ho[1], Gerard P. Irzyk[1], Szilveszter C. Jando[1], Maria L. I. Alenquer[1], Thomas P. Jarvie[1], Kshama B. Jirage[1], Jong-Bum Kim[1], James R. Knight[1], Janna R. Lanza[1], John H. Leamon[1], Steven M. Lefkowitz[1], Ming Lei[1], Jing Li[1], Kenton L. Lohman[1], Hong Lu[1], Vinod B. Makhijani[1], Keith E. McDade[1], Michael P. McKenna[1], Eugene W. Myers[2], Elizabeth Nickerson[1], John R. Nobile[1], Ramona Plant[1], Bernard P. Puc[1], Michael T. Ronan[1], George T. Roth[1], Gary J. Sarkis[1], Jan Fredrik Simons[1], John W. Simpson[1], Maithreyan Srinivasan[1], Karrie R. Tartaro[1], Alexander Tomasz[3], Kari A. Vogt[1], Greg A. Volkmer[1], Shally H. Wang[1], Yong Wang[1], Michael P. Weiner[4], Pengguang Yu[1], Richard F. Begley[1] & Jonathan M. Rothberg[1]

Nature (2004)

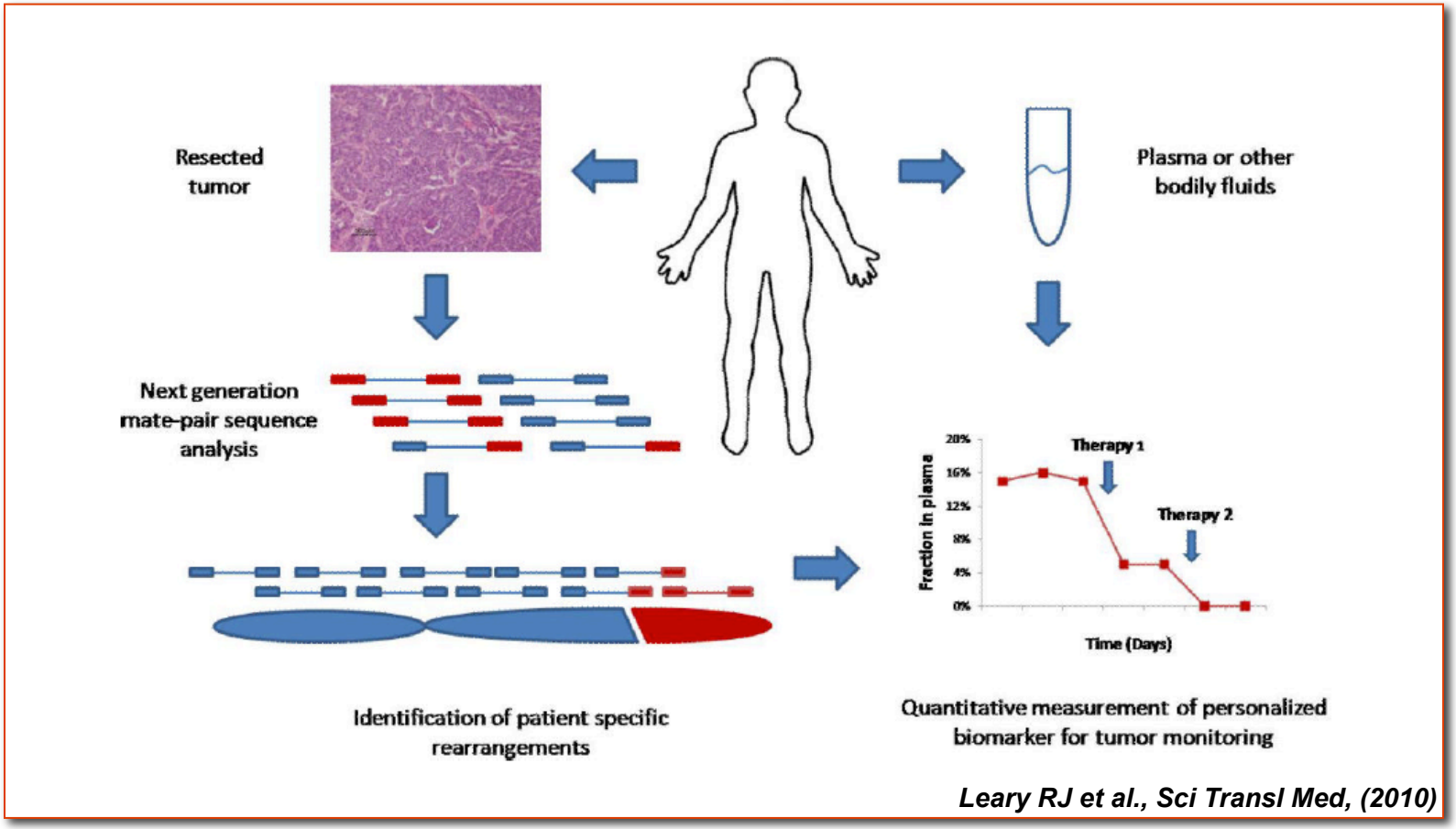
individual vs. personalized Dx Approaches

(Personalized Analysis of Rearranged Ends [PARE])

Histopathology
molecular Pathology

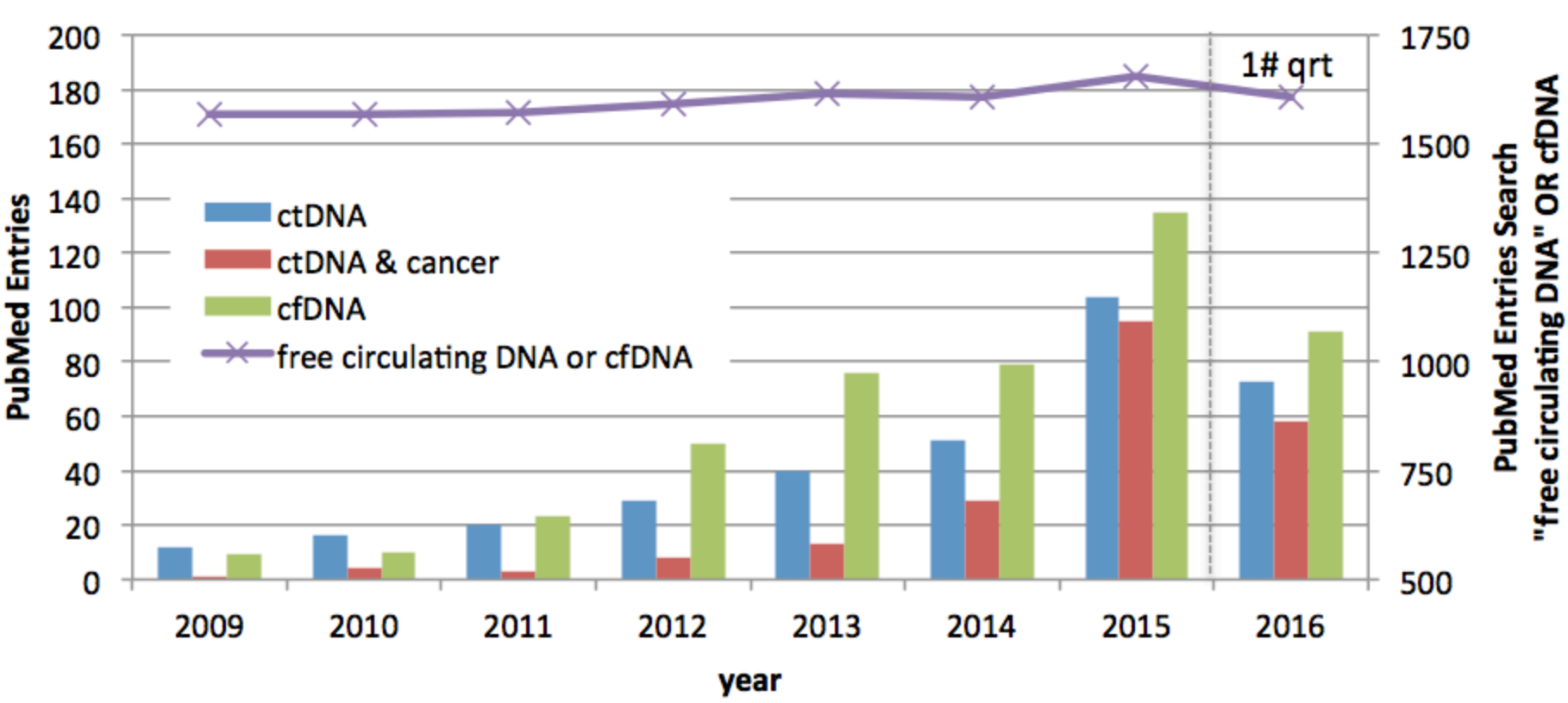
Marker

Clinical Laboratory
monitoring in Plasma



Leary RJ et al., Sci Transl Med, (2010)

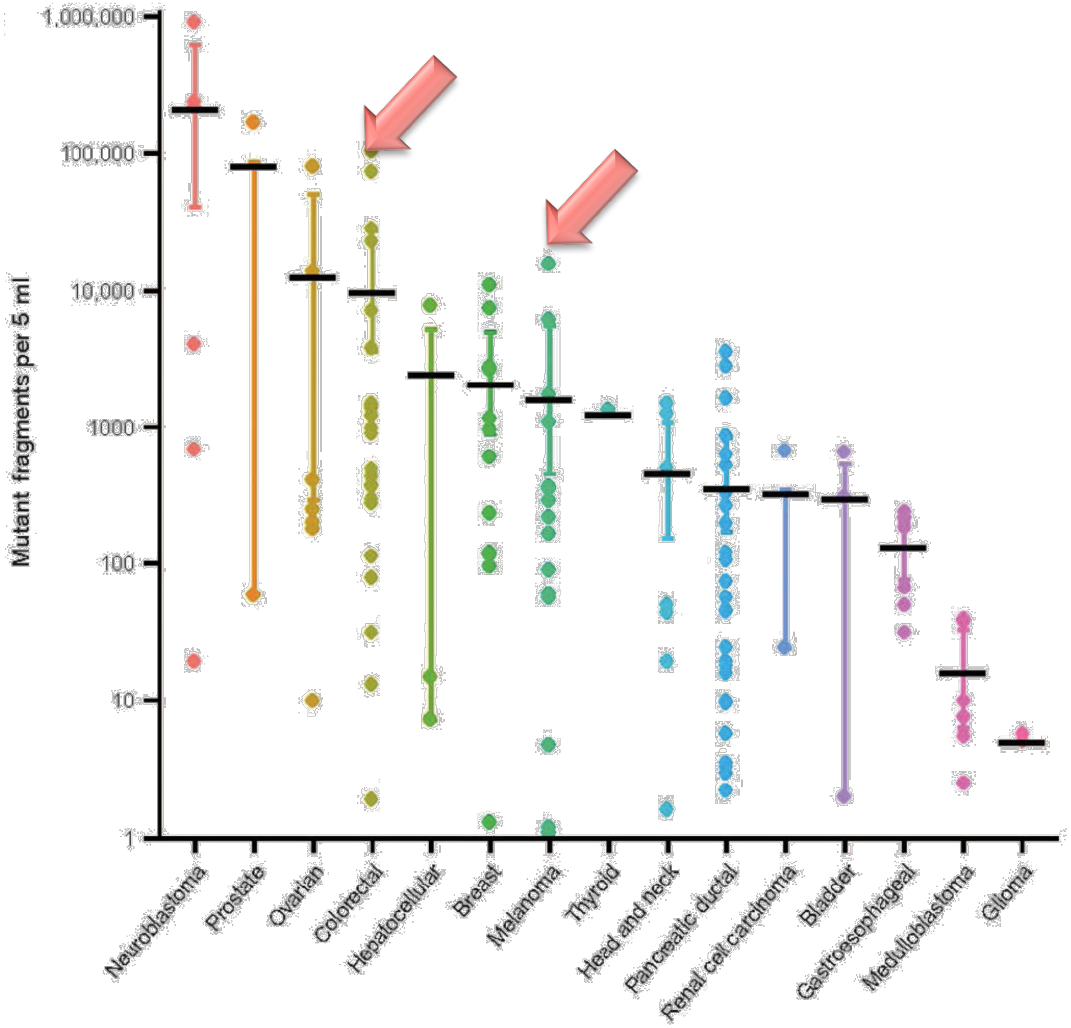
PubMed (5/2016)

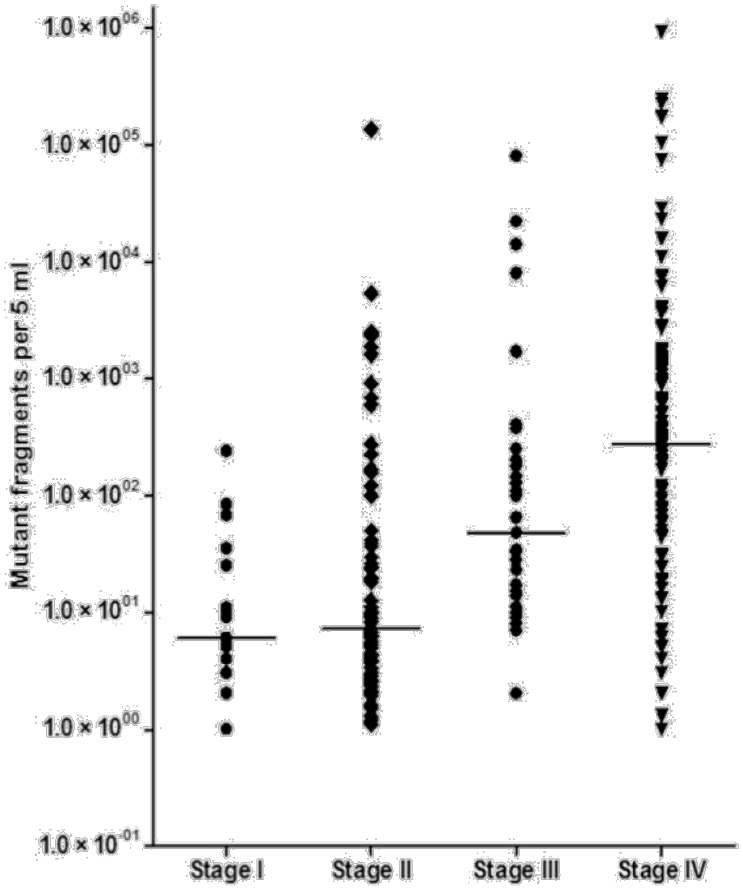


recent Literature (excerpts) on cfDNA/ctDNA

- *Newman A et al., Nature Biotech. (2016)* iDES on CaPPSeq; 37 NSCLC pat.
- *Garcia-Murillas I et al. Sci. Transl. Med. (2015)* Breast Ca; follow-up 55 pat.
- *Morelli MP et al., Ann Oncol. (2015)* selection/resistance after aEGFR treatment
- *Schütz E et al., Clin Chem. (2015)* chromosomal aberration in PCa
- *Bettegowda C et al., Sci. Transl. Med. (2014)* various tumors (n=640) by NGS
- *Newman A et al., Nature Med. (2014)* Cancer Pers. Profil. by Deep Seq. (CaPPSeq)
- Murtaza M et al., Nature (2013) resistance testing by NGS
- *Forshew T et al., Sci. Transl Med (2012)* resistance testing by NGS
- *Punnoose EA et al., Clin. Cancer Res. (2012)* CTC vs. ctDNA in NSCLC
- *Higgins MJ et al., Clin. Cancer Res. (2012)* PIK3CA in blood vs. tissue
- *Holdhoff M et al., Clin.Cancer Res. (2011)* resection margins
- ***Leary RJ et al., Sci. Transl. Med. (2010)*** translocations in solid tumors
- *Li M et al., Nature Meth. (2006)* analytical sensitivity of BEAMing

very low Concentrations of ctDNA: Tumor Type/Tumor Stage related Release



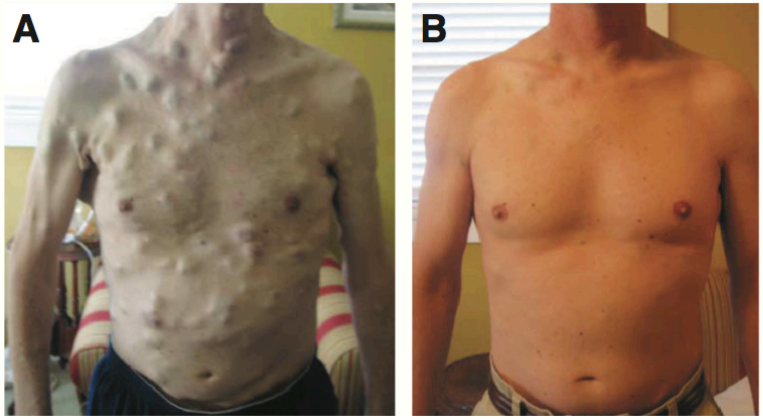


Bettegowda C. et al., SciTransl. Med. (2014)

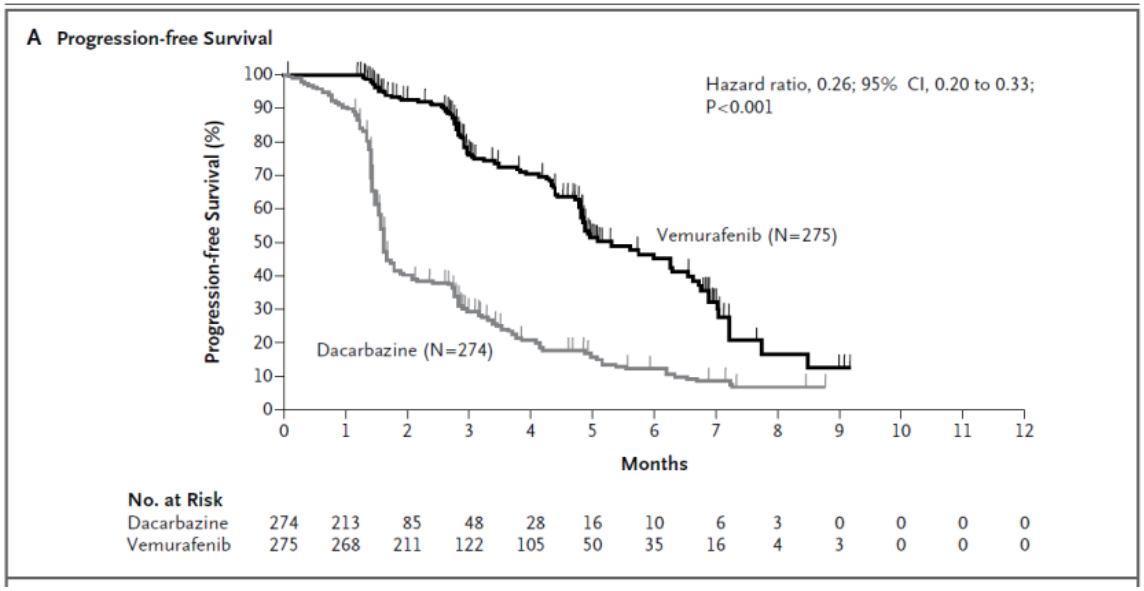
Example: BRAF-Inhibitor Therapy in malignant Melanoma

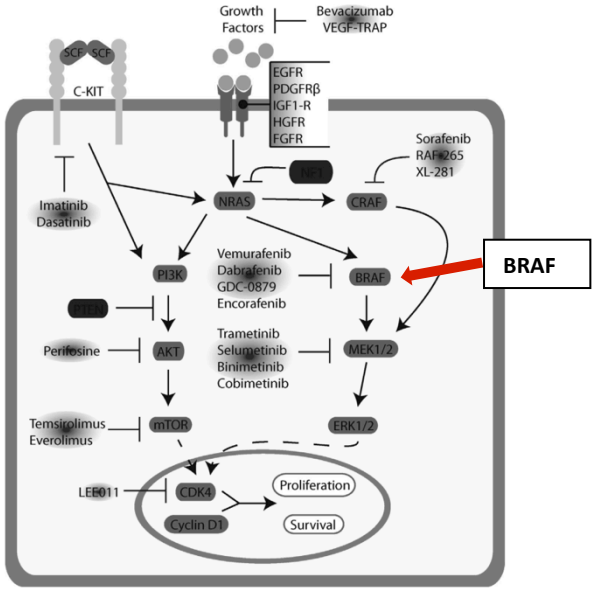
before treatment

15 weeks into treatment



Chapman et al 2011, NEJM
Hirth et al. 2012, Nat Drug Discov
Wagle et al., JCO 2011



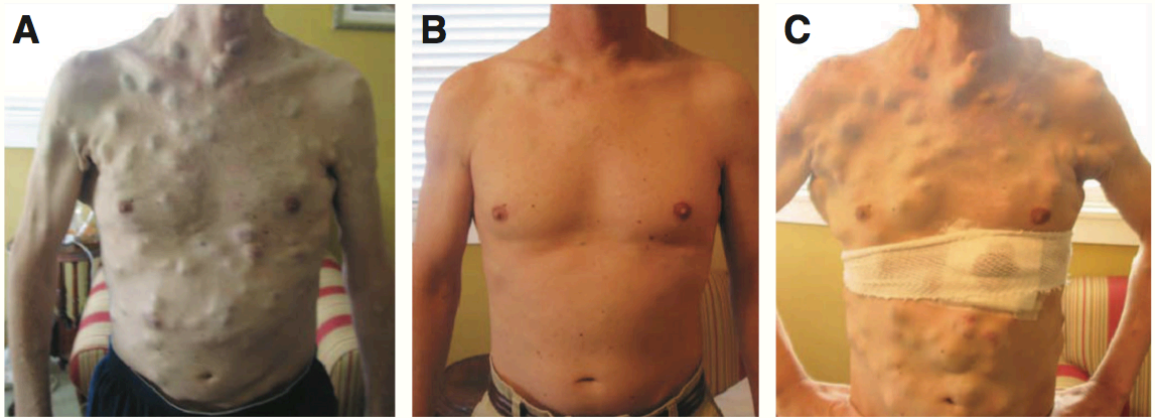


Example: BRAF-Inhibitor Therapy in malignant Melanoma

before treatment

15 weeks into treatment

23 weeks into treatment



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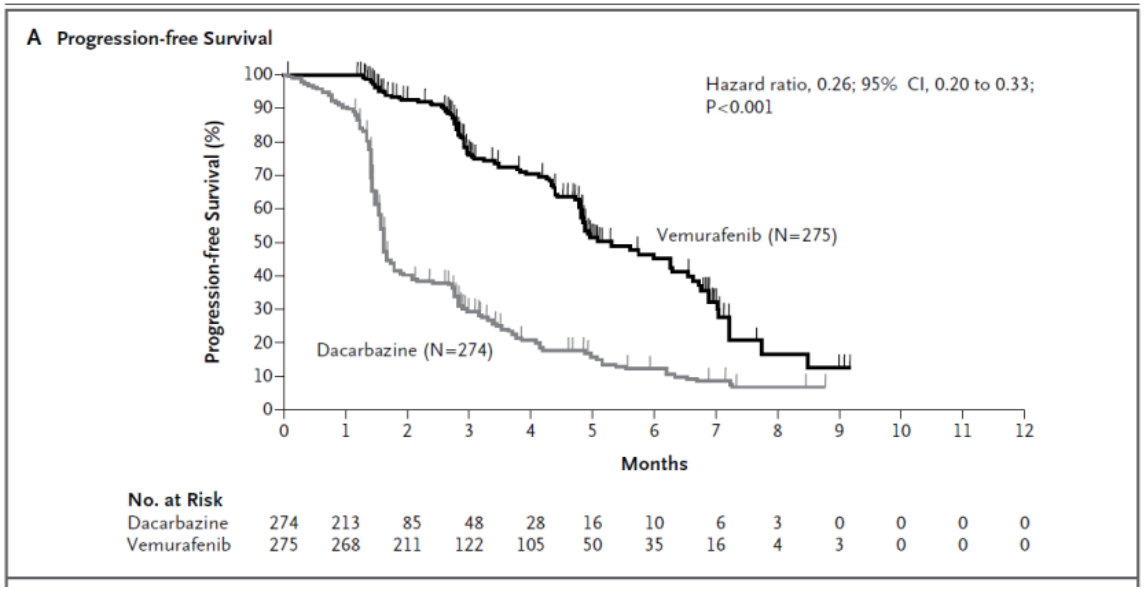
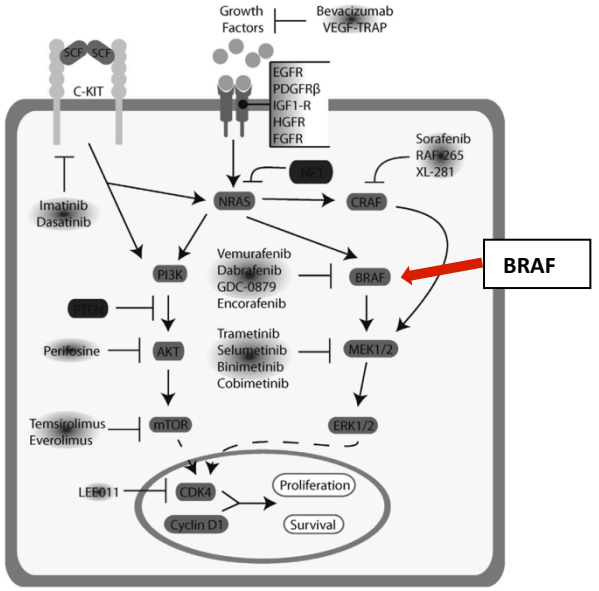
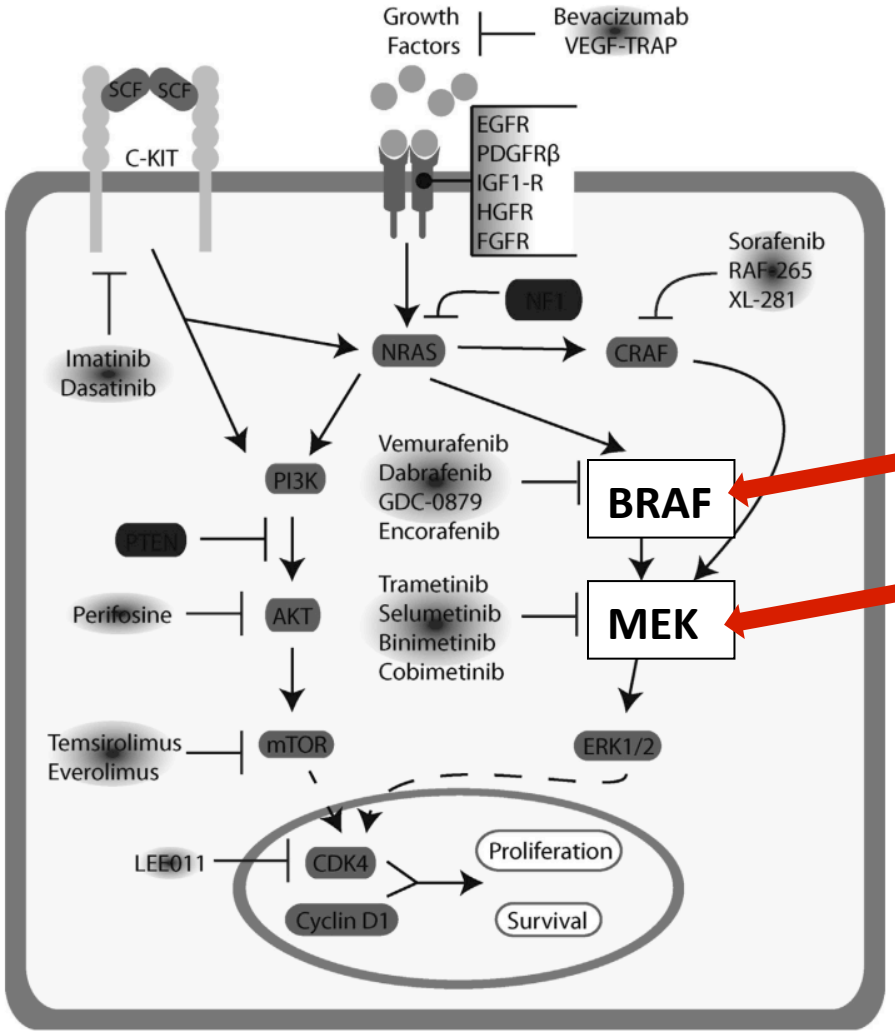




Example: Combination Therapy in malignant Melanoma

Dabrafenib + Trametinib (COMBI-d Study)



Vemurafenib Approval 2012

Cobimetinib Approval 2015

Dabrafenib Approval 2014

Trametinib Approval 2015

Flaherty et al 2012, NEJM
Ribas et al., 2014, Lancet Oncol
Long et al., 2014, NEJM
Larkin et al., 2014, NEJM
Robert et al., 2015, NEJM
Long et al., 2015, Lancet

Example: Combination Therapy in malignant Melanoma

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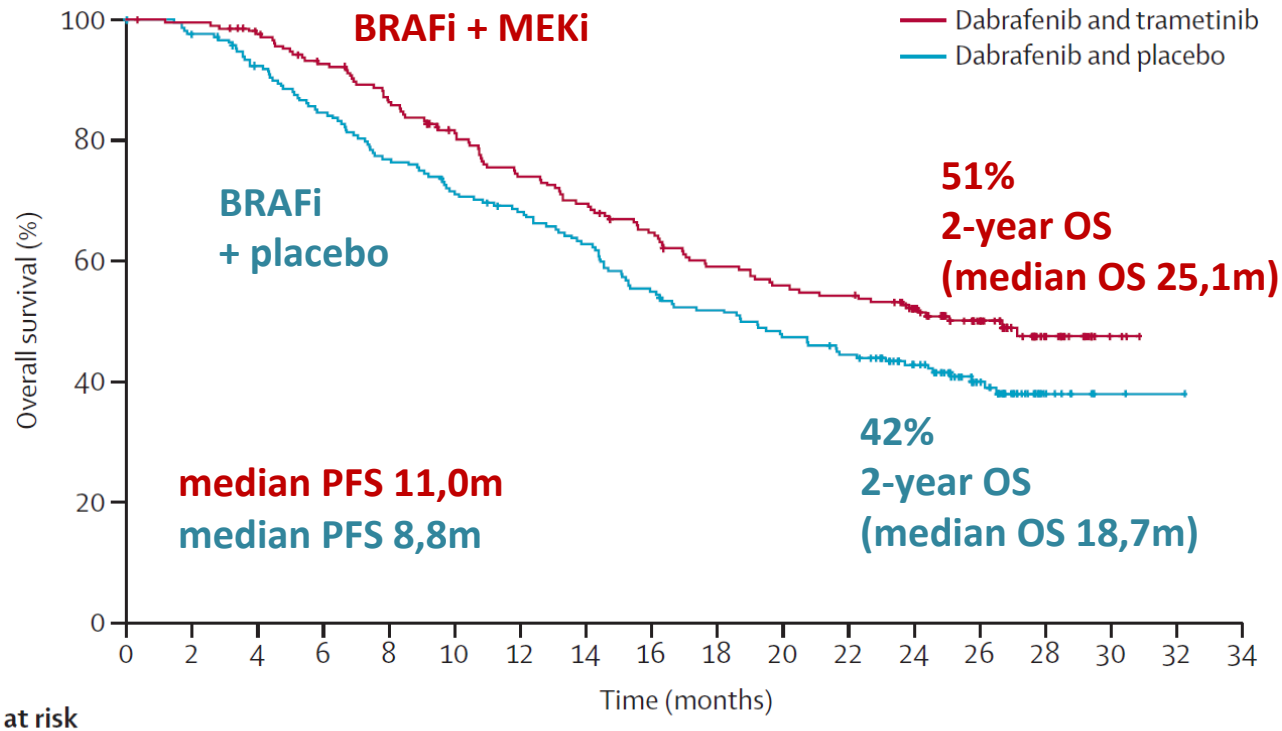
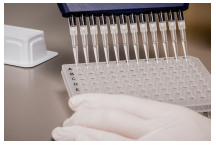


Number at risk

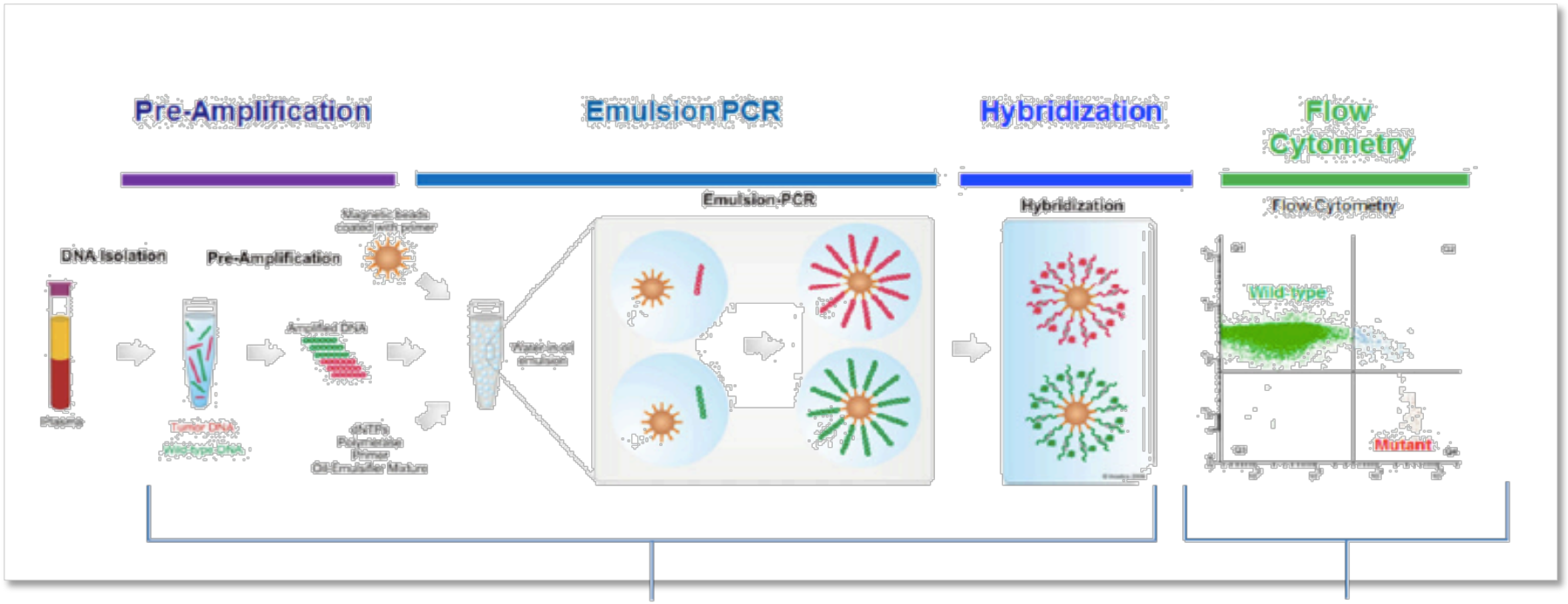
Dabrafenib and trametinib	211	208	200	187	174	159	144	135	124	112	106	103	88	53	21	3	0	0
Dabrafenib and placebo	212	206	191	175	159	147	138	127	111	104	95	88	70	42	10	2	1	0

Long et al., the Lancet (2015)



BEAMing* for metastatic CRC

(34 Mutations in *KRAS* and *NRAS*)



*) Beads Emulsion Amplification Magnetics

sysmex.co.jp

Study 2 (Follow Up; liquid Profiling Melanoma)

- **Total Cohort:**
  - **1,402 samples from 304 Patients (Stage I-IV), time of Analysis between 10/2011 and 6/2014**
  - **Analysis of Concordance: 131 Patients (Stage IIIC to IV) had BEAMing over a Period of 3 Years**

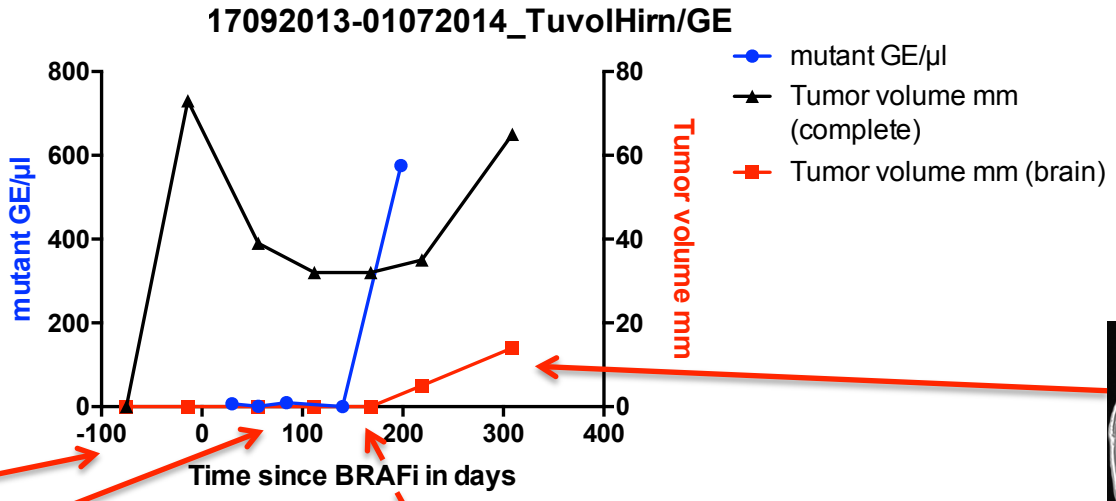
		mol. Pathology (BRAFV600E)		
		Positive	Negative	Total
BEAMing (BRAF V600E)	Positive	48	5*	53
	Negative	3	75	78
	Total	51	80	131

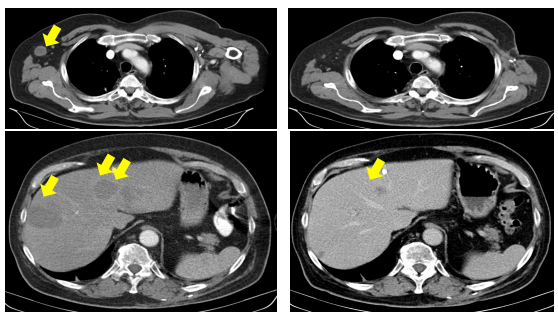
PATH-negs: * 5/10 Patients with secondary *BRAF*-pos. Tumour (e.g. second MM, hairy cell leukemia, CRC); remaining did not receive *BRAF*i Therapy -> genotype conversions or false negs during sampling?

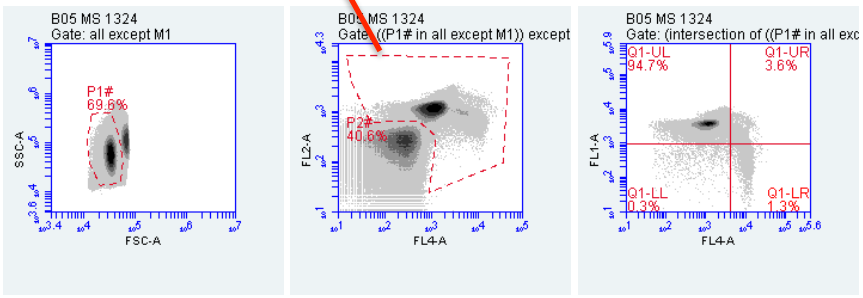
LAB-negs: 2/3 no Response under *BRAF*i Therapy -> false positives
1/3 retested -> true false negative

Haselmann et al., in submission

Study 2 (Follow Up; liquid Profiling Melanoma)



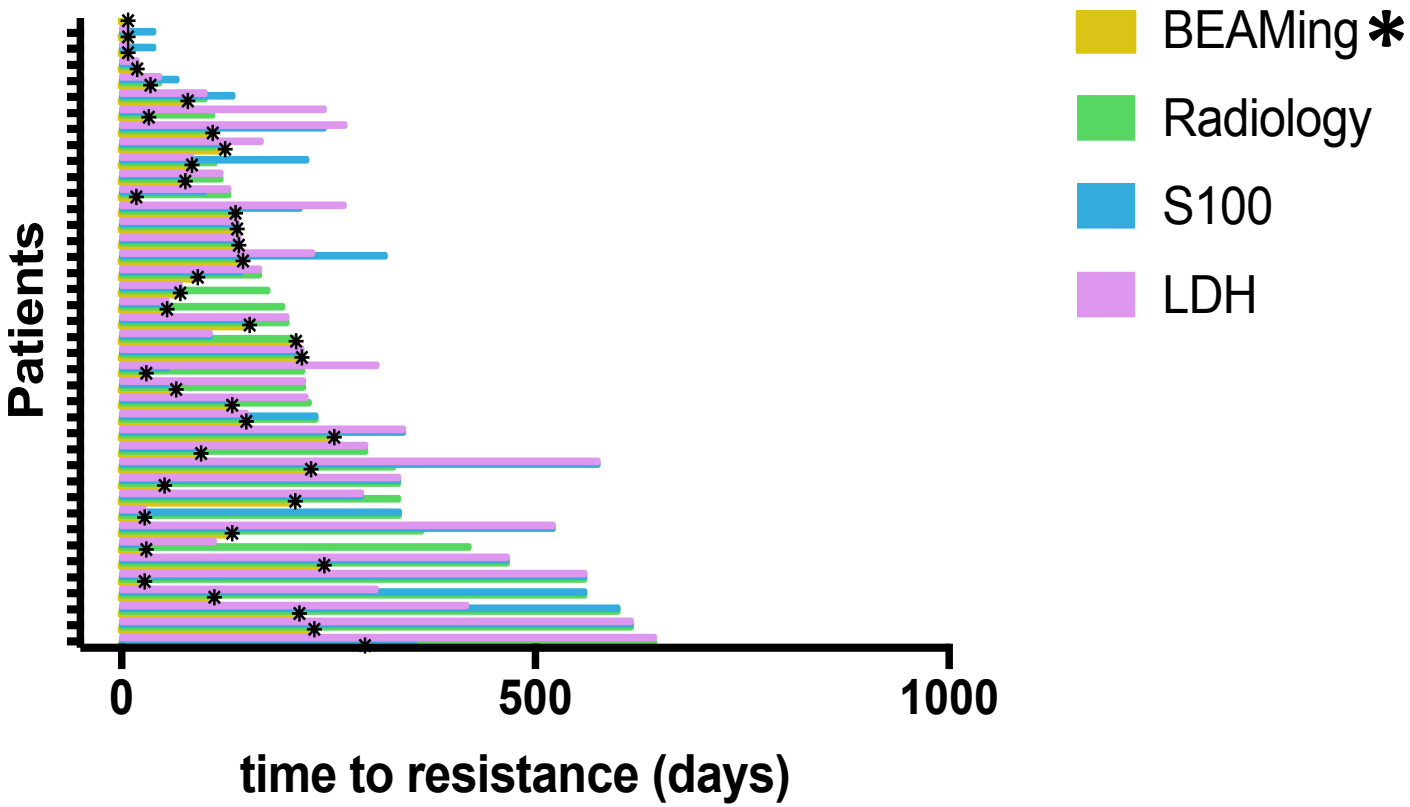




Total Single Beads	Extended Beads	Percent Extended	Wildtyp Beads (Q2)	Mutant Beads (Q4)
651.960	264.909	40,63	250.941	3.457
Sample Name	**Mutant Fraction**			
Plot 1: B05 MS 1324: Gate	1,3589			

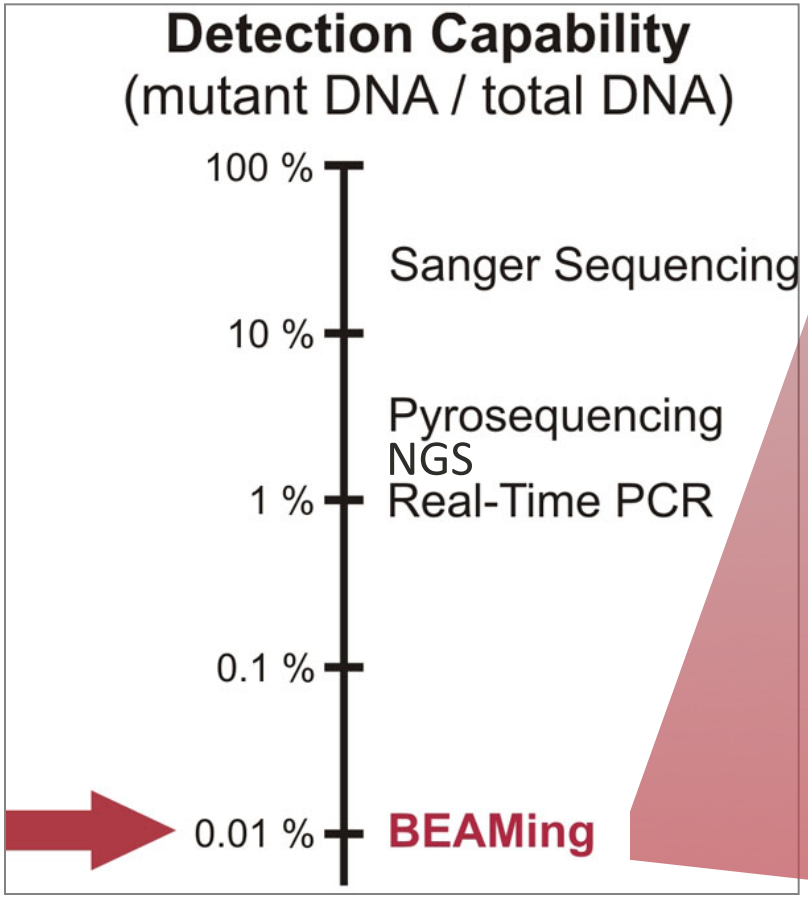
Haselmann et al., in submission

Detecting Tumour Resistance to *BRAF*i Therapy: Time-to-Positivity of Markers



Haselmann et al., in submission

Molecular Profiling of ctDNA for Companion Dx
(LLoD for different Methods)



Quelle: www.inostics.com

KRAS (10) G34A, G34C, G34T, G35A, G35C, G35T, G37T, G38A, A183C, G436A

BRAF (1) T1799A

PIK3CA (8) G1624A, G1633A, A1634G, C1636A, G3129T, C3139T, A3140G, A3140T

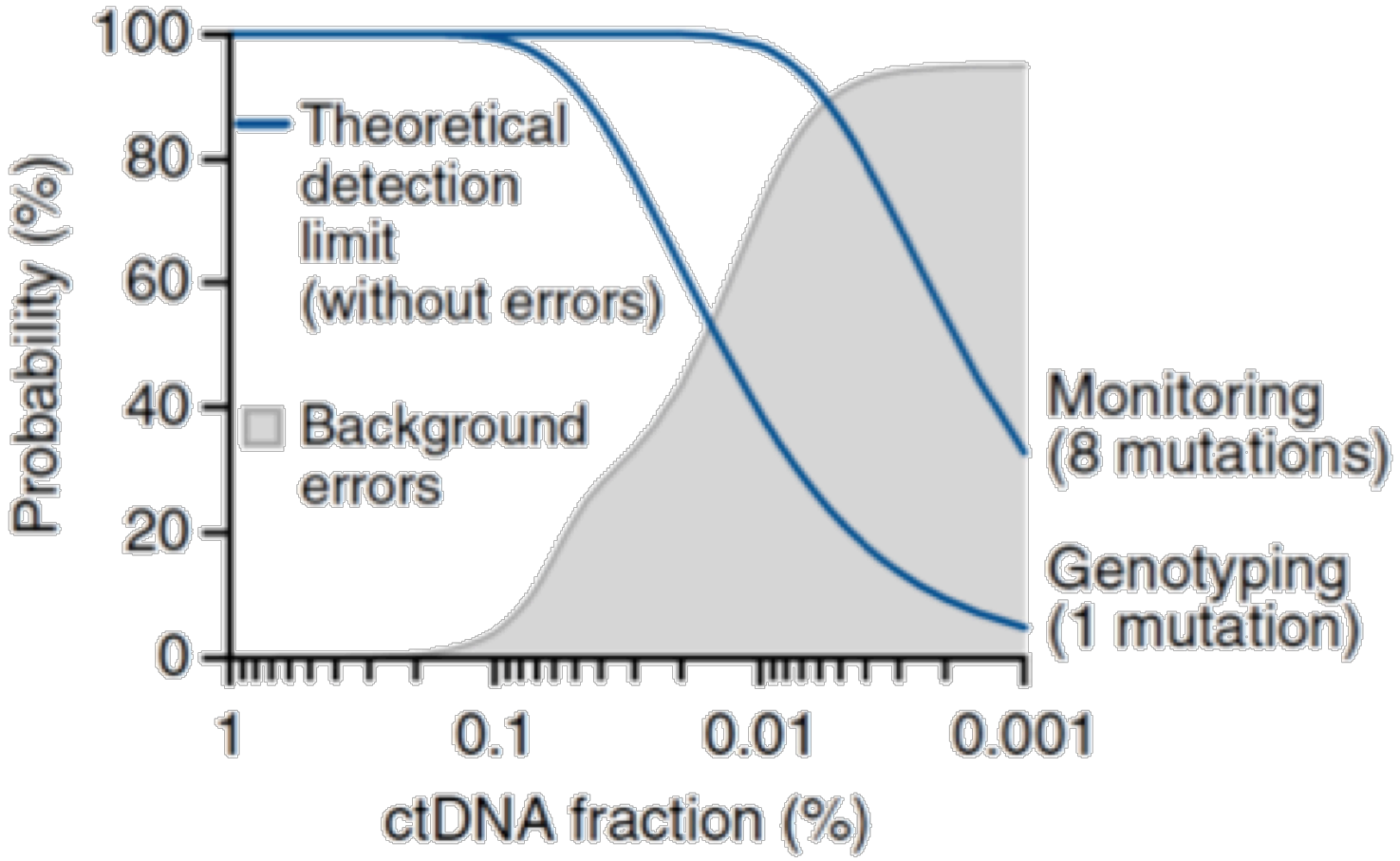
AKT1 (1) G49T

APC (16) C2626T, G3826T, 3927_3932del5, G3964T, C3980G, C4012T, C4031A,C4132T, G4135T, G4189T, C4285T, C4348T, 4461delT, 4465delT, 4467delA, 4661insA

TP53 (11) G524A, G524T, C535T, G730A, G733A, G733T, C742T, G743A, C817T,G818A, C844T

Diehl F et al., PNAS (2005)

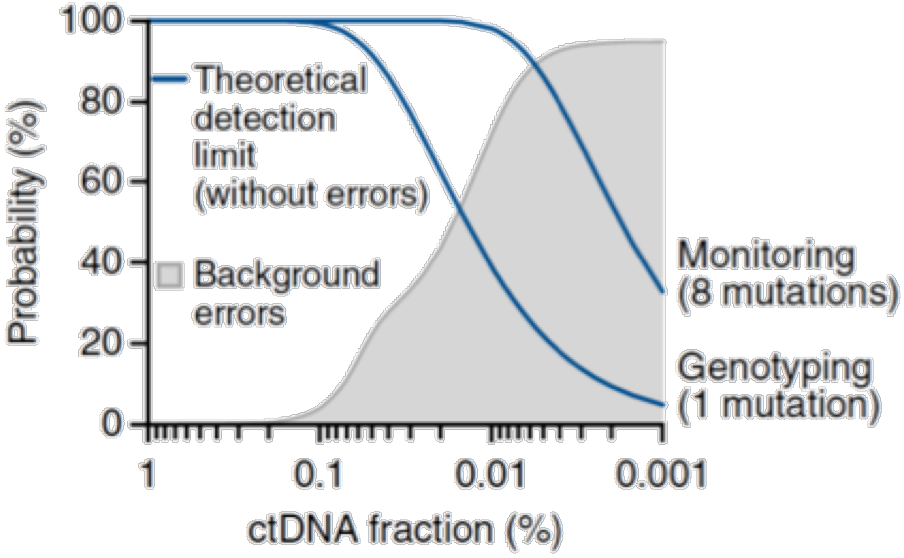
Development of Laboratory Diagnostics in Oncology: Improvement of MPS Data Interpretation*

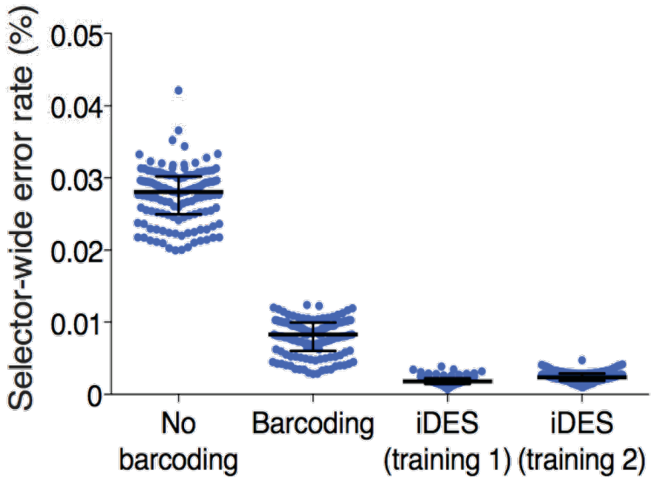


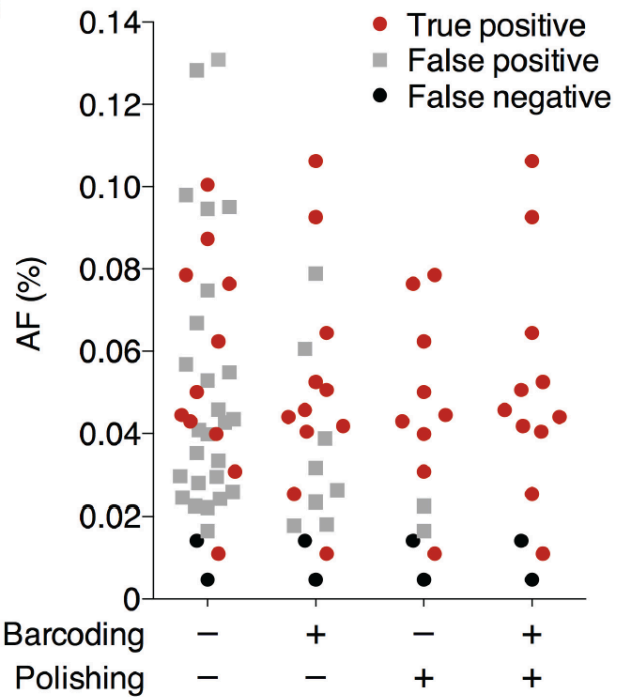
*) integrated Digital Error Suppression (**iDES**) in CaPP-Seq

Newman A et al., Nature Biotech., (2016)
Newman A et al., Nature Biotech., supplement (2016)

Development of Laboratory Diagnostics in Oncology: Improvement of MPS Data Interpretation*







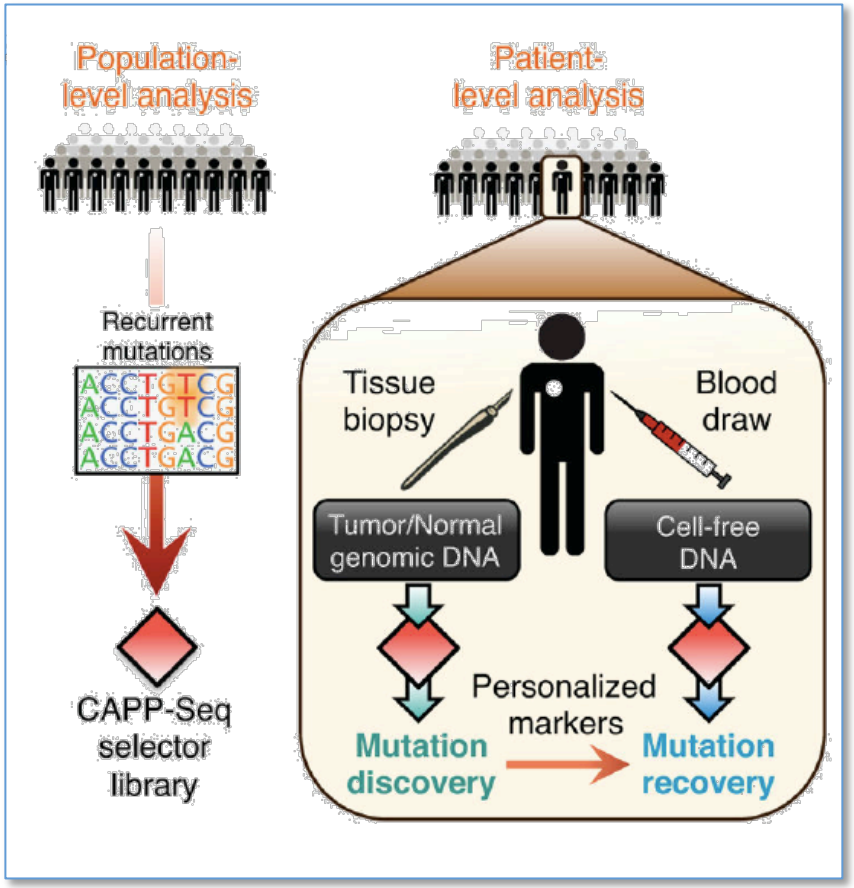
Sn	84.6%	84.6%	69.2%	84.6%
*Sn	100.0%	100.0%	80.0%	100.0%
Sp	97.7%	99.3%	99.8%	100.0%
PPV	27.8%	55.6%	80.0%	100.0%
NPV	99.8%	99.8%	99.7%	99.8%
*NPV	100.0%	100.0%	99.8%	100.0%

*Detection-limit-adjusted

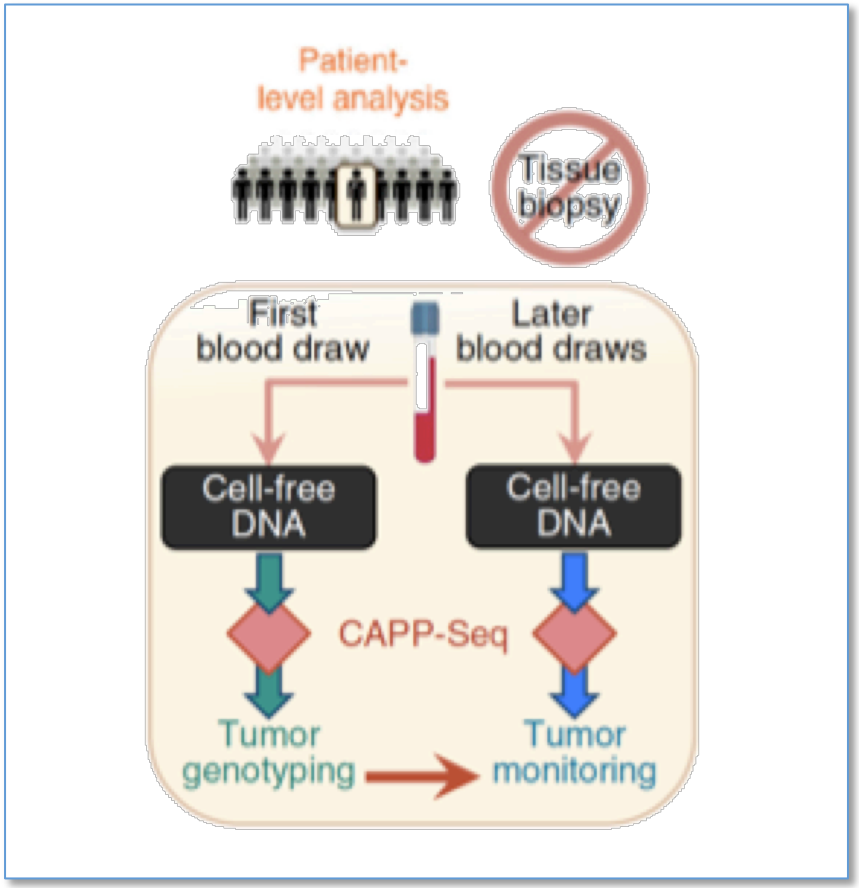
*) *integrated Digital Error Suppression (**iDES**) in CaPP-Seq*

Newman A et al., Nature Biotech., (2016)
Newman A et al., Nature Biotech., supplement (2016)

Changes of molecular diagnostic Strategies predicted by improved Performance of liquid Profiling of ctDNA in peripheral Blood



A Newman et al., Nature Med (2014)



A Newman et al., Nature Biotech (2016)

Conclusions
Modern Laboratory Diagnostics in Oncology.....

- detects the „biologic Achilles Heel“ of Tumours (driver defects) thus defining/ changing Therapy Regimens.
- identifies Resistance to Therapy with Lead-Time (up to 10 Months reported).
- detects minimal residual Disease (MRD) from Blood Samples (using digital Droplet PCR Methods - MPS is getting there, too).
- will reduce the Need for Tissue Sampling due to Availability of genetic Footprints (by liquid Profiling/liquid Biopsy) in the Blood.

Thank you for your kind Attention!

michael.neumaier@umm.de

Acknowledgement

Institut für Klinische Chemie, UMM

Dr. Verena Haselmann
Ingrid Brechtel
Angelika Duda

Klinik für Dermatologie, UMM
DFKZ, Heidelberg

Prof. Dr. Jochen Utikal
Dr. Christoffer Gebhardt
Dr. Tim Holland-Letz

Klinik für Dermatologie, UK-Essen

Prof. Dr. Dirk Schadendorf
Antje Sucker