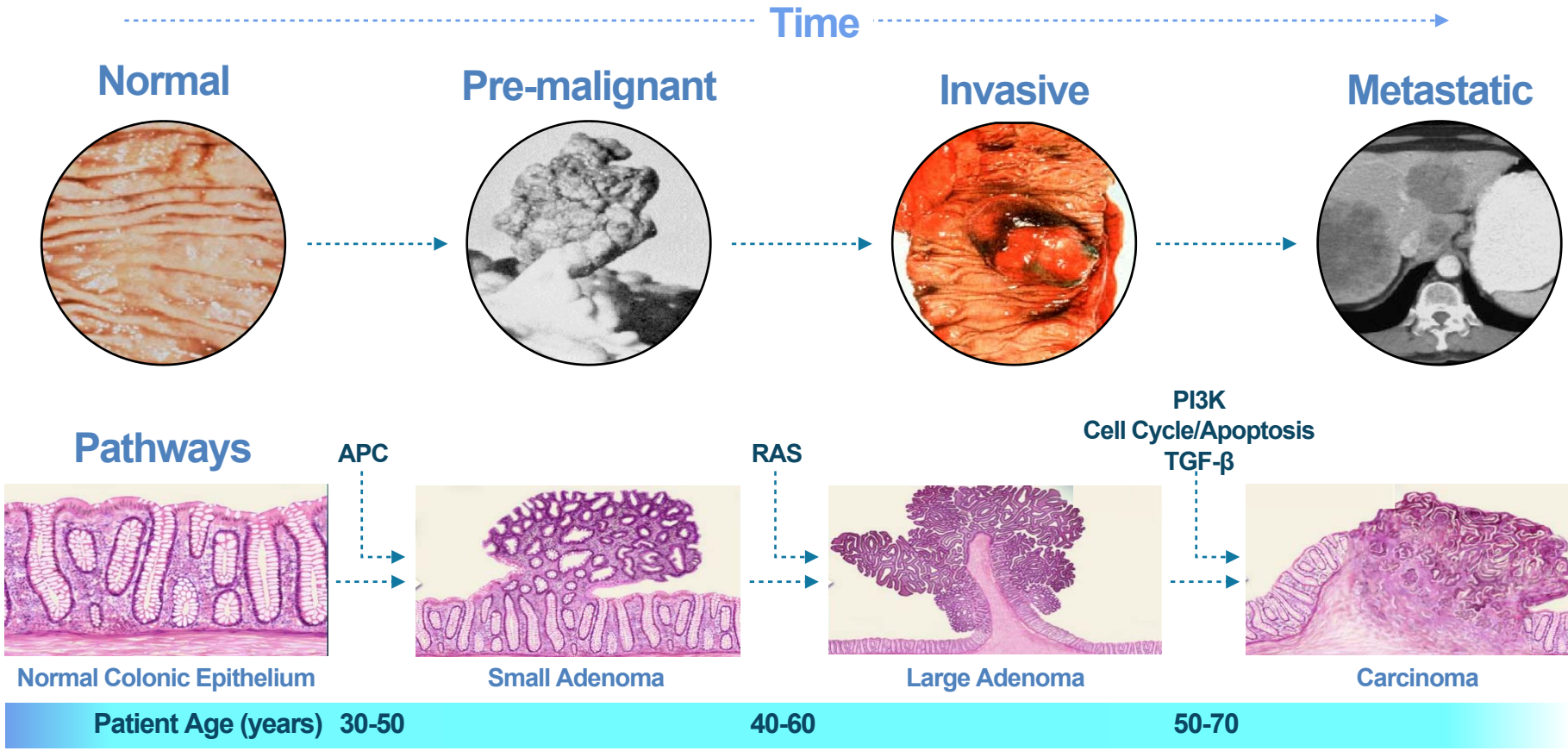
Opportunities and Limitations of ctDNA as a Clinical Biomarker in Cancer Management: New Insights in the Clinical Application of ctDNA

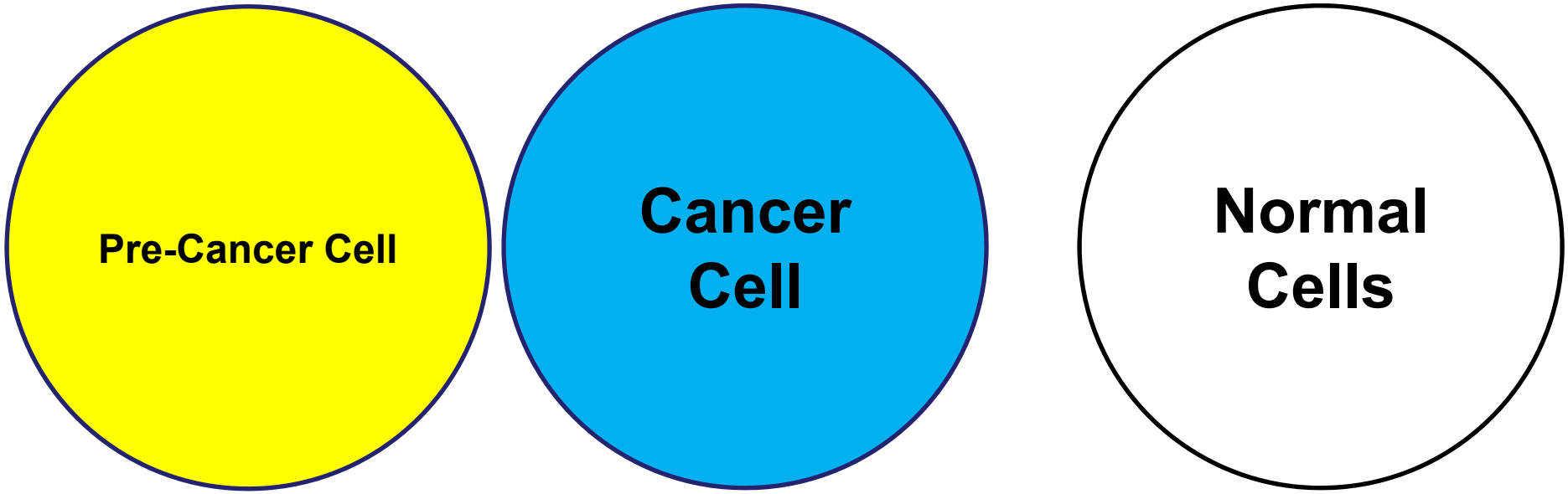
Luis A. Diaz, M.D.
Head, Division Solid Tumor Oncology
Memorial Sloan Kettering Cancer Center
October 4, 2018

Disclosures of potential COI

Company	Relationship
Jounce Therapeutics	Board of Directors
Personal Genome Diagnostics, Inc.	Board of Directors, Consultant and Stock
PapGene, Inc.	Stock
Merck	Consultant
Phoremost	SAB, Consultant
Lyndra	Consultant (not active)
Caris	Consultant (not active)
Genocea	Consultant (not active)
Cell Design Labs	Consultant (not active)
15 Patents – multiple managed by Johns Hopkins and MSK conflict of interest office	Royalties related to applications of ctDNA analysis and mismatch repair deficiency for diagnosis and therapy

How Does Cancer Evolve?

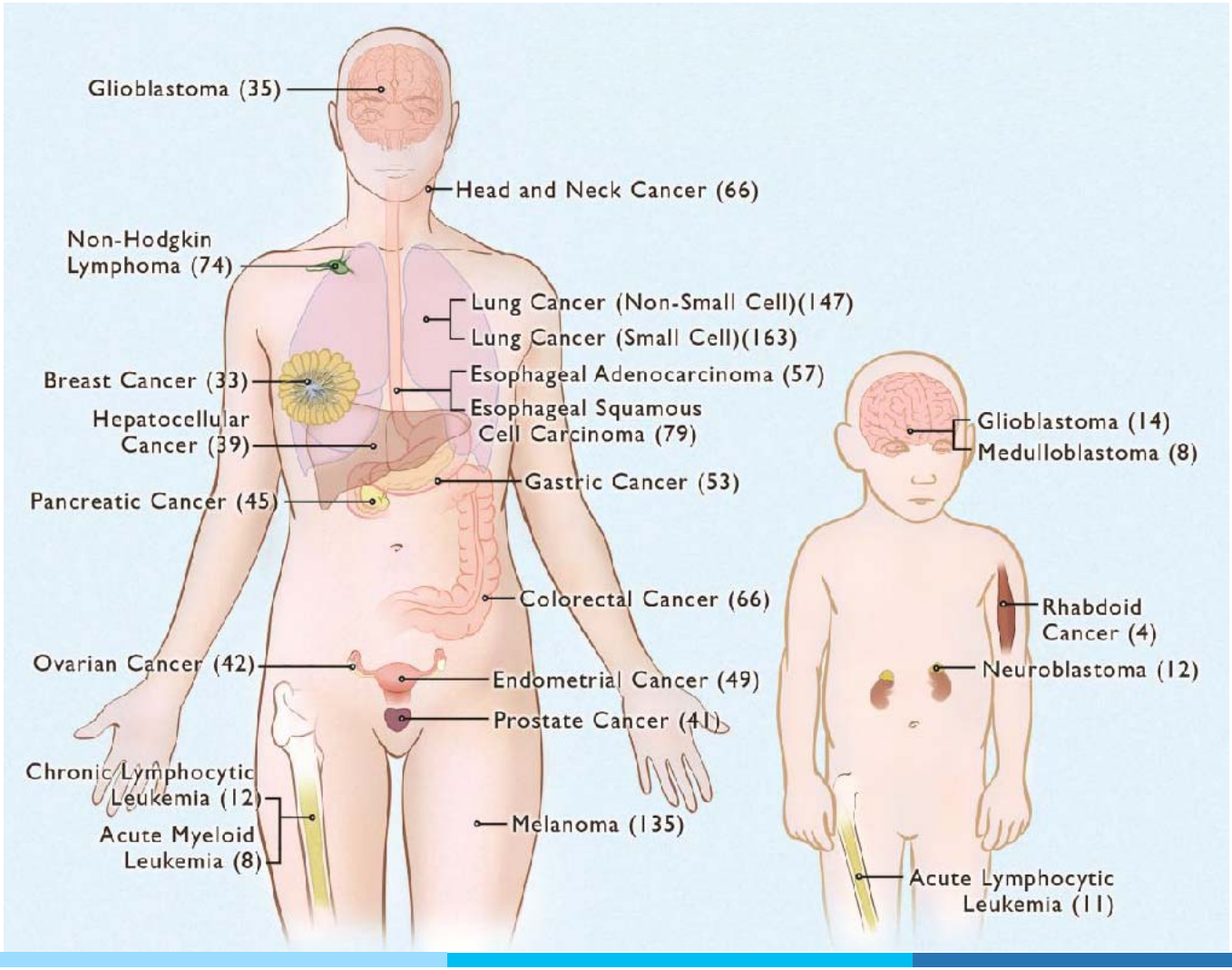


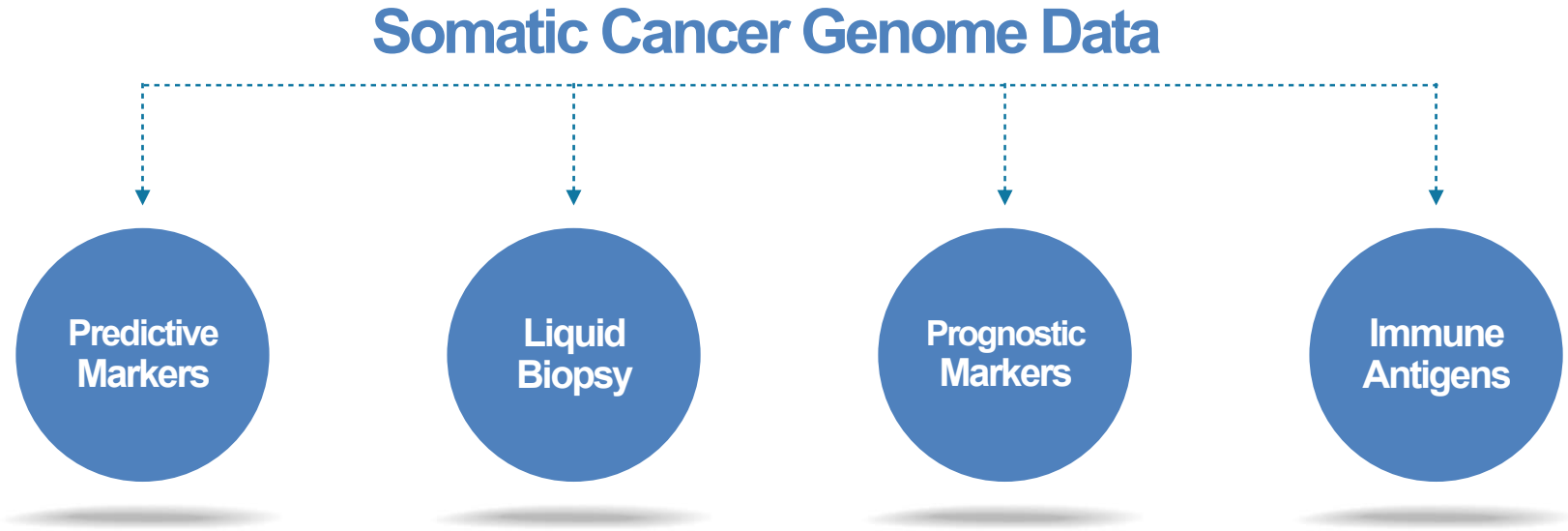


Mutations

No Mutations

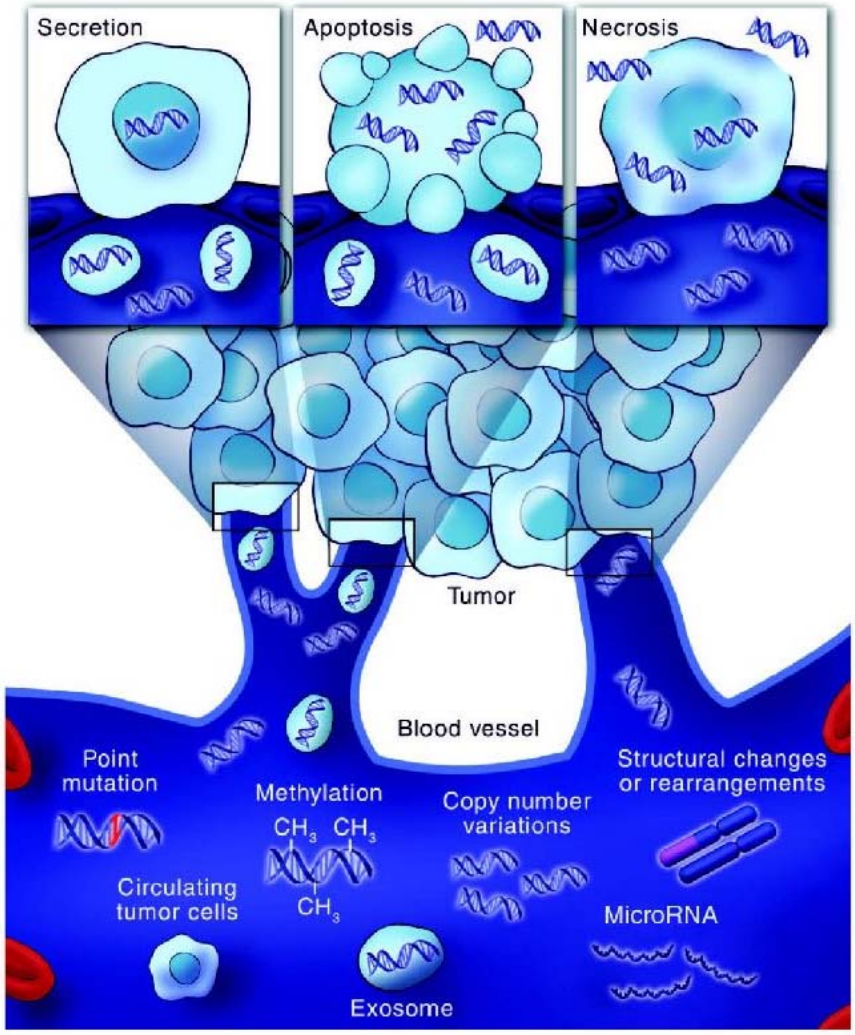
Human Cancer Exomes Sequenced




Somatic Cancer Genome Data
Predictive Markers
Liquid Biopsy
Prognostic Markers
Immune Antigens

Liquid Biopsies

- DNA fragments of 120-200 bp with half life of ~2 hours
- Real-time, non-invasive, multi-lesions, potentially cheaper (considering cost of biopsies)
- Often very low amount of ctDNA in the sea of wild type DNA - ”Needle in a farm”
- Specific to tumor



Access to Somatic Mutations

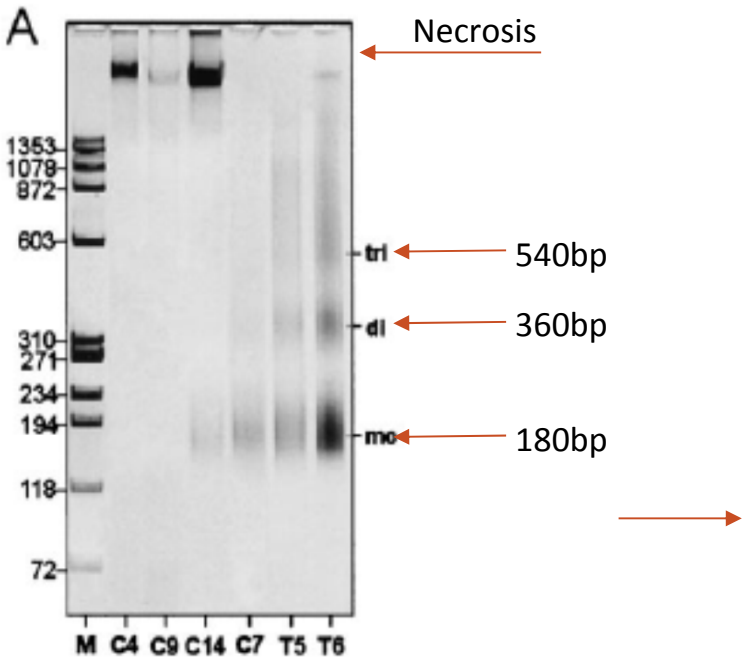
Tumor Tissue

- Formalin Fixed Parrafin-Embedded (FFPE)
- Frozen tissue

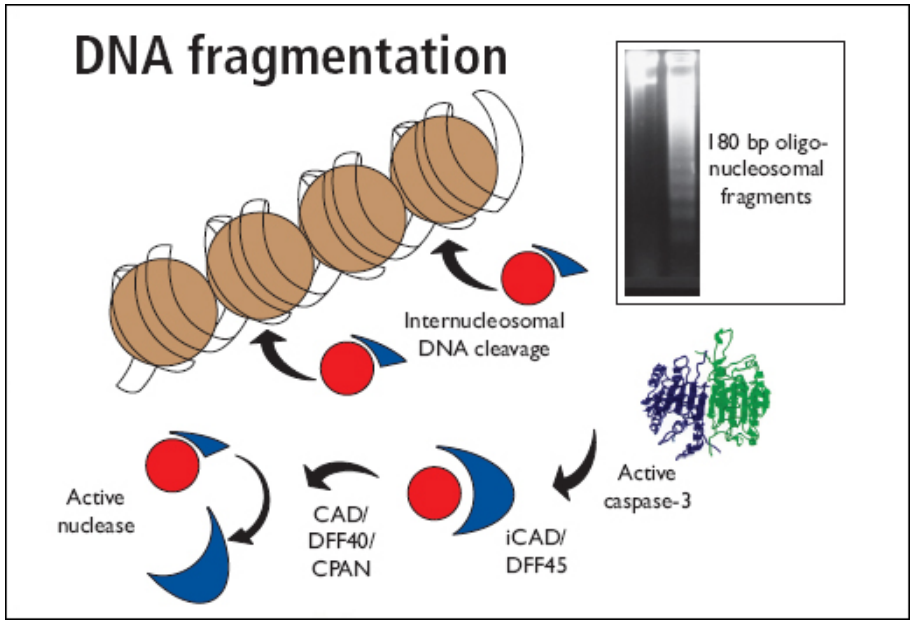
Blood & Other Bodily Fluids

- Cell-free DNA
- Circulating tumor cells (CTCs)

Cell-free DNA – origin

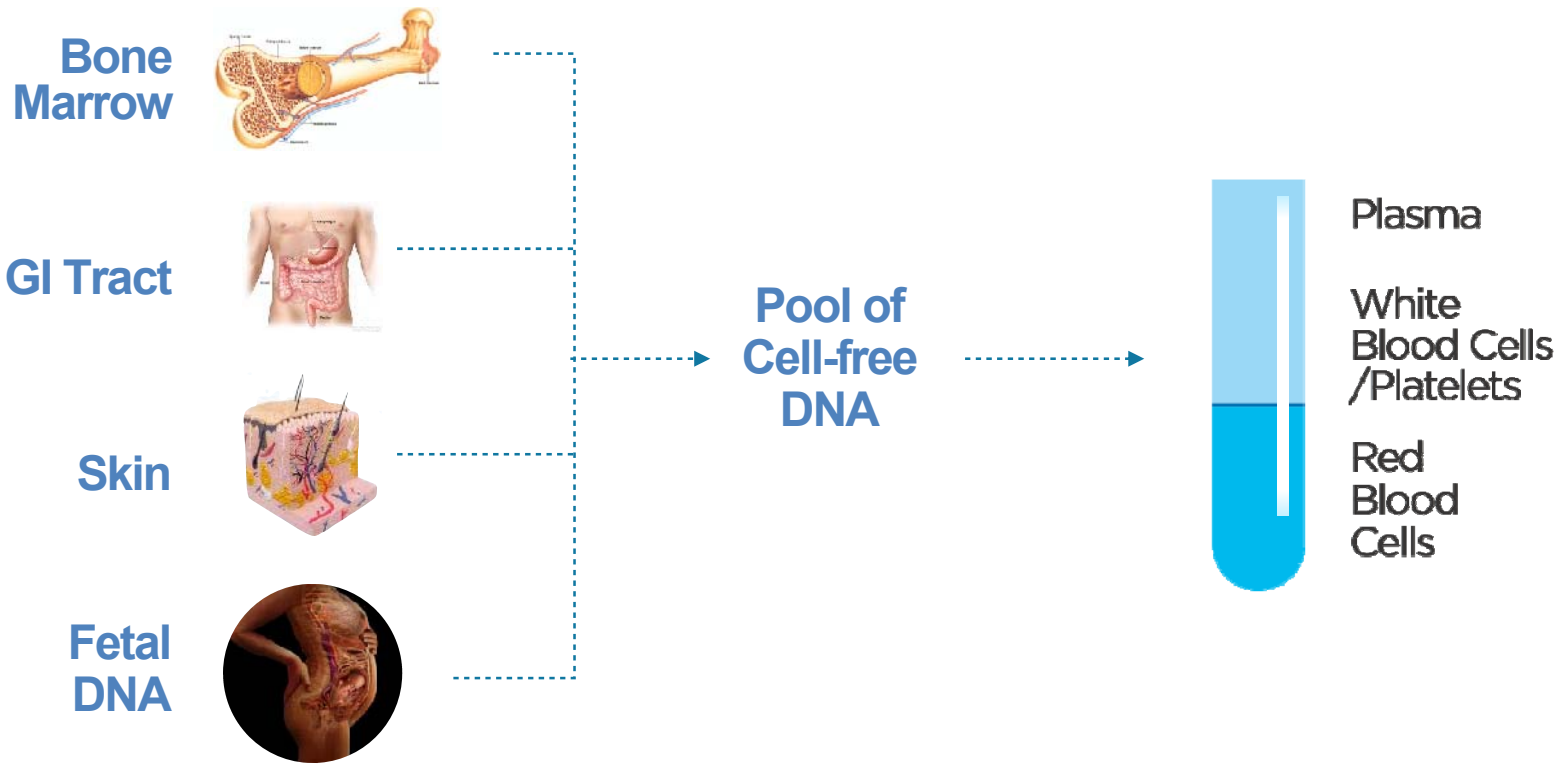


Apoptosis



Jahr, S. Cancer Res, 2001

Source Circulating Cell-Free DNA



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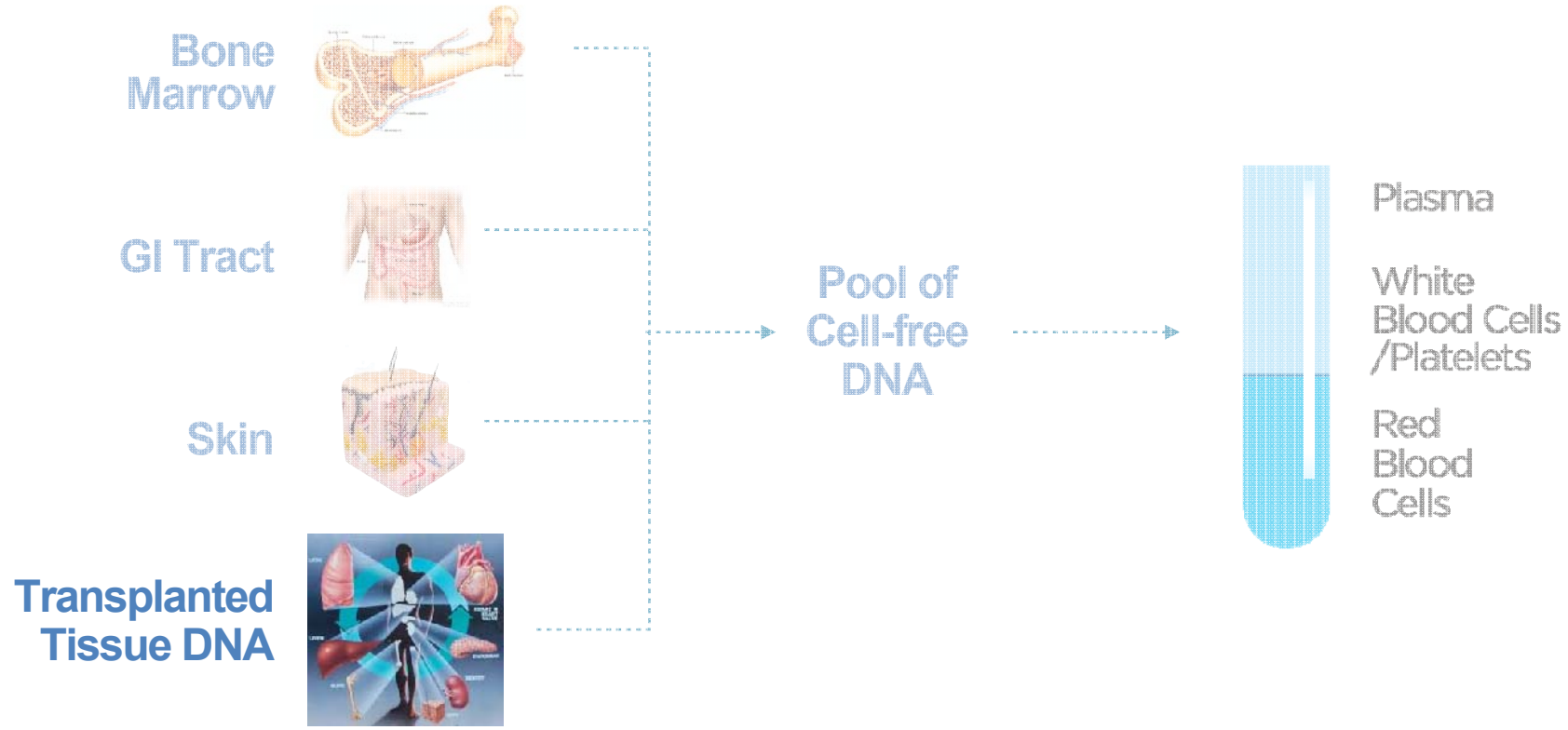
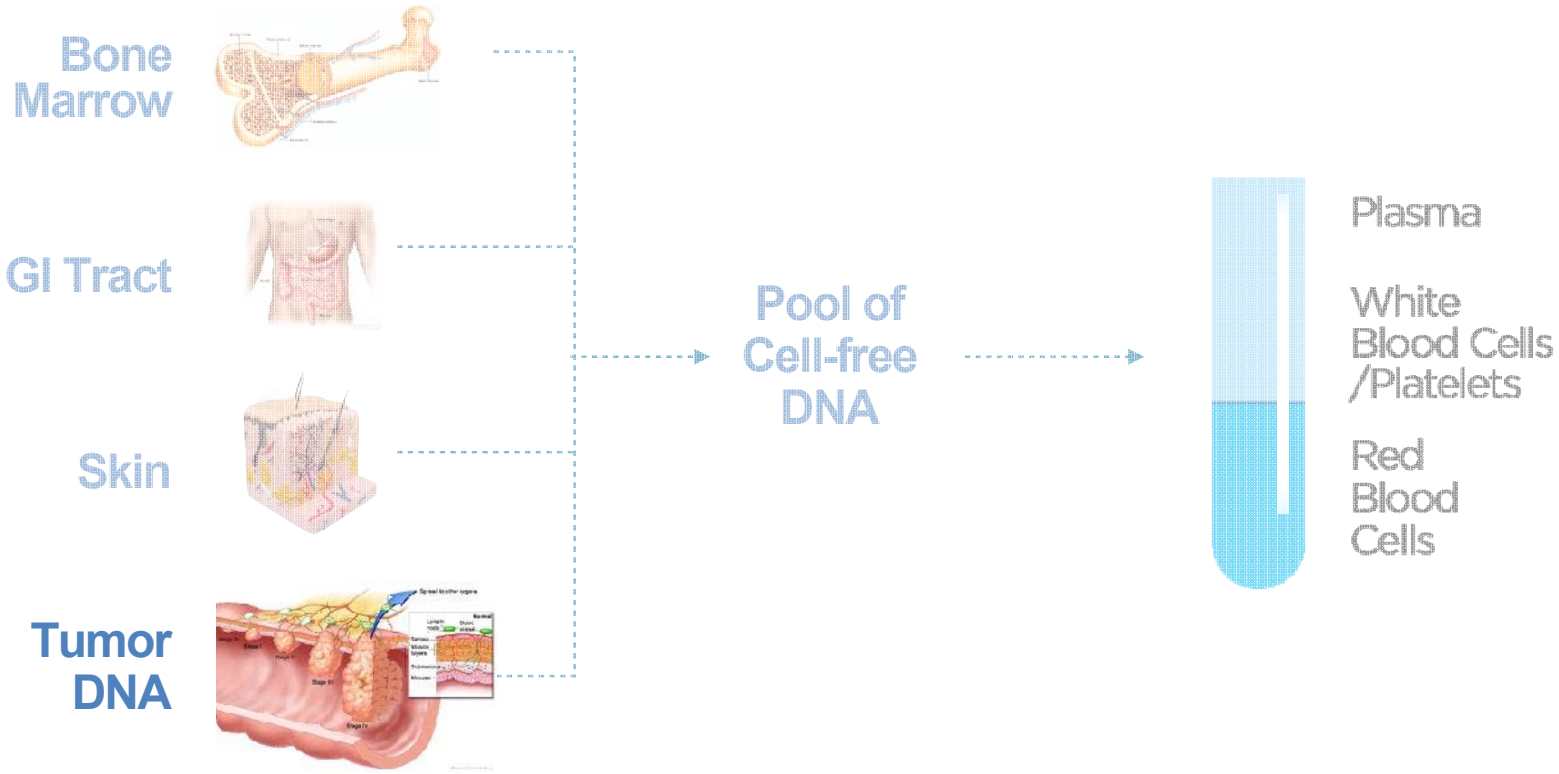
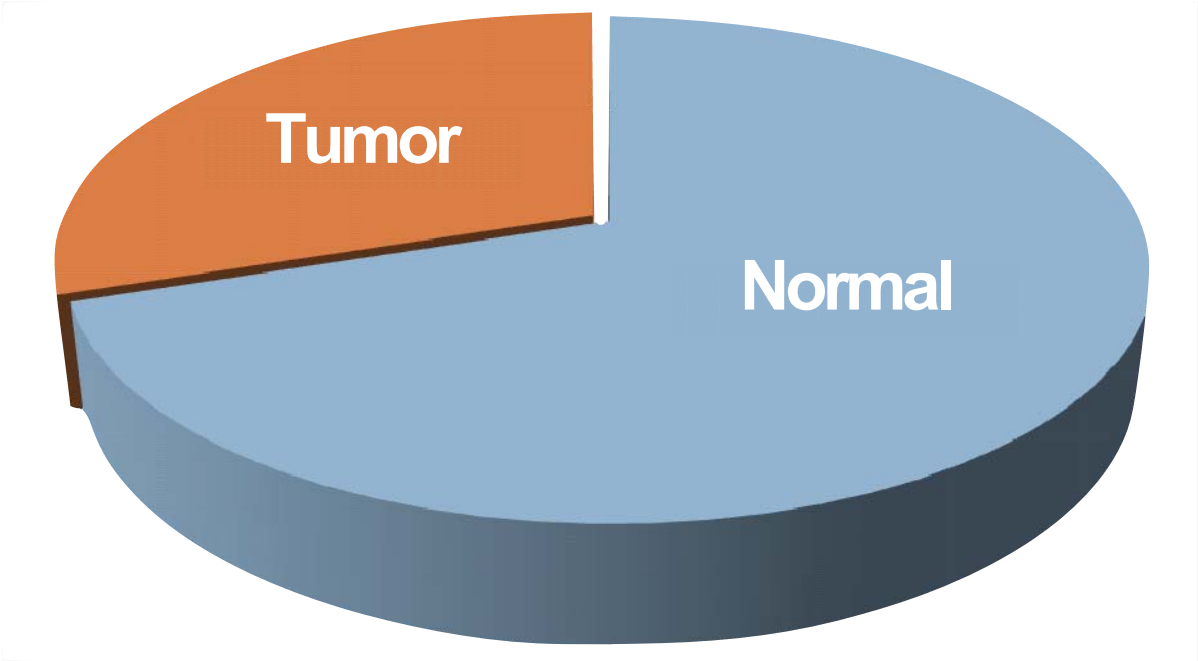


Source Circulating Cell-Free DNA



Circulating Cell-Free DNA in a Cancer Patient



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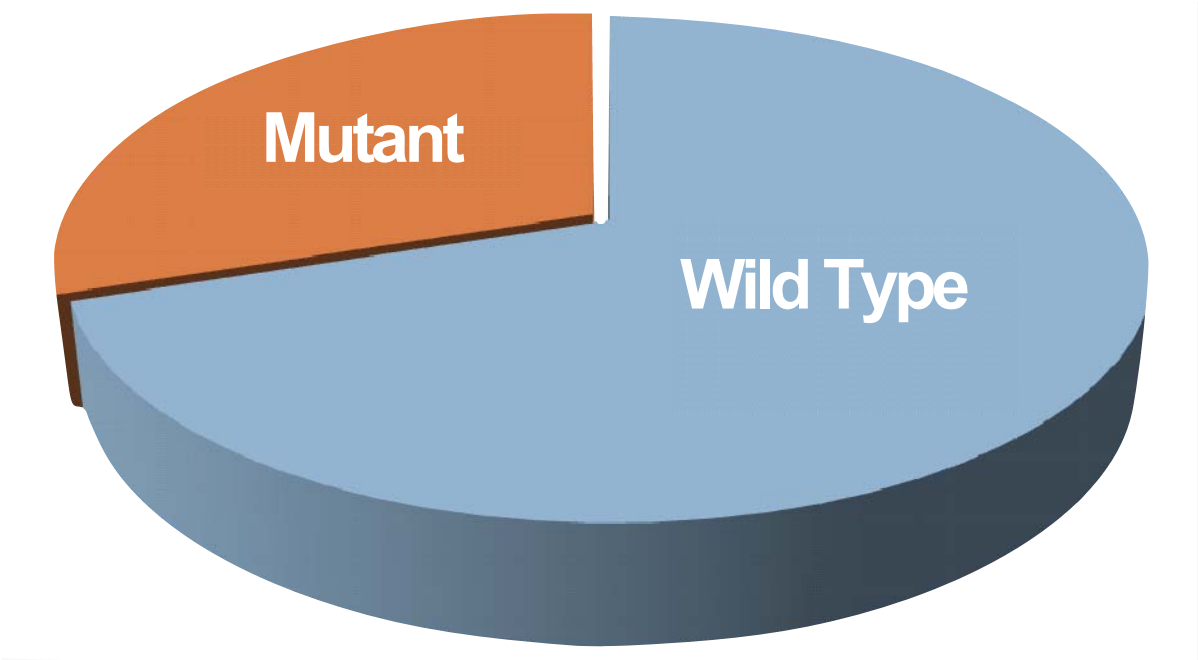


Technology To Assess Circulating Tumor DNA

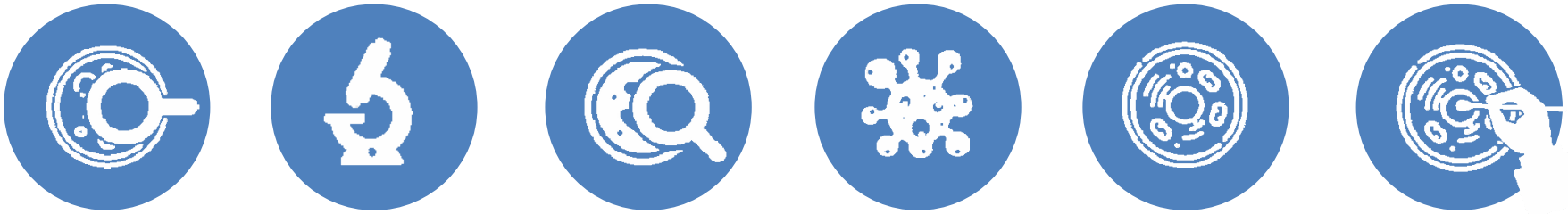
Digital PCR

- Best for individual point mutations but can be used for crude copy number analysis
- Mutation needs to known ahead of time (ie BRAF v600e)
- Sensitivity is dependent on specific mutation and assay optimization
- Multiplexing assay is possible
- Fast and highly reproducible – results in hours
- Minimal bioinformatics needs
- Inexpensive

Next-generation Sequencing

- Evaluates genomic regions of interest using PCR or capture-based methods
- Has been used for point mutations, rearrangements, genomic amplification, aneuploidy, whole exome and whole genome sequencing
- High false discovery rate that requires pre-sequencing barcoding and post-sequencing bioinformatics for error suppression
- Expensive
- Turnaround time 1-2 days at best

Potential of Liquid Biopsies in Precision Medicine



Mutations as Biomarkers

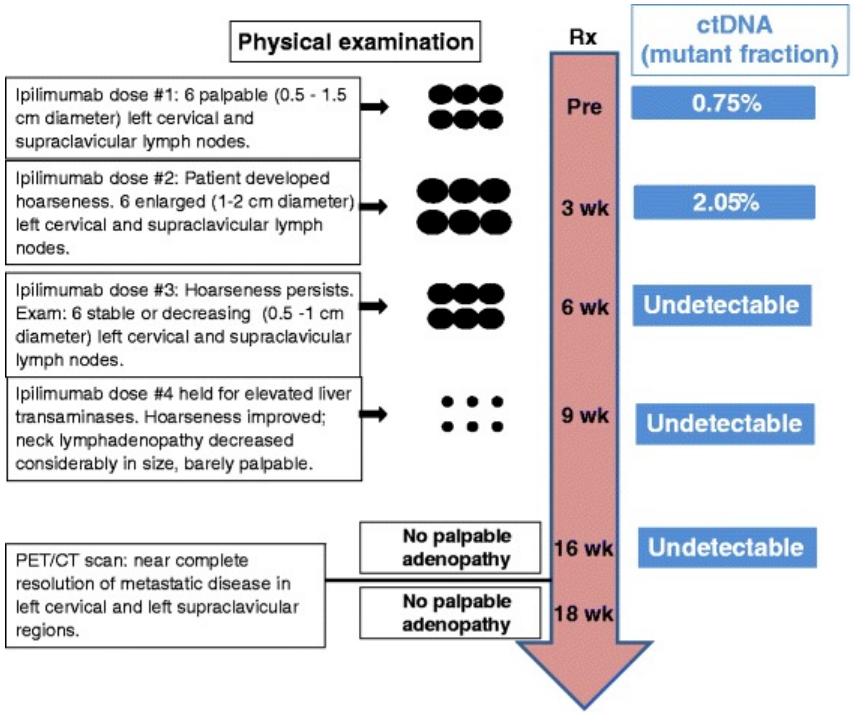
Monitoring Tumors

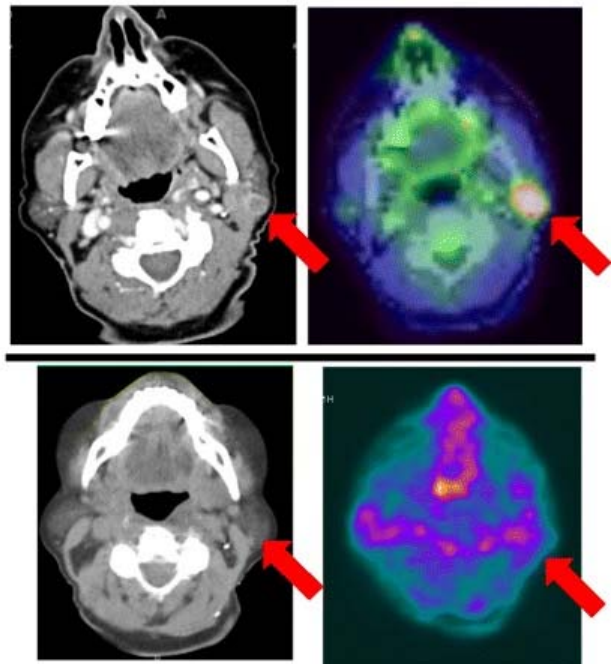
Tracking Resistance

Minimal Residual Disease

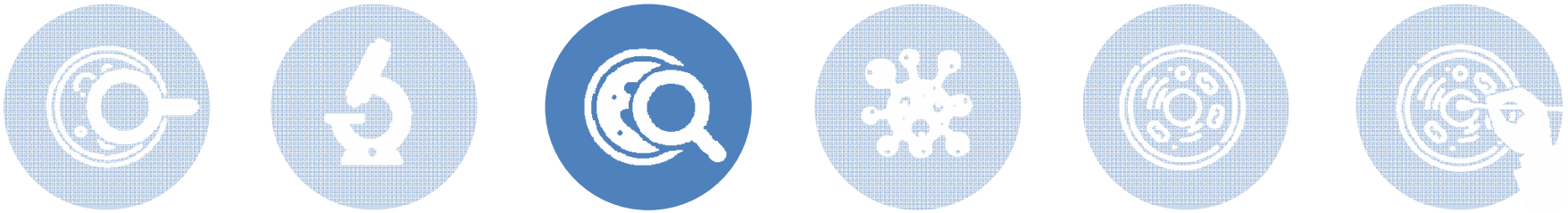
Molecular Remission

Early Detection


Physical examination
Rx
ctDNA (mutant fraction)
Ipilimumab dose #1: 6 palpable (0.5 - 1.5 cm diameter) left cervical and supraclavicular lymph nodes.
Pre
0.75%
Ipilimumab dose #2: Patient developed hoarseness. 6 enlarged (1-2 cm diameter) left cervical and supraclavicular lymph nodes.
3 wk
2.05%
Ipilimumab dose #3: Hoarseness persists. Exam: 6 stable or decreasing (0.5 -1 cm diameter) left cervical and supraclavicular lymph nodes.
6 wk
Undetectable
Ipilimumab dose #4 held for elevated liver transaminases. Hoarseness improved; neck lymphadenopathy decreased considerably in size, barely palpable.
9 wk
Undetectable
No palpable adenopathy
16 wk
Undetectable
PET/CT scan: near complete resolution of metastatic disease in left cervical and left supraclavicular regions.
No palpable adenopathy
18 wk


2 months pretreatment
4 months post-treatment

Potential of Liquid Biopsies in Precision Medicine



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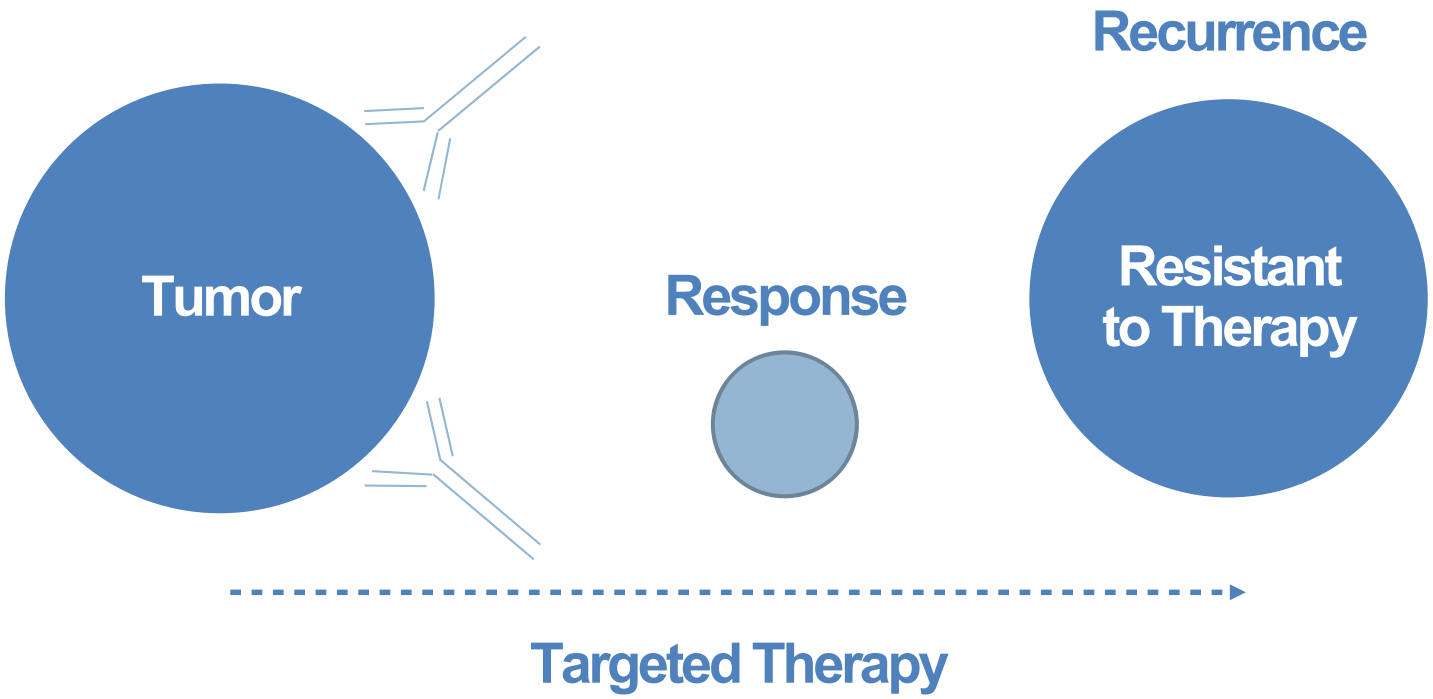


Diaz, et. al. Nature, March 2012

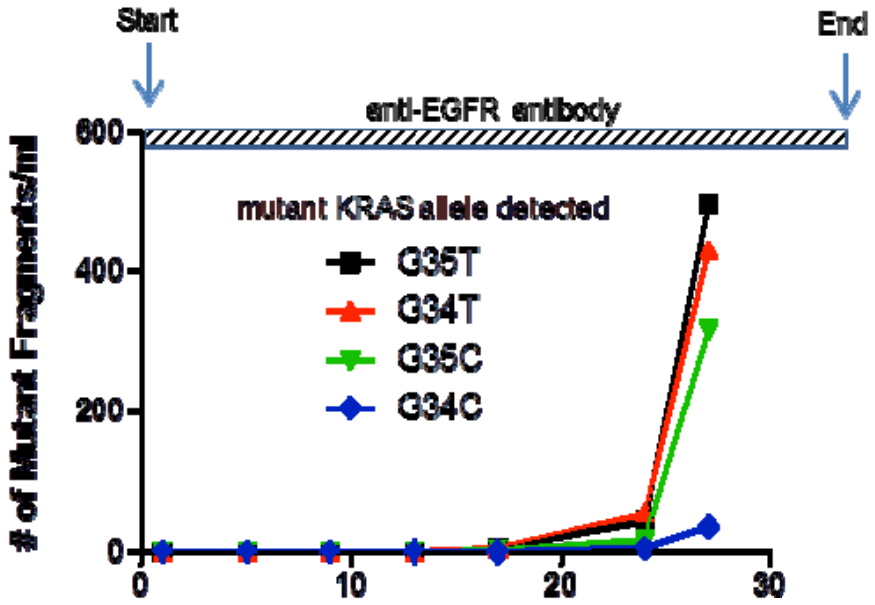
Genetic Heterogeneity

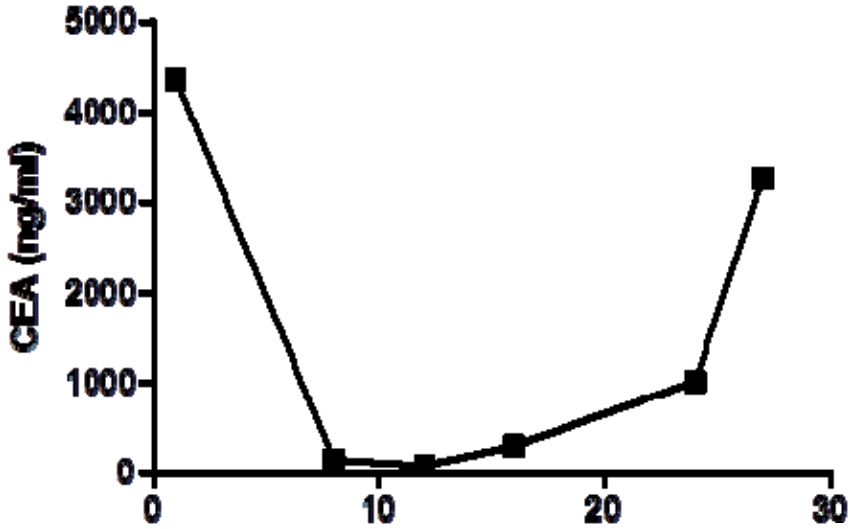
EGFR blockade in Colorectal Cancer

Primary resistant	Exon 12 or 13 KRAS mutation
Response Rate	17%
Secondary resistance	Mutations in KRAS, NRAS, EGFR and Amplification in MET

Tracking Resistance

Monitoring the emergence of resistant mutations in KRAS WT patients treated with EGFR blockade



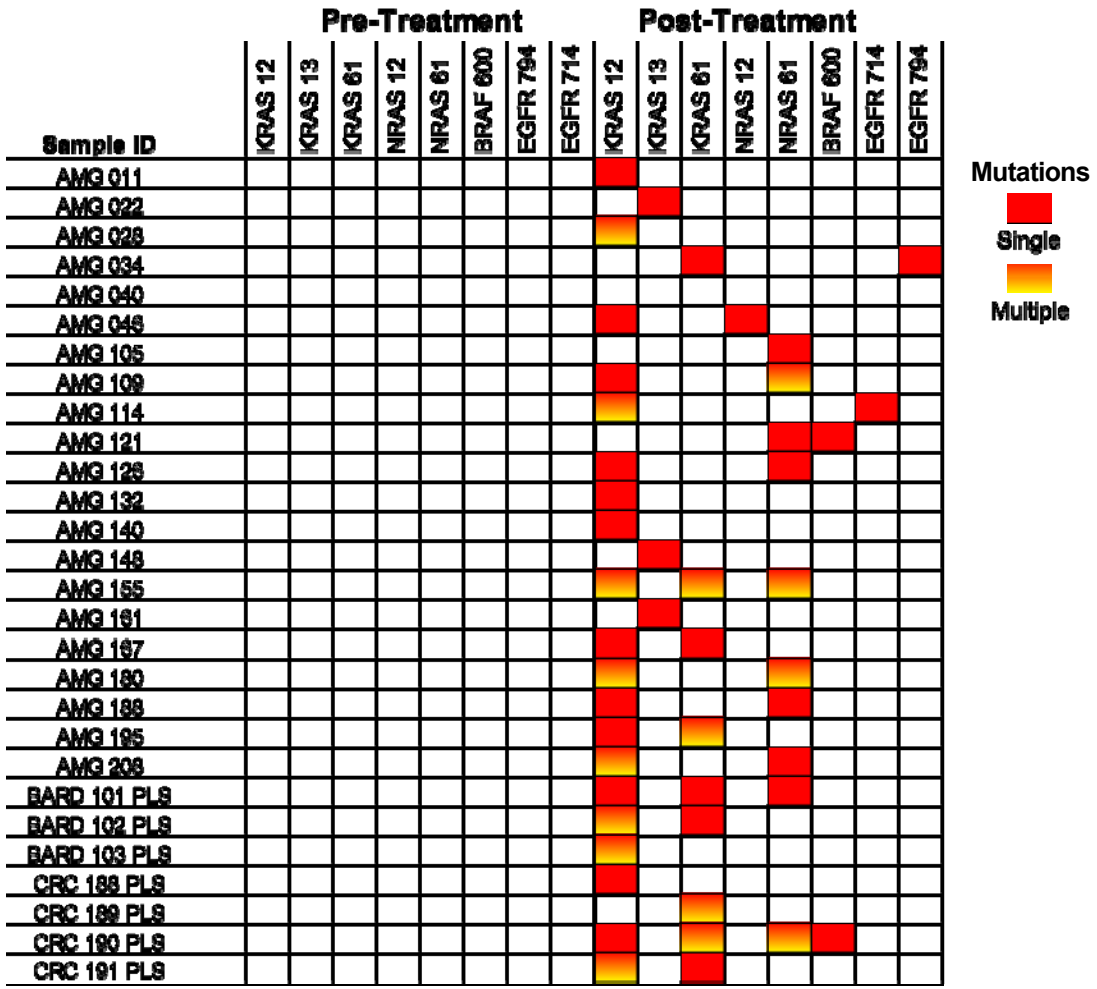


Diaz, et. al. Nature, 2012

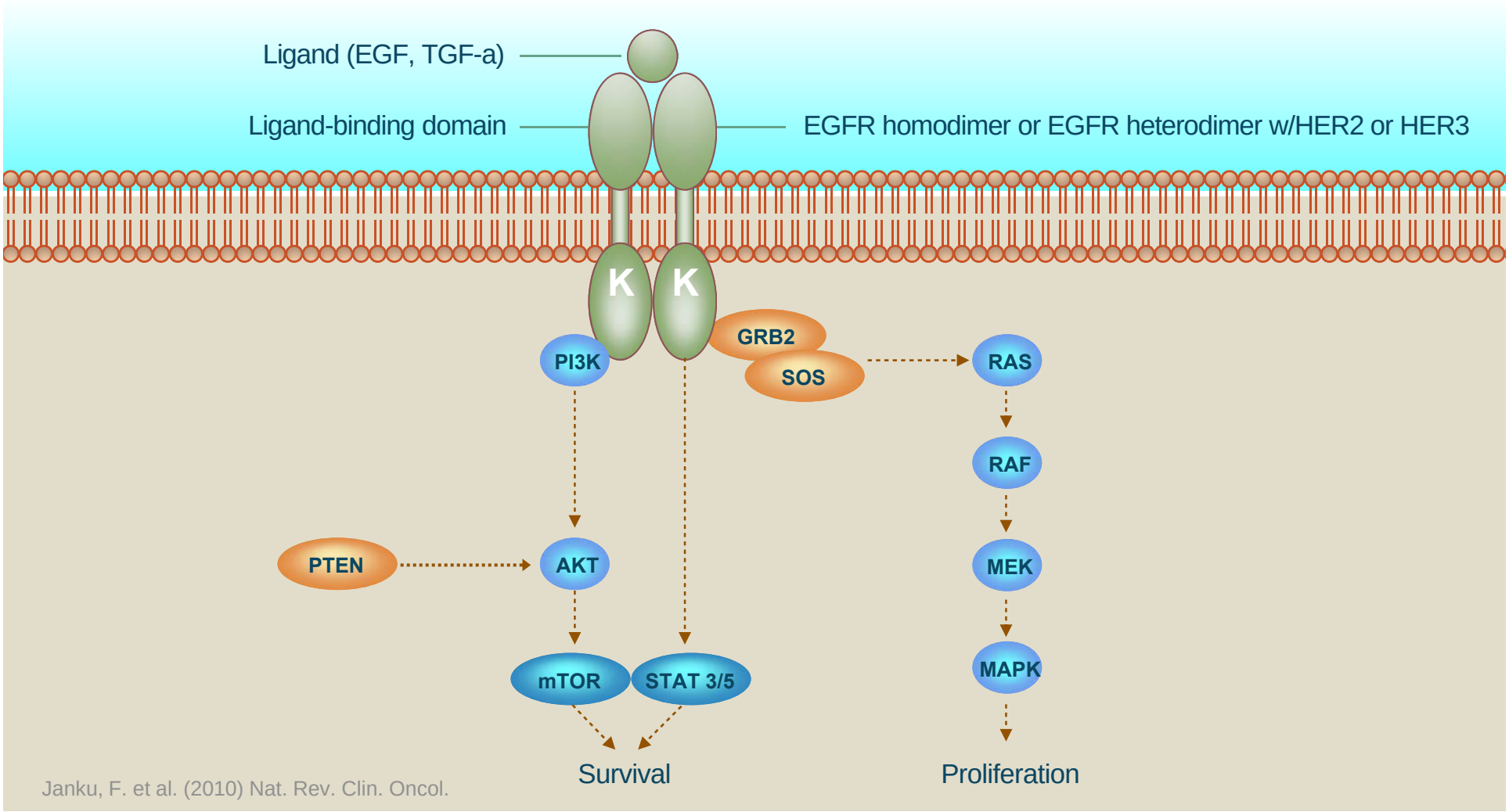
Tracking Resistance

Interrogated all exons of KRAS, NRAS, BRAF, PIK3CA and EGFR

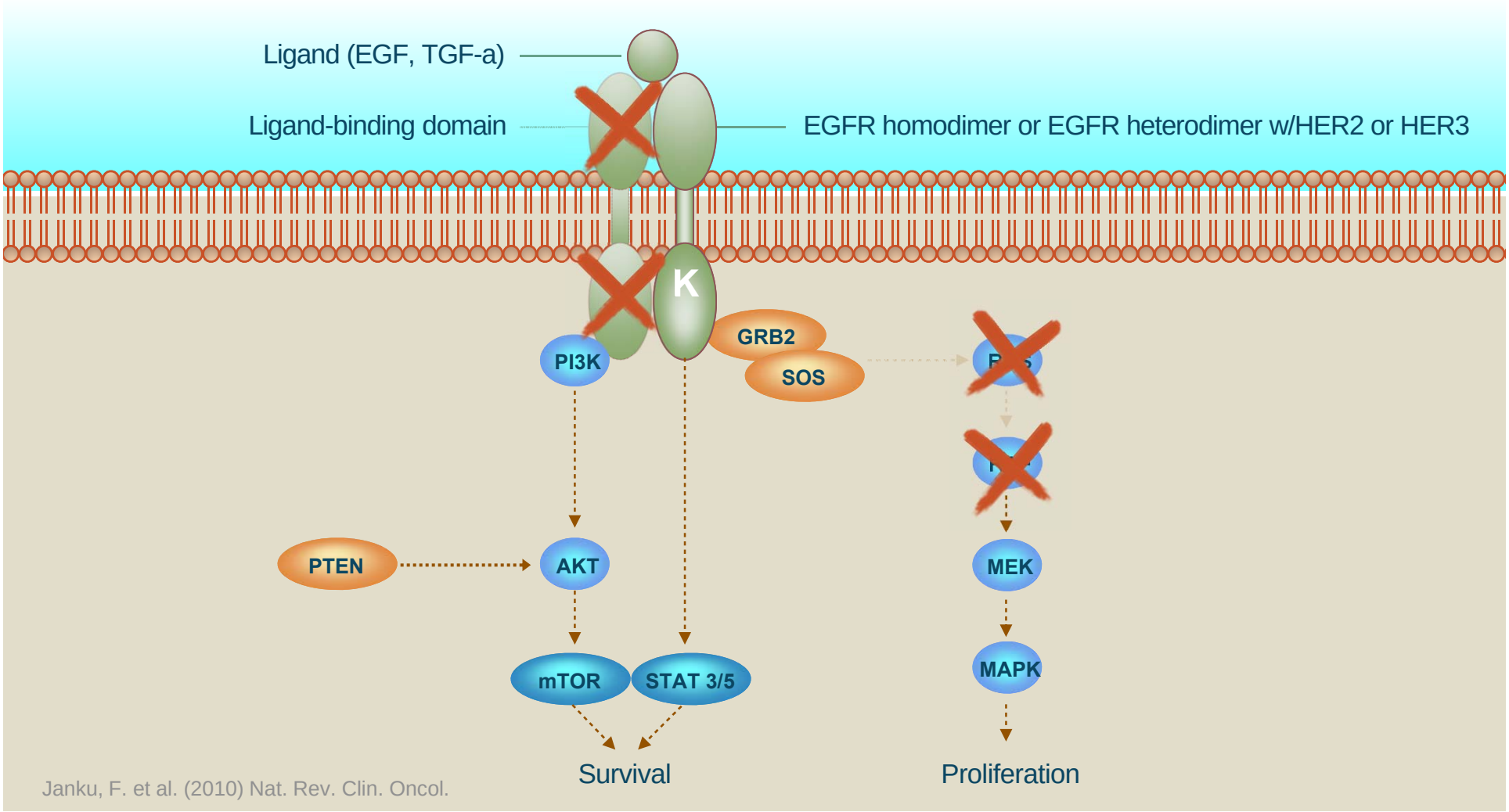
96% of cases had at least 1 mutation KRAS or NRAS



Bettegowda et al, Sci Tran Med 2014 (in press)



Janku, F. et al. (2010) Nat. Rev. Clin. Oncol.



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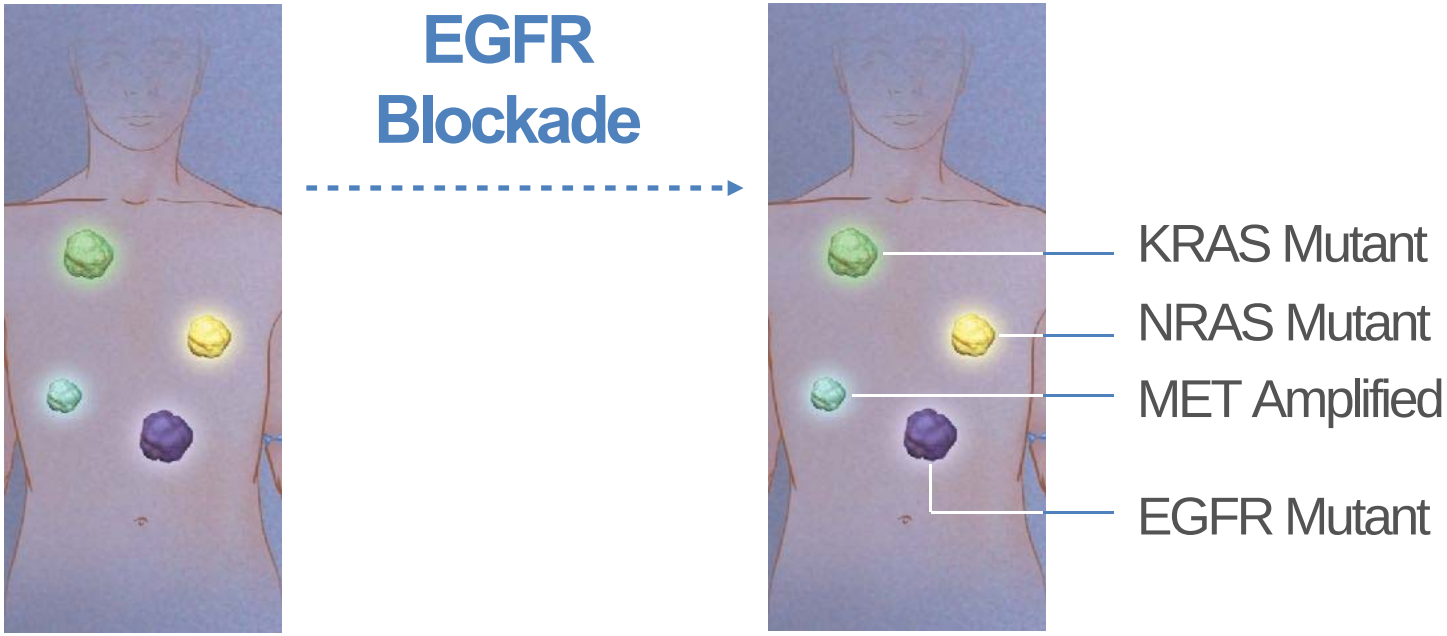
Tracking Resistance



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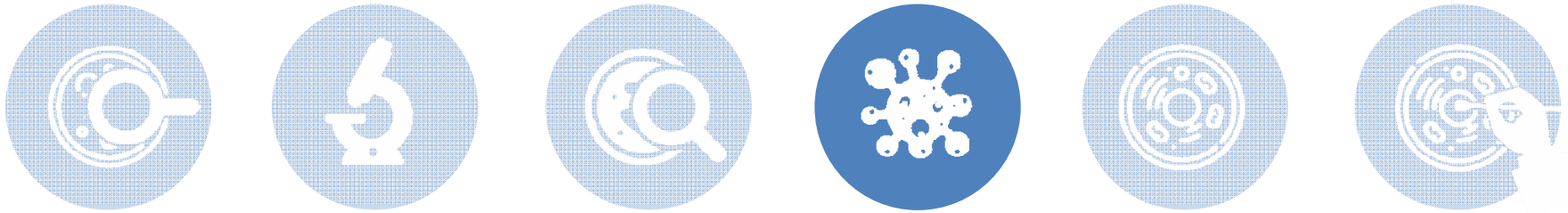

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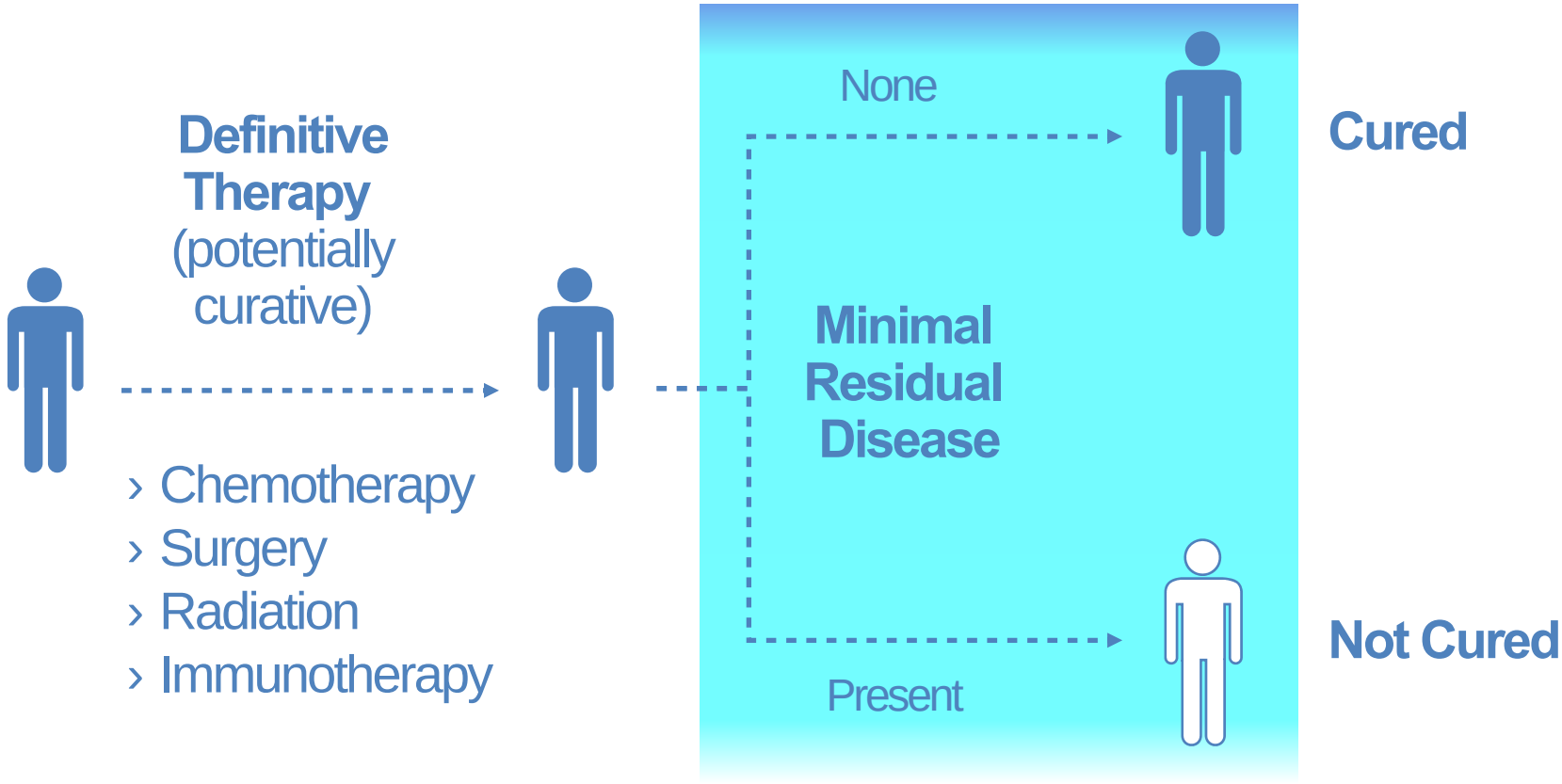
Early Detection

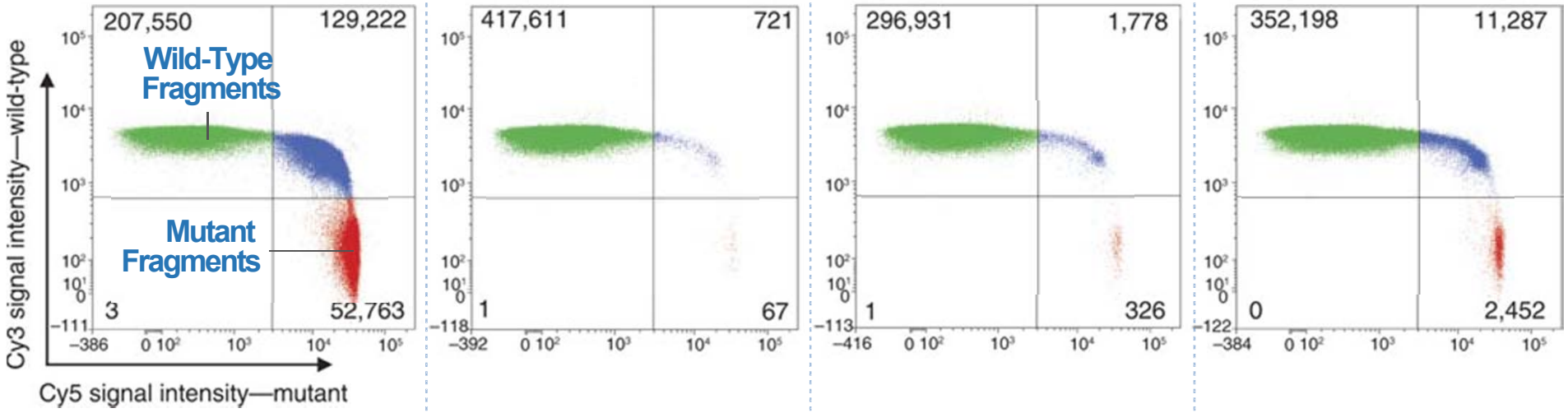
Minimal Residual Disease (MRD) Defined



Systemic Approaches to Detect MRD

Imaging (FDG-PET or CT Scan)	› Poor sensitivity for microscopic disease › Variable specificity
Protein Biomarkers (e.g. CA19-9, CEA, CA-125)	› Long half-life › Often Non-specific
CTCs	› Poor sensitivity for microscopic disease › Does not localize disease
Circulating Nucleic Acids	› Does not localize disease › Highly specific

Surgery	Pre	Post		
Day	0	1	42	244
			CT scan negative	CT scan positive

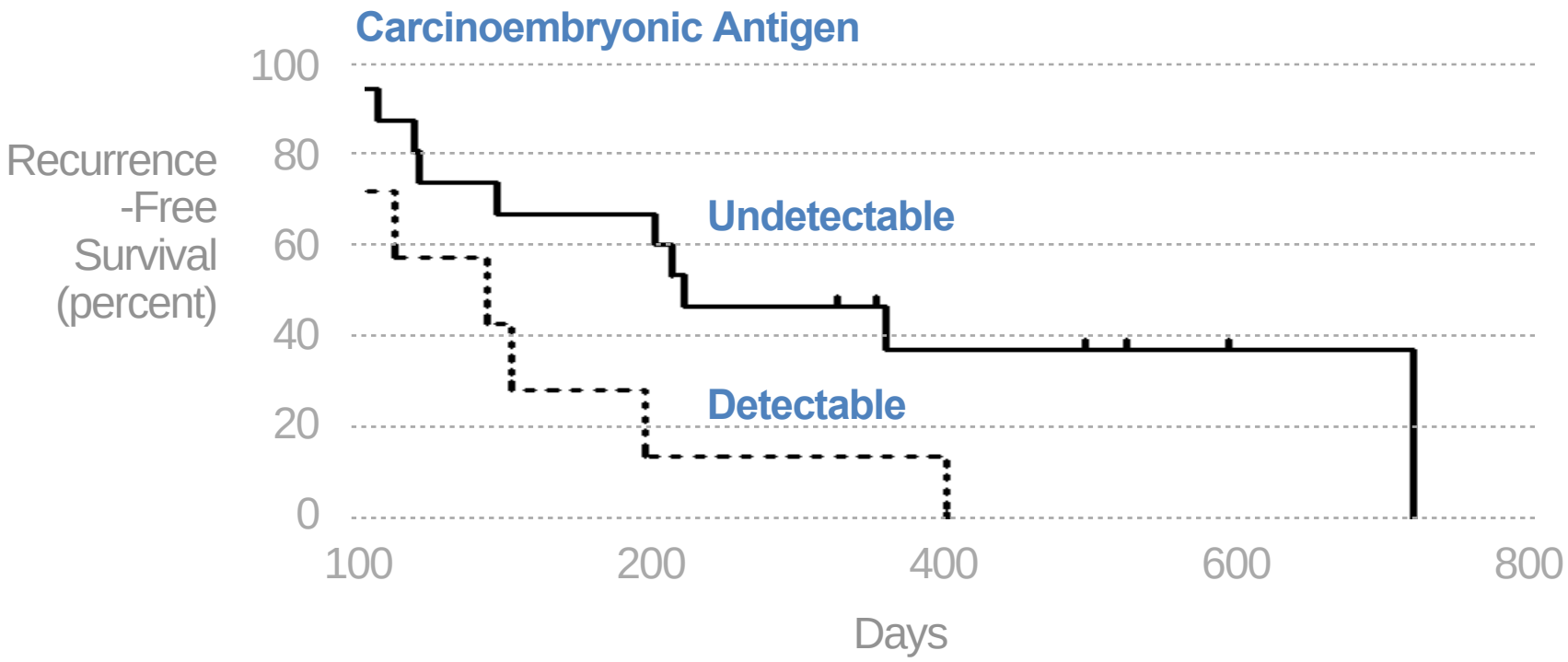


13.4 %	0.015 %	0.11 %	0.66 %

Percent Mutant

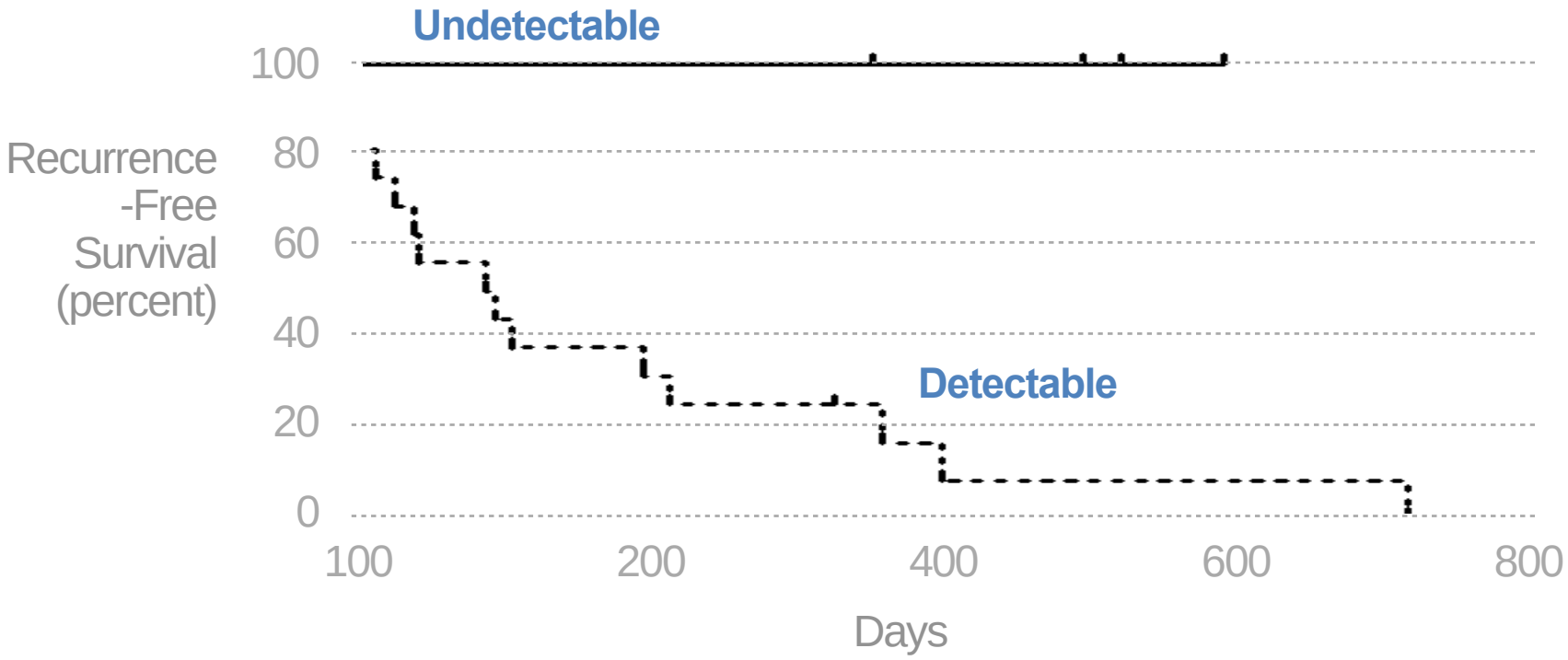
Diehl et al Nature Medicine, 2008

Carcinoembryonic Antigen (CEA) measured 6-8 weeks following curative resection of metastatic Colorectal Cancer (mCRC)



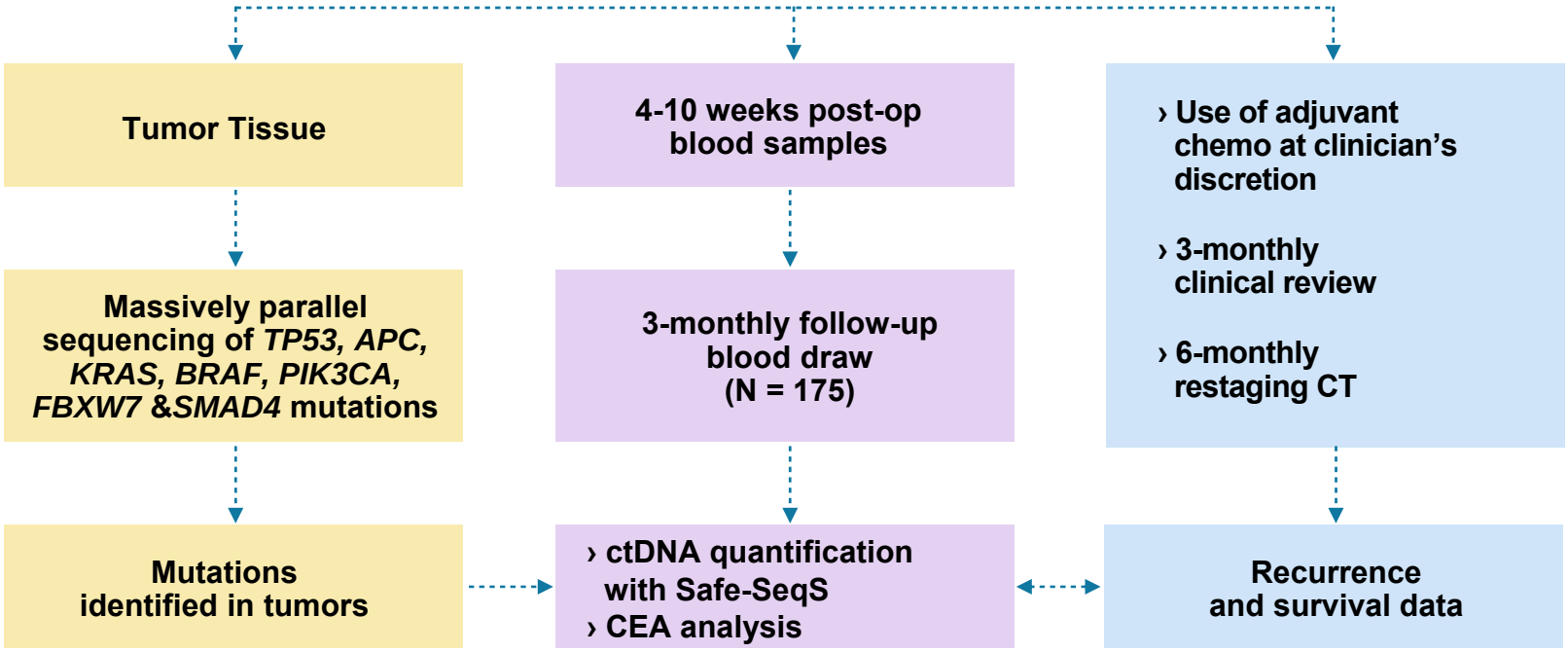
Diehl et al Nature Medicine, 2008

ctDNA measured 6-8 weeks following curative resection of mCRC



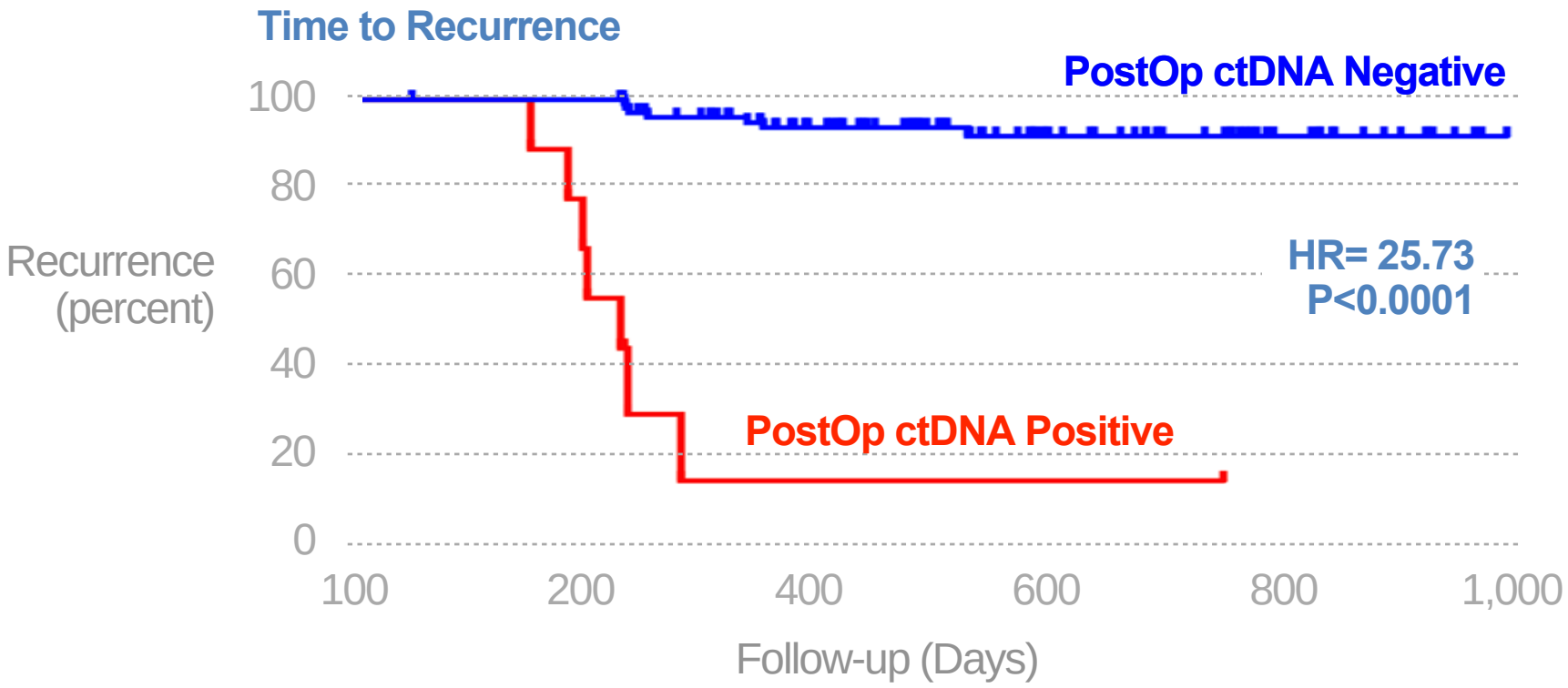
Diehl et al Nature Medicine, 2008

250* patients with Stage II Colon Cancer



J. Tie & Peter Gibbs

ctDNA Measured 6-8 weeks following curative resection of Stage II CRC



J. Tie and Peter Gibbs, ASCO 2015

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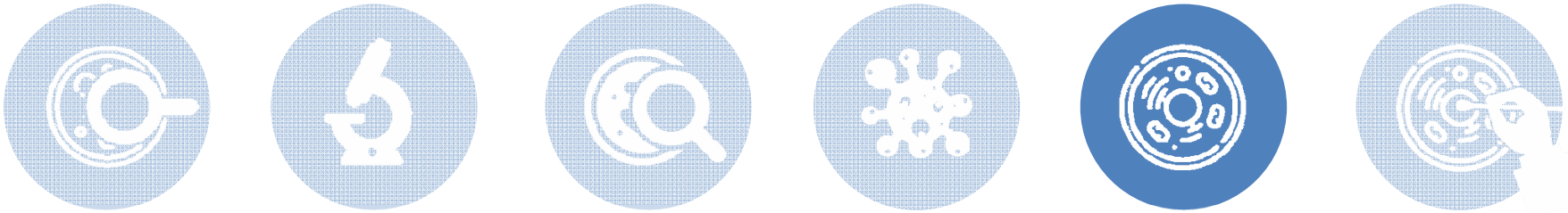

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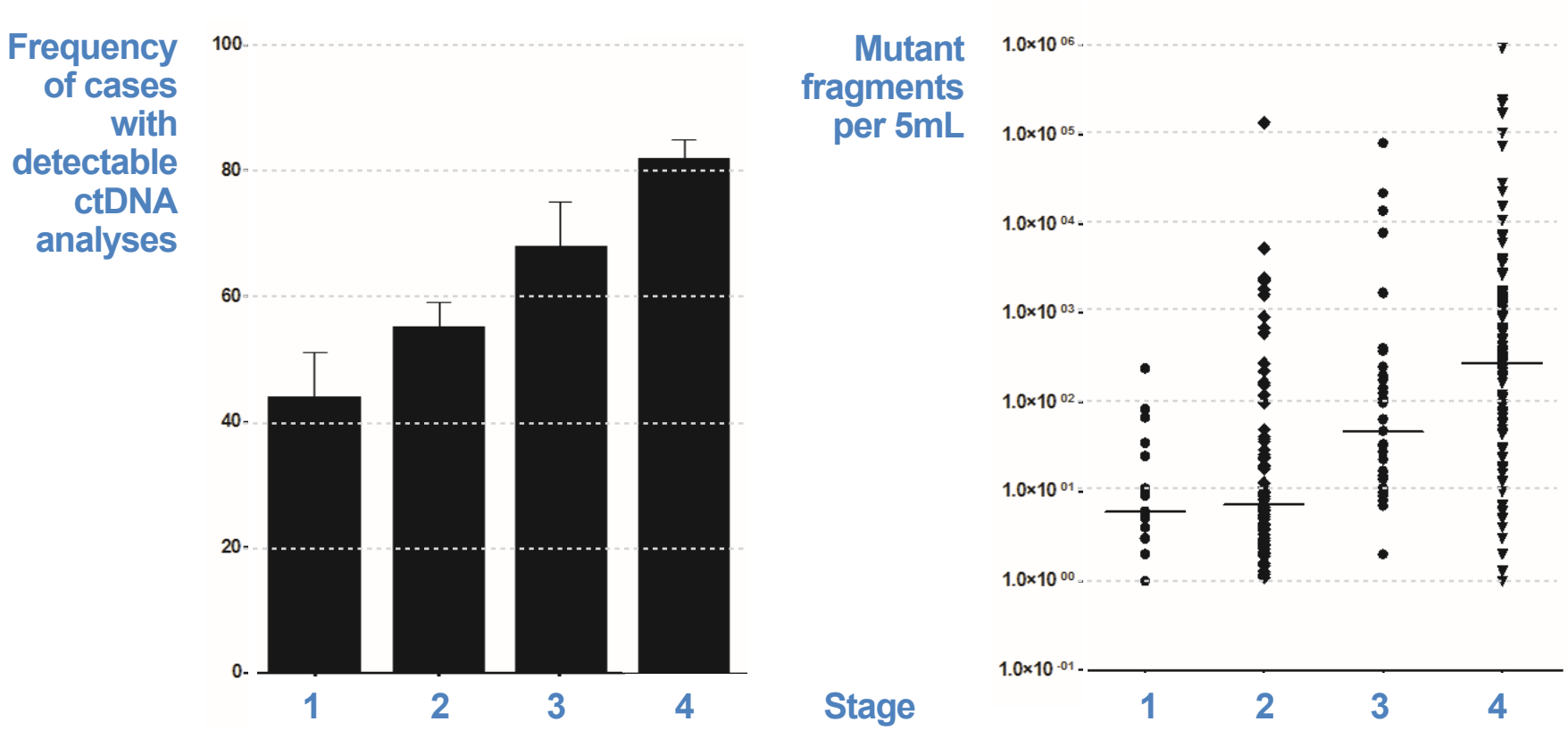
Early Detection

Early Detection using ctDNA Analyses

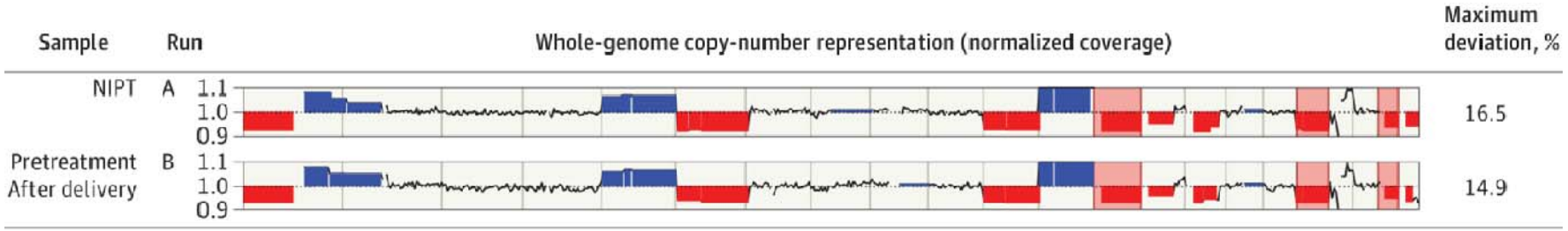


14 Tumor types (n = 684)

Bettegowda et al, Sci Tran Med 2014

Detection of Occult Malignancy from Analyses of cell free Fetal DNA

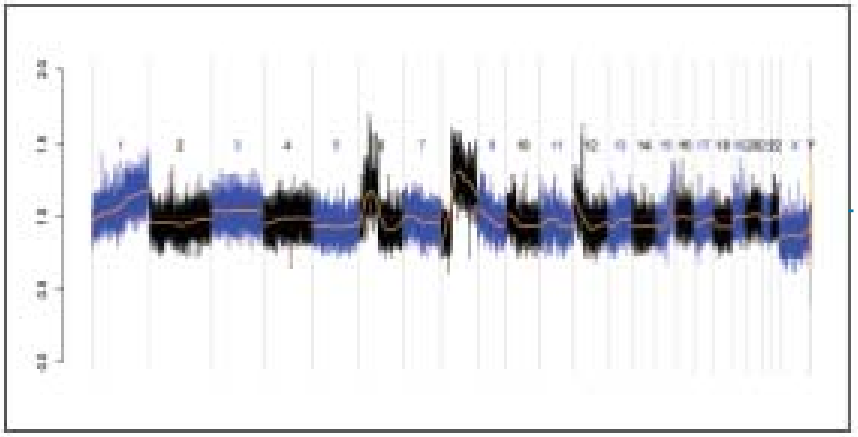
Non-Invasive Prenatal (NIPT) Tests	125,426
Positive for 1 or More Aneuploidies	3757 (3%)
Cases of Maternal Cancer Identified	10



36 year old female at 20 weeks gestation
Monosomy in Chromosomes 21, 18 and 13 persisted post-delivery
Diagnosed with Stage IIA Hodgkin disease

Bianchi, et. al. JAMA 2015

Detection of Occult Malignancy from Analyses of cell free Fetal DNA



400,000 NIPT Tests
38 confirmed Aneuploidies with Neoplasm
17 Mutant, 15 Benign, 6 Unclassified

Type and frequency of maternal malignancies identified adventitiously by NIPT.

Diagnosis	No. of Cases
Hodgkins Lymphoma	2
Non-Hodgkin's Lymphoma	2
Follicular Lymphoma	1
Multiple Myeloma	1
Breast Carcinoma	3
Angiosarcoma	1
Colon Carcinoma	2
Uterine Leiomyoma	11
Uterine Leiomyosarcoma	1
Teratoma (Dermoid Cyst) of Right Ovary	1
Mass on Right Fallopian Tube	1
Non-Reportable, Clinical Feedback Pending	12
Total	38

Dharajiya et al. AMP Abstract 2015

sequenom laboratories

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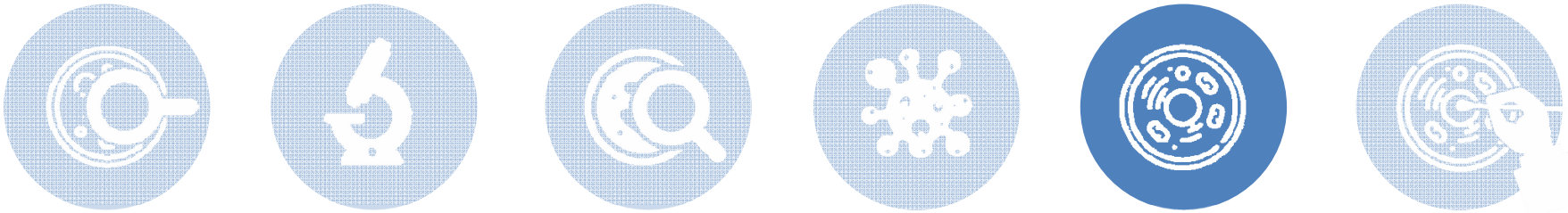

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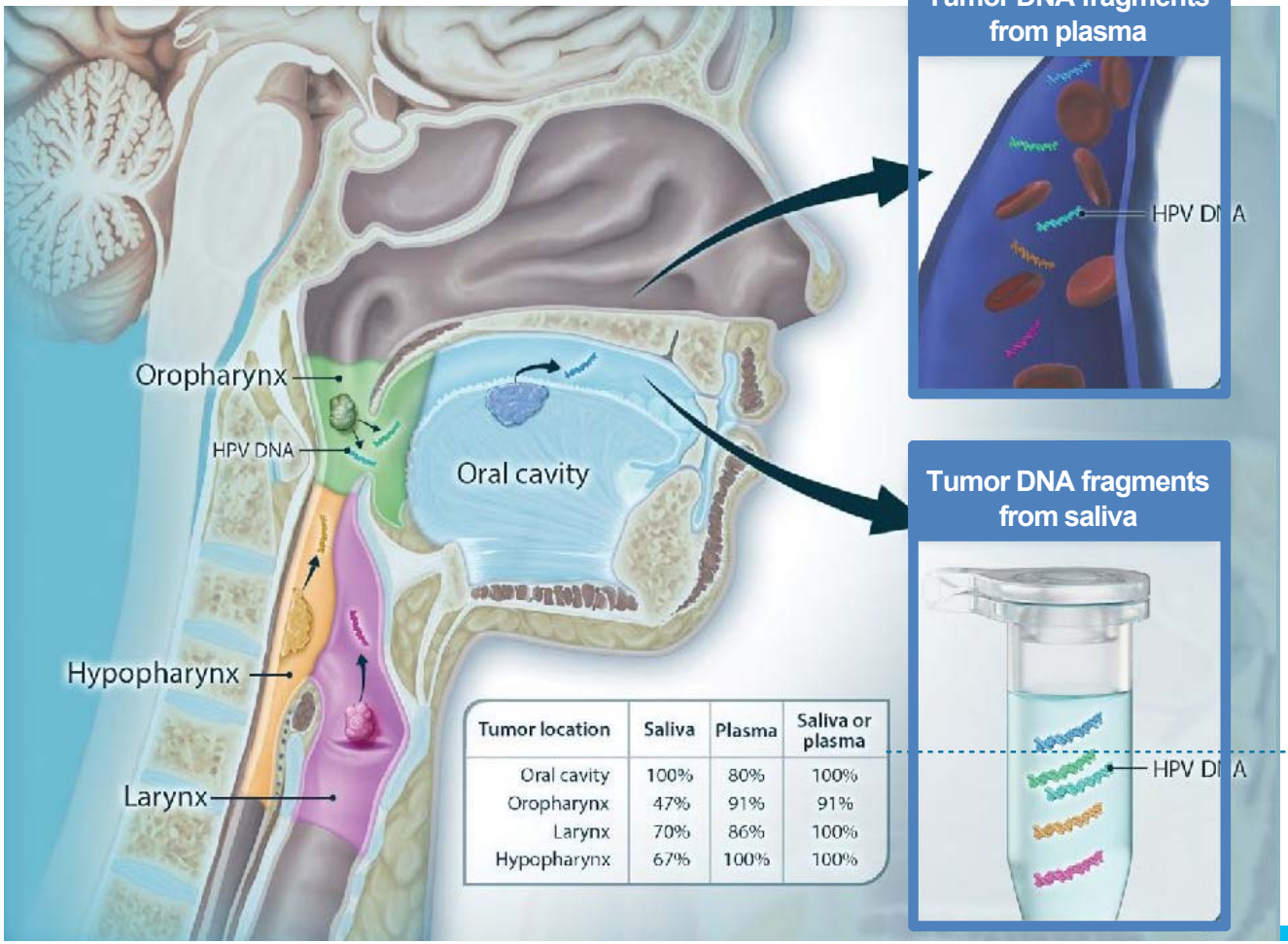
Tumor DNA in Saliva / Plasma



Tumor location	Saliva	Plasma	Saliva or plasma
Oral cavity	100%	80%	100%
Oropharynx	47%	91%	91%
Larynx	70%	86%	100%
Hypopharynx	67%	100%	100%

Tumor DNA fragments from plasma

Tumor DNA fragments from saliva

Sensitivity

Tumor location	Saliva	Plasma	Saliva or plasma
Oral cavity	100%	80%	100%
Oropharynx	47%	91%	91%
Larynx	70%	86%	100%
Hypopharynx	67%	100%	100%

Wang et al., Sci Transl Med 2015

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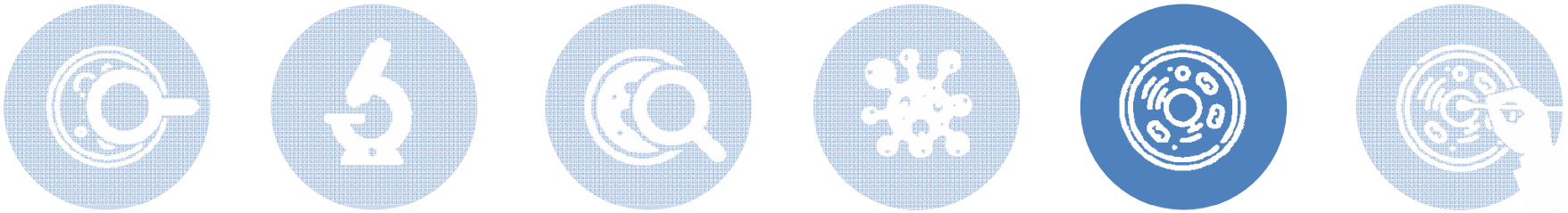

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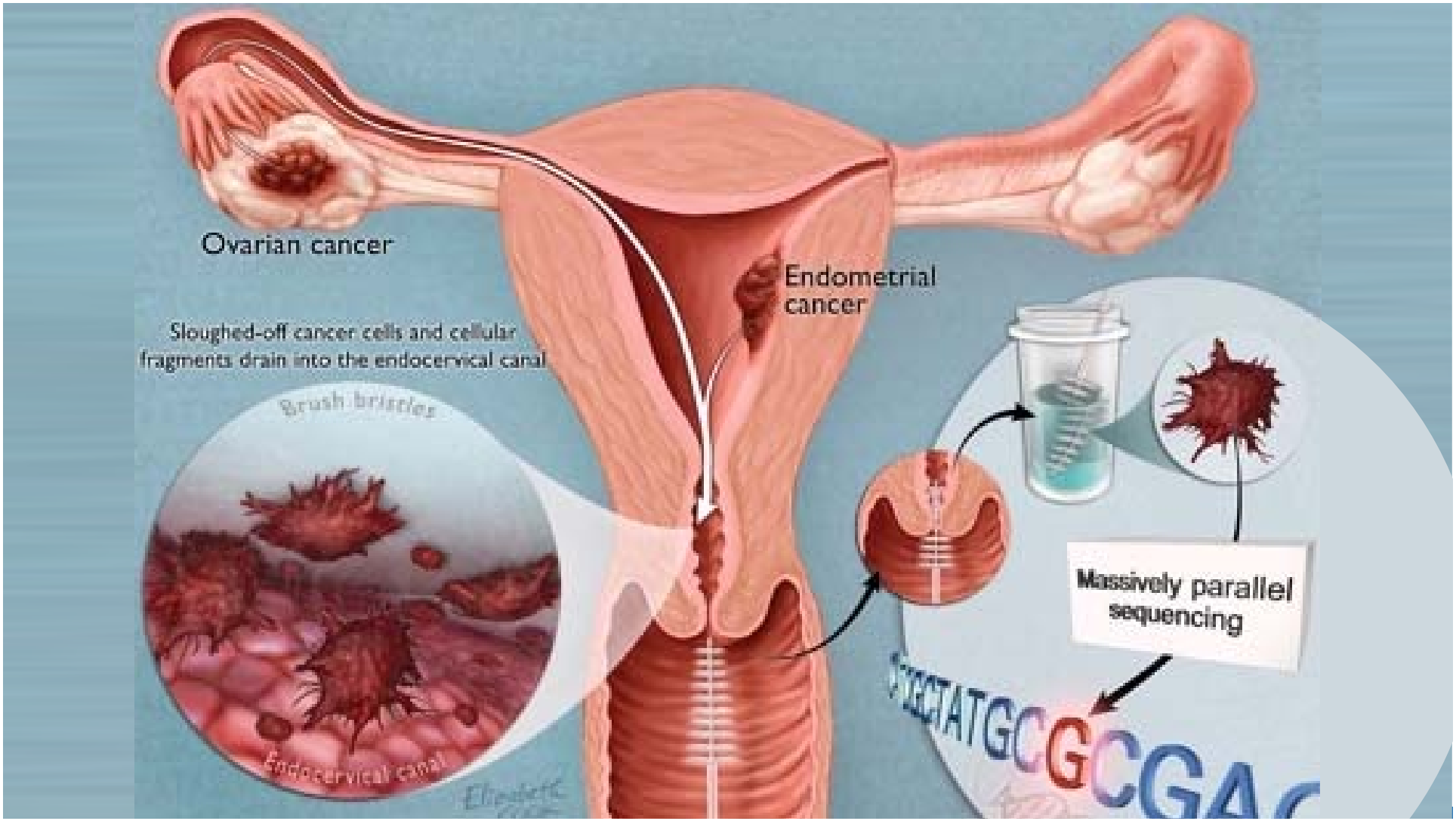
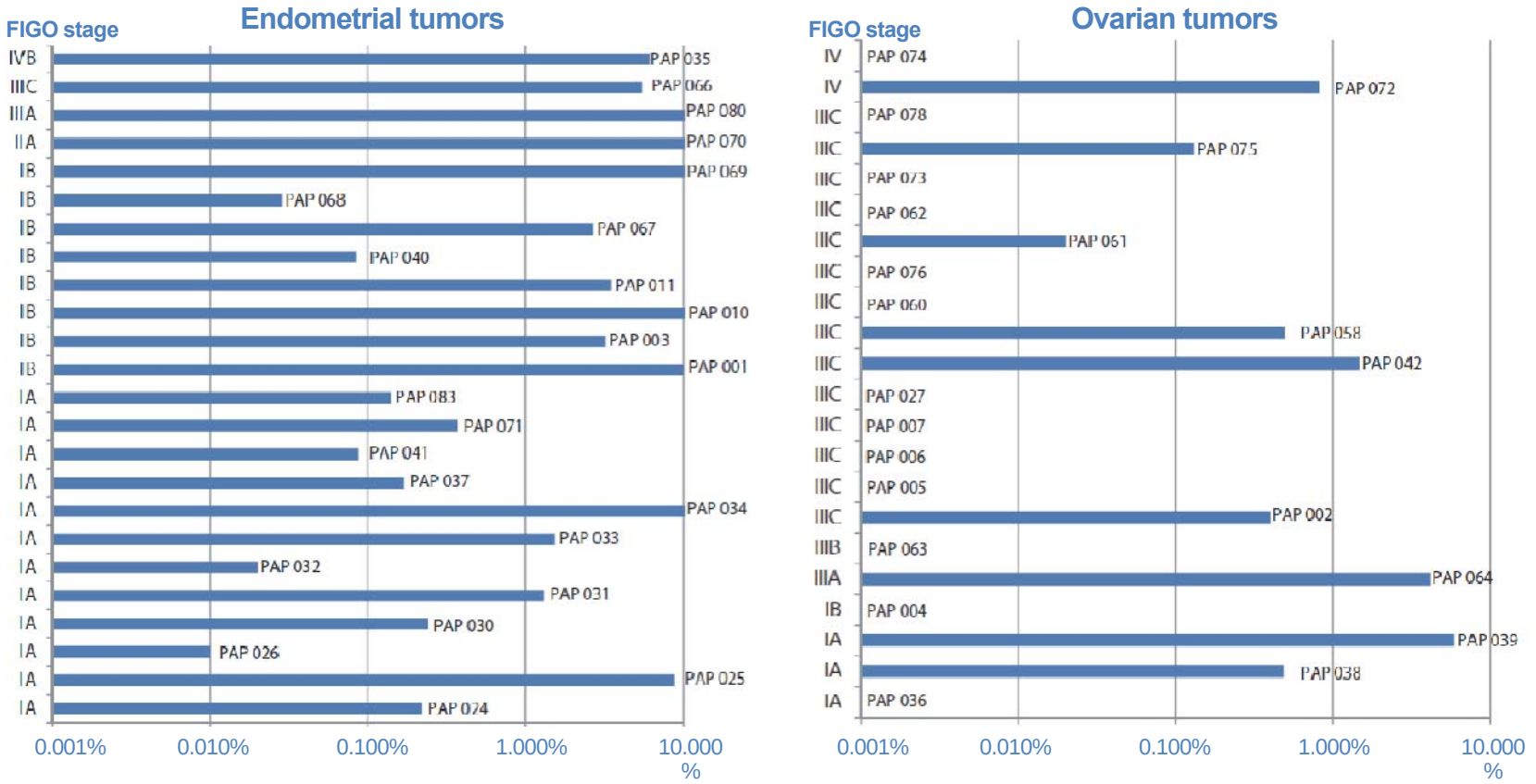
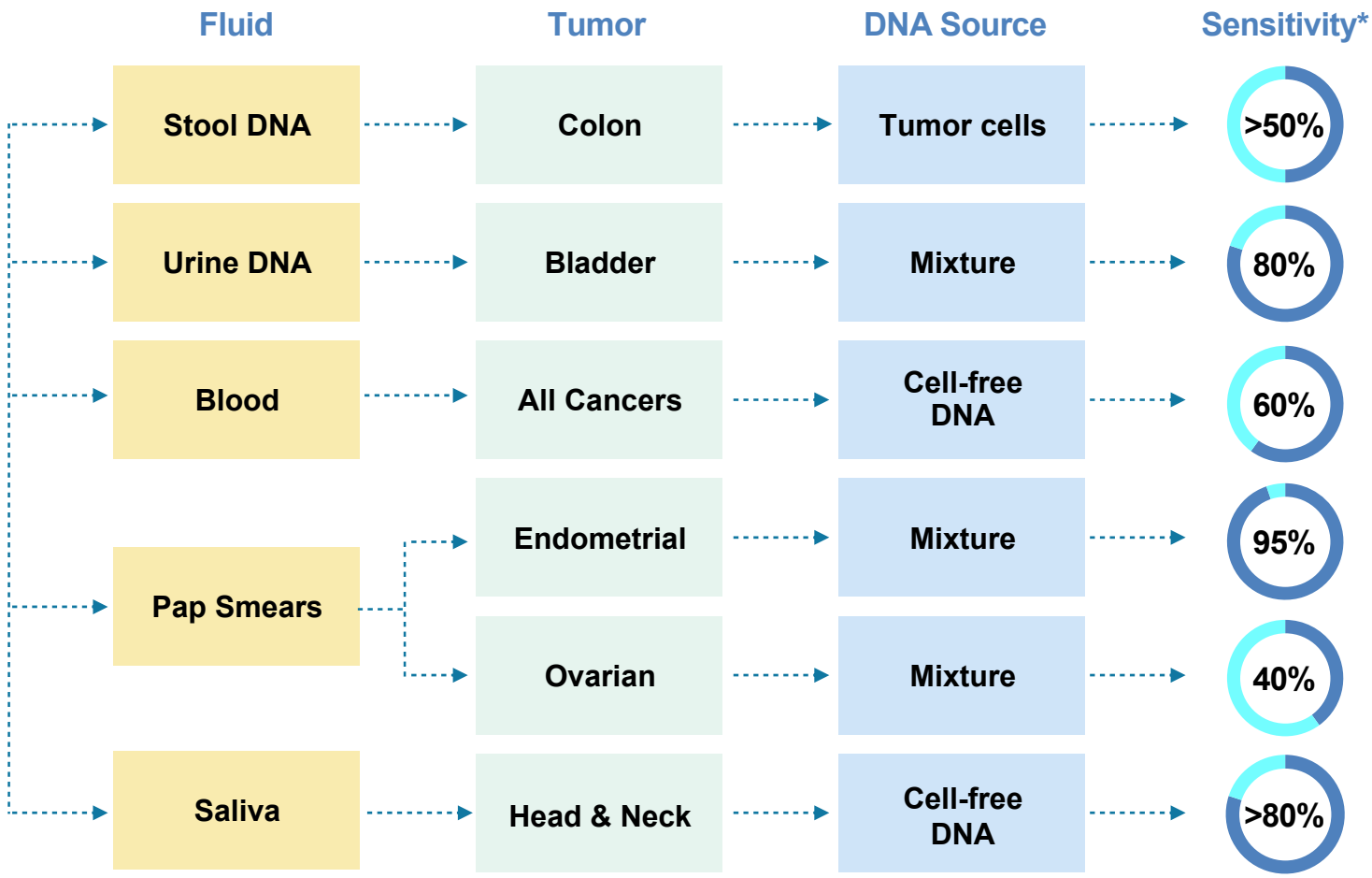

Ovarian cancer
Endometrial cancer
Sloughed-off cancer cells and cellular fragments drain into the endocervical canal
Brush bristles
Massively parallel sequencing

Early Detection - PapGene



Somatic Mutation as Biomarkers



*Stage I and II Disease

Challenges

Not all clonal events are cancer

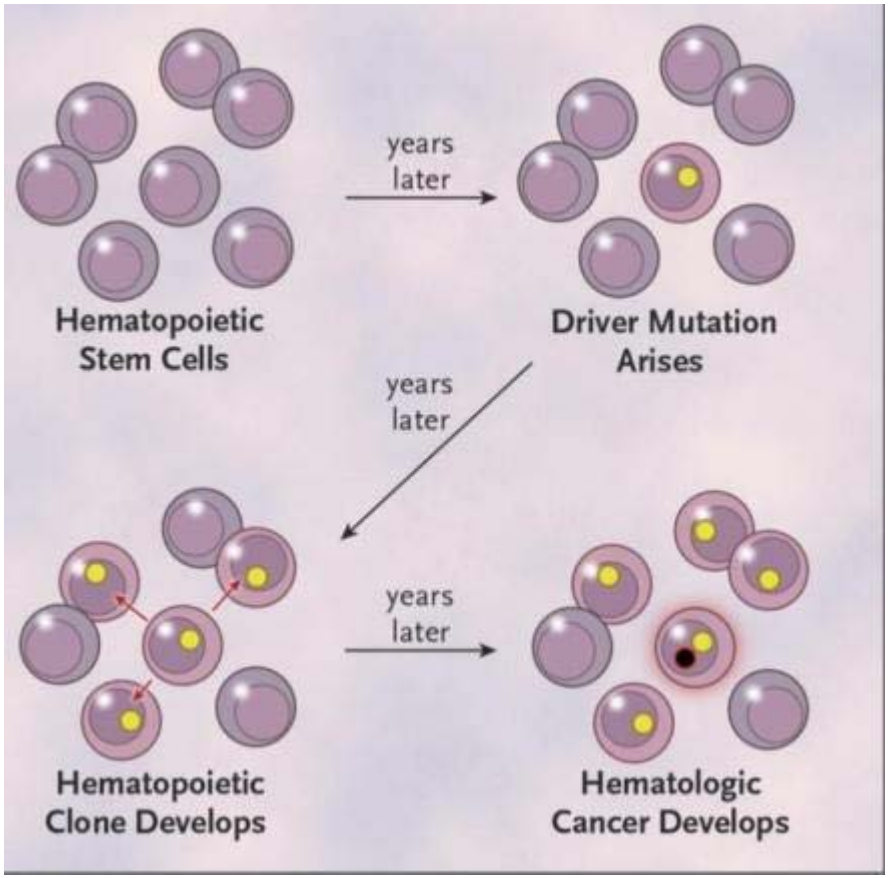
The NEW ENGLAND JOURNAL of MEDICINE

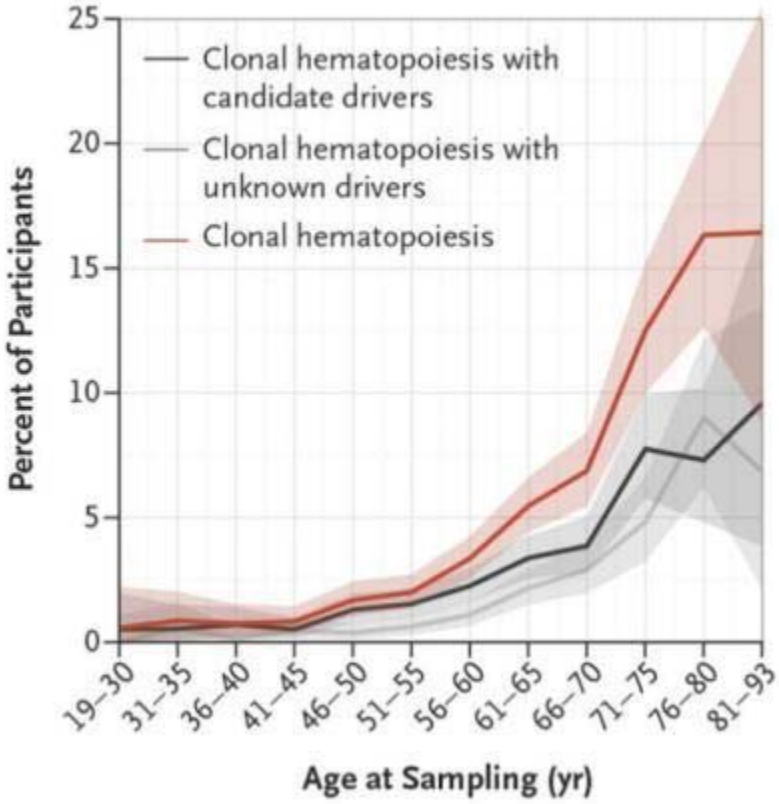
ORIGINAL ARTICLE

Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Whole-exome sequencing of DNA in peripheral-blood cells from 12,380 persons → somatic mutations characteristic of hematologic malignancies were observed in 10% of persons older than 65 years of age

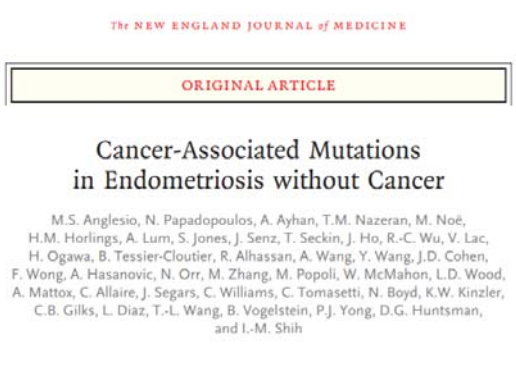
Genovese et al., N Engl J Med 2014; 371:2477-2487





Genovese G et al. N Engl J Med 2014;371:2477-2487.

NOT ALL SOMATIC MUTATIONS ARE CANCER

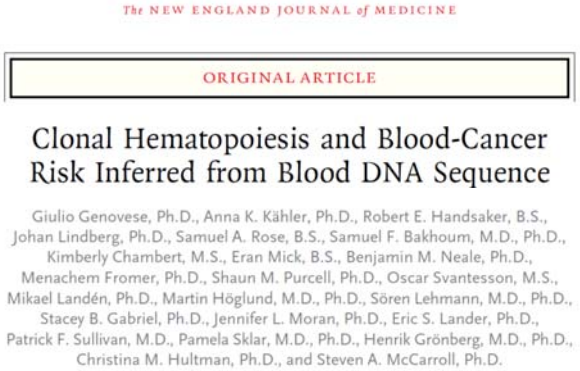


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cancer-Associated Mutations in Endometriosis without Cancer

M.S. Anglesio, N. Papadopoulos, A. Ayhan, T.M. Nazeran, M. Noë, H.M. Horlings, A. Lum, S. Jones, J. Senz, T. Seckin, J. Ho, R.-C. Wu, V. Lac, H. Ogawa, B. Tessier-Cloutier, R. Alhassan, A. Wang, Y. Wang, J.D. Cohen, F. Wong, A. Hasanovic, N. Orr, M. Zhang, M. Popoli, W. McMahon, L.D. Wood, A. Mattox, C. Allaire, J. Segars, C. Williams, C. Tomasetti, N. Boyd, K.W. Kinzler, C.B. Gilks, L. Diaz, T.-L. Wang, B. Vogelstein, P.J. Yong, D.G. Huntsman, and I.-M. Shih

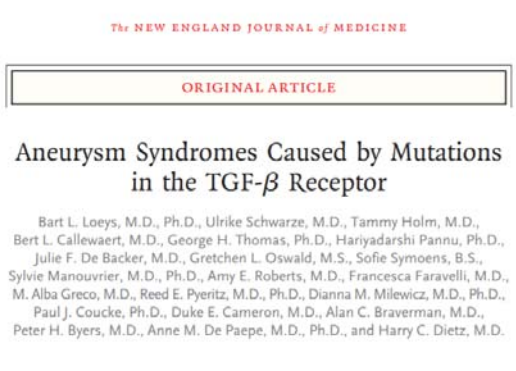


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence

Giulio Genovese, Ph.D., Anna K. Kähler, Ph.D., Robert E. Handsaker, B.S., Johan Lindberg, Ph.D., Samuel A. Rose, B.S., Samuel F. Bakhoum, M.D., Ph.D., Kimberly Chambert, M.S., Eran Mick, B.S., Benjamin M. Neale, Ph.D., Menachem Fromer, Ph.D., Shaun M. Purcell, Ph.D., Oscar Svantesson, M.S., Mikael Landén, Ph.D., Martin Höglund, M.D., Ph.D., Sören Lehmann, M.D., Ph.D., Stacey B. Gabriel, Ph.D., Jennifer L. Moran, Ph.D., Eric S. Lander, Ph.D., Patrick F. Sullivan, M.D., Pamela Sklar, M.D., Ph.D., Henrik Grönberg, M.D., Ph.D., Christina M. Hultman, Ph.D., and Steven A. McCarroll, Ph.D.



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Aneurysm Syndromes Caused by Mutations in the TGF-β Receptor

Bart L. Loeys, M.D., Ph.D., Ulrike Schwarze, M.D., Tammy Holm, M.D., Bert L. Callewaert, M.D., George H. Thomas, Ph.D., Hariyadarshi Pannu, Ph.D., Julie F. De Backer, M.D., Gretchen L. Oswald, M.S., Sofie Symoens, B.S., Sylvie Manouvrier, M.D., Ph.D., Amy E. Roberts, M.D., Francesca Faravelli, M.D., M. Alba Greco, M.D., Reed E. Pyeritz, M.D., Ph.D., Dianna M. Milewicz, M.D., Ph.D., Paul J. Coucke, Ph.D., Duke E. Cameron, M.D., Alan C. Braverman, M.D., Peter H. Byers, M.D., Anne M. De Paepe, M.D., Ph.D., and Harry C. Dietz, M.D.

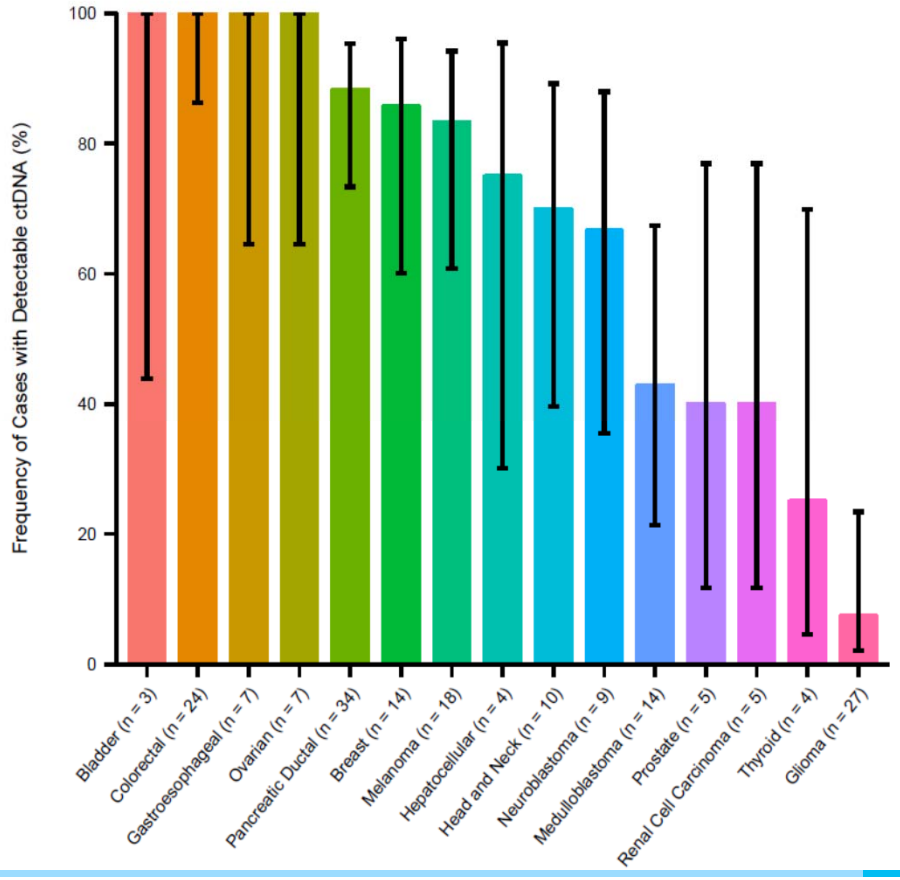
Localization

CASE: A 55 year old male was found to have a persistent KRAS mutation (G12D) in ctDNA at 0.8%

CT Scan, PET Scan, Colonoscopy and PSA are normal.

What is this? Cancer of the Lung, Colon Pancreas? Precursor?

Heterogeneity



- ~80% late stage tumors shed ctDNA
- Anatomic barriers to tumor DNA release into circulation
- Heterogeneity in shedding

Biological Limitations

- Not many mutant molecules in blood
- On average 1,000-3,000 genome equivalents per mL
- Need at least 3 molecules to call a positive result
- Sensitivities are limited by insufficient mutant molecules

Challenges for ctDNA in the future

Biology

- Not all clonal events are cancer
- Heterogeneity
- Localization
- Very few molecules in blood

Lack of focus on feasible umet clinical need

- Few targeted therapeutics
- Cost does not match clinical benefit or need

Future for ctDNA

Incremental improvements in technology

- Increase in comprehensive panels
- Limited by biology more that technology
- Need a biologic based discovery to drive dramatic improvement

Clinical Application

- Tumor genotyping in plasma will be integrate into routine practice – based on concordance studies
- High impact applications that drive improvements in SURVIVAL will require prospective clinical trials and partnership with FDA.

Ludwig Center for Cancer Genetic and Therapeutics

SWIM ACROSS AMERICA
★ MAKING WAVES TO FIGHT CANCER ★

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Acknowledgments

Ludwig Center at Johns Hopkins
Bert Vogelstein
Ken Kinzler
Nick Papadopoulos
Shibin Zhou
Isaac Kinde
Yuxuan Wang
Chetan Bettegowda
Nishant Agrawal
Cherie Blair
Kathy Romans-Judge
A-Team
Kerstin Schmidt
Rebecca Leary

Johns Hopkins Cancer Center
Matthias Holdhoff
Hao Wang

Brandon Luber

Pathology at Hopkins
Ralph Hruban
Laura Wood
George Netto

Ludwig Australia
Jeannie Tie
Peter Gibbs

Inostics
Frank Diehl
Philip Aggencort

Swim Across America Laboratory
Bjarne Bartlett
Aleksandra Eyrling

UT Southwestern
Michael Choti

Harvard
Martin Nowak

Stanford
Steven Goodman

Amgen
Kelly Oliner

Memorial Sloan Kettering Cancer Center

Opportunities and Limitations of ctDNA as a Clinical Biomarker in Cancer Management: New Insights in the Clinical Application of ctDNA

QUESTIONS