Μοριακή Παθολογική Ανατομική

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2022

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

Μοριακή Διάγνωση

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
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- Η μοριακή διαγνωστική είναι ένας σχετικά νέος κλάδος της σύγχρονης ιατρικής που στοχεύει στην **ασφαλή** και **γρήγορη** διάγνωση διαφόρων παθήσεων, συμπεριλαμβανομένου του καρκίνου των συμπαγών οργάνων (σαρκώματα, καρκινώματα, νεοπλάσματα κεντρικού νευρικού συστήματος).

- Βασίζεται στην ανάλυση **γενετικού υλικού** ή/και **πρωτεϊνών** από κύτταρα ή/και ιστούς ασθενών με νεοπλασματική νόσο

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

Ρόλος και σημαντικότητα

- Χρησιμοποιεί πολύ **μικρή ποσότητα** υλικού για να θέσει σίγουρη και ασφαλή **Διάγνωση**
- Προσδιορίζει σε αρκετές περιπτώσεις τα **αίτια** των νεοπλασιών (π.χ. σε αντιμεταθεσεις, απώλεια ετεροζυγωτίας κ.λ.π.)
- Δίνει σημαντικές πληροφορίες για τη **φυσική πορεία** και την εξέλιξη της νόσου
- Οι πληροφορίες έχουν άμεση κλινική εφαρμογή αφού μπορούν να χρησιμοποιηθούν τόσο για τη **θεραπεία** όσο και για την αξιολόγηση της **ανταπόκρισης** σε θεραπευτικά σχήματα

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
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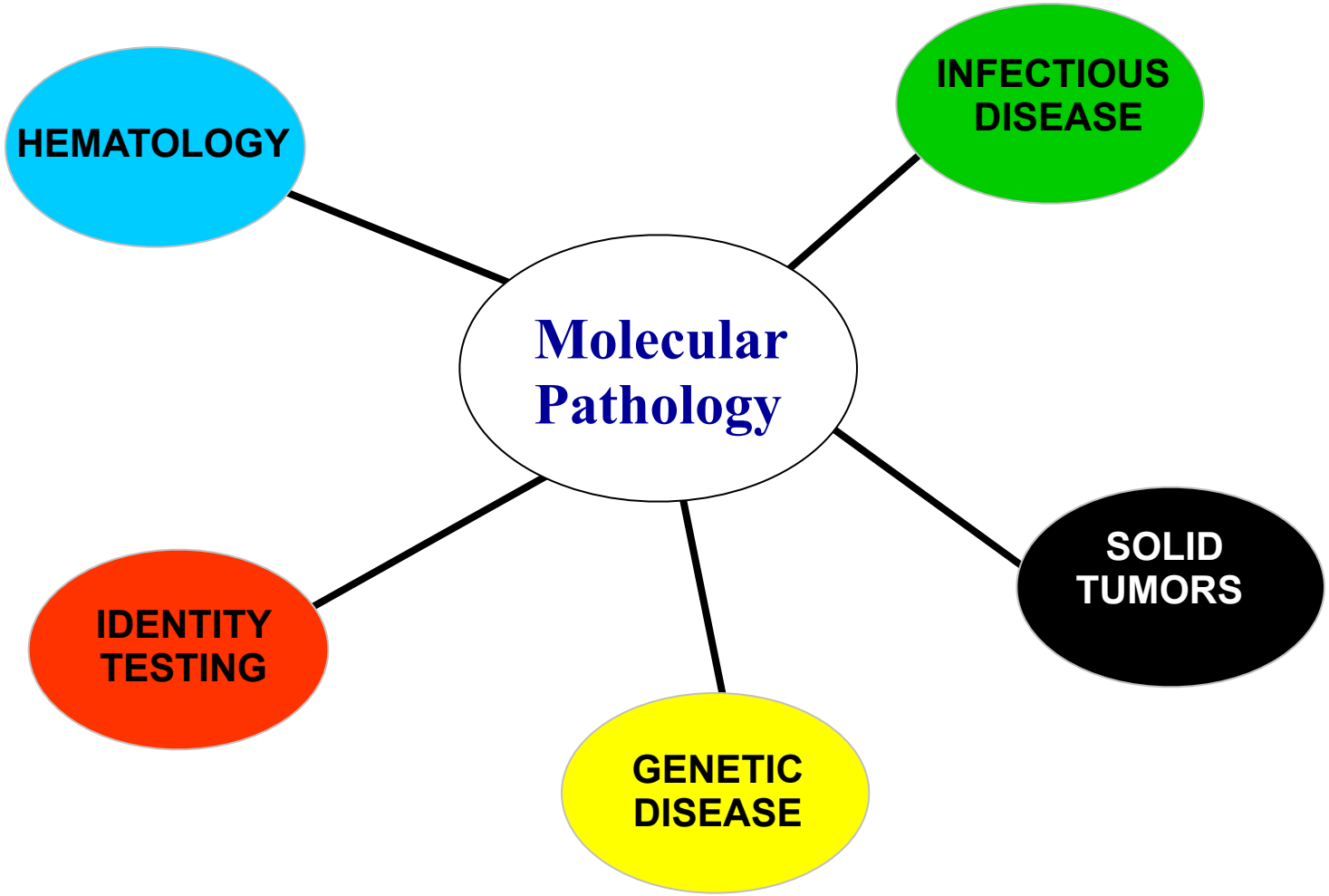
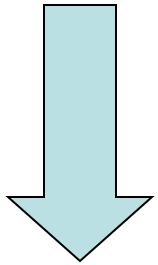
Molecular Pathology

A Universal Discipline of Laboratory Medicine



Υπόθεση

- Η φαινοτυπική ποικιλομορφία ενός όγκου συνοδεύεται από αντίστοιχη ποικιλία στο προφίλ της γονιδιακής έκφρασης → ανίχνευση με μεθόδους ΜΔ



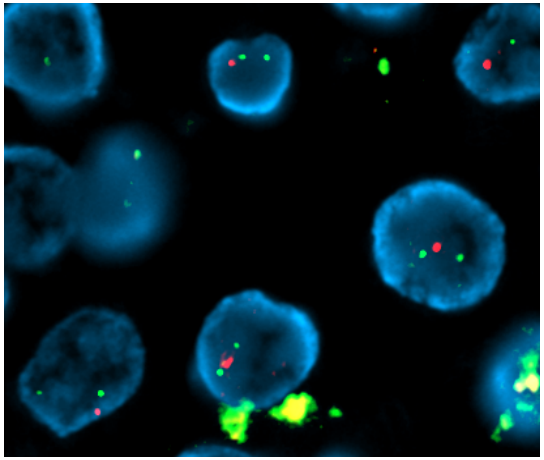
- Ταυτοποίηση του γονιδιακού προφίλ ενός όγκου αποτελεί τη βάση για σωστή διάγνωση και ταξινόμηση

Αλγόριθμος προσέγγισης

Ασθενής που πάσχει από νεοπλασματική νόσο

Χειρουργείο / Βιοψία

Μοριακή επεξεργασία ιστού



Καθορισμός μοριακού προφίλ του όγκου

Πρόγνωση

Ακριβής διάγνωση

Εξατομικευμένη/στοχευμένη Τχ

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

Άξονες ΜΔ

- Επιστημονικό υπόβαθρο
- Μεθοδολογία και τεχνολογική υποδομή
- Υπακοή στους κανόνες βιοηθικής

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
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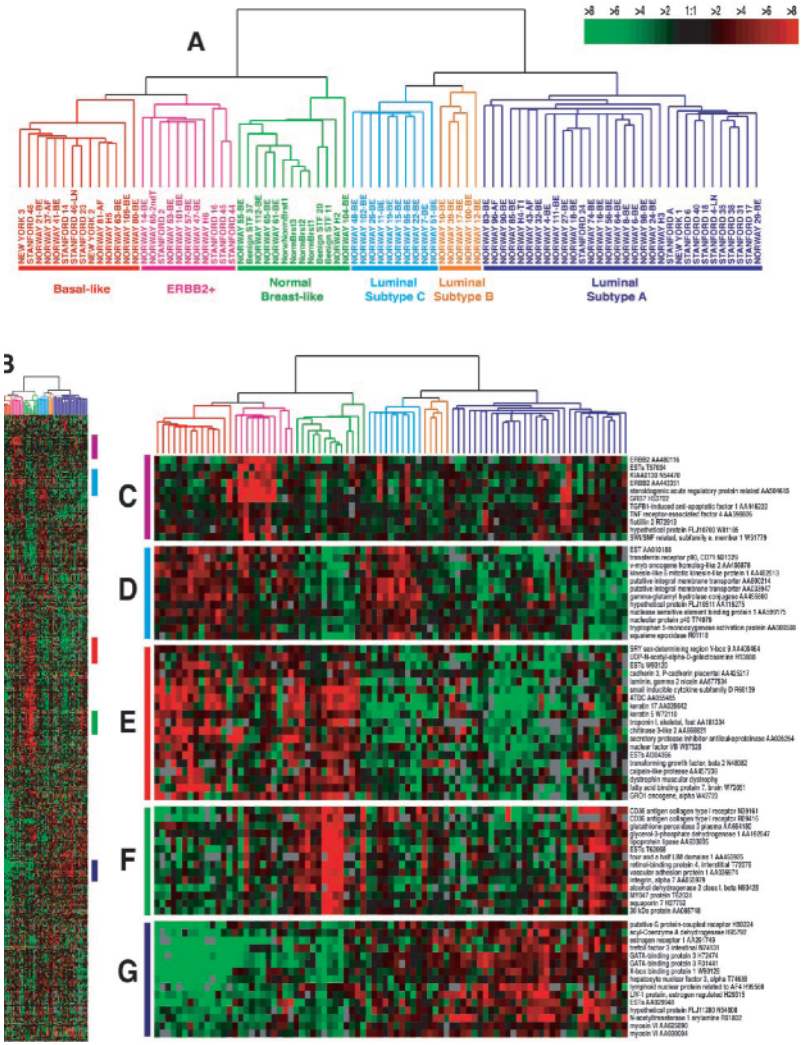
Επιστημονικό Υπόβαθρο

Καθορισμός του γονιδιακού προφίλ των ΣΟ

Το παράδειγμα του καρκίνου του μαστού

Καθορισμός του γονιδιακού προφίλ των ΣΟ: το παράδειγμα του καρκίνου του μαστού



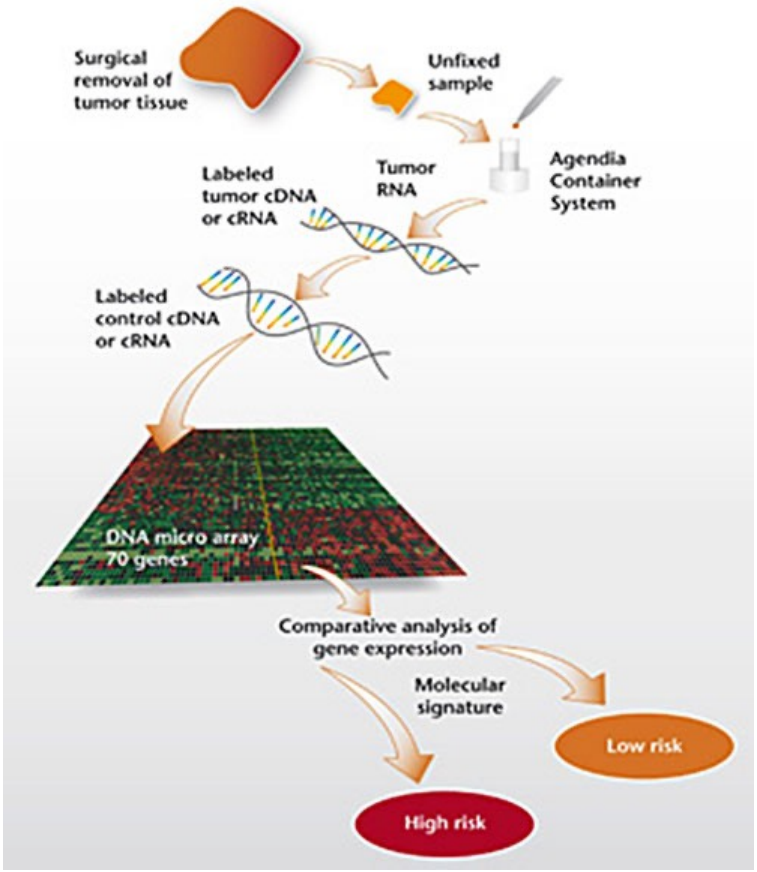
78 καρκινώματα, 3 ινοαδενώματα, 4 φυσιολογικά δείγματα

456 γονίδια

Basal-like και Cerb-b2+: χειρότερη Px

Sorlie et al, PNAS 2001

Multiplexed Molecular Dx **MammaPrint®: Px test**



FDA

agendia™

(Agendia BV, The Netherlands)

FDA approved December 12, 2007

DNA micro array-based in vitro diagnostic laboratory service that measures the activity of 70 genes

The assay are focuses primarily on proliferation with additional genes associated with invasion, metastasis, stromal integrity and angiogenesis

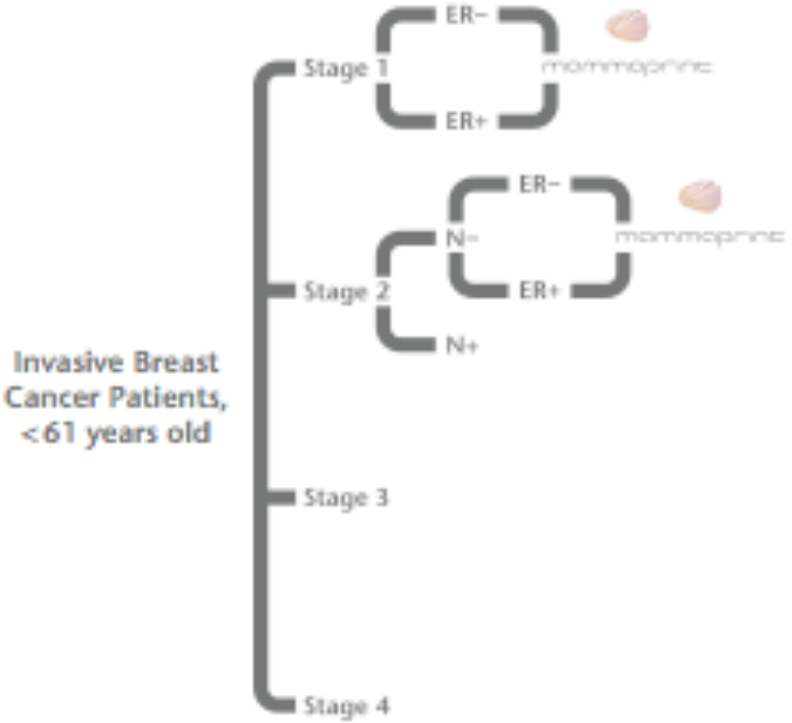
Expert Rev Mol Diagn. 2009;
Cancer Genomics Proteomics. 2007

MammaPrint®

- **MammaPrint® is currently available for breast cancer patients who are:**

1. <61 years old
2. stage I or II disease
3. with a tumor size ≤ 5 cm
4. lymph node negative
5. without any limitation in treatment.

- Eligibility will be broadened in the near future.
- FFPET applications (from June 2007)



ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
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Treatment tailoring by profiling

Premenopausal, lymph node-negative

GENE EXPRESSION PROFILING

60%

Poor Signature

~ 56 % metastases at 10 yrs
~ 50 % death at 10 yrs

Adjuvant chemo - and hormonal therapy

40%

Good Signature

~ 13 % metastases at 10 yrs
~ 4 % death at 10 yrs

No adjuvant therapy or hormonal therapy only

Mammaprint *vs* Oncotype Dx

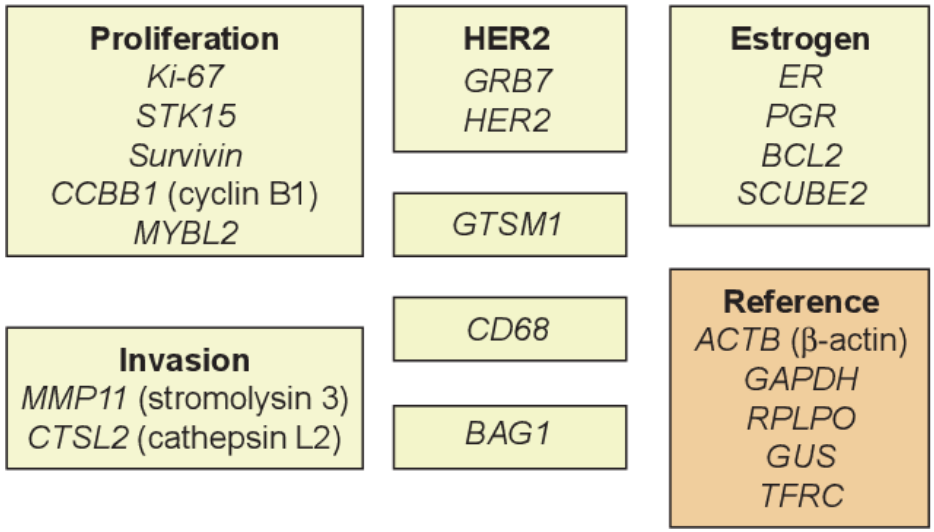
- **Oncotype DX™** is a 21-gene RT-PCR assay; Genomic Health)
- The Oncotype DX Breast Recurrence Score Test for people diagnosed with **early-stage**, **ER+**, **HER2-negative** invasive breast cancer
- The Oncotype DX Breast DCIS Score Test for people diagnosed with **DCIS** (ductal carcinoma in situ)
- The Oncotype DX **Breast Recurrence Score Test** analyzes the activity of a group of genes that can affect how an early-stage breast cancer is likely to behave and respond to treatment
-

Oncotype DX

- the likelihood that the **breast cancer will return**
- possible **benefit from chemotherapy** to treat early-stage invasive breast cancer

- The Oncotype DX Breast **Recurrence Score Test** is used in two ways:

1. to help doctors figure out a **person's risk** of early-stage, estrogen-receptor-positive breast cancer coming back in a part of the body away from the breast (distant recurrence)
2. to help figure out if a person will **benefit from chemotherapy**



TAILORx **N0**

N=9,719

The Oncotype DX® test is the only predictive marker, and it provides precise chemotherapy benefit estimates[1-4]

	Recurrence Score® result				
	0–10	**11–15**	**16–20**	**21–25**	**26–100**
Age >50 years n=6,665 (69%)	**No CT Benefit** n=1,190 (12%)	**No CT Benefit** n=1,572 (16%)	**No CT Benefit** n=1,789 (18%)	**No CT Benefit** n=1,134 (12%)	**CT Benefit** n=980 (10%)
Age ≤50 years n=3,054 (31%)	**No CT Benefit** n=429 (4%)	**No CT Benefit** n=801 (8%)	**~1.6%CT Benefit** n=923 (9%)	**~6.5%CT Benefit** n=492 (5%)	**CT Benefit** n=409 (4%)

	Patients ≤50 years	
Low clinical risk[a]	7% of all patients **No CT benefit**	3% of all patients **~6.4% CT benefit**
High clinical risk[b]	2% of all patients **~6.5% CT benefit**	2% of all patients **~8.7% CT benefit**

Based on an exploratory analysis of TAILORx study.

The Oncotype DX is a test that may predict how likely it is that your breast cancer will return.
It also predicts whether you will benefit from having chemotherapy in addition to hormone therapy.
The test results can help you and your doctors make a treatment plan that's right for you.

This test can be done on early-stage breast cancers (stage 1 or 2) that:

- Have receptors for estrogen (estrogen-receptor positive)
- Don't have large amounts of the human epidermal growth factor protein (HER2 negative)

Next Generation Sequencing (NGS)

- Modern high-throughput DNA sequencing technologies
- parallel, rapid
- Decreasing price, time, workflow complexity, error rate
- Increasing data quantity and quality, read lenght (data storage capacity), repertoire of bioinformatics tools
- Wide range of applications

- Third Generation Sequencing (single molecule, real time, *in situ* ...)

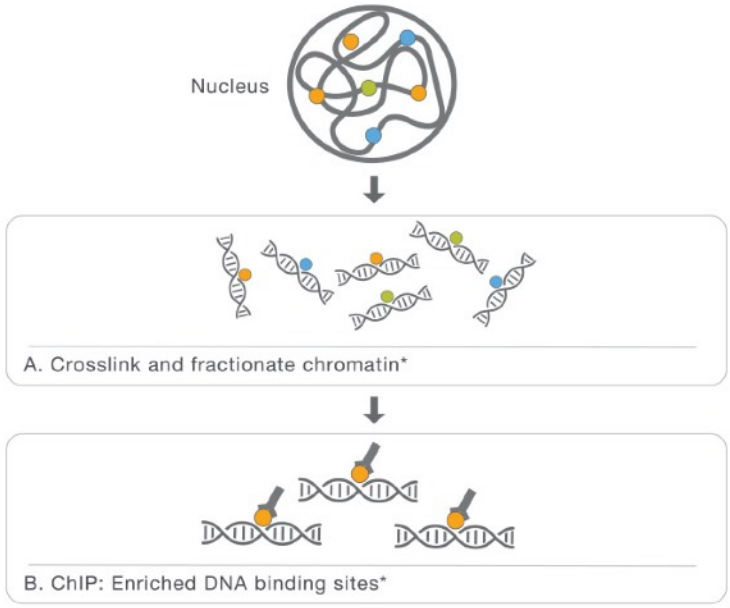
Next Generation Sequencing (NGS)

- Starting material:

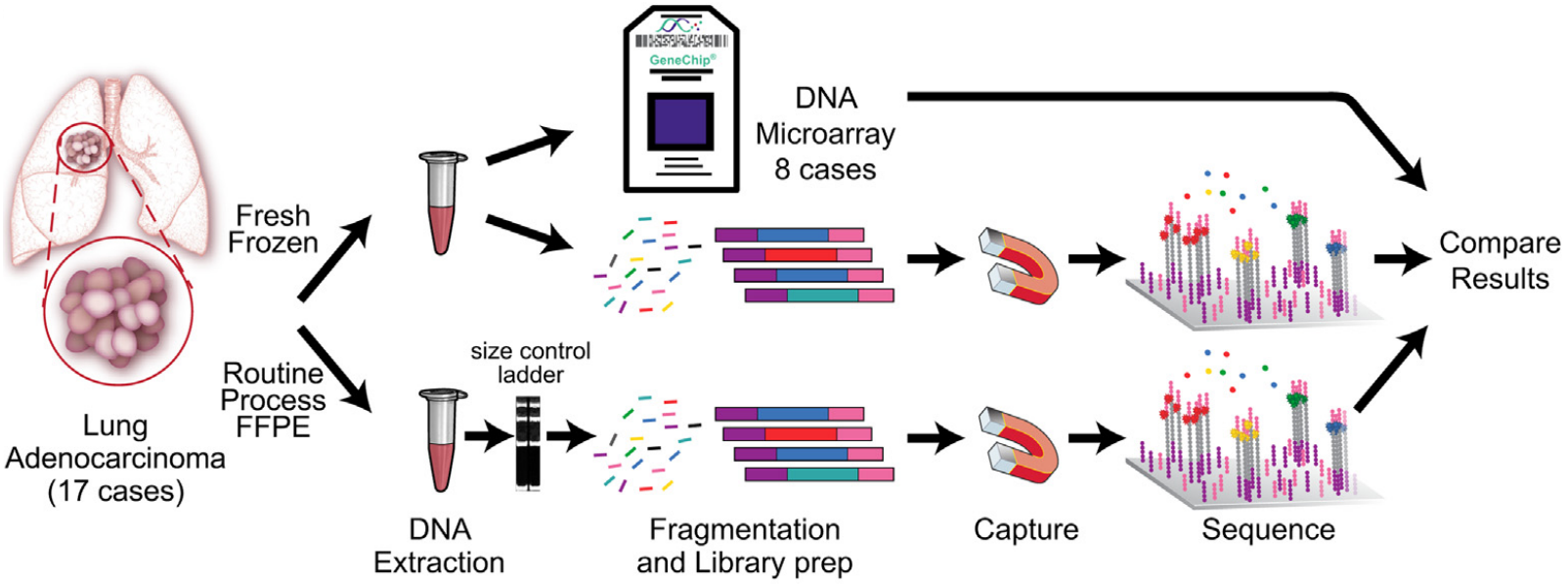
  - DNA (DNA-seq)

  - RNA (RNA-seq)

  - DNA fragments bound to selected protein – to analyse the sequences of DNA-binding sites of protein of interest or localisation of histone modifications (ChIP-seq)



Next Generation Sequencing (NGS)

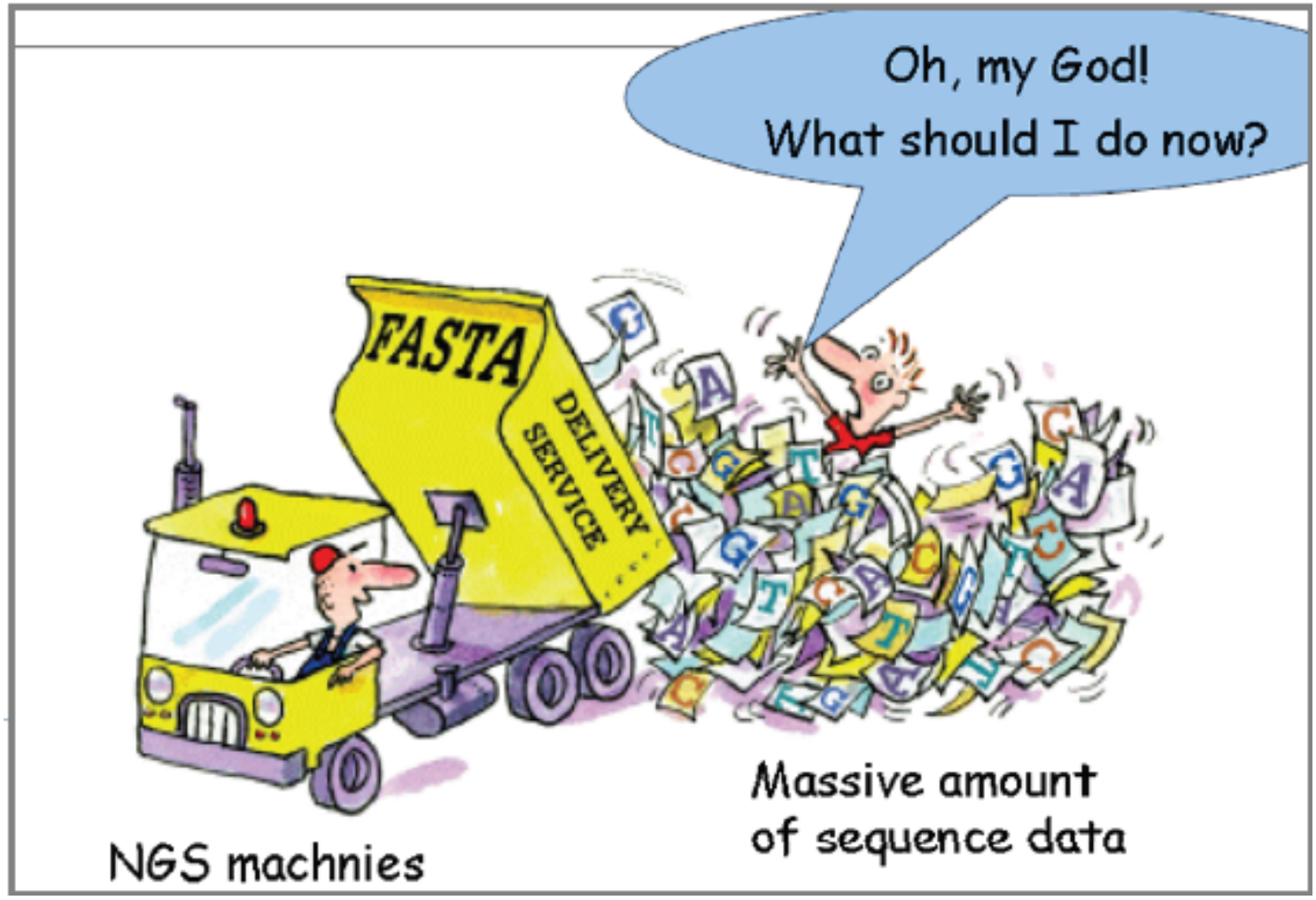


Αναδιατάξεις που αναλύονται: ATF1-EWSR1, CHIC2-ETV6, COL1A1-PDGFB, COL1A1-USP6, ETV6-ABL1, ETV6-ABL2, ETV6-ACSL6, ETV6-ARNT, ETV6-BAZ2A, ETV6-CDX2, ETV6-FLT3, ETV6-GOT1, ETV6-ITPR2, ETV6-JAK2, ETV6-LYN, ETV6-MDS2, ETV6-MECOM, ETV6-MN1, ETV6-NTRK3, ETV6-PDGFRA, ETV6-PDGFRB, ETV6-PER1, ETV6-PRDM16, ETV6-RUNX1, ETV6-RUNX1_AML1, ETV6-SYK, EWSR1-ATF1, EWSR1-CREB1, EWSR1-DDIT3, EWSR1-ERG, EWSR1-ETV1, EWSR1-ETV4, EWSR1-FEV, EWSR1-FLI1, EWSR1-NFATC2, EWSR1-NR4A3, EWSR1-PATZ1, EWSR1-PBX1, EWSR1-SMARCA5, EWSR1-SP3, EWSR1-YY1, EWSR1-ZNF384, EWSR1-ZNF444, FUS-DDIT3, LINC00598-ETV6, MN1-ETV6, MNX1-ETV6, NFATC2-EWSR1, NTRK3-ETV6, PAX3-FOXO1, PAX3-NCOA1, PAX3-NCOA2, PAX5-ETV6, PAX7-FOXO1, SS18-SSX1, SS18-SSX4, SYK-ETV6, YY1-EWSR1 Βιβλιογραφία

Mertens F, Antonescu CR, Mitelman F. Gene fusions in soft tissue tumors: Recurrent and overlapping pathogenetic themes. Genes Chromosomes Cancer. 2016 Apr;55(4):291-310.

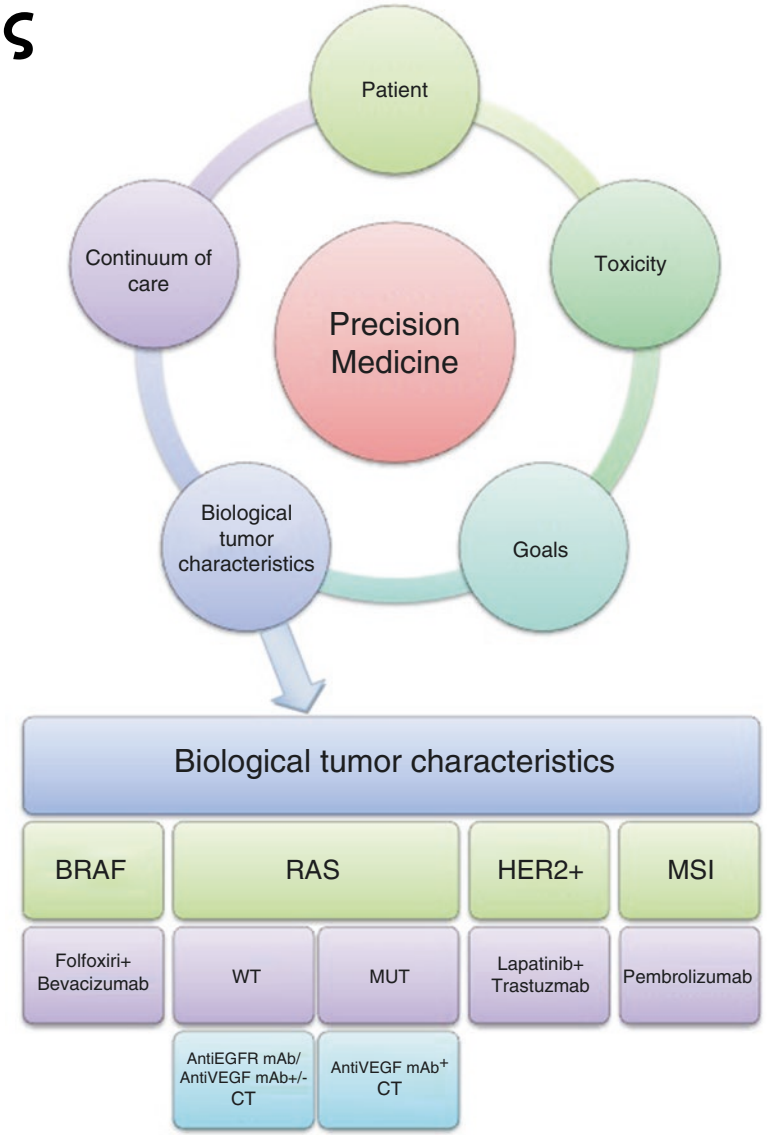
***__Σημείωση__ : Κάθε ανάλυση έχει εσωτερική πιθανότητα λάθους 0,5-1%. Αυτό οφείλεται σε σπάνια γεγονότα και παράγοντες που εμπλέκονται στην παρασκευή και ανάλυση των δειγμάτων.

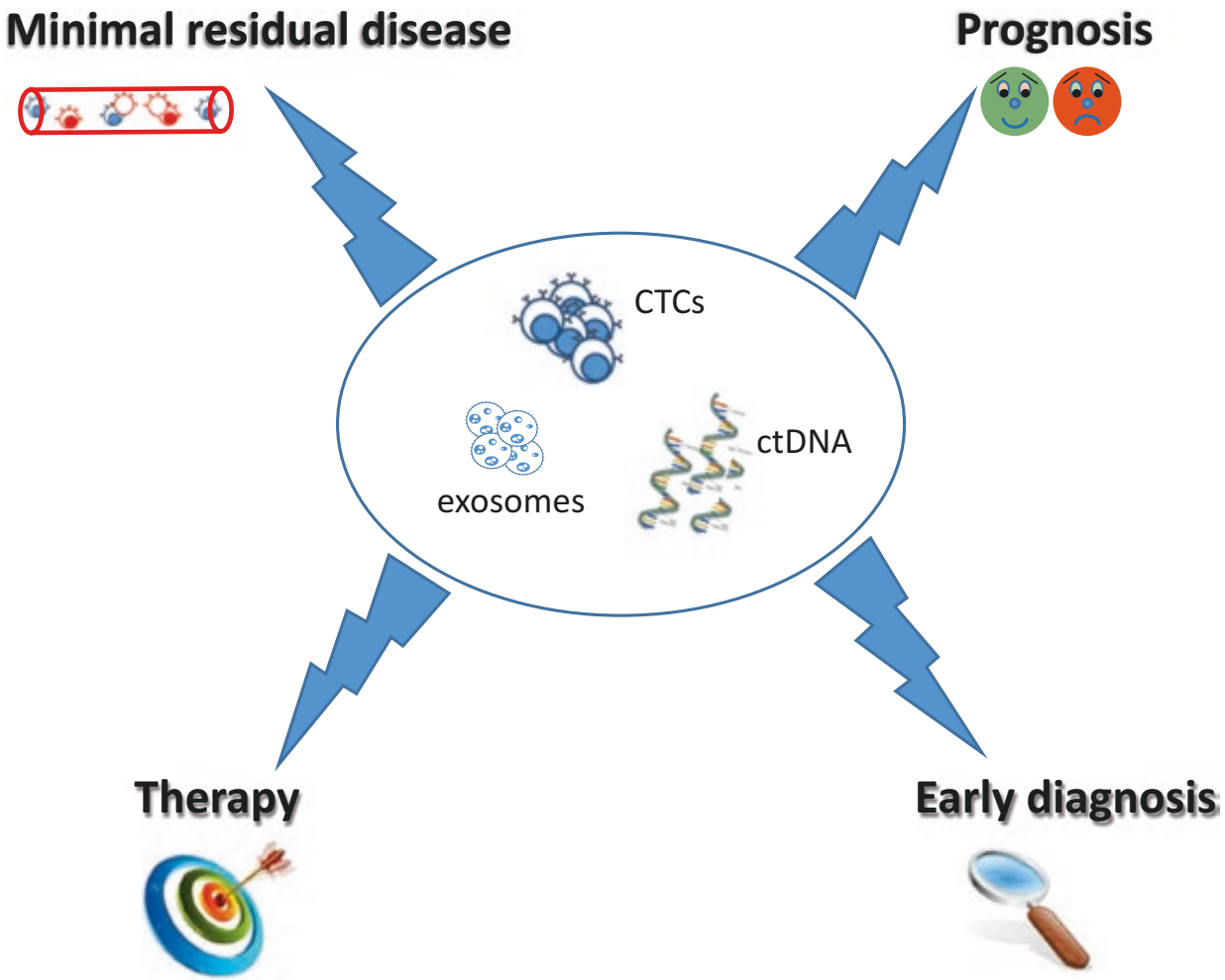
- Δεν είναι Ewing's
- Δεν είναι epithelioid sarcoma
- Δεν είναι extraskeletal CHS
- Δεν είναι clear cell sarcoma

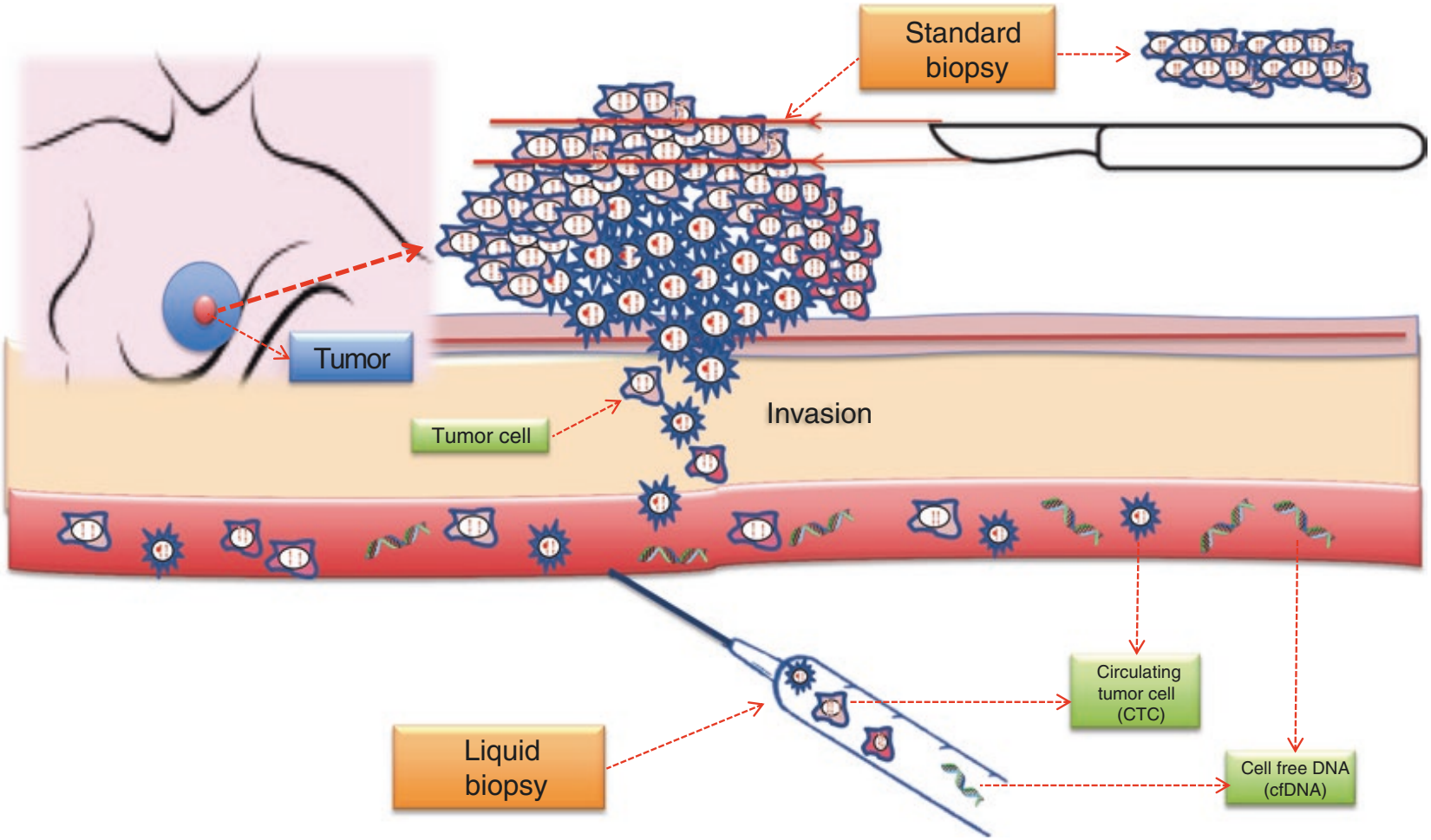

Oh, my God!
What should I do now?
FASTA
DELIVERY SERVICE
NGS machnies
Massive amount
of sequence data

Ιατρική Ακριβείας

1.γονίδια
2.περιβάλλον
3.τρόπος ζωής




Minimal residual disease
Prognosis
CTCs
ctDNA
exosomes
Therapy
Early diagnosis


Standard biopsy
Tumor
Tumor cell
Invasion
Circulating tumor cell (CTC)
Liquid biopsy
Cell free DNA (cfDNA)

Liquid Bx

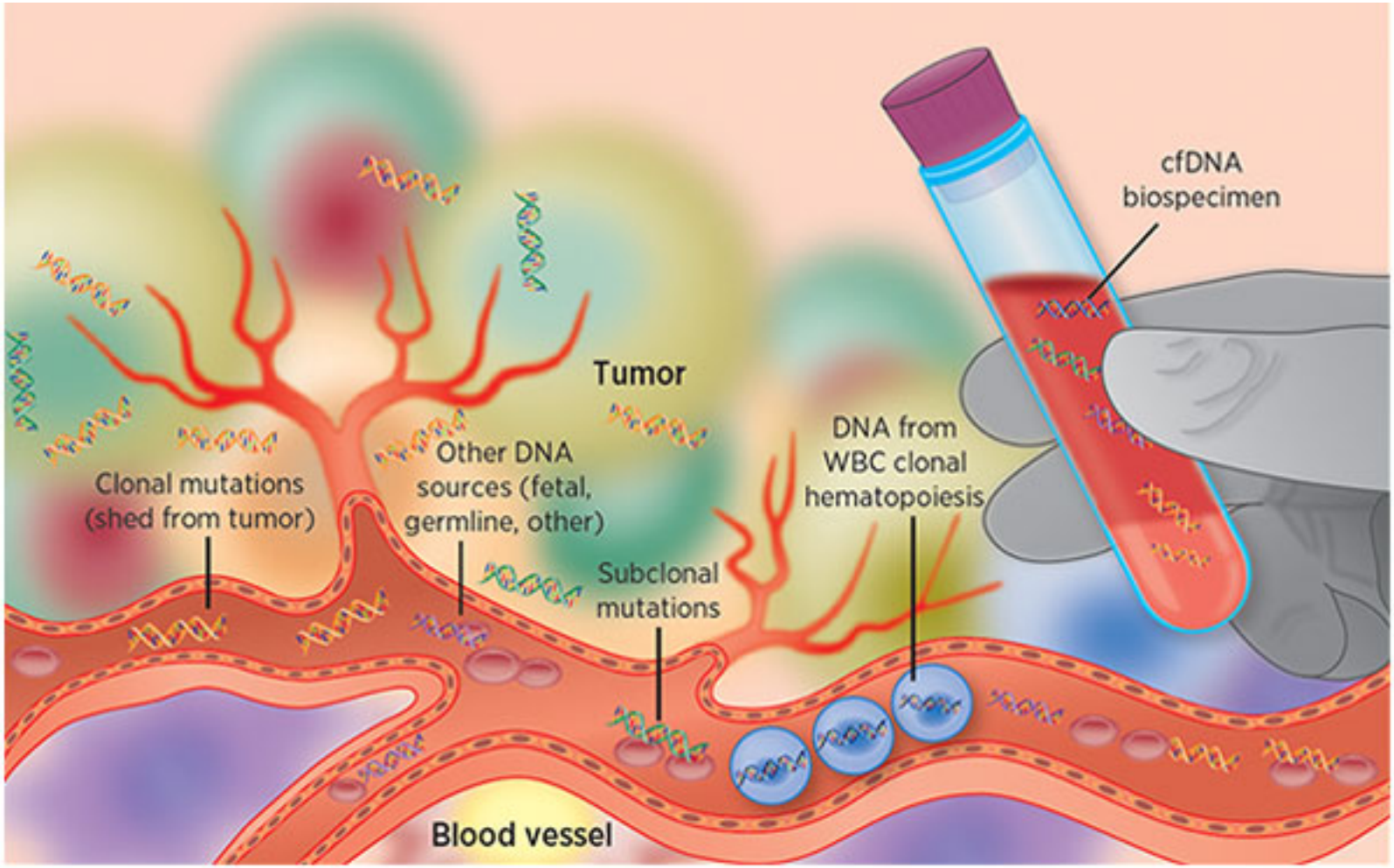
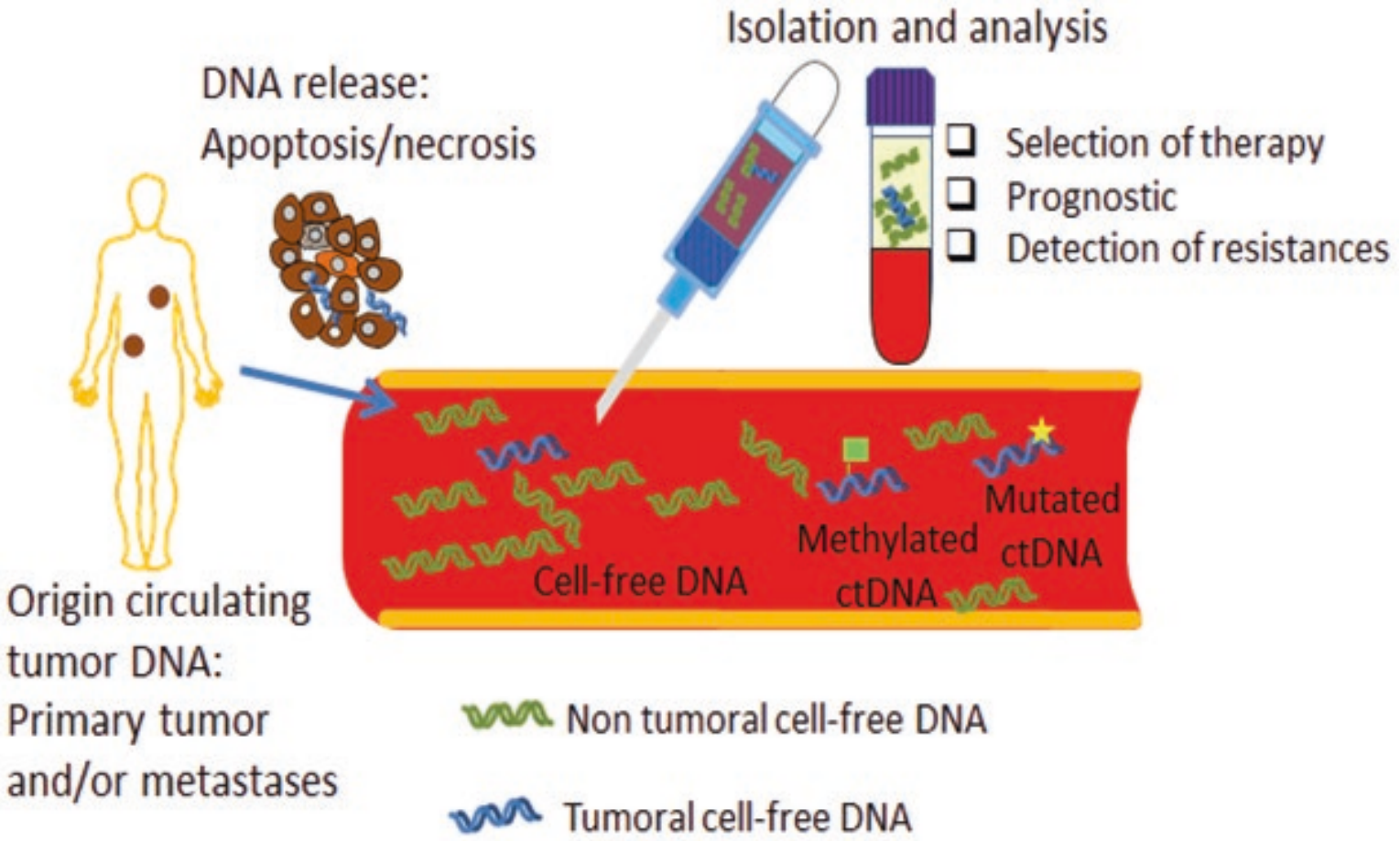
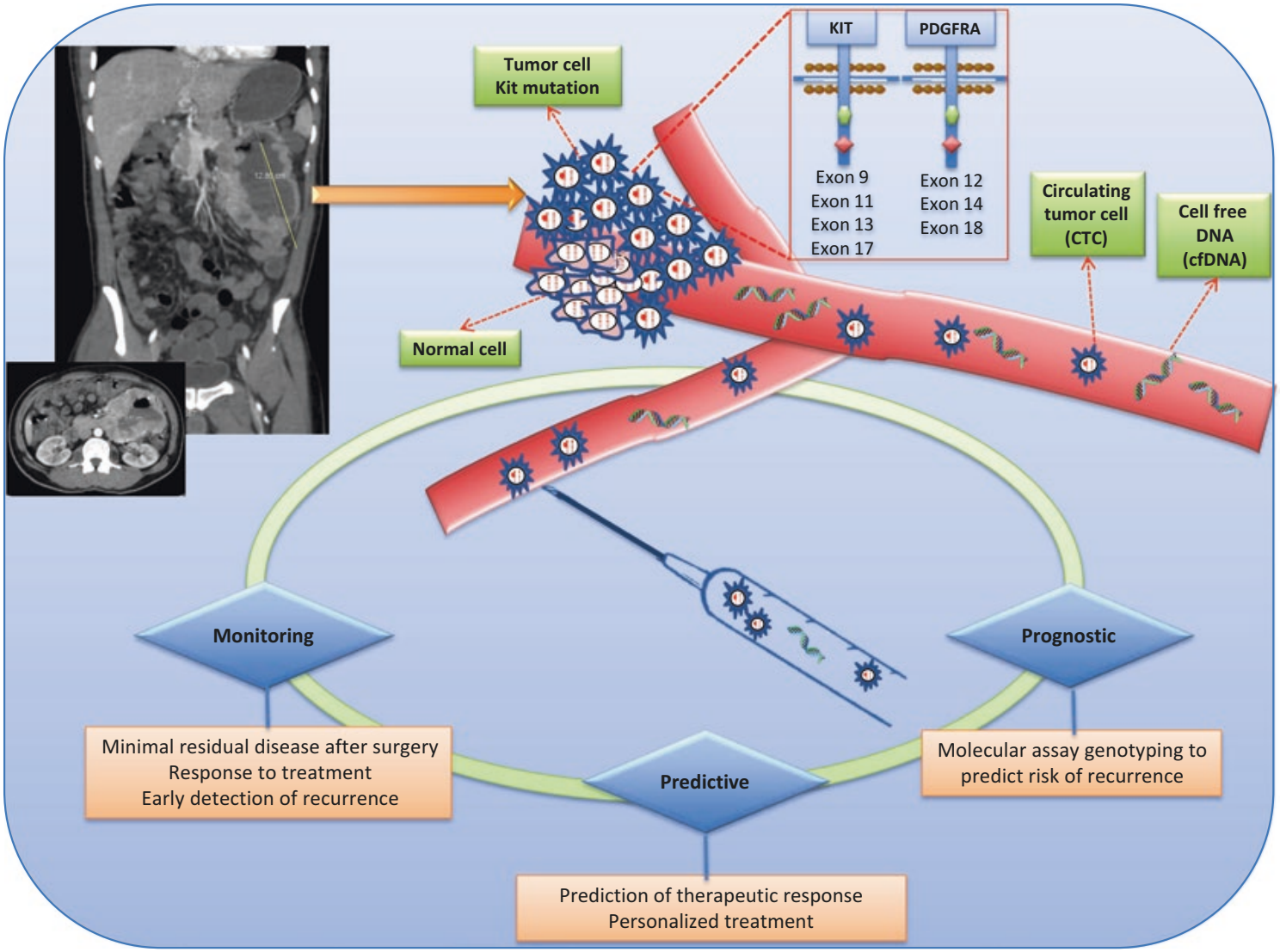
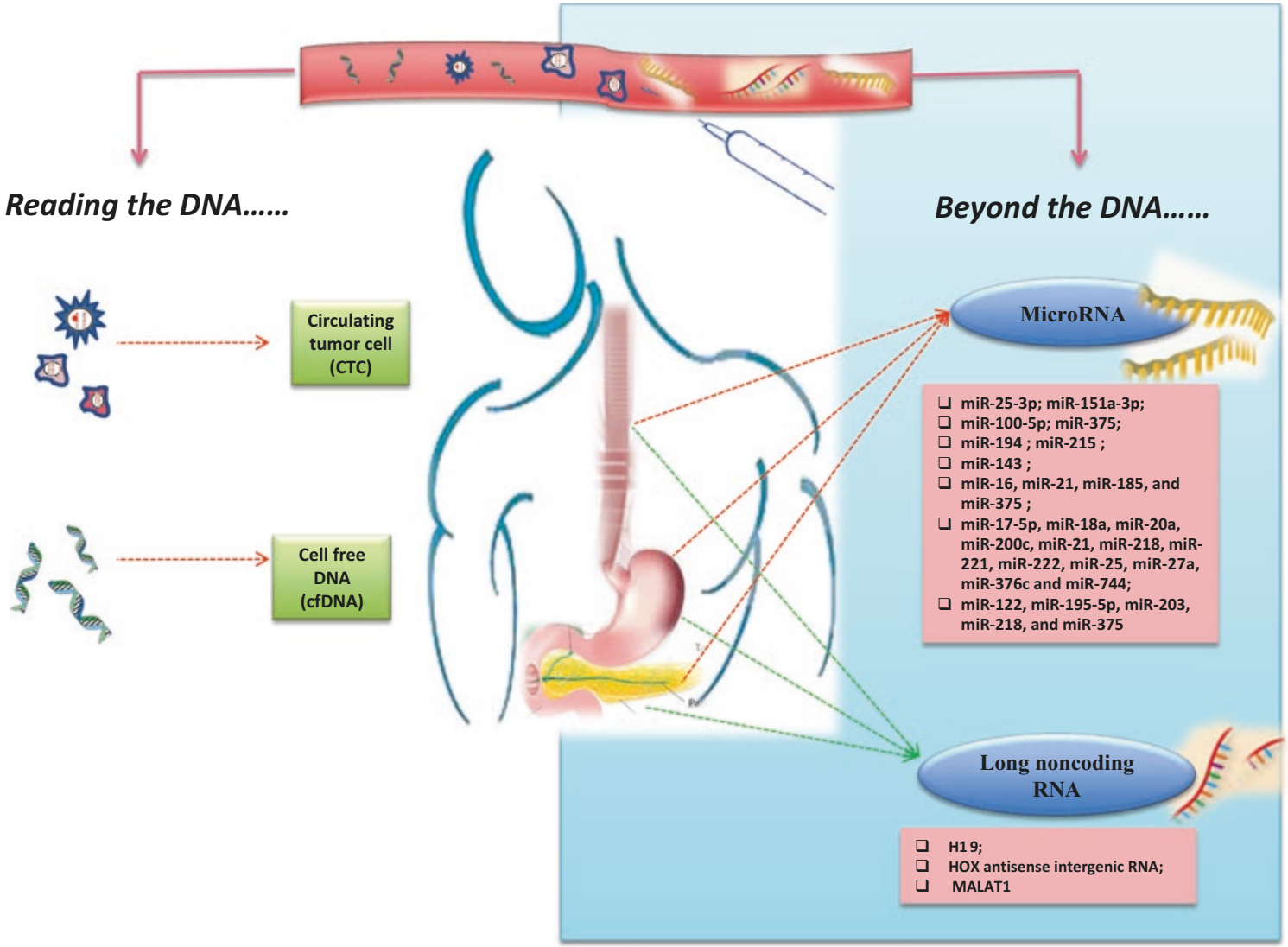


Figure originally published in Clin Cancer Res; Published OnlineFirst May 10, 2018.


Isolation and analysis
DNA release:
Apoptosis/necrosis
Selection of therapy
Prognostic
Detection of resistances
Cell-free DNA
Methylated
ctDNA
Mutated
ctDNA
Origin circulating
tumor DNA:
Primary tumor
and/or metastases
Non tumoral cell-free DNA
Tumoral cell-free DNA


Tumor cell
Kit mutation
KIT
PDGFRA
Exon 9
Exon 11
Exon 13
Exon 17
Exon 12
Exon 14
Exon 18
Circulating tumor cell (CTC)
Cell free DNA (cfDNA)
Normal cell
Monitoring
Minimal residual disease after surgery
Response to treatment
Early detection of recurrence
Prognostic
Molecular assay genotyping to predict risk of recurrence
Predictive
Prediction of therapeutic response
Personalized treatment

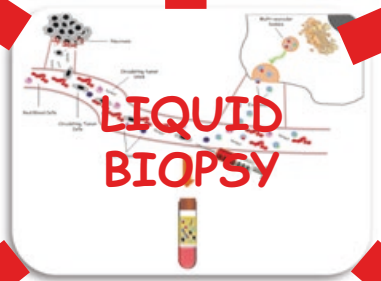
What the genome can tell us……



Molecular characterization of the tumor (early diagnosis)

Metastasis development

Prognostic information



Identification of therapeutic targets and resistance mechanism

Real-time monitoring of disease (MRD and PD)

Circulating tumor DNA

PROs

- Minimally invasive prognostic marker
- Early detection of drug resistance development
- Driver mutation detection from blood samples
- Solving the issue regarding "insufficient material for analysis"

CONs

- Lack of standardized and widely approved methods for analysis
- Contamination with cfDNA from healthy cells
- Low levels of ctDNA (False Negative)
- Accurate quantification of the mutant allele in the sample

A.

Circulating Tumor Cells

PROs

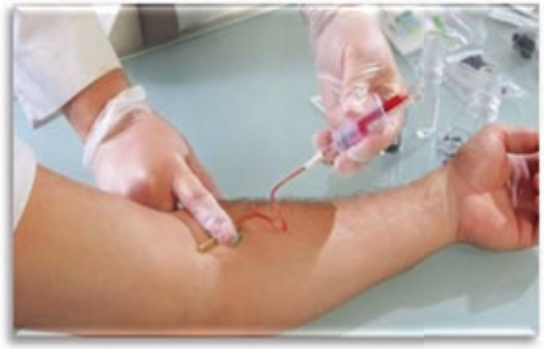
- Minimal invasive prognostic marker
- Therapeutic management
- Comprehension of mechanisms of drug resistance
- Availability of FDA-approved method for isolation

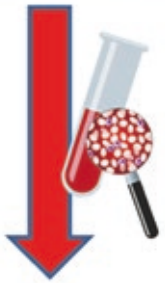
CONs

- Filtration of large or clustering CTCs in smaller capillaries (FN)
- Presence of benign circulating epithelial cells (FP)
- Heterogeneity

B.

Tissue Biopsy	vs.	Liquid Biopsy
• Allows histological diagnosis and staging		• Does not allow tumor histotype specification and staging
• Often difficult and invasive		• Non-invasive procedure
• Not always representative for the entire variety of malignant clones: TUMOR HETEROGENEITY		• Representative of the different localization of the malignant clones: TUMOR HETEROGENEITY
• Multiple sampling are not always feasible		• Easily repeatable and highly reproducible
• Single snapshot over time and space		• Real-time monitoring of disease (MRD and PD)
• Still the gold standard for tumor characterization		• Lack of standardization, still used mainly in translational research





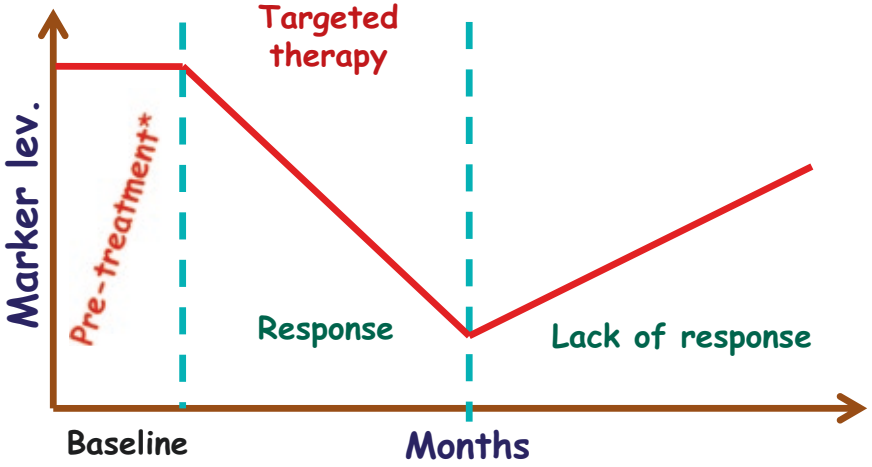

Targeted therapy
Marker lev.
Pre-treatment*
Response
Lack of response
Baseline
Months

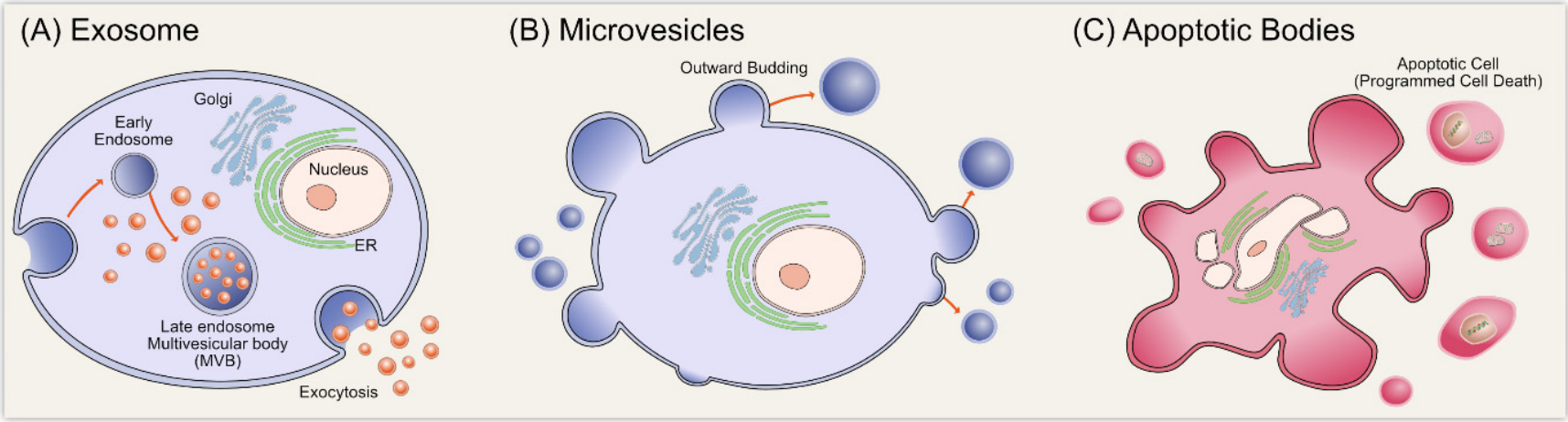
Table 8.1 Actionable cancer targets tested in liquid biopsy analysis

Actionable target (Dx or Rx)	% (*n*) of mutated samples with single-base mt/insertion/deletion	Current use (Dx:Rx value)	Validation in liquid biopsy (source of liquid biopsy)	Analytic method	References
JAK2	20.9 (32,692)	Not established	Not determined	–	–
BRAF	15.5 (24288)	Rx, melanoma	(ctDNA) (ctDNA) (ctDNA)	ddPCR PCR ddPCR	[3] [4] [5]
KRAS	14.9 (23261)	Dx, multiple	(exosomes) (ctDNA) (CTC & ctDNA) (ctDNA)	dPCR PCR ddPCR NGS	[6] [7] [8] [9]
TP53	9.2 (14438)	Dx, multiple	(ctDNA) (exosomes) (ctDNA)	dPCR dPCR NGS	[10] [6] [11]
FLT3	7.4 (11520)	Rx under development	Not available	–	–
EGFR	6.8 (10628)	Rx, multiple	(ctDNA) (cfDNA) (cfDNA)	NGS Seq NGS	[12] [13] [14]
KIT	3.0 (4720)	Rx, GIST, AML	(ctDNA)	NGS	[30]
PIK3CA	2.9 (4560)	Dx, breast	(cfDNA) (ctDNA) (CTC)	NGS dPCR NGS	[15] [16] [17]
IDH1	2.9 (4509)	Not established	Not validated	–	–
CTNNB1	2.1 (3262)	Dx, multiple	No (ctDNA)	NGS	[18]
FGFR3	1.9 (2948)	Rx under evaluation	(ctDNA)	NGS	[19]
NRAS	1.8 (2738)	Dx, multiple	(ctDNA)	ddPCR	[5, 20]
APC	1.6 (2561)	Dx, colon	(ctDNA) (ctDNA) (ctDNA)	NGS&dPCR NGS&dPCR NGS	[21] [22] [23]
NPM1	1.6 (1471)	Not established			
PTEN	1.1 (1719)	Rx under evaluation	(CTC) (ctDNA)	NGS NGS	[24] [25]
VHL	0.8 (1287)	Dx, VHL syndrome	(CTC)	NGS	[26]
IDH2	0.7 (1029)	Not established	(ctDNA)	NGS	[25]
CDKN2A	0.6 (968)	Dx, multiple	(ctDNA) (ctDNA)	MPS NGS	[27] [28]
TET2	0.6 (864)	Not established	–	–	–
ABL1	0.5 (851)	Rx, CML	–	–	–
HRAS	0.5 (812)	Dx under evaluation	–	–	–
DNMT3A	0.5 (788)	Not established			–
NOTCH1	0.4 (661)	Not established	(exosomes) (ctDNA)	NGS NGS	[29] [12]
PDGFRA	0.4 (653)	Rx under evaluation, GIST	(ctDNA)	NGS	[30]
NF2	0.4 (609)	Dx, neurofibromatosis, mesothelioma	(ctDNA) (ctDNA)	NGS NGS	[31] [28]
MPL	0.3 (531)	Not established			
SF3B1	0.3 (516)	Dx under evaluation	(ctDNA)	NGS	[32]
RET	0.3 (500)	Dx under evaluation	(ctDNA)	NGS&dPCR	[22]

The actionable targets in the table originate from Vogelstein et al. (2013) (source: COSMIC open database) and represent single-base mutated driver genes (both oncogenes and tumor suppressor genes) found most frequently in cancer with a mutation hit >500/tumor. Bolded correspond to targets with clinically available therapeutics. The complete list available through the cited reference

ctDNA circulating tumor DNA, *CTC* circulating tumor cell, *NGS* next-generation sequencing, *dPCR* digital PCR, *ddPCR* droplet digital PCR, *Dx* diagnostic, *Rx* therapeutic

Extracellular vesicles



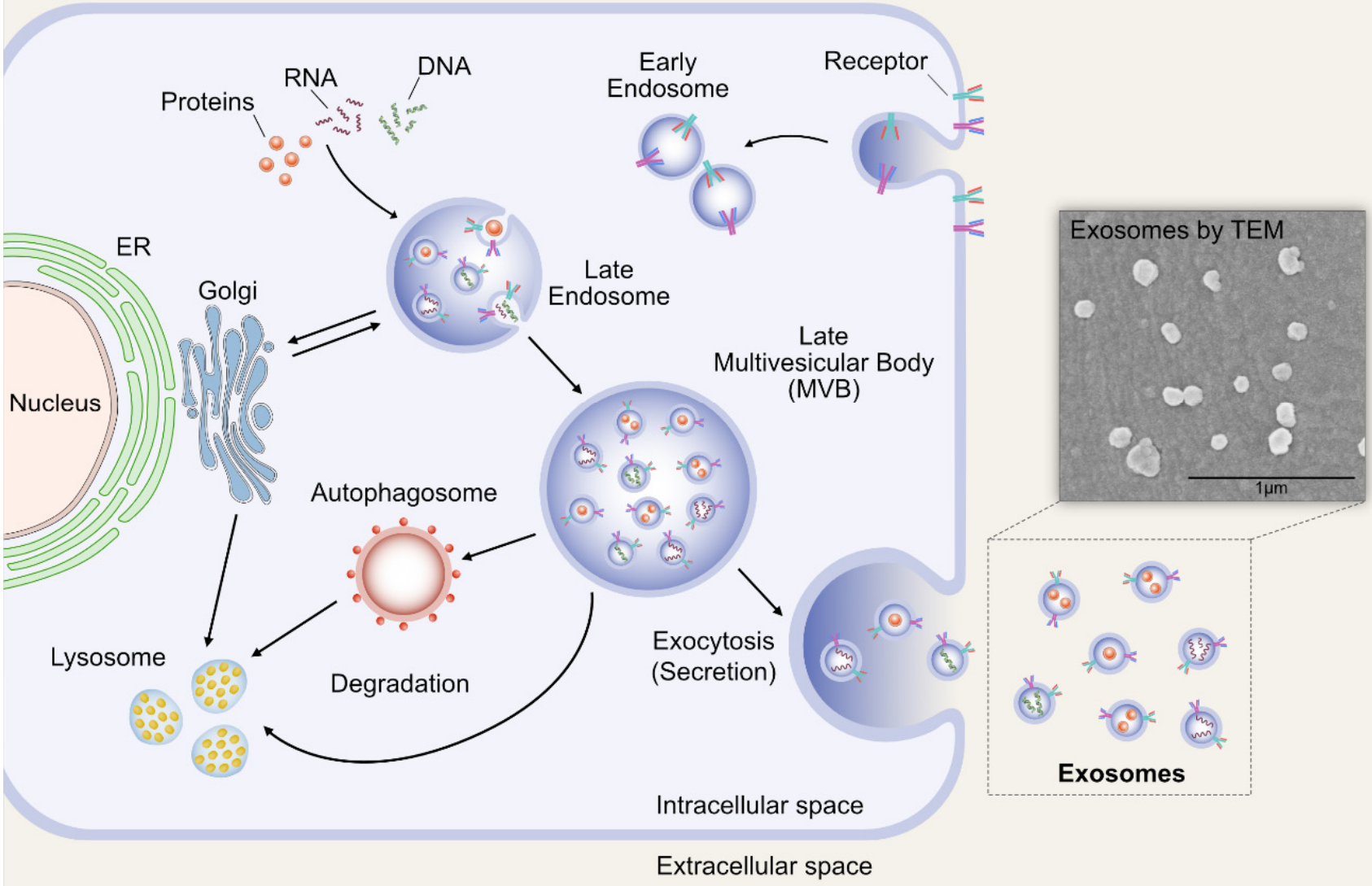
<150 nm

100-1000nm

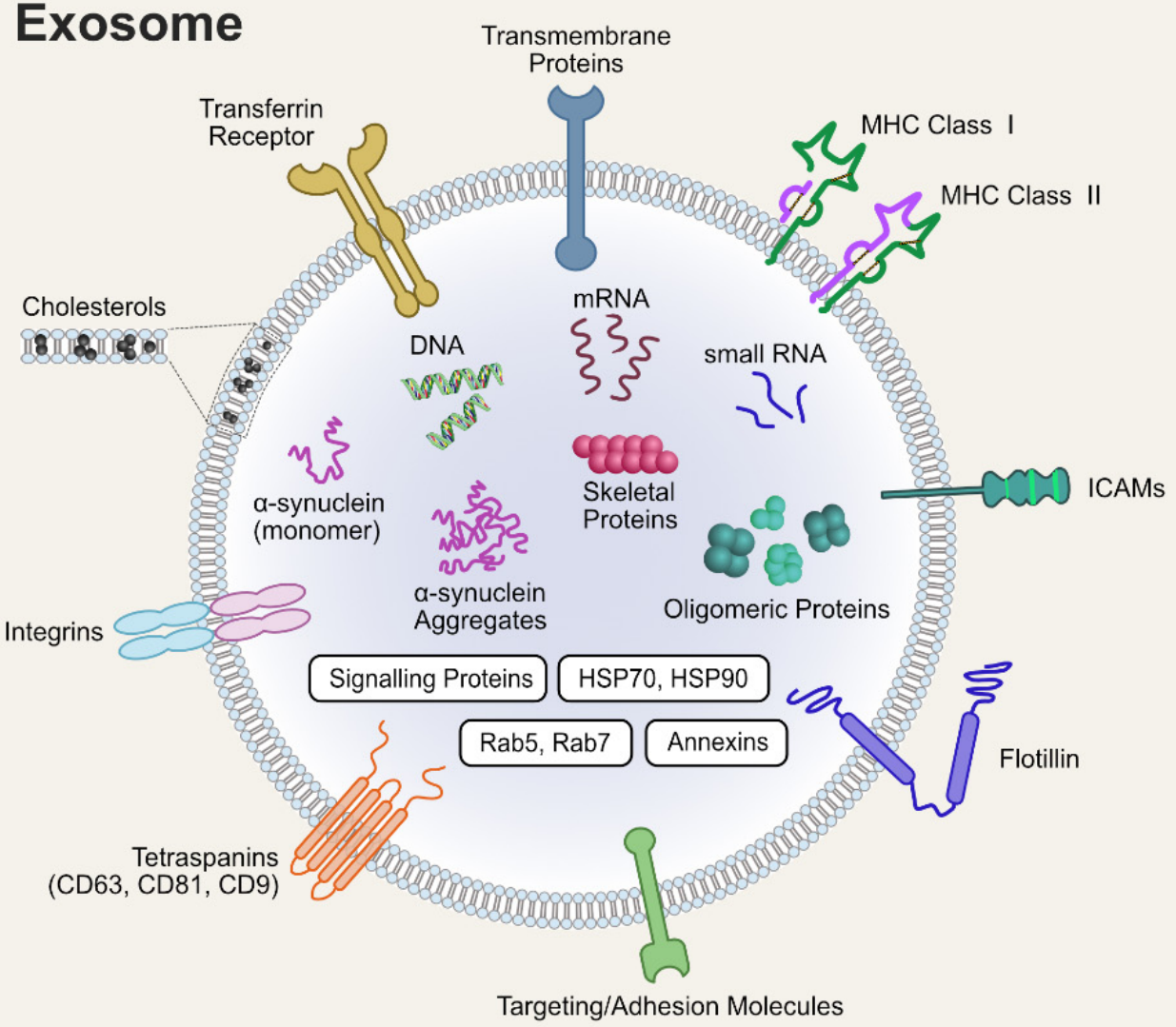
>1000 nm

Gurunathan et al, Cells 2019

Exosome Biogenesis

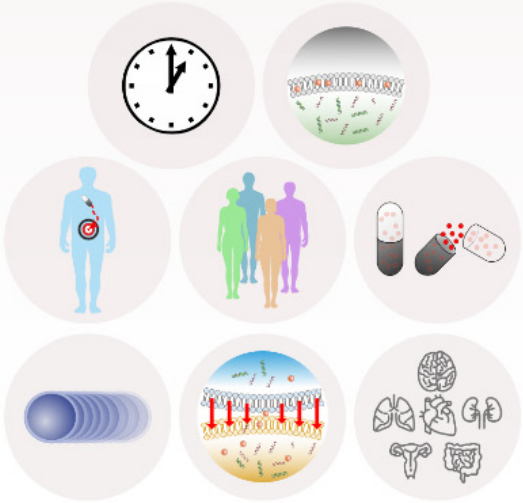


Exosome

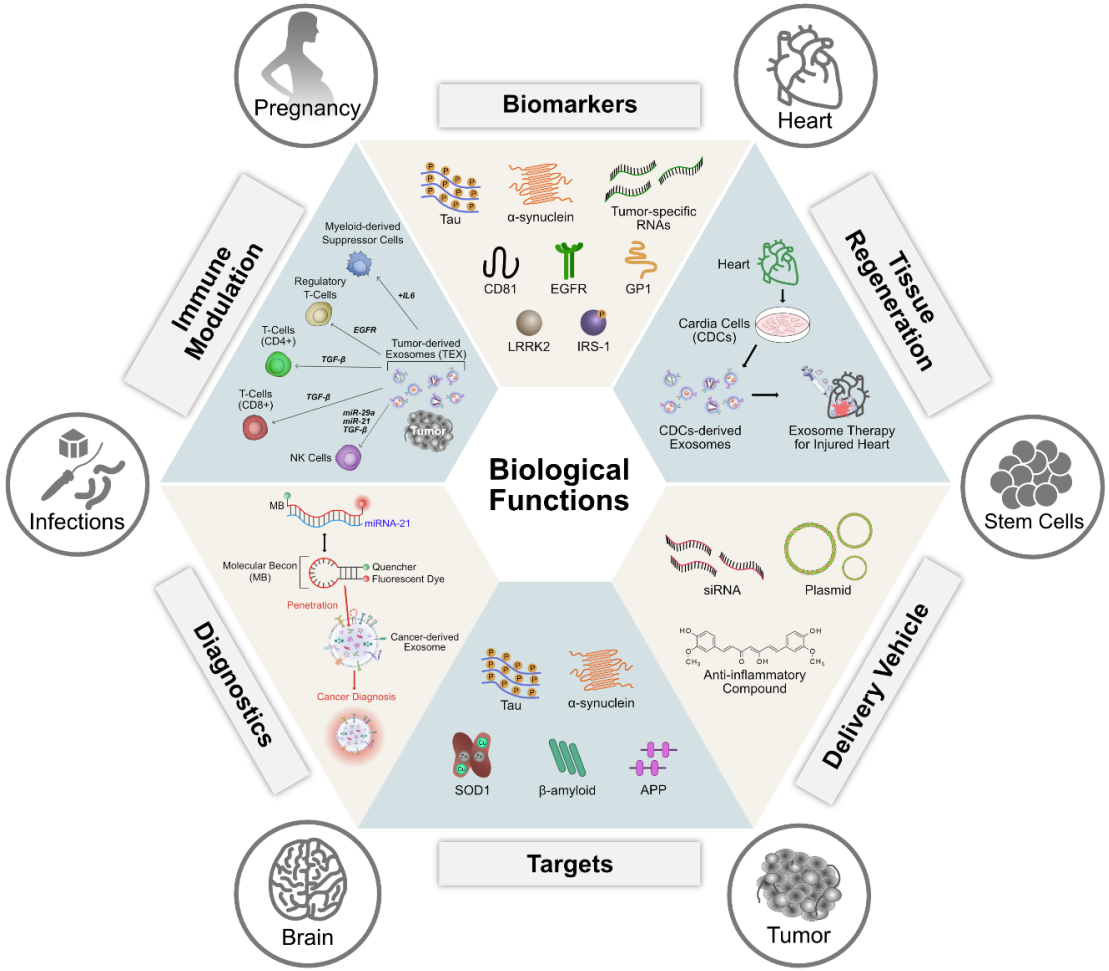


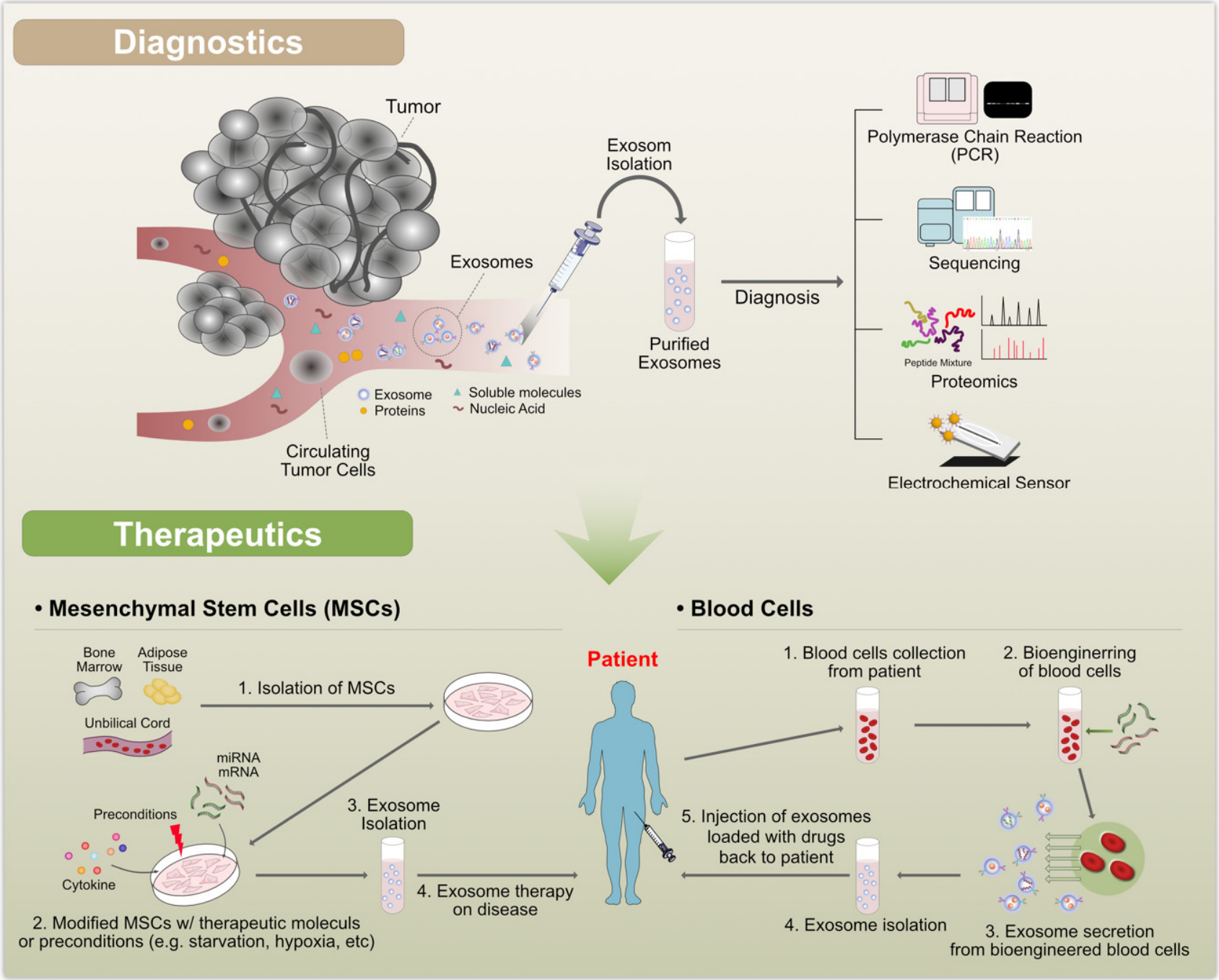
PROPERTIES

- ***Long Body Circulations***
- ***Compartmentalization***
- ***Targeted Delivery***
- ***Biocompatiability & Safe***
- ***Cargo Protection & Encapsulation***
- ***Hight Penetration***
- ***Biodegradable***
- ***Enhanced Biodistribution***



Exosomes Biological Functions




Diagnostics
Tumor
Exosomes
Exosom Isolation
Purified Exosomes
Diagnosis
Polymerase Chain Reaction (PCR)
Sequencing
Peptide Mixture
Proteomics
Electrochemical Sensor
Exosome
Proteins
Soluble molecules
Nucleic Acid
Circulating Tumor Cells
Therapeutics
• Mesenchymal Stem Cells (MSCs)
Bone Marrow
Adipose Tissue
Unbilical Cord
1. Isolation of MSCs
miRNA
mRNA
Preconditions
Cytokine
2. Modified MSCs w/ therapeutic moleculs or preconditions (e.g. starvation, hypoxia, etc)
3. Exosome Isolation
4. Exosome therapy on disease
Patient
• Blood Cells
1. Blood cells collection from patient
2. Bioenginerring of blood cells
3. Exosome secretion from bioengineered blood cells
4. Exosome isolation
5. Injection of exosomes loaded with drugs back to patient

324 genes, TMB, MSI and LoH

Assesses the four main classes of genomic alterations* in 324 cancer-relevant genes and reports TMB, MSI and LoH[1,2]

Extensively validated

Based on our analytically and clinically validated, FDA-approved comprehensive platform†[3,4]

Supports clinical decision-making

Clear, in-depth report provides insights on the genomic profile of your patient as well as associated targeted therapies, immunotherapies (ranked alphabetically within NCCN therapy categories)‡ and relevant clinical trials[5]

EU report

Reports vary according to regional differences, e.g. EU reports list EU-approved therapy options to support clinical decision-making§[5]

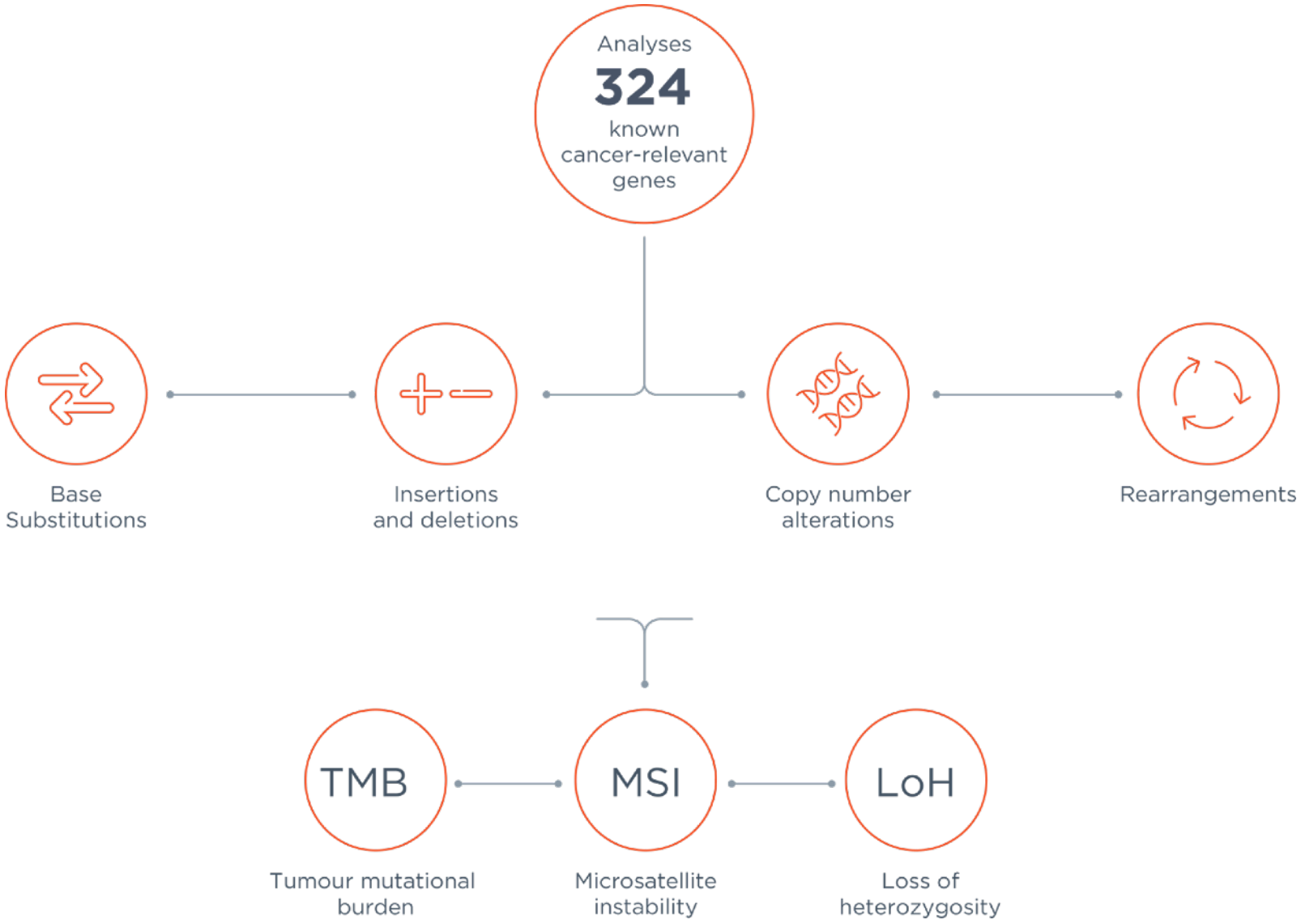
Opens possibilities

Potentially expands your patient's treatment options¥[1,6–10]

Provides insights that can help support treatment decisions and may improve clinical outcomes¥[2,11–14]

A single, tissue- and time-saving test

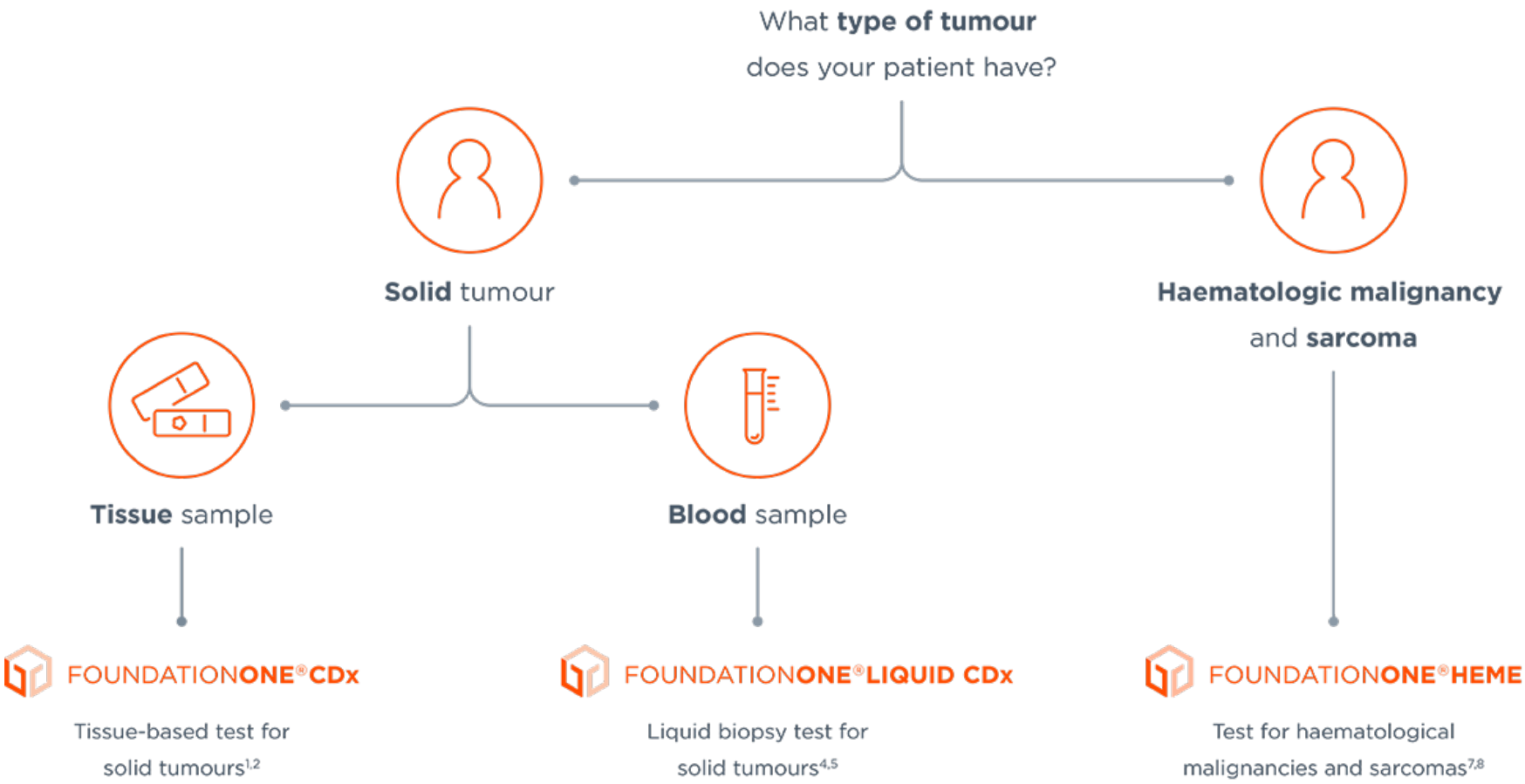
Delivers all insights at once in a single test, thus saving tissue and time versus sequential biomarker testing[1,2,6]


Analyses
324
known
cancer-relevant
genes
Base
Substitutions
Insertions
and deletions
Copy number
alterations
Rearrangements
TMB
MSI
LoH
Tumour mutational
burden
Microsatellite
instability
Loss of
heterozygosity

Single biomarker testing

FoundationOne CDx

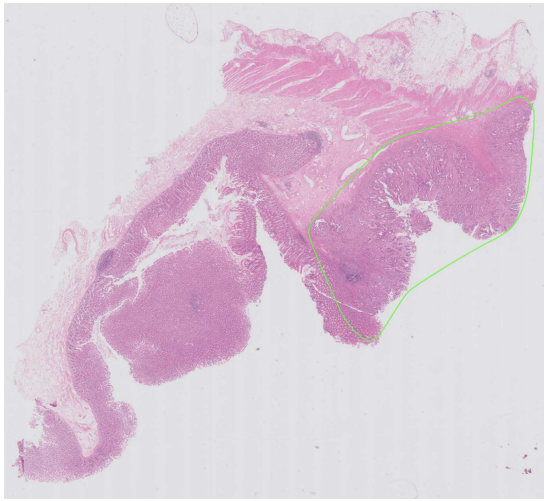
FOUNDATION**ONE CDx**



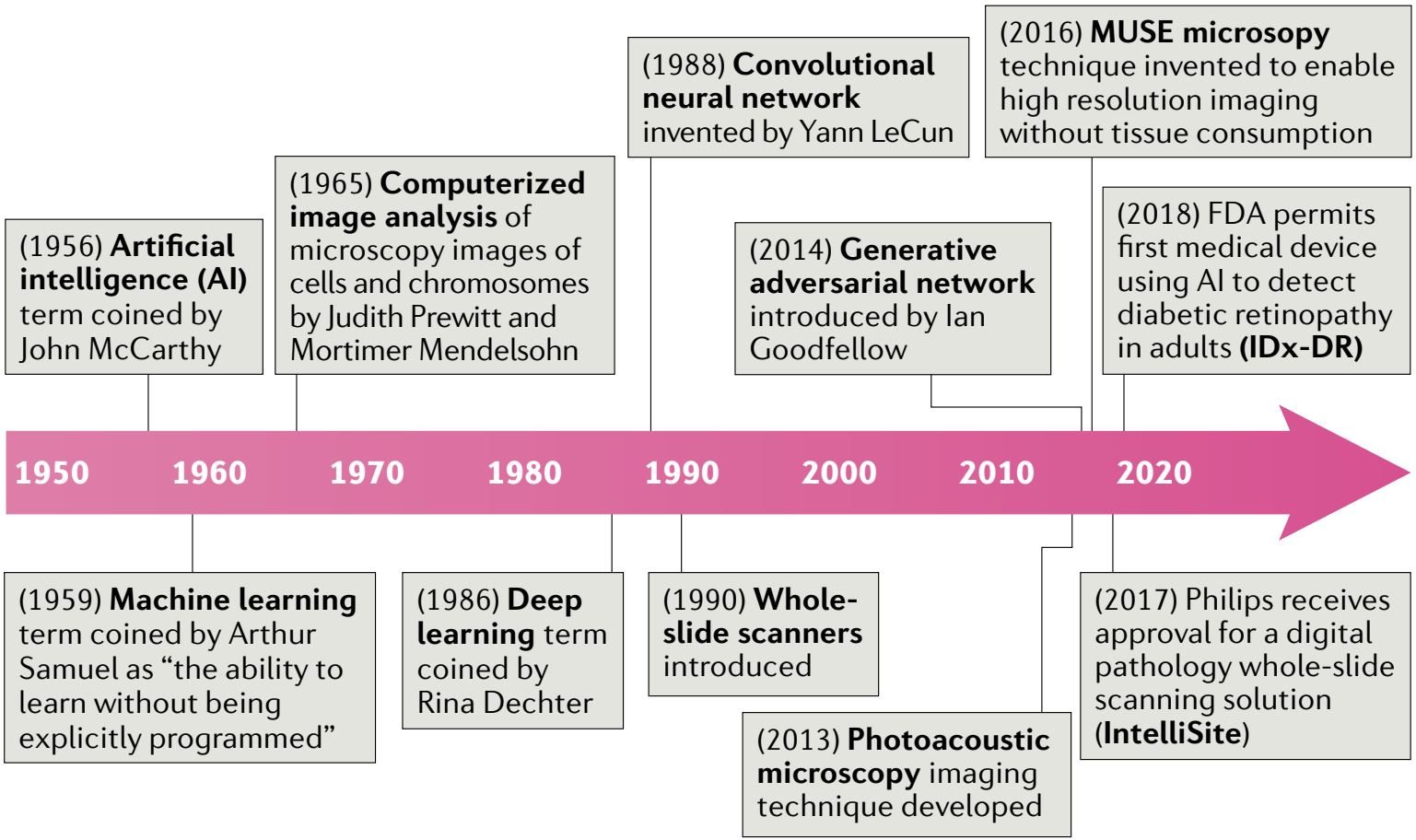

AI
In digital pathology

Non-Digital Pathology

- Routine pathology → glass slide

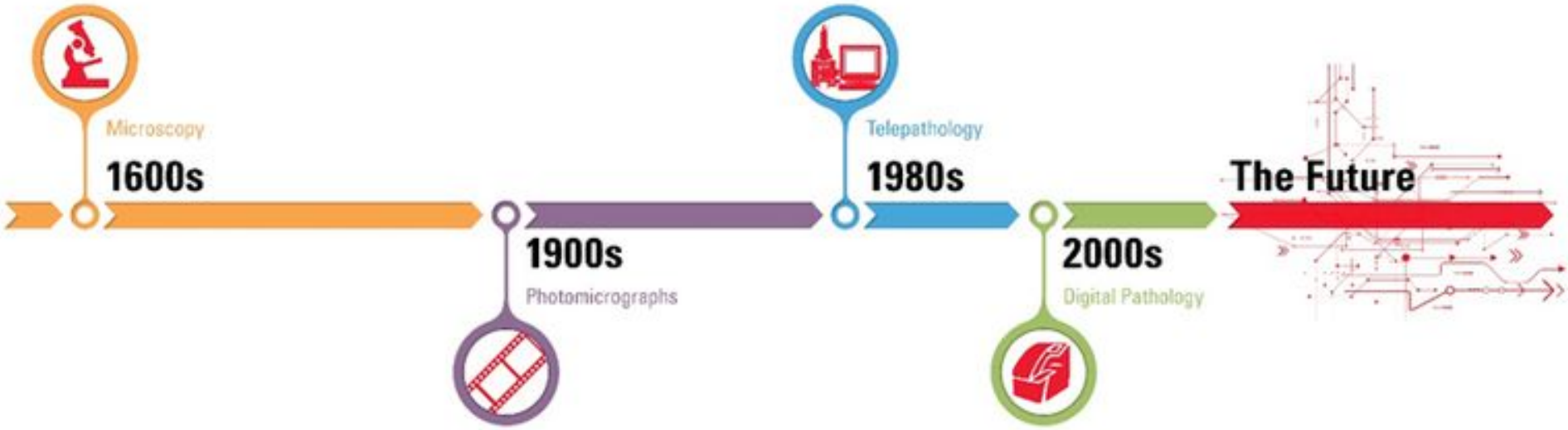


Dx: time consuming / expensive / subjective

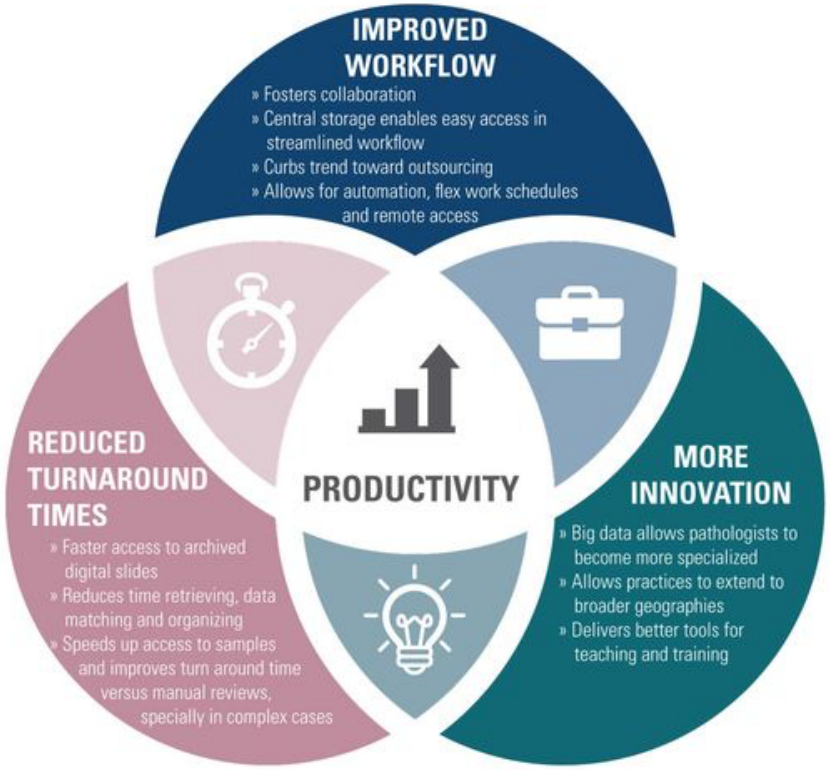


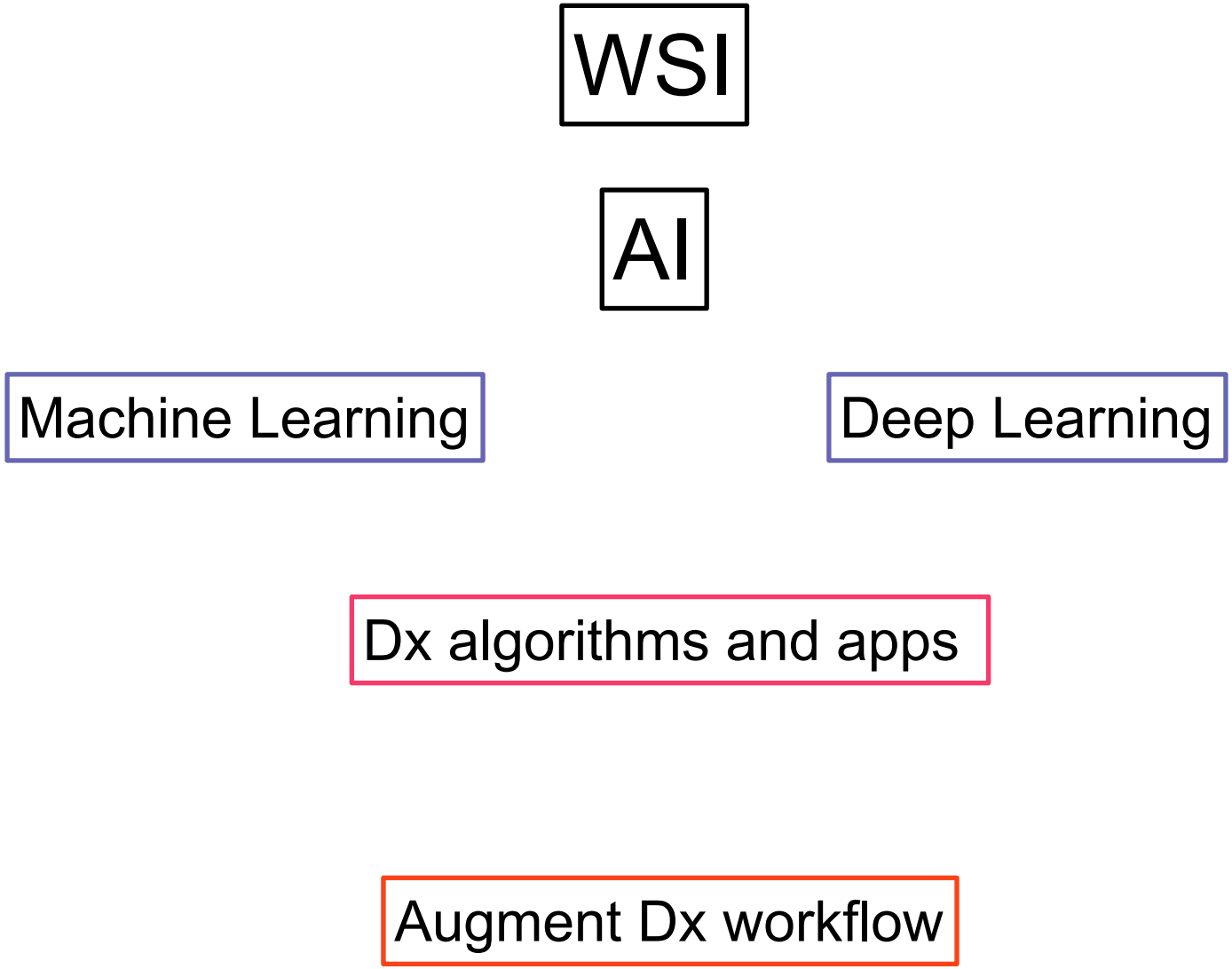
Bera et al, Nat Clin Oncol 2019

The evolution of Digital Pathology



Digital Pathology

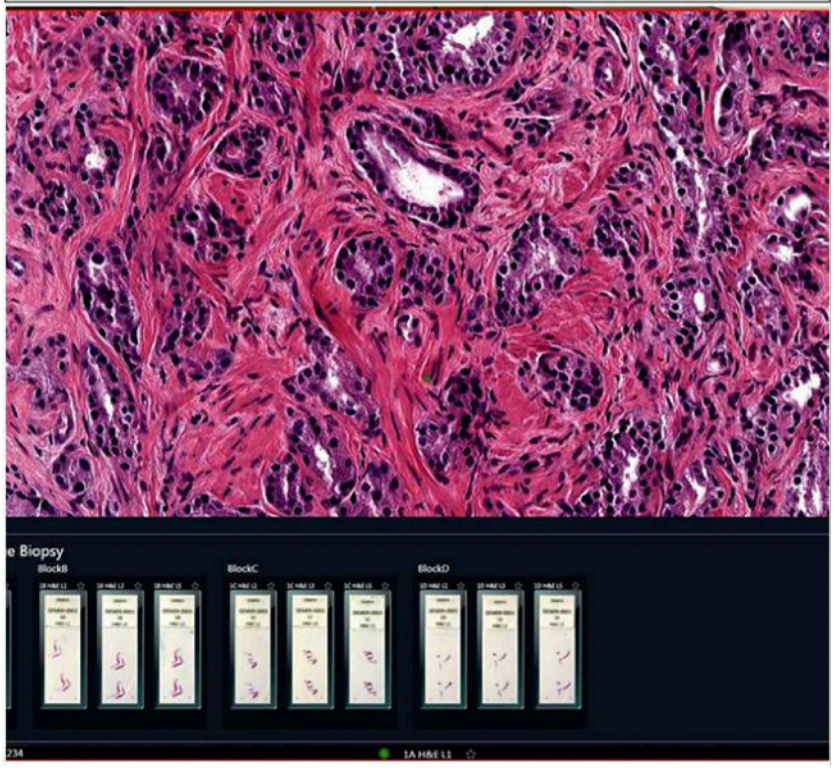


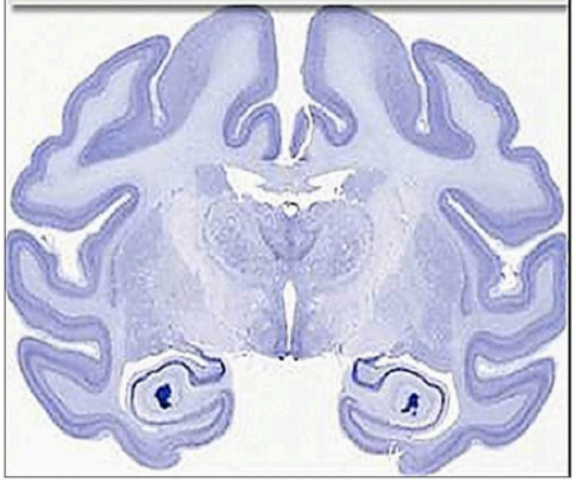

WSI
AI
Machine Learning
Deep Learning
Dx algorithms and apps
Augment Dx workflow

Digital Pathology Era


Omnyx VL120


Huron Technologies


e Biopsy
BlockB
BlockC
BlockD
234
1A H&E L1

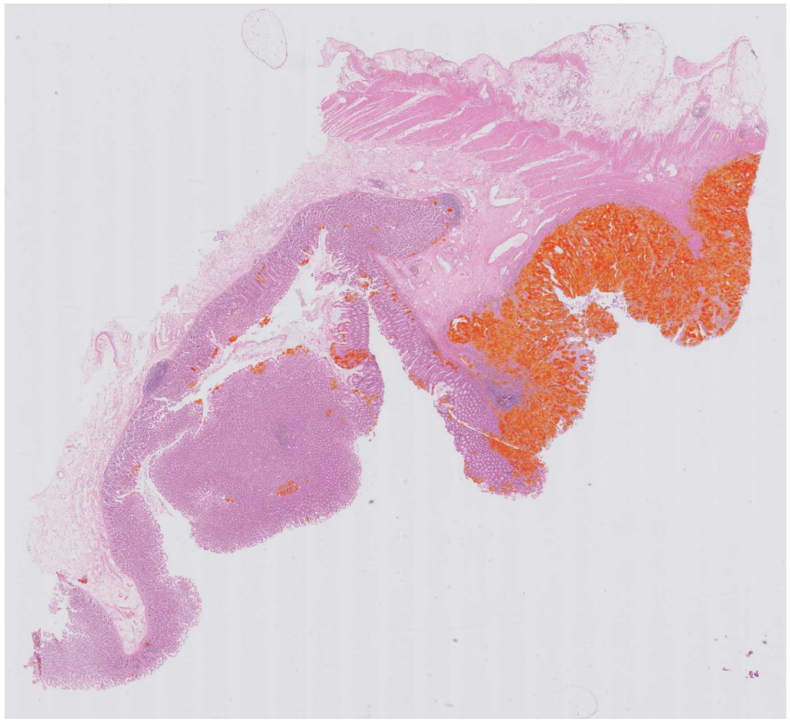


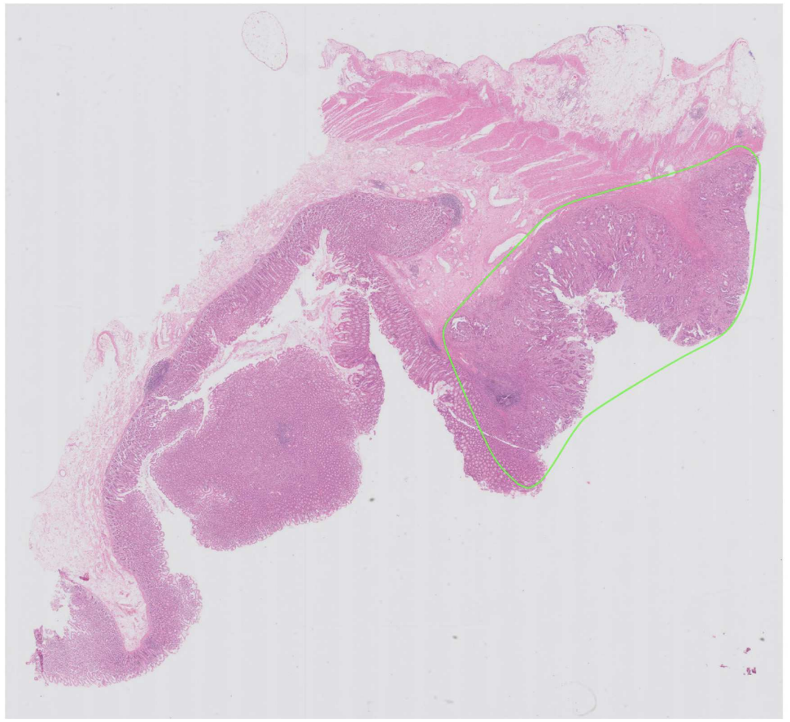
Combining WSI with image analysis tools allows users to leverage technology to perform tasks that were previously too cumbersome or even impossible for humans to undertake manually. Examples include:

- high-throughput morphologic analysis of cases to quantitatively and reproducibly measure histologic structures such as tumors
- automated grading of tumors to reduce variability encountered with manual grading
- automated selection of desired regions of interest, such as hot spots (most active areas in proliferative rate)
- detecting mutations and perform tumor subtyping from H&E imaging using deep learning approaches

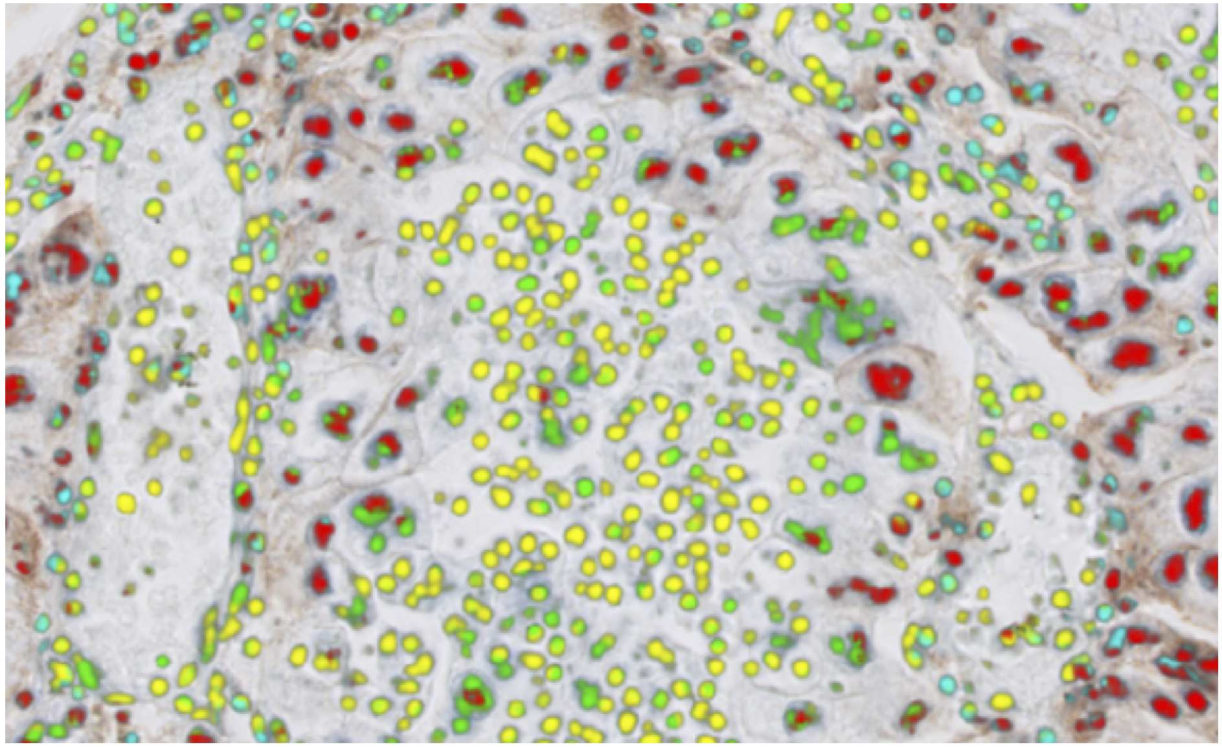
Farhani et al, 2015

Automated tumor identification





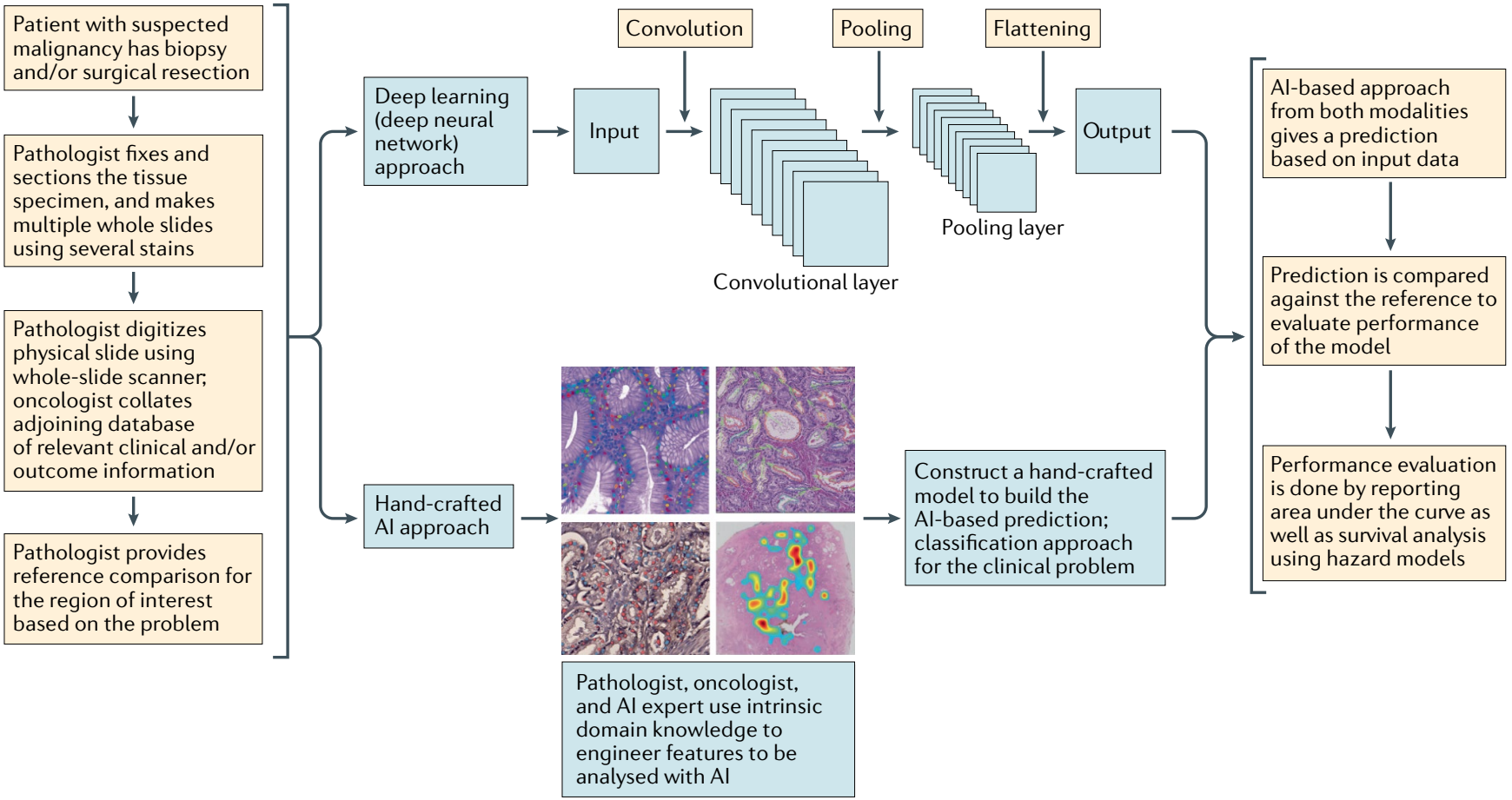
PD-L1 imaging in lung cancer WSI/AI



Pos / Neg tumor cells, Inflammatory cells

Serag et al, 2019

Advanced level of DP/AI

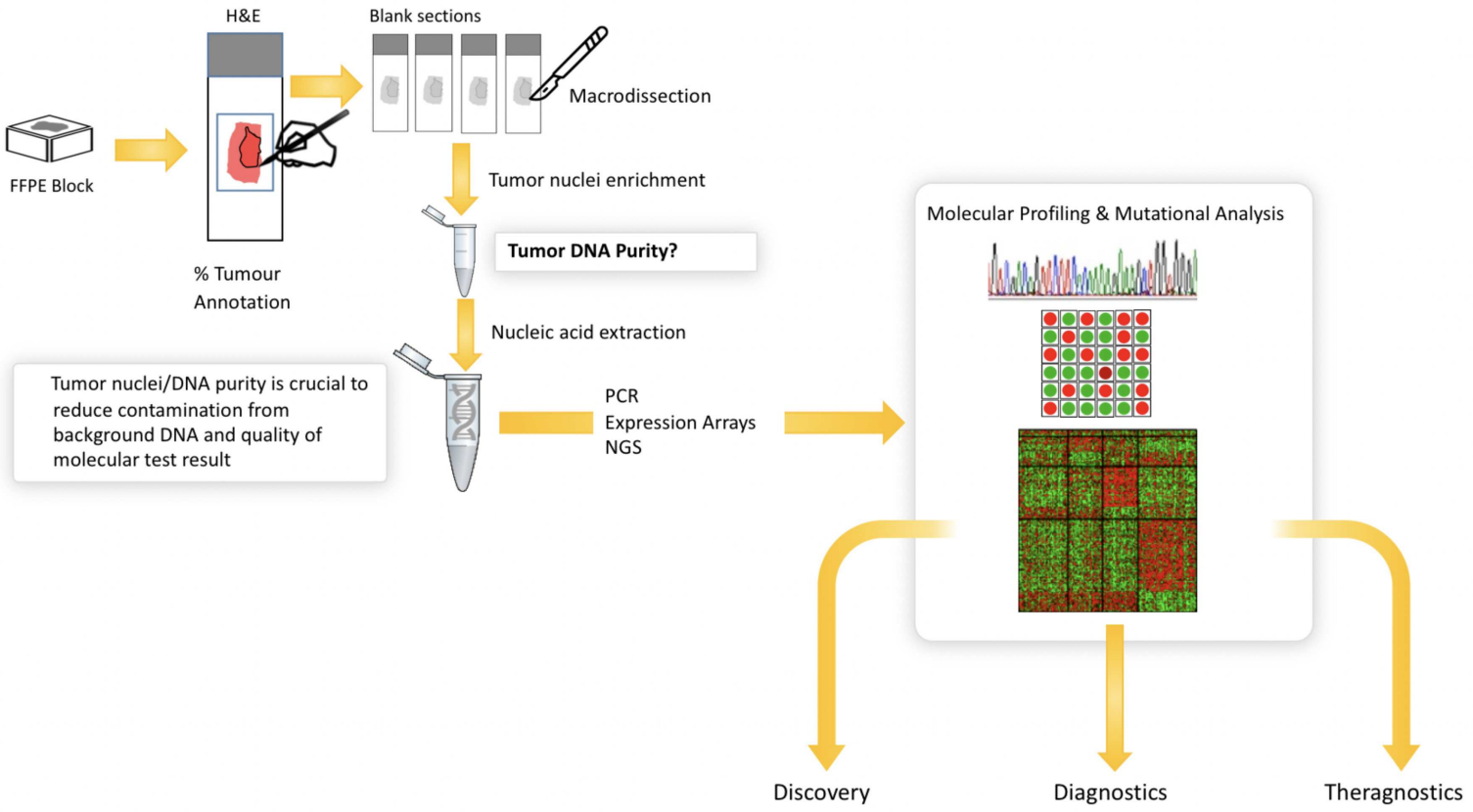


- WSI: whole slide imaging
- interpreting diagnostic, prognostic and therapeutic data from very large patient populations
- providing real-time guidance on risk, clinical care options and outcome
- provide up-to-date medical information from journals, textbooks, and clinical practices to inform proper patient care
- reduce diagnostic and therapeutic errors that are inevitable in conventional human clinical practice
- 3D images

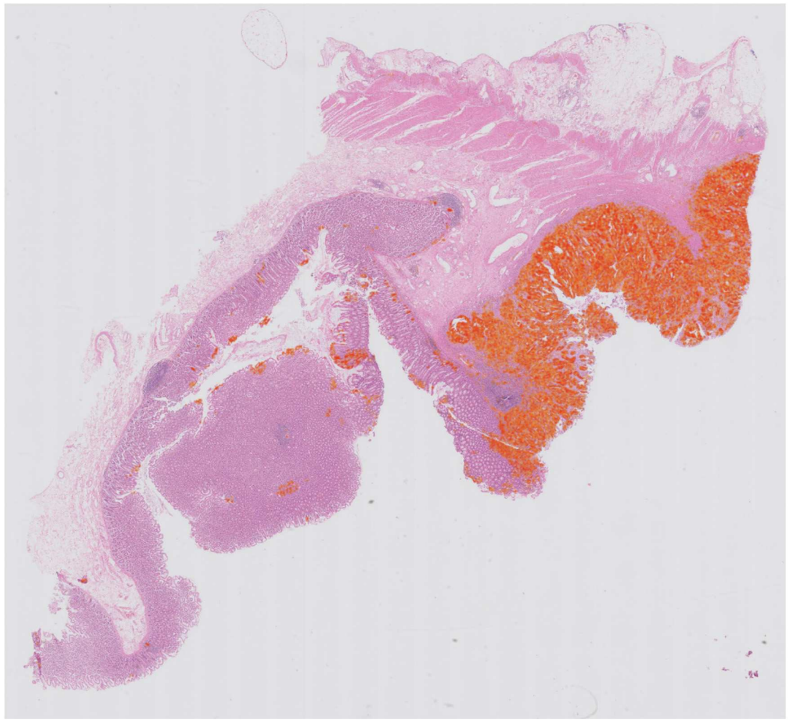
Digital Pathology Applications

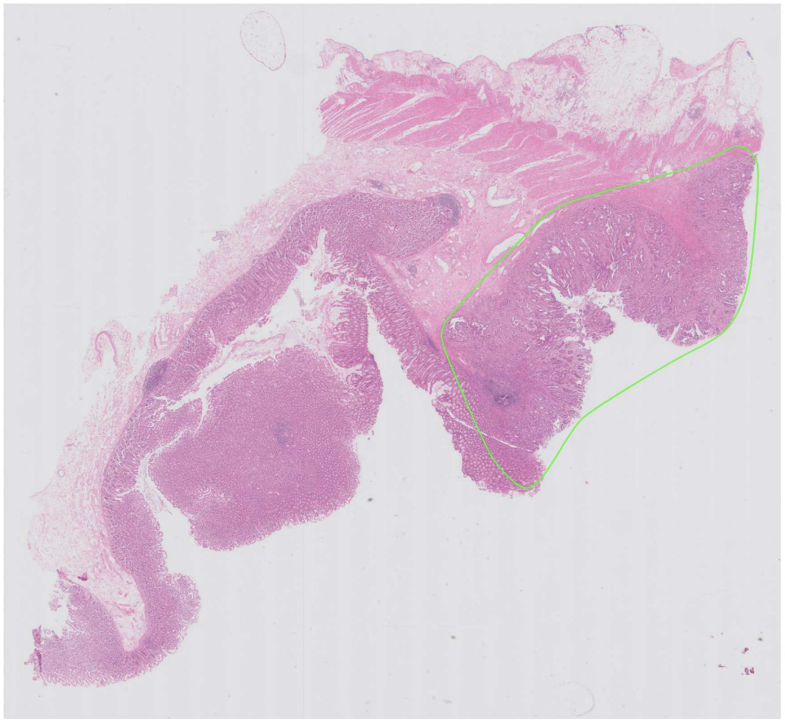
- Prostate cancer grading
- Metastasis detection in LNs
- Mitosis count
- Ki67 scoring
- IHC evaluation (eg PD-L1)
- Tumor detection for molecular analysis
- AI works best in well-defined domains, overcoming the issue of standardization
- 75% of routine pathology (**BUT extremely unsophisticated and boring!!!!)**
- **Intelligence Augmentation (IA) instead of AI in Pathology—> remove noise, but extract useful data**

AI: Identifying the boundary of tumor

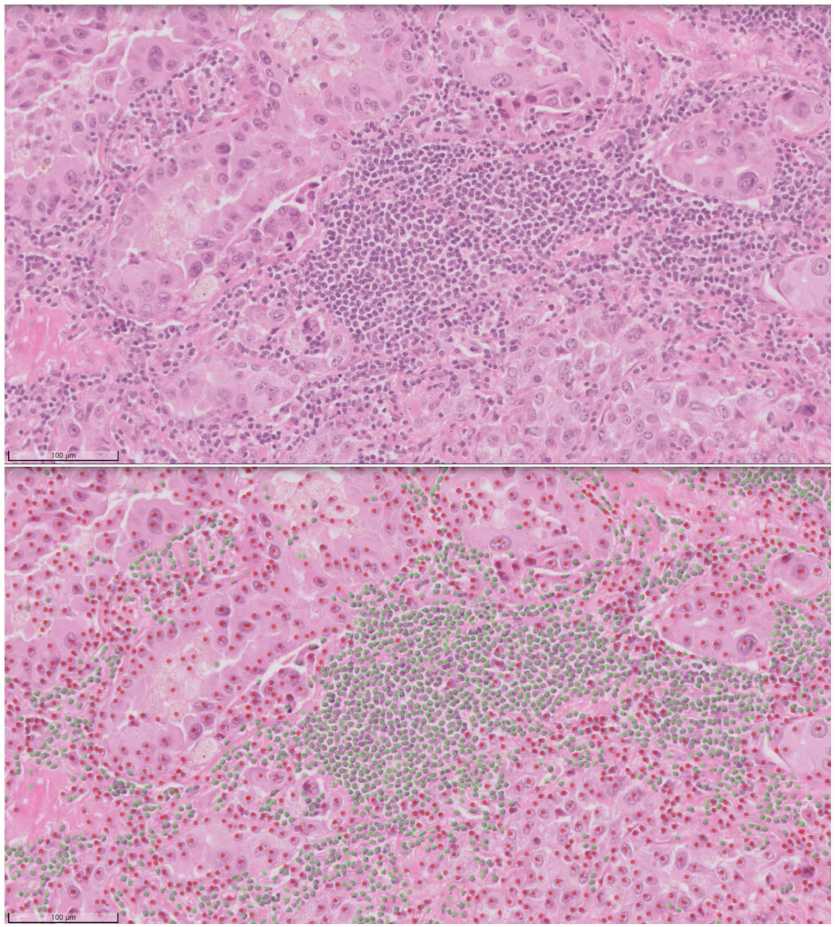


Automated tumor identification tumor quantification





Automated analysis of cellular content in H&E using deep learning



HE

WSI/AI system

Problems/key challenges

- limiting technology/image quality/storage
- Algorithms are slow to run
- Properly define protocols for training and evaluation
- shortcomings to scan all materials (eg, cytology, microbiology)
- the cost of these systems /Lack of health economics
- their inability to handle high-throughput routine work, regulatory barriers in certain countries, user-unfriendly ergonomics
- pathologists' reluctance to use WSI

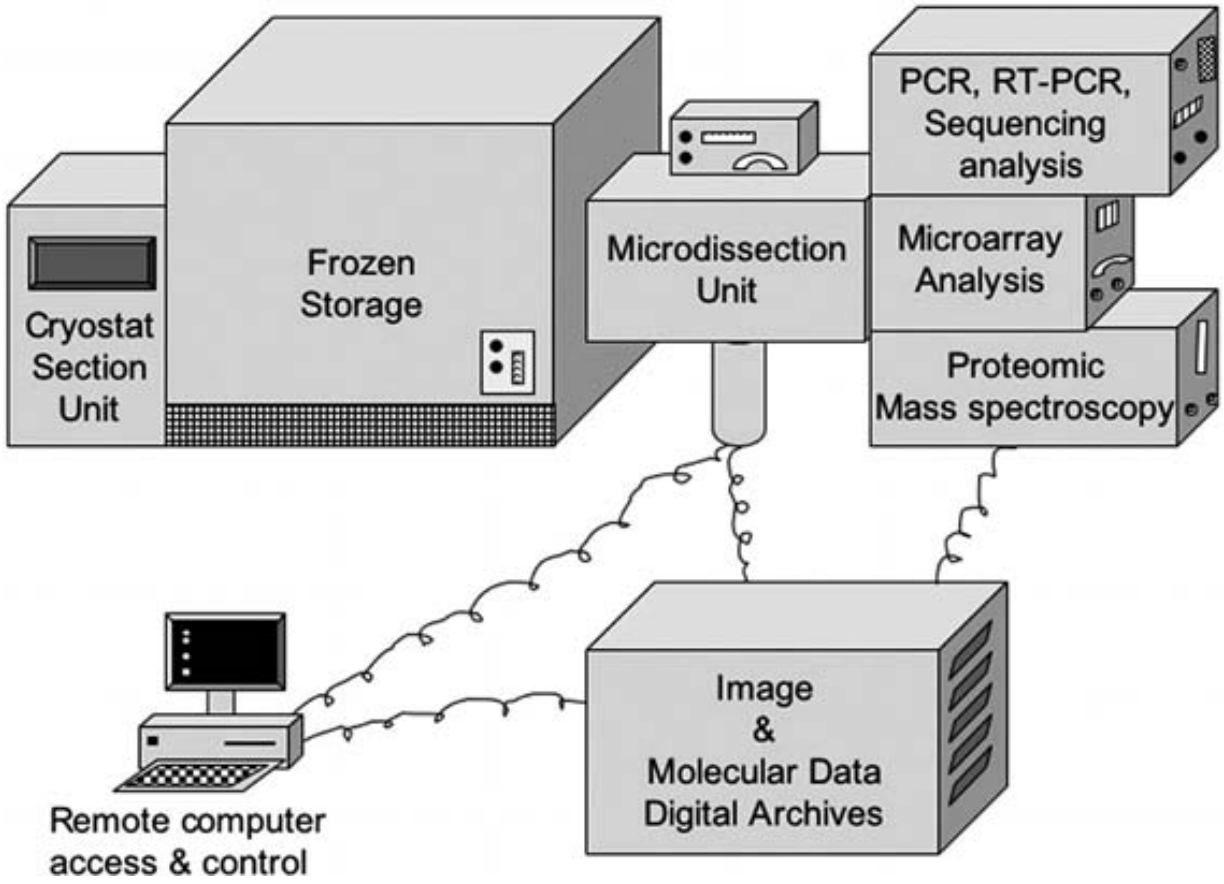
ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

AI: …to conclude

The field of pathology AI is still **young** and will continue to mature as researchers, clinicians, industry, regulatory organizations, and patient advocacy groups **work together** to innovate and deliver new technologies to health care providers: technologies which are **better, faster, cheaper, more precise, and safe for the pt!!**

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

Το μέλλον της ΜΔ




THE
END