

ESMO ADVANCED COURSE ON BIOMARKERS FOR PRECISION MEDICINE:

Multiplex Molecular Pathology

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Zürich, 28-29 November 2019

University Côte d'Azur and Inserm U1081 CNRS 7284, Nice, France



DISCLOSURE OF INTEREST

I herewith declare that,

I have the following potential conflict(s) of interest to report:

Receipt of grants/research supports:

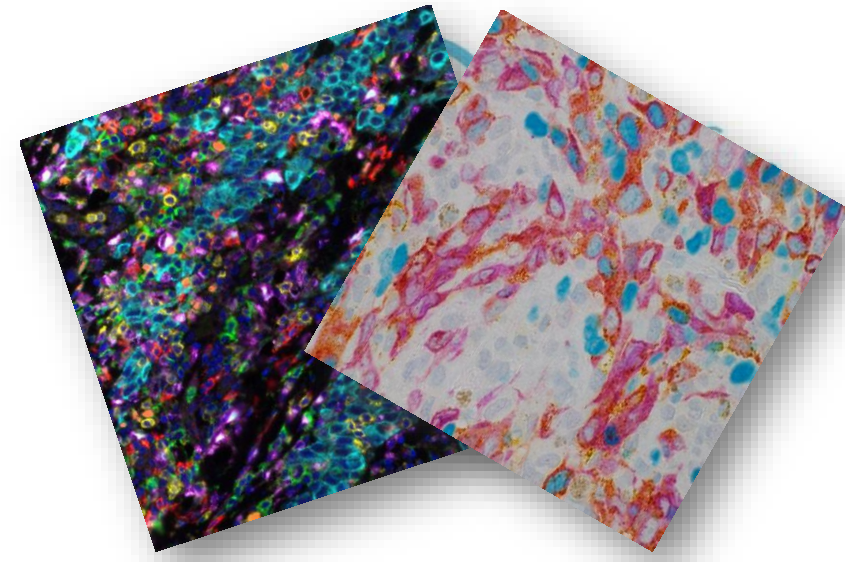
Bristol-Myers Squibb

Receipt of honoraria or consultation fees:

Bristol-Myers Squibb, Thermo Fisher Scientific, Qiagen, Illumina, AstraZeneca, Roche, AbbVie, Pierre Fabre, Boehringer Ingelheim, Novartis



Road map



Short background

Multiplex IHC: Technical principles

Chromogenic *versus* fluorescent multiplex IHC : What is the best choice?

The digital and image analysis: A pivotal issue for the pathology labs

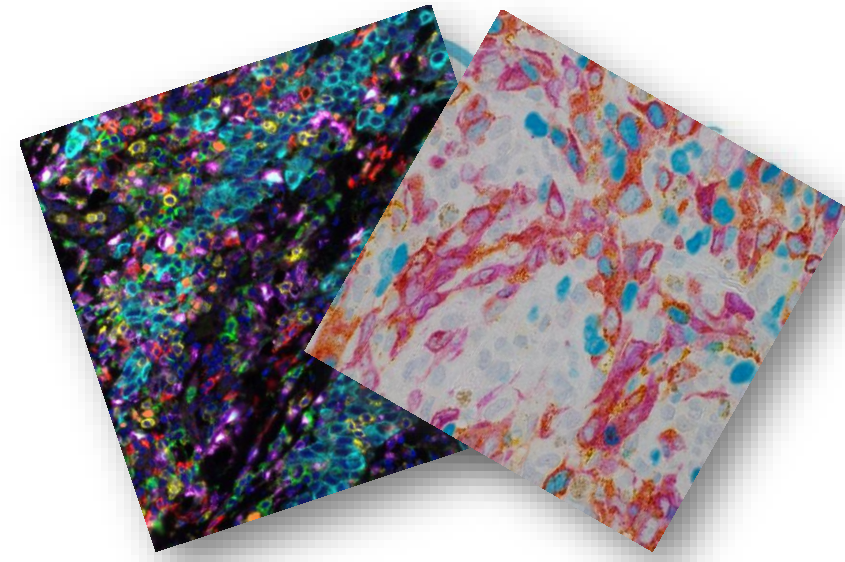
Multiplex IHC in translational research: Many promises in I-O

Multiplex IHC in routine clinical practice: Why and how?

Take away message



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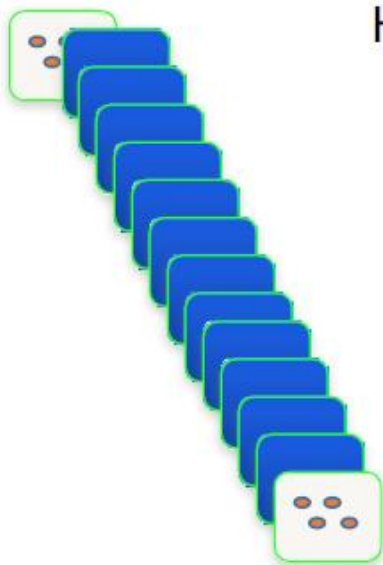
Multiplex IHC in routine clinical practice: Why and how?

Take away message

The three main challenges for the pathologist in thoracic oncology



More analysis from one biopsy



HE first

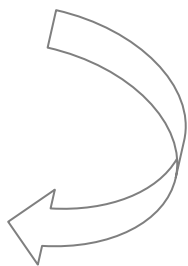
Diagnostic stain TTF1
Diagnostic stain mucin
Diagnostic stain p63/p40

Predictive stain ALK IHC
Predictive stain ROS1 IHC

Predictive stain PD-L1 IHC, etc

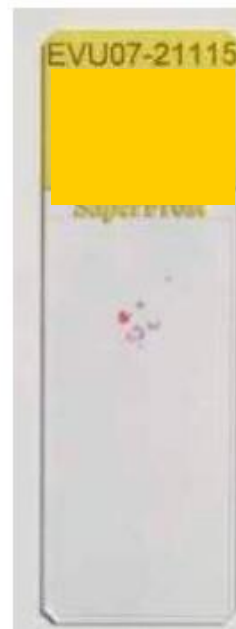
DNA isolation EGFR
DNA isolation

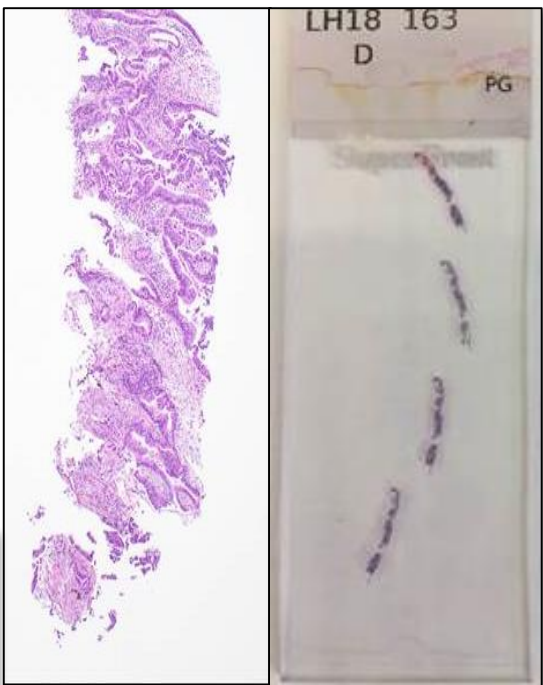
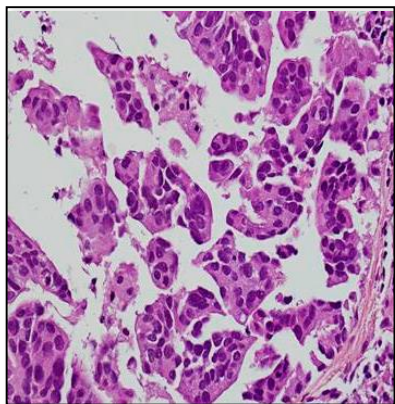
HE last



NGS

- miseq 40-250 ng
- Ion torrent 10 ng



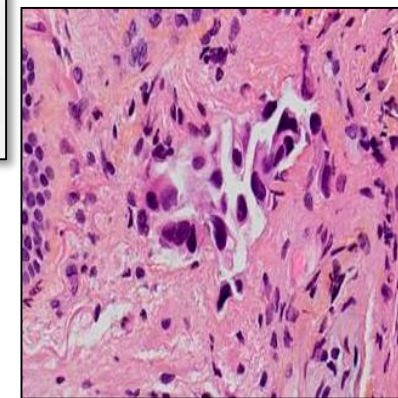


1

DNA quantity

2

DNA quality



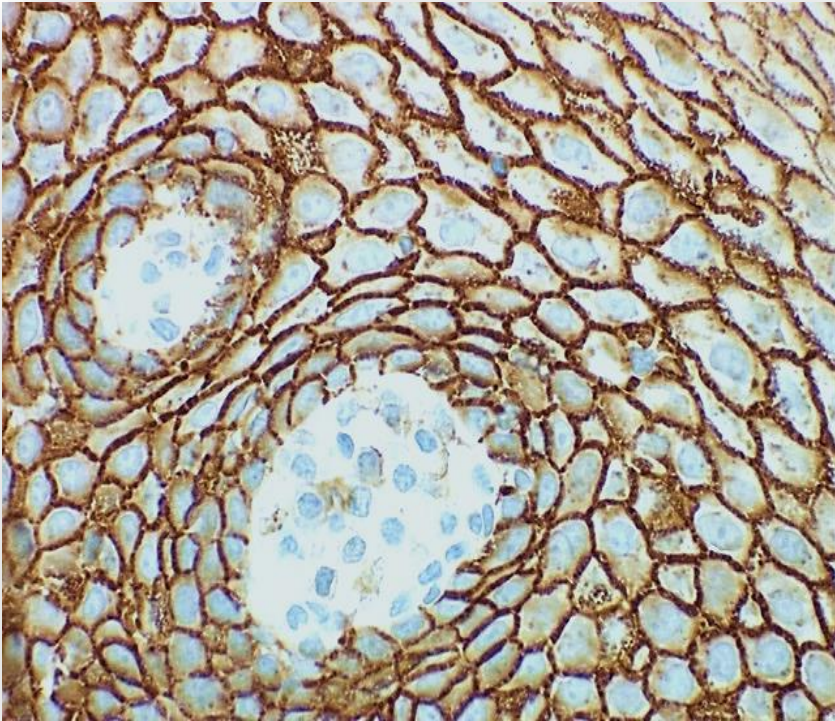
One major challenge in thoracic oncology





IHC PD-L1

The **only validated test** as a predictive biomarker of I-O first line



Main current issues

- Patients with 0% of PD-L1 TC positivity can be good responders
- Patients with $\geq 50\%$ of PD-L1 TC positivity can be non responders
- Heterogeneity of PD-L1 staining limits its interpretation in small biopsies
- Inter & intra operator variability in PD-L1 IHC assessment
- Many PD-L1 clones, different devices, different performances
- Many cut off ($>1\%$, $> 25\%$, $\geq 50\%$) according to the anti-PD1/PD-L1 molecule
- Clinical value of positive immune cells for PD-L1 is debated in NSCLC
- N-linked glycosylation of PD-L1 hinders its recognition by PD-L1 antibodies

IHC PD-L1

In the real world, how viable an option is first line immunotherapy in NSCLC according to the PD-L1 IHC status ?

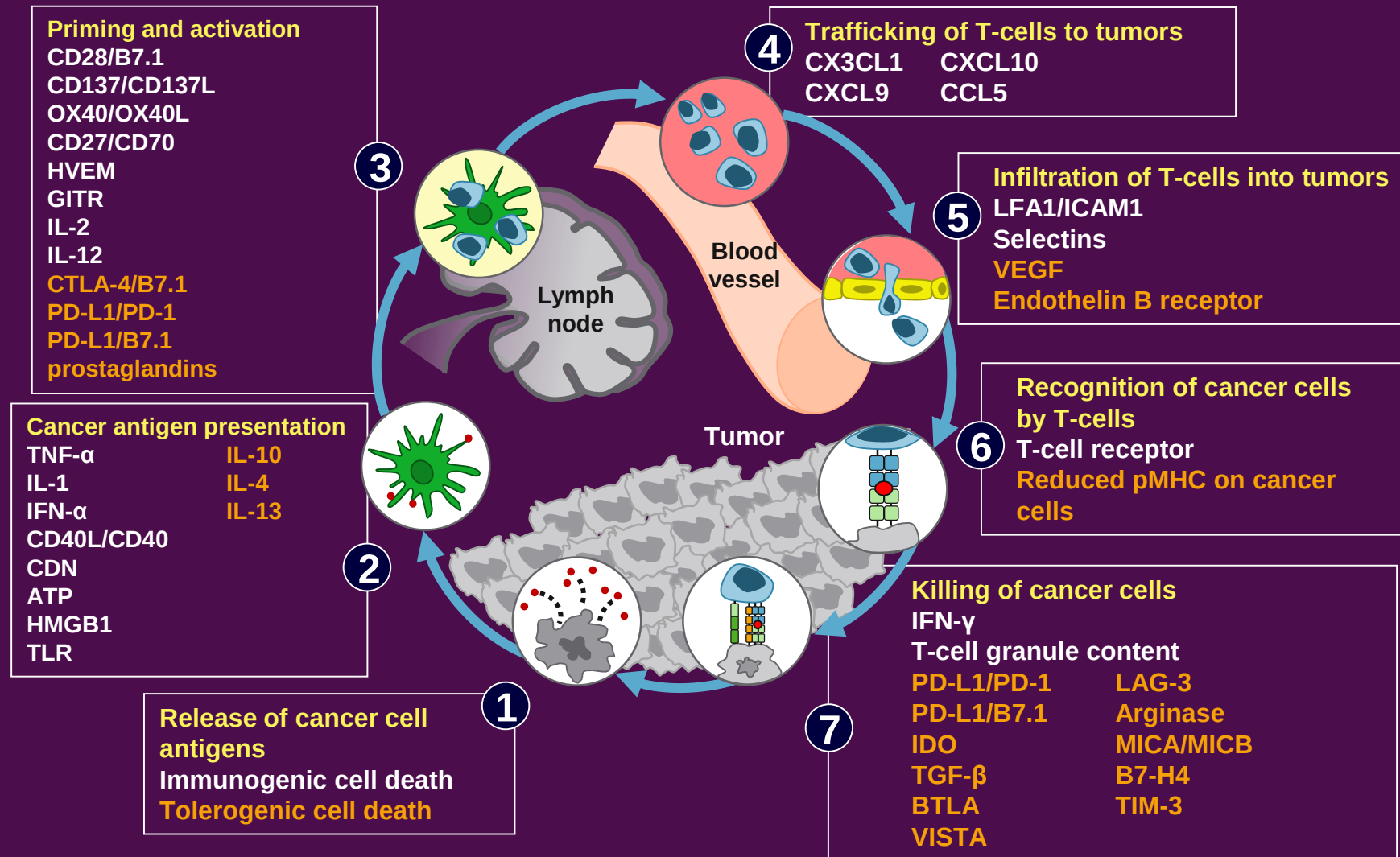
- Patients with tumors $\geq 50\%$ PD-L1: 30% patients
- Patients with poor PS not eligible for treatment: 34% patients
- Patients with either *EGFR* mut or *ALK/ROS1* fusion: 18% patients
- Patients with preexisting autoimmune disease: 13% patients

Patients currently eligible for immunotherapy first line in clinical routine practice:

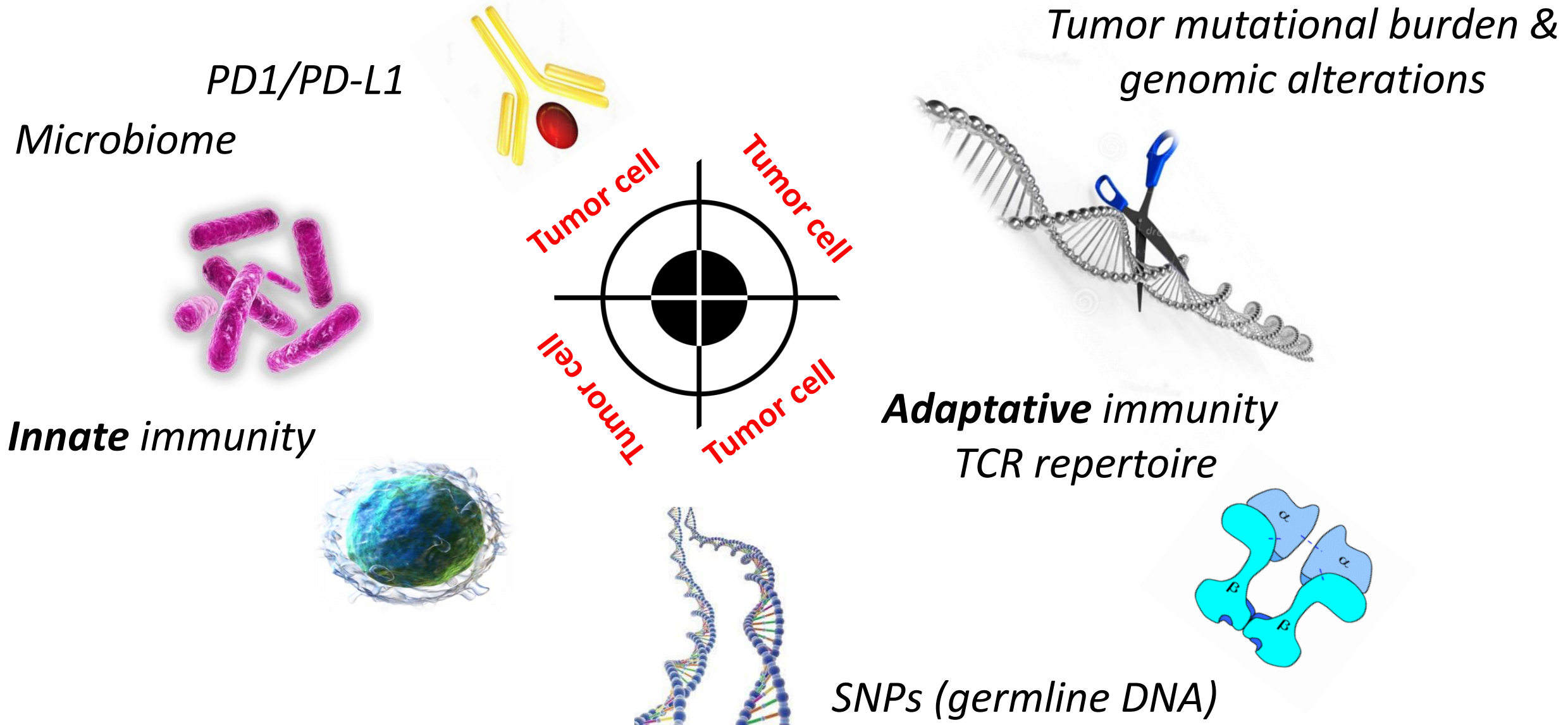
Around 10% of stage IIIB/IV NSCLC patients !



Many other antigens of strong interest



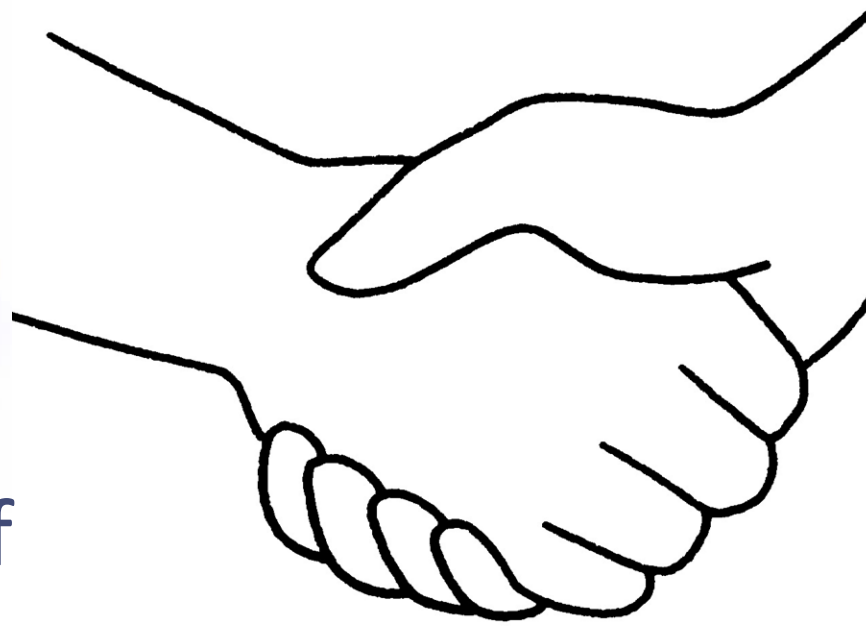
Urgent need for using better predictive biomarkers in oncologywhich could be use in small tissue specimens



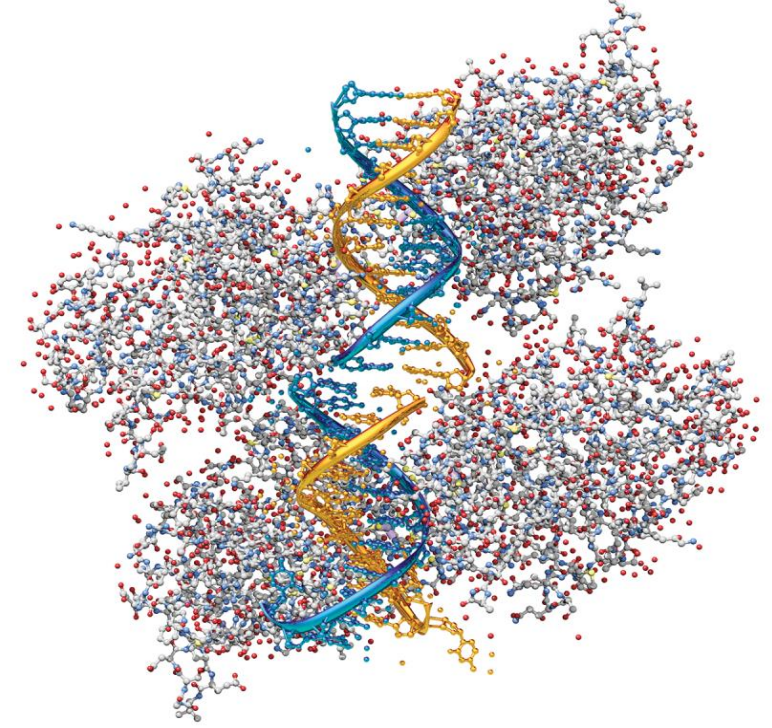
And moreover.....



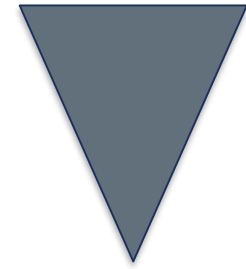
Combination of
therapies



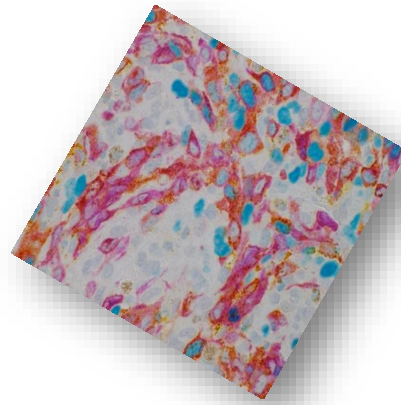
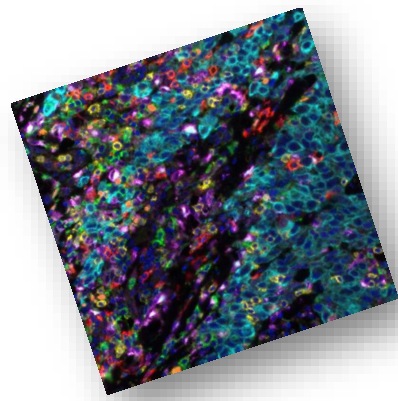
Why and how?



Combination of
biomarkers



How to assess them?



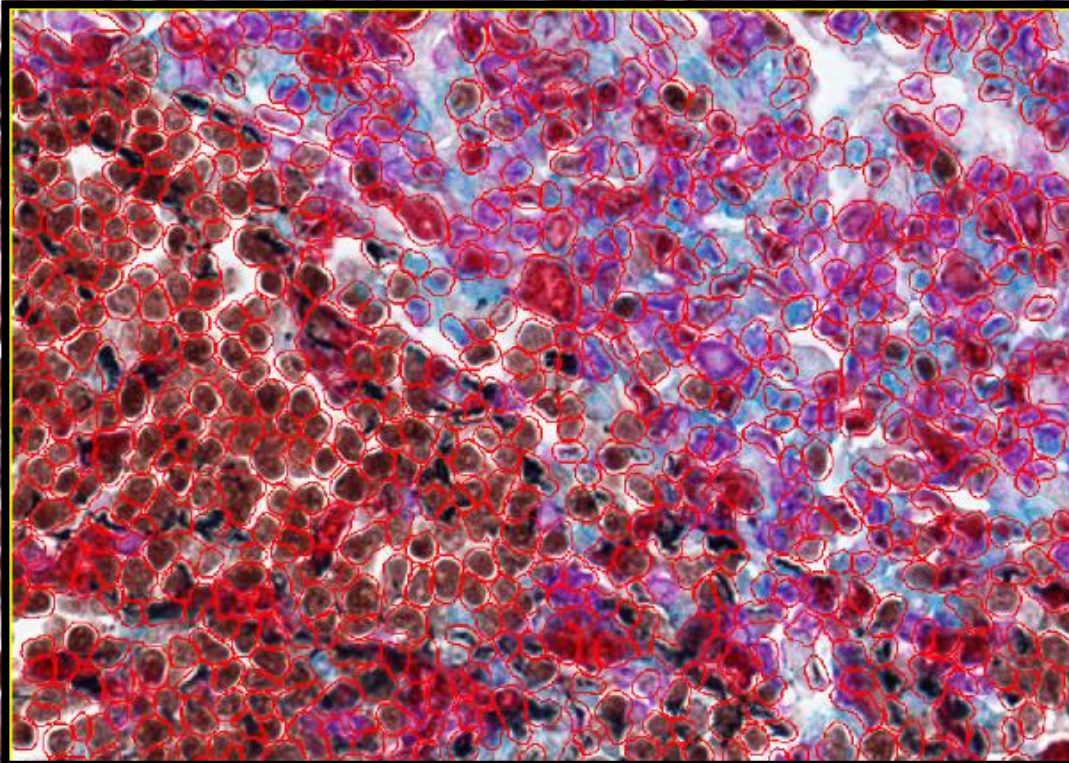
Multiplexed immunohistochemistry may be one solution

A sudden recent renewed interest in pathology lab due to I-O development

Number of biomarkers

Bioresource available

Multiplex Immunohistochemistry (MplHC-Multiplex Molecular Pathology)



1. Detect expression of
different biomarkers at the
same time

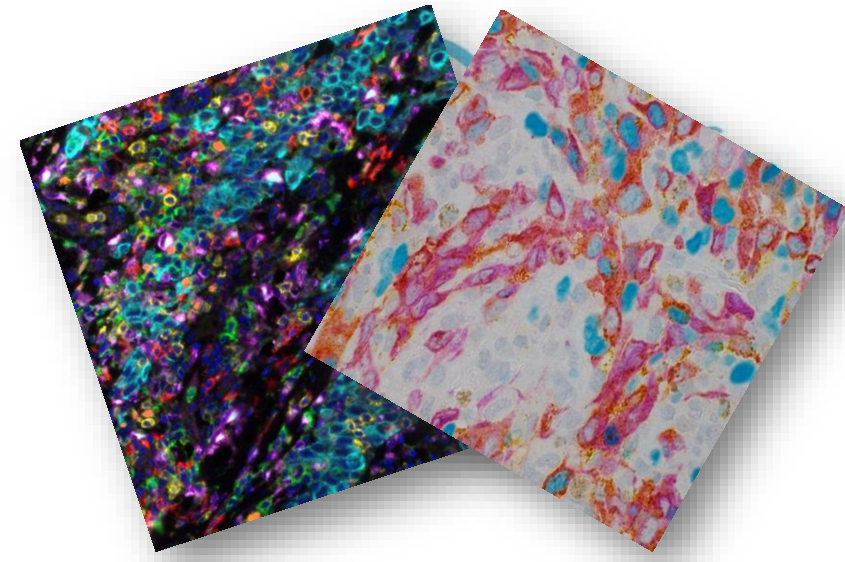
2. Enhanced interrogation
Immune cells signature

3. Spatial analysis
Cell-Cell interaction

4. Accurate scoring
Quantification



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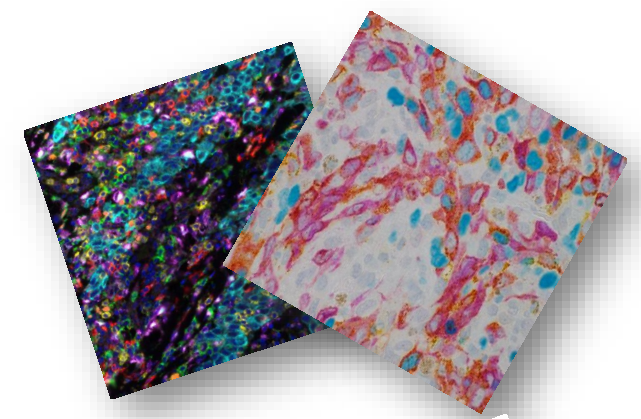
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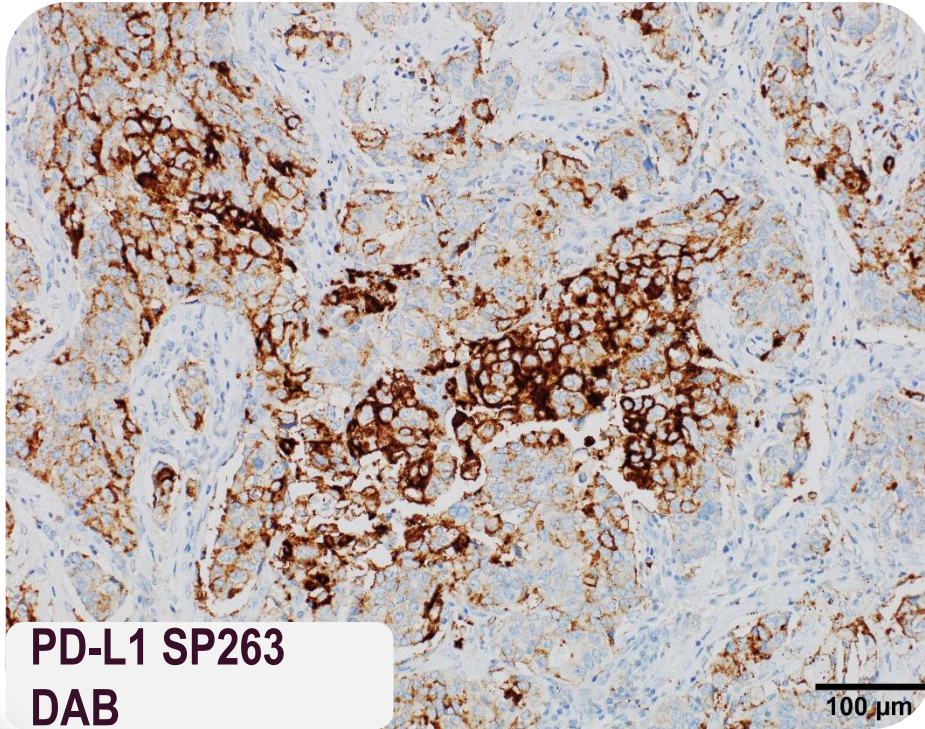
Multiplexed immunohistochemistry

Many complex technologies !



Multiplex IHC principle

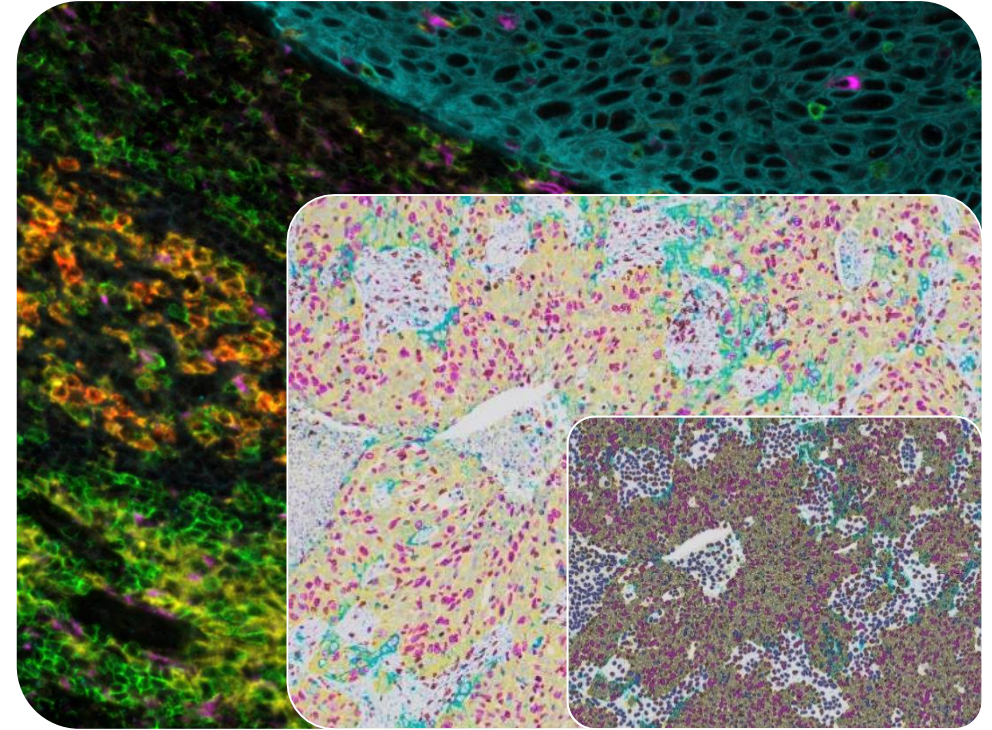
Current analysis in routine



THE TISSUE IS THE ISSUE

- Still the gold standard
- Consumption of small biopsy clinical samples
- Difficult correlation of protein expression/topography from one slide to another
- Reduced availability of samples for additional analyses (e.g. clinical trials, research)

Quantitative and multi-cell analysis*

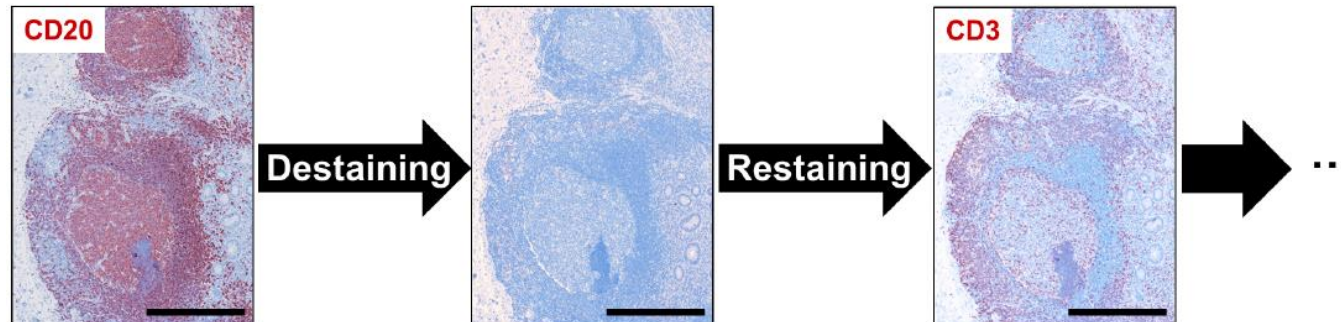
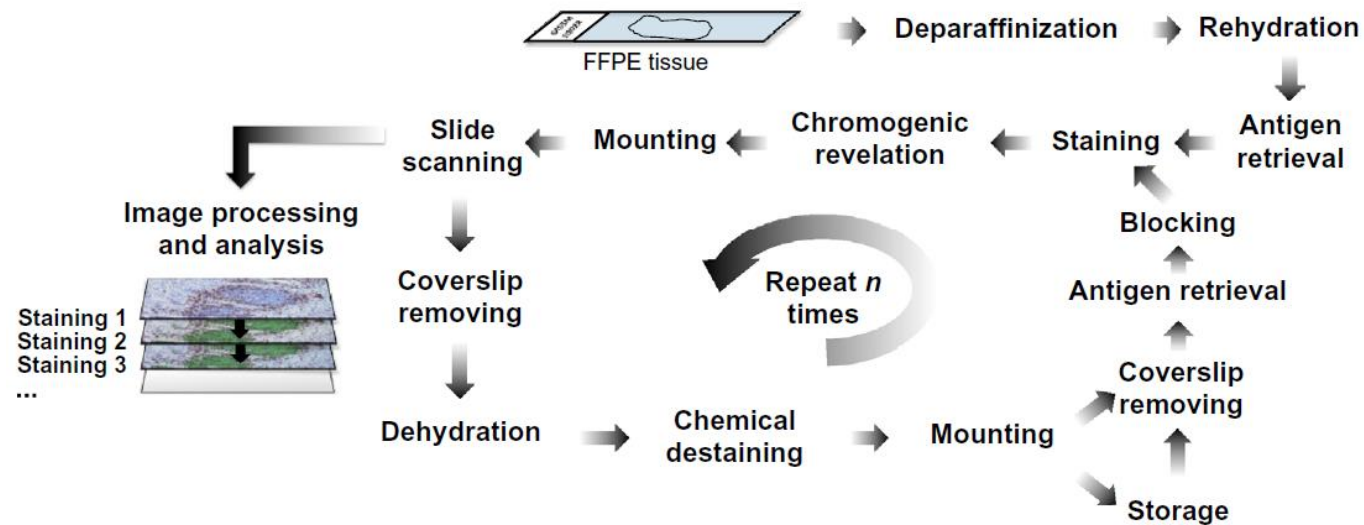


* Need for digital analysis

Examples of technologies

1) Antigen retrieval by serial stripping

MICSSS technology : Multiplexed immunohistochemical consecutive staining on single slide



First developed technology

*Time consuming
Repeatability?
Research use only !*

2) Fluorescence technologies (e.g. Vectra 3 approach)

Opal™ (Perkin Elmer)^{2,3}

Heat



Antigen A
Antigen B
Antigen C

Antibody A (Rabbit)
Antibody B (Rabbit)



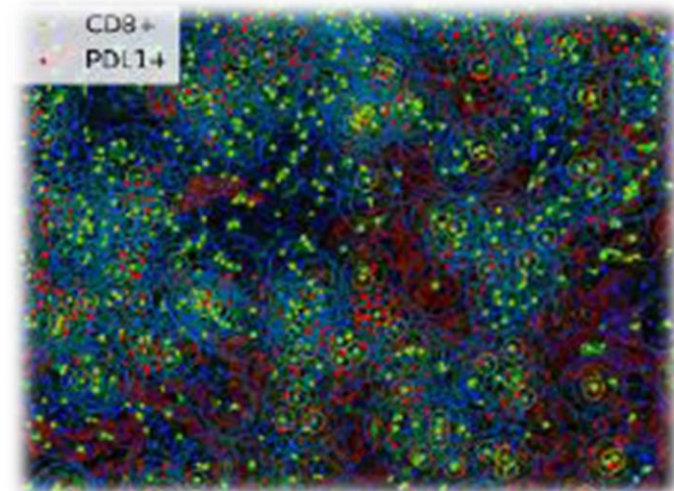
HRP-conjugated
Anti-Rabbit IgG
or Superpicture



Fluorochrome -
tyramide



Scan Fluo slides
Vectra 3 PerkinElmer
Phenochart



3) Digital fluorescence analysis

Scanners (fluo / spectral)

- Multiples fluorochromes (even instable, ex. phycoerythrine)
- > 7 antibodies / slide



Vectra

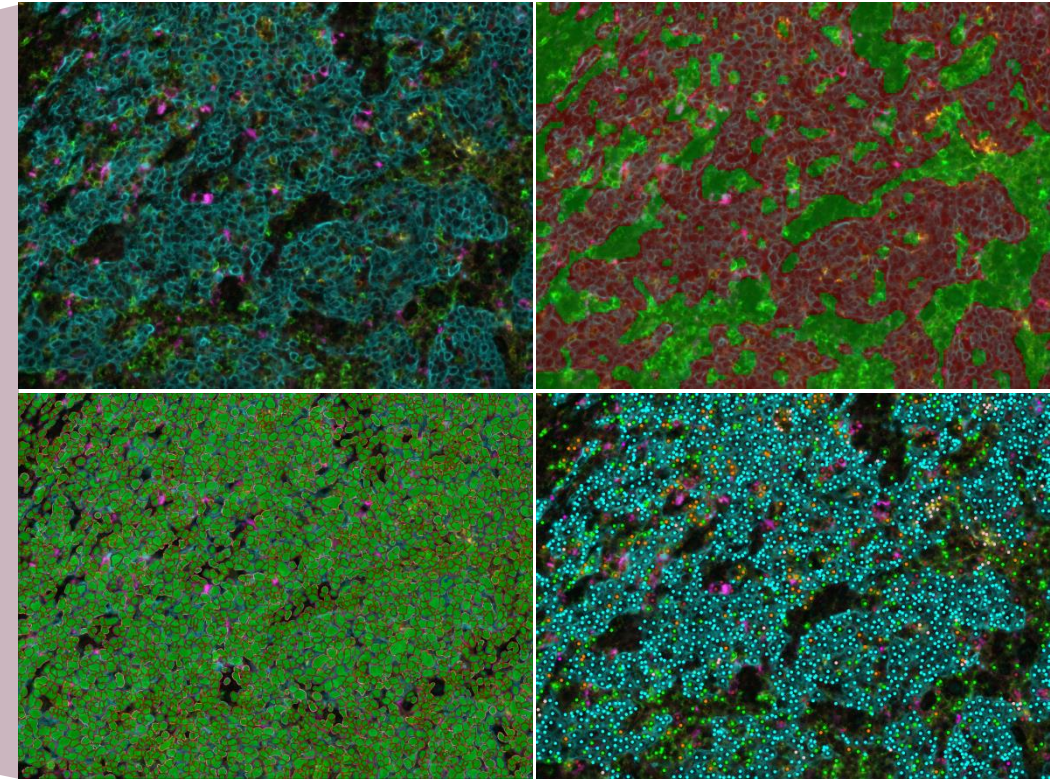


Polaris

InForm software

Autofluorescence Reduction Technology[®]
(ART[™])

- Spectral resolution 420nm - 900 nm
- Precise quantitative analysis/cell-by-cell
- Bioinformatics interpretation
- Tissue/cell segmentation
- Phenotyping
- Co-localization



pan-Cytokeratin

CD4

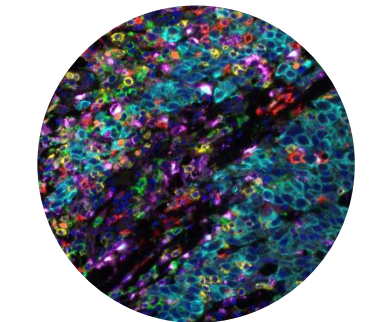
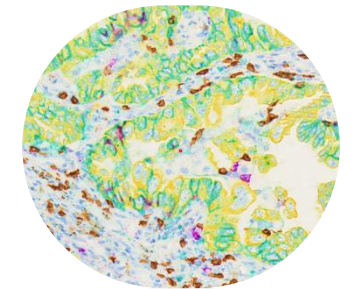
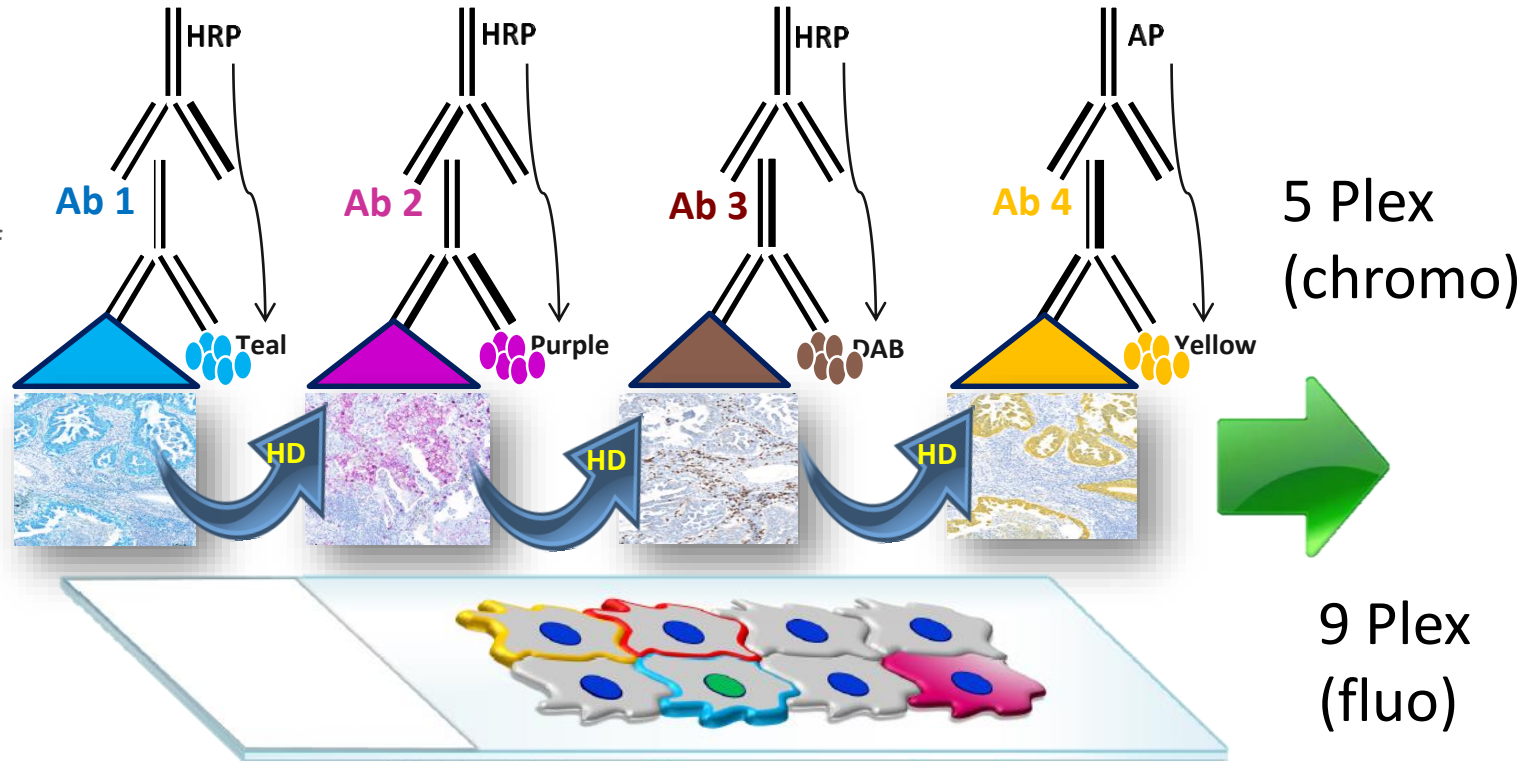
CD68

PD-1

PD-L1

4) Heat deactivation (HD)

- HRP catalyzes tyramide into highly reactive free radicals
- Tyramide conjugated to chromogen remains stable
- HRP-activated tyramid molecules covalently bind to the amino acids of antigens
- Resistant to thermochemical denaturation



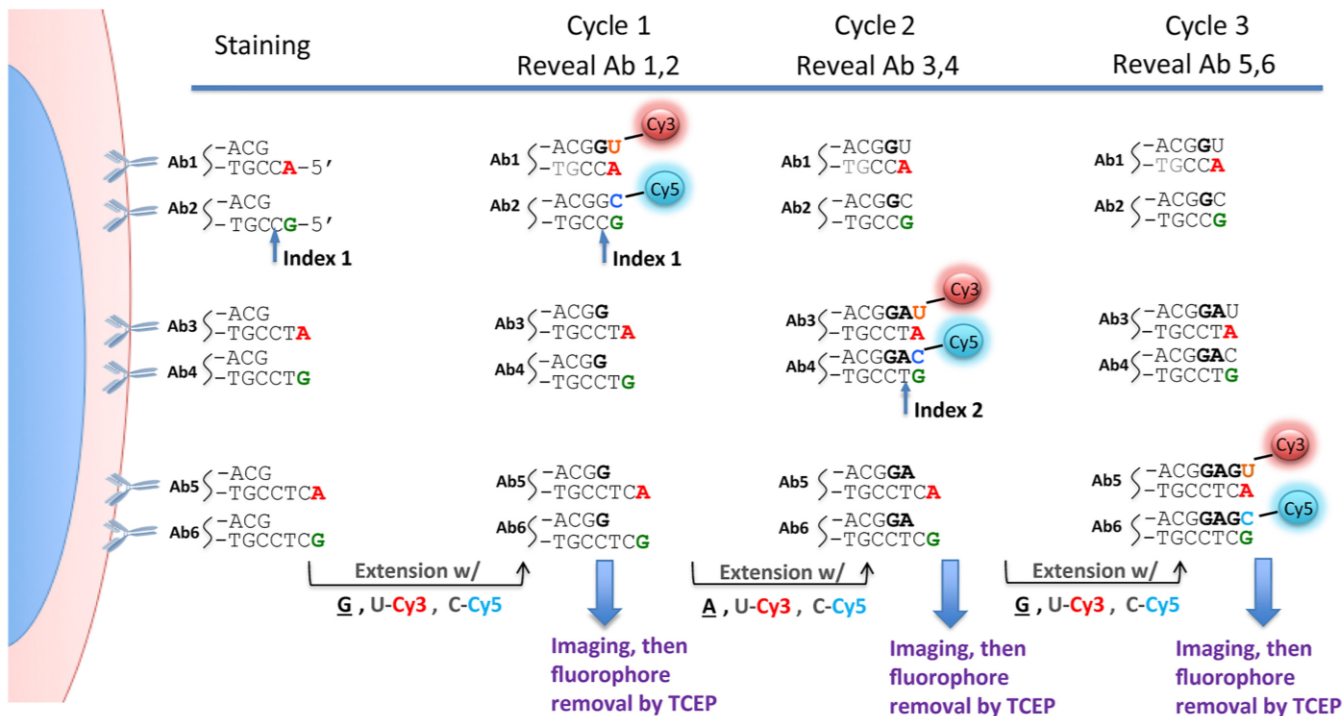
1. Zhang W, et al. Fully automated 5-plex fluorescent immunohistochemistry with tyramide signal amplification and same species antibodies. *Lab Invest.* 2017.
2. Ilie M, et al. Automated chromogenic multiplexed immunohistochemistry assay for diagnosis and predictive biomarker testing in non-small cell lung cancer. *Lung Cancer.* 2018.
3. Hofman P, Badoual C, Henderson F, Berland L, Hamila M, Long-Mira E, Lassalle S, Roussel H, Hofman V, Tartour E, Ilié M. Multiplexed Immunohistochemistry for Molecular and Immune Profiling in Lung Cancer-Just About Ready for Prime-Time? *Cancers (Basel).* 2019 Feb 27;11(3).

And even more...!

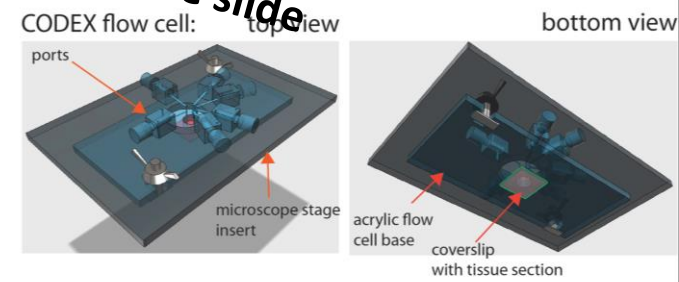
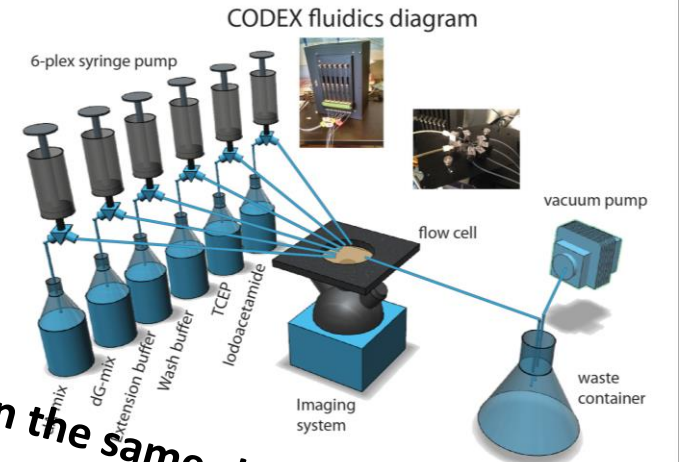
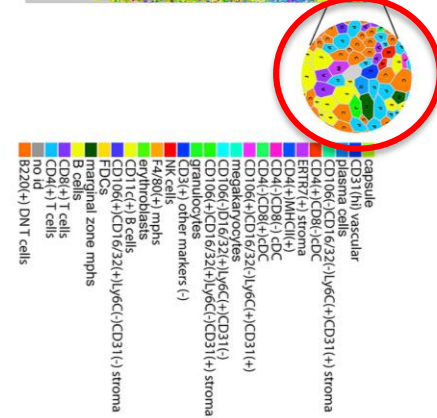
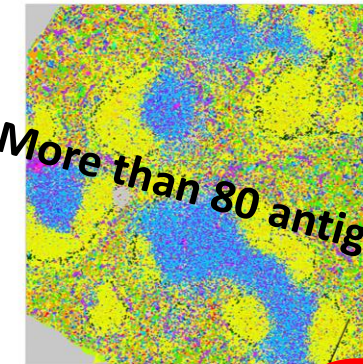
New technologies: many targets but high complexity

5) Co-detection by indexing (CODEX)

CODEX iteratively visualizes antibody binding events using, **1) DNA barcodes**, **2) fluorescent dNTP analogs**, and **3) an in situ polymerization-based indexing procedure**



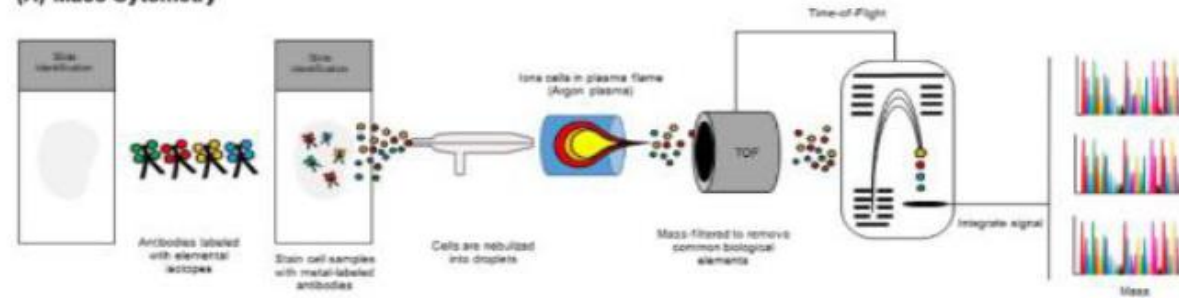
More than 80 antigens in the same slide



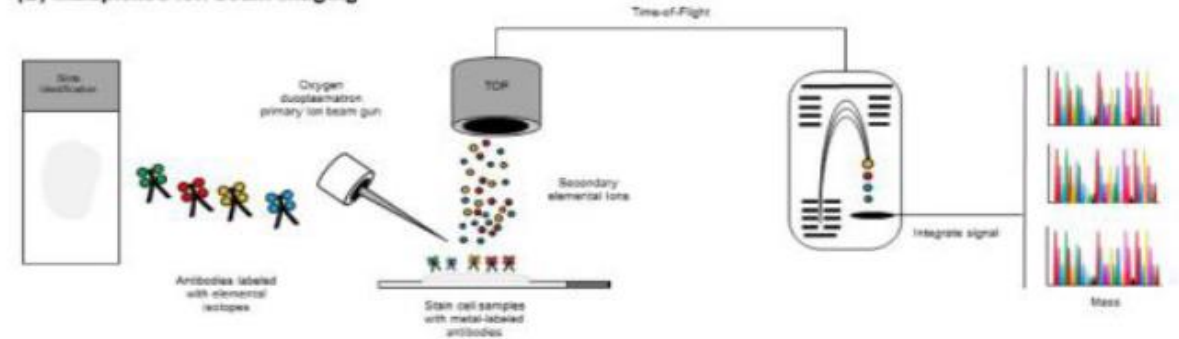
6)

Mass cytometry-based techniques

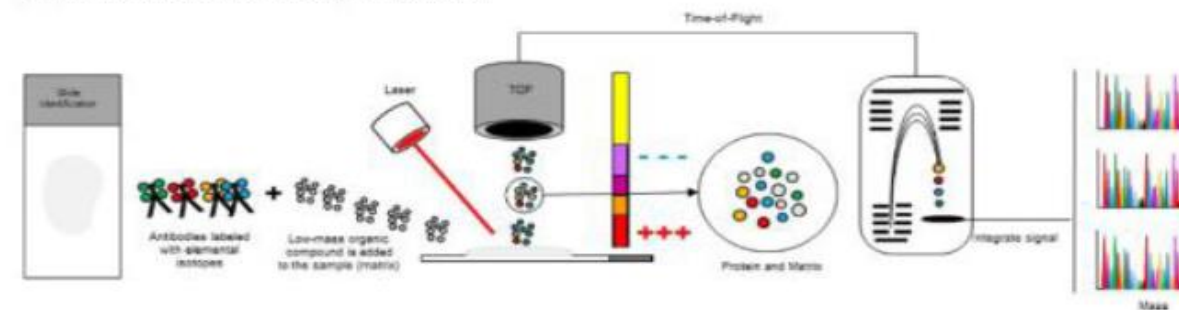
(A) Mass Cytometry



(B) Multiplexed ion beam imaging



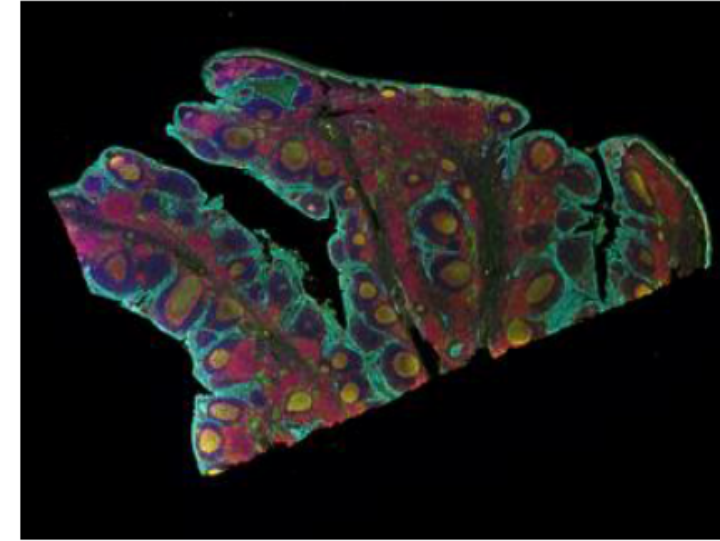
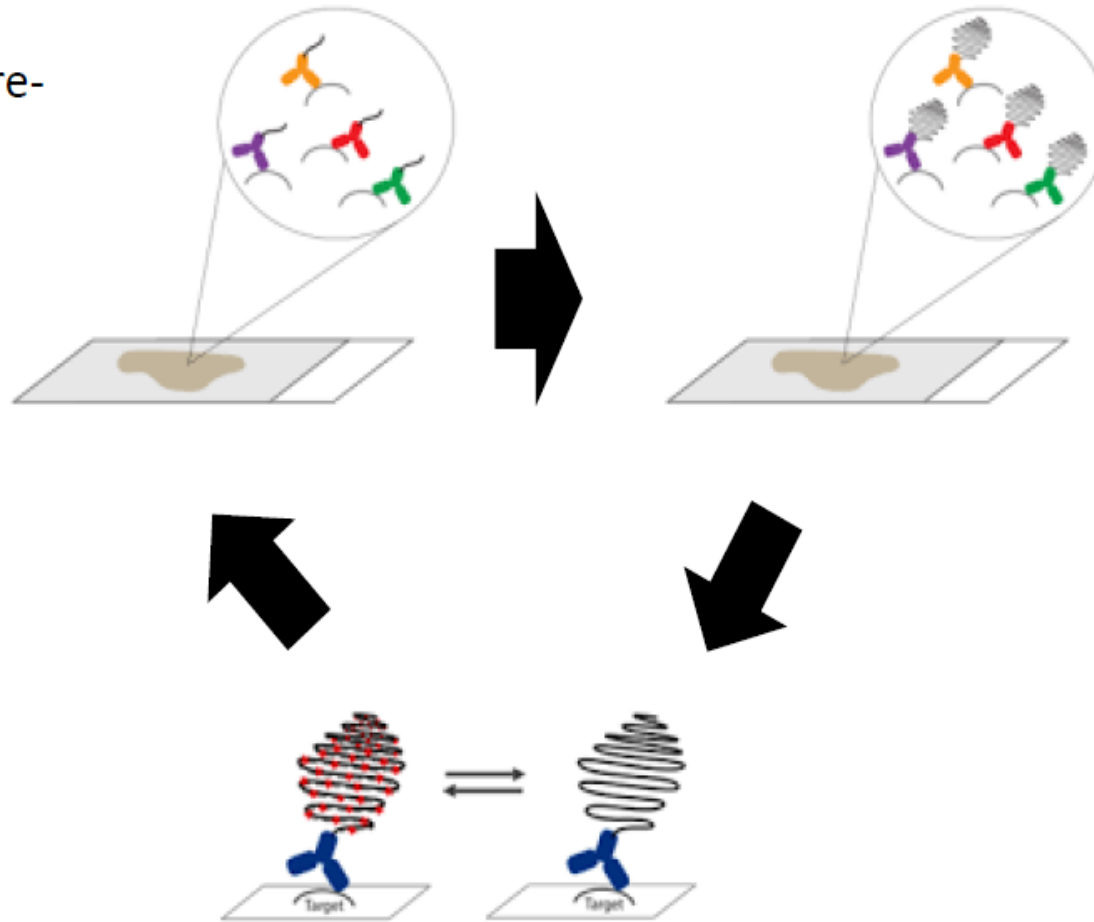
(C) Matrix-assisted laser desorption/ionization



E. Parra, 2018

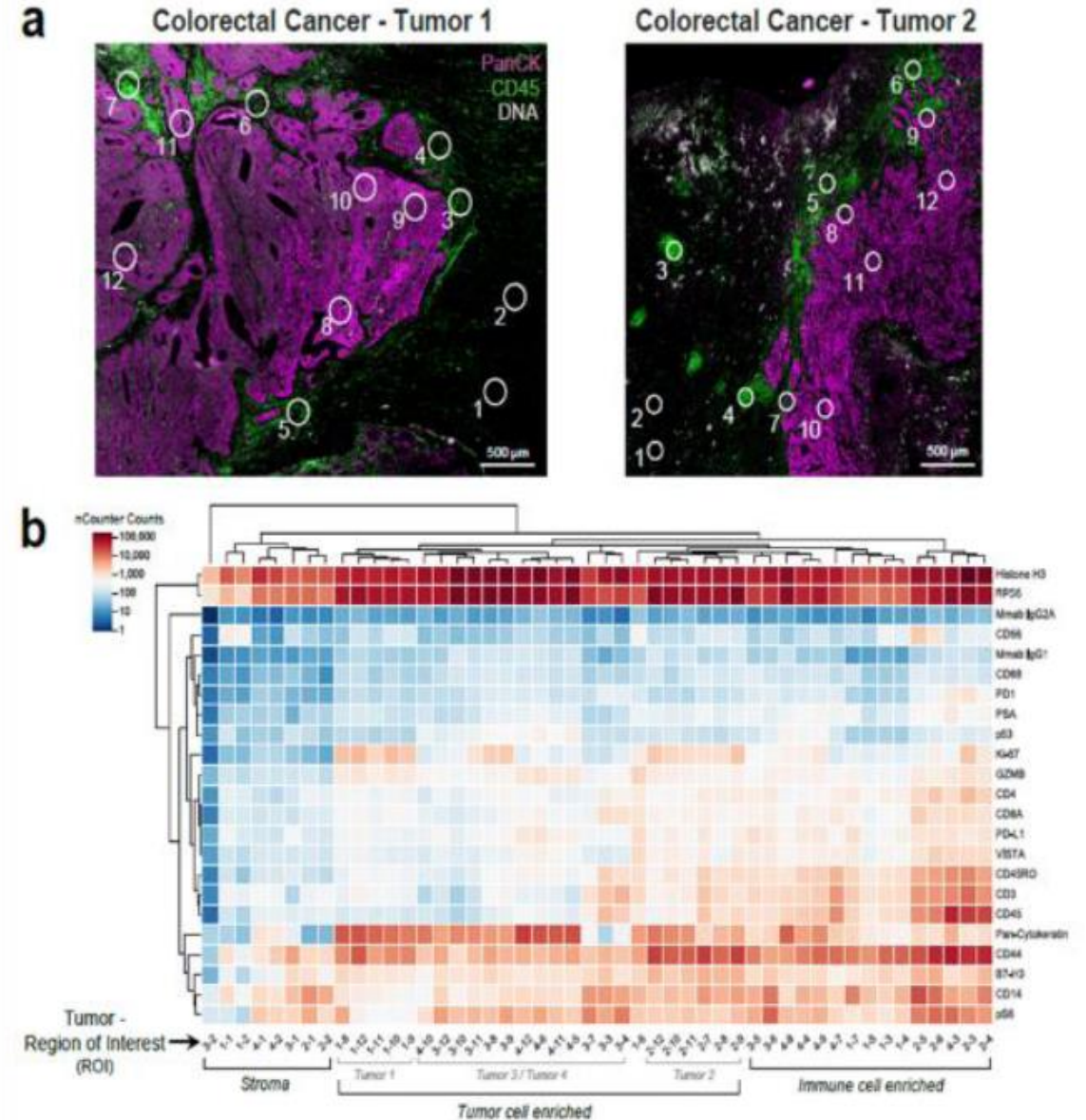
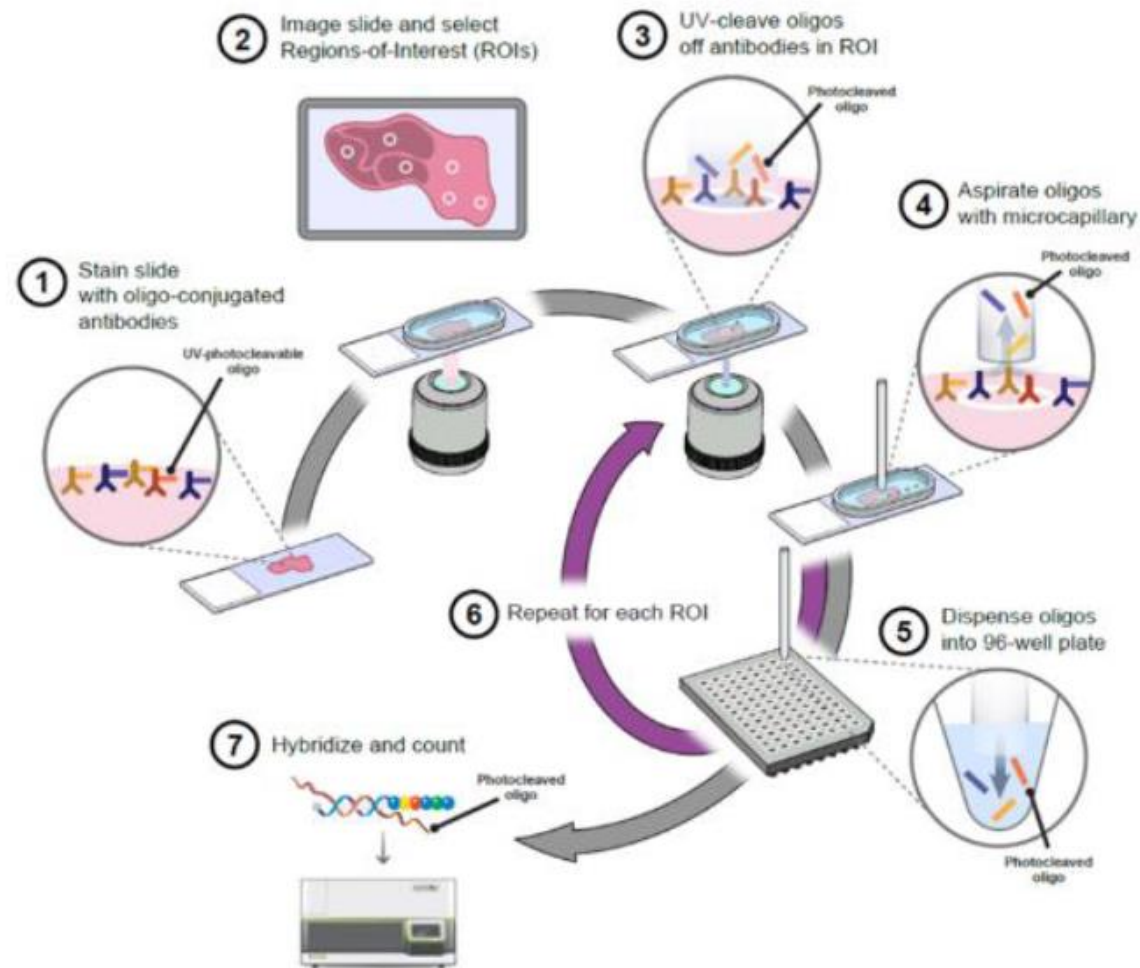
7) InSituPlex Technology (Ultivue)

- DNA barcode conjugated to primary antibodies
- Complementary fluorophore-labeled probes attach to fluorophores
- Specific, sensitive and reversible



ZEISS Axio Scan.Z1

8) Digital spatial profiling



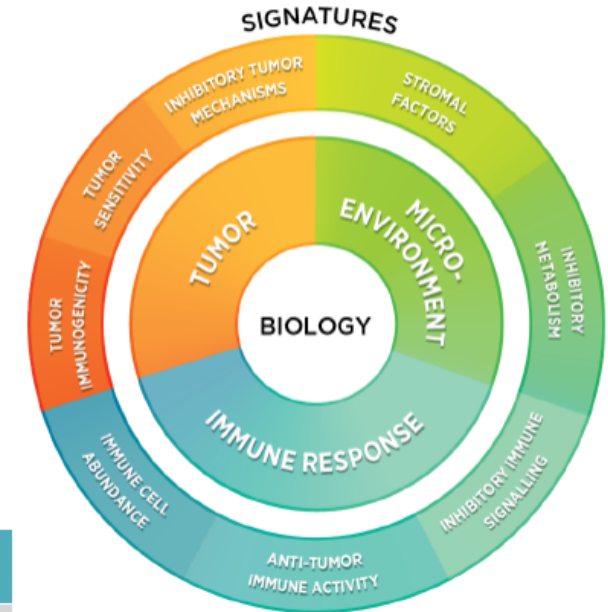
From complex protein analysis to complex RNA analysis

Tumor inflammation signature

PanCancer IO 360™ Gene Expression Panel and Analysis

360 = Holistic view Tumor-Microenvironment-Immune Response

- 770 human genes total (customizable)
- Including 20 reference genes
- Designed for use with FFPE tumor tissue



Tumor (20%)	Microenvironment (20%)	Immune Response (60%)
-------------	------------------------	-----------------------

16 Pathways and Processes

34 Signatures Measuring Immune and Tumor Activity

14 Cell Type Abundance Scores

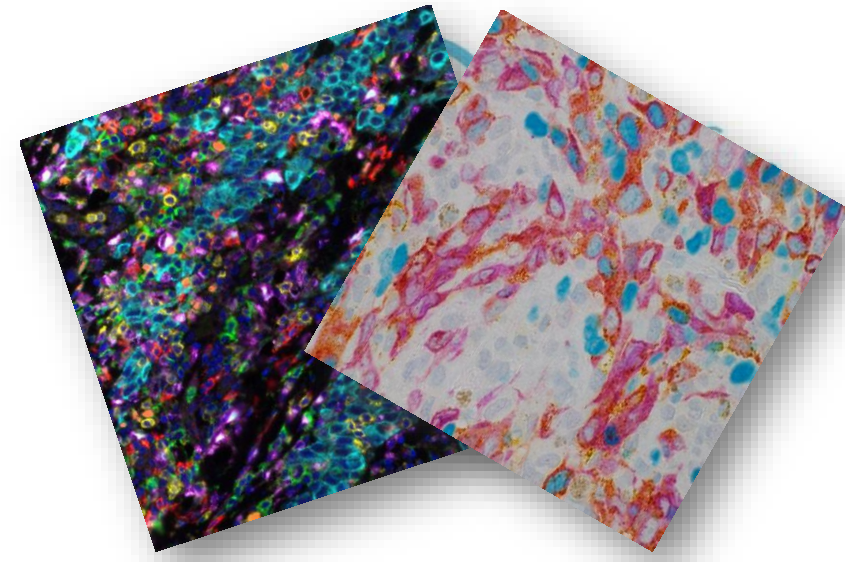
18-Gene Tumor Inflammation Signature (TIS): Measures peripherally suppressed adaptive immune response
--

PanCancer IO 360 Biological Signatures

Tumor Immunogenicity	Tumor Sensitivity to Immune Attack	Inhibitory Tumor Mechanisms	Stromal Factors	Inhibitory Metabolism	Anti-Tumor Immune Activity	Inhibitory Immune Signaling		Immune Cell Population Abundance	
Antigen Processing Machinery	Apoptosis	IDO1 Gene Expression	Endothelial Cells	Glycolysis	Tumor Inflammation Signature (TIS)	CTLA4 Gene Expression	PD-L2 Gene Expression	B Cell Abundance	Mast Cell Abundance
Antigen Presenting Machinery Expression Loss	Tumor Proliferation	PD-L1 Gene Expression	Stromal Tissue Abundance	Hypoxia	Cytotoxicity	IL10 Gene Expression	TIGIT Gene Expression	CD45+ Cell Abundance	Neutrophil Abundance
Immunoproteasome	JAK-STAT Pathway Gene Expression Loss	B7-H3 Gene Expression			Interferon Gamma Signaling	Inflammatory Chemokines	ARG1 Gene Expression	CD8+ T Cell Abundance	NK CD56dim Cell Abundance
MAGE Genes Expression		TGF-Beta Gene Expression			Interferon Signaling Response	Myeloid-Derived Inflammatory Signaling	NOS2 Gene Expression	Cytotoxic Cell Abundance	Natural Killer Cell Abundance
Loss of Mismatch Repair Gene Expression					Lymphoid Compartment Activity	PD-1 Gene Expression		Dendritic Cell Abundance	T Cell Abundance
Hypermutation					MHC Class II Antigen Presentation			Exhausted CD8 Cell	TH1 Cell Abundance
MSI Predictor					Myeloid Compartment Activity			Macrophage Abundance	Treg Abundance



Road map



Short background

Multiplex IHC: Technical principles

Chromogenic *versus* fluorescent multiplex IHC : What is the best choice?

The digital and image analysis: A pivotal issue for the pathology labs

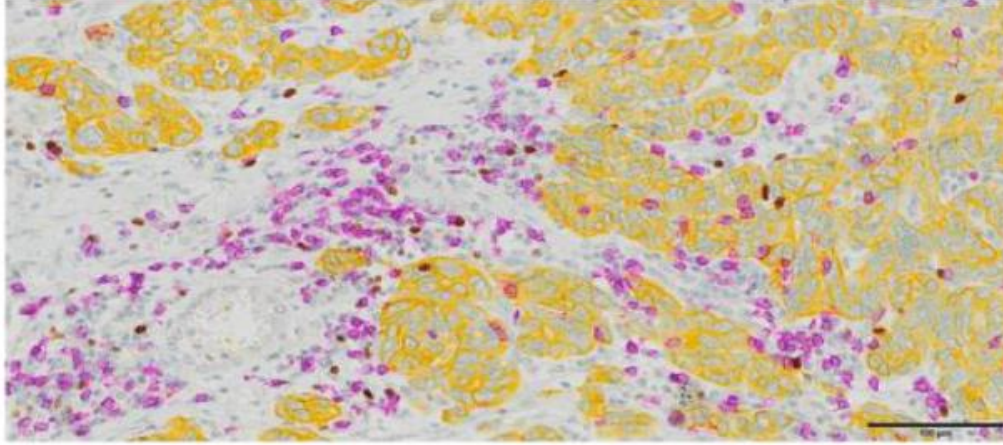
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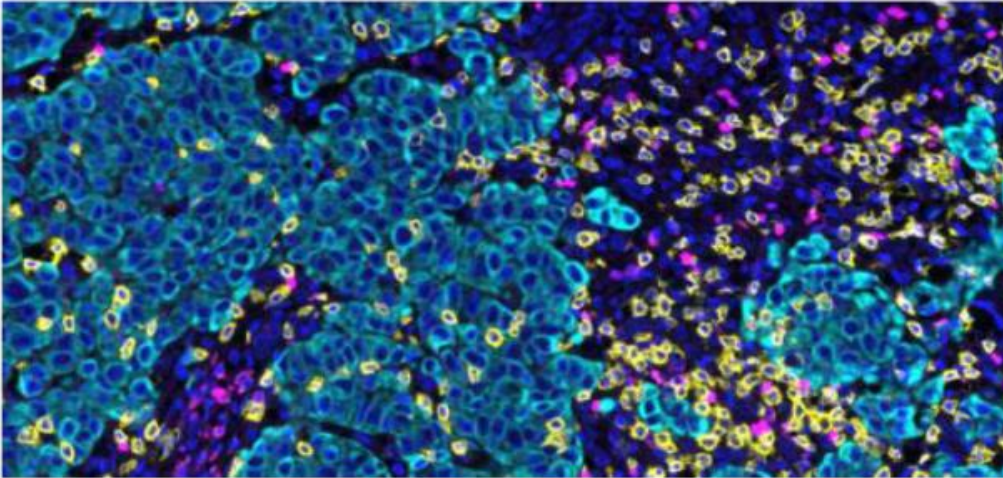
Chromogenic versus fluorescent multiplexed staining

Chromogenic
multiplex IHC



- Brightfield imaging, easy to manage in pathology labs
- Pathological assessment
- Cheaper
- Limited to one marker by cell compartment

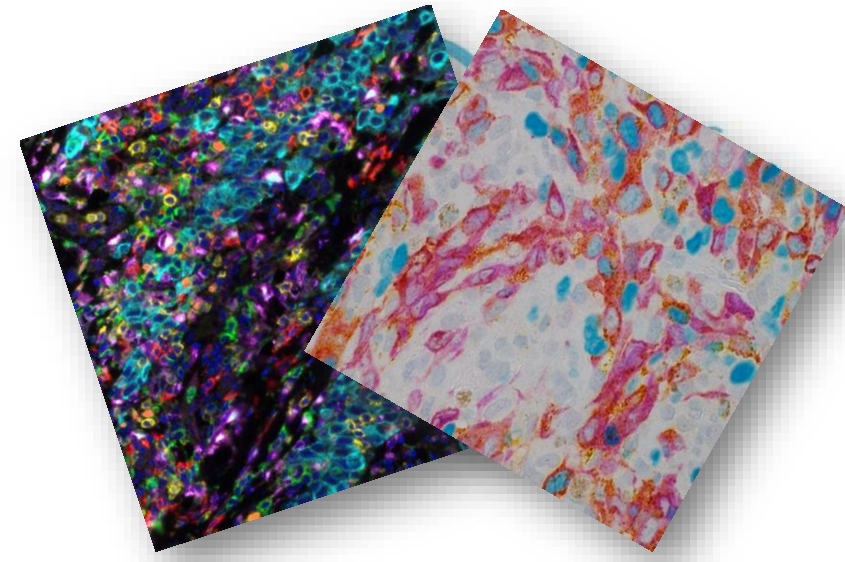
Fluorescent
multiplex IHC



- Multiple markers in a cell compartment
- More quantitative assessment of protein expression
- Availability and complexity of fluorescence imaging
- Complex validation process
- Expensive



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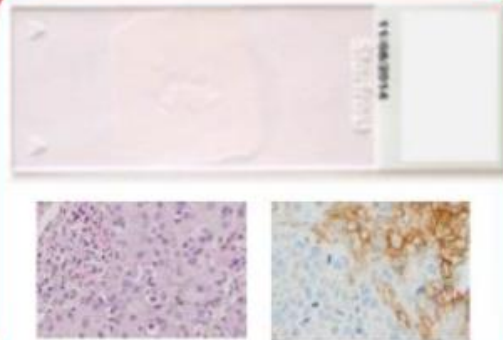
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FFPE tissue sample



Tissue sections

H&E, IHC, ISH

Multiplex technologies

Spatial resolution

Tumor heterogeneity

Cell phenotyping



Digital pathology

Availability
Harmonization
Storage
Workflow, cost

Image analysis

Machine learning/deep learning

Supervised/unsupervised

Hand-crafted features/deep neural networks

Data analysis

Image analysis platforms

Vendor	Program name	Method	Availability
FARSIGHT	Nucleus Editor	Multichannel-based object identification/toolkit	Free
Queen's University Belfast	QuPath	Color-based, interactive segmentation	Free
Tribvn	Calopix	Signal intensity, area, counting objects	Licensed
Visiopharm	Visimoph Tissuemorph	Signal intensity, area, counting objects, statistical analysis	Licensed
Spot Imagine	Spot advanced	Color-based colocalization	Licensed
HistoRx	AQUAnalysis	Signal intensity per unit area and per layer	Licensed
CompuCyte	iCyte	Nucleus segmentation or phantom contouring, measuring associated signals	Licensed

Commercialized image analysis products (chromo/fluo)

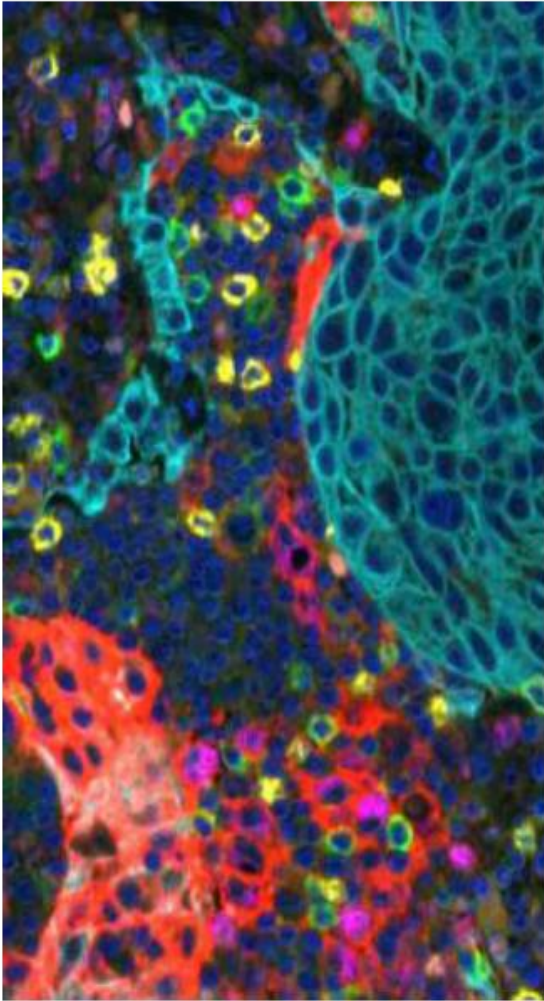
Whole slide

- 5 colors
 - **HALO**
Indica Labs
- 4 colors
 - **Mirax HistoQuant**
3DHistech
 - **BLISS workstation**
Bacus Laboratories
- 3 colors
 - **Vectra Polaris/inForm**
PerkinElmer
 - **Aperio Color Deconvolution Algorithm/SlidePath**
Leica Biosystems
 - **Automated Cellular Imaging System**
ACIS III, Dako
- 2 colors
 - **Tissue Studio® 4.0**
Definiens

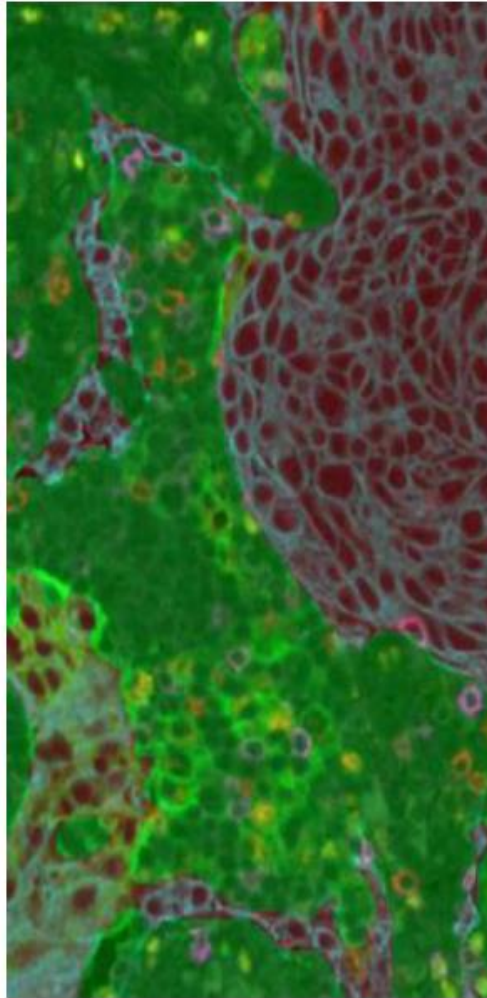


Fluorescent multiplex immunohistochemistry

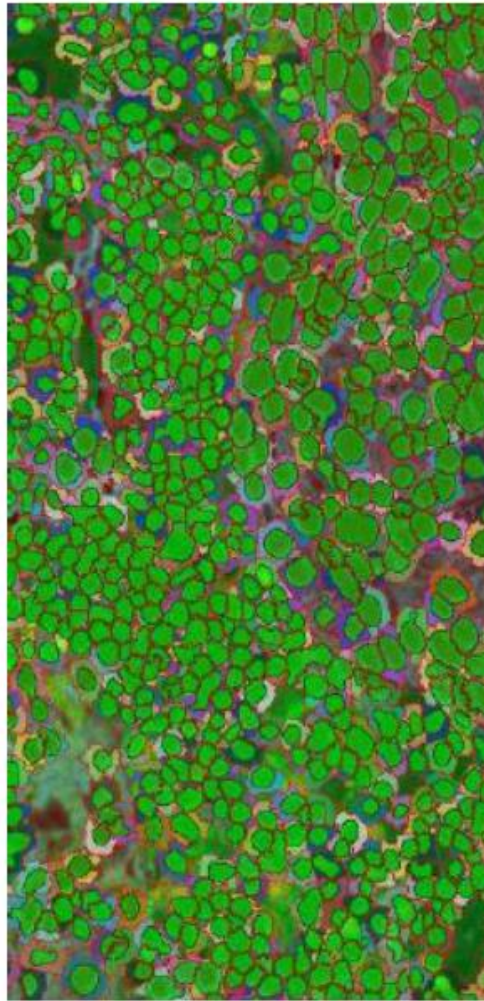
Multispectral
imaging



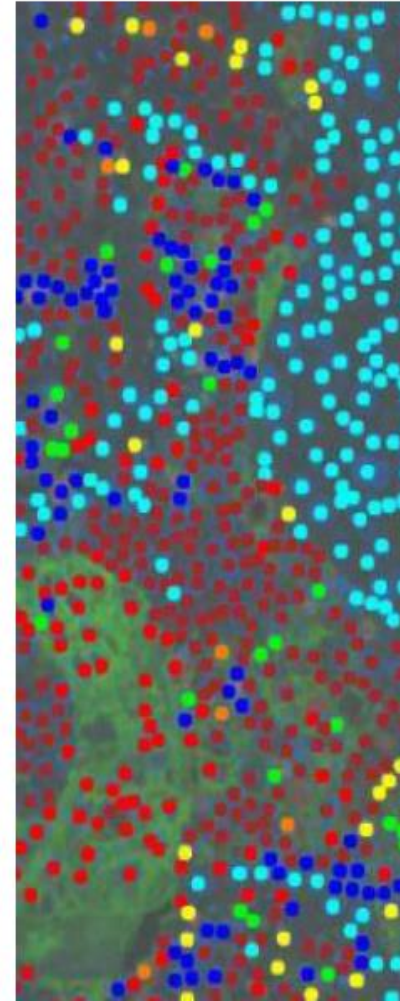
Tissue
segmentation



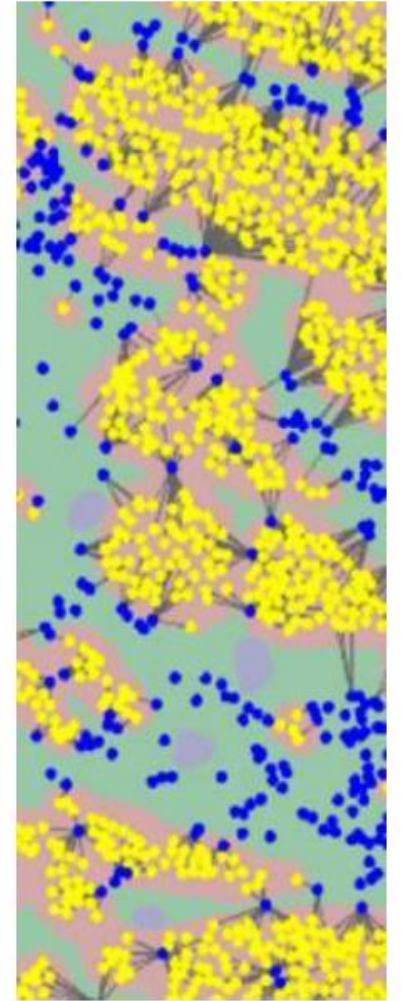
Cell
segmentation



Phenotyping



Spatial features



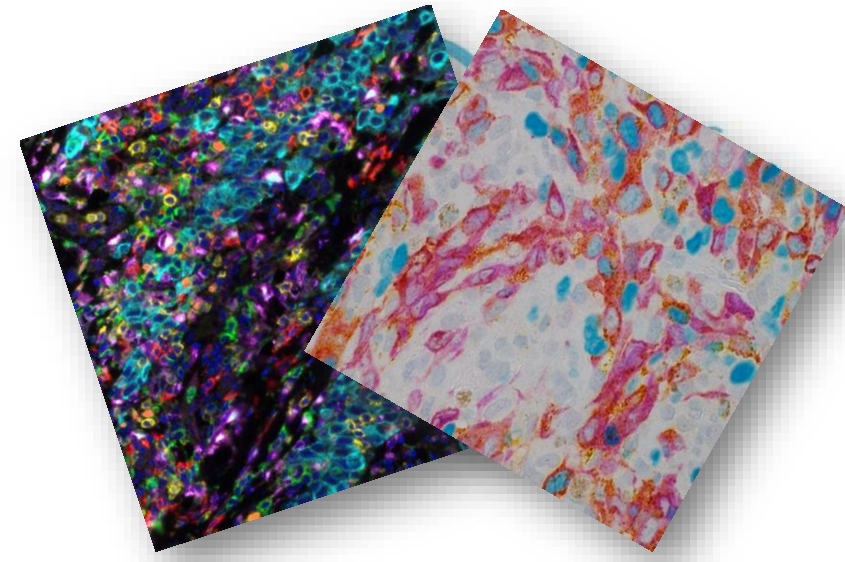
Courtesy Dr J Adam

Beware of « picking up trash ».... or how to make the good choice?





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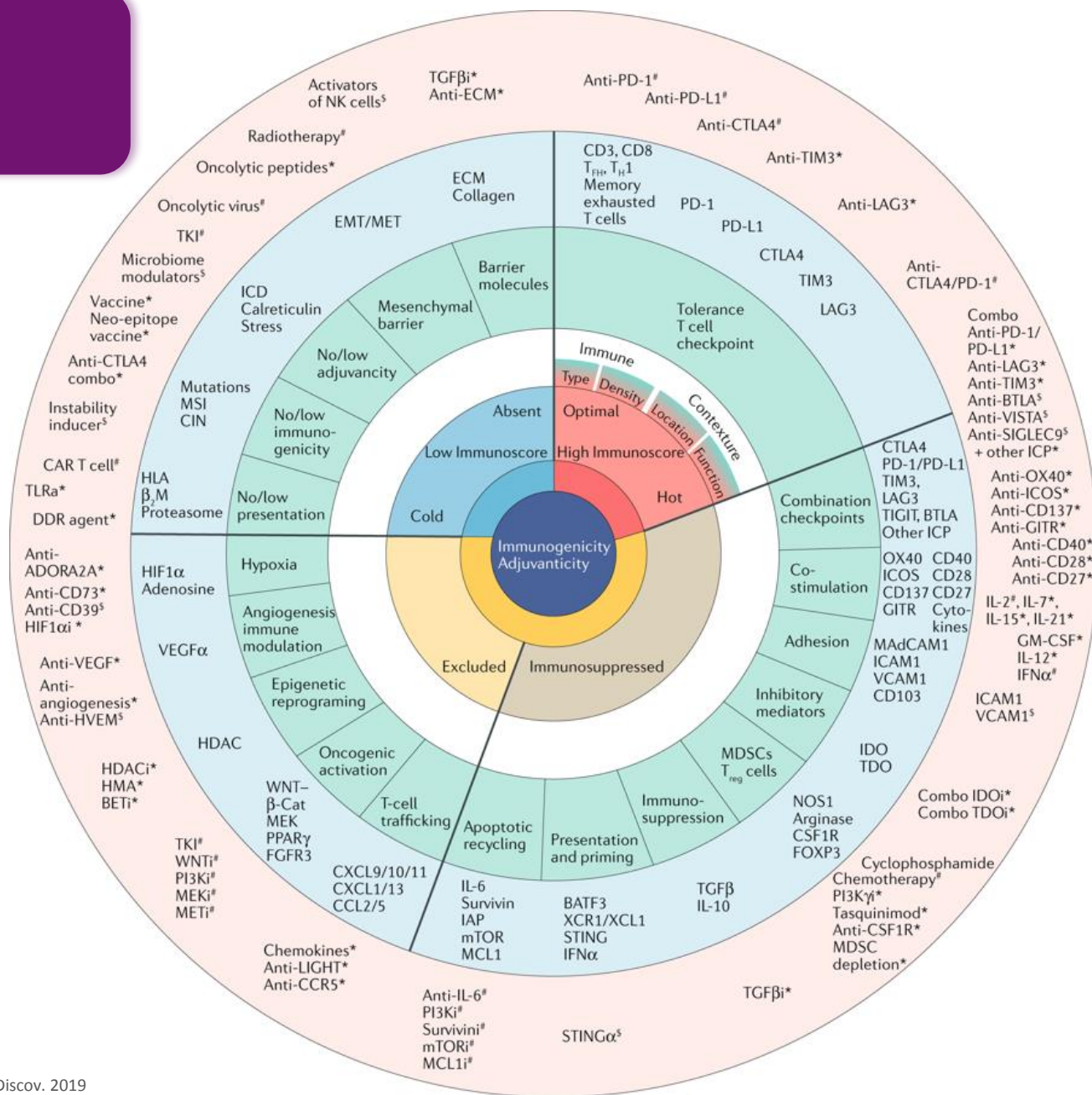
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Take away message

Multiparameter integration

Immune contexture

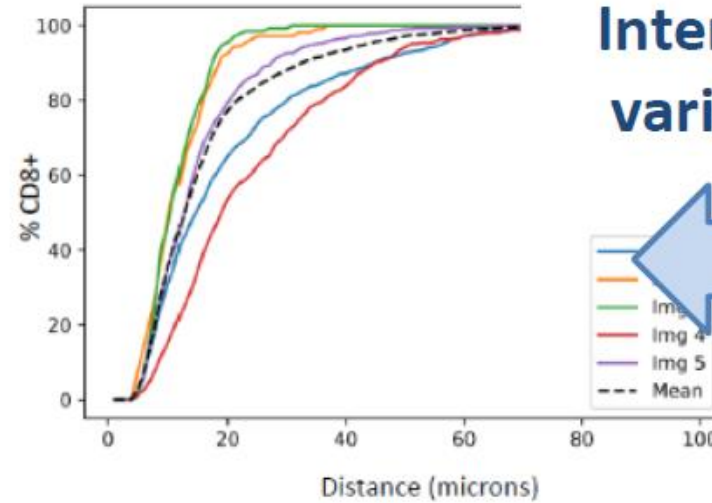
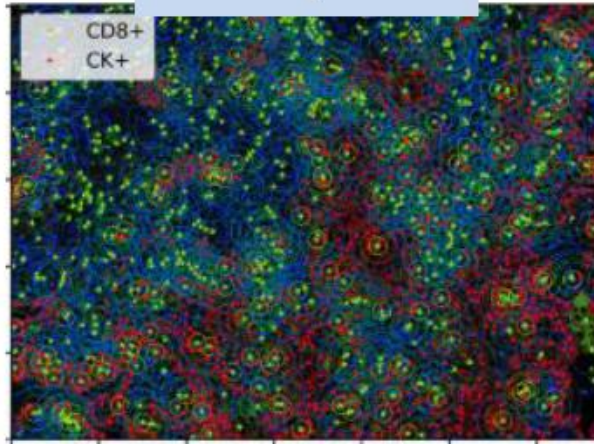
Immune contexture	Parameters: positive association with survival
Type	CTLs (CD3 ⁺ CD8 ⁺) Memory T cells (CD45RO ⁺)
Location	Core of the tumour Invasive margin
Density	Number of cells per mm² 1 10 100 1,000 10,000 CD3⁺_{CT} CD3⁺_{IM} CD8⁺_{CT} CD8⁺_{IM} CD45RO⁺_{CT} CD45RO⁺_{IM}
Functional orientation	T _H 1 cell-associated factors (IFN γ , IL-12, T-bet and IRF1) Cytotoxic factors (granzymes, perforin and granulysin) Chemokines (CX3CL1, CXCL9, CXCL10, CCL5 and CCL2) T _H 17 cells, T _{Reg} cells and T _H 2 cells have a variable effect on survival, depending on tumour type
TLS	Presence and quality



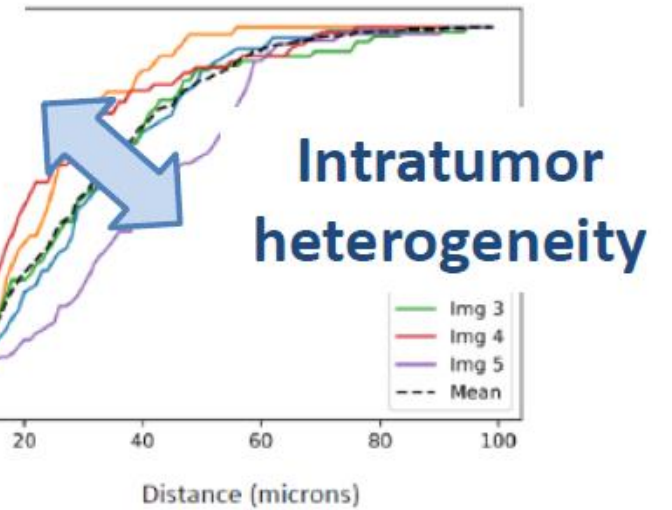
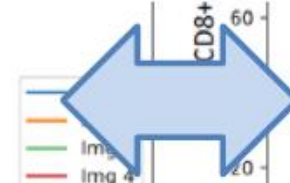
1. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. *Nat Rev Drug Discov.* 2019
2. Fridman, W. H. et al. The immune contexture in cancer prognosis and treatment. *Nat. Rev. Clin. Oncol.* 2017
3. Anichini A, et al. The non-small cell lung cancer immune landscape: emerging complexity, prognostic relevance and prospective significance in the context of immunotherapy. *Cancer Immunol Immunother.* 2018

Distance analyses

CD8+ / CK+



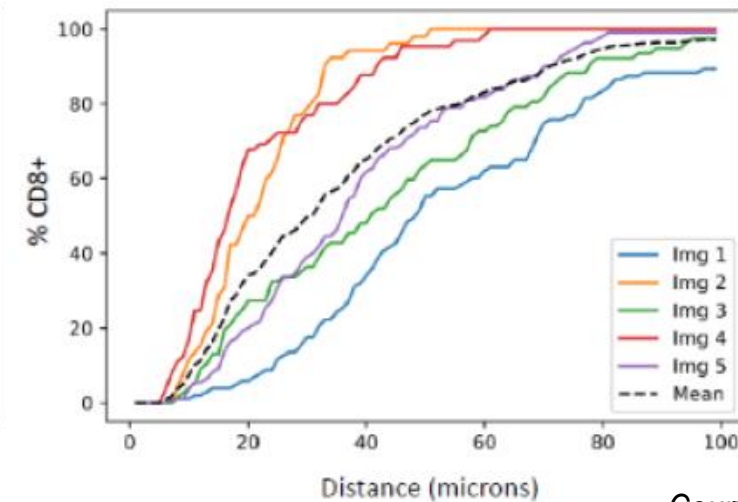
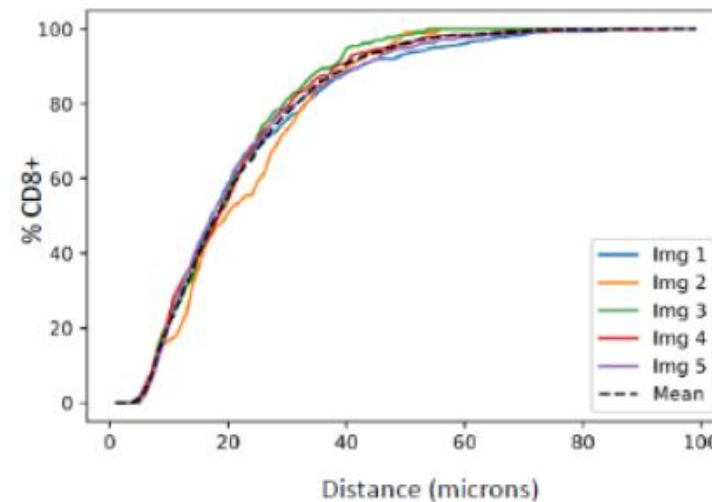
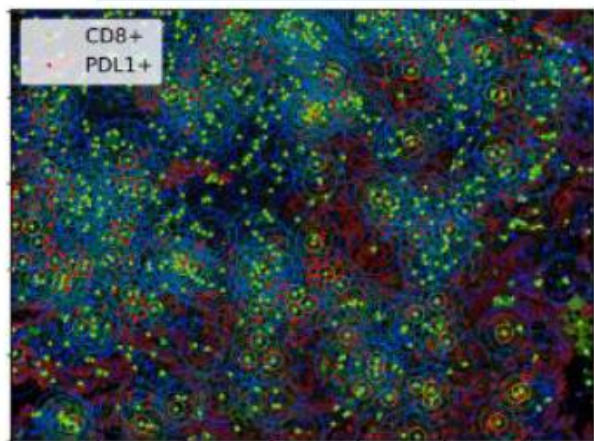
Intertumor variability



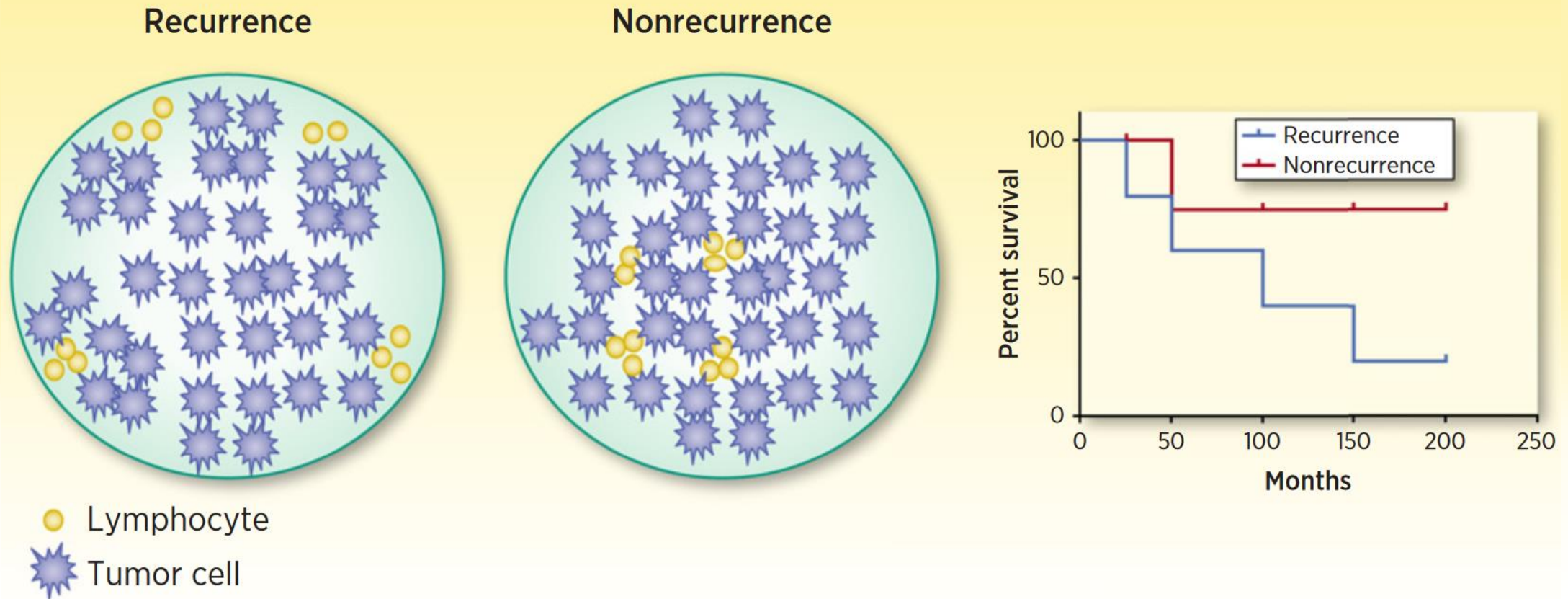
Intratumor heterogeneity



CD8+ / PD-L1+



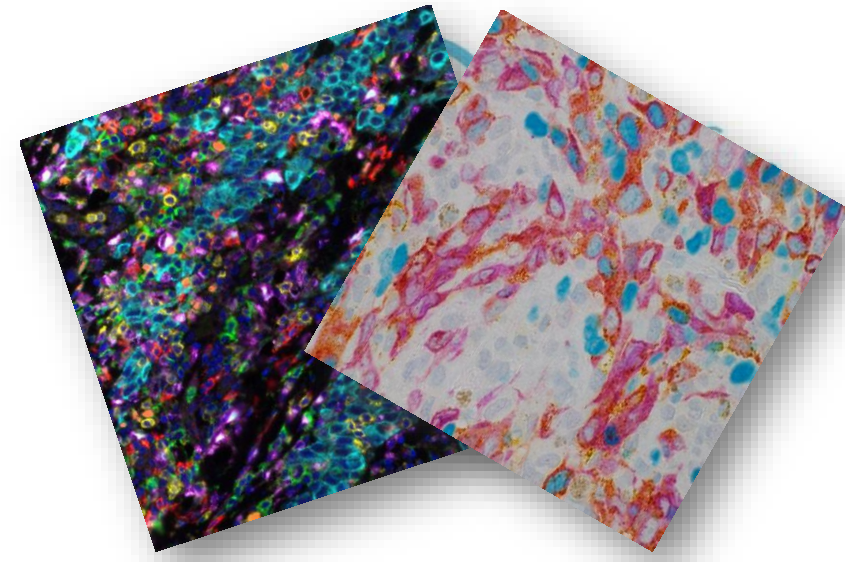
Spatial analysis of immune cells



1. Corredor G, et al. Spatial Architecture and Arrangement of Tumor-Infiltrating Lymphocytes for Predicting Likelihood of Recurrence in Early-Stage Non-Small Cell Lung Cancer. Clin Cancer Res. 2019 Mar 1
2. Peled M, Onn A, Herbst RS. Tumor-Infiltrating Lymphocytes-Location for Prognostic Evaluation. Clin Cancer Res. 2018



Road map



Short background

Multiplex IHC: Technical principles

Chromogenic *versus* fluorescent multiplex IHC : What is the best choice?

The digital analysis: A pivotal issue for the pathology labs

Multiplex IHC in translational research: Many promises in I-O

Multiplex IHC in routine clinical practice: Why and how?

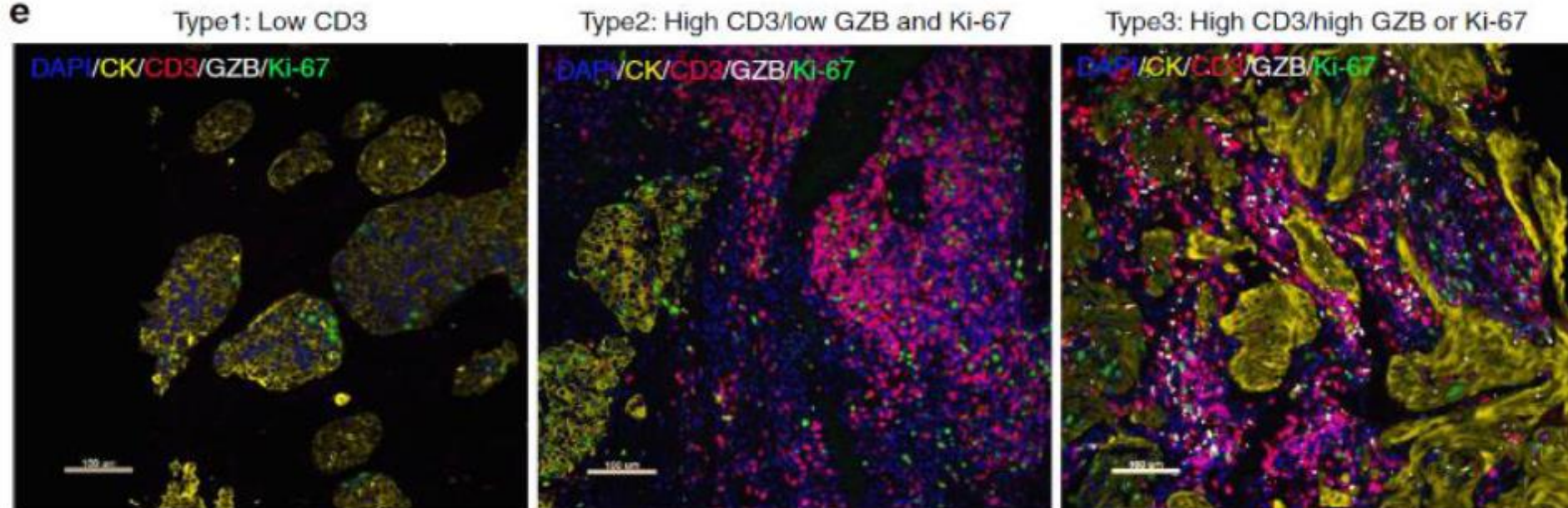
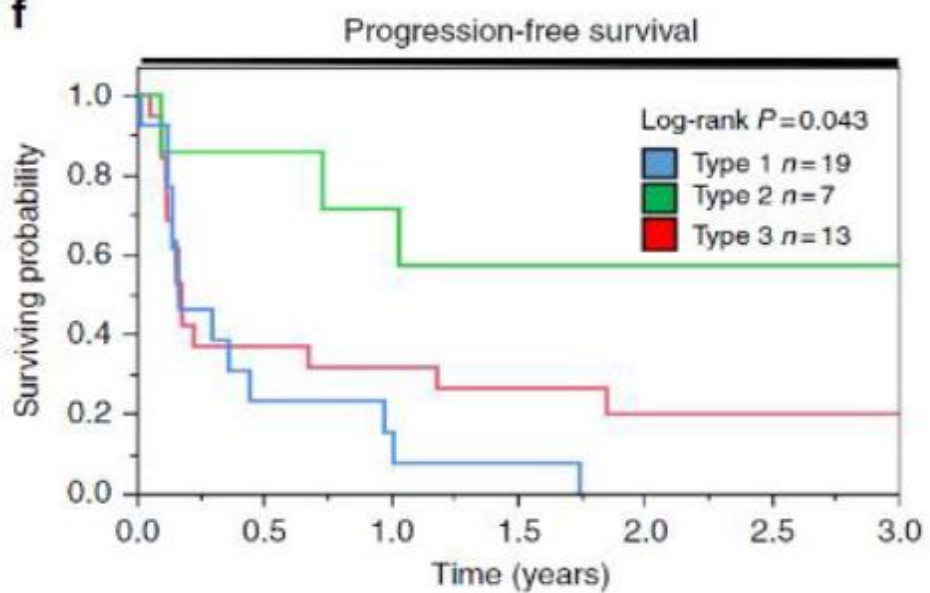
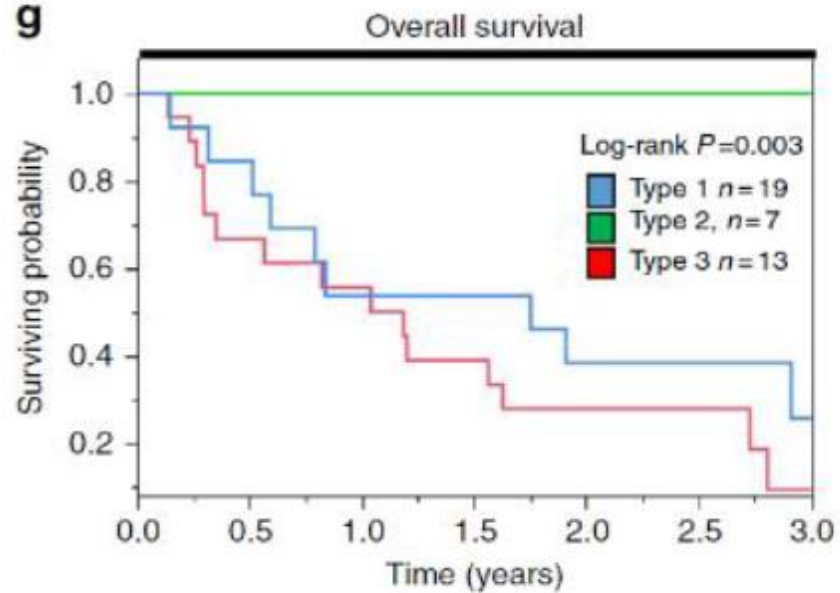
Take away message

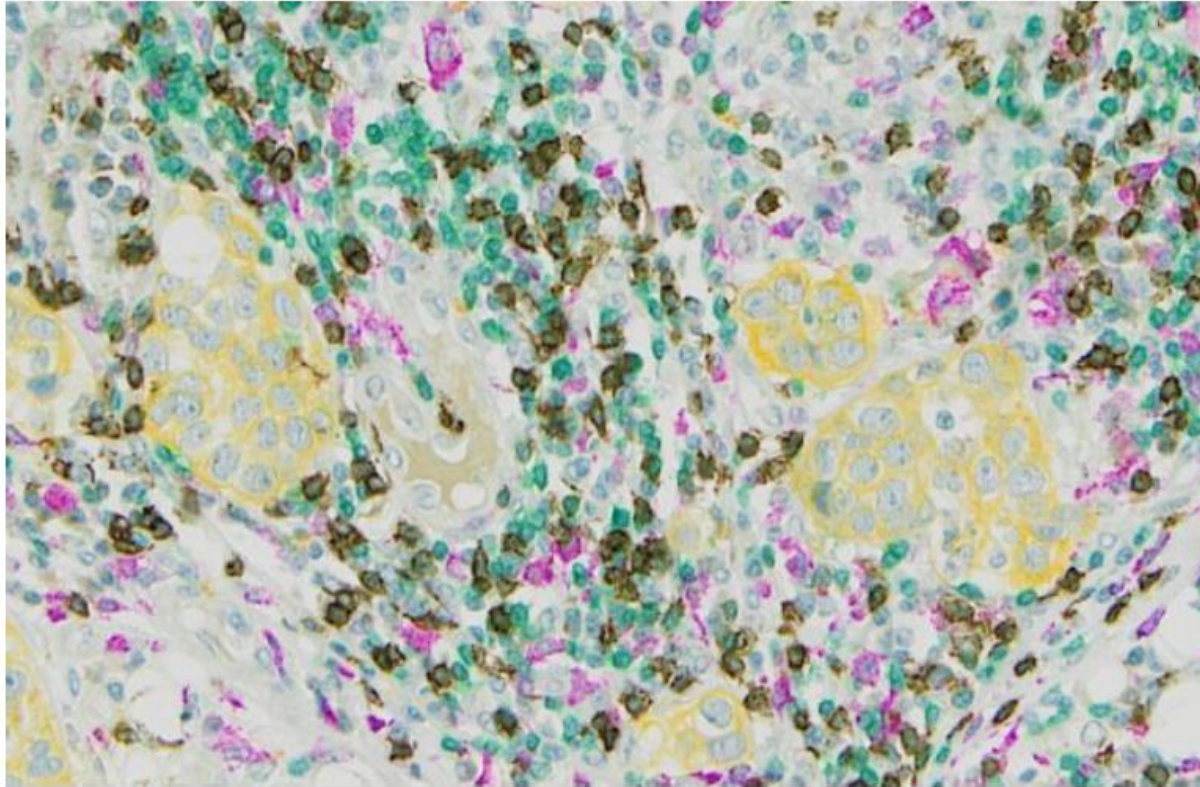
Improving the response rate to ICIs

Most of the immunotherapy biomarkers explored to-date are proxy measures of tumor inflammation

Increasing Directness of Measure of Tumor Immunogenicity						
	TMB	PD-L1	Gene Expression Signatures	Neoantigens / Neoepitopes	Tumor-Infiltrating Immune Cells	Immune Status / Antigen-Specific Immunity
Biomarker Function	• Suggestive of the presence of specific, potentially immunogenic tumor neoantigens	• Suggestive of tumor-infiltrating immune cells / adaptive immune resistance	• Suggestive of immune cell presence / activation / function	• Indicative of the presence of specific, potentially-immunogenic tumor neoantigens	• Indicative of the presence of tumor-infiltrating cells	• Indicative of antigen-specific immunity / immune response
Assay Technologies	• NGS	• IHC	• NGS (RNA-seq) • Hyb. arrays (e.g., microarrays, NanoString) • PCR • Other	• IHC • NGS (WES + WTS) • Computational Informatics • Mass Spec or Proteomics	• IHC / IF (single- and multiplex) • Digital Pathology • Other Multiplex Spatial Tissue Analysis platforms	• IGRA / ELISPOT, • ELISA • Flow cytometry • PCR • NGS

Measurable via digital pathology and/or multiplex spatial tissue analysis

e**f****g**



Immune microenvironment
characterization and interaction with
tumor cells

CD8+

CD4+

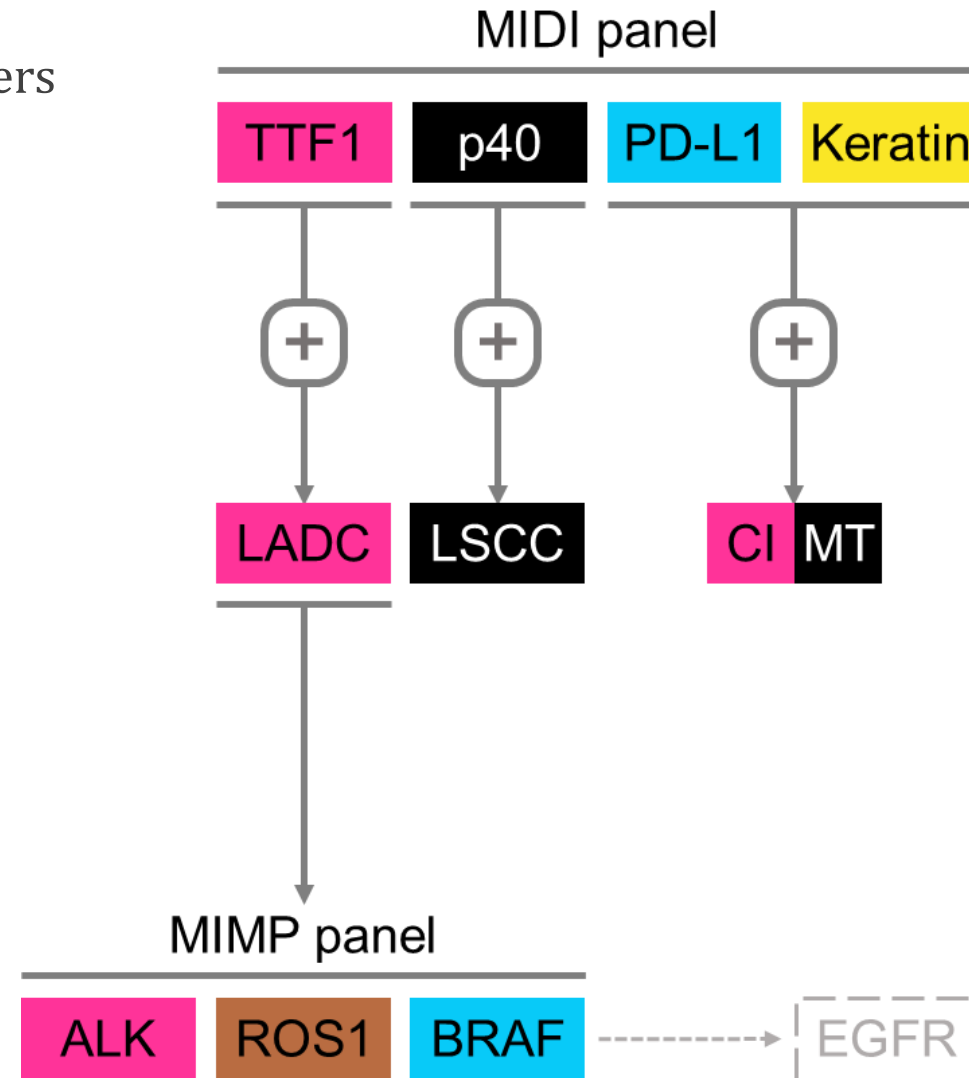
CD163+

Cytokeratins+(TC)

Chromogenic mIHC for diagnosis and predictive biomarker testing in non-small cell lung cancer



- FDA-cleared diagnosis & predictive biomarkers
- No antigenicity loss
- No steric interference
- No increased cross-reactivity
- Standard antigen retrieval
- Automated staining protocols
- Limits the need for validation strategies
- Method of choice in routine setting
- Therapeutic decision making in NSCLC
- Unique sample-sparing tool to characterize limited tissue samples



Chromogenic mIHC for diagnosis and predictive biomarker testing in non-small cell lung cancer



MIDI panel

mIHC for **D**iagnosis &
Immunophenotyping



TTF1 (SP141)



p40 (BC28)



PD-L1 (SP263)



AE1/AE3 (PCK26)



MIMP panel

mIHC for **M**olecular **P**rofilng



ALK (D5F3)

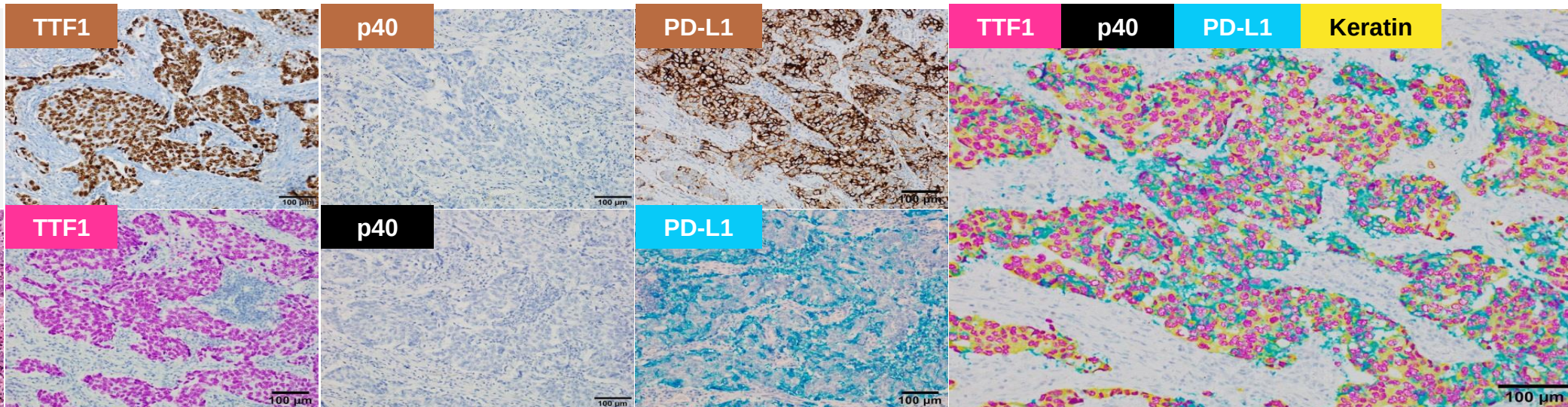


ROS1 (D4D6)

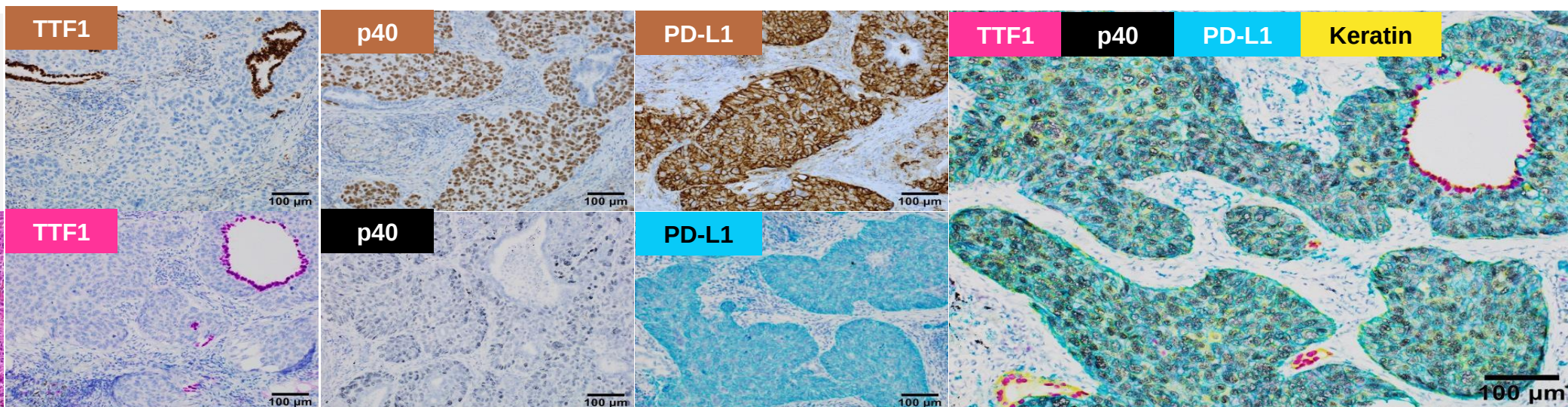


BRAFV600E (VE1)

Adenocarcinoma



Squamous cell carcinoma



MIDI Panel

ALK positive adenocarcinoma

ALK

ROS1

BRAF

ALK

ROS

BRAF

HES

ALK

ROS1

BRAF

100 µm



MIMP Panel

ROS1 positive adenocarcinoma

ALK

ROS1

BRAF

ALK

ROS

BRAF

HES

ALK

ROS1

BRAF

100 µm



MIMP Panel

**BRAF V600E
positive
adenocarcinoma**

ALK

ROS1

BRAF

ALK

ROS

BRAF

HES

ALK

ROS1

BRAF

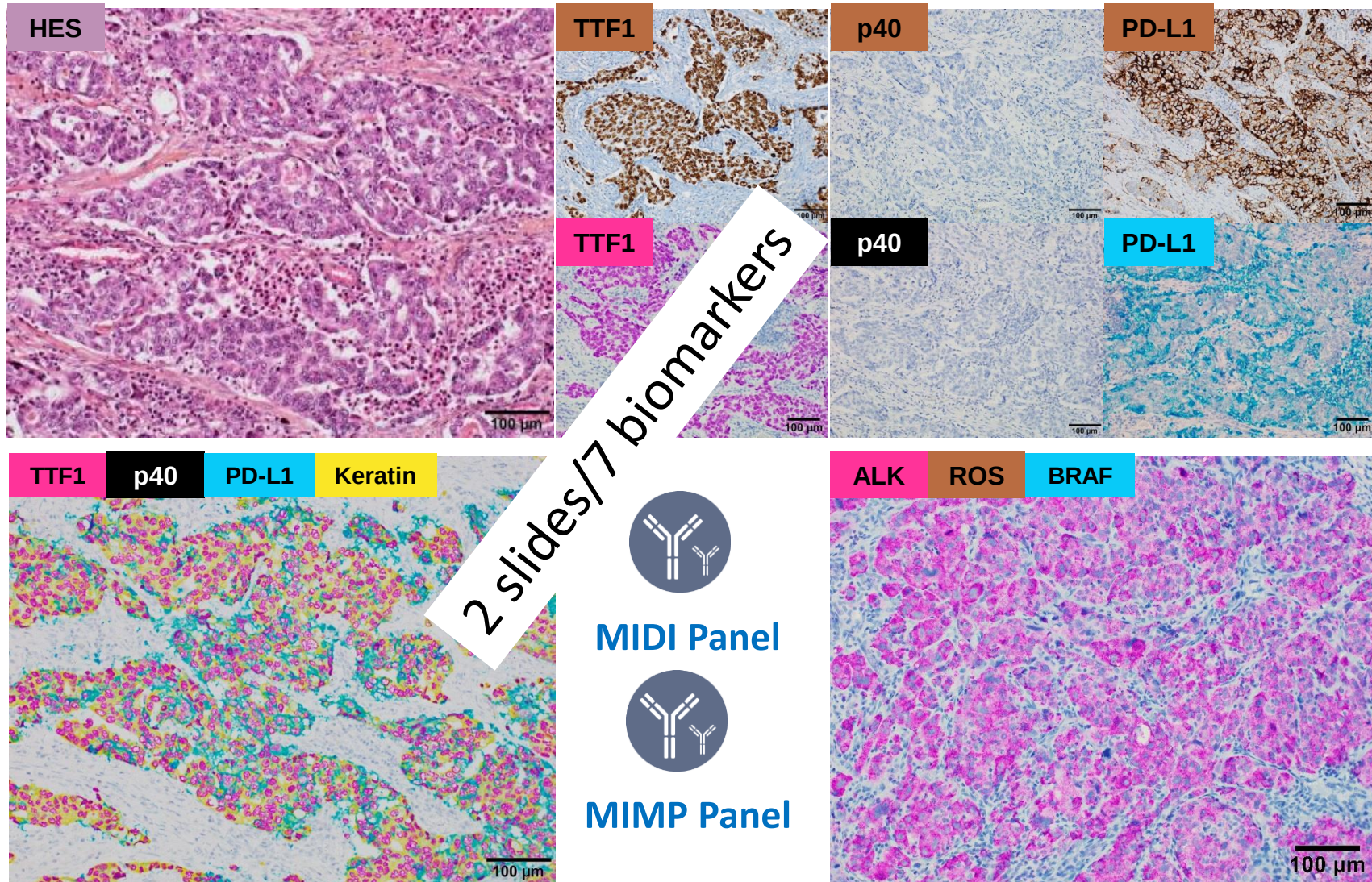
100 μ m



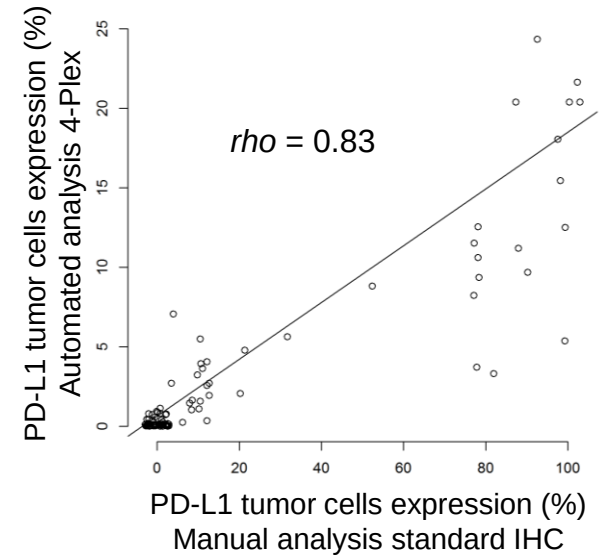
MIMP Panel

Lung adenocarcinoma

TTF1+ PD-L1+ ALK+ // p40 — ROS1— BRAF V600E—



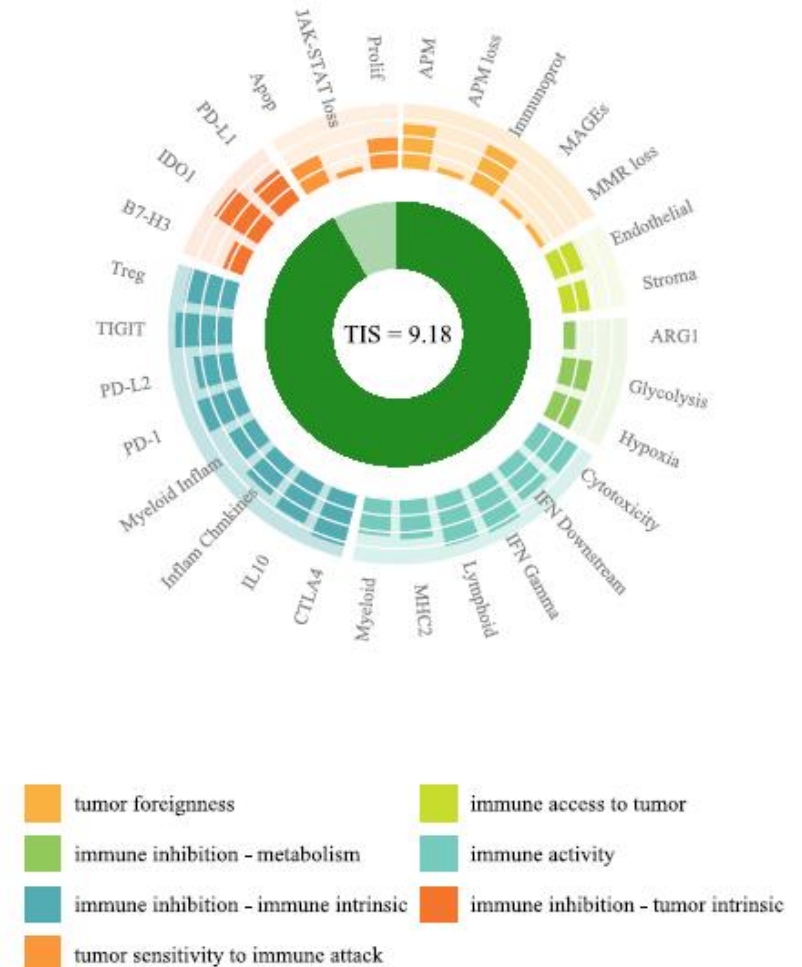
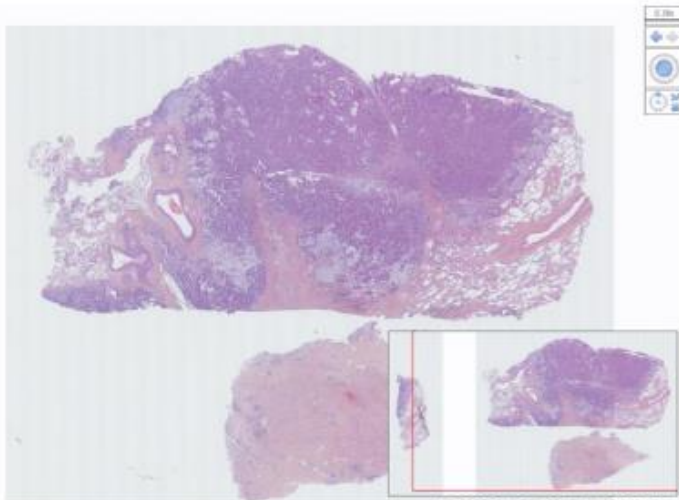
Tumor cells only, exclude immune cells



NSCLC Data with IO Outcome – Case Study

Case Study – Patient

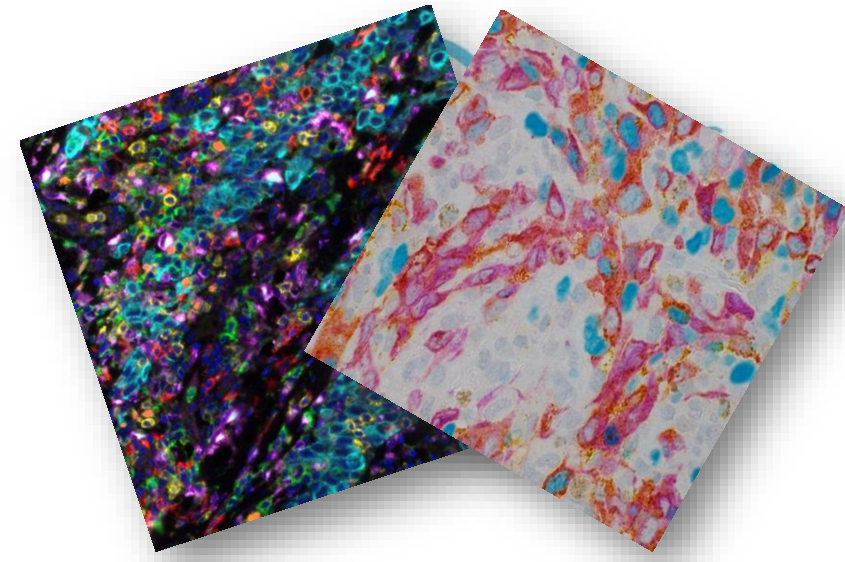
- Adenocarcinoma
- Sample is a CR on Nivolumab
- PD-L1 IHC negative
 - Low gene expression by NanoString – likely due to PD-L1 expression on immune cells not tumor cells
- IO360 gene expression data reveals high expression of many immune signatures and low expression of tumor related signatures



FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.



Road map



Short background

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Chromogenic *versus* fluorescent multiplex IHC : What is the best choice?

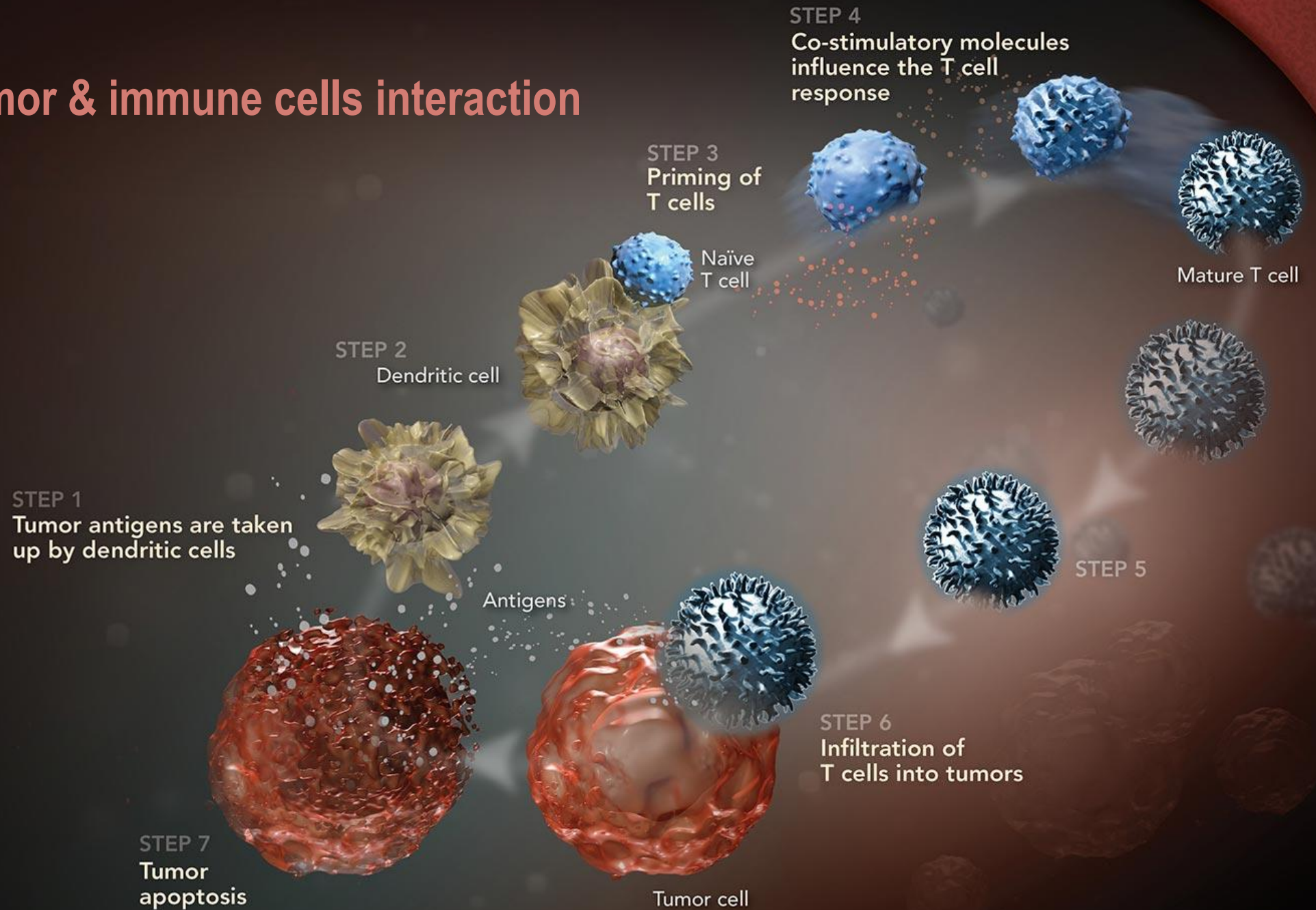
The digital analysis: A pivotal issue for the pathology labs

Multiplex IHC in translational research: Many promises in I-O

Multiplex IHC in routine clinical practice: Why and how?

Some take away messages

Complexity of tumor & immune cells interaction



Multiplex IHC chromogenic vs. fluorescent

- **Visualization**

Chromogenic: optical microscope

IF : > 4 colors = spectral microscope

- **Slides storage**

Chromogenic: > 10 years

Fluorescence: loss of intensity $f(t)$: quick analysis !

- **Multiplexing capacity**

High in fluorescence

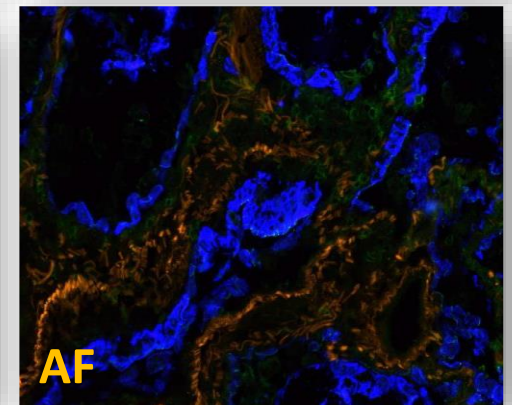
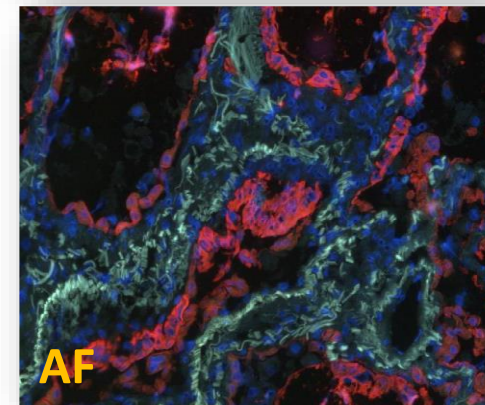
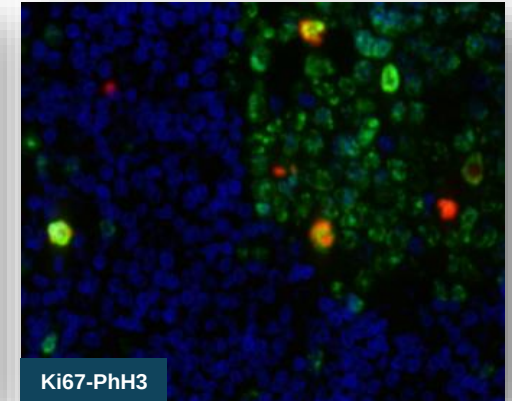
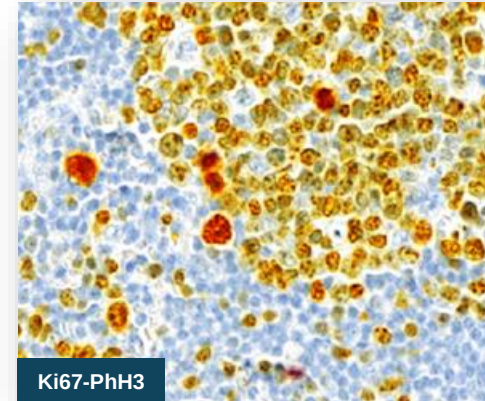
Fluorochromes: extended color library

Interpretation of co-localization easier in fluorescence

Prevalent autofluorescence in purple, blue and green

- **Costs**

Fluorescence less expensive than chromogenic

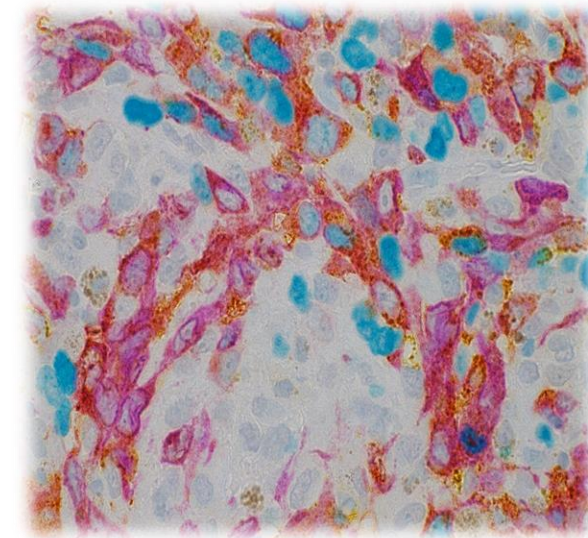
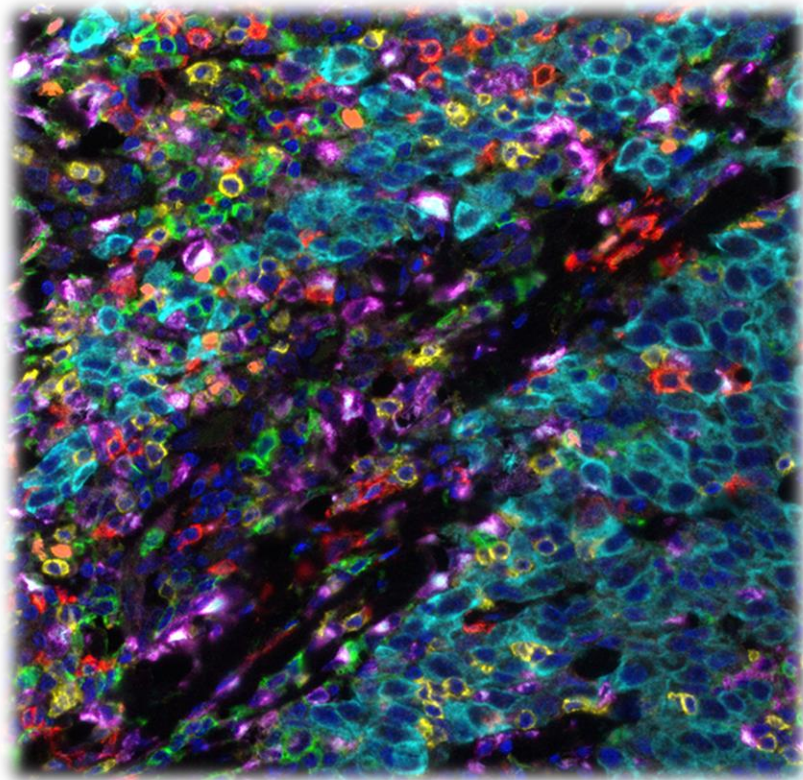


Review

Multiplexed Immunohistochemistry for Molecular and Immune Profiling in Lung Cancer—Just About Ready for Prime-Time?

Paul Hofman ^{1,2}, Cécile Badoual ^{3,4}, Fiona Henderson ⁵, Léa Berland ¹, Marame Hamila ¹, Elodie Long-Mira ^{1,2}, Sandra Lassalle ^{1,2}, Hélène Roussel ^{3,4}, Véronique Hofman ^{1,2} , Eric Tartour ^{4,6}  and Marius Ilié ^{1,2,*} 

A few open questions (among others):



Which targets need to be assessed?

Which methods?

How many fields to assess per tumor?

Primary and/or metastatic site(s)?

How to quantify the different signals?

How to assess the different IHC cut off?

Budget evaluation if transfer in routine practice?

Turnaround time to get the results in daily life?

Data/images storage?

QC/QA (ISO15189)?

How to integrate the genomic associated data?

Growing interest in the tumor microenvironment analysis

Explosion of providers of instruments, reagents, software, and service solutions for multiplexed tissue analysis

Scanners / Image Acquisition



Leica

HAMAMATSU

PerkinElmer

VENTANA

PHILIPS

OLYMPUS
Your Vision, Our Future

OptraScan
On Demand Digital Pathology

ZEISS

VISIOPHARM[®]
Augmented Pathology

3DHISTECH

The Digital Pathology Company

HURON
Digital Pathology

RARECYTE

Multiplex Analytical Systems



nanoString

FLUIDIGM

AKOYA
BIOSCIENCES

PerkinElmer

IONpath

MACS
Miltenyi Biotec

ACD

10X GENOMICS[®]

READCOOR

CANOPY
BIOSCIENCES

Kits / Assays (3rd party, off-the-shelf)*



ULTIVUE

PerkinElmer

Cell IDx

Image Analysis Products / Services**



DEFINIENS[®]
the tissue phenomics company

Lunit

halio
dx

NEO
GENOMICS

PROSCIA

PathAI

OracleBio
Image Analysis Solutions

TEMPUS

indica labs
informed pathology

VISIOPHARM[®]
Augmented Pathology

Flagship

HISTALIM

3DHISTECH

aiforia[®]

PAIGE

deep
lens

Keen Eye

PROTEAN
BIODIAGNOSTICS

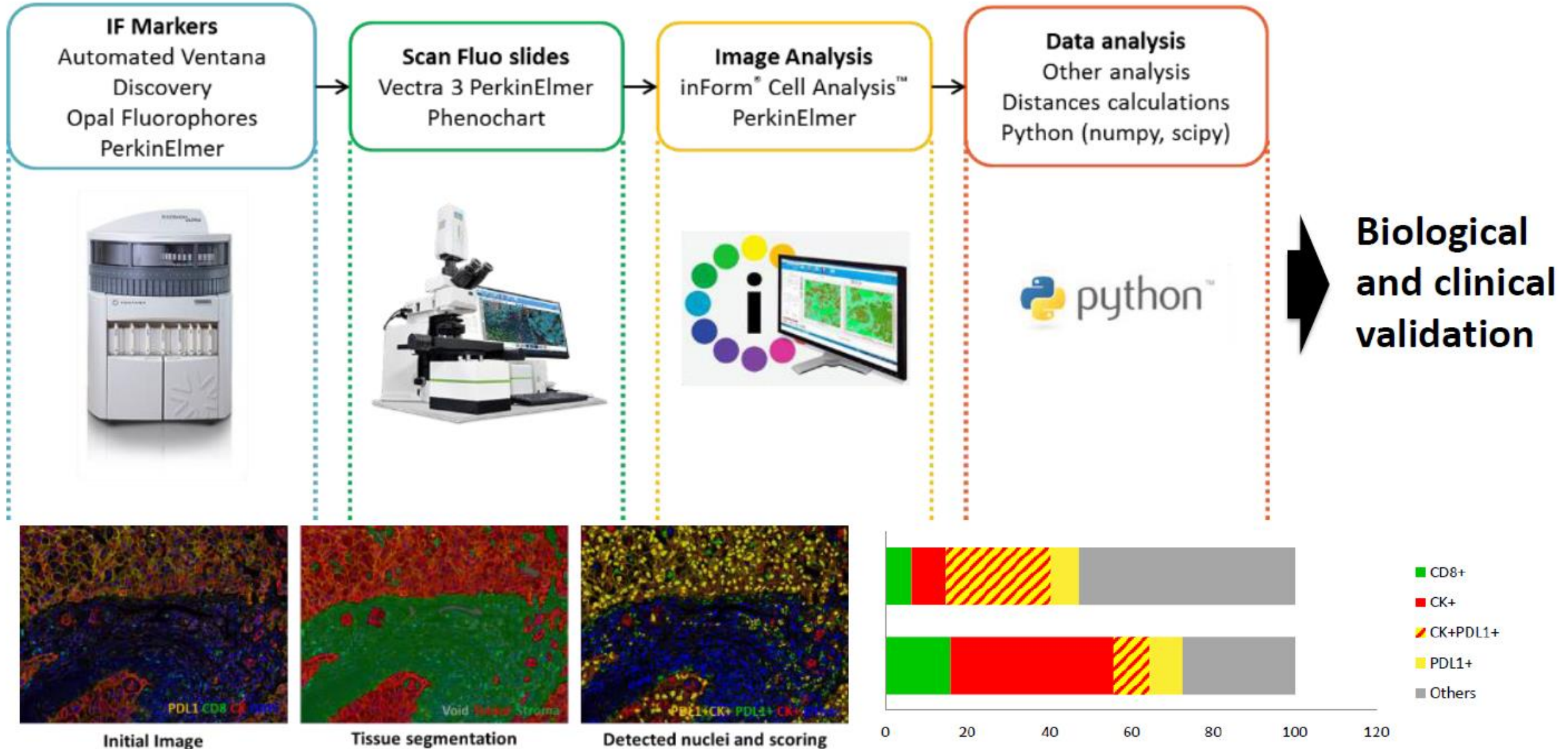
SECTRA

VISIKOL

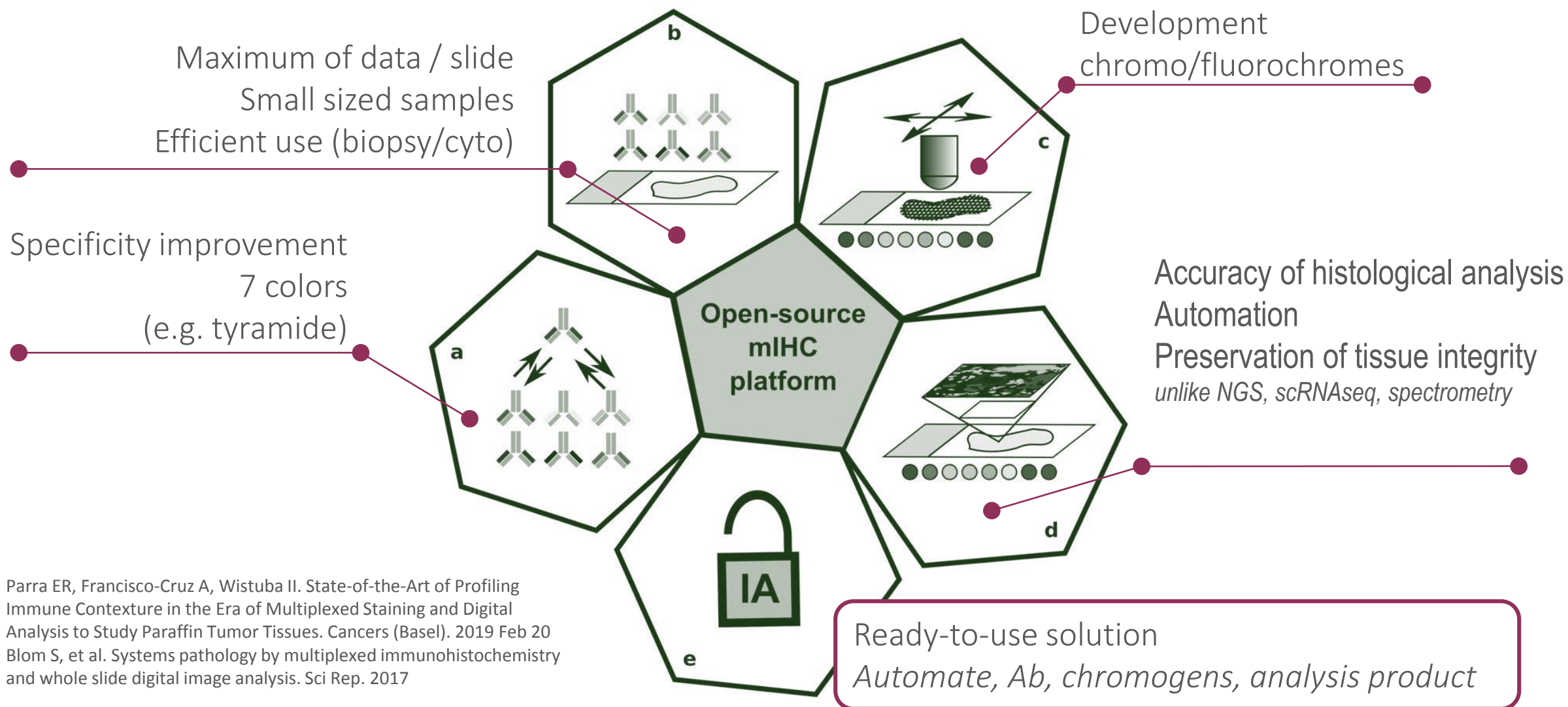
ContextVision

Invicro
A Konica Minolta Company

Example: workflow

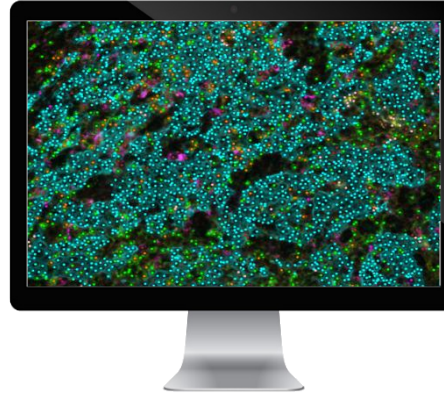
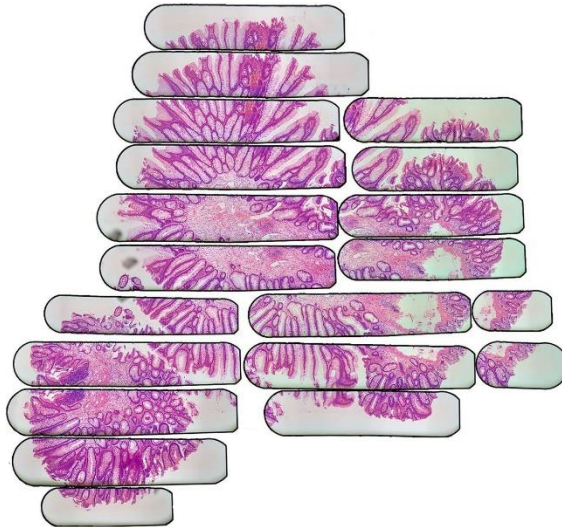
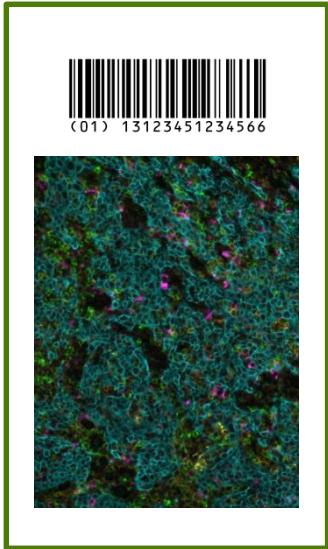


Advantages



1. Parra ER, Francisco-Cruz A, Wistuba II. State-of-the-Art of Profiling Immune Contexture in the Era of Multiplexed Staining and Digital Analysis to Study Paraffin Tumor Tissues. *Cancers (Basel)*. 2019 Feb 20
2. Blom S, et al. Systems pathology by multiplexed immunohistochemistry and whole slide digital image analysis. *Sci Rep*. 2017

Current limitations – Digital analysis & multiplex IHC



« à priori » analysis

- Manual annotations
Imperfect

Without « à priori »

- Check accuracy
- Robustness

Resolution

Scanners (acquisition, visualization, speed)

Images (storage, sharing, visualization)

Quantification and interpretation

Inter-sample variability

Inter-lab variability

Management of data volume and variability

Inter-software reproducibility

Costs: software, staff

Regulation and reimbursement

1. Schwen LO, et al. Data-Driven Discovery of Immune Contexture Biomarkers. Front Oncol. 2018 Dec 18.
2. Litjens G, et al. A survey on deep learning in medical image analysis. Med. Image Anal. 2017

Resection specimen

Peritumor tissue

Exploratory biological studies
Proof of concept studies
Adjuvant treatments biomarkers

Intratumor
heterogeneity

Preamanalytical variability
Sample/images size
Processing time
Resectable stage only

$10^{-3/-4}$



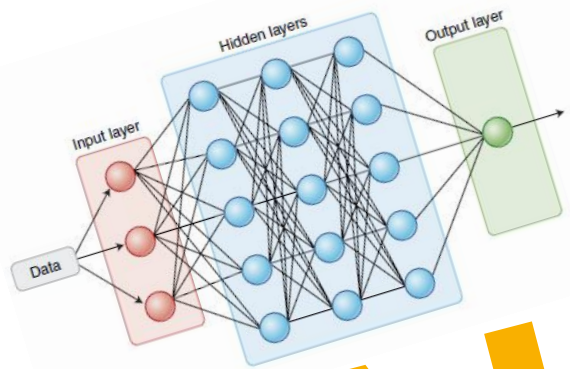
Biopsy

Late stage disease
Metastatic sites
Temporal heterogeneity
Pre/post treatment

Sampling bias: impact of spatial heterogeneity
Sampling artifacts
Sample availability

NSCLC
lobectomy

needle biopsy



AI

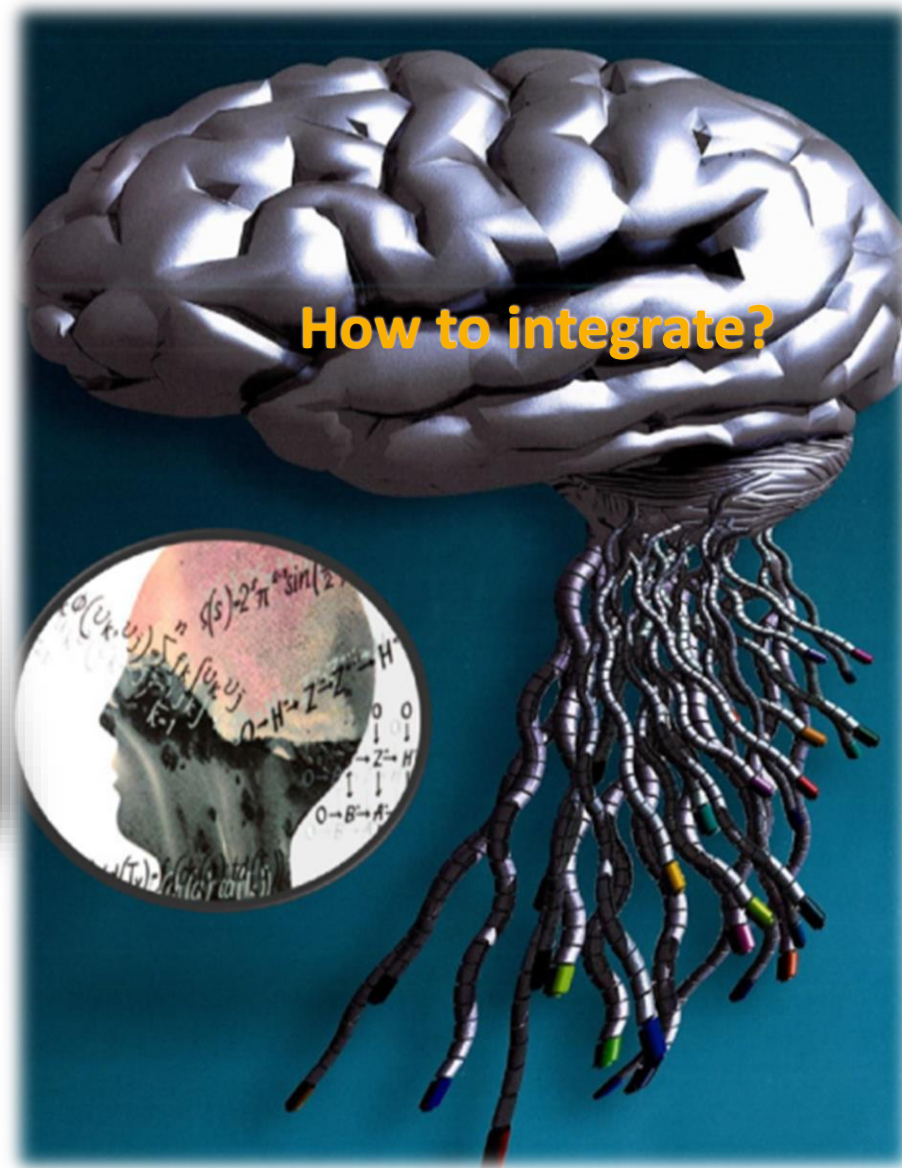


REVIEW ARTICLE | FOCUS
<https://doi.org/10.1038/s41591-018-0300-7>

High-performance medicine: the convergence of human and artificial intelligence

Eric J. Topol 

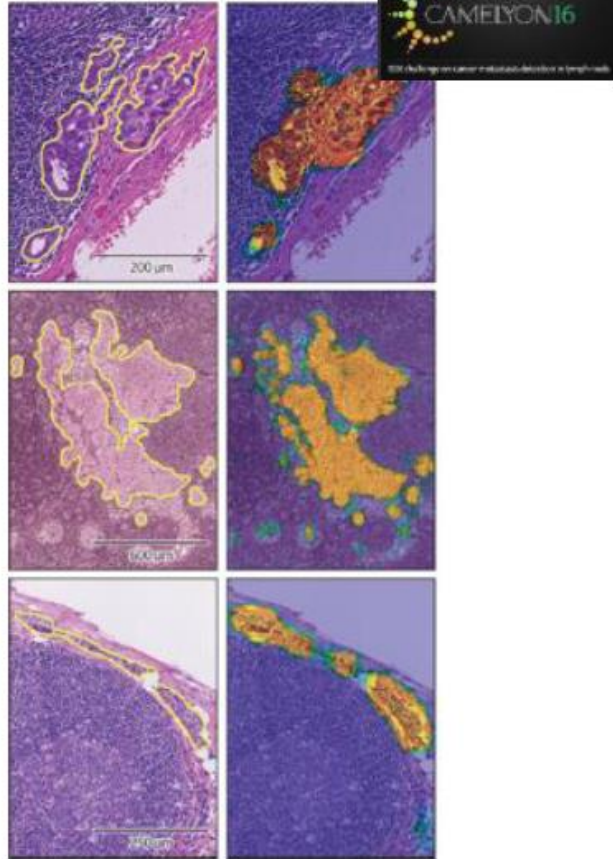
nature
medicine



How to integrate?

Computer aided diagnosis

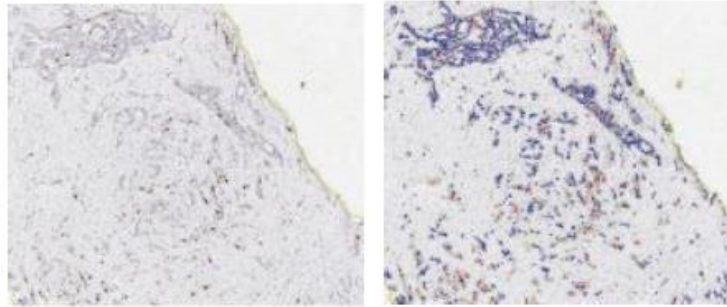
Lymph node metastases



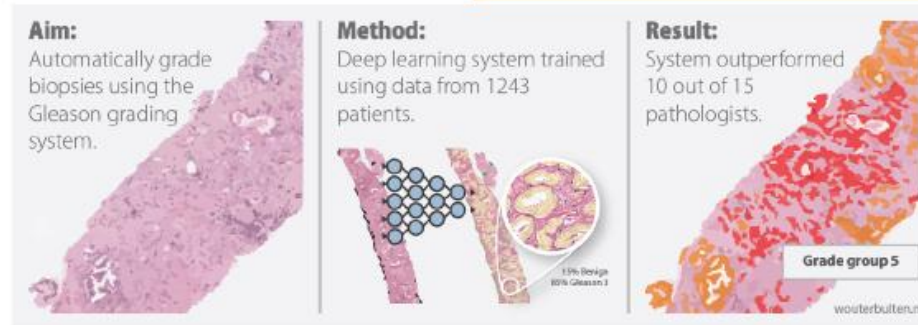
Allows pathologists to concentrate on more complex tasks and interpretation

Automation of quantitative biomarkers assessment

Ki67 immunohistochemistry

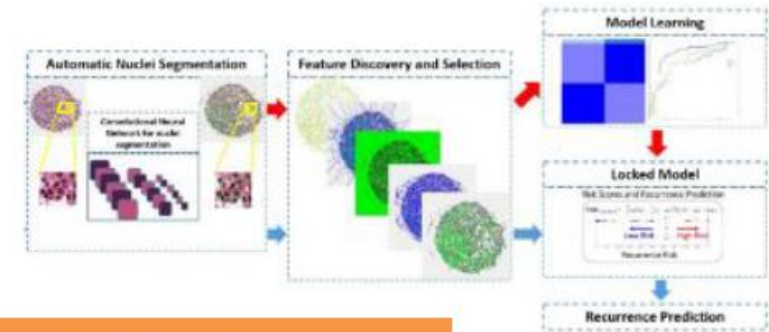


Gleason grading

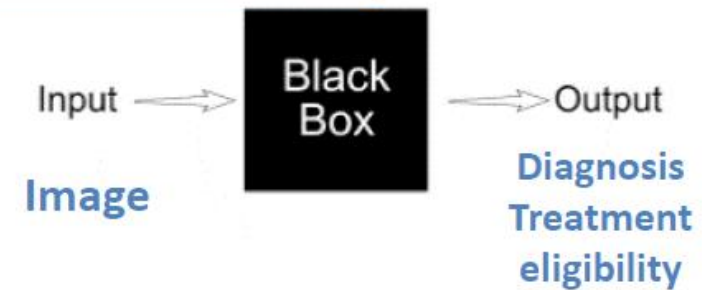


Annotation by pathologists: variability
Under/overfitting

Deep learning based approaches



Correlation to clinical outcome



Large amount of data for training
Non explainable decision



Research

JAMA Oncology | **Original Investigation**

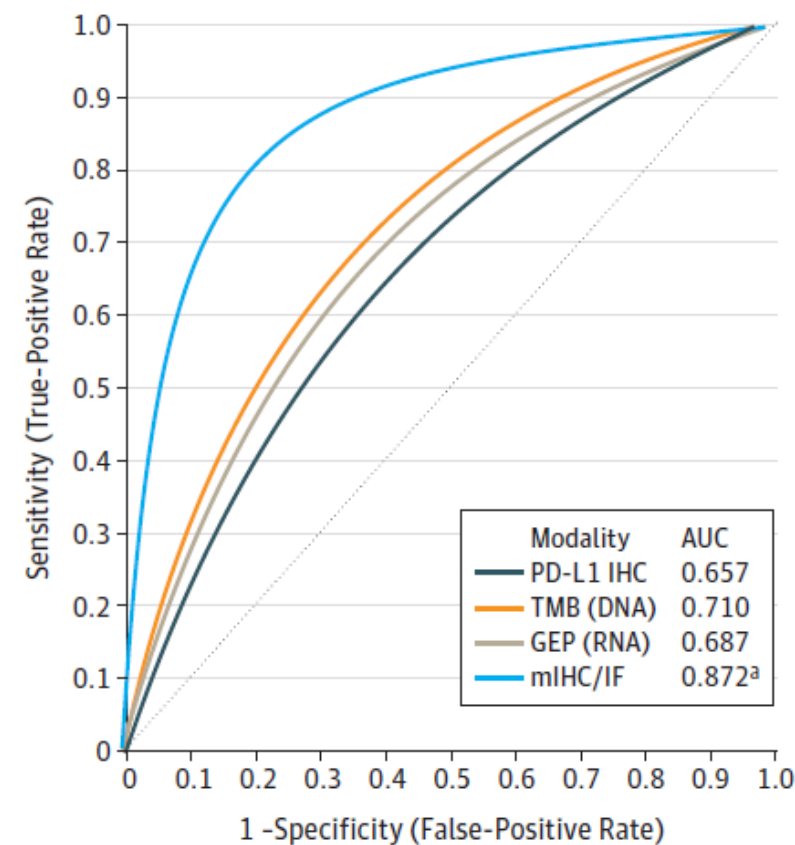
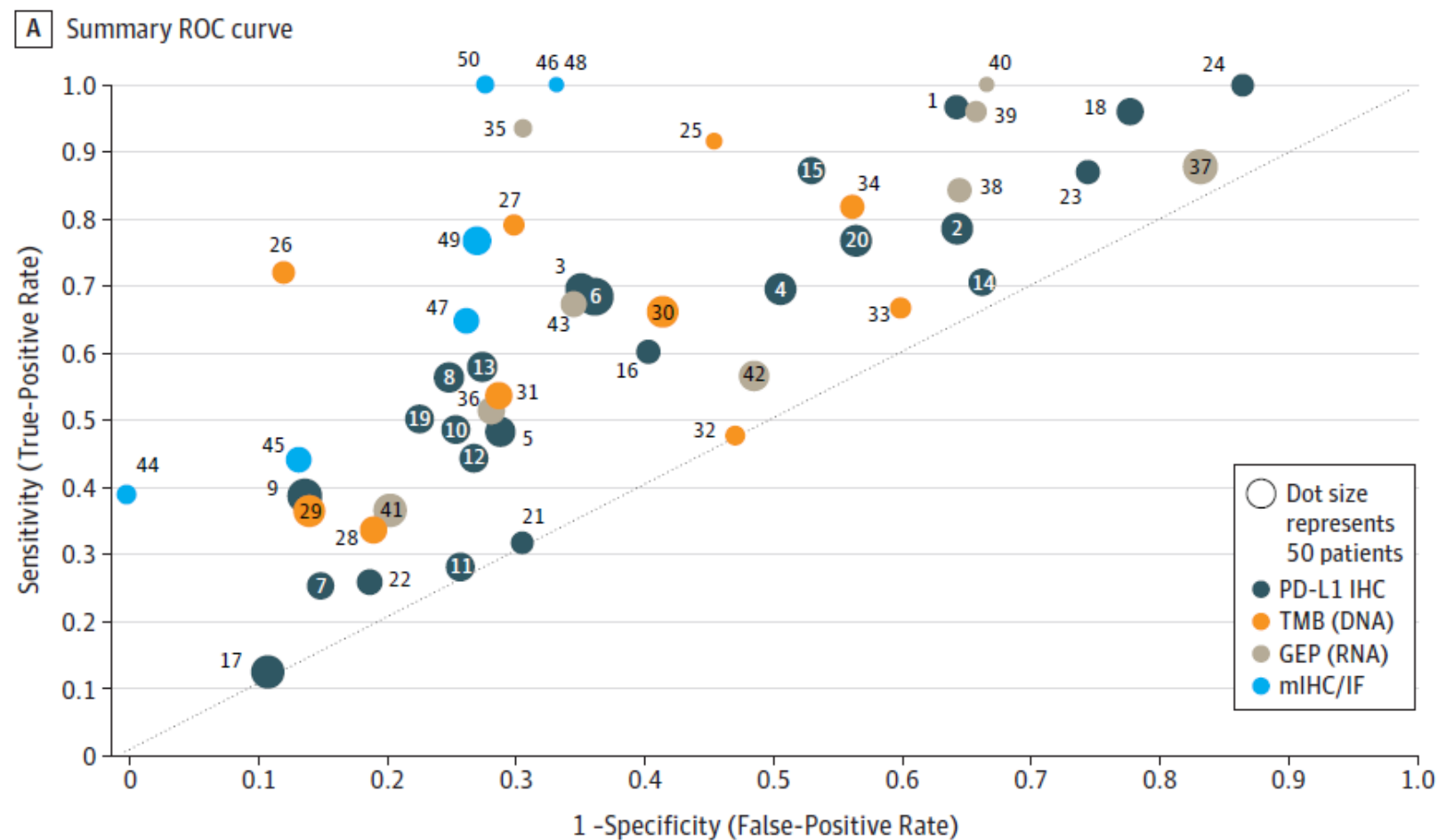
Comparison of Biomarker Modalities for Predicting Response to PD-1/PD-L1 Checkpoint Blockade

A Systematic Review and Meta-analysis

Steve Lu; Julie E. Stein, MD; David L. Rimm, MD, PhD; Daphne W. Wang, MS; J. Michael Bell; Douglas B. Johnson, MD; Jeffrey A. Sosman, MD; Kurt A. Schalper, MD, PhD; Robert A. Anders, MD, PhD; Hao Wang, PhD; Clifford Hoyt, MS; Drew M. Pardoll, MD, PhD; Ludmila Danilova, PhD; Janis M. Taube, MD

CONCLUSIONS AND RELEVANCE In this meta-analysis, tumor mutational burden, PD-L1 IHC, and GEP demonstrated comparable AUCs in predicting response to anti-PD-1/PD-L1 treatment. Multiplex immunohistochemistry/IF and multimodality biomarker strategies appear to be associated with improved performance over PD-L1 IHC, TMB, or GEP alone. Further studies with mIHC/IF and composite approaches with a larger number of patients will be required to confirm these findings. Additional study is also required to determine the most predictive analyte combinations and to determine whether biomarker modality performance varies by tumor type.

Figure 2. Summary Receiver Operating Characteristic Curve Analysis by Assay Modality for Responders vs Nonresponders



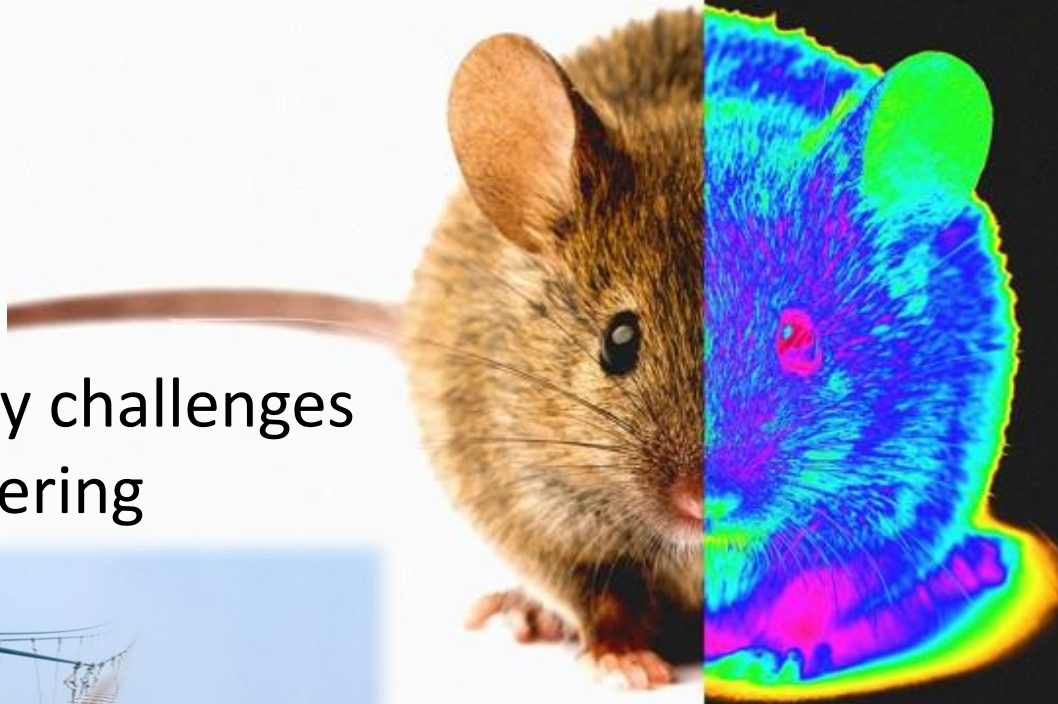
Conclusion

Multiplex Molecular Pathology in practice.....

The 4V

- Variability
 - inter-samples
 - inter-labs
 - inter-sofwarees
 - inter-data
- Velocity of data production
- Volume of data
- Validation mPlex clinical panels

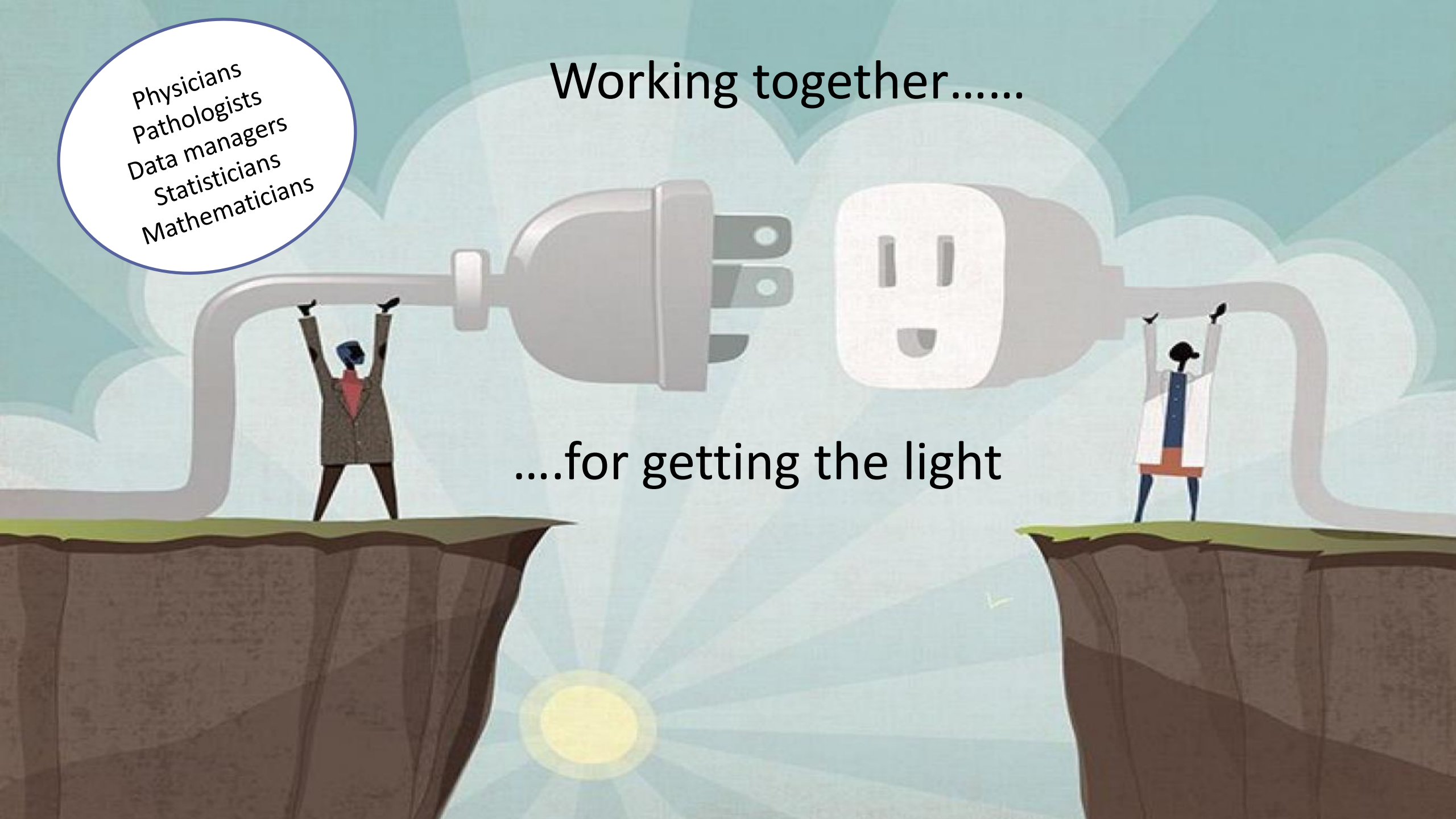
There are still many challenges
to be mastering



Physicians
Pathologists
Data managers
Statisticians
Mathematicians

Working together.....

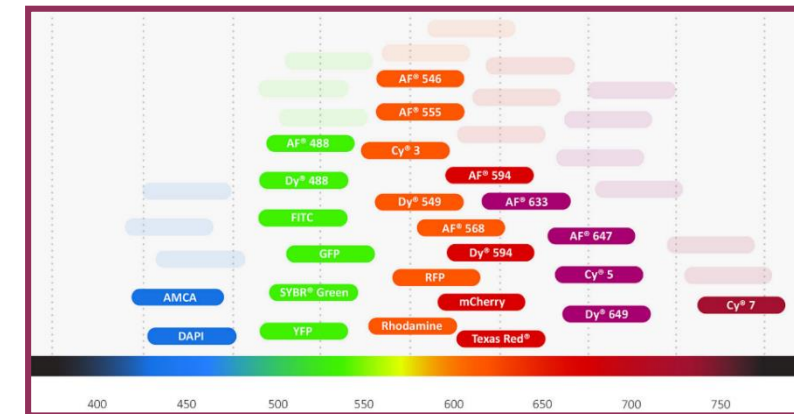
....for getting the light





Current limitations

- Same limits as conventional IHC
 - Pre-analytical (ischemia, fixation, dehydration, embedding)
- Limited number of chromogens (7) and limitation of combinations (signal saturation)
- Fluorescent mPlex:
 - Fluorochromes - research advantage (rare events, co-expression)
 - Complex setup
 - No clinical routine use
 - Low signal reproducibility (despite automation)
- Increase in the number of Mplex combinations
 - Ab, complex classifiers ➦ risk of random results
- Intrinsic costs: equipment, reagents, personnel
- Biomarkers costs ➦ : chromogens, automate, software



1. Parra ER, Francisco-Cruz A, Wistuba II. State-of-the-Art of Profiling Immune Contexture in the Era of Multiplexed Staining and Digital Analysis to Study Paraffin Tumor Tissues. Cancers (Basel). 2019 Feb 20
2. Hofman P, Badoual C, Henderson F, Berland L, Hamila M, Long-Mira E, Lassalle S, Roussel H, Hofman V, Tartour E, Ilié M. Multiplexed Immunohistochemistry for Molecular and Immune Profiling in Lung Cancer-Just About Ready for Prime-Time? Cancers (Basel). 2019 Feb 27;11(3).