In situ Biomarker analysis in cancer Immunotherapy : development of quantitative multiplex IHC

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Objectives

There is now growing evidence that the immune contexture influences cancer progression and clinical outcome of patients. The tumors microenvironment (TME) is the bed of cancer progression and the target of increasing drugs in development. The objective is to develop and partially validate multiplex IHC panels to analyze the human immune TME. The developed panels enabled to analyze the lymphocyte compartment (TIL), the macrophage status (M1 versus M2) and the presence of Tertiary Lymphoid Structures (TLS) in clinical sample. Multiplex method and quantitative analyses allowed to obtain maximum information about TME in precious clinical sample (biopsies).

Material and Method

Single and Multi-stainings were performed using OPAL system from Perkin Elmer on FFPE Human tumor section : Breast carcinoma, Lung carcinoma …
Assessment of three panels : TILs (Tumors Infiltrating Lymphocytes): CD4-CD8-CD20
TLS (Tertiary Lymphoid Structure) CD3-CD20-DCLamp
TAMs : (Macrophages differentiation) CD163-CD68

Used Antibodies

Primary antibodies	References	Suppliers
CD4	NCL-CD4-368	Novocastra
CD8	M 7103	Dako
CD20	M 0755	Dako
CD3	A0452	Dako
CD208 DCLamp	DDX0191	Dendritics
CD68	M 0814	Dako
CD163	NCL-CD163	Leica

IHC process OPAL

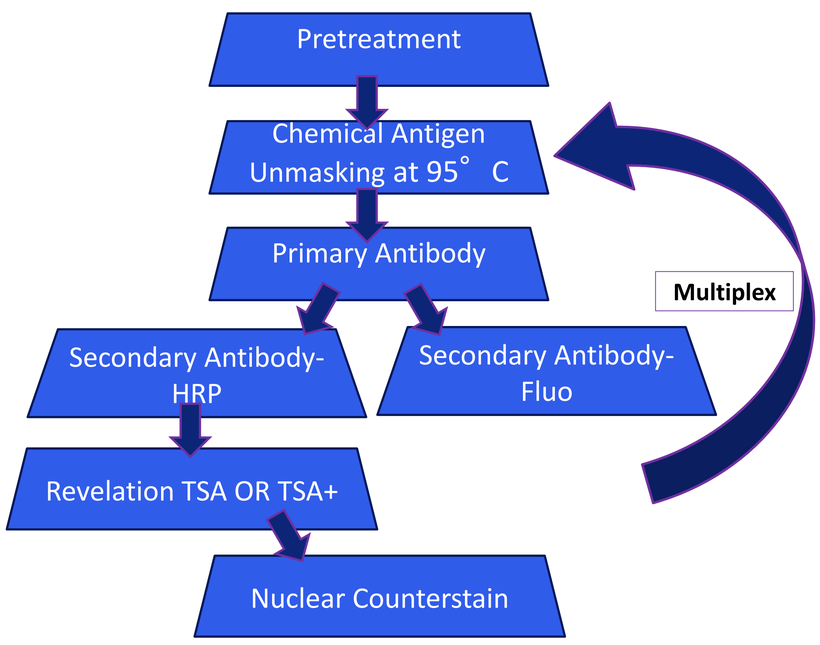
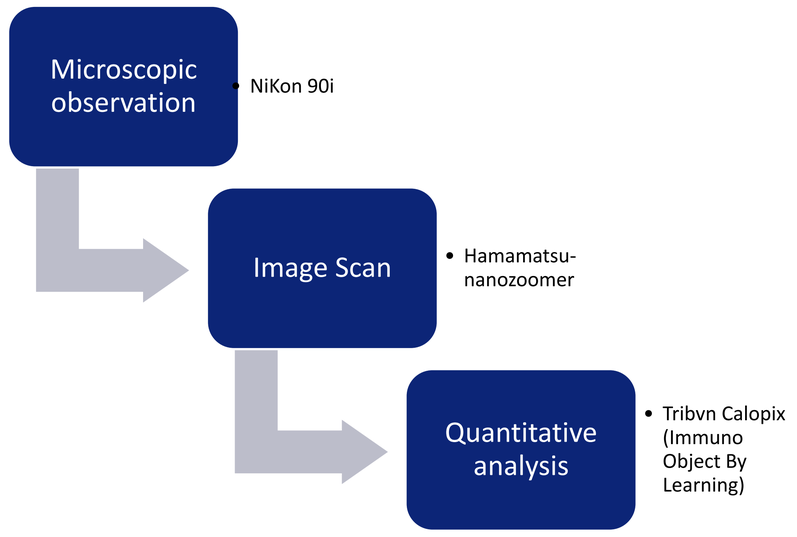
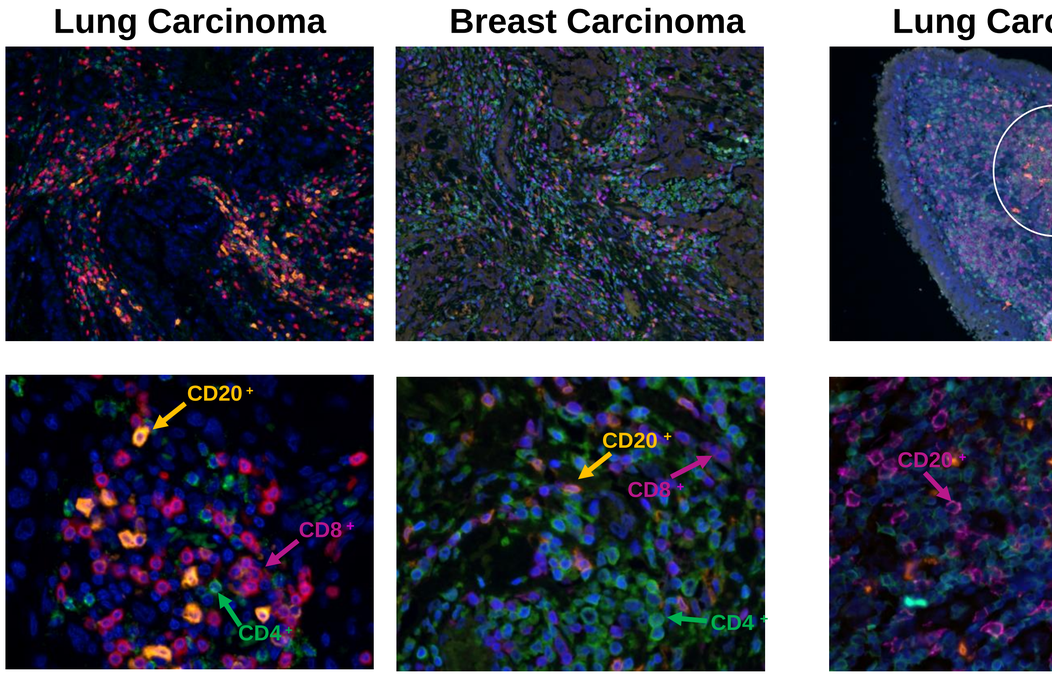


Image Analysis assay

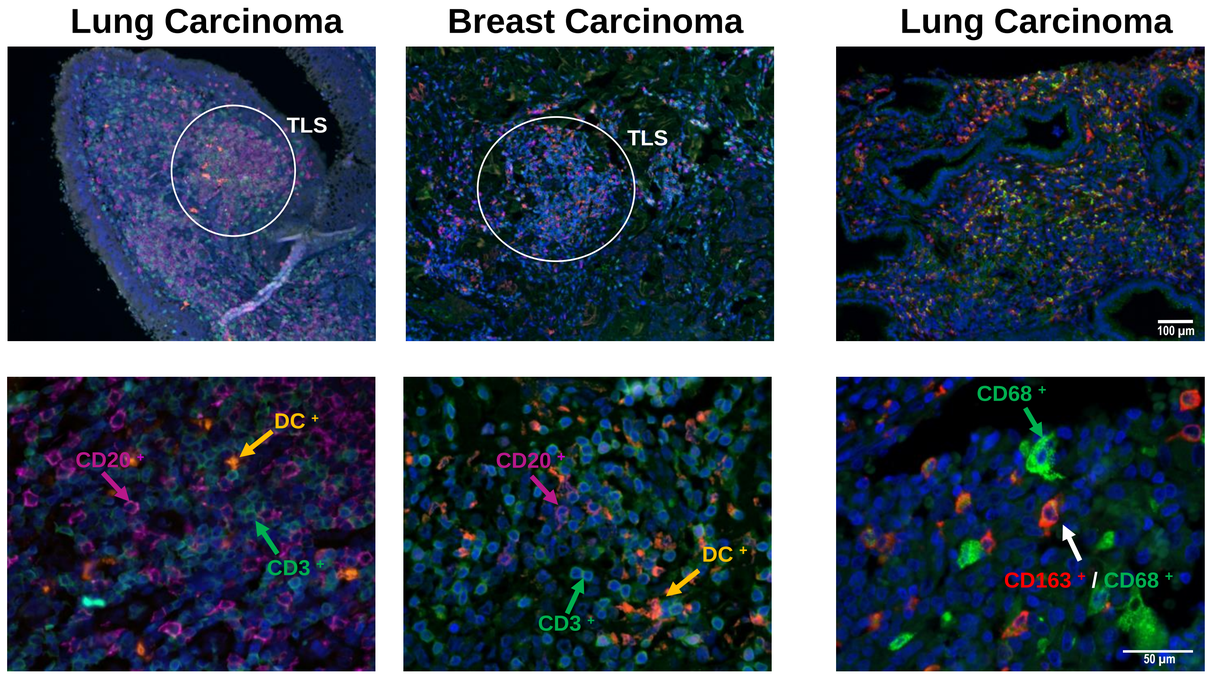


Results

TILs CD4-CD8-CD20

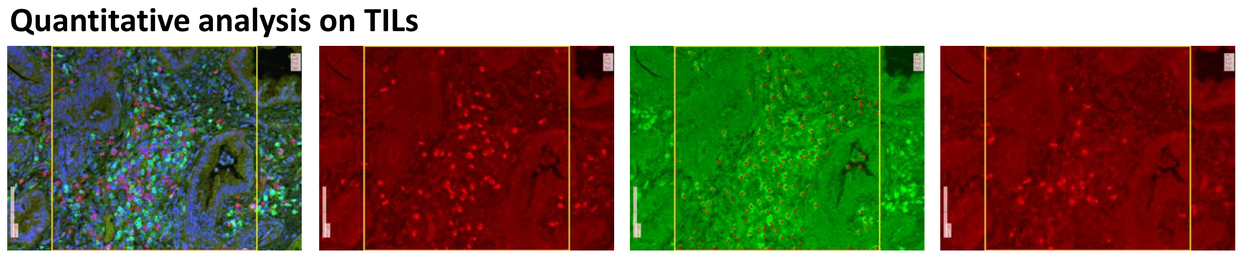


TLS CD3-CD20-DCLamp



TAMs CD163-CD68

Quantitative analysis on TILs



		Breast Carcinoma Cells/mm²		Pancreas Carcinoma Cells/mm²		Glioblastoma Cells/mm²		HCC Cells/mm²		NSCLC Cells/mm²	
	Staining	Triple	Single	Triple	Single	Triple	Single	Triple	Single	Triple	Single
Operator 1	CD4	621	690	300	236	214	365	23	73	993	862
	CD8	306	297	343	280	37	5	60	86	383	356
	CD20	122	98	63	51	24	16	46	53	231	175
Operator 2	CD4	410	882	96	289	179	712	106	274	882	856
	CD8	380	371	468	399	30	9	136	69	401	312
	CD20	168	136	147	39	130	24	306	38	252	148

Validation process (ongoing on TIL)

- Objectives
  - Multiplex versus simplex labelling
  - Quantification method via Calopix
    - Repeatability : repeat process by one operator
    - Reproducibility : repeat process by two operators

- First observations (raw data) : Variability induced by methods:
  - Between simplex and multiplex
  - ROA definition : tumor versus parenchyma/necrosis…
  - Tumor type: difficult for HCC, easier for NSCLC …

Conclusion and Next Steps

Development of new *in situ* biomarkers is essential to understand the influence of TME on tumor progression for immunotherapy. The assessment of multiplex IHC panels allows the immune "phenotyping" of this TME. In addition, we start to develop a validated process to quantify these biomarkers

Next step :

- Complete validation of quantification: increasing the repeatability and the reproducibility data (including a third operator)
- Describe an harmonized quantification process for future analysis
- Other markers :
  - tumor architecture via Pan Cytokeratin , CD31, MHC I…
  - Innate immunity : NK, Neutrophil, regulatory cells Treg, Marker M1 : iNOS

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