2023 ASCO ANNUAL MEETING

GINECO

In situ immune impact of neo-adjuvant nivolumab + ipilimumab combination (ICB) before standard chemoradiation therapy for FIGO IB3-IVA cervical squamous carcinoma patients.

COLIBRI trial, a GINECO study.

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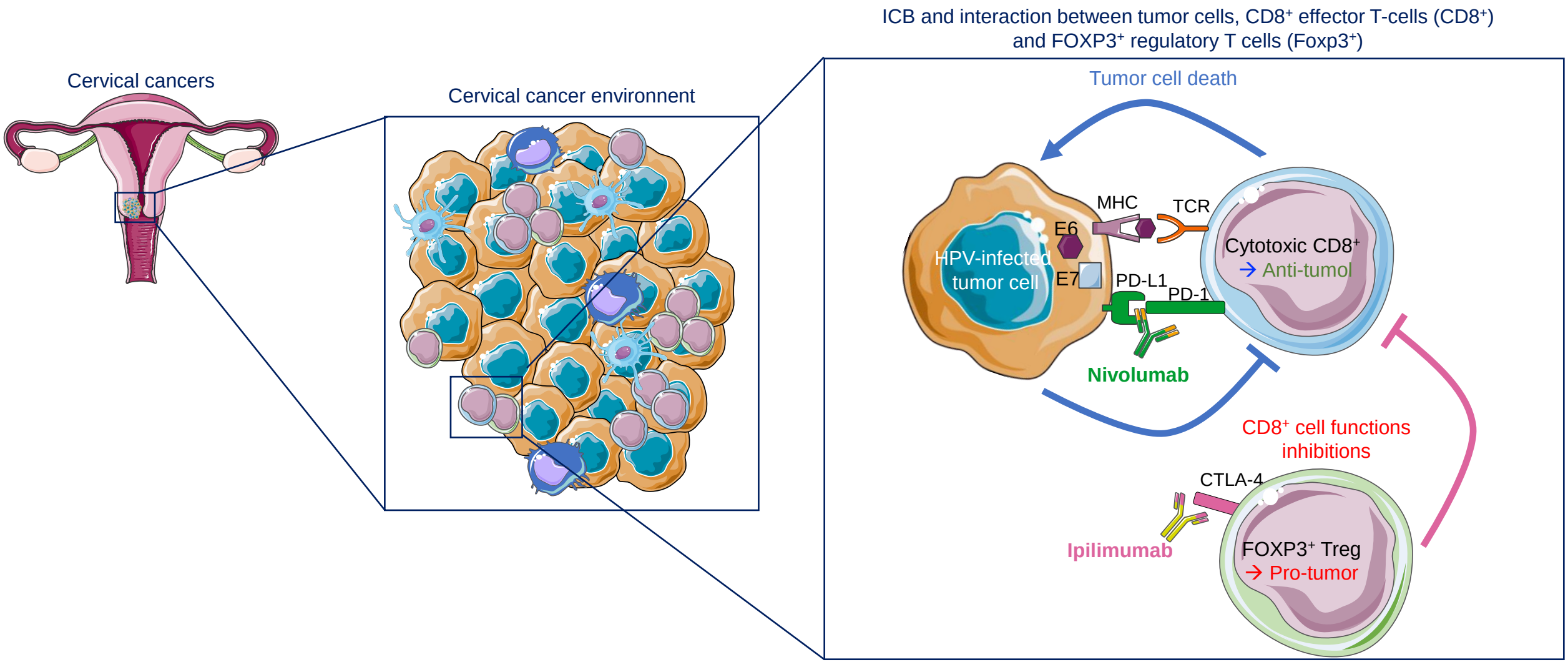
Background

- Locally-advanced cervical cancer (LACC) remains an unmet therapeutic need, with more than 40% rate of recurrence despite treatment with the standard of care chemoradiation (RTCT)[1]
- Common prognostic factors include FIGO stage, pathological tumor type, LVSI
- A high tumor CD8+/FOXP3+ cell ratio is associated with better clinical outcome after neoadjuvant chemotherapy in cervical cancer patients[2]
- Immune checkpoint blockade (ICB) represents a new treatment option in cervical cancer, with survival benefits in the recurrent setting[3,4]
- However, Durvalumab, in combination with and following RTCT, did not significantly improved PFS in patients with high-risk LACC compared with RTCT alone in CALLA trial[5]
- **Alternative neo-adjuvant ICB and differential sequencing of radiation therapy and ICB are worth exploring**

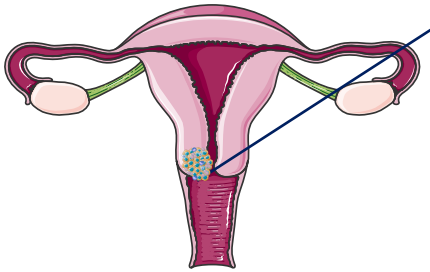
[1]Morris MD, et al. *N Engl J* Med. 2009;340:1137-1143.
[2]Liang Y, et al. *Diag Pathol*. 2018;13:93.
[3]Tewari KS, et al. *N Engl J Med*. 2022;386:544-555.
[4]Colombo N, et al. *N Engl J Med.* 2021;385:1856-1867.
[5]Monk B, et al. *ICGS*. 2022.

Rationale

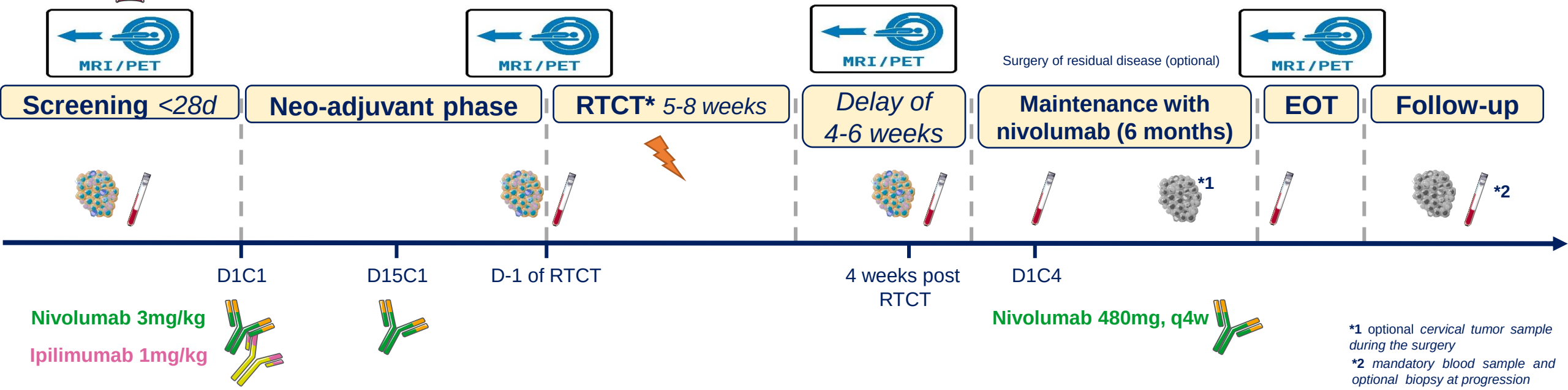


1. How does neo adjuvant dual PD-1/CTLA4 blockade impact immune response in cervical cancer ?
2. Is sequencing ICB before and after RTCT impacting on immune response and anti tumor efficacy ?

COLIBRI inclusion criteria & Study design



Cervical cancer
- Women aged ≥18 years
- Histologically confirmed cervical (adeno)squamous carcinoma
- LACC (FIGO 2018) stage IB3-IVA
- ECOG performance status of 0 or 1
- Multicentric single arm pilot study



***1** optional *cervical tumor sample during the surgery*

***2** *mandatory blood sample and optional biopsy at progression*

Tumor biopsy

Blood sample

***Chemoradiotherapy Regimen**

Platinum agent	Cisplatin 40 mg/m^2 or carboplatin AUC2 q1w × 5 weeks
EBRT	45 Gy in 25 fractions at 1.8 Gy/fraction, 5 fractions per week
Brachytherapy	High-dose rate: 27.5–30 Gy; Low/pulsed-dose rate: 35–40 Gy

Objectives of the COLIBRI trial

- **Primary objective:**

  To measure the $CD8^+/FOXP3^+$ lymphocyte ratio in pre- versus post-ICB therapy biopsies in patients treated with neo-adjuvant combination of nivolumab + ipilimumab, before starting standard RTCT.

  → ***Primary endpoint:*** *$CD8^+/FOXP3^+$Treg cell relative change between pre- and post-ICB biopsies by multiplex-immunofluorescence (multi-IF) tissue imaging*

- **Secondary objectives:**
  - Evolution of the immune microenvironment ($CD8^+$, $FOXP3^+$Treg, DCs, MPs,…) before & after RTCT, and at progression → multi-IF and HTG
  - Objective Response Rate (ORR) by RECIST 1.1 criteria before & after RTCT, and at EOT for local tumor and global response
  - Correlation between clinical activity assessment and biological changes of the immune microenvironment
  - Safety
  - *Progression Free Survival and Overall Survival at 3 years*
  - *Other exploratory translational research on immune microenvironment and HPV molecular signatures*

2025

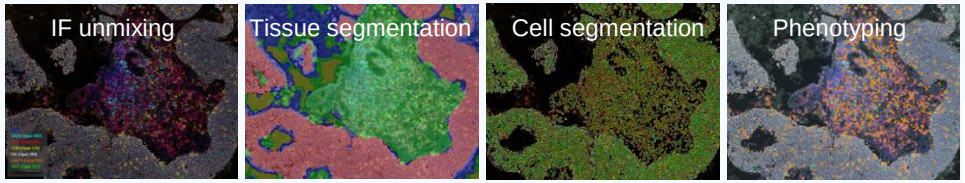
Methods – multi-IF and HTG

7-colors multiplex immunofluorescence tissue imaging (multi-IF)[1,2]:

CD3-CD8-CD20-Foxp3-Ki67-CK-DAPI

↓

Digital image analysis by machine learning



↓

Densities (cells/mm²)
- Total and proliferating (Ki67⁺) CD8⁺ (CD3⁺CD8⁺Foxp3⁻)
- Total Foxp3⁺ (CD3⁺Foxp3⁺)

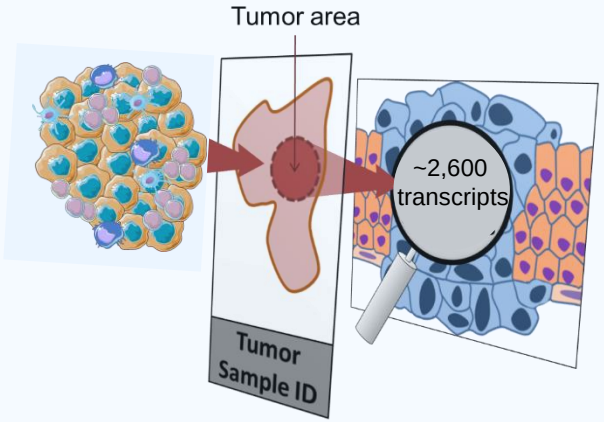
Ratio
- Total CD8⁺ / Total Foxp3⁺
- Proliferating CD8⁺ / Proliferating Total Foxp3⁺

[1]Small M, et al. *Acta Neuropath*. 2018;135:569-579
[2]Plaschka M, et al. *J Immunother Cancer*. 2022

Transcriptomic analysis

High-Throughput Genomic sequences (HTG):

Evaluation of the 'HOT' score as biomarker for immunologically active tumors which may benefit from immunotherapies[3]



HOT signature gene list: CCL19, CCR2, CCR4, CCR5, CD27, CD40LG, CD8A, CXCL10, CXCL11, CXCL13, CXCL9, CXCR3, CXCR6, FASLG, FGL2, GZMA, GZMH, IDO1, IFNG, IRF8, LAG3, LYZ, MS4A1, PDCD1, TBX21, TLR7, TLR8

[3]Foy JP, et al. *Eur J Can*. 2022;174:287-298.

Statistical methodology

- **Sample size**: Assuming a standard deviation of 14 units, **40 patients were enrolled** providing a 95% confidence interval with a precision of 5 units around the mean estimation of the $CD8^+/FOXP3^+$ relative change of lymphocytes from pre- to post-treatment biopsies.

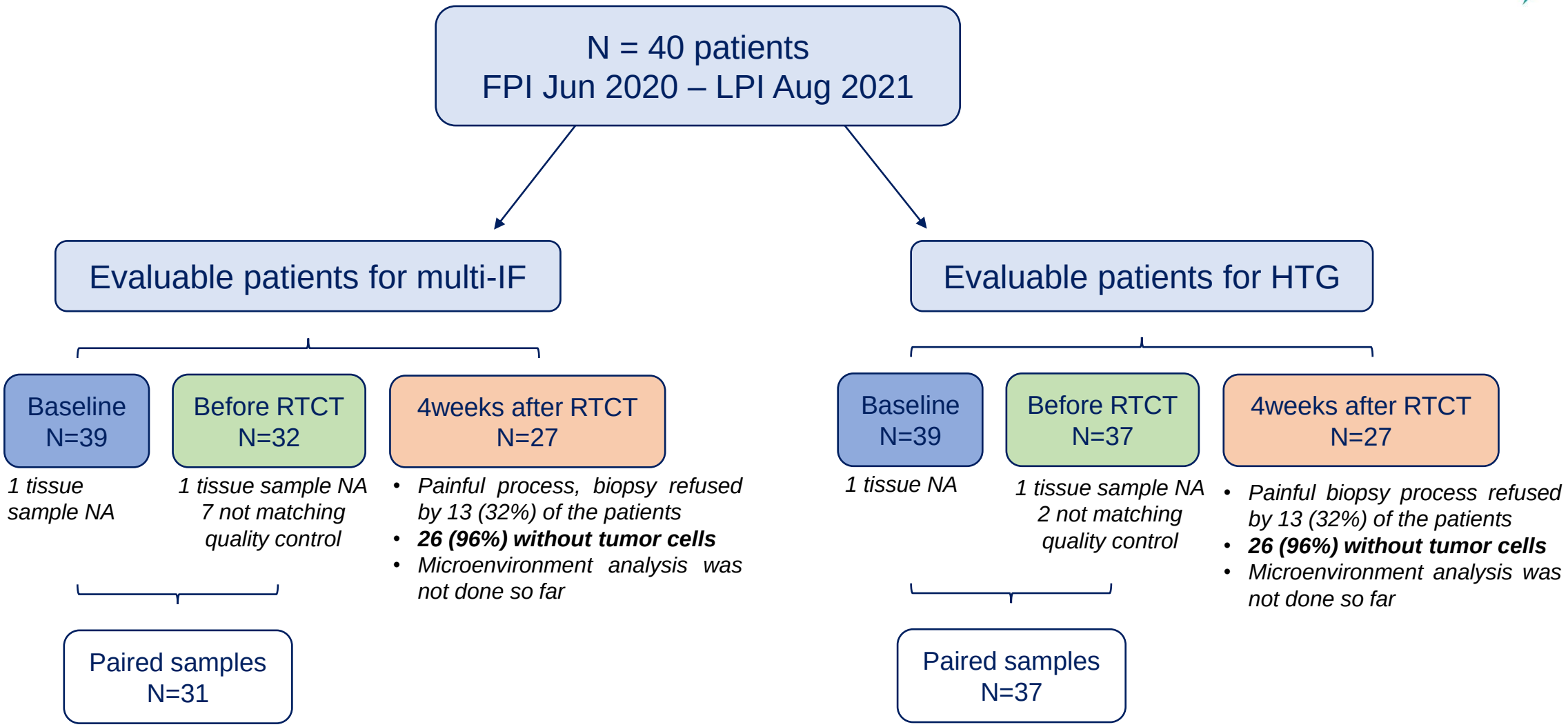
- Depending on the distribution of the ratio, Student paired t-tests or Wilcoxon signed rank tests were used to compare pre and post treatment biopsies.

- Association between multi-IF or HTG data and the tumoral responses was assessed using Wilcoxon Mann-Whitney or Fisher exact tests.

- P values less than 0.05 were considered to indicate statistical significance in all tests.

- All analyses were performed using SAS® v9.4 (SAS Institute Inc., Cary, NC, USA) and GraphPad Prism 9

Available tumor samples



NA = not available

Post dual ICB = before RTCT

PRESENTED BY: Prof. Isabelle RAY-COQUARD

Patients characteristics

	Analysis population (N=40)
Median follow-up, weeks (min; max)	**38 (24; 71)**
Median age, years (min; max)	**55.0 (31.0; 77.0)**
Weight (kg)	**60.5 (40.0; 90.0)**
ECOG performance status, n(%)	
0	**26 (65.0%)**
1	**14 (35.0%)**
FIGO 2018 stage tumor, n (%)	
IB3	**1 (2.5%)**
IIA2	**1 (2.5%)**
IIB	**19 (47.5%)**
IIIB	**1 (2.5%)**
IIIC1	**8 (20.0%)**
IIIC2	**6 (10.0%)**
IVA	**4 (10.0%)**
Histology, n(%)	
Squamous cell carcinoma	**38 (95%)**
Adeno squamous carcinoma	**2 (5.0%)**
PD-L1 expression*, (SP263)	
< 1	**3 (7%)**
≥ 1	**37 (93%)**

	Analysis population (N=40, (%))
Treatment exposure, n (%)	
Neo-adjuvant ICB phase	
Nivolumab 3mg/kg C1D1 & C1D15	**40 (100)**
Ipilimumab 1mg/kg C1D1	**40 (100)**
Chemotherapy during RT	
Cisplatin	39 (97.5)
Carboplatin	3 (7.5)
EBRT delivered, per protocol	40 (100)
Brachytherapy delivered	36 (90)*
Radiotherapy delivered in ≤ 55 days	**39 (97.5)**
Maintenance therapy	**39 (97.5)**
Completed 6 cycles with Nivolumab (480 mg total dose each cycle)	34 (85)**

*+ 4 patients who received external tumor boost with EBRT
**early discontinuation: 2 pts for progression, 2 for AEs and 2 for patient's decision

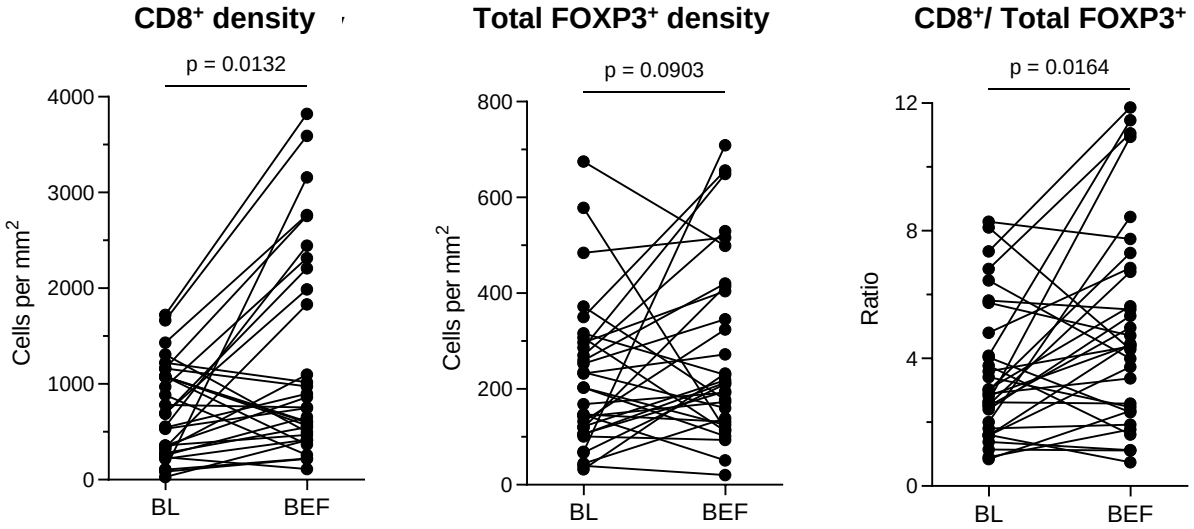
Safety- adverse events

	Neo-adjuvant ICB N = 40 (%)	RTCT N = 40 (%)	Maintenance N = 39 (%)
Any AE	**33 (82.5)**	**38 (95)**	**35 (87.5)**
Any AE of CTCAE grade ≥ 2	**14 (35)**	**33 (82.5)**	**17 (42.5)**
Any TRAE of CTCAE grade 3 or 4 *	**1 (2.5)**	**11 (27.5)**	**8 (20)**
Possibly related to Nivolumab Possibly related to Ipilimumab Possibly related to RTCT	1 (2.5) 1 (2.5) NA	3 (7.5) 3 (7.5) 10 (25)	6 (15) 1 (2.5) 5 (12.5)
Any AE with outcome of death	**0 (0)**	**0 (0)**	**0 (0)**
Any AE leading to discontinuation of ICB	**0 (0)**	**NA**	**2 (5)**
Possibly related to ICB			**1 (2.5)**
Any AE leading to discontinuation of RTCT	**NA**	0 (0)	NA
Possibly related to ICB	NA	0 (0)	NA

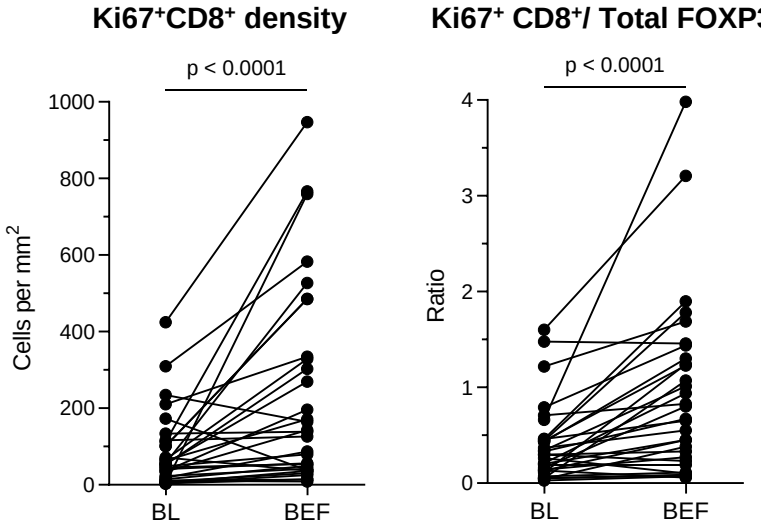
*Grade 3 or 4 related to ICB are lymphopenia, neutropenia, asthenia, muscular squeletal pain, cutaneous rash, proctitis, liver enzymatic abnormalities

Relative changes before/after ICB by multi-IF

Neo-adjuvant dual ICB significantly increases tumor-associated CD8⁺T cells and CD8⁺/FOXP3⁺ ratio



Similar results with **proliferative** CD8⁺ T cells & CD8⁺/FOXP3⁺ ratio



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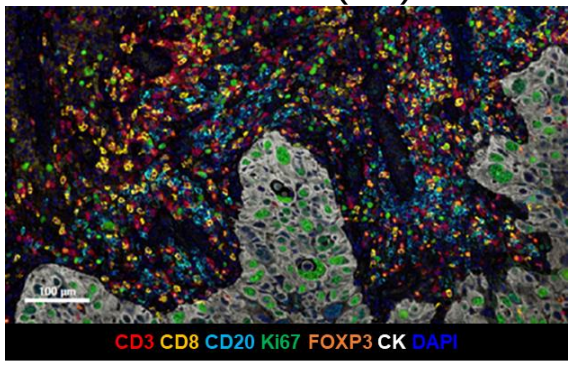
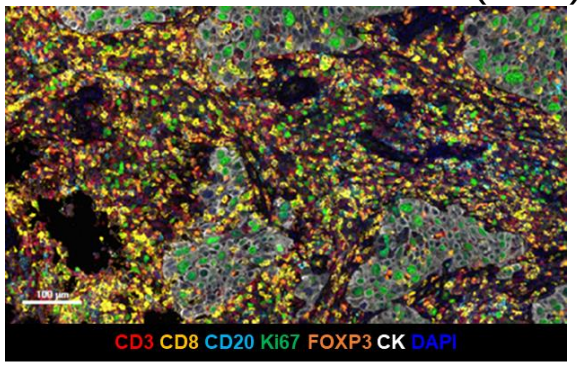
Baseline (BL)



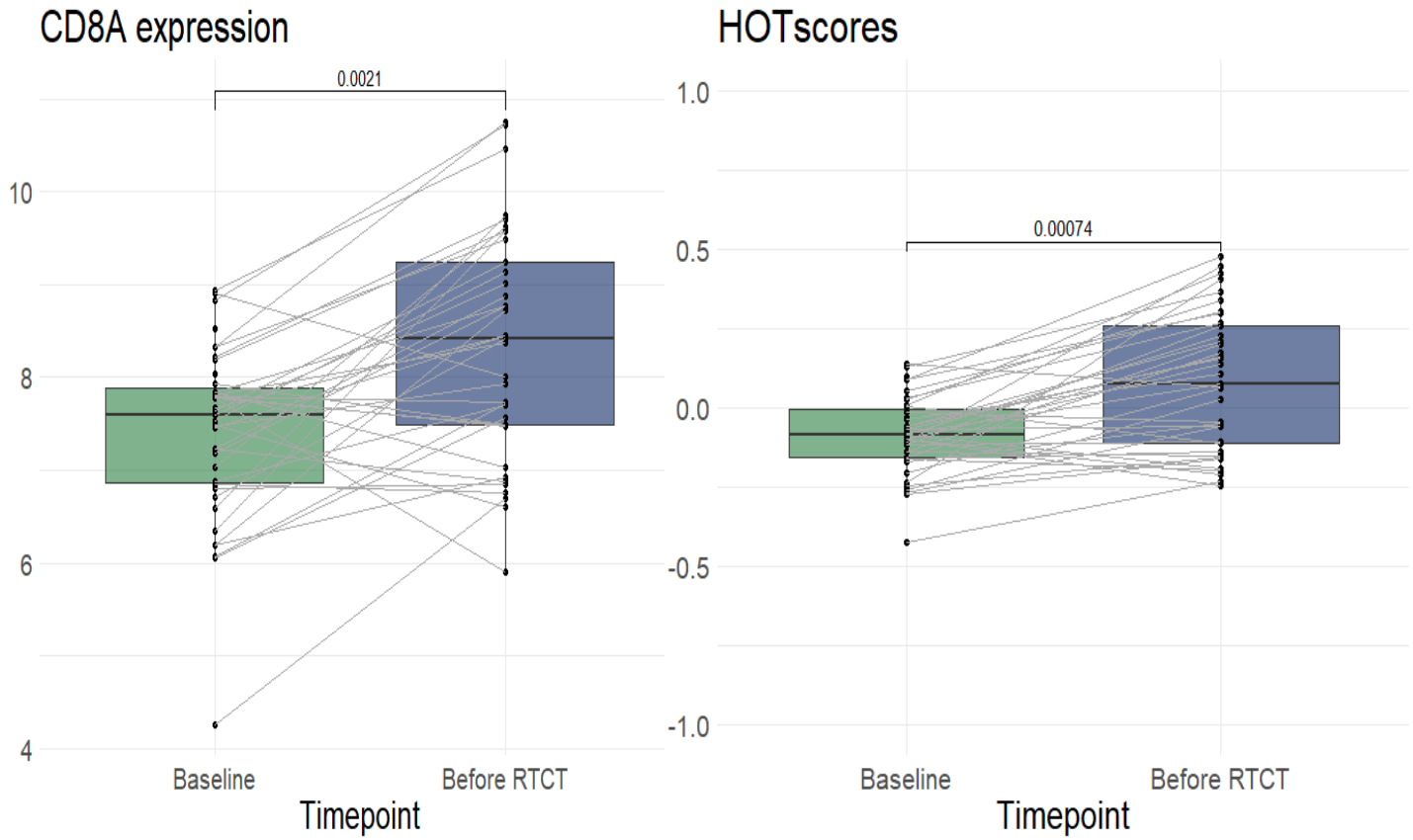
Ipi + Nivo

After ICB = Before RTCT (BEF)

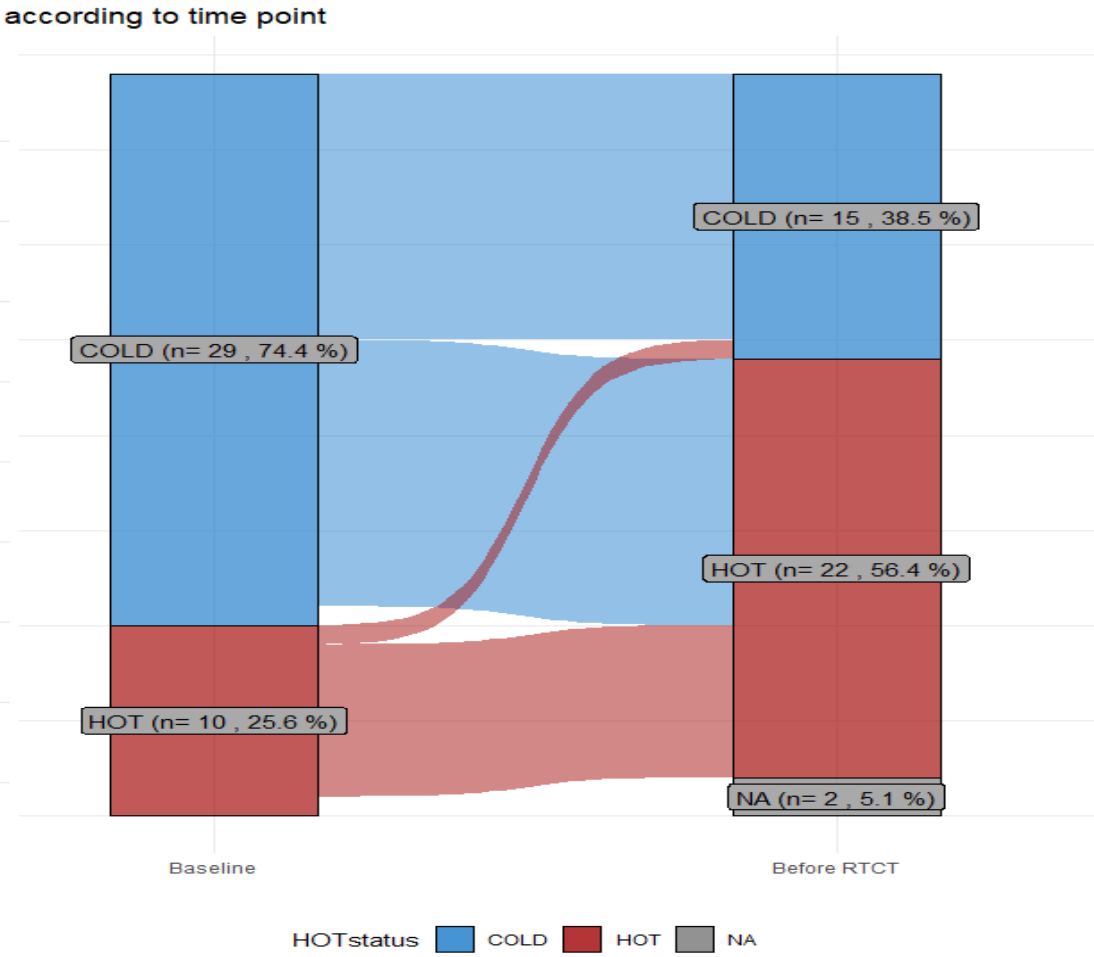


Results – HTG

Significant increase of the *CD8A* gene expression and the 'HOT' score after ICB was observed



Evolution of the 'HOT' score after neo-adjuvant ICB



Efficacy by response rate
After neo-adjuvant ICB, post RTCT and end of maintenance

RESPONSE	RR	Before RTCT N (%)		Post RTCT N(%)		End of maintenance	
Local control	CR	-		27	(68)	34	(85)
	PR	**6**	**(15)**	12	(30)	3	(8)
	SD	32	(80)	1	(2)	1	(2)
	PD	**2**	**(5)**	-		2	(5)
Global response	CR	-		26	(65)	31	(78)
	PR	5	(13)	13	(33)	5	(12)
	SD	33	(82)	1	(2)	-	
	PD	2	(5)	**-**		**4**	**(10)**

3 pts with initial FIGO IIIC
4 pts have no change before/after ICB for:
- CD8+ infiltrate
- CD8+/Foxp3 ratio
- Cold 'HOT' score

RESPONSE	FIGO STAGE	COMPLETE RESPONSE RATE
Global response	FIGO I/II	81%
	FIGO III/IV	74%

CR complete response, PR partial response, SD stable disease, PD progressive disease

Correlation of the CD8+/FOXP3+ ratio with response rate by multi-IF

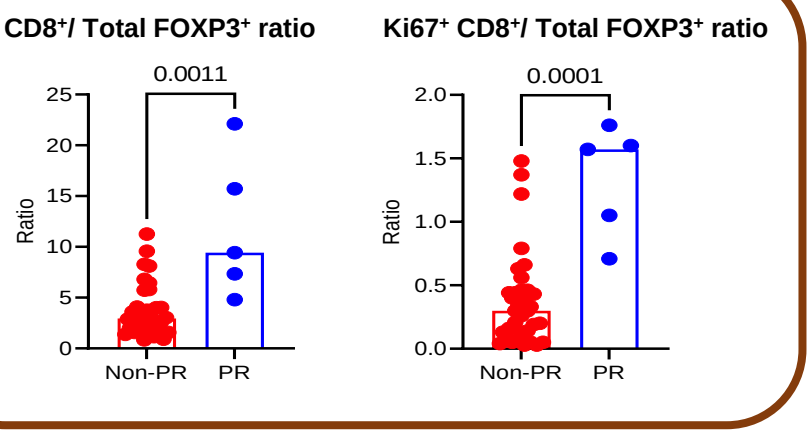
Elevated CD8+/FOXP3+ ratio at baseline correlate with partial response before RTCT

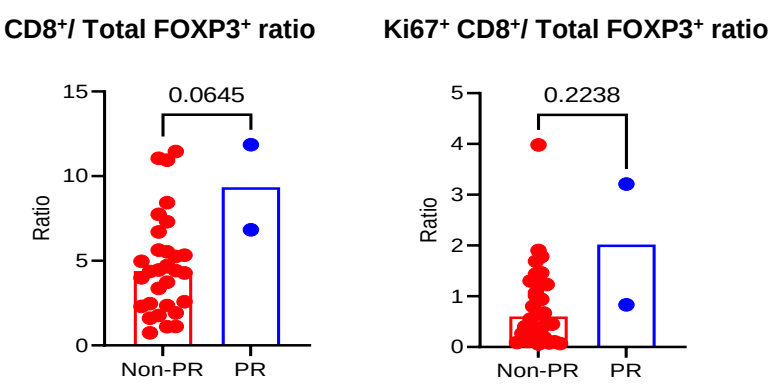
Elevated proliferative CD8+/FOXP3+ ratio correlate with CR at the end of maintenance therapy

Baseline

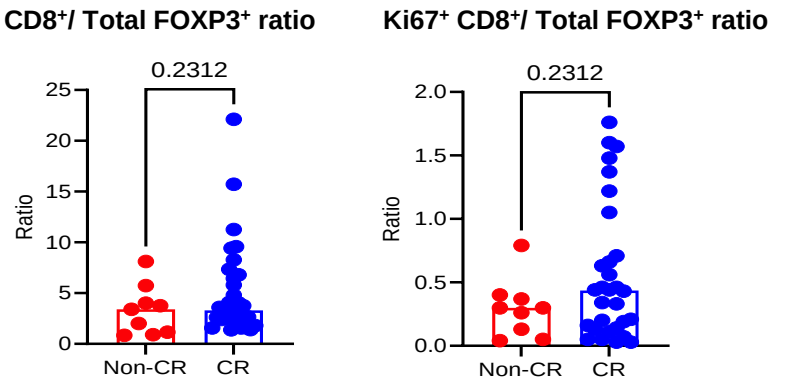
Before RTCT

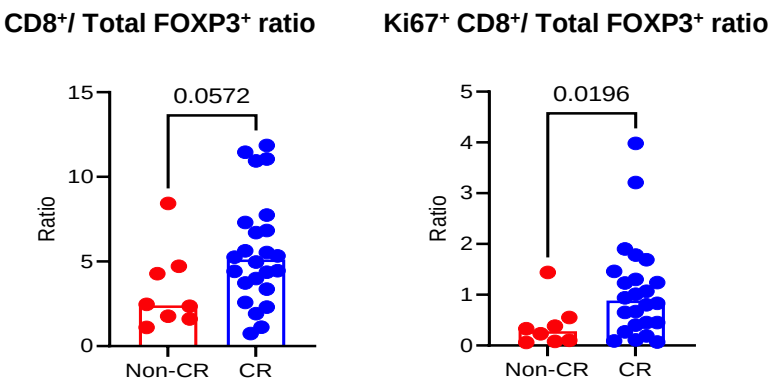
Response before RCT





Response at EOT



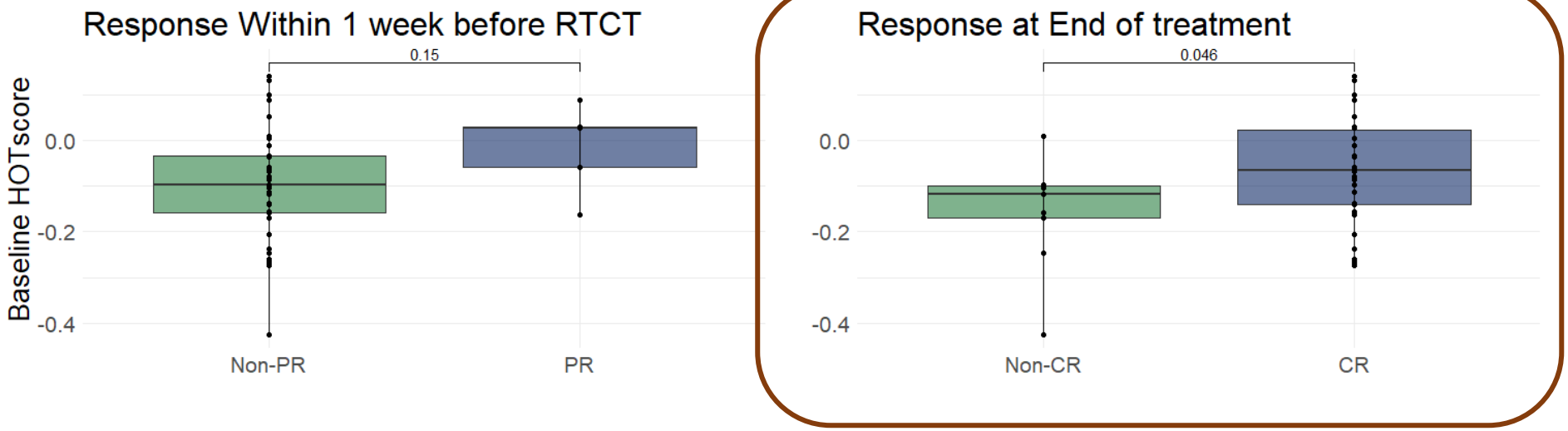


PR, partial response
CR, complete response

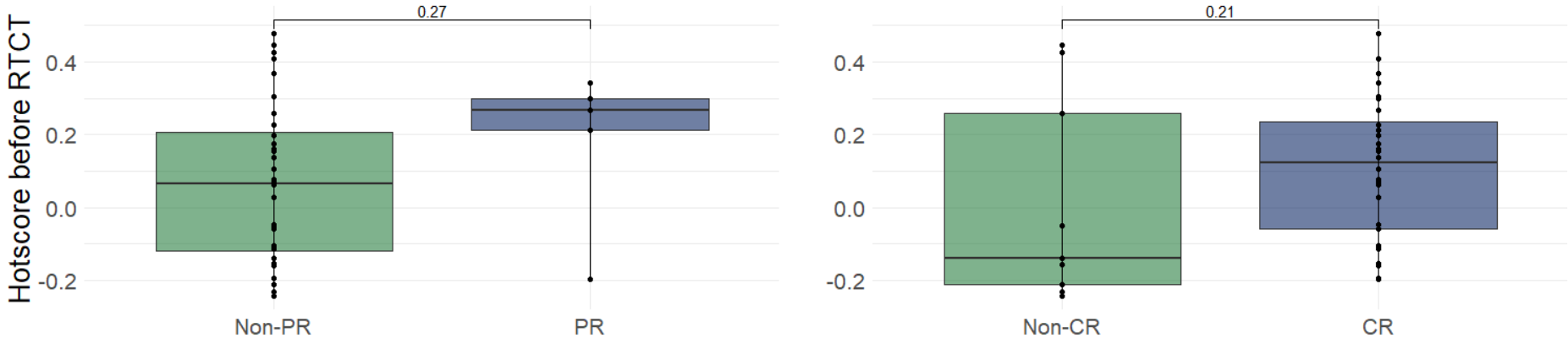
Correlation of the ‘HOT’ score with response rate

The ‘HOT’ score at baseline correlates with complete response at the end of maintenance therapy

Baseline



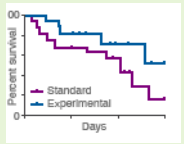
Before RTCT



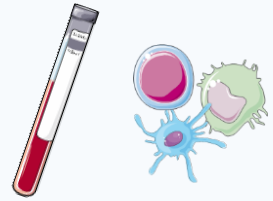
PR, partial response
CR, complete response

Perspectives

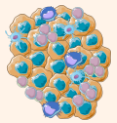
1. Clinical end points (2025)
→ PFS
→ OS

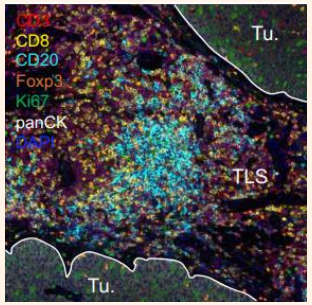


4. To analyze the systemic immune response by characterizing the phenotype and activation status of immune cells and anti-HPV T-cell response:
→ Multiparametric flow cytometry (mFC)
→ ELISA spots

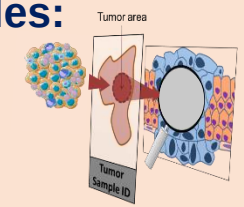


2. To evaluate the neighborhood of $CD8^+$ and $FOXP3^+$ immune cells and spatial localization of TLS, DCs, MPs and PD-L1 expression
→ Multi-IF
→ IHC





3. To analyze gene expression profiles:
→ RNA-sequencing
→ Whole Exome Sequencing (WES)



5. To characterize HPV molecular status, integration sites and viral genes deletion to correlate with prognosis and response to ICB
→ Capture HPV technics and NGS

To analyze HPV circulating tumor DNA (ctDNA) to correlate HPVctDNA kinetics with treatment response
→ Digital droplet PCR (ddPCR)

Conclusion

- The COLIBRI trial demonstrates the acceptability and safety of a dual ICB with Nivolumab+Ipilimumab in the neo-adjuvant setting pre RTCT followed by Nivolumab monotherapy as maintenance therapy post RTCT
- 90% of LACC in COLIBRI are in complete or partial response at EOT suggesting no detrimental effect from sequencing ICB before RTCT
- Using multi-IF or HTG methods, expansion of $CD8^+$ cells (including proliferative ones), elevated $CD8^+/FOXP3^+$ ratio and 'HOT' score were significantly increased post neo-adjuvant dual ICB
- Both the CD8+/FOXP3+ ratio defined by multi-IF and the 'HOT' score correlated with response to treatment
- **These data, combined with on-going translational research, offer support for further studies with neo-adjuvant sequencing strategies to evaluate ICB alternative therapies in LACC**

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