Different flavors of regulatory T-cell subsets in patients with cancer and their role in tumor escape

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Treg subsets promote tumor escape from the host immune system

- Types of regulatory T cells:
  - **nTreg**: $CD4^{+}CD25^{high}Foxp3^{high}$
    - thymus derived
    - suppress immune responses against “self” by mechanisms involving contact inhibition
  - **Tr1 cells**: $CD4^{+}CD25^{neg}IL10^{+}TGF\text{-}\beta_1^{+}$
    - induced in the periphery upon Ag presentation
    - suppress immune responses through IL-10 and TGF- β_1 secretion

- an increased frequency of Treg in the tumor and in the peripheral circulation of patients with HNSCC was previously reported by us:

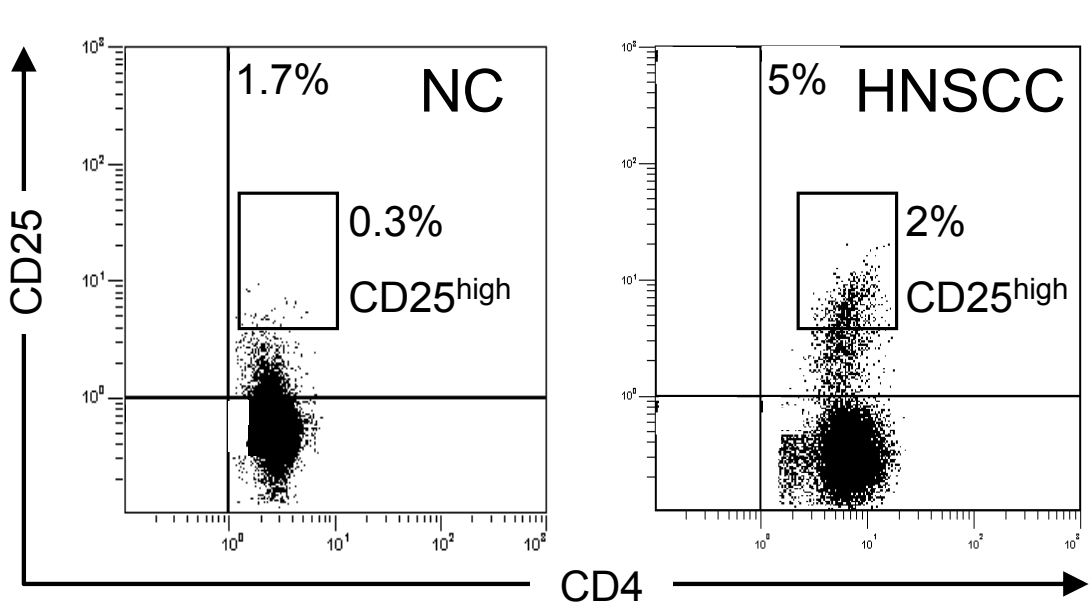
  Albers AE *et al.,* Cancer Immunology Immunotherapy, 2005;54: 1072-81
  Schaefer C *et al.,* British Journal of Cancer, 2005;92: 913-20

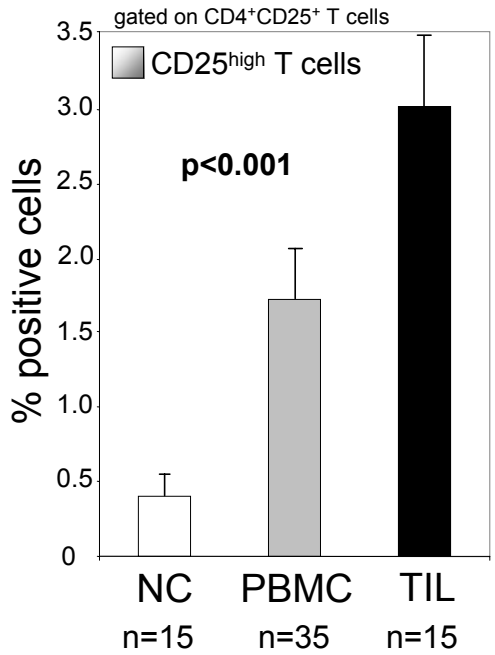
- In patients with ovarian cancer, accumulations of Treg at the tumor site were associated with shorter survival (Curiel, Nat Med 2004)

Methods

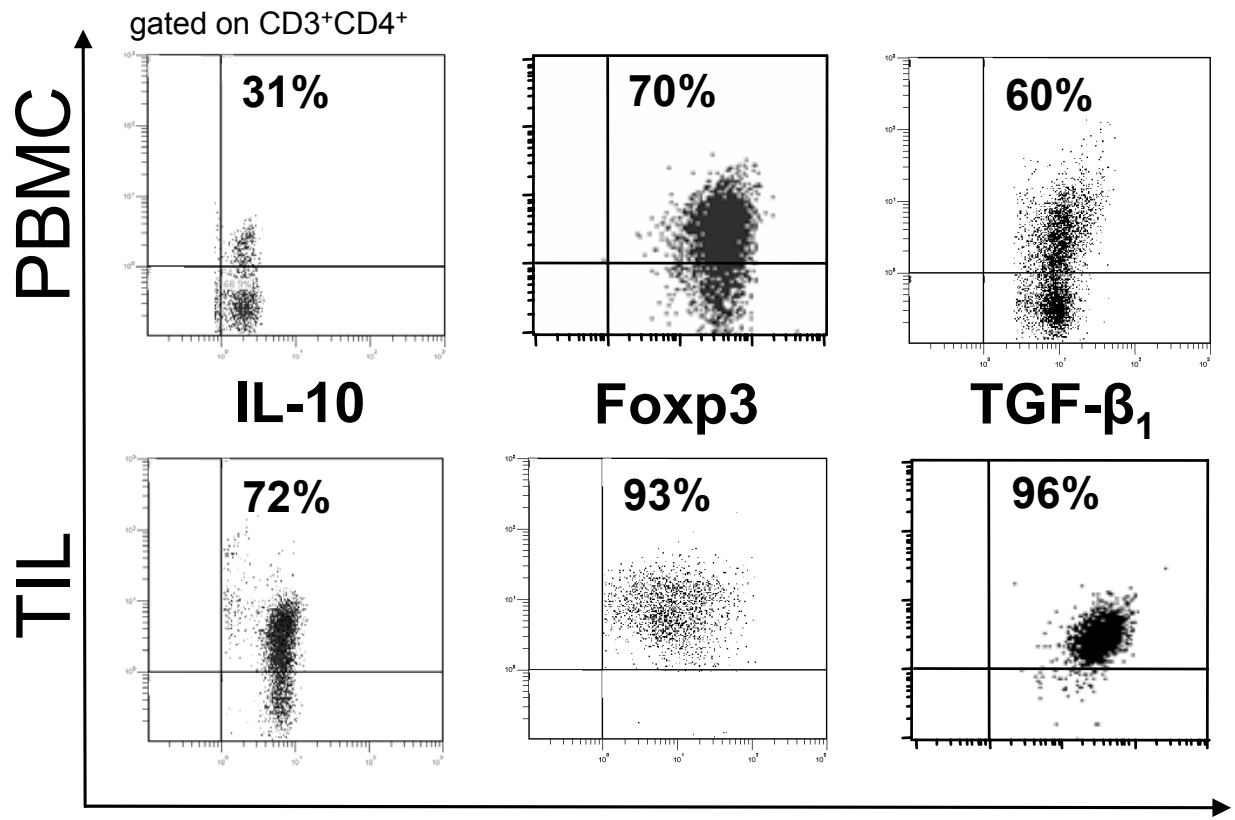
- Cell source: PBMC and TIL from HNSCC patients or PBMC from NC
- Single-cell sorting: **$CD4^+CD25^{high}$**
  $CD4^+CD25^{neg}$
- Phenotype: gate on $CD3^+CD4^+$ (Tr1) or $CD4^+CD25^{high}$ (nTreg)
  rare-event multicolor flow cytometry
  multicolor immunofluorescence microscopy
- Suppressor function:
  CFSE-labeled autologous $CD4^+CD25^{neg}$ responder cells (R) + Treg (S)
  added at 1S:1R, 1S:5R, 1S:10R ratios
- Mechanisms of suppression:
  - Transwell system
  - neutralizing antibody in suppressor assays
  - IL-10, TGF-β_1 in cells, in supernatants (Flow, Luminex)
- Associations with the disease stage and/or progression

CD25^{high} nTreg are expanded in HNSCC patients vs. NC





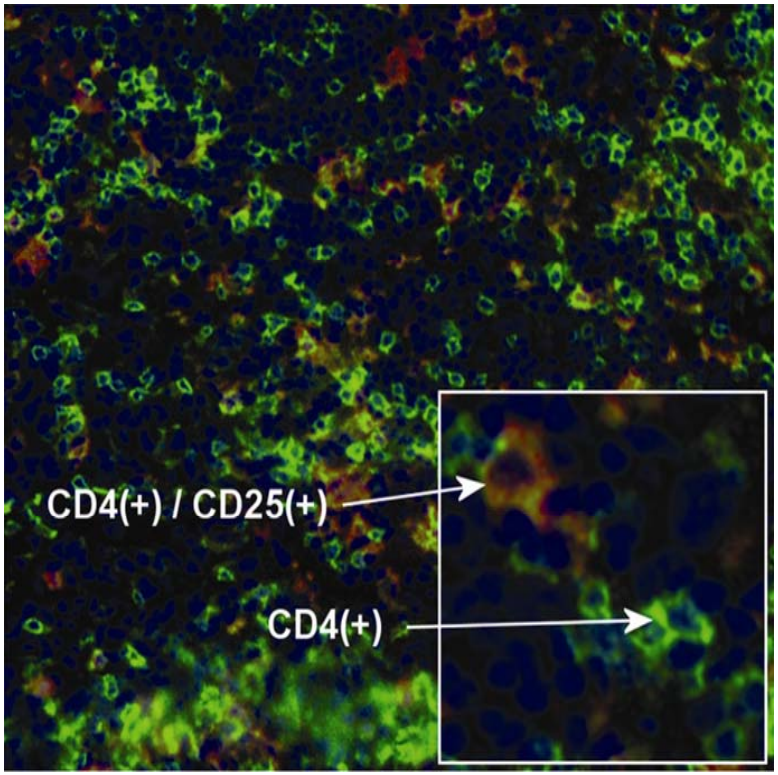
Phenotypic characteristics of CD25^{high} nTreg in different compartments (HNSCC patients)

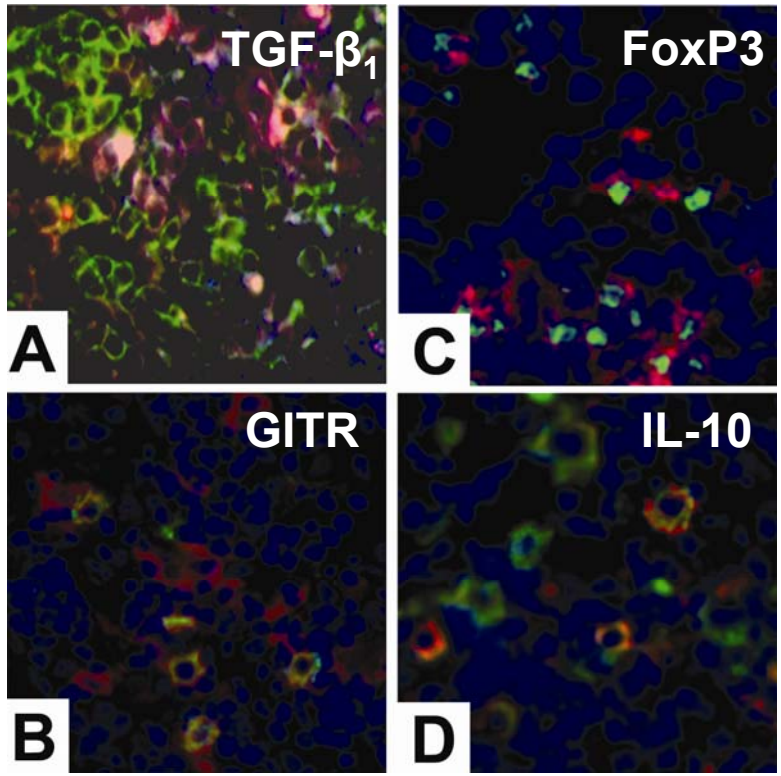


CD4+**CD25high**
Foxp3+**CD62L+CCR7+**

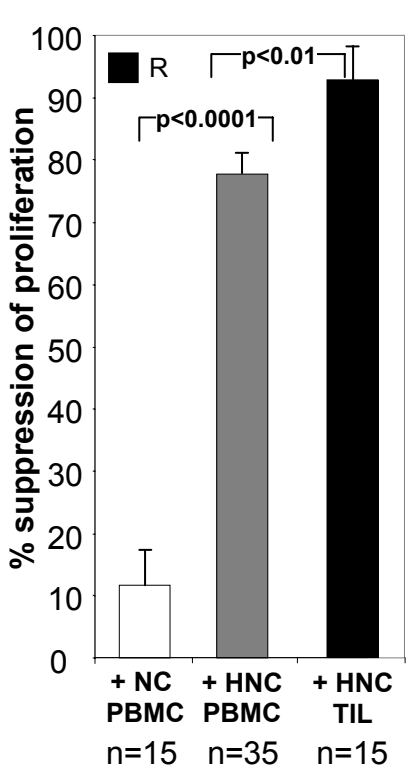
CD4+**CD25high**
Foxp3+**GITR+IL-10+TGF-β1+**

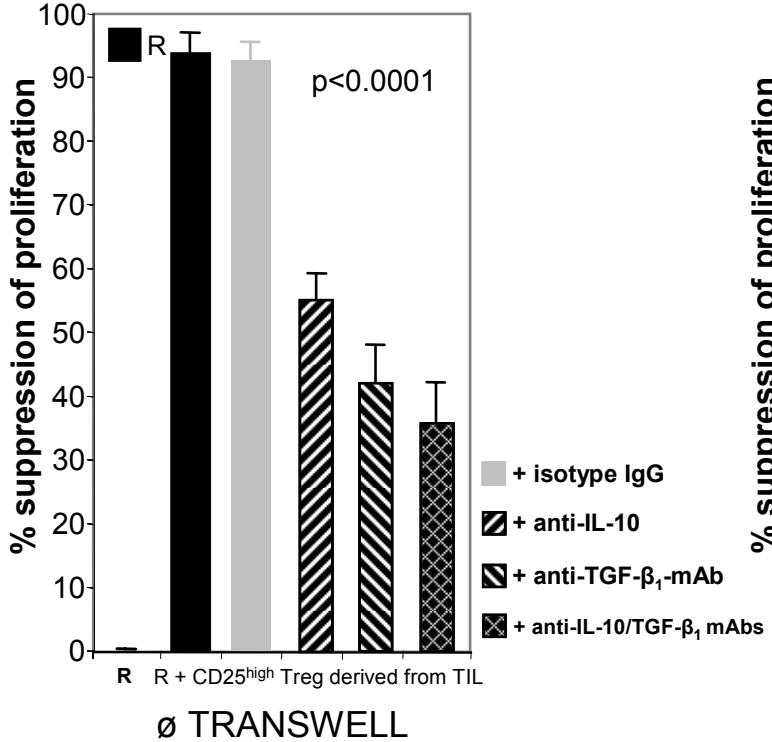
$CD4^+CD25^+$ nTreg among TIL at the tumor site

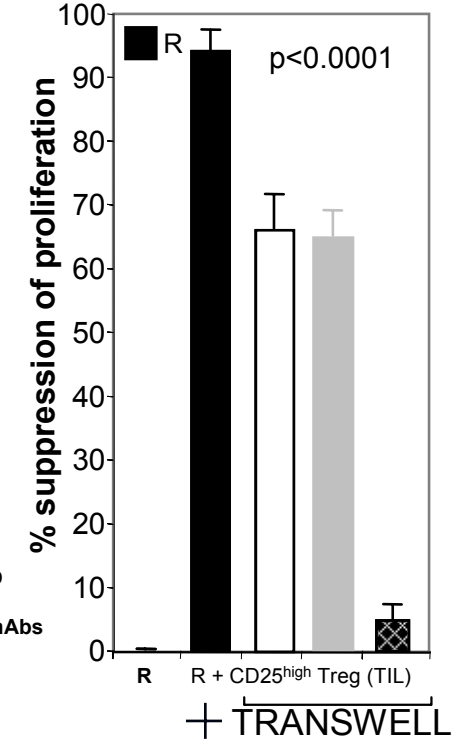




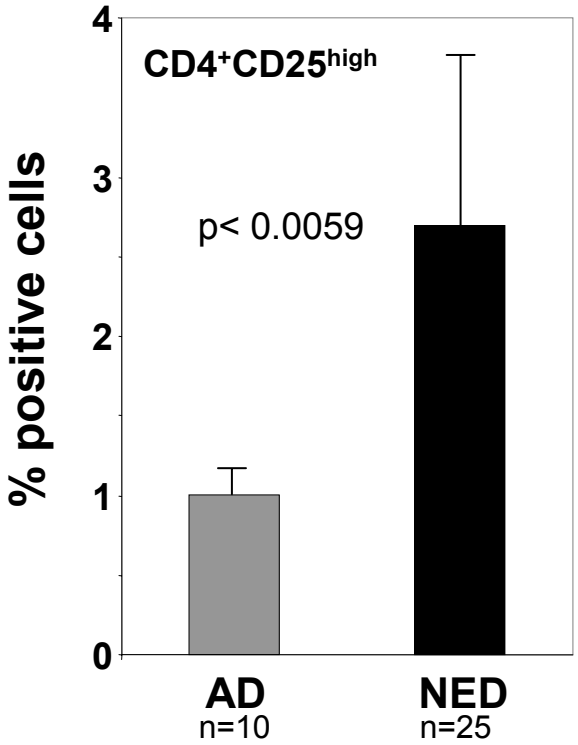
Suppressor function of $CD4^{+}CD25^{high}$ nTreg is cell contact- and cytokine- dependent

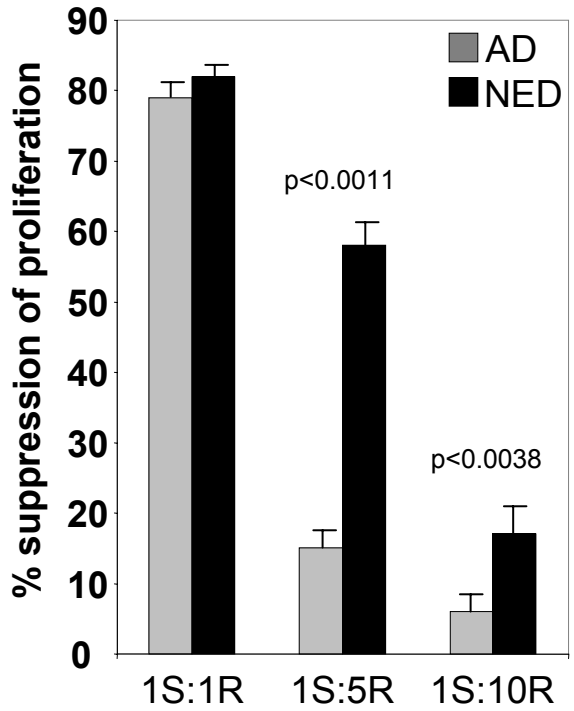






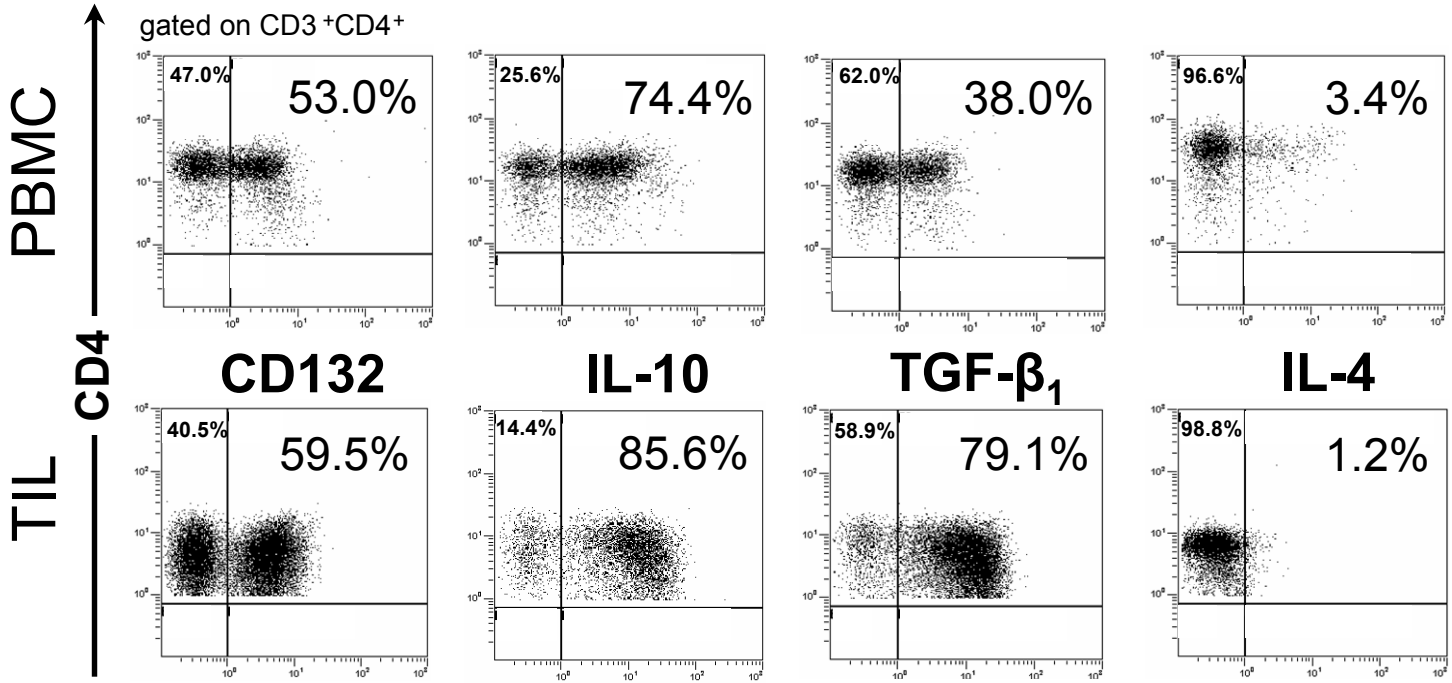
$CD4^+CD25^{high}$ Treg in PBMC of HNSCC patients expand after oncologic therapy



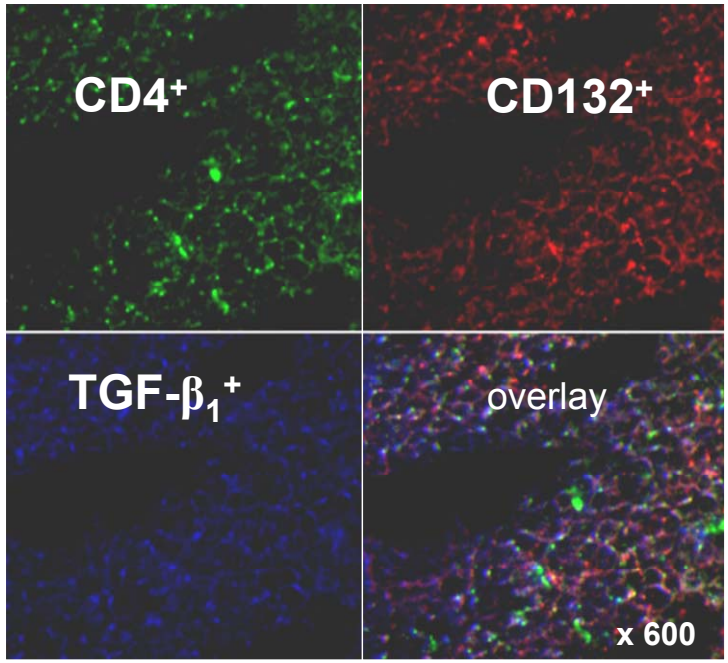


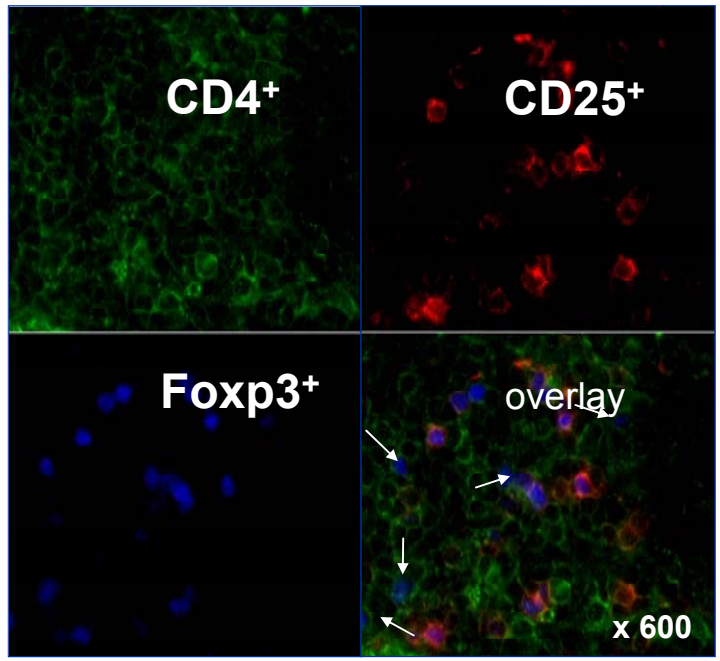
Phenotypic characteristics of CD4+ Tr1 cells in the circulation or TIL in HNSCC patients

PBMC: CD4+**CD25neg**Foxp3+CD122+**IL-10+TGF-β1+**
TIL: CD4+**CD25neg**CD132+**IL-10+TGF-β1+**

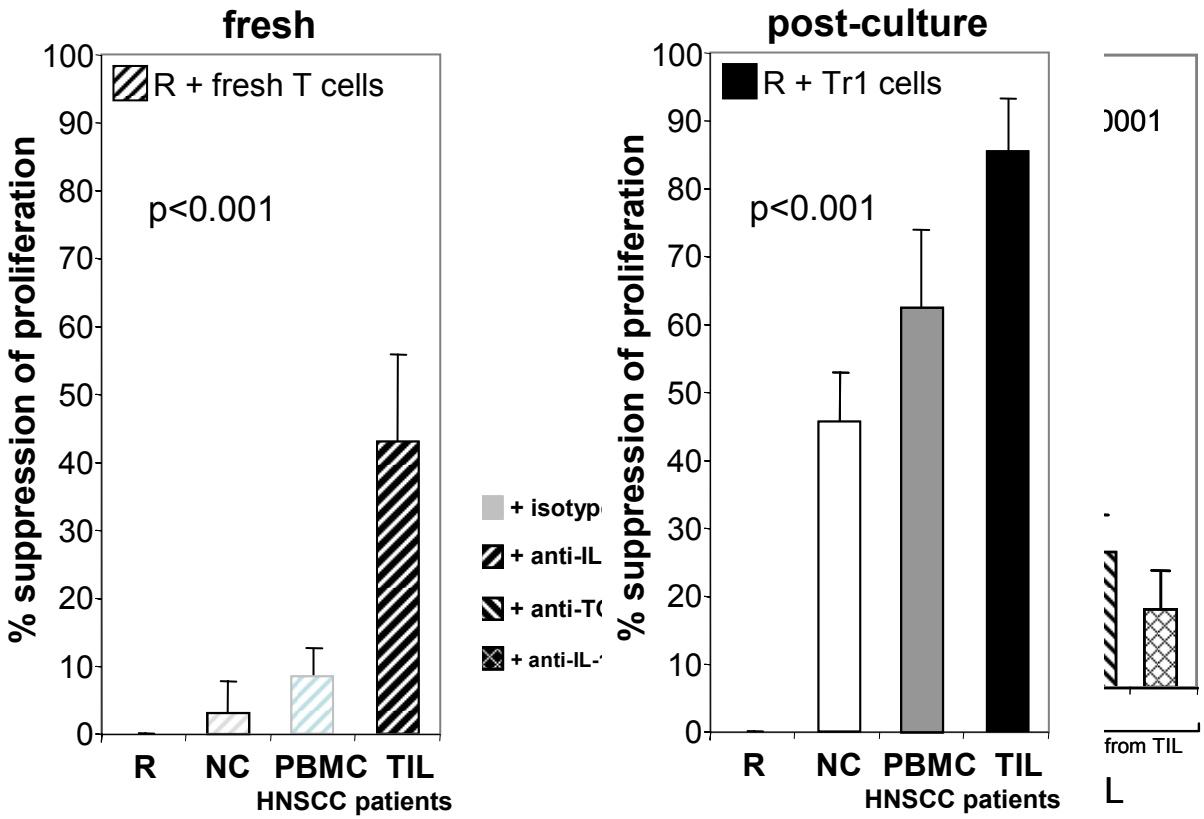


Tr1 precursors *in situ* at the tumor site expressing suppressive molecules



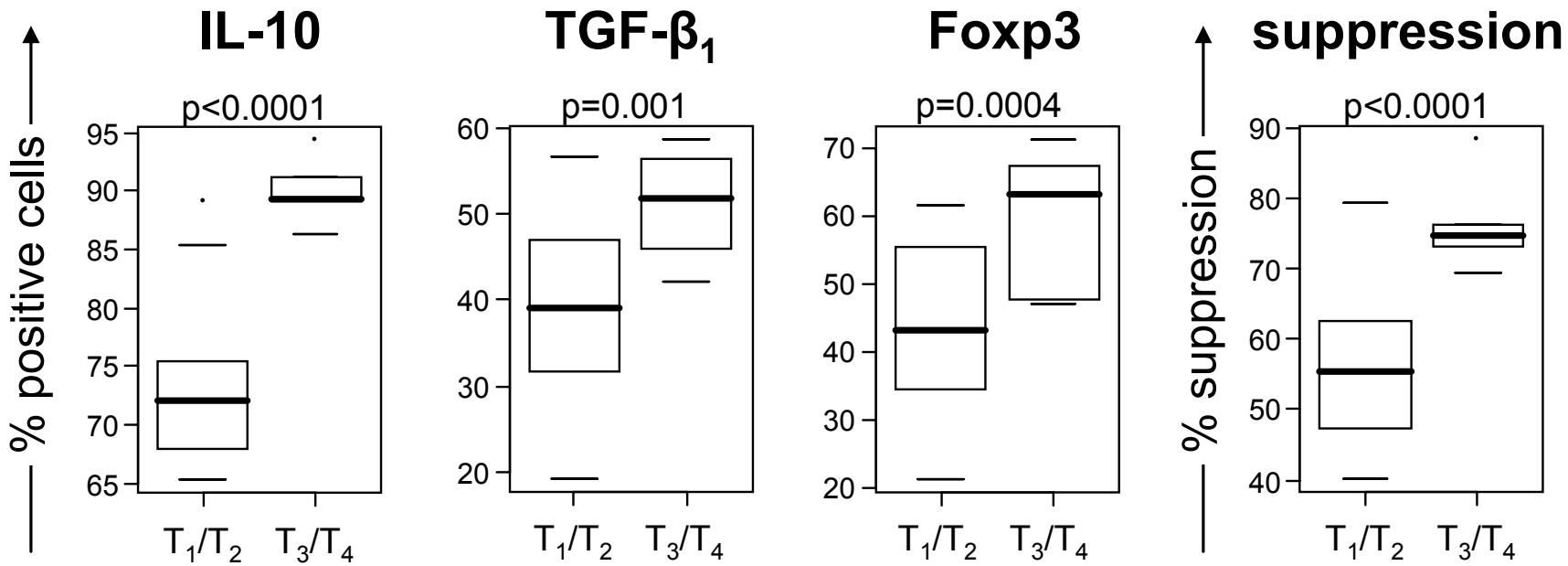


Suppressor activity of Tr1 precursors or Tr1 cells is cytokine-dependent but cell contact-independent



NC n=15 **HNSCC** PBMC n=16; TIL n=10

Marker expression and function of Tr1 cells in HNSCC patients is associated with the T stage



Conclusions

❖ Treg in the blood and in the tumor of patients with HNSCC have a distinct phenotype and elevated suppressor activity relative to Treg in NC

❖ Both nTreg and Tr1 assemble at the tumor site

❖ nTreg mediated suppression is contact dependent while that mediated by Tr1 is cytokine (IL-10 and TGF-ß) dependent

❖ In HNSCC patients

- **nTreg** expansion and regulatory activity is higher in NED than in AD
- **Tr1** expansion and regulatory activity increase with tumor stage

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