Preliminary evaluation of Unconventional T cells in Renal Cell Carcinoma (RCC) patients.

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BACKGROUND and AIMS:

Renal Cell Carcinoma (RCC) is an immune-mediated tumor that it has proven refractory to conventional treatment modalities such as chemotherapy and radiotherapy. The Double negative T cells (DNTs) have emerged as new subset of T cells that contribute specifically to anti- tumor immunity since they are involved in immune regulation and tolerance acting as regulatory T cells (Treg)and/or cytotoxic T cells. DNTs express either αβ or γδ T-cell receptors (TCR) and lack CD4,CD8 and CD56. No data are available on the role of DNT cells in human RCC.

The aim of this study was to assess the frequency and the functional attitude of circulating DNTs in RCC patients and healthy donors as controls, in order to assess the role of DNTs on clinical outcome and progression.

METHODS

Peripheral blood (PB) samples of 23 RCC pts, were selected. As control PB samples of 16 healthy donors and 90 patients with lymphoma (Ly) were collected. Circulating DNT subsets (TCRαβ+ and TCRγδ+) were characterized for their ontogeny, tolerogenic or cytotoxic attitude and TCR clonality by staining with the following conjugated monoclonal antibodies (MoAbs) for surface markers: CD3, CD4, CD8, CD56, CD45, TCRαβ, granzyme B and IFN-gamma. Isotype-matched MoAbs were used as staining controls. Data were acquired using an 8-colour flow cytometer and analyzed using Kaluza software. Data were compared among the groups using the Mann-Whitney non-parametric test or Kruskal–Wallis one-way analysis of variance.

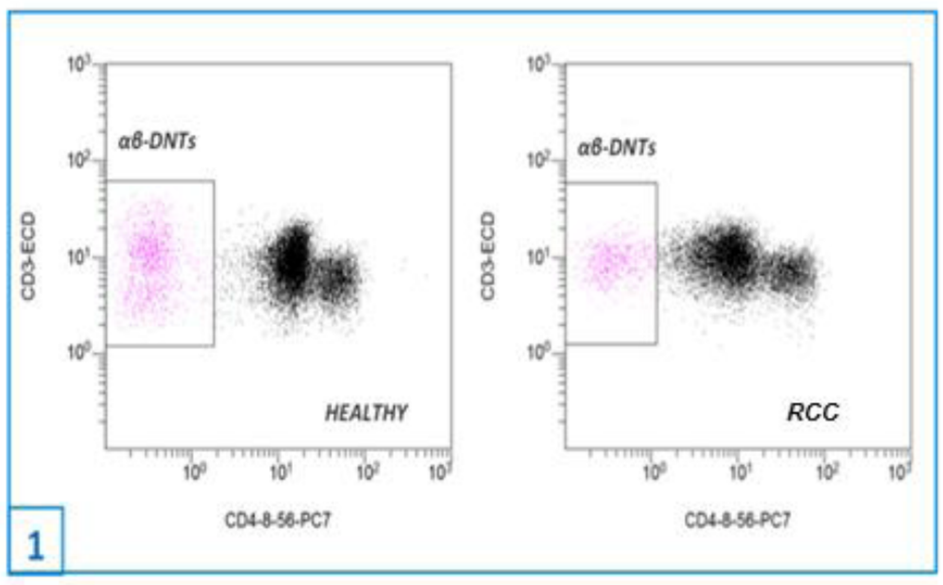

Fig.1 Circulating αβ-DNTs. Typical pattern of DNT frequencies on gated CD3+T cells. Representative staining of an healthy donor and RCC patient shown.

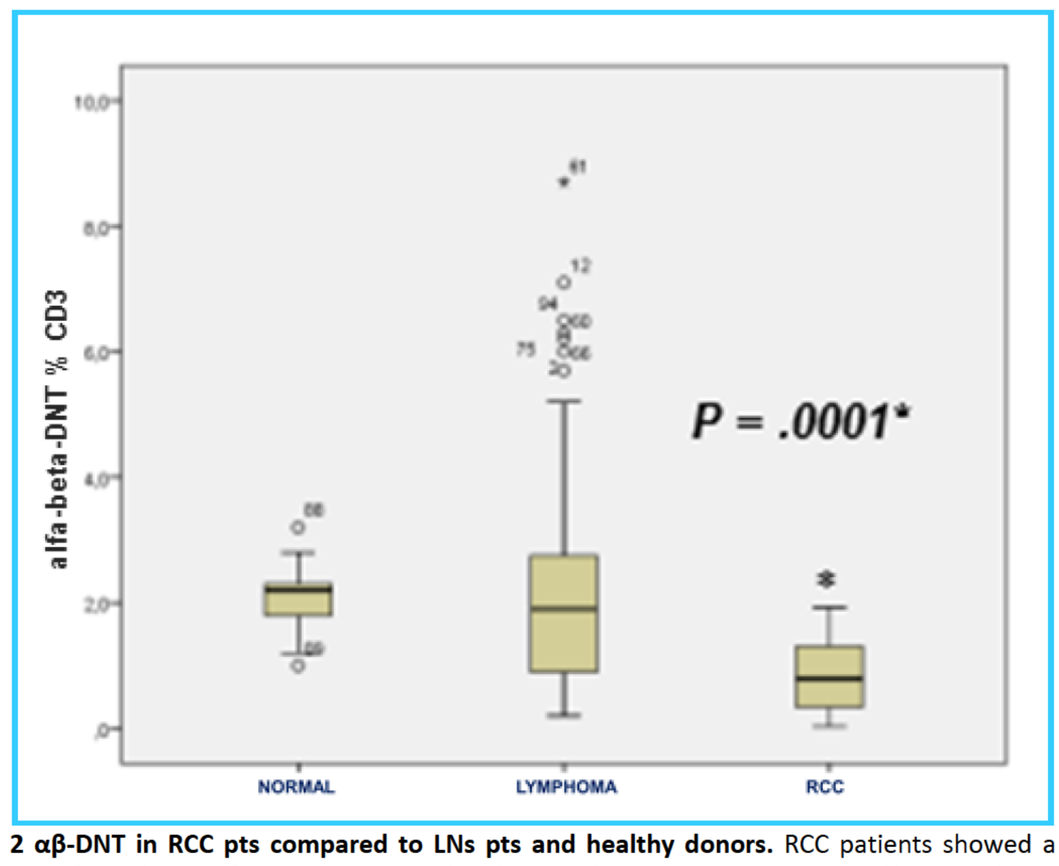

Fig. 2 αβ-DNT in RCC pts compared to LNs pts and healthy donors. RCC patients showed a lower number of circulating αβ-DNT as compared with controls or Lymphoma patients (*p=0.001)

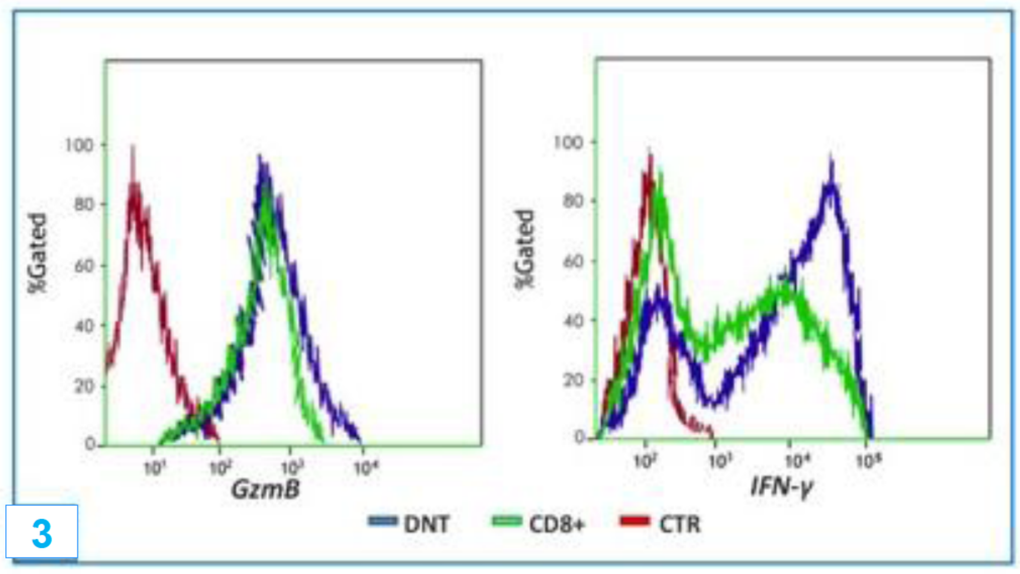

Fig.3 Expression of GzmB and IFN-γ (as MFI median florescence intensity) in ex-vivo expanded DNTs from RCC patients as compared with autologous CD8+ T cells.

RESULTS

In 22 RCC pts evaluated, the αβDNTs significantly decreased as compared with healthy controls (p=0.001) and this reduction was higher when compared with that observed in Ly pts (p=0.001) (fig.1,2), given the greater immunological impairment of RCC tumor burden. *Ex vivo* expanded DNTs acquired an immunomodulatory cytokine profile which is characterized by the secretion of IFN-gamma and Granzyme B (GzmB) that is comparable to that autologous $CD8^+$ T cells (fig.3).

CONCLUSIONS

To date, no data have been reported on DNT phenotypic and functional characterization in RCC patients. Our results has demonstrated for the first time that DNTs could play an important role in the development and the progression of RCC.

More functional studies in malignant RCC are required to better understand the role of DNT in RCC pathogenesis. In addition, based on our preliminary results, it is likely that *ex-vivo* expanded DNTs exert an anti-tumor activity, thus, suggesting their possible use as a new strategy for adoptive immune-therapies.

References

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