

# Plasmacytoid dendritic cells infiltrating ovarian cancer are associated with poor prognosis

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**Abbreviations:** pDC, plasmacytoid dendritic cell; TApDC, tumor-associated pDC; IFN, interferon; OC, ovarian cancer; TMA, tissue micro-array

Using two different and complementary approaches (flow cytometry and immunohistochemistry) on two independent cohorts of ovarian cancer patients, we found that accumulation of plasmacytoid dendritic cells (pDC) in tumors is associated with early relapse. This deleterious effect of tumor-associated pDC was evident when they are present in cancer epithelium but not in lymphoid aggregates.

Plasmacytoid dendritic cells (pDC) are one of the two main subsets of DC in human blood. They play a major role in anti-viral and autoimmune responses through their unique capacity to produce massive amounts of IFN $\alpha$  in response to both viral and self nucleic acids, respectively, following TLR7 and TLR9 engagement.<sup>1</sup> However, their role in antitumor immunity has not been clarified yet. Emerging evidence indicates that tumor infiltration by pDC may have clinical importance, as underlined by their identification in melanoma, head and neck, lung, ovarian and breast cancers.<sup>1</sup>

Ovarian cancer (OC) is the most frequent and aggressive gynecologic cancer. This is due, at least in part, to its diagnosis at advanced stages (III/IV) in the majority of patients with peritoneal carcinosis and malignant ascites. In a recent report,<sup>2</sup> we investigated the clinical significance of the presence of pDC in tumor mass and malignant ascites by conducting a systematic comparison of tumor-associated (TA) and ascites pDC. We observed an accumulation of pDC in most of malignant ascites and their presence at high frequency (highest tertile) in 36% of

tumors whereas they were profoundly depleted in blood. Importantly, only accumulation of pDC in tumors was an independent prognostic factor associated with early relapse while their presence in malignant ascites was not deleterious.

These initial results obtained on 33 patients in whom pDC were identified by flow-cytometry as CD4<sup>+</sup>BDCA2<sup>+</sup>CD123<sup>+</sup> cells<sup>2</sup> were confirmed on a larger series (n = 97 patients) treated for OC in Centre Leon Berard between 1997 and 2009 by immunohistochemistry (IHC). Thus, TApDC were identified as BDCA2<sup>+</sup> cells (clone 104C12, Dendritics) on formalin-fixed, paraffin-embedded tissues using tissue microarray allowing the analysis of impact of the presence of pDC in both cancer epithelium and lymphoid aggregates present in cancer stroma. BDCA2<sup>+</sup> cells frequency was scored using a semi-quantitative method as 0 (no cells) (Fig. 1A), + ( $\leq 5$  cells) (Fig. 1B) and ++ ( $> 5$  cells) (Fig. 1C). BDCA2<sup>+</sup> TApDC were present in only 18/97 tumors (18%) with a majority (73%) of score +. The median follow-up of the cohort was 34.1 mo. In univariate analysis, the presence of TApDC within cancer epithelium

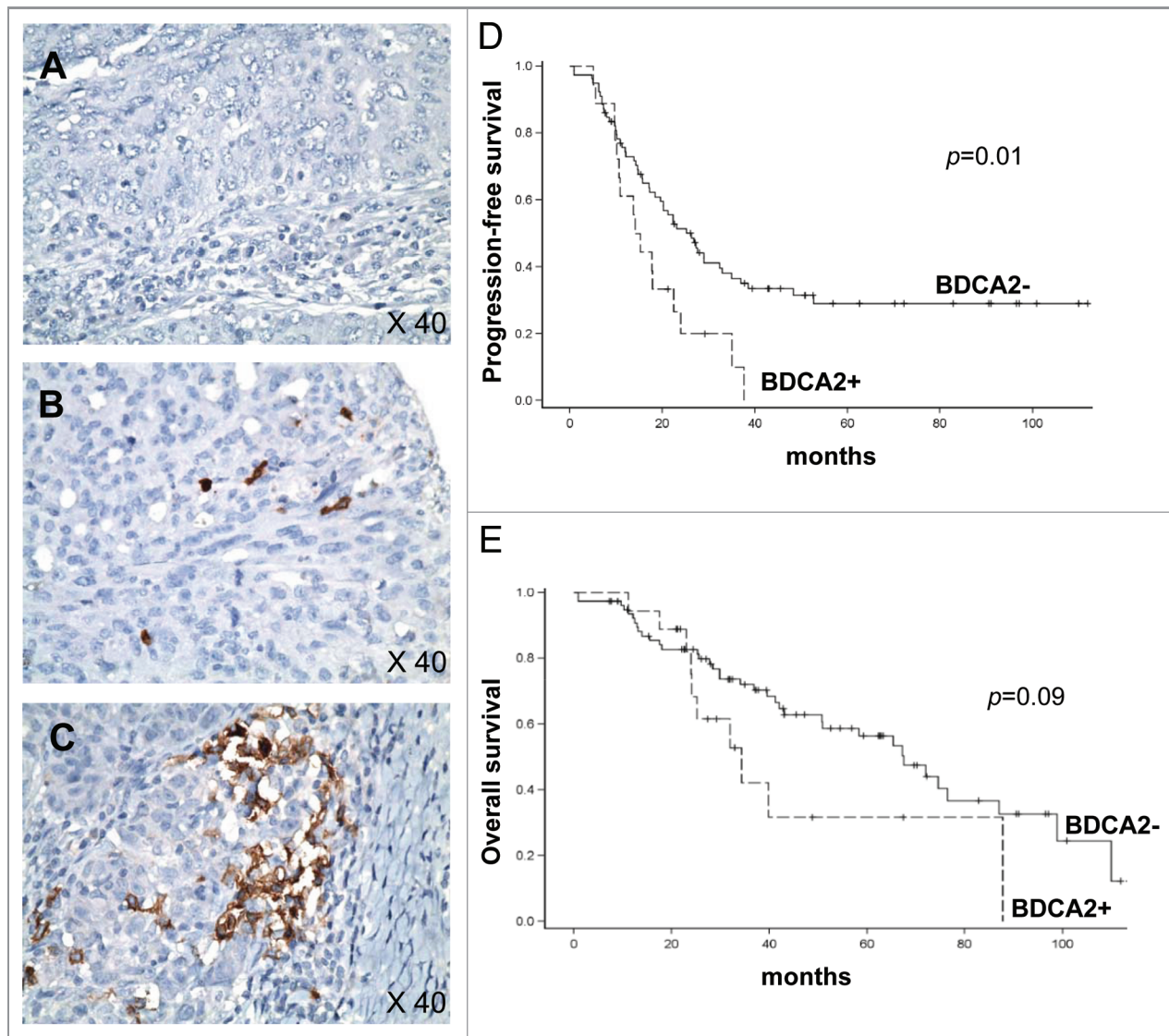
was associated with early relapse, as median progression-free survival (PFS) was estimated to 14.6 mo, compared with 26.2 mo in the absence of TApDC (p = 0.01, Fig. 1D). Similarly, median overall survival was shorter in the presence of TApDC, although not statistically significant (34.3 mo compared with 67.5 mo, p = 0.09; Fig. 1E). In multivariate analysis, in addition to clinical prognostic factors (advanced stage, debulking surgery and residual tumor), the presence of TApDC remains an independent prognostic factor associated with shorter PFS (HR = 2.19, 95%IC = 1.1–4.07, p = 0.02).

Interestingly, the presence of TApDC into lymphoid aggregates had no impact on clinical outcome, suggesting a direct interaction between TApDC and tumor cells favoring tumor progression. Thus, using two different and complementary methods (flow-cytometry and IHC) on two independent cohorts, we observed a deleterious impact of the presence of TApDC within tumors on OC patient's outcome. These data corroborate our findings in breast cancer<sup>3</sup> and others in melanoma<sup>4</sup> showing that TApDC accumulation correlates with poor prognosis. Collectively, these results

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**Figure 1.** The presence of plasmacytoid dendritic cells (pDC) in ovarian cancer (OC) epithelium is associated with early relapse. (A–C) IHC analysis on paraffin-embedded ovarian tumor sections was performed using anti-BDCA2 (brown). Representative pictures of BDCA2<sup>+</sup> pDC infiltration of OC epithelium scored as 0 (A), + (B) and ++ (C) are shown. Original magnification: x40. (D) Progression-free survival (PFS) according to the presence (+/++) or absence (0) of BDCA2<sup>+</sup> pDC in OC epithelium (n = 97 OC patients). (E) Overall survival (OS) according to presence (+/++) or absence (0) of BDCA2<sup>+</sup> pDC in OC epithelium (n = 97 OC patients). PFS and OS were calculated using Kaplan–Meier method.

suggest that TApDC may contribute to immune tolerance and tumor progression.

In our initial report in OC,<sup>2</sup> we showed that, unlike ascites pDC, TApDC (1) expressed a semi-mature phenotype as evidenced by high levels of CD40 and CD86 and (2) were strongly affected for their IFN $\alpha$  production in response to CpG-A (TLR9 ligand) known to induce huge amounts of Type I IFNs. The alteration of IFN $\alpha$  production by pDC in response to TLR ligands has been previously described in chronic viral infections such as HIV and HCV contributing

to the failure of an efficient immune response.<sup>5</sup> In the context of cancer, previous works have also reported this alteration in breast,<sup>3</sup> lung and head and neck cancers<sup>1</sup> and chronic myeloid leukemia (CML).<sup>6</sup> Type I IFNs promote immunosurveillance through the activation of innate immune cells (reviewed in ref. 7) and inhibition of regulatory T cells.<sup>8</sup> The critical role of type I IFNs in anti-tumor immunosurveillance is also well demonstrated in IFN $\alpha$  receptor 1-deficient mice where tumor growth is exacerbated.<sup>7</sup> IFN $\alpha$  has also direct

antitumor activities in OC in vitro and in vivo either in nude mice when associated to paclitaxel or in patients with minimal residual tumor<sup>9</sup> by inhibiting OC cell proliferation, tumor growth and angiogenesis. Thus, the inhibition of IFN $\alpha$  production by TApDC in tumor microenvironment might confer a selective advantage for tumor cells allowing their progression.

In addition to altered immunosurveillance function, a direct contribution of pDC to tumor progression has been reported in multiple myeloma (MM) and

ovarian cancer.<sup>1</sup> Indeed, pDC in the bone marrow microenvironment promote MM cell growth, survival and drug resistance and pDC isolated from ovarian malignant ascites induce tumor angiogenesis in vivo in immunodeficient mice.<sup>1</sup> In this context, it would be interesting to evaluate the impact of TApDC on tumor cell cycling and angiogenesis in situ in ovarian tumors.

Currently, we assist to a renaissance of IFN therapy for the treatment of myeloid malignancies. Preliminary results of a Phase III clinical trial in CML showed that a pegylated form of IFN $\alpha$ 2a (pegIFN $\alpha$ 2) in association with imatinib doubled molecular response (30%) in comparison to imatinib alone (14%,  $p = 0.001$ ).<sup>10</sup> By analogy to CML, the association of

pegIFN $\alpha$ 2a to chemotherapy could improve outcome in OC. Thus, restoring pDC capacity to produce Type I IFNs represents an attractive therapeutic strategy in cancer. In this context, the treatment of skin cancers with imiquimod (TLR7 ligand) resulted in IFN $\alpha$  production by TApDC that correlates to local immune reaction and destruction of tumor lesions.<sup>1</sup>

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