analyze tumors and tumor-draining lymph nodes (tdLN). With various immunological in vitro and in vivo assays we determined the functional role of DC in tumor immunity.

**Results**
A loss of skin DC has previously been reported for primary melanoma lesions and we here show that melanoma progression in the tg(Grm1)EPv mouse model coincides with a gradual decrease in the skin cDC2 subset and an upregulation of the inhibitory ligands PD-L1 and galectin-9. Monotherapy with anti-PD-L1 could not delay tumor growth, suggesting that this is a good model to study resistance to checkpoint blockade. We hypothesized that by boosting DC numbers and function we would restore responsiveness to checkpoint blockade. By administering a treatment consisting of systemic Flt3L and intratumoral polyI:C/anti-CD40, we were able to rescue the numbers and function of skin cDC2. Analysis of the treated tumors by flow cytometry showed that the DC boost regimen led to an increased tumor infiltration of activated CD4+ and CD8+ T cells. An in vitro T cell proliferation assay revealed that dermal cDC2 that had migrated to the tdLN, played a crucial role in this process, since these were able to cross-present endogenous gp100 antigen more efficiently than migratory Langerhans cells and dermal cDC1. CD4+ and CD8+ T cells recruited in the tumors of the DC boost treated mice, expressed PD-1 and TIM-3. Therefore, combination therapy with checkpoint blockade of these molecules resulted in increased cytotoxic activity within the tumor and eventually delay of tumor growth.

**Conclusions**
Our results demonstrate that skin DC shape the tumor microenvironment upon immunotherapy and thus, therapies that aim to enhance responsiveness to checkpoint blockade may well benefit from a component that boosts the numbers and the function of skin DC.

**Disclosure Information**
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**P04 Vaccine Therapy**

**P04.01 DENDRITIC-CELL BASED IMMUNOTHERAPY TARGETING PANCREATIC AND NSCLC CANCER STEM CELLS**

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**Background**
The field of cancer immunotherapy is growing at a fast pace, with new developments in this field leading to a change in cancer therapy. Dendritic cells (DCs) are one of the central tools in cellular anti-tumour immunotherapy and the production of clinical grade monocyte-derived DCs (Mo-DCs) is the most frequent approach for antitumor vaccines production. However, there is a large space for improvement of protocols and a clear need for the establishment of clinical standard operating procedures (CSOP). Cancer stem cells (CSCs) are a recently identified small cell population present in the tumour, resistant to radio/chemotherapy and known to be responsible for disease recurrence. Here, we aim to contribute to the standardization of CSOPs and to target and eradicate CSCs by developing a DC-based immunotherapy vaccine for pancreatic and non-small cells lung cancer (NSCLC), comparing DC loading with CSCs vs. classical tumour lysates.

**Materials and Methods**
CSCs from Panc-1 (pancreatic cancer) and A549 (NSCLC) cell lines were isolated and characterized by RT-PCR and flow cytometry. CSCs resistance to chemotherapy was also assessed. In vitro anti-tumour cytotoxicity assays were performed. We also defined and compared the effect of 4 culture media during human Mo-DCs production. Three Good Manufacturing Practice (GMP) serum-free culture media for clinical use were tested - DendriMACS, AIM-V and X-VIVO 15. RPMI was used as a comparative term given that it is largely used in pre-clinical research. We characterized DC viability, differentiation, maturation, internalization of tumor lysates, cytokines production and autologous T cell stimulatory capacity, as well as metabolic profiles by Nuclear Magnetic Resonance (NMR) spectroscopy.

**Results**
CSCs from Panc-1 and A549 cell lines were successfully isolated and overexpressed the stem-like markers NANOG, OCT4, SOX2 and CD133, with resistance to gemcitabine. In terms of differentiation, maturation, antigen uptake capacity and metabolic profiles, AIM-V and X-VIVO 15 present similar results. However, the use of X-VIVO 15 shows an enhanced DC production of IL-12. DCs cultured in X-VIVO 15 and AIM-V media are able to induce a superior stimulation of T cells (CTLs and Th1 responses) while DCs cultured in DendriMACS are more prone to induce Treg polarization. Our data show that X-VIVO 15 and AIM-V culture media are preferable to support the differentiation of DCs to be used in immunostimulatory approaches such as in cancer immunotherapy.

**Conclusions**
Overall, our results demonstrate that blood mononuclear precursors present considerable plasticity allowing a tailored differentiation of DCs just by changing the nutritional support. This highlights the need of critically defining the culture medium to be used in DC cancer immunotherapy, attaining the desired cell characteristics and consequent robust clinical responses. We are now assessing in vitro antitumour cytotoxicity to evaluate if DC loading with CSC antigens can be an efficient immunotherapy strategy to target and eliminate this specific and resistant cancer cell population.

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