Co-injection of human monocytes improves the in vivo antitumoral activity of Bevacizumab in two NSCLC PDX models

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1 Introduction

Nowadays, an increasing number of monoclonal antibodies (mAbs) that specifically target malignant cells or interfere with different compartments of the tumor microenvironment are available for cancer therapy. They take effect via different modes of action including the initiation of a tumor-targeting immune response. Preclinical platforms such as PDX models have to be improved to better recapitulate all possible modes of action for mAbs as well as other immune-modulating agents. In the current study, we evaluated the antitumoral activity of Bevacizumab in two NSCLC PDX growing subcutaneously in NMRI nu/nu mice with and without co-injection of human monocytes.

2 Materials and Methods

Two NSCLC PDX, LXFA 2478 and LXFA 677, were subcutaneously implanted into 4-6 weeks old female NMRI nu/nu mice (Harlan, Denmark). Tumor models were chosen based on their VEGFA expression level. When median tumor size reached 150 – 300 mm³, mice were equally distributed to treatment groups (n=4/group). Animals were treated once weekly for 7 cycles with a) control vehicle b) control vehicle + $5x10^6$ human monocytes c) Bevacizumab at 40 mg/kg/d and d) Bevacizumab 40 mg/kg/d + $5x10^6$ human monocytes. Tumor volume was determined twice weekly by caliper measurement. At the end of the study, tumor and lymphatic organs of the animals were harvested and subsequent IHC analysis for human CD14, CD49, CD68 and CD163 was performed to detect human monocytes/macrophages.

3 Results

Characteristics of tumor models used in this study are shown in Table 1.

Table 1: Overview of tumor models used in this study

tumor	stage	histology	site of origin	patient	VEGF expression	mutation
LXF 2478	T2aN2M1	adeno-squamous	brain metastasis	female 44y	high 12,11	EGFR M766X ins
LXFA 677	T1N1Mx	adeno	lung primary	male 62y	high 11,91	BRAF D549A TP53 V173fsX1

Figure 1: Heatmap of median of optimal T/C values

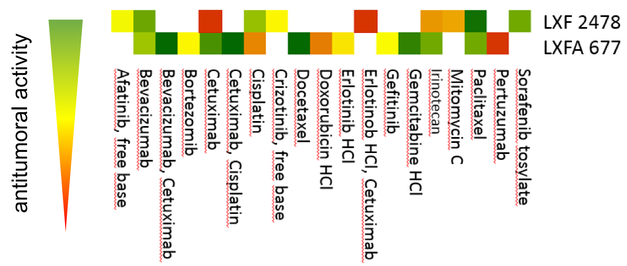
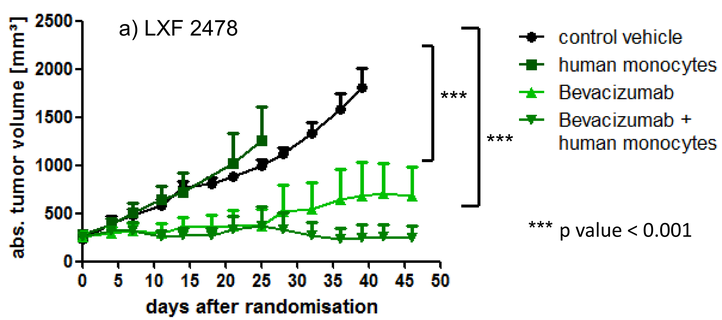
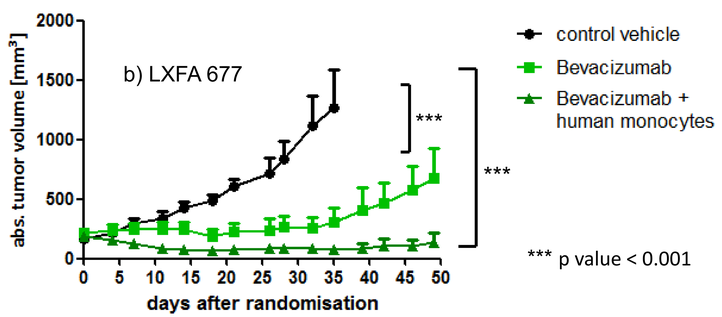


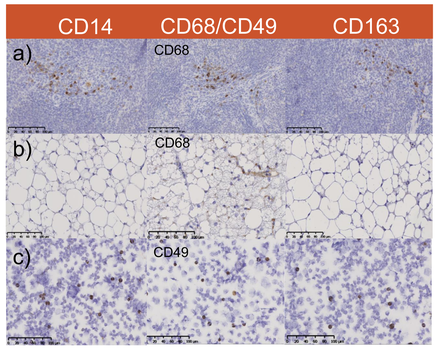
Figure 2: Tumor growth of a) LXF 2478 and b) LXFA 677 during therapy





The highest antitumoral activity for LXF 2478 is obtained by Cetuximab whereas Erlotinib and Paclitaxel show no effect in this model (Figure 1). For LXFA 677 best activity is shown by Pertuzumab whereas no activity is achieved by Cetuximab, Docetaxel or Gemcitabine. In both investigated tumor models, Bevacizumab showed significant antitumoral activity ($p<0.001$) with a maximal tumor load reduction of 71% (LXF 2478) and 84 % (LXFA 677) as compared to untreated controls on days 39 and 35 (Figure 2). The co-injection of human monocytes markedly enhanced the therapeutic effect of Bevacizumab in both NSCLC PDX: maximal tumor load reduction was 89% in LXFA 2478 and 95 % in LXFA 677 in combination groups. The injection of monocytes alone did not affect tumor growth as compared to untreated control. This antitumoral activity was increased by 18% and 11% for LXF 2478 and LXFA 677, respectively, through the additional injection of monocytes (Figure 2).

Figure 3: IHC analysis of mouse lymphatic organs for human monocyte/ macrophage markers: a) spleen; b) lymph node; c) bone marrow



By sequential IHC analysis no human monocytes/macrophages could be detected in mouse tissue (peripheral blood, lung, Peyer's patch) or human tumor (data not shown). However in mouse lymphoid organs (spleen, lymph node, bone marrow) human monocytes/ macrophages could be detected (Figure 3).

4 Conclusions

Monocytes and macrophages have been reported to induce antibody-dependent cytotoxicity and phagocytosis of tumor cells in the presence of IgG anti-tumor mAbs, like Bevacizumab. Our results confirm these observations in a PDX based NSCLC in vivo model. Therefore, the study highlights the suitability of PDX for immuno-oncology approaches by supplementation of the murine host with human immune cells. This advanced PDX approach will lead to more predictive preclinical data for innovative mAb´s and other compounds acting via the activation of monocytes and related immune cells.