

Bavituximab modulates tumor microenvironment and activates CD8+ tumor infiltrating lymphocytes in a patient-derived 3D ex vivo system of lung cancer

Soner Altiock^{1, 2}, Melanie Mediavilla-Varela^{1, 2}, Jenny Krehling^{1, 2}, David Noyes^{1, 2}, Tiffany N. Razabdouski^{1, 2}, Nikoletta L.

Kallinteris³, Joseph Shan³ and Scott Antonia^{1, 2}

¹Moffitt Cancer Center, Tampa, FL 33612, ²Nilogen Oncosystems, Tampa, FL 33647, ³Peregrine Pharmaceuticals Inc., Tustin, CA 92780

NILOGEN
ONCOSYSTEMS

ABSTRACT

Bavituximab is a monoclonal antibody directed against the membrane phospholipid phosphatidylserine exposed on the outer leaflet of tumor and vascular endothelial cells of the tumor microenvironment. Bavituximab modulates the tumor microenvironment by blocking PS-mediated immune suppression and activating cytotoxic T lymphocyte anti-tumor responses.

In this study, we tested the immunomodulatory effect of bavituximab using a proprietary 3D ex vivo tumor microsphere technology. Upon obtaining informed consent, fresh tumor tissue from lung cancer patients were collected at the time of surgical resection. Tissue was processed for characterization of the tumor microenvironment and potential immunosuppressive mechanisms such as expression of PD-1, CTLA4, LAG3, TIM3, BTLA, and Adenosine A2AR. 3D tumor microspheres were prepared and cells were treated ex vivo with f(ab)2 version of bavituximab, bavituximab, docetaxel, and a combination of bavituximab and docetaxel for 36 hours within the 3D tumor microsphere simulating an intact tumor microenvironment made up of tumor infiltrating lymphocytes (TIL) and myeloid cells. At the end of the treatment, TILs were analyzed by flow cytometry for cell activation and changes in CD4, CD8 and Treg (CD25+/CD127-) subpopulations. A multiplex human cytokine assay was used to simultaneously analyze the differential secretion of cytokines, including human IFN γ , in culture media as a surrogate of TIL activation.

Preliminary results indicate the combination of bavituximab and docetaxel can induce TIL activation as demonstrated by a significant increase in IFN γ secretion when compared to tumors treated with control or either agent alone. Flow cytometry analysis revealed that this effect was associated with low PD-1 expression on CD8 cells, but did not correlate with other known immune-modulating receptors. Complete results on a number of patients will be presented.

This lung patient derived ex-vivo approach indicates that bavituximab in combination with docetaxel can elicit a tumor specific immune response in human adenocarcinoma of the lung. This effect involves, at least in part, activation of CD8+ TIL and increased inflammatory cytokine production by lymphoid and myeloid cells. In addition, we have observed low PD1 expression as a potential prognostic biomarker of positive response to bavituximab treatment. Furthermore, these data suggest that the interruption of the PD-1/PD-L1 axis may enhance the bavituximab activity in lung cancer.

BAVITUXIMAB MECHANISM OF ACTION

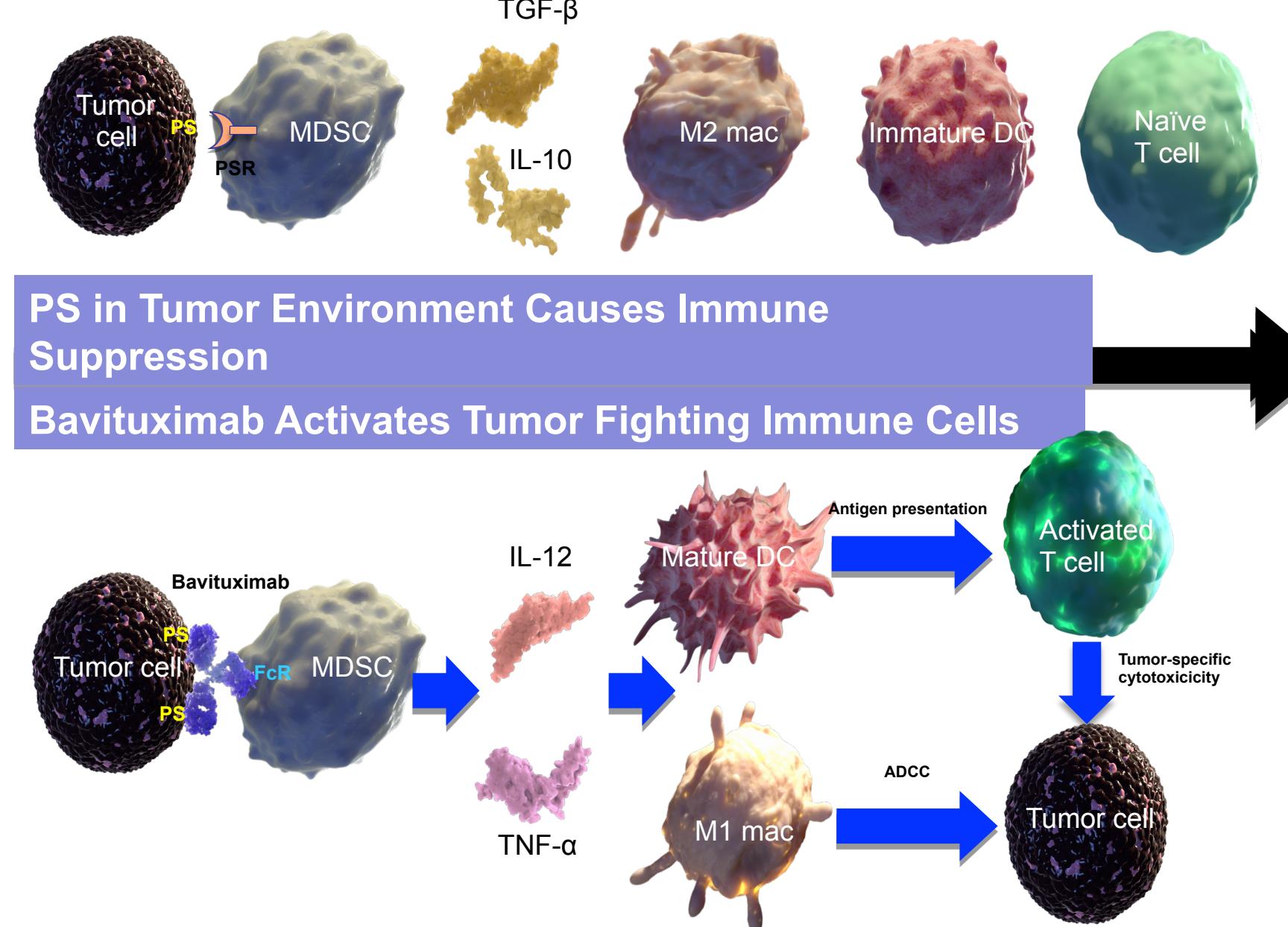


Figure 1. Summary: Bavituximab Activates Tumor Immunity. Bavituximab blocks the immunosuppressive effect of phosphatidylserine (PS) in tumor microenvironment. PS blockade with bavituximab reduces the level of MDSCs, M2 macrophages and Tregs and increases the presence and activity of mature dendritic cells, M1 macrophages, and cytotoxic T cells.

NILOGEN'S DRUG DISCOVERY PLATFORM

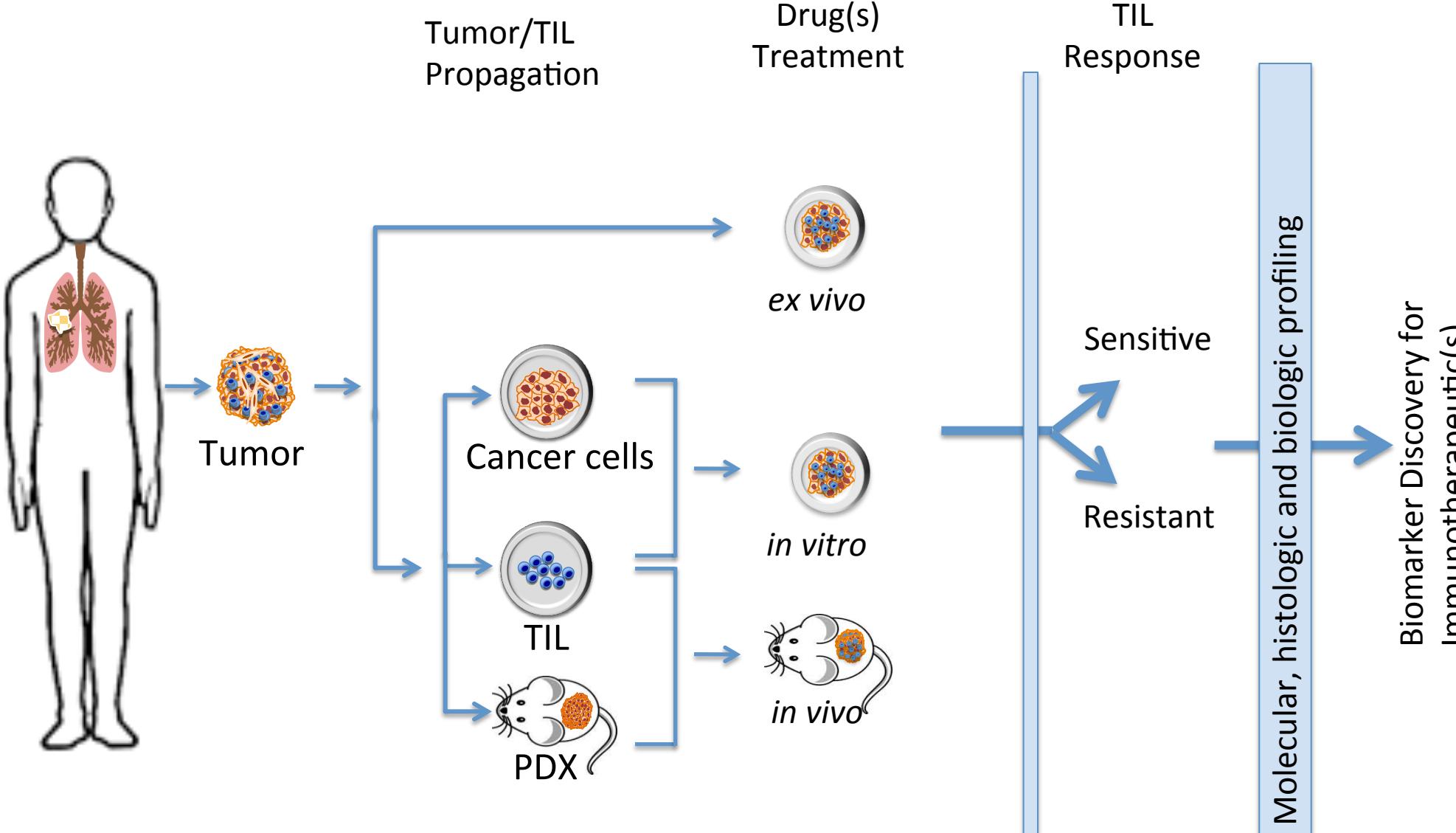


Figure 2. Nilogen's ex-vivo model for immunotherapy drug discovery. Freshly resected patient samples were used to produce a 3D ex-vivo model in which tumor response to different drugs could be elucidated. After collection, the tumor sample was divided into two: 1. Sample was disaggregated to characterize the immune checkpoint proteins, and 2. Sample was disaggregated into 3D microspheres for drug discovery. After a 36 hour treatment, the 3D microspheres were disaggregated into single cell solution, and the immune components (CD3, CD4, CD8 and Tregs) were characterized. Additionally, matching TIL and cancer cell lines as well as patient-derived xenograft (PDX) models were generated for future *in vitro* and *in vivo* studies.

RESULTS

Patient	Gender	Smoker	Type	Stage
Tumor 1	Female	No	Adenocarcinoma	T1aN0M0
Tumor 2	Female	Yes	Adenocarcinoma	T2aN0M0
Tumor 3	Female	Yes	Adenocarcinoma	T2bN2M0
Tumor 4	Female	Yes	Adenocarcinoma	T2aN0M0
Tumor 5	Female	No	Adenocarcinoma	T1bN2M0
Tumor 6	Female	Yes	Adenocarcinoma	T2aN0M0

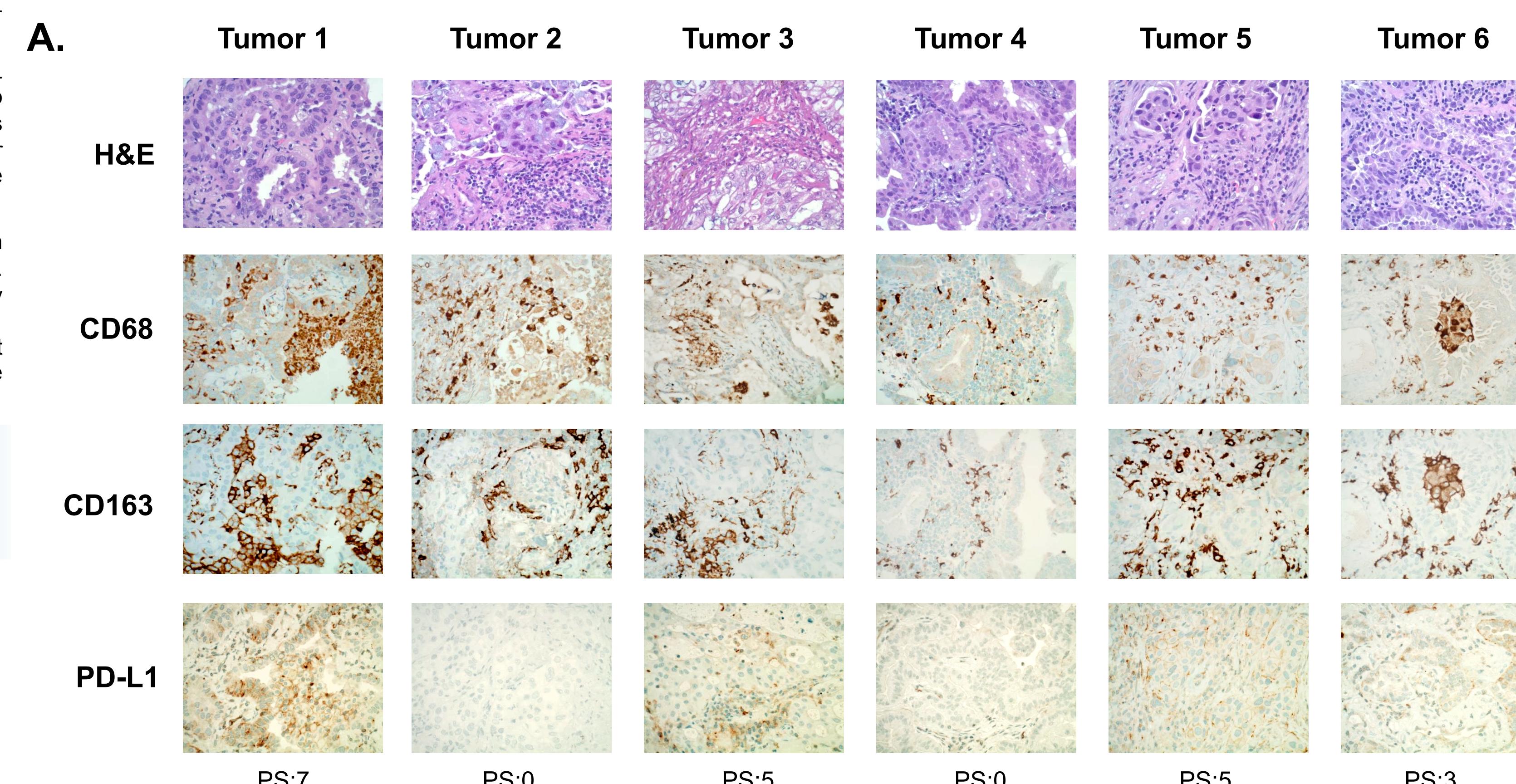


Figure 3. Ex-vivo characterization of immune checkpoints and M Φ /M2 markers in adenocarcinoma of the lung patient samples. A. IHC analysis of fresh tissue from each patient for H&E, CD68 (M Φ), CD163 (M2) and PD-L1. The Allred scoring system was used to evaluate the percentage of PD-L1 positive stained cells and staining intensity. A proportion score (PS) was assigned representing the proportion of positively stained tumor cells (0 = none; 1 = <1/100; 2 = 1/100 to <1/10; 3 = 1/10 to 1/3; 4 = 1/3 to 2/3; and 5 = >2/3). Average intensity of staining in positive cells was assigned as an intensity score (0 = none; 1 = weak; 2 = intermediate; and 3 = strong). B. Fresh tumor tissue from lung cancer patients were collected at the time of surgical resection. The tumors were disaggregated and flow cytometric analysis was performed for all of the immune checkpoint proteins (LAG-3, PD-1, BTLA, CTLA-4, TIM-3, and A2AR) in both CD4+ and CD8+ cells as well as regulatory T-cell (Treg) in CD4+ cells. The gating strategy is as follows: Morph gate on lymphocyte population, live population (negative for NearIR Live/Dead), and CD3+ population. Tumors 2, 4 and 6 that are sensitive to Bavi treatment express lower PD-L1 expression than Bavi resistant tumors 1, 3 and 5.

Ex-vivo Treatments	Tumor 1			Tumor 2			Tumor 3			Tumor 4			Tumor 5			Tumor 6		
	Treg	CD4	CD8															
Tumor	13.0%	61.6%	35.5%	6.8%	58.1%	31.8%	27.0%	59.9%	36.1%	20.1%	58.2%	38.1%	21.3%	73.5%	23.9%	16.0%	88.1%	11.5%
Tumor + IL2	18.2%	54.6%	42.1%	17.2%	50.0%	45.4%	27.6%	53.3%	43.0%	25.0%	67.4%	29.6%	31.0%	70.6%	26.8%	22.7%	89.3%	9.2%
Tumor + IL2 + Fab	19.1%	56.3%	40.7%	16.2%	49.1%	44.4%	36.2%	54.0%	42.0%	30.3%	67.1%	29.4%	32.3%	69.2%	28.1%	24.7%	89.1%	9.7%
Tumor + IL2 + Bavi	18.6%	54.1%	42.5%	20.2%	50.5%	44.0%	36.7%	55.9%	40.0%	32.0%	62.5%	34.1%	32.7%	68.0%	29.6%	24.7%	89.3%	8.9%
Tumor + IL2 + Doce	18.0%	55.2%	41.6%	18.2%	50.9%	43.3%	30.2%	49.7%	45.2%	25.0%	65.9%	30.4%	31.8%	71.4%	25.8%	21.3%	88.7%	10.3%
Tumor + IL2 + Bavi + Doce	19.7%	52.0%	44.6%	17.0%	49.0%	45.3%	35.6%	56.2%	40.3%	31.4%	63.0%	33.0%	31.8%	69.9%	27.8%	25.3%	89.8%	9.5%

Figure 4. Characterization of CD4, CD8 and Tregs after treatment of 3D microspheres with Bavituximab and Docetaxel in adenocarcinoma of the lung patient samples. A. 3D microspheres were seeded in a 24-well plate and treated with IL2 (6000 U/ml), Bavituximab (Bavi, 12.5 μ g/ml) and/or Docetaxel (Doce, 3 μ M) for 36 hours. After treatment, the 3D microspheres were disaggregated and flow cytometric analysis of CD4, CD8 and Tregs was performed. The gating strategy is as follows: Morph gate on lymphocyte population, live population (negative for NearIR Live/Dead), and CD3+ population.

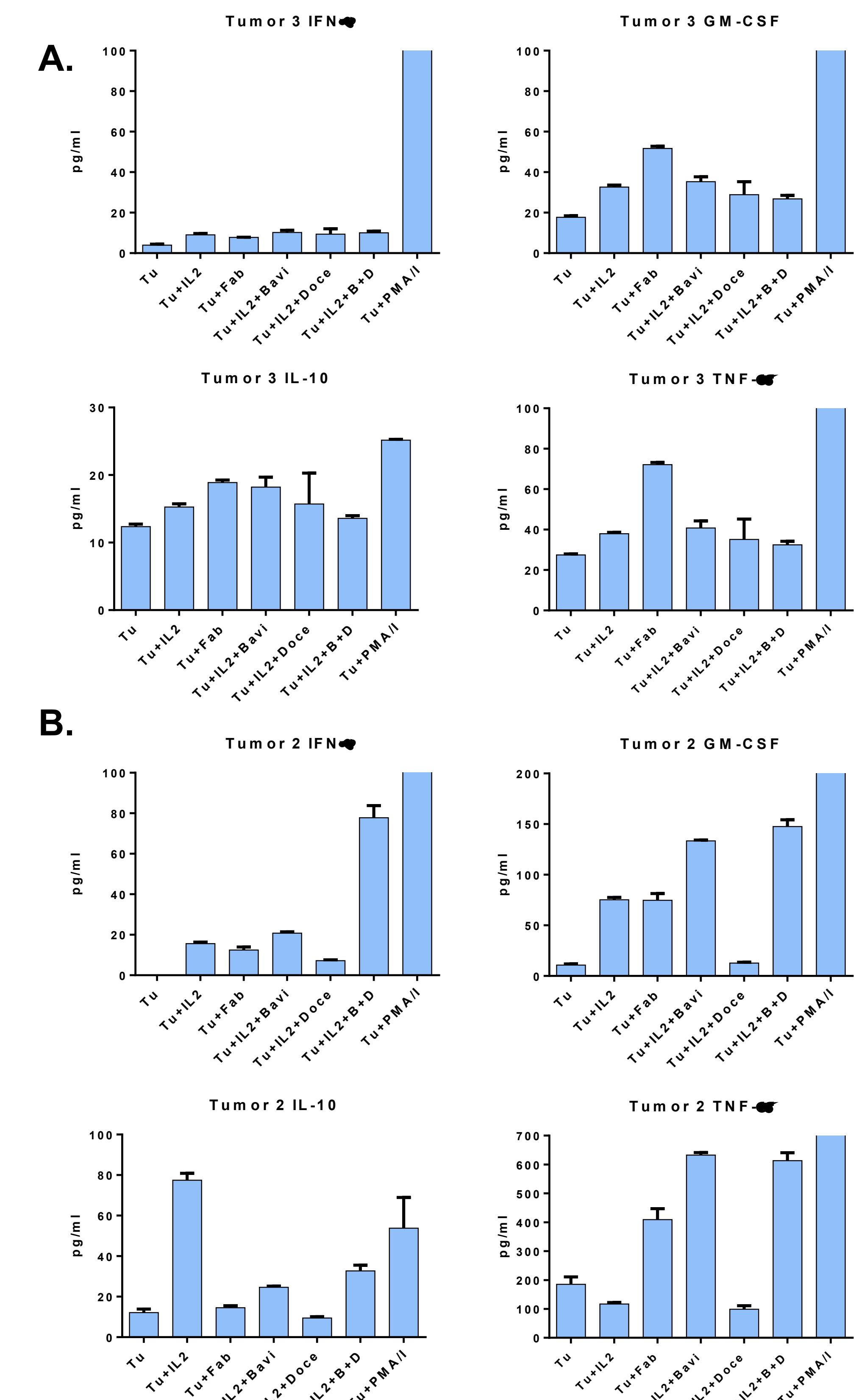


Figure 5. Milliplex analysis of cytokines in treated 3D microspheres with Bavituximab and Docetaxel. Expression of IFN γ , GM-CSF, IL-10 and TNF- α in two representative patients, one negative (A) and one positive (B). Culture media obtained from ex vivo-treated 3D microspheres were analyzed using the Milliplex Immunology Multiplex Assay for cytokine secretion. All experiments were performed in triplicate, and the means and standard deviations were plotted. Combination of Phorbol myristate acetate (PMA) and Ca $^{2+}$ ionophore (I) was used as positive control to activate TILs.

SUMMARY

- Nilogen's ex-vivo system is reliable to demonstrate drugs effect on the tumor immune microenvironment in fresh patient samples.
- Bavituximab, alone and in combination with Docetaxel, induces TIL activation in 3 out of 6 patient samples.
- Bavituximab's response appears to correlate with low PD-L1 expression in the tumor sample.
- Combination of Bavituximab with PD1/PD-L1 inhibitors may enhance the immunomodulatory efficacy in lung cancer.

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Nilogen contact: