Bavituximab Activates CD8+ TILs in a 3D Ex Vivo System of Lung Cancer Patient Derived Tumors With Negative PD-L1 Expression

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Introduction

- Cancer is a leading cause of death worldwide. Lung cancer is the most common cause of cancer death with 1.59 million deaths every year.
- Traditional chemotherapy fails to provide long-term benefit for many patients.
- Immunotherapy has emerged in recent years as a promising therapeutic approach in lung cancer.
- Antibody blockade of the PD1/PD-L1 pathway demonstrated durable responses and tolerability in a subset of patients.

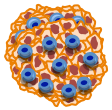
Elements of Inhibition of Immune Responses in the Tumor Microenvironment

There are multiple potential mechanisms whereby tumors evade rejection by the immune system.

- **Surface membrane proteins- checkpoints**
  - PD1, CTLA4, LAG3, TIM3, BTLA, Adenosine A2AR
- **Soluble factors**
  - IDO, Arginase, IL10, TGF-β, Adenosine
- **Inhibitory cells**
  - Cancer Associated Fibroblasts, Treg, MDSC, TAM
- **Externalization of phosphatidylserine in the tumor microenvironment**
  - The phosphatidylserine-specific antibody bavituximab (Peregrine Pharmaceuticals) demonstrated promising results in a phase II trial of advanced NSCLC

Shtivelband, M. et al. *J. Clin. Oncol.* 31 (Suppl.), 8095 (2013).

Ex vivo drug study



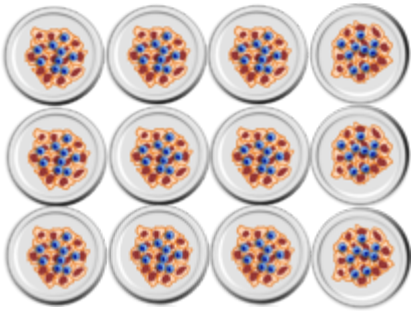
Drug(s) Treatment

Control

Bavi

Doce

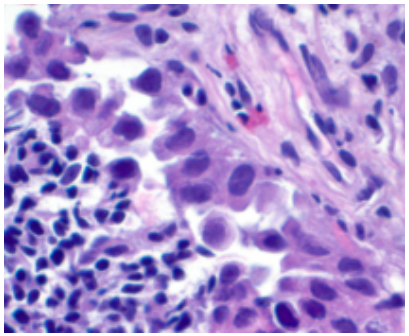
Doce+Bavi



Ex vivo (36h)

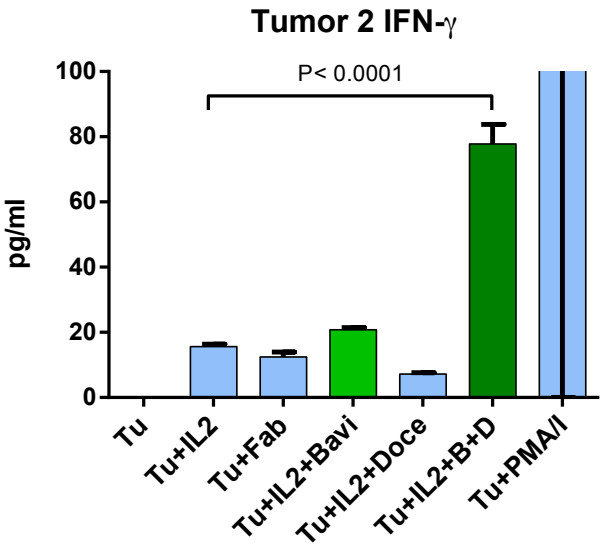
Tumor Response

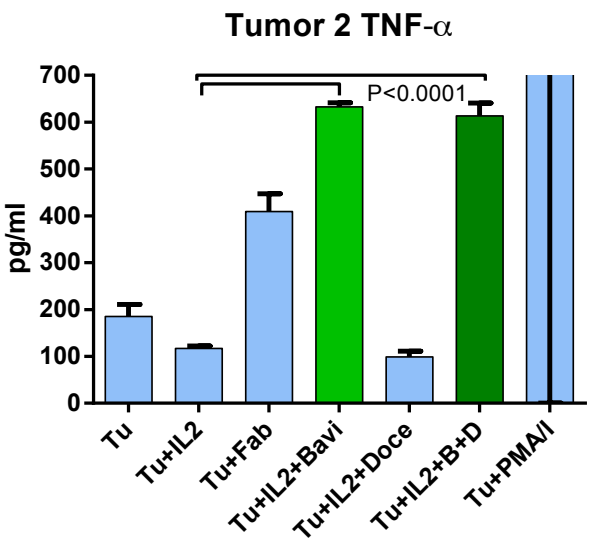
- Cytokine profile
- Flow cytometry
- IHC
- Immune gene expression analysis (NanoString)

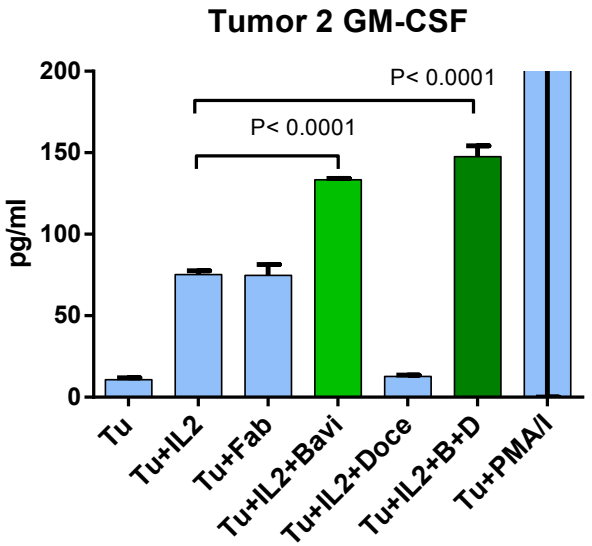


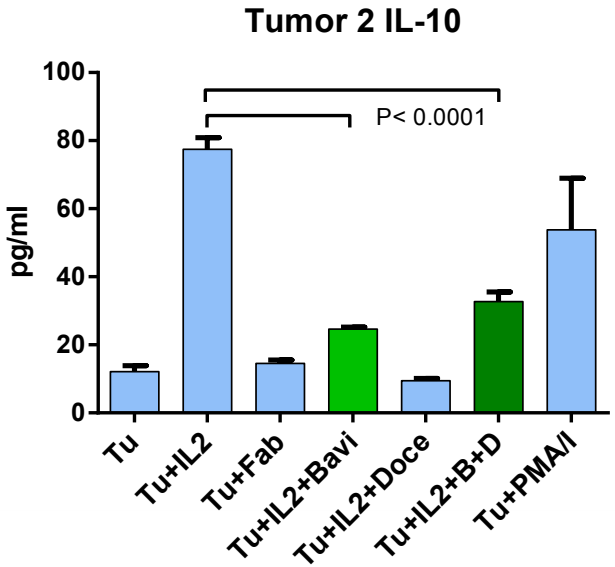
Bavi: Bavituximab
Doce: Docetaxel

Cytokine Analysis in ex vivo Treated 3D Microspheres – Immune Responder

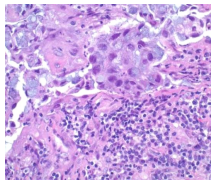


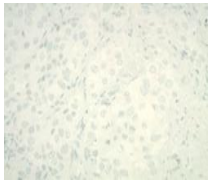


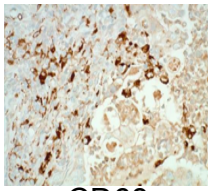


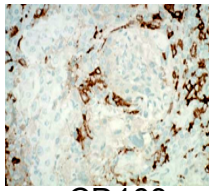


Profile at Tumor Resection


H&E


PD-L1

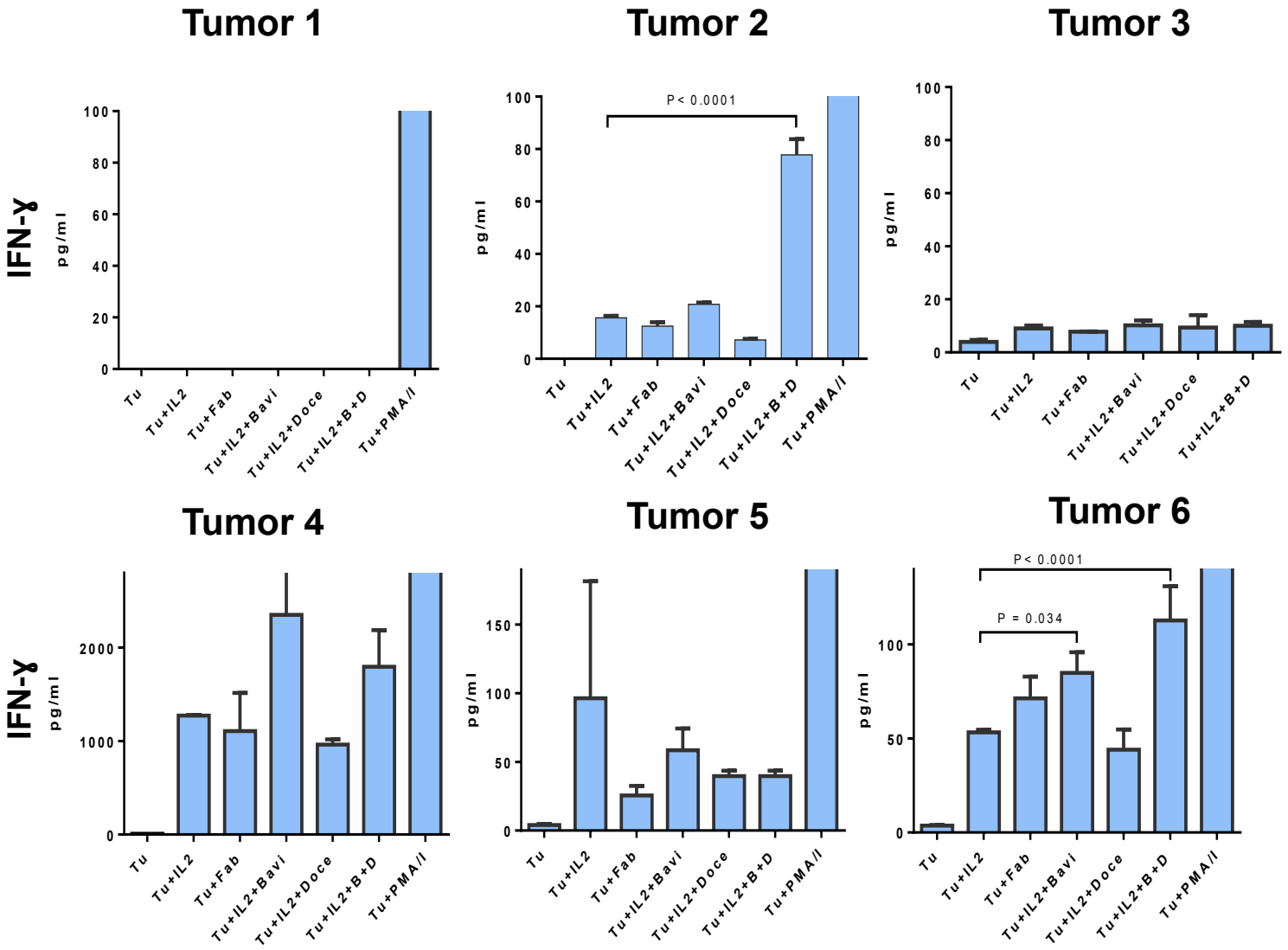

CD68


CD163

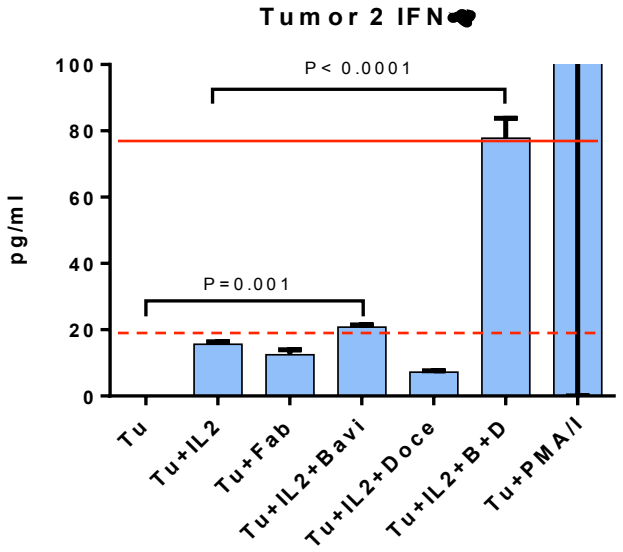
CD8 Cell Profile at Start of *Ex Vivo* Culture

PD-1 CD8-cells	Lag3 CD8-cells	CTLA-4 CD8-cells	Tim3 CD8-cells	BTLA CD8-cells	A2aR CD8-cells
6.8%	62.6%	0.5%	0%	51.5%	95.9%

IFN-γ expression levels of *ex vivo* drug treated tumor samples



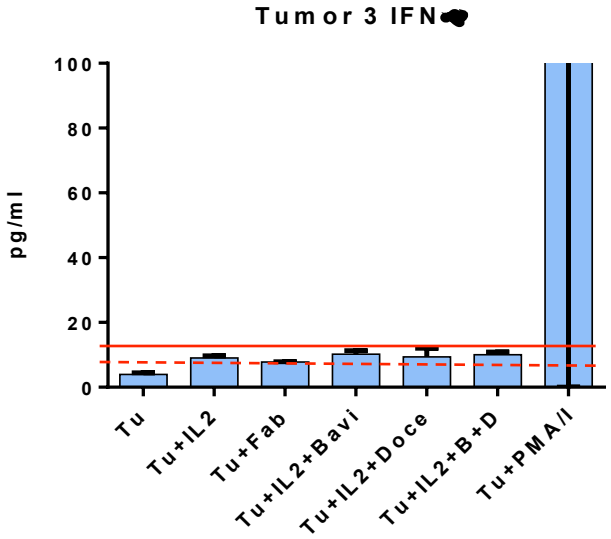
Tumor 2 and 6 were immune-responsive to
ex vivo drug treatment with bavituximab in combination with docetaxel



TIL	CD4+ 53.8%	CD8+ 44.0%
Lag 3	1.0%	0.8%
PD-1	11.1%	20.7%
BTLA	79.3%	6.6%
CTLA-4	0.5%	0.4%
TIM3	3.2%	2.9%
A2aR	98.2%	8.0%
Tregs	11.0%	-

Treg: CD25+/CD127-

Ex Vivo Treatments	CD4+ Tregs	CD4+	CD8+
Tm	13.0%	61.6%	35.5%
Tm+IL2	18.2%	54.6%	42.1%
Tm+IL2+F	19.1%	56.3%	40.7%
Tm+IL2+B	18.6%	54.1%	42.5%
Tm+IL2+D	18.0%	55.2%	41.6%
Tm+IL2+B+D	19.7%	52.0%	44.6%

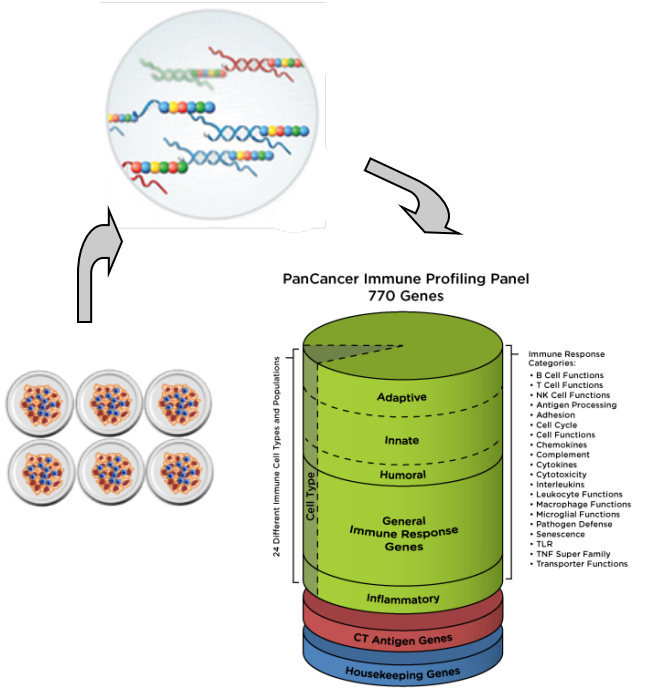


TIL	CD4+ 42.4%	CD8+ 52.9%
Lag 3	31.5%	62.6%
PD-1	44.5%	6.8%
BTLA	98.5%	51.5%
CTLA-4	7.3%	0.5%
TIM3	0%	0%
A2aR	99.8%	95.9%
Tregs	27.2%	-

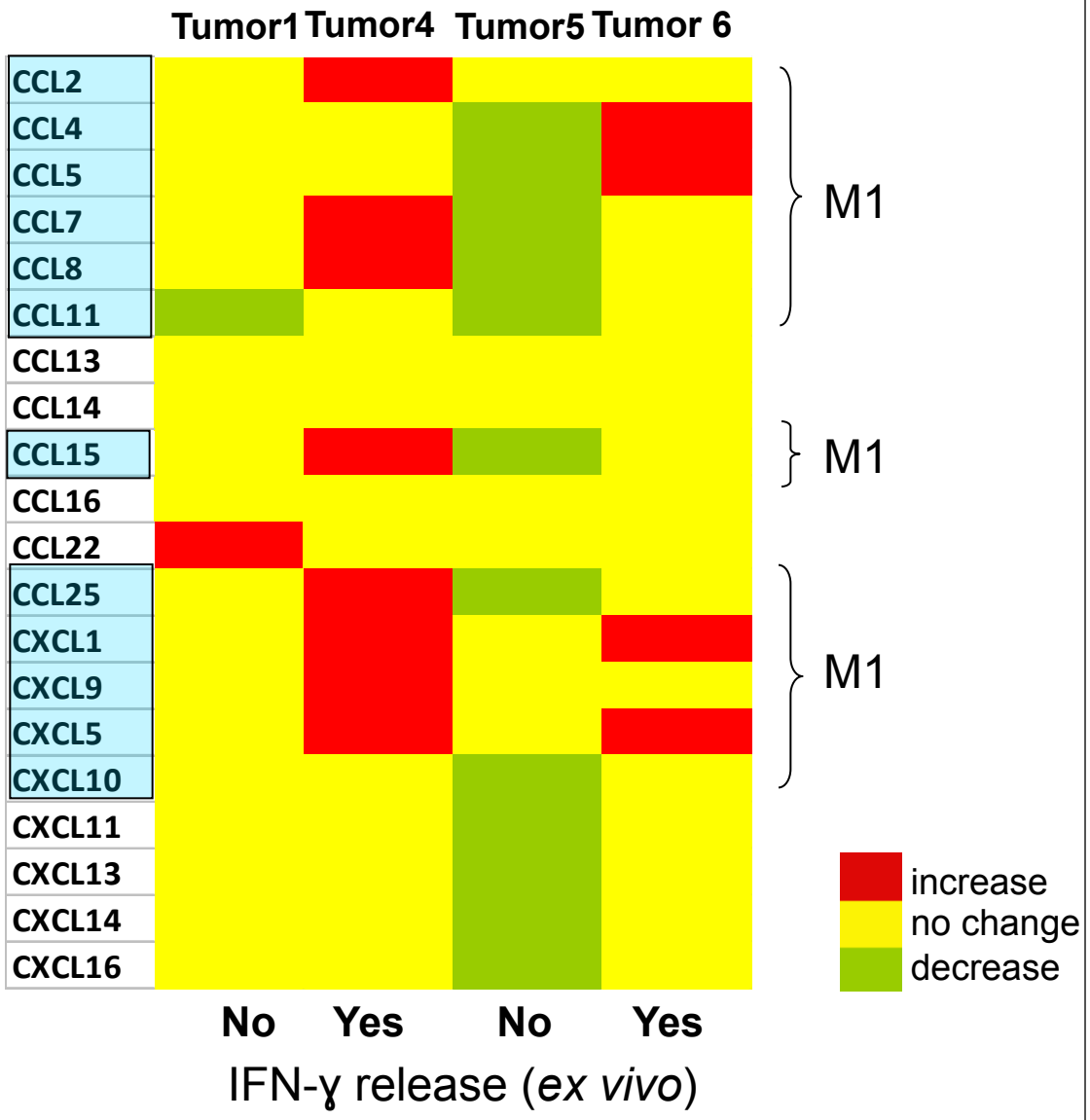
Ex Vivo Treatments	CD4+ 58.1% Tregs	CD4+	CD8+
Tm	6.8%	58.1%	31.8%
Tm+IL2	17.2%	50.0%	45.4%
Tm+IL2+Fab	16.2%	49.1%	44.4%
Tm+IL2+B	20.2%	50.5%	44.0%
Tm+IL2+D	18.2%	50.9%	43.3%
Tm+IL2+B+D	17.0%	49.0%	45.3%

B: bavituximab
D: docetaxel

Ex-vivo characterization of immune checkpoints in adenocarcinoma samples

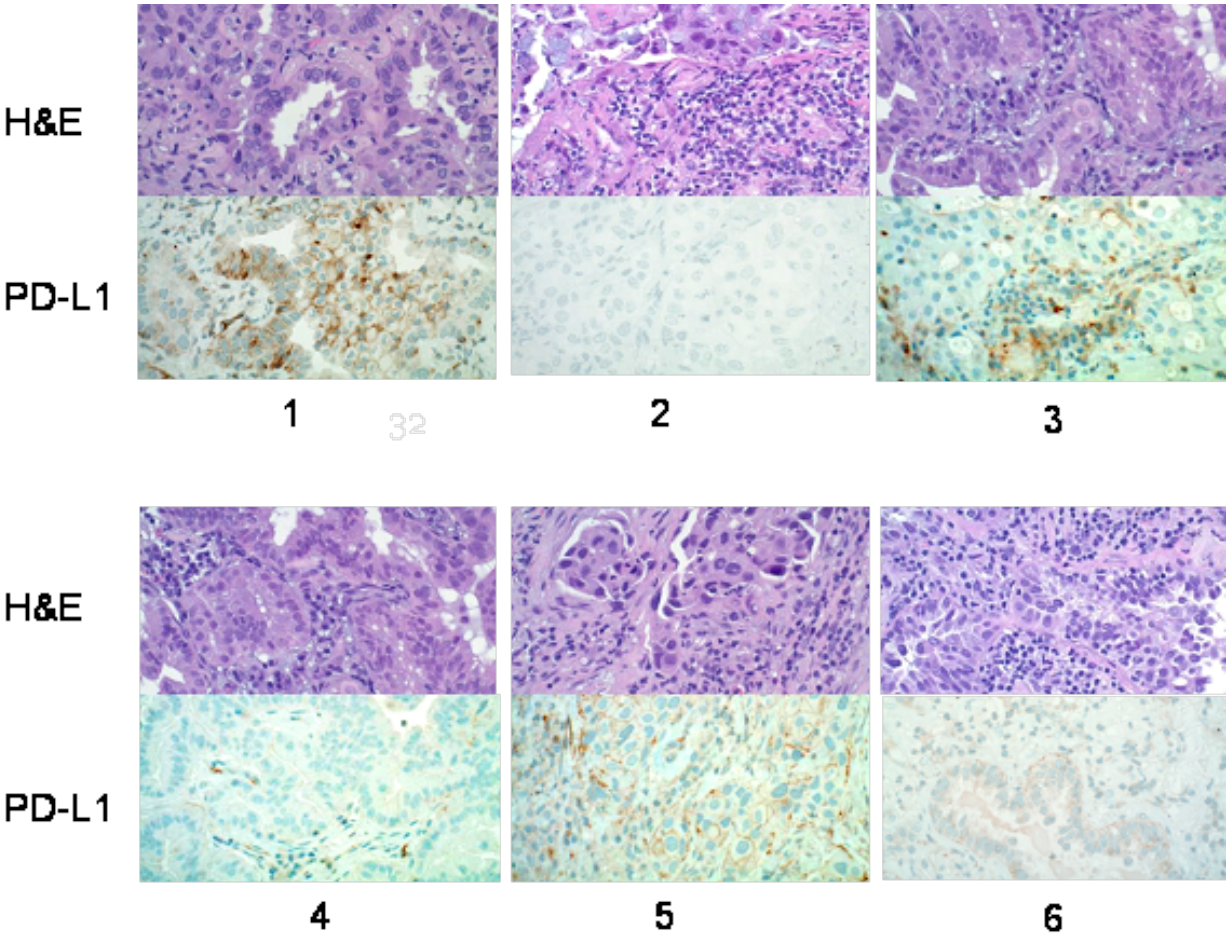


PanCancer Immune Profiling Panel by Nanostring



M1 polarization of tumor associated macrophages is likely involved in bavituximab-mediated activation of tumor infiltrating lymphocytes.

PD-L1 expression in tumor samples



Patient Derived	PD-L1	IFN-ɣ increase ex vivo
Tumor 1	Positive	No
Tumor 2	**Negative**	**Yes**
Tumor 3	Positive	No
Tumor 4	**Negative**	**Yes**
Tumor 5	Positive	No
Tumor 6	**Negative**	**Yes**

Tumor response to bavituximab appears to correlate with low PD-L1 expression

Conclusions

- The *ex-vivo* system is reliable to demonstrate drug combination effects on the tumor immune microenvironment of fresh patient samples.
- Bavituximab, alone and in combination with docetaxel, induces activation of tumor infiltrating lymphocytes as demonstrated by a significant increase in IFN-ɣ, TNF-α, and GM-CSF with corresponding decrease in IL-10 secretion.
- Bavituximab's response appears to correlate with low PD-L1 expression in the tumor samples.
- M1 polarization of tumor associated macrophages is likely involved in bavituximab-mediated activation of tumor infiltrating lymphocytes.
- Combination of bavituximab with PD-1/PD-L1 inhibitors may enhance the immunomodulatory efficacy in lung cancer.