Changes in Intratumoral Immune Cell Infiltrates, FoxP3, and Indoleamine 2, 3-Dioxygenase (IDO) Expression with the CTLA4 Blocking mAb CP-675,206

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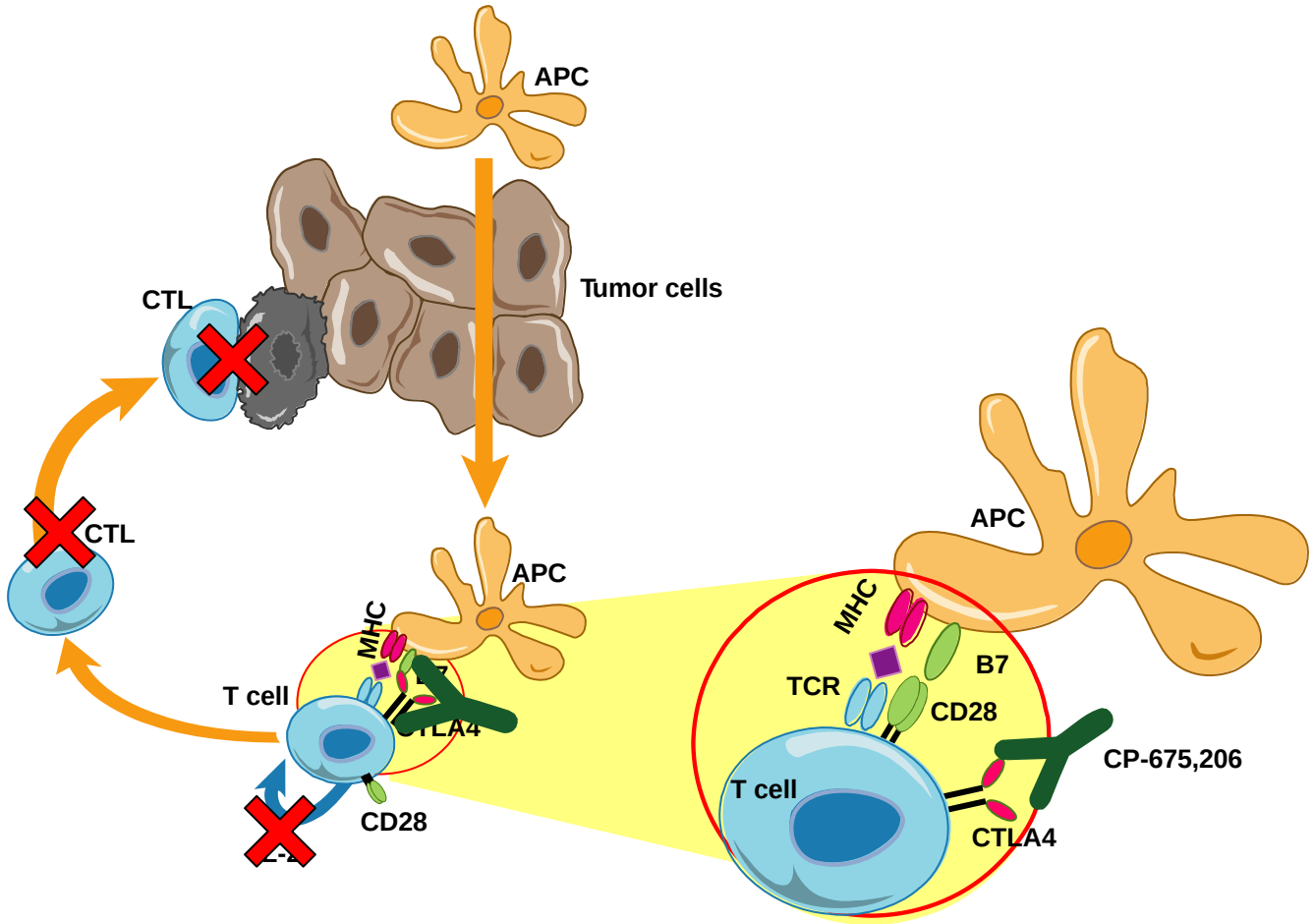
Rationale for CTLA-4 Blockade to Induce Antitumor Immune Responses

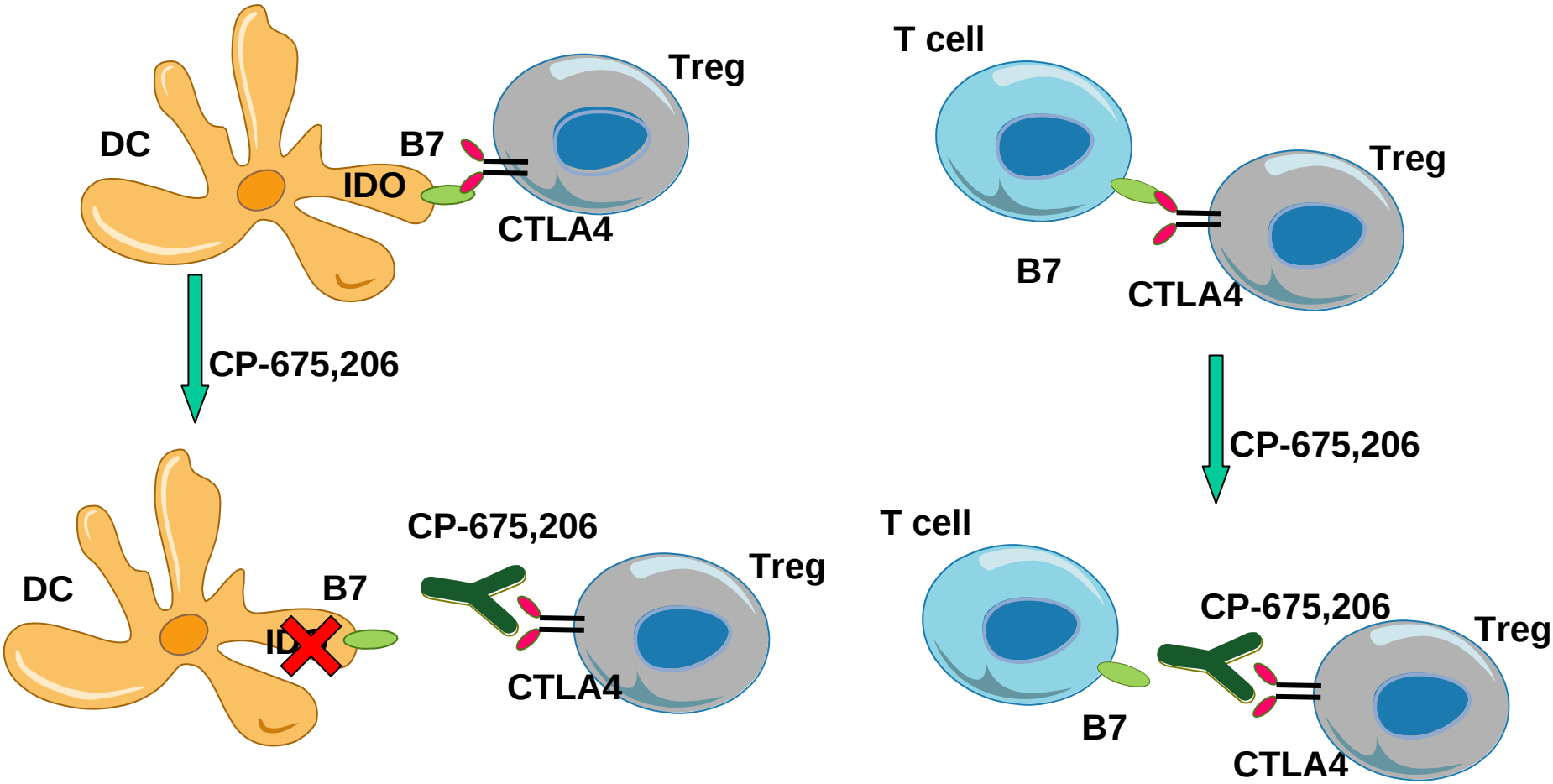


Leach, D. R., Krummel, M. F., and Allison, J. P. Enhancement of antitumor immunity by CTLA-4 blockade. Science, *271:* 1734-1736., 1996.

Lee, K. M., *et al*. Molecular basis of T cell inactivation by CTLA-4. Science, *282:* 2263-2266, 1998.

Korman, A. J., Peggs, K. S., and Allison, J. P. Checkpoint blockade in cancer immunotherapy. Adv Immunol, *90:* 297-339, 2006.

Additional Potential Mechanisms of Action of CTLA4 Blocking Monoclonal Antibodies



Grohmann, Fallarino *et al*. Nat Immunol. *3*, 1097 (2002), Nat Immunol. *4*, 1206 (2003).
Munn, Mellor *et al*. J Clin Invest. *114*, 280 (2004), Int Immunol. *16*, 1391 (2004).
Paust, Cantor *et al*. Proc Natl Acad Sci U S A. *101*, 10398 (2004).

Background

- Role of immune cell subsets in melanoma responses to anti-CTLA4 antibodies:
  - CD4 and/or CD8 cells:
    - Involved: Hodi *et al*. PNAS 03, Phan *et al*. PNAS 03, Attia *et al*. JCO 05, Sanderson *et al*. JCO 05, Maker *et al*. J Immunol 05, Reuben *et al*. Cancer 06, Maker *et al*. J Immther 06
  - Treg:
    - Involved: Reuben *et al*. Cancer 06.
    - Not involved: Maker *et al*. J Immunol 06, Comin-Anduix *et al*. iSBTc 06
  - IDO pDC: No reports to date.
- Where should immune activation be studied?
  - Peripheral blood: Maker *et al*. J Immunol 06, Comin-Anduix *et al*. iSBTc 06
  - Lymph nodes: No reports to date
  - Tumor: Anecdotal reports to date

Materials and Methods

- 89 patients have received dosing with CP-675,206 at UCLA, and a small subset of patients underwent tumor biopsies
- Samples collected for:
  - Diagnostic or therapeutic need (more likely on progressing patients)
  - Research purposes under UCLA IRB# 02-08-067 (more likely on responding patients)
- IHC staining for:
  - Melanoma markers: S-100, HMB45, MART-1, tyrosinase
  - Immune cell subset markers: CD1a, CD3, CD4, CD8, CD20
  - Treg marker: FoxP3
  - Immune suppressive DC marker: IDO
- IHC scoring by Dr. Alistair Cochran:
  - Frequency (0-3+) of reactive cells
  - Distribution (diffuse or patchy) of reactive cells

Patient Characteristics and Timing of Biopsies

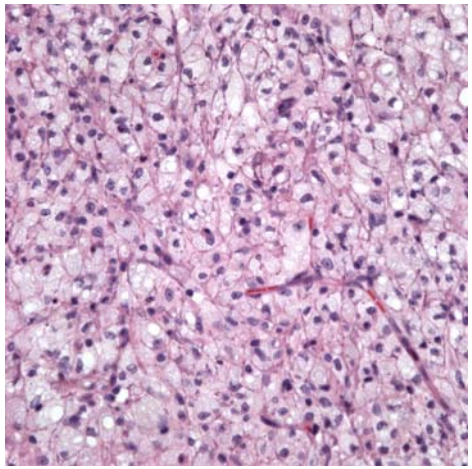
Patient No.	Age	Sex	CP-675,206 Regimen	Response	Toxicity	Timing of Biopsy (first dose/last dose)
1	39	M	15 mg/kg q3mo	PR	-	Pre
						Post (3 mo/3mo)
2	78	M	10 mg/kg qmo	PR	G2 arthritis	Pre
						Post (2 mo/1 mo)
3	64	M	10 mg/kg qmo	pPR	G2 asthenia	Pre
						Post (9 mo/1 mo)
4	90	M	10 mg/kg qmo	PR	-	Post Progressing
						Post Stable
						Post Responding (8 mo/1 mo)
5	57	F	10 mg/kg qmo	PD	-	Post (4 mo/1 mo)
6	62	M	10 mg/kg qmo	PD	G3 diarrhea	Post (8 mo/6 mo)

Patient 1: PR (26+ mo) to skin, adrenal, liver and lung metastasis

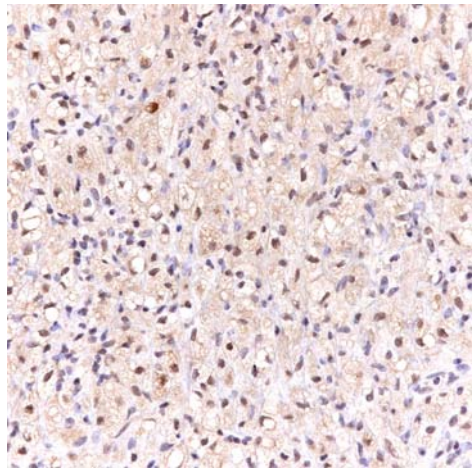
6/04 Pre-Dose

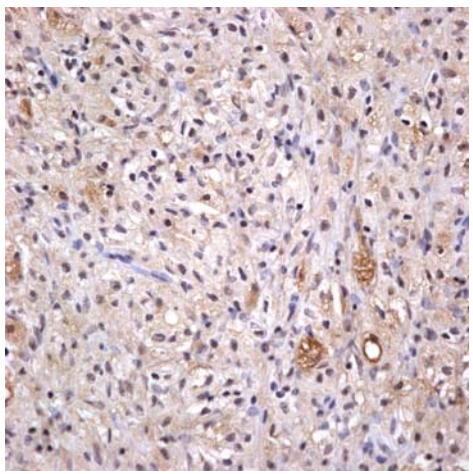
10/04 Post-Dose

H&E

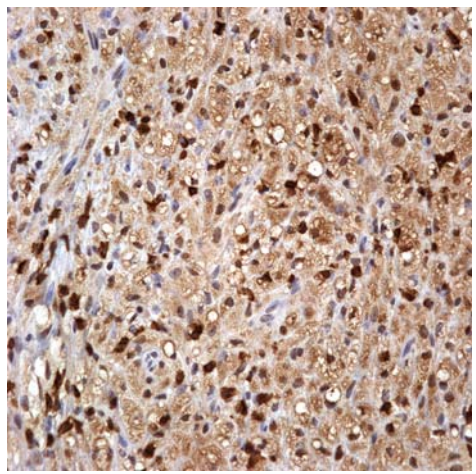


MART-1





CD4



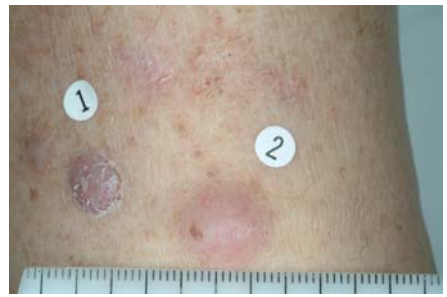
CD8

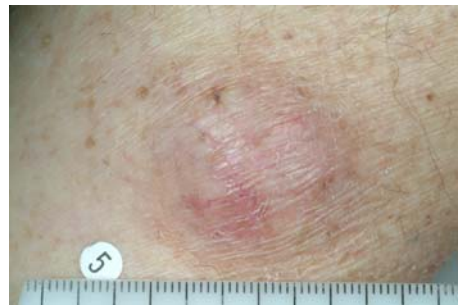
40x

Patient 2: PR (11+ mo) to in-transit metastasis

Pre: 11/05

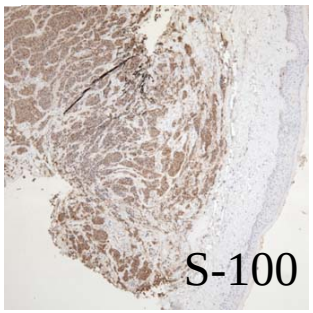
Post: 1/06

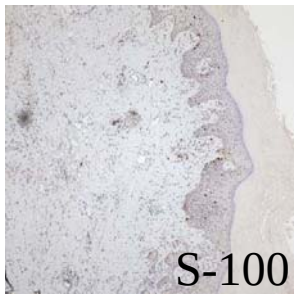


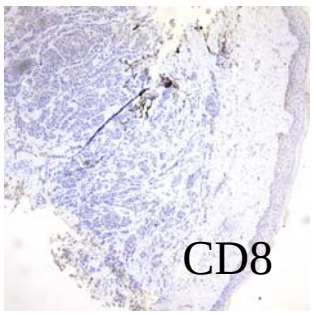


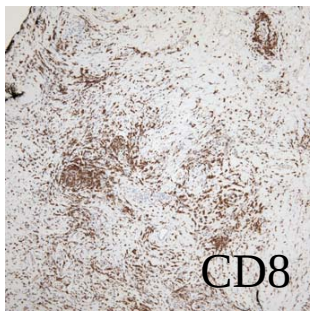
Pre

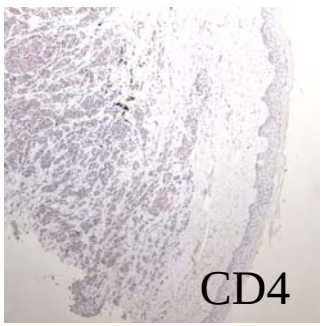
Post

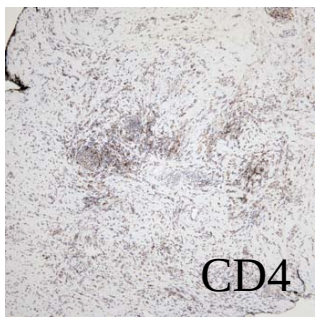






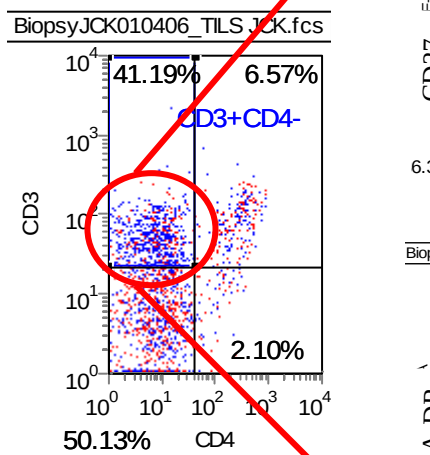


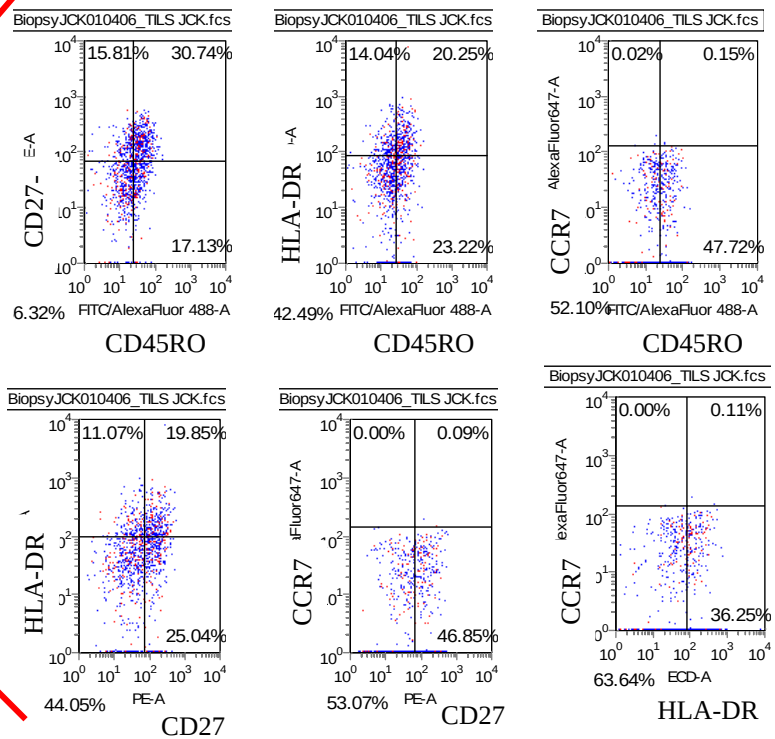




Color	Marker
AlexaFluor405/PacificBlue	CD3
FITC	CD45RO
PE	CD27
PC5/7AAD (Dump Channel)	CD19/CD56
ECD	HLA-DR
APC/AlexaFluor647	CCR7
APC-Cy7/APC-AF750	CD4

CD8^{+}: 41%, CD4^{+}: 6.5%





Phenotype CD8^{+}: HLA-DR^{+}CD45RO^{+++}CD27^{++}CCR7^{-}
(T early memory)

Begonya Comin-Anduix, Ph.D.

Patient 3: PET and Pathological PR (pPR)

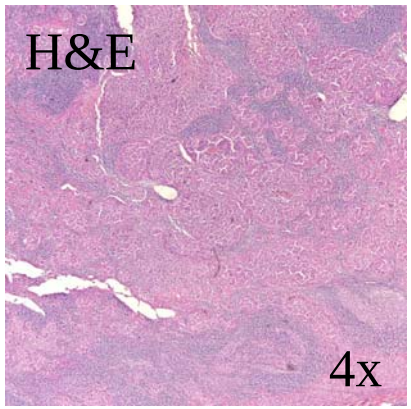
Pre

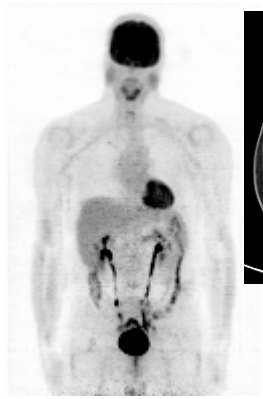
Post

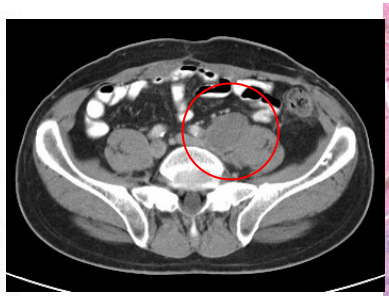
3/05

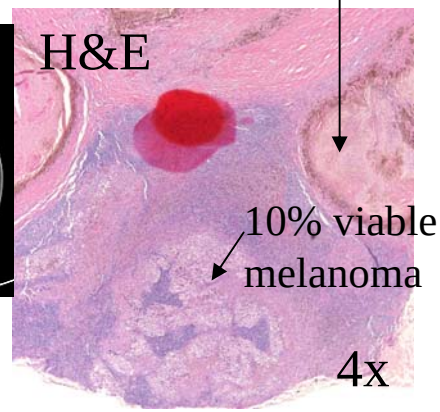
7/05

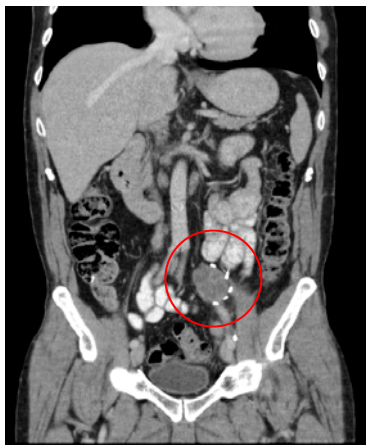
1/06

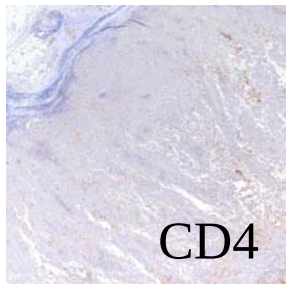


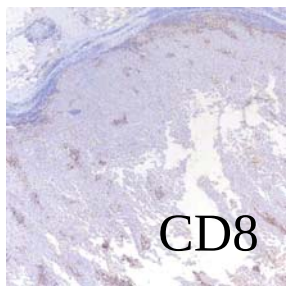


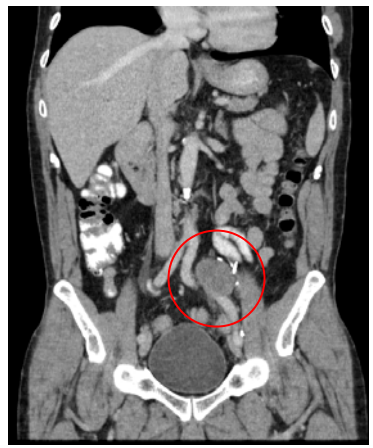


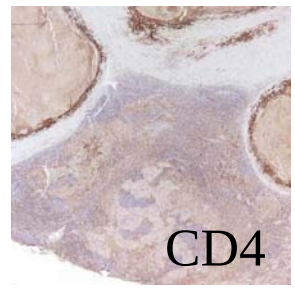


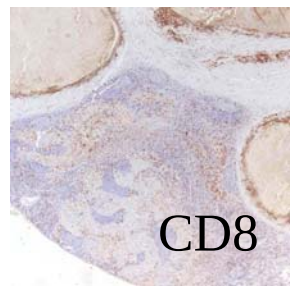






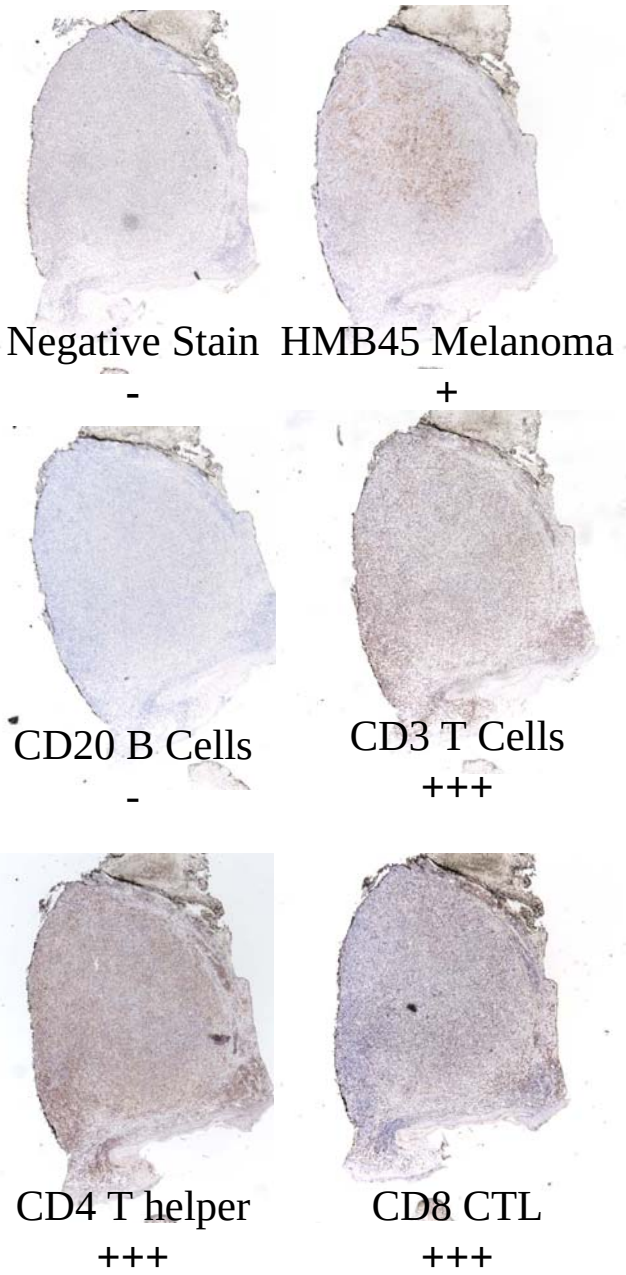






Patient 4: PR (19+ mo) to bulky in-transit metastasis

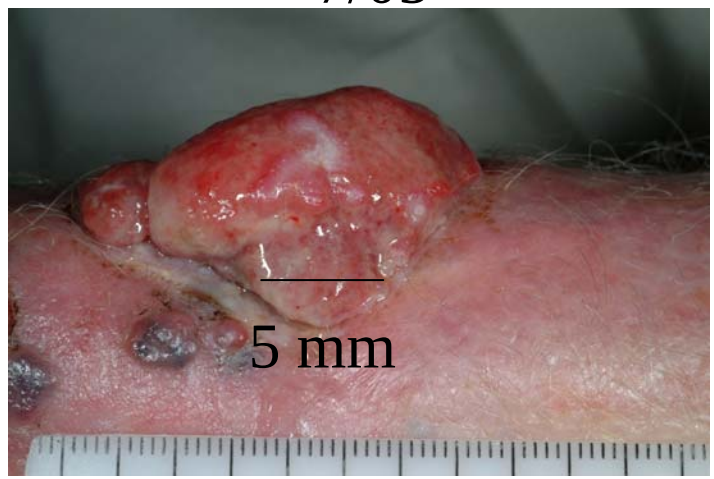
Post



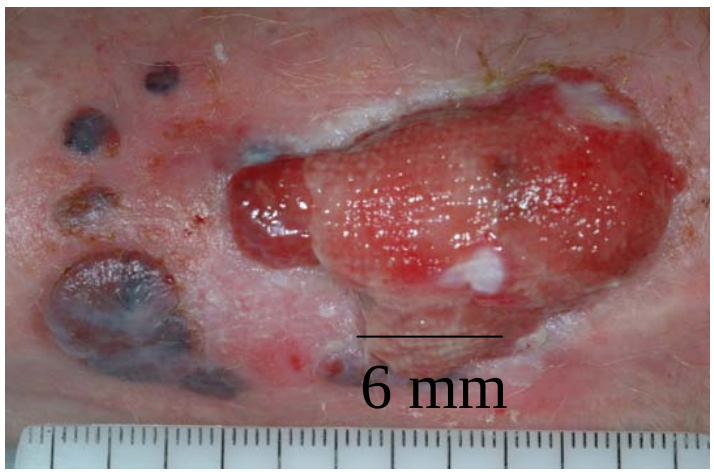
Patient 4 (PR): Co-existing responding and progressing lesions

6/05

7/05

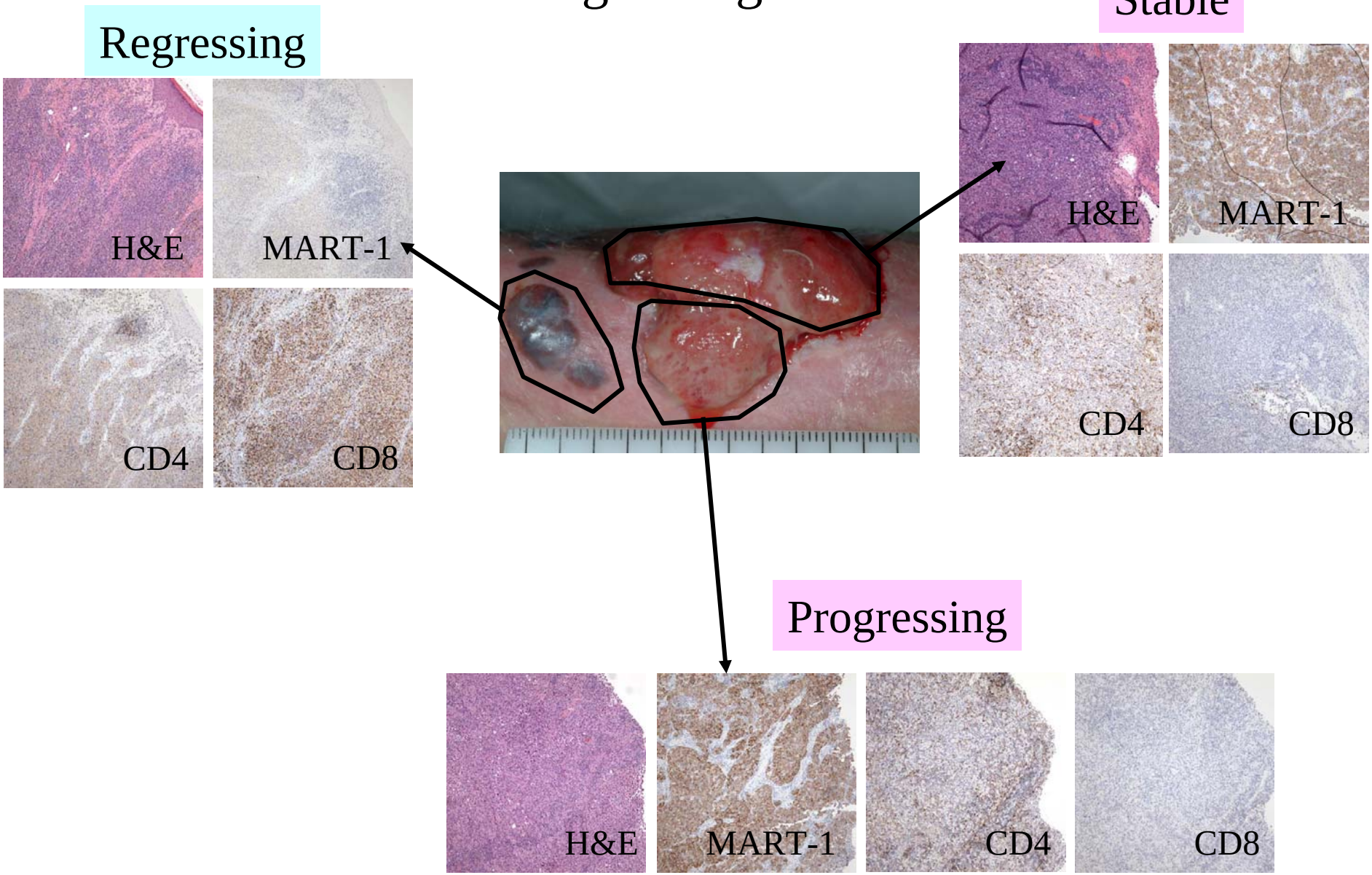


8/05



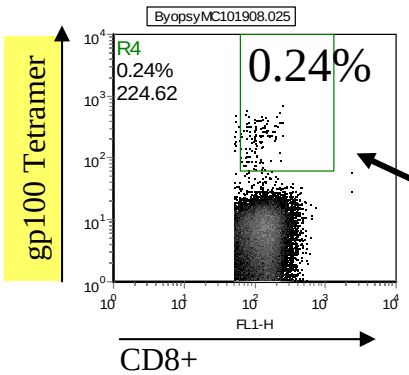
9/05

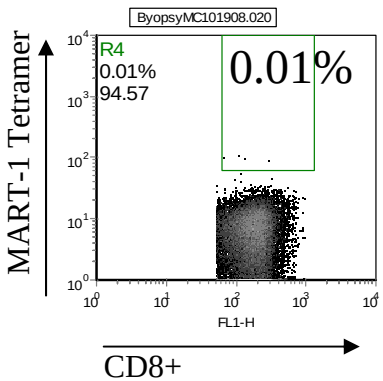
Patient 4 (PR): Simultaneous Analysis of Regressing and Progressing Lesions



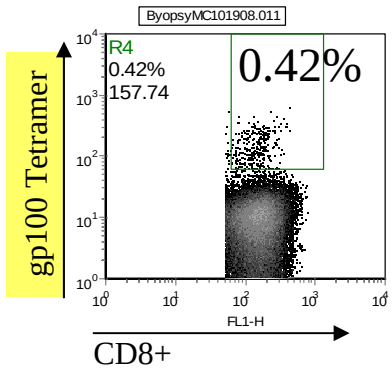
Patient 4 (PR): gp100 Tetramer Analysis

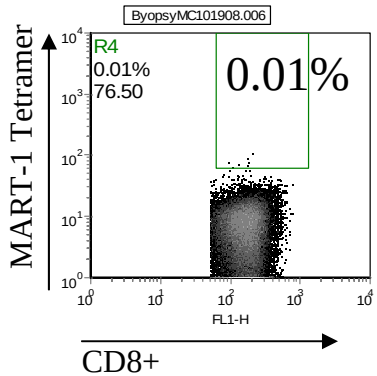
Regressing



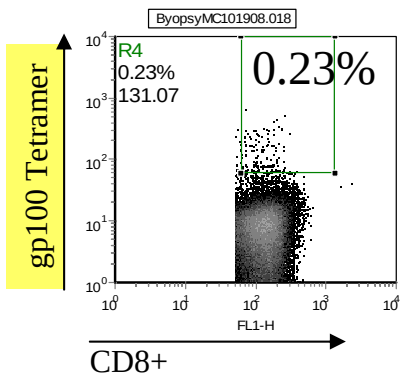


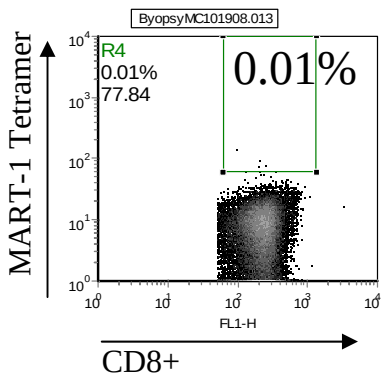
Stable



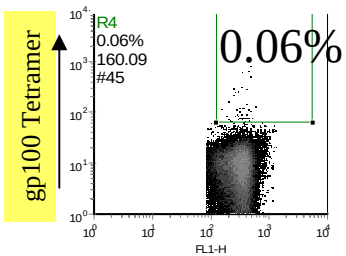


Progressing





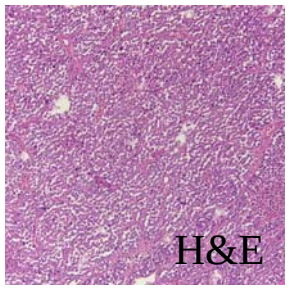
Peripheral blood

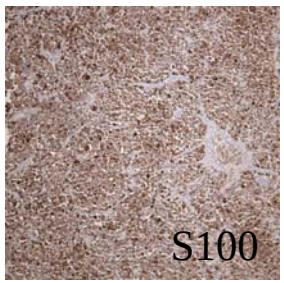


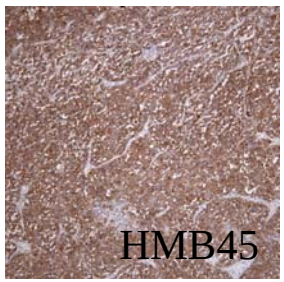
Patient 5 (PD): Progressive Abdominal Mass

Pre

Post



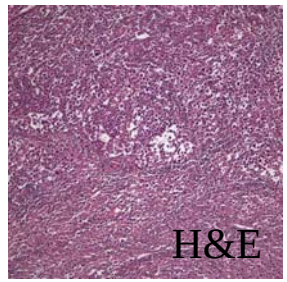


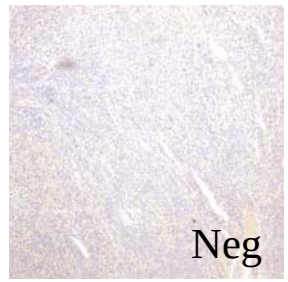


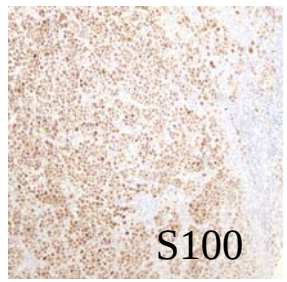
10x

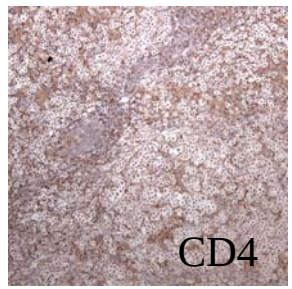
Patient 6 (PD): Progressive Lymph Node Metastasis

Post





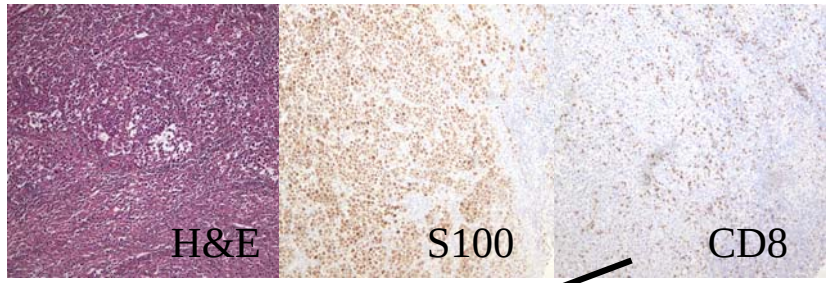




10x

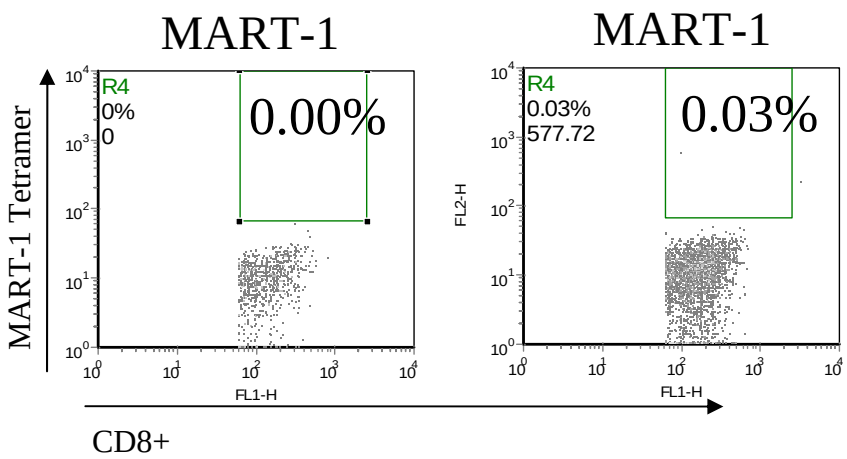
Patient 6 (PD): Tetramer Analysis in PBMC and TIL

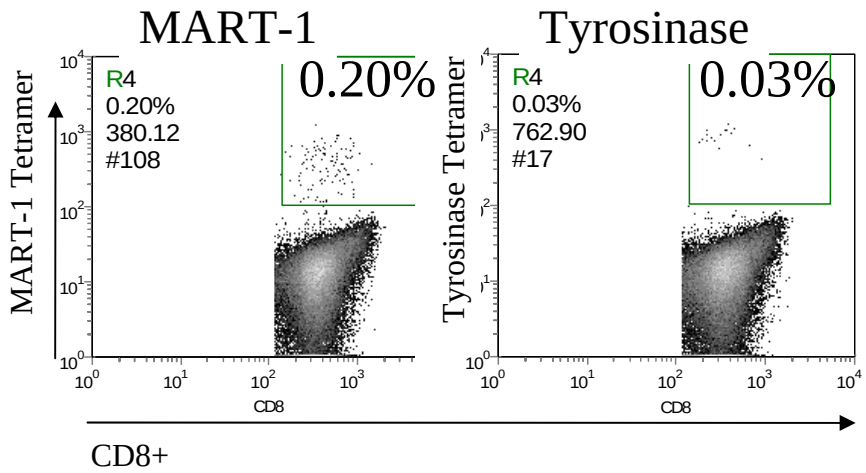
10x



Peripheral Blood

TIL:

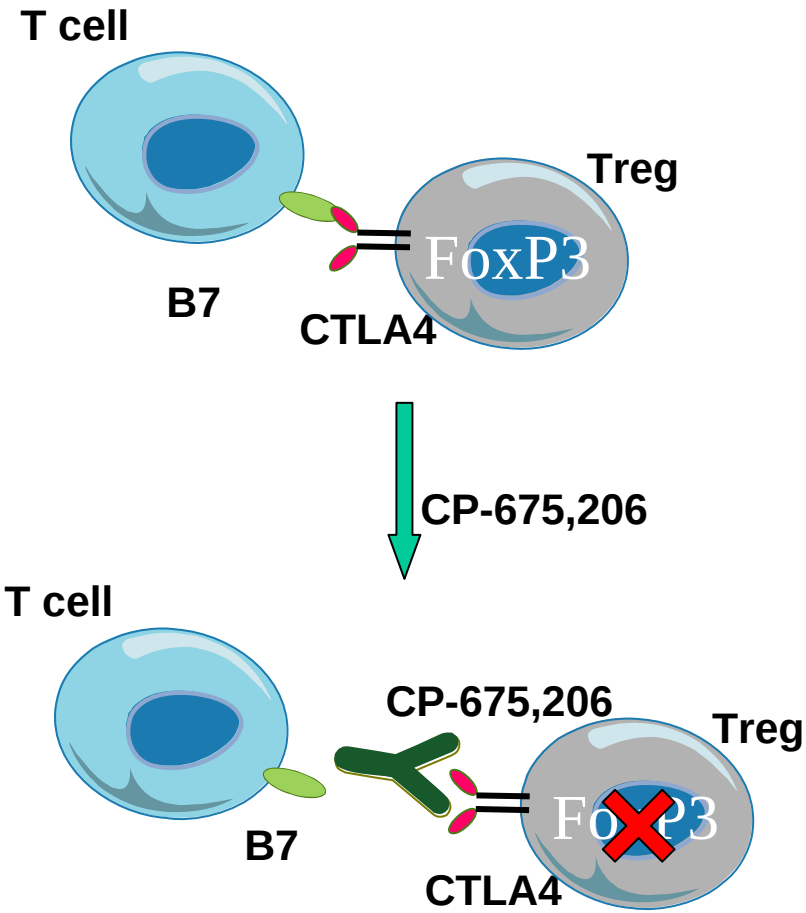




Intratumoral CD8 and CD4 Cells

Patient No.	Response	Timing of Biopsy (first dose/last dose)	CD8	CD4	Change
1	PR	Pre	0	NA	
		Post (3 mo/3mo)	+++ diffuse	++ diffuse	↑
2	PR	Pre	0	0	
		Post (2 mo/1 mo)	++ diffuse	+ diffuse	↑
3	pPR	Pre	++ patchy	+ patchy	
		Post (9 mo/1 mo)	++ diffuse	+++ diffuse	↑
4	PR	Post Progressing	+ patchy	+++ diffuse	
		Post Stable	+ patchy	+++ diffuse	
		Post Responding (8 mo/1 mo)	+++ diffuse	++ diffuse	↑ CD8 ↓ CD4
5	PD	Post (4 mo/1 mo)	+/-	++ diffuse	
6	PD	Post (8 mo/6 mo)	+ diffuse	+++ diffuse	

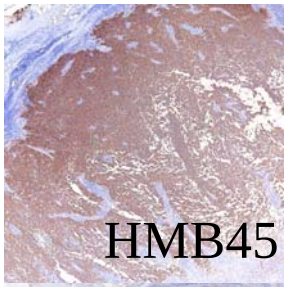
Treg Depletion with CTLA4 Blocking Monoclonal Antibodies

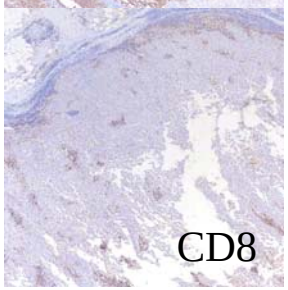


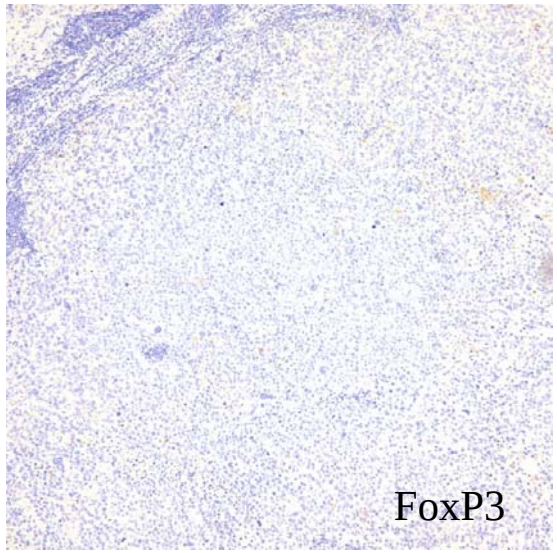
- Treg depletion in peripheral blood with anti-CTLA4 mAb:
  - Reuben *et al*. Cancer 2006
- No Treg depletion in peripheral blood with anti-CTLA4 mAb:
  - Maker *et al*. J Immunol 2005
  - Comin-Anduix *et al*. iSBTc 2006

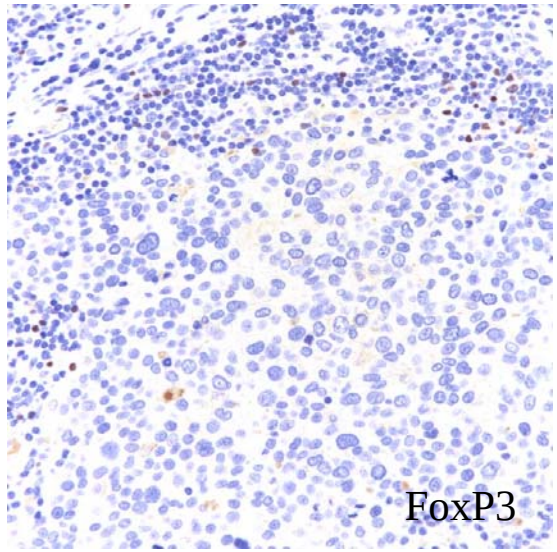
Patient 3 (pPR): FoxP3 Pre and Post CP-675,206

Pre

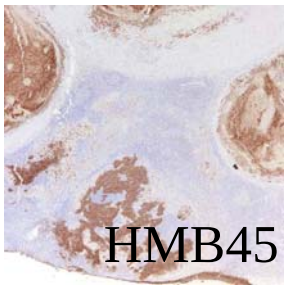


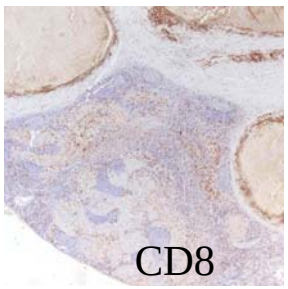


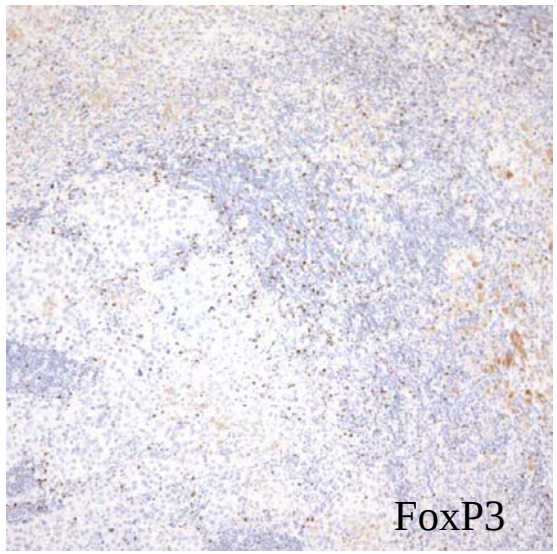


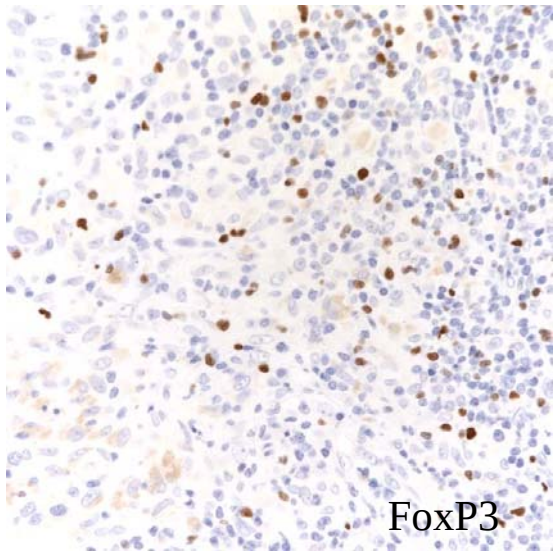


Post



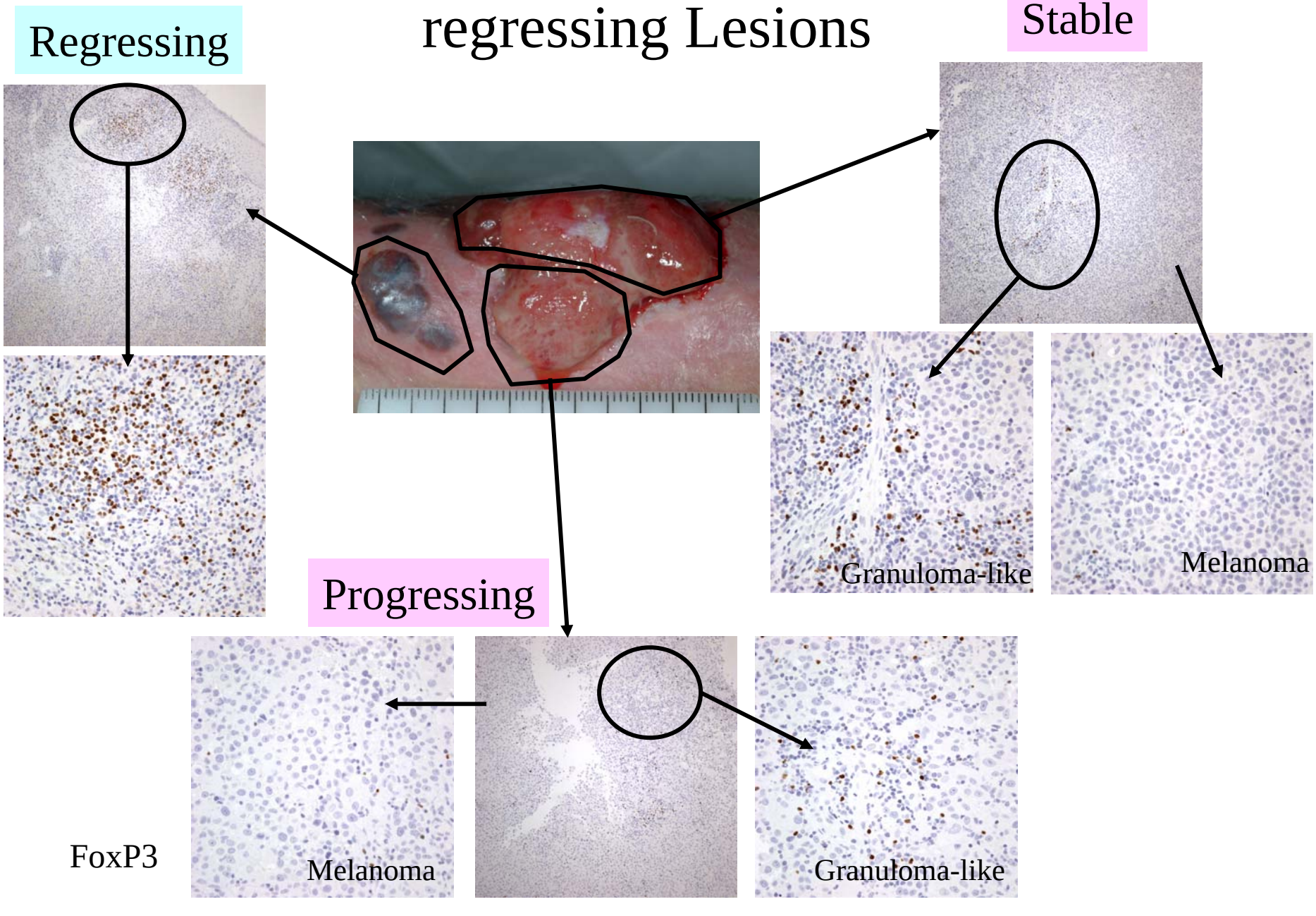






4x 10x 40x

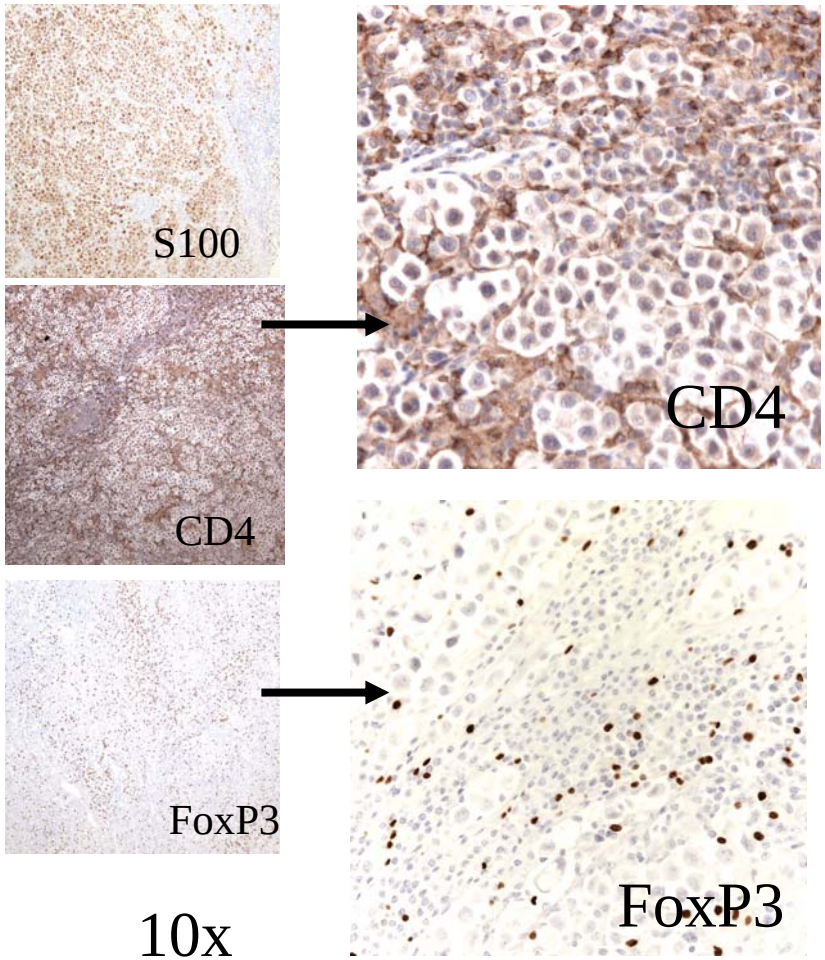
Patient 4 (PR): FoxP3 in Regressing and Non-regressing Lesions



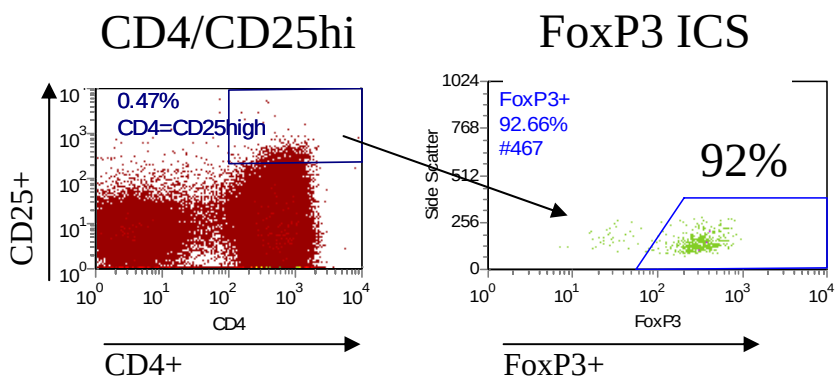
Patient 6 (PD): FoxP3 by IHC or ICS in TIL

Post

FoxP3 in TIL by IHC



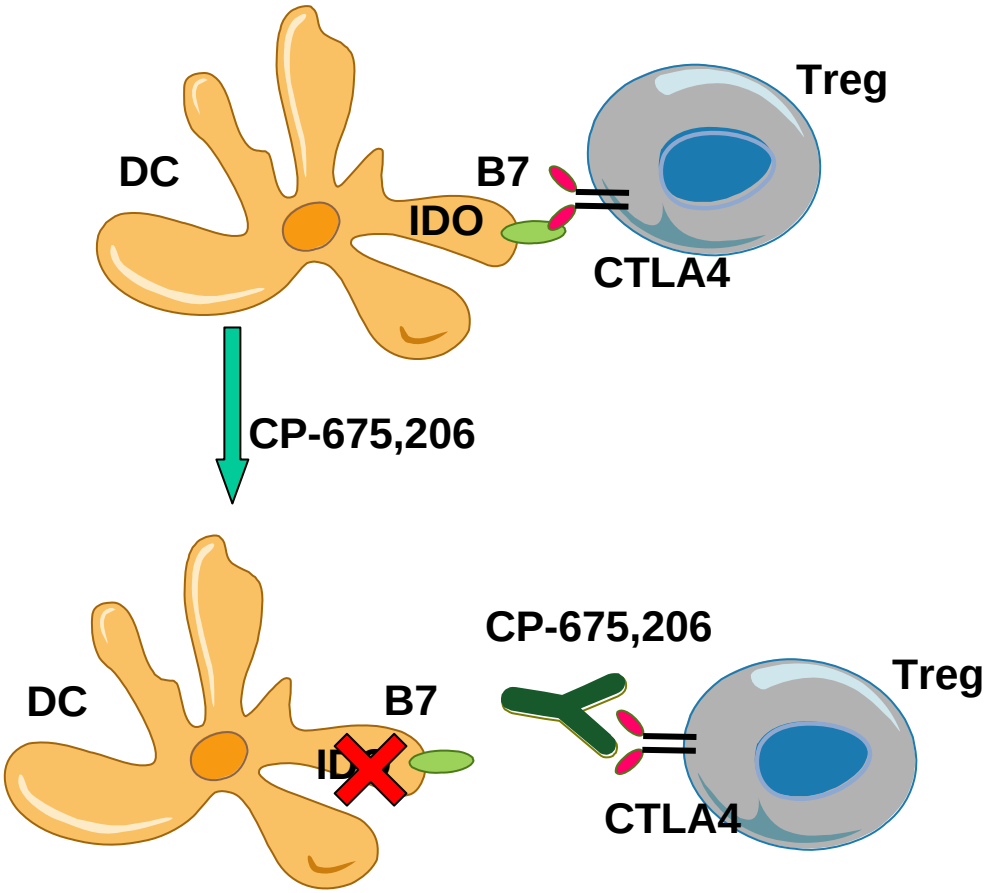
FoxP3 in TIL by ICS



Intratumoral FoxP3+ Treg

Patient No.	Response	Timing of Biopsy	FoxP3	Change
1	PR	Pre	0	
		Post (3 mo/3mo)	+ patchy	↑
2	PR	Pre	0	
		Post (2 mo/1 mo)	+ patchy	↑
3	pPR	Pre	+ patchy	
		Post (9 mo/1 mo)	++ patchy	↑
4	PR	Post Progressing	+ patchy	
		Post Stable	+ patchy	
		Post Responding (8 mo/1 mo)	+ patchy	-
5	PD	Post (4 mo/1 mo)	+/-	
6	PD	Post (8 mo/6 mo)	+++ diffuse	

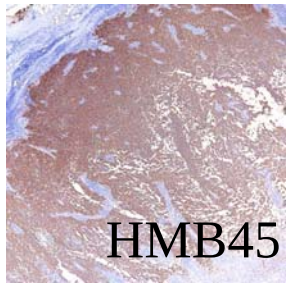
Inhibition of IDO by CTLA4 Blocking Monoclonal Antibodies

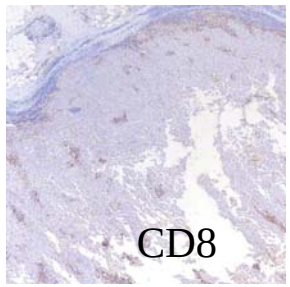


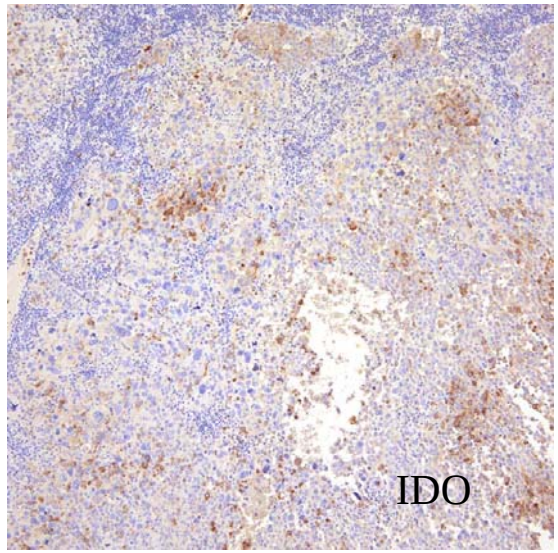
- Grohmann, Fallarino *et al*. Nat Immunol. *3*, 1097 (2002)
- Grohmann, Fallarino *et al*. Nat Immunol. *4*, 1206 (2003)
- Munn, Mellor *et al*. J Clin Invest. *114*, 280 (2004)
- Munn, Mellor *et al*. Int Immunol. *16*, 1391 (2004)

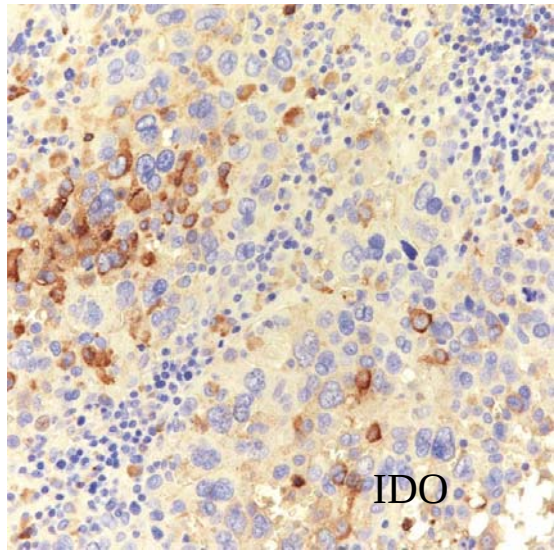
Patient 3 (pPR): IDO Pre and Post CP-675,206

Pre

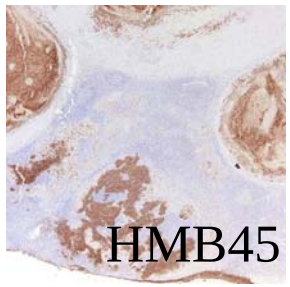


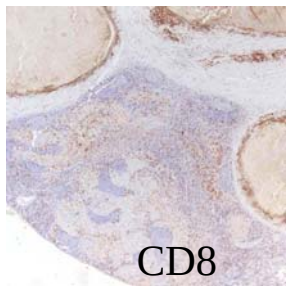


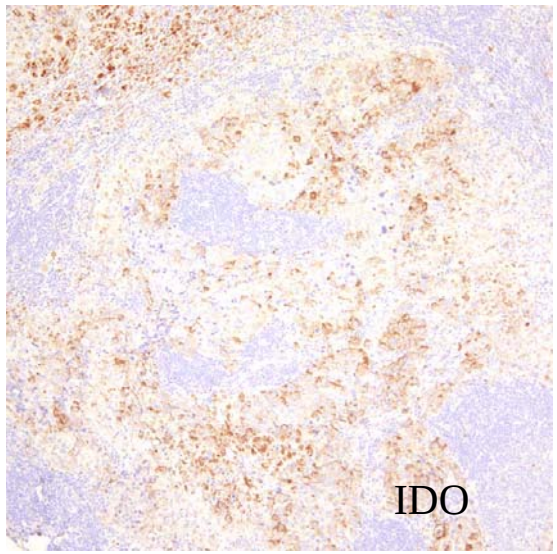


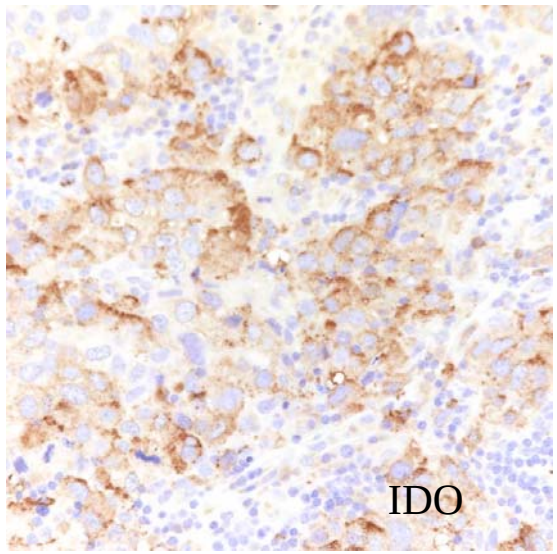


Post







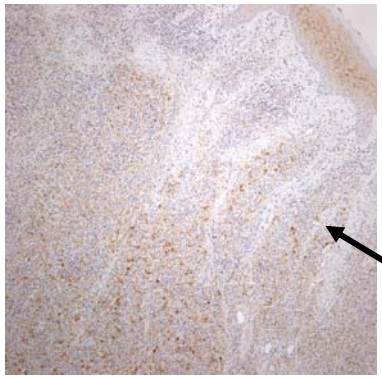


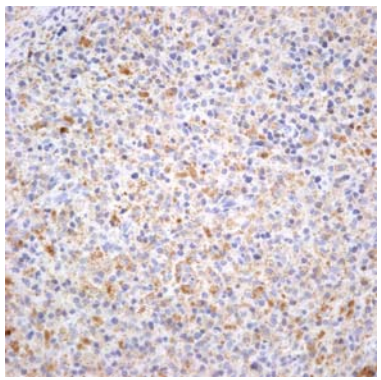
4x 10x 40x

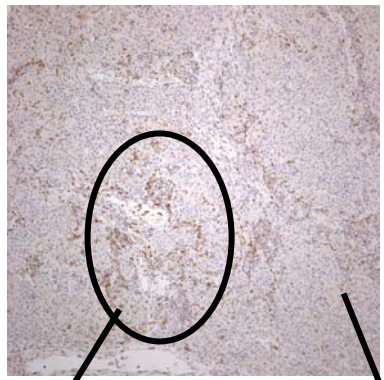
Patient 4 (PR): IDO in Regressing and Non-regressing Lesions

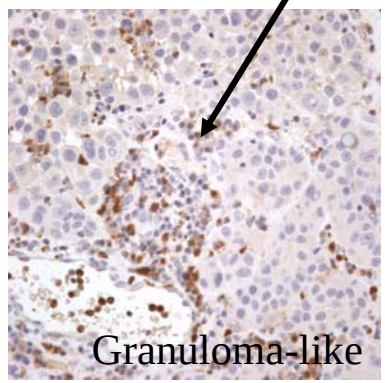
Regressing

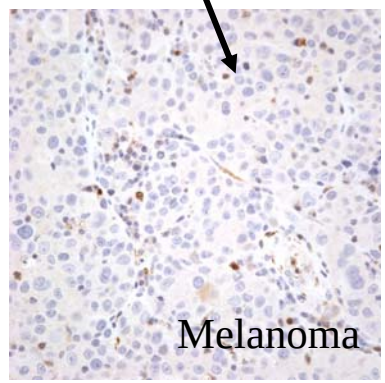
Stable



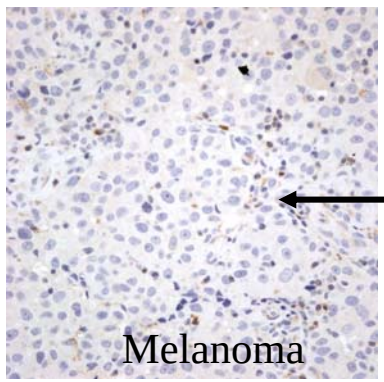


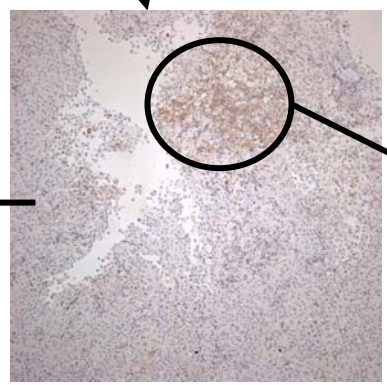


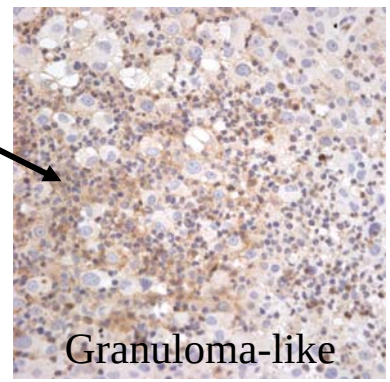




Progressing







IDO

Intratumoral IDO+ Cells

Patient No.	Response	Timing of Biopsy	IDO	Change
1	PR	Pre	++ diffuse	
		Post (3 mo/3mo)	+ patchy	↓
2	PR	Pre	+ patchy	
		Post (2 mo/1 mo)	+ patchy	-
3	pPR	Pre	+ patchy	
		Post (9 mo/1 mo)	+ patchy	-
4	PR	Post Progressing	++ patchy	
		Post Stable	++ patchy	
		Post Responding (8 mo/1 mo)	+++ patchy	↑
5	PD	Post (4 mo/1 mo)	++ patchy	
6	PD	Post (8 mo/6 mo)	+ patchy	

Conclusions

- Regressing lesions after CTLA4 blockade with CP-675,206 have:
  - Dense intratumoral infiltrates by CD8+ CTL, and variable CD4+ T helper infiltrates
  - No consistent decrease in intratumoral infiltrates by FoxP3+ Tregs and no inhibition of IDO expression
- Contrary to the posed hypothesis, FoxP3 and IDO positive cells may cluster at areas of active immune response against melanoma

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  - Cecile A. Bulanhagui, MS
  - Margaret Marshall, MD
  - Jesus Gomez-Navarro, MD

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