Special Symposium
Melanoma Therapy: From frustration to enthusiasm

Biomarkers for checkpoint protein inhibitors

37th ESMO-ECCO 2012
Vienna, Austria

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Protocol Schema MCC15241

- **Resected stage IIIC/IV melanoma**

  - 75 HLA-A*0201 patients received ipilimumab at 3 mg/kg or 10 mg/kg for 1 year, then were able to be boosted every 3 months for up to 5 years

  - Peptide vaccine only given to 50 A *0201 patients; every 2 weeks times 6, then every 4 weeks times 4, then every 12 weeks times 2 for a total of 12 vaccinations over 1 yr

  - PBMC collected at 3, 6 and 12 months, and 55 had sufficient cells for biomarker analysis

Demographics and treatment related variables of all patients in this biomarker study (n=55)
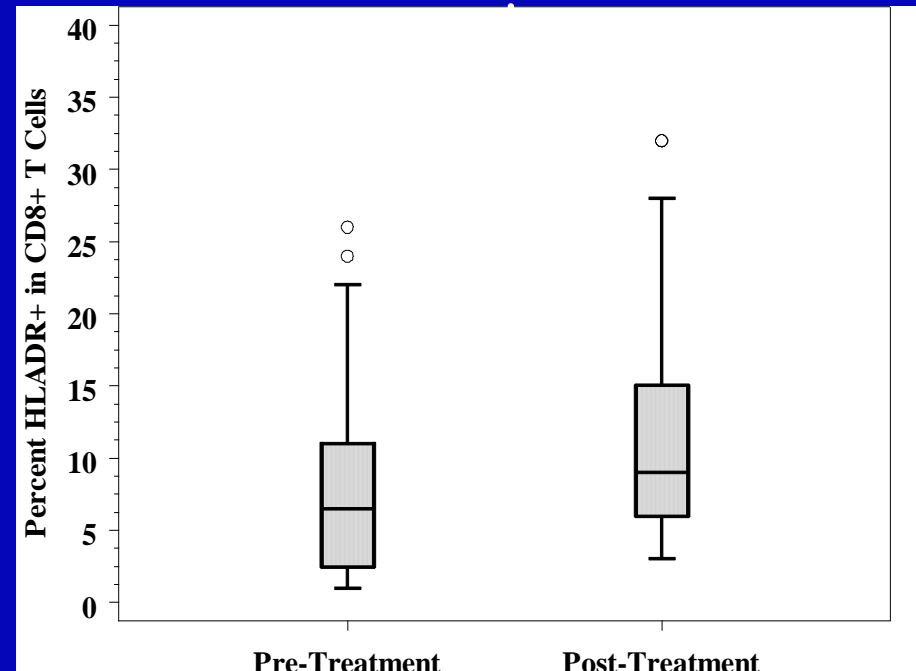
Variable	Level	n (%)
gender	F	21 (38.2)
	M	34 (61.8)
stage	IIIc	24 (43.6)
	IV	31 (56.4)
HLA A2	A2+	34 (61.8)
	A2-	21 (38.2)
dosage	10 mg/kg	40 (72.7)
	3 mg/kg	15 (27.3)
irAE	N	31 (56.4)
	Y	24 (43.6)
Outcome	NED	35 (63.6)
	Relapse	20 (36.4)

Increased CD4+DR+ and CD8+DR+ T cells after ipilimumab
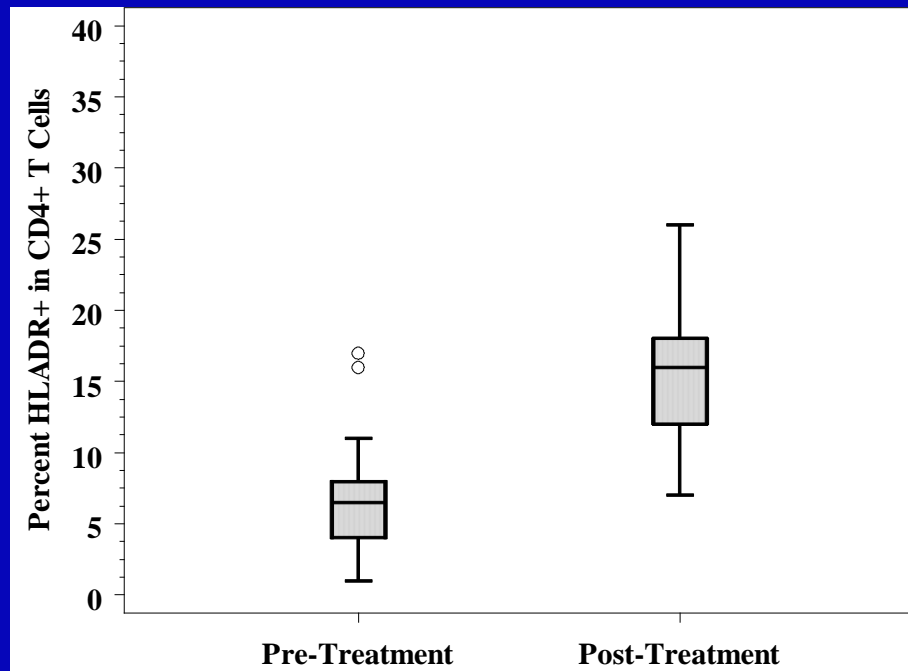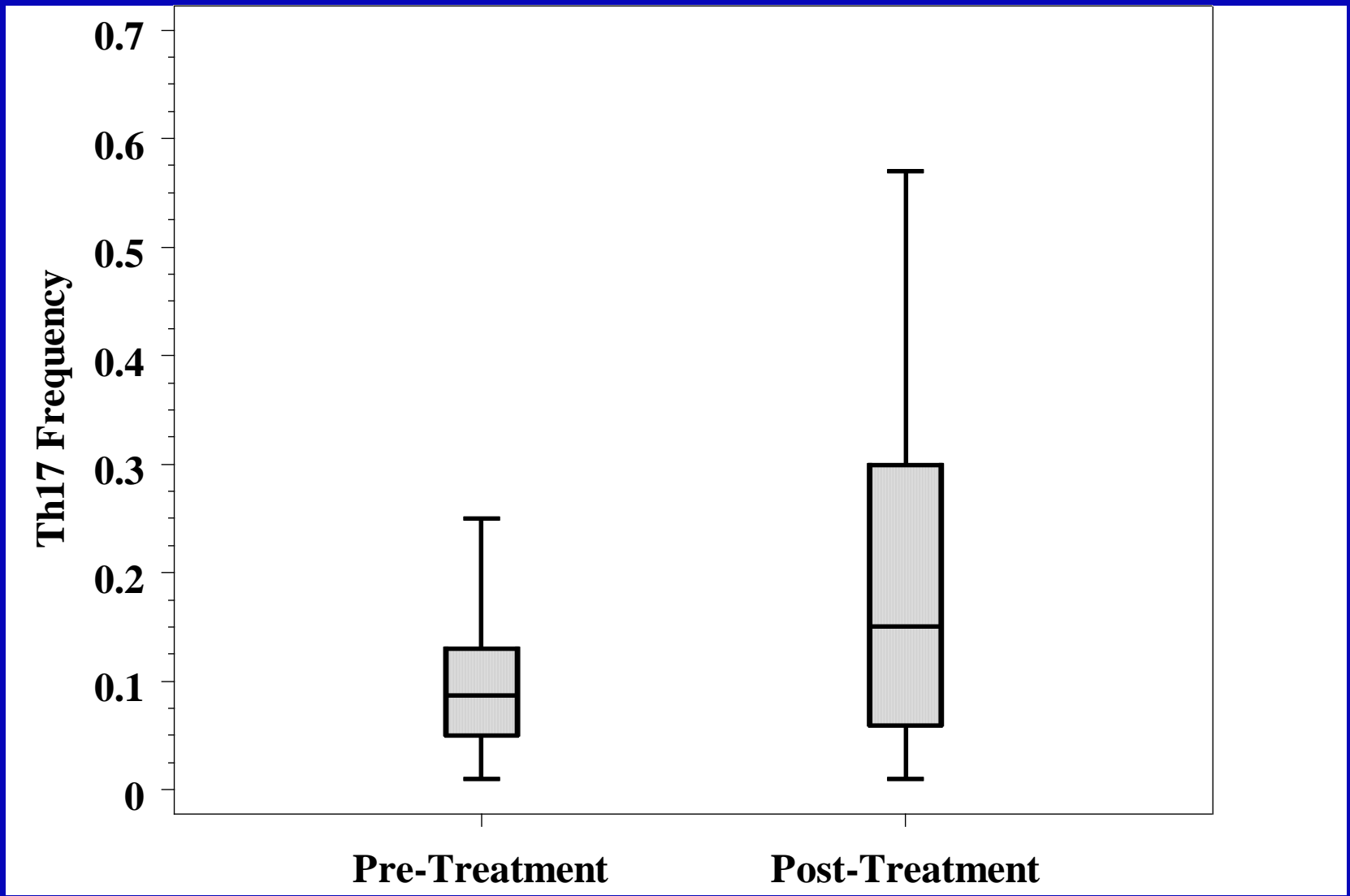

CD4 T cells

CD8 T cells

From Sarnaik et al, CCR 2010
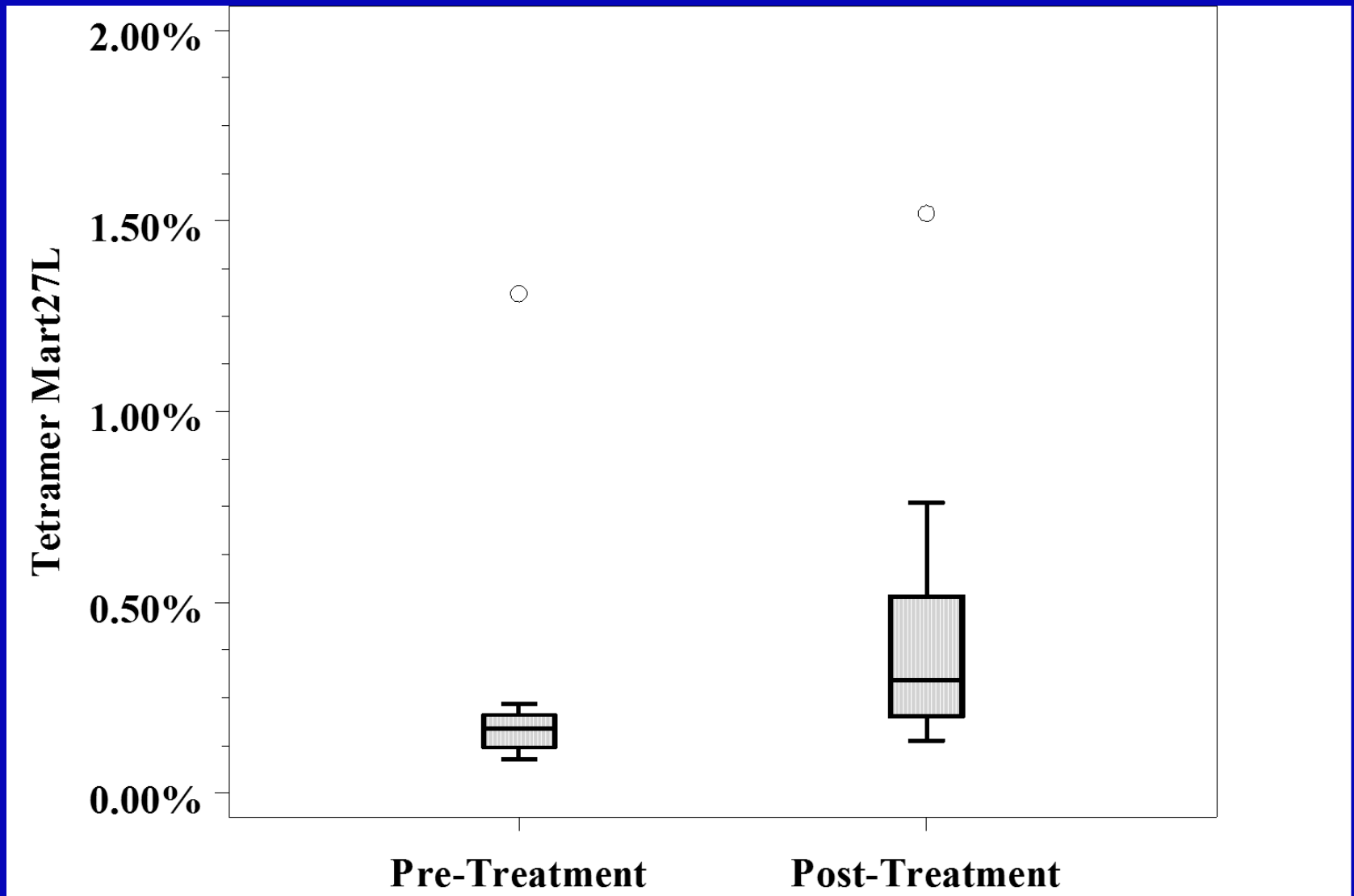
* Wilcoxon p < 0.001

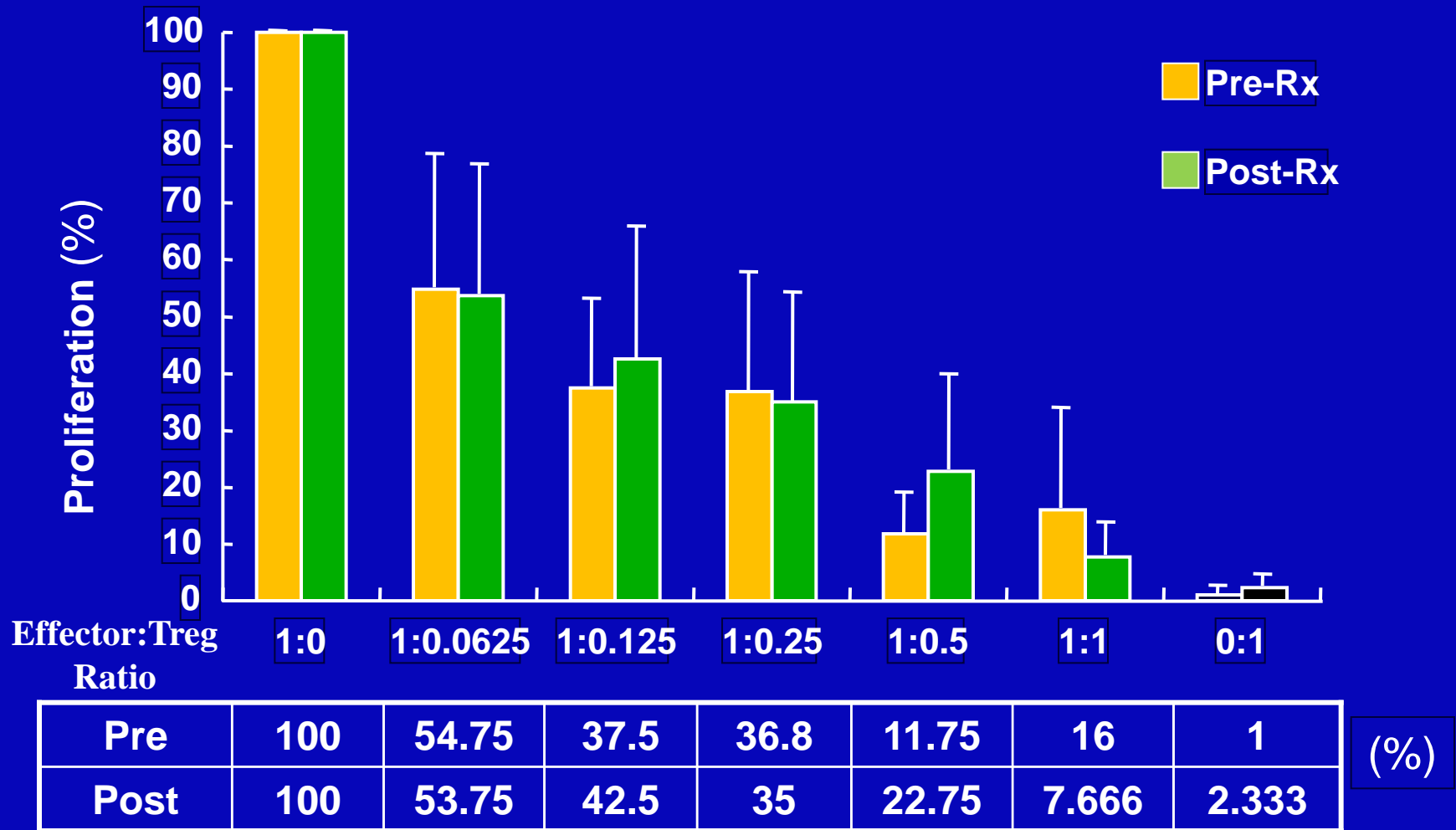# Increased induction of Th17 T cells after ipilimumab

From Sarnaik, A et al CCR 2011

* Wilcoxon p <0.0001

Increased MART-1 (27L) specific T cells after ipilimumab + peptide

* Wilcoxon p < 0.0001

T reg function is <u>not</u> associated with ipilimumab treatment

Effector:Treg Ratio	1:0	1:0.0625	1:0.125	1:0.25	1:0.5	1:1	0:1	(%)
Pre	100	54.75	37.5	36.8	11.75	16	1	
Post	100	53.75	42.5	35	22.75	7.666	2.333	

From Sarnaik, A et al CCR 2011

Statistical analysis of changes in biomarkers at 3 months

| Biomarker	n	Median change * (Q1, Q3)	Wilcoxon p-value	Median fold-change * (Q1, Q3)	Wilcoxon p-value
%-ICOS-CD4	25	13.00 (1.15, 20.69)	<.0001	1.35 (0.23, 3.62)	<.0001
%-ICOS-CD8	25	4.19 (2.07, 7.75)	<.0001	2.90 (1.25, 6.72)	<.0001
%-Ki67-CD4	24	3.62 (0.74, 6.94)	<.0001	1.50 (0.18, 2.64)	<.0001
%-Ki67-CD8	24	1.49 (-0.10, 4.86)	0.0009	0.54 (-0.03, 2.20)	0.0003
%-CCR7-CD8	25	-3.98 (-11.40, 1.30)	0.0122	-0.14 (-0.27, 0.04)	0.018
%-CD25-CD8	25	-1.60 (-5.68, -0.70)	<.0001	-0.42 (-0.56, -0.25)	<.0001

*(Q1, Q3) are first and third interquartiles.

Statistical analysis of changes in biomarkers at 6 months

Biomarker	n	Median change * (Q1, Q3)	Wilcoxon p-value	Median fold-change * (Q1, Q3)	Wilcoxon p-value
%-ICOS-CD4	37	9.45 (3.13, 14.57)	<.0001	1.66 (0.70, 2.42)	<.0001
%-ICOS-CD8	37	3.07 (1.62, 5.36)	<.0001	1.34 (0.63, 3.30)	<.0001
%-Ki67-CD4	35	2.33 (1.37, 6.97)	<.0001	0.87 (0.29, 2.12)	<.0001
%-Ki67-CD8	36	1.88 (0.13, 4.91)	0.0004	0.55 (0.04, 1.52)	<.0001
%-Gata3-CD4	28	3.76 (0.28, 8.04)	0.0004	0.94 (-0.01, 1.62)	<.0001
%-Gata3-CD8	28	2.07 (0.59, 4.02)	0.0006	0.59 (0.09, 1.23)	<.0001
%-CCR7-CD4	37	-2.90 (-6.80, -0.40)	0.0017	-0.03 (-0.09, -0.01)	0.006

*(Q1, Q3) are first and third interquartiles.

Changes in PD-1on CD4 T cells induced by ipilimumab at 3 and 6 months

PD-1 induction by ipilimumab treatment on CD4+ T cells
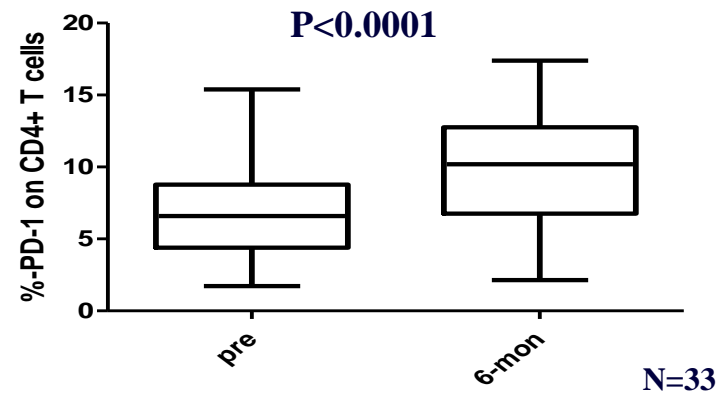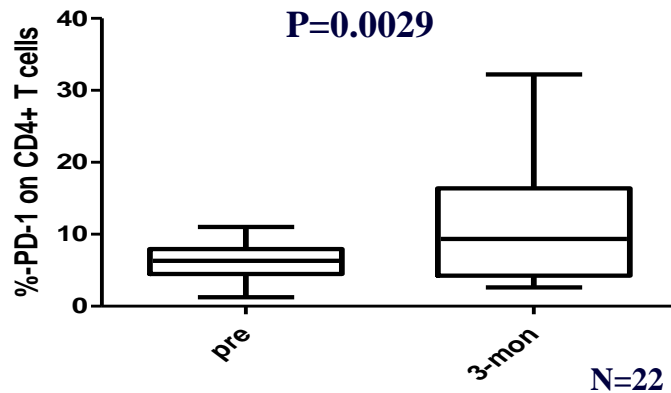
Association between outcome (Relapse/NED) and dichotomized baseline biomarkers by median

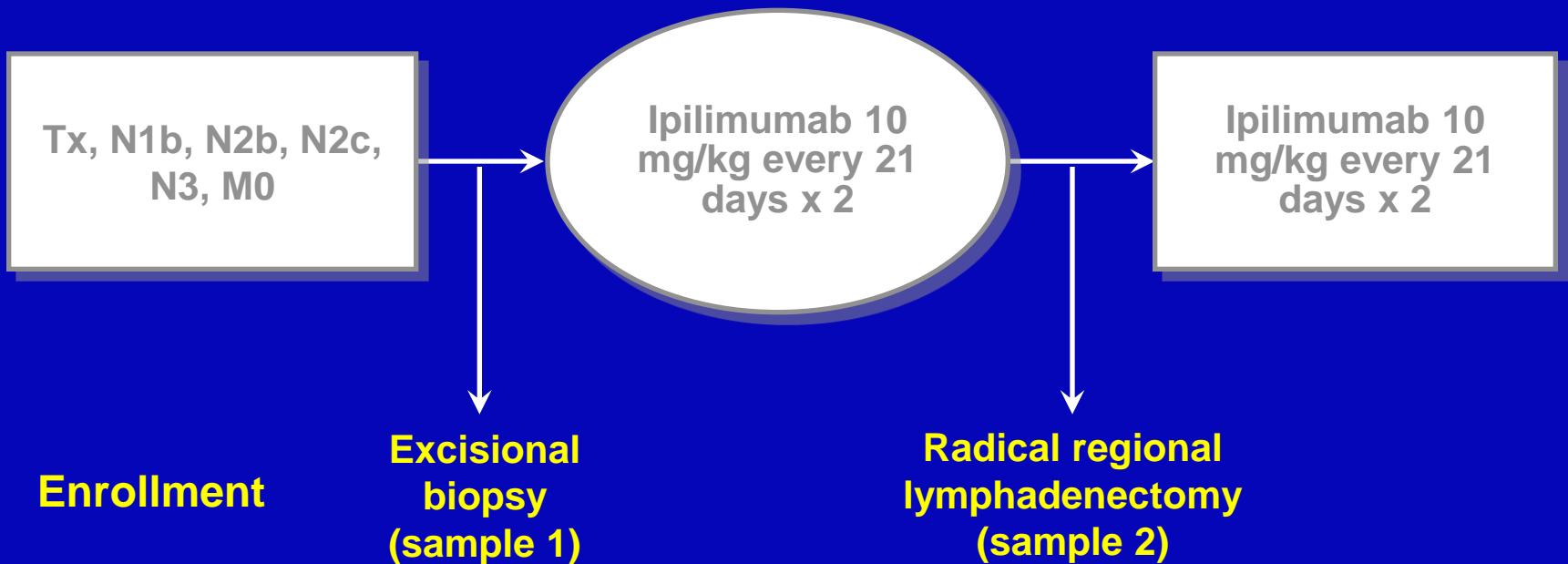| Biomarker	Outcome	n (%)	Odds Ratio (95% CI)	Fisher p-value
%-Ki67+EOMES+CD8+ (n=39)			11.25 (2.52, 50.27)	0.0012
<=2.11	Relapse			
<=2.11	NED	5 (25.0)		
>2.11	Relapse	4 (21.1)		
>2.11	NED			
%-EOMES+CD8+ (n=54)			3.77 (1.16, 12.27)	0.0473
<=55.6	Relapse	14 (51.9)		
<=55.6	NED	13 (48.1)		
>55.6	Relapse	6 (22.2)		
>55.6	NED	21 (77.8)		

From Wang, W. et al, J Trans Med 2012

Kaplan-Meier curves for association of baseline biomarkers with relapse free survival
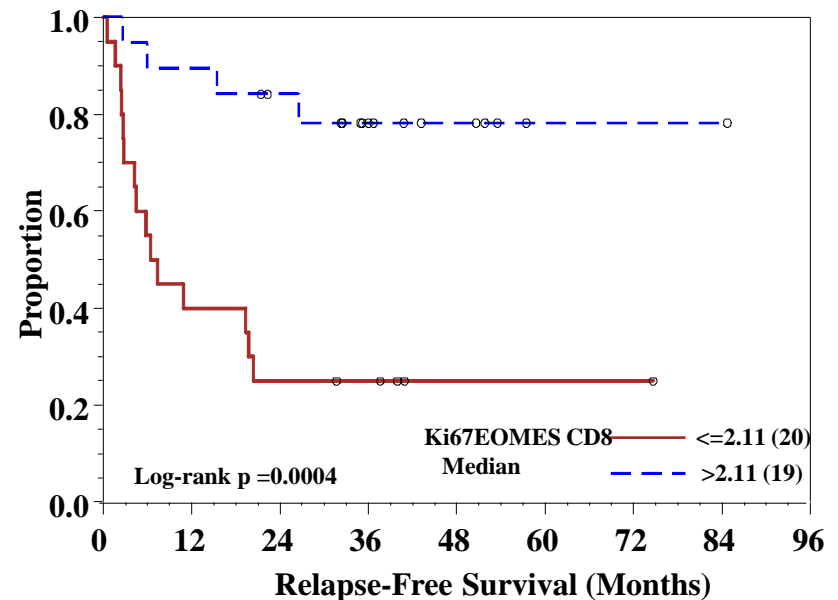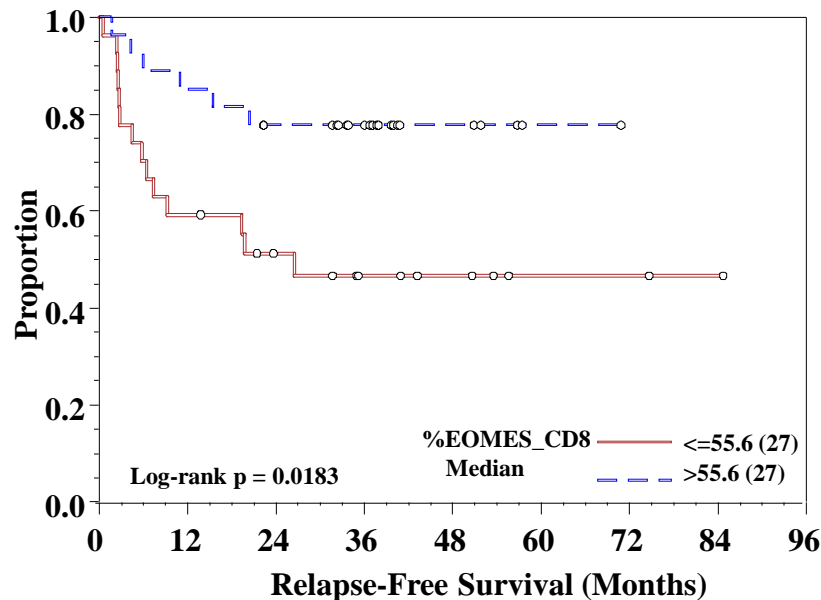
Association between irAE (Yes/No) and dichotomized baseline biomarkers by median

Biomarker	irAE	n (%)	Odds Ratio (95% CI)	Fisher p-value
%-Ki67+EOMES+CD4+ (n=39)			8.00 (1.74, 36.70)	0.0079
<=0.446	Yes	12 (60.0)		
<=0.446	No	8 (40.0)		
>0.446	Yes	3 (15.8)		
>0.446	No	16 (84.2)		

# Neoadjuvant Ipilimumab in N1b,2b, N2c, N3 Melanoma   UPCI 08-144

Tx, N1b, N2b, N2c, N3, M0

Ipilimumab 10 mg/kg every 21 days x 2

Ipilimumab 10 mg/kg every 21 days x 2

Enrollment

Excisional biopsy (sample 1)

Radical regional lymphadenectomy (sample 2)
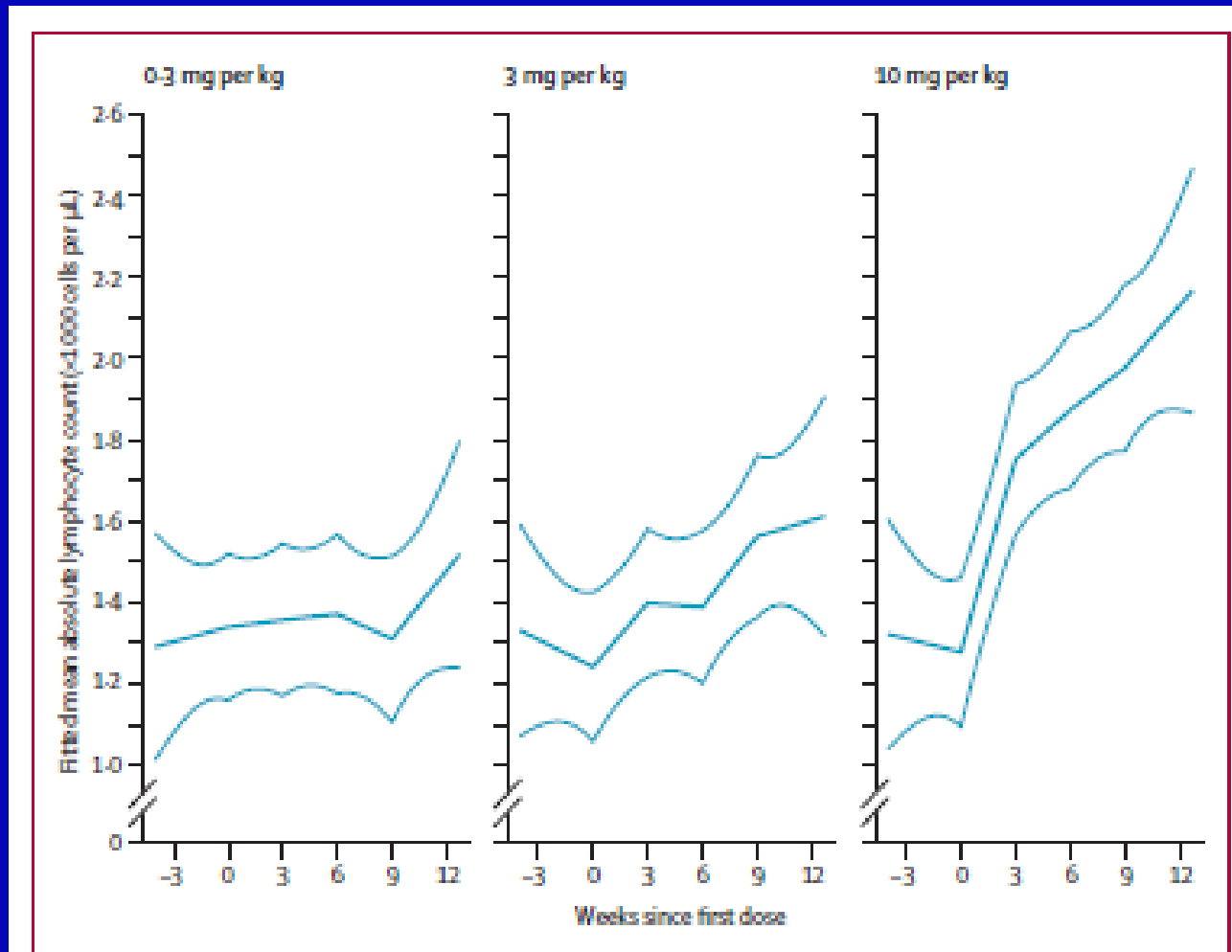
From Tarhini , A et al ASCO 2012

Tumor TIL by IHC (N=24)

TIL	Median Δ (Wk6 – Baseline) with 95%CI	P-value (paired)
CD8+ T Cells	19 (4.79,39.25)	0.019

Conclusion: Significant increase in cytotoxic CD8+ TIL after Ipi
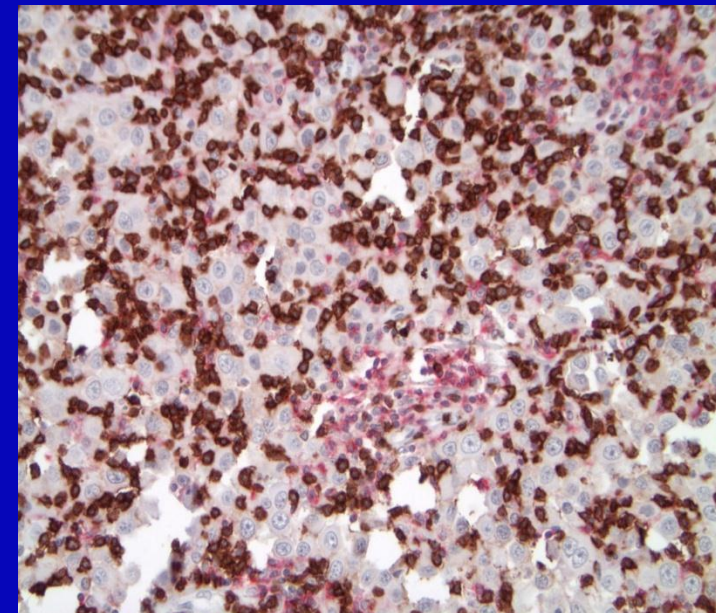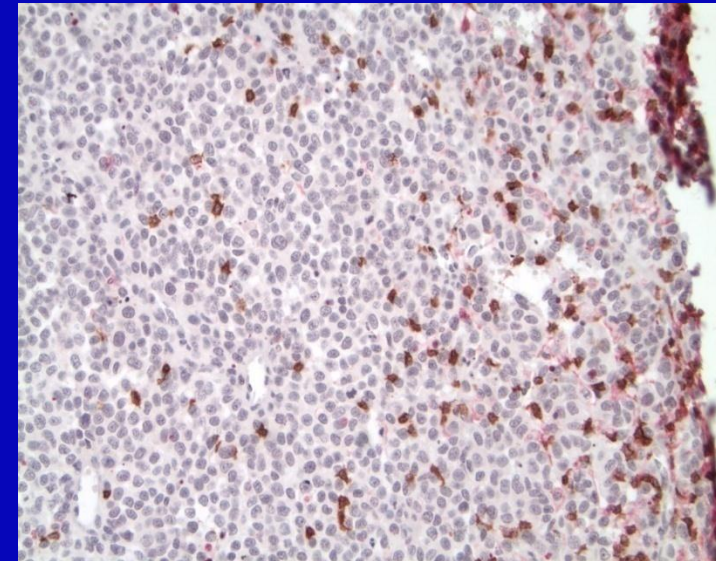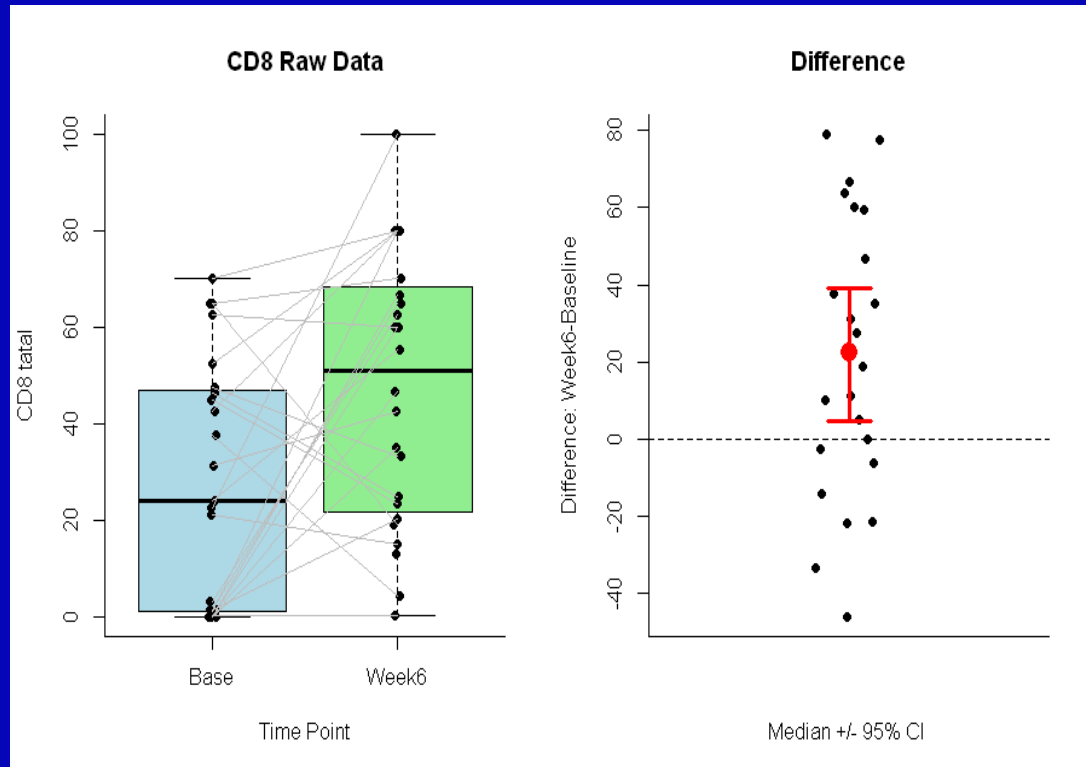
From Tarhini A et al, ASCO 2012

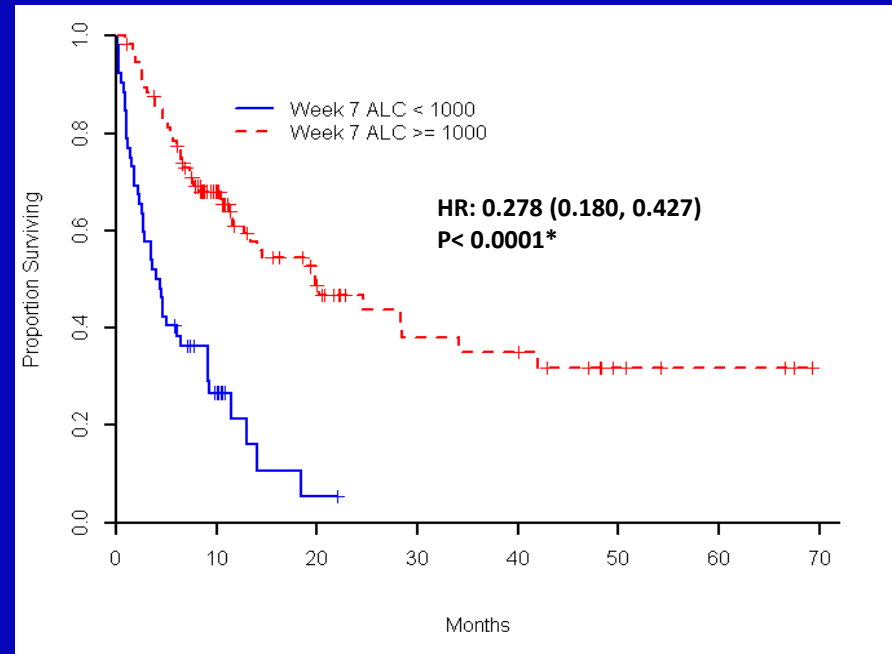# Pharmacodynamic Effects on the Absolute Lymphocyte Count

Wolchok et al. *Lancet Oncology* 2010

Kaplan-Meier Curves for OS (p-values reflect univariate analysis)

Pre-Treatment Baseline
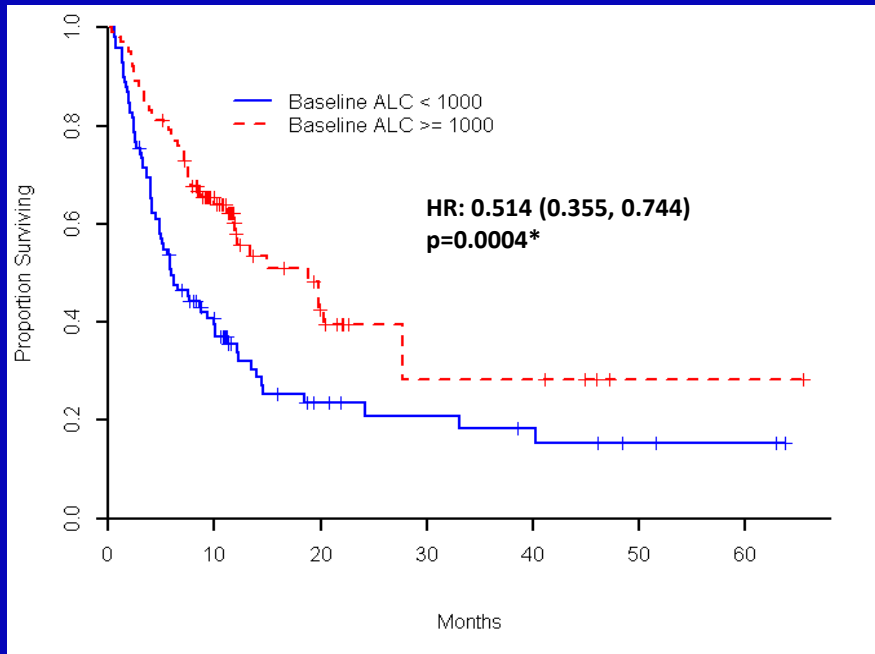
Week 7

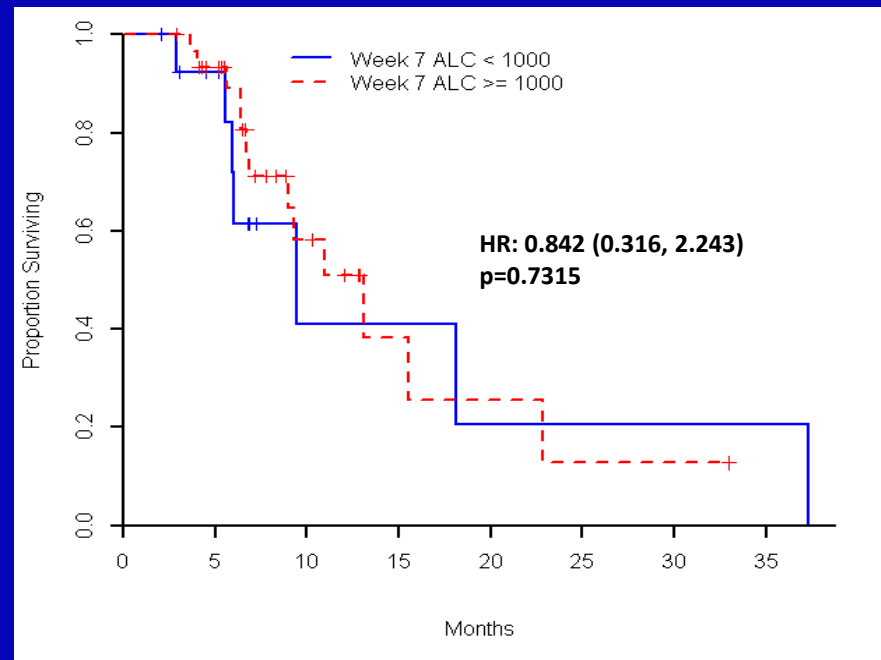**All ipi pts at 10mg/kg and 3mg/kg**

n=201

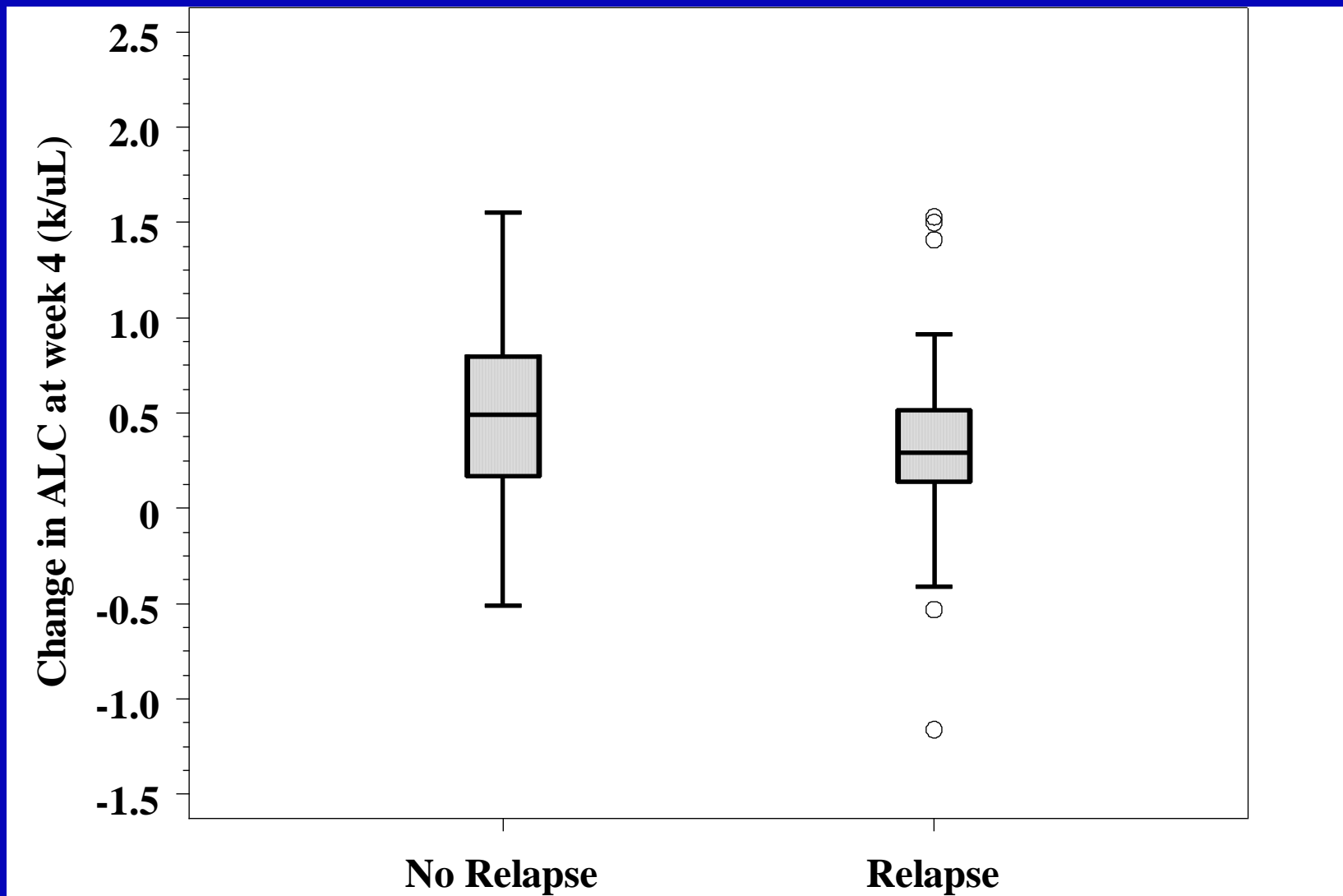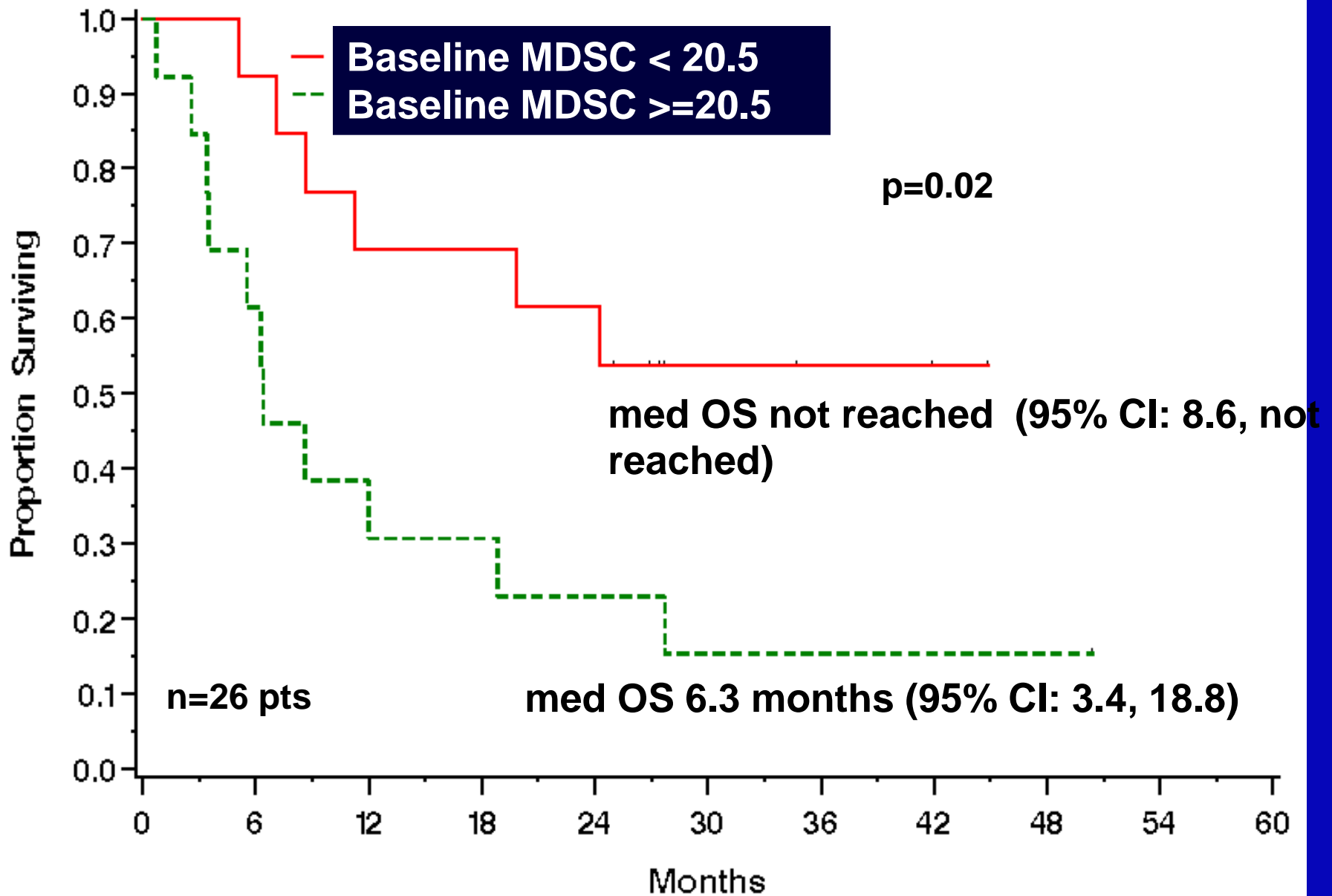# Kaplan-Meier Curves for OS: BRAF inhibitors

Pre-Treatment Baseline
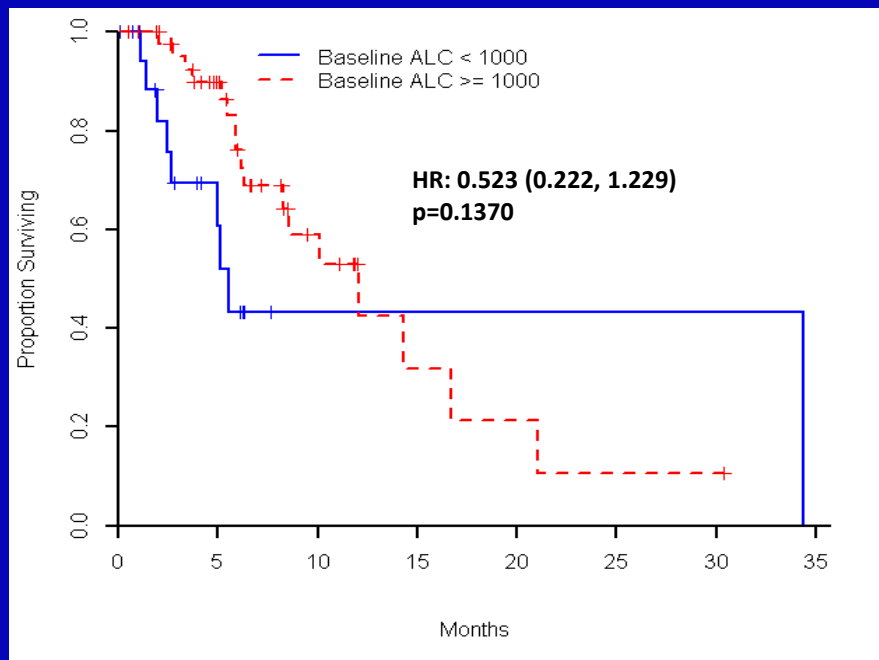
Week 7

n=74, p-values reflect univariate analysis

**No association of change in ALC with relapse after adjuvant ipilimumab**

Y-axis: Change in ALC at week 4 (k/uL)

X-axis categories: No Relapse, Relapse

Sarnaik, A et al CCR 2011

* T test (Satterwaithe) p = 0.19

Kitano S, Postow M, et al. ASCO 2012

Study Design: Phase I Multi-dose Regimen of PD-1 antibody BMS 936558

8-wk treatment cycle

Rapid PD or clin. deterioration → Off Study

Unacceptable toxicity → Follow-up every 8 wks x 6 (48 wks)

CR/PR/SD or PD but clinically stable → Treat to confirmed CR, worsening PD, unacceptable toxicity, or 12 cycles (96 wks)

Day 1   15   29   43   57 SCANS

* * * *

*Dose administered IV Q2wk

Eligibility: Advanced MEL, RCC, NSCLC, CRC, or CRPC with PD after 1-5 systemic therapies

From Hodi, S et al ASCO 2012

PD-L1 Expression in Melanoma

- PD-L1-positive metastatic lesions correlated with improved survival in 56 patients with stage III-IV melanoma[1]

- In patients with MEL, NSCLC, CRC, RCC, or CRPC treated with BMS-936558 (n=42), PD-L1 expression in pretreatment tumor biopsies correlated with clinical outcomes[2]

- Further studies in melanoma patients are planned to define the role of PD-L1 as a potential molecular marker of response to BMS-936558
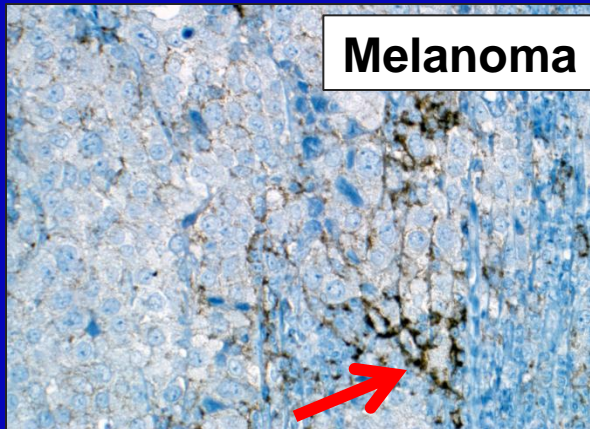
Melanoma

Immunohistochemical staining with anti-PD-L1 monoclonal antibody 5H1 of melanoma lymph node metastasis

[1] Taube JM, et al. *Science Transl Med.* 2012;4:127ra37 [2]Topalian SL et al. 2012 ASCO abs 2509

Phase I Trial Of Vaccine With PD-1 Antibody BMS 936558 With Vaccine

- **34 patients were treated at 1, 3 and 10 mg/kg every 2 weeks for 12 weeks with a multi-peptide vaccine**
- **Up to two cycles given for 24 weeks, then patients that are stable or better boosted every 12 weeks for 2 years**
- **New cohorts include those who are HLA A2 positive and had either no, or dose limiting prior ipi toxicity, or are A2 negative and had no ipi dose limiting irAEs**
- **The drug is very well tolerated for up to 84 weeks, with no MTD reached; one episode of grade three optic neuritis and one grade three pulmonary toxicity observed, one DLT at 3 mg/kg**
- **Thyroiditis, hypophysitis, fatigue, pulmonary toxicity, rash and fevers observed**

Changes in T_{reg} and CTLA-4 expressing cells are associated with response with BMS 936558

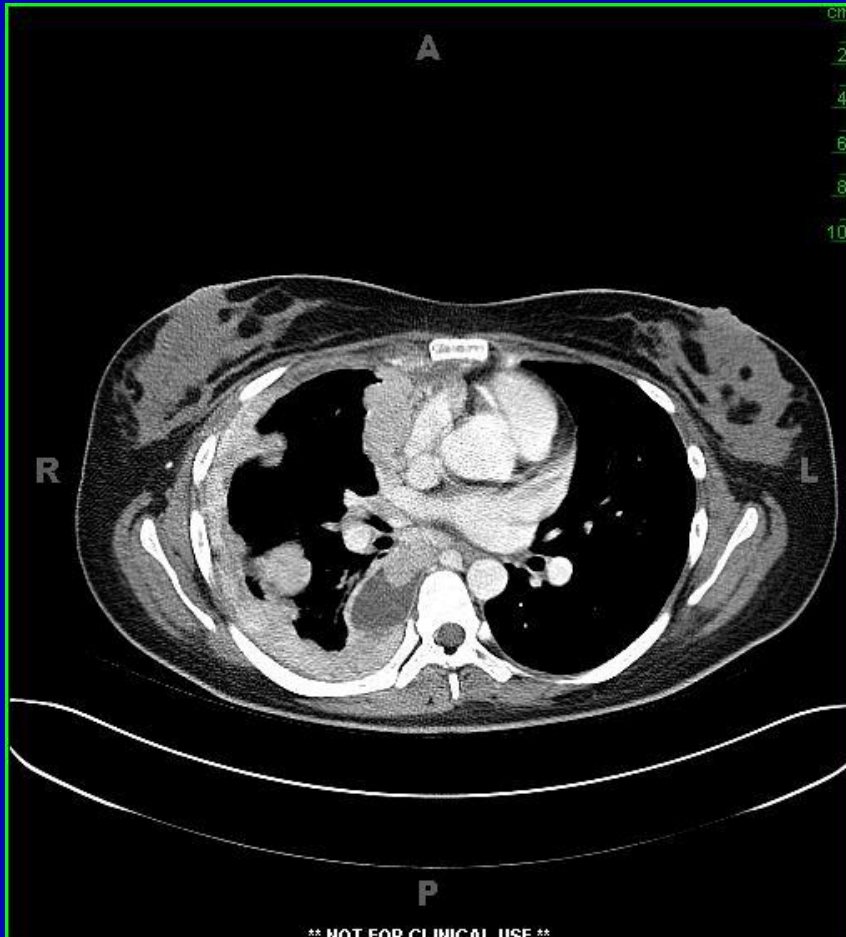**Decreased T regs and response p=0.001**

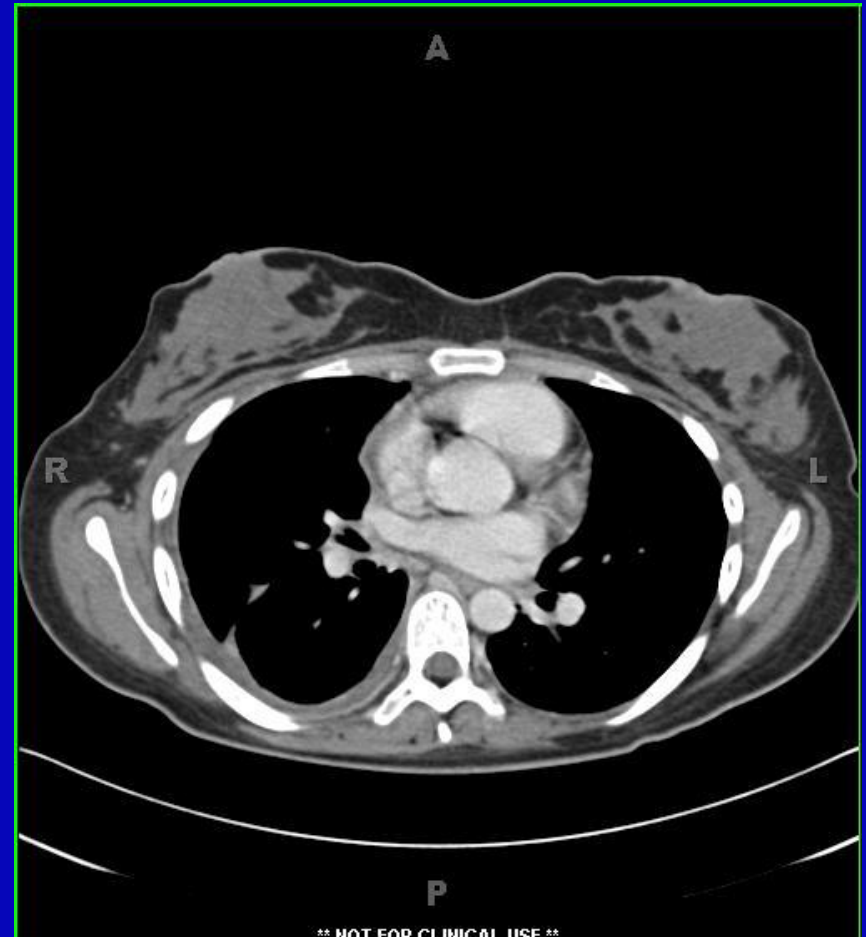**Decreased CTLA-4/CD8 T cells and response p=0.01**
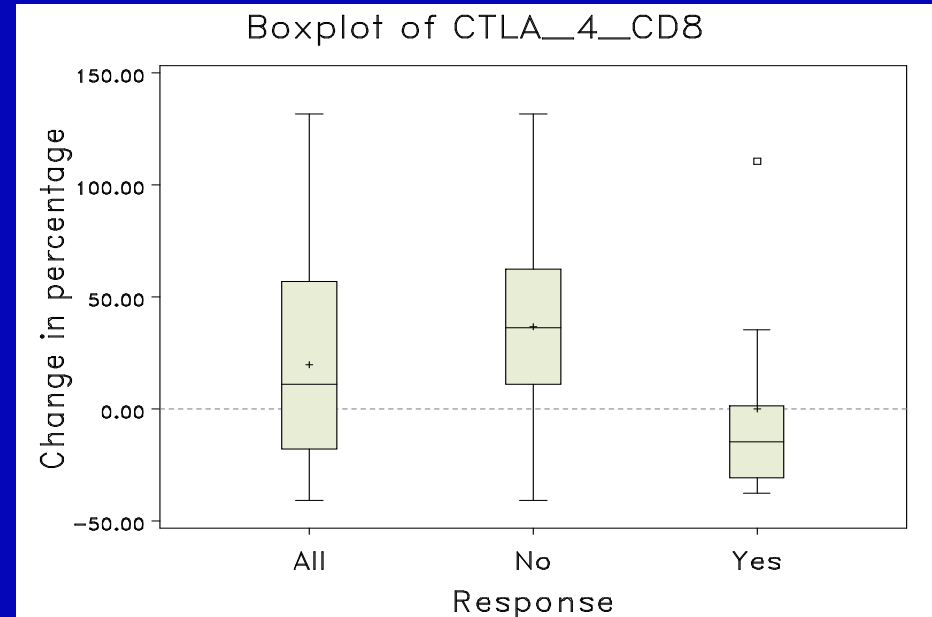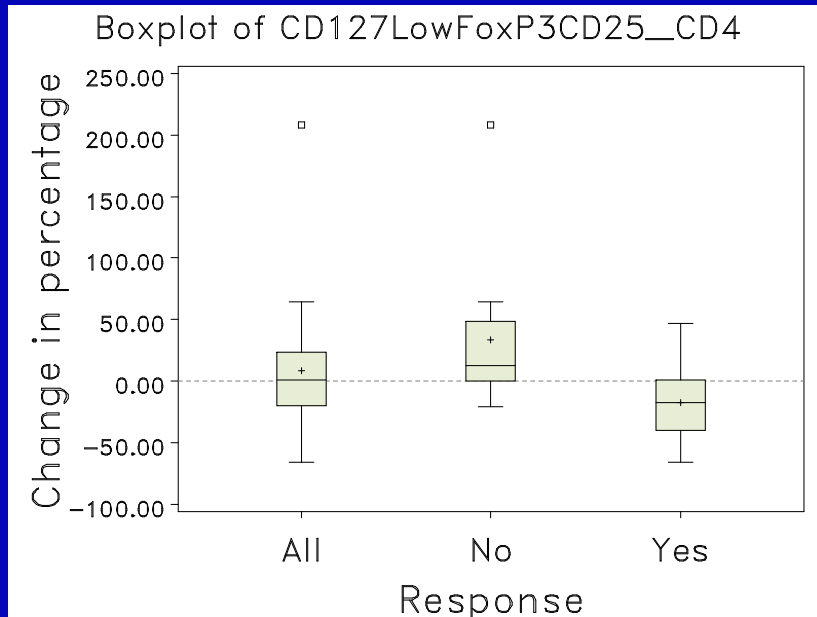
From Kudchadkar R et al ASCO 2012

Regression With Ipilimumab After Failing Anti-PD-1 Ab

Pretreatment 5-18-11

Postcycle 1 ipilimumab 8-16-11

From Kudchadkar R et al ASCO 2012

Conclusions

- Ipilimumab induces a marked tumor T cell CD8 infiltrate in which PD-1 expressing cells may predominate; it induces circulating ICOS+ CD4 and CD8 T cells

- Pre-treatment levels of CD8/EOMES/Ki67 and post treatment CD8/EOMES/granzyme B expressing T cells are associated with outcome after ipilimumab

- Increase in ALC may be an ipi PD biomarker for OS

- Preliminary data support an association of PD-L1 expression in pretreatment tumor biopsies with clinical outcomes after PD-1 antibody; to be further explored

- These biomarker data support sequencing trials of PD-1 antibody and ipilimumab and suggest predictive markers