

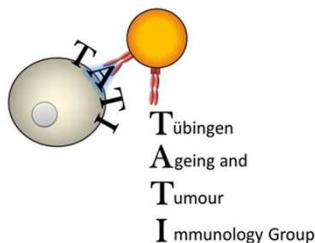
# Relationship between the peripheral immune profile and tumour antigen T cell responses in breast cancer patients

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Progress In Vaccination Against Cancer  
(PIVAC)

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## **Presenter Disclosure Information**

No Relationships to Disclose

## Immune features as prognostic markers

- NY-ESO and Melan A reactive T cells are independent prognostic factors in late stage melanoma
- High MDSC levels independently correlate with survival in melanoma
- Role of suppressor and effector cells in breast cancer still emerging
- Aims of the current study:
  - Evaluate peripheral immune parameters incl. suppressor and effector cells as prognostic markers in breast cancer
  - Investigate relationships between these features

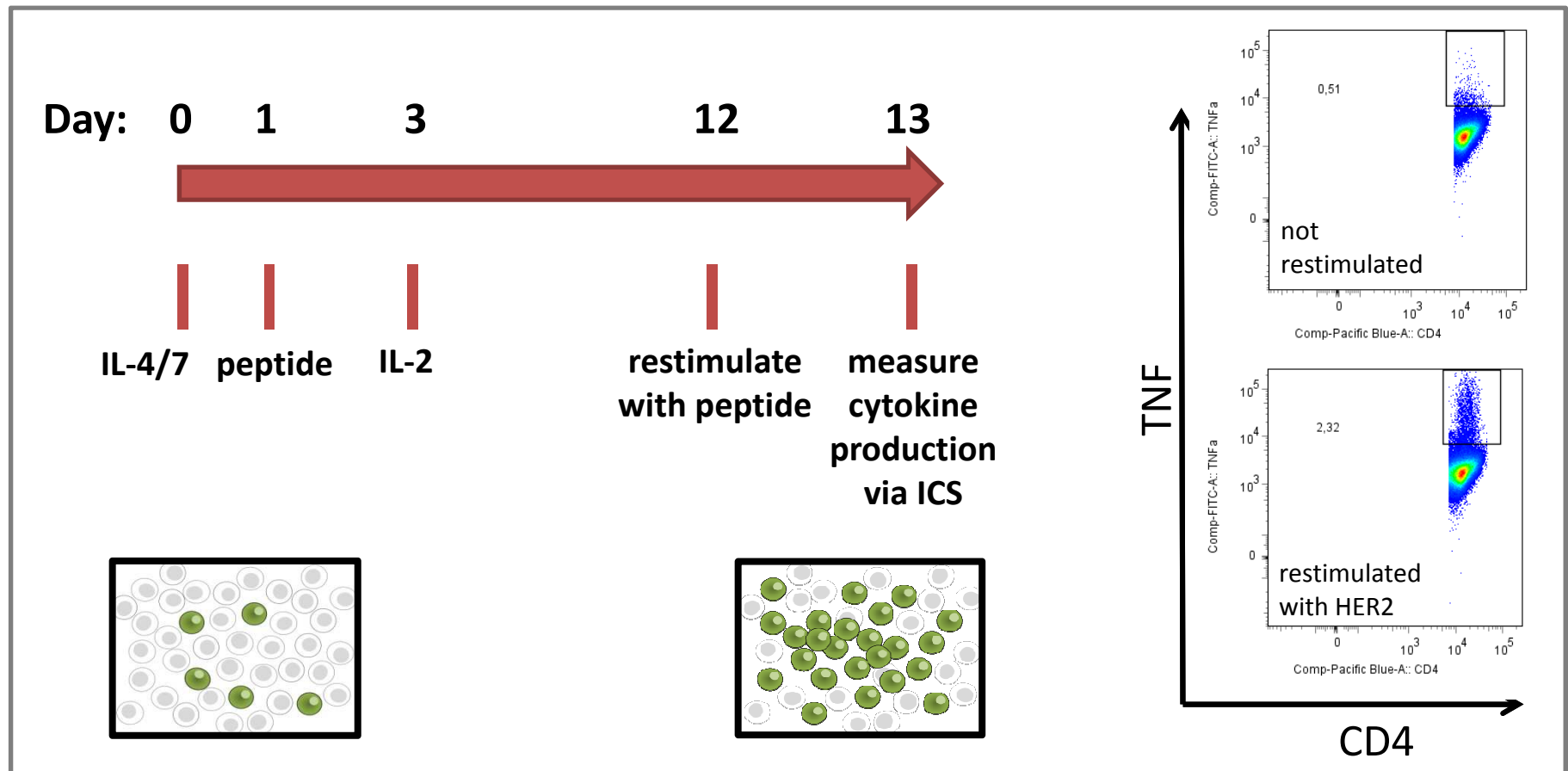
Weide, *et al.* 2012 (JCO)

Weide, *et al.* 2014 (CCR)

## Immune features as prognostic markers in breast cancer

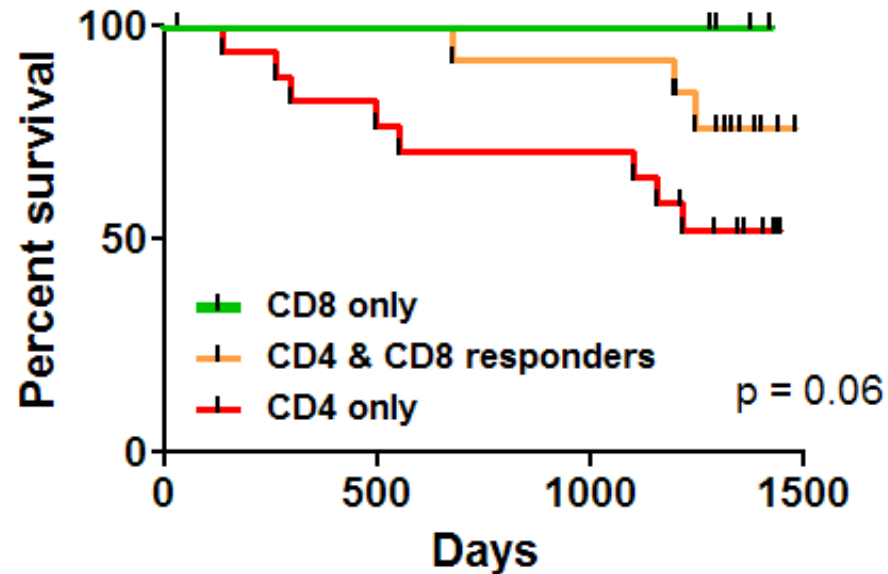
- **Cohort 1** (N = 75) tested for polyfunctional CD4 and CD8 T cell responses (TNF, IFN $\gamma$ , IL-2/5/10/17) to the HER2 tumour-associated antigen

## Detection of antigen-reactive T cells



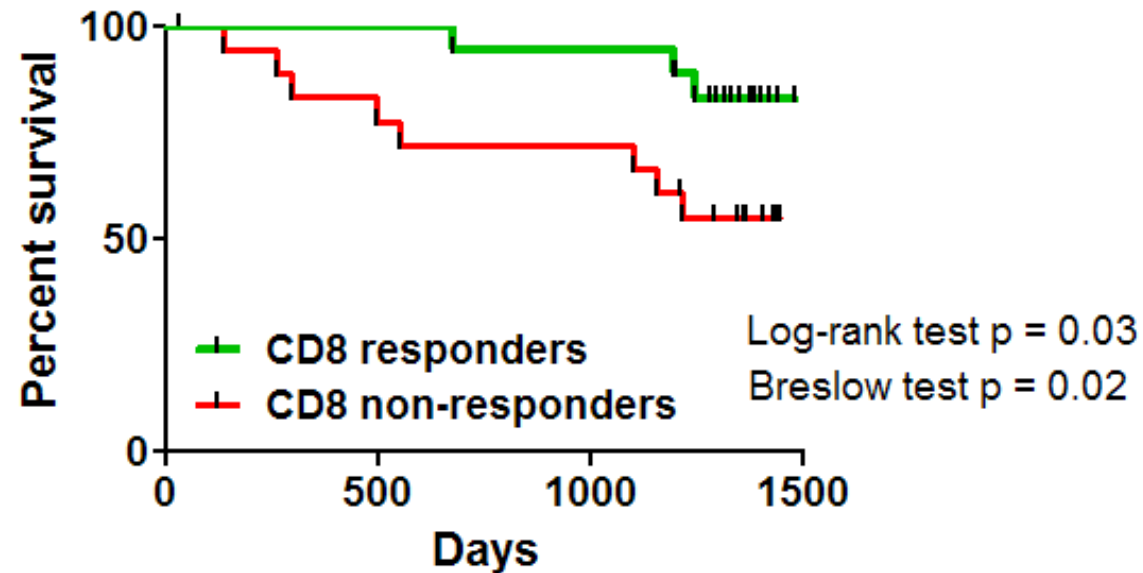
- Criteria for positive response:**
- 1) Clear positive population present (IFN $\gamma$ , TNF- $\alpha$ , IL-2, IL-5, IL-10, IL-17)
  - 2) Positive population has to be 2x the unstimulated sample
  - 3) Flu control must be positive

## HER2 reactive T cells predict survival in breast cancer (Cohort 1)



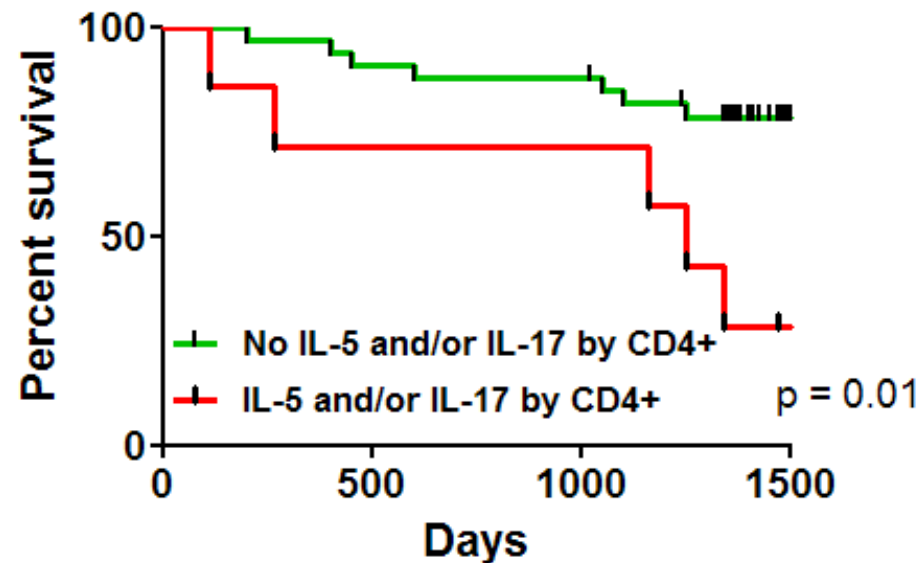
*Bailur JK et al., Breast Can Res 2015*

## HER2 reactive T cells predict survival in breast cancer (Cohort 1)



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*Bailur JK et al., Breast Can Res 2015*



## **Investigation of immune features as prognostic markers in breast cancer (Cohort 2, ISPE-BREAST)**

- 50 early stage patients with invasive ductal carcinoma (Cohort 2)
- Peripheral features (PI G. Pawelec. University Hospital Tübingen):
- Immunophenotyping:
  - Myeloid cells (including monocytes, MDSCs, pDCs and mDCs)
  - T cells (including Tregs and markers of differentiation and proliferation)
- Polyfunctional CD4 and CD8 T cell responses (TNF, IFN $\gamma$ , IL-2/5/10/17) to tumour-associated antigens (HER2, MUC-1 and Survivin)

## **Investigation of immune features as prognostic markers in breast cancer (Cohort 2, ISPE-BREAST)**

- Intra-tumoural features (PI C. Baxevanis. St. Savas Cancer Hospital, Athens)
  - T cells (CD4, CD8, FOXP3)
  - Macrophages (CD163)
  - Serum cytokines (IL-1Ra, IL-9, IL-10, TGF $\beta$ 1, RANTES)

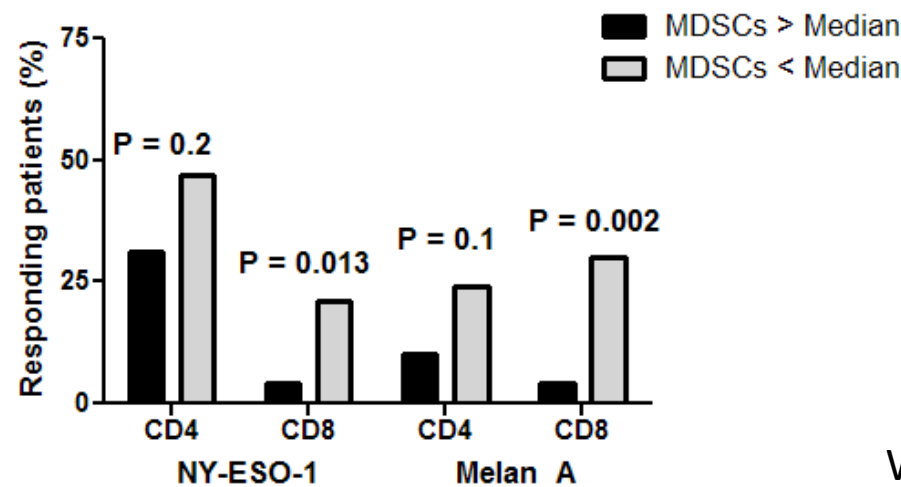
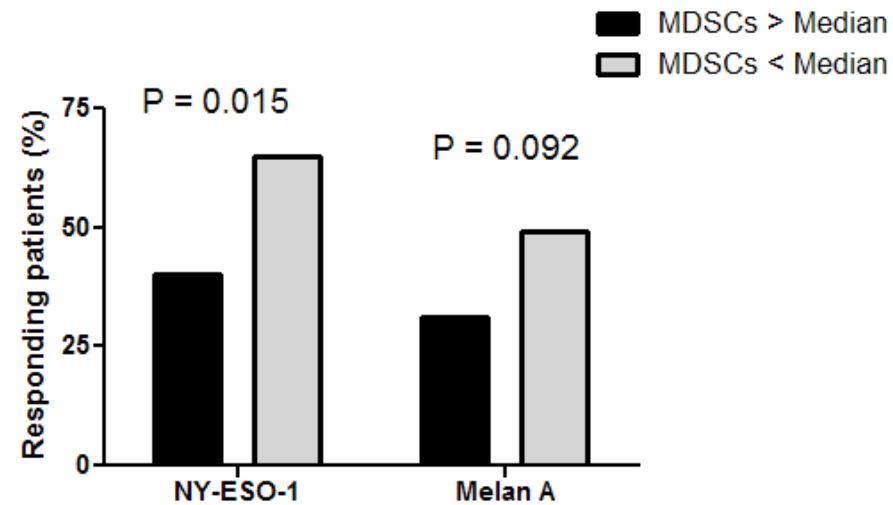
## **Investigation of immune features as prognostic markers in breast cancer (Cohort 2, ISPE-BREAST)**

- Few studies have compared peripheral and intra-tumoural immune features
- Evaluation of these features as prognostic markers

## **Can the peripheral immune profile predict T cell responses to tumour-associated antigens?**

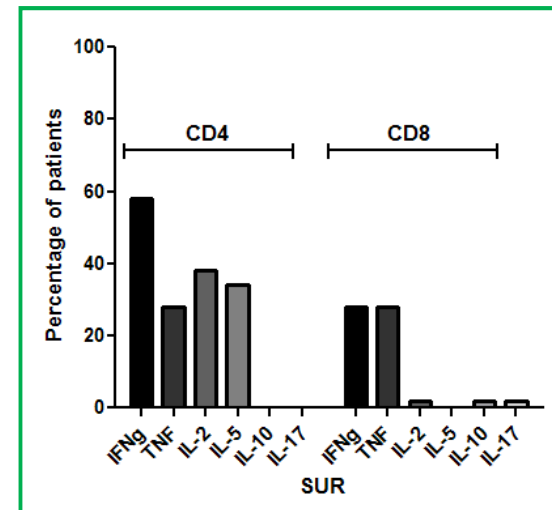
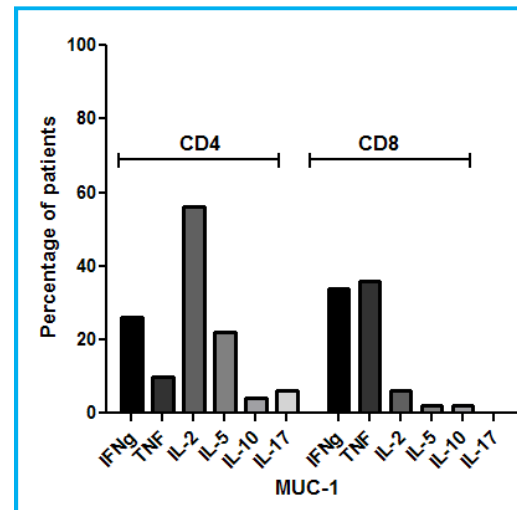
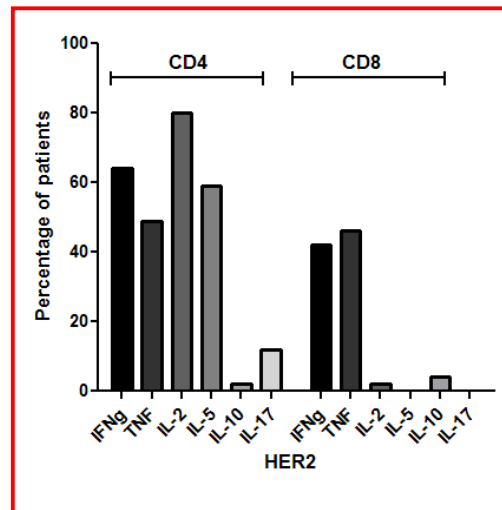
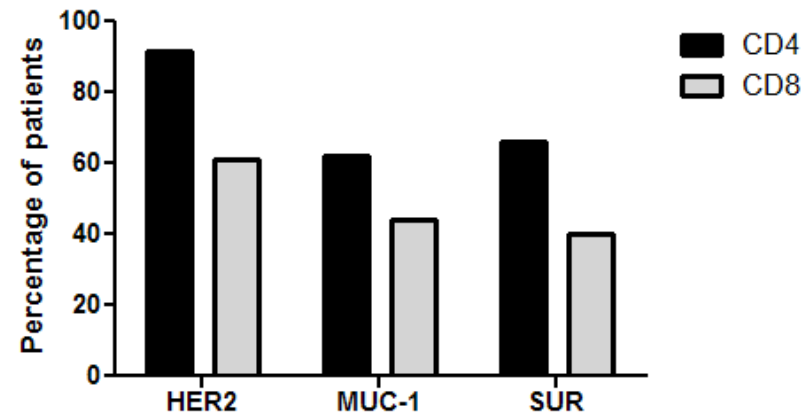
- TAA responses are prognostic, but resource intensive to perform
- Measurement of immunomodulatory cells relevant to T cell TAA responses?

## MDSC levels in blood correlate with T cell responses to TAAs in melanoma



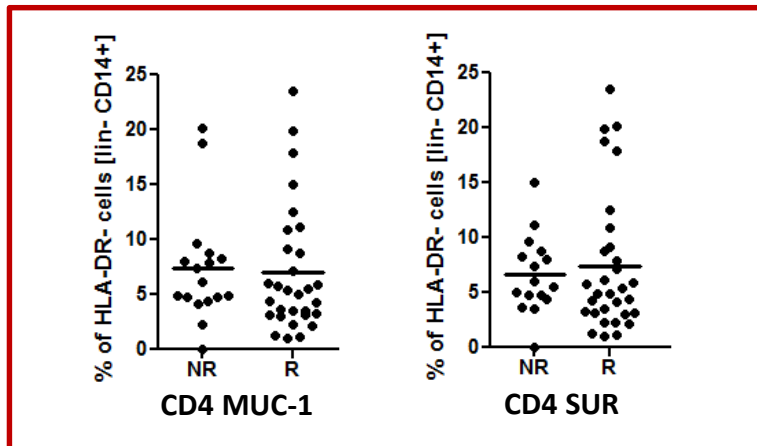
Weide, *et al.* 2014 (CCR)

## T cell responses to HER2, MUC1 and SUR (Cohort 2)

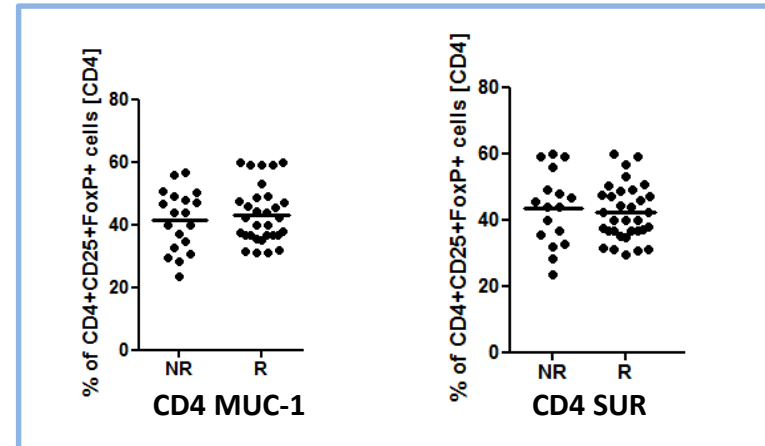


## Association between peripheral immune populations and T cell responses (Cohort 2)

- 23 myeloid populations, 30 T cell populations and NK cells tested for association with T cell responses to HER2, MUC-1 and SUR:
  - CD4 or CD8 responses by any cytokine
  - Patients who responded by CD4 and CD8 Vs those who did not respond by either
  - Comparing response to all three antigens collectively



MDSC-like cells

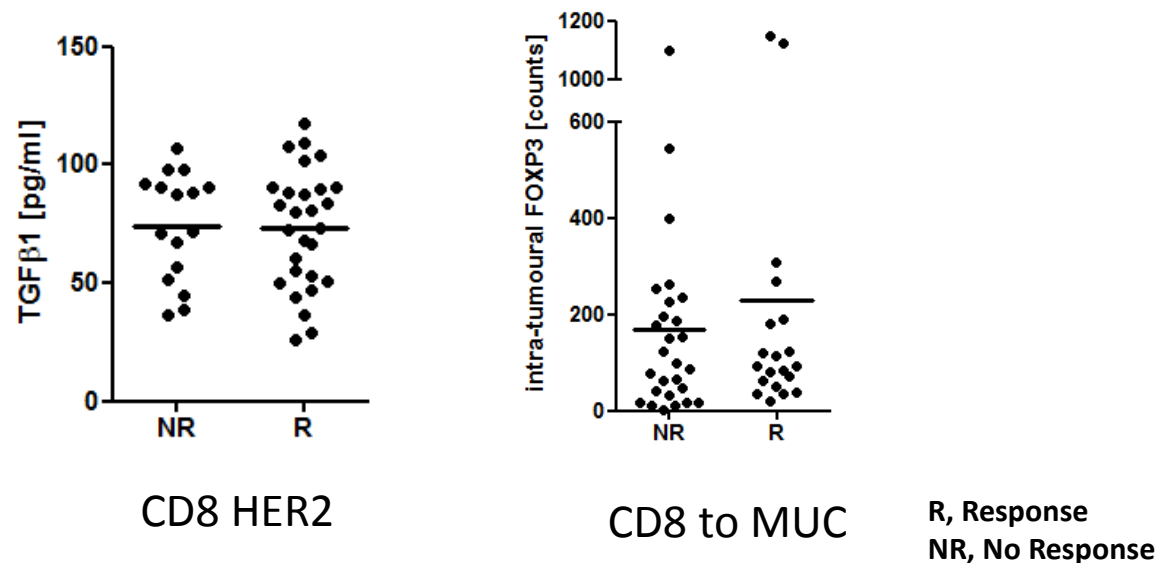


Tregs

R, Response  
NR, No Response

## If T cell responses to TAAs are not associated with peripheral immune cells, are they associated with TILs or serum cytokines? (Cohort 2)

- T cell responses to HER2, MUC-1 and SUR tested for association with:
  - Tumour infiltration by CD4, CD8 FOXP3 T cells and 163+ macrophages
  - Serum levels of IL-1Ra, IL-9, IL-10, RANTES and TGF $\beta$ 1

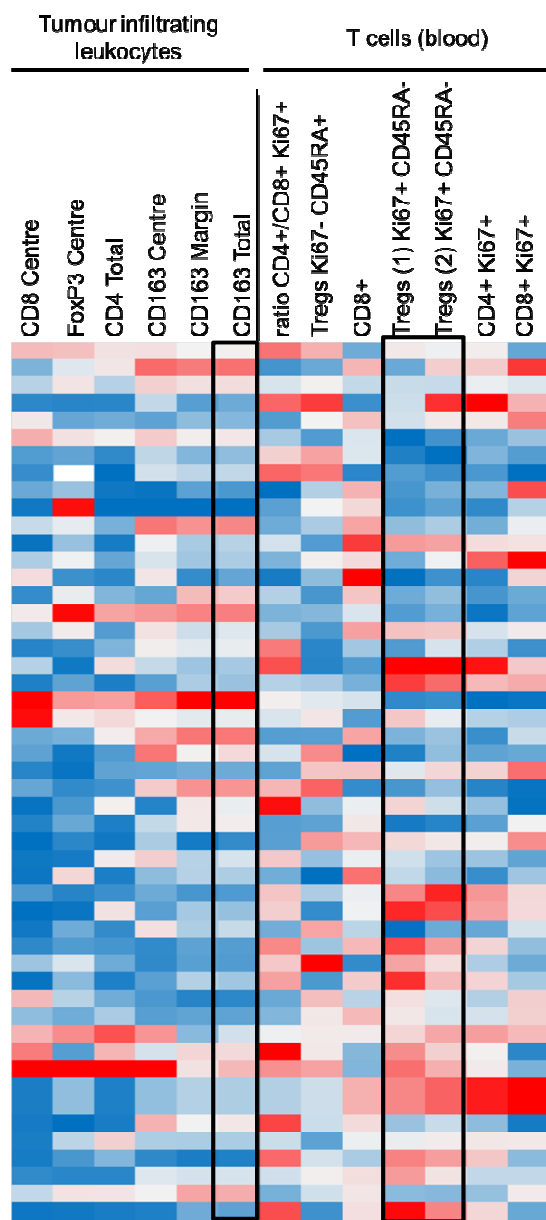




## **Does the immune profile in blood relate to that in the tumour? (Cohort 2)**

- TAA responses are not related to the peripheral immune or intra-tumoural immune profile
- Is there a relation between the intra-tumoural and peripheral immune profile?

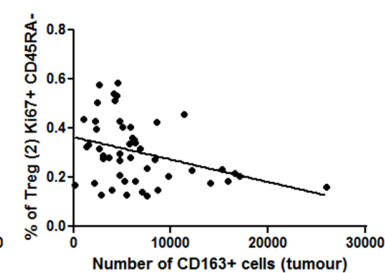
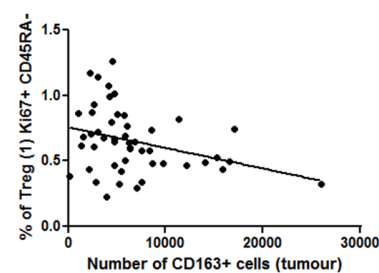
## Tumour infiltrating leukocyte levels correlate with T cells in peripheral blood (Cohort 2)



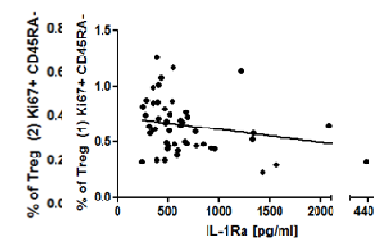
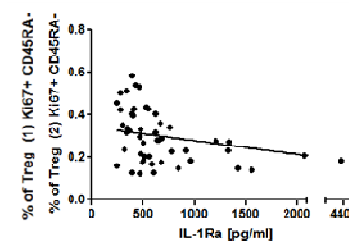
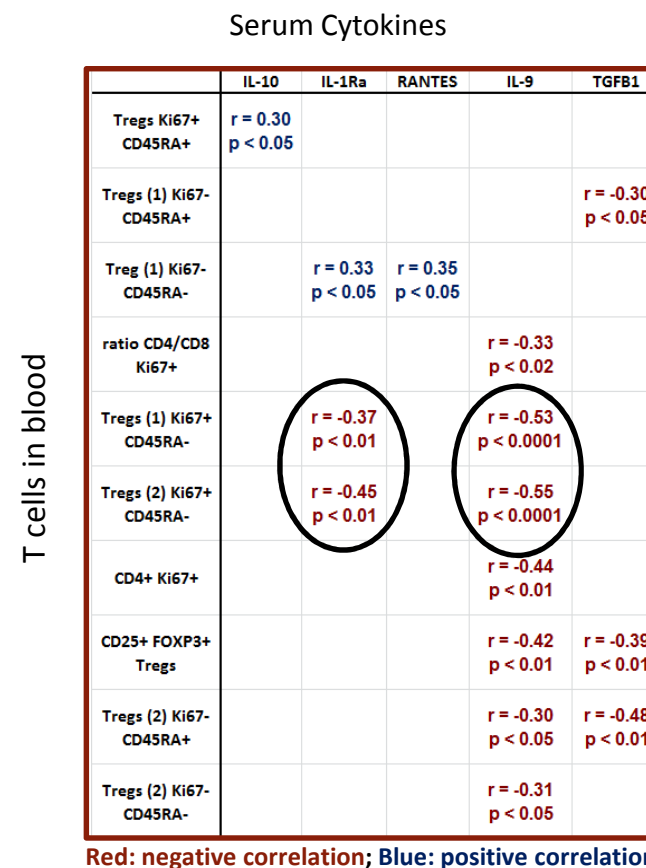
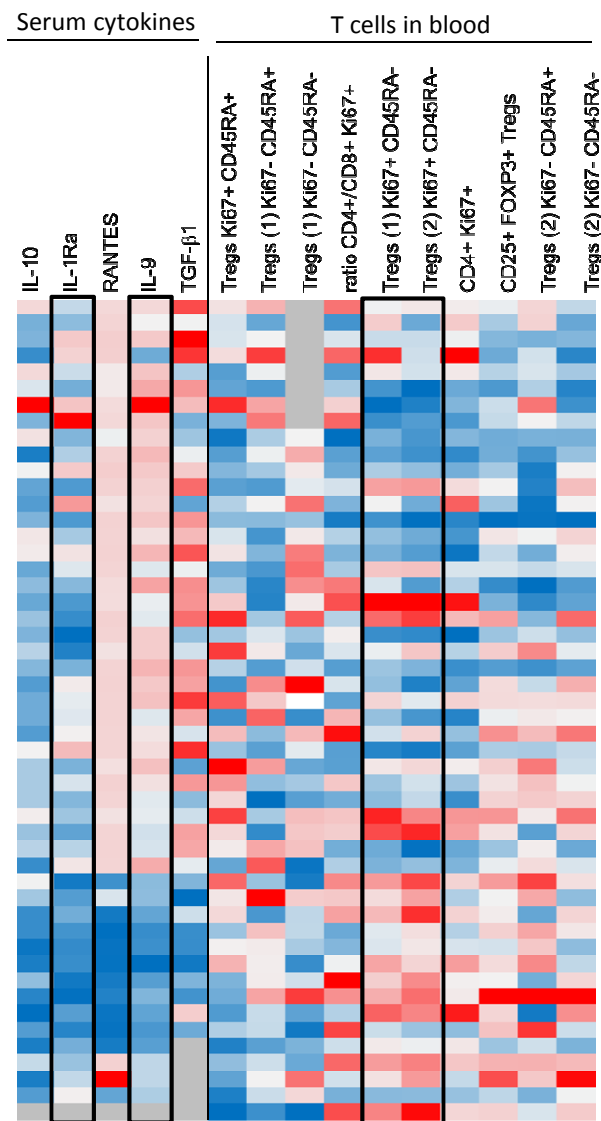
Tumour Infiltrating Leukocytes

	CD8 Centre	FoxP3 Centre	CD4 Total	CD163 Centre	CD163 Margin	CD163 Total
ratio CD4/CD8 Ki67+		$r = -0.31$ $p < 0.05$				
Tregs Ki67- CD45RA+	$r = 0.28$ $p < 0.05$					
CD8+			$r = -0.29$ $p < 0.05$			
Tregs (1) Ki67+ CD45RA-		$r = -0.31$ $p < 0.05$		$r = -0.35$ $p < 0.02$	$r = -0.34$ $p < 0.02$	
Tregs (2) Ki67+ CD45RA-				$r = -0.40$ $p < 0.01$	$r = -0.29$ $p < 0.05$	$r = -0.34$ $p < 0.02$
CD4+ Ki67+				$r = -0.41$ $p < 0.01$		$r = -0.39$ $p < 0.01$
CD8+ Ki67+				$r = -0.43$ $p < 0.01$		$r = -0.40$ $p < 0.01$

Red: negative correlation; Blue: positive correlation



## Serum levels of IL-1Ra, IL-9, IL-10, RANTES and TGFβ are associated with T cell phenotypes in peripheral blood (Cohort 2)



## Tumour infiltrating leukocyte levels correlate with peripheral myeloid and NK cells (Cohort 2)

### Tumour Infiltrating Leukocytes

	CD4 Centre	CD8 Centre	CD8 Margin	CD8 TOTAL	CD163 Centre	CD163 Margin	CD163 TOTAL	FOXP3 Centre	CD3 Centre	CD3 TOTAL
CD14	$r = -0.30$ $p < 0.05$								$r = -0.30$ $p < 0.05$	
mDCs	$r = -0.33$ $p < 0.05$		$r = -0.30$ $p < 0.05$	$r = -0.31$ $p < 0.05$	$r = -0.36$ $p < 0.02$	$r = -0.32$ $p < 0.05$	$r = -0.35$ $p < 0.02$		$r = -0.37$ $p < 0.02$	
pDCs		$r = -0.36$ $p < 0.02$		$r = -0.31$ $p < 0.05$				$r = -0.34$ $p < 0.05$	$r = -0.39$ $p < 0.01$	$r = -0.31$ $p < 0.05$
ratio mDCs/HLADR- pDCs		$r = 0.31$ $p < 0.05$				$r = -0.30$ $p < 0.05$				
lin- CD14+	$r = -0.31$ $p < 0.05$						$r = -0.29$ $p < 0.05$		$r = -0.30$ $p < 0.05$	
ratio NK cells/monocy- tes						$r = 0.35$ $p < 0.02$	$r = 0.33$ $p < 0.05$	$r = 0.32$ $p < 0.05$		
ratio NK cells/mDCs					$r = 0.40$ $p < 0.01$	$r = 0.43$ $p < 0.01$	$r = 0.44$ $p < 0.01$			
ratio NK cells/pDCs		$r = 0.32$ $p < 0.05$					$r = 0.30$ $p < 0.05$	$r = 0.37$ $p < 0.02$	$r = 0.29$ $p < 0.05$	
lin- CD14+ HLA-DR-									$r = -0.30$ $p < 0.05$	

Red: negative correlation; Blue: positive correlation

## Conclusions

- T cell responses to HER2 are independent prognostic features in breast cancer
- Peripheral (T cells, myeloid cells, NK cells and serum cytokines) or intra-tumoural immune features (T cells and macrophages) are not related to T cell responses to HER2, MUC-1 and SUR
- Peripheral T cells, myeloid and NK cells are associated with serum cytokines as well as intra-tumoural leukocytes
- Pending clinical follow-up will reveal associations between these intra-tumoural and peripheral immune features in relation to patient prognosis

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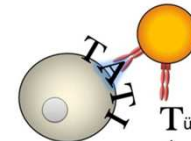
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