

# **ERB B2 (Her 2/neu)**

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**Member of the epidermal growth factor receptor (EGFR) family**

**Ligand induced interaction results in activation of tyrosine kinase intracellular domain signaling cascades that mediate cell growth, differentiation and survival**

**Herceptin (Trastuzumab) is a monoclonal antibody that binds to the extracellular domain of the receptor**

**High expression in some cancers:**

**breast**

**ovarian**

**colorectal**

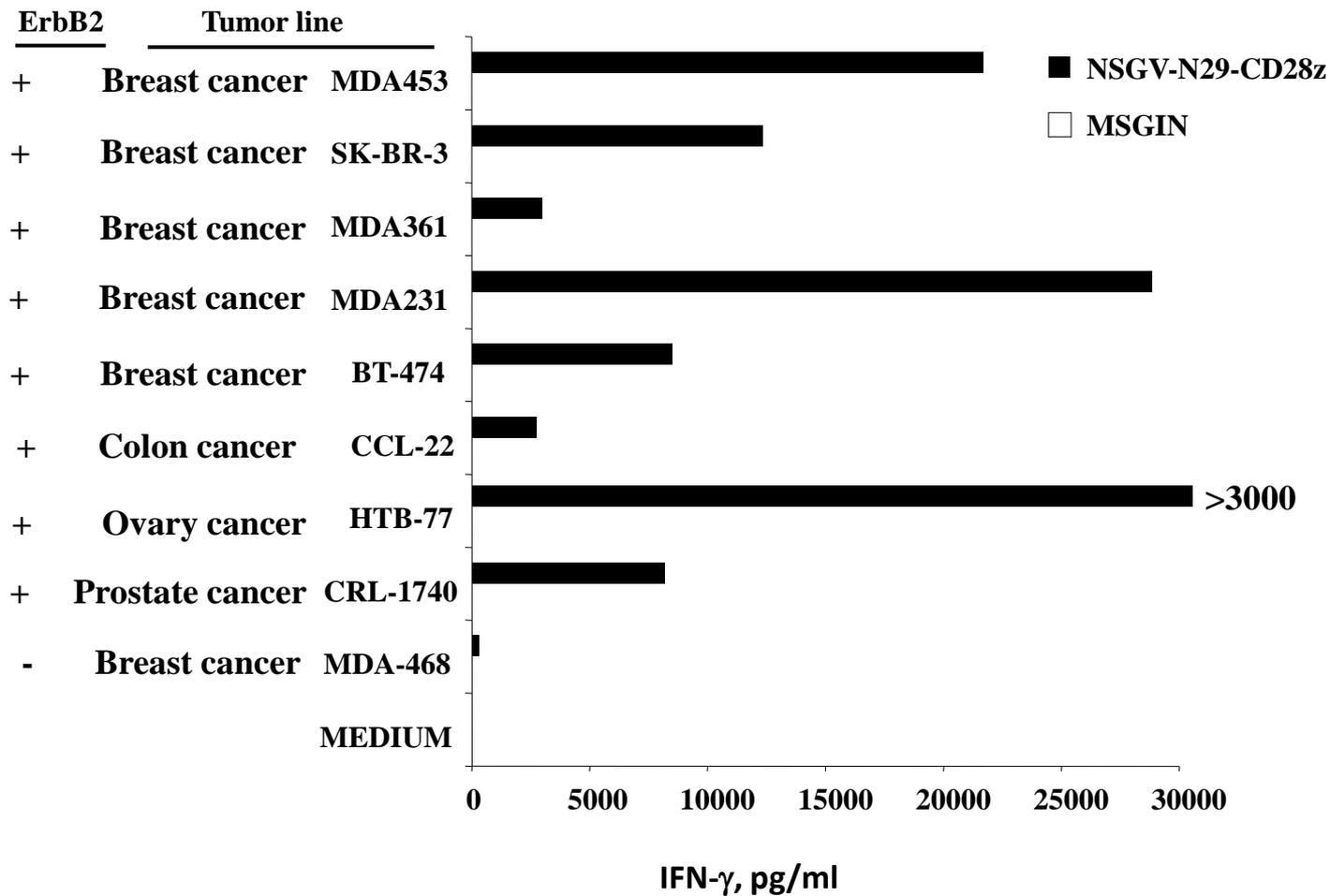
**stomach**

**NSCLC**

**and others**

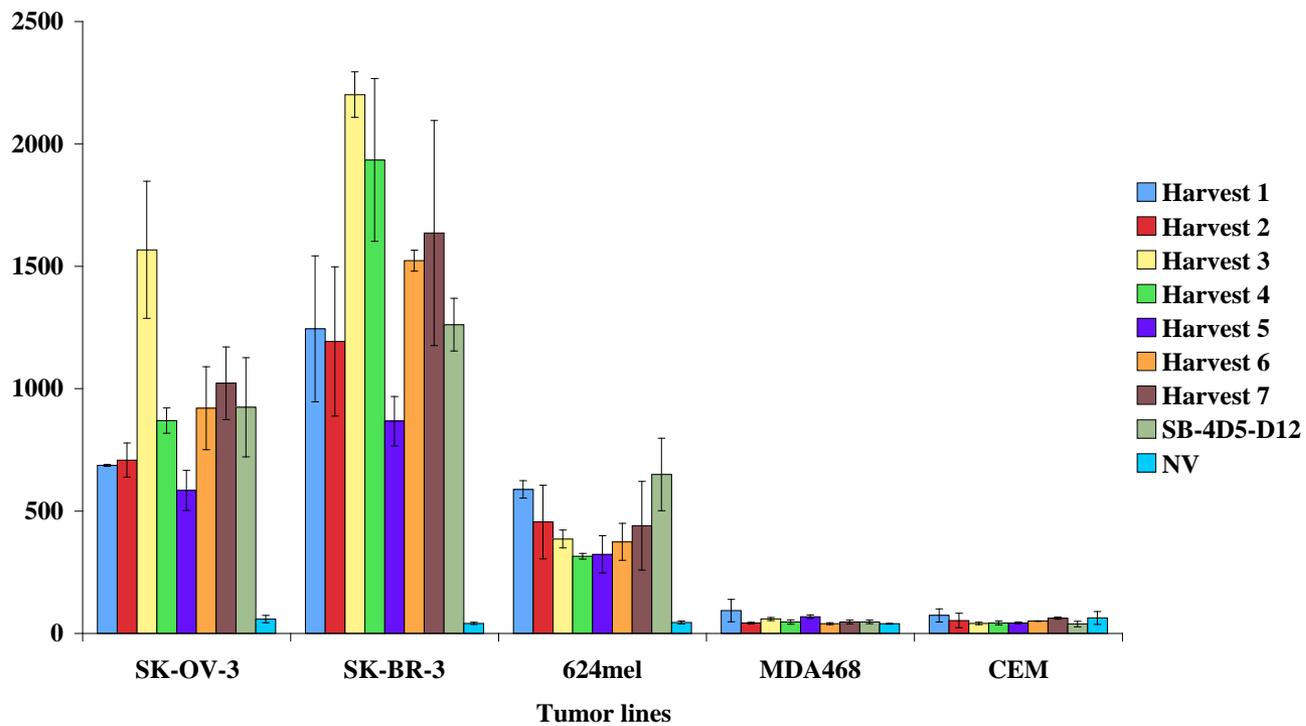
# Recognition of Non-melanoma Tumors by HER-2 Chimeric Receptor Transduced

PBL



### Testing Clinical Grade Herceptin-CAR (4D5-D12) Supernatant

1:1 dilute



# **Safety Considerations in the Use of an Anti-Her-2 CAR**

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**>100,000 women have received trastuzumab (Herceptin; anti-Her-2)**

**Dozens of published studies of vaccination of humans against Her-2 without toxicity**

**Published study of autologous anti-Her-2 T cells administered to cancer patient; no toxicity  
(Bernhard et al, Cancer Immunol. Immunother., 57:271-280, 2008)**

**Extensive in vivo models in mice targeting Her-2 with CAR transduced T cells; no toxicity  
(Pinthus et al, Cancer Res. 63:2470-2476, 2003)  
(Kershaw et al, J. Immunol. 173:2143-50, 2004)**

## Adoptive transfer of autologous, HER2-specific, cytotoxic T lymphocytes for the treatment of HER2-overexpressing breast cancer

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**Abstract** The human epidermal growth factor receptor 2 (HER2) has been targeted as a breast cancer-associated antigen by immunotherapeutical approaches based on HER2-directed monoclonal antibodies and cancer vaccines. We describe the adoptive transfer of autologous HER2-specific T-lymphocyte clones to a patient with metastatic HER2-overexpressing breast cancer. The HLA/multimer-based monitoring of the transferred T lymphocytes revealed that the T cells rapidly disappeared from the peripheral blood. The imaging studies indicated that the T cells accumulated in the bone marrow (BM) and migrated to the liver, but were unable to penetrate into the solid metastases. The disseminated tumor cells in the BM disappeared after the completion of adoptive T-cell therapy. This study suggests the therapeutic potential for HER2-specific T cells for eliminating disseminated

HER2-positive tumor cells and proposes the combination of T cell-based therapies with strategies targeting the tumor stroma to improve T-cell infiltration into solid tumors.

**Keywords** Tumor immunity · Human · Cytotoxic T cells · Antigens/peptides/epitopes · MHC

### Abbreviations

HER2	Human epidermal growth factor receptor 2
<sup>111</sup> In	Indium-111
SPECT	Single photon emission computed tomography
MRI	Magnetic resonance tomography
FDG-PET	[ <sup>18</sup> F] Fluorodeoxyglucose positron emission tomography
MNC	Mononuclear cell

(Total of 2.7e9 autologous HER-2 reactive cells infused. No toxicity. Response in bone marrow but not liver).

# Her-2 Clinical Protocol

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## Eligibility

≥18 years old

metastatic cancer; tumors express Her-2

non-responsive to or recurred after standard treatment

## Treatment

day -7, day -6      cyclophosphamide 60mg/kg

day -5 to day -1    fludarabine 25mg/kg

day 0                cells

IL-2 720,000 IU/kg q8h

## Cohorts

I:  $1 \times 10^9$  to  $1 \times 10^{10}$

II:  $1.1 \times 10^{10}$  to  $3 \times 10^{10}$

III:  $3.1 \times 10^{10}$  to  $1 \times 10^{11}$

## History of Patient S.A.

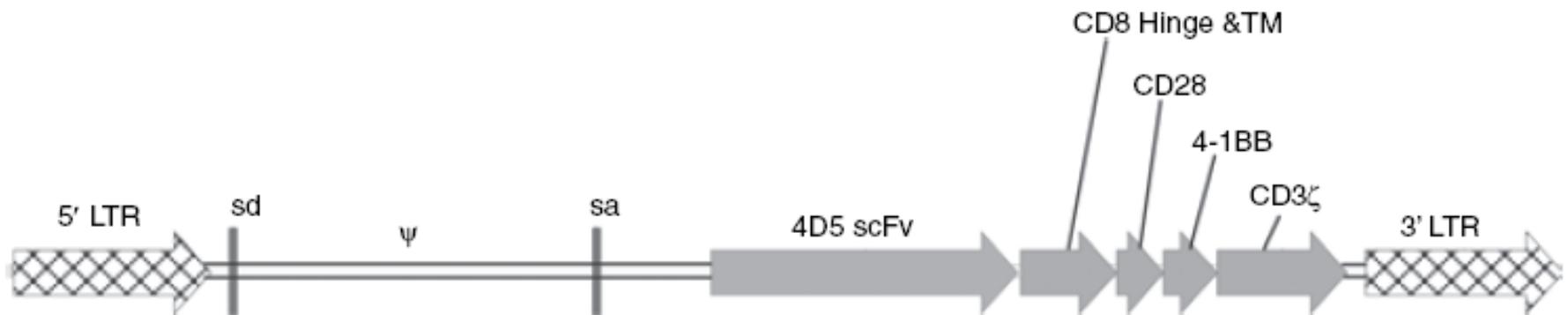
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**39 year old female with metastatic colon cancer to liver and lungs**

- 10/06 Sigmoid resection for cancer of colon; 6/21 lymph nodes positive; synchronous liver metastases present**
- 2/07 Chemotherapy with 5-fluorouracil, leukovorin (Fu/L), oxaliplatin, bevacizumab**
- 6/07 Severe allergy to oxaliplatin; this drug omitted**
- 2/08 Progressive growth of liver metastases; switched to Fu/L plus irinotecan, bevacizumab**
- 12/08 Progressive disease in liver; switched to xeloda, oxaliplatin (after desensitization), bevacizumab**
- 3/09 Progressive multiple metastases in liver and lungs**
- 4/09 To NCI for evaluation**

## Design of the Anti-Her-2 CAR Vector

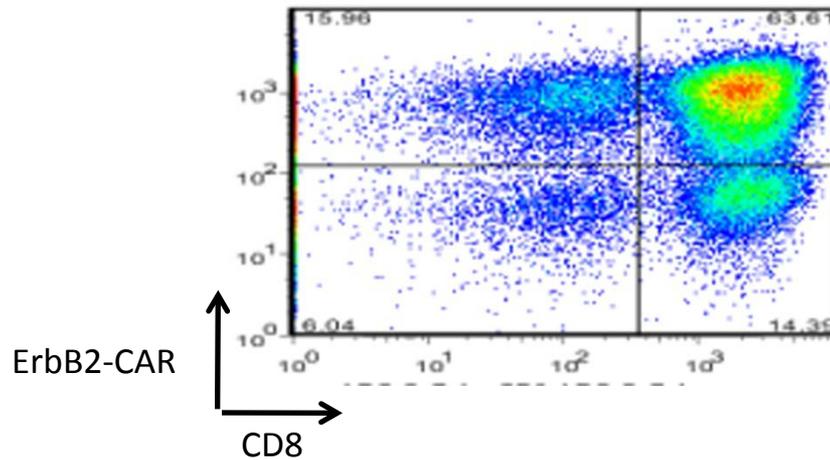
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# Gene transfer efficiency, patient 1

5 Color Stain

Summary		Patient#1 UT R2d10	Patient#1 Her-2 5% R2d10
% of total	CD4+	24	22
	CD8+	78	78
	CD3+	99	99
% of CD8	ErbB2	0	82
% of CD4	ErbB2	0	78
% of CD3	ErbB2	1	80



# COA-reactivity assay, patient 1

	<u>None</u>	<u>Melanoma Cell Line (all Her-2 +)</u>				<u>Non-melanoma Cell Line</u>				
		<u>A2-</u>		<u>A2+</u>		<u>Her2-</u>		<u>Her-2+</u>		
		<u>888</u> <u>A1,24</u>	<u>938</u> <u>A1,24</u>	<u>526</u> <u>A2,3</u>	<u>624</u> <u>A2,3</u>	<u>CEM</u>	<u>MDA468</u>	<u>MDA361</u>	<u>SK-BR3</u>	<u>SK-OV3</u>
<u>Controls</u>										
None	24	49	68	44	66	37	99	57	143	82
DM5	26	37	35	<u>3700</u>	<u>4900</u>	29	55	37	68	47
RA Her-2 Control	82	<u>&gt;35660</u>	<u>&gt;24870</u>	<u>&gt;24310</u>	<u>&gt;26370</u>	186	138	<u>&gt;49530</u>	<u>&gt;54780</u>	<u>&gt;58690</u>
<u>patient #1 Her-2 CAR PBL</u>										
R2d10	27	<u>13140</u>	<u>11950</u>	<u>11650</u>	<u>&gt;20340</u>	101	113	<u>&gt;24520</u>	<u>&gt;34080</u>	<u>&gt;58380</u>
	65	<u>12350</u>	<u>14470</u>	<u>11210</u>	<u>&gt;21790</u>	127	155	<u>&gt;29110</u>	<u>&gt;32690</u>	<u>&gt;58580</u>
UT PBL R2d10	46	69	81	93	127	117	103	82	144	146

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- 3/09 Progressive multiple metastases in liver and lungs**
- 4/09 To NCI for evaluation**
- 4/17/09 Start cyclophosphamide (2d) and fludarabine (5d) preparative regimen**
- 4/24/09 Cell infusion**

# Clinical Course: HER-2 CAR

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## April 24, 2009

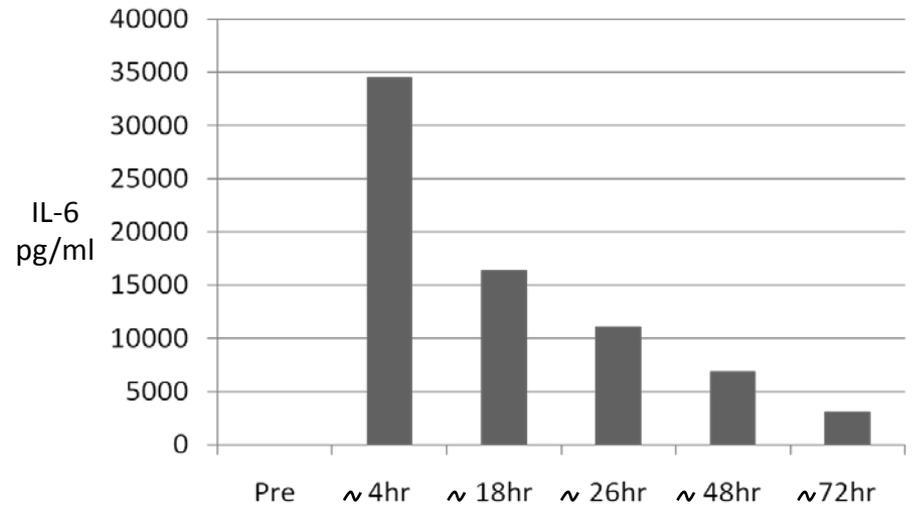
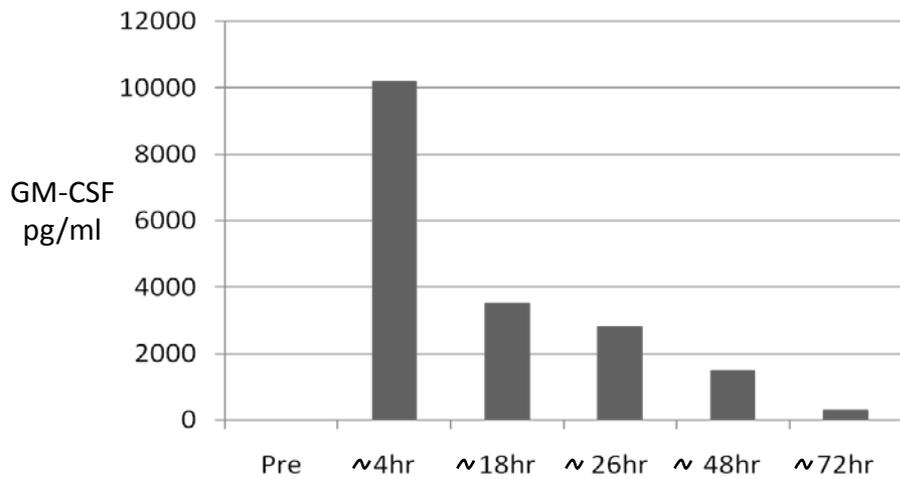
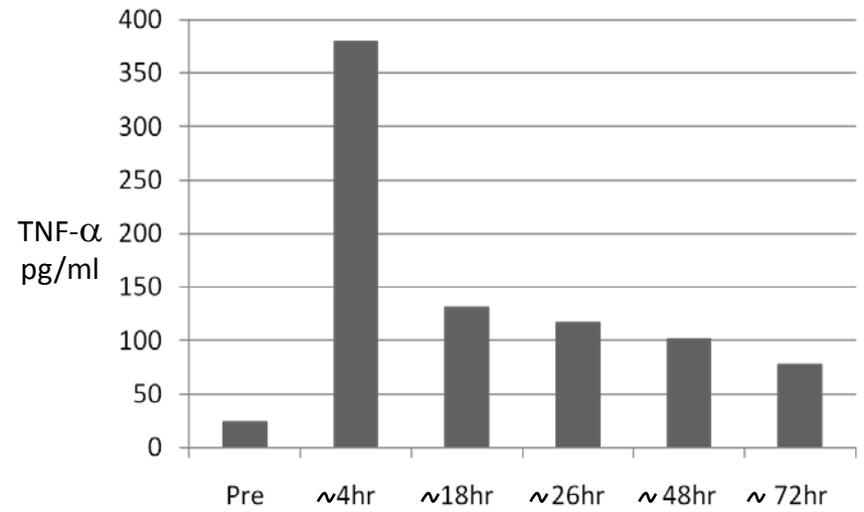
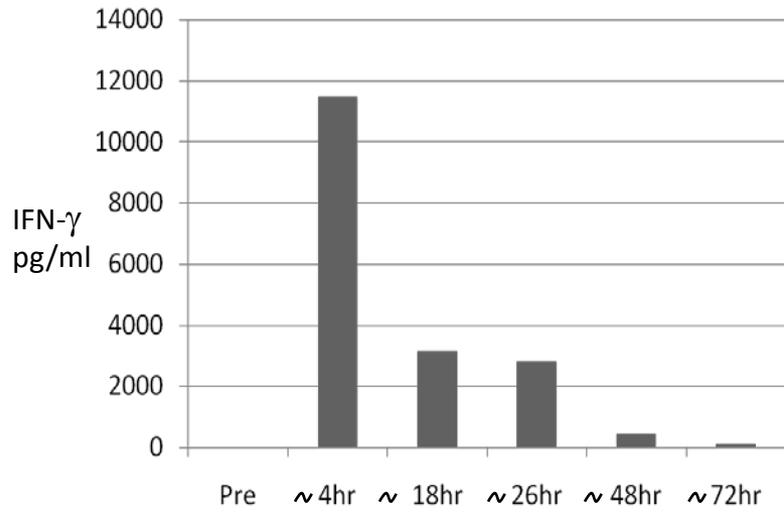
- Noon:** 1x10<sup>10</sup> cells administered  
Respiratory distress within 15 minutes; to ICU  
Chest x-ray showed early pulmonary edema  
Intubated electively within 1 hour  
Progressive hemodynamic instability requiring multiple  
vasopressors  
Multiple antibiotics and intensive monitoring  
Hemodialysis
- 5:35 p.m.:** Cardiac arrest, CPR  
Dexamethasone  
Progressive deterioration of pulmonary function

## April 29, 2009

**3:00 p.m.:** Died despite intensive support

**Autopsy:** Widespread hemorrhage  
Metastatic carcinoma in lungs and liver

# Serum cytokines, patient 1



## **Likely cause of this complication**

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**Recognition of very low levels of HER-2 on lung epithelium leading to:**

**cytokine release**

**pulmonary capillary leak**

**hypotension**

# Surgery Branch experience with administration of TCR gene-modified lymphocytes

<u>Target cell</u>	<u>Transgene</u>	<u>Conditioning</u>	<u>Number of patients</u>	<u>TRM</u>
PBL	MART-1 (F4)	NMA	31	0
TIL	MART-1 (F4)	NMA	3	0
PBL	MART-1 (F4)	NMA+1200TBI	4	0
PBL	gp100 (209)	NMA	11	0
TIL	gp100 (209)	NMA	3	0
PBL	p53	NMA	11	0
PBL	MART-1 (F5)	NMA	24	0
TIL	MART-1 (F5)	NMA	3	0
PBL	gp100 (154)	NMA	19	0
TIL	gp100 (154)	NMA	2	0
PBL	gp100(154)+MART-1(F5)	NMA+600TBI	4	0
PBL	MART-1 (F5)-ALVAC	NMA	2	0
PBL	gp100 (154)-ALVAC	NMA	2	0
PBL	Her2/neu-CAR	NMA	1	1
PBL	NY-ESO-1	NMA	19	0
PBL	2G1-RCC	NMA	2	0
PBL	CEA	NMA	3	0
PBL	CD19-CAR	NMA	2	0

**Total: 146 1**

