

# Multi-Institution Trials and the Eastern Cooperative Oncology Group (ECOG)

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Which vaccines are superior and should be moved forward?

vaccine A: IFN $\gamma$ /CD8 $^+$  ELISPOT of 20 spots/10e5

vaccine B: IFN $\gamma$ /CD8 $^+$  ELISPOT of 200 spots/10e5?

## **Scientific Goal:**

To identify important immune biomarkers which might be predictive of clinical outcome, or ability to respond to an intervention:

Need: reliable, standardized measures of immune response.

**CLIA** (Clinical Laboratory Improvements Amendments) rules:

Test Accuracy (close agreement to the true value),

Precision (agreement of independent results: same day, different day),

Reproducibility (intra-assay and inter-assay)

Reportable range (limits of detection)

Normal ranges (pools of healthy donors, accumulated patient samples),

Personnel competency testing

Equipment validation, monitoring

Reagent tracking

# Advantages of a Central Laboratory

Quality and reliability—external QA/QC

State-of-the-art assay development, standardization  
and validation

Decreased cost of immune monitoring: essential for  
large scale protocols

Assay consultation and result interpretation in  
conjunction with analysis by biostatisticians

Banks of samples for normal controls and comparisons

# ECOG Central Immunology Laboratory

Clinical Site

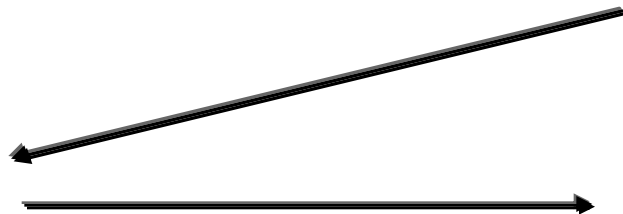
Central Lab

Screen or enrollment:  
fax blood kit request



Kit prepared and  
shipped ground

Pt. blood draw  
mailed O/N to lab



Blood processed and  
banked according to  
SOPs within 24 hours



Assays performed per  
SOPs, send results

ECOG coordinating  
center gathers data;  
transfers to ECOG  
biostatistics



Results to  
trial PI



# ECOG Samples and Assays: 2004-2008

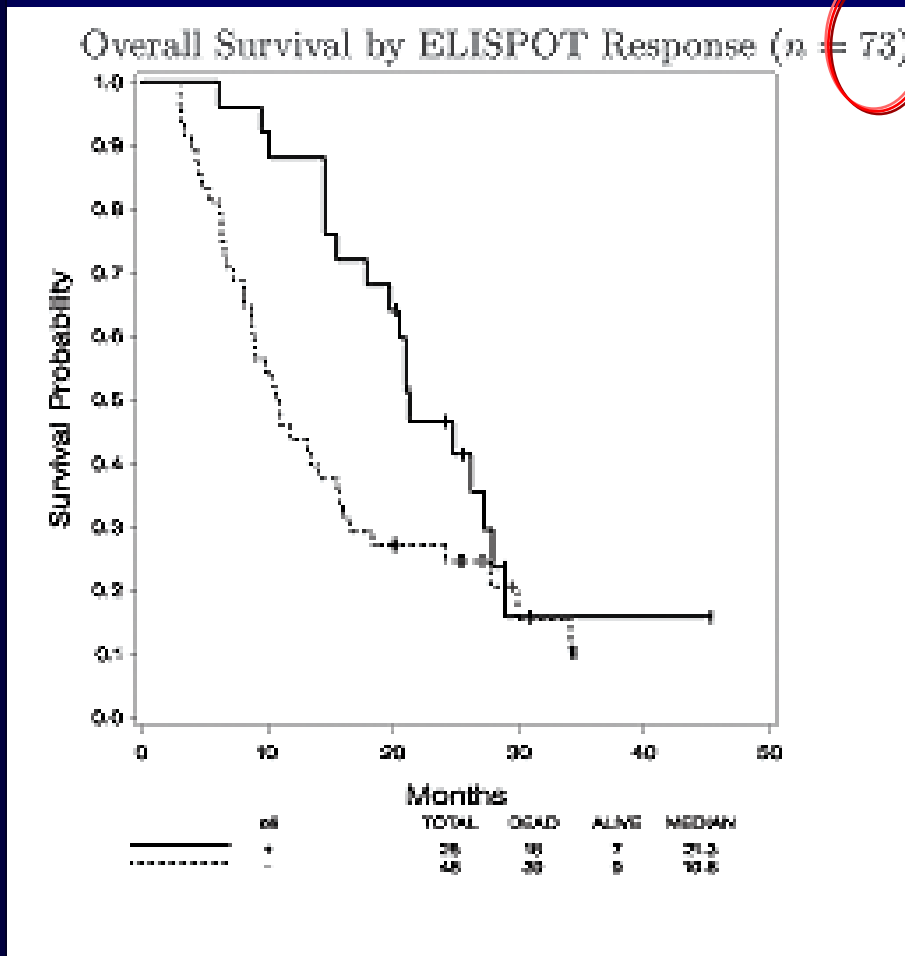
<u>Protocol</u>	<u>Samples</u>	<u>Assays</u>
•E4697	>1800	>5,800 (HLA typing, Dendritic Cell blood progenitors, ELISPOT T cell frequency)
•E1602	>750	(screening sera for cytokines)
•E1603	10	
•E1696	1	>350 (MHC tetramer)
•E1697	85	(accruing, assays planned)
•E1402	12	
•E1902	23	
•E9802	71	(ELISPOTs and anti-PSA antibody)

Totals: 2,970 specimens from 1,238 patients (>2,900 kits sent to sites); 18,036 assays completed; 55,577 vials cryopreserved.



# Immune Response Correlates with Overall Survival

Multiple melanoma antigen peptide vaccine ± GM-CSF ± IFN $\alpha$ 2b



The Kaplan-Meier plot for OS by immune response status is shown for E1696 (Phase II).

*There was a significant difference in OS by immune response status. Immune responders lived longer than the non-immune responders (median OS 21.3 versus 10.8 months, p=0.033).*

*(Kirkwood, J.M., Clin. Cancer Res. 2009)*



# ELISPOT Assays: intra-assay and inter-assay variability

## E1696

Date	pt.	Control	triplicates	%CV	
1/16/2003	16010	<b>93726</b>	421 412 363	6% CV	
1/14/2003	16002	<b>93726</b>	364 332 284	10% CV	
1/21/2003	16028	<b>93726</b>	360 285 238	17% CV	
			Positive control PMA/I		

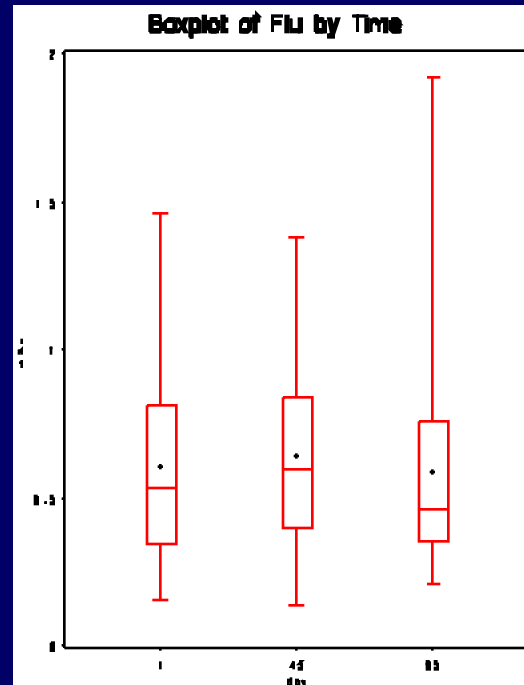
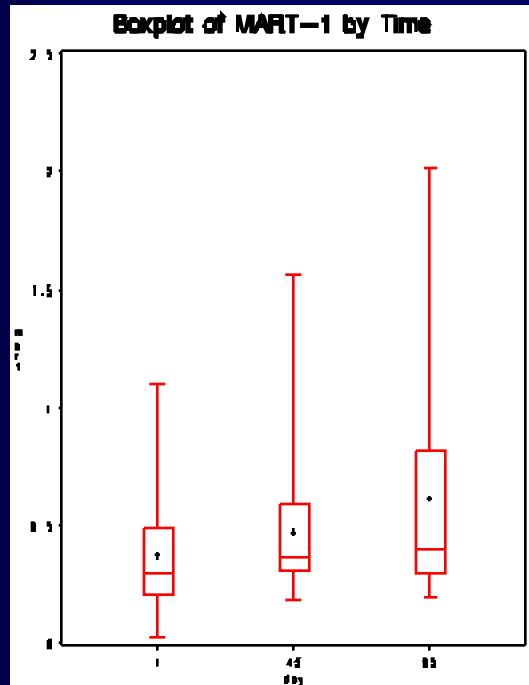
# ELISPOT Assays

**E1696** (*n=20, 2002-2003, intra-assay variation*)

	<u>spontaneous</u>	<u>PMA/I (+)/PHA</u>
Healthy control ave.:	5.4 (56%CV)	284 (15.5 %CV)
Patient ave.:	19 (40.5%CV)	171 (18.8 %CV)

# Immune Response: E1696

## Melanoma antigen peptide-specific CD8+ T cells



%MART-1  
CD8+ cells: .29% .36% .39%

.53% .53% .43%

%effector  
cells: 10% 16% 18%  
(p=0.048)

17% 17% 16%  
(p = ns)

### MHC Tetramer Analysis:

The frequency of vaccine peptide-specific CD8+ T cells was measured by MHC tetramers, showing significant increases for all 3 melanoma antigen peptides (not Flu).

The MART-1 and gp100-specific cells differentiated towards effector cells with vaccination.



# Ongoing Studies

- E4697: Phase III Vaccine +/- GM-CSF adjuvant: *GM-CSF adjuvant significantly improves DFS (p=0.034).*

*Impact of circulating DC modulation by GM-CSF (significantly reduced circulating DC with GM-CSF).*

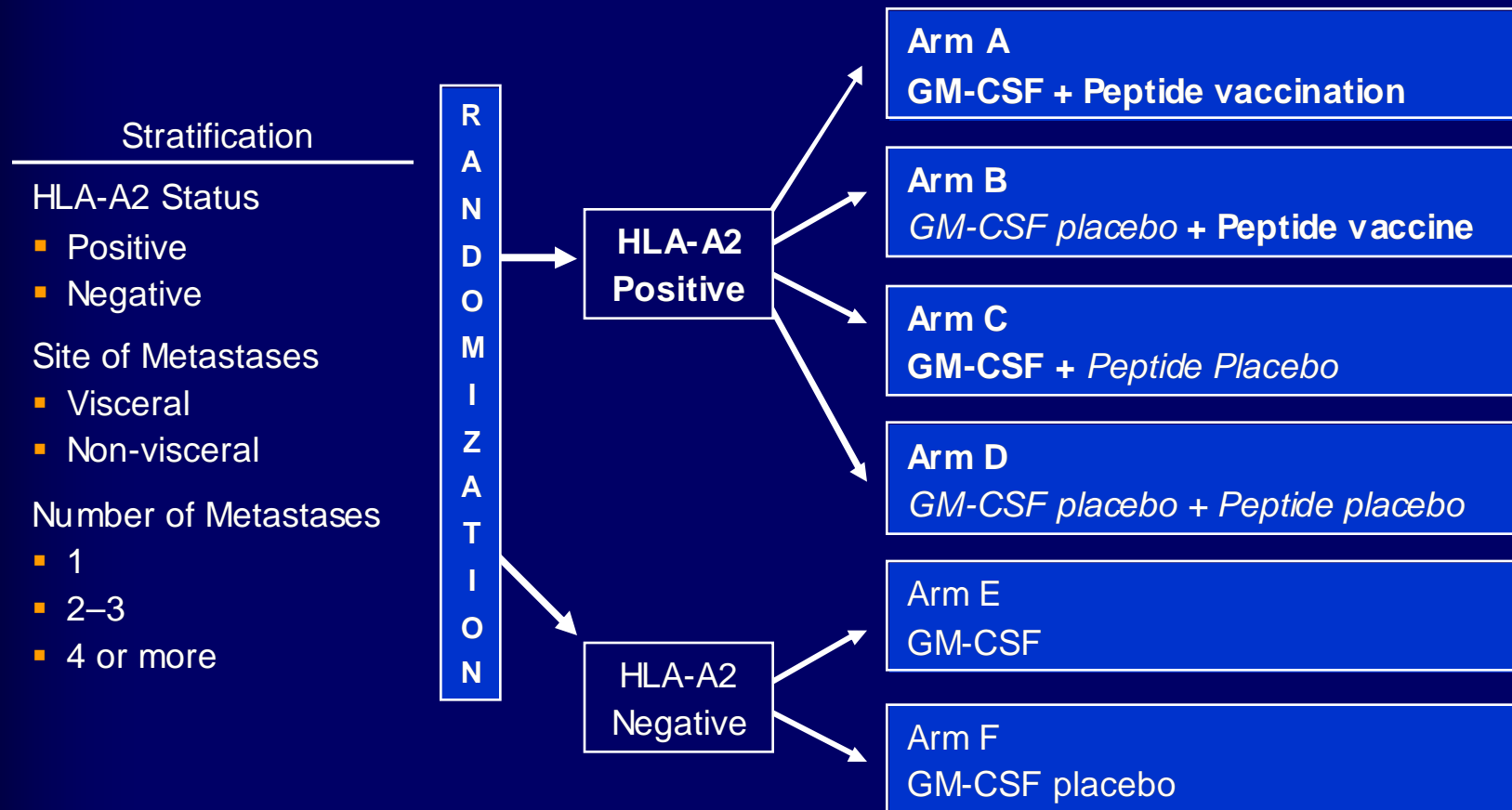
- E4697: 2<sup>nd</sup> immunological endpoints pending statistical analysis (largest data set *(from 283 pt.)* of standardized ELISPOT assays to date): *impact of immune response to vaccine on clinical outcome;*



# E4697 Intergroup Trial in High Risk Resected Stage III-IV

**Hypothesis: GM-CSF and/or multi-epitope peptide vaccine will improve OS by 33%, acting upon T-cells and/or dendritic cells of stage III-IV melanoma patients**

Chair: D. H. Lawson



# ELISPOT Assays: intra-assay and inter-assay variability

## E4697

Date	pt.	Control	triplicates	%CV	
1/5/2009	46267	<b>125041</b>	473 337 241	27% CV	
12/15/2008	46360	<b>125041</b>	399 434 389	5% CV	
12/8/2008	46318	<b>125041</b>	388 495 343	16% CV	

# ELISPOT Assays

## **E4697** (*n=20, 2008-2009*)

	<u>spontaneous</u>	<u>PMA/I (+)/OKT3</u>
Healthy control	ave.: 4.9 (54%CV)	304 (19.2%CV <i>intra-assay</i> ) (48% CV inter-assay)
Patient	ave.: 0.7 (35%CV)	81 (38.7 %CV)

## **E1696** (*n=20, 2002-2003*)

	<u>spontaneous</u>	<u>PMA/I (+)/PHA</u>
Healthy control	ave.: 5.4 (56%CV)	284 (15.5%CV <i>intra-assay</i> ) (51% CV inter-assay)
Patient	ave.: 19 (40%CV)	171 (18.8 %CV)

E1696: PI: J.M. Kirkwood, S. Lee statistics

E4697: PI: D.H. Lawson, S. Lee statistics

University of Pittsburgh Cancer Institute

Immunologic Monitoring Laboratory

(T. L. Whiteside; L. H. Butterfield)