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: IFNg/CD8+ ELISPOT of 20 spots/10e5

vaccine B: IFNg/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

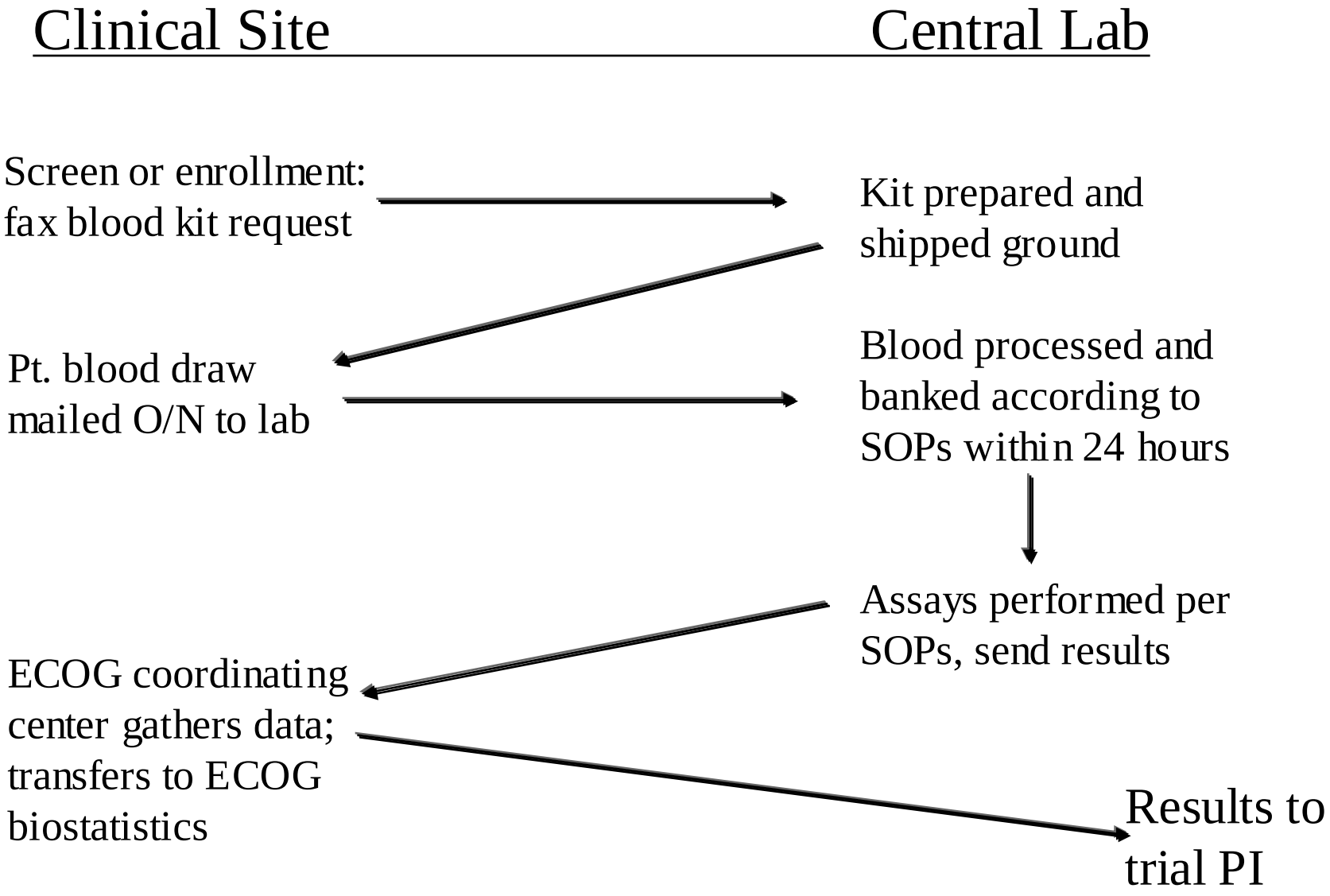
State-of-the-art assay development, standardization and validation

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



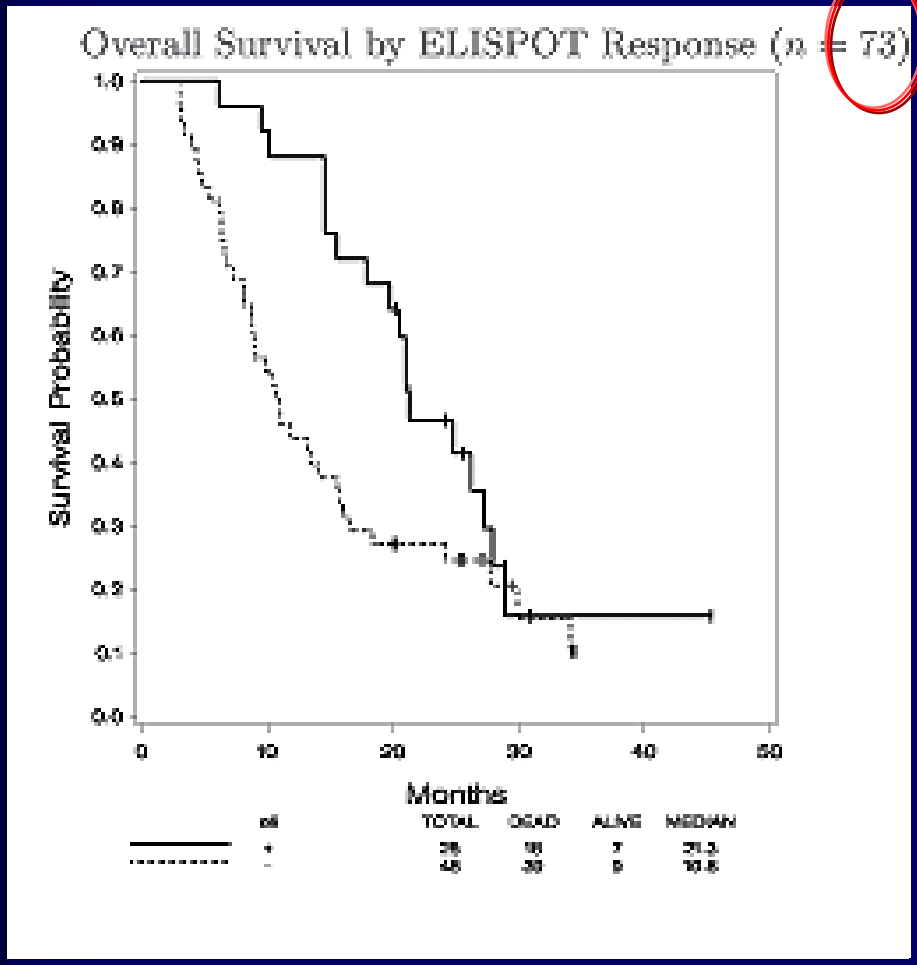
ECOG Samples and Assays: 2004-2008

Protocol	Samples	Assays
•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 ± IFNα2b



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	**93726**	421 412 363	6% CV	
1/14/2003	16002	**93726**	364 332 284	10% CV	
1/21/2003	16028	**93726**	360 285 238	17% CV	
			Positive control PMA/I		

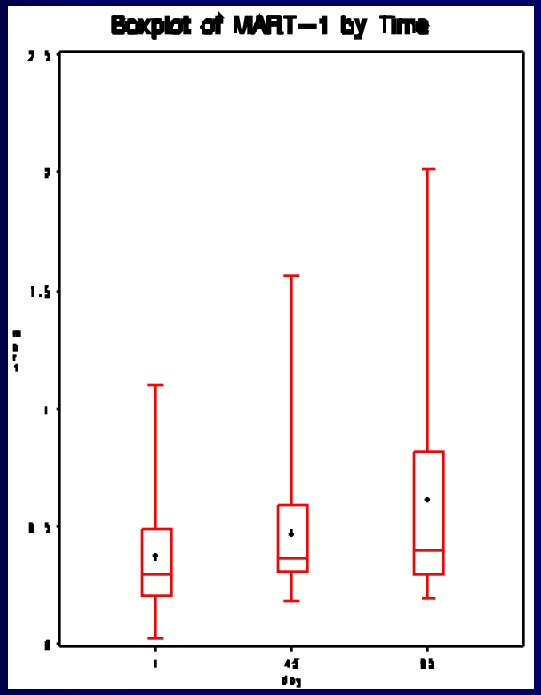
ELISPOT Assays

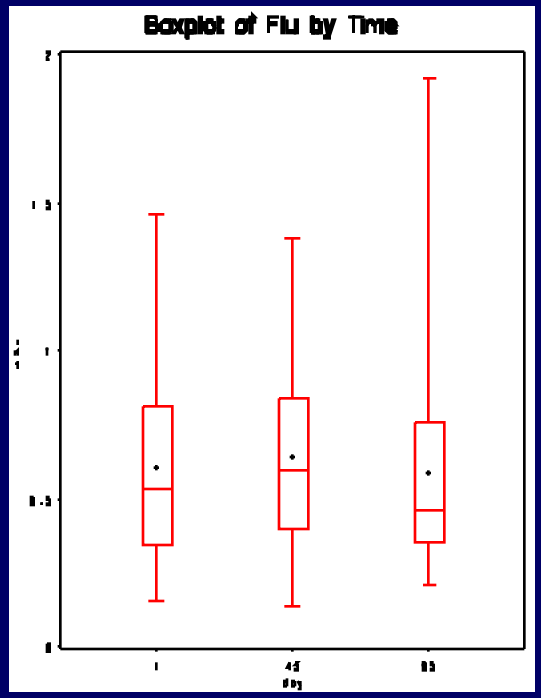
E1696 *(n=20, 2002-2003, intra-assay variation)*

		spontaneous	PMA/I (+)/PHA
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%	17%	17%	16%
		(p=0.048)			(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)*.

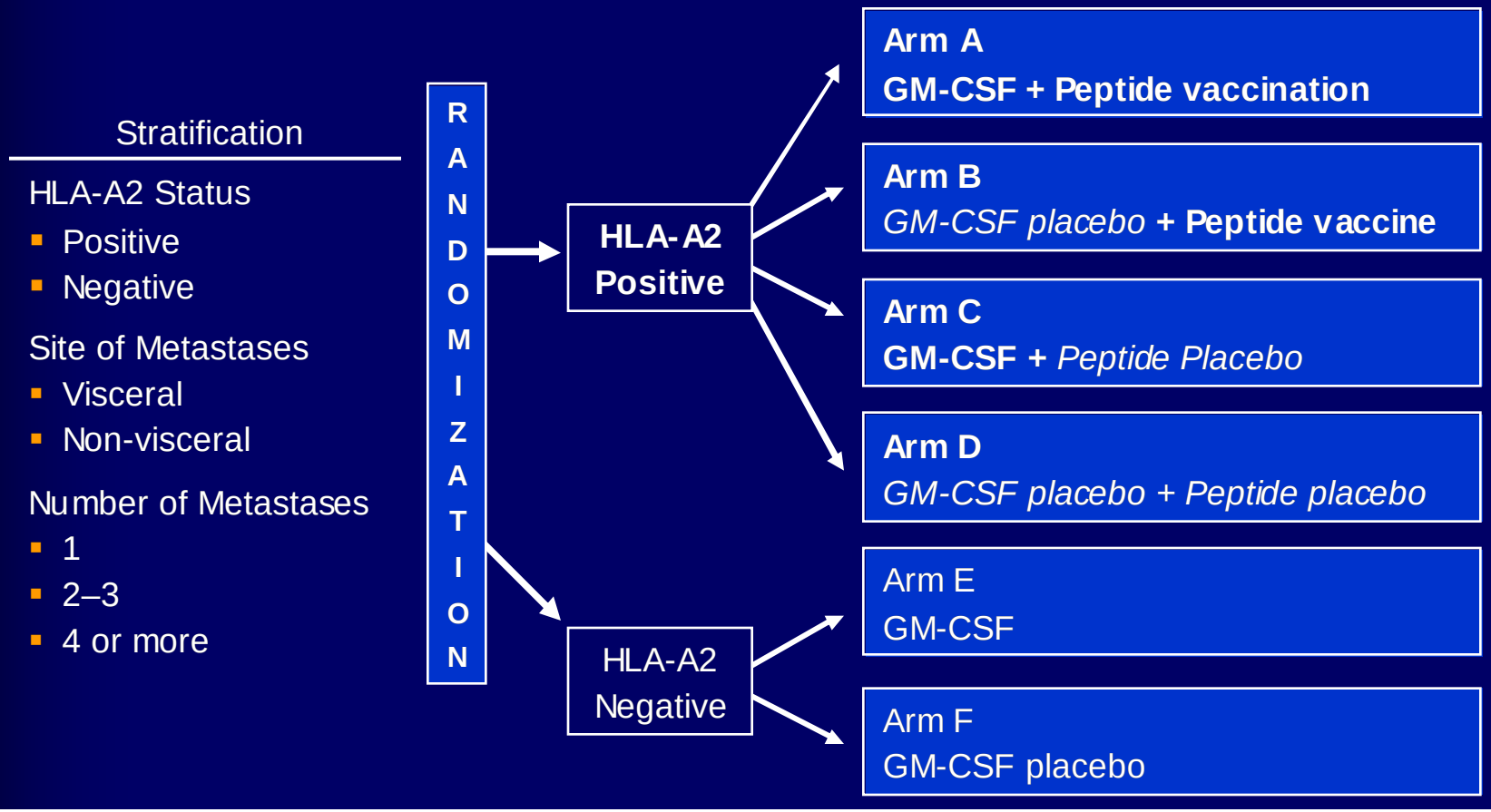
  Impact of circulating DC modulation by GM-CSF (significantly reduced circulating DC with GM-CSF).

- E4697: 2nd 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	**125041**	473 337 241	27% CV	
12/15/2008	46360	**125041**	399 434 389	5% CV	
12/8/2008	46318	**125041**	388 495 343	16% CV	

ELISPOT Assays

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

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

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

		spontaneous	PMA/I (+)/PHA
Healthy control	ave.:	5.4 (56%CV)	284 (15.5%CV *intra-assay*) (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)