Systemic adjuvant therapy of patients with high-risk melanoma: Progress to 2003 Promise for the Future

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Acknowledgements:
ECOG, SWOG, and CALGB participants and members of the University of Pittsburgh Cancer Institute Melanoma Center
Current status of melanoma therapy 2003

- No properly tested therapy demonstrates capability to prolong survival of populations with metastatic disease
  - Promising new therapies may be better evaluated in high-risk setting without active disease
- Relapse and death risk are accurately predicted by microstage/ulceration and regional [sentinel] node evaluation
  - Molecular markers of disease risk are needed
- Immunological responses to tumor are measurable and inducible, and may be correlated with disease outcome
Intergroup E3695: Survival data for aggressive biochemotherapy do not support its use

p = 0.696

Atkins, et al., Proc ASCO 2003
Adjuvant therapy decisions for patients with high-risk melanoma

- Evidence-based: randomized, controlled, multicenter (RCT) trials are our best evidence—with endpoints
  - survival (OS)
  - relapse interval (RFS)
  - quality of life (QOL)
- Molecularly defined interventions, intermediate endpoints
  - host immune response, tumor cell death (apoptosis), vascularization (angiogenesis)
- Paradigm shift: advanced -> adjuvant -> precursor focus
Adjuvant Therapy of Melanoma

Questions

- Definition of effective therapy?
- Acceptable risk-benefit ratio?
- Does benefit differ for some subsets (e.g., patients grouped by stage)?
- Is there a relationship of dose and response?
- Are there alternatives?
Adjuvant Therapy of Melanoma

Potential definitions of efficacy

Survival
- Survival prolongation of 12 mos. (median)
- Durability of survival benefits at 5 yr or more
- Cure of at least 5% of patients

Disease-free status
- Prolongation relapse-free interval by >12 mos.
- Durability of relapse-free interval gains (≥ 10y)

Improved quality-adjusted survival
Among therapies evaluated in RCT, only high-dose IFNα2 has ever shown durable survival prolongation and reduction of relapses

- Nonspecific Immunostimulants (BCG, C. parvum, OK432)
- Chemotherapy & Chemobiotherapy
- Interferons & Cytokines
  - IFNα2
  - IFNγ
  - IL-2
  - GM-CSF
- Vaccines and Adoptive Cellular/Passive Ab Transfer
  - Antibody (B cell)-inducing (Gangliosides)
  - Effector T cell-inducing (Peptides proteins, DNA...
### Published trials of adjuvant IFNα2 for high-risk T3-4/node (+) melanoma (AJCC stage IIB/III)

<table>
<thead>
<tr>
<th>Cooperative group/PI</th>
<th>Eligibility</th>
<th>n</th>
<th>Treatment agent dosage duration</th>
<th>Significant Impact on DFS</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG 1684 Kirkwood</td>
<td>T4, N1</td>
<td>287</td>
<td>IFNα2b 20 MU/M2/D IVx1 mo 10 MU/M2 SC TIW for 11 mos</td>
<td>+</td>
<td>+</td>
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<tr>
<td>NCCTG 837052 Creagan</td>
<td>T3-4, N1</td>
<td>262</td>
<td>IFNα2a 20 MU/M2/D IM TIW x3 mos</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WHO #16 Cascinelli</td>
<td>N1-2</td>
<td>444</td>
<td>IFNα2a 3 MU/D SC TIWx3 yrs</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>EORTC 18871 Kleeberg</td>
<td>T3-4, N1</td>
<td>830</td>
<td>IFNα2b 1 MU/D SC QODx1 yr vs IFNg 0.2 mg/D SC QODx1yr</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E1690 Intergroup Kirkwood</td>
<td>T4, N1</td>
<td>642</td>
<td>IFNα2b 20 MU/M2/D IVx1 mo 10 MU/M2 SC TIWx11 mos vs 3 MU/D SC TIWx2 yrs</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>E1694 Intergroup Kirkwood</td>
<td>T4, N1</td>
<td>880</td>
<td>IFNα2b 20 MU/M2/D IVx1 mo 10 MU/M2 SC TIWx11 mos vs GMK vaccine x 96 wks</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ECOG 2696 Kirkwood</td>
<td>T4, N1, M1</td>
<td>107</td>
<td>GMK + IFN or --&gt;IFN vs GMK</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Analysis of Adjuvant Trials:
Hazard Ratios for comparison of *rates of events* over time using all Kaplan-Meier data (Log-rank analysis)

Hazard ratio for relapse or death expresses comparison of outcomes for arm not treated with IFN vs. arm treated with IFN in a single number

- $1 = \text{no difference between arms}$
- $>1 = \text{high-dose IFN is better}$
- $<1 = \text{observation better}$
E1684: Study Design

Randomization
N = 287
(within 56 days)

Observation
52 wk

IFN-α2b

Induction
4 wk

Maintenance
48 wk

Induction: 20 MIU/m² IV 5× weekly × 4 wk
Maintenance: 10 MIU/m² SC TIW × 48 wk
Design: Exponential model, hazard ratio analysis
Stratification: AJCC stage groupings

E1684: Relapse-Free Survival (Eligible Cases) at 6.9 yr Median Follow-up

![Graph showing relapse-free survival with treatment groups.

- **Treatment groups (n = 280)**
  - IFN-α2b
  - Observation

- **Hazard Ratio for relapse w/o IFN** 1.43
  - Significant at p=.002

<table>
<thead>
<tr>
<th></th>
<th>No. patients</th>
<th>No. relapsed</th>
<th>Median yr</th>
<th>P value</th>
<th>5-yr RFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α2b</td>
<td>143</td>
<td>90</td>
<td>1.72</td>
<td>&lt;.01</td>
<td>37%</td>
</tr>
<tr>
<td>Observation</td>
<td>137</td>
<td>103</td>
<td>0.98</td>
<td></td>
<td>26%</td>
</tr>
</tbody>
</table>

Impact of HDI versus Observation at 6.9 yr median follow-up

- Relapse Free Survival significantly improved ($P_1 = 0.0023$)
- Estimated 5-yr RFS: 37% versus 26%
- Overall hazard ratio for relapse: 1.43

Intergroup E1690 Phase III Trial of High or Low Dose IFN-α2b Versus Observation

Randomization
N = 642 (within 70 days)

- High-dose IFN-α2b × 1 yr
- Low-dose IFN-α2b × 2 yr
- Observation

- Goal: Determine if low-dose IFN-α2b for 2 yr is effective as high-dose IFN-α2b for 1 yr
- Design: Cure rate model, hazard ratio analysis
- Stratification: AJCC stage groupings and number of positive nodes

E1690: Relapse-Free Survival

Treatment groups (n = 608)
- High-dose IFN
- Low-dose IFN
- Observation

Hazard Ratio for Relapse w/o HD IFN = 1.24
Significant at p = .05

Intergroup E1694 Phase III Study of Ganglioside GM2 Vaccine (GMK) Versus High-Dose IFN-α2b

Randomization
N = 880 (within 70 days)

GMK SC Days 1, 8, 15, 22, and Weeks 12, 24, 36 48, 60, 72, 84, 96,
versus
HDI 20 MIU/m² IV 5× weekly × 4 wk
10 MIU/m² SC TIW × 48 wk

- Goal: Determine if GMK is superior to HDI
- Design: Cure rate model
- Stratification: Number of involved nodes
- First Intergroup US Trial adopting HDI as a reference standard for adjuvant therapy of high risk melanoma

E1694: Relapse-Free Survival

Treatment groups (n = 774)

IFN-α2b

GMK

Hazard Ratio for Relapse w/o IFN 1.33
Significant at p= .002

Months and no. events/no. at risk

<table>
<thead>
<tr>
<th>Group</th>
<th>0-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>40-50</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α2b</td>
<td>74/385</td>
<td>19/178</td>
<td>3/84</td>
<td>2/25</td>
<td>0/1</td>
</tr>
<tr>
<td>GMK</td>
<td>110/389</td>
<td>32/188</td>
<td>7/90</td>
<td>2/23</td>
<td>0/1</td>
</tr>
</tbody>
</table>
Updated Relapse-Free Survival Is Highly Significant for Trials E1684-1690-1694-2696

E1684

IFN vs Observation: $p_2=0.02$, $p_1=0.01$, HR=1.38

E1690

IFN vs Observation: $p_2=0.09$, HR=1.24

E1694

IFN vs GMK: $p_2=0.006$, HR=1.33

E2696

GMK + Concurrent IFN vs GMK Alone: $p_2=0.18$, HR=1.56
GMK + Sequential IFN vs GMK Alone: $p_2=0.14$, HR=1.64
Does High Dose IFNα2b improve overall survival?

Two large multicenter randomized trials of high-dose IFNα2b show a significant overall survival advantage

- ECOG Trial E1684
  - Compared to observation
- US Intergroup Trial E1694
  - Compared to most promising vaccine available for intergroup study in 1995 (ganglioside GM2)
Impact of HDI versus Observation at 6.9 yr median follow-up

- Median OS significantly improved \( (p_1 = .0237) \)
- Estimated 5-yr OS: 46% versus 37%
- Overall hazard ratio for death w/o IFN: 1.32

E1684: Overall Survival at 6.9 yr Median Follow-up


### Treatment groups (n = 280)

- **IFN-α2b**
  - No. patients: 143
  - No. dead: 81
  - Median yr: 3.82
  - P value: < .047
  - 5-yr survival: 46%

- **Observation**
  - No. patients: 137
  - No. dead: 90
  - Median yr: 2.78
  - P value: < .047
  - 5-yr survival: 37%

Hazard Ratio for Death w/o IFN 1.32
Significant at p=.023
E1694: Overall Survival

Treatment groups (n = 774)
- IFN-α2b
- GMK

Hazard Ratio for Death w/o IFN 1.38
Significant at p=.009

Time interval, mo

<table>
<thead>
<tr>
<th>Group</th>
<th>0-10</th>
<th>10-20</th>
<th>20-30</th>
<th>30-40</th>
<th>40-50</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α2b</td>
<td>11/385</td>
<td>29/258</td>
<td>11/125</td>
<td>1/38</td>
<td>0/1</td>
</tr>
<tr>
<td>GMK</td>
<td>23/389</td>
<td>42/264</td>
<td>14/132</td>
<td>2/38</td>
<td>0/2</td>
</tr>
</tbody>
</table>
E1694: Updated Overall Survival (ITT at 2.1 years Median Follow-up)

Log-rank test $P_2 = .04; P_1 = .02$

Updated HR for death w/o IFN 1.32

Treatment groups (n = 743)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Dead</th>
<th>Alive</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>GMK</td>
<td>439</td>
<td>127</td>
<td>312</td>
<td>3.8</td>
</tr>
<tr>
<td>High-dose IFN</td>
<td>438</td>
<td>102</td>
<td>336</td>
<td>NR</td>
</tr>
</tbody>
</table>
Overall Survival

**E1684**

IFN vs Observation: \( p_2 = 0.16, p_1 = 0.09, HR = 1.22 \)

**E1690**

IFN vs Observation: \( p_2 = 0.98, HR = 1.00 \)

**E1694**

IFN vs GMK: \( p_2 = 0.04, HR = 1.32 \)

**E2696**

GMK + Concurrent IFN vs GMK Alone: \( p_2 = 0.65, HR = 1.20 \)

GMK + Sequential IFN vs GMK Alone: \( p_2 = 0.64, HR = 1.20 \)
What is the balance of risk-benefit ratio for treatment:

- Interferon toxicity
- Quality-adjusted time without symptoms or toxicity
- Cost-efficacy

Common Grade 3/4 Adverse Events in Patients Treated With High-Dose IFN-α2b

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>E1684</th>
<th>E1690</th>
<th>E1694</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>23</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>Fever</td>
<td>18</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Myalgia</td>
<td>17</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Nausea</td>
<td>–</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>–</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Anorexia</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Myelosuppression</td>
<td>26</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>Liver (increased SGOT)</td>
<td>14</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>Depression</td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

§Based on data in the prescribing information from 143 patients treated with high-dose IFN-α2b in Trial E1684.
||Category includes neurologic manifestations characterized as depression, neuropsychiatric, or neuropsychological toxicity.
Safety Summary for High-Dose IFN-α2b

- All patients receiving HDI therapy experience mild-to-moderate side effects
- Some patients experience more severe side effects, including severe fatigue and flu-like symptoms, abnormal blood counts or liver functions, and depression
- Discontinuation of treatment due to adverse events
  - 24% of patients in E1684
  - 13% of patients in E1690
  - 10% of patients in E1694
- The majority of patients can tolerate a full course of 52 weeks with appropriate supportive measures and dose modifications

*Kirkwood et al., J.Clin. Oncol. 2002*
Interferon toxicity: *Quality-of-Time Analyses (‘Q-TWiST’)*

Node-positive patients

Toxicity is not paramount

Relapse time is of lesser quality

Quality-adjusted time improved significantly


A majority of patients state they would accept

1. Mild-moderate IFN$\alpha$-2b side effects for at least a 4% improvement in 5 yr DFS

2. Severe IFN$\alpha$-2b side effects for at least a 10% improvement in 5 yr DFS
Applications

- Patient-defined utilities are useful to decide value of quality-adjusted survival benefit of adjuvant IFNα-2b for specific patients

- Methods include Q-TWiST analysis and decision time-utility analysis
Does the benefit of HDI differ according to the stage (nodal status)?

- Early data suggested greatest benefit in patients with nodes involved (N+)
- Subsequent data has favored groups with few nodes involved (N2)
- Most recent and largest trial shows greatest benefit in those without clinical evidence of nodal involvement (N0)
### Subset analysis of benefit by number of positive nodes in completed published phase III studies

<table>
<thead>
<tr>
<th>Number of nodes positive</th>
<th>E1684 (ITT)</th>
<th>E1690 (eligible)</th>
<th>E1694 (eligible)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.53</td>
<td>1.46</td>
<td>2.07 (\dagger)</td>
</tr>
<tr>
<td>1</td>
<td>2.29 (\S)</td>
<td>1.00</td>
<td>1.44</td>
</tr>
<tr>
<td>2 – 3</td>
<td>1.24</td>
<td>1.92 (|)</td>
<td>1.16</td>
</tr>
<tr>
<td>(\geq) 4</td>
<td>1.18</td>
<td>1.15</td>
<td>1.47</td>
</tr>
</tbody>
</table>

*Hazard ratios for E1684 are based on intent-to-treat, whereas the hazard ratios given for E1690 and E1694 are based on eligible cases, reflecting OBS or GMK risk over IFN.*

\(\S P = .0015\); \(\| P = .02\); \(\dagger P = .01\).
Does the benefit of HDI differ according to the tumor burden, stage, or nodal status?

Conclude:

- No trial has been of adequate size (power) to evaluate the real impact in disease subsets
- Relative risk reduction is probably equivalent across disease stage-defined subsets
Conclusion: Efficacy of adjuvant IFN in high-risk melanoma is established and a foundation for progress

- Highest level of evidence based on analysis of primary endpoints of prospective randomized multicenter cooperative group trials
  - High-dose IFN-α2b benefit is consistent in terms of RFS and OS, compared to observation and to GMK
  - Hazard for relapse without IFN rises 1.24-1.38 fold
  - Hazard for mortality rises 1.22-1.32 fold
  - No differential stage-specific effects are meaningful
No less toxic regimen is effective

- Very low dose interferon (1 MU SC QOD)
  - EORTC 18871
- Low dose interferon (3 MU SC TIW)
  - WHO Trial 16, ECOG 1690, UK AIM-High, & Scottish trial
- Intermediate-dose interferon (SC)
  - EORTC 18952
  - EORTC 18991
Are there leads with Intermediate doses of interferon?

- Previous possibility from EORTC 18952 testing two intermediate doses of 5 MU sc 3x/wk for 2 years or 10 MU sc 3x/wk for 1 year
  - Total dose ~1/2 of E1684
  - No intravenous induction phase
  - Reported improvement in disease-free survival at median of 1.9 years (2001) -- now without overall survival benefit
  
  - Data reported to 3rd Int. Congress on Melanoma Research 26 May 2003 by Eggermont, AMM
Current Active Adjuvant Melanoma Trials

- **Improve therapeutic index of HDI using induction IFN only, or pre-operative ‘neoadjuvant’ application**
  - Intergroup E1697: 1 month IV HDI vs. Obs for intermediate risk stage IIA[US], IIB/IIIA[CA-AU]
  - Univ. Pittsburgh Melanoma Center trial 00-008 1 month IV HDI given as a neoadjuvant

- **Evaluate more aggressive empiric combinations of chemobiotherapy**
  - Intergroup S0008: CVD-IFN-IL-2 × 3 months vs HDI for 1 yr. In stage III B/C

- **Introduce new cytokines and more specific peptide vaccine interventions**
  - Intergroup E4697: Adjuvant evaluation of GM-CSF and multiple epitope peptide vaccine in resected stage IIIB,C & M1
E1697 - A randomized study of four weeks of high-dose interferon alpha-2b in stage T3-T4 or N1 (microscopic) melanoma

Hypothesis: Induction IV IFN is necessary and sufficient to achieve durable adjuvant benefit in intermediate-risk melanoma patients

STRATIFICATION
Pathologic Lymph Node Status
- Known
- Unknown

Lymph Node Staging Procedure
- Sentinel Lymph Node Procedure
- Elective Lymph Node Dissection
- No Lymphadenectomy

Breslow Depth
- 1.5 - 3 mm
- 3.1 - 4 mm
- > 4 mm

Ulceration of Primary Lesion
- Yes
- No

Disease Stage
- Lymph Node Positive
- Lymph Node Negative

Arm A:
Observation

Arm B:
4 week high-dose IFN alfa-2b (Intron A)
20 MU/m²/d qd IV for 5 consecutive days out of 7 (M-F) every week times 4 weeks
**S0008** - Phase III trial of high-dose interferon alpha-2b vs cisplatin, vinblastine, DTIC + IL-2 and interferon in patients with high-risk melanoma

**Hypothesis:** Chemobiotherapy will have an adjuvant effect superior to HDI in high-risk melanoma

**Randomization**

Arm 1
One year high-dose IFN alpha-2b (Intron A)

*Induction Therapy* - Weeks 1-4

*Maintenance* - Weeks 5 - 52

Arm 2
Cisplatin (CDDP)
Dacarbazine (DTIC)
Vinblastine
Interleukin 2 (IL-2)
Interferon alfa-2b
G-CSF

Repeat cycle every 21 days
Maximum of 3 cycles

Stratification by newly diagnosed versus recurrent disease; further stratification by ulceration of the primary tumor and nodal status for newly diagnosed disease
Are there vaccine alternatives?

- Allogeneic, polyvalent vaccines
- Autologous tumor vaccines
- Defined antigen vaccines
  - Gangliosides
  - Peptides
- Dendritic cell-based vaccines
- Genetically modified tumor vaccines
**T Cell Recognition of Tumor Epitopes →CD8 (killer) and CD4 (helper) Vaccines**

- **TCR**
- **MHC I**
- **MHC II**
- **Lysis**
- **Cytokines**
- **Tumor Regression**

**Diagram:**
- Anti-Tumor CD8+ CTL
- Anti-Tumor CD4+ T Cell
- MHC I
- MHC II
- Class I
- Class II
- Tumor Antigen
- Tumor
Progressive Paraneoplastic Vitiligo

Immune recognition and response to melanosomal markers that may be harnessed to treat melanoma

Nordlund, Kirkwood 1983; Bloasberg, O’Day 2003 Proc ASCO 2857
Peptide antigens of melanoma available for CD8 and CD4 T cell stimulation

**Differentiation Antigens**

- HLA-A2 and DR-4 bound epitopes of
  - Melan A/MART-1 (multiple)
  - gp100 (HMB45) (multiple, both mutated and native)
  - Tyrosinase (internal, leader)

**Cancer-Germline Antigens**

- HLA-A1 and other epitopes of
  - MAGE-1, 3

- HLA-A2 and Pan-DR bound epitopes of
  - ESO-1
Immunodominant epitopes of several lineage/differentiation antigens are identified, and prepared for evaluation in cooperative group trials

- MART-1/Melan-A (27-35),
- gp100 (209-217, 210M)
- tyrosinase (368-376, 370D)

No large studies of immunological response and clinical response to vaccination with multiple epitopes of the differentiation class yet conducted

Kirkwood et al., Proc ASCO 2003
GM-CSF and IFN α2 are both modulators of antigen presenting cell (DC) function
- GM CSF has effects locally administered with vaccines, and may have benefit systemically with vaccines
  - clinically evaluated regimen of 250 mcg daily s.c./d for 14 day q. 28 days
- IFN α2 has been studied in vitro, in experimental animals, and in human adjuvant trials
  - clinically most effective IV and SC at dosages of ≥10 Mu/M2/d
Potential Functions of GM-CSF and IFNα upon DC Subsets and Polarization of the Immune Response

GM-CSF

IFN α

Th0

Th1

Th2

Tr/Th3

Cell-Mediated Anti-Tumor Immunity

Humoral Anti-Tumor Immunity

GM-CSF

IFN α

IL-12

IL-18

IFN-γ

IL-4

IL-5

IL-10

IL-10

TGF-β?

DC1

DC2

iDC

iDC
E1696 Goal

- To evaluate the immunological and antitumor efficacy of multi-epitope peptide vaccine MGT for HLA-A2+ patients with metastatic melanoma
- To compare the immunological and antitumor efficacy - vaccination q 2 weeks with three melanosomal peptides M/G/T
  - (A) alone
  - (B) +GM-CSF 250 mcg daily for 14/28 days each month
  - (C) +IFNα2b10 MIU/M2 TIW
  - (D) +both IFNα2b and GM-CSF at above dosages

Kirkwood et al., Proc ASCO 2003
E1696 Immunological Endpoints

Initial assay
- ELISPOT
  - Developed in the context of 3 prior UPCI peptide vaccination trials against lineage antigen peptides (n=83)
ELISPOT assay detecting production of interferon gamma by individual T cells from the peripheral blood of a patient after vaccination with Melan-A/MART 1 peptide
**E1696 Eligibility and Demographics**

- Measurable metastatic melanoma
- Normal laboratory values
- Prognosis of > 3 mos OS
- Entered: 120 patients
- Evaluable at 3 months with laboratory assays completed
  - 60 patients
  - Pending laboratory analysis, 30 patients

- Prior therapy with
  - Chemotherapy 33
  - Radiotherapy 18

*Kirkwood et al., Proc ASCO 2003*
E1696: Multiepitope Immunization
+ IFNα2b + GMCSF in Metastatic Measurable Melanoma

Eligibility
1. Measurable Metastatic Melanoma
2. HLA-A2+
3. PS 0-1
4. Labs

Peptide Vaccination in All Groups:
- Melan A/MART-1:27-35 AAGIGLTV
- gp100:209-217 (210M) IMDQVPFSV
- Tyrosinase: 368-376 (370D) YMDGTMSQV

<table>
<thead>
<tr>
<th>ARMS</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IFNα2b</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
E1696 Initial Analysis:
Immune response to peptides is not correlated with IFN, GM-CSF therapy

- Immunologic Response Data (n=60)
  ELISPOT (-): 40/60=67%
  ELISPOT (+): 20/60=33%

- ELISPOT (+) % by IFN Treatment (n=60)
  IFN: 9/31=29%
  No IFN: 11/29=38%

- ELISPOT(+)% by GM-CSF Treatment (n=60)
  GM-CSF: 10/27=37%
  No GM-CSF: 10/33=30%

Elispot performed directly on blood lymphocytes: (+) if >26 spots increment over baseline at d43, d85 (95% CI response to nonvaccine epitopes in 83 patients previously studied at UPCI)
E1696 Initial Analysis: Immune Response by Elispot is Correlated with Overall and Progression-Free Survival

ELISPOT (-)
- OS (n=40)
  Median 13.4 mo
  95% CI (8.7, 15.7)
- PFS (n=28)
  Median 2.8 mo
  95% CI (2.7, 3.0)

ELISPOT (+)
- OS (n=20)
  Median 21.3 mo
  95% CI (10.7, 21.3)
- PFS (n=18)
  Median 2.5 mo
  95% CI (2.5, 4.8)

Kirkwood et al., Proc ASCO 2003
E1696: Correlation of Overall Survival
and CD8 IFNγ Elispot Response

Survival Probability

<table>
<thead>
<tr>
<th>elispot</th>
<th>Months</th>
<th>TOTAL -</th>
<th>DEAD</th>
<th>ALIVE</th>
<th>MEDIAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>20</td>
<td>8</td>
<td></td>
<td>12</td>
<td>21.3</td>
</tr>
<tr>
<td>-</td>
<td>40</td>
<td>19</td>
<td></td>
<td>21</td>
<td>13.4</td>
</tr>
</tbody>
</table>

P_2 = 0.203
Immunity to CD8 epitopes of three lineage antigens is possible to induce in 1/3 of patients with measurable metastatic disease

- ELISPOT assays without *in vitro* stimulation

Immune response to lineage antigens is associated with trend to longer median survival

Immune response to lineage antigens is associated with a trend to non-progression of melanoma

Protocol closed to accrual 5/14/03 with 120 patients; 3 month data for all will be available within 6 months for complete analysis of this trial

*Kirkwood et al., Proc ASCO 2003*
Future Prospects

- Extrapolation to the adjuvant sphere: E4697 trial of identical multi-epitope peptide vaccine (Lawson)
- Extrapolation to larger numbers of peptides targeting both CD8 (12) and CD4(6): E1602 (Slingluff)
- Optimization of immunological adjuvants beyond ISA-51: DC, DC1, and pDC (CpG), using CD4 and CD8 peptides
Peptide antigens of melanoma available for induction of CD8 (CD4) T cell response

**Differentiation Antigens**

- HLA-A2 bound epitopes of
  - Tyrosinase (internal, leader)
  - gp100 (HMB45) (multiple, both mutated and native)
  - Melan A/MART-1 (multiple)

**Cancer-Germline Antigens**

- HLA-A1 and other epitopes of
  - MAGE

- HLA-A2 and Pan-DR bound epitopes of
  - ESO-1
E4697 Intergroup- A randomized, placebo-controlled phase III trial of yeast derived GM-CSF vs peptide vaccination vs GM-CSF plus peptide vaccination vs placebo in patients with “no evidence of disease: after complete surgical resection of “locally advanced” and /or stage IV melanoma

Hypothesis: GM-CSF will prolong survival in patients with resected stage III-IV melanoma multi-epitope peptide vaccine will augment this benefit, and be modulated by GM-CSF, acting to improve antigen presentation by dendritic cells

Stratify:
- HLA-A2 Status¹
  1. Positive
  2. Negative
- Site of Metastases
  1. Visceral
  2. Non-visceral
- Number of Metastases
  1. 1
  2. 2 - 3
  3. 4 or more
Adjuvant therapy trials for melanoma should build systematically upon the evidence

- Further progress will come from trials of adequate size, evaluating clinical and laboratory endpoints, building upon current evidence
- Immunotherapy with specific peptide vaccines, and induction of CD4/CD8 T cell responses
- Anti-angiogenic approaches assessing intermediate endpoints (bFGF, VEGF)
- Reversal of molecular processes of progression—anti-apoptotic, invasion, as targets and interventions are identified