Systemic Management of Melanoma

From explorations of advanced disease to adjuvant high-risk and precursor arenas in search of better understanding and therapeutic success

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Dermatology	**Pathology**	**Biostatistics/ Protocol Office**
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Z You	D Becker	J. Cramer
W. Storkus		**Informatics**
		S. Urda

Laboratory Clinical Combined

UPCI Melanoma Program

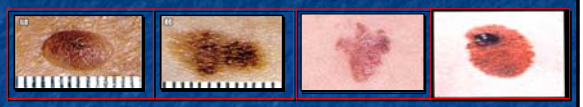
- Advanced disease interventions that are both immunologically specific and effective
  - Zarour, Storkus, Falo, Geskin, Butterfield, Kirkwood
- Regional nodal high-risk disease intervention studies building upon high-dose IFNα with molecular, immunologic, and pathologic analyses
  - Kirkwood, Edington, Agarwala, Moschos, Wang
- Primary and precursor lesion studies to define the markers of progression with novel optical imaging and expression array analyses:
  - Geskin, Edington, Kirkwood, Becker, Wang

General Introduction
Melanoma-- Platform for Immunological Intervention

Incidence:

- 2% of all new cancer, greatest rise in incidence
- 97% of fatal skin cancers

Clinical features of melanoma progression are widely recognized, but molecular markers remain incompletely understood



Normal nevocellular nevus | **Atypical nevocellular nevus** | **Radial invasion in melanoma arising in a nevocellular nevus** | **Vertical invasion in melanoma arising in a nevocellular nevus**

General Introduction
Melanoma-- Platform for Immunological Intervention

Incidence:

- 2% of all new cancer, greatest rise in incidence
- 97% of fatal skin cancers

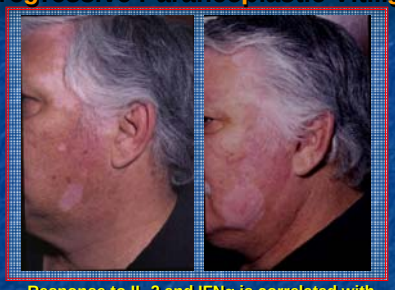
Stage IV survival <5% at 5+ years

- Only agent approved in modern era for stage IV is HD bolus IL-2, with
  - -<5% durable CR/PR based on phase II trials

Prognosis of earlier stages I-III highly predictable by sentinel LN biopsy

- Only approved adjuvant therapy of high-risk stage IIB-III is high-dose IFNα2b
  - 10% durable disease-free survival benefit based on multiple phase III cooperative group trials

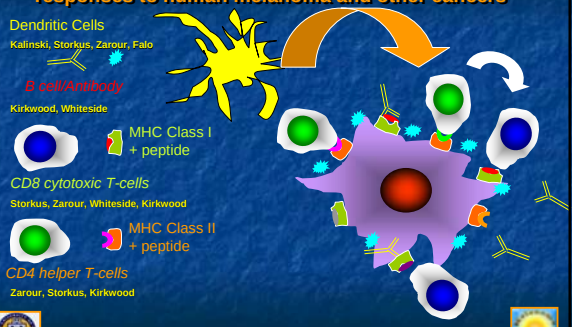
Progressive Paraneoplastic Vitiligo



Response to IL-2 and IFNα is correlated with the development of autoimmune responses
Question: can immune recognition and response to (melanosomal) markers of melanoma be harnessed

Nordlund, Kirkwood J Am Acad Derm 198:102, 1983

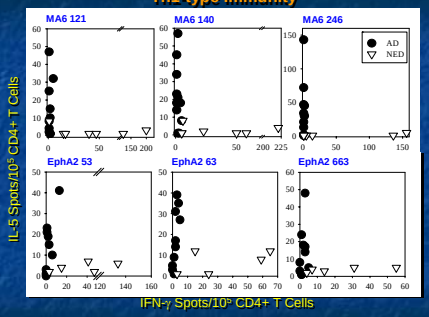
Scientific basis of the Melanoma Program of UPCI, ECOG: Novel approaches to induction of more effective immune responses to human melanoma and other cancers



Tolerance is established and may be difficult to reverse in stage IV disease

- In advanced stage IV melanoma, immune responses are Th2-biased
  - associated with immune tolerance (IL-4, 5)
- In earlier stages of disease immune responses are Th1 biased (IFN γ, TNF)
- Polarization of the immune response is demonstrable at level of DC (DC1) and T cell (Th1)

Patients with active stage IV melanoma display Th2-type anti-MAGE-A6, Anti-EphA2 responses, while patients with NED exhibit Th1-type Immunity



*AD = Active Disease; NED = No evidence of Disease.
Patients exhibited Th1-type immunity to Flu/EBV Th Epitopes
Tatsumi et al., J. Exp. Med. 196:619 (2002); Tatsumi et al., Cancer Res. 2003.

Rationale for evaluation of immunotherapy in adjuvant setting

- Time required for therapy to induce intermediate effects (immunity)
- Susceptibility of the host to intervention
- Expression of antigens by the tumor

Adjuvant treatment modalities evaluated in randomized controlled trials for melanoma

- Chemotherapy & Chemobiotherapy
- Nonspecific Immunostimulants (BCG-E1673; C. parvum-SEG; OK432)
- Vaccines, Adoptive Cellular/Passive Ab Transfer
  - Antibody (B cell)-inducing Gangliosides (E1694)
  - Effector T cell-inducing peptides (E1696; E4697); proteins, DNA
- Interferons & Cytokines
  - IL-2 (S0008)
  - GM-CSF (E4697)
  - IFNγ (E4687, S8710)
  - IFNα2

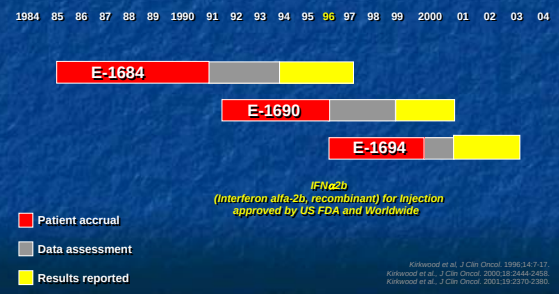
Decisions in the development of adjuvant therapy for melanoma

- Evidence-based medicine: trials that are randomized, controlled, multicenter, and reproducible with endpoints of
  - survival (OS)
  - relapse interval (RFS)
  - quality of life (QOL)
- Molecularly defined interventions and intermediate immunological,, proteomic, or genomic endpoints
  - host immune response, tumor cell apoptosis, vascularization
- Paradigm shift:Advanced -> adjuvant ->precursor disease

Published trials of adjuvant IFNα2 for high-risk T3-4/node+ resected melanoma

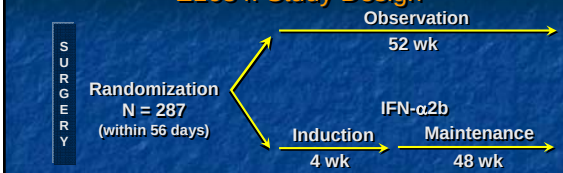
Cooperative group/PI	Eligibility	n	Treatment agent/ dosage/duration	Impact on DFS	Impact on OS
NCCTG 837052 Creagan	T3-4, N1	262	IFN×2a 20 MU/m²/D IM TIW x3 mos	-*	-
ECOG 1684 Kirkwood	T4, N1	287	IFN×2b 20 MU/m²/D IVx1 mo 10 MU/m² SC TIW for 11 mos	+ @6.9 –12.6 yrs	+
E1690 Intergroup Kirkwood	T4, N1	642	IFN×2b 20 MU/m²/D IVx1 mo 10 MU/m² SC TIWx11 mos vs 3 MU/D SC TIWx2 yrs	+ @ 4.3 – 6.6 yrs	-
WHQ #16 Cascinelli	N1-2	444	IFN×2a 3 MU/D SC TIWx3 yrs	-	-
EORTC 18871 Kleeberg	T3-4, N1	830	IFN×2b 1 MU/D SC QODx1 yr vs IFNg 0.2 mg/D SC QODx1yr	-	-
E1694 Intergroup Kirkwood	T4, N1	880	IFN×2b 20 MU/m²/D IVx1 mo 10 MU/m² SC TIWx11 mos vs GMK vaccine x 96 wks	+ @1.3 –2.1 yrs	+
ECOG 2696 Kirkwood	T4, N1, M1 107		GMK + IFN or -->IFN vs GMK	+ @ 1.4 – 2.6 yrs	-
UKCCR Aim-High Hancock	T4, N1	674	IFN×2a 3 MU/D SC QODx2 ysr	-	-

HDI Trials Timeline



Kirkwood et al. *J Clin Oncol.* 1996;14:7-17.
Kirkwood et al., *J Clin Oncol.* 2000;18:2444-2458.
Kirkwood et al., *J Clin Oncol.* 2001;19:2370-2380.

E1684: Study Design

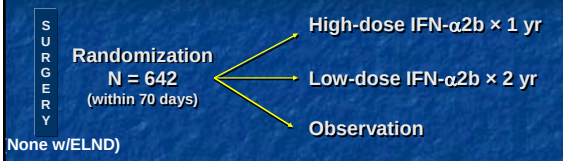


(All with ELND)

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

Kirkwood JM, et al. *J Clin Oncol.* 1996;14:7-17.

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

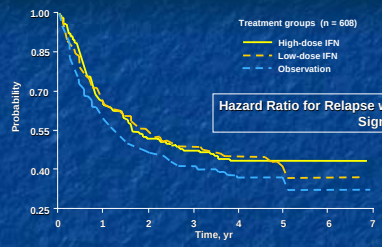


None w/ELND)

- 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

Kirkwood JM, et al. *J Clin Oncol.* 2000;18:2444-2458.

E1690: Relapse-Free Survival (Eligible Cases)



Time interval and no. events/no. at risk

Group	0-1	1-2	2-3	3-4	4-5	5-6	6-7
HDI	70/203	24/127	9/100	6/77	0/53	0/28	0/9
LDI	69/203	22/132	12/110	5/86	3/56	2/21	0/5
Obs	78/202	24/112	10/87	6/71	1/45	2/22	0/3

Kirkwood JM, et al. *J Clin Oncol.* 2000;18:2444-2458.

Intergroup E1694 Phase III trial: GM2-KLH/QS-21 (GMK vaccine) vs. High-dose IFNα2b

SURGERY

880 Patients
T4 N0 or
T(any)N1-2
None w/ELND)

GM2-KLH/QS-21 (GMK)x96 wks

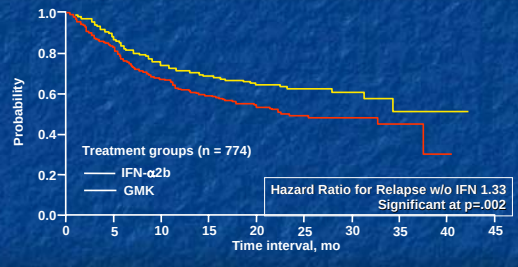
High-dose IFNα2b x 52 wks

Primary endpoints: Survival, Relapse-free interval

Secondary Analysis: Antibody responses, in relation to clinical course

Kirkwood et al., J. Clin.Oncol. 2001

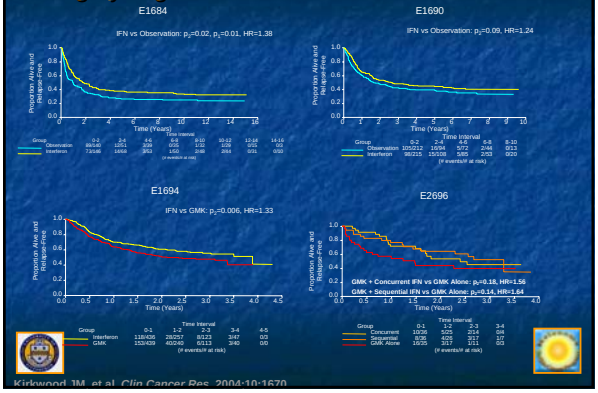
E1694: Relapse-Free Survival



Months and no. events/no. at risk

Group	0-10	10-20	20-30	30-40	40-50
IFN-α2b	74/385	19/178	3/84	2/25	0/1
GMK	110/389	32/188	7/90	2/23	0/1

Updated Durable Relapse-Free Survival Is Highly Significant for E1684-1690-1694-2696

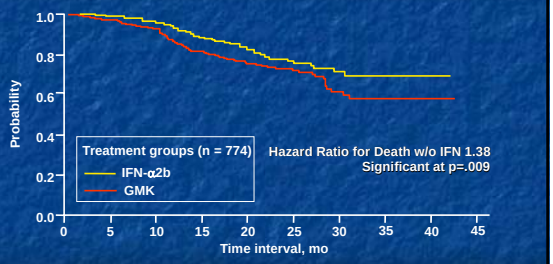


Kirkwood JM, et al. *Clin Cancer Res.* 2004;10:1670

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

- ECOG Trial E1684
  - vs. observation
- US Intergroup Trial E1694
  - vs. GMK ganglioside vaccine

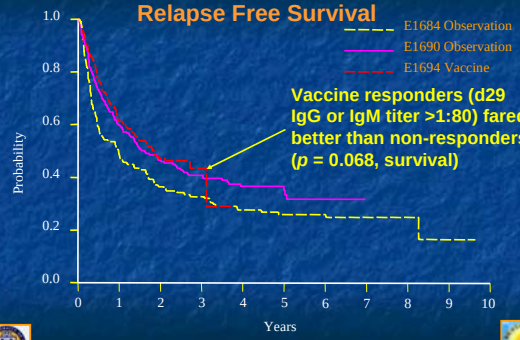
E1694: Overall Survival



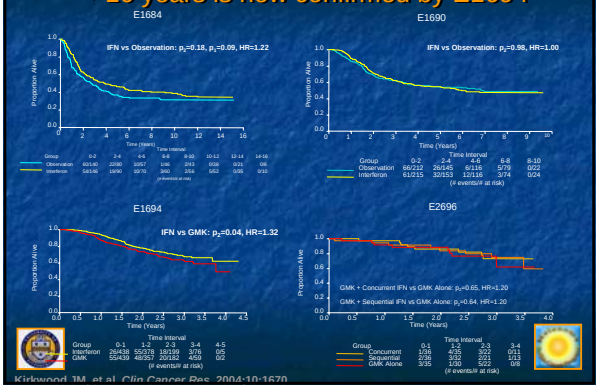
Months and no. events/no. at risk

Group	0-10	10-20	20-30	30-40	40-50
IFN-α2b	11/385	29/258	11/125	1/38	0/1
GMK	23/389	42/264	14/132	2/38	0/2

E1684 & E1690 Observation Arms vs E1694 Vaccine Arm Relapse Free Survival

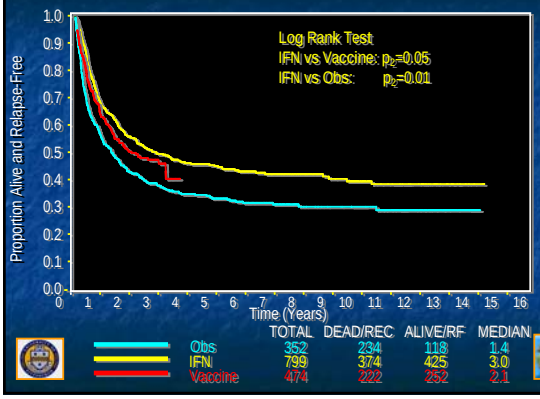


Overall survival benefit for pivotal E1684 to >10 years is now confirmed by E1694



Kirkwood JM, et al. *Clin Cancer Res.* 2004;10:1670

Relapse-Free Survival by Treatment



Conclusions from the Primary Trial Data: IFN in High-Risk Melanoma

- Highest level of evidence, based on analysis of the primary endpoints of prospective randomized multicenter cooperative group trials demonstrate:
  - Consistent high-dose IFN-α2b benefit for RFS and OS compared to observation and GMK
  - Hazard for relapse without IFN increases 1.24-1.38 fold
  - Hazard for mortality without IFN increases 1.22-1.32 fold
  - No differential stage-specific effects

No less toxic IFNα 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 (pending)

Current Intergroup Adjuvant Trials of ECOG, SWOG, CALGB

- Improve therapeutic index of HDI using induction IFN only, or neoadjuvant application
  - Intergroup E1697: 1 month IV HDI *vs.* Obs for intermediate risk stage IIA[US], IIB/IIIA[CA-AU]
  - UPCI 00-008 1 month IV HDI neoadjuvant
- Improve DC number/function
  - Intergroup E4697: Adjuvant evaluation of GM-CSF and multi-epitope peptide vaccine in resected stage IIIB,C & M1) with GM-CSF, multi-epitope peptide vaccine
  - UPCI 03-107, 00-079, and 04-020 test new plasmacytoid DC stimulant CpG

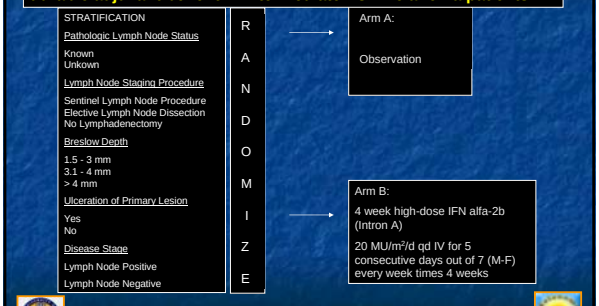
How to improve the therapeutic index?

→Dissect role of induction versus maintenance

- All positive trials of IFNα have given one month of IV induction therapy at 20MU/m^2 (C_{max} >10,000u/ml)
- Is one month of intravenous IFNα2b necessary and sufficient?
  - Intergroup E1697
  - Sunbelt Melanoma Trial (PCR-positive, histologically negative sentinel nodes)

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



Current Intergroup Adjuvant Trials of ECOG, SWOG, CALGB

- Improve therapeutic index of HDI using induction IFN only, or neoadjuvant application
  - Intergroup E1697: 1 month IV HDI *vs.* Obs for intermediate risk stage IIA[US], IIB/IIIA[CA-AU]
  - UPCI 00-008 Neoadjuvant trial of 1 month IV HDI
- Introduce more Specific Peptide Vaccination and Improve DC number/function with GM-CSF
  - Intergroup E4697: Adjuvant evaluation of GM-CSF and multi-epitope peptide vaccine in resected stage IIIB,C & M1)

Understanding of the mechanism of high-dose IFNα is critical to progress

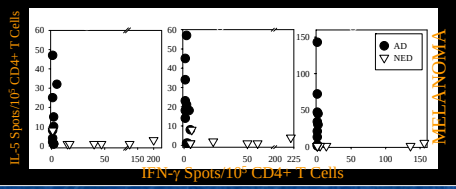
- Immunomodulatory effect on tumor (↑MHC class I, II, or costimulatory molecules)
- Immunomodulatory effect upon host (polarization of CD4/CD8 or dendritic cell function, or resistance to tumor-induced apoptosis)
- Antitumor cytotoxic effect
- Antiproliferative cytostatic effect
- Antivascular antiangiogenic effect

Molecular biology and immunology of melanoma progression are increasingly understood; difficult to evaluate in postoperative adjuvant setting

- Molecular events in melanoma progression
  - Constitutive activation of Stat3→tolerance (triggers VEGF, IL-10)
  - Anti-apoptotic mechanisms: bcl-2, bax, bcl/xl
- Immunologic events
  - Loss of MHC class I, II molecule and costimulatory molecule expression
  - Polarization of host response—DC, CD4 and CD8 T cell toward tolerance rather than effector function

Tumor-Induced T Cell Functional Modulation

Melanoma Patients With Active Disease Display Th2, not Th1-type CD4+ T Cell Responses to Tumor Antigens



Th1 → *DTH, CTL*

Th2 → *Ab*

Tatsumi et al., J. Exp. Med. 2002

How to improve the therapeutic index? Move induction before surgery as 'neoadjuvant'

- Improve therapeutic index of HDI using induction IFN only, or neoadjuvant application
  - Intergroup E1697: 1 month IV HDI *vs.* Obs for intermediate risk stage IIA[US], IIB/IIIA[CA-AU]
  - UPCI 00-008 neoadjuvant trial of 1 month IV HDI prior to surgery
- Improve DC number/function with GM-CSF, multi-epitope peptide vaccine
  - ECOG E1696 and Intergroup E4697: Therapeutic and adjuvant evaluations of multi-epitope peptide vaccines, GM-CSF
- Combine IL-2 and IFN with CVD: chemobiotherapy
  - Intergroup S0008: CVD-IFN-IL-2 × 3 months *vs* HDI for 1 yr. In stage III B/C

Neoadjuvant Trial UPCI 00-008

Stage IIIB/C

→ Surgical Biopsy

→ IFNa2b 20Mu/m2/d x 5d/wk x 4

Sample 1

→ Lymphadenectomy

Sample 2

- Tumor analysis for T cell & DC infiltrate, molecular studies at d1, 29, ± relapse
- Blood lymphocyte phenotypic and functional immune analysis at 7 time points d0, 1, 15, 29, 36, 57, 365
- Blood serum for immunological and proteomic analysis

Neoadjuvant therapy with IFNa2b in Stage III melanoma

Accrual to Dec 2004: 17 subjects

Toxicity precluded completion of IFN in 2

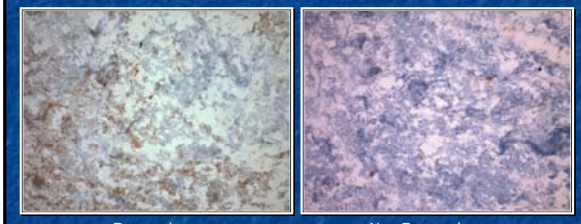
Induction therapy completed in 15

Clinical antitumor response in 9 (53%):

7 PR and 2 CR (by clinical radiologic and pathologic evaluation at day 29)

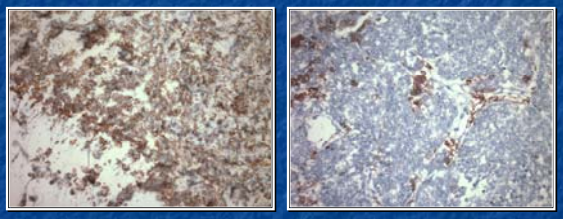
Tumor tissue biopsies adequate for analysis in 15 pre and 12 post-therapy

Endo-Tumoral Infiltrate (CD8)



Responder | Non-Responder

Endo-Tumoral Infiltrate (CD4)



Responder | Non-Responder

IFN α therapy significantly augments tissue CD4 infiltrate

Prognostic and Predictive Markers of Melanoma Outcome/Response are Needed

- CD4 - CD8 T cell Primary Tumor Infiltrates may be prognostic of disease outcome
  - Clemente & Mihm, 1996
  - Rao, Mihm, Kirkwood 2005 in preparation
- CD4 and CD8 T cell Infiltrates of regional nodal metastasis predict IFNα2b benefit
  - Hakannson et al 1996
  - Moschos, Rao, Kirkwood 2005

Current Adjuvant Trials of UPCI and ECOG, SWOG, CALGB, Intergroup

- Improve therapeutic index of HDI using induction IFN only, or neoadjuvant application
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E1696: Multiepitope Immunization ± IFNα2b ± GMCSF in Metastatic Measurable Melanoma

Eligibility

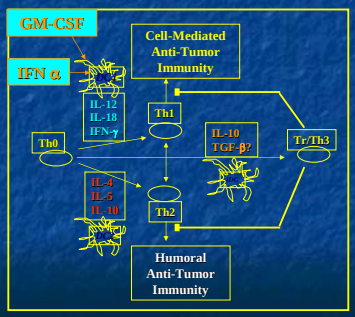
1. Measurable Metastatic Melanoma
2. HLA- A2+
3. PS 0-1
4. Labs

RANDOMIZE

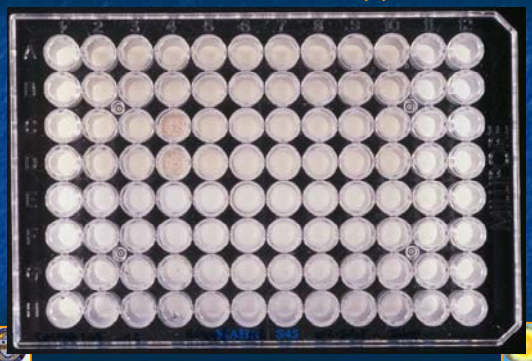
Peptide Vaccination in All Groups:
Melan A/MART-1:27-35 AAGIGLTV
gp100:209-217 (210M) IMDQVPFSV
Tyrosinase: 368-376 (370D) YMDGTMSQV

ARMS	A	B	C	D
GM-CSF	-	-	+	+
IFNα2b	-	+	-	+

Potential Functions of GM-CSF and IFNα upon DC Subsets and Polarization of the Immune Response

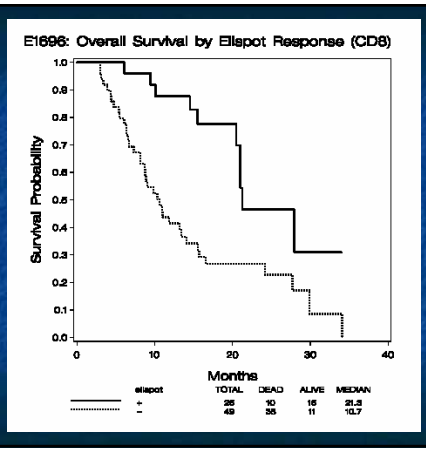


ELISPOT assay detecting production of interferon gamma by individual T cells from the peripheral blood of a patient after vaccination with Melan-A/MART 1 peptide



E1696 Analysis: Immune response to one or more peptide, and modulation with IFN, GM-CSF therapy

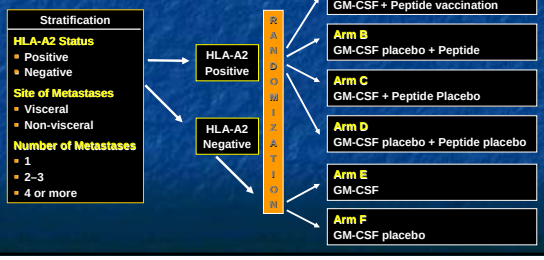
- Immunologic Response Data (n=75)
  - (+) ELISPOT : 26/75= 35% 95% CI (24%, 47%)
  - (-) ELISPOT : 49/75= 65% 95% CI (53%, 76%)
- ELISPOT Reactivity by Treatment with IFN (n=75) (p_1=.075)
  - (+) IFN: 16/37= 43% 95% CI (27%, 61%)
  - (-) IFN: 10/38= 26% 95% CI (13%, 43%)
- ELISPOT Reactivity by Treatment with GM-CSF (n=75)
  - (+) GM-CSF: 14/36= 39% 95% CI (23%, 56%)
  - (-) GM-CSF: 12/39= 31% 95% CI (17%, 48%)



E4697- Adjuvant trial for high risk resected stage III-IV melanoma

Hypothesis: GM-CSF and/or multi-epitope peptide vaccine will be of therapeutic benefit, acting upon T-cells or through dendritic cells in resected stage III-IV melanoma

E4697 Intergroup Trial: 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

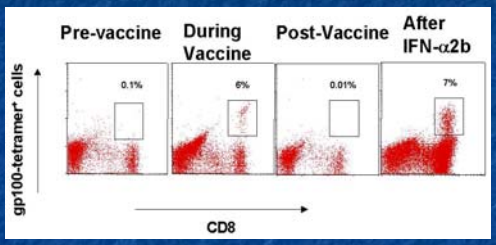


Direct evidence of recall - immunopotentiation of vaccine responses with IFN following vaccination vs. gp 100 for melanoma

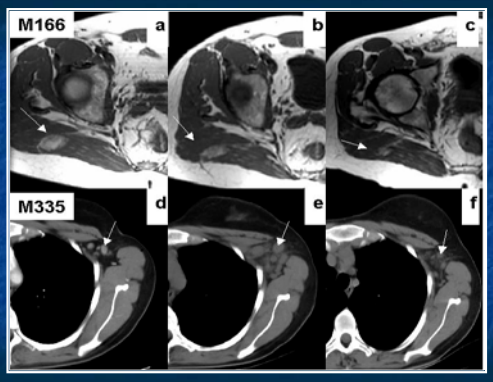
- ALVAC-2 gp100 vaccine experience*
  - 27 melanoma patients vaccinated with ALVAC-2
  - transient response to vaccine, no clinical effects
- Post vaccination treatment of 7 patients with IFN (*ad hoc* at 1.5-9 months)
  - Recall augmentation of durable immune responses (4)
  - Objective antitumor response 2/2 pts with measurable disease
  - T cell cytotoxicity to gp100+ tumor in both

*Astsaturov et al., Clin Cancer Res. 2003

HDI following gp100 vaccination enhances numbers of T cells by flow cytometry



Astsaturov et al, 2003



Rigorous analysis of the role of IFN as an immune modulator of vaccines

- Need prospective analysis of HDI following specific vaccine trials
- UPCI Trial 04-125 is prospective sequel for ongoing vaccine trials
  - Mart/Melan-A CD4 and CD8 epitope trial (UPCI 99-088)
  - ESO-1 protein/CD4-CD8 epitope trial (UPCI 00-079)
  - αDC1 adoptive transfer (UPCI 03-118)

Conclusions

- Immunological approaches to adjuvant high-risk resectable melanoma have achieved the only real successes in past 30 years
- High-risk disease is benefited with adjuvant high-dose IFNα
  - improved relapse-free survival in all studies
  - Improved overall survival in 2 multicenter ph III RCT
  - Immunological mechanism is leading candidate

Adjuvant therapy trials for melanoma should build systematically upon the evidence

- Further progress will come from trials of adequate size, incorporating clinical and laboratory endpoints, building upon current evidence
- Immunotherapy with specific peptide vaccines, and induction of CD4/CD8 T cell responses
- Reversal of tumor cell molecular processes of progression—
  - anti-apoptosis, invasion, angiogenesis
- Reversal of host response lesions of immune tolerance and immunoregulation
  - Surgery to debulk host may prepare for vaccines
  - IFN, IL-12, IL-18 to repolarize host immune system
  - Anti-CTLA-4 antibody to unbrake T cell effector and T regulatory (suppressor) cells
  - Adoptive transfer of polarized DC, NK, or T cells
    - Minitransplant?

Leading current candidates for adjuvant application in combination with IFNα2b

- Anti-GD3 monoclonal chimeric antibody KW2871: UPCI 04-193
  - Cytotoxic to melanoma at pcg/ml quantities
  - ADCC a major mechanism of action
    - Enhancement by IFN well documented
  - Half-life of 2+ weeks after IV dosing
  - Non-immunogenic (no HACA) with repeated dosing (Scott, J. Clin Onc 2001)

Leading current candidates for adjuvant application in combination with IFNα2b

- Anti-CTLA4 is a potent immunotherapeutic approach to melanoma
  - Single agent: UPCI 04-065 will test dosing at 10mg/kg every 3 weeks vs 15 mg/kg once (and then potentially after 3 months)
  - Combined with peptide vaccination: UPCI 04-125 will test dosing in conjunction with gp100 vaccine
  - Potentially most interesting application of anti-CTLA4 antibody will be in the adjuvant setting
  - Combination with IFNα2b is in design

Evaluation of the effects of IFN and other agents is feasible in precursor atypical nevi

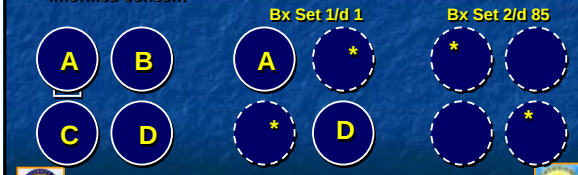
- Feasible given frequent appearance of atypical nevi in patients with melanoma
- Will allow more complete identification of molecular & immunologic markers of progression
- Provides an avenue to analyze the mechanism of IFN in melanocytic processes

Analysis of Effects of High-Dose IFNa2b upon the Morphology, Histopathology, Molecular and Immunologic Features of Atypical Nevi in Patients with Melanoma (UPCI 95-71)

Eligibility

- **High-risk resected melanoma**
- **≥4 atypical nevi**
- **Planned IFNa2b**
- **Informed consent**

Photographic Documentation d 1, d 29, d 57, d 85



*Random selection by Statistical Office