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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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

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 IIIB-III is high-dose IFNα/2b
  —10% durable disease-free survival benefit based on multiple phase III cooperative group trials

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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)

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 & Chemotherapy
- 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 (S0003)
  - GM-CSF (E4697)
  - IFN (E4687, S8710)
  - IFNα2

Expression of antigens by the tumor

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

Question: can immune recognition and response to (melanosomal) markers of melanoma be harnessed in stage IV disease to reverse autoimmunity?
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

**HDI Trials Timeline**

- E-1684: Study Design
  - Observation: 52 wk
  - Randomization: N = 287 (within 56 days)
    - Induction: High-dose IFN-α2b
    - Maintenance: 48 wk
  - (All with ELND)
  - Induction: 20 MIU/m² IV x weekly x 4 wk
  - Maintenance: 10 MIU/m² SC TIW x 48 wk
  - Design: Exponential model, hazard ratio analysis
  - Stratification: AJCC stage groupings

- **E1694 Intergroup Phase III Trial of High or Low Dose IFN-α2b Versus Observation**
  - Randomization: N = 642 (within 70 days)
    - High-dose IFN-α2b x 1 yr
    - Low-dose IFN-α2b x 2 yr
    - Observation
  - Stratification: AJCC stage groupings and number of positive nodes
  - 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

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

<table>
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<tr>
<th>Cooperative group</th>
<th>Eligibility</th>
<th>Treatment arms</th>
<th>Comparison</th>
<th>Impact on</th>
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<tr>
<td>NCCTG 837052</td>
<td>T3-4, N1</td>
<td>IFN-α2b 10 MU/m² SC TIW x 11 mos vs @ 4.3 – 6.6 yrs</td>
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<td>UKCCR Aim-High</td>
<td>T4, N1</td>
<td>IFN-α2b 10 MU/m² SC TIW x 11 mos vs @ 6.9 – 12.6 yrs</td>
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<td>GMK + IFN vs GMK + IFN</td>
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<tr>
<td>WHO #16</td>
<td>N1-2</td>
<td>IFNg 0.2 mg/D SC QOD x 1 yr</td>
<td>DFS, OS</td>
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<td>T4, N1</td>
<td>IFN-α2b 10 MU/m² SC TIW x 11 mos vs @ 4.3 – 6.6 yrs</td>
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**E1690 Phase III Trial of High or Low Dose IFN-α2b Versus Observation**

- Randomization: N = 642 (within 70 days)
  - High-dose IFN-α2b x 1 yr
  - Low-dose IFN-α2b x 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
Two randomized trials of high-dose IFN-α2b show a significant overall survival advantage

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

![Graph showing overall survival with treatment groups.]

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

![Graph showing overall survival comparison between groups.]

Relapse-Free Survival by Treatment

![Graph showing relapse-free survival by treatment.]

Vaccine responders (d29
IgG or IgM titer >1.80) fared better than non-responders
(p = 0.068, survival)

![Graph showing relapse-free survival comparison by treatment groups.]

Log Rank Test

IFN vs Vaccine: p2=0.05
IFN vs Obs:        p2=0.01

![Statistical tests results for relapse-free survival.]

E1694: Overall Survival

![Graph showing overall survival with time intervals and events.]

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

![Graph showing relapse-free survival comparison between groups.]

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

![Statistical hazard ratio for death without IFN.]

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

![Graph showing relapse-free survival comparison between groups.]

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(p = 0.068, survival)

![Graph showing relapse-free survival comparison by treatment groups.]

Overall survival benefit for pivotal E1684 td
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![Graph showing overall survival comparison between groups.]

E1684 Observation
E1690 Observation
E1694 Vaccine

![Graph showing overall survival comparison between groups.]

E2696

![Graph showing overall survival comparison between groups.]

GMK + Concurrent IFN vs GMK Alone: p2=0.65, HR=1.20
GMK + Sequential IFN vs GMK Alone: p2=0.64, HR=1.20

![Statistical tests results for relapse-free survival.]

Log Rank Test

IFN vs Vaccine: p2=0.05
IFN vs Obs:        p2=0.01

![Statistical tests results for relapse-free survival.]

Kirkwood JM,
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

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 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

E1697 - A randomized study of four weeks of high-dose interferon alpha-2b in stage IIB-IV or N1 (melanoma melanoma)

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<td><strong>Randomization</strong></td>
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<tr>
<td>4 week high-dose IFN alpha-2b (Intron A)</td>
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<tr>
<td>20 MU/m2/day IV for 5 consecutive days then 2 MU/m2/day every week for 4 weeks</td>
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<td><strong>Arm A:</strong></td>
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<tr>
<td>Observation</td>
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<td><strong>Arm B:</strong></td>
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<tr>
<td>4 week high-dose IFN alpha-2b (Intron A)</td>
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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)

Dissect role of induction versus maintenance

- All positive trials of IFNα have given one month of IV induction therapy at 20MU/m² (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)

How to improve the therapeutic index?

- Intergroup E1697
- SWOG, CALGB

Current Intergroup Adjuvant Trials of ECOG, SWOG, CALGB

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  - UPCI 00-008 Neoadjuvant trial of 1 month IV HDI
- Improve DC number/function with GM-CSF
  - EORTC 18871
  - WHO Trial 16, ECOG 1690, UK AIM-High, & Scottish trial
- Introduce more Specific Peptide Vaccination and Improve DC number/function with GM-CSF
  - EORTC 18952
  - Scottish trial

SWOG, CALGB
Understanding of the mechanism of high-dose IFNa 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

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

- Polarization of host response—DC, CD4 and CD8 T cell toward tolerance rather than effector function
- Loss of MHC class I, II molecule and costimulatory molecule expression
- Anti-apoptotic mechanisms: bcl-2, bax, bcl-xl
- Constitutive activation of Stat3

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 vaccines
  - ECOG E1696 and Intergroup E4697: Therapeutic and adjuvant evaluations of multi-epitope peptide vaccines, GM-CSF
- Combine IL-2 and IFN with CVD: chembiotherapy
  - Intergroup S0008: CVD-IFN-IL-2 × 3 months vs HDI for 1 yr. In stage III B/C

Neoadjuvant Trial UPCI 00-008

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

- Tumor analysis for T cell & DC infiltrate, molecular studies at d1, 29, + relapse
- Blood lymphocyte phenotypic and functional immune analysis at 7 time points d6, 1, 15, 29, 36, 57, 365
- Blood serum for immunological and proteomic analysis
**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
  - Intergroup E1697: 1 month IV HDI vs. Obs for intermediate risk stage IIA[US], IIIB[CA-AU]
  - UPCI 00-008: 1 month IV HDI neoadjuvant

- 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 S0006: CVD-IFN-IL-2 × 3 months vs HDI for 1 yr. in stage III BIC

**E1696: Multiepitope Immunization + IFNα2b + GMCSF in Metastatic Measurable Melanoma**

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

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

**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: 20/75 = 35% 95% CI (24%, 47%)
    - (-) ELISPOT: 49/75 = 65% 95% CI (53%, 76%)
  - ELISPOT Reactivity by Treatment with IFN (n=75) (p = 0.075)
    - (+) 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%, 46%)

**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

**Stratification**

- **HLA-A2 Status**
  - Positive
  - Negative
- **Site of Metastases**
  - Visceral
  - Non-visceral
- **Number of Metastases**
  - 1
  - 2-3
  - 4 or more

**Arm A**
- GM-CSF + Peptide vaccination

**Arm B**
- GM-CSF Placebo + Peptide

**Arm C**
- GM-CSF + Peptide Placebo

**Arm D**
- GM-CSF Placebo + Peptide Placebo

**Arm E**
- GM-CSF Placebo

**Arm F**
- GM-CSF Placebo

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

*Aikaturov et al., Clin Cancer Res. 2003*
HDI following gp100 vaccination enhances numbers of T cells by flow cytometry

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)

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?

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 IFNα2b
  - Improved relapse-free survival in all studies
  - Improved overall survival in 2 multicenter ph III RCT
- Immunological mechanism is leading candidate

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

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Analysis of Effects of High-Dose IFNα2b upon the Morphology, Histopathology, Molecular and Immunologic Features of Atypical Nevi in Patients with Melanoma
(UPCI 95-71)

Eligibility:
- Photographic Documentation d 1, d 29, d 57, d 85
- High-risk resected melanoma
- ≥4 atypical nevi
- Planned IFNα2b
- Informed consent

Bx Set 1/d 1
Bx Set 2/d 85

*Random selection by Statistical Office*