Systemic Therapy of Melanoma
Current Approved Options

Stage IIb-III (high risk) Melanoma

- Only High-dose IFNα is approved by US FDA

Stage IV (inoperable) survival <2% at 5+ years

- Only one cytotoxic agent is approved by FDA
  - Dacarbazine (Temozolomide) with 6.8-12% response in modern trials, rarely durable
- Only one biologic approved in modern times
  - High-dose IL-2, with 15% response and 5% durable responses

Opportunities from Chemotherapy and Biological Agents

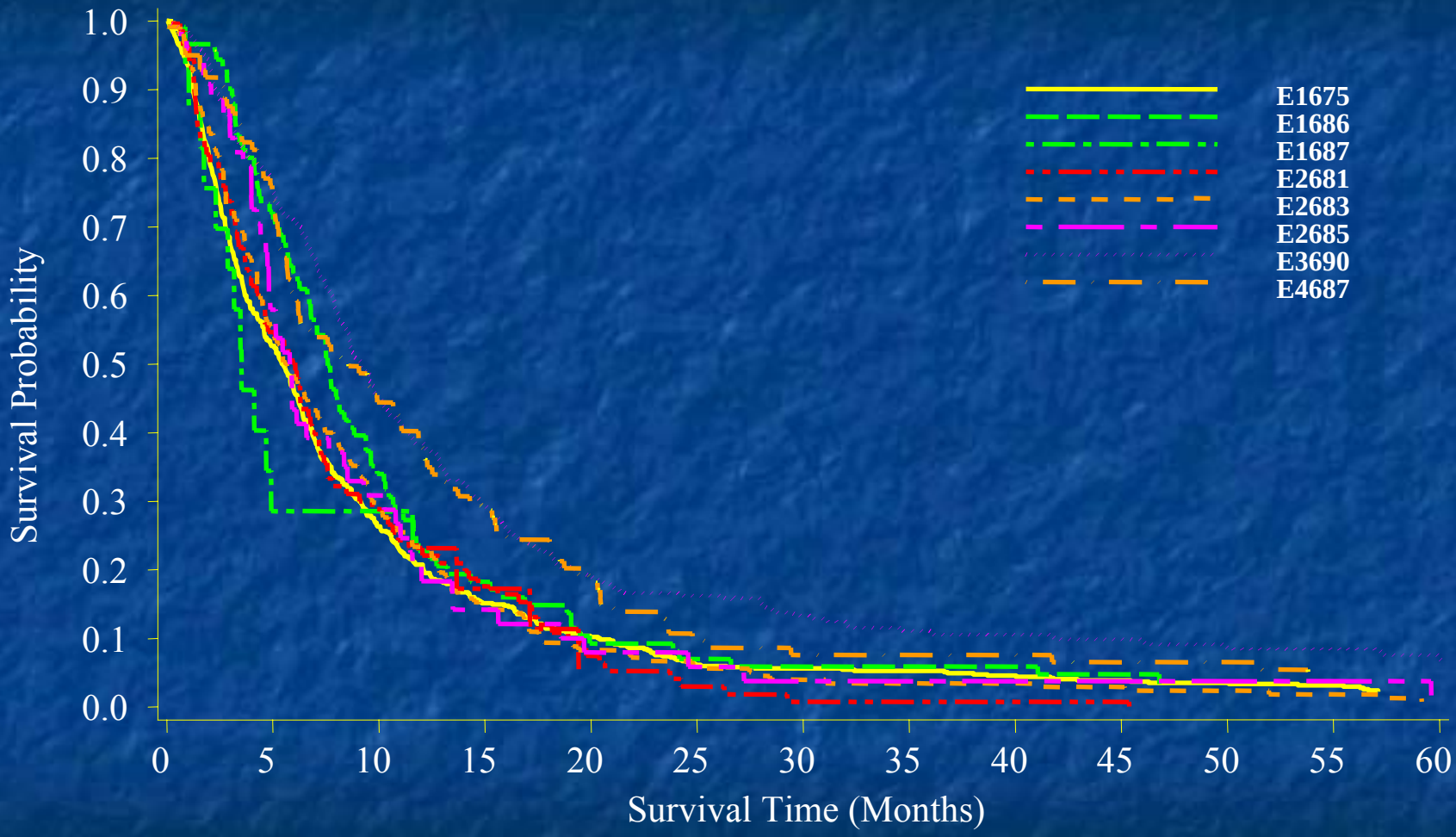
- Tumor bulk reduction in and of itself may facilitate biological therapy
- Chemotherapy may reduce Treg and other elements of immunosuppressive environment
- Chemotherapy may serve as means to release tumor antigen

- Each of these needs to be assessed rigorously to establish proof of principle

Chemotherapy of Melanoma: Little impact to date

- **Tumor cell resistance to cytotoxic drugs (Dacarbazine, TMZ):**
  - I. Alkyl guanine alkyl transferase expression (AGAT) → Resistance
    Patrin, an oral AGAT inhibitor doubles toxicity of DTIC/TMZ
    - Tawbi et al., Proc ASCO 2006
  - II. Mismatch Repair Required for Responsiveness to Alkylators
    - Sobol, et al Proc ASCO 2006
  - III. Base Excision Repair -- β pol and other mechanisms now understood
    PARPi of strong interest:
    - Plummer et al., Proc ASCO 2006 #8013
- **Tumor cell resistance to apoptosis**
  - BCL2
  - Survivin
  - UBC9
  - XIAP
- **Tumor induced immunosuppression**
  - STAT3
  - PDL1
  - FASL

Overall Survival by Protocol



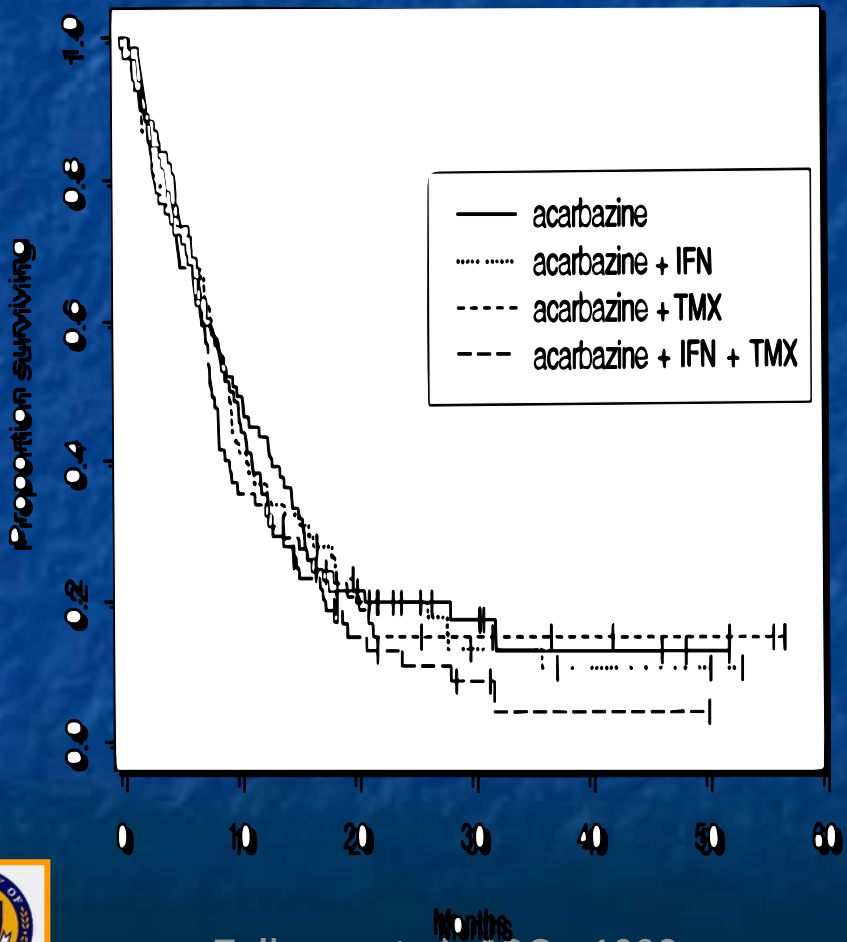
Manola, et al., J. Clinical.Oncology 1999

Empiric Combinations without Intermediate Surrogate Markers have Failed to Improve Outcome in Large Phase III Trials

- Dacarbazine and IFN
- Dacarbazine and Tamoxifen
- CBD and Tamoxifen
- CVD and IL-2 IFN

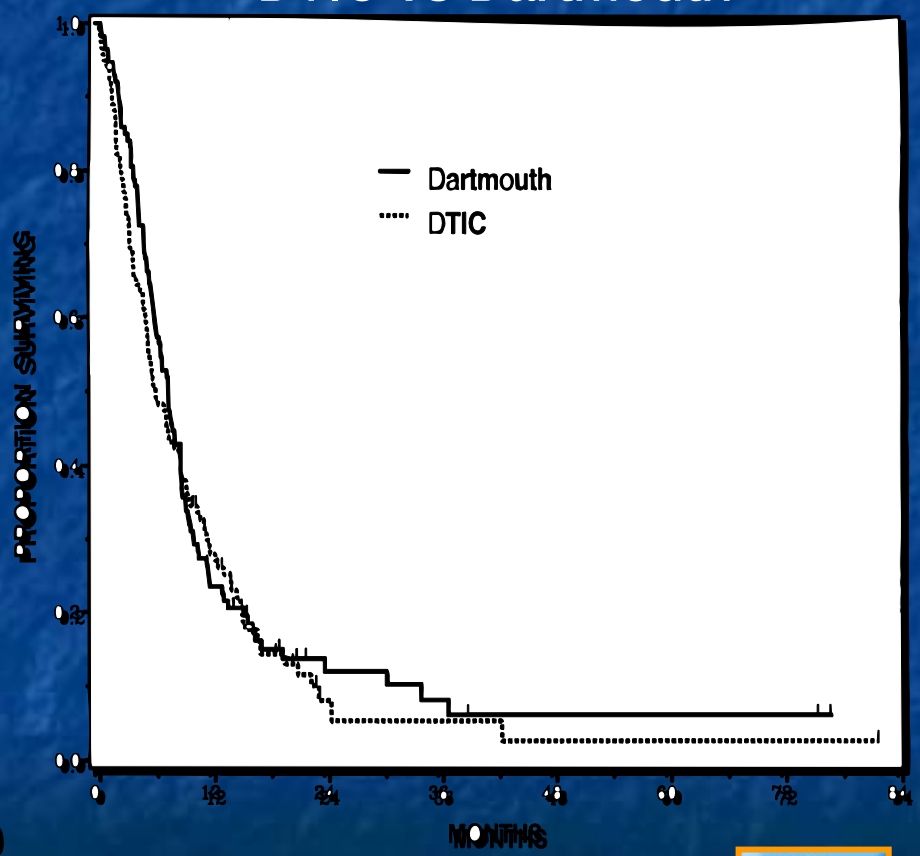
Recent Phase III Trials of Chemotherapy Combinations

DTIC +/- IFN +/- Tam



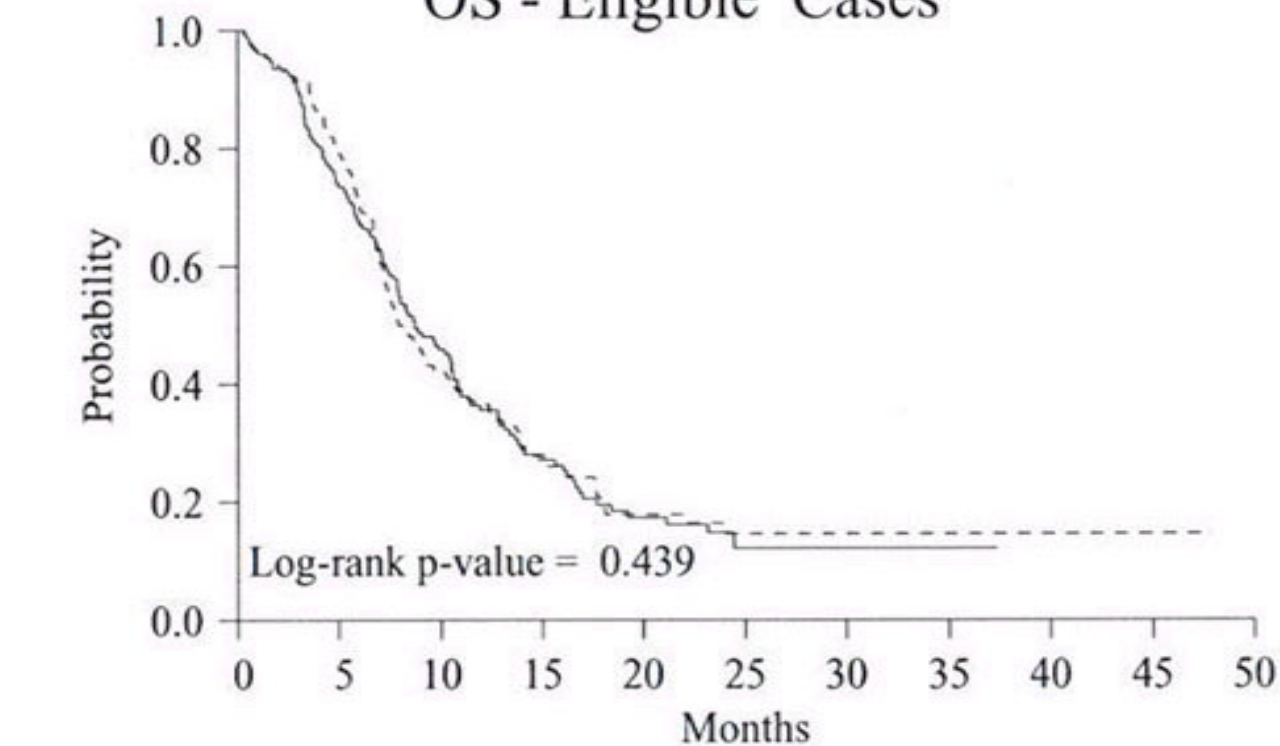
Falkson et al: JCO ; 1998

DTIC vs Dartmouth



Chapman et al: JCO 1999

E3695: Survival Data



Group	Time Interval 0-10	10-20	20-30	30-40	40-50
CVD	85/192	34/63	4/16	0/4	0/0
CVD-BIO	89/194	29/58	2/15	0/7	0/2

(# events/# at risk)

CTEP-sponsored studies of combination targeted therapy

NCI solicited studies in renal cell, glioma, and melanoma have not incorporated chemotherapy (yet)

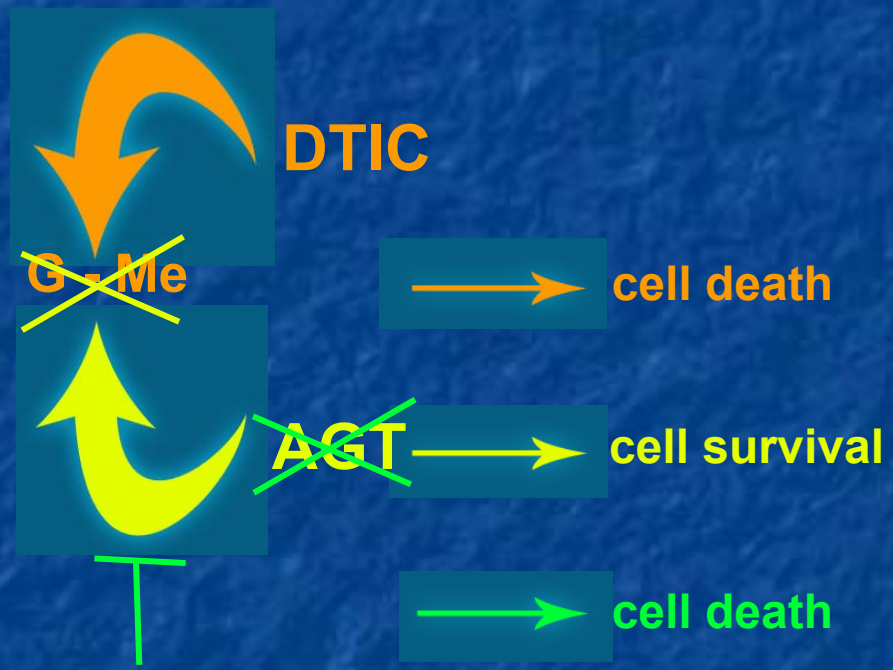
- Melanoma targets of interest: VEGF, Raf, Ras, mTOR
  - Tipifarnib + Sorafenib
  - Bevacizumab + Sorafenib
  - CCI-779 + Sorafenib
  - CCI-779 + Bevacizumab
- In all studies, tumor and surrogate tissue samples to be collected for biologic studies & banked.

Phase I trial of Lomeguatrib (Patrin) combined with dacarbazine for treatment of patients with melanoma

- The DNA repair enzyme O6-Alkylguanine Alkyl transferase (AGT) reverses O6-methylguanine (O6-MeG) base lesion induced by DTIC
- Depletion of AGT using O6-MeG analogs enhances DTIC cytotoxicity in preclinical and phase I trials of O6-Benzylguanine
- Lomeguatrib (Patrin) is orally bioavailable potent O6-MeG analog well-tolerated as a single agent
- First phase I experience for PN combined with DTIC reported in patients who failed prior CTx

Tawbi et al., #8016 24:457 2006

Mechanism of Action of Patrin



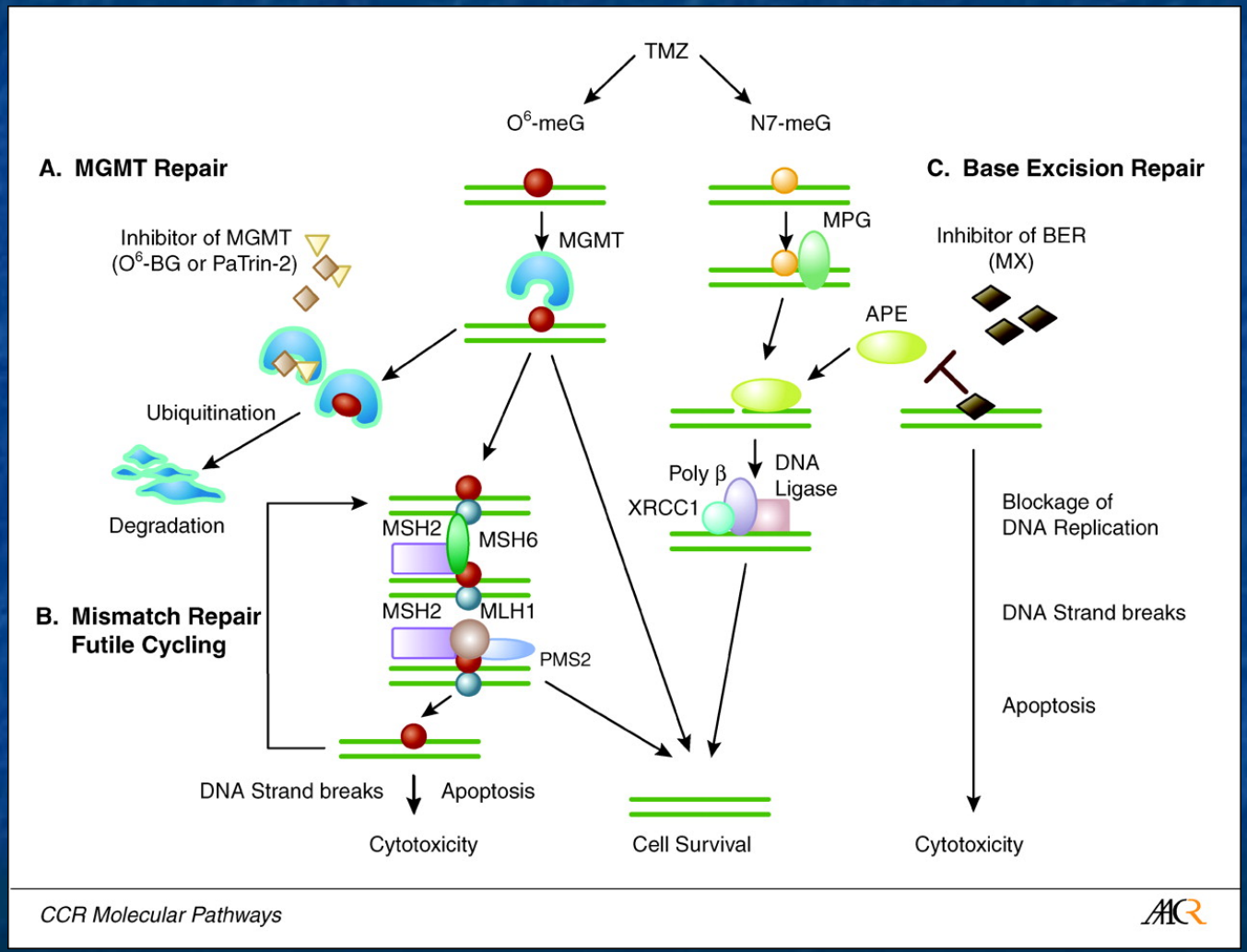
Results

- Adverse events observed in ≥ 50% of pts:
  - grade 1-2 nausea and vomiting,
  - grade 1-2 fatigue
- Hematologic toxicity prominent:
  - gr3-4 neutropenia in 14 pts (47%)
  - gr3-4 thrombocytopenia in 4 pts (13%)
  - even at doses of DTIC 50% of usual clinical doses
- Prolonged neutropenia represented all dose-limiting toxicities (DLT) observed so far
- The MTD not yet reached: hematologic toxicities requiring dose modification of DTIC frequent (42% of all cycles administered).

Conclusions

- Oral Patrin alters toxicity and tolerability of DTIC
  - induces prolonged hematologic toxicity at dosages that are <50% of routine dosages tolerated w/o Patrin
- Results consistent with phase I studies of O^6-MeG analogs but with major advantage of oral route
  - Patrin allows for schedules of administration that provide maximal and prolonged depletion of AGT.
  - In this study Patrin was tolerated at 40 mg bid for 10 days
- MTD of Patrin combined with DTIC to be refined and efficacy in first line therapy defined in ongoing study

To Die or not to Die: DNA Repair Pathways



Liu, L. et al. Clin Cancer Res 2006;12:328-331

Mechanism of Resistance II: MMR

- Functional MMR is required for cytotoxicity of DTIC
- MMR deficiency associated with resistance
- Decreased expression of MLH1, MSH2 and MSH6 related to decreased response
- Epigenetic silencing by promoter gene hypermethylation leads to loss of MMR

Mechanism of Resistance II: MMR

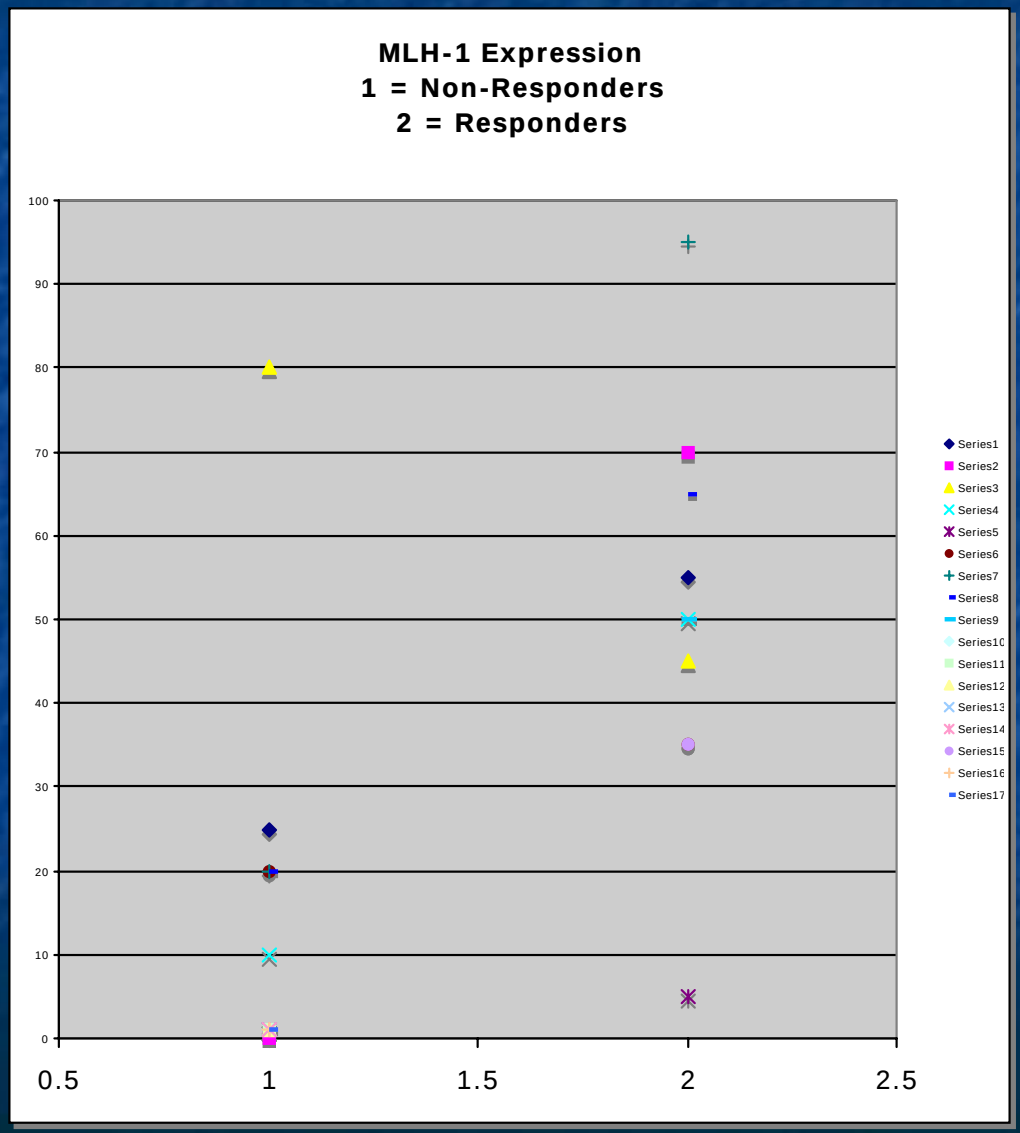
- Pilot study of tumor tissues from DTIC/TMZ treated patients with response or nonresponse:
  - Tumor tissues from 17 patients with metastatic melanoma treated with alkylator-based therapy at the UPCI Melanoma Program examined

- Analysis of clinical response vs MLH1:
  - responder vs non-responder
  - IHC performed for MLH1

- 7/9 sensitive tumors exhibit high MLH1 expression compared with 1/7 resistant tumors (p=0.015)

Sobol, Tawbi, Kirkwood Proc ASCO 2006

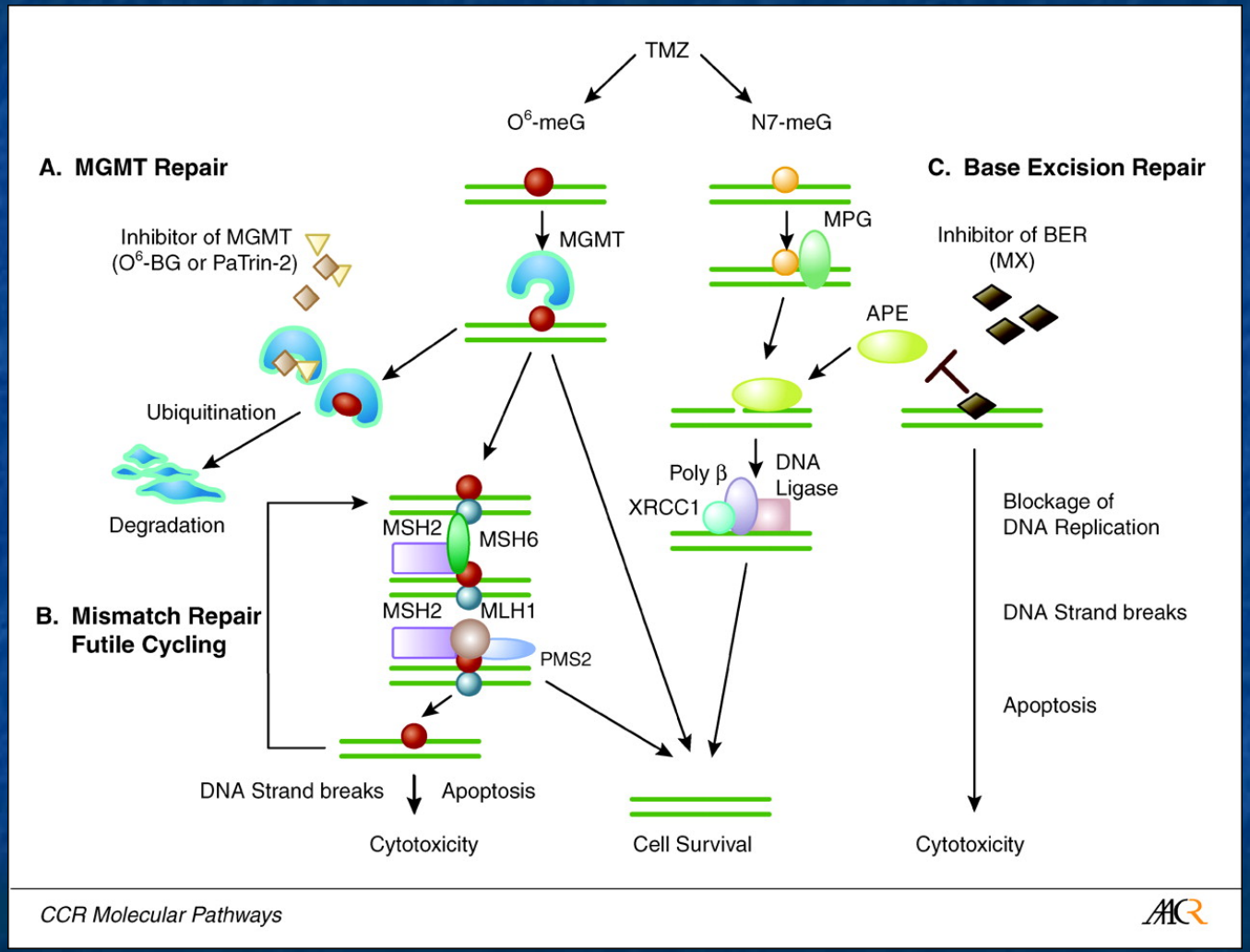
Mechanism of Resistance: MMR
MLH1 expression



Abrogating Resistance II: Reactivate MMR

- Reversal of promoter hypermethylation of MMR genes increases alkylating agent sensitivity
- 5-aza-2'-deoxycytidine re-activates MMR pathway
- TMZ + Patrin + 5-aza-2'-deoxycytidine shows enhanced cytotoxicity in drug-resistant ovarian cancer cell lines
  - *→Dual DNA repair modulation of interest*
- 5-azacytidine is a demethylator already in clinical use for acute leukemia and MDS

To Die or not to Die: DNA Repair Pathways

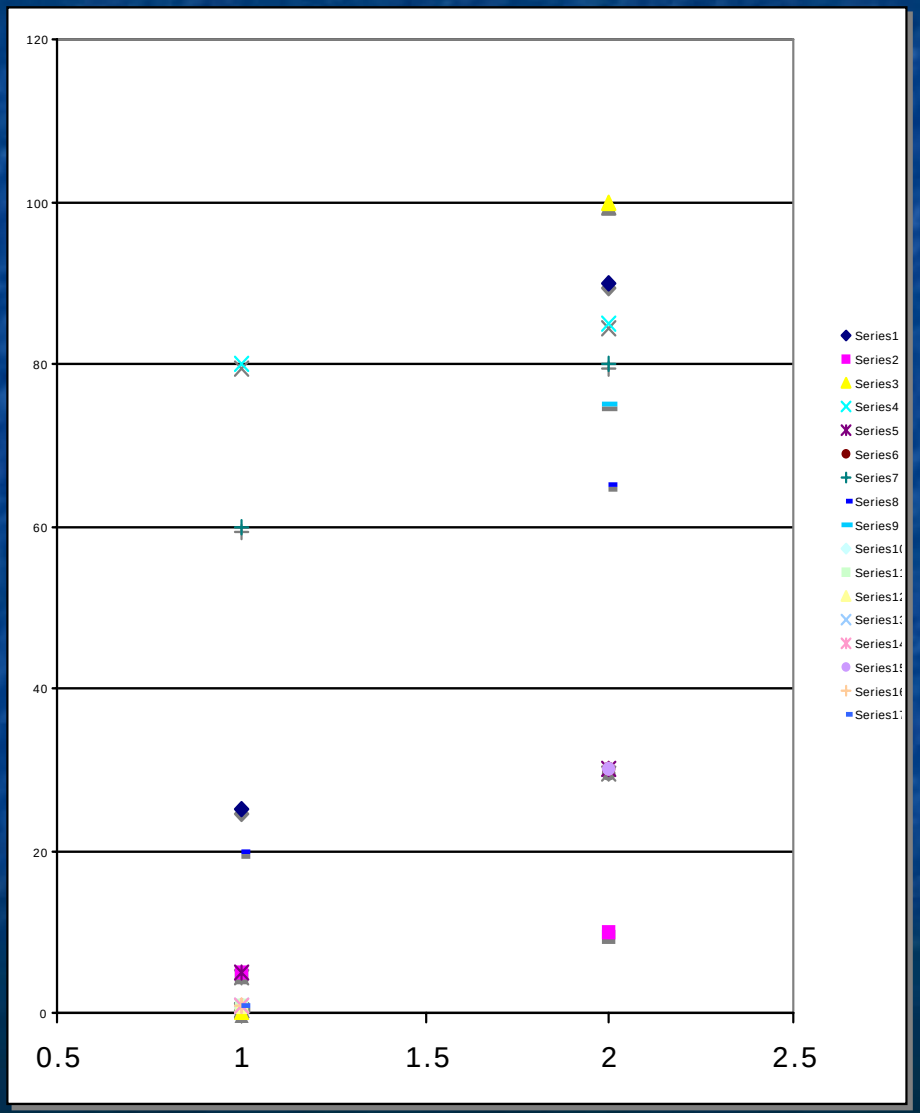


Liu, L. et al. Clin Cancer Res 2006;12:328-331

Mechanism of Resistance III: BER

- N^7-MeG is recognized by DNA glycosylases that initiate BER.
- DNA intermediate (5'-dRP) induces DNA replication block and triggers cellular toxicity.
- 5'-dRP is substrate removed by DNA polymerase β (*pol-β*)
- Alkylating agents induce increased cytotoxicity in *pol-β* deficient cells

Mechanism of Resistance III: BER
Pol-β expression



Abrogating Resistance III: Targeting BER

- Pol-β expression can be inhibited with siRNA, but no small molecule inhibitor is yet available
- Methoxyamine an alternate route to inhibit BER
- Poly (ADP)-Ribose Polymerase (PARP) is an enzyme recruited early at site: negatively charged riboses push DNA strands apart allowing BER proteins to access DNA
- PARP inhibitors are already in phase I trials (UK)

Phase II study of poly (ADP-ribose) polymerase inhibitor (PARPi) AGO14699 in combination with TMZ

- 40 patients with measurable cutaneous MM
  - AGO14699 12 mg and
  - TMZ 200 mg/m2 5x daily q 4 weeks

  Two stage study powered to detect 25% improvement in response to TMZ with 27→40 patients at 3 responses

  Toxicity:

  Grade IV-- ↓Platelets 12% and ↓ANC 15%;

  Gr V: 1

  Response:

  PR in 4 and prolonged SD in 4 of 20 eval; 20 TE

Plummer et al., 2006 #8013 24:457

Chemotherapy/Drug Resistance Conclusions

- Alkylating agent efficacy is decreased due to tumor resistance based upon DNA repair
- Small molecule inhibitors are available or in development for each known pathway
- Future is likely to be multi-targeted approach: combining inhibitors and protecting stem cells
- Individualized therapy is possible based on assessment of DNA repair potential

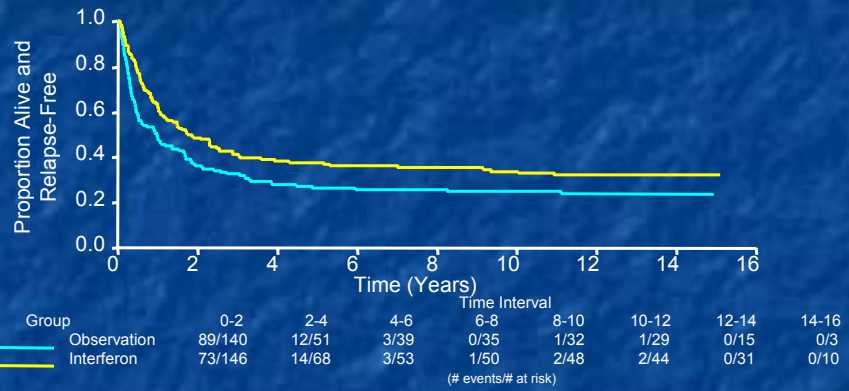
Common themes of successful interventions

- Immunomodulation
- Induction of autoimmunity
  - –anti-pigmentary and other phenomena

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

E1684

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

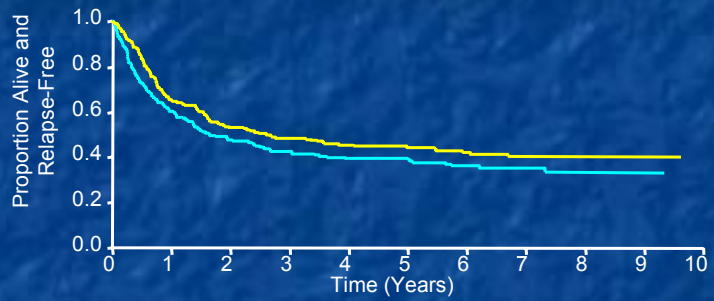


Group	Time Interval 0-2	2-4	4-6	6-8	8-10	10-12	12-14	14-16
Observation	89/140	12/51	3/39	0/35	1/32	1/29	0/15	0/3
Interferon	73/146	14/68	3/53	1/50	2/48	2/44	0/31	0/10

(# events/# at risk)

E1690

IFN vs Observation: p_2=0.09, HR=1.24

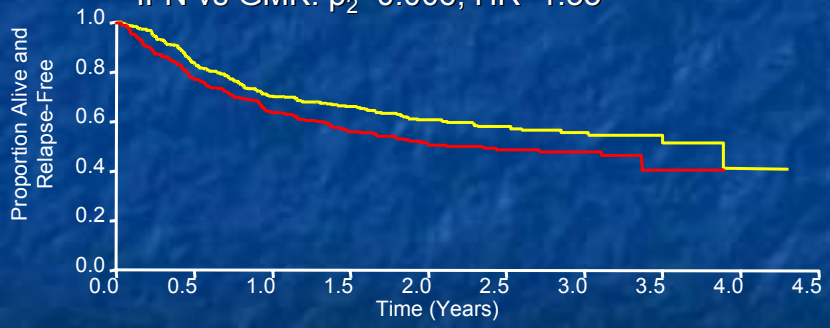


Group	Time Interval 0-2	2-4	4-6	6-8	8-10
Observation	105/212	16/94	5/72	2/44	0/13
Interferon	98/215	15/108	5/85	2/53	0/20

(# events/# at risk)

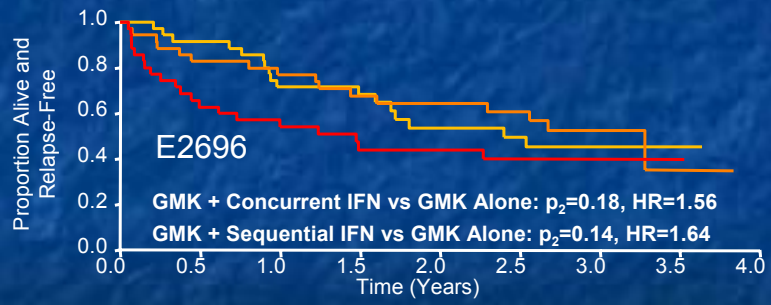
E1694

IFN vs GMK: p_2=0.006, HR=1.33



Group	Time Interval 0-1	1-2	2-3	3-4	4-5
Interferon	118/436	28/257	8/123	3/47	0/3
GMK	153/439	40/240	6/113	3/40	0/0

(# events/# at risk)



Group	Time Interval 0-1	1-2	2-3	3-4
Concurrent	10/36	5/25	2/14	0/4
Sequential	8/36	4/26	3/17	1/7
GMK Alone	16/35	3/17	1/11	0/3

(# events/# at risk)

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

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
Unkown

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

RANDOMIZE

→ Arm A:

Observation

→ Arm B:

4 week high-dose IFN alfa-2b (Intron A)

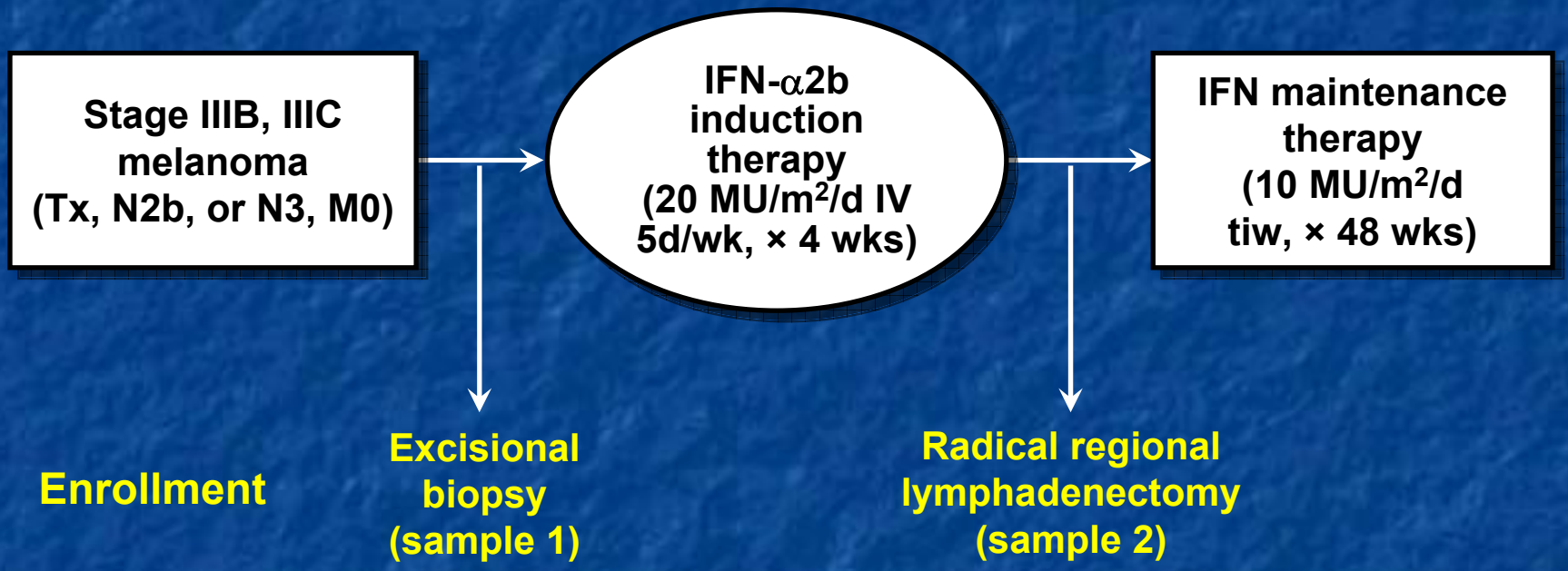
20 MU/m^2/d qd IV for 5 consecutive days out of 7 (M-F) every week times 4 weeks

Neoadjuvant Therapy of Stage III UPCI 00-008 Study

- Biomarker discovery
  - predict treatment efficacy
  - correlate with long-term disease impact
- Define molecular mechanisms of IFN action
  - Which of multiple known direct pro-apoptotic, indirect immunomodulatory, anti-angiogenic effects are critical?
- Measure clinical response early at 4 weeks to determine correlation with RFS and OS

Moschos, Kirkwood Proc ASCO 2005; J. Clin Onc July 1, 2006

UPCI 00-008 Schema



UPCI 00-008 Results

- 20 patients (age: median 59, range 40-78, 13 males)
- 11 with recurrent disease
- 15 completed 4 weeks of HDI
- At 4 weeks of treatment:

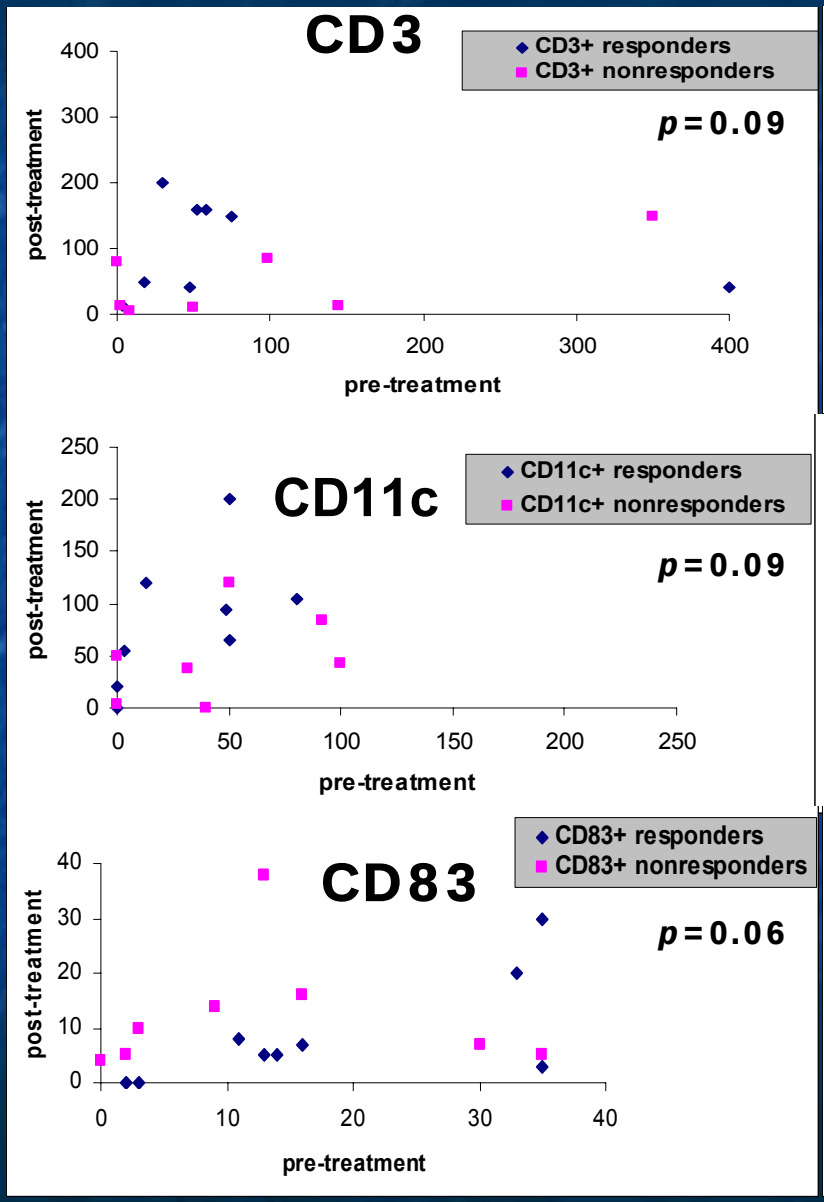
  Clinical responses

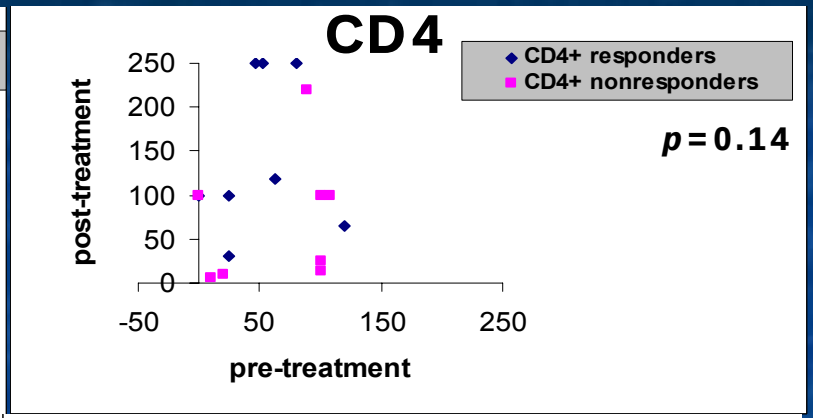
  1 complete, 10 partial

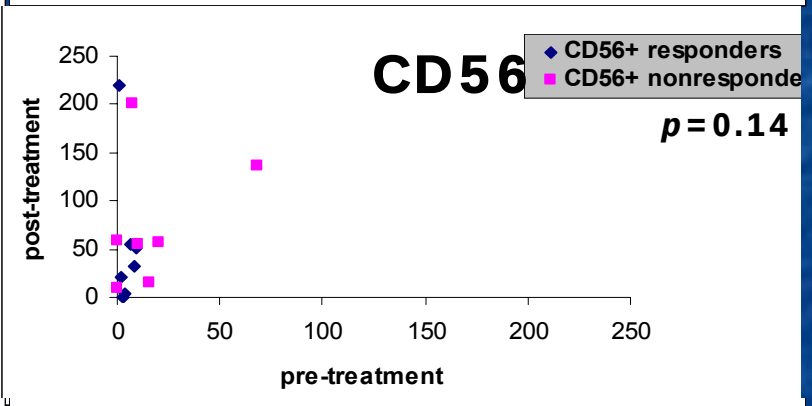
  Pathologic responses

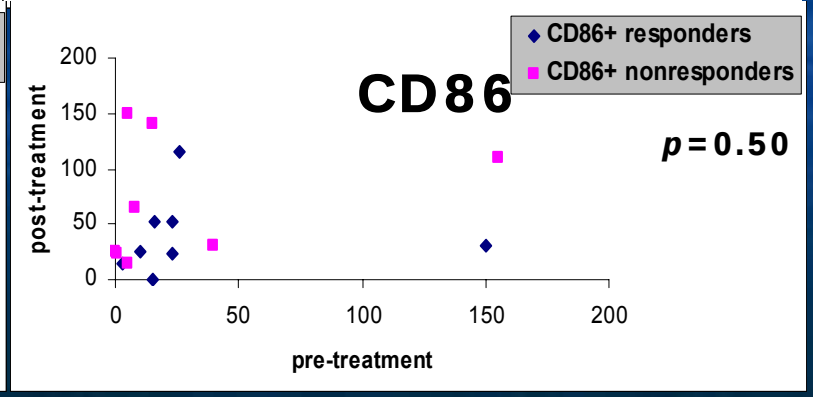
  3 complete, 2 microscopic residual disease

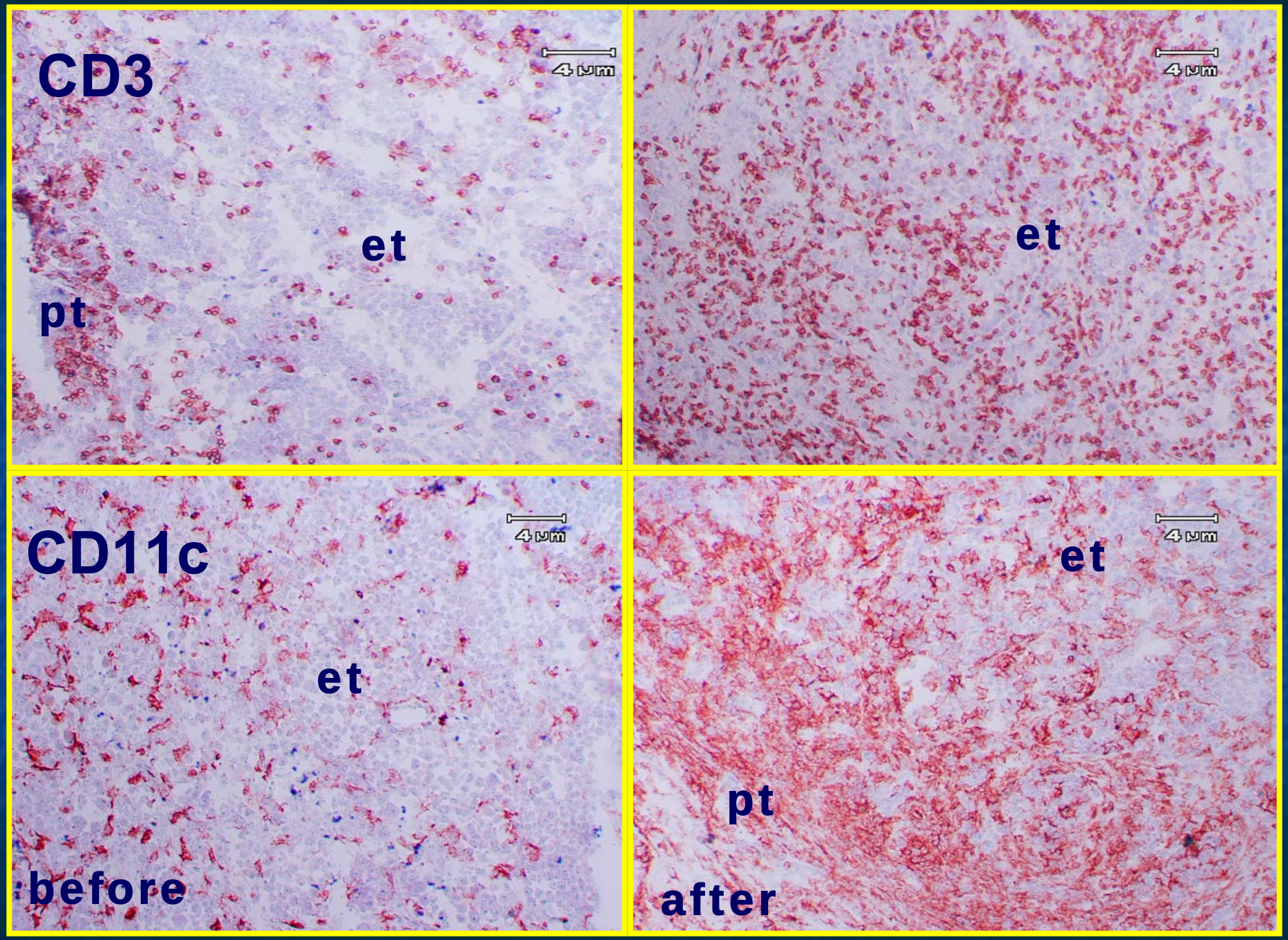
HDI increases the number of immunologically relevant cells infiltrating regional lymph node metastatic tumor










CD3
4 μm
et
pt
4 μm
et
CD11c
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before
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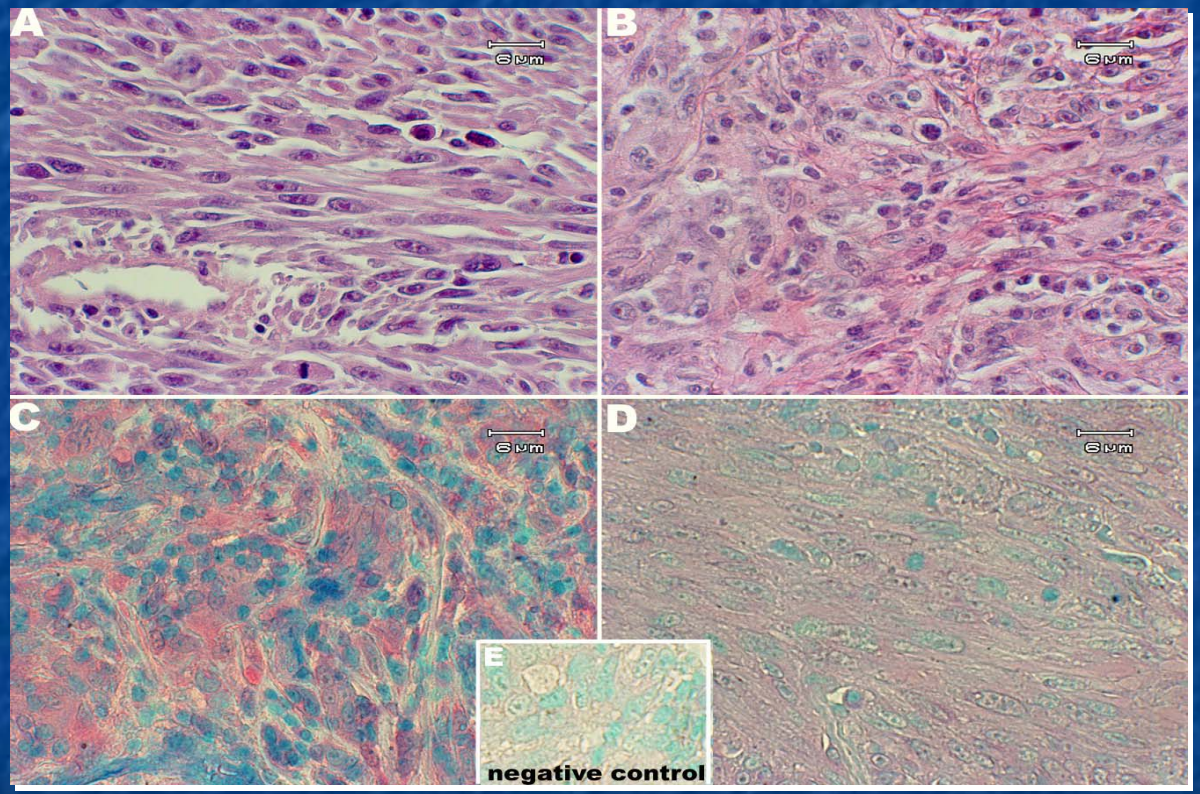
after

HDI Down-Regulates pSTAT3 Tyr705 And STAT3 Expression in Tumor Cells

Pretreatment **Post treatment**

H&E

IHC



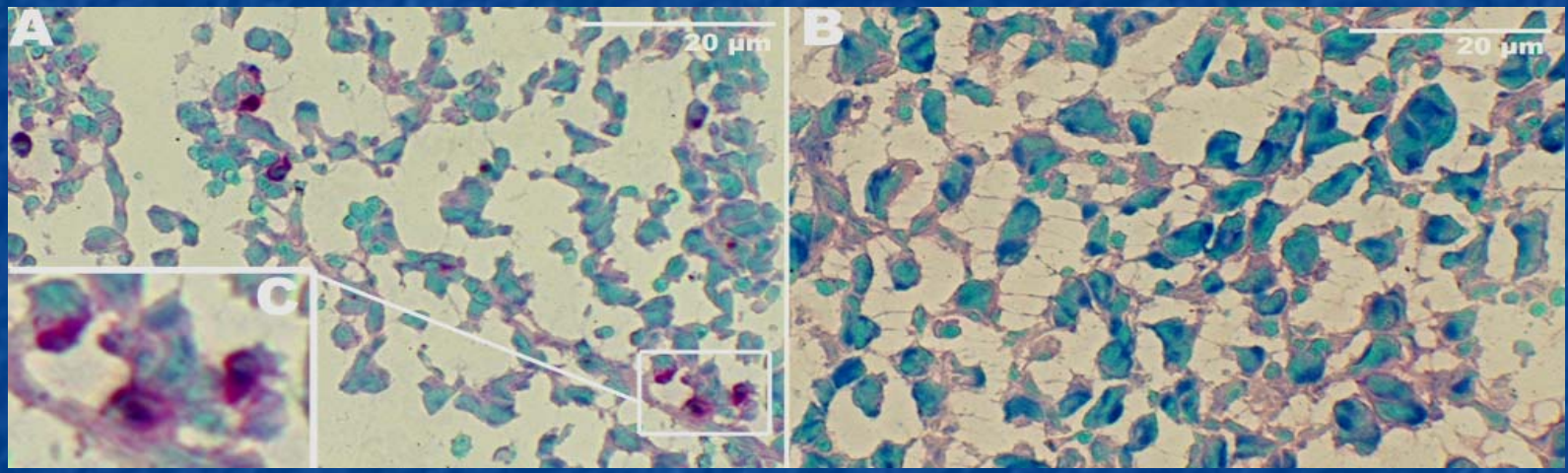
Blue = pSTAT3tyr705
Red = STAT3

HDI Down-Regulates pSTAT3 Tyr705 and STAT3 in Regional Lymph Node Melanoma

HDI Up-Regulates pSTAT1 Tyr701 as it Down-Regulates pSTAT3 Tyr705 and therefore Alters STAT1/STAT3 Balance

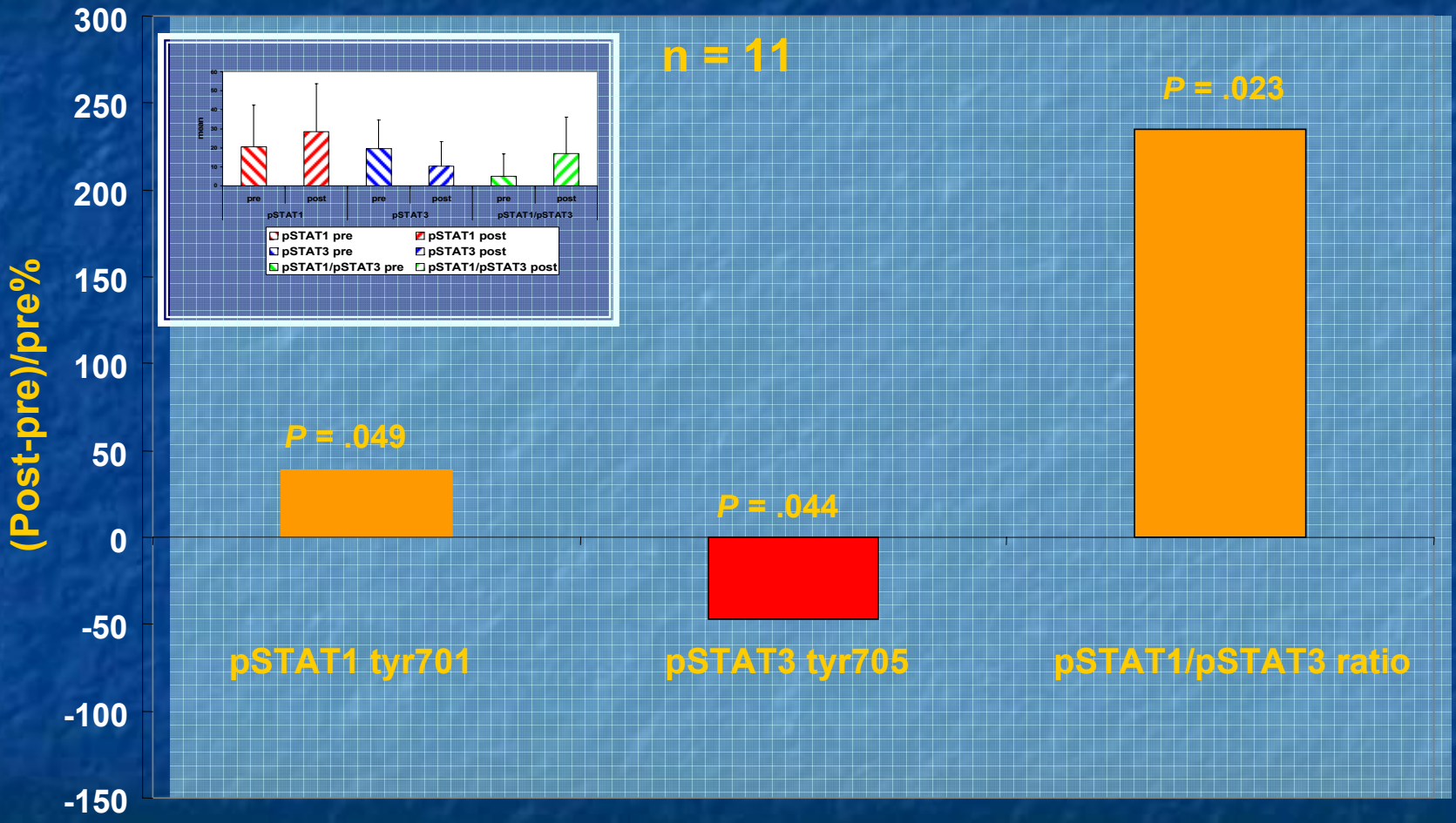
Snap-Frozen Regional Lymph Node Tumor

Pretreatment **Post treatment**



Blue = pSTAT1 tyr701
Red = pSTAT3 tyr705
Frozen section IHC

HDI Up-regulates pSTAT1 Tyr701 and Down-regulates pSTAT3 Tyr705 in Melanoma



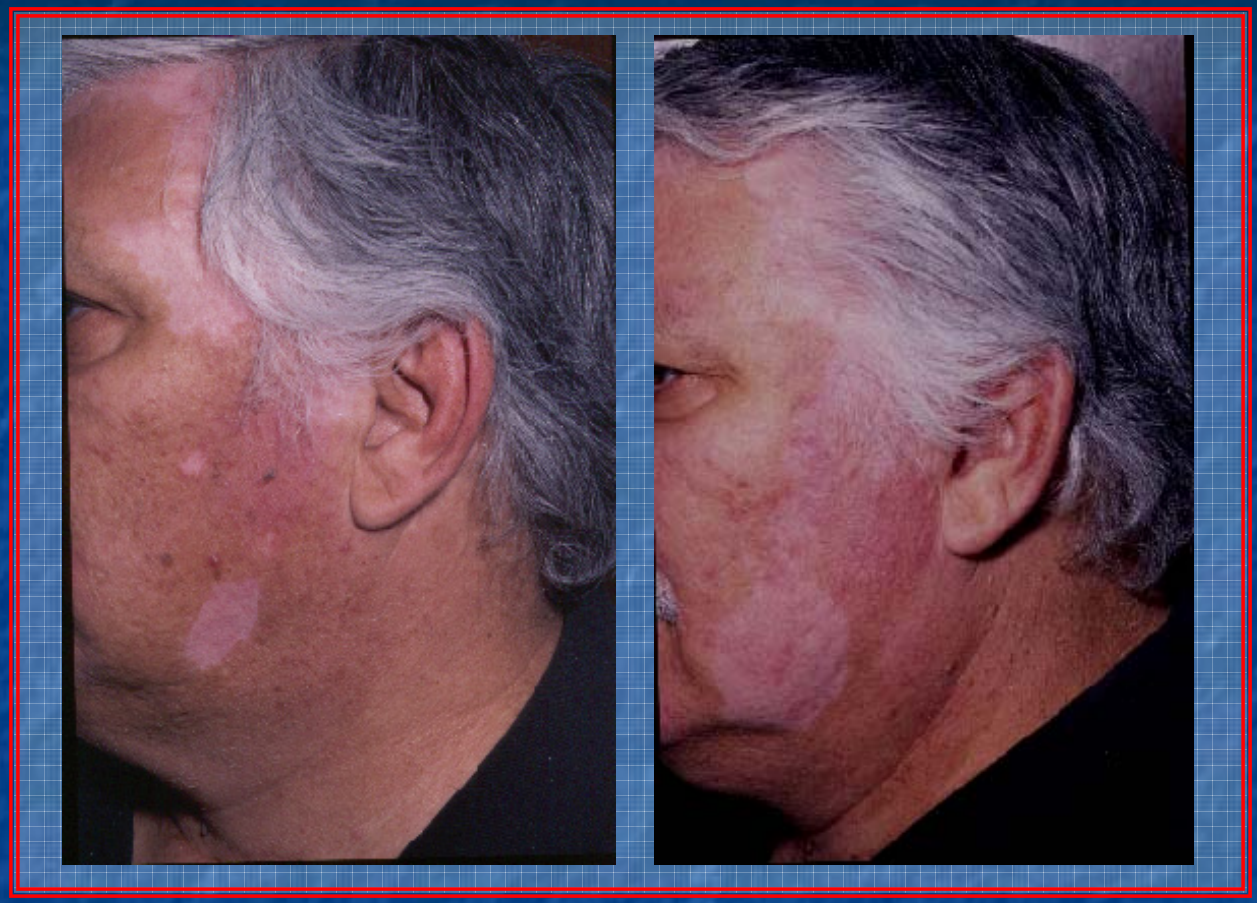
Conclusions of Neoadjuvant High-Dose IFN-α2b Trial UPCI 00-008

- Improved clinical response at day 29
  - 55% of patients with objective response
  - Radiographic and pathologic criteria
  - Relapse-free and overall survival data too early for final assessment
- Molecular and immunologic impact including:
  - ↓ pSTAT3/STAT3, IFNAR2
  - ↑ pSTAT1, pSTAT1/3 ratio, and TAP2
  - ↑ CD3 T cell and CD11c dendritic cell populations in tumor

Current Approaches to Improve Results with Adjuvant Therapy

- Hellenic Oncology Group Trial comparing Induction alone vs 1 year of modified HDI
  - Autoantibody response predicts RFS and OS (Gogas 2006)
- IMI trial of HDI Induction q. 2 mos x 4 (80 doses, n=300, Endpoint=RFS, OS)
- DeCOG trial of HDI Induction q. 4 mos x 1 year (60 doses, n=800, Endpoint=OS)
  - Accrual is now ~15/month with 14 sites
  - Intermediate endpoint MX protein induction

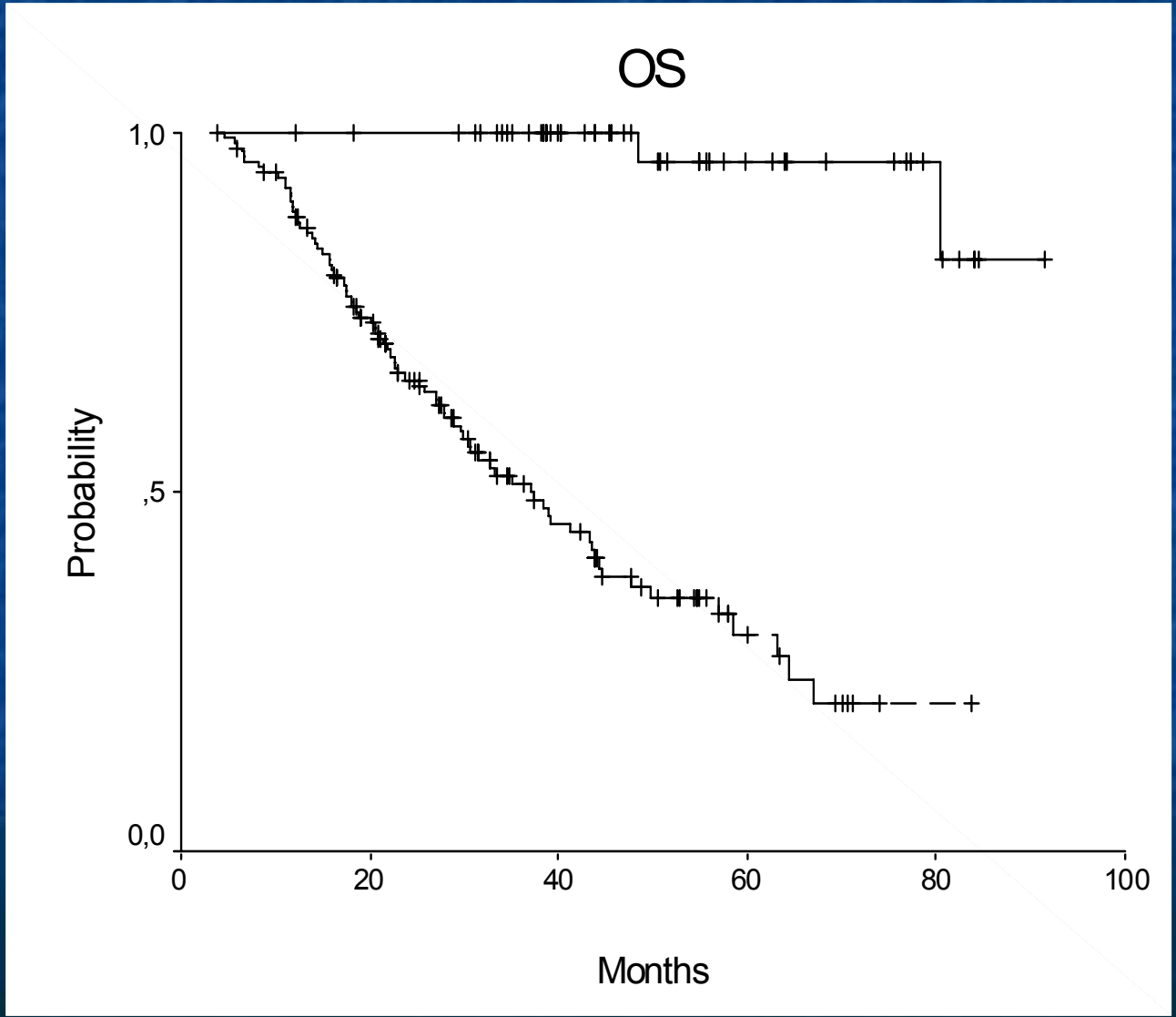
Progressive Paraneoplastic Vitiligo



Immune recognition and response to melanosomal markers of melanoma may be harnessed therapeutically

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

Overall survival plot by antibodies



Current Trials to Build upon IFN

- IFN combined with other agents for stage IV
  - Peptide and DC-based Vaccines (04-125)
  - Antibodies to GD3 UPCI 04-193/LUD 04-012
  - Anti-CTLA4 CP 675-206 (UPCI 05-125)
- Neoadjuvant approach is reasonable for exploration of all new agents of promise
  - Define influence GM-CSF, anti-CTLA4, and other immunomodulators in tumor

Mechanism of Anti-CTLA4 is likely relevant to several biologicals

- Autoimmune reactions seen at a greater frequency with anti-CTLA4 antibody than with any other biological agent
- Autoimmune responses with
  - High-dose IL-2 (Atkins, NEJM 1987) and
  - High-dose IFNα (Gogas NEJM 2006) are correlated with durable antitumor benefit

Progress in the Combined Modality Therapy of Melanoma

- Multiple scientifically valid approaches to therapy & trials that are currently in study
- Analysis of relevant endpoints --both
  - Clinical (PFSR and OS) and
  - Laboratory; Neoadjuvant designs are powerful
- Power sufficient to detect relevant differences
- Patience in awaiting mature results to avoid underestimation of the magnitude & durability of the results