Circulating Melanoma Cells in Melanoma Patients: Feasibility and Preliminary Findings

Anthony Lucci, MD

Professor, Department of Surgical Oncology

The University of Texas MD Anderson Cancer Center

Background

- The incidence of melanoma has increased significantly over the last 20 years, with an estimated 76,250 new cases in 2012

- Patients often develop recurrent disease despite complete excision of local/regional melanoma
  - Need for novel markers to more accurately stage patients and estimate recurrence risk

Limited data suggests that circulating melanoma cells (CMCs) predict recurrence in melanoma patients. There is a need for a sensitive, reproducible, and standardized identification technique.

CMC detection using molecular approaches

Hoshimoto, et al. *J Clin Oncol.* 2012 Nov 1;30(31):3819-26

- Using a multimarker RT-qPCR CTC assay (*MART1*, *MAGE-A3*, and *GalNAc-T*), stage III patients positive for 2+ markers had significantly worse DFS

Koyanagi, et al. *Clin Cancer Res.* 2010 Apr 15;16(8):2402-8

- Using a 5 gene biomarker RT-qPCR CTC assay, stage IV patients with increased biomarker expression exhibited poor response to biochemotherapy and worse OS

CMC Detection using CellSearch® in metastatic melanoma

Khoja, et al. *J Invest Dermatology* 2013 Jun;133(6):1582-90

- 26% had ≥2 CMCs
- ≥2 CMCs at baseline and throughout treatment was prognostic for shortened OS

Rao, et al. *Inter. J Oncology 2011* 38:755–760

- 23% had ≥2 CMCs
- ≥2 CMCs was prognostic for shortened OS

Hypothesis and Aims of the Study

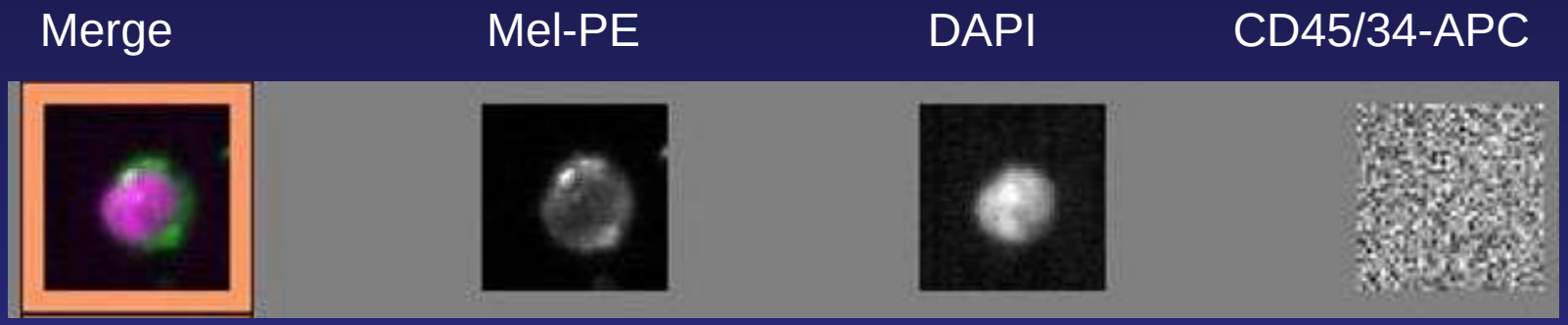
Given the high positivity rate and clinical significance of CMCs in stage IV patients, we hypothesized that CMCs can also be identified in non-metastatic patients, *and* CMCs are prognostically significant.

1. To determine whether CMCs could be identified in stage I-IV melanoma patients
2. To identify if presence of CMCs correlates with known prognostic factors
3. To determine if CMC assessment provides important prognostic information

Patients and Methods

- 200 stage I - IV melanoma patients seen at UT MD Anderson Cancer Center
  - Stage I- SLN eligible only were included
- 2/12 to present
- Mean age = 56 years (range: 19-90 yrs)
- 116 men / 84 women
- Longitudinal blood draws every 6 months
- CMC detection with CellSearch® CMC

CellSearch® CMC Assay



- 7.5 mL blood collected in CellSave® tubes and processed within 96 hours

- CD146+ cells are immunomagnetically enriched
- Isolated cells are fluorescently stained with:
  - DAPI
  - anti-molecular weight melanoma-associated antigen (HMW-MAA)
  - anti-CD45 and CD34

CMC Assay Quality Control

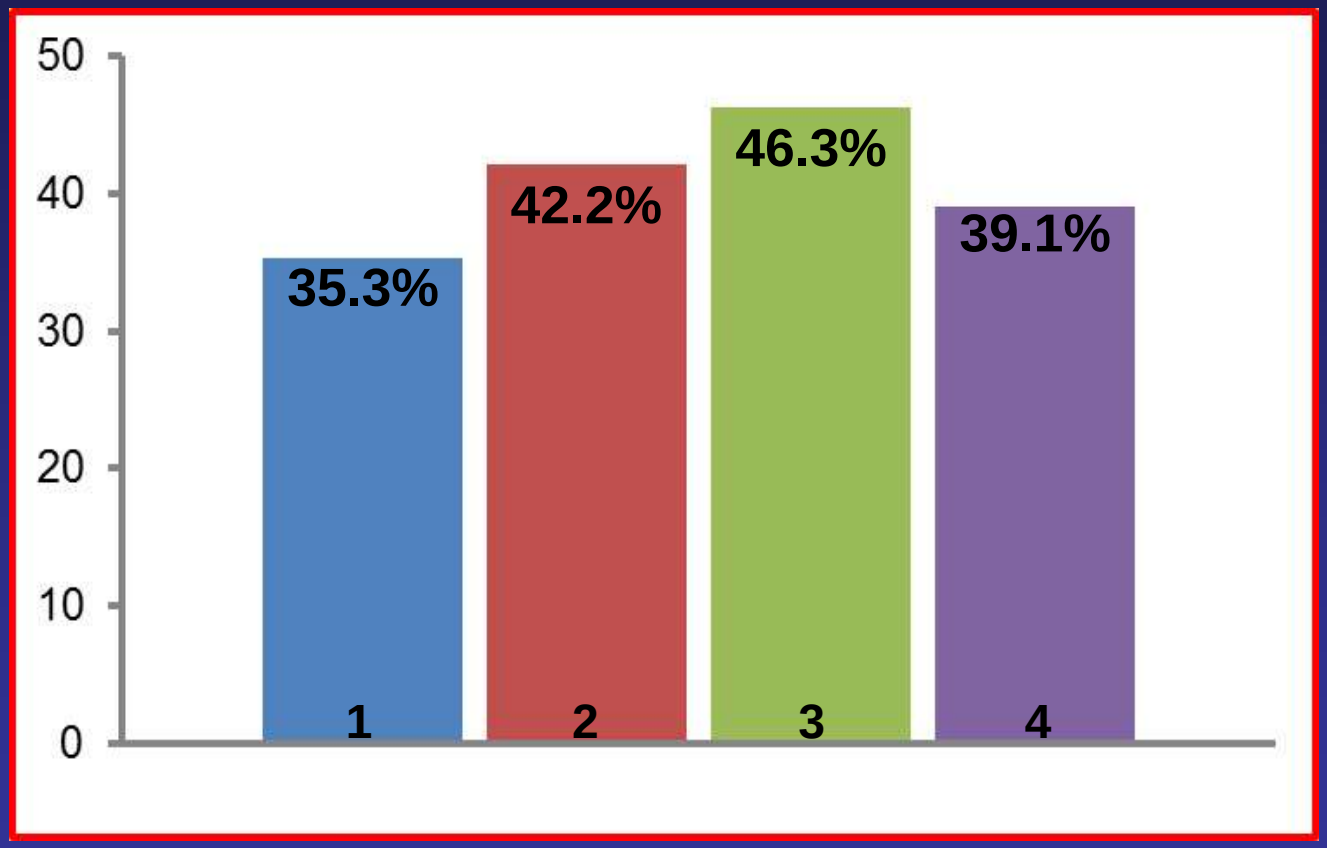
- All CMC data were reviewed by trained laboratory personnel who were blinded to all patient data

- Kappa Inter-rater variance (N=32 samples) = 0.88

CMC Identification in Healthy Controls

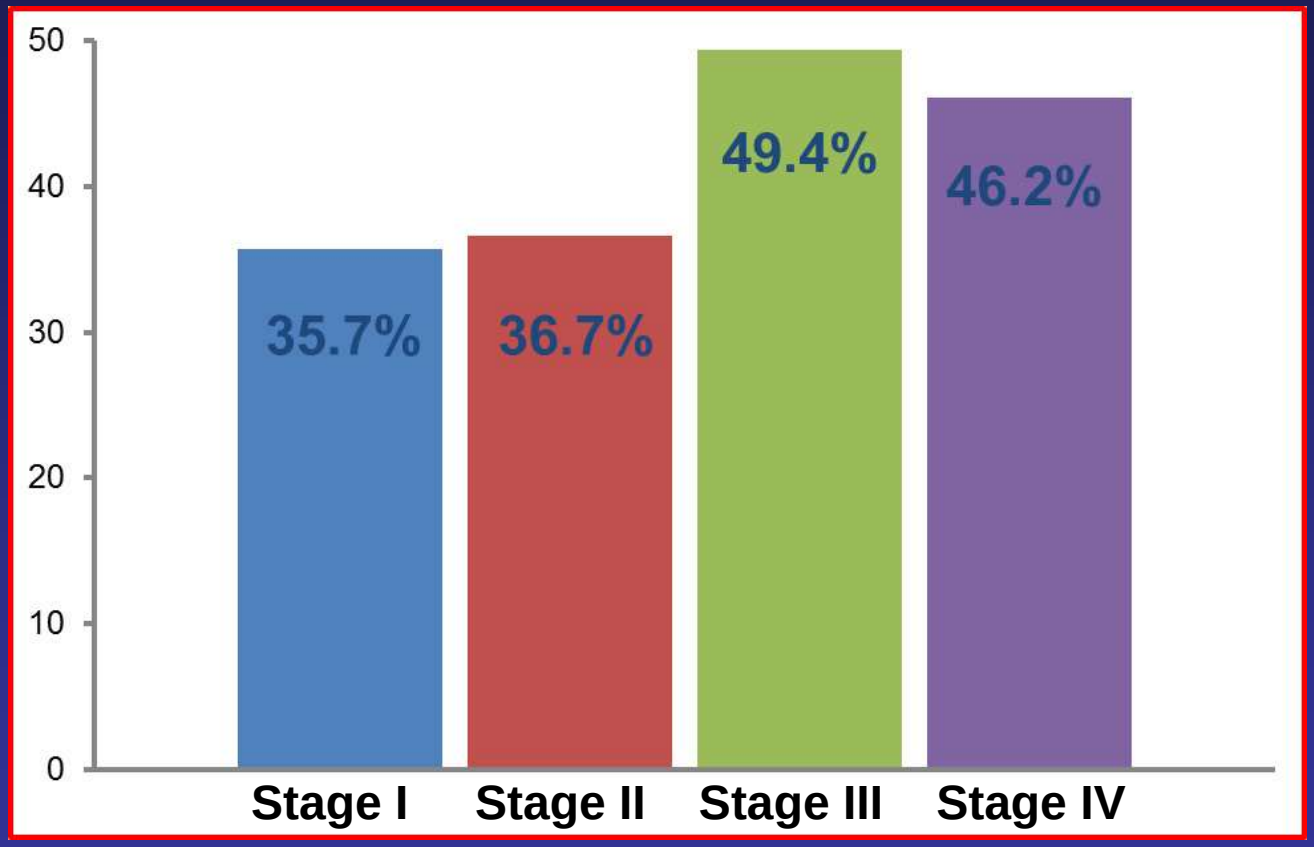
		%
CMC + Patients	**2/28**	**7.1**
CMC - Patients	**26/28**	**92.9**

CMC Positivity and Breslow Score



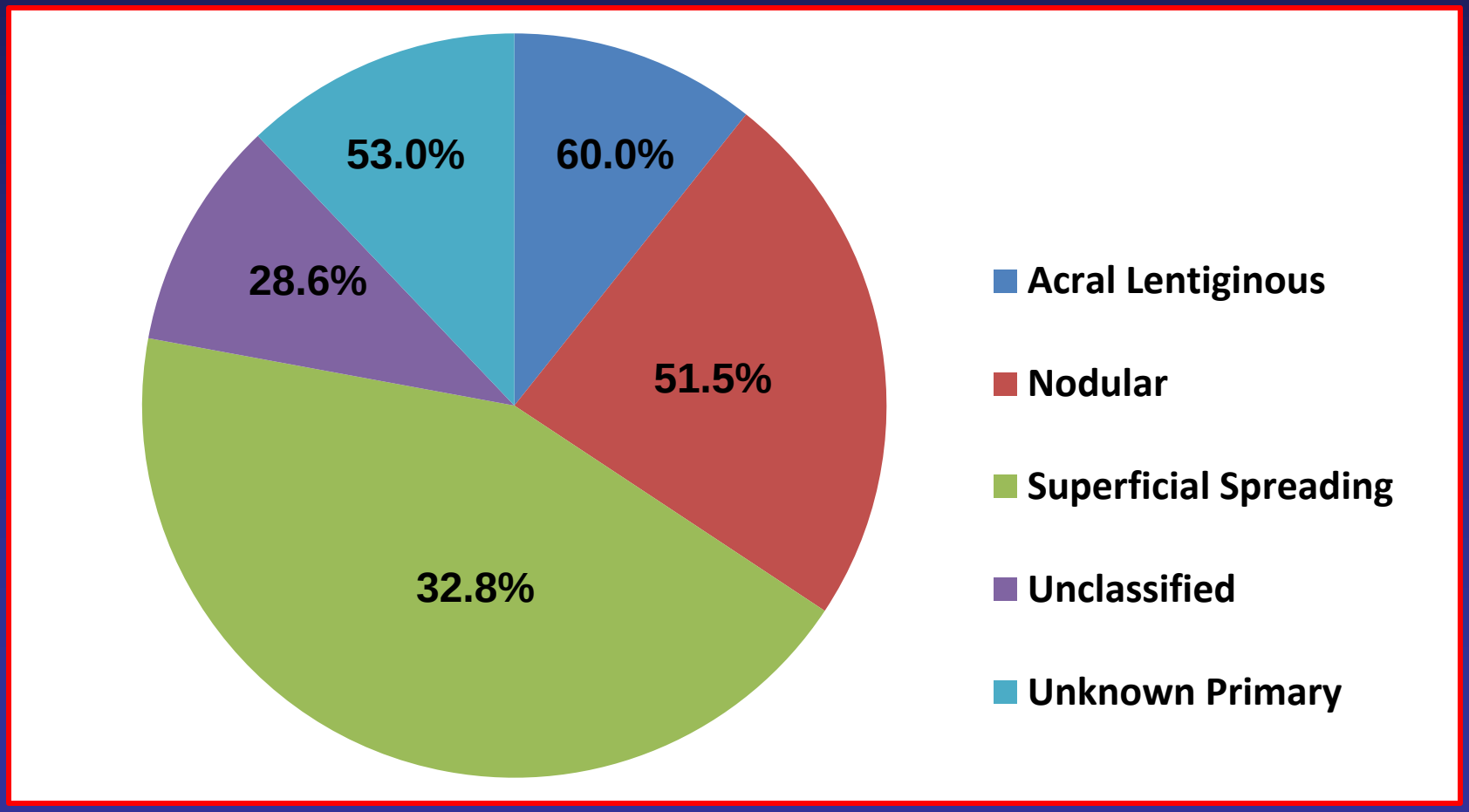
N=34 N=45 N=41 N=23

CMC Positivity and Path TMN Stage



Overall Positivity Rate = 44.2%

CMC positivity in histopathological subtypes



CMCs versus standard variables

Variable	0 CMCs	1 or more CMC	Significance testing
Age (mean) years	56.67	55.27	NS
Sex (Males)	62/108	47/80	NS
Braslow Thickness (stage 4-5)	36/84	28/59	NS
Mitotic index (positive)	64/75	48/51	0.155 (exact)
Ulceration (positive)	28/74	20/53	NS

CMC Positivity and Mitotic Index

	CMC + Patients	%
Mitotic Figures: No	**3/14**	**21.4**
Mitotic Figures: Yes	**48/112**	**42.9**

P=0.155

CMC Positivity and Ulceration

	CMC + Patients	%
Ulceration: No	**33/79**	**41.8**
Ulceration: Yes	**20/48**	**41.7**

P=0.991

Numbers of CMCs identified

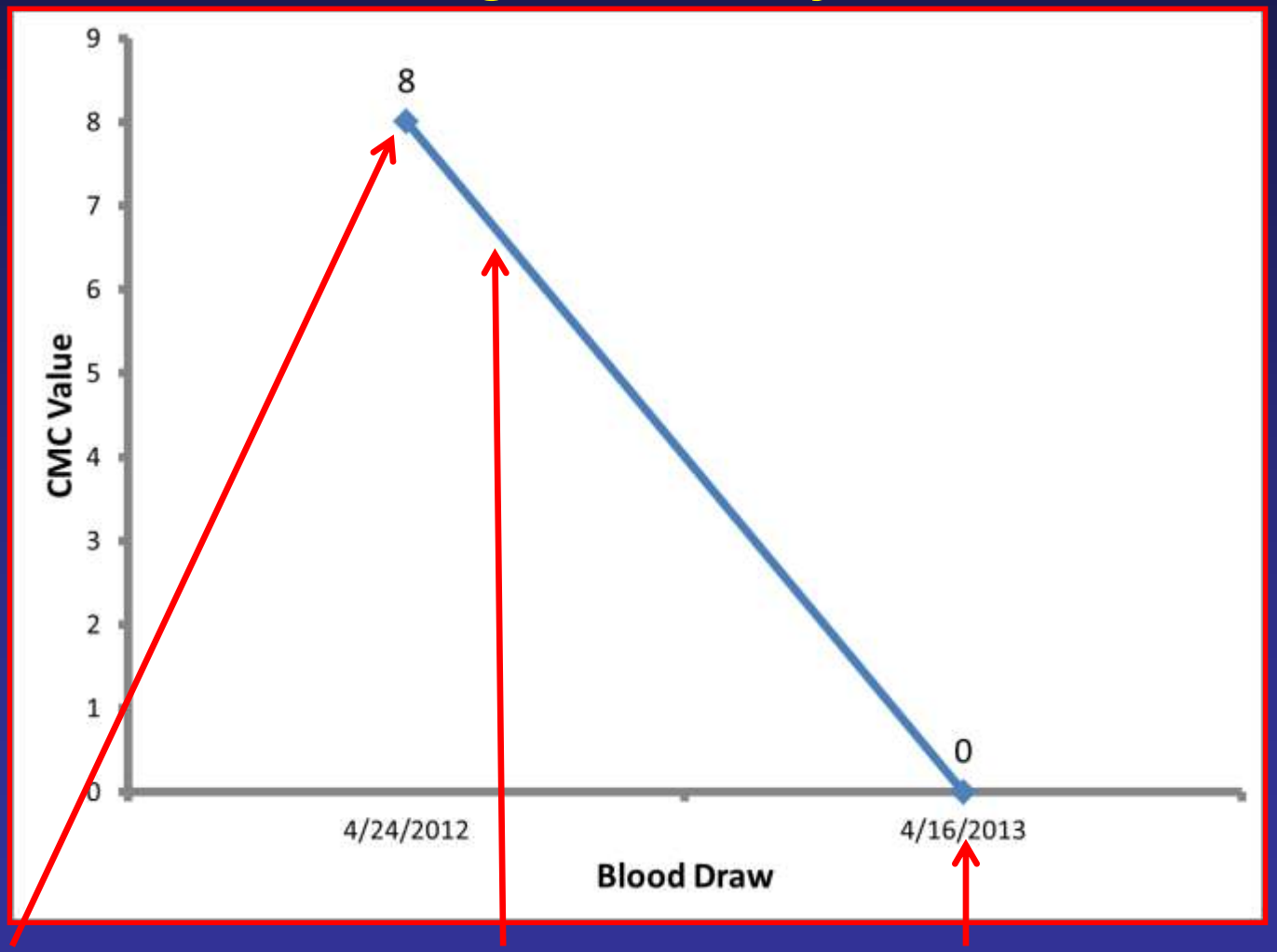
CMC=0	108			
CMC=1	29			
CMC=2	10			
CMC>2	8			

Interferon Therapy and CMC Counts

	Number of Patients	%
CMC Count Decreased	**6/15**	**40%**
CMC Count Unchanged	**7/15**	**47%**
CMC Count Increased	**2/15**	**13%**

P=0.03

Representative Case Study
Dx Stage IB, 59 yrs old

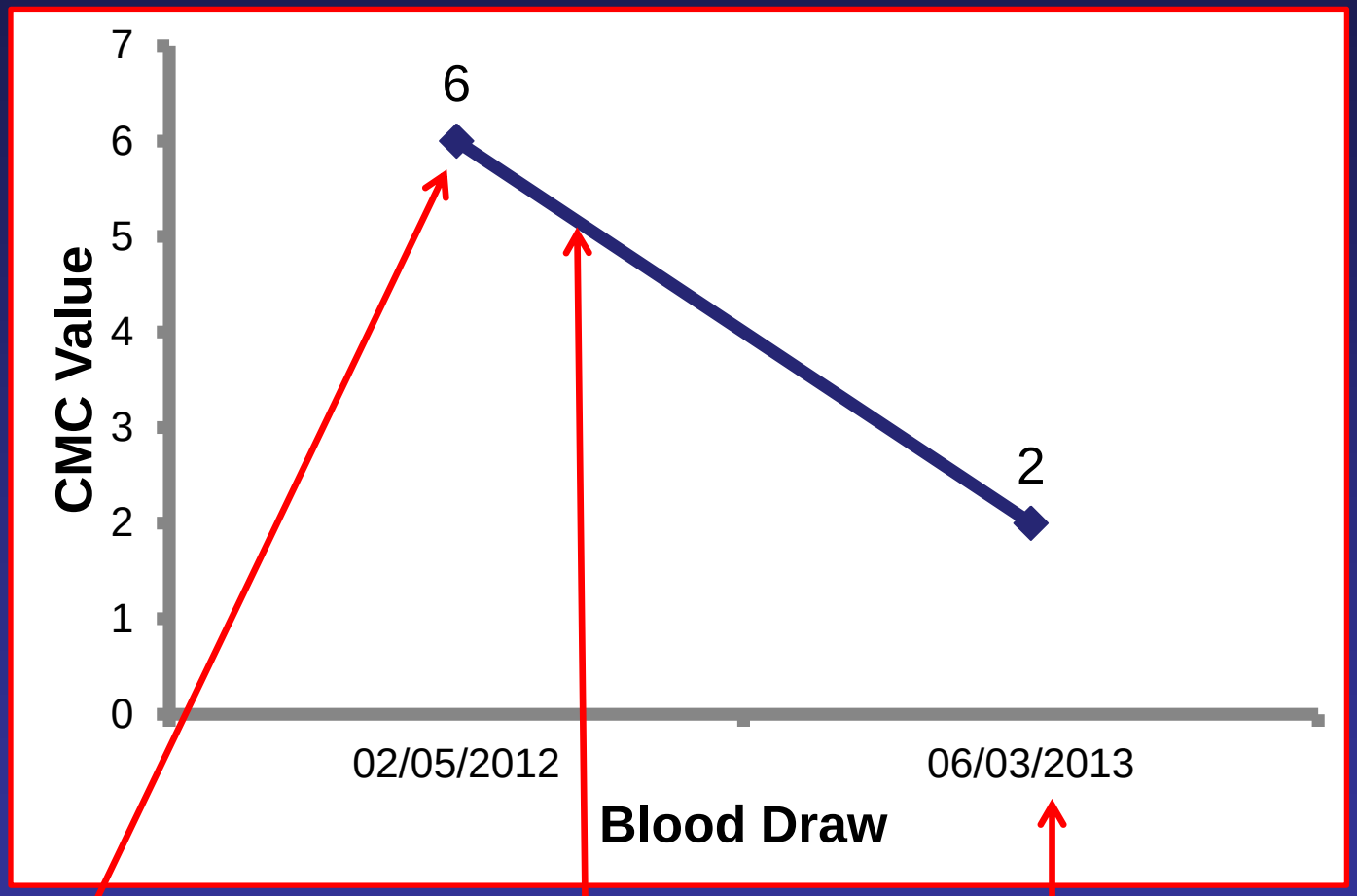


Stage at CMC#1=IIIA

Interferon

Stage at CMC#2=IIIA

Representative Case Study
Dx Stage IIB, 55 yrs old

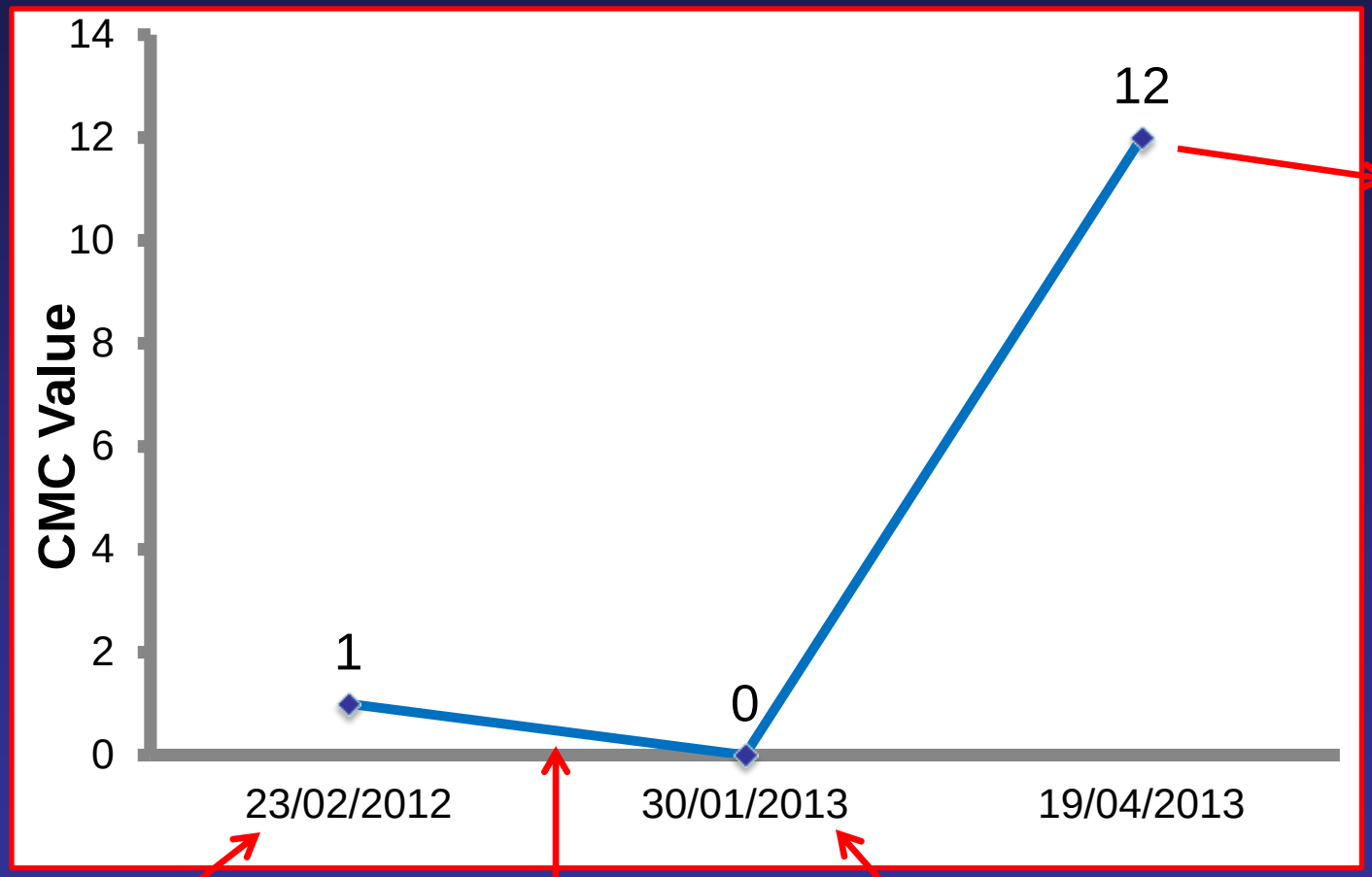


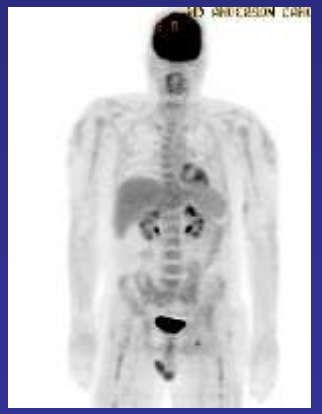
Stage at CMC#1=IIB

Interferon

Stage at CMC#2=IIB

Representative Case Study
Dx: Stage IIA, 43 yrs old





Stage at CMC#1=IIIA

Biochemotherapy
Radiation
TLR Vaccine
IFN, IL-2

Excision of Mets

Conclusions

- CMCs were detected in a significant number of stage I-IV melanoma patients
- CMC detection was not associated with standard prognostic markers
- Longitudinal CMC assessment might identify patients at higher risk for disease progression

Future Studies

- Clinical trial involving CMC assessment before and after neoadjuvant B-raf inhibitor

- Collaboration with Dr. Dave Hoon (JWCI) involving nucleic acid assessment in pair-matched serum samples

Acknowledgements

- Dr. Merrick Ross
- Dr. Carolyn Hall
- Barbara Laubacher
- Joshua Upshaw
- Amber Anderson
- Our patients at UT MD Anderson CC