Cytokines: Interferons, Interleukins and Beyond

Sanjiv S. Agarwala, MD
Professor of Medicine, Temple University
Chief, Oncology & Hematology
St. Luke's Cancer Center
Bethlehem, PA, USA

Overview

- What is a Cytokine?
- Clinical Applications of Cytokines
  - IFN
    - Adjuvant Therapy of Melanoma
    - RCC
  - IL-2
    - Metastatic melanoma and RCC
- Beyond Cytokines

What is a Cytokine?

- The term "cytokine" is derived from a combination of two Greek words - "cyto" meaning cell and "kinos" meaning movement.
- Cytokines are proteins that at as cell signaling molecules for cell to cell communication in immune responses
- They stimulate the movement of cells towards sites of inflammation, infection and trauma.

Cytokines

- Polypeptides secreted by living cells that act non-enzymatically to regulate cellular functions
- Regulate
  - Immune function: interleukins, interferons
  - Hematopoiesis: G-CSF, GM-CSF
  - Cell proliferation and differentiation: EGF, TGF, FGF

Examples of Cytokines and Their Clinical Relevance

TABLE 1. EXAMPLES OF CYTOKINES AND THEIR CLINICAL RELEVANCE.*

CYTOKINE	CELLULAR SOURCES	MAJOR ACTIVITIES	CLINICAL RELEVANCE
Interleukin-1	Macrophages	Activation of T cells and macrophages; promotion of inflammation	Implicated in the pathogenesis of septic shock, rheumatoid arthritis, and atherosclerosis
Interleukin-2	Type 1 (Th1) helper T cells	Activation of lymphocytes, natural killer cells, and macrophages	Used to induce lymphokine-activated killer cells; used in the treatment of metastatic renal-cell carcinoma, melanoma, and various other tumors
Interleukin-4	Type 2 (Th2) helper T cells, mast cells, basophils, and eosinophils	Activation of lymphocytes, monocytes, and IgE class switching	As a result of its ability to stimulate IgE production, plays a part in mast-cell sensitization and thus in allergy and in defense against nematode infections
Interleukin-5	Type 2 (Th2) helper T cells, mast cells, and eosinophils	Differentiation of eosinophils	Monoclonal antibody against interleukin-5 used to inhibit the antigen-induced late-phase eosinophilia in animal models of allergy
Interleukin-6	Type 2 (Th2) helper T cells and macrophages	Activation of lymphocytes; differentiation of B cells; stimulation of the production of acute-phase proteins	Overproduced in Castleman's disease; acts as an autocrine growth factor in myeloma and in mesangial proliferative glomerulonephritis
Interleukin-8	T cells and macrophages	Chemotaxis of neutrophils, basophils, and T cells	Levels are increased in diseases accompanied by neutrophilia, making it a potentially useful marker of disease activity
Interleukin-11	Bone marrow stromal cells	Stimulation of the production of acute-phase proteins	Used to reduce chemotherapy-induced thrombocytopenia in patients with cancer
Interleukin-12	Macrophages and B cells	Stimulation of the production of interferon-γ by type 1 (Th1) helper T cells and by natural killer cells; induction of type 1 (Th1) helper T cells	May be useful as an adjuvant for vaccines
Tumor necrosis factor α	Macrophages, natural killer cells, T cells, B cells, and mast cells	Promotion of inflammation	Treatment with antibodies against tumor necrosis factor α beneficial in rheumatoid arthritis
Lymphotoxin (tumor necrosis factor β)	Type 1 (Th1) helper T cells and B cells	Promotion of inflammation	Implicated in the pathogenesis of multiple sclerosis and insulin-dependent diabetes mellitus
Transforming growth factor β	T cells, macrophages, B cells, and mast cells	Immunosuppression	May be useful therapeutic agent in multiple sclerosis and myasthenia gravis
Granulocyte–macrophage colony-stimulating factor	T cells, macrophages, natural killer cells, and B cells	Promotion of the growth of granulocytes and monocytes	Used to reduce neutropenia after chemotherapy for tumors and in ganciclovir-treated patients with AIDS; used to stimulate cell production after bone marrow transplantation
Interferon-α	Virally infected cells	Induction of resistance of cells to viral infection	Used to treat AIDS-related Kaposi's sarcoma, melanoma, chronic hepatitis B infection, and chronic hepatitis C infection
Interferon-β	Virally infected cells	Induction of resistance of cells to viral infection	Used to reduce the frequency and severity of relapses in multiple sclerosis
Interferon-γ	Type 1 (Th1) helper T cells and natural killer cells	Activation of macrophages; inhibition of type 2 (Th2) helper T cells	Used to enhance the killing of phagocytosed bacteria in chronic granulomatous disease

*AIDS denotes acquired immunodeficiency syndrome.

The NEW ENGLAND JOURNAL of MEDICINE

Cytokines in Clinical Practice

- Interferons
- Interleukins

Interferons

- First described in 1956 as substances that interfered with viral replication
- Are proteins with multiple biologic activities
- Immune system modulation
- Direct antitumor effects
- Antiangiogenic

Interferons

- Type I
  - IFN-α
  - IFN-β
  - Others: IFN-τ, IFN-ω
- Type II
  - IFN-γ

Interferon-α

- Three main subspecies
  - IFN-α2a
  - IFN-α2b
  - IFN-α2c
- Share a common receptor
- Differ minimally in amino acid sequence

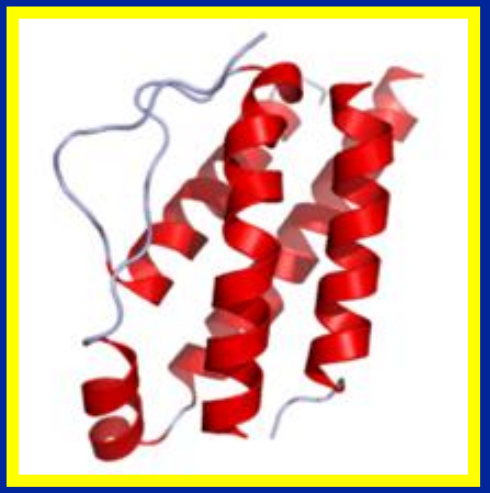
- In melanoma, IFN-α2b has been most widely investigated

Interleukins

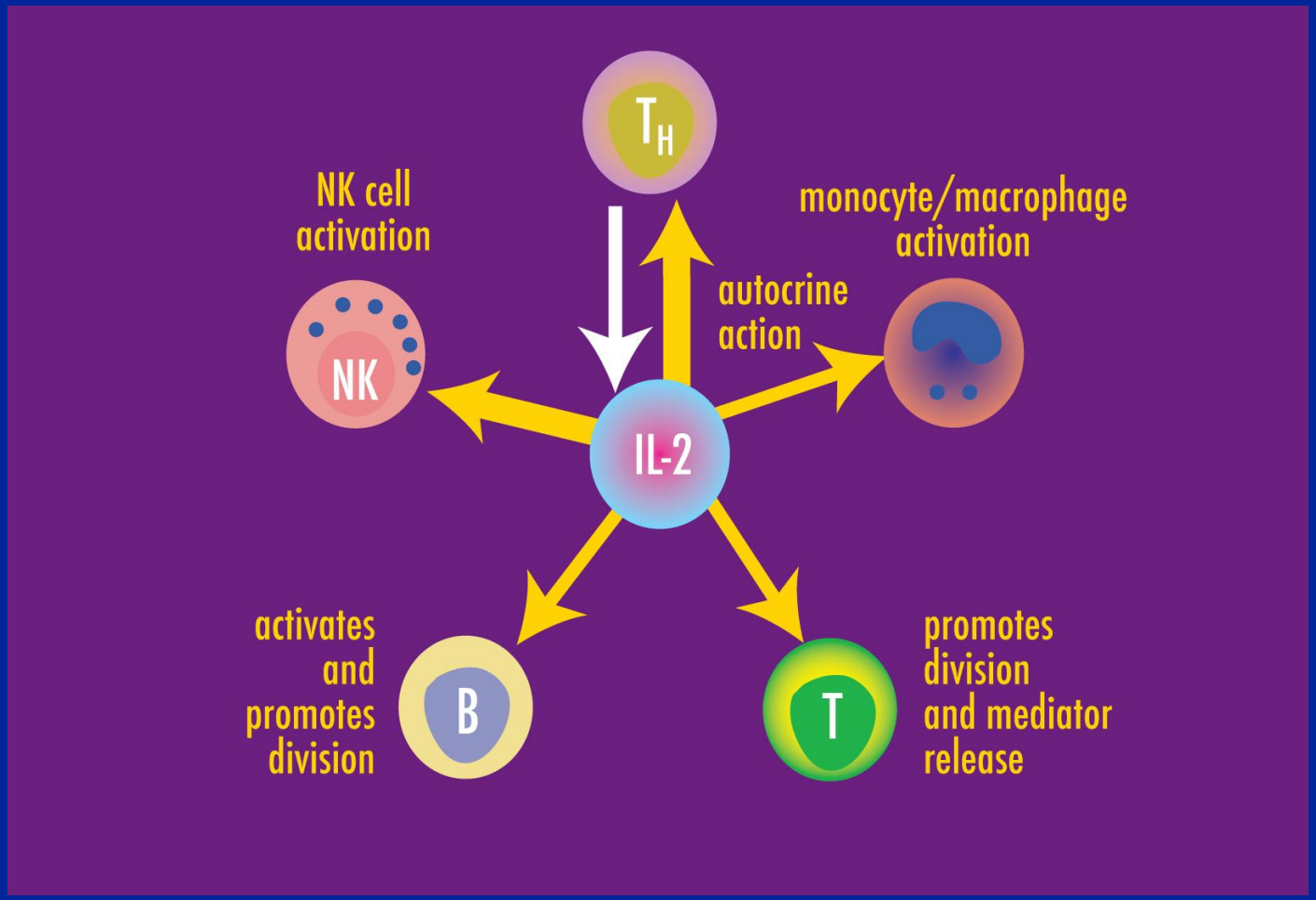
- Cytokines produces by leukocytes that have effects predominantly on other leukocytes
- Produced by lymphocytes, macrophages
- Act on T and B-cells, NK cells

What is IL-2?

- IL-2 is an immunotherapy
- Discovered in 1976 and described as a protein that stimulated growth of T cells[1]
- Recombinant IL-2 first cloned in 1983[1]
- Phase II clinical trials began in 1985[1]



Role of IL-2 in the Immune Response



Roitt I, et al. *Immunology*. 1996;8:11.

Overview

- What is a Cytokine?
- Clinical Applications of Cytokines
  - IFN
    - Adjuvant Therapy of Melanoma
    - RCC
  - IL-2
    - Metastatic melanoma and RCC
- Beyond Cytokines

High Dose Interferon Therapy

Induction:

- 20 MIU/m2/dose x 4 weeks IV (Monday - Friday)

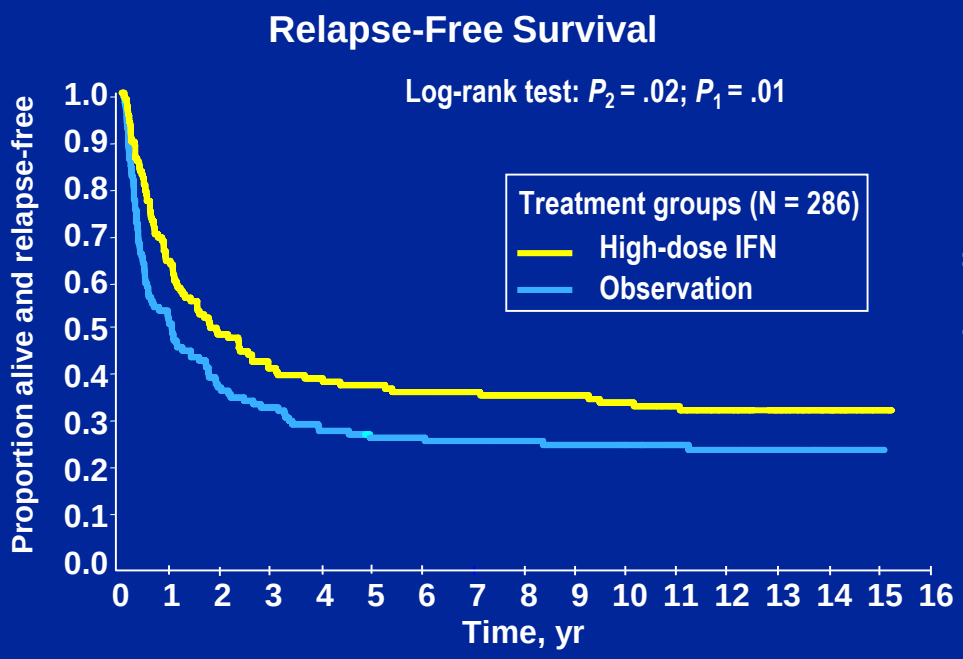
Maintenance:

- 10 MIU/m2/dose x 48 weeks SQ (Every Monday, Wednesday and Friday)

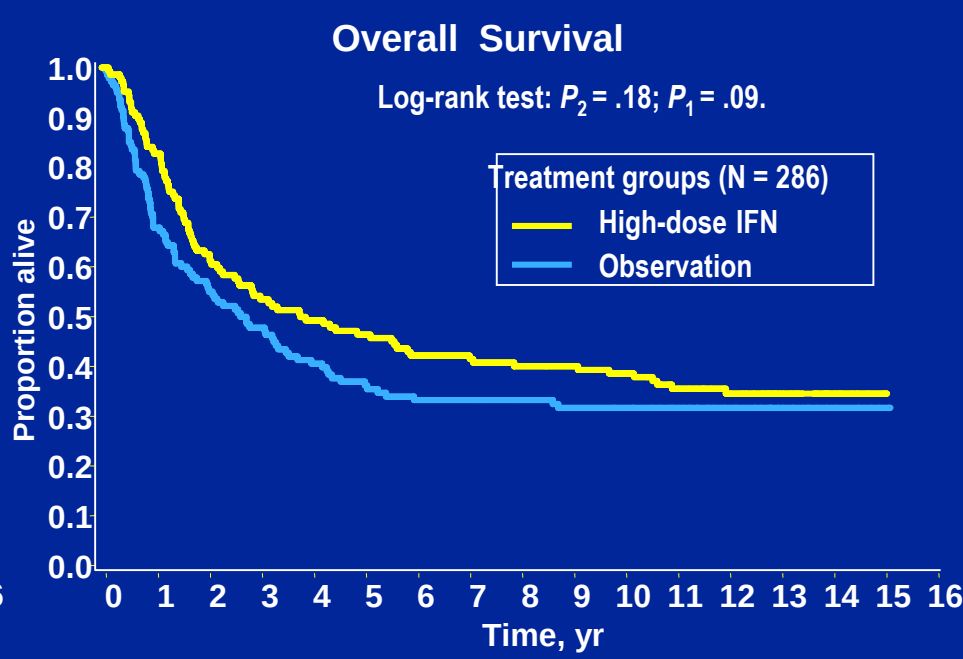
Adjuvant IFN-α Regimens

Schedule	Dose	Frequency	Duration
Low Dose			
	3 MIU	3 x weekly	18 – 24 months
Intermediate Dose			
Induction	10 MIU	5 x weekly	4 weeks
Maintenance	10 MIU	3 x weekly	12 -24 months
	5 MIU	3 x weekly	24 months
High Dose			
Induction	20 MIU/m^2	5 x weekly	4 weeks
Maintenance	10 MIU/m^2	3 x weekly	11 months
Short Course			
Induction X 1	20 MIU/m^2	5 x weekly	4 weeks
Intermittent			
Induction X 3	20 MIU/m^2	20 MIU/m^2	5 x weekly for 4 weeks Q 4 months

E1684: Updated Efficacy (ITT at 12.6 yr Median Follow-up)



	Total	Dead or relapsed	Alive or relapsed-free	Median
Observation	140	106	34	1.0
High-dose IFN	146	95	51	1.7



	Total	Dead	Alive	Median
Observation	140	95	45	2.7
High-dose IFN	146	93	53	3.8

Short Duration (Induction only) vs. Prolonged Duration (PEG-IFN)

- Hypothesis that much of the benefit of HDI may be driven by the one month IV induction phase
- Other trials have suggested that longer duration of treatment with a lower dose may be beneficial

- Short duration intensive therapy

vs.

- Long duration less intensive therapy

Study design: ECOG 1697

Patients with intermediate- and high-risk melanoma

Defined as T3:

Breslow thickness >1.5 mm (AJCC 6th ed)
>2.0 mm (AJCC 7th ed)

or

Any thickness with microscopically positive nodal disease (N1a–N2a)

RANDOMIZATION

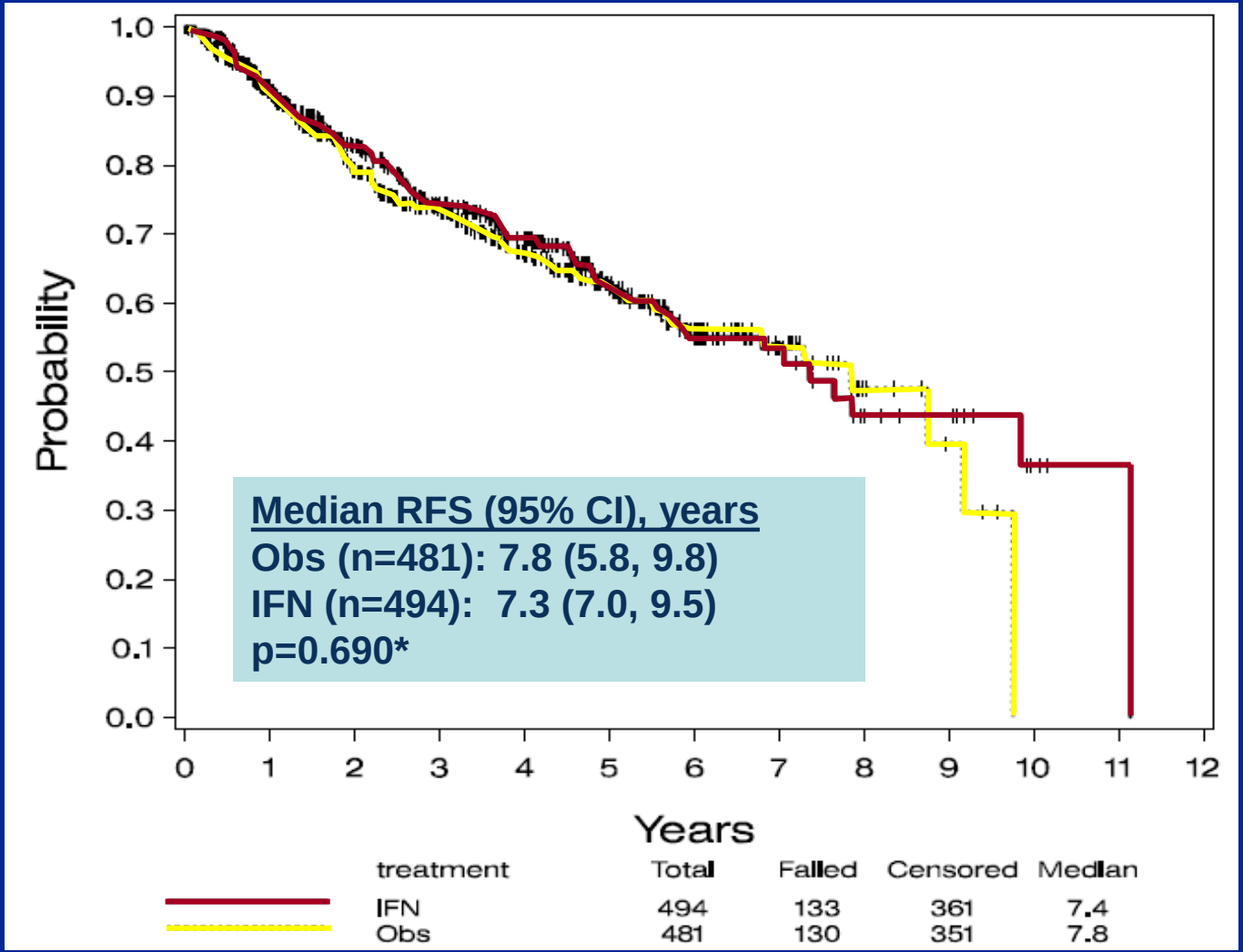
1:1

Postoperative adjuvant IFN alfa-2b 20 MU/m^2/day for 5 days/week × 4 weeks

Observation

Agarwala SS et al. Abs #8505

Relapse-free survival (n=975)



*Stratified log-rank test

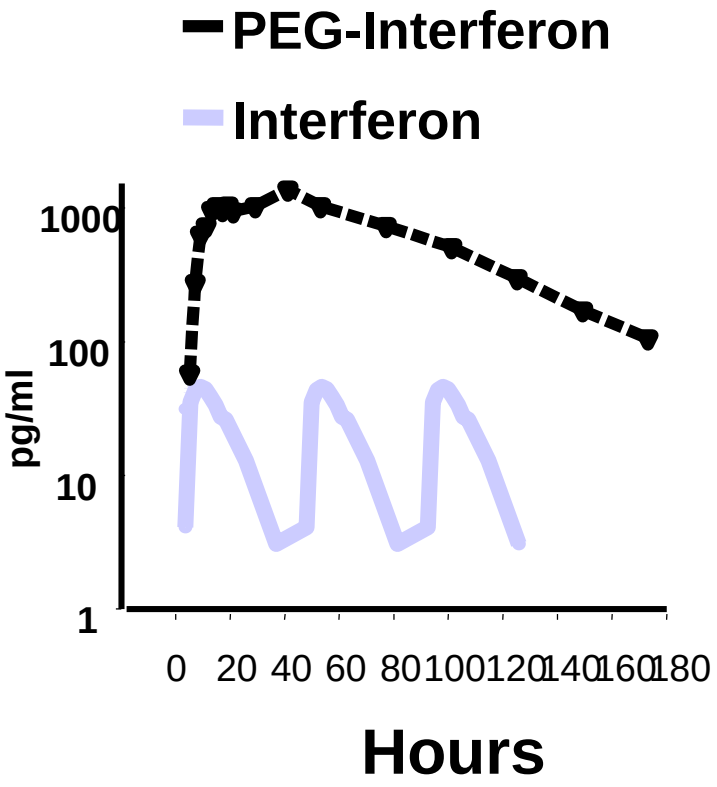
Agarwala SS et al. Abs #8505

IV Induction Alone

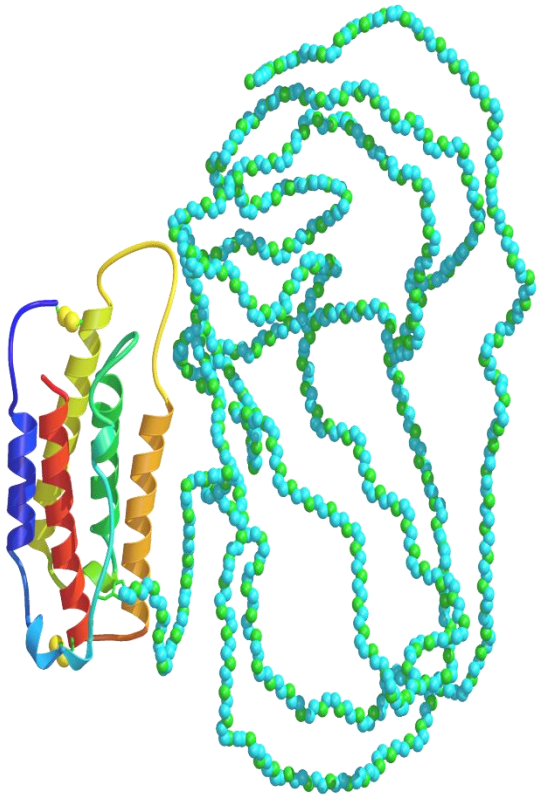
- Not effective by itself (without maintenance)
- If HDI is used, it must be the approved induction followed by maintenance and should not be shortened

"Does IV Induction matter at all?"

Serum Levels of Pegylated vs. Conventional Interferons



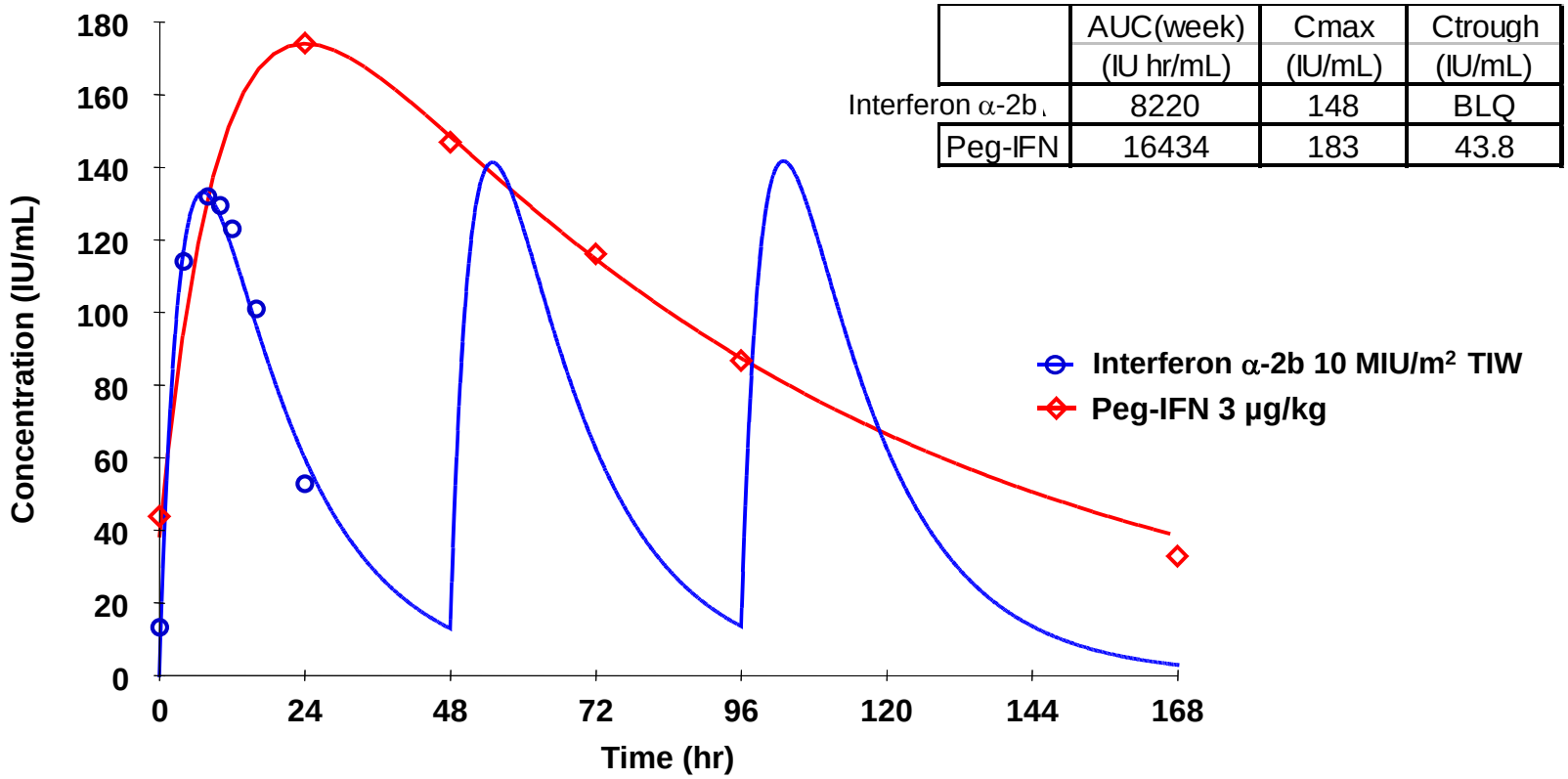
PegIFN α-2b



Pegylated IFN-α

Schedule	Dose	Frequency	Duration
Induction	6 µg/kg SC	Q weekly	8 weeks
Maintenance	3 µg/kg SC	Q weekly	up to 5 years

Concentration-time Profiles of IFN SC 10 MIU/m^2 TIW vs Peg-IFN Alfa-2b 3 μg/kg/Week in Melanoma Subjects



	AUC(week) (IU hr/mL)	Cmax (IU/mL)	Ctrough (IU/mL)
Interferon α-2b	8220	148	BLQ
Peg-IFN	16434	183	43.8

Peg-IFN mean concentrations at 3 μg/kg/week Week 12 was converted to IU/mL based on the specific activity with a model fit line

Interferon α-2b mean concentrations at 2nd and 3rd dosing were simulated based on the pharmacokinetic model of 10 MIU/m^2 SC at Day 52

Design

Patients (n=1,256):
Resected TxN1-2M0 melanoma, within 7 weeks of lymphadenectomy

Randomization

Stratified by:
- **Microscopic (N1) *vs.* palpable (N2)**
- 1 *vs.* 2-4 *vs.* 5+ nodes
- Breslow
- **Ulceration**
- Gender
- Site

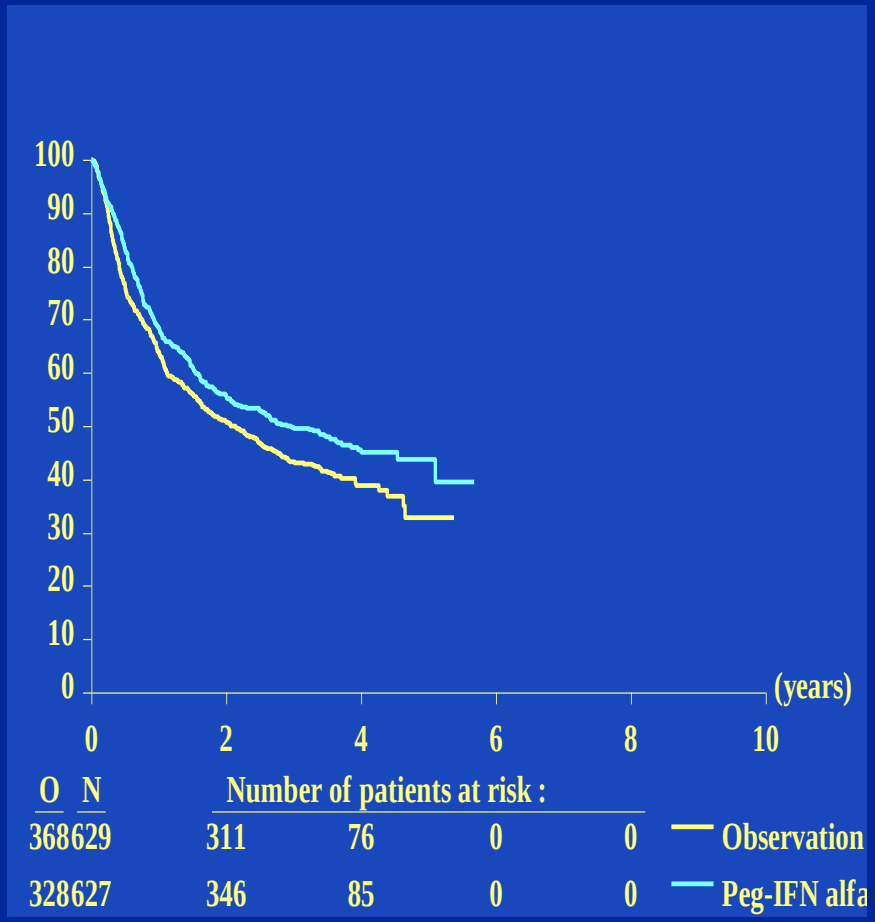
Observation

Peg-IFN alfa-2b
- Induction (8 weeks) 6 µg/kg/week
- Maintenance (5 years or distant metastasis) 3 µg/kg/week
- Dose reduction to 3, 2, 1 to maintain performance status

Primary Endpoints:
- Relapse-free survival (RFS)
- Distant metastasis-free survival (DMFS)

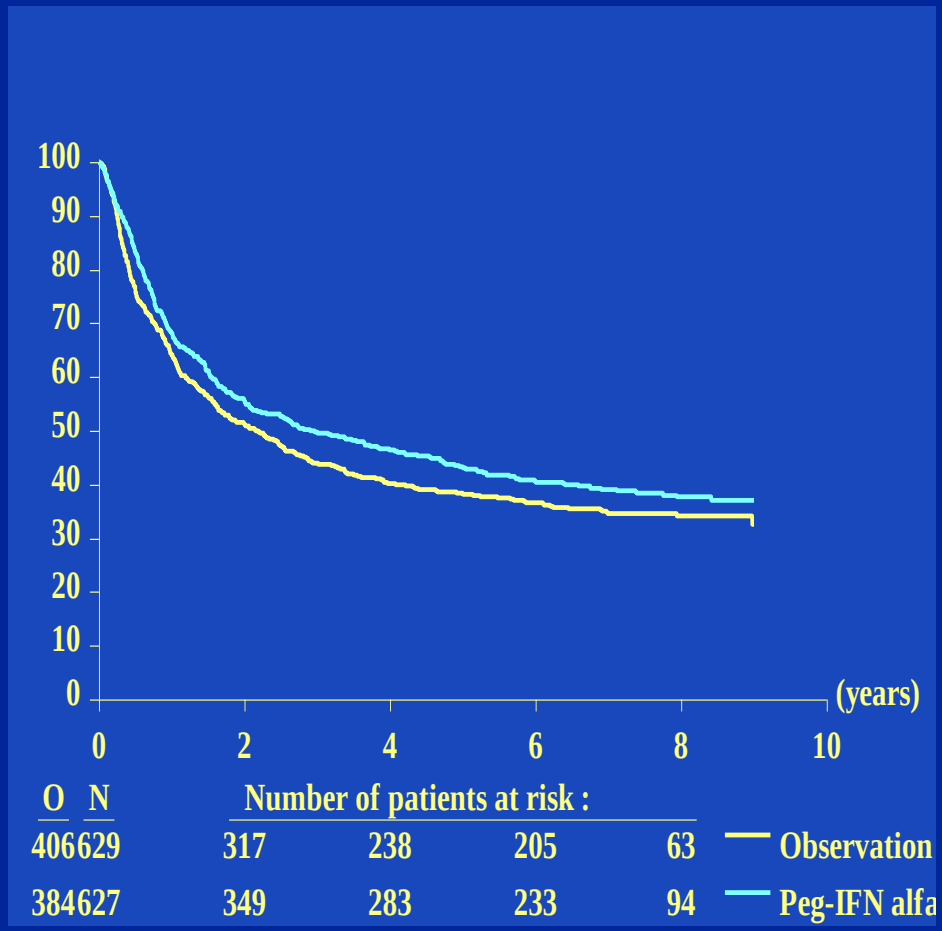
Relapse-Free Survival (ITT)

2007 evaluation



P=0.01 HR = 0.82 (95% CI 0.71 , 0.96)

2011 evaluation



P=0.05 HR = 0.87 (95% CI 0.76 , 1.00)

Overview

- What is a Cytokine?
- Clinical Applications of Cytokines
  - IFN
    - Adjuvant Therapy of Melanoma
    - RCC
  - IL-2
    - Metastatic melanoma and RCC
- Beyond Cytokines

IFN α Monotherapy in Advanced RCC: Cochrane Review

- Meta-analysis evaluating the use of immunotherapy in RCC
  - Primary endpoint studied: OS
  - Secondary endpoint studied: Remission
- Analysis included a total of 58 studies involving 6880 patients
  - 4 studies of interferon α in 644 patients
- Interferon α provided a reduced risk for mortality vs control therapy
  - ↓ 46% at 1 year
  - ↓ 36% at 2 years
- Concomitant therapy with a variety of agents produced no additional survival effect compared with interferon α alone

IFN α = interferon-alfa; RCC = renal cell carcinoma

Coppin C, et al. *Cochrane Database of Systematic Reviews;* 2008, Issue 4.

Overview

- What is a Cytokine?
- Clinical Applications of Cytokines
  - IFN
    - Adjuvant Therapy of Melanoma
    - RCC
  - IL-2
    - Metastatic melanoma and RCC
- Beyond Cytokines

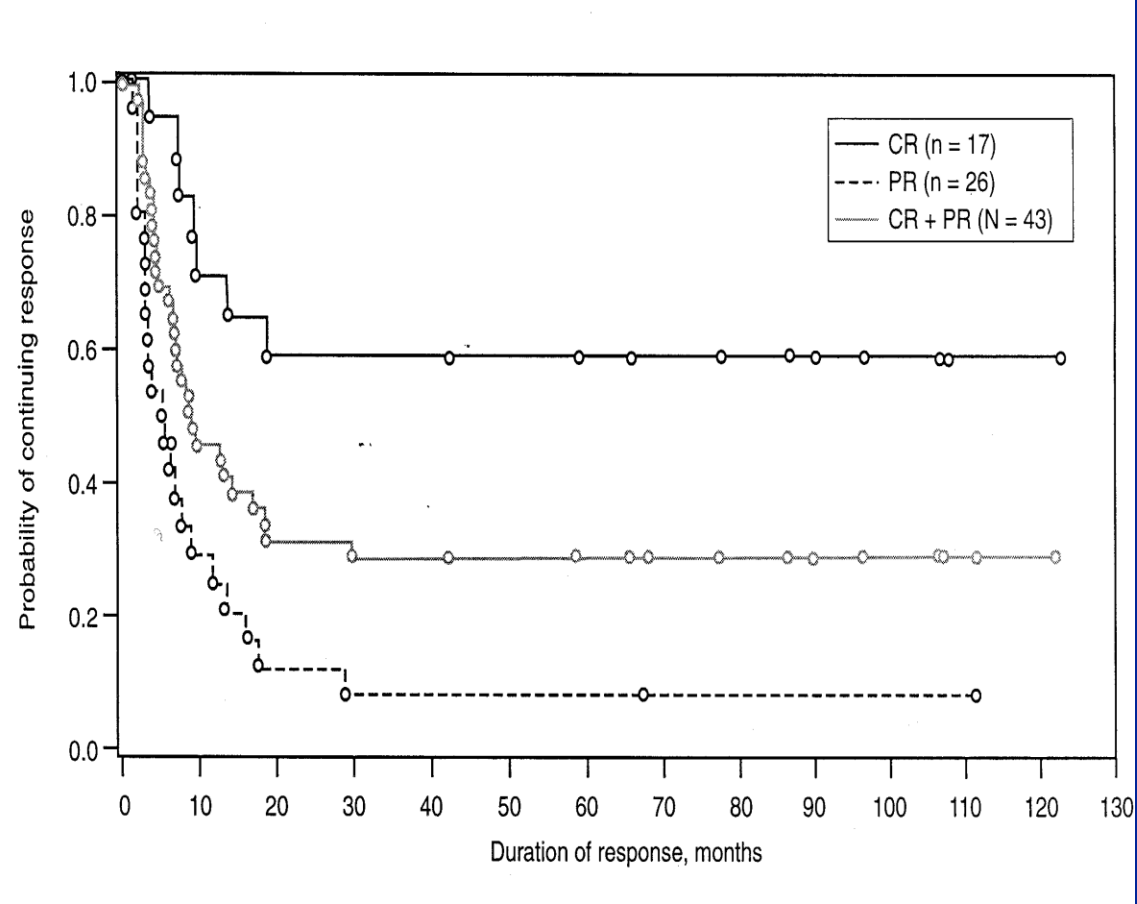
High-Dose IV Bolus rIL-2 Schedule

Course of Treatment

- Median number of doses per course: 7 per first treatment cycle

Fyfe G, et al. *J Clin Oncol*. 1995;13:688-696.
Kammula US, et al. *Cancer*. 1998;83:797.

High Dose IL-2 Therapy



***Atkins et al JCO, 1999 (N=270)**

- RR: 16% (43 / 270)
- Durable responses 6%
  - Median 8.9 mos
  - CR: not reached

Atkins et al., JCO 1999

Single-Agent IL-2 in RCC

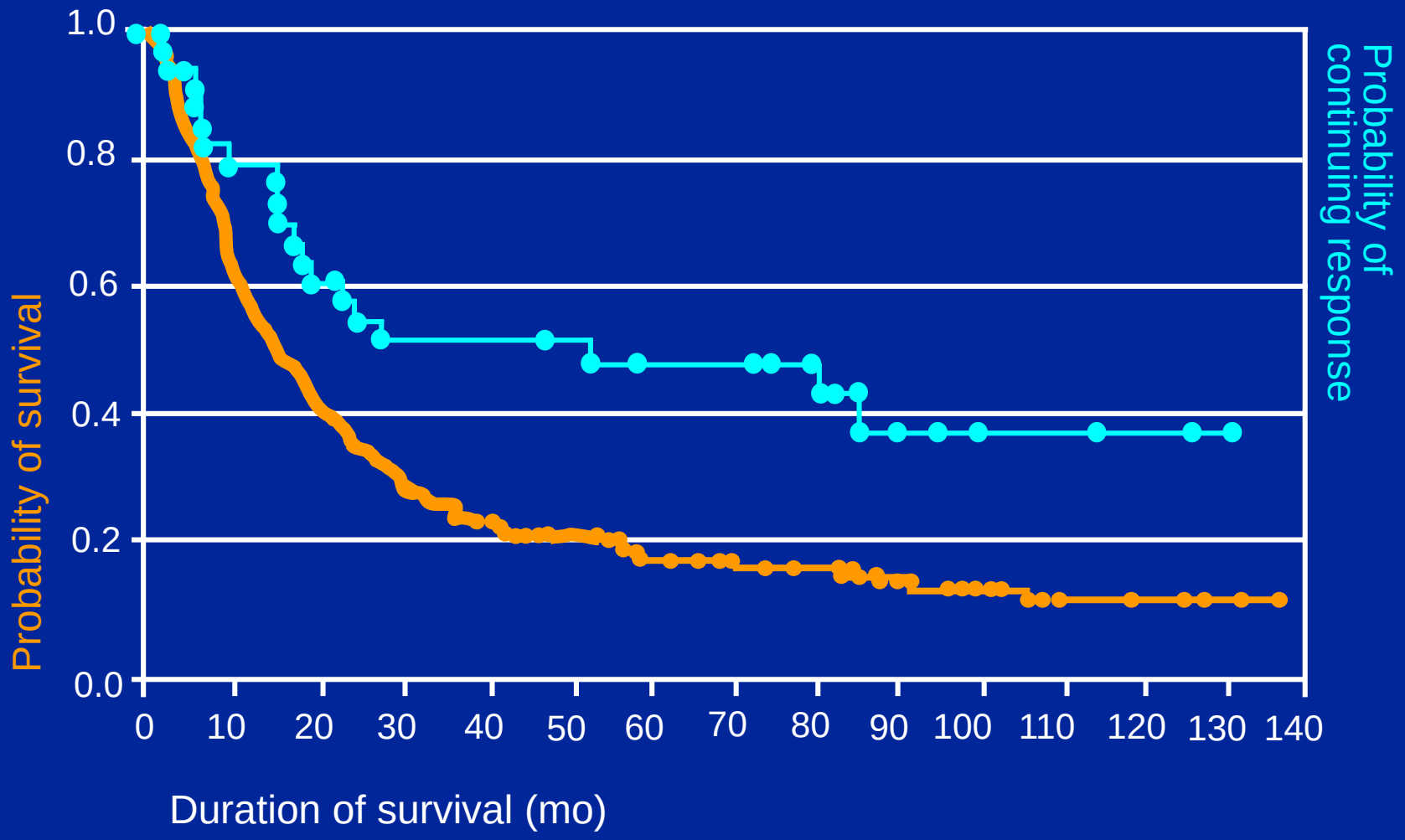
Author	*N*	*Dose*	*OR*	*MDR*	*MS*
Fisher[1]	255	6 or 7.2x10^5 IU/kg q8h IV X 14	15% (7/8)	54 m	16.3 m
Gold[2]	123	18-22 MIU/m^2/day 1-5 6-8 MIU/m^2/day 10-19	18.7% (7.3/11.4)	-	19 m

1: Median response duration for all CRs not reached, but at least 80 months (range: 7-131+ m)

2: 7/ 9 CRs in continuing complete response at 43 to 109 months

IL-2 Response Duration: All Responding Patients

Kaplan-Meier Estimate



Fisher R, et al. C*ancer J Sci Amer*. 2000;6(suppl 1):S55-S57.

Phase III CWG RCC Trial Schema
HD IL-2 vs LD IL-2/IFN

STRATIFY

- Bone or liver metastases
- Performance status 0–1
- Primary tumor in place

RANDOMIZE

ARM A (Outpatient)

IL-2 / IFN-α2b

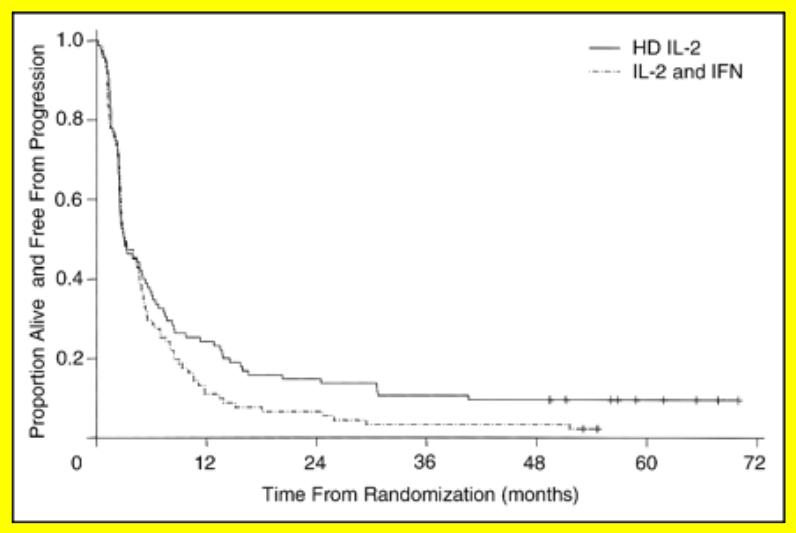
ARM B

High-dose IL-2

McDermott D, et al. *J Clin Oncol*. 2005;23:133-141.

Low-Dose IL-2 Not as Effective as High-Dose IL-2 in mRCC

Randomized phase III trial of high-dose (HD) IL-2 vs outpatient low-dose IL-2 + interferon-alpha in patients (N=192) with mRCC*



- Overall response rate was 23% for HD arm vs 19% for low-dose arm (*P*=.018)
- Survival was superior for patients with bone or liver metastases in the HD arm (*P*=.001)

*High-dose IL-2 (600,000 U/kg/dose intravenously every 8 hours on days 1 through 5 and 15 to 19 [maximum 28 doses])

Low-dose IL-2 (5 MIU/m^2 subcutaneously 3 times per week for 4 weeks) every 6 weeks.

McDermott DF et al. *J Clin Oncol*. 2005;23:133-141.

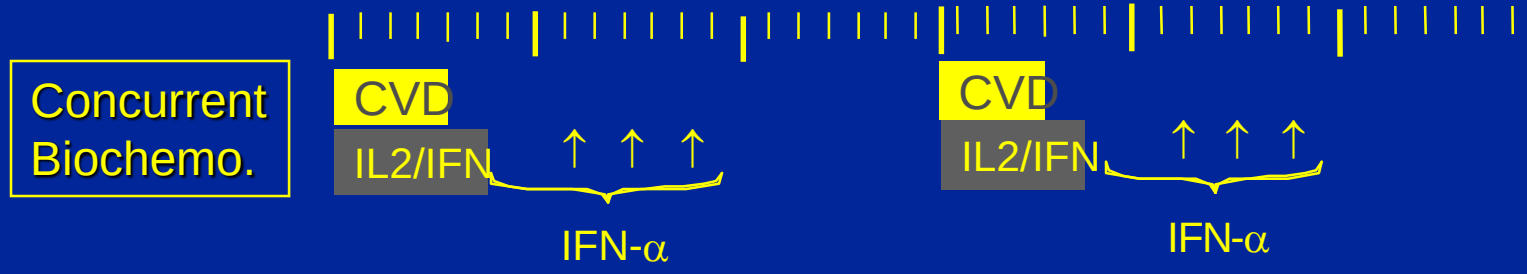
Biochemotherapy

- Combination of immunotherapy (biologic therapy) with chemotherapy
- Concept of non cross-resistance
- Sequential or concurrent
- Usually in-patient administration

- Phase II studies: RR 40-55%; long-term remissions in 9%

Concurrent Biochemotherapy

Regimen

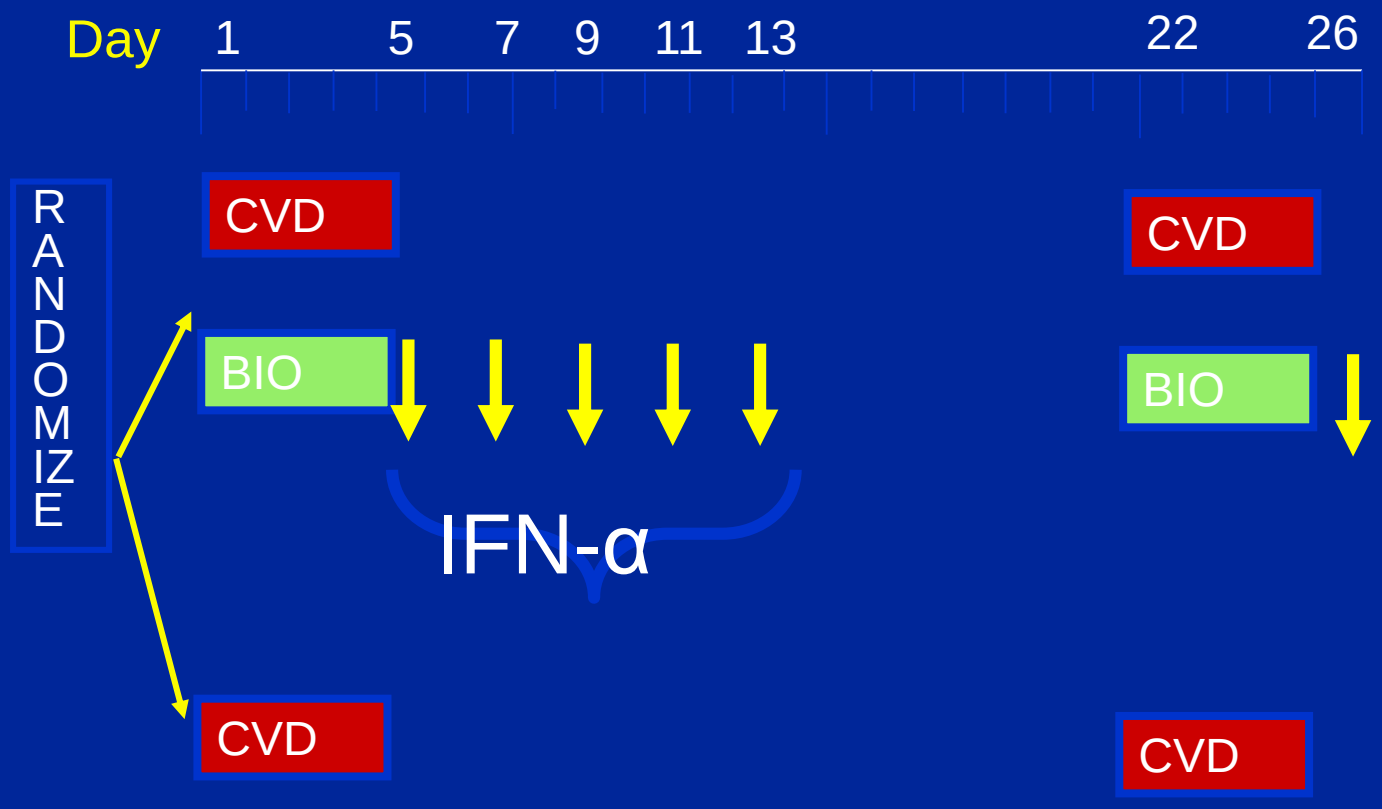


Advantages:

- Only 5 days of hospitalization per 21 day cycle
- No increased organ toxicity
- Similar activity

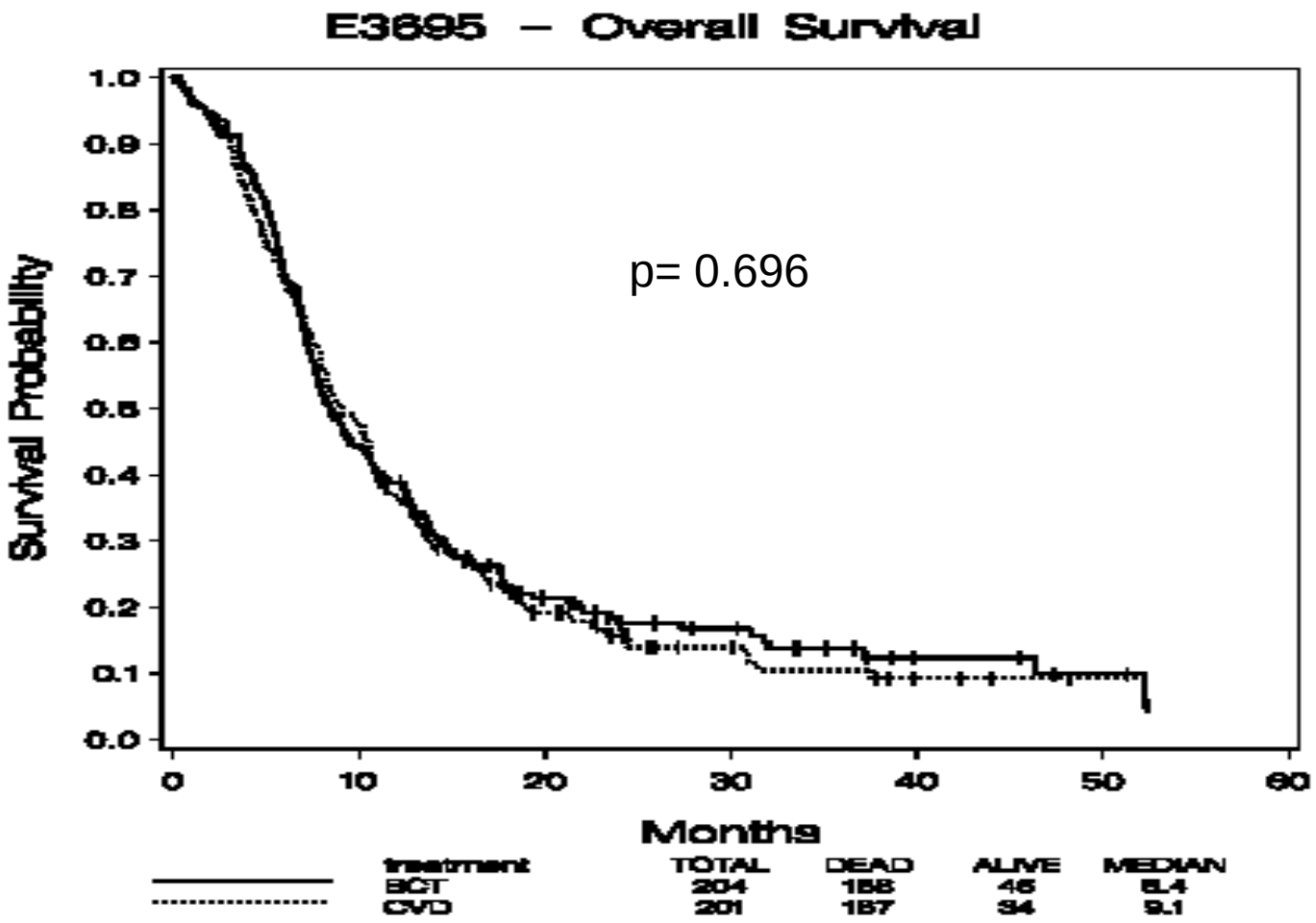
Legha et al

Intergroup Trial E 3695: Schema

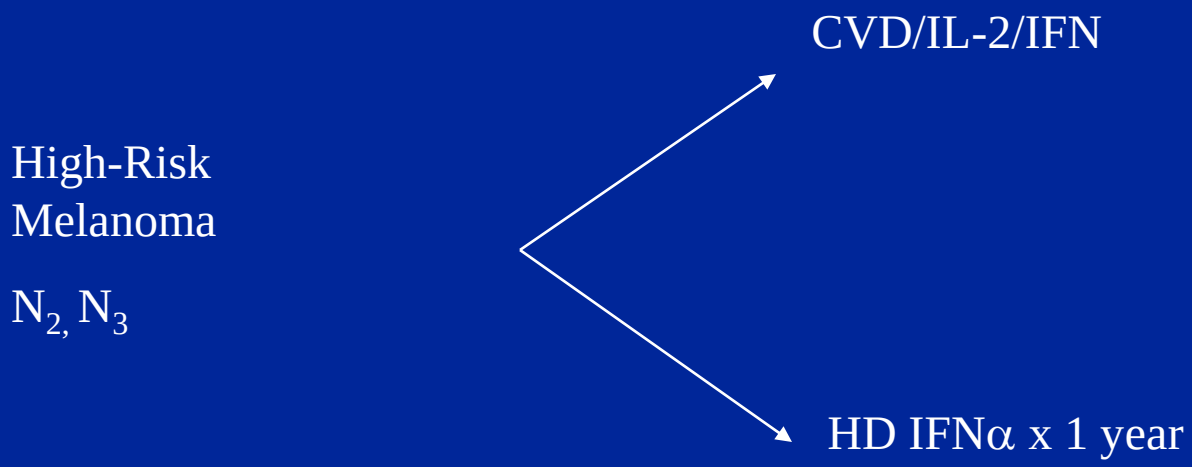


C = cisplatin; V = vinblastine; D = DTIC; BIO = IL-2/IFN-α

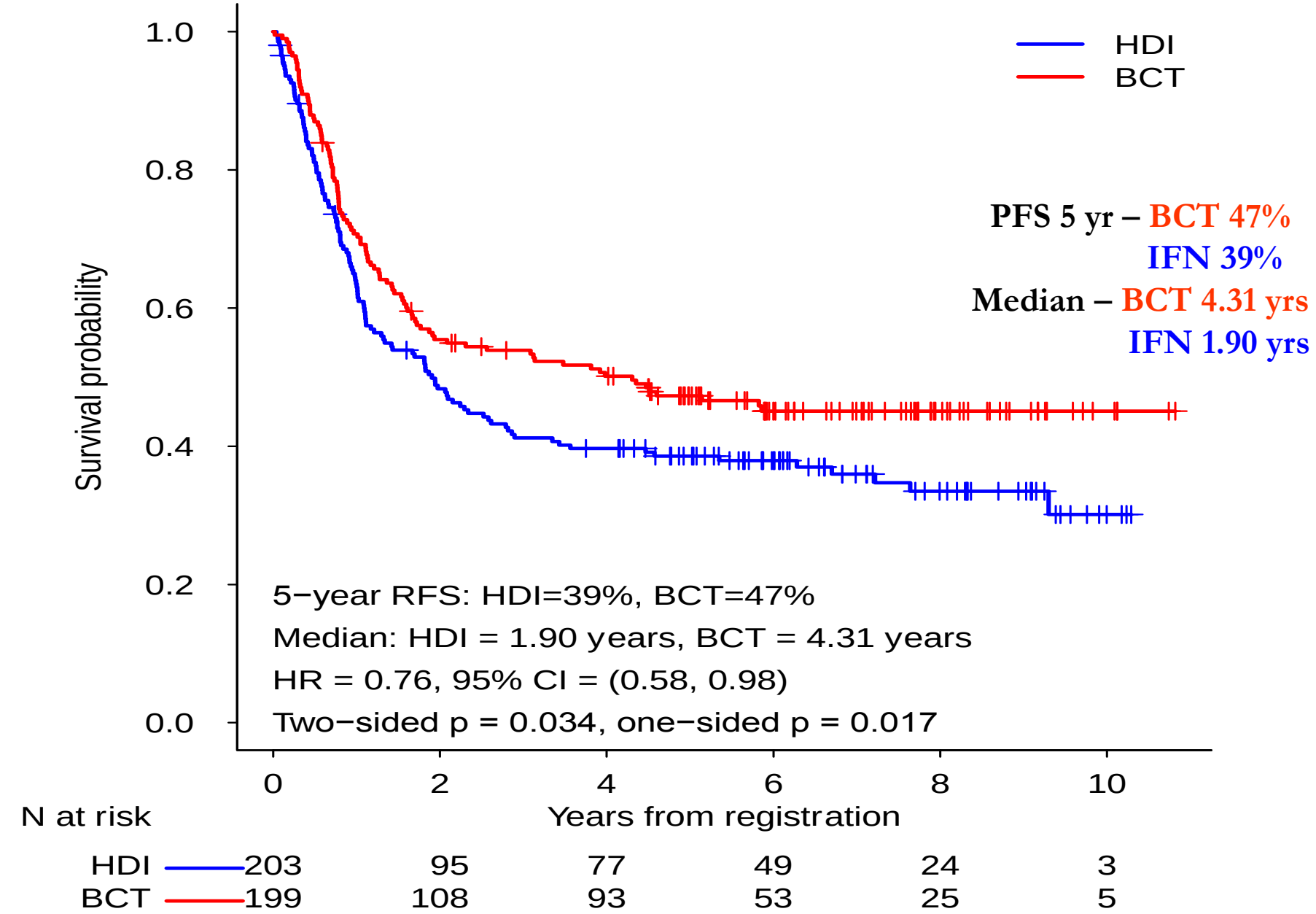
E3695: Survival Data

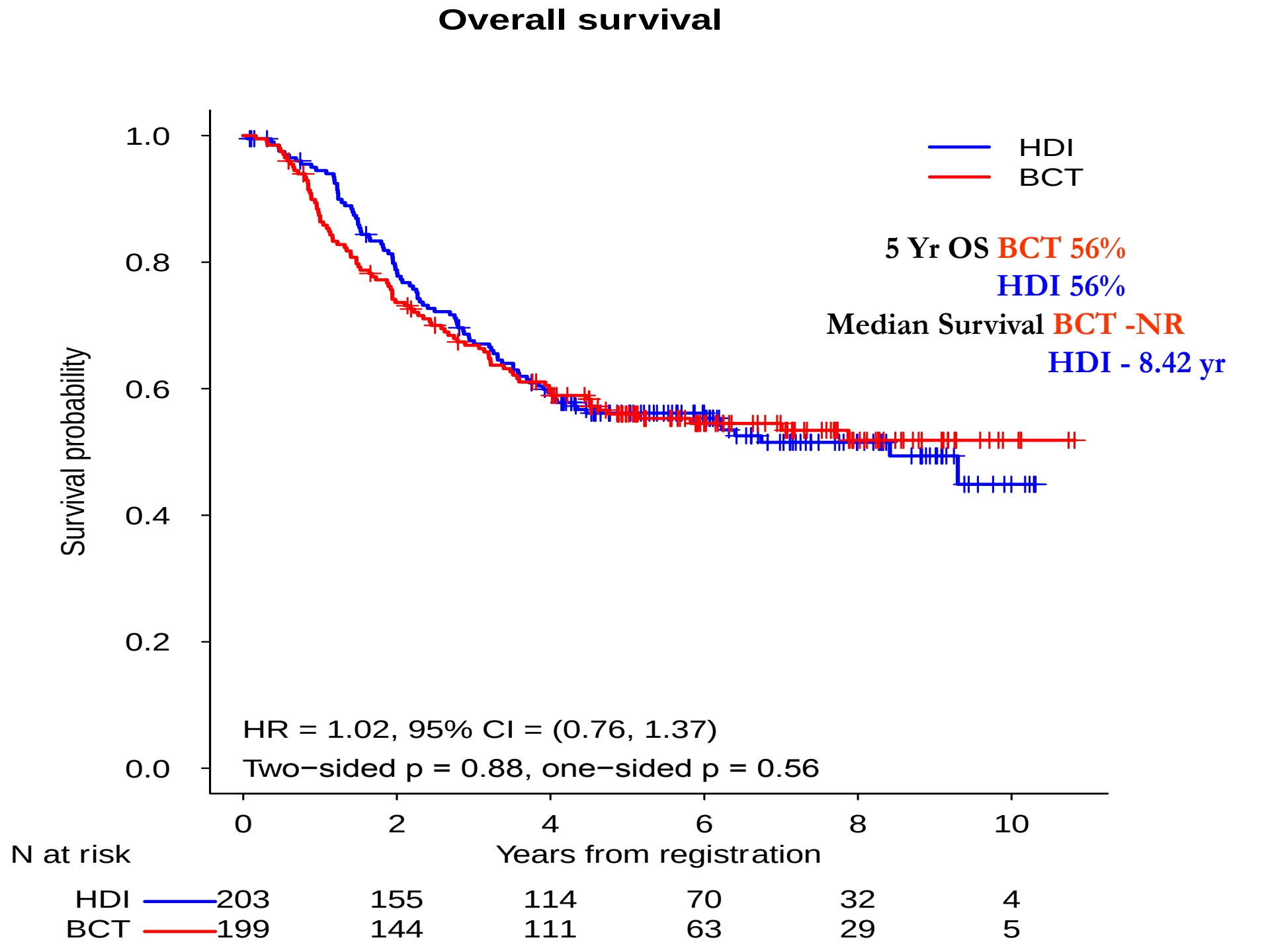


Testing IL-2 in Adjuvant Therapy SWOG/ECOG 0008



Relapse−free survival

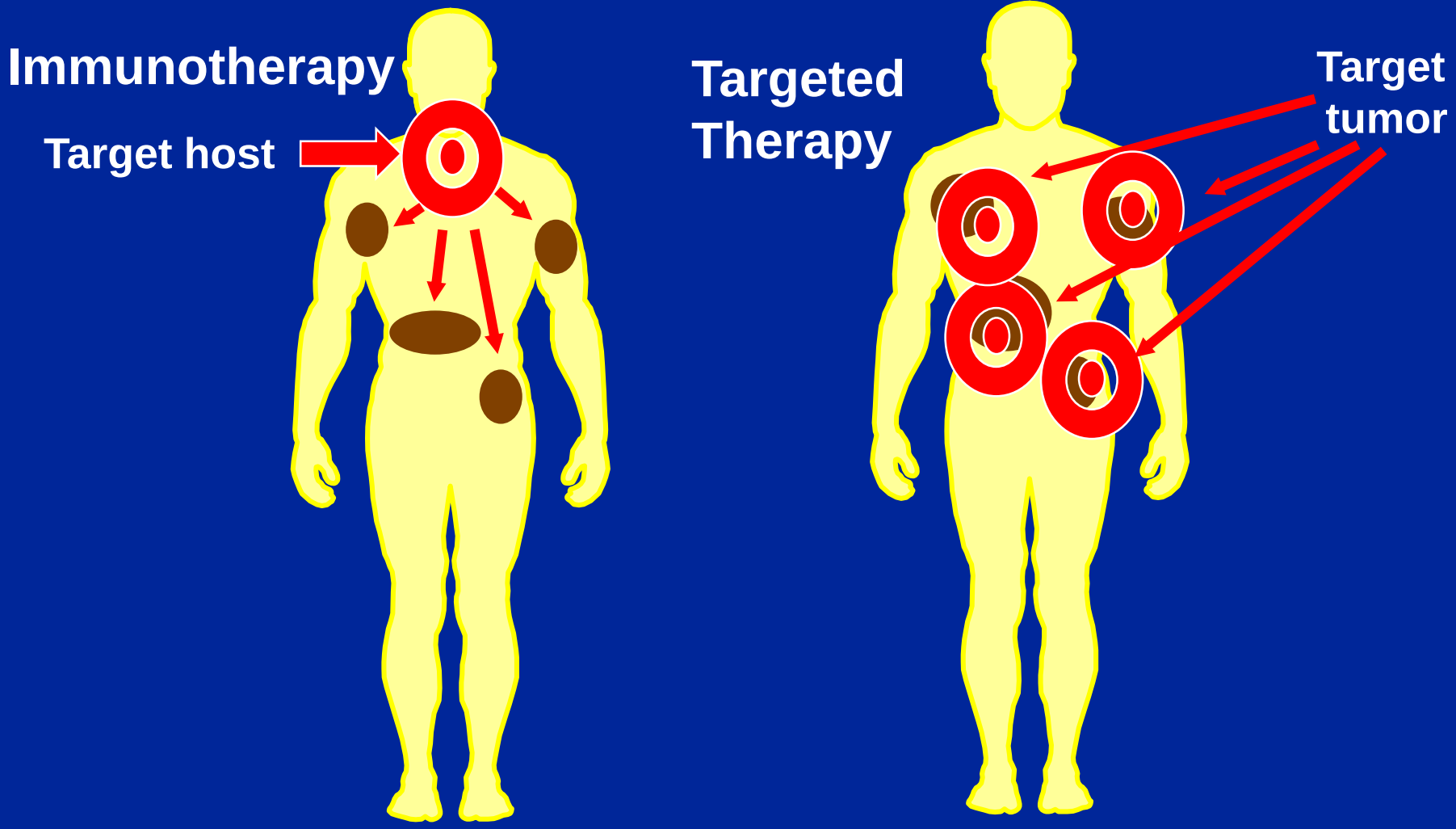



Overall survival
HDI
BCT
5 Yr OS BCT 56%
HDI 56%
Median Survival BCT -NR
HDI - 8.42 yr
Survival probability
1.0
0.8
0.6
0.4
0.2
0.0
HR = 1.02, 95% CI = (0.76, 1.37)
Two−sided p = 0.88, one−sided p = 0.56
0
2
4
6
8
10
Years from registration
N at risk
HDI 203 155 114 70 32 4
BCT 199 144 111 63 29 5

Overview

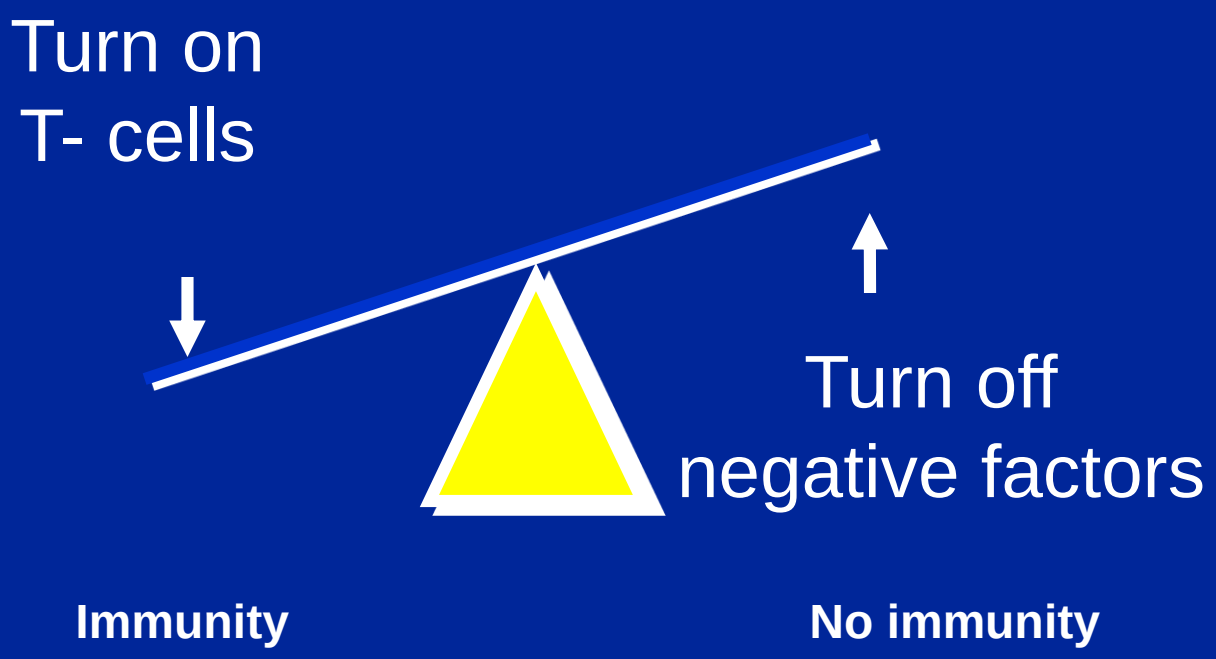
- What is a Cytokine?
- Clinical Applications of Cytokines
  - IFN
    - Adjuvant Therapy of Melanoma
    - RCC
  - IL-2
    - Metastatic melanoma and RCC
- Beyond Cytokines

New Paradigm in the treatment of melanoma



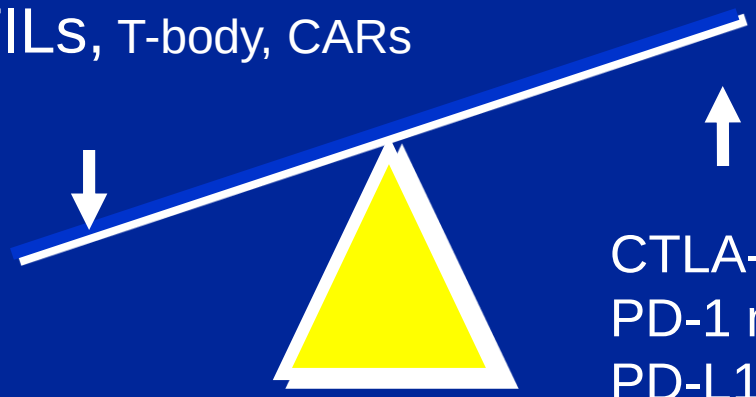
Courtesy, Axel Hauschild, MD

Strategies to Tip the Balance of Immunity



Strategies to Tip the Balance of Immunity

Cytokines
Vaccines
CD40, CD137, OX40 mAbs
Adoptive TILs, T-body, CARs



CTLA-4 mAb
PD-1 mAb
PD-L1 mAb
IDOi
CD25 mAb
Cyclophosphamide
TGF-beta mAb

Summary & Conclusions

- Cytokines are proteins with an important role in the immune system
- Cytokines in clinical use include IFN and IL-2 for both melanoma and RCC
- Their clinical efficacy is limited but still significant
- We are now moving “beyond cytokines” not only in melanoma but other cancers