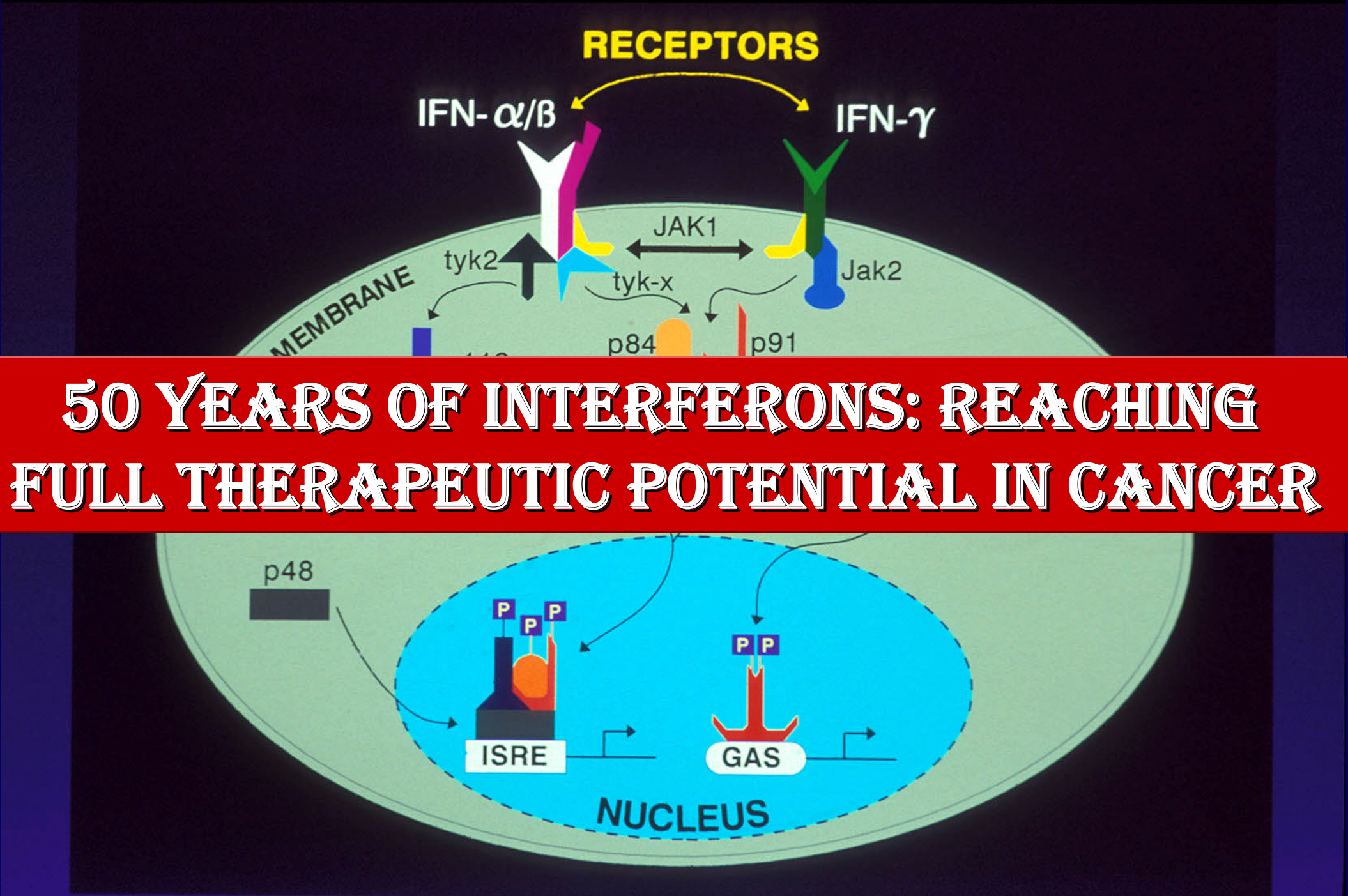
Taussig Cancer Center
Lerner Research Inst
Cleveland Clinic
Foundation
Case Comprehensive
Cancer Center

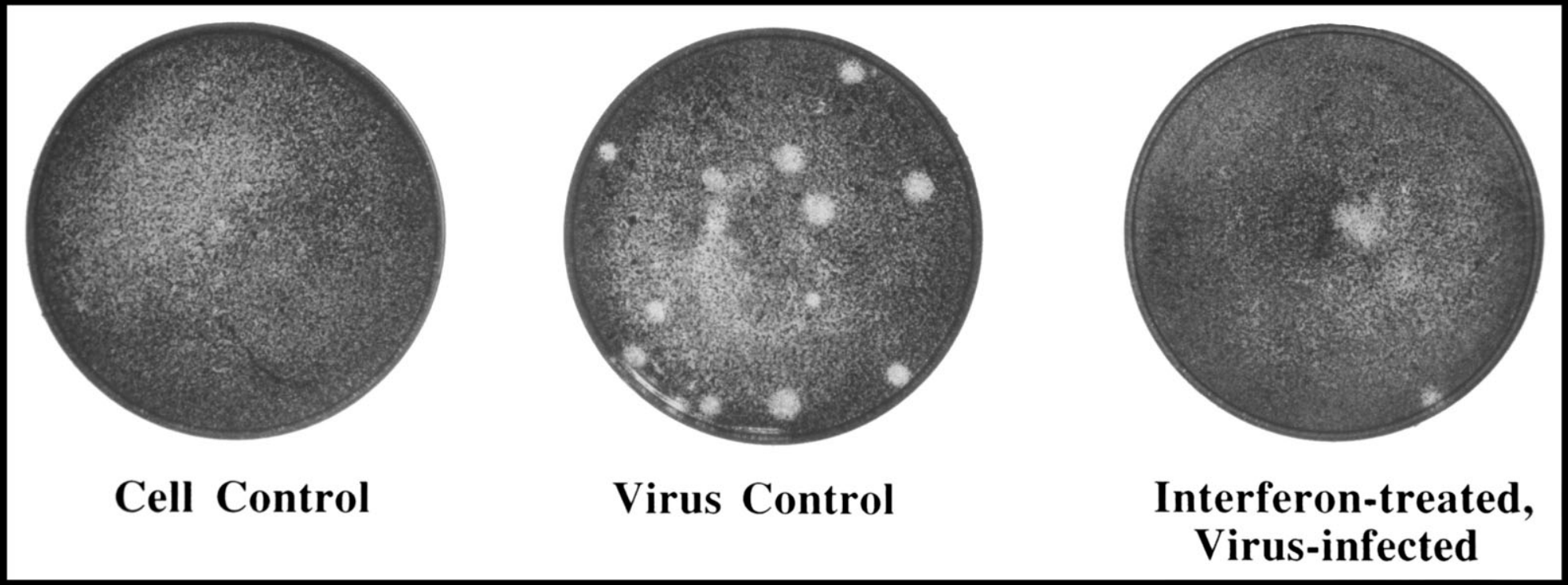
Center

NO PERCEIVED CONFLICTS OF INTEREST RELATED TO PRODUCTS DISCUSSED



50 YEARS OF INTERFERONS: REACHING FULL THERAPEUTIC POTENTIAL IN CANCER

1957 A ISAACS AND J LINDENMANN ANTIVIRAL EFFECTS OF INTERFERONS



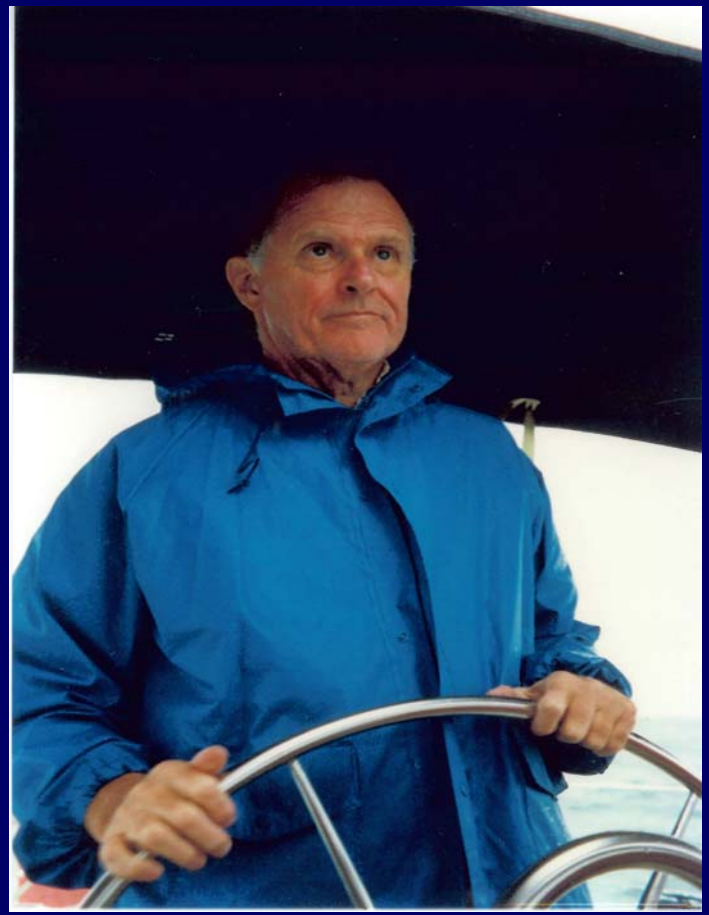
Heat inactivated influenza virus added to allantoic membrane for 24 hrs and fresh membrane incubated in supernate before live influenza virus challenge

INTERFERONS AND CANCER

- 1967-2007
  - Antitumor effects
    - "…interferon preparations increased survival of mice inoculated with RC19 and EL4 tumor cells. Only 3.7% untreated mice survived >22d…whereas 98% of interferon…"
      - Gresser I et al, PNAS 63:51-57, 1969
  - Decrease cancer morbidity and mortality
    - Hematologic malignancies
    - Solid tumors
      - Most broadly active cytokine for cancer treatment
- How did it happen?

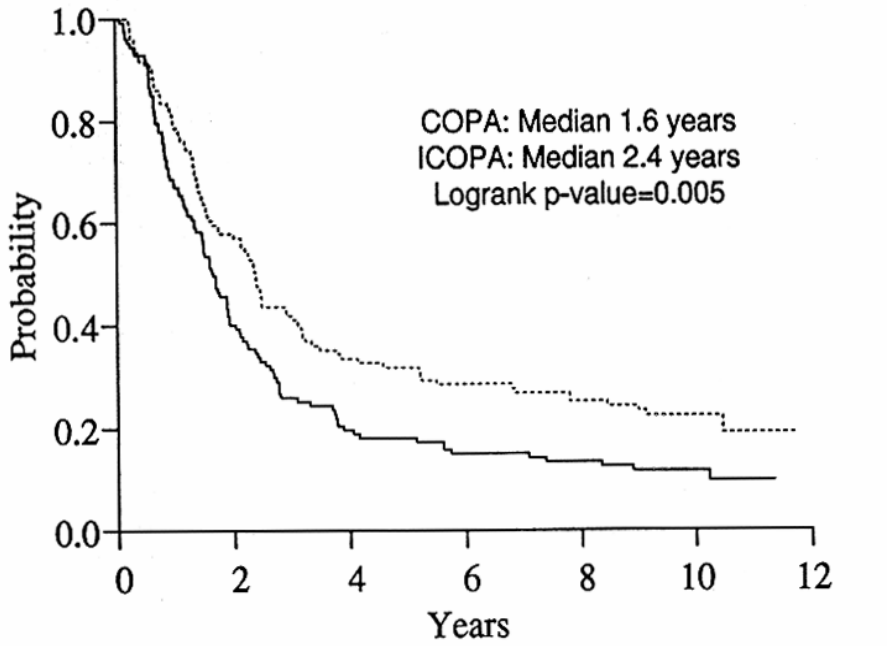
NCI BIOLOGICAL RESPONSE MODIFIERS PROGRAM

- Robert Oldham 1980 Director BRMP, NIH
- Needed Firm Hand on Rudder
  - Richard V. Smalley MD
    - Professor of Medicine Temple
- University of Wisconsin Comprehensive Cancer Center 1984-1990



DISEASE FREE AND OVERALL SURVIVAL OF I-COPA OR COPA INTERMEDIATE PROGNOSIS (NPDL, NM, NH, DPDL) LYMPHOMAS

PFS

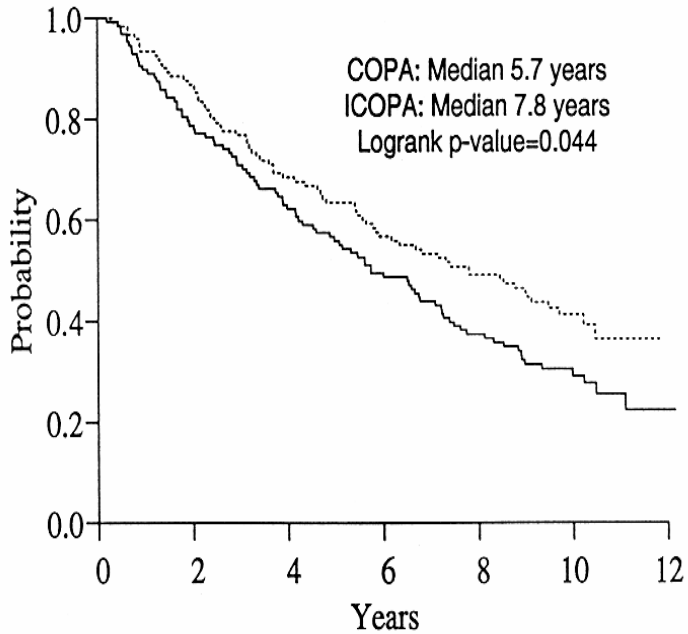


Group	Time Interval 0-2	2-4	4-6	6-8	8-10	10-12
COPA	76/127	26/51	6/25	2/19	2/16	1/7
I-COPA	51/122	29/69	6/40	4/34	3/30	1/14

(# events/# at risk)

Smalley RV et al. N Engl J Med. 1992;327:1336

OVERALL



Group	Time Interval 0-2	2-4	4-6	6-8	8-10	10-12	12-14
COPA	27/127	21/100	16/79	15/62	9/46	3/22	0/1
I-COPA	16/122	22/104	14/82	9/68	8/58	2/25	0/0

(# events/# at risk)

Smalley RV et al Leukemia 2001

INTERFERONS AND CANCER
WHAT HAVE WE LEARNED 1977-2007

- **CAN CAUSE REGRESSIONS AND PROLONG SURVIVAL**

- ***IN VITRO* EFFECTS ALSO OCCUR IN PATIENTS**
  - Signaling ⇒ Gene induction
  - Immunomodulation
  - Antiproliferative/Apoptosis
  - Anti-angiogenic

- **ENHANCE EFFECTS OF OTHER TREATMENTS**
  - Surgery
  - Chemotherapy

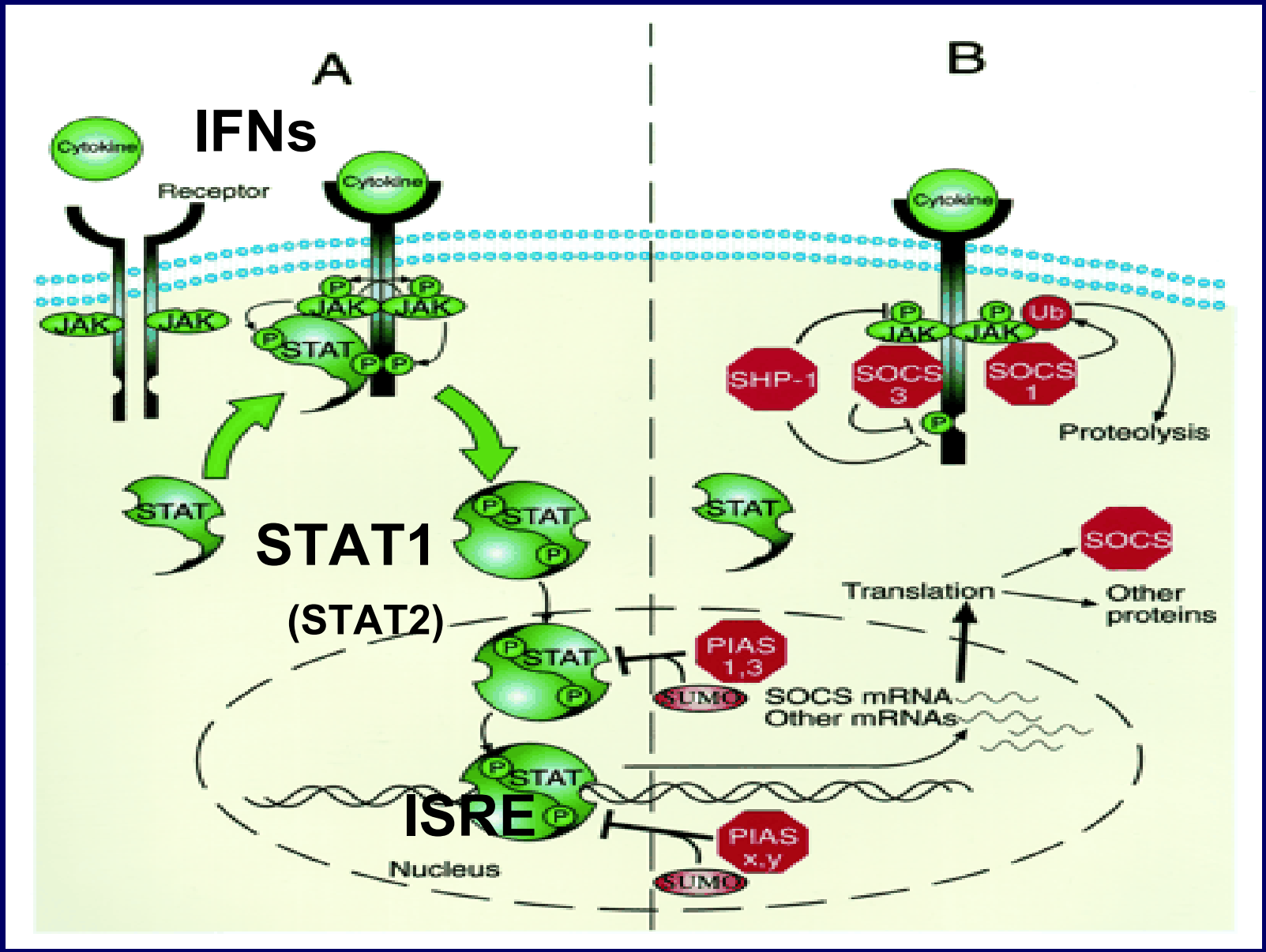
WHERE FROM HERE?

WHERE FROM HERE?

- **REGULATION AND FUNCTION OF >300 INDUCED GENES IN PATHOGENESIS AND RESPONSE**
  - **Apoptosis Immune Modulating Angiogenesis Inhibition**
- **DESIGN INTERVENTIONS TO OVERCOME RESISTANCE**
  - **Second Generation (Therapeutic Index)**
  - **TLR Agonists and Inducers**
- **INTEGRATION WITH OTHER THERAPIES**
  - **Phase I/II/III Clinical Trials**

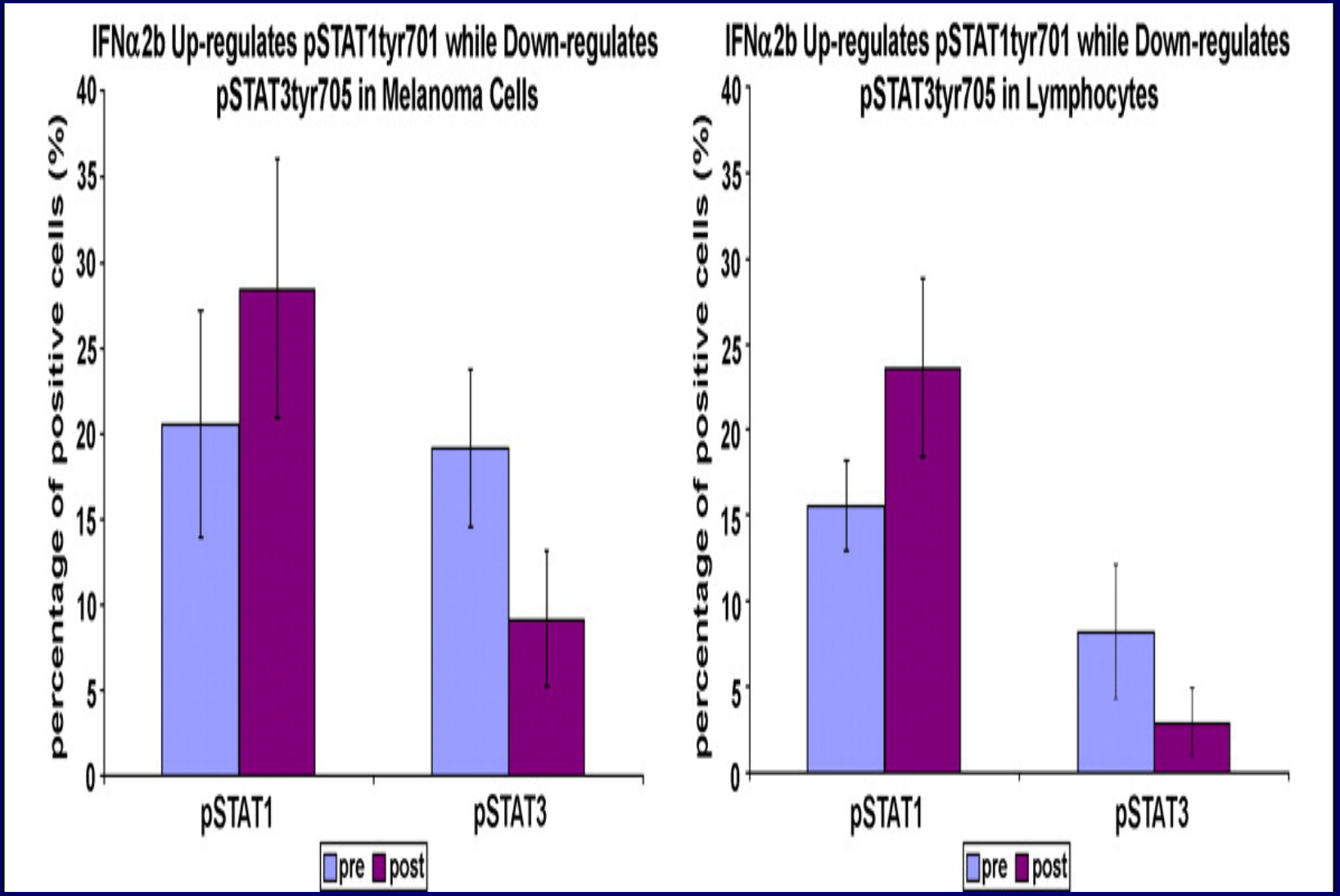
REVIEW: PUB MED►BOOKSHELF► *SEARCH*: INTERFERONS BORDEN

IFN SIGNALING



Hilton DJ JBC 279: 821, 2004

STAT1/STAT3 RATIO FROM IFN-α2



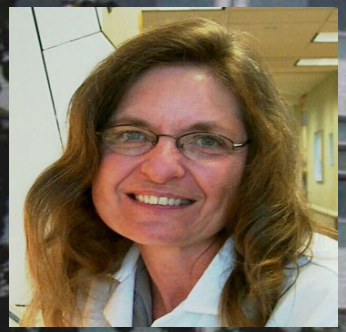
Basal
hi v. lo ratio

OS p<0.05 MEL
OS p= NS PBL

W Wang, J Kirkwood et al Clin Can Res 2007

CLEVELAND CLINIC CYTOKINES AND INTERFERONS

Taolin Yi, Thomas Hamilton, Andrew Larner, Xiaoxia Li, Richard Ransohoff, Ganes Sen, Robert Silverman, George Stark, Daniel Lindner, Doug Leaman (UTol), Pierre Triozzi, James Finke, Ronald Bukowski, Bryan Williams (Monash U), Paul Elson,



Barbara Jacobs

Venu Cheriyath

SooIn Bae

Fred Reu

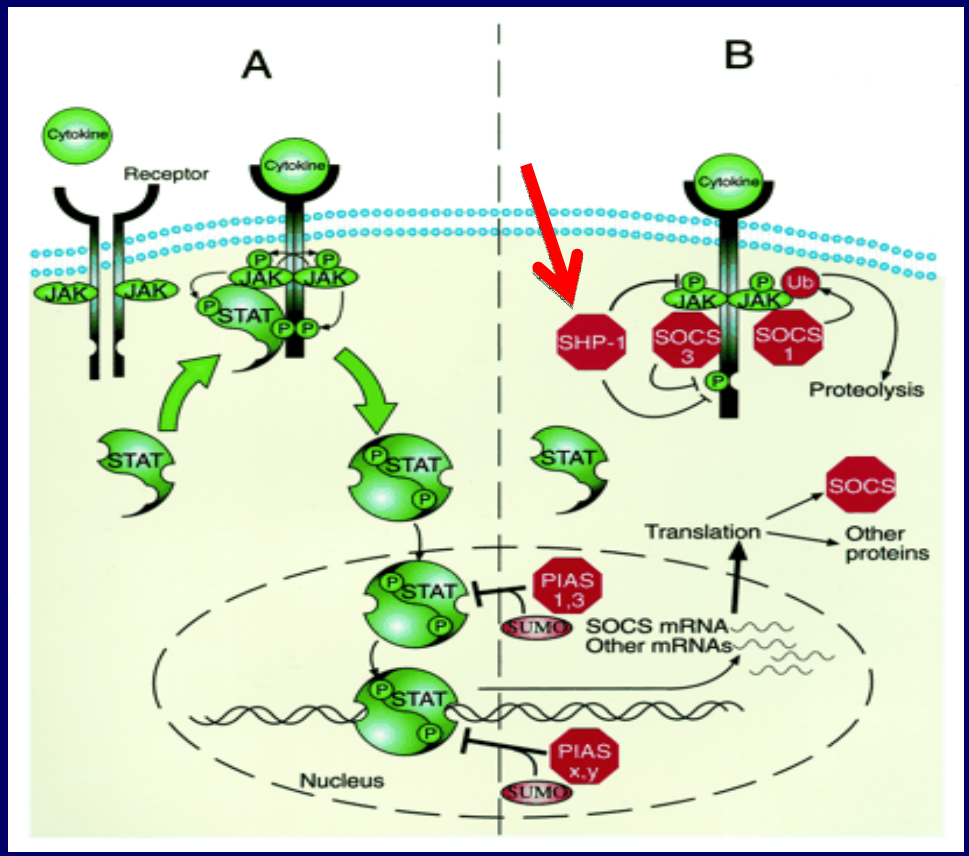
Emese Hollovary

Rebecca Haney

Taussig Cancer Center Laboratories and Lerner Research Institute

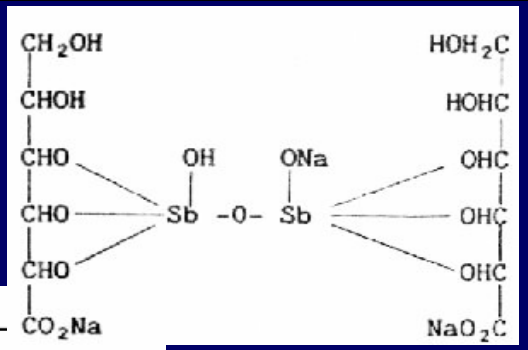
A NEW PARADIGM FOR TARGETED MODIFICATION OF SIGNALING

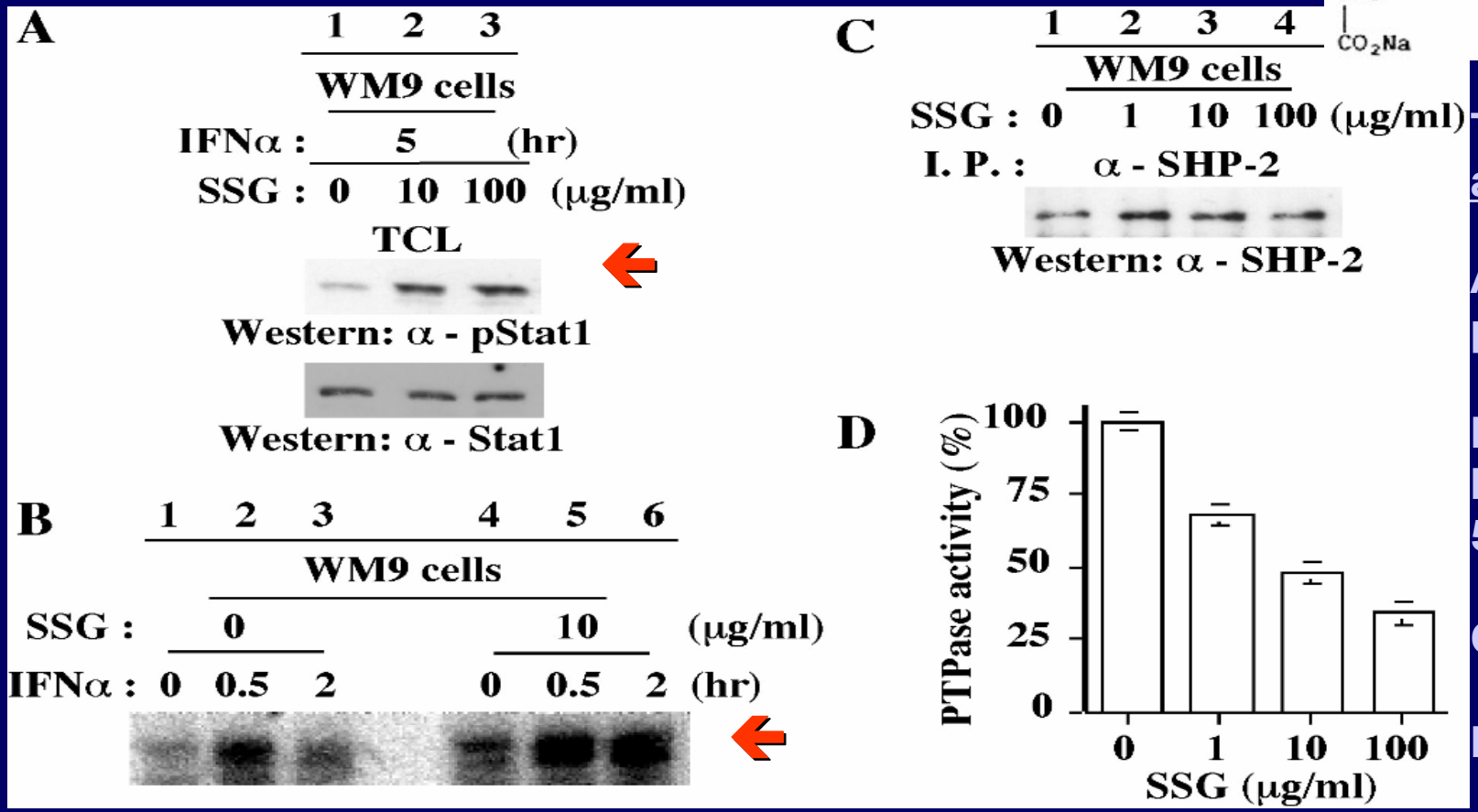
- **INHIBITORS OF PHOSPHATASES**
  - Substrates: Phosphatases for tyrosine kinases
  - Stibogluconate (SSG) prototype: other low MW by library screening
- **SHP-1 AND SHP-2**
  - SHP-1 mostly in hematopoietic cells; SHP-2 all cell types
    - SHP-1: ↓ activated T cell signaling
  - ≈SHP-1 negative regulator of signaling; ≈SHP-2 positive role



Poole and Jones, Cell Signaling 2005; Tsui et al, Immunol Revs 2006

STIBOGLUCONATE AUGMENTATION OF STAT 1 PHOSPHORYATION





Total cell lysates after SSG IFN-α2

A: persistence pSTAT1

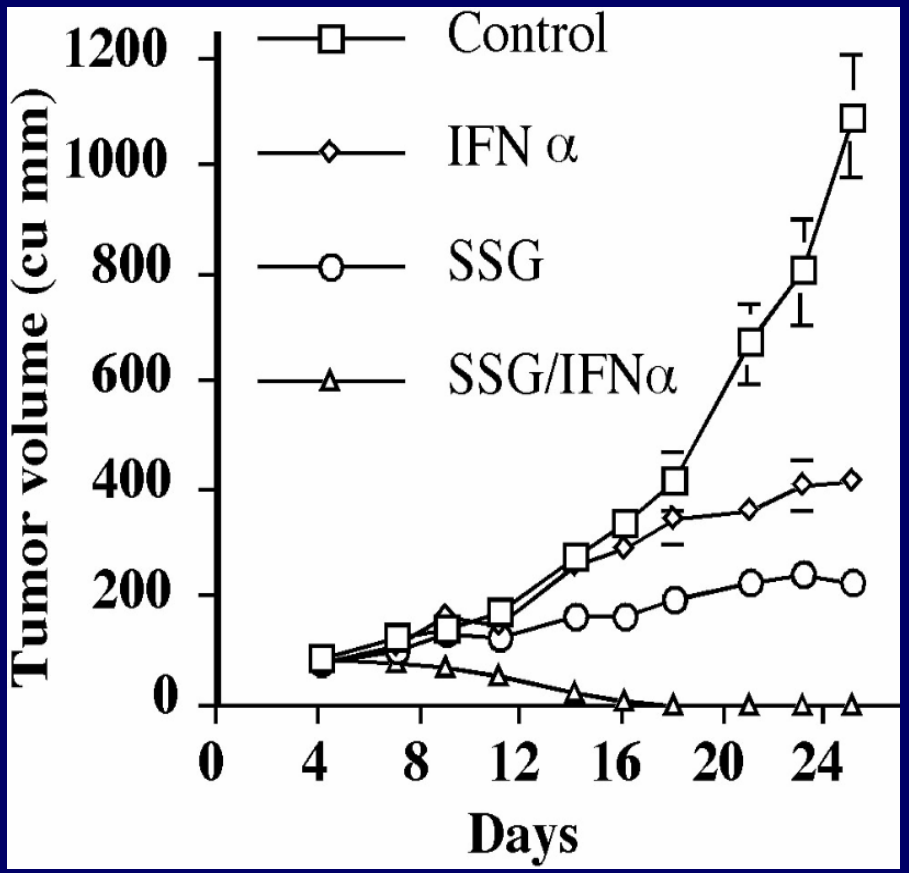
B. Augmentation ISGF3: EMSA 561ISRE

C. Stable SHP-2

D. Inhibition SHP-2

Yi et al, J Immun 169: 5978, 2002

PHASE I CLINICAL TRIAL OF STIBOGLUCONATE AND IFN-α2

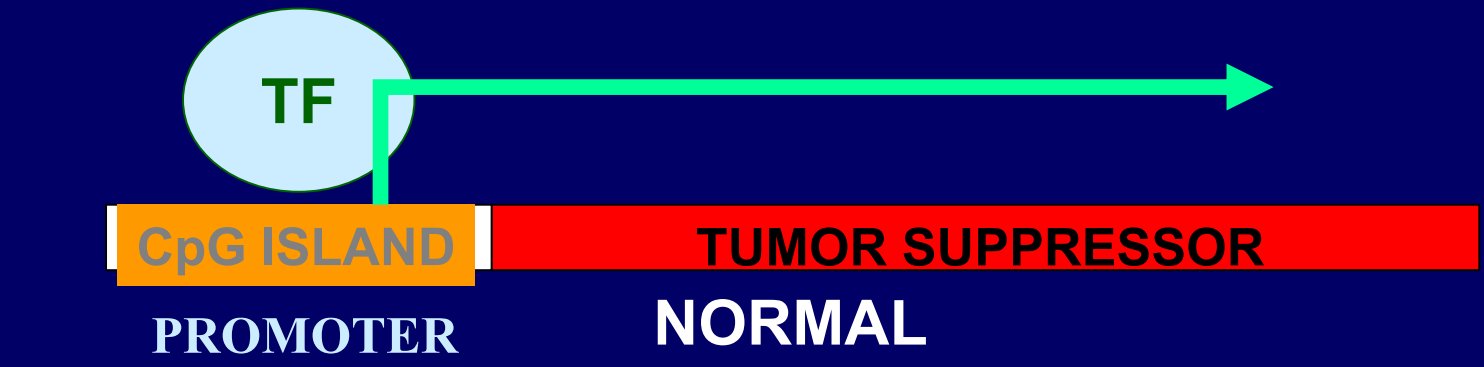


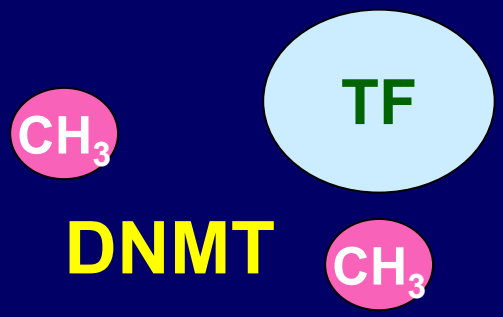
Proof of concept

-SHP-1 inhibition in PBMC
-ISG augmentation
-Safety of combination
-Design with constant IFN-α2 ($3x10^6 U/m^2$ qd) and escalating SSG ($400mg/M^2$ iv, Albert David, Calcutta)
-US IND #68881

WM9 human melanoma; IFN-α2: 5x105U and SSG 12mg/kg; n=8

EPIGENETIC
Heritable DNA Hypermethylation



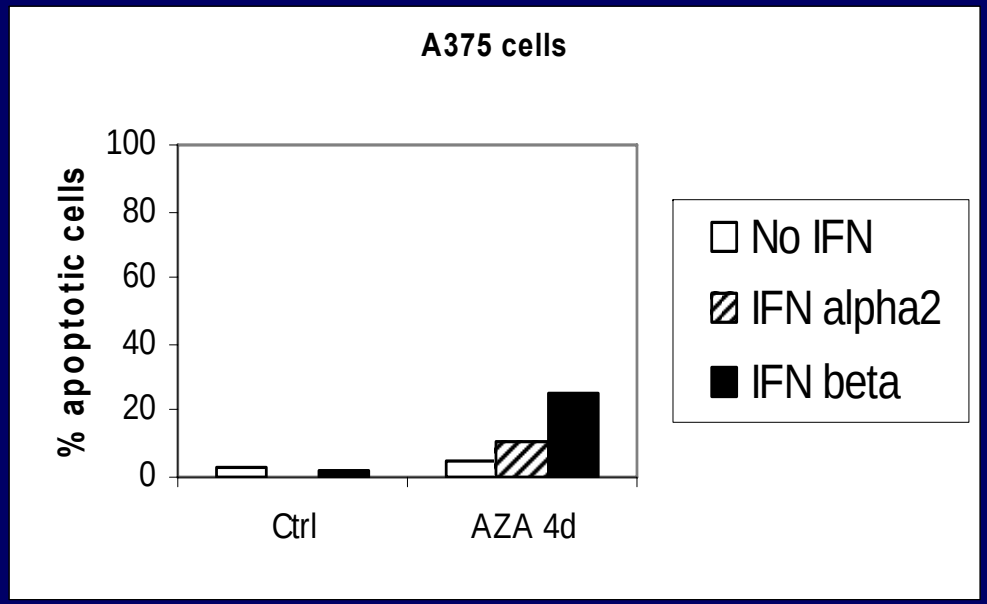


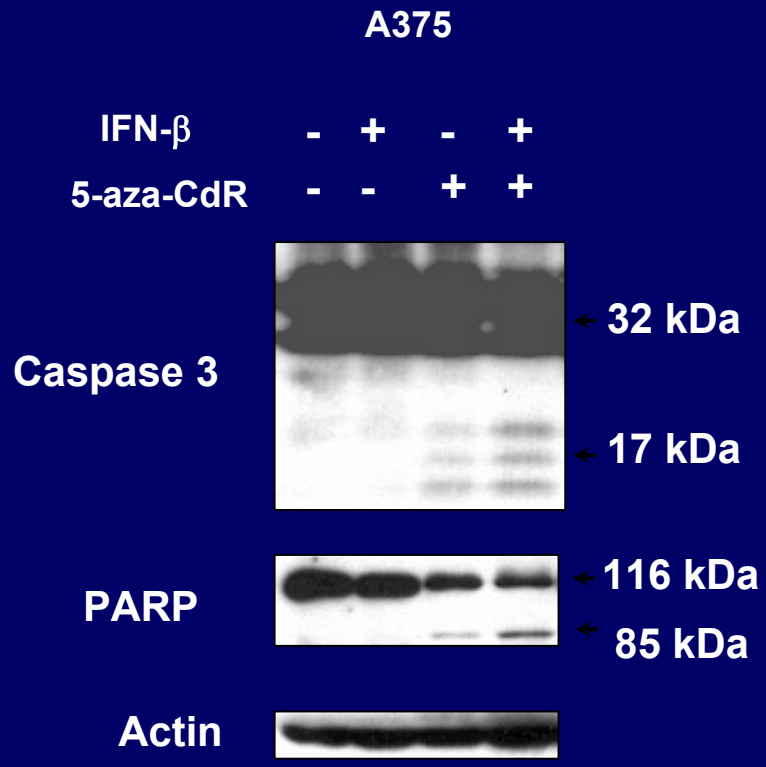
Hypothesis: Aberrant gene methylation of promoter CpG islands may be functionally involved in resistance to IFNs

DNA stability and repair (p16, MGMT)

Proliferation Apoptosis (PTEN, RASSF1A)

SENSITIVITY TO IFN-INDUCED APOPTOSIS IN DNMT1 DEPLETED A375 MELANOMA CELLS





All cells resistant to apoptosis up to 1500 U/ml of IFN-α2 or IFN-β (50 to 100 U/ml IFN-α2 or IFN-β over 4-5d).
Pretreatment with 200 nM 5-Aza-dC daily over 2 or 6 d before IFNs.
5-Aza-dC markedly decreased DNMT1 protein in A375 cells.

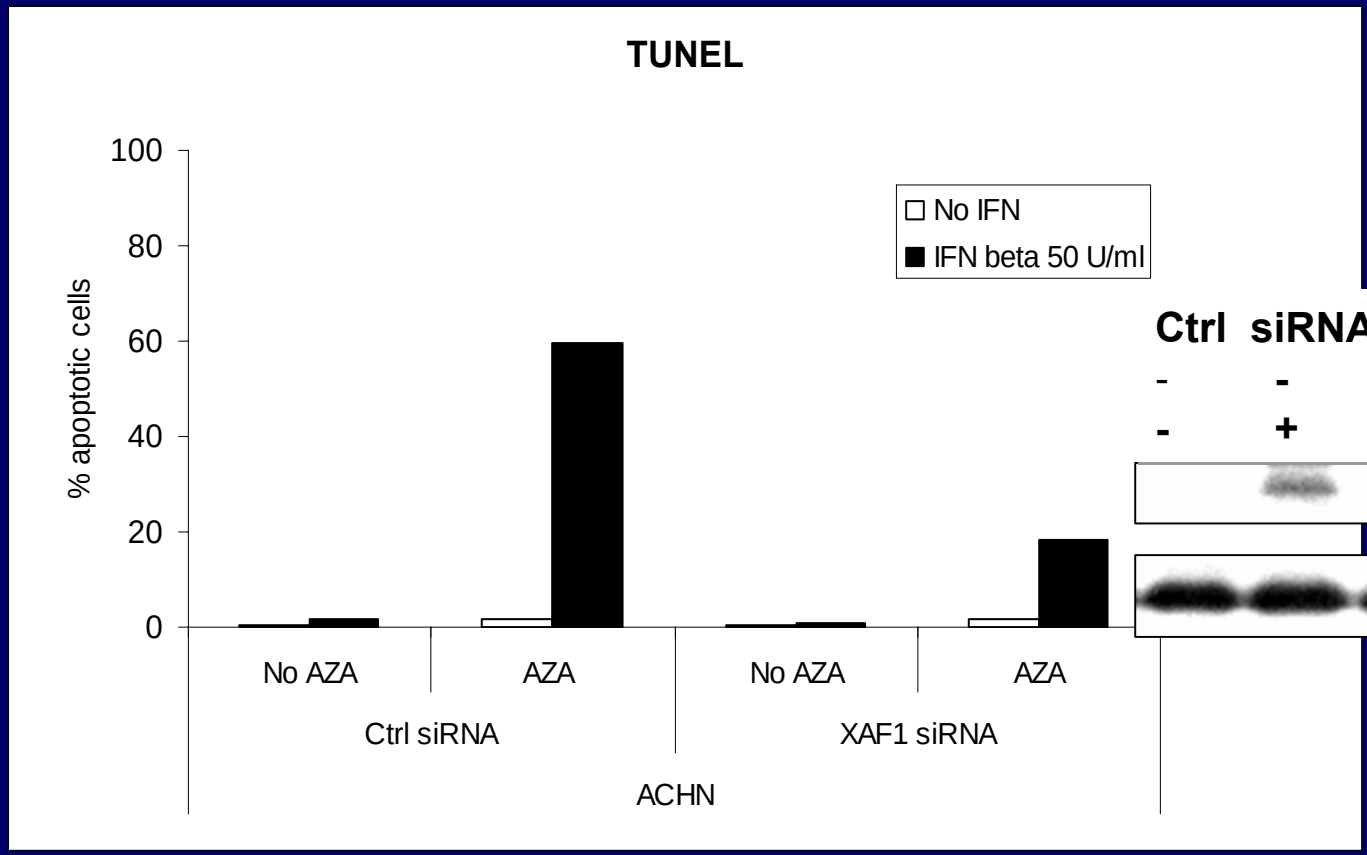
NO SIMILAR EFFECT IN MELANOCYTES

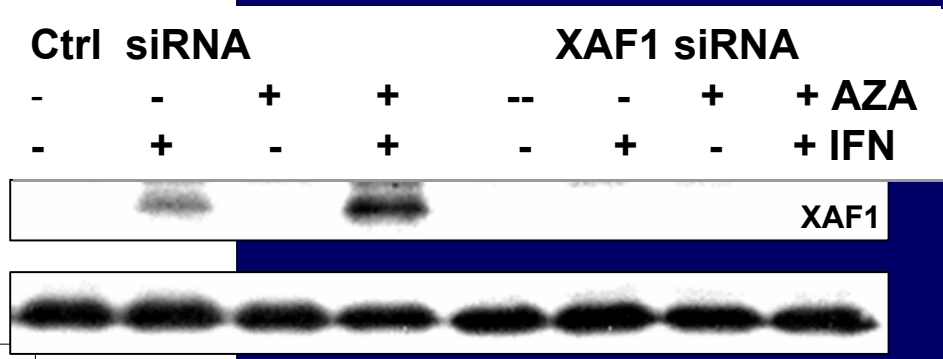
Reu et al Can Res 2006

PRO-APOPTOTIC ISGs

- **TRAIL and XAF1 both required for apoptosis**
  - Chawla-Sarkar M et al Clin Cancer Res 2001
- **XAF1 identified as XIAP binding protein**
  - **Blocked XIAP inhibition of apoptosis**
  - **Implicated as tumor suppressor gene**
    - Byun et al Can Res 2003
- **Induced by IFNs 50x in cells sensitive to apoptosis**
  - **Induced 5x in apoptosis resistant cell line**
  - **Presence necessary for IFN-induced apoptosis**
    - Leaman et al JBC 2002 Leaman et al JICR 2003
- **Increased by 5-Aza-dC in 5/9 melanoma cell lines**
  - **25-150x augmented by qRT-PCR**
    - Reu et al JCO 2006

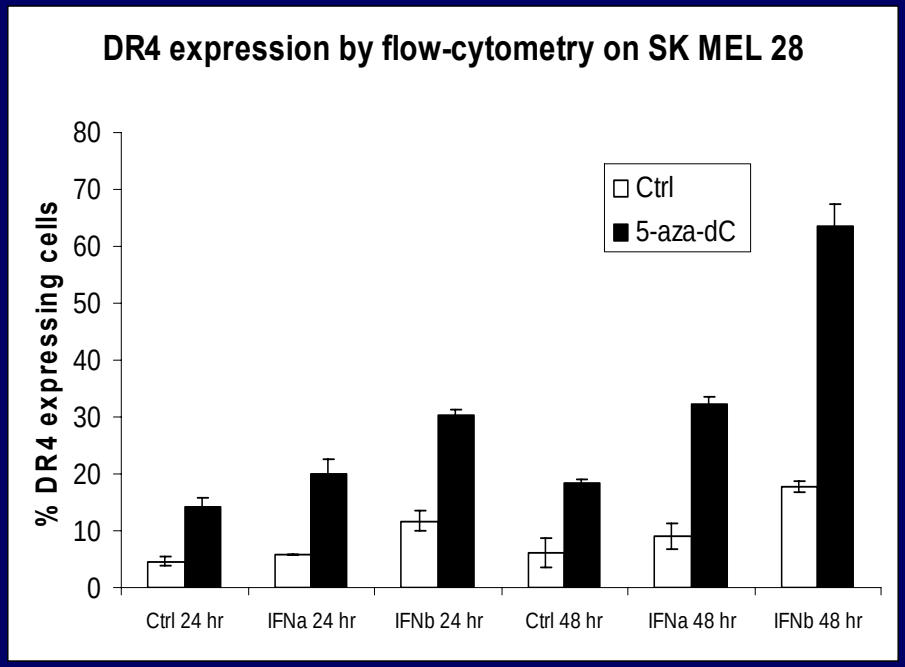
SENSITIVITY TO IFN-INDUCED APOPTOSIS IN DNMT1 DEPLETED CELLS REDUCED BY XAF1 siRNA





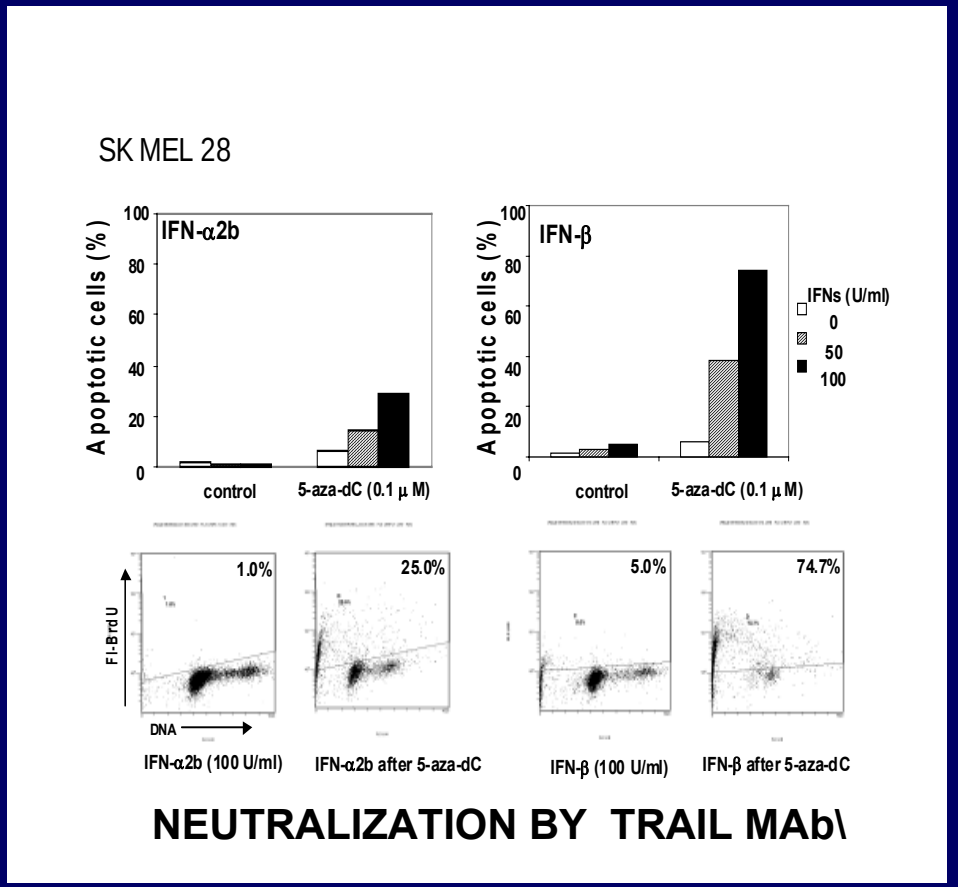
Cotreatment of ACHN cells with XAF1 si RNA (40 nM daily over 2 days, lipofectamine) and 5-AZA-dC (200 nM, 2 days). IFN-β (50 U/ml +24h).

TRAIL R1 METHYLATION: PROTEIN INCREASE AND APOPTOSIS



0.1 μM of 5-aza-dC (96 h). IFNs (100 U/ml) over 24 or 48 hrs.
mRNA increased 30x at 96 hrs by 5-Aza-dC by qRT-PCR

Bae et al Oncogene 2007



Cells treated with 50 or 100 U/ml of IFN-α2b or IFN-β for 96 h after 5-aza-dC treatment (0.1uM) for 96 h. Apoptosis positive cells were assessed by TUNEL assay.

METHYLATION SILENCING OF IFN ACTIONS

- **Constitutive ISGs suppressed in malignant cells**
  - **compared to normal**
    - **(↑ in PBMCs by 5-Aza-dC: Gollob et al, Clin Cancer Res 2006)**
- **DNMT1 inhibitors increase silent pro-death genes**
  - **XAF1, RASSF1A, and TRAIL R1 with functional effects**
  - **Gene re-expression of many other ISGs--functional effects?**
- **5-Aza-dC synergistic with IFN-α2 and IFN-β**
  - **apoptosis *in vitro* antitumor effects *in vivo***
- **5-Aza-dC can induce apoptosis in resistant melanomas**
  - **TRAIL CDDP** **Borden Cytokine Growth Factor Revs 2007**

VARIABILITY IN PATIENT RESPONSE

- **"If it were not for the great variability among individuals, medicine might as well be a science and not an art."**

- **Sir William Osler 1892**
  The Practice of Medicine

WHERE FROM HERE?

- **REGULATION AND FUNCTION OF >300 INDUCED GENES IN PATHOGENESIS AND RESPONSE**
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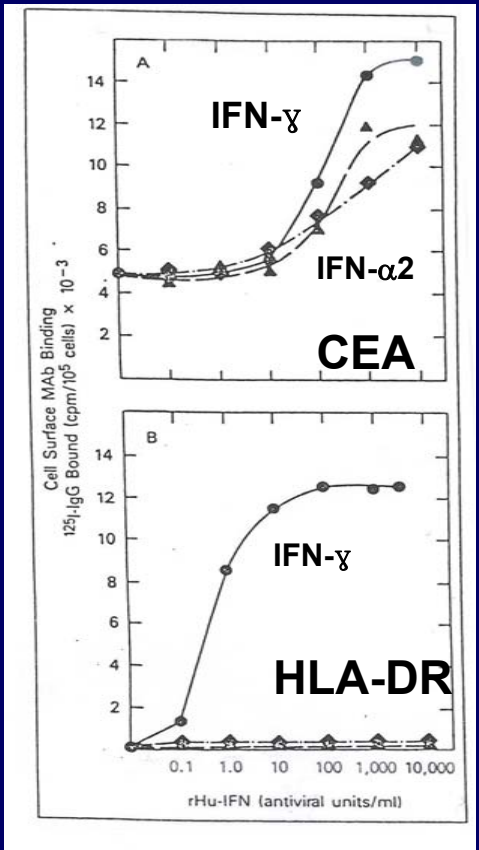
  - Phase I/II/III Clinical Trials

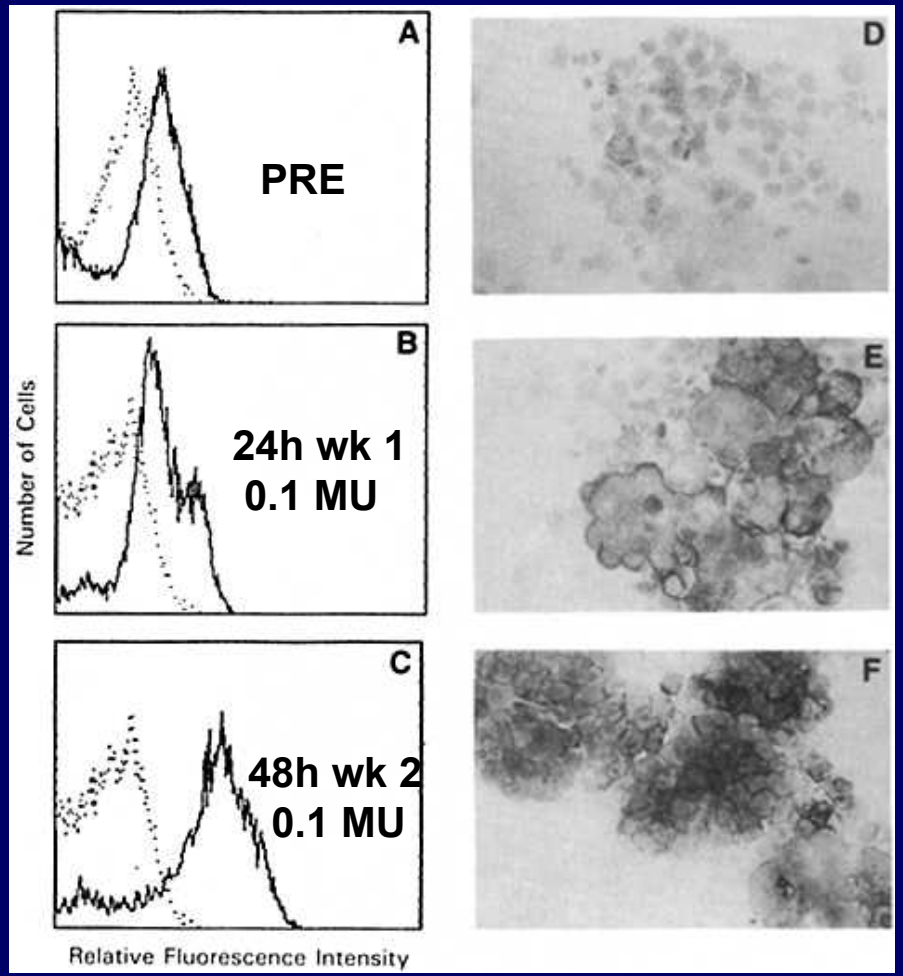
INCREASE IN CELL TAA BY IFNs

In Vitro



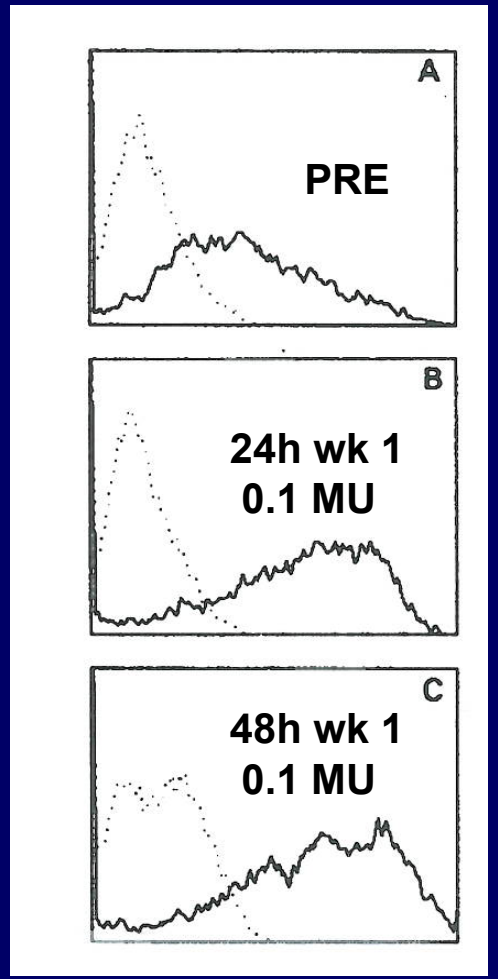
IFNs↑ RIA>50%
CEA 13/22 (59%)
TAG72 27/35 (77%)

In Vivo TAG72



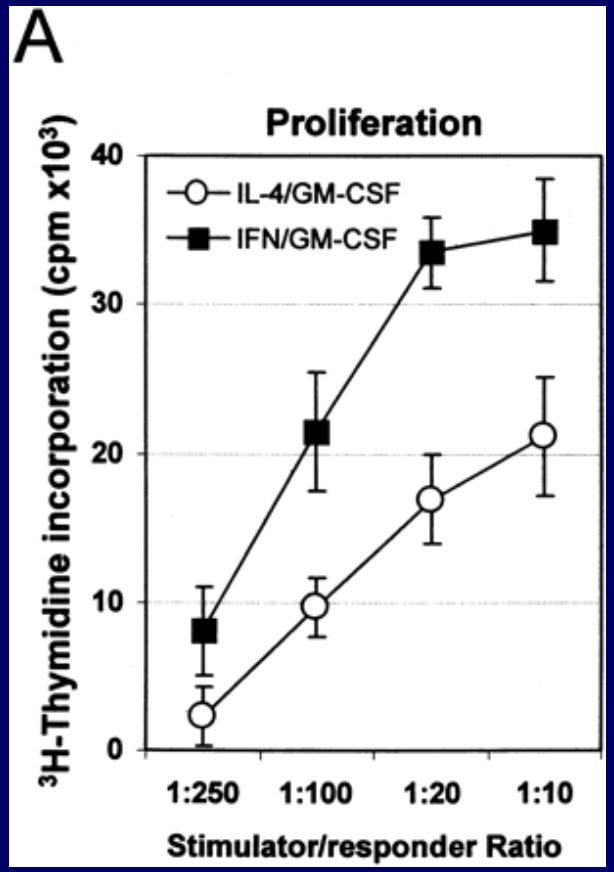
IFN-γ ip wkly 0.1-100MU
Peak *in vitro* ≈48h
Ascites 24h≈5U at 0.1MU

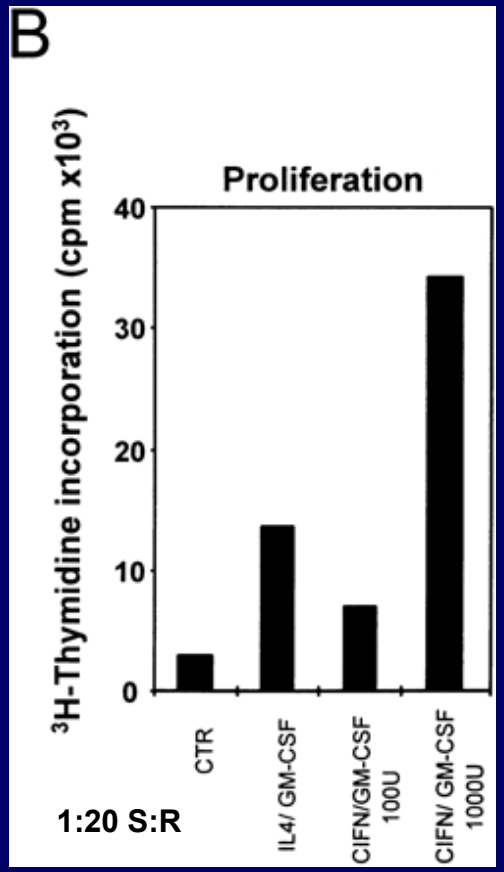
In Vivo CEA

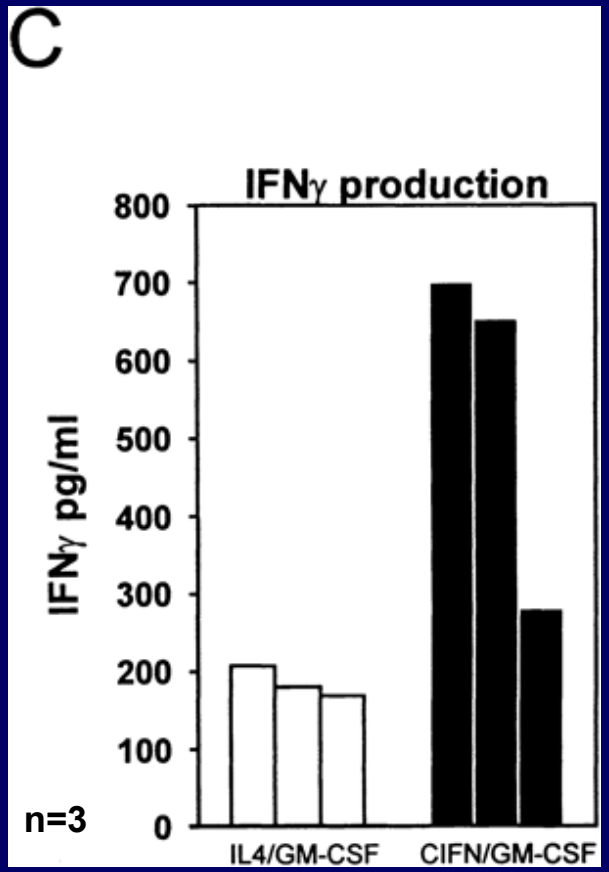


Guadnagni,Schlom,Smalley et al JNCI 1989
Greiner, Smalley, Schlom et al JCO 1992

IFN-α_{CON} GENERATION OF EFFECTIVE ALLOANTIGEN PRESENTATION BY DENTRITIC CELLS







DCs incubated with 1000U IL-4 or IFN and GM-CSF (500U)x3d. Monocyte depleted alloPBLs added

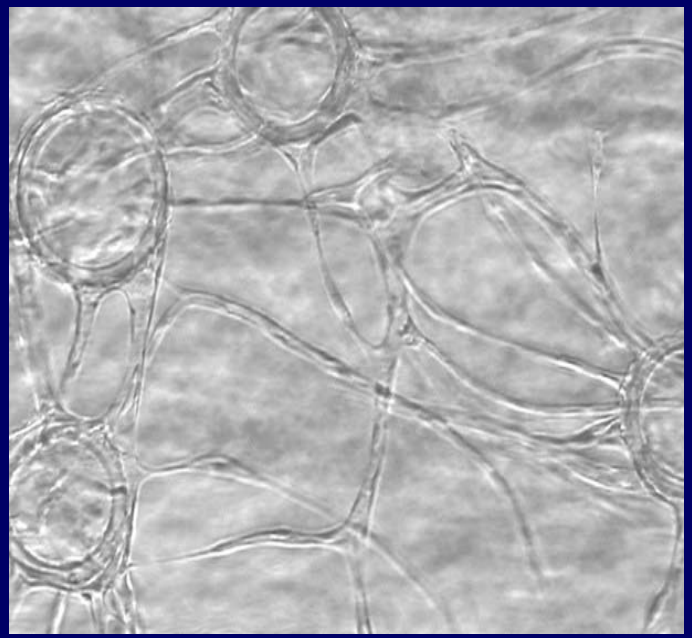
Four ISGs ↑ >10 x in DCs: IFIT1, ISG15 (G1P2), IP10, MxA: Schlaak J et al, JBC 2002

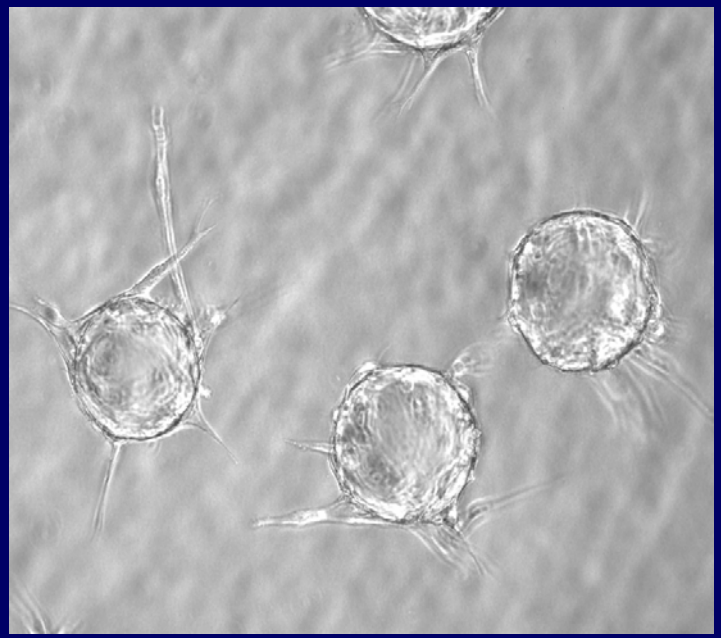
TRAIL mediated cytotoxicity of Jurkat cells also after IFN.

Santini et al JEM 2000

ANTI-DIFFERENTIATIVE EFFECT OF IFN FOR CAPILLARY NETWORK FORMATION

- **IFN-α2 INDUCED GENES**
  - CIG5 1361x
  - IFIT 1 722x
  - CXCL11 459x
  - CXCL10 373x
  - IFI44L 298x
  - OASl 204x
  - MX2 269x
  - MX1 198x
  - IFIT4 193x
  - IFIT2 173x
    - HUVEC
    - Affy U133
    - 1000 U/ml 5hr
      - Indraccolo
      - JImmun 2007





CONTROL IFN-β

Microcarrier beads (cytodex 3) with HUVECs embedded in fibrin gel co-cultured with normal human fibroblasts in EGM2 growth factor enriched media with 1000 units IFN-β

--Lindner Taylor Borden, unpublished 2006

LIFE-THREATENING HEMANGIOMA TREATED WITH WITH IFN-α2

Ezekowitz, Folkman NEJM 1994

PHASE III TRIAL OF IFN-α2 OR IFN-α2 AND BEVACIZUMAB FOR METASTATIC RENAL CARCINOMA (RCC)

Response	IFN/BEV n=306*	IFN(n=289)*
CR/PR	31%	13%**
PFS (med)	10m	5m**

*IFN- α2 9 million units 3x/wk sub Q; BEV 10mg/kg q2w; No prior Rx

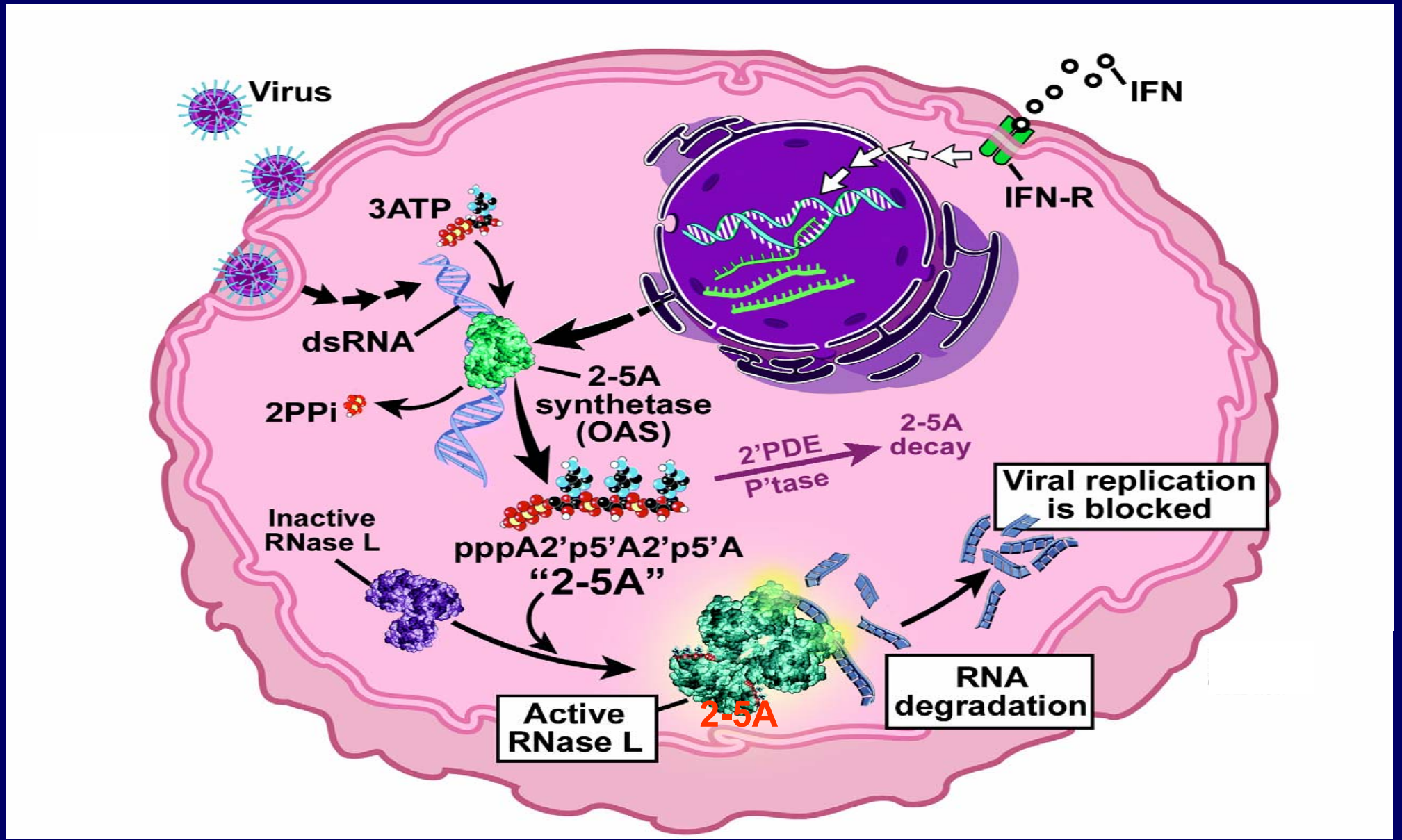
**p<0.0001

Bev alone CR/PR=13%; PFS=8 m

Bukowski et al Proc ASCO, 2006

Escudier B et al International Roche RCC Study Group Proc ASCO #3; p2s, 2007

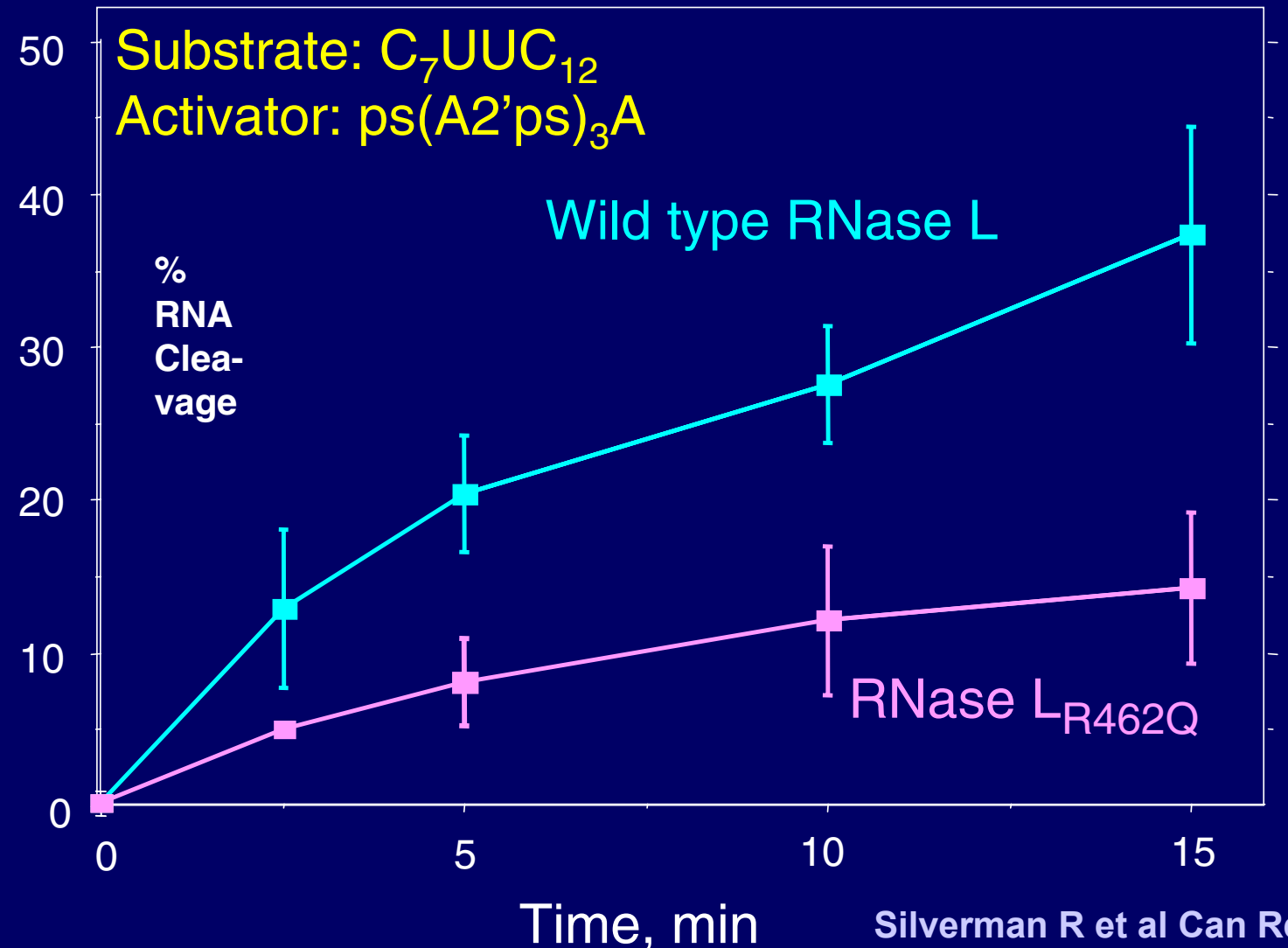
RNase L Activation Pathway



R462Q VARIANT OF RNase L WITH DECREASED ACTIVITY AND HERIDITARY PROSTATE CARCINOMA (HPC)

HPC <55
HPC1=RNaseL
60%allelic freq in germline
R462Q 13% unselected
Hetero 50%↑
Homo 200%↑

Casey G et al
Nature Gen 2002
Silverman R
Biochem 2003



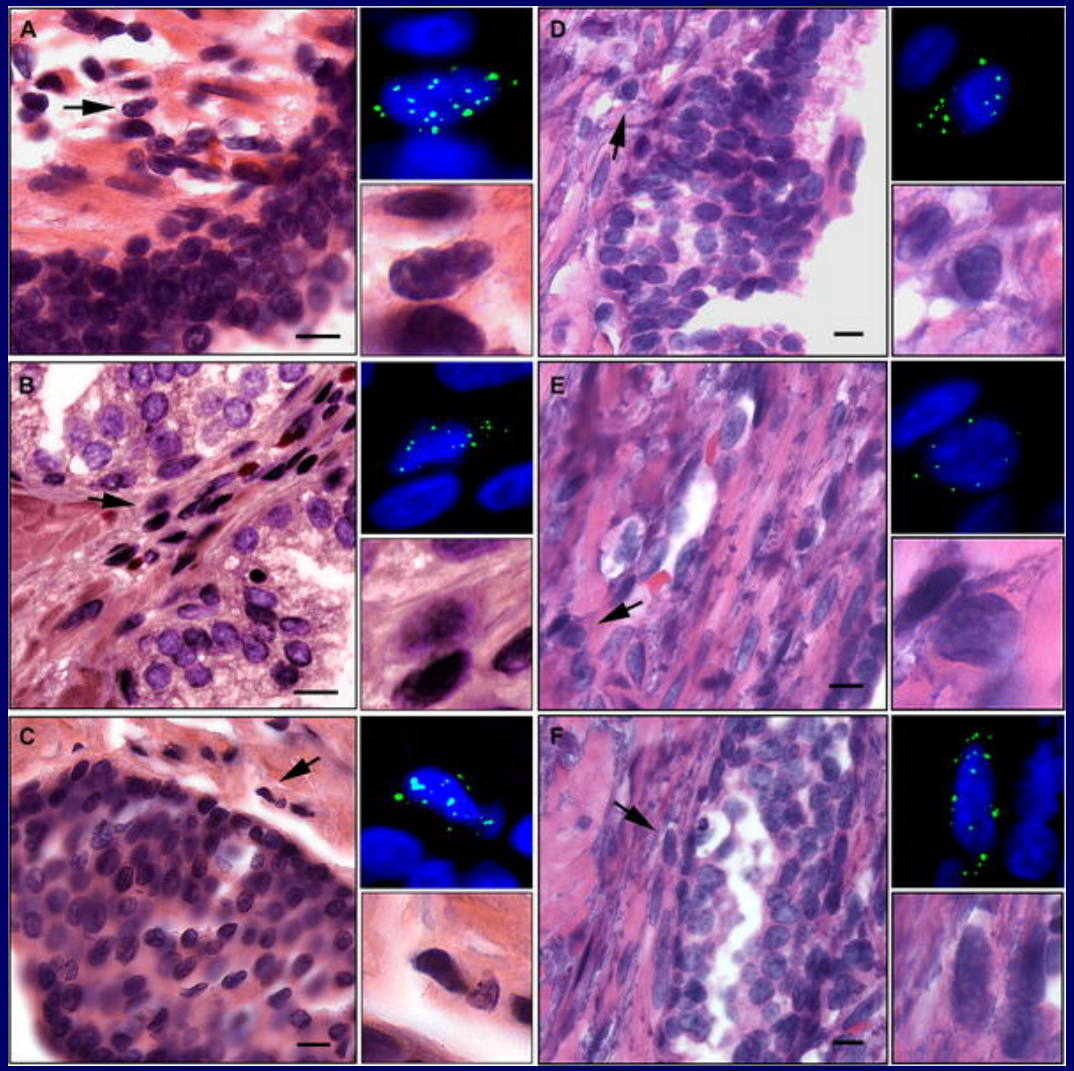
Silverman R et al Can Res 2003

IDENTIFICATION OF A NOVEL GAMMARETROVIRUS IN PROSTATE TUMORS HOMOZYGOUS FOR R462Q *RNASEL* MUTATION

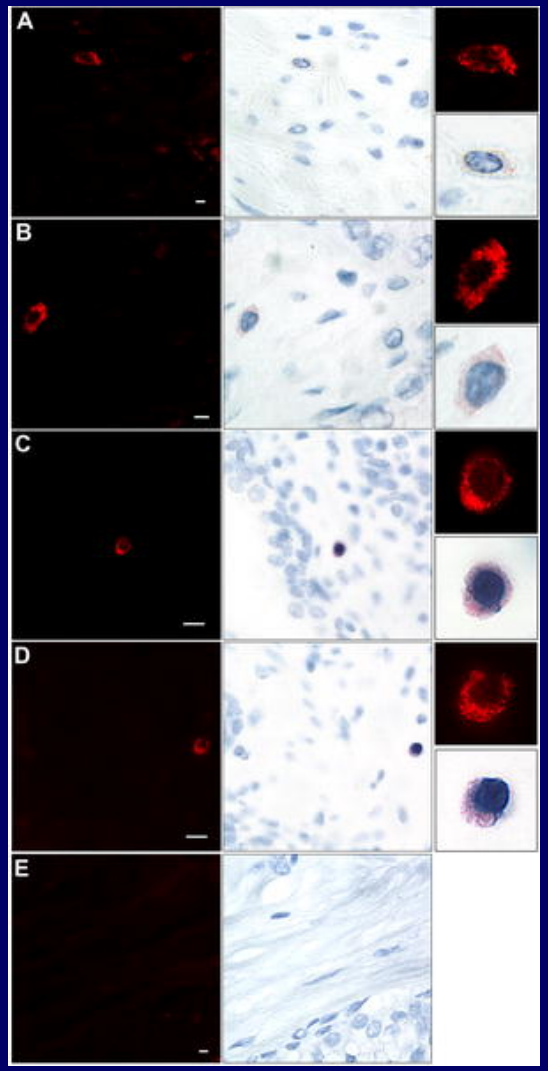
- **VIRAL DETECTION**
  - DNA OLIGO ARRAY OF ALL KNOWN VIRUSES
  - GAMMA RETROVIRUS 8/20 QQ PATIENTS
    - 1.5% RQ OR RR (n=66)
    - NEW XENOTROPIC MURINE RETROVIRUS:XMRV
    - FULL LENGTH GENOME FROM 3 PATIENTS
    - FISH AND IHC IN 1% STROMAL CELLS
      - » Urisman Klein Silverman DeRisi et al PLOS Pathol 2006
- **MOLECULAR CLONAL VIRUS REPLICATES**
  - INHIBITED BY IFN-β IN DU145 CELLS
    - NOT IN RNaseL siRNA DEFICIENT OR LNCaP
  - PROVIRUS INTEGRATION SITES
    - TRANSCRIPTION FACTORS NFATc3 AND CREB5
    - ANDROGEN RECEPTOR TRANSACTIVATOR SUPPRESSOR
      - » Dong Klein DeRisi Silverman et al PNAS 2007

IDENTIFICATION OF A NOVEL GAMMARETROVIRUS IN PROSTATE TUMORS HOMOZYGOUS FOR R462Q *RNASEL* MUTATION

FISH



IHC



Urisman, Silverman, DeRisi et al PLOS Pathol 2006

INTERFERON SYSTEM IN MALIGNANT PATHOGENESIS

- ISGs in melanoma and other tumor cell lines
  - decrease in constitutive expression
  - increase correlates with improved prognosis
  - RNase L (HPC1) mutation increases prostate cancer risk
- Murine tumor development
  - Ab to murine IFN hastens tumor emergence
  - IFNs decrease carcinogen-induced tumors
- Role in T cell and dendritic cell maturation
- Methylation silencing of genes critical for IFN actions
  - ISGs (XAF1)
  - RASSF1A MAGE1 TRAIL R1

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SYMPTOMS AFTER IFN-α1a AND IFN-α2a

15 µg		45 µg	
IFN-α1	IFN- α 2	IFN- α1	IFN- α2

Total number of side effects in each patient compared by paired *t* test.

9	21	11	24
p < 0.01		p < 0.01	

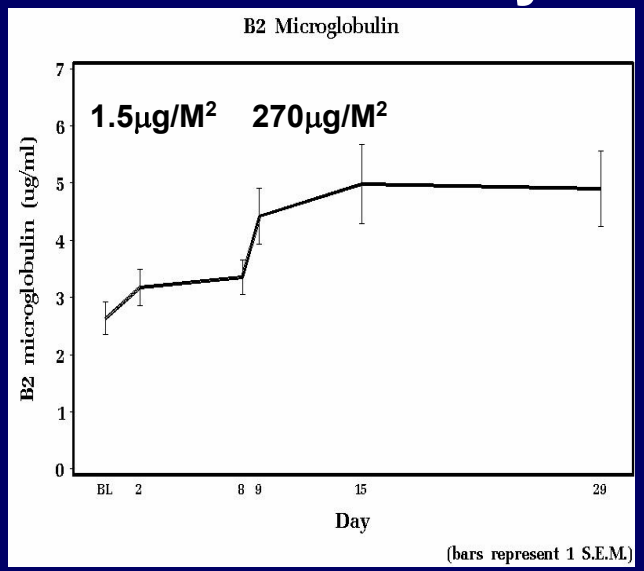
Peak Temperature

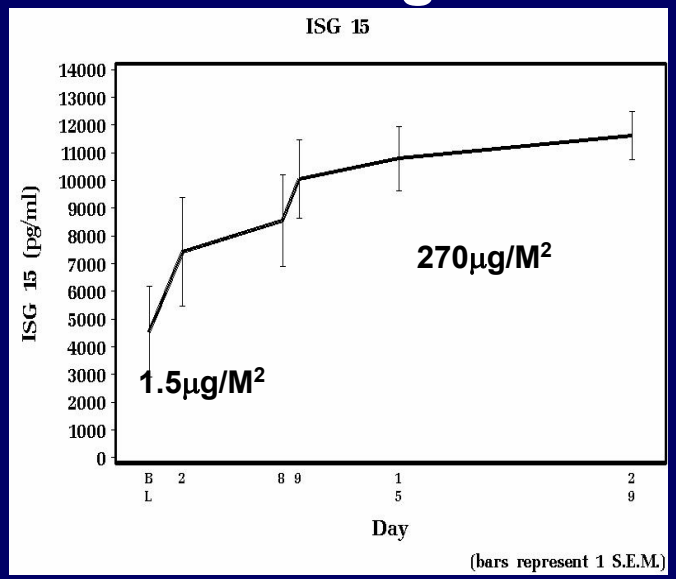
37.4 ± 0.2 °C	37.6 ± 0.2 °C	38.4 ± 0.2 °C	38.8 ± 0.2 °C
p<0.05		p<0.001	

n=8 Randomized double blind crossover

CONCLUSIONS

- Clinical side effects of two recombinant interferons, IFN-α2a and IFN-α1a differed significantly
- Pharmacokinetics same but biological potency of IFN α2a and IFN α1a was equivalent: ISG 2-5 AS and NK cell activity
  - » J Clin Oncol 2: 221-6 1984
- ⇒IFN-α1b from Ministry Public Health Shanghai: IND#8790



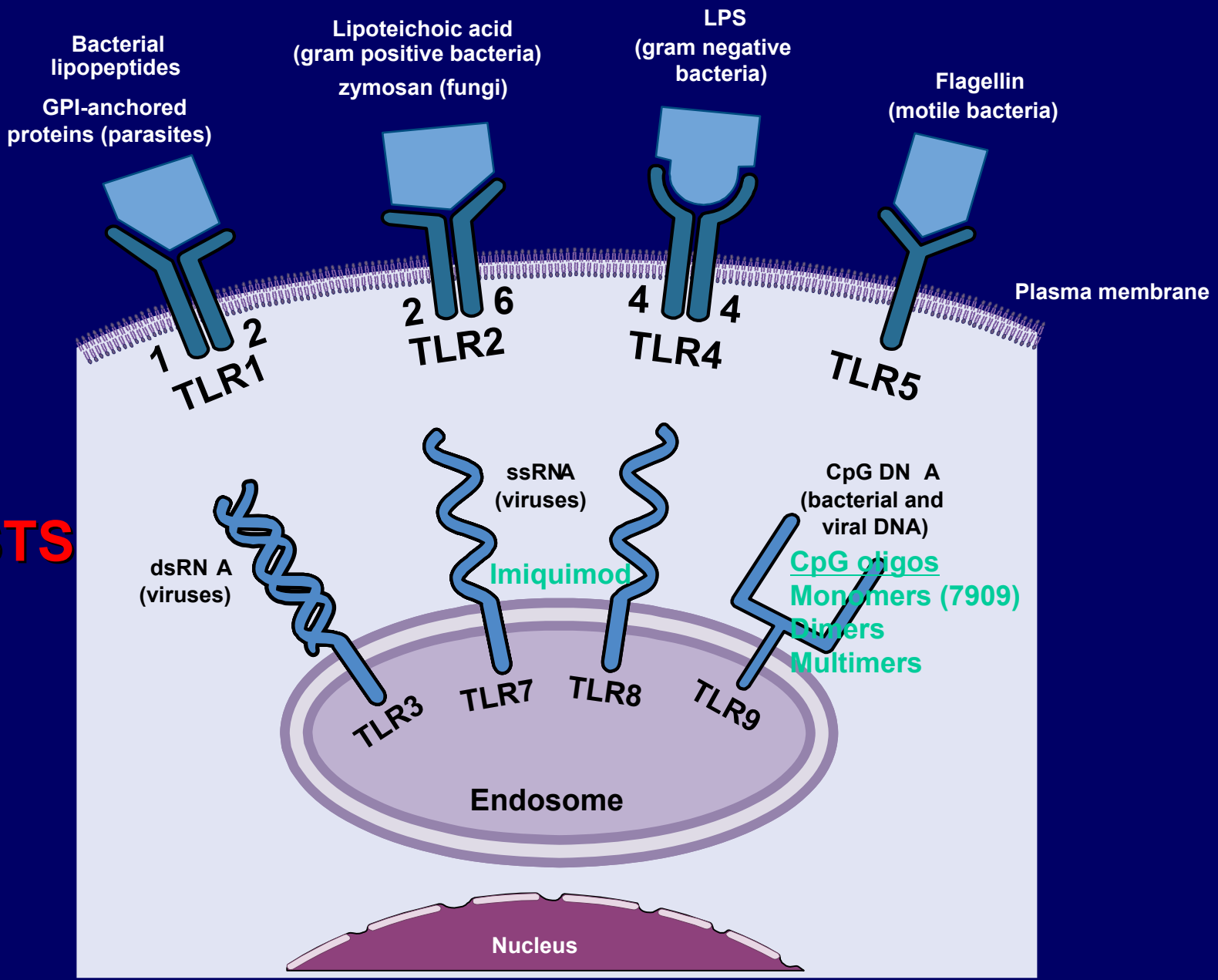


Conclusions IFN-α1b

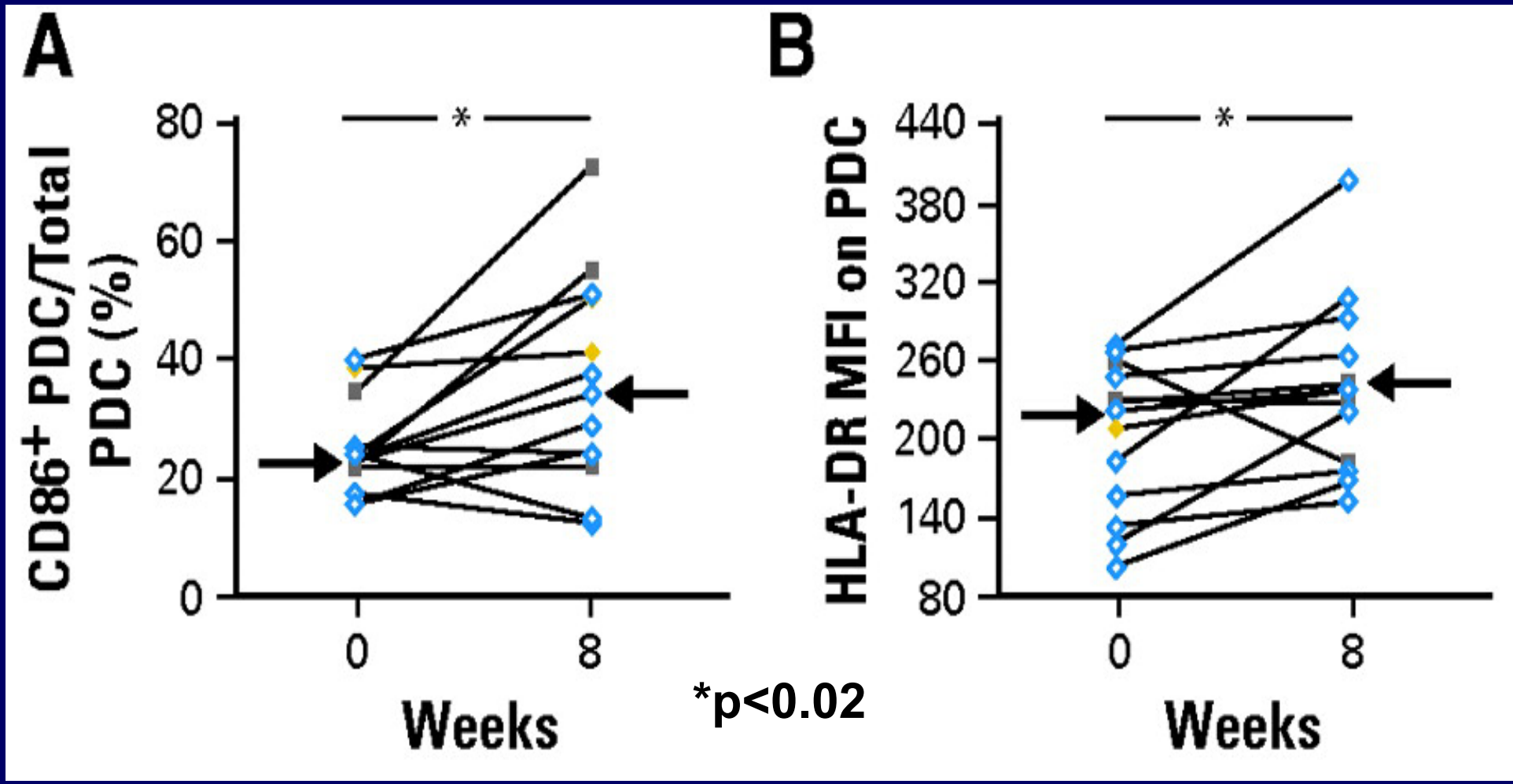
- **Biologically and clinically active IFN-α isoform**
  - 18x range
  - ↑ ISGs and new ISGs not previously induced in patients
    - ISG54 GEM GTPase CIG5
  - ↑ISGs and ↓PMN at 15,000 Hu antiviral units
  - Two patients on IFN-α1b for >12 mos and two RCC PRs
  -
- **Safe to develop phase II studies**
  - Only limiting III toxicity—fever and rigors d1 at highest dose No Grade IV toxicity
  - Probably less fatigue and anorexia than IFN-α2

Masci P et al Clin Pharmcol Ther 2007

TLR AGONISTS (Pathogen-Associated Molecular Patterns)



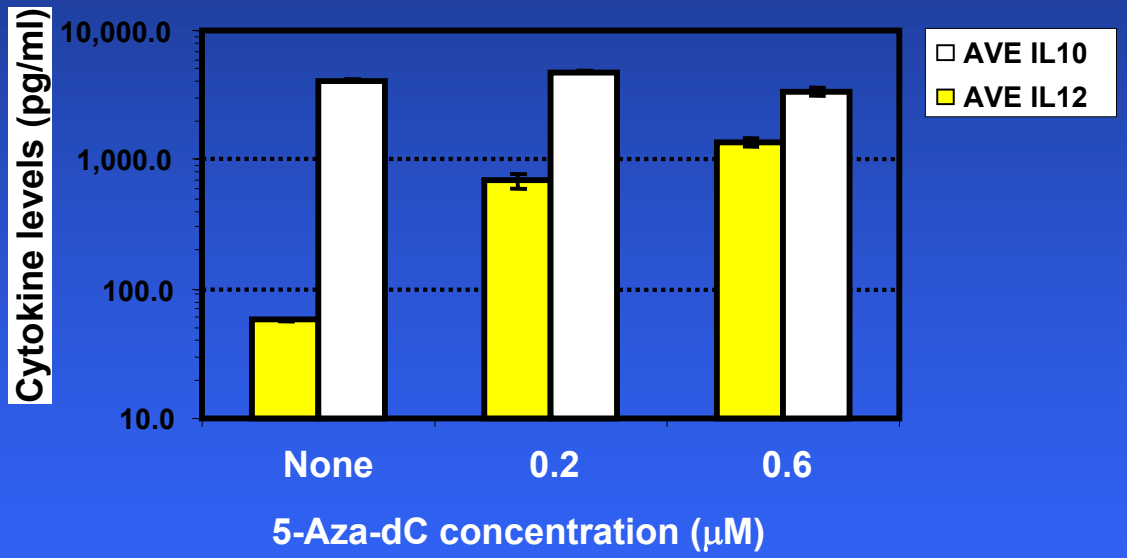
CPG7909 TLR9 AGONIST EFFECTS ON pDENDRITIC CELLs IN MELANOMA PATIENTS

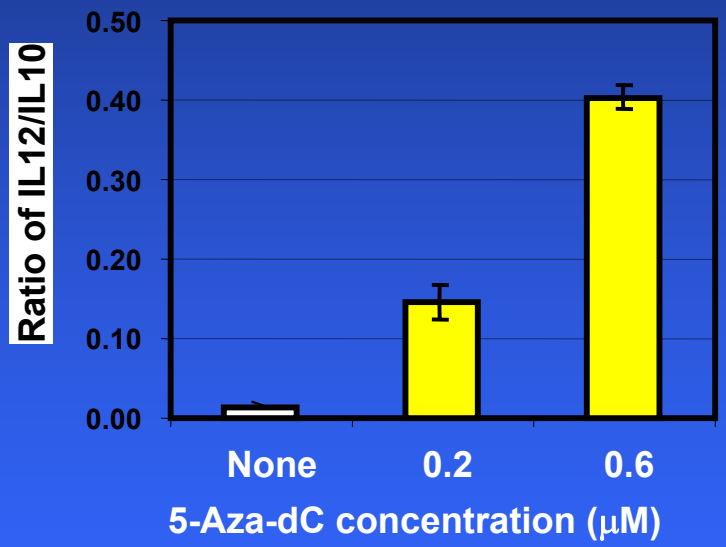


6 mg sq wkly; %CD86+/BDCA2

Pashenkov et al J Clin Oncol 36: 2006

AUGMENTATION OF EFFECTS OF TLR3 ACTIVATION BY METHYLATION INHIBITOR





Human myeloid DCs were matured from peripheral blood with CSF-GM and IL-4, treated with 5-Aza-dC (0.1 uM) for 4d, and then poly I:poly C (10μg/ml), as a representative ligand for TLR3.

WHY IFNs WILL NOT PREMATURELY AGE: REACHING FULL POTENTIAL FOR CANCER

- **Mechanism(s) of Action**
  - Regulation and effects of >300 induced genes
    - Which ISG(s) are most important?
  - Define and overcome resistance mechanisms
    - How can effects be enhanced through modification of signaling?
- **Second Generation IFNs and TLR inducers**
  - TLRs, IFNARs, ISGs
    - What more (and oral) inducers and activators?
  - Side effects: What genes and protein products?

Borden et al Nature Revs: Drug Discovery Dec 2007

WHERE FROM HERE?

“More shall come after…than have gone before; the world [of interferon] is only middle-aged."

--Herman Melville 1850.

TOP 10 REASONS iSBT HAS BECOME AN IMPORTANT SCIENTIFIC FORCE

10. OLDHAM VISION
9. SMALLEY ATTENTION TO DETAIL
8. OTHER FOUNDING MEMBERS

Herberman Fidler Bast Borden Griffin Koprowski Krim Krown Lister Whisnant Mastrangelo Oettgen Ritz Royston Sarna Abrams Gutterman Foon Hersh

7. SECOND GENERATION LEADERSHIP

Parkinson, Lotze, Dillman, Atkins, Keilholz…Withingham……

6. MOLECULAR TUMOR IMMUNOLOGY
5. PROMISE OF ANTIGEN-SPECIFIC THERAPIES
4. IFNs WORK
4. IL-2 WORKS
4. RITUXIMAB WORKS
1. EXCELLENT RESEARCH OF MEMBERS