NO PERCEIVED CONFLICTS OF INTEREST RELATED TO PRODUCTS DISCUSSED
50 YEARS OF INTERFERONS: REACHING FULL THERAPEUTIC POTENTIAL IN CANCER
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...”

  – 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

OVERALL


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

IFNα2b Up-regulates pSTAT1tyr701 while Down-regulates pSTAT3tyr705 in Melanoma Cells

IFNα2b Up-regulates pSTAT1tyr701 while Down-regulates pSTAT3tyr705 in Lymphocytes

Basal hi v. lo ratio
OS p<0.05 MEL
OS p= NS PBL

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 Triozi, James Finke, Ronald Bukowski, Bryan Williams (Monash U), Paul Elson,
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 PHOSPHORYRATION

A: persistence
B. Augmentation
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 (3x10^6U/m² qd) and escalating SSG (400mg/M² iv, Albert David, Calcutta)
- US IND #68881

WM9 human melanoma; IFN-α2: 5x10^5U 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 siRNA (40 nM daily over 2 days, lipofectamine) and 5-AZA-dC (200 nM, 2 days). IFN-β (50 U/ml +24h).
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 (0.1μM) 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
  – 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
INCREASE IN CELL TAA BY IFNs

**In Vitro**

- IFN-γ, IFN-α2, CEA, HLA-DR

**In Vivo TAG72**

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

**In Vivo CEA**

- Guadagni, Schlam, Smalley et al JNCI 1989
- Greiner, Smalley, Schlam et al JCO 1992

IFNs ↑ RIA>50%
CEA 13/22 (59%)
TAG72 27/35 (77%)
IFN-α Generation of Effective Alloantigen Presentation by Dendritic Cells

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

Four ISGs >10x 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
  – OAS1  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 IFN-α2

Ezekowitz, Folkman NEJM 1994
## PHASE III TRIAL OF IFN-α2 OR IFN-α2 AND BEVACIZUMAB FOR METASTATIC RENAL CARCINOMA (RCC)

<table>
<thead>
<tr>
<th>Response</th>
<th>IFN/BEV n=306*</th>
<th>IFN(n=289)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR/PR</td>
<td>31%</td>
<td>13%**</td>
</tr>
<tr>
<td>PFS (med)</td>
<td>10m</td>
<td>5m**</td>
</tr>
</tbody>
</table>

*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

Virus

3ATP

dsRNA

2PPI

Inactive RNase L

pppA2’p5’A2’p5’A

“2-5A”

2-5A synthetase (OAS)

2’PDE

P’tase

2-5A decay

Viral replication is blocked

Active RNase L

2-5A

RNA degradation

IFN

IFN-R
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

Substrate: C7UUC12
Activator: ps(A2’ps)3A

Wild type RNase L
RNase L_{R462Q}

% RNA Cleavage

Time, min

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

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
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
<table>
<thead>
<tr>
<th></th>
<th>15 µg</th>
<th>45 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α1</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>IFN-α2</td>
<td>21</td>
<td>24</td>
</tr>
</tbody>
</table>

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

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α1</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>IFN-α2</td>
<td>21</td>
<td>24</td>
</tr>
</tbody>
</table>

*p* < 0.01

**Peak Temperature**

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α1</td>
<td>37.4 ± 0.2 °C</td>
<td>38.4 ± 0.2 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-α2</td>
<td>37.6 ± 0.2 °C</td>
<td>38.8 ± 0.2 °C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p* < 0.05

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

Bacterial lipopeptides
GPI-anchored proteins (parasites)
Lipoteichoic acid (gram positive bacteria)
zymosan (fungi)
LPS (gram negative bacteria)
Flagellin (motile bacteria)

TLR AGONISTS
(Pathogen-Associated Molecular Patterns)

Endosome
Nucleus
Plasma membrane
CPG7909 TLR9 AGONIST EFFECTS ON pDENDRITIC CELLS IN MELANOMA PATIENTS

6 mg sq wkly; %CD86+/BDCA2

*p<0.02

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?

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