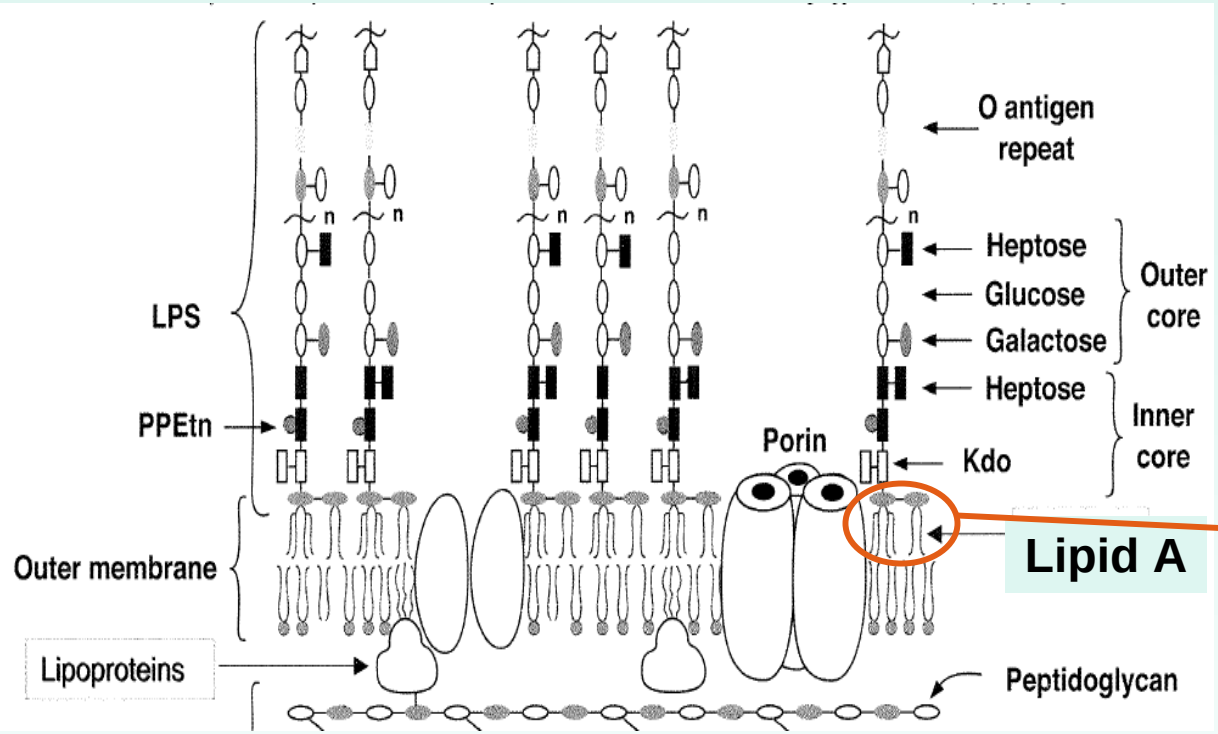
Monophosphoryl Lipid A (MPL or MPLA)

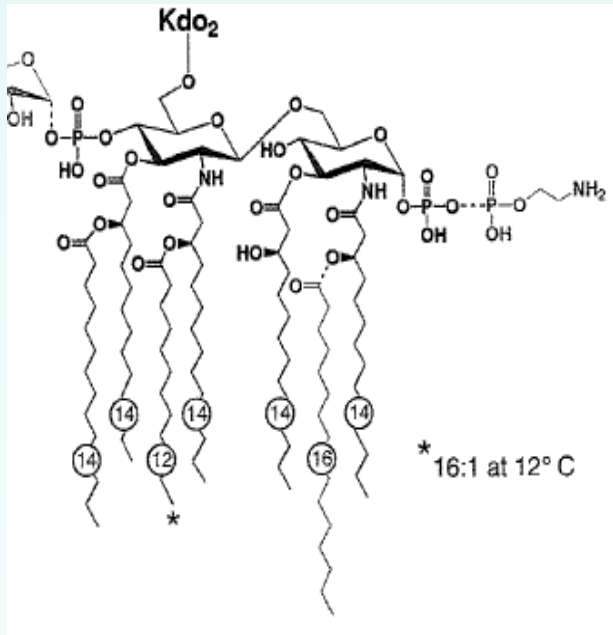
- Martin A. “Mac” Cheever, M.D.
- Member and Director of Solid Tumor Research, Fred Hutchinson Cancer Research
- Professor of Medicine, University of Washington

MPL (Component of LPS)

- TLR4 agonist
  - LPS was the first microbial molecule identified as a TLR agonist
    - Too toxic for clinical use
- MPL is modified LPS of *Salmonella Minnesota*
  - Significantly reduced toxicity and pyrogenicity
  - Maintained adjuvant effect and immunopotentiating characteristics
- Contains several closely related molecules
    - Differs only in the degree and type of fatty acid acylation, including the major hexaacyl component

Gram-Negative Bacteria—Outer Cell Wall





Mechanisms of Action: (MPL = TLR4 Agonist)

- Activates macrophages
  - Enhances respiratory burst and phagocytic activity
  - Production of soluble mediators, including cytokines and chemokines
    - IL-8 and MIP-1β enhance cellular interactions
      - Recruitment of neutrophils, macrophages, dendritic cells, and NK cells
    - TNF-α and IL-1β
      - Induces activation and maturation of dendritic cells
- Activates dendritic cells
  - Production of IL-12, IFN-γ, and IL-5
  - Stimulates both Th1 and Th2 responses
- Upregulates multitude of intracellular and cell surface markers
- Local production of chemokines and cytokines

Preclinical Summary: MPL as Vaccine Adjuvant

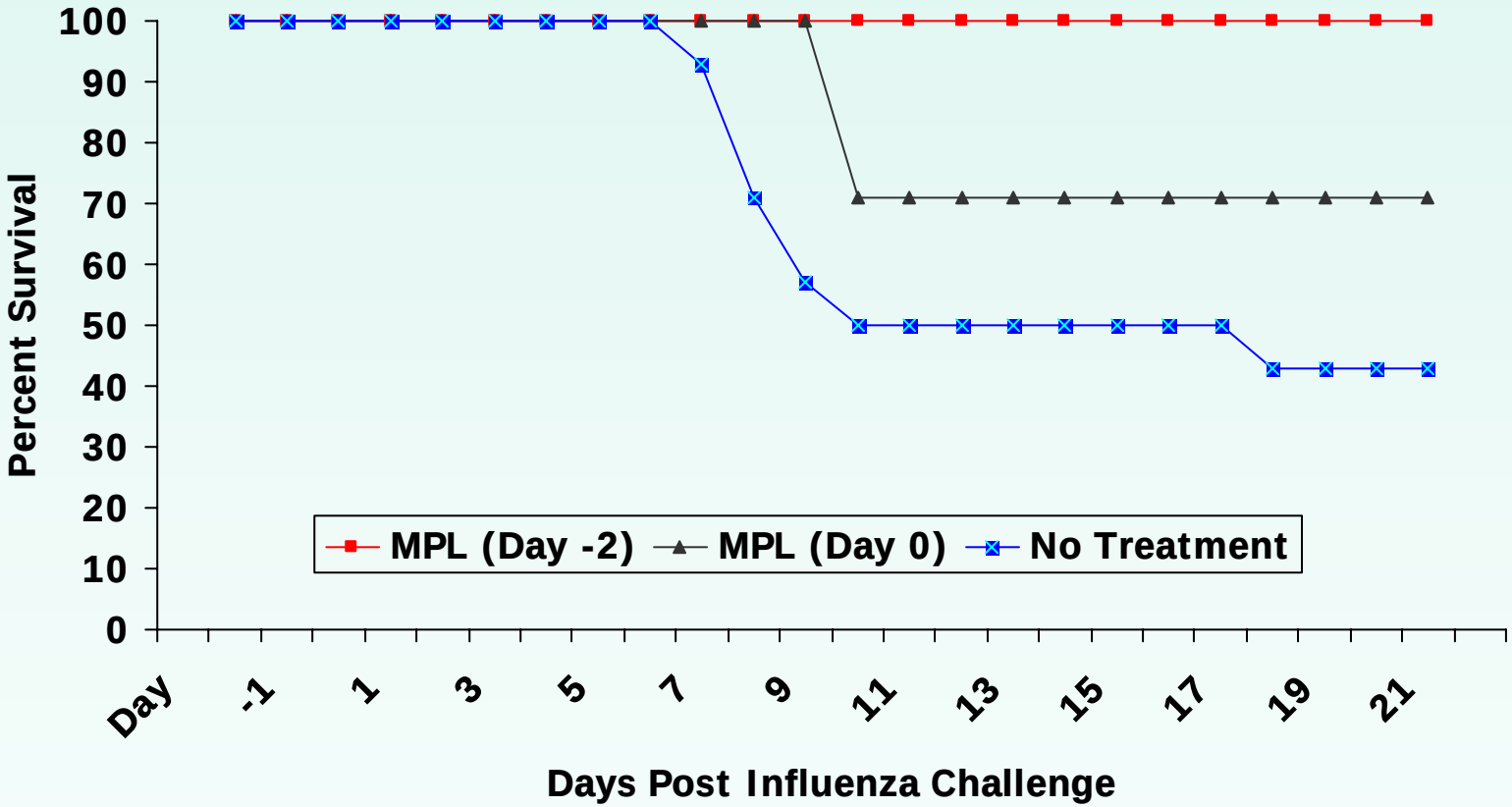
- Shown to produce adjuvant responses to relatively nonimmunogenic antigens
  - e.g., malarial sporozoite antigen, gangliosides, polysaccharides, short synthetic peptides, and viral proteins
- Effective by multiple, different routes
  - IV, SC, ID, intranasal, and oral
- Adjuvant antibody and T-cell responses including
  - Th1 and Th2 responses
- Induces systemic and mucosal immunity
- Multiple formulations possible
  - e.g., aqueous, oil in water, liposomal, and nanoparticles
- Effective in combinations with other adjuvants
  - e.g., alum, QS21, and CpG

Preclinical Summary: MPL = Activator of Innate Immune Responses: Prophylactic Administration Increases Resistance to a Variety of Agents Including:

- Gram-Negative Bacteria
  - *Escherichia coli*
  - *Salmonella enteritidis*
  - *Klebsiella pneumonia*
  - *Pseudomonas aerugenosa*
- Gram-Negative Bacterial Products
  - Endotoxin
- Candida albicans
- Influenzavirus A

- Gram-Positive Bacteria
  - *Listeria monocytogenes*
  - *Staphylococcus epidermidis*
  - *Staphylococcus aureus*
- Gram-Positive Bacteria
  - Staphylococcal enterotoxin B
  - Streptococcal pyrogenic exotoxin
  - Toxic shock syndrome toxin
- Toxoplasma gondii
- Pneumocystis carinii

Example: Nonspecific Protection Against Lethal Influenza Challenge: [Intranasal MPL 2 days before influenza infection]



[Persing, et al. *Trends Microbiol.* 2002;10(10 Suppl):S32-7]

MPL: Clinical Summary

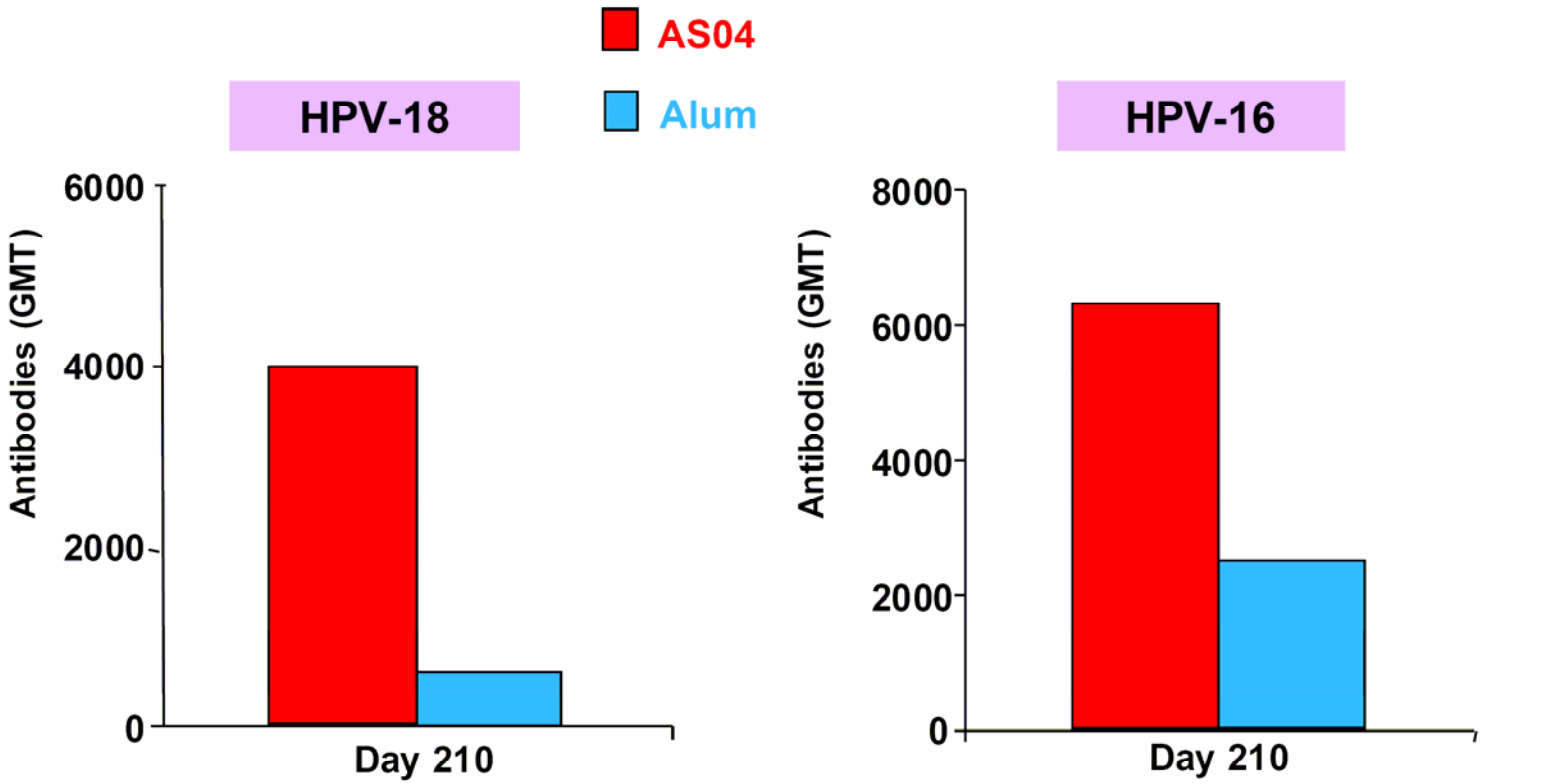
- >120,000 doses administered to
  >50,000 human subjects
- Safe and effective vaccine adjuvant
  - Safety data profile equivalent to alum
    - [JT Evans, et al., *Expert Rev Vaccines* 2:219–29, 2003]
- Approved in Europe
  - HBV vaccine approved in Europe (GSK-Fendrix)
    - MPL + Alum
    - >10,000 patients with demonstrable safety and increased immunogenicity
  - Named patient use in allergy vaccines
  - Phase III testing completed
    - HPV (MPL + alum)(aka, Cervarix)

HBV Vaccine

- Standard GSK vaccine (Energix)
  - HBV surface antigen with alum
- New GSK vaccine (Fendrix)
  - HBV surface antigen with alum + MPL
  - Approved in Europe for renal dialysis patients
- Normal with 1 injection (Fendrix vs. Energix)
  - Seropositivity rate = 77% vs. 37%
  - Seroprotection rate = 34% vs. 13%
- Dialysis patients with 3 monthly injections
  - Seroprotection rate = 74% vs. 52% at 3 months

HPV Vaccine

Higher antibody levels with GSK Adjuvant (AS04)
[AS04 = Alum + MPL]



(AS04) is a novel proprietary adjuvant developed by GSK in collaboration with Corixa.
GMT = Geometric Mean Titers

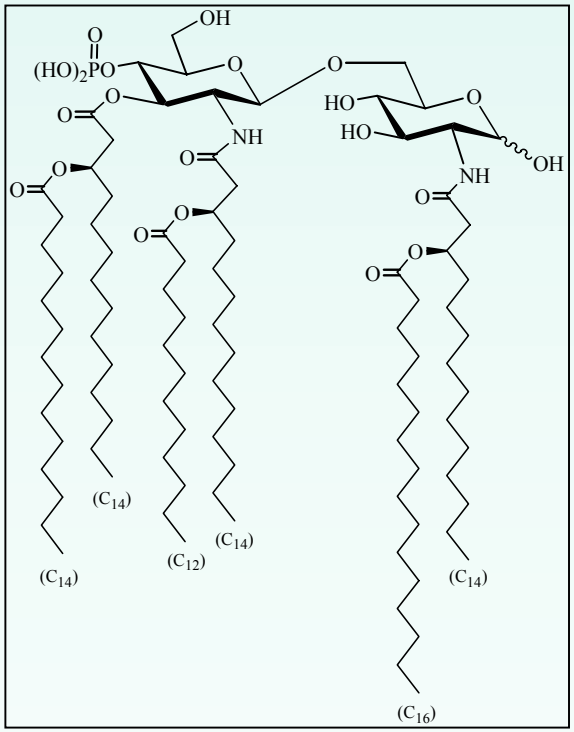
[JP Garnier, GSK CEO, Corporate Media Presentation Feb, 2005]

MPL Adjuvant Combinations: Cancer Vaccines

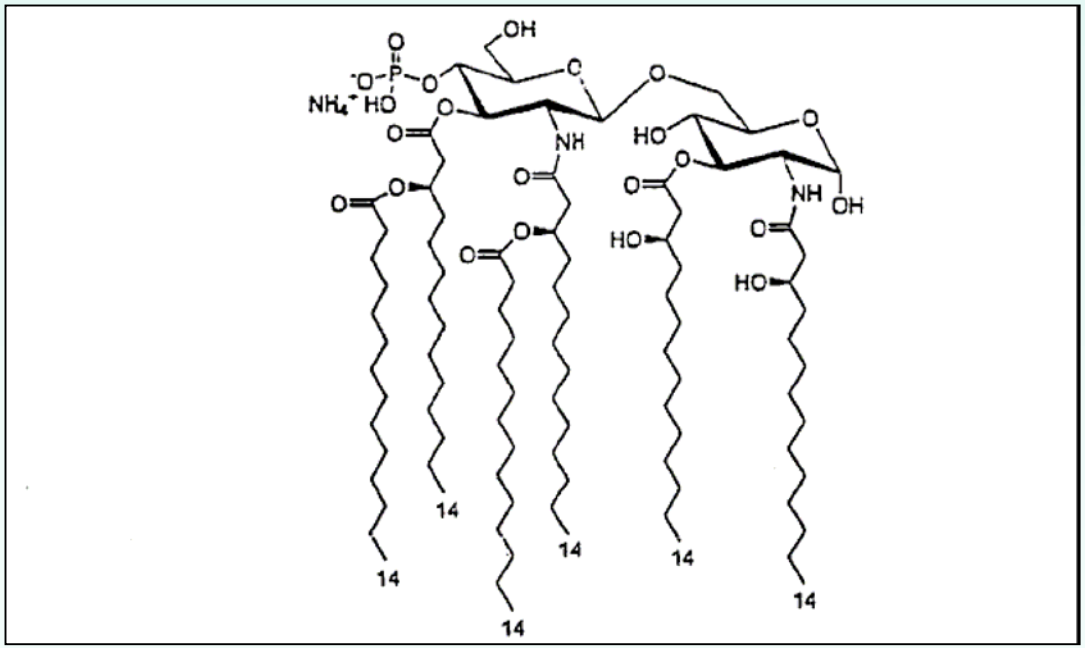
- MPL + QS21 in oil-in-water emulsion
  - MAGE-A2 protein in melanoma
  - Antibody responses (IgG) after 4 vaccinations
    - 96% (23/24) of patients
  - T-cell responses (increased IFN-g production)
    - 30% (5/16) of patients
      - [Vantomme, et al. *J. Immunother.* 27:124–35, 2004]
- MPL + QS21 + CpG
  - HER2 protein in breast cancer
  - Antibody after 6 vaccinations
    - 100% (15/15) of patients
  - T-cell responses
    - 62% (8/13) of patients
      - [Limentani ASCO Abst #2520, 2005]

MPL Is a Purified Biologic and Contains Several Closely Related Molecules: A Pure Synthetic Form Is Available—GLA

MPL



Glucopranosyl Lipid (GLA)



(S. Reed, Infectious Disease Research Institute, Seattle)

MPL: Contemplated Uses

- Adjuvant for vaccines
  - Safe and effective, appropriate as a single adjuvant or combined with other adjuvants
    - Multiple, different types of antigens
    - Multiple, different formulations
    - Multiple, different routes of administration
    - Multiple, adjuvant combinations
- Activation of innate immune responses

MPL: Comparison of Agents

- “Work Horse” adjuvant for new GSK vaccines
- Producible as a purified biologic or as a synthetic compound

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TLR9 Agonist

- Ellis L. Reinherz, M.D.
- Professor of Medicine
- Harvard Medical School
- Chief, Laboratory of Immunobiology
- Director, Cancer Vaccine Center
- Dana-Farber Cancer Institute

TLR9 Agonist: Background (1)

- Category of drug
  - Immunomodulator, vaccine adjuvant
- Molecular or physical characterization of agent
  - CpG oligodeoxynucleotides (CpG-ODN)
    - **PF-3512676 (CPG 7909, Pfizer/Coley):** TCGTCGTTTTGTCGTTTTGTCGTT
    - **1018 ISS (Dynavax):** TGACTGTGAACGTTCGAGATGA
    - **CpG-28:** TAAACGTTATAACGTTATGACGTCAT
    - **IMO-2055 (Idera):** ODN consisting of 3'-3'-linked structure and synthetic CpR (R = 2'-deoxy-7-deazaguanosine) motif
    - **dSLIM (Mologen):** CpG motif-containing circular ODN
  - Phosphodiester (PO) or phosphorothioate (PS) backbone
  - Well-known chemistry and analytic methods
  - Excellent aqueous solubility and stability
  - GMP-grade synthesis and purification is simple and economical
  - Well-characterized absorption, distribution, metabolism, and elimination properties
  - Sequences and secondary structures affect activities (A-class, B-class, C-class)

TLR9 Agonist: Background (2)

- Target
  - TLR9 on B cells and plasmacytoid DC (pDC)

    * TLR9 expression patterns vary between different species (i.e., mice express on B cells, monocytes, and all DCs)

- Biology of target
  - TLR9 detects the unmethylated CpG dinucleotides prevalent in bacterial and viral DNA but not in vertebrate genomes
  - TLR9 localizes to endosome and lysosome compartment
  - TLR9 complexes with [MyD88 + IRAK1 + IRAK4 + TRAF6] upon CpG binding

    -----> activation: IRF7, MAPKs, and NF-κB activation pathways

- Biology of agent-target interaction
  - B cells: proliferation and differentiation to plasma cells, IgG isotype switching, and antibody secretion
  - pDC: maturation, type I IFN secretion
  - Th1-T cell responses, CTL activation
  - NK cell activation (enhancement of ADCC activity), monocyte, neutrophil activation
  - Pro-inflammatory cytokines and chemokines (IL-6, IL-12, IFN-γ, TNF-α, IP- ˜ı) secretion
  - Treg generation, IDO expression, anti-inflammatory (IL-10) cytokine secretion (counter-regulatory feedback)

TLR9: Preclinical Summary (1)

Efficacy in animal models

1) Monotherapy

- Variable effects depending on tumor characteristics
- Particularly effective in small tumors (up to 2–3 mm in size)
- Injection site: peri- or intra-tumor > systemic
- (CD8+) T cell–dependent or NK cell–dependent

2) Vaccine adjuvant

- Peptide or protein, DC, autologous tumor cells, etc.
- Stronger Th-1–promoting effects and CTL responses than any other TLR ligands
- Synergistic with GM-CSF or other adjuvants, such as alum, lipid emulsions, nanoparticles

3) Combination therapies (show improvement over CPG-ODN alone)

- CpG-ODN + anti–IL-10 receptor mAb (+ CCL16 or + Treg depletion)
- CpG-ODN + anti–CD40 mAb (agonistic)
- CpG-ODN + anti–CTLA-4 mAb + vaccine
- CpG-ODN + FLT3 ligand + vaccine
- CpG-ODN + TLR3 agonist or TLR5 agonist
- CpG-ODN + IL-18
- CpG-ODN + IL-13 exotoxin fusion protein
- CpG-ODN + chemotherapy (cyclophosphamide, topotecan, 5-FU, gemcitabine, pemetrexed, coramsine, etc.)
- CpG-ODN + radiation
- CpG-ODN + cryoablation

TLR9: Preclinical Summary (2)

Safety and toxicities issues

1) PS-ODN
   - Mononuclear cell infiltrate in liver, kidney, spleen, bone marrow
     - Only in rodents (different TLR9 expression pattern)
     - Dose-dependent
     - Liver: Kupffer cell activation, basophilic granulation
     - Kidney: degeneration (necrosis) in proximal tubules
   - Cytokine storm: proinflammatory cytokines increase in serum
   - Activation of alternative complement pathway: leukocyte activation, vascular permeability change, cardiovascular collapse
   - Inhibition of coagulation by binding to thrombin: prolonged APTT

2) Autoimmunity
   - Not reported, but enhanced autoimmunity is observed in lupus, MS, colitis, arthritis mouse models

TLR9 Agonist: Phase I/II Trial (Reported)

Treatment		Cancer type	Phase of trial (number)	Adverse events* (> Grade 3/4)	Efficacy
TLR9 agonist	Other reagents				
1) Monotherapy					
CPG 7909 (iv)		NHL	Phase I (23)	Fatigue (1), ALT elevation (1)	No response
PF-3512676 (sc)		Melanoma	Phase II (20)	Laboratory data (6; Uric acid, Na, Cl, PT, PTT, neutrophils)	2 PR
PF-3512676 (peri-tumor)		Melanoma	Phase II (23)	No	(not determined)
CPG 7909 (sc)		Cutaneous T lymphoma	Phase I/II (28)	No	3 CR, 6 PR
CPG 7909 (intra- or peri-tumor)		BCC (4), melanoma (4)	Phase I (8)	No	BCC: 1 CR, 2 PR, melanoma: no response
CPG 7909 (sc)		RCC	Phase I (35)	No	2 PR
CpG-28 (intra-tumor)		Glioblastoma (recurrent)	Phase I (24)	Lymphopenia (7), fever (1), ALT (2), hyponatremia (1)	2 minor response
IMO 2055 (sc)		Advanced solid tumors	Phase I (23)	Pain/nausea (1), hypoxia/chills (1), anemia (2)	No response
2) Vaccine adjuvant					
CPG 7909 (sc)	Melan-A peptide, ISA-51	Melanoma	Phase I (8)	No	No response
CPG 7909 (im)	MAGE-3 protein	Melanoma	Phase I/II (13)	No	1 PR
CPG 7909 (sc)	Autologous tumor cells, GM-CSF, IFN-α	RCC	Phase I/II (12)	No	3 PR
CPG 7909 (sc)	NY-ESO-1 protein, ISA-51	Various tumors	Phase I (18)	No	(not determined)
dSLIM (sc)	CAP-1, IL-2, (chemotherapy)	Colorectal	Phase I/II (17)	No	(not determined)
3) Combination therapy					
CPG 7909 (sc)	± Dacarbazine (DTIC)	Melanoma	Phase II (184)	Anaphylactoid reaction (1), vasovagal reaction (1), MI (1)	4 PR in combination, 2 PR in DTIC alone
CPG 7909 (sc)	± Taxane/platinum	NSCLC	Phase II (112)	Neutropenia, thrombocytopenia	ORR: 19% vs. 38% 1-Y survival: 33% vs. 50%
CPG 7909 (sc or iv)	Rituximab	NHL	Phase I (50)	Lymphopenia (2), neutropenia, (2), diarrhea (1), dehydration (1)	4 CR, 8 PR
1018 ISS (sc)	Rituximab	NHL	Phase I (20)	Atypical pneumonia (2)	1 CR, 5 PR

* Most common adverse events: local injection site reactions and systemic flu-like illness

TLR9 Agonist: Phase I/II Trial (Ongoing)

1) Monotherapy
   - CPG 7909: CLL (2nd line), phase I (48)
   - IMO-2055: RCC, phase II (1st line, 46; 2nd line, 46)
   - CpG-28 (intra-tumor): glioblastoma, phase II (80)

2) Vaccine adjuvant
   - CPG 7909 + MAGE-3.A1 peptide: melanoma (2nd line), phase I/II (14)
   - CPG 7909 + Melan-A, tyrosinase peptides + Montanide ISA-51: melanoma, phase I (27)
   - CPG 7909 + autologous PBMCs + Melan-A peptide in IFA + cyclophosphamide + fludarabine: melanoma, phase I (12)
   - CPG 7909 (SB-AS15 adjuvant) + MAGE-3-His fusion protein: melanoma, phase II (68)
   - CPG 7909 (SB-AS15 adjuvant) + HER2 recombinant protein: breast, phase I/II (~40)

3) Combination therapies
   - Paclitaxel/carboplatin/bevacizumab ± PF-3512676: NSCLC (1st line), phase II (140)
   - Erlotinib ± PF-3512676: NSCLC (2nd line), phase II (130)
   - Pemetrexed ± PF-3512676: NSCLC (2nd line), phase II (130)
   - Docetaxel ± PF-3512676: breast (1st line), phase II (100)
   - CPG 7909 + KLH, tetanus toxoid (following autologous SCT): leukemia/lymphoma/multiple myeloma, phase I (25)
   - CPG 7909 (intra-tumor) + local radiation: NHL (recurrent), phase I/II (30)
   - CPG 7909 + rituximab + yttrium Y 90 ibritumomab tiuxetan: NHL (2nd line or recurrent), phase I (30)
   - 1018 ISS + rituximab: NHL (2nd line), phase II (30)
   - 1018 ISS + irinotecan + cetuximab: Colorectal (2nd line), phase I (15)
   - IMO-2055 + gemcitabine/carboplatin: refractory solid tumors, phase I (12-18); NSCLC, phase II

TLR9 Agonist: Phase III Clinical Trial

1) Randomized trial of gemcitabine/cisplatin + PF-3512676 vs. gemcitabine/cisplatin alone in patients with advanced NSCLC (Pfizer/Coley)
2) Randomized trial of paclitaxel/carboplatin + PF-3512676 vs. paclitaxel/carboplatin alone in patients with advanced NSCLC (Pfizer/Coley)

- **Study design:** treatment, randomized, open label, active control, parallel assignment, safety/efficacy study
- **Primary endpoint:** overall survival
- **Secondary endpoints:** overall confirmed objective response rate, duration of response, progression-free survival, time to tumor progression, overall safety profile, patient-reported outcomes, pharmacokinetics
- **Eligibility:** 18 years and above**,** advanced NSCLC stage IIIB with pleural effusion or stage IV, no prior systemic treatment for NSCLC (first-line treatment), ECOG PS 0 or 1
- **Total enrollment:** 800 **Enrollment completed**: 1) November 2005–December 2006
  2) November 2005–April 2007
- **Safety and efficacy results:** not available

3) Adjuvant therapy with recombinant MAGE-A3 protein + CPG7909 in MAGE-A3–positive patients with early stage, completely resected stage IB, II, or IIIA NSCLC (GlaxoSmithKline/Coley)

- **Not started** (announced on June 5, 2007)
- **Study design:** adjuvant, randomized, double-blind, placebo-control, safety/efficacy study
- **Primary endpoint:** disease-free survival
- **Patients:** after surgery ± standard chemotherapy ± immunotherapy
- **Total enrollment:** 2270

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Resiquimod, 852A

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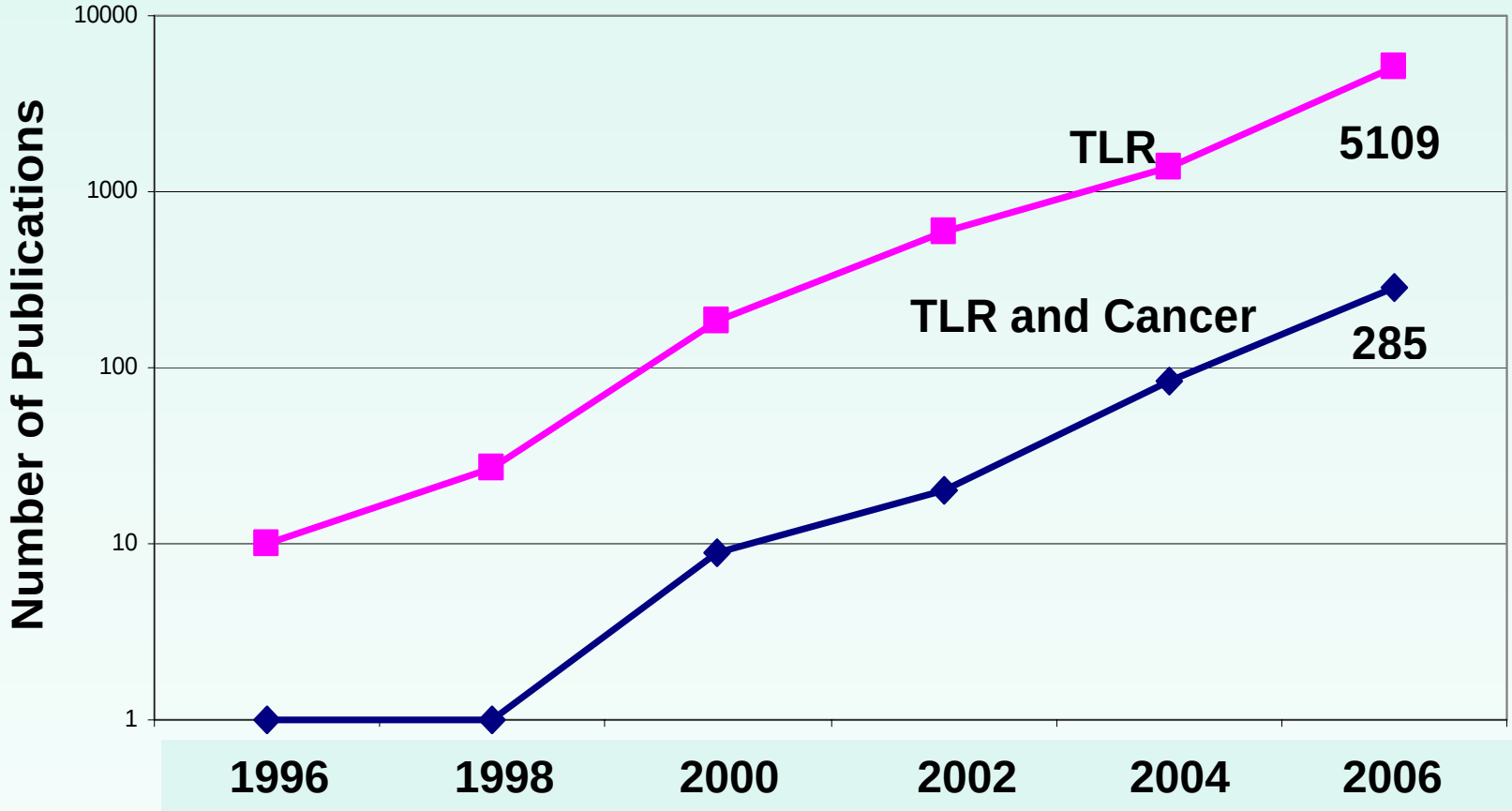
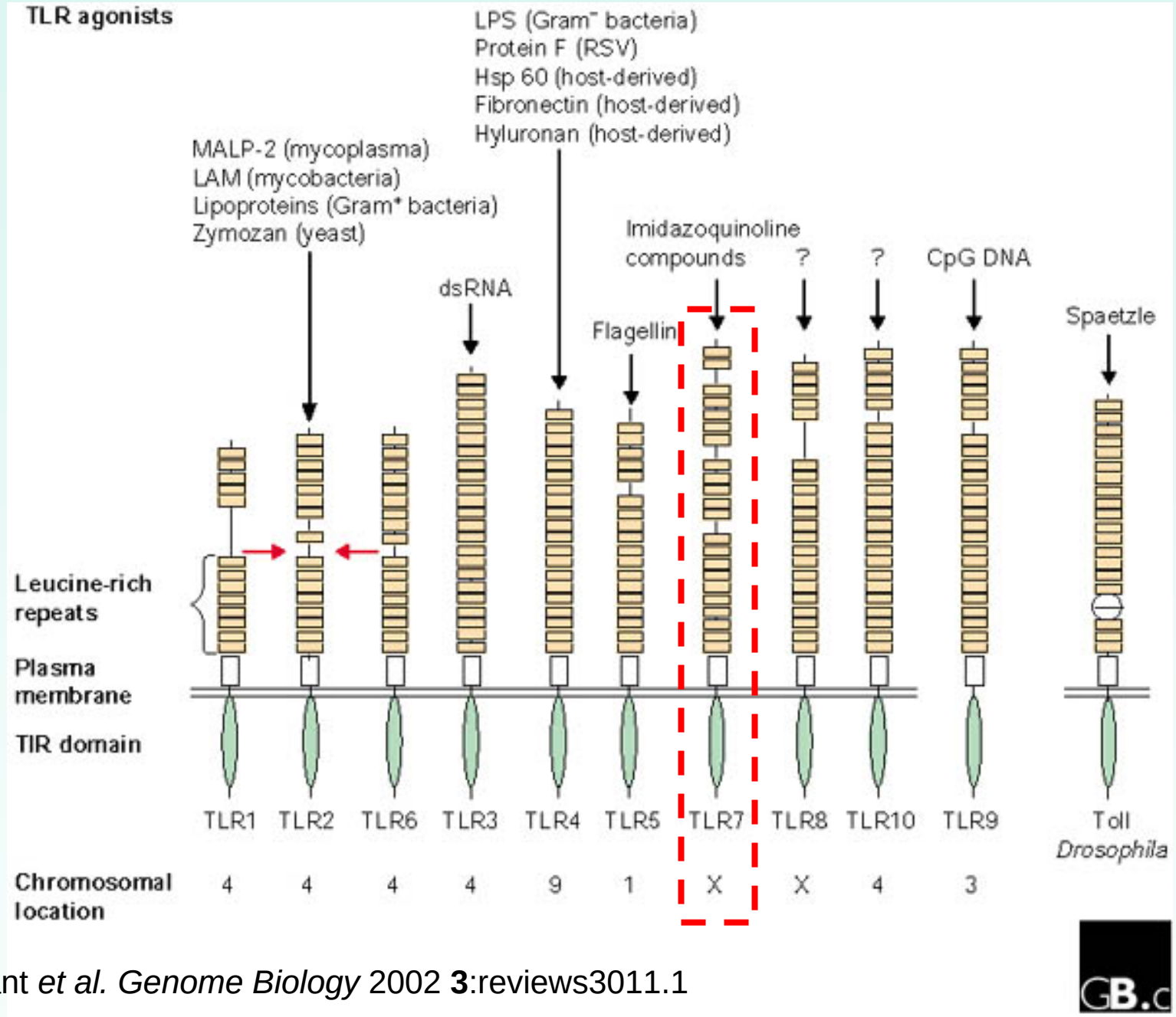
- Vice President, Translational Research, and Chairman, Department of Medical Oncology
- Fox Chase Cancer Center

Publications on TLR

1996 - 2006



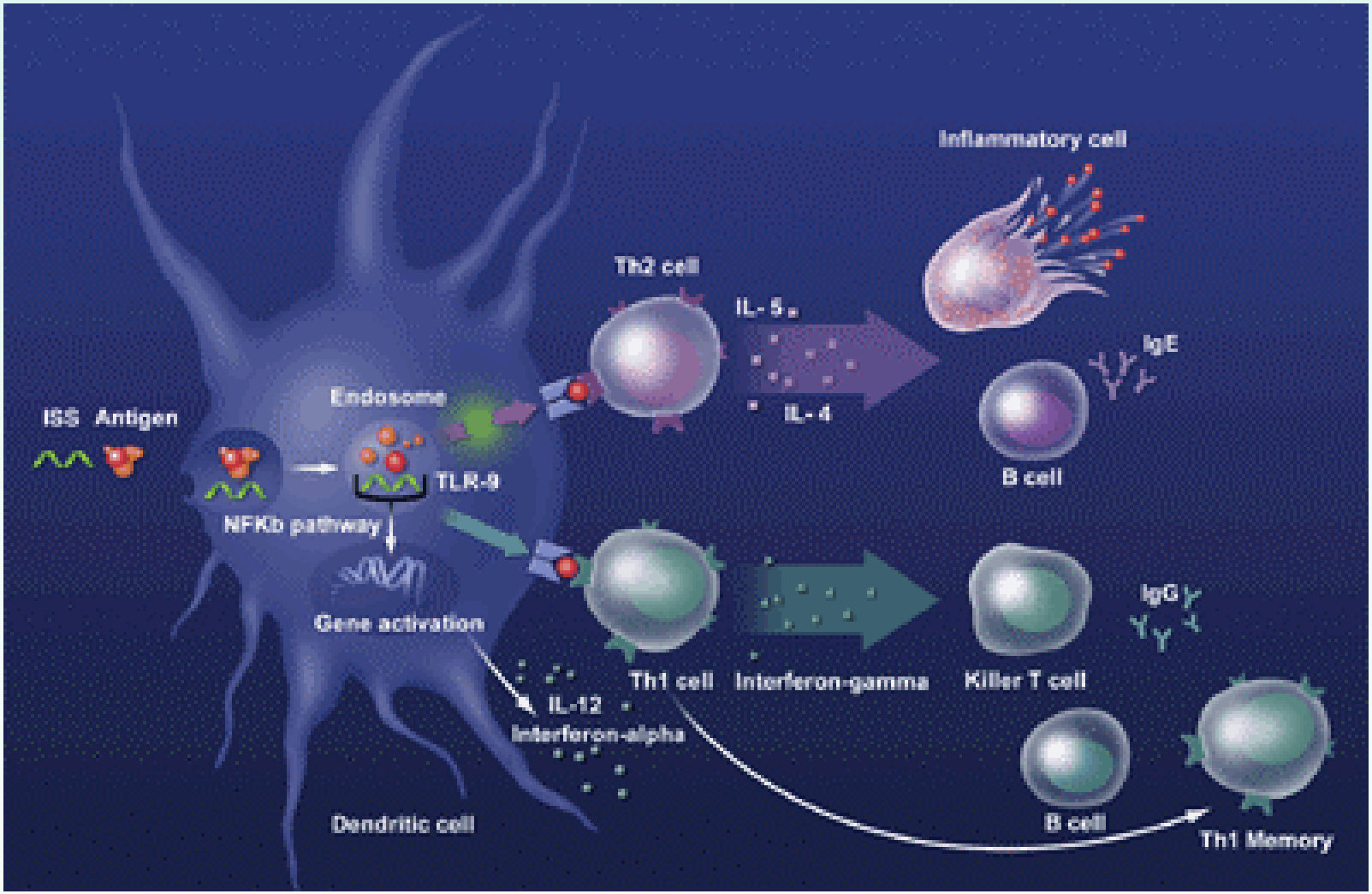


Armant *et al. Genome Biology* 2002 **3**:reviews3011.1

Resiquimod, 852A

- Toll Receptor 7/8 Agonists – similar biology as imiquimod
  - Topical imiquimod is currently FDA approved for the treatment of basal cell skin cancer
  - Many studies of topical use of imiqumod in skin CA, including in situ melanoma and invasive melanoma
  - Anecdotal reports have also shown that imiquimod is useful in the management of some cases of melanoma with cutaneous metastases

TLR Agonists Induce Innate and Adaptive Immune Responses



Schmidt, Nature Biotech. 24, 230 - 231 (2006)

Resiquimod Clinical Summary

- Phase I & II data
  - Imidazoquinolinamine
  - Induces production of IFN-α, IL-12, IL-6, IL-8, and TNF-α from dendritic cells, monocytes, and macrophages
  - Activation stimulates the innate immune response and leads to a subsequent Th1 cell–mediated immune response
- Phase III data
  - Administered as topical gel
  - Negative results in Phase III genital herpes recurrence study
  - Safe

Resiquimod Contemplated Uses

- Monotherapy
  - Immune activation
  - Unlikely to be useful
  - Topical administration required
- Combination with chemotherapy agents
- Combination with antibodies
- Vaccine adjuvant

Resiquimod

- Potential to be generally useful when employed as a vaccine adjuvant
- Applicable to virtually all vaccines that are administered intradermally, subcutaneously, or peritumorally
- Could be a valuable addition to existing immune-adjuvant portfolio

Resiquimod vs. Imiquimod

- Why Resiquimod?
  - Similar biological properties with respect to TLR activation and immune activation properties
  - Resiquimod has the potential to be formulated orally
  - Otherwise, relatively few differences from imiquimod

852A

- Another 3M TLR7 Agonist
  - Stimulates plasmacytoid dendritic cells
  - Similar biological properties with respect to TLR activation and immune activation properties
  - IV administration (TIW)
    - MTD = 1.2 mg/m^2; no effect dose < 0.60 mg/m^2; DLT defined by constitutional symptoms
  - Clinical responses seen in carcinoid tumor, melanoma, breast cancer (Dudeck et al, Proc. ASCO, Abstract 2525, 2005)

Dudeck, et al., Proc. ASCO, Abstract 2525, 2005

Summary

- TLR 7 agonists would add to vaccine adjuvant options
- Topical and systemic formulations could be useful
- Resiquimod may not be sufficiently distinct from imiquimod to warrant development unless a parenteral formulation is possible
- 852A is a promising new agent that merits careful consideration for future clinical development
  - Potent immune activation and some single-agent activity in a Phase I clinical trial

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Flt3 Ligand

- Drew M. Pardoll M.D., Ph.D.
- Seraph Professor of Oncology
- Sidney Kimmel Cancer Center, Johns Hopkins University School of Medicine

Flt3 Ligand: Background

- Hematopoietic growth factor
- Binds to Flk2/Flt3-receptor tyrosine kinase in the c-kit/fms family
- Involved in development of both myeloid and lymphoid lineages, particularly dendritic cells and NK cells
- Induces expansion and differentiation of all DC progenitors—particularly IKDC and pDC

Flt3 Ligand: Preclinical Summary-1

- Systemic administration of Flt3L protein increases DC numbers in blood, secondary lymphoid tissues, and tumors
- Some reports of antitumor activity with systemic Flt3L protein alone, though effects very variable
- Number of reports demonstrating synergy between Flt3L and DC activators such as anti-CD40 in generating antitumor activity

Flt3 Ligand: Preclinical Summary-2

- Flt3L-transduced tumor cells demonstrate enhanced vaccine potency in various tumor models
- Flt3L-mobilized DCs from spleen or blood serve as effective vaccines in various tumor models
- Systemically administered Flt3L can enhance activity of various tumor vaccines
- Reports that Flt3L can induce AML cells to differentiate into “leukemic DCs” that can present their own antigens to T cells

Flt3L: Clinical Summary-1

- Scattered Phase I/II reports using Flt3L alone, together with peptide vaccines, DC stimulators (CpG, sCD40L), and after BMT
- CEA-loaded Flt3L-mobilized DC vaccine for colon cancer reported increased CD8 priming and clinical responses. Mage-loaded Flt3L-mobilized DC vaccine (with CD40L *in vitro* DC activation for melanoma reported increased CD8 responses)

Flt3L: Clinical Summary-2

- Flt3L well tolerated to 20 ug/kg though “autoimmune phenomena” reported in one study
- Increases in circulating DCs consistently reported
- Increased T cell responses commonly reported, though significance unclear
- Studies generally too small and variable to make significant statements about clinical efficacy
- Immunex terminated trials after lack of obvious efficacy as single agent or with sCD40L

Flt3L: Contemplated Uses

- Systemic use in combination with vaccines, BMT, BMT + vaccine, adoptive T-cell therapy, TLR agonists and other immune stimulants
- Local administration (topical, admixture with vaccine preparations, intratumoral injection) to enhance DCs
- Transduction of Flt3L gene into engineered tumor vaccines or *in vivo* delivery (i.e., plasmid vaccination or in viral carriers
- DC mobilization for *ex vivo* DC vaccines

Flt3L: Perceived Need

- Ability to enhance DC numbers systemically and locally makes Flt3L an attractive adjunct to any immunotherapy in which enhanced DC activity a mechanistic component (i.e., vaccines)
- Possibly important for *ex vivo* DC vaccine efforts
- Preclinical studies suggest interesting opportunities in combination with sCD40L (or agonist anti-CD40 Ab) or TLR ligands

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Poly I: C and Poly-ICLC

- A. Karolina Palucka, M.D., Ph.D.
- Investigator
- Baylor Institute for Immunology Research

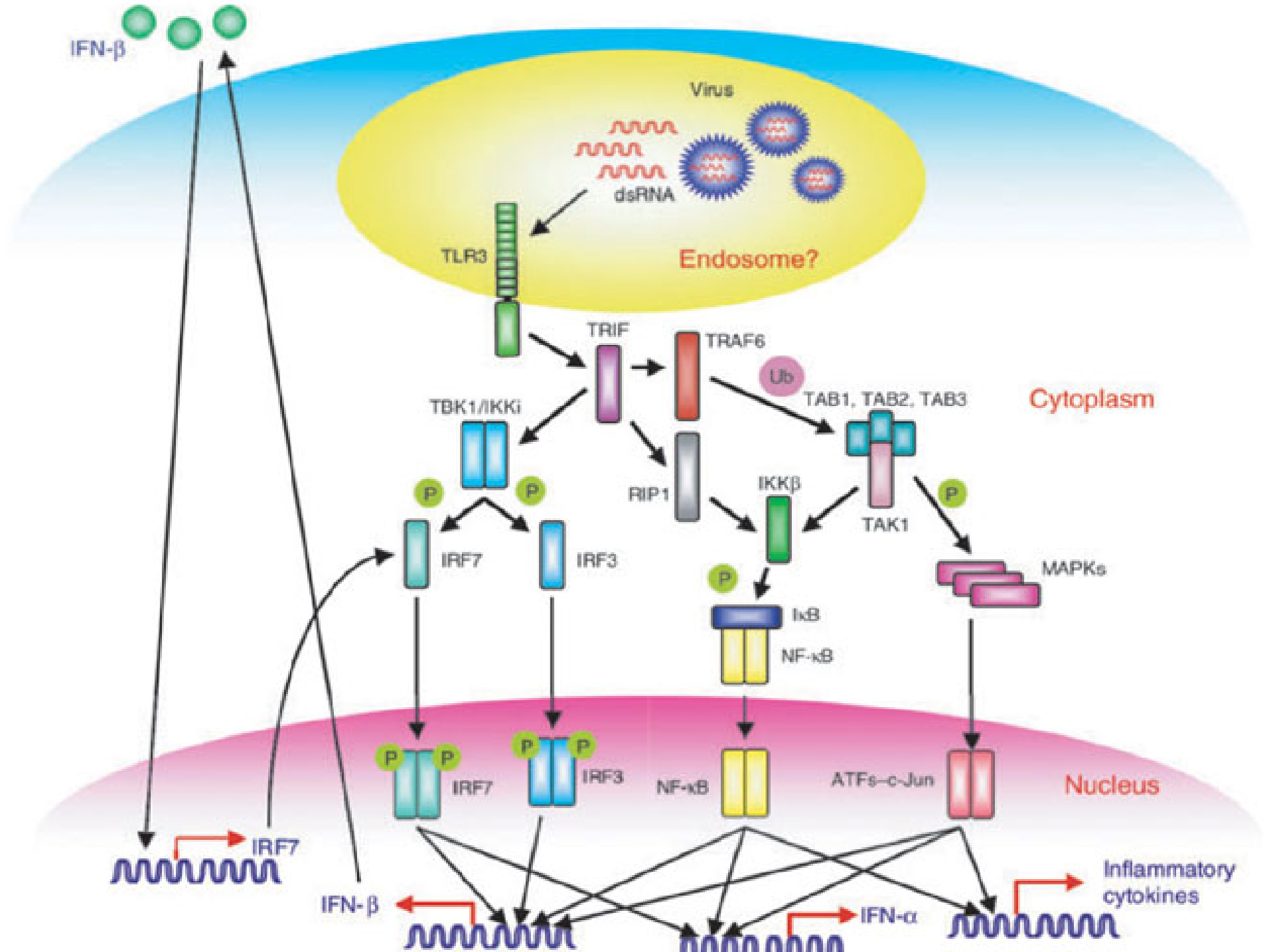
Poly I: C and Poly-ICLC: Background

- Category: Adjuvants
- Molecular characterization:
  - dsRNA polyinosinic: polycytidylic acid (Poly I:C)
  - Stabilized with poly-Llysine and carboxymethylcellulose (poly-ICLC)
- Target:
  - Toll-like receptor 3 (TLR-3)
  - dsRNA-dependent protein kinase (PKR); retinoic acid induced protein I (RIG-I) helicases and melanoma differentiation associated gene-5 (mda-5)

Poly I: C and Poly-ICLC: Background

- **Biology of target**
  - TLR3 localized in endosomes in myeloid DCs; also fibroblasts and epithelial cells
  - PKR, RIG-I, and mda-5 localized in cytoplasm

- **Biology of agent-target interaction**
  - Possibly binds TLR3 ectodomain
  - TLR3 signaling activates IRF3 and NF-kb leading to secretion of type 1 IFN and inflammatory cytokines
  - PKR, RIG-I, and mda-5 are essential in type 1 IFN responses


IFN-β
Virus
dsRNA
TLR3
Endosome?
TRIF
TRAF6
Ub
TAB1, TAB2, TAB3
Cytoplasm
TBK1/IKKi
RIP1
IKKβ
TAK1
P
IRF7
IRF3
MAPKs
IκB
NF-κB
IRF7
IRF3
NF-κB
ATFs–c-Jun
Nucleus
IRF7
IFN-β
IFN-α
Inflammatory cytokines

Poly I: C and Poly-ICLC: Preclinical Summary

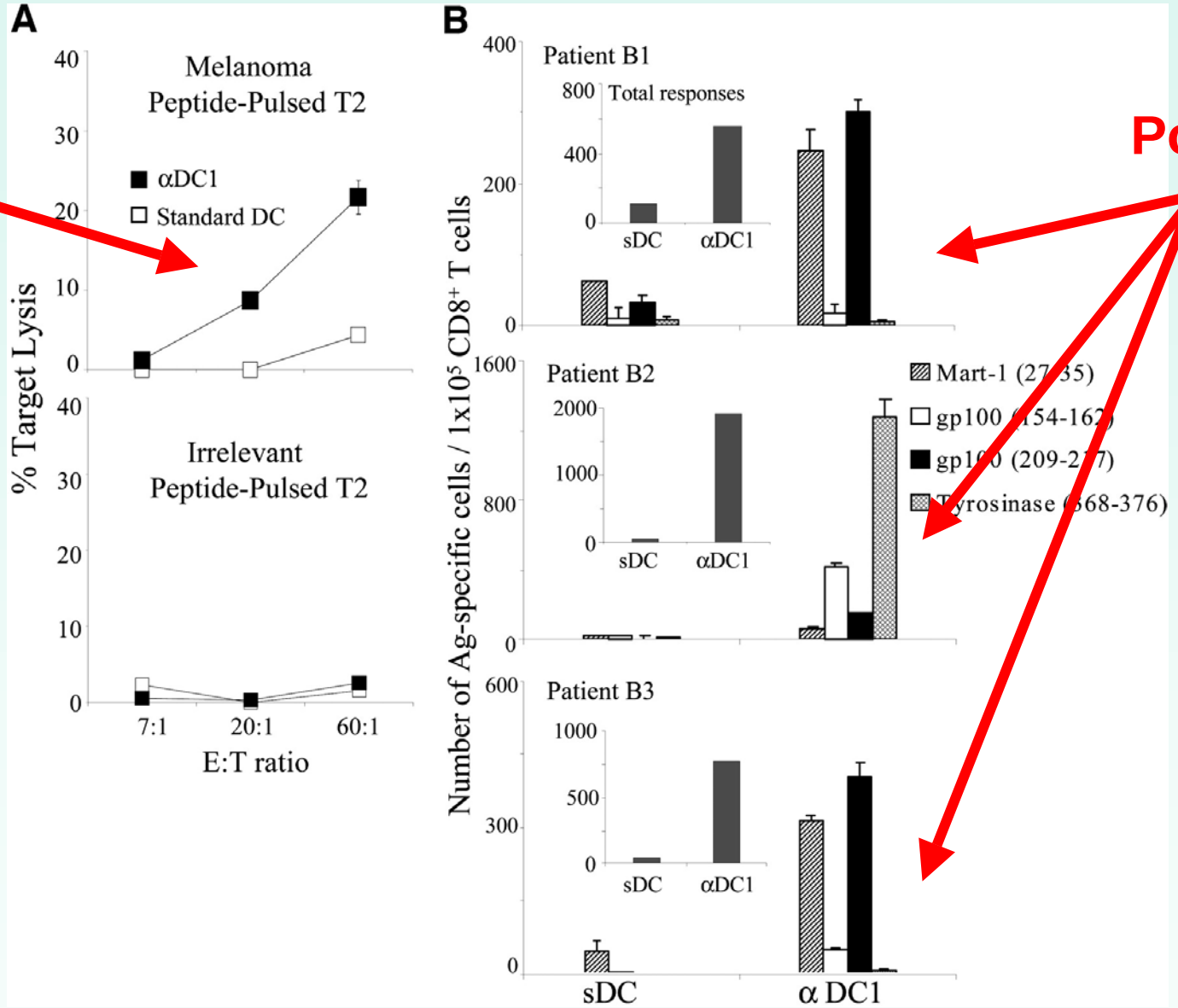
- **Efficacy *in vitro***
  - Activation of human DCs
  - Improved antigen presentation
  - Enhanced Th1 polarization

- **Efficacy *in vivo* in animal models**
  - Adjuvant effect *in vivo* in murine models of vaccination in cancer and infectious diseases
  - Improved cross-priming *in vivo*
  - Activation of NK cells

Poly IC: Improved DC Function *In Vitro*

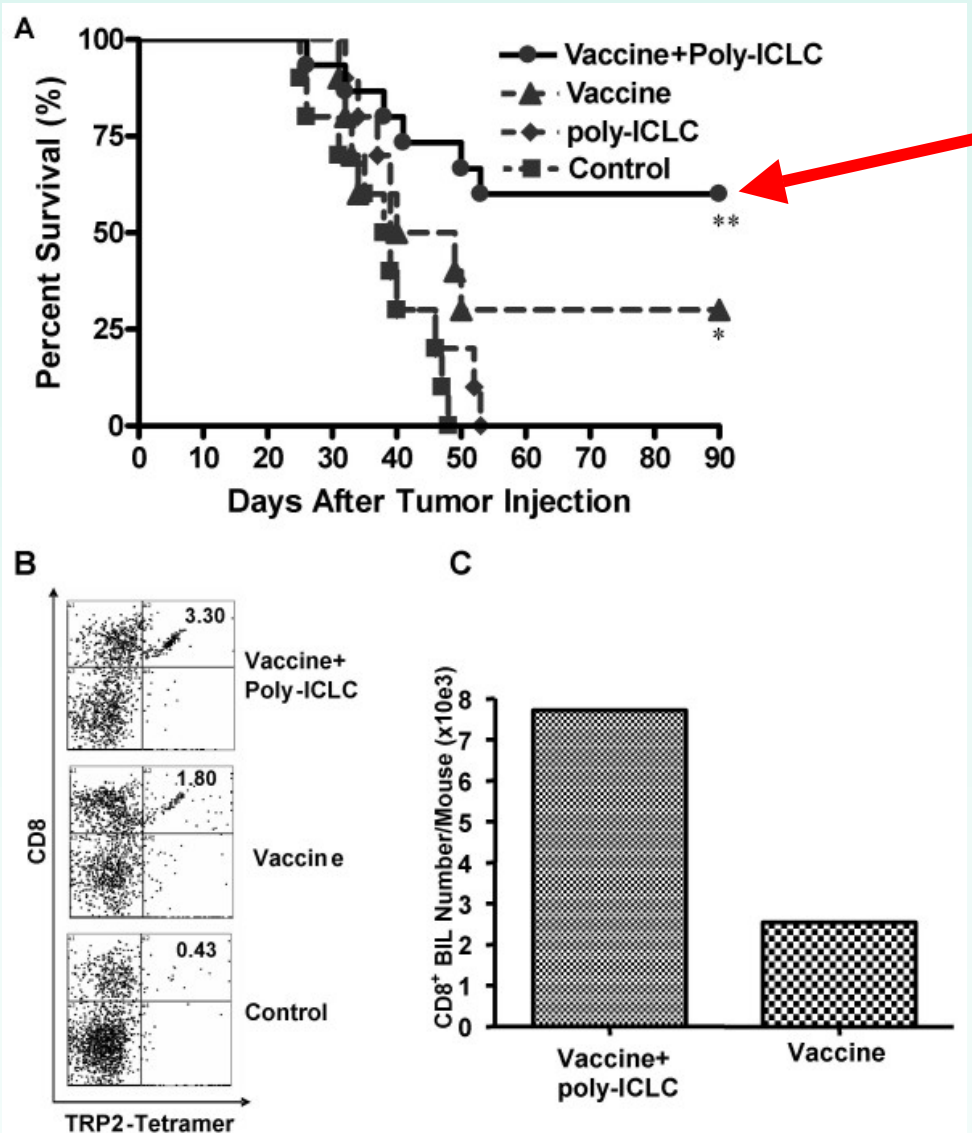
Poly IC

Poly IC



Mailliard, R. B., et al., Cancer Res 2004;64:5934-5937

Poly IC: Cancer Vaccine Adjuvant *In Vivo*

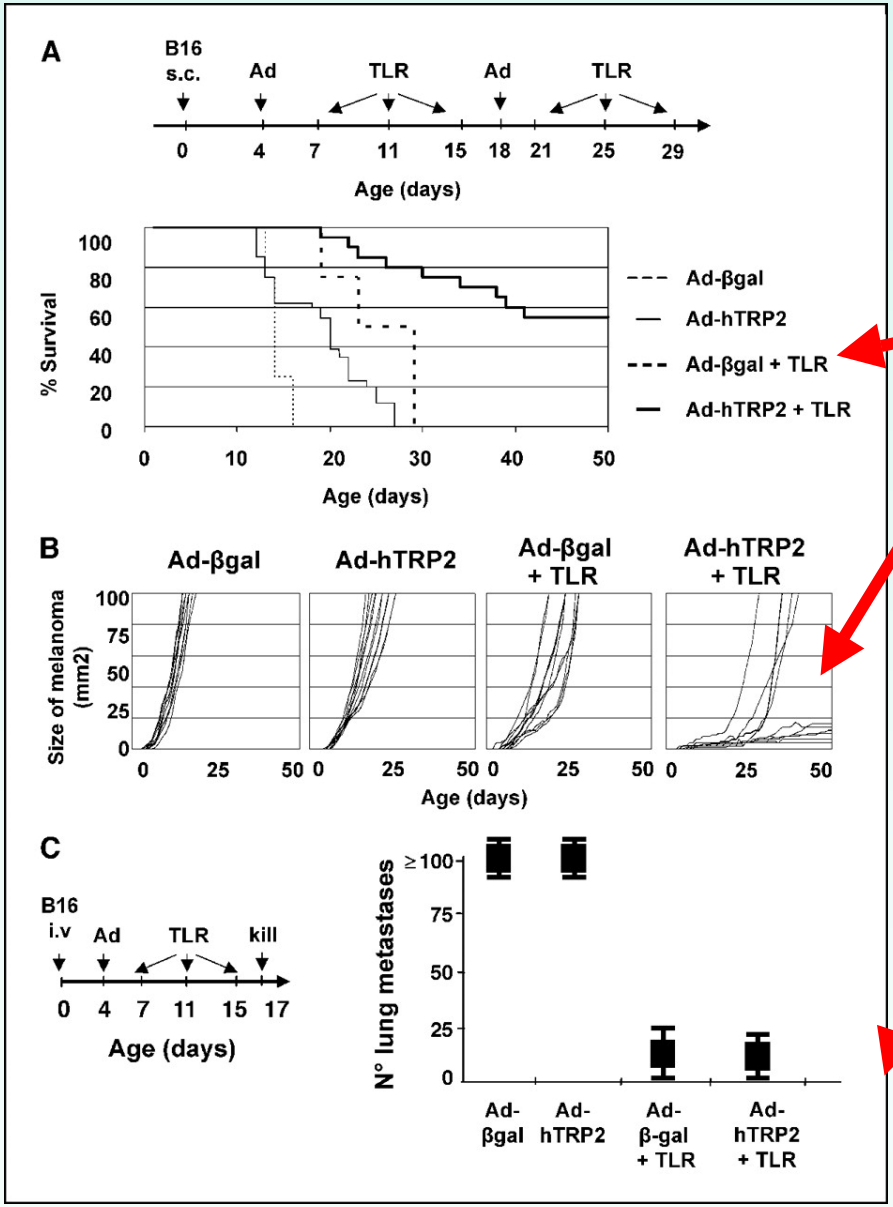


Poly IC

Zhu, et al., J Transl Med. 2007; 5:10

Toll-like receptor-3 ligand poly-ICLC promotes the efficacy of peripheral vaccinations with tumor antigen-derived peptide epitopes in murine CNS tumor models

Poly IC: Cancer Vaccine Adjuvant *In Vivo*



TLR=
CpG + poly IC

Tormo D, et al., Cancer Res 2006;66:5427-5435

Poly I: C and Poly-ICLC: Clinical Summary

- Early phase I and II data
  - Multiple monotherapy trials in healthy volunteers and HIV patients, and various types of cancer since at least 1971
  - MTD: variable, several hundred-fold dose range
  - Induction of type 1 IFN but limited immune and clinical efficacy:
    - Ewel, et al., Cancer Res 52, 3005-3010, 1992
    - Giantonio, et al., Invest New Drugs 19:89-92, 2001

Poly I: C and Poly-ICLC: Clinical Summary

- More recent phase II data
  - Multiple trials in HIV, chronic fatigue syndrome, and cancer
  - Limited and transient toxicity–low dose
  - Induction of type 1 IFN
  - *In vivo* activation of innate immune cells
  - Anti-viral activity

Poly I: C and Poly-ICLC: Clinical Summary

- Ampligen (poly I: polyC12U)
  - Induction of type 1 IFN and activation of RNase-L (antiviral)
  - Tested broadly in viral infections in trials in many countries:
    - HIV, HCV, HPV, CFS, and SARS
    - Clinical trials.gov: currently accruing HIV and chronic fatigue syndrome (CSF)
  - Trials in melanoma

Poly I: C and Poly-ICLC: Clinical Summary

- Hiltonol (poly-ICLC)
  - Induction of type 1 IFN and activation of innate immunity
  - Ongoing phase I/II trials in malignant gliomas
  - Ongoing trial in prostate cancer testing adjuvant effect to MUC1 100-mer peptide vaccine (clinicaltrials.gov)

Poly I: C and Poly-ICLC: Clinical Summary

- Efficacy in cancer
  - Limited when used as monotherapy or in combination with chemotherapy or cytokines
  - No clinical data yet on the intended use as adjuvant to cancer vaccines

Poly I: C and Poly-ICLC: Contemplated Uses

ADJUVANT TO CANCER VACCINES

- *Ex vivo* activation of cancer vaccines based on *ex vivo* DCs
- *In vivo* as adjuvant to cancer vaccines
- *In vivo* as adjuvant to vaccines based on DC targeting
- Therapeutic vaccination
- Preventive vaccination in adjuvant setting

Poly I: C and Poly-ICLC: Perceived Need

- Adjuvant to vaccines possibly in combination with other immunomodulators, for example, Cytoxan or other TLR ligands, for example, CpG
- Application to any cancer amenable to vaccine therapy
- Multiple investigators

Comparison of Agents

- Poly I: C and Poly-ICLC; Poly-ICLC has been shown more stable and, therefore, more active

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Interleukin-12

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- Professor of Interdisciplinary Oncology and Head, Donald A. Adam Comprehensive Melanoma Research Center
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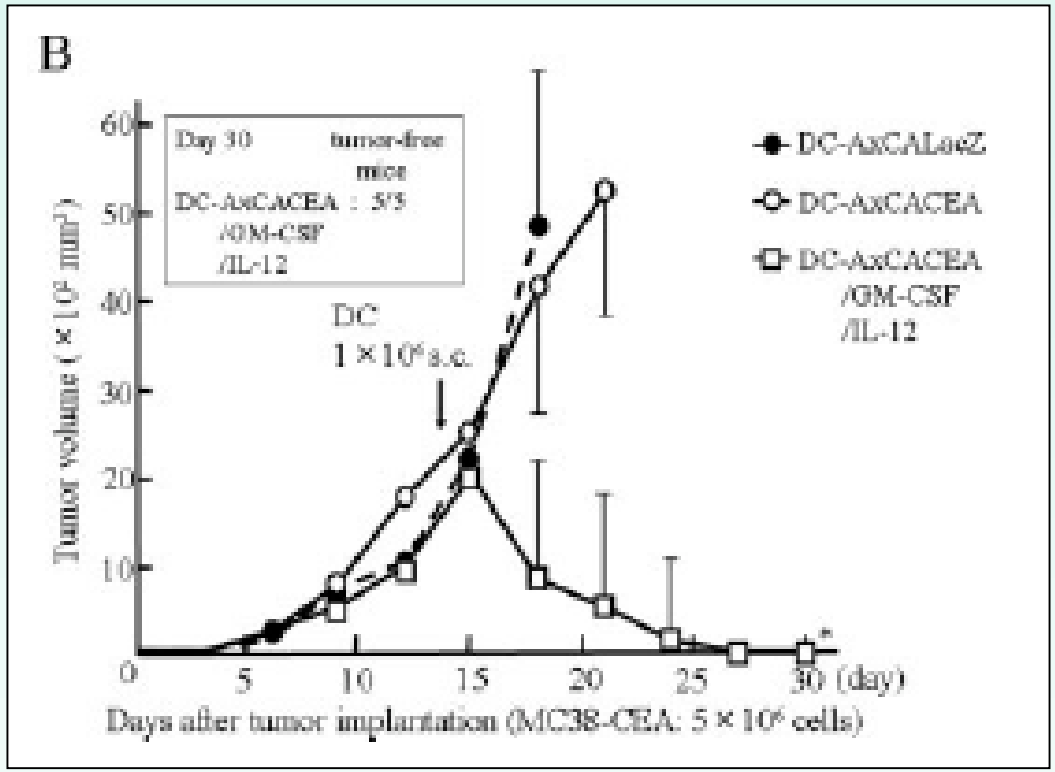
Interleukin-12: Background

- Cytokine, consists of two chains, p35 and p40
- Glycoprotein of 70kD; shares p40 subunit with IL-23, which induces Th17 cells, and also shares IL-12Rß1 with IL-23
- Binds to IL-12R on NK, T cells, dendritic cells, and macrophages
- Promotes gamma interferon release by IL-12R expressing T and NK cells, and induces Th1 polarization, as well as proliferation of gamma interferon expressing T cells
- Also has anti-angiogenic activity

Interleukin-12: Background

- Primarily produced by activated DC, but also by monocytes, macrophages, and microglia
- Predominant signaling via Stat 4
- Plays a central role in resistance to mycobacterial and intracellular pathogens such as parasites
- Important role in anti-cancer development and immunity in animal systems
- IL-12 appears to have a role in autoimmunity, but IL-23 is, likely, the main player; anti-p40 MoAbs cause regression of some autoimmune diseases

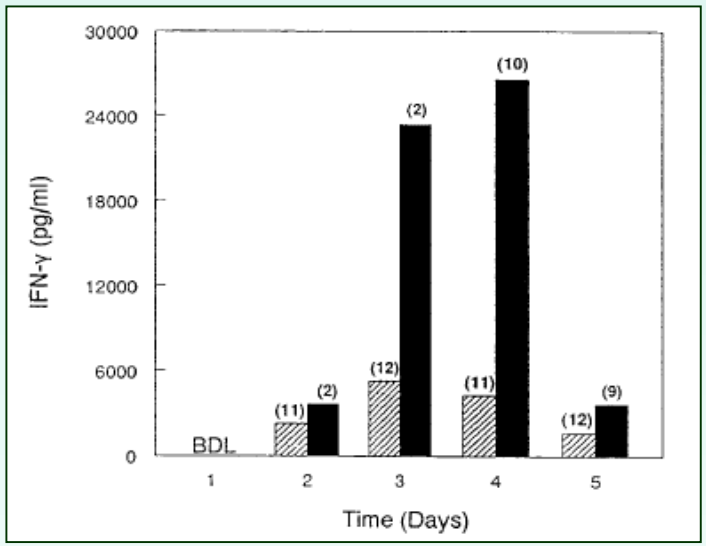
Interleukin-12



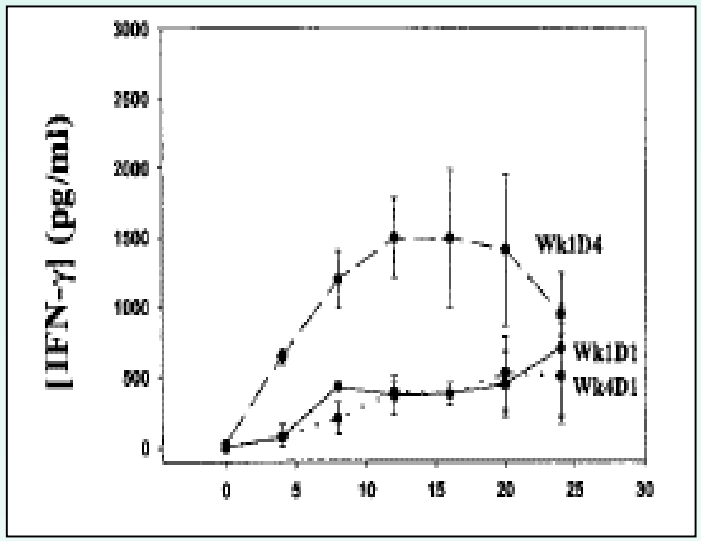
Ojima, et al. *Int J Cancer* 120: 585, 2006.

IL-12 is an exceedingly potent immune adjuvant.

Interleukin-12



Leonard J, et al. Effects of single-dose interleukin-12 exposure on interleukin-12 associated toxicity and interferon-g production. *Blood* 90: 2541, 1997.



Gollub T, et al. Twice-weekly intravenous interleukin-12 in metastatic renal cell cancer or malignant melanoma. *Clin Cancer Res* 6: 1678, 2000.

IL-12 was initially developed as a systemic cytokine, but proved challenging to administer safely.

Interleukin-12

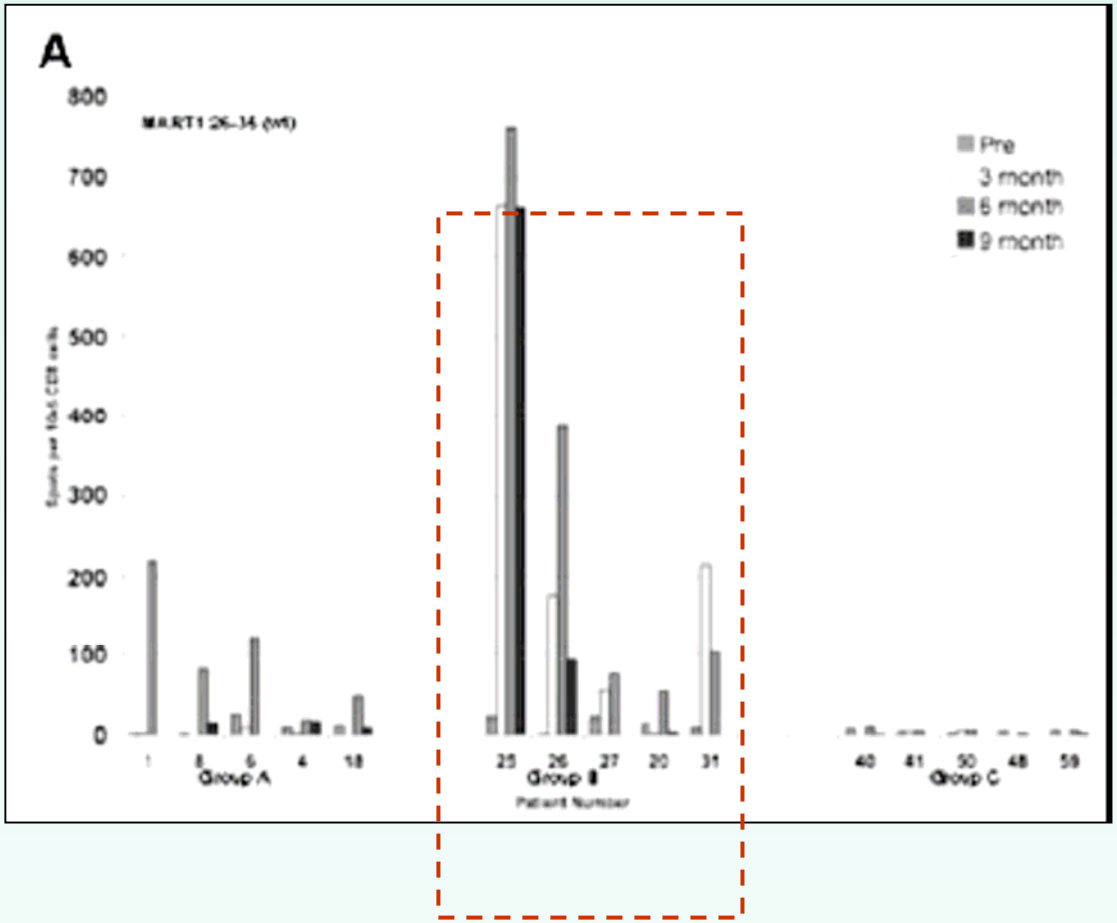
Table 4 Relation between pattern of IFN-γ induction by IL-12 during cycle 1, clinical response, and cytopenias

IFN-γ induction pattern	Disease	Cycle 1 Peak IFN-γ level (pg/ml)			IL-12 dose level (ng/kg)	Response	Cytopenia
		Wk1D1[a]	Wk1D4	Wk4D1			
Type I							
Patient 10	RCC	1600	2560	1200	500	SD @48 wk	None
13	RCC	1100	2235	1340	500	PR @36 wk	None
18	RCC	450	1290	400	700	SD @20 wk	Hemolytic anemia
22	RCC	1000	1840	850	500	SD @24 wk	Agranulocytosis
Type II							
Patient 24	RCC	2365	4960	220	500	PD @12 wk	None
26	RCC	2040	9060	248	500	PD @6 wk	None
28	Mel	1560	5918	77	500	PD @12 wk	None
Type III							
Patient 21	Mel	2229	1476	159	500	PD @6 wk	None
23	Mel	1256	951	90	500	PD @6 wk	None
19	RCC	1800	890	125	700	PD @6 wk	None

[a] Wk1D1, week 1 day 1; Wk1D4, week 1 day 4; Wk4D1, week 4 day 1; RCC, renal cell cancer; Mel, melanoma; SD, stable disease; PD, progressive disease.

IL-12 had insufficient clinical activity to warrant further clinical development.

Interleukin-12



IL-12 adsorbed to Alum gives higher CD8+ T-cell responses to melanoma antigens.

Vaccine: Melanoma Peptides

Adjuvants:

Montanide ISA-51

GM-CSF (Group C)

IL-12 – 30ng/kg (Groups A, C)

– 100 ng/kg (Group B)

Hamid, et al. *Clin Cancer Res* 13: 215, 2007.

This recently reported trial demonstrates the potency of IL-12 as an immune adjuvant.

Interleukin-12: Clinical Summary

- Phase I & II data
  - Very modest efficacy suggested alone; handful of melanoma and RCC responses; might be more impressive with a vaccine
    - MTD defined at 500 ng/kg IV once/twice weekly
  - Benefit may have been associated with elevated gamma interferon levels
  - Safety: hepatitis, fevers, and “cytokine storm”
  - One septic death seen in first Phase I study
- No Phase III data available; was development stopped too early?

Interleukin-12: Contemplated Uses

- Excellent potential as either adjunctive cytokine therapy or adjuvant in a vaccine approach based on murine and human data
- Delivery locally via viral or other plasmid vectors may be useful
- Its use as an adjuvant may both polarize Th1 responses and augment CD8 responses in any antigen-specific strategy

Interleukin-12: Perceived Need

- Might be useful as a vaccine adjuvant in any tumor setting; breast, colon, prostate, lung, and gliomas are histologies with active vaccine trials
- Tom Gajewski, I. Lou Weiner, and Bob Seeger all have had IL-12 trials halted because of lack of supply

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Interleukin-4 (IL-4)

- Theresa L. Whiteside, Ph.D., ABMLI
- Professor of Pathology, Immunology, and Otolaryngology and Laboratory Director
- University of Pittsburgh School of Medicine and University of Pittsburgh Cancer Institute

Interleukin-4 (IL-4): Background

- Cytokine (ligand of IL-4R)
- Glycoprotein (15kDa) produced by activated T cells, NK-T cells, mast cells, eosinophils, and basophils
- Coded for by the gene located on chr. 5q23-31
- IL-4 structure (left-handed four α-helical bundles with short stretches of β sheets) resembles that of GM-CSF, M-CSF, and growth hormone
- Has 20% homology with IL-13
- Targets a broad variety of cells expressing IL-4R: B, T, NK cells, monocytes, and various tissue cells

Interleukin 4 (IL-4): Biologic Effects

- **B cells**: induces isotype switching (IgG and IgE), up-regulates surface markers
- **T cells**: promotes Th2 and Th1 cell differentiation and growth
- **Myeloid cells**: with GM-CSF promotes differentiation of b.m. precursors to DC, matures myeloid DC
- Induces histamine release from **mast cells**
- Up-regulates VCAM-1 on **EC**
- Suppresses *in vitro* growth of **IL-4R$^+$ cancer cells**, but may also promote it (HNSCC)

IL-4 Receptor Complex

- The classical (type I) IL-4R expressed on hematopoietic cells consists of IL-4Rα (140kDa) and γ_c (65 kDa)
- The alternative (type II) form expressed on cancer cells consists of IL-4Rα and IL-13Rά chains; binds IL-4 and IL-13
- Upon dimerization of the receptor by IL-4, Jak1, Jak3, and Tyk3 are phosphorylated; STAT6 and p170/IRS-2 are activated, leading to stimulation of the PI3K pathway

Interleukin-4 (IL-4): Effects of Knock-in and Knock-out

- **IL-4 TG**: increased IgE levels and allergic-like disease; inflammation in the eye
- **IL-4 KO**: CD8+ T cells cannot mediate anti-tumor immunity after immunization with tumor; no CTL; reduced cutaneous DTH, no protective Th1 immunity to *Candida;* defective Th1 responses
- **IL-4R KO**: diminished Th2 responses, lack of IFN-γ responses, sensitivity to worms and *L. major* infections
- **STAT6 KO**: failure of B cells to mature; lymphocytes fail to up-regulate MHC class II and IL-4R and to proliferate in response to IL-4; T cells do not differentiate into Th2 in response to IL-4 or IL13, failure to develop memory responses

Interleukin-4 (IL-4) in Animal Cancer Models

- Anti-tumor effects: CD8+/NK-mediated, dose-dependent decrease in lung mets in RENCA; tumor rejection in other models after local sc or intralesional delivery; anti-angiogenic effects reported
- Suppression of anti-tumor immunity by systemic delivery of IL-4 in many models; splenomegaly and PMN accumulations
- Gene therapy with IL-4-transduced cancer cells: tumor rejection accompanied by MØ, PMN, and CD8+ T cells, and also DC infiltrations into tumor
- Dose-limiting toxicities in monkeys: death, cardiac inflammation/necrosis, hepatitis and DIC; no dose-limiting toxicity at 5μg/kg/day in 1–6 mo sc studies

Interleukin-4: Clinical Use (1)

- Only Phase I & II data available :
  - **Recombinant hIL-4** as monotherapy (variety of cancers: sc, iv, or intratumor)
    - MTD defined at 4–5μg/kg/day for iv or sc or 10 μg/kg when given 3x/wk
    - The toxicity profile established: flu-like symptoms, GI problems, including ulcerations and bleeding, and transient hypotension
    - Safe and well-tolerated when given at modest doses
    - In over 300 cancer patients with advanced disease; no efficacy was demonstrated
  - **Combined immunotherapy:IL-4 and GM-CSF** (metastatic disease)
    - MTD: GM-CSF 2.5 μg/kg/d and IL-4 6.0 μg/kg/d
    - BAD: GM-CSF 2.5 μg/kg/d and IL-4 4.0 μg/kg/d
    - Toxicity: grade I and II; rare grade III (hepatic)
    - Anti-tumor effects: 1PR, 8SD (8.5 mo), and 12 PD
    - Immunologic effects: expansion and activation of type-1 DC

Interleukin-4: Clinical Use (2)

- **IL-4 gene therapy**: tumor cells (auto or allo) transfected with the IL-4 gene or IL-4-transfected fibroblasts + tumor cells (GBM, melanoma) mild side effects; immunologic responses observed in some patients; one glioma patient had a transient response and survived for 10 months
- **IL-4 toxin therapy**: IL-4 fusion toxin = IL-4 protein conjugated to mutated forms of *Pseudomonas* exotoxin or to diphtheria toxin. These fusion proteins are highly toxic to tumor cells. Anti-tumor activity in animal models
  - Used in patients with CNS or non-CNS IL-4+ tumors
  - MTD defined at 0.016 mg/m^2 given daily x 5
  - Hepatotoxicity (iv) or CNS toxicity (intratumor)
  - No objective tumor responses
- Polarization and transfer of donor CD4+ T cells for augmentation of allogeneic hematopoietic cell transplantation
- Generation of clinical-grade MDC for adoptive transfers

Interleukin-4: Contemplated Uses

- As an adjuvant to anti-cancer vaccines in combination with other cytokines to increase the number and activity of APC
- In HCT to ameliorate GVHD and to augment anti-tumor Th1/Th2 responses
- In chronic inflammatory conditions, to modulate Th1/Th2 balance and explore anti-inflammatory activities of IL-4

Agent Name: Perceived Need

- Potentially useful as adjuvant in cancer or in management of inflammatory disorders as part of multiple therapy regimens in many clinical settings
- Potentially needed by multiple independent clinical investigators
- Needed by all for *ex vivo* culture of MDC or IL-4 polarized CD4+ T cells

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IL-15

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IL-15: Background

- Cytokine and T-cell growth factor
- 4-helix bundle cytokine similar to IL-2
- Made by dendritic cells, macrophages, and stromal cells, NOT T cells
- Acts on $CD8^+$ T cells, $CD4^+$ T cells, and NK cells; also mast cells
- Uses unique IL-15Rα chain but shares β and γ chains of IL-2 receptor. IL-15Rα binds IL-15 to cell surface of DCs and presents IL-15 in trans to IL-2/15 β and γ chain receptor

IL-15: Preclinical Summary

- Necessary for NK cell development and for maintenance of $CD8^+$ T-cell memory
- Inhibits antigen-induced cell death of T cells, in contrast to IL-2 that promotes AICD
- In vaccines, promotes induction of longer-lived and higher avidity $CD8^+$ T cells, more efficacious at killing tumor cells
- Can reverse T-cell anergy
- In differentiation of monocyte-derived DCs *in vitro,* promotes differentiation into Langerhans similar to DCs that are more potent inducers of $CD8^+$ T cells
- Helps sustain T cells in adoptive immunotherapy
- Overcomes lack of $CD4^+$ T-cell help in CTL induction
- Can induce tumor regression with intratumoral injection or hydrodynamic delivery in mice

IL-15: Clinical Summary

- Phase I and II data
  - None available
- Phase III data
  - None available

IL-15: Contemplated Uses

- As a vaccine adjuvant to induce longer-lived, higher avidity, more efficacious $CD8^+$ T cells
- As a single agent to overcome T-cell anergy
- As a T-cell growth factor in conjunction with adoptive T-cell immunotherapy
- As an agent to differentiate DC precursors into Langerhans-like DC, more efficacious for $CD8^+$ T-cell induction, for DC-based vaccines
- As a systemic or intratumoral cytokine therapy for cancer

IL-15: Perceived Need

- Needed for multiple cancers, for both cancer vaccines and adoptive immunotherapy, as well as for use as direct therapy *in vivo* and for DC differentiation *in vitro* for DC vaccine therapy
- At least 10 independent clinical investigators have applied for or are actively working to obtain GMP IL-15 for clinical use

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Interleukin-7

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IL-7: Background

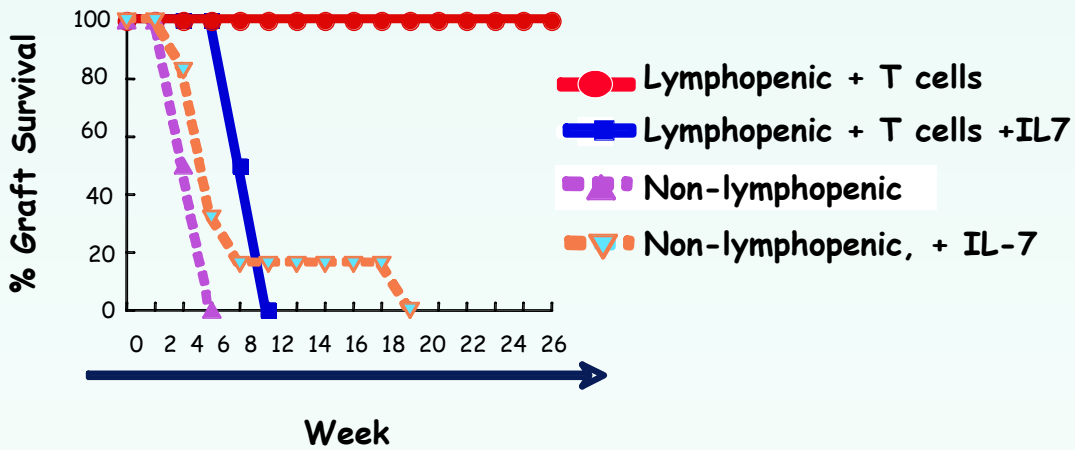
- 25 kD cytokine
  - Lymphopoietic: required for T-cell development in humans
  - Required for naïve T-cell survival in the periphery
  - T-cell growth factor, costimulates TCR signaling
- Produced by stromal cells, epithelium, keratinocytes, some APCs, not T cells
- Signals via IL-7R comprised of γc and IL-7Rα
  - γc shared with IL2, IL9, IL15, IL21
  - IL-7Rα shared with TSLP
- IL-7R expression
  - Common lymphoid progenitors
  - DN thymocytes
  - Naïve T cells, early memory T cells, not effectors or senescent cells
  - Immature B cells, germinal center B cells, not most B cells
  - $\gamma\delta$ progenitors
  - Low levels on APCs and stroma

IL-7: Background

- IL-7 signaling
  - Jak1, Jak3/STAT5
  - PI3K activation, mTOR activation
    - Inhibited by rapamycin
- IL-7 signaling and/or T-cell activation results in receptor down-regulation
  - Opposite regulation compared to IL-2, IL-15
- IL-7 accumulates during lymphopenia as a result of diminished utilization
- IL-7 signaling on mature T cells
  - Responsible for homeostatic expansion of naïve cells during lymphopenia
  - Can substitute for IL-15 for homeostatic expansion of memory cells during lymphopenia
  - IL-7Rα expression marks cells destined to become memory during the evolution of the immune response (Kaech, Nat Imm 2003)

IL-7: Preclinical Summary

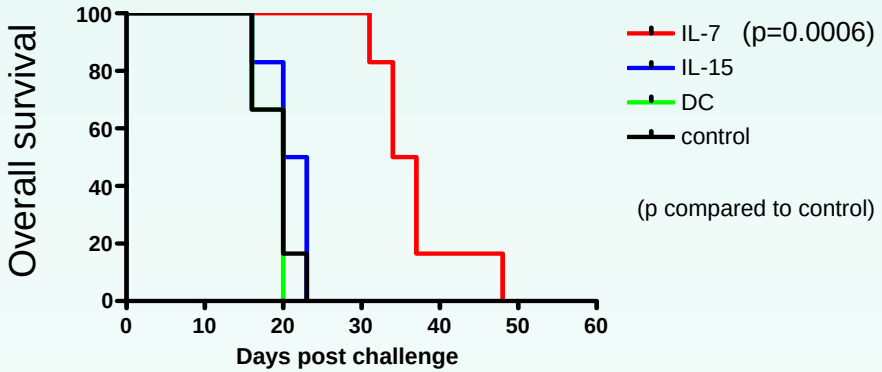
- IL-7–treated mice show enhanced marrow engraftment, mobilize stem cells and myeloid progenitors (Boerman, J Leuk Bio 1995)
- IL-7–treated mice (Geiselhart, J Leuk Bio 2001) and primates (Fry, Blood 2003) show dramatic expansions in peripheral T-cell numbers without obvious toxicity
- IL-7Tg mice show lymphoproliferation and autoimmunity dermatitis mediated by $\gamma\delta$ cells (Uehera, Int Immunol 2003)
- IL-7 increases the rate and degree of immune reconstitution following bone marrow transplantation in mice (Bolotin, Blood 1996, Mackall, Blood 2001) and primates (Storek, Blood 2002)
- IL-7 normalizes immune function in athymic lymphopenic mice (minor Ag disparate skin graft rejection)



Fry, Blood 2001

IL-7 Preclinical Summary

- IL-7 is a vaccine adjuvant (Melchionda, JCI 2005)
  - Enhances CD4+ and CD8+ effector and CD8+ memory populations
  - Most dramatic effects on subdominant responses
  - Enhanced tumor protection following vaccination with DCs plus IL-7 vs. DCs + IL-15 or DCs alone



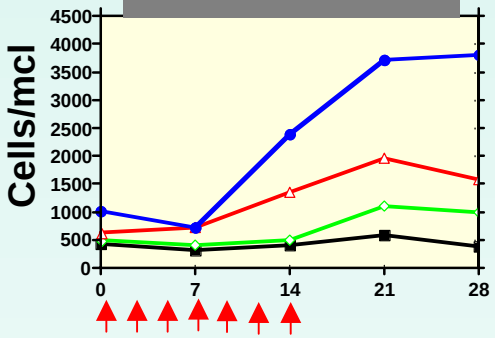
- IL-7 is essential for the augmented antitumor effects observed during lymphopenia (Gattinoni, JEM 2005)

IL-7: Clinical Summary

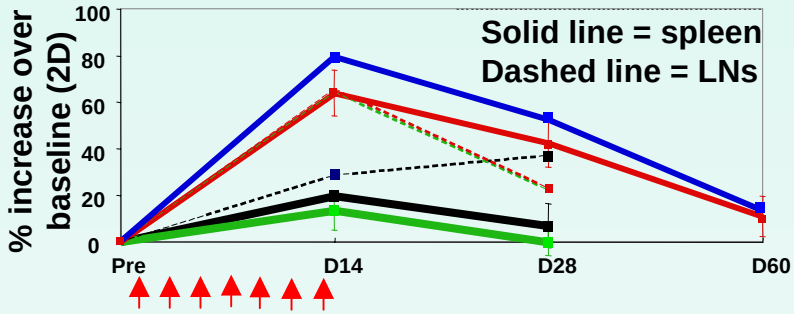
- Two phase I/II trials completed in patients with cancer (Rosenberg et al., J Immunother 2006, Sportes et al., submitted)
  - Proof of principle established
    - Dramatic increases in total body CD4+ and CD8+ T cells
    - Modest increases in NK cells and $\gamma\delta$ cells, no change in mature B cells
    - No selective increase in Tregs
    - Associated with enhanced cell cycling and diminished programmed cell death
  - Preferential expansion of naïve T cells
    - Like related to cycling of recent thymic emigrants
    - Increased T-cell repertoire diversity
    - Anti-aging effect
  - Favorable safety profile
    - No significant toxicity observed

IL-7: Clinical Summary

Peripheral Blood CD4+ T cells

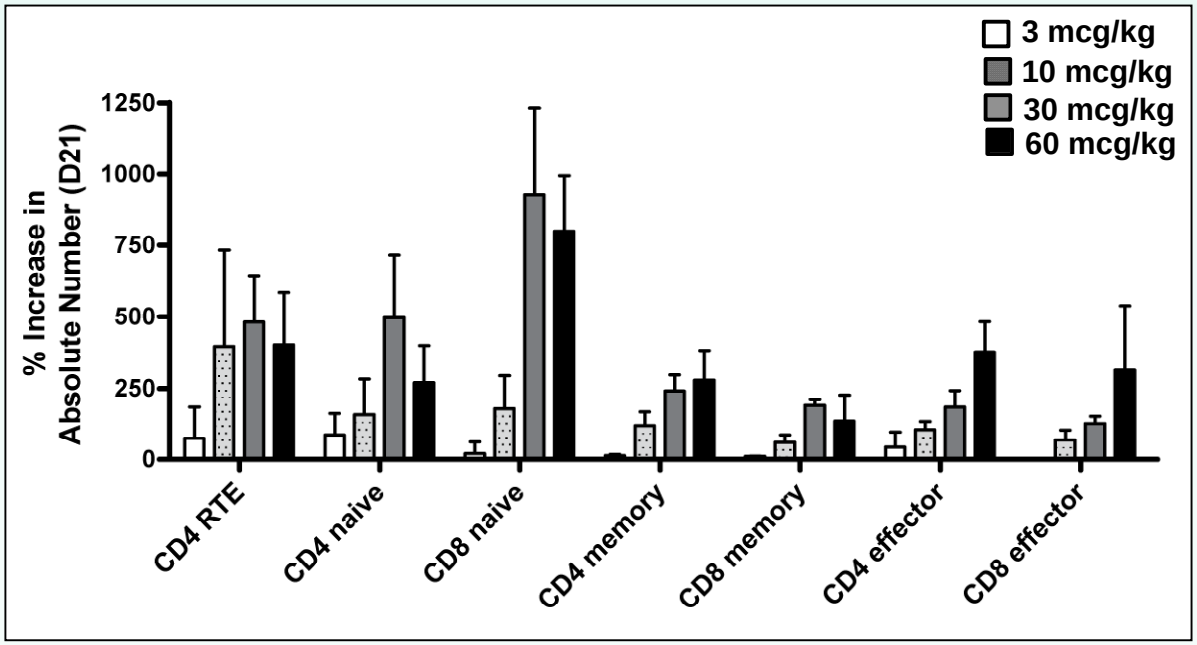


Spleen & Lymph Node Size



IL7 Dose (⇧): QOD SQ x 8 doses

- 3 ucg/kg (n=3)
- 10 mcg/kg (n=3)
- 30 mcg/kg (n=6)
- 60 mcg/kg (n=3)

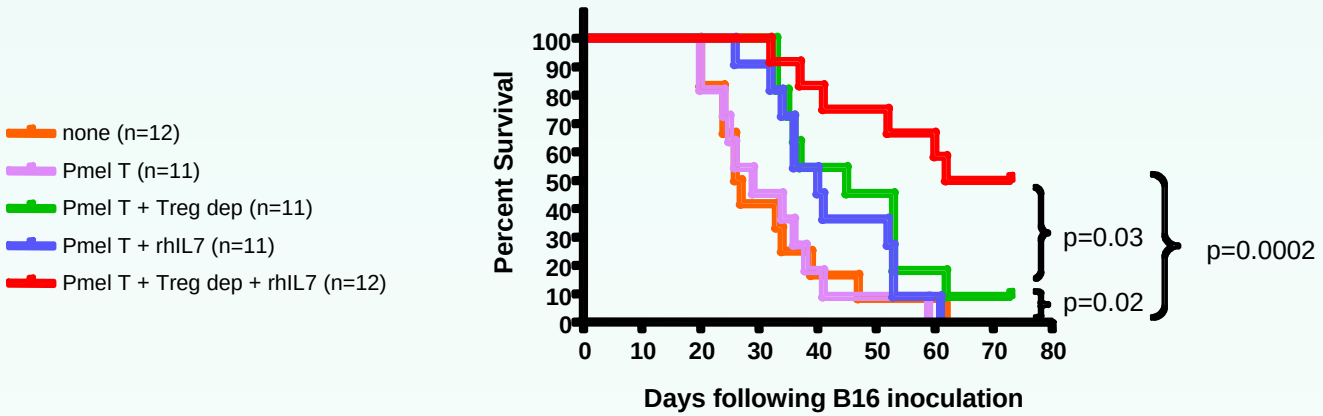


IL-7: Contemplated Uses

- Vaccine adjuvant
  - Tumor antigens are self antigens for which reactive T cells have low affinity
  - During lymphopenia, IL-7 induces widespread T-cell cycling to self antigens
  - Adjuvant studies show the most potent effect on subdominant antigens
  - IL-7's properties should enhance the effectiveness of tumor vaccines targeting weak self antigens

IL-7: Contemplated Uses

- Immunorestorative
  - Chronic T-cell depletion following bone marrow transplantation for hematologic malignancy remains a real problem
  - Improving immune reconstitution in this setting may diminish leukemic relapse
  - Whether improving immune reconstitution following intensive chemotherapy might diminish tumor recurrence
- IL-7 plus Treg depletion
  - Presumably, the favorable environment of lymphopenia relates to the combination of diminished Tregs and increased homeostatic cytokines
  - Combining IL-7 with Treg depletion shows therapeutic benefit when using adoptive immunotherapy for B16 melanoma (unpublished)



IL-7: Perceived Need

- Of interest to the tumor immunology community, BMT community, and HIV community
- Challenge: licensing will require identifying a tumor where the “boost” in immunity mediated by IL-7’s adjuvant effect results in a survival difference

Comparison of IL-15 and IL-7

- Superficial similarity only
  - Both are vaccine adjuvants but likely work at different phases to T-cell activation
    - IL-7 signals resting T cells
    - IL-15 preferentially signals activated T cells
  - Both alter T-cell homeostasis but work on different populations
    - IL-7 preferentially expands naïve T cells, leading to increased repertoire diversity
    - IL-15 preferentially expands effector/memory populations
- More work is needed to identify optimal ways to combine these agents: additive vs. synergistic effects in animal models

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IL-21

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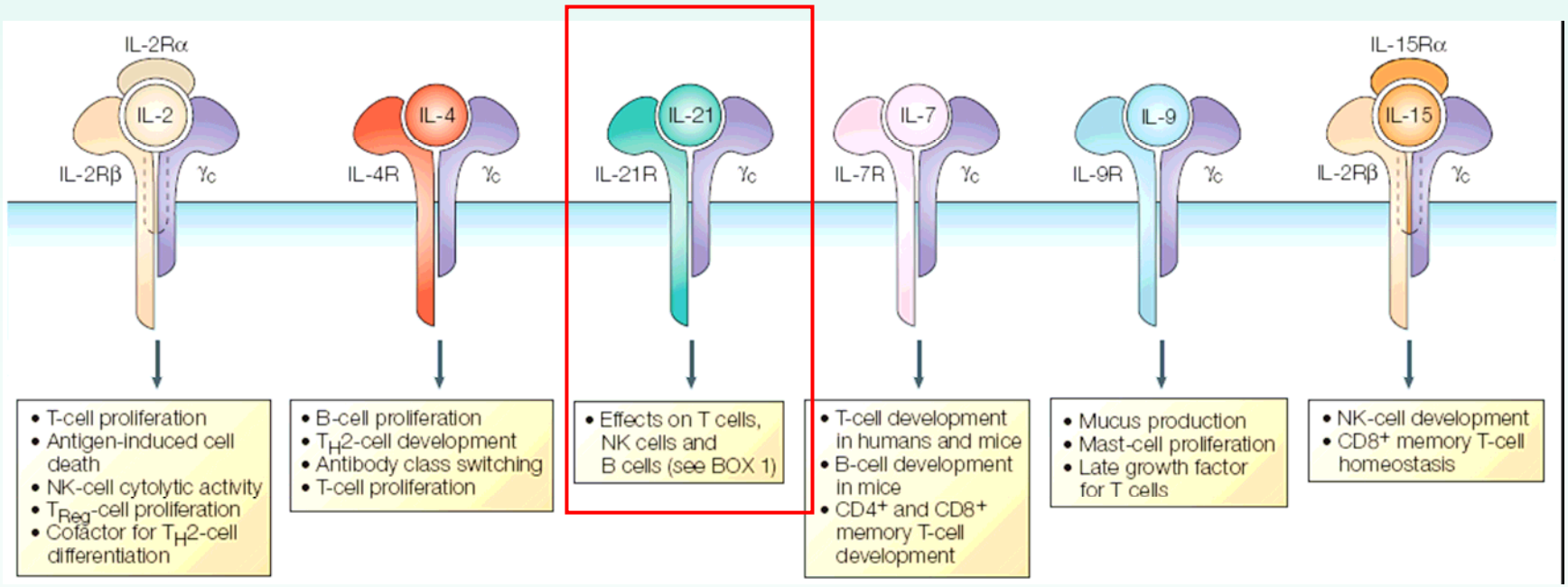
- Genentech, Inc.

Any opinions expressed by me during this presentation and during this workshop are my own, and not intended to represent those of Genentech, Inc.

IL-21: Background

- Member of common γ-chain family of cytokines (**IL-2**, **IL-4**, IL-7, IL-9, and **IL-15**)
- Produced primarily by $CD4^+$T cells
- IL-21Rα expressed by T cells, B cells, NK cells, DC/myeloid cells, and non-immune cells



Leonard and Spolski (2005) Nat Rev Immunol 5:689.

IL-21: Preclinical Summary (1)

- Effects on T cells
  - Stimulates memory and naïve $CD8^+$ expansion synergistically with IL-7 or IL-15
  - Induces/preserves $CD8^+$ CD28 expression
  - Improves degree of expansion and affinity of antigen-specific CTL clones generated *in vitro*
  - Induces differentiation of pro-inflammatory murine $CD4^+$ T_H17 cells
- Effects on NK cells
  - Augments expansion/differentiation and anti-tumor activity (ADCC)
  - Can induce apoptosis (terminal differentiation)

IL-21: Preclinical Summary (2)

- Effects on DC
  - Inhibits maturation, activation, and cytokine production of immature DC
  - Induces an immunosuppressive phenotype
- Effects on B cells
  - Plasma cell differentiation
  - Class switching to IgG; suppression of IgE
  - Apoptosis of naïve or “incompletely activated” B cells

IL-21: Preclinical Summary (3)

- *In vitro* anti-tumor models
  - Promotes apoptosis of B-CLL cells
  - Induces proliferation and inhibits apoptosis of acute T-cell leukemia and multiple myeloma cells

- *In vivo* anti-tumor models
  - IL-21 gene-transduced tumor cells, recombinant IL-21, IL-21 vector
  - Activity in multiple tumor types (e.g., melanoma, fibrosarcoma, mammary, colon, and pancreatic)
  - Demonstrate tumor rejection, prevention of metastases, and immune memory
  - Activate predominantly NK or T-cell–dependent responses (model dependent)

IL-21: Clinical Summary (1)

- Phase I, open label (US)
  - Dose escalation and expansion cohort (#34) at MTD 30 μg/kg (Thompson, et al., ASCO 2006, #2505)
    - Indications: metastatic melanoma and renal cell cancer
    - Administration: IV daily x 5 (+ 9 days rest) per cycle
    - Toxicities: rash, fatigue, fever, chills, muscle aches; thrombocytopenia, lymphopenia; acute hepatotoxicity (transient)
    - Lab findings: increases in soluble CD25, IL-15, and IL-18
    - Activity: 1 partial remission in RCC, 1 complete remission in melanoma (<10% each), and majority with stable disease
- Phase I (Australia)
  - Alternative dosing regimen tested (3x/week x 6 weeks)
  - <10% response rate in renal cell cancer and melanoma
- Phase IIa (Australia; Davis, et al., ASCO 2007, #3055)
  - 5+9 dosing, metastatic melanoma
  - Seven evaluable patients (Jan 07), 1 CR
  - Enrollment ongoing

IL-21: Clinical Summary (2)

- Other ongoing/planned studies
  - Phase I combination with rituximab (relapsed NHL)
    - preclinical enhancement of *in vitro* and *in vivo* ADCC by rituximab
  - Phase I/II combination with sorafenib (metastatic RCC)
    - preclinical additive effects of TKI and IL-21 (*in vivo* RCC model)
  - Phase I (U. Glasgow) combination with cetuximab (anti-EGFR) in metastatic CRC
  - Phase II (US) planned in metastatic melanoma

IL-21: Contemplated Uses

- Systemic immunomodulator
  - single agent in melanoma and renal cell
  - combination therapy
    - TKI (sorafenib)
    - ADCC (CD20, EGFR, HER2, and DR5)
- Vaccine therapy adjuvant
  - allogeneic/autologous tumor cells
  - antigen (protein, peptide, and DNA/RNA)
- Adoptive immunotherapy
  - *in vitro* generation/expansion of antigen-specific CTL lines/clones
  - *in vivo* enhancement of infused CTL activity
  - synergy with IL-15

IL-21: Perceived Need

- Current clinical trials
  - single agent systemic immunomodulator
  - combination therapy: NHL, renal, and colon
- Potential uses in multiple tumors with vaccine strategy or adoptive immunotherapy
  - tumor or antigen vaccine adjuvant
  - *in vitro* CTL generation
  - *in vivo* post-infusion administration
  - combination with IL-15

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Anti-CTLA-4 Monoclonal Antibody

- Steven A. Rosenberg, M.D.,Ph.D.
- Chief of Surgery
- National Cancer Institute, Surgery Branch
- Ipilimumab
- MDX-010, Medarex, Inc.

Cytotoxic T-Lymphocyte-Associated Antigen-4 (CTLA4; CD152)

- Ig superfamily member cloned from a murine CTL cDNA library (Brunet et al., 1986)
- Inducible receptor that is engaged by the B7 family of ligands (CD80, CD86) and inhibits CD4+ and CD8+ T-cell activation
- Knockout mice lacking CTLA4 develop lymphoproliferative disease

Pre-Clinical Anti-CTLA4 Abs

- Hamster anti-mCTLA4 antibody active in tumor prevention models
- Combinations of anti-CTLA4 and vaccines more effective in tumor prevention setting and can slow growth in more advanced models
- No evidence of autoimmunity in monkeys given ipilimumab

Prognostic Factors Related to Clinical Response in Patients with Metastatic Melanoma Treated by CTLA-4 Blockade

Stephanie G. Downey[1], Jacob A. Klapper[1], Franz O. Smith[1], James C. Yang[1], Richard M. Sherry , Richard E. Royal, Udai S. Kammula[1], Mary Beth S. Hughes[1], Tamika E. Allen[1], Catherine L. Levy[1], Michael Yellin[3], Geoffrey Nichol[3], Donald E. White[1], Seth M. Steinberg[2], and Steven A. Rosenberg[1]

(Submitted to *Clin Can Res*)

- 139 Patients:
  - 54 pts 02-C-0106 (Trial 1)
    - median f/u 50 months
  - 85 pts 04-C-0083 (Trial 2)
    - median f/u 29 months
- Inclusion:
  - Measurable metastatic melanoma, ECOG ≤ 2, no clinical evidence or h/o autoimmune disease, > 3 weeks from prior therapy

- 144 patients initially enrolled
  - 5 patients excluded from analysis
    - 2 – diagnosis not confirmed
    - 3 – recent adoptive cell transfer, relatively immune suppressed
- Treatment
  - Trial 1: 3 mg/kg initial dose, 1 or 3 mg/kg subsequent doses, concomitant gp100 peptide vaccine
  - Trial 2: intra-patient dose escalation, starting at 3 or 5 mg/kg, HLA A201 patients randomized to peptide vaccination

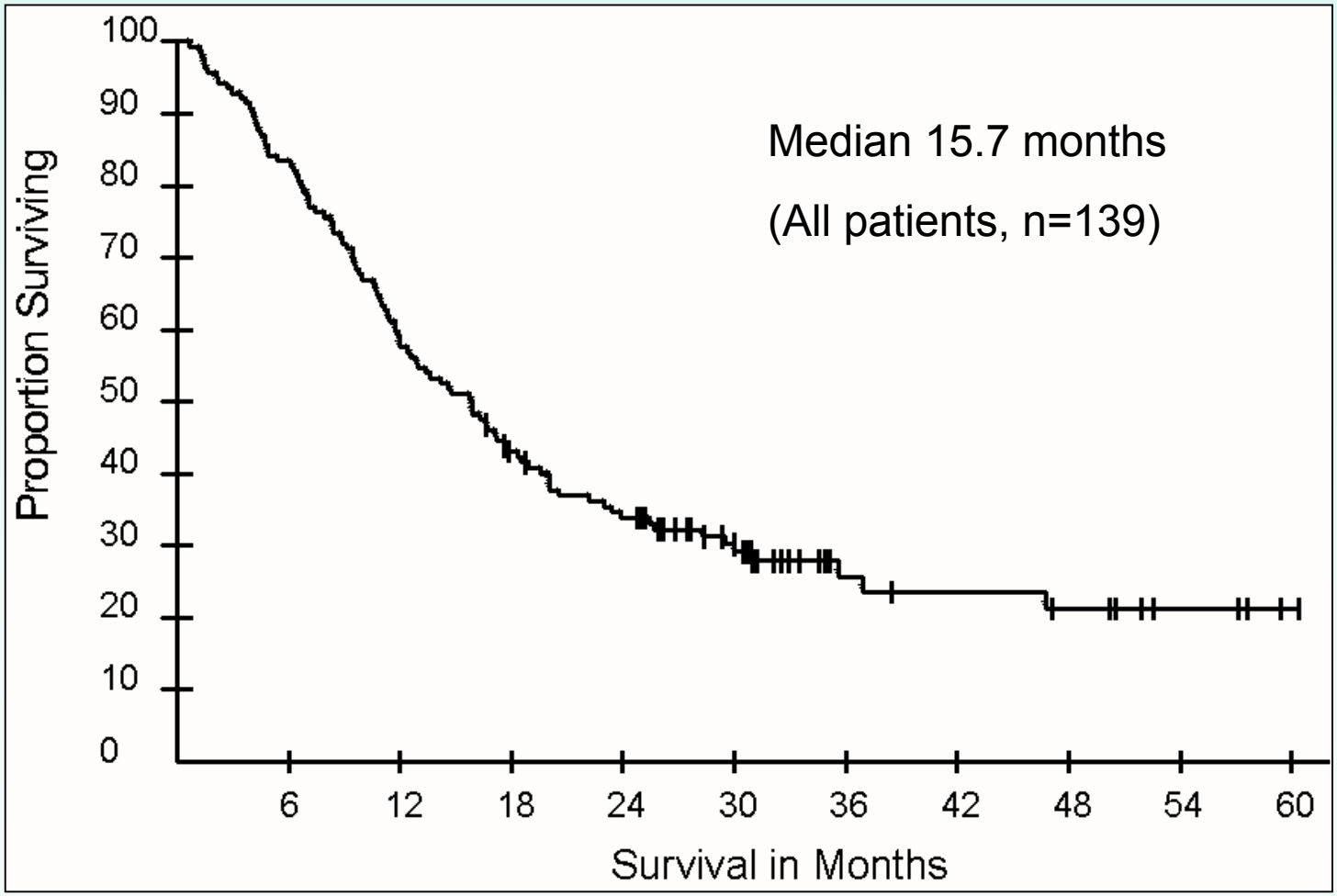
Duration of Response

(As of 4/1/07)

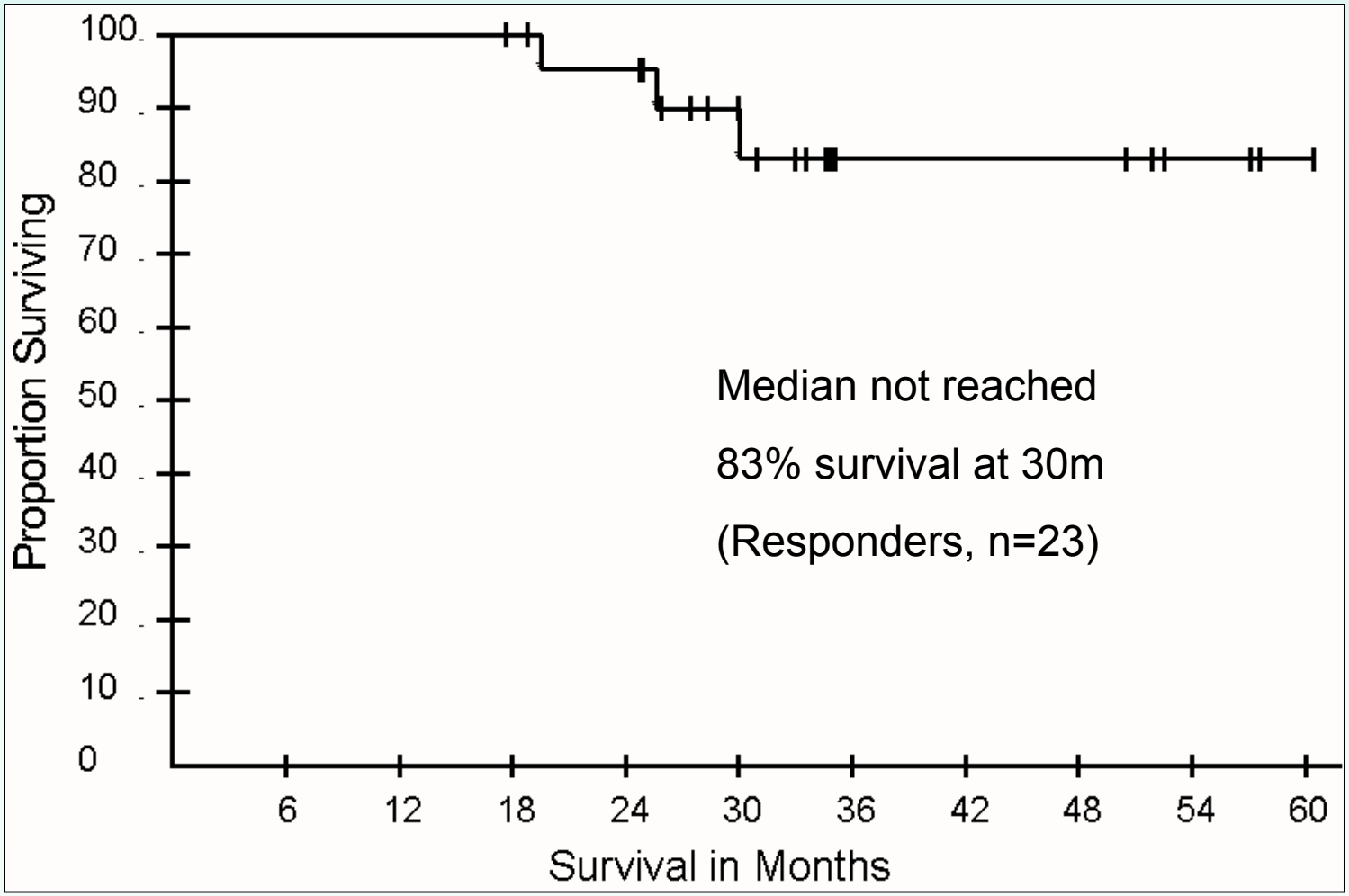
	Total	PR	CR	PR+CR
All patients	139	20	3	23 (17%)
Trial 1	54	5	2	7 (13%)
Duration (months)		4, 5, 43, 47+, 50+	52+, 53+	
Trial 2	85	15	1	16 (19%)
Duration (months)		6, 6, 7, 9, 10, 10, 11, 17+, 17+, 18+, 19, 22+, 28+, 30+, 31+	29+	

+ indicates ongoing response

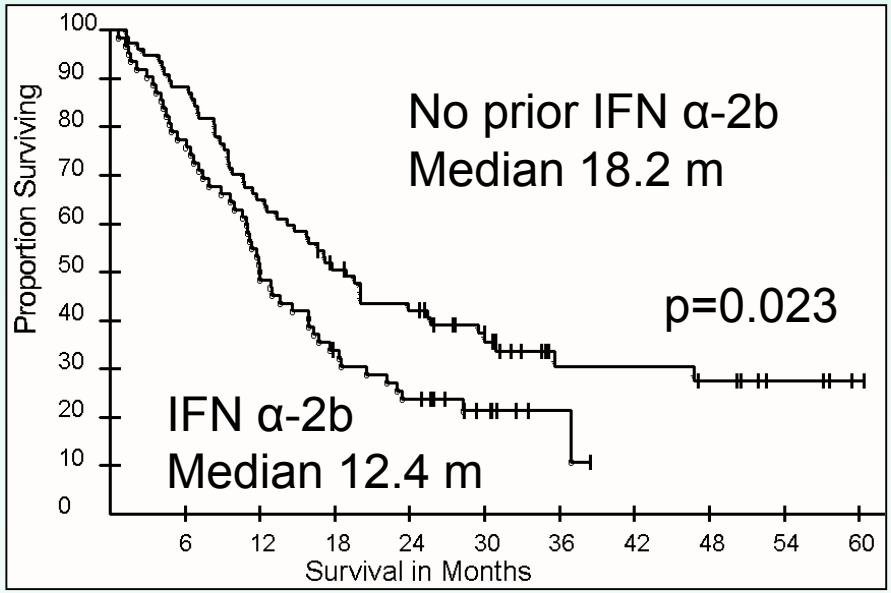
Overall Survival

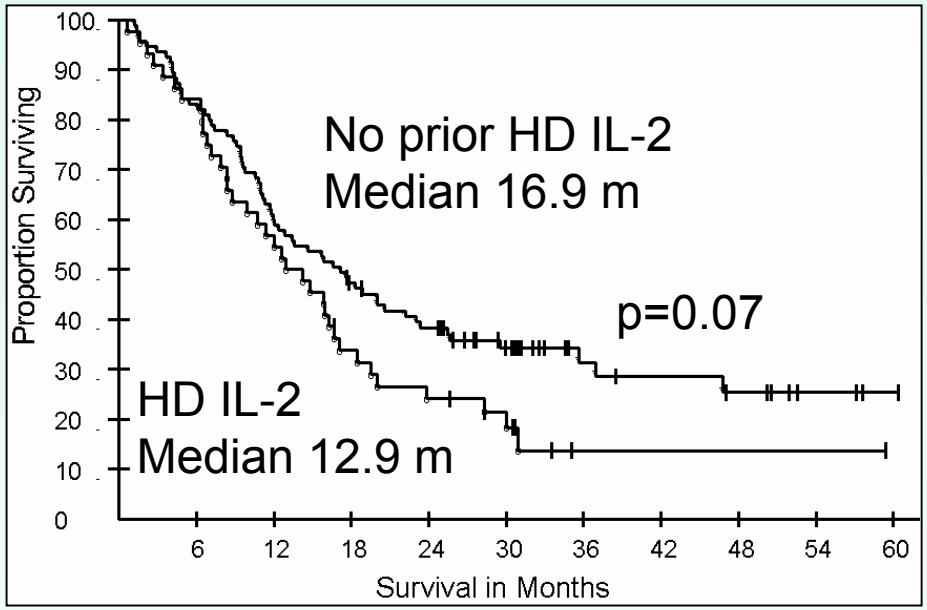


Survival – Responding Patients



Effect of Prior Therapy on Survival





Frequency of Immune-Related Adverse Events

	Gr I/II	Gr III/IV
	n=139	n=139
None	76 (55%)	89 (64%)
1 or more	63 (45%)	50 (36%)
Alveolitis	1 (1%)	1 (1%)
Arthralgia	11 (8%)	3 (2%)
Conjunctivitis	2 (1%)	0
Dermatitis	40 (29%)	8 (6%)
Enterocolitis	4 (3%)	24 (17%)
Hepatitis	0	2 (1%)
Hypophysitis	0	13 (9%)
Hypothyroidism	3 (2%)	0
Episcleritis	0	1 (1%)
Nephritis	0	1 (1%)
Pruritis	35 (25%)	0
Uveitis	1 (1%)	3 (2%)
Death	0 (0%)	0 (0%)

Relationship Between IRAEs and Response

	All	NR	PR+CR	p value	Duration of Response Median (Range) in months
IRAE					
None	53	52	1 (2%)	0.0004	18+
Only Gr I/II	36	28	8 (22%)		11 (4-30+)
Gr III/IV	50	36	14 (28%)		35 (7-53+)

Duration of Response in Patients Requiring Steroid Administration

	# of pts	Duration of Response	Median	p value
All responders	n=23		30.6 months	0.23*
Steroids	12	6, 7, 9, 10, 11, 19, 28+, 29+, 31+, 43, 47+, 52+	19.3	
No Steroids	11	4, 5, 6, 10, 17+, 17+, 18+, 22+, 30+, 50+, 53+	not reached	

* by time varying covariate analysis

Ipilimumab in Patients with Melanoma

- No clear dose/response relationship
- Overall OR 17%
- Grade III/IV Autoimmune Toxicity (IRAE) 36%
  - Colitis 17%
  - Hypophysitis 9%
- Responses are highly durable
- Strong relationship between induction of autoimmune toxicity and likelihood of response

MDX-010 for RCC

- Patients with metastatic RCC
- Cohort #1: q3wk dosing starting at 3 mg/kg and then 1 mg/kg
- Cohort #2: 3 mg/kg q3wks
  - Patients with previous IL-2
  - Patients without previous IL-2
- Repeated dosing to limiting toxicity or PD
- Phase II study with response endpoint

Association of Clinical Responses and Major Immune-Mediated Events

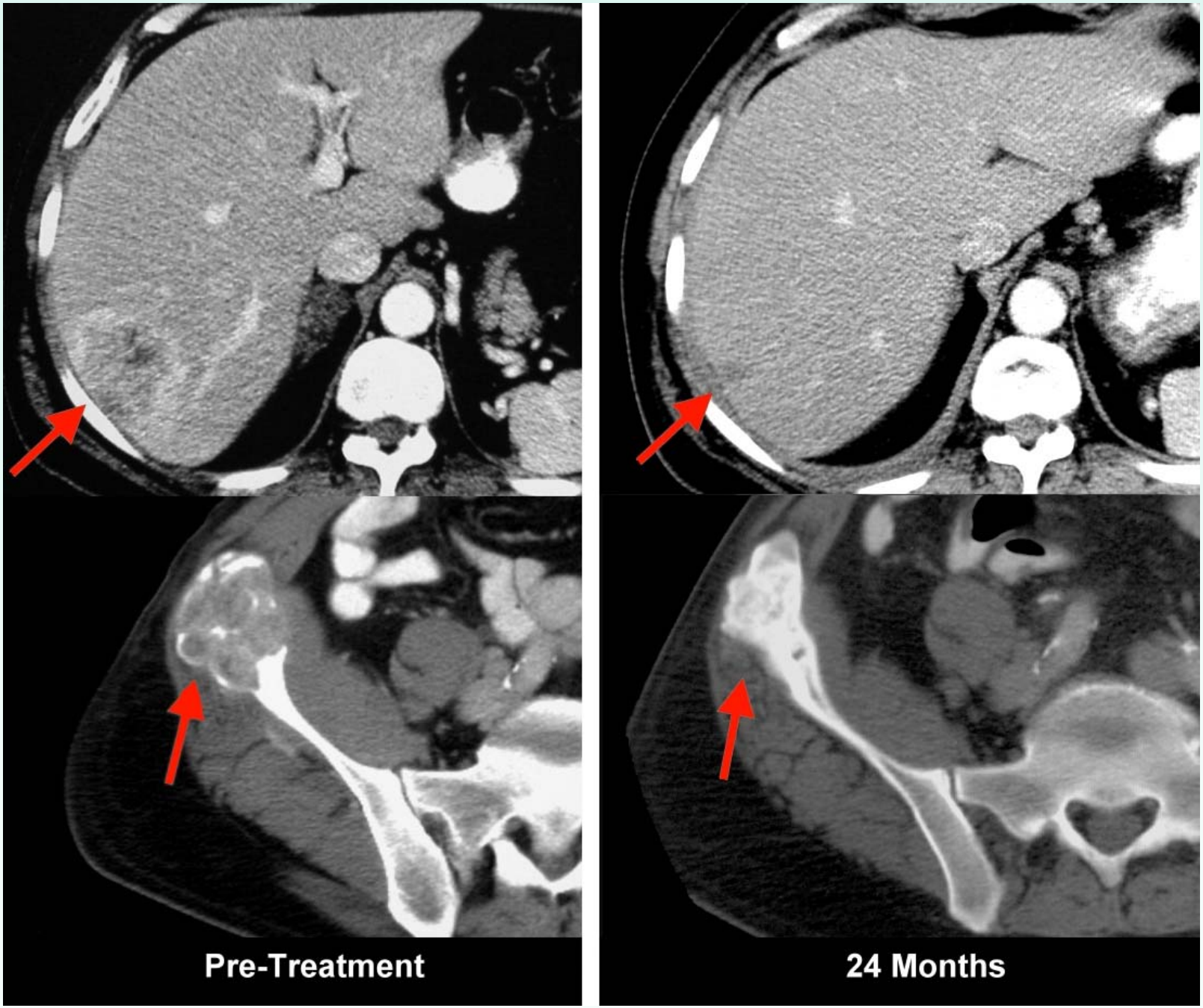
Cohort	Major Immune-Mediated Event	
	Yes	No
3 mg + 1 mg		
Responses	1/3	0/18
3 mg + 3 mg		
Responses	5/17	0/23
Total	6/20	0/41

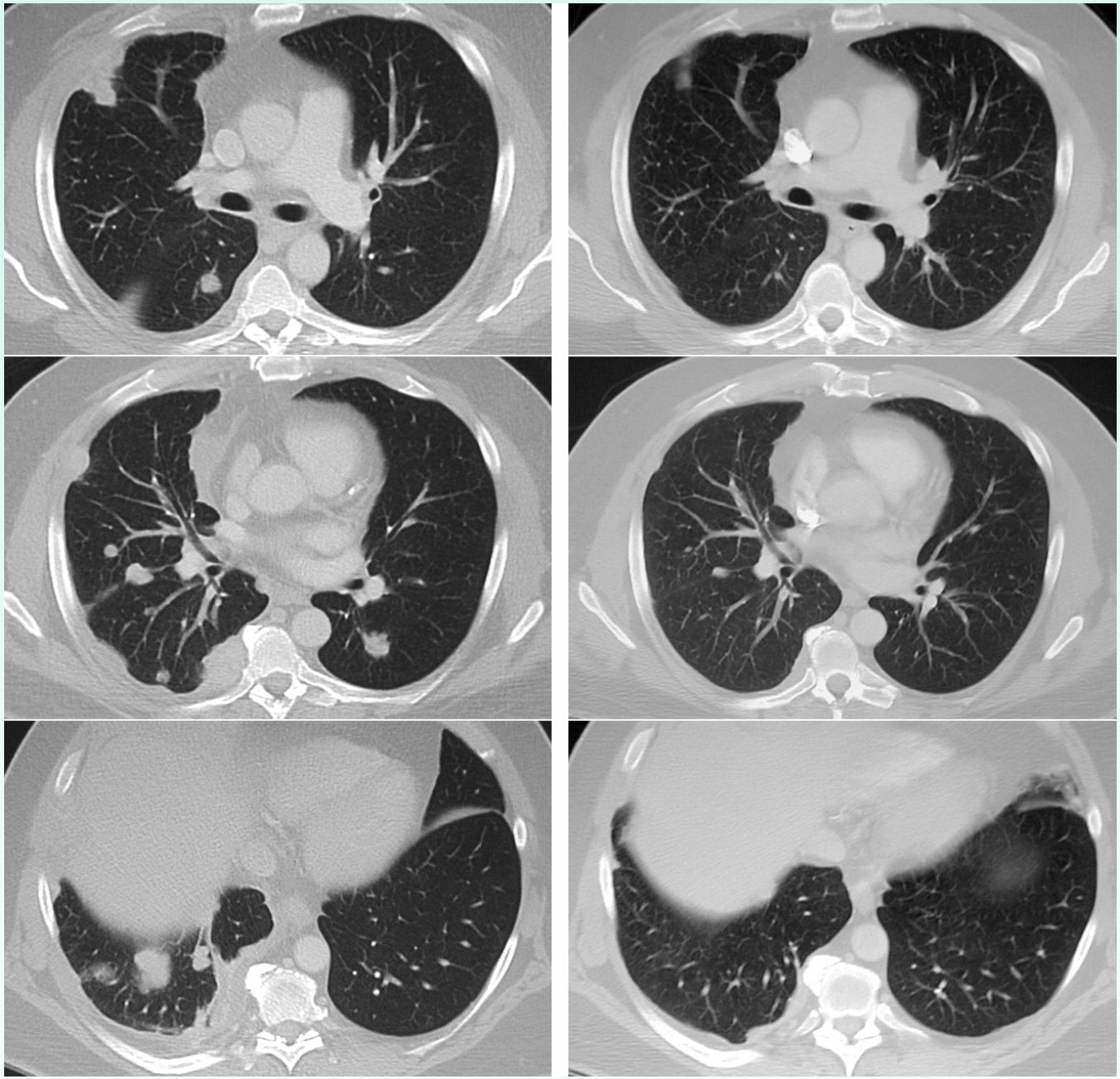
p=0.0007

May 2007

Summary

- The major toxicities of ipilimumab in patients with RCC appear immune mediated
- The incidence of AI may be higher at 3 mg/kg than at 3+1 mg/kg (Cohort 1 14%, Cohort 2 43%, p=.043)
- The response rate was 12.5% at 3 mg/kg
- All responses were partial and durations ranged from 7-21 months
- There was a highly significant association between responses and AI

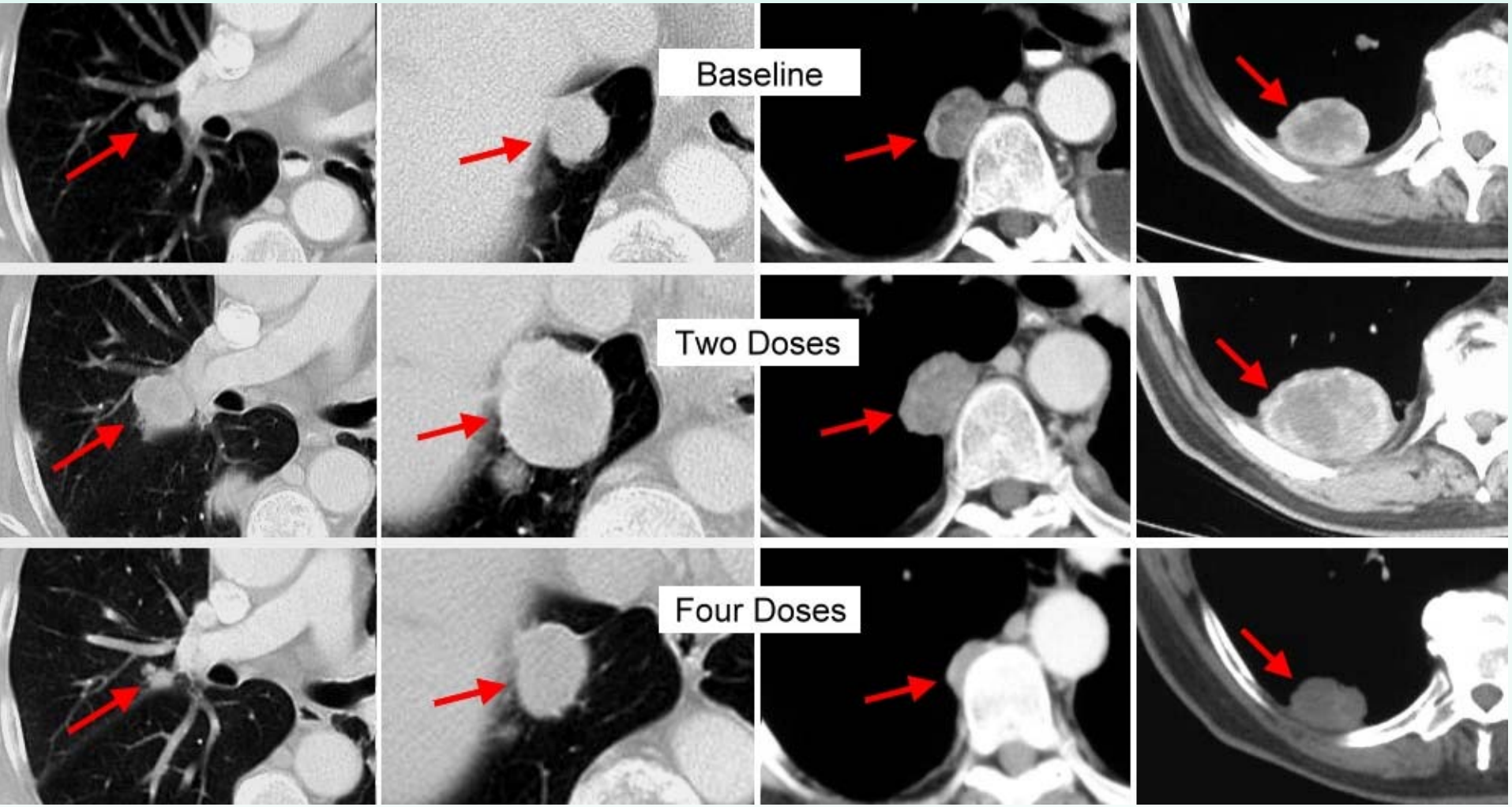

Pre-Treatment
24 Months



Pre-Treatment

12 months

Delayed Regression After Episode of Colitis



Patient Demographics

Protocol	Trial 1	Trial 2	Total
Total	54	85	139
Sex			
M	36	56	92 (66%)
F	18	29	47 (34%)
Age			
Median (Range)	53 (21-67)	49 (24-69)	50 (21-69)
ECOG			
0	43	54	97 (70%)
1 - 2	11	31	42 (30%)
HLA			
A*0201	54	40	94 (68%)
Other	0	45	45 (32%)
Prior Therapy			
None	15	5	20 (14%)
HD IL-2	13	31	44 (32%)
IFN α-2b*	23	39	62 (45%)
Biochemotherapy	10	19	29 (21%)
Chemotherapy	6	27	33 (24%)
Other Biologic	11	31	42 (30%)
2 or more	18	45	63 (45%)
Stage of Disease			
M1a (only subcutaneous, lymph node)	8	12	20 (14%)
M1b (lung sole site of visceral met)	11	19	30 (22%)
M1c (visceral met, or elevated LDH)	35	54	89 (64%)

*6 of these 62 patients received IFN α-2b after resection of distant metastases

Treatment Characteristics

# cycles			
Median (range)			
1-2	3.5 (1-12)	4 (1-10)	4 (1-12)
3-4	23	25	48 (35%)
5-6	20	25	45 (32%)
7-8	5	23	28 (20%)
> 8	3	7	10 (7%)
	3	5	8 (6%)
Total Dose (mg/kg) Median (range)	6 (3-24)	28 (3-70)	12 (3-70)
Peptide Administration			
Yes	54	19	73 (53%)
No	0	21	21 (15%)
Not eligible (non-A2)	0	45	45 (32%)

Pretreatment Factors Versus Response

	Total	PR	CR	PR+CR	p value
Sex					
M	92	15	2	17 (18%)	0.39
Age					
Median (Range)	50 (21-69)			54 (35-67)	0.03*
ECOG					
0	97	16	3	19 (20%)	0.14
1-2	42	4	0	4 (9%)	
HLA	94	14	3		0.63
A*201	45	6	0	17 (18%)	
nonA2				6 (13%)	
Prior Therapy					
None	20	3	2	5 (25%)	0.33
HD IL-2	44	5	0	5 (11%)	0.26
IFN α-2b	62	5	0	5 (8%)	0.016
Biochemotherapy	29	3	1	4 (14%)	0.78
Chemotherapy	33	6	0	6 (18%)	0.77
Other Biologic	42	10	0	10 (24%)	0.13
2 or more	63	7	0	7 (11%)	0.12
Sites of Disease					
No evidence of visceral disease	30	6	0	6 (20%)	0.58
Presence of visceral disease	109	14	3	17 (16%)	
No evidence of brain metastases	129	18	2	20 (15%)	0.37
Presence of brain metastases	10	2	1	3 (30%)	
Stage of Disease					
M1a	20	5	0	5 (25%)	0.35
M1b	30	5	0	5 (17%)	
M1c	89	10	3	13 (15%)	

* p>0.05 in logistic regression analysis after adjusting for any IRAE and prior interferon

Treatment Factors Versus Response

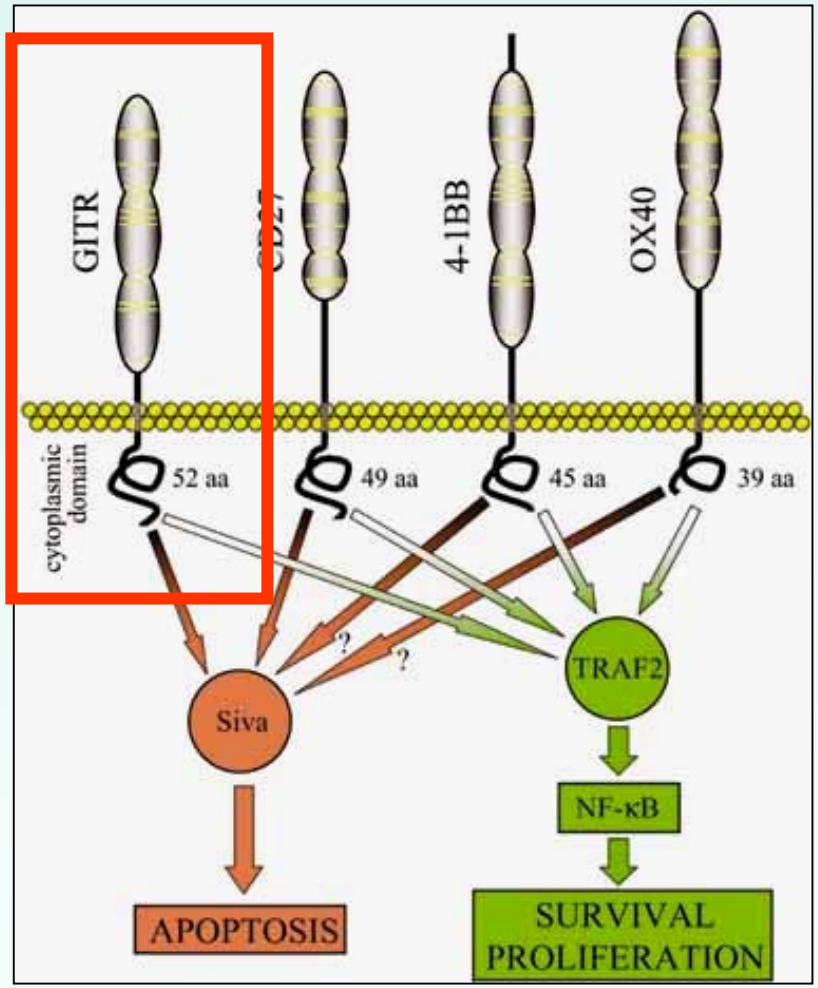
	All	NR	PR+CR	p value
Protocol				
Trial 1	54	47	7 (13%)	0.36
Trial 2	85	69	16 (19%)	
Peptide†		60	13 (18%)	1.00 *
Yes	73	17	4 (19%)	
No	21	39	6 (13%)	
Non-HLA A*0201	45			
# cycles ^				
Median (range)	4 (1-10)	4 (1-10)	6.5 (3-10)	<0.0001
1-2	25	25	0 (0%)	
3-4	25	23	2 (8%)	
5-6	23	17	6 (26%)	
7-8	7	3	4 (57%)	
> 8	5	1	4 (80%)	
Total Dose ^ (in mg/kg)				
Median (range)	28 (3-70)	23 (3-70)	35.5 (19-70)	0.0005

† Within Trial 2, 6 of 19 (32%) patients receiving peptides were responders (p=0.18)
* Non-HLA A*0201 patients excluded
^ Due to different dosing strategies, # cycles and total dose only compared for patients in Trial 2 (n=85)

Agonist Anti-GITR Ligand and mAb

- Alan N. Houghton, M.D.
- Ludwig Chair in Clinical Investigation, Member and Attending Physician
- Memorial Sloan-Kettering Cancer Center
- Professor of Medicine and Immunology
- Weill Medical College of Cornell University

GITR Glucocorticoid–Induced TNF Receptor Family Related Protein



- Expressed constitutively at high levels by regulatory T cells (CD4+CD25+Foxp3+ T cells, Tregs)
- Expressed minimally by naïve CD4+ and CD8+ T cells
- Upregulated following T-cell activation

From Nocentini and Riccardi, Eur J Imm 35:1016, 2005

Signaling through GITR

1. Abrogates suppressive activity of CD4+CD25+Foxp3+ Tregs *in vitro*
2. Co-stimulatory for effector CD4+ and CD8+ T cells
3. Induces resistance of effector T cells to Treg suppression
4. Enhances tumor immunity and rejects tumors

Anti-GITR Agonists

Experimental agents

- Agonist monoclonal antibody
- Agonist GITR ligand-immunoglobulin Fc domain fusion molecule

Potential clinical agents

- Chimeric or humanized agonist mAb
- Recombinant GITR ligand (e.g., multimer with leucine zipper, Ig Fc

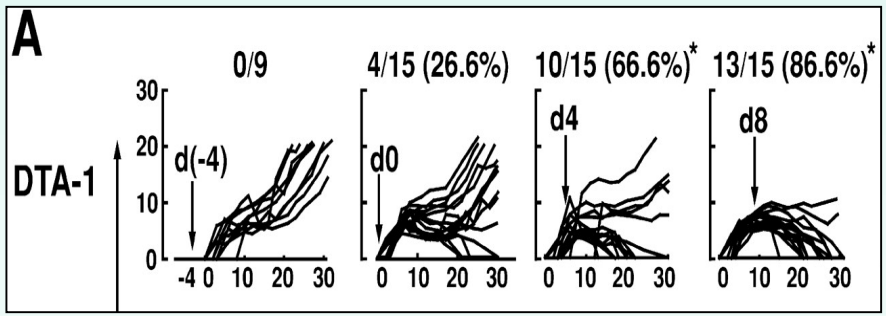
Anti-GITR Agonists

- GITR expressed by tumor-infiltrating CD4+ Foxp3+ regulatory T cells
- GITR signaling in tumor-infiltrating Tregs markedly reduces or abrogates suppressive activity
- GITR upregulated in CD8+ and CD4+ T cells early in T-cell activation
- GITR signaling co-stimulates activated CD8+ and CD4+ T cells and NKT cells, including self-reactive T cells against cancer antigens
- GITR signaling may induce resistance of T cells to suppression by Tregs

Anti-GITR Agonists

- GITR ligation promotes immune responses to cancer antigens through suppression of Tregs and co-stimulation of effector T cells

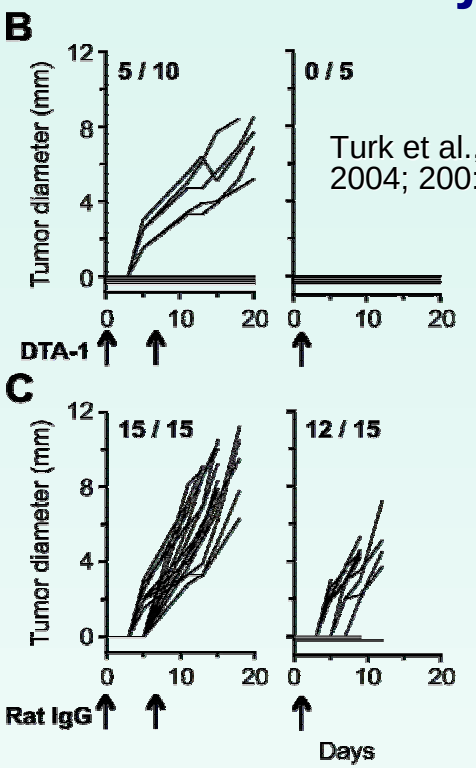
- Directly induces cancer immunity



- Synergizes with anti-CTLA-4 blockade therapy

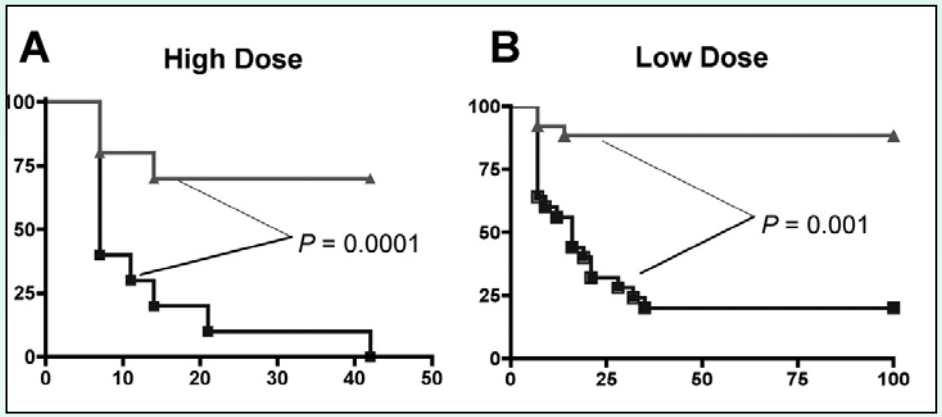
- Augments cancer immunity in combination with vaccines against cancer antigens

Tumor Rejection with Anti-GITR mAb Alone



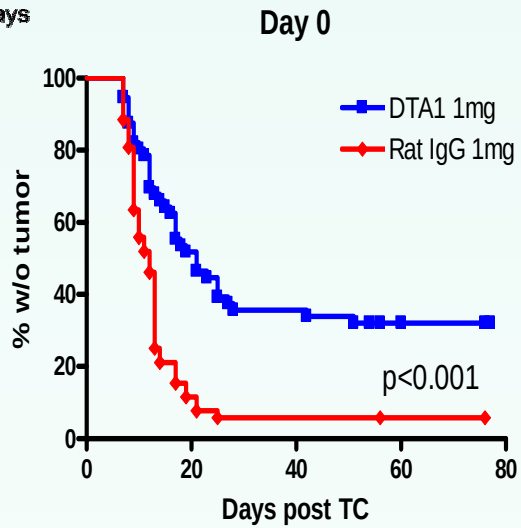
Turk et al., JEM 2004; 200:771

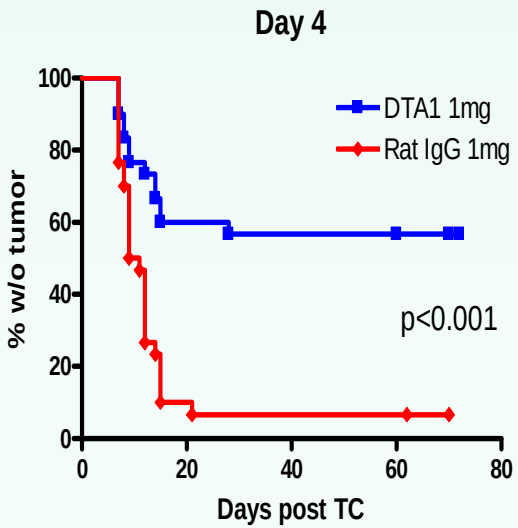
B16 melanoma



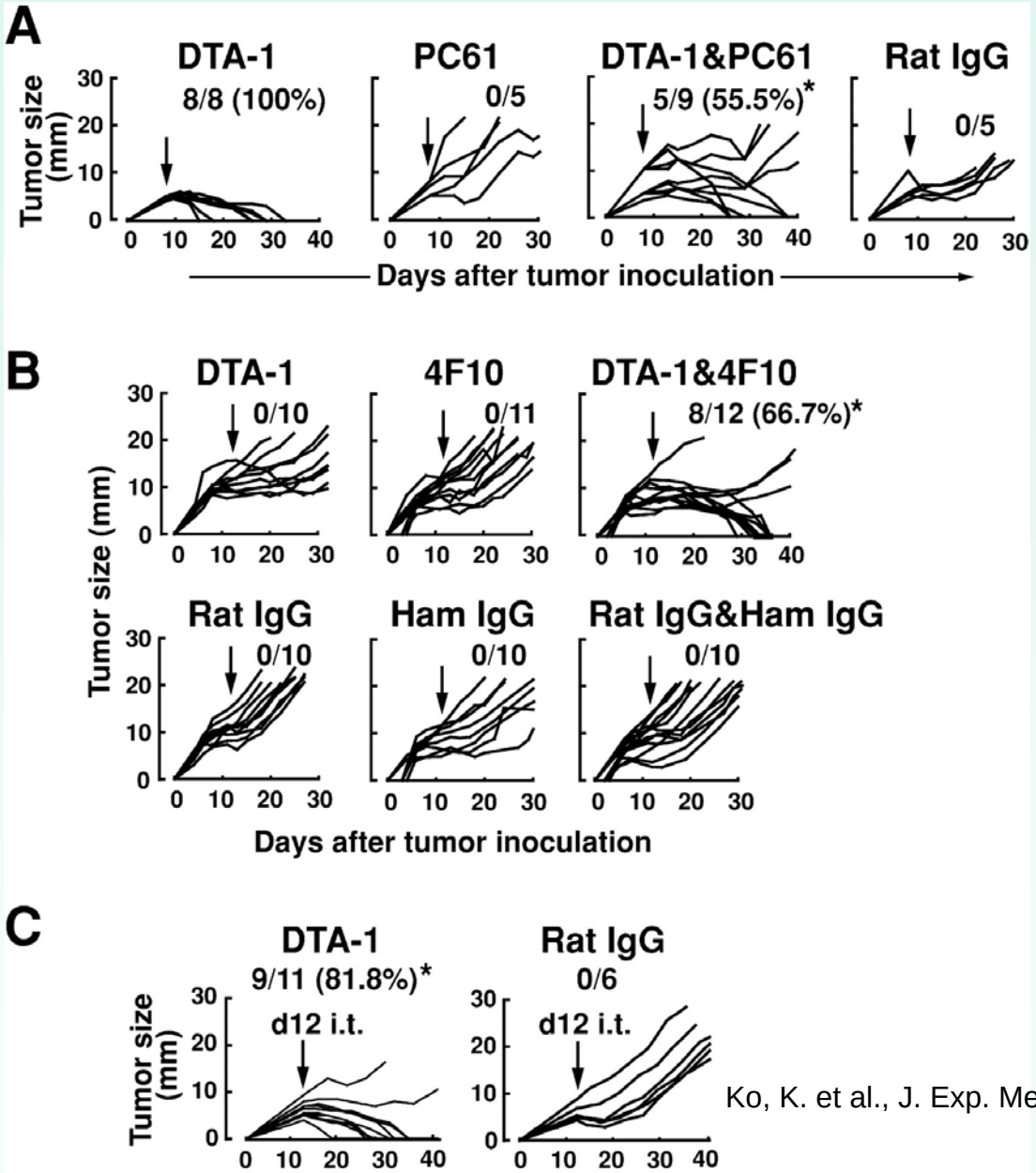
Ramirez-Montagut et al., J Immunol 2006;176: 6434

Cohen, Houghton unpublished





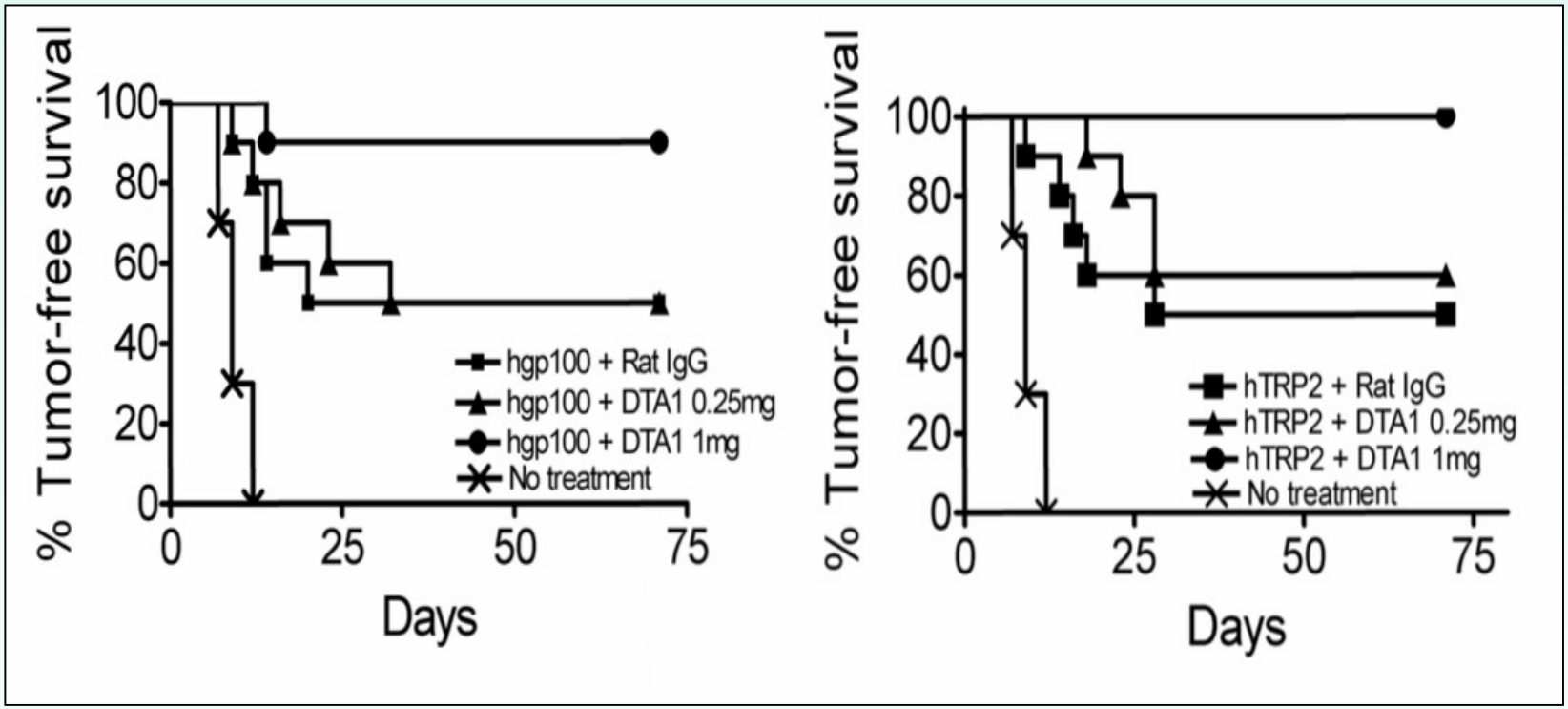
Synergistic Antitumor Effect of Anti-GITR and Anti-CTLA-4, but Not Anti-GITR and Anti-CD25



Ko, K. et al., J. Exp. Med. 2005;202:885-891

JEM

Additive or Antitumor Effects with DNA Vaccination



Cohen, et al., Cancer Res 2006; 66:4904

Anti-GITR Agonists

- Preclinical data
  - Efficacy in animal models
  - Safety issues
  - Can exacerbate autoimmunity in animal models, e.g., colitis, arthritis, vitiligo, and atopy

Agent Name: Clinical Summary

None

Agent Name: Contemplated Uses

- Systemic therapy alone or in combination
- Potential application across multiple tumor types (not sure about use in GITR+ hematopoietic malignancies)
- Established disease and adjuvant therapy
- Combinations with vaccines, CTLA-4 blockade, and chemotherapy

Agent Name: Perceived Need

Potential application in combination with:

- CTLA-4 blockade and other immune modulation
- Cancer vaccines
- Chemotherapy and radiation therapy

Comparison of Agents

- Agonist anti-GITR monoclonal antibody (Tolerx, other?)
- GITR ligand/Ig Fc (Academic investigators)
  - Strong agonist properties, non-antagonist
  - Protein folding, avidity for receptor
  - Pharmacokinetics, pharmacodynamics

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Anti-OX40 Ligand and mAb

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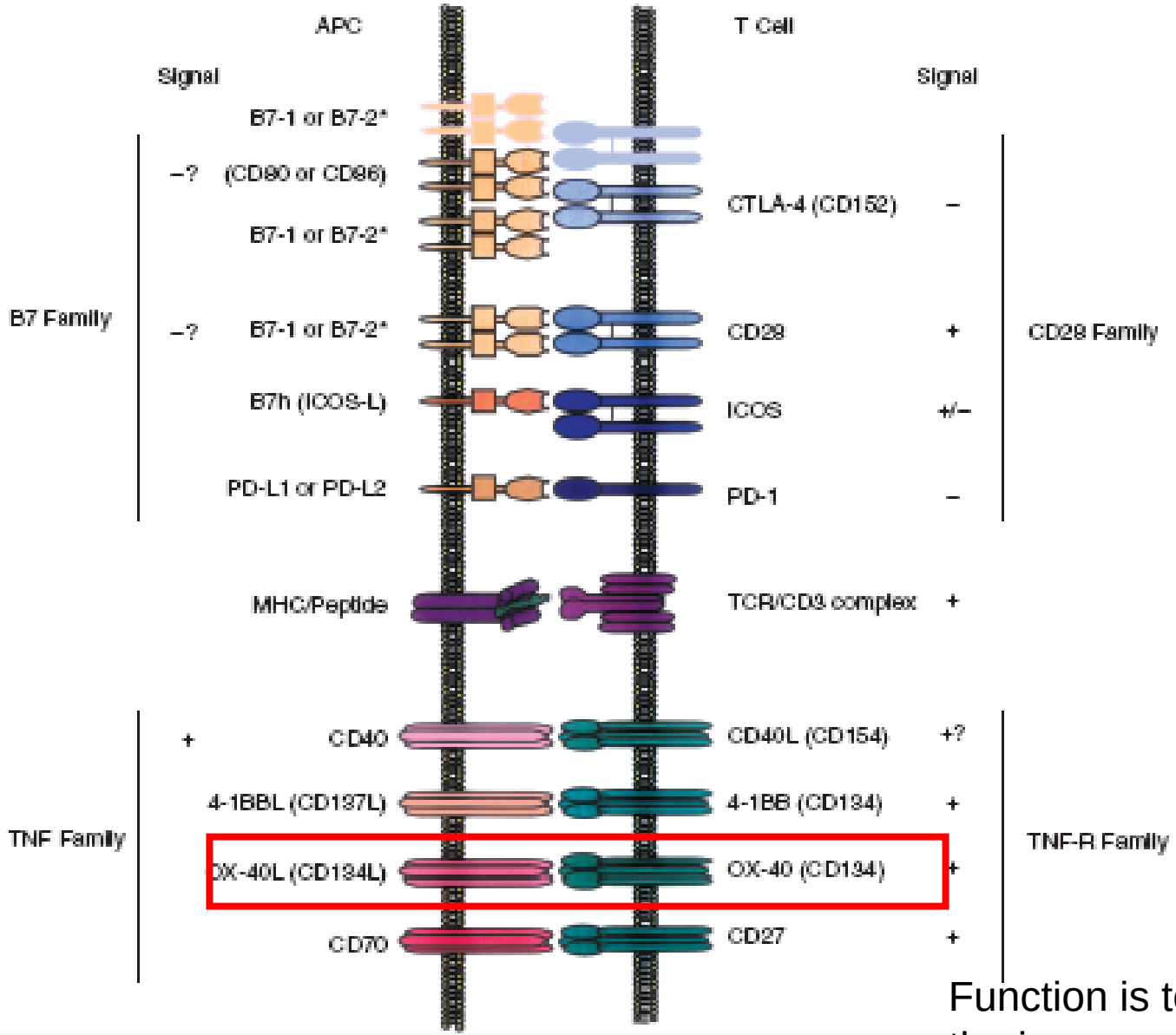
- Ludwig Chair in Clinical Investigation, Member and Attending Physician
- Memorial Sloan-Kettering Cancer Center
- Professor of Medicine and Immunology
- Weill Medical College of Cornell University

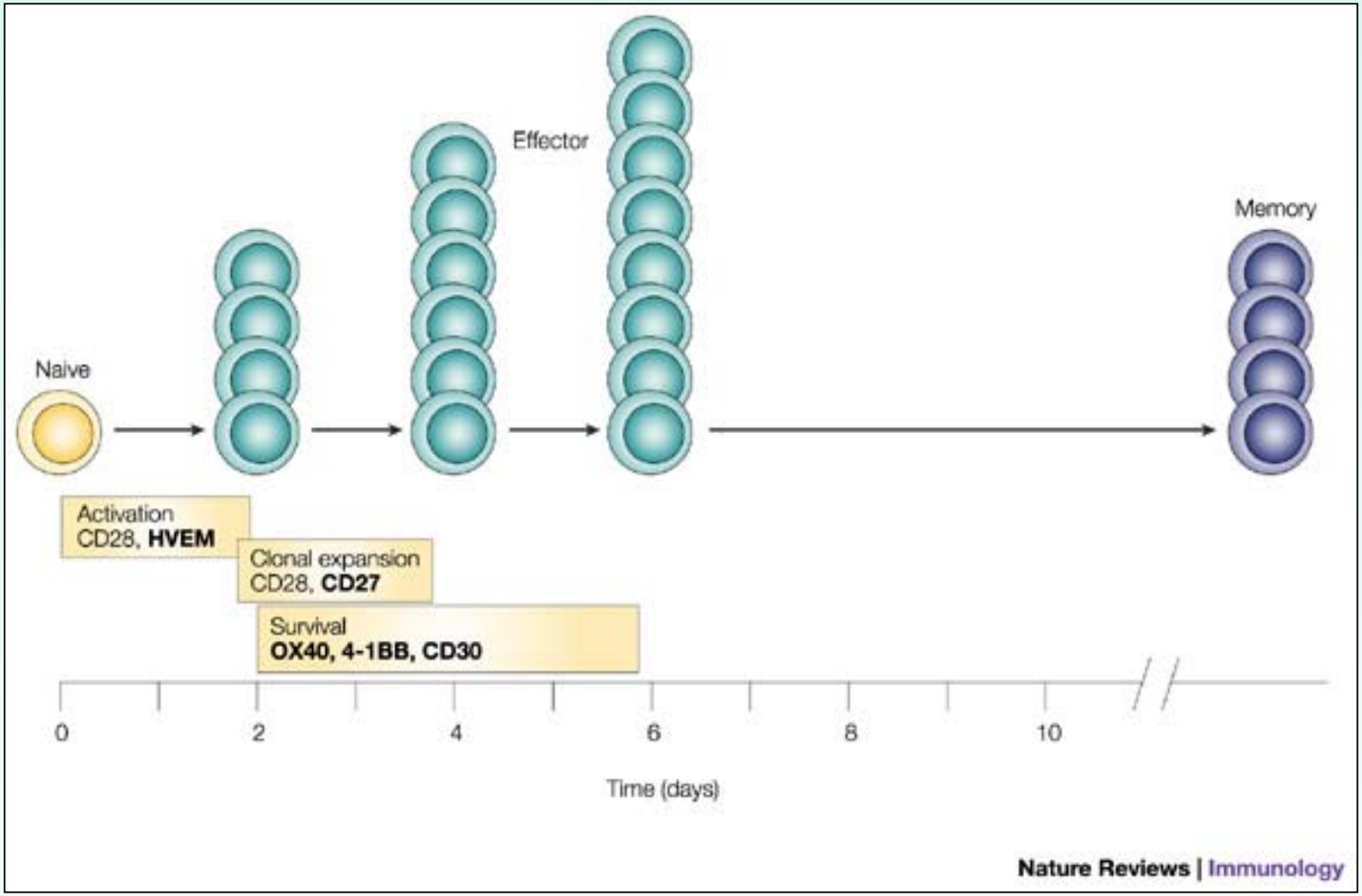
Agent Name: Anti-OX40 (CD134)

- Co-stimulatory receptor for CD4+ (and CD8+) T cells
- Signaling for T-cell survival, generation of memory T cells, and reactivation of memory T-cell responses
- Inhibition of suppression by regulatory T cells (Tregs) *in vitro*



Function is to “fine tune” the immune response

Rothstein and Sayegh, Immunol Rev. 2003 Dec;196:85-108



Croft M. Nat Rev Imm 3:609, 2003

Anti-OX40: Preclinical

Table 3 | **Immunotherapy of tumours through targetting TNFR co-stimulation**

TNFR molecule targeted	Method	Tumour type	T-cell response augmented*	References
HVEM	Tumour transfection with LIGHT	Breast carcinoma	CD8/CD4 ?	121
	Tumour transfection with LIGHT	Mastocytoma	CD8 and CD4	28
CD27	Tumour transfection with CD70	Sarcoma	CD8/CD4 ?	39
	Tumour transfection with CD70	Mastocytoma	CD8	122
	Tumour transfection with CD70	Adenocarcinoma	CD8/CD4 ?	40
OX40	Agonist OX40L–Ig fusion protein and OX40-specific antibody	Sarcoma, melanoma	CD4	123
	Agonist OX40-specific antibody	Sarcoma, glioma	CD4 and CD8	124
	Agonist OX40-specific antibody	Colon carcinoma	CD4	125
	Tumour transfection with OX40L	Colon carcinoma	CD4 and CD8	126
4-1BB	Agonist 4-1BB-specific antibody	Sarcoma, mastocytoma	CD8 and CD4	55
	Tumour transfection with 4-1BBL	Sarcoma, mastocytoma	CD8	56
	Tumour transfection with 4-1BBL	Lymphoma	CD8	58
	Agonist 4-1BB-specific antibody	Melanoma	CD4/CD8	127
	Tumour transfection with 4-1BBL	Squamous cell carcinoma	CD8	128
	Agonist 4-1BB-specific antibody	Lung carcinoma, melanoma	CD8	129
	Tumour transfection with Fv of 4-1BB-specific antibody	Melanoma	CD4	130

All mouse studies, carried out by tumour-cell transfection with tumour-necrosis factor (TNF) ligands or injection of agonist TNF receptor (TNFR) reagents. *Indicates which T-cell response was enhanced. ?, not clear whether $CD4^+$ or $CD8^+$ T-cell responses are enhanced. HVEM, herpes-virus entry mediator; Ig, immunoglobulin; L, ligand.

Croft M. Nat Rev Imm 3:609, 2003

Anti-OX40: Preclinical Summary

- Safety issues
  - Autoimmune sequelae, exacerbation of atopy
  - Rhesus macaque study
    - Well-tolerated
    - Enlarged gut-associated lymph nodes, splenomegaly, resolved over 28 days
    - Increased antibody titers and T-cell responses against SIV gp130 following immunization

Agent Name: Clinical Summary

- Phase I and II data
  - Mouse mAb in phase I clinical study at Providence Cancer Center, Portland, Oregon
- Phase III data
  - None

Agent Name: Contemplated Uses

- Systemic therapy for established cancers
- Adjuvant therapy

Agent Name: Perceived Need

- Systemic therapy against multiple cancer types
- Combination therapy with vaccines, other immune modulators, and chemotherapy

Comparison of Agents

- Agonist anti-OX40 mAb
- Agonist multimeric OX40 ligand agent (e.g., fusion with leucine zipper-immunoglobulin Fc domains)
- Relative agonist versus antagonist properties
  Protein folding, avidity for receptor
  Pharmacokinetics, pharmacodynamics

Anti-OX40: Preclinical/Clinical Development

- Andy Weinberg, Walter Urba, and colleagues at Providence Cancer Center, Portland
- Institute of Biotechnology and Clinical Immunology Research Laboratory of Jiangsu Province, Soochow University
- Celtic Pharmaceutical Holdings, L.P., [a holding company in Bermuda which acquired a monoclonal antibody from Xenova]
- Genentech and CellTech antagonist anti-OX40 agents (mAb, OX40 ligand) for autoimmune diseases

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PD-1 (Programmed Death-1)

- Jeffrey Weber, M.D., Ph.D.
- Professor of Interdisciplinary Oncology and Head, Donald A. Adam Comprehensive Melanoma Research Center
- H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida

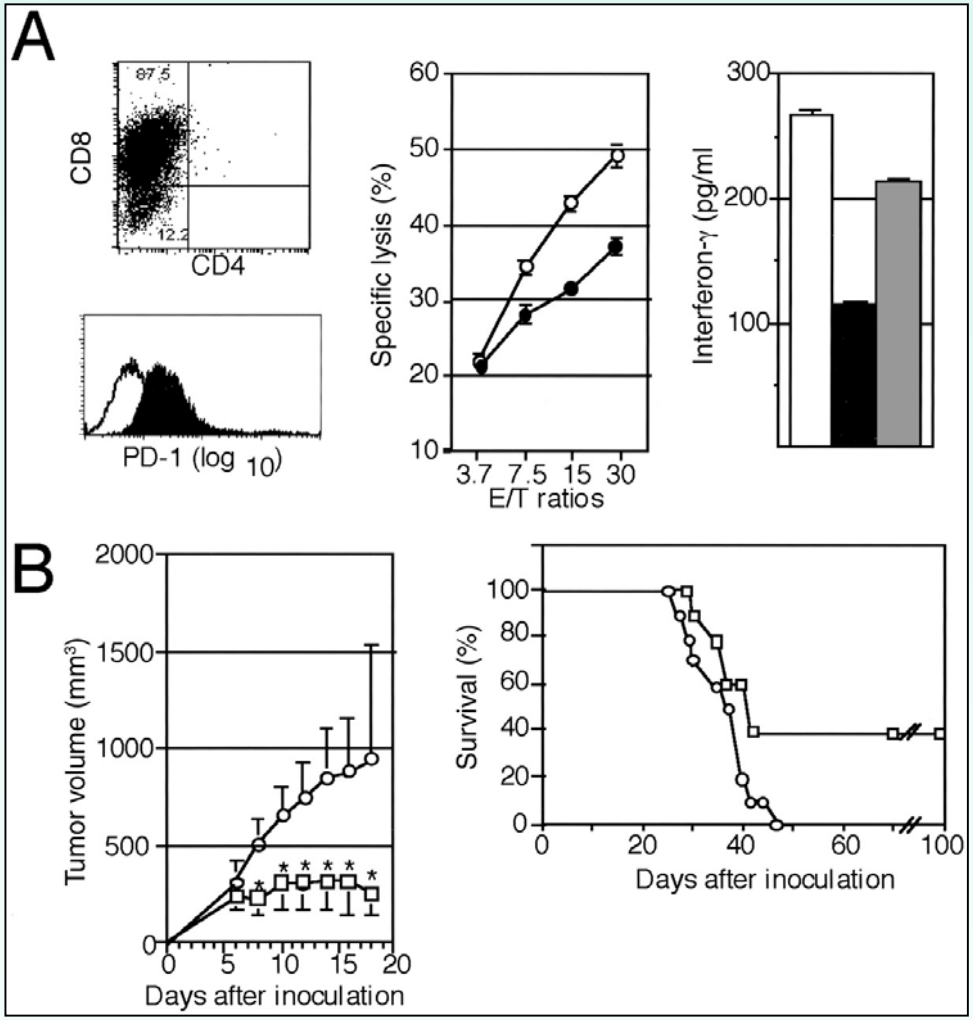
PD-1 Antibody: Background

- PD-1 is a receptor that is a member of the immunoglobulin superfamily
- Structurally related to CTLA-4 and CD28
- The abrogating antibody is a human IgG4
- PD-1 is up-regulated on activated T and B cells and monocytes
- It binds to PD-L1 on T and B cells, macrophages, and DC, as well as parenchymal and tumor cells
- PD-L2 is only on DC and macrophages

PD-1 Antibody: Preclinical Summary

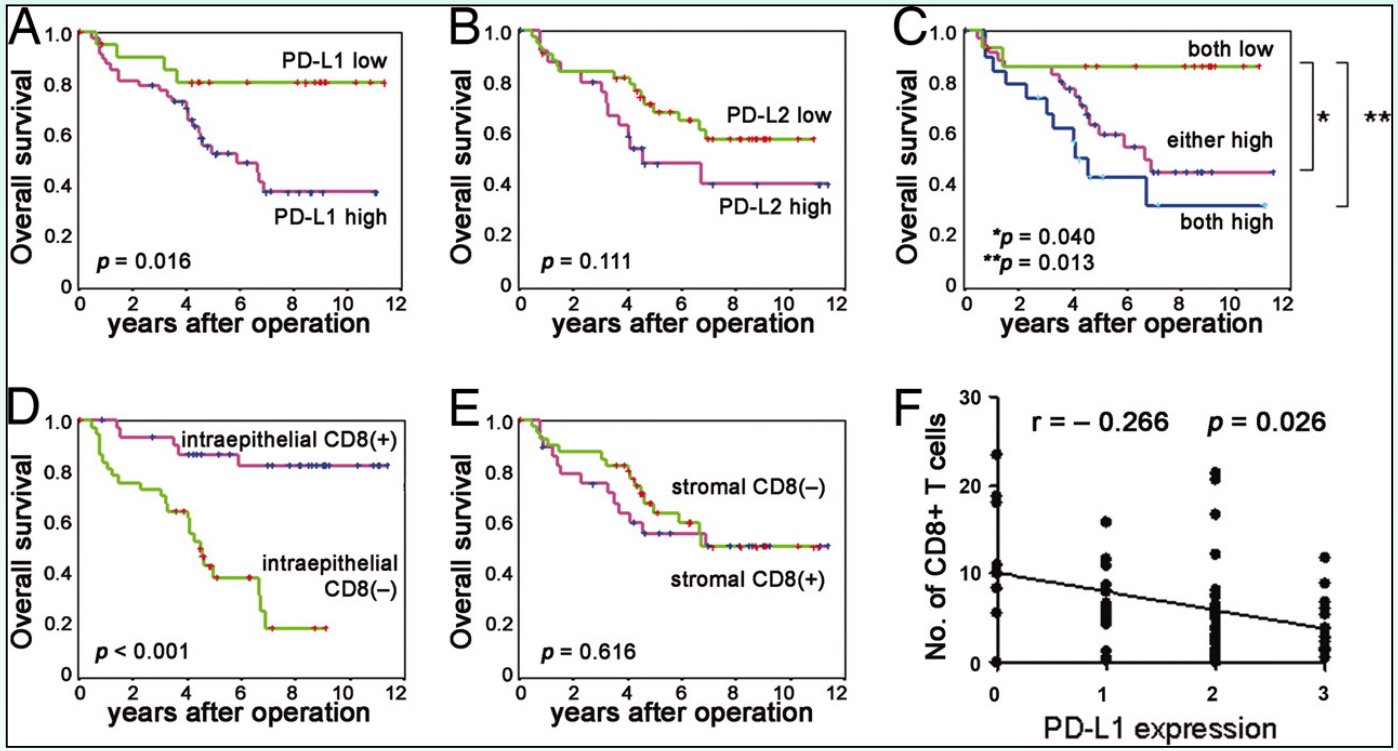
- PD-1 is a negative regulator of T-cell function
- It is implicated in tolerance induction in mice
- PD-L1 expression on many human tumors is associated with a reduced T-cell infiltrate and a poorer prognosis; not so for PD-L2
- Blockade of PD-L1 and also PD-1 in murine tumor models results in long-lasting tumor regression
- PD-L1 expression by tumors appears to “protect” them from immune attack by CTL

Effects of Anti–PD-L1 on P815-PD-l1 Tumor Growth in Mice



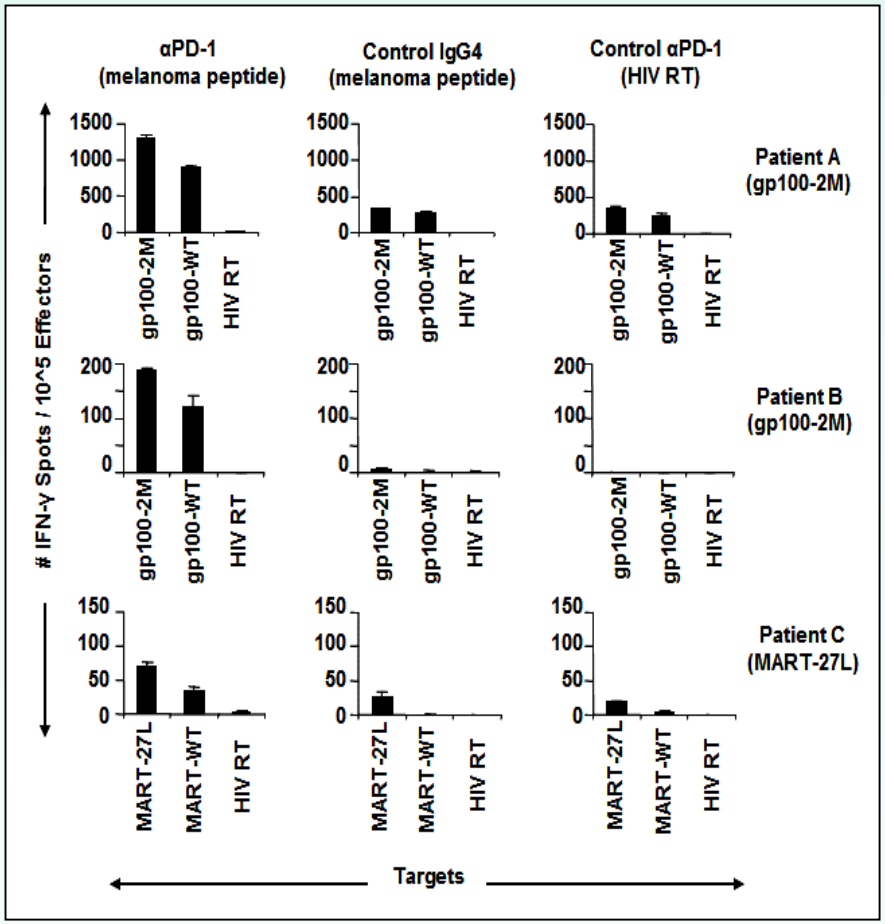
Iwai, Yoshiko et al. (2002) Proc. Natl. Acad. Sci. USA 99, 12293–12297

Overall Survival Analyses of Patients with Ovarian Cancer According to the Expression of PD-Ls and the Presence of Tumor-Infiltrating CD8+ T Lymphocytes

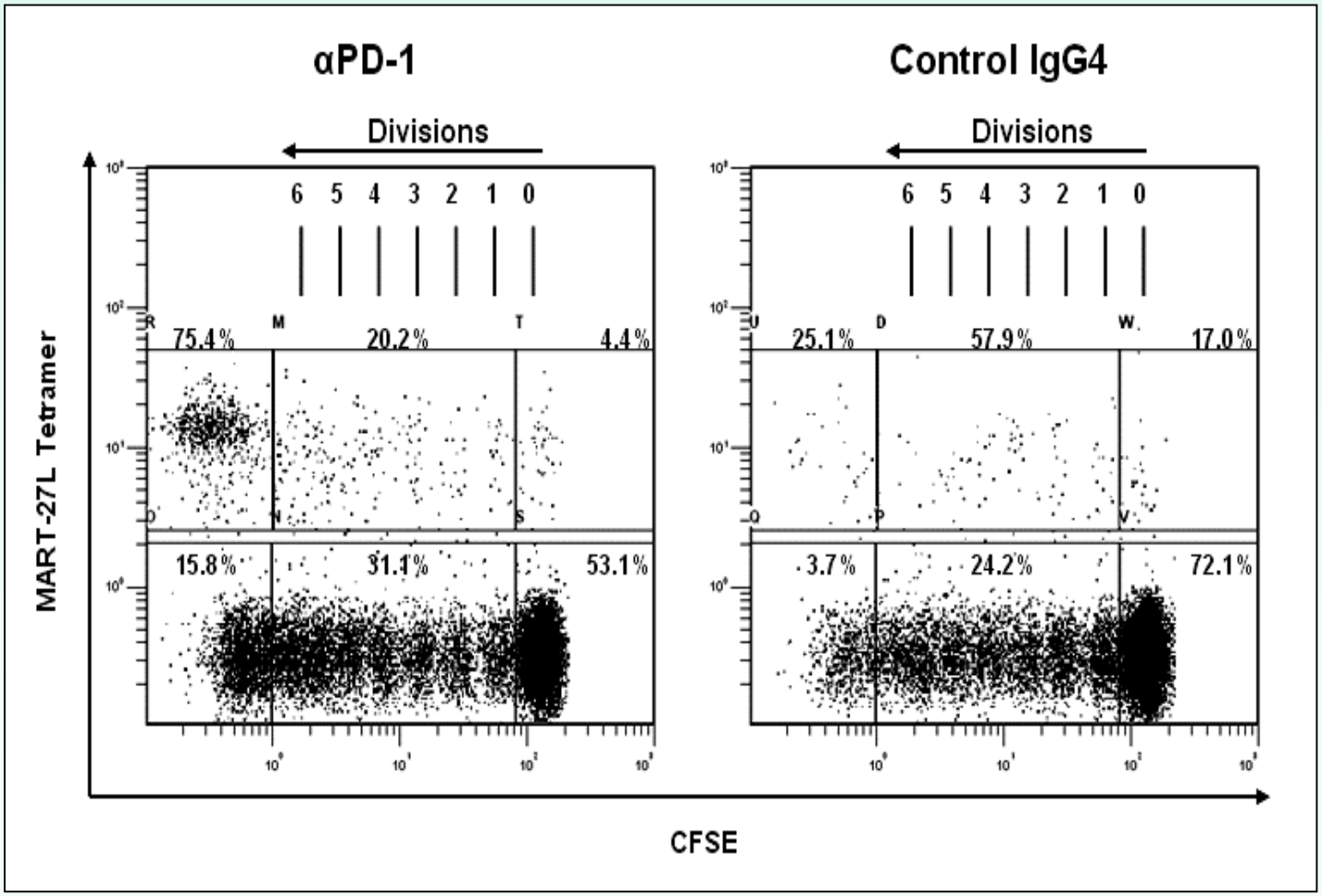


Hamanishi, Junzo et al. (2007) Proc. Natl. Acad. Sci. USA 104, 3360–3365

PD-1 Abrogation Increases Numbers of Functional Cytokine Secreting CTL



Anti–PD-1 Ab Increases Melanoma Specific CTL Proliferation by CFSE



Anti–PD-1: Clinical Summary

- Phase I data:
  - First-in-human trial started in colon cancer based on promising *in vivo* murine data
  - Dosing has been at 0.3 and 1 mg/kg
  - Single dosing escalation will continue to MTD
- Phase II study planned, awaits definition of MTD and assessment of toxicities

Anti–PD-1: Contemplated Uses

- Pre-clinical data suggest that squamous esophageal, colon, lung, and ovarian cancer as well as melanoma should be targets for interruption of the PD-1/PD-L1 axis; they express high levels of PD-L1 by IHC
- Anti–PD-1 alone, anti–PD-1 with a vaccine, and anti–PD-1 with anti–CTLA-4 are reasonable possibilities

Anti–PD-1: Perceived Need

- If PD-1 up-regulation is shown to be as common on activated tumor-specific T cells as is suspected, then T-cell “exhaustion” as in Rafi Ahmed’s HIV work may be a common immune suppression mechanism in melanoma and other cancers
- PD-1 abrogation may be an important way to disinhibit antitumor T-cell immunity

Comparison of Anti–PD-1 to Anti–PD-L1 Blockade

- Anti–PD-L1 antibody with blockade at the tumor site may also be useful
- Targeting the tumor may be difficult if saturation of tumor tissue is needed
- Anti–PD-L1 may alter parenchymal tissue and increase its recognition, increasing autoimmunity
- PD-1 not highly expressed on resting cells

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BMS663513, Human Agonistic Anti–CD137 (Anti–4-1BB) Antibody

- Kim Margolin, M.D.
- Associate Director
- Clinical Research, Division of Medical Oncology, City of Hope, Duarte, CA

BMS663513 Background (1)

- Category of drug
  - Nonblocking functional MoAb to CD137/4-1BB
  - Co-stimulation/proliferative/anti-apoptotic
- Molecular characterization of agent
  - Fully human IgG4, T cell EC_{50} ~ 1 μg/mL
  - $T_{1/2}$ ~360 hours
- Target = CD137
  - TNF-superfamily receptor
  - Receptor on activated T (all), NK, NKT, innate
  - Receptor not on tumors
- Ligand = TNF-like glycoprotein on T, APC

BMS663513 Background (2)

- Biology of target
  - Adaptive
    - Induced upon activation of T cell (3rd signal)
    - Signaling via NFκB, JNK/SAPK, p38
    - ↑ Th1 secretion of GM-CSF, γ-IFN (→IDO), ↑ cytotoxic granules
    - ↓ apoptosis, AICD; ↑ anti-tumor effects
  - Innate
    - Induced upon cell-specific activating signal
    - ↑ secretion of IL-6, IL-12
    - ↑ APC function, expression of class I, co-stim molecules
- Biology of agent-target interaction
  - Enhance activation
    - Suboptimal→optimal (memory response > primary effector response)
    - Effects on other cells system-dependent; autoimmune modulation (B cell/Ab suppressive effects) under active parallel investigation
  - Essential role of γ-IFN
    - γ-IFN KO host
    - Neutralizing Ab to γ-IFN

BMS663513: Preclinical Summary (1)

- *In vitro* co-stimulation of anti-CD3 suboptimally stimulated T cells
- Vax: enhanced cellular response to SIV gag DNA
- *In vivo* tumor models with murine anti-CD137
  - Cures in P815 (immunogenic), AG104 (poorly) 3- and 12-day models
  - P815, EMT-6 regressors had memory response to re-exposure
  - Enhanced anti-tumor effect of whole tumor cell vax (several lines)
  - Synergism with RT in M109 lung and EMT mammary tumors

BMS663513: Preclinical Summary (2)

- Safety/toxicity
  - Murine—hepatic target related to NKT cell predominance in liver
    - Hepatic necrosis, inflammatory infiltrate
    - Elevated transaminases
  - Monkey
    - Minimal toxicity up to 10 mg/kg dose
    - Occasional mild colitis possibly related to high number of activated lymphocytes in intestinal mucosa

BMS663513: Clinical Summary

- Phase I study—single-agent (BMS CA186001)
  - Study design: multiple-dose Rx, patients with advanced CA
    - Primary objective: safety, MTD
    - Secondary objectives: PK, exploratory biomarkers, activity screen
  - Therapy
    - Doses 0.3, 1, 3, 6, 10, 15 mg/kg q 3 wks x 4
    - Retreatment for CR, PR, SD (“with benefit”)
  - MTD/RP2D
    - Doses through 10 mg/kg safe with activity in melanoma across levels
    - Drug (dose)–related toxicities: hepatic, skin, neutrophils
  - Preliminary biomarker data show CD8, Treg, NK cell effects
- Phase II randomized screening (extension of CA186001)
  - Doses 1, 3, 10 mg/kg on same schedule
  - Histologies: melanoma, renal CA, ovarian CA 30 patients/3 doses each

BMS663513: Contemplated Uses

- Ongoing
  - Screening single-agent Rx in 3 advanced CAs—broad net, high bar
  - Combinations with approved Rxs
    - Phase I with paclitaxel and carboplatin (BMS CA186004)—active
    - Phase I with RT or chemoradiotherapy (BMS CA186005)—pending
- Anticipated
  - Ag-specific strategies
  - Combinations with cytokines
  - Autoimmune modulation—implications for combinations (e.g., with CTLA-4Ab)

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B7-H1 Antagonist

- Walter J. Urba, M.D., Ph.D.
- Director, Cancer Research
- Earle A. Chiles Research Institute
  Providence Portland Medical Center

B7-H1 Antagonist: Background (1)

- Anti-checkpoint
- Agent is an antibody to B7-H1
- Target—B7-H1 (PD-1 ligand)
- Blocks B7-H1: PD-1 interaction
- PD-1 expressed on activated CD4, CD8, NK cells, and monocytes

B7-H1 Antagonist: Background (2)

- B7-H1 present on many normal tissues/cancers
- B7-H1 negatively regulates immune responses (inhibits T-cell proliferation and cytokine production)
- B7-H1 expression increased by IFNγ
- Blockade B7-H1/PD-1 enhances T-cell immunity

B7-H1 Antagonist: Preclinical Summary (1)

- Blockade enhances autoimmunity—diabetes mellitus, colitis, and EAE
- Blockade disrupts fetal maternal tolerance and increased abortion rate
- Blockade boosts viral immunity
- Minimal effects when anti-B7-H1 is administered alone in murine tumor models
- Most active when combined with other immunotherapy (anti-CD137)

B7-H1 Antagonist: Preclinical Summary (2)

- Blockade enhances type 1 immune responses
- Blockade improved long-term memory
- Blockade increased expansion of tumor-specific T cells for adoptive immunotherapy
- Strong correlation of B7-H1 expression on human tumor cells and survival (RCC and esophageal)

B7-H1 Antagonist: Clinical Summary

- Human anti-B7-H1 antibody produced by Medarex
- No clinical data

B7-H1 Antagonist: Contemplated Uses

- Possible activity as a single agent
- Combination therapy with vaccines and other immunomodulatory agents
- Enhances T-cell expansion *ex vivo* for adoptive immunotherapy
- Potential as prognostic or predictive tool

B7-H1 Antagonist: Perceived Need

- Potentially useful for any cancer type
- Most useful in combination with other agents, T-cell transfer
- Likely to be useful to multiple investigators

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B7-H4 Antagonist

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- Earle A. Chiles Research Institute
  Providence Portland Medical Center

B7-H4 Antagonist: Background (1)

- Anti-checkpoint
- Agent is an antibody to B7-H4
- Target—B7-H4
  - Identified by search human cDNA ESTs with homology for Ig V and C domains of B7-1,2
  - Structure similar to B7-1,2 but lacks binding sequences for CTLA-4 or CD28
  - Expressed on multiple nonlymphoid tissues
  - Expressed on activated T, B, M, DC, and particularly tumor–associated macrophages (TAMs)
  - Highly expressed in variety of cancers

B7-H4 Antagonist: Background (2)

- Biology of target
  - B7-H4 binds to an unknown receptor on activated but not naïve T cells
  - Negatively regulates T-cell immunity in peripheral tissues: may regulate tolerance and promote evasion of tumor immunity
- Biology of agent/target intervention
  - Anti-B7-H4 restores proliferation and cytokine production by activated T cells
  - Antibody blockade increases allogenic CTL activity
  - Soluble B7-H4Ig inhibits T-cell responses *in vivo* (not suitable for blockade)

B7-H4 Antagonist: Preclinical Summary

- T-regulatory cells enable APC suppressive activity by increasing B7-H4 expression (IL-10 dependent
- B7-H4 blockade (antisense oligos) reduced suppressive activity of Treg-conditioned APCs
- B7-H4^{+} tumor-associated macrophages suppress tumor antigen-specific T-cell immunity (IL-6/IL-10)
- GM-CSF/IL-4 inhibits B7-H4 expression
- B7-H4 blockade increased T-cell proliferation and reduced tumor volumes *in vivo*

B7-H4 Antagonist: Clinical Summary

- Human anti-B7-H4 antibody produced by Medarex
- No clinical data

B7-H4 Antagonist: Contemplated Uses

- Possible single agent activity
- Use in combination with vaccines or other immunomodulatory agents

B7-H4 Antagonist: Perceived Need

- Potentially useful for any cancer type
- Most useful in combination with other agents
- Likely to be useful to multiple investigators

Comparison of Agents B7-H1 Antagonist vs. B7-H4 Antagonist

- Both agents would be potentially beneficial
- B7-H1 blockade has more supportive preclinical data
- B7-H4 blockade has added benefit of possibly interfering with Treg function
- Lieping votes for antibodies to B7-H1

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Anti–IL-10 Abs

- Theresa L. Whiteside, Ph.D., ABMLI
- Professor and Laboratory Director
- University of Pittsburgh Cancer Institute

Anti–IL-10 Abs: Background

- For effects in cancer only pre-clinical data available
- Used clinically for therapy of SLE and rheumatoid arthritis
- Potential clinical use based on neutralization of IL10 activities. IL-10 is known to:
  - Exert direct growth-inhibitory effects on tumor cells *in vitro* and *in vivo*
  - Be a growth factor for B lymphoma and melanoma cells
  - Stimulate as well as suppress immune cells

Anti–IL-10 Abs: Background

- IL-10 is produced by tumor cells, B cells, TAM, TIL, and Treg in tumors/cancer patients' blood
- IL-10 signals through STAT1 and STAT 3 in most cells (STAT 3 is constitutively activated in tumor cells) but also involves other signaling pathways
- Anti–IL-10 Abs that neutralize human IL-10 may be a "**double sword**"
- *In vitro,* Anti–IL-10 Abs sensitize tumors to chemotherapeutic drugs, suggesting that IL-10 is a anti-apoptotic/protective factor possibly acting via Bcl-2 modulation
- Studies with the NZB/NZW murine SLE model: continuous administration over time of Anti–IL-10 Abs to NZB/W F1 mice protected the animals from autoimmune disease development and prolonged their survival; IL-10 administration accelerated the onset of autoimmunity

Anti–IL-10 Abs: Clinical Studies

- In an open pilot study, Anti–IL-10 Abs (e.g., murine IgG1Ab B-N10) were given to 6 steroid-dependent SLE patients by daily IV infusions for 21 days (20mg/day)
- Monitoring on days 1, 7, 14, and 21 was complete and included immunologic evaluations (e.g., HAMA, B-N10, and IL-10 plasma levels)
- Therapy was safe; no SAE
- Clinical improvements observed in all patients: SLE was inactive in 5/6 patients after therapy; the SLEDAI score decreased ($p < 0.001$), and patients required less corticosteroids ($p < 0.005$)
- MAb plasma levels exceeded those of IL-10 at all time points after 9 days of treatment: clinical improvement likely to be due to neutralization of endogenous IL-10
- IL-10 serum levels remained elevated above those reported for normal individuals, but patients' PBMC showed decreased release of IL-10 after therapy
- This is the first report of Anti–IL-10 Ab therapy in humans

Anti–IL-10 Abs: Contemplated Uses

- It may be first necessary to separate immunosuppressive from immunostimulatory activities of IL-10 before contemplating the use of antagonists
- Potential use in the sensitization of resistant tumors to chemotherapeutic drugs
- Potentially useful in:
  - Elimination of Treg
  - Direct inhibition of tumor proliferation
  - Up-regulation of antigen processing in APC
  - Down-regulation of tumor-associated inflammation
  - Elimination of tumor escape

Anti–IL-10 Abs: Perceived Need

- Potential uses of humanized clinical-grade Anti–IL-10 Abs would encompass many different settings and tumor types
- Such Abs could potentially be used in multiple therapy regimens
- There is great interest and potential need for such Abs by multiple independent clinical investigators

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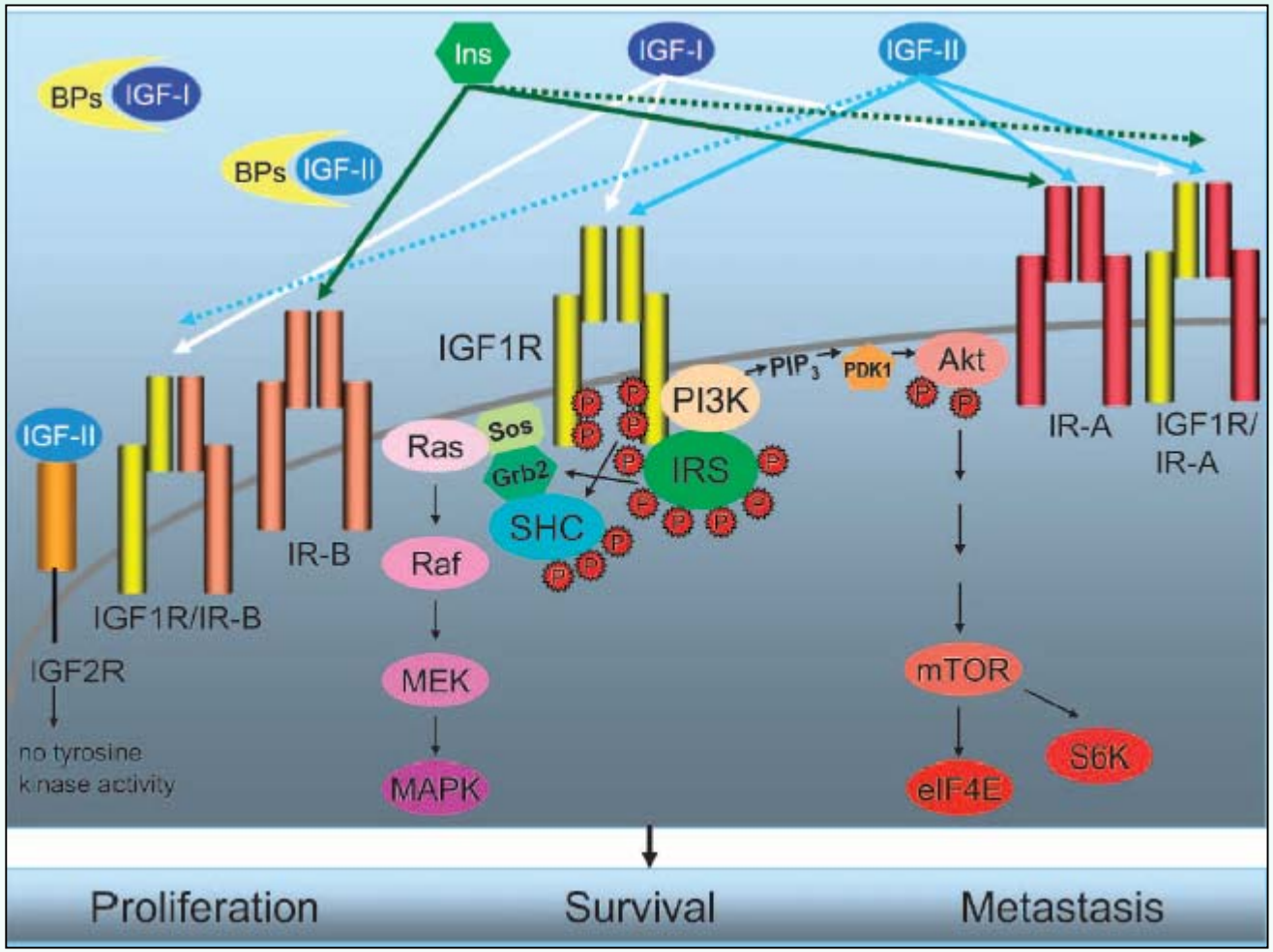
Anti-IGFII

- Nora L. Disis, M.D.
- Professor/Member
- University of Washington/Fred Hutchinson Cancer Research Center

Anti-IGFII: Background



IGFII

- Binds IGF1R
- Binds IR
- Binds hybrids
- Associated with BP
- Increases signal via IGF1R

Sachdev D., et al., Mol Cancer Ther, 2007

Anti-IGFII: Background

- Implicated in the proliferative potential of a broad category of malignancies
  - Breast cancer, lung cancer, colon cancer, MM, prostate cancer
  - Enhance metastatic progression
  - Hormone resistance
- Potential synergy with anti-IGF1R
- Preclinical effects both with Moab to IGFII as well as soluble IGFII receptor *(Harper J., et al., Cancer Res, 2006)*
- Current agent under discussion: functional humanized IGFII specific antibody (m610)
- Preclinical data only to assess efficacy; no assessment of toxicity

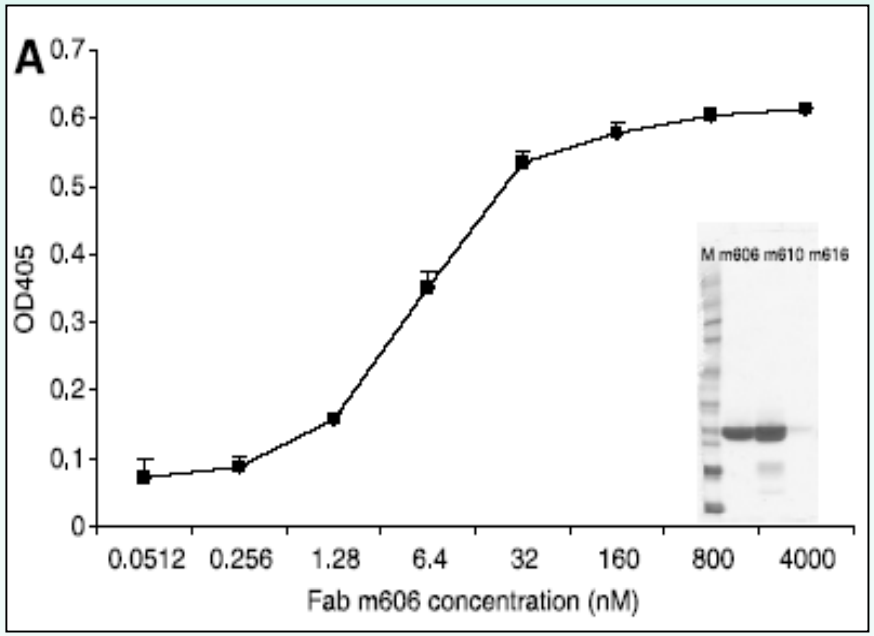
Anti-IGFII: Preclinical Summary

- Ab against IGFI and II (KM1468) inhibited new bone tumors and progression of established bone tumors *(Goya M., et al., Cancer Res, 2004)*
  - Bound to human IGFI and human/murine IGFII, not to insulin
  - Blocked autophosphorylation IGF1R
  - Similar effects in a bone model using MM *(Araki K., et al., Int J Cancer, 2006)*

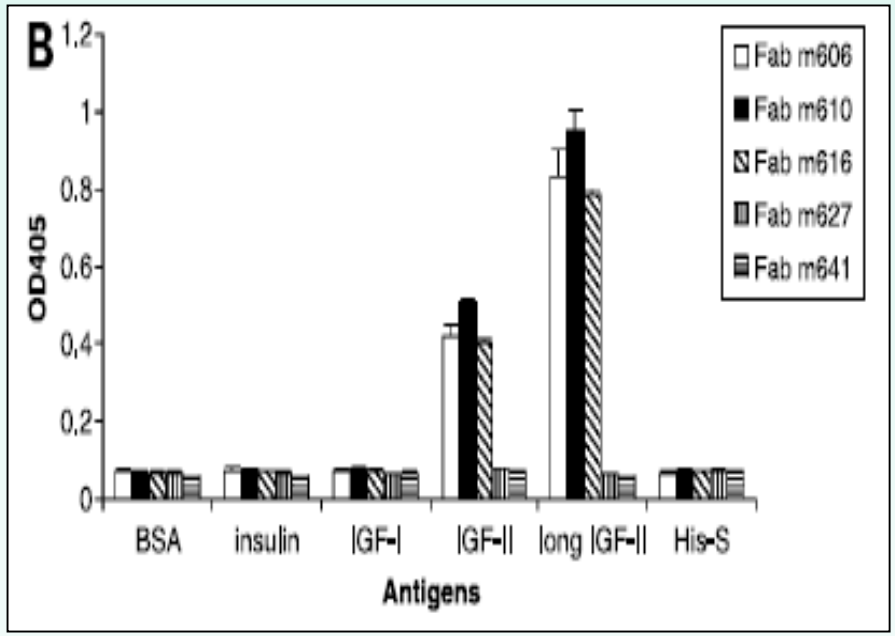
- Humanized anti-IGFII Moabs: high affinity to IGFII, non-cross-reactive with IGFI and insulin (most potent IgG1 m610) *(Feng Y., et al., Mol Cancer Res, 2006)*
  - Inhibited signal via IGF1R
  - Inhibit AKT signaling
  - Inhibit growth of DU145 (prostate cancer)
  - Inhibit migration of MCF-7 (breast cancer)

Anti-IGFII: Preclinical Summary

M610: IgG1 Humanized Moab to IGFII



Binding Affinity m610



Specificity m610

Feng Y., et al., Mol Cancer Ther, 2006

Anti-IGFII: Clinical Summary

- No clinical data available
- No animal toxicity data published
  - IGFII-deficient mice: growth failure but not as severe as IGFI$^{-/-}$
  - IGFII-deficient mice: higher percentage if immature DC, severe impairment of allogeneic T-cell proliferation
- Unknown clinical development process
- Agent suggested by Dimiter Dimitrov
  (Feng Y., et al., Mol Cancer Ther, 2006)

Anti-IGFII: Contemplated Uses

- Primary therapy for a variety of common cancers
- Combination with agents targeting IGF1R
- Combination with chemotherapy (potentiate effects, e.g., trastuzumab?)

Anti-IGFII: Perceived Need

- Potential for use in cancer therapy: HIGH
- Potential for use in more than one clinical setting: HIGH
- Perceived need: HIGH (especially in combination)
- Broad availability: NO
- Commercially available: NO

Little information available demonstrating clinical feasibility

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CP-751,871 (anti-IGF-1R mAb)

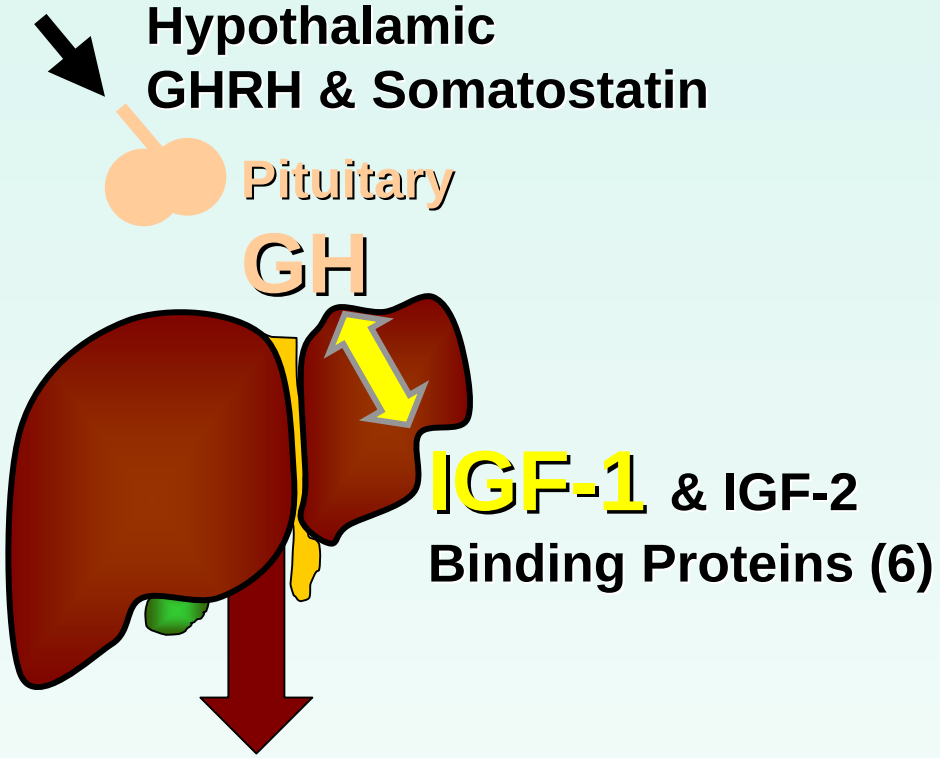
- Michael J. Morin, Ph.D.
- Vice President, Discovery
- Antibacterials, Immunology, and Cancer
- Pfizer Global R&D (Groton, CT)

CP-751,871 (anti-IGF-1R mAb): Background

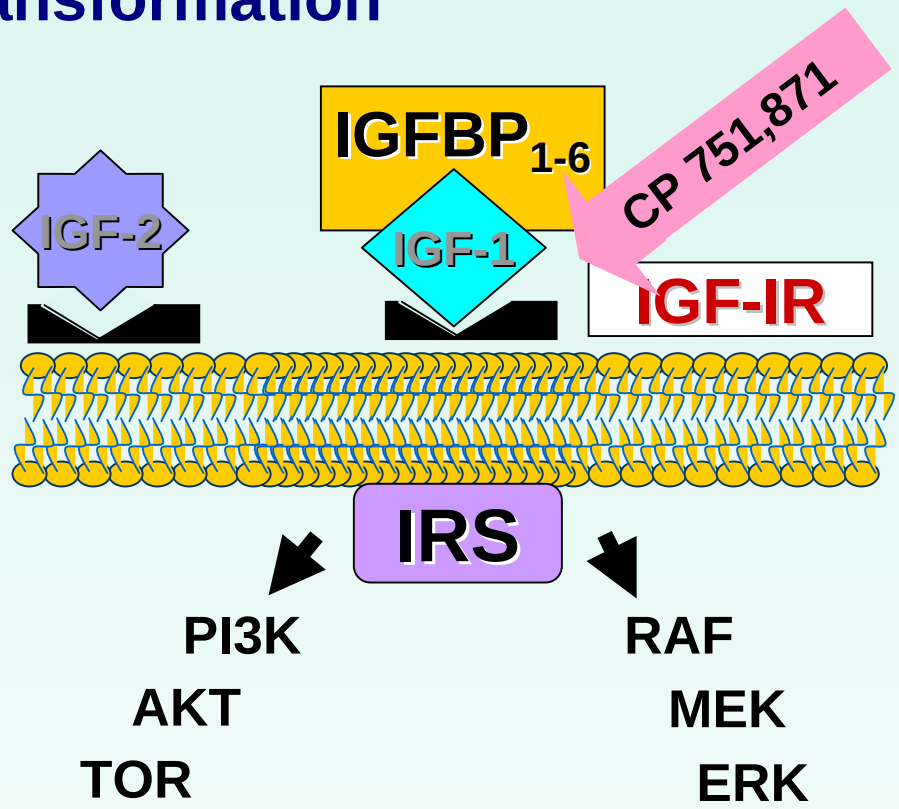
Category of drug

- Not an immune modulator per se
- For this toolbox exercise- if this belongs, why not Herceptin, Erbitux, Avastin, etc.?
- Selectively binds to and blocks function of IGF-1R
- Binding leads to internalization and degradation
- Spares Insulin Receptor expression/function
- No hyperglycemia seen in P1
- P2 population had higher background hyperglycemia (taxol-related steroid use) that may be exacerbated by CP-751,871 (blocks hypoglycemic function of IGF-1)

Insulin-like Growth Factor Receptor 1 (IGF-1R) Plays a Key Role in Transformation



- Tyrosine Kinase Activation
- 70% Homology with Insulin
- Controls Cell & Body Size
- Growth Stimulation
- Inhibition of Apoptosis

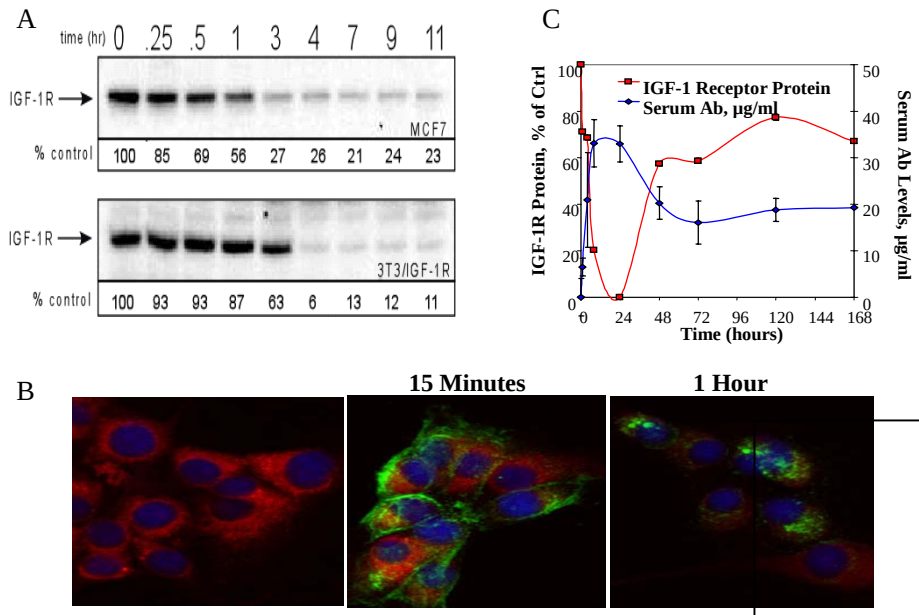


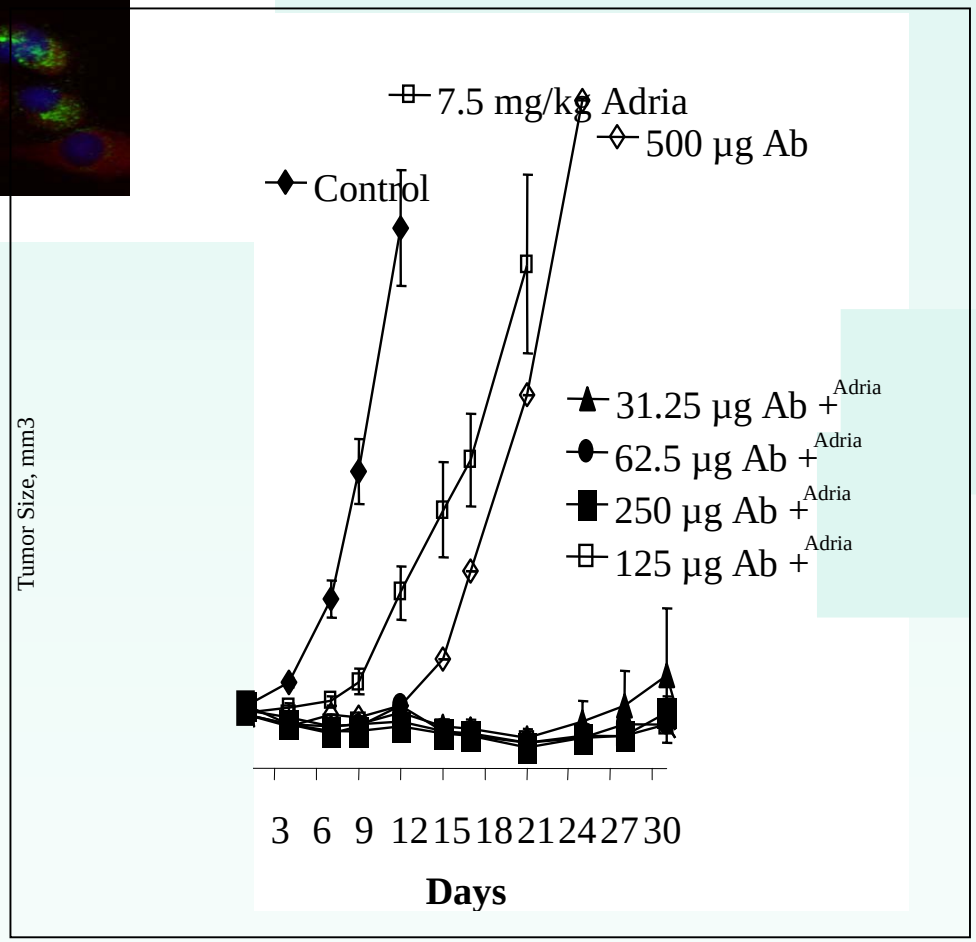
Autocrine Growth Factor
Cell Adhesion
Increased Motility
Cell Survival

IGF-1R as a Drug Target

- Receptor tyrosine kinase
- IGF family: IGF-1R, IGF-1R ligands (IGF-1, IGF-2), IGF binding proteins 1-6 (IGFBPs)
- IGF-1,-2 serum levels associated cancer risk (breast, prostate, lung, colon, and cervix)
- IGF-1R ligands promote cell growth and survival *in vitro* and mediate tumor resistance to treatment *in vivo*

CP-751,871
Pre-clinical



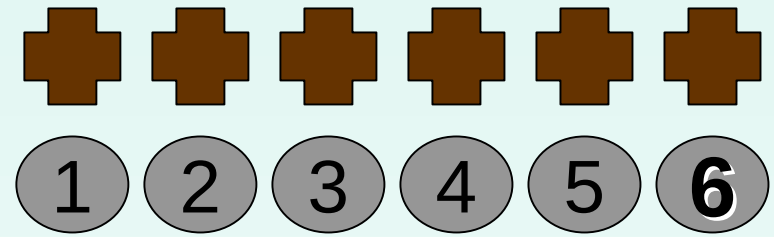


- First fully human monoclonal antibody (IgG2) with high specificity for IGF-1R to enter clinical trials
- *In vitro*, inhibits IGF-1 binding and down-regulates IGF-1R by receptor internalization
- *In vivo*, found to significantly increase the anti-tumor activity of cytotoxic agents, (including the taxanes, adriamycin, 5-FU, etc.) as well as targeted agents (tamoxifen, Tarceva, etc.)

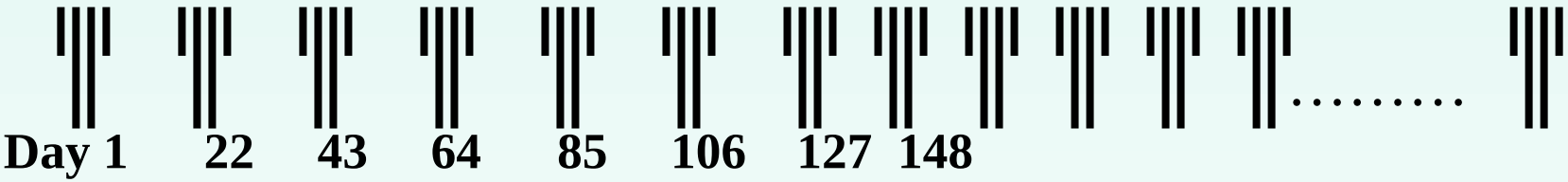
Randomized Phase 2 – anti-IGF-1R (CP-751,871) mAb in Untreated Advanced NSCLC

Arm A – 48pts

2:1 randomization



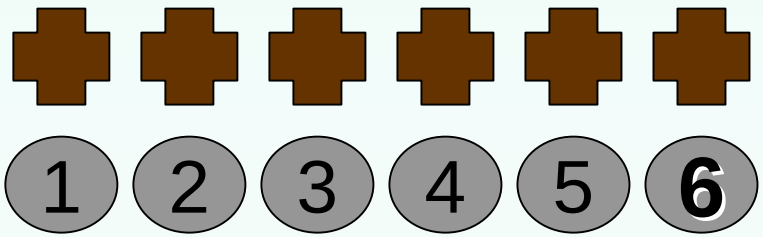
Maximum 17 doses of study drug



3wk intervals……

If no response or progressive disease after 2 (two) courses, physician allowed to add CP 751,871 or give as single agent.

ARM B – 25pts



= Taxol 200mg/m2

= CarboPlat AUC = 6

= CP-751,871 Antibody

Response Rate (RR) by Histology

Patients	TCI (44% adenocarcinoma)	TC (40% adenocarcinoma)
TOTAL (N = 73)	22/48 (46%)	8/25 (32%)
Adenocarcinoma (n = 31)	8/21 (38%)	3/10 (30%)
Non-adenocarcinoma (n = 42)	14/27 (52%)	5/15 (33%)

- **46% RR satisfied criterion (> 40%) to proceed further**
- **TCI RR higher than TC across the parameters of age, stage, and smoking class groups**

Hyperglycemia / Dehydration Most Common Adverse Events (AEs) but Easily Manageable

	Grade 3: 251 - 500		Grade 4: > 500		All grades (%)	
	TCI	TC	TCI	TC	TCI	TC
Dehydration	**4**	0	0	0	12	0
Hyperglycemia	**10**	**12**	**10**	0	58	44

- **Mechanism of Hyperglycemia**
  - Increased HGH[1]
  - Diabetic pt / Steroids
  - Loss of *hypo*glycemic effect of IGF-1
- **Other AEs were mild**
  - mucositis, cough, hemoptysis, dizziness, anemia, etc.
  - no special measures required

[1]**Haluska P, et al. ASCO 2007. Abstract 3586.**

CP-751,871: Anticipated Uses and Issues

Potential for broad therapeutic utility

- Lung, breast, prostate, etc.
- IGF-1R axis may be particularly important in ER^- PR^- $her2^-$ breast cancer (“triple negative”)
- P3 in NSCLC (non-adenos?) 2008
- CTC with high IGF-1R expression may predict best responders (de Bono, ASCO, 2007)

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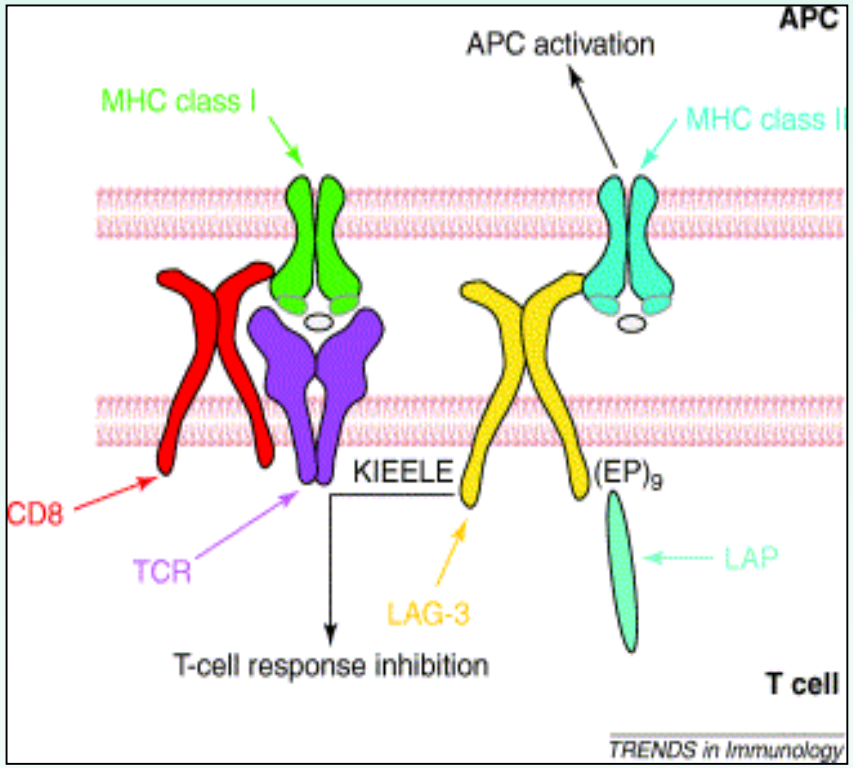
Anti-LAG3 and sLAG

- Elizabeth M. Jaffee, M.D.
- Professor of Oncology
- Johns Hopkins University

Lymphocyte activation gene-3 (LAG3 or CD223)

Lag-3 is a negative regulator of activated T-cells

- T-cell homeostasis regulator and potential T-regulatory marker
- Expressed on activated NK and T-cells but not resting lymphocytes
- Negative regulatory function depends on binding to MHC Class II molecules and signaling through a conserved KIEELE motif
- Binds to MHCII with higher avidity than CD4
- Inhibition via T-cell intracellular signaling rather than disrupting the CD4-MHCII interactions (binds at a different site)
- LAG-3 is selectively up-regulated on Tregs and is involved in mediating Treg function in murine models
- A soluble LAG-3 fragment is released by activated T-cells and is found in the sera



Anti-LAG3 antibody

Rat IgG1anti-mouse LAG-3 (generated to D1 30aa loop)

- Activity: blocks LAG-3 function without interfering with its ability to bind to MHC Class II molecules *in vitro*
- Blocks Treg activity based on *in vitro* supression assays and *in vivo* enhanced T-cell expansion to a level comparable to that in LAG-$3^{-/-}$ cells
- Use: checkpoint inhibitor (blocks T-regulatory cells)

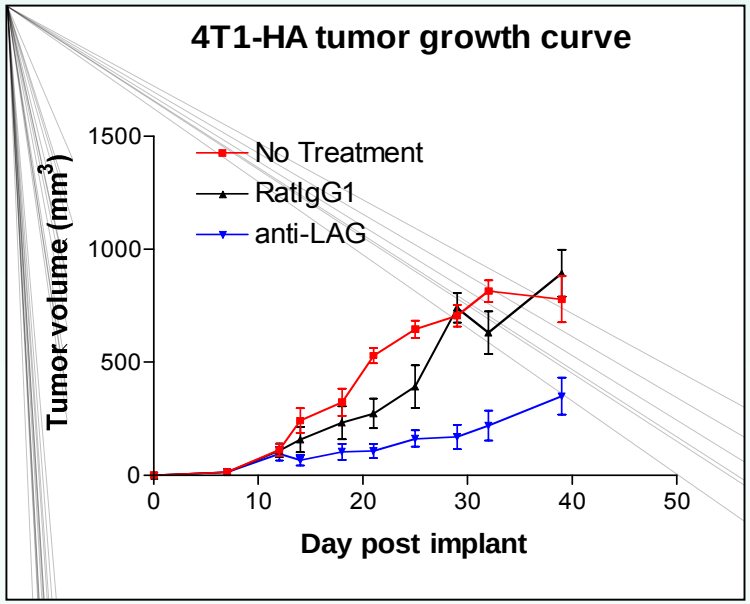
sLAG-3

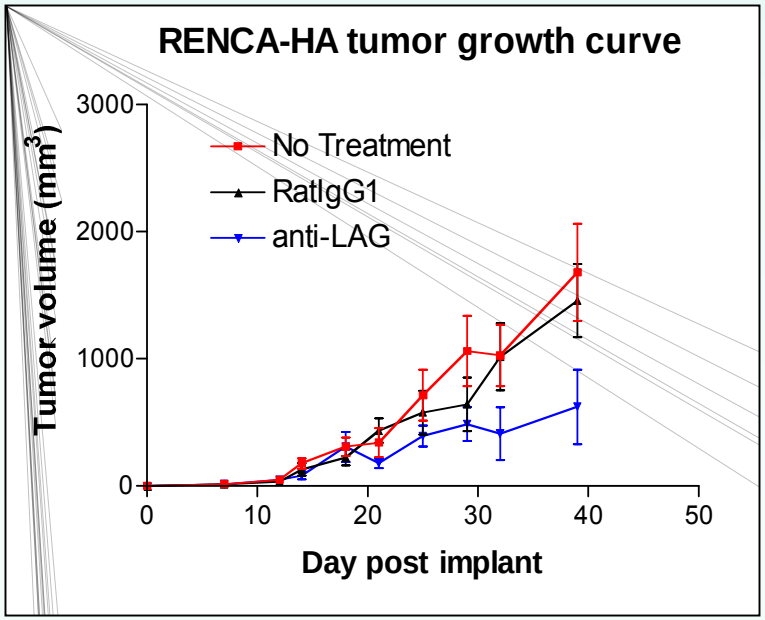
IMP321 (D1-D4 extracellular domains of human LAG-3 fused to the Fc tail of human IgG1)

- Activity: binds to MHC Class II on APCs
- Induces secretion of chemokines (MDC,TARC) and Th1 cytokines needed for DC migration to secondary lymphoid organs
- Use: potential candidate adjuvant for cancer vaccines

Preclinical Data
Anti-LAG3 antibody

- Blocks T-regulatory activity *in vitro* and *in vivo*
- Anti-LAG-3 mAb treatment impairs LAG-3 control of T-cell homeostasis *in vivo* mimicking what is observed in the LAG3$^{-/-}$ mice
- Prevents tumor growth when injected 6 days post-tumor challenge in the 4T1-HA (mammary carcinoma) and Renca-HA mouse tumor model
- Improved survival when combined with a Listeria-based vaccine in a CT26 liver metastasis model (Drake,C; JHU; Personal Comunication)





Preclinical Data

sLAG-3 (IMP321)

***In vitro* studies**

- Induces phenotypic maturation of human DC and the release of chemokines and proinflamatory cytokines by APCs
- The addition of sLAG-3 to PBMCs cultures from melanoma patients increased the antigen-specific proliferation and induced a significant amplification of IFN-γ release even in the absence of Tregs

***In vivo* studies**

- sLAG-3 molecule used as a vaccine adjuvant elicits greater humoral and cellular immune responses to both particulate and soluble antigens
- Induced antigen/tumor-specific CTL and CD4 Th1 responses *in vivo* when administered as vaccine adjuvant together with the antigen or the tumor cells subcutaneously
- Enables DNA vaccination to establish effective protection against mammary carcinogenesis in Her-2-neu transgenic BALB/c mice by enhancing cross-presentation of the DNA-coded antigen
- IMP321 alone or co-injected with antigen is well tolerated (long-term systemic or local effects)

sLAG-3 Clinical Summary

- High levels of sLAG-3 in sera correlates as a Th1 marker to resistance to tuberculosis
- Disease-free and overall survival rates are greater in breast cancer patients with estrogen/progesterone+ tumors who have elevated sLAG-3 at diagnosis
- Two concluded phase I studies to access safety and T-cell responses
  - use of influenza and HBsAg as model antigens
  - well tolerated
  - may increase both CD4Th1 and CD8Tc1 antigen-specific T-cells
  - Increase CD4 and CD8 antigen-specific T-cells
- Ongoing clinical studies
  - IMP321 Phase 1 Trial in Metastatic Renal Cell Carcinoma (MRCC)
  - IMP321 Phase I Breast Carcinoma
  - Immunization of Disease-Free Melanoma Patients With Different HLA-A2 Peptides

sLAG Influenza Study

- Phase I single-blinded, randomized, controlled clinical trial
  - 20 volunteers received 3, 10, 30, or 100 ug IMP321 alone
  - 40 volunteers recruited into 4 consecutive cohorts of 10 subjects, randomly assigned to receive the flu vaccine+3, 10, 30, or 100 ug IMP321 or vaccine +saline control
- Post-vaccination humoral responses measured at day 29 and 57 by assay of hemagglutinin inhibition activity were similar for all groups
- 10, 30, and 100 ug of IMP321+vaccine resulted in higher levels of Th1-type flu-specific CD4+ T-cell responses

sLAG+HBsAG Vaccine Study

- Phase I single-blinded, randomized, controlled clinical trial
  - 48 seronegative healthy volunteers received 10ug HBsAg mixed with saline (control) or with 3, 10, 30, or 100 ug of IMP321(0,4, and 8 wks)
  - 40 volunteers recruited into 4 consecutive cohorts of 10 subjects, randomly assigned to receive the flu vaccine+3, 10, 30, or 100 ug IMP321 or vaccine +saline control
- Post-vaccination HBsAg-specific Abs appeared sooner and were higher at 8 and 12 weeks in IMP321 groups
- Post-vaccination HBsAg-specific CD4 Th1 and CD8 Tcl antigen-specific T-cells were increased in number in the IMP321 groups

Anti-LAG3 & sLAG Contemplated Uses

- Cancer Vaccine Adjuvant for priming of immune response (sLAG)
- As a checkpoint inhibitor
  - alone (some activity in preclinical models)
  - in combination with vaccine (best choice)

Anti-LAG3 & sLAG Perceived Need

- Tregs are a formidable barrier to effective anti-cancer immunity induced by all types of immunotherapy (in preclinical models and human tumors)
- Tregs in ascites of advanced ovarian cancer patients are associated with decreased survival
- Tregs have been isolated from lung, pancreatic, ovarian, and breast cancers
- LAG-3 expression by TILs associated with suppression of $CD8^+$ T-cell function in Hodgkin's lymphoma
- Depletion of Tregs via monoclonal antibody to CD25 or low-dose cyclophosphamide alters tumor growth *in vivo*
- Anti-LAG3 and sLAG mediate similar enhanced anti-tumor function *in vivo*.
- No single cell surface marker uniquely identifies Tregs—no good depleting agents
- LAG-3 is the best surface marker candidate for differentiating Tregs
- Useful against different tumor types and as part of multiple therapy regimens

Comparison of Agents

- As a checkpoint inhibitor, for blocking of Tregs, the data favors the use of anti-LAG-3 antibody

- As a vaccine adjuvant, preclinical and early clinical data strongly support the use of sLAG-3

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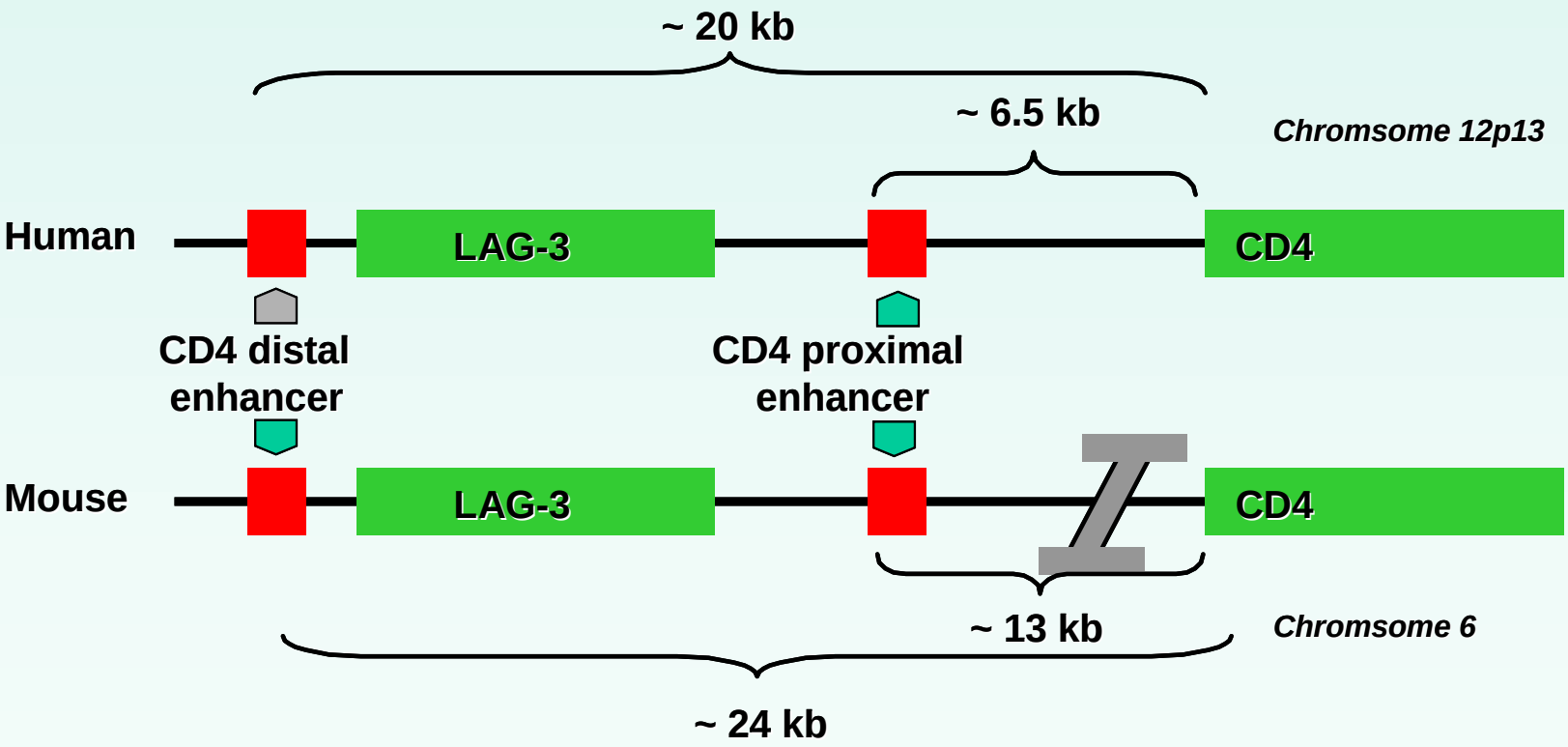
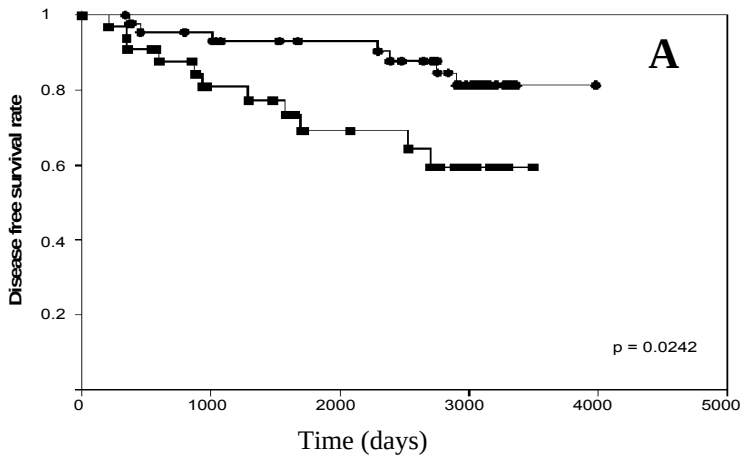
Additional Slides

LAG-3 Gene Cloned in 1990

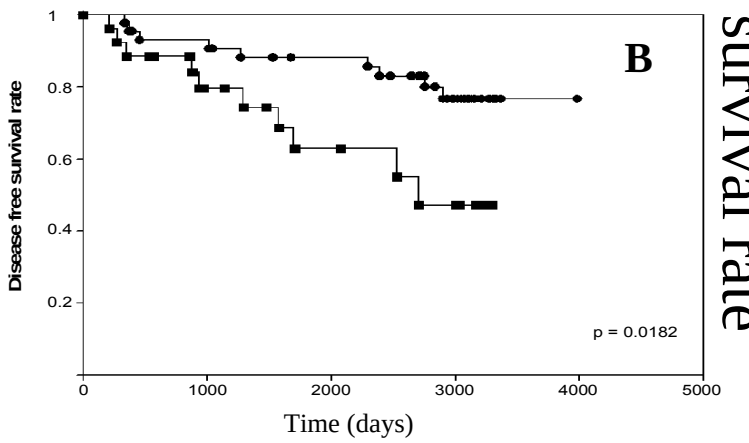


Disease-free Survival Based on Pre-treatment sLAG-3 Concentration



Disease-free survival rate

A estrogen receptor-positive tumors: ▪, sLAG-3 < 120 pg/ml (detection limit), n=33; and ●, >120 pg/ml, n=45.



Disease-free survival rate

B progesterone receptor-positive tumors: ▪,sLAG-3 < 120 pg/ml, n=26; and ●,>120 pg/ml, n=43.

Adv-CCL21

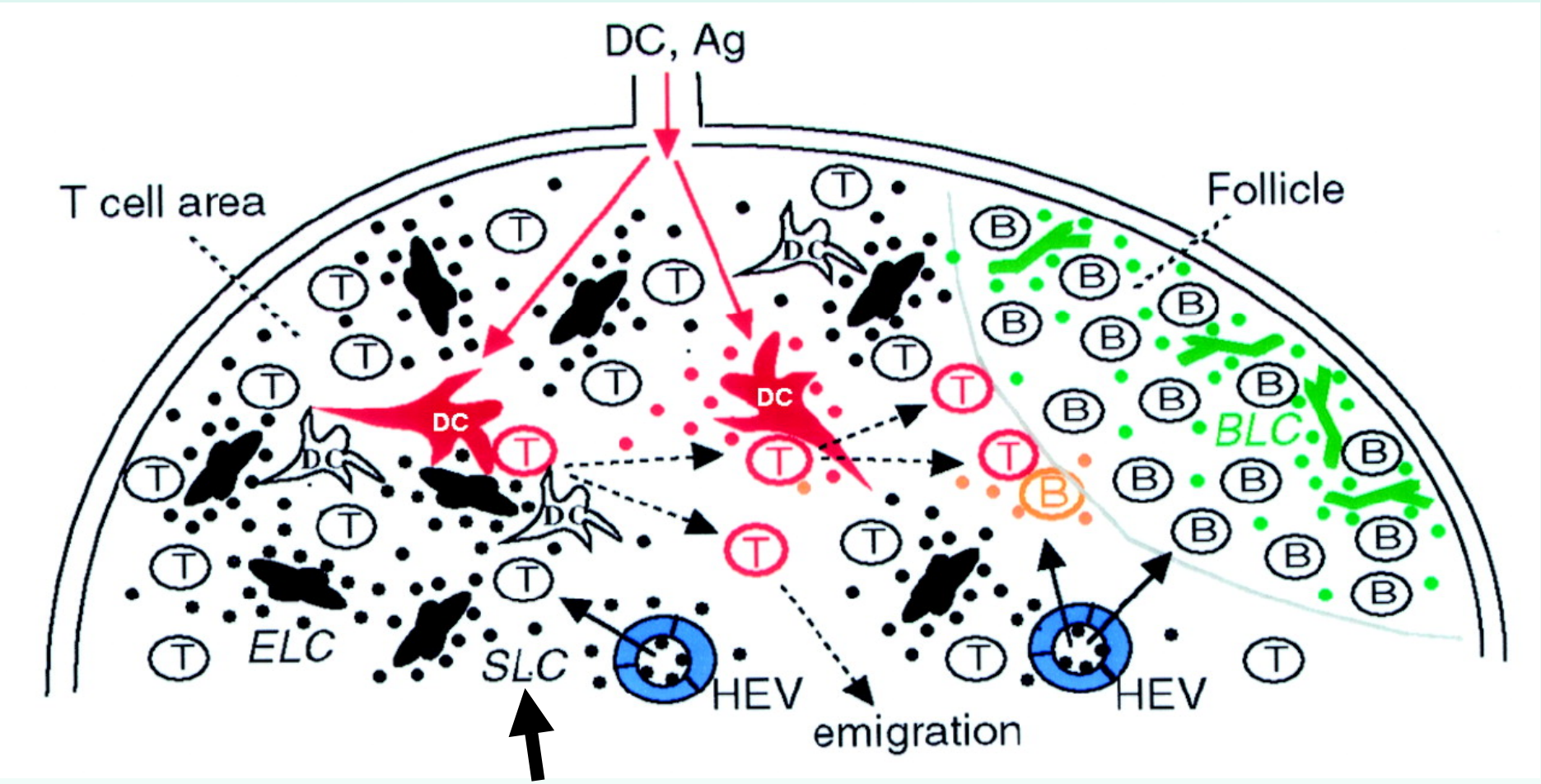
- A. Karolina Palucka, M.D., Ph.D.
- Investigator
- Baylor Institute for Immunology Research

CCL21: Background

- Category: Chemokines
- Molecular characterization:
  - CC chemokine
  - Also known as secondary lymphoid tissue chemokine (SLC); Exodus-2; thymus derived chemotactic agent 4, or 6CKine
- Target:
  - CCR7 in lymphoid tissues: receptor expressed on naïve T cells and mature dendritic cells
  - CXCR3 in CNS

CCL21: Background

- **Biology of agent-target interaction**
  - CCL21 is expressed by high endothelial venules and in T-cell zones of spleen and lymph nodes, strongly attracts naïve T cells and mature dendritic cells via interaction with CCR7


DC, Ag
T cell area
Follicle
DC
DC
DC
DC
T
B
BLC
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HEV
HEV
emigration

CCL21

Adv-CCL21: Preclinical Summary

- Efficacy *in vitro*
  - Human DCs transduced with Adv-CCL21:
    - produce large amounts of CCL21;
    - attract T cells and DCs

      (Riedl et al. Mol Cancer 2003)
    - prime naïve T cells

      (Terando et al. Cancer Gene Therapy 2004)

Adv-CCL21: Preclinical Summary

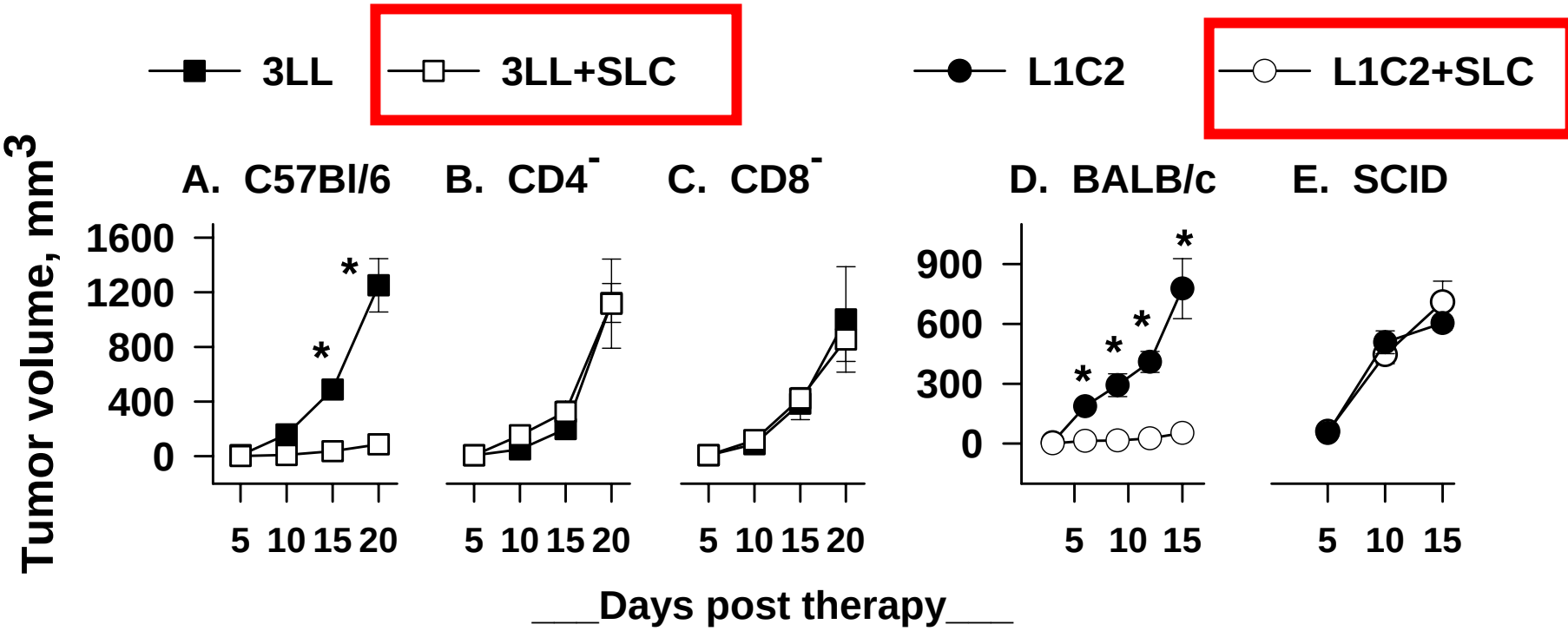
- Efficacy *in vivo* in animal models
  - Intratumoral injection: CD4– and CD8–dependent antitumor response in both localized and metastatic disease accompanied by infiltration of DC and lymphocytes within resolving primary tumors at both the primary (injected) as well as metastatic sites
  - CCL21 transduced DCs effective in transgenic mice that develop bronchoalveolar carcinoma spontaneously

    (Sharma, et al. J Immunol 2000; Cancer Res 2001; Yang, et al. Clin Cancer Res 2004; Cancer Res 2006)

Adv-CCL21: Preclinical Summary

- Efficacy *in vivo* in animal models
  - Adjuvant for TERT-DNA vaccine in breast cancer model
    (Yamano, et al. Cancer Gene Therapy 2007)
  - Immunologically mediated regression of pancreatic tumors in mice upon intratumoral delivery
    (Turnquist, et al. Int J Oncol 2007)
  - Improved survival and therapeutic efficacy of adoptive T-cell transfer in mouse model of melanoma
    (Thanarajasingam, et al. Cancer Res 2007)

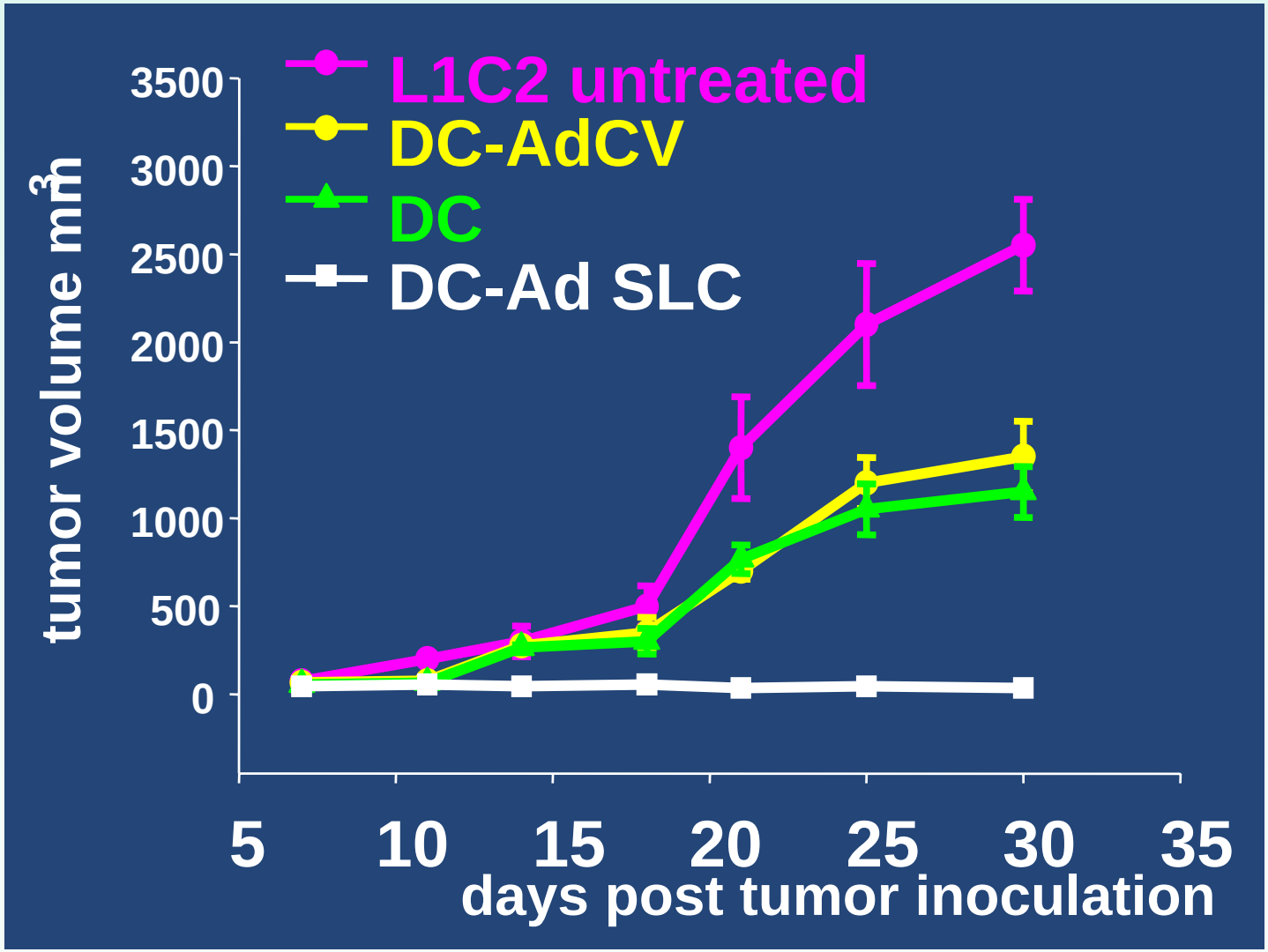
CCL21 (SLC) Mediates Antitumor Responses *In Vivo*: Requirement for CD4 and CD8 Lymphocytes



J Immunol. 2000 May 1;164:4558

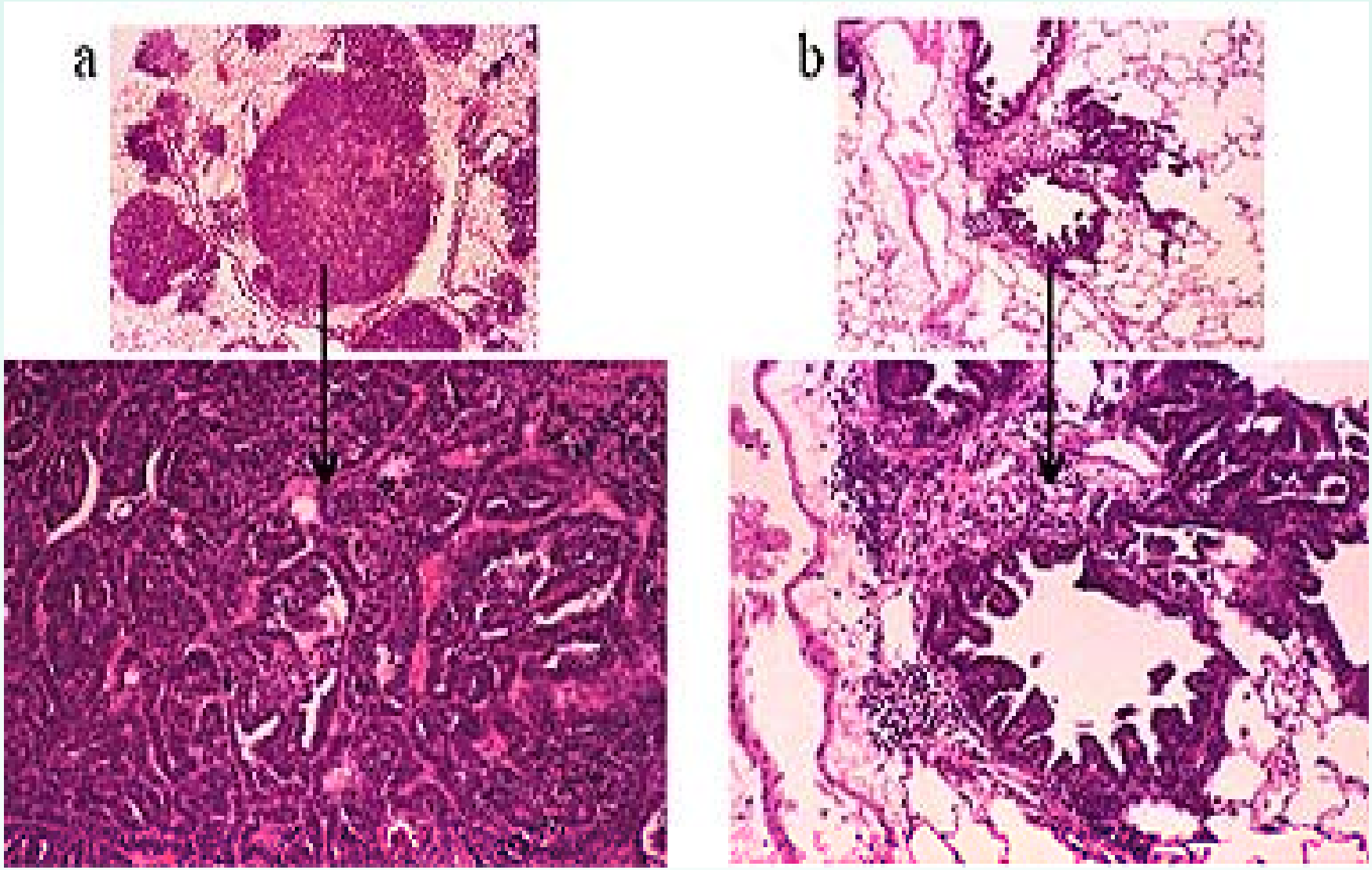
S. Dubinett, UCLA

DC-Ad CCL21 (SLC) Induces Potent Anti-Tumor Responses *In Vivo*



S. Dubinett, UCLA

DC-Ad CCL21 (SLC) Induces Potent Anti-Tumor Responses *In Vivo*



S. Dubinett, UCLA

“A Phase I Trial of Intratumoral Administration of Secondary Lymphoid Chemokine Gene-Modified Autologous Dendritic Cells in Advanced Non-Small Cell Lung Cancer”

Protocol #0610-807

Principal Investigator: Steven M. Dubinett, M.D.

UCLA School of Medicine

Approved by UCLA IRB, RAC, and FDA

Adv-CCL21: Contemplated Uses

ADJUVANT TO CANCER VACCINES

- *Ex vivo* transduction of cancer vaccines based on *ex vivo* DCs or cell lines, for example, GVAX
- *In vivo* as adjuvant to cancer vaccines
- *In vivo* for intratumoral gene therapy

Adv-CCL21: Perceived Need

- Could be evaluated in numerous malignancies
- Multiple investigators

Potential Limitations

- Adv: limitations due to high immunogenicity and generation of neutralizing antibodies upon intratumoral/adjuvant delivery or possible CTL responses upon DC transduction leading to elimination of vector or of transduced DCs

Alternative Formulations

- CCL21 recombinant protein
- CLL21 RNA

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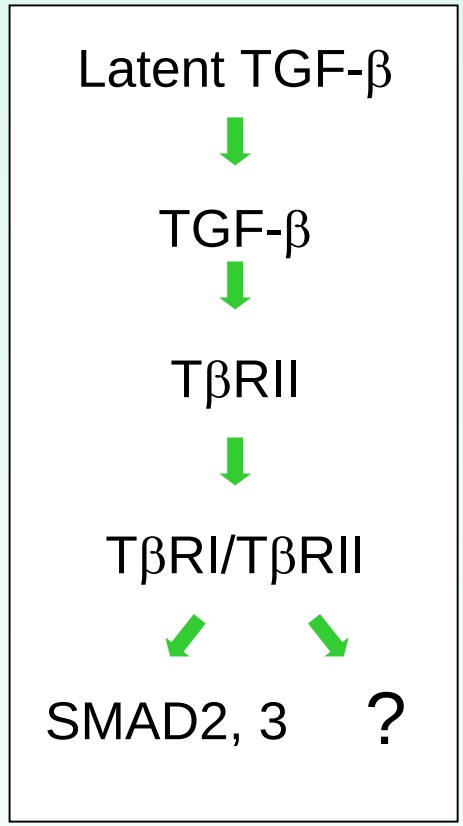
TGF-β Inhibitors
Anti-TGF-β Ab and TβRII (ECD)-huFc

- Frank Calzone, Ph.D.
- Executive Scientific Director
- Amgen, Inc.

TGF-β Targeted Inhibitors: Mechanism

- SMAD-dependent TGF-β signaling is well understood
  - Ligands: TGF-β1, 2, and 3
  - Signaling Receptors: TβRI and TβRII
  - Co-receptor: TβRIII
  - Transcription Factors: SMAD2, 3, and 4
- Alternative TGF-β signaling is **not** well understood
- An Ab/TbRII therapeutic should neutralize TGF-β without cross-reacting with latent ligand



Some TGF-β Targeted Inhibitors

Agent Name	Drug Category	Description	Status	Activated Ligand Binding Affinity			Activated Ligand Bioassay (IC50)[1]		
				TGFb1	TGFb2	TGFb3	TGFb1	TGFb2	TGFb3
TβRII-B-huFc	Soluble Receptor	TβRII splice variant B ectodomain fused to human IgG1 Fc	Preclinical	38 pM	None	38 pM	360 pM	None	360 pM
TβRII-huFc	Soluble Receptor	TβRII full length-ectodomain fused to human IgG1 Fc	Preclinical	75 pM	None	75 pM	660 pM	None	360 pM
Lerdelimumab CAT(AZ)-152	Ab	Human recombinant monoclonal IgG4 raised against TGFβ2	Phase III Inactive	None	890 pM	10 nM	ND	1-2 nM	ND ?
Metelimumab CAT(AZ)-192	Ab	Human recombinant monoclonal IgG4 raised against TGFβ1	Phase I/II Inactive ?	Binds	?	?	Blocks	?	?
GC-1008/1D11 CAT/AZ	Ab	Humanized 1D11 murine monoclonal IgGX pan-specific TGF-β neutralizing Ab	Phase II	Binds	3 nM	Binds	130 pM	Blocks	Blocks
2G7 Genentech	Ab	Human or Humanized Ab raised against TGF-β1 ?	Preclinical	Binds			Blocks		

[1] A Mv1Lu CAGA12 MPL-Luc reporter assay containing 100 pM active TGF-β ligand was used to determine IC50 for soluble receptors. A TF1 human erythroleukaemia cell line proliferation assay was used to determine TGF-b2 IC50 for CAT-152. Binding affinities of GC-1008 where listed are for the 1D11 parent. Inhibition of mIL-4-dependent 3H-thymindine incorporation by 10 pM TGFb1 was used for 1D11 bioassay.

TGF-β Targeted Inhibitors: Preclinical Summary

Cancer Immunotherapy

- Transgenic mice expressing a T-cell specific TβRII dominant negative reject antigenic, syngeneic tumors
- Disruption of TGF-β signaling by gene disruption of TGF-β1, TβRII dominant negative, or conditional TβRII K/O results in a multifocal inflammatory response

Direct Tumor Efficacy

- TGF-β, TβRI, TβRII, overexpression or constitutive activation promote EMT, invasion, and metastasis in experimental models
- TGF-β pathway inhibition with antibodies, TβR soluble receptors and dominant negative receptors, and TβR kinase inhibitors inhibit tumor growth or metastasis in animal tumor models

TGF-β Targeted Inhibitors: Cancer Risk

Tumorigenesis

- SMAD pathway inhibition could increase cancer risk (carcinomas) that might only become apparent long after drug approval and wide clinical acceptance

Evidence

- TβRI, TβRII, and SMAD4 are frequently inactivated by mutation in human pancreatic and biliary cancers
- Experimentally, TGF-β is a potent, negative regulator epithelial cell proliferation (normal cells and nonaggressive cancers)

Comparison of Agents

	TGF-β Ab GC-1008	**TβRII(ECD)-huFc**
Specificity	Pan-Specific	TGF-β 1,3 Active Only
Process Development	Standard (1 g/L)	To be developed
Human PK	Longer Lived (wks)	Shorter Lived (days)
Potential for anti-TβRII response	~ Nill	Significant
Human Safety	Phase I Cancer Study Underway CAT 152 and CAT 192 PI-III Clinical Fibrosis Programs	**No Human Data**

- TGF-β Ab (GC-1008) is preferred over TβRII(ECD)-uFc for several reasons

GC-1008: Clinical Summary

Phase I Genzyme/AZ (In progress)

- Primary objective: Assess MTD and safety in patients with locally advanced metastatic renal cell carcinoma or malignant melanoma
- Secondary objectives: PK, biomarkers, tumor responses, etc
- IV dosing: 0.1 to 15 mg/kg on days 0,28, 42, 56 (course 1)
- Results not yet published

CAT-192: Clinical Summary

Phase I AZ (Completed)

- Objective: To assess MTD and safety in patients with early-stage diffuse, cutaneous systemic sclerosis
- IV dosing: 0.5, 5.0, 10.0 mg/kg; 0, 6 wk, 12 wk, and 18 wk
- Results: 45 patients were enrolled. More AE and SAE in treatment group (skin, gastro, musculo, and lung) Frequency was not dose-dependent. Generally, well-tolerated. No efficacy

GC-1008: Contemplated Uses

- A single agent to amplify or unmask natural immunosurveillance
- An agent to amplify the efficacy of an anti-cancer vaccine aimed at inducing CTL-mediated tumor regression
- An agent to enhance T-cell adoptive immunotherapy cancer

TGF-β Ab GC-1008: Perceived Need

- No intentions to evaluate the clinical utility of TGF-β inhibition in cancer immunotherapy have been announced
- A clinical study of TGF-β blockade for cancer immunotherapy will require special expertise to address multiple mechanisms of tumor inhibition and disease heterogeneity

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CD40 Agonists

- Paul M. Sondel, M.D., Ph.D.
- Professor of Pediatrics
- Head, Division of Hematology/Oncology
- Vice Chair, Research, Department of Pediatrics
- Associate Director, UW Paul P. Carbone Cancer Center
- University of Wisconsin, Madison

CD40 Agonists: Background

- **Category of drug:** recombinant trimeric ligand or monoclonal antibody
- **Molecular or physical characterization of agent:**
  - Recombinant CD40 ligand trimer is CD154 (expressed on activated T cells and platelet) engineered with an isoleucine zipper motif
  - CP-870,893 is a fully human and selective CD40 agonist mAb
- **Target:** CD40
- **Biology of target:** CD40 (TNF receptor superfamily member 5) is a costimulatory protein found on APCs and binds to CD40L on T cells
- **Biology of antigen-target interaction:**
  - Agonistic CD40 antibodies substitute for T-cell help provided by CD4+ lymphocytes and trigger effective immune responses against tumor-associated antigens in tumor-bearing hosts
  - Also, CD40 is expressed on many tumor cells and its ligation can mediate a direct cytotoxic effect that results in apoptosis *in vitro* and impaired tumor growth *in vivo*

CD40 Agonists: Preclinical Summary (1)

APC activation and induction of T-cell immunity

- *In vitro* (Grewal, van Kooten)
  - Ligation of CD40 on APCs enhances the expression of MHC and costimulatory molecules such as CD86
  - CD40 ligation stimulates the production of pro-inflammatory cytokines such as IL-12
  - This leads to T-cell activation and cell-mediated immunity
- *In vivo*
  - In mice, agonist CD40 mAb mimics the signal of CD40L and substitutes for the function of CD4+ lymphocytes (Bennett)
  - Agonist CD40 mAb can also overcome T-cell tolerance in tumor-bearing mice, evoke effective CTL responses, and enhance the efficacy of antitumor vaccines (French)

CD40 Agonists: Preclinical Summary (2)

Direct tumor inhibition

- *In vitro*
  - Engagement of CD40 on tumor cells *in vitro* inhibits the growth of solid tumor cells and high-grade B-cell lymphoma lines [a direct cytotoxic effect resulting in tumor regression through apoptosis and necrosis] (Funakoshi)
- *In vivo*
  - CD40-mediated tumor inhibition has been observed *in vivo,* including inhibition of breast carcinoma or B-cell lymphoma xenografts in immunocompromised mice (Funakoshi)

CD40 Agonists: Preclinical Summary (3)

Other mechanisms

- Anti-angiogenic effect
  - CD40 can be expressed on endothelial cells, and activation might block (or enhance) neoplastic angiogenesis (Chiodoni)
- Induction of antitumor innate immunity
  - Indirect activation of tumor-reactive NK cells (Turner)
  - Activation of tumor-reactive macrophages (Buhtoiarov)

Toxicity issues

- Cytokine release syndrome

CD40 Agonists: Clinical Summary

- Phase 1 data (Vonderheide) CP-870,893—Fully human IgG2 mAb
  - Efficacy—4 PR (melanoma) out of 15 melanoma and 14 other solid tumor patients
  - MTD—0.2 mg/kg
  - Proof of principle
    - Rapid dose-related up-regulation of CD86 costimulatory molecule
    - Single well-studied case where tumor-specific T cells induced
  - Safety profile—cytokine response syndrome (CRS), liver/hematologic toxicity

CD40 Agonists: Clinical Summary

- Phase 1 data (Vonderheide) rhuCD40L—recombinant human trimeric CD40 ligand
  - Efficacy—2 PR out of 32 solid tumors or non-Hodgkin lymphoma
  - MTD—0.1 mg/kg/d based on elevations of serum liver transaminases
  - Proof of principle—in 76% of the patients, decrease in the percentage of circulating CD19 B lymphocytes on day 5 compared with baseline, possibly related to the peripheral clearance of these CD40+ cells by binding to rhuCD40L
  - Percentage of CD4+ T lymphocytes increased during this time in 81% of patients
  - Safety profile—increased AST/ALT, injection site reactions

CD40 Agonists: Contemplated Uses

- Single-agent systemic use for induction of innate and adoptive immunity to $CD40^+$ and $CD40^-$ tumors
- Single-agent systemic use for direct inhibition of $CD40^+$ tumors
- Great potential for CD40 agonists in combination with other agents. Preclinical models suggest
  - Chemotherapy
  - Radiotherapy
  - Tumor vaccines (local or systemic CD40 agonist)
  - Toll-like receptor agonists
  - Cytokines
  - TNF receptor family agonists such as DR5 and CD137 mAb

CD40 Agonists: Perceived Need—Tumor Type

- Tumors expressing high CD40—nearly 100% of B-cell malignancies have high levels of CD40 (based on flow cytometry)
- Up to 70% of solid tumors may express CD40 (i.e., melanoma, neuroblastoma, ectodermally derived tumors, others) based largely on flow with cell lines (no large systematic immunohistochemistry confirmation has been published)

Comparison of CD40 Agonists

- Recombinant trimeric ligand (was developed by Immunex-Amgen), no longer in development
- CP-870893, humanized IgG2 **agonist** being actively developed by Pfizer
- SGN-40, humanized IgG1 **agonist** being actively developed by Genentech
- HCD-122, humanized IgG1 **antagonist** being actively developed by Novartis/XOMA

CD40 Agonists: Perceived Need

- All 3 mAb are being actively developed by pharma in industry-led and investigator-initiated trials
  - No clear need for NCI production or distribution
- Recombinant trimeric ligand (was developed by Immunex-Amgen), no longer in development
  - May have certain advantages for repeat injection (especially locally as vaccine adjuvant)
  - May be advantageous for *ex vivo* DC activation or for preclinical work, but is not available
  - May be appropriate for NCI production/distribution

CD40 Agonists: Toxicity Summary

- Toxicity issues—CD40L trimer
  - Hepatic toxicity (bilirubin and ALT/AST)
- Toxicity issues—CP-870,893
  - Common adverse event was cytokine release syndrome (CRS)
    - Transient chills, rigors, and fevers on the day of infusion
    - Associated with elevations of serum TNF-alpha and IL-6
  - Liver toxicity—elevated total bilirubin and ALT/AST
  - Hematologic toxicity—coagulopathy (elevated D-dimers) and decrease in absolute lymphocyte count (ALC), absolute monocyte count (AMC), and platelets

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This presentation was put together with helpful consultative input from:

- **Robert H. Vonderheide, M.D., D.Phil.,** Assistant Professor of Medicine, University of Pennsylvania
- **Richard Yang, MD/PhD student,** University of Wisconsin

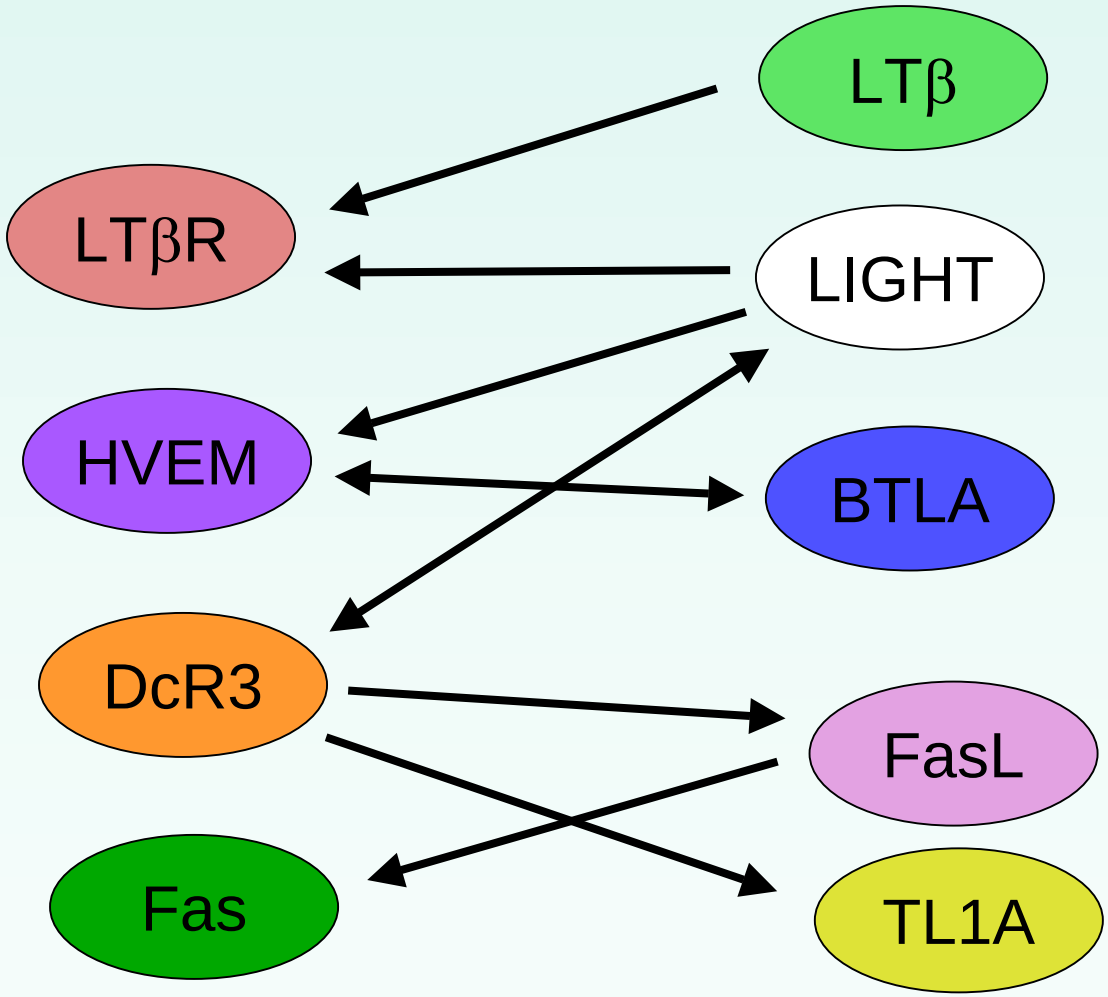
LIGHT

- Drew M. Pardoll M.D., Ph.D.
- Seraph Professor of Oncology
- Sidney Kimmel Cancer Center, Johns Hopkins University School of Medicine

LIGHT: Background

- TNF family member
- Normally a membrane-bound trimer, cleaved into a soluble form similar to TNF
- 2 known receptors - LTβR, HVEM
- Part of a complex multicomponent receptor-ligand network
- LIGHT-LTβR interactions mediate costimulation of CD8 T cells
- LIGHT-HVEM interactions mediate GVHD

LIGHT: Part of a Complex Receptor-Ligand Network



LIGHT: Preclinical Summary

- LIGHT-transduced tumors eliminated in immunocompetent mice
- LIGHT-transduced tumors induce increased CD8 T-cell responses
- Soluble LIGHT costimulates CD8 T cells while CD8 responses diminished in LIGHT KO mice
- Soluble LIGHT can enhance vaccine-induced antitumor immunity
- LIGHT is a major mediator of hepatitis induced by viruses and other agents
- Blockade of LIGHT or HVEM ameliorated GVHD

LIGHT: Clinical Summary

- No clinical data

LIGHT: Contemplated Uses

- Soluble LIGHT for systemic administration alone or in combination with vaccines
- LIGHT-expressing vectors for transduction of tumor cells
- Anti-LIGHT antibodies (or anti-HVEM) for treatment of GVHD

LIGHT: Perceived Need

- Potentially useful for any cancer type
- Could be used as an adjunct to vaccination, adoptive CD8 transfer
- Paracrine administration via direct injection into tumors or transduced tumor vaccines

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1MT
(1-methyl-tryptophan)

- George C. Prendergast, Ph.D.
- Professor and President/CEO
- Lankenau Institute for Medical Research (LIMR)

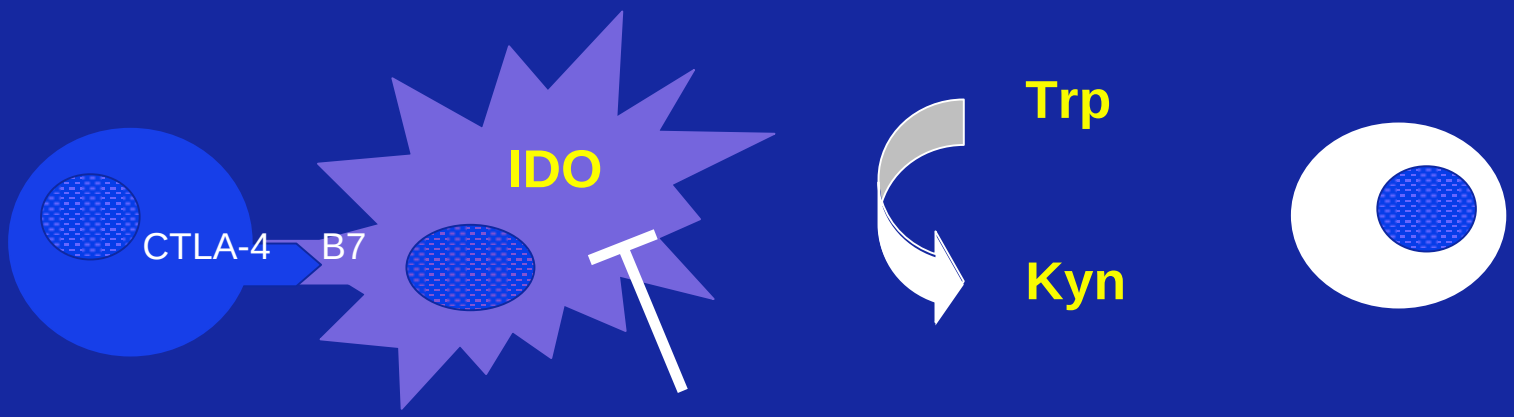
COI Statement

1MT Background

- Small molecule
- Inhibits immunosuppressive enzyme IDO (indoleamine 2,3-dioxygenase), also IDO2
- Micromolar Ki, IC50
- Outstanding preclinical PK + Tox profile (mouse, rat, dog)
- Widely studied as D,L-1MT racemic mixture
  - D-1MT more biologically active isomer
  - L > D against IDO ... D > L against IDO2
  - D-1MT chosen for clinical translation (NewLink Genetics Corporation and NCI)

Target Background: General Model IDO Suppresses T-Cell Activation Via Tryptophan Catabolism

T reg cell APC T effector cell



1MT

- Ki ~ 35 µM
- Excellent PK
- Virtually nontoxic

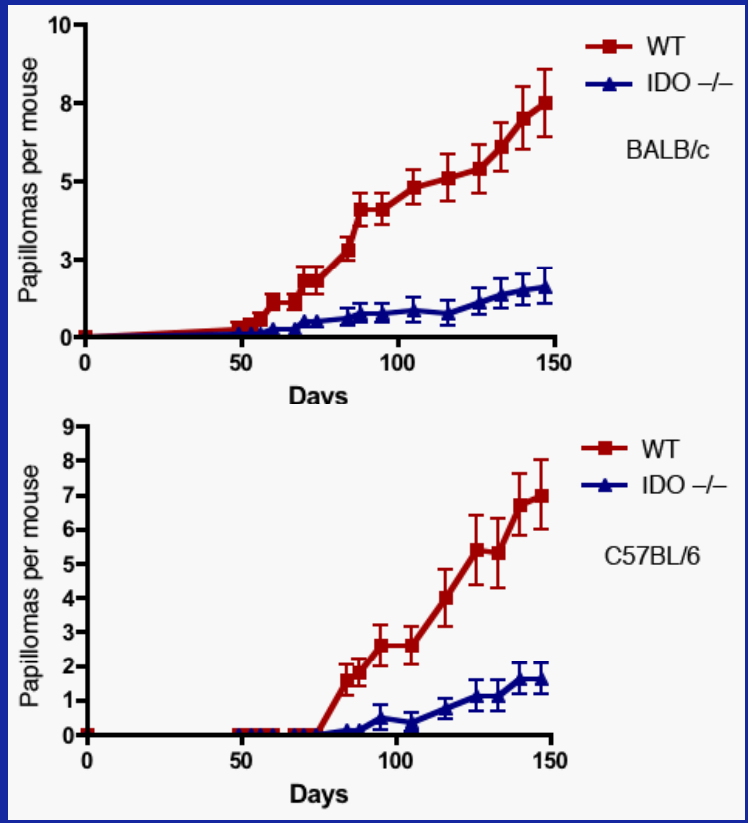
Munn, Mellor, et al.

IDO Background (1MT Target)

- Pathological function
  - Limits Ag-induced T-cell activation
  - Mediates immunosuppression in cancer
- Highly expressed in tumor cells and pDCs in TDLNs
- Mediates tumor formation in mice caused by attenuation of tumor suppressor gene Bin1
- IDO KO mouse:
  - Viable, fertile, lacks autoimmune disease
  - Resistant to inflammatory carcinogenesis
- No data on IDO2 as yet (expressed pDCs only?)

IDO Knockout Mice Offer an Initial Genetic Validation in Cancer

Skin carcinogenesis
(DMBA + TPA)



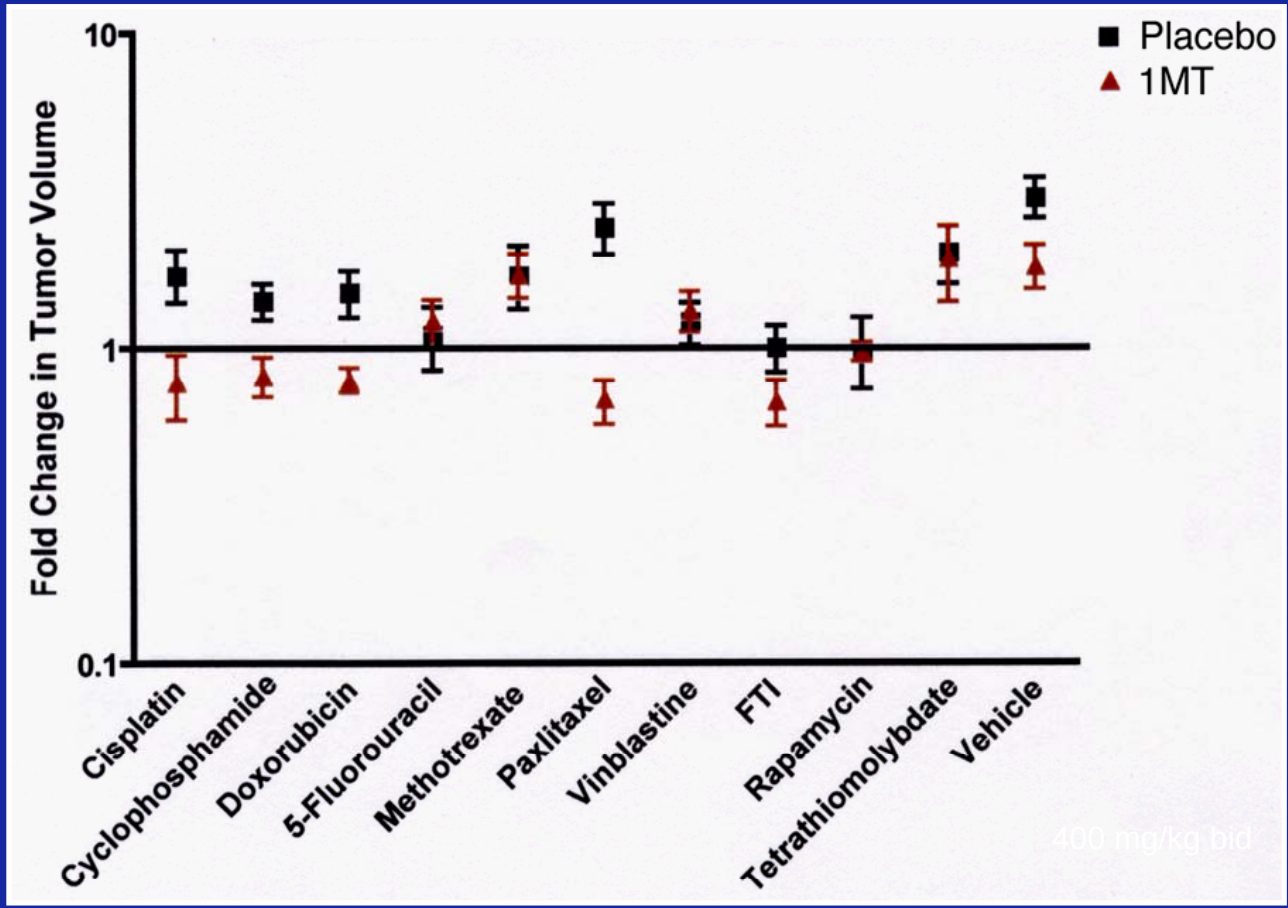
IDO KO mice lack evidence of autoimmune disease up to 10 mos of age

(Prendergast, Muller, et al., unpublished)

Preclinical 1MT Biology

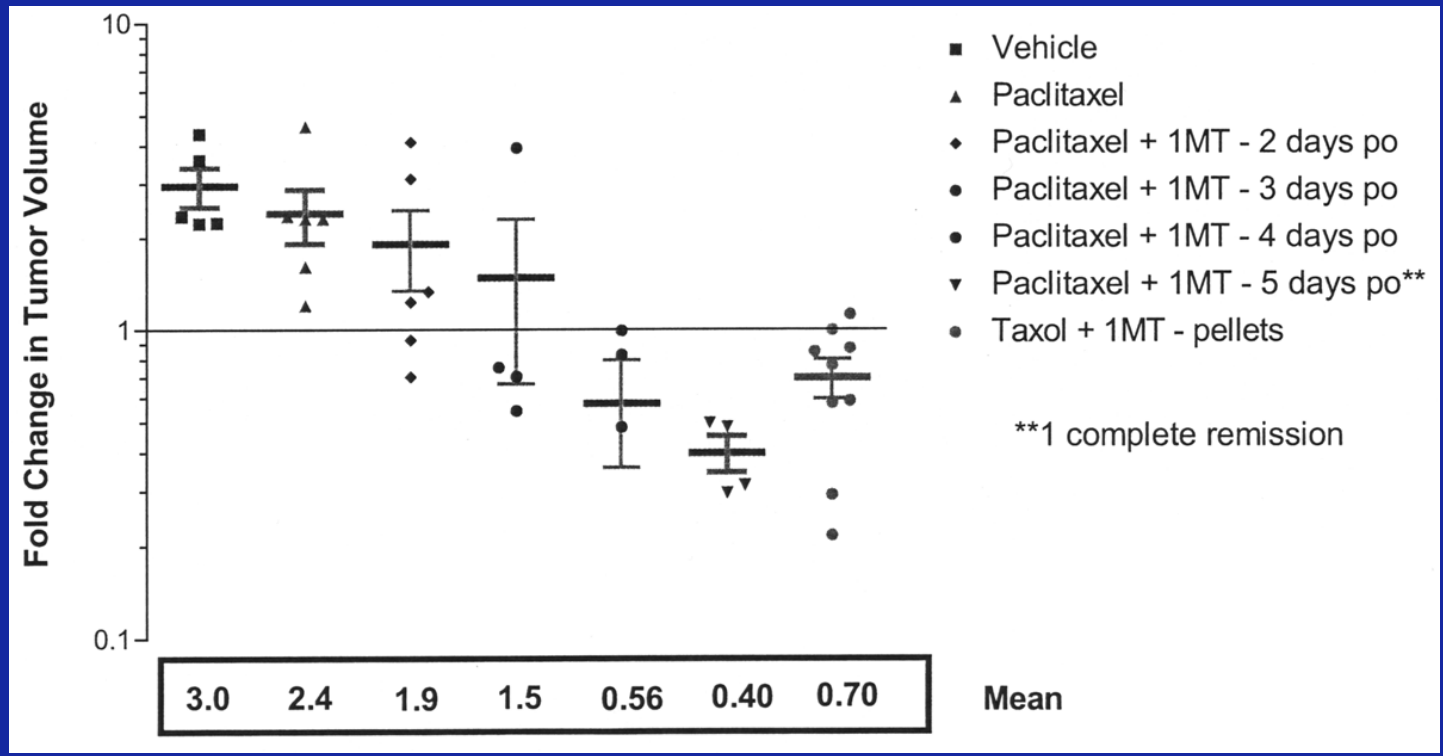
- No side effects or autoimmunity evident
- Limits tumor growth and regresses tumors in combination with cytotoxic chemotherapy
  - Grafted tumors (B16, 4T1)
  - Transgenic “immunoedited” tumors (MMTVneu)
- Antitumor activity is CD4+ T cell–dependent
- Short exposure (days) yields efficacy (weeks)?
- D > L for antitumor activity in most models
- IDO knockout abolishes antitumor effect

IDO Inhibition Cooperates Broadly with Chemotherapy
(MMTVneu Oncomouse Model)



- MMTVneu 2-week trial
- 3x/week MTD (cytotoxics) or daily per literature (MCTs)

Transient Exposure to 1MT is Sufficient for Efficacy in Combination with Paclitaxel (MMTVneu Oncomouse Model)



2-week trial

Is IDO2 a Target of “Lead” Clinical Candidate D-1MT?

- D-1MT isomer is generally more active than L-1MT isomer for antitumor activity
- IDO knockout in mice blunts D-1MT antitumor activity
- BUT as a biochemical inhibitor, D-1MT blocks IDO2 much better than IDO!
- IDO2 may be a relevant target *in vivo*
- There appears to be significant genetic variation of IDO2 in human populations

1MT: Clinical Summary

- IND presently completed (D-1MT)
- Phase I study—traditional dose escalation
- Possible safety concerns (not observed in animals)
  - Eosinophilia-myalgia syndrome?
  - Autoimmunity?
  - Toxoplasma gondii susceptibility?

1MT: Contemplated Uses

- General adjuvant for cancer therapy that acts to relieve a mechanism of tumoral immune suppression
- Combine with
  - Cytotoxic chemotherapy
  - Tumor vaccines
  - TLR agonists (e.g., CpG oligo)
  - Radiation
  - Mabs or drugs that target other mechanisms of immune suppression (e.g., OX40, PD-L1, etc.)

1MT: Contemplated Uses (II)

- Prevention or quality-of-life issues
  - Promote clearance of chronic infections associated with cancer?
  - Relieve cancer-associated depression in patients?
- Application in paraneoplastic syndromes?

1MT: Perceived Need

- Preclinical studies suggest possible utility in combination with a variety of antitumor agents
  - Biological, chemo, radio
- Interest among investigators still small but emerging rapidly at present

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