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Letter From the President

Dear Colleagues,

Welcome to the SITC Primer on Tumor Immunology and Cancer Immunotherapy™ From Biology to Treatment. We are in a pivotal time for this field given the recent FDA approvals of two new immunotherapeutic strategies and evidence for clinical activity with several additional approaches being tested clinically. Increased excitement and success with immunotherapy has been driven by a deeper understanding of the mechanisms by which the immune system can destroy cancer, as well as the resistance mechanisms employed by the tumor that need to be overcome. The long durability of clinical benefit with immune-based therapies is an attractive feature that is not usually seen with most other cancer treatments. As such, immunotherapy is now being viewed as a valuable treatment option and a welcome addition to the armamentarium of cancer therapeutics.

In response to this heightened enthusiasm, the Society for Immunotherapy of Cancer (SITC) has expanded its educational offerings and is pleased to offer this primer for all professionals interested in the field of cancer immunotherapy. The SITC Primer on Tumor Immunology and Cancer Immunotherapy™ From Biology to Treatment is a half-day educational program designed for clinical fellows, practicing oncologists, and other oncology health care professionals who wish to solidify their understanding of recent advances and the biologic basis of tumor immunology and immunotherapy. This program will provide a framework of basic to advanced immunology principles and an understanding of how these concepts are translated into the clinic towards improved patient outcomes.

The SITC cancer immunotherapy primer is relevant for a variety of professionals in the field of oncology and hematology. Whether you are just starting out and need to understand the basic concepts of cancer immunotherapy or if you've been in the field for years but want to gain a better perspective on cancer immunotherapy as a growing field of research, you can benefit from the SITC cancer immunotherapy primer.

On behalf of SITC, I'm excited to welcome everyone to a program that will be highly valuable in advancing our field and training the next generation of cancer immunologists and immunotherapists.

Sincerely,

Thomas F. Gajewski, MD, PhD

SITC President

Society Overview

SITC Profile

The Society for Immunotherapy of Cancer (SITC) was established in 1984 to facilitate the exchange and promotion of scientific information about the use of biological cancer therapies. SITC is a 501(c)(3) not-for-profit organization of medical professionals with a constituency of academic, government, industry, clinical, and basic scientists from around the world. The Society was founded on the belief that new systemic therapeutic treatments would continue to complement chemotherapies and move into the mainstream in the fight against cancer. To aid in this effort, SITC provides intimate channels for the discussion of current clinical trial results and methodologies, as well as means to collaborate on new initiatives in tumor immunology and biological therapy. It is these key interactions and innovations that help advance the progress of cancer research and therapies and lead to better patient outcomes.

Mission Statement

It is the mission of the Society for Immunotherapy of Cancer to improve cancer patient outcomes by advancing the science, development and application of cancer immunology and immunotherapy through our core values of interaction/integration, innovation, translation and leadership in the field.

Core Values

- Interaction/Integration: Facilitate the exchange of information and education among basic and translational researchers, clinicians, young investigators, societies and groups sharing the mission of SITC
- Innovation: Challenge the thinking and seek the best research in the development of cancer immunotherapy
- Translation: Facilitate the transfer of cancer immunology and immunotherapy research from the bench to the clinic and back
- Leadership: Define what is new and important and effectively communicate it to all relevant stakeholders

Members and Meeting Attendees

Society membership continues to grow and now includes more than 550 influential leaders and scientists engaged in immunotherapy/biological therapy of cancer, including academicians, senior researchers, clinicians, students, government representatives, and industry leaders from around the world. SITC's members represent 17 medical specialties and are engaged in research and treatment of at least a dozen types of cancer. With major developments and recent FDA approvals in the field of cancer immunotherapy, the SITC Annual Meeting & Associated Programs attendance is growing as well, attracting over 800 of the brightest minds in the field. Both scientists and clinicians alike from around the globe convene at SITC to share data, hear the most recent advances in the field and find collaboration opportunities.

Disease States Represented by SITC Constituents

SITC covers the full spectrum of both solid tumors and hematologic malignancies including:

- Breast
- Colorectal
- Head & Neck
- Hepatocellular
- Kidney
- Leukemia
- Lung
- Lymphoma
- Melanoma
- Neuroblastoma
- Ovarian
- Prostate
- Renal Cell

Medical Specialties Represented by SITC Constituents

- Cell Biology
- Dermatology
- Genetics
- Gynecologic Oncology
- Hematology
- Immunotherapy
- Internal Medicine
- Medical Oncology
- Microbiology
- Molecular Biology
- Pediatric Oncology
- Pharmacology/Toxicology
- Radiation Oncology
- Radiology
- Stem Cell Biology
- Surgical Oncology
- Transplantation

2012 Educational Activities and Initiatives

Live Educational Events

A major component of the SITC Strategic Plan is an emphasis on providing education and training about the principles and practice of cancer immunotherapy to a broad audience. By continuing with our tradition of facilitating the exchange and promotion of scientific information at our meetings with the aim of expediting the safe transfer of both basic and applied research to the clinical setting, SITC is becoming widely recognized as the leading scientific voice in the field.

NEW! SITC Primer on Tumor Immunology and Cancer Immunotherapy™ From Biology to Treatment

Leading into the American Society for Clinical Oncology (ASCO) Annual Meeting
June 1, 2012 ~ Chicago, IL

The SITC Primer leading into the American Society for Clinical Oncology (ASCO) Annual Meeting is designed to provide a foundation for understanding core immunology principles as they relate to basic and clinical research in immunology and the immunotherapy of malignancy. It aims to advance successful immune-based treatments provided by practicing oncologists quickly and efficiently in point-of-care patient applications for positive outcomes. Thus, the audience for this half-day educational program includes clinical oncologists and other oncology health care practitioners who wish to solidify their understanding of recent advances and the biologic basis of tumor immunology and immunotherapy.

SITC 27th Annual Meeting

October 26-28, 2012 ~ Bethesda, MD

The SITC Annual Meeting is a two and a half day educational activity that allows for scientific exchange of the most cutting-edge preclinical and clinical data on immunotherapies and biological therapy of cancer. The program includes dynamic presentations, interactive panel discussions and scientific posters on the most timely topics in cancer immunotherapy as well as vital information on clinical trial design and regulatory issues to advance collaboration and translation of cancer immunotherapies. Session and abstract topics include:

- Adoptive T Cell Transfer and Cell Therapy as Cancer Immunotherapy (CARS)
- Combining Immunotherapy and Other Therapies
- DC Subsets / Cancer Vaccines
- Immunity of Oncolytic Viruses
- Immunotherapy Combinations
- Innate Immunity in Cancer
- Single Cell High Throughput Technologies Immune Monitoring
- T Cell Manufacture and Potency Testing
- T Cell Modulating Strategies
- Targeted Therapies and Anti-Tumor Immunity
- Targeting Immune Suppression
- Therapeutic Monoclonal Antibodies in Cancer
- Tumor Microenvironment
- Tumor Vasculature, Chemokines and Lymphocyte Trafficking to the Tumor

NEW! Professional Development Session

October 24, 2012 ~ An Associated Program of the SITC Annual Meeting

This half-day event is intended to educate attendees about relevant career development topics that lead to successful scientific careers in cancer immunotherapy. The intended audience for this educational program includes graduate, medical, and post baccalaureate students; clinical fellows; postdoctoral fellows; assistant professors; and other early career professionals. Topics include: Getting a Grant, Managing The Lab / Preparing For Tenure, and The Business Of Research as well as a panel discussion with professionals from academia, industry and government.

SITC Workshop – Focus on the Target: The Tumor Microenvironment

October 24-25, 2012 ~ An Associated Program of the SITC Annual Meeting

The development of cancer has historically been attributed to genomic alterations of normal host cells, with cancer treatments historically targeting the malignant cell itself. It is now clear that tumor growth and development is a complex process that involves both malignant transformation and the influence of normal host cells, including fibroblasts, endothelial cells, lymphocytes, monocytes, and macrophages. The tumor microenvironment has emerged as a critical target for cancer diagnosis, prognosis, and therapy. SITC's two-day workshop on the tumor microenvironment will include presentations from thought leaders in the field, and cover topics from basic tumor

immunobiology to clinical immunotherapy trials that incorporate agents that modulate the tumor microenvironment. It will end with a presentation of progress on the development of the Immunoscore, an ongoing initiative to promote the incorporation of an analysis of immune infiltrates within primary tumors as part of their standard pathologic evaluation for cancer diagnosis, prognosis, and therapy.

SITC Primer on Tumor Immunology and Cancer Immunotherapy™

October 25, 2012 ~ An Associated Program of the SITC Annual Meeting

Understanding of tumor immunobiology has increased dramatically in recent years, leading to the successful development of new immune-based treatment options to improve cancer outcomes. The SITC Primer on Tumor Immunology and Cancer Immunotherapy™ is designed to provide a foundation for understanding core immunology principles as they relate to basic and clinical research in immunotherapy of cancer. Prominent investigators will summarize central themes and recent research in tumor immunology and cancer immunotherapy. Topics include: Innate Immunity, Dendritic Cells, T Cell Differentiation, Antibody Therapy, and the Tumor Microenvironment as well as Recent Advances in the Clinical Application of Cancer Vaccines, Coinhibition and Costimulation of Immune Cells for Immunotherapy, Adoptive Immunotherapy, and Immune Monitoring in Clinical Trials of Cancer Immunotherapies.

"Meet-the-Expert" Breakfast

October 27, 2012 ~ An Associated Program of the SITC Annual Meeting

This breakfast session will focus on unique issues related to the career development of early career scientists. Key leaders in the field will facilitate roundtable discussions on particular areas of interest. Experts will answer questions and lead informal dialogues to help provide guidance and direction. The intended audience for this educational program includes graduate, medical, and post baccalaureate students; clinical fellows; postdoctoral fellows; assistant professors; and other early career professionals. Topics include: Developing Successful Collaborations, Finding Your Niche, Grant Writing, Publishing Papers, Translational Research, Managing a Research Lab, and Work-Life Balance.

NEW! Online Education

As part of the expanded offerings on the newly redesigned SITC website, SITC intends to offer web-based learning opportunities as part of the "education portal" that is in the final stages of development. Information on the following planned activities will be updated as made available.

Webinars

Two webinars are planned for 2012. These programs will be live educational activities that will also be archived on the SITC website for future learning. The intended audience(s) and program topics are currently under development.

Online Courses

The following two courses are currently under development and are planned to be offered via the SITC website:

- Primer for Clinical Oncologists
- Primer for Immunologists

NEW! SITC Initiatives

SITC initiatives are projects that have been developed based on the goals outlined in the SITC Strategic Plan, are designed to promote the Society's mission, and are focused on issues of major importance to the field. The following activities are examples of current and planned SITC Initiatives that are supported by the Society and general contributions to the SITC Trust. For more information about these and other SITC initiatives and collaborations, please inquire with the SITC office at 414-271-2456.

- Cancer Immunotherapy Guidelines Development
- Immunoscore Project
- Professional Development and Merit Awards
- International Collaboration and Outreach
- Immunotherapy Ambassadors Program
- Professional Resource Materials
- Patient Education About Immunotherapy
- Young Investigator Awards and Programs

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Education and Meetings Coordinator

Haley Haas
Administrative Coordinator

SITC Membership Information

Overview

The Society for Immunotherapy of Cancer invites your support for our organization, its activities, and events by becoming a member. SITC fills its membership with those from industry, academia, and government, serving as clinical and basic scientists and industry representatives. Your contributions as a member can help shape SITC policy as we continue in our efforts to advance the development and application of cancer immunotherapy.

Through membership in SITC, you will be a member of an organization that is actively engaged in facilitating the implementation of timely, cutting-edge translational clinical research in cancer biotherapy.

SITC Membership Benefits

- One year subscription to the *Journal of Immunotherapy*, an official journal of SITC
- One year, online full-text access to the *Journal of Immunotherapy*
- Reduction in submission fees and opportunity for rapid publication in SITC's subsection of the open-access *Journal of Translational Medicine*
- Reduction in registration fees for the SITC Annual Meeting and Associated Programs
- Free access to speaker presentations and slide sets from past SITC live events
- Online directory of SITC members
- Access to "Members Only" section of SITC website: www.sitcancer.org
- Eligibility to serve on SITC committees
- Eligibility to serve on SITC Board of Directors (Regular members)
- Discount on SITC enduring materials

Additional Benefits:

- Access to the best science in the field
- Early access to timely information on what is new and relevant to biological approaches for the treatment of cancer
- Opportunities to participate in and shape discussions that guide progress in the field
- Opportunities to network with colleagues to develop new ideas, establish new collaborations to advance your work, and participate in active scientific exchange
- Access to luminaries in the field, including leading scientists and clinical researchers
- Guidance on relevant and timely issues
- The opportunity to advance your career

Membership Types

Regular Membership ($220 annual dues) Available to individuals with an MD or PhD in a biological science or the equivalent who are active, bona fide representatives of the international scientific community with a specialty or interest in a field related to the biological therapy of cancer. Regular membership includes the right to vote.

Affiliate Membership ($220 annual dues) Available to individuals active or otherwise interested in the biological therapy of cancer. Affiliate membership does not include the right to vote.

Scientist-in-Training (Student) Membership ($50 annual dues) Available to individuals enrolled in MD or PhD academic programs or those participating in postdoctoral fellowships and residency programs who show a demonstrated interest in biological therapy of cancer. Student membership includes an online only subscription to the *Journal of Immunotherapy*, but does not include the right to vote.

Application Requirements

Regular applicants:

- Curriculum Vitae or educational resumé

Affiliate applicants:

- Business or educational resumé or Curriculum Vitae

Scientist-in-Training (Student) applicants:

- Proof of enrollment
- Letter of recommendation or Curriculum Vitae

SITC Membership Application

Please check the membership category you are applying for:
☐ Regular ☐ Affiliate ☐ Scientist-in-Training (Student)

Name: _____

Academic Degree: (please circle) MD PhD RN MS NP PharmD Other: _____

Institution/Company: _____

Title: _____ Dept: _____

Mailing Address: _____

City: _____ State: _____ Postal Code: _____

Country: _____ Email: _____

Phone: _____ Fax: _____

Work Sector (check one):
☐ Academia ☐ Government ☐ Industry/Corporate ☐ Not-for-Profit Organization

Practice or Work Setting (check one):
☐ Clinic ☐ Government ☐ Hospital ☐ Lab ☐ Lab & Clinic (translational) ☐ Medical School/University
☐ Pharmaceutical/Biotech ☐ None

Field(s) of specialty (check all that apply):

☐ Cell Biology	☐ Immunotherapy	☐ Pediatric Oncology	☐ Stem Cell Biology
☐ Dermatology	☐ Internal Medicine	☐ Pharmacology/ Toxicology	☐ Surgical Oncology
☐ Genetics	☐ Medical Oncology	☐ Radiation Oncology	☐ Transplantation
☐ Gynecologic Oncology	☐ Microbiology	☐ Radiology	☐ Others _____
☐ Hematology	☐ Molecular Biology		_____

Disease state(s) (check those most affiliated with your research or practice):

☐ Breast	☐ Hepatocellular	☐ Lung	☐ Neuroblastoma	☐ Renal Cell
☐ Colorectal	☐ Kidney	☐ Lymphoma	☐ Ovarian	☐ Others _____

Application Requirements

Regular applicants:
☐ I will email my CV or educational resumé to info@sitcancer.org.
☐ My CV or educational resumé is enclosed.

Affiliate applicants:
☐ I will email my business or educational resumé to info@sitcancer.org.
☐ My business or educational resumé is enclosed.

Scientist-in-Training (Student) applicants:
☐ I will email my letter of recommendation or CV and proof of enrollment to info@sitcancer.org.
☐ My letter of recommendation or CV and proof of enrollment are enclosed.

Membership applications are reviewed throughout the year. Applicants will be contacted upon acceptance. Membership is valid from the date dues are paid in full until the end of that calendar year.

Membership Fee:
☐ Regular/Affiliate ($220) ☐ Scientist-in-Training (Student) ($50)
☐ Check (enclosed) Make checks payable to SITC in U.S. dollars drawn from a U.S. bank.
☐ VISA ☐ MasterCard ☐ American Express ☐ Discover

Card Holder: _____

Card Number: _____ Exp.: _____

Signature: _____ Date: _____

Return this form to: SITC • 555 E. Wells St., Suite 1100 • Milwaukee, WI 53202-3823
Tel: 414-271-2456 • Fax: 414-276-3349 • Email: info@sitcancer.org • Web: www.sitcancer.org

SITC Primer on Tumor Immunology and Cancer Immunotherapy™ From Biology to Treatment

June 1, 2012
8:00 am - 1:00 pm
Conference Center, First Floor, Room 10 ABC
Hyatt Regency McCormick Place in Chicago, IL

Program Purpose
The SITC Primer on Tumor Immunology and Cancer Immunotherapy™ From Biology to Treatment is designed to provide a foundation for understanding core immunology principles as they relate to basic and clinical research in immunology and the immunotherapy of malignancy. It aims to advance successful immune-based treatments provided by practicing oncologists quickly and efficiently in point-of-care patient applications for positive outcomes.

Prominent investigators will summarize central themes and recent research advances in such areas as innate immunity and inflammation in cancer biology, dendritic cells, T cell function, antibody therapy, and the host-tumor relationship as well as recent advances in the clinical application of cancer vaccines, coinhibition and costimulation of immune cells, and adoptive cellular therapy of cancer. These topics will be addressed in a series of lectures by thought leaders in the field and through interactive question and answer sessions.

Organizers
Kim A. Margolin, MD - *Seattle Cancer Care Alliance*
Walter J. Urba, MD, PhD - *Earle A. Chiles Research Institute*

Intended Audience
The audience for this half-day educational program is clinical oncologists working directly with patients as well as other oncology health care practitioners who wish to solidify their understanding of recent advances and the biologic basis of tumor immunology and immunotherapy. In addition, other audiences who would find this program of value are basic scientists and clinical investigators (clinicians, researchers, graduate students, postdoctoral fellows, and allied health professionals) from academic institutions, industry, and regulatory agencies.

Program Goals
- Provide a framework of basic immunology for clinical oncologists and facilitate understanding of more sophisticated principles of tumor immunology and immunotherapy
- Facilitate the translation of cancer immunotherapy research into medical practice by clinical oncologists
- Provide a common terminology and knowledge base for clinical oncologists from all oncology disciplines and sub-specialties
- Review the biology of innate immunity, dendritic cells, T cell differentiation and intracellular signaling, and the tumor microenvironment as related to recent advances in cancer immunotherapies
- Summarize the principles of and recent advances in the application of tumor antigens for immunization, coinhibition and costimulation of immune cells, and adoptive immunotherapy of malignant disease
- Provide the opportunity for dialogue and professional interactions that promote collaboration and scientific exchange

Expected Learner Outcomes
Upon completion of this program, the participants will be able to:
- Interpret the key principles of tumor immunology and immunotherapy
- Describe the role of tumor biology, antigens, T cell differentiation and signaling, and the host-tumor relationship in cancer immunotherapies
- Discuss recent research and clinical applications of tumor immunization, immune coinhibition and costimulation, adoptive immunotherapy, and biomarkers
- Participate in scientific exchange with colleagues and collaborators on research and application of cancer immunotherapies to improve outcomes of patients with cancer

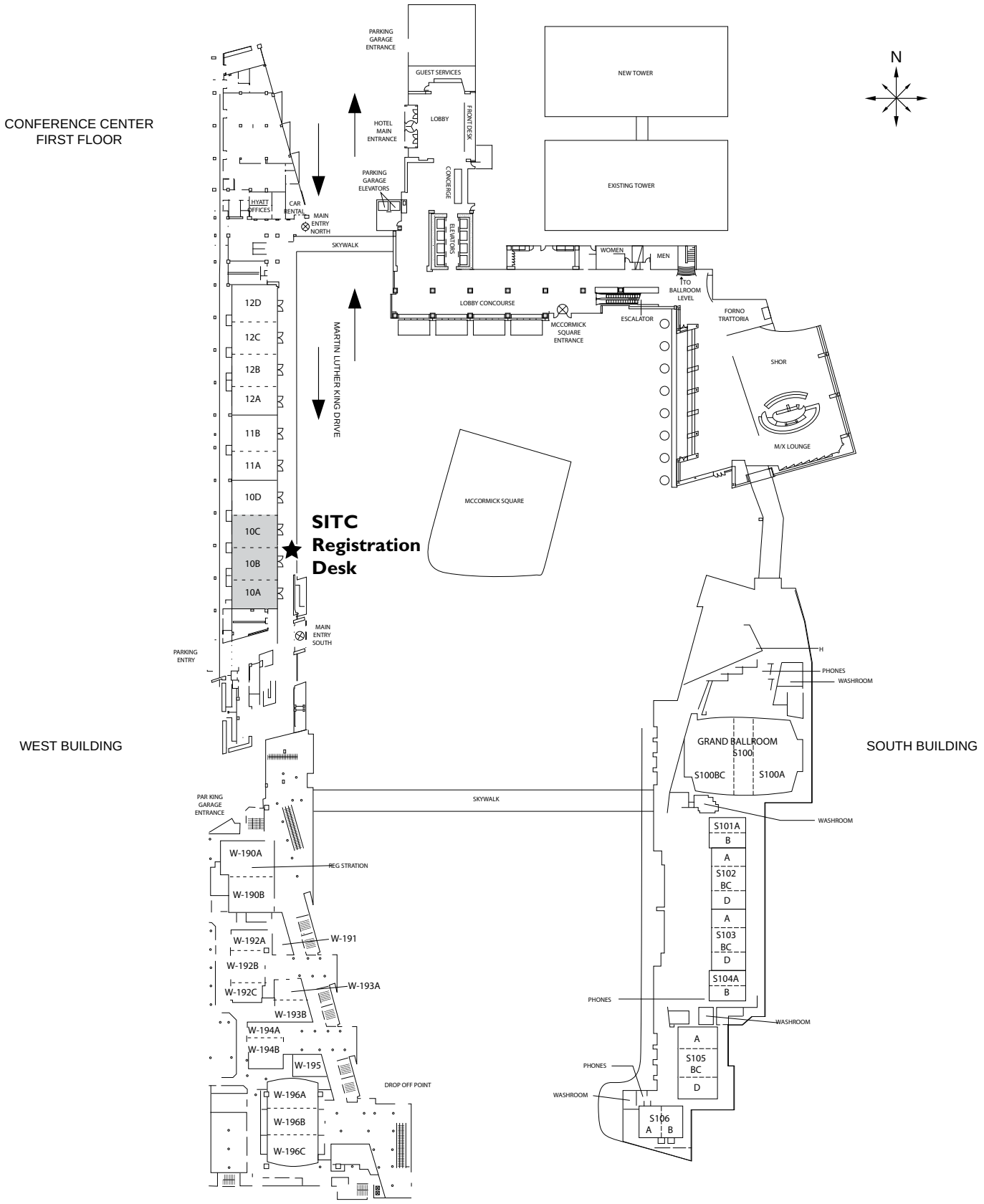
Faculty
Laurence J.N. Cooper, MD, PhD - *MD Anderson Cancer Center*
Leisha A. Emens, MD, PhD - *Johns Hopkins University*
Justin P. Kline, MD - *University of Chicago*
Robert J. Kreitman, MD - *National Institutes of Health*
Augusto Ochoa, MD - *Louisiana State University Health Sciences Center*
Drew M. Pardoll, MD, PhD - *Johns Hopkins University School of Medicine*

Primer Schedule

As of May 23, 2012

7:00 am – 8:00 am	**Registration and Continental Breakfast**
8:00 am – 8:05 am	**Welcome and Introductions** Kim A. Margolin, MD - *Seattle Cancer Care Alliance*
8:05 am – 8:40 am	**Basic Immunology** Justin P. Kline, MD - *University of Chicago*
8:40 am – 8:45 am	Audience Response/Questions and Answers
8:45 am – 9:20 am	**Host Tumor Relationship** Augusto Ochoa, MD - *Louisiana State University Health Sciences Center*
9:20 am – 9:25 am	Audience Response/Questions and Answers
9:25 am – 10:00 am	**Tumor Antigens and Cancer Vaccines** Leisha A. Emens, MD, PhD - *Johns Hopkins University*
10:00 am – 10:05 am	Audience Response/Questions and Answers
10:05 am – 10:40 am	**Adoptive Immunotherapy with T Cells** Laurence J.N. Cooper, MD, PhD - *MD Anderson Cancer Center*
10:40 am – 10:45 am	Audience Response/Questions and Answers
10:45 am – 11:10 am	*Refreshments and Networking*
11:10 am – 11:45 am	**Coinhibition and Costimulation** Drew M. Pardoll, MD, PhD - *Johns Hopkins University School of Medicine*
11:45 am – 11:50 am	Audience Response/Questions and Answers
11:50 am – 12:25 pm	**Antibody Based Immunotherapy** Robert J. Kreitman, MD - *National Institutes of Health*
12:25 pm – 12:30 pm	Audience Response/Questions and Answers
12:30 pm – 12:50 pm	**Final Questions and Answers**
12:50 pm – 1:00 pm	**Closing Comments** Walter J. Urba, MD, PhD - *Earle A. Chiles Research Institute*

Venue Map


CONFERENCE CENTER
FIRST FLOOR
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GUEST SERVICES
NEW TOWER
HOTEL MAIN ENTRANCE
LOBBY
FRONT DESK
EXISTING TOWER
PARKING GARAGE ELEVATORS
CONCIERGE
HYATT OFFICES
CAR RENTAL
MAIN ENTRY NORTH
SKYWALK
ELEVATORS
WOMEN
MEN
TO BALLROOM LEVEL
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S103
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Disclosures

Presenter Financial Disclosure Information

It is the policy of SITC to ensure balance, independence, objectivity and scientific rigor in all educational activities. All presenters participating in any SITC sponsored activity are required to disclose to SITC any real or potential conflict of interest that may have a direct bearing on the subject matter of the activity. This pertains to relationships with pharmaceutical companies, device manufacturers, or other corporations whose products or services are related to the subject matter of the presentation topic.

In assurance of independence, objectivity, and balance, the disclosures are critically reviewed to ensure any conflicts of interest are resolved prior to the educational program taking place.

The disclosures provided are inclusive of all faculty, organizers, and planners for this educational program.

Disclosures

Sharon Kayne Chaplock, PhD
SITC Staff
No relevant financial relationship to disclose

Laurence J.N. Cooper, MD, PhD
MD Anderson Cancer Center
InCellerate, Inc, Ownership, Founder

Nadine M. Couto, CMP
SITC Staff
No relevant financial relationship to disclose

Leisha A. Emens, MD, PhD
Johns Hopkins University
Genentech, Grant, Regional Advisory Board; Roche, Inc, Xeloda in Breast Cancer, Consultant/Advisor; Biosante, Patent Royalty/ Intellectual Property Rights; Bristol-Myers Squibb, Nothing, Advisory Role; US Food and Drug Administration, Nothing, Advisory Committee

Haley Haas
SITC Staff
No relevant financial relationship to disclose

Angela Kilbert
SITC Staff
No relevant financial relationship to disclose

Justin P. Kline, MD
University of Chicago
Genentech, Consulting Fee, Advisory Committee, Bristol-Myers Squibb, Access to Clinical Trials and Patient Samples, Co-Principal Investigator

Amanda Knack
SITC Staff
No relevant financial relationship to disclose

Robert J. Kreitman, MD
National Institutes of Health
Coinventor of Patent for Moxetumomab pasudotox, Royal Payments from the National Institutes of Health, Work for National Institutes of Health

Margaret Lecey
SITC Staff
No relevant financial relationship to disclose

Kim A. Margolin, MD
Seattle Cancer Care Alliance
Genentech, Honorarium, Consultant; GlaxoSmithKline, Honorarium, Advisory Board; Bristol-Myers Squibb, Honorarium, Consultant; Hoffman-LaRoche, Honorarium, Consultant; Cell Therapeutics, Consulting fee, Review Regulatory Documents

Augusto Ochoa, MD
Louisiana State University Health Sciences Center
No relevant financial relationship to disclose

Drew M. Pardoll, MD, PhD
Johns Hopkins University School of Medicine
Amplimmune, Consulting Fee, Consultant; Aduro, Consulting Fee, Consultant; Immune Excite, Consulting Fee, Consultant

Celeste Stroh
SITC Staff
No relevant financial relationship to disclose

Walter J. Urba, MD, PhD
Earle A. Chiles Research Institute
Bristol-Myers Squibb, Consulting Fee and Honoraria, Independent Contractor, Speaking/ Teaching, Member Advisory Board; DC Prime, Consulting Fee, Scientific Advisory Board; SAIC-Frederick, Consulting Fee, Member Advisory Board; National Cancer Institute, Consulting Fee, Member Advisory Board

Jennifer Warren
SITC Staff
No relevant financial relationship to disclose

Tara Withington, CAE
SITC Staff
Executive Director, Inc, Ownership Interest, Employment/Partner

Basic Immunology

Justin P. Kline, MD
University of Chicago

Dr. Kline is currently an Assistant Professor of Medicine, Section of Hematology/Oncology at the University of Chicago. He trained as a postdoctoral fellow in the lab of Dr. Thomas Gajewski. His laboratory is interested in studying immune evasion mechanisms operational in pre-clinical models of acute leukemia, and is currently developing a genetic model of acute myeloid leukemia (AML), which will more accurately recapitulate the development of this lethal disease as it occurs in humans. This model and others like it will someday serve to increase our knowledge of the outcome of T cell – tumor cell interactions in vivo. Dr. Kline's clinical interest is in the treatment of hematological malignancies and in stem cell transplantation.

Pre-Test Questions
Basic Immunology

1. Cells of the innate immune system include all listed below, except:

A. Natural killer (NK) cells
B. Dendritic cells
C. B cells
D. Macrophages

2. CD4+ T cells are capable of differentiating into all of the subsets below, except:

A. Induced regulatory T cells
B. TH1 cells
C. TH17 cells
D. Cytotoxic T lymphocytes (CTL)

3. The development and regulation of regulatory T cell function is dependent upon which transcription factor?

A. T-bet
B. FoxP3
C. ROR-γt
D. GATA-3

4. Which of these cytokines is necessary for naïve T cell survival under homeostatic conditioins?

A. IL-7
B. IL-12
C. TGF-β
D. IL-4

Basic immunology for the non-immunologist

Justin Kline, M.D.
Assistant Professor of Medicine
University of Chicago

The following relationships exist related to this presentation

Honoraria/Consulting - Genentech

Outline

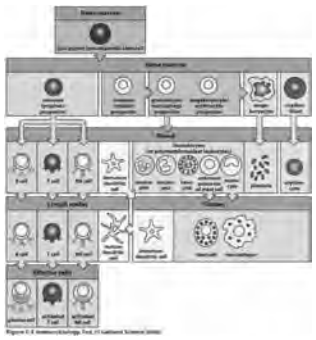
- **Immune system – development**
- **Innate immune system**
  - Pattern recognition receptors
  - Dendritic cells
  - NK cells
- **Adaptive immune system**
  - T cell development/maturation
  - T cell subsets
  - T cell activation/differentiation
  - Regulatory T cells
  - Homeostatic T cell cytokines
- **Cancer immunology – brief introduction**

Immunology – basic principles

- Attributed to Edward Jenner (late 1700s)
  - Found that inoculation with cowpox virus conferred protection against smallpox
    - Coined the term "vaccination"
- The immune system evolved to provide protection against invasive pathogens
- Consists of a wide variety of cells and proteins whose purpose is to generate immune responses against micro-organisms
- Whether the immune system provides active surveillance against malignant cells is debatable

Immune system - developement

- All immune cells are produced in the bone marrow
- **T cells** mature in the thymus
- **B cells** mature in the marrow
- **Primary lympoid organs** (bone marrow, thymus) – where immune cells are produced/matured
- **Secondary lymphoid tissues** (lymph nodes, spleen, mucosal lymphoid tissues) – where immune responses are initiated

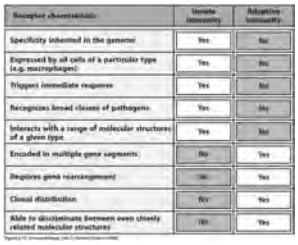


Immune system – a division of labor

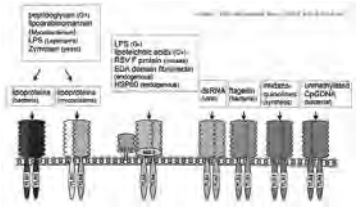
- Immune system is comprised of:
  - Innate immune system
  - Adaptive immune system
- Innate immune system
  - Provides initial recognition of self vs non-self
  - Comprised of ***cells*** (granulocytes, monocytes, dendritic cells and NK cells) and ***proteins*** (complement)
  - Recognize non-self via pathogen-associated molecular patterns (PAMPs)
    - conserved structures (i e. LPS) in microbes
  - Pattern recognition receptors (PRRs) expressed on innate immune cells recognize PAMPs
  - Necessary for priming adaptive immune responses
  - Does not provide immunological memory

Innate immunity – on the front line of host defense

- Classes of PRRs
  - Toll-like receptors
  - NOD proteins
  - C-type lectin receptors
- Differential expression of PRRs on innate immune cells determines "functionality"



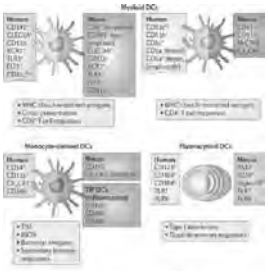
Innate immunity – the Toll-like Receptors



- TLRs originally described in Drosophila
  - Bruce Beutler received Nobel prize in 2011 for discovering that LPS bound TLR4
- 10 expressed TLR genes in humans
- Present on extracellular or intracellular membranes
- Binding of TLR by ligand induces signalling through MyD88 adaptor protein
  - leads to NF-kB activation
  - upregulation of MHC molecules
  - costimulatory molecules
  - cytokines (TNF-α, IFN-β, IL-12) and chemokines

Innate immunity – dendritic cells

- Ralph Steinman (1970s) hematopoietic cells which excelled at antigen presentation and T cell activation
  - Nobel prize in 2011 for discovery of DC
- DC classified functionally in 2 groups
  - Conventional DC
    - Antigen presentation
    - T cell activation
  - Plasmacytoid DC
    - Type I IFN production
    - Important for immune responses against viruses



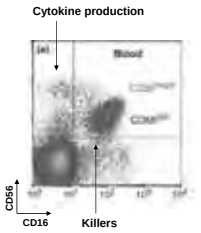
Colin et al Nat Rev Immunol 2011

Innate immunity – dendritic cells

- DC receive signals through PRRs and other receptors (i.e. CD40) to become activated
  - Activation/licensing of DC results in differentiation
    - Upregulation of MHC
    - Upregulation of costimulatory and cell adhesion molecules
    - Production of pro-inlfammatory cytokines (IL-12, TNF-α, type I FNs
    - Alteration of chemokine receptor expression
    - Migration
  - Only licensed DC will activate naïve T cells
  - Non-licensed DC fail to activate T cells and can induce peripheral tolerance (T cell deletion or anergy)

Innate immunity – NK cells

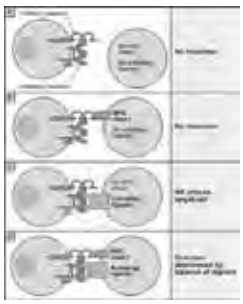
- Natural killer cells (NK cells – $CD3^{-}CD56^{+}CD16^{+/-}$ lymphocytes)
  - Develop in bone marrow from CLP
  - Circulate in blood
  - Functionally identified by ability to kill lymphoid tumor cell lines in vitro without need for prior activation
  - Mechanism of killing – production of cytotoxic granules containing perforin and granzymes
    - Also express Fc receptors - effectors of ADCC
  - Important for early host recognition of infected host cells
    - HSV and Leishmania
  - NK cells are "activated" in response to Type I IFNs, TNF-α and IL-12
    - killing capacity and production of IFN-γ



Cooper et al Trends Imunol 2004

Innate immunity – NK cell receptors

- 2 families of NK receptors
  - Killer lectin-like receptors (KLRs)
  - Killer cell Ig-like receptors (KIRs)
- Both KLRs and KIRs can act as activating or inhibitory receptors
  - Makes the study of NK cell activation complicated
  - Further complicated by the fact that KIR genes are also polymorphic
- Missing self hypothesis:
  - NK cells do not kill self cells due to MHC class I expression
  - NK cell do kill target cells which lack MHC class I



Lanier L Ann Rev Immunol 2005

Innate immunity – NK cells and cancer

- NKG2D - C-type lectin receptor on NK cells
  - Recognizes RAE proteins and MICA and MICB
    - RAE and MICA/B are MHC class I-like molecules expressed on virally-infected cells and some malignant cells
    - Recognition of ligands by NKG2D on NK cells serves as a "danger" signal, resulting in "costimulation" of NK cells
    - Leads to lysis of targets and production of IFN-γ
- KIRs and graft-versus-leukemia effect following allogeneic SCT
  - Donor vs recipient KIR mismatches provided GVL effect and protected from GVHD
    - Ruggeri et al Science 2002.
- Other retrospective analyses have confirmed that K R mismatched donor vs host allografts led to decreased risk of AML relapse following alloSCT
  - Ongoing studies are evaluating the efficacy of adoptive KIR-mismatched NK cell therapy in myeloid leukemias

Interest in NK cell therapy is growing

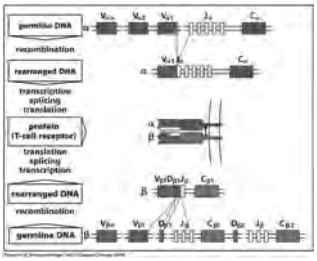
	Transplant	Graft	Outcome
Ruggeri et al, Science 2002	Haploidentical KIR-L mismatch	TCD	Benefit
Davies et al, Blood 2002	URD KIR-L mismatch	UBM	No Benefit
Geibel et al, Blood 2003	URD KIR-L mismatch	In Vivo TCD	Benefit

Adaptive immunity – lymphocytes

- Adaptive immune system evolved in vertebrates to provide an almost unlimited diversity of antigen receptors to protect the host from infection
- Comprised of B and T lymphocytes
- Each B and T cell expresses a unique antigen receptor comprised of a combination of variable and constant gene segments
  - Diversity (in part) and antigen specificity is provided by Complimentary Determining Regions (CDR) of the BCR and TCR
    - CDR regions are located at the joining segments of the BCR or TCR
  - 10^8 unique lymphocyte receptors present in humans!
- B cell receptor = antibody – recognizes intact extracellular antigens
  - Proteins/glycoproteins
- T cell receptor – recognizes peptides in the context of MHC class I and II proteins
- Because T cells appear to be more important in the response to malignant cells, we will focus our attention here

Adaptive immunity – T cell development and maturation

- T cells develop in the bone marrow and mature in the thymus
- T cell receptor gene rearrangement occurs in the thymus (RAG prinicple enzyme)
- The TCR is comprised of the TCR-α and TCR-β chains
  - TCR-α = VαJαCα
  - TCR-β = VβDβJβCβ
- Successful rearrangement of TCR-α and B chains permits survival and is followed by positive and negative selection

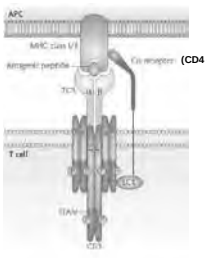


Adaptive immunity – Thymic selection of T cells

- Thymic T cells with rearranged TCR will undergo both positive and negative selection prior to leaving the thymus as mature, naïve T cells
- Positive selection – developing T cell **must** recognize host MHC (determined by CDR1 and CDR2 regions of the TCR)
  - If no MHC binding affinity, T cell undergoes apoptosis
- Negative selection – developing T cell **must not** recognize MHC:peptide with strong affinity
  - If yes, then T cell is deleted (apoptosis)
  - Mechanism of central (thymic) tolerance – assures that strongly auto-reactive T cells do not escape the thymus
  - Developing T cells exposed to a wide variety of host proteins expressed in the thymus via AIRE (autoimmune regulator)
    - Transcription factor expressed in thymic medullary stromal cells – induced expression of tissue-specific proteins
    - AIRE mutations lead to severe autoimmune syndromes
- Only a small percentage of thymic T cells ever leave the thymus as mature T cells

Adaptive immunity – CD4 and CD8 T cell subsets

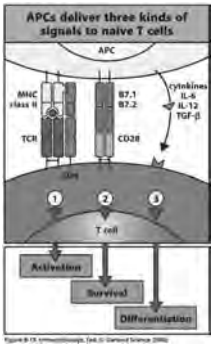
- 2 main "flavors" of mature T cells
  - $CD8^+$ T cell
  - $CD4^+$ T cell
- $CD8^+$ T cells recognize peptide (7-9aa) presented by MHC class I
  - Cytosolic antigens (intracellular pathogens and self peptides) are presented by MHC class I and recognized by $CD8^+$ T cells
- $CD4^+$ T cells recognize peptide (20aa) presented by MHC class II
  - Exogenous antigens are processed and presented by MHC class II molecules and recognized by $CD4^+$ T cells



Gascoigne et al Nat Rev Immunology 2008

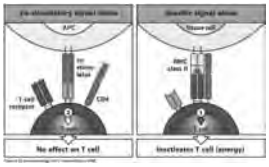
Adaptive immunity – Activation of naïve T cells

- Naïve T cells survive long-term in absence of encounter with cognate (specific) antigen
  - Dependent on cytokines (IL-7) and non-specific MHC TCR interactions
- Initial T cell encounter with antigen causes
  - Entry into cell cycle
  - Production of IL-2 (growth factor)
  - Upregulation of IL-2 receptor (autocrine loop)
- Full activation of naïve $CD4^+$ or $CD8^+$ T cell requires (at least) 2 signals provided by APC
  - MHC/peptide TCR (signal 1)
  - B7 CD28 (signal 2)
  - Cytokines (IL-12) (signal 3)
- Activated T cells proliferate and differentiate into effectors that do not require co-stimulation to act



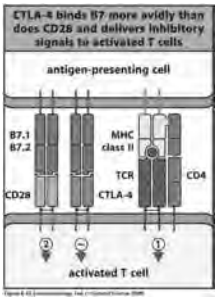
Adaptive immunity – Activation of naïve T cells

- If a naive T cell receives signal 1 in absence of signal 2….
  - Clonal deletion
  - Anergy (non-responsive state)
  - Major mechanism of peripheral tolerance
- The maturation state of APC is important
  - Quiescent APC – poor costimulation → tolerance
  - Activated APC – strong costimulation → T cell activation



Adaptive immunity – Positive and negative costimulatory receptors

- Serve to modulate the magnitude of T cell activation and effector function
- Positive costimulatory ligands
  - CD28 (classical)
  - ICOS (inducible costimulator)
  - CD27 (TNF family receptor)
- Negative costimulatory ligands
  - CTLA-4 (cytotoxic lymphocyte antigen – 4)
  - PD-1 (programmed death -1)
  - TIM-3 (T cell immunoglobulin mucin -3)
- CTLA-4 and PD-1 blocking mAbs are effective in preventing tumor-induced T cell dysfunction in humans with cancer (melanoma).



Adaptive immunity – $CD8^+$ T cell differentiation and effector function

- Following activation, $CD8^+$ T cells differentiate into cytotoxic lymphocytes (CTL)
  - Functions
    - 1) killing via release of cytoplasmic granules containing granzymes and perforin which induce target cell apoptosis
    - 2) release of effector cytokines (IFN-γ, LT-α, TNF-α)

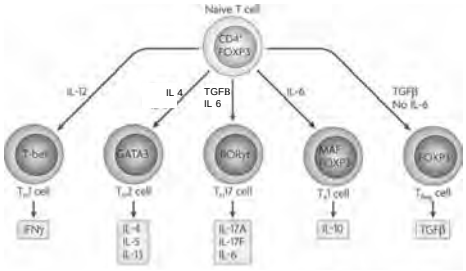
Adaptive immunity – $CD4^+$ T cells are "helpers" in the immune response

- Similar to $CD8^+$ T cells, activated $CD4^+$ T cells proliferate and acquire effector functions
- Classical functions of $CD4^+$ T cells include
  - Production of IL-2 to promote proliferation of activated $CD8^+$ T cells
  - Licensing of dendritic cells through CD40-CD40L interactions
  - Production of effector cytokines (T_H subtype-dependent)
  - ? Lysis of target cells

Adaptive immunity – $CD4^+$ T cell differentiation and effector function

- Differentiation pathways for $CD4^+$ T cells are more complicated than for $CD8^+$ T cells
- 4 subsets of $CD4^+$ T cells (a.k.a. T_H cells)
  - **T_H1** – Typical bacterial infection, viral infection, tumor immunity
  - **T_H2** - allergy
  - **T_H17** – gut homeostasis, autoimmunity
  - **Regulatory T cells (Tregs)** – suppress conventional T cells, peripheral tolerance
- Which of these pathways a $CD4^+$ T cells follows depends on
  - Antigen specificity
  - Local environment - signal 3 received (IL-12, TGF-B, L-6, L-4)
- Each $CD4^+$ T cell subset acquires a unique effector program (cytokine production) and drives a different type of immune response

Adaptive immunity – CD4+ T cell differentiation



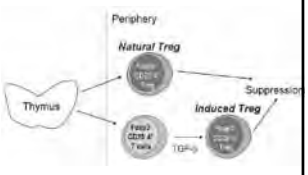
Hooper et al Nat Rev Immunol 2010

Adaptive immunity – Regulatory T cells

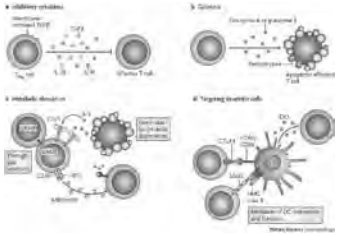
- Subset of CD4+ T cells with natural suppressive function
  - Definitively described in 1998 by Sakaguchi and colleagues
  - Immunophenotype: $CD4^+CD25^+FoxP3^+$
  - Represent ~ 5-10% of the circulating CD4+ T cell population
  - Development and functional program of Tregs is controlled by the FoxP3 transcription factor
    - Necessary for Treg development and maintenance of the functional properties of Treg
    - Mice and humans with mutations of FoxP3 expression develop severe autoimmune disorders
    - Highlights the important role of Treg in maintaining peripheral tolerance

Adaptive immunity – Regulatory T cell development

- 2 main subsets of Treg
  - Natural Treg (nTreg)
    - Develop in thymus
    - Recognize self-Ag
    - Stable phenotype
  - Induced Treg (iTreg)
    - Exit thymus as $CD4^+FoxP3^-$ naïve CD4 T cells
    - In the presence of TGF-B, induced to express FoxP3
  - Both nTreg and iTreg have potent suppressive capability in vivo



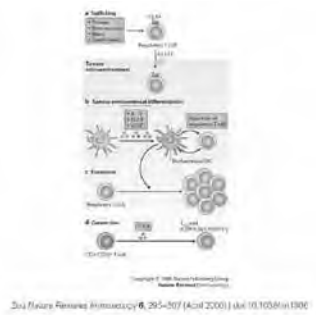
Adaptive immunity – Regulatory T cells – suppressive mechanisms



- Treg suppress conventional T cell function through a number of diverse mechanisms
  - Secretion of suppressive cytokines (TGF-B, IL-10, L-35)
  - Act as cytokine sinks (Binding of local IL-2)
  - Secrete granzymes to kill effector T cells and DC
  - Block costimulatory ligands on DC

Adaptive Immunity – Regulatory T cells and cancer

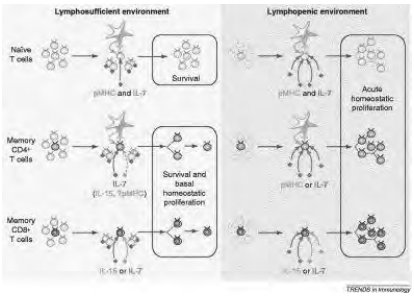
- Regulatory T cells expand in patients with a variety of malignancies
  - In several malignancies, expansion of Tregs correlates with reduced survival
- Induced Treg may also play a role in suppression of anti-tumor immune responses
- Depletion of Treg in pre-clinical cancer models almost universally leads to enhanced anti-tumor immune responses
- Strategies to deplete or inhibit Treg in humans
  - Denileukin Diftitox
  - Daclizumab
  - CTLA-4 blockade
  - Cyclophosphamide



Adaptive immunity – Naïve and memory T cell homeostasis

- In absence of infection the size and composition of the peripheral T cell pool remains relatively constant
- Naïve and memory T cells can survive for long periods of time in the host
  - Slow proliferation balanced by death
- Homeostasis of the T cell compartment depends on:
  - Cytokine signals (IL-7, IL-15)
    - Upregulate pro-survival genes and cell cycle-dependent genes
  - Interaction with self-MHC:peptide

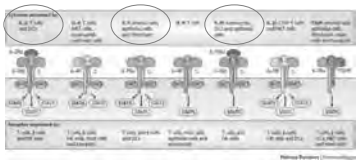
Adaptive immunity – Cytokines are required for T cell survival and proliferation



Baccala R et al Trends Immunol 2006

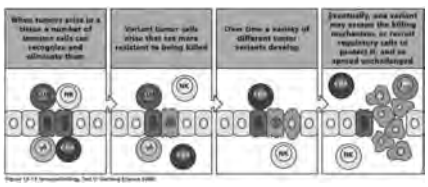
Adaptive immunity – Receptors for homeostatic cytokines

- Homeostatic cytokines signal through a family of common receptor subunits
  - IL-2Rα – CD25
  - IL-2Rβ – CD122
  - IL-7Rα – CD127
  - IL-15Rα – CD215
  - Common γ-chain – CD132



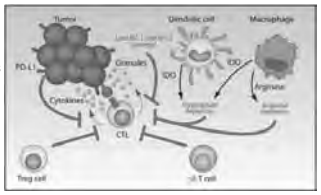
Cancer and Immunity

- 50-60 years ago, observation: rejection of transplanted tumor cells in syngeneic mice
- 20 years ago, tumor antigens recognized by T cells began to be identified
- More recently, components of the immune system which are necessary for rejection of transplanted tumors have been clarified
  - For most tumor cell lines, both innate and adaptive immunity must be functional for tumor rejection to occur
- The concept of immune surveillance of cancer has been developed (Bob Schreiber)
  - Based on clinical observation that immunosuppressed individuals have a higher cancer risk
  - 3 phases of immune surveillance
    - E imination
    - Equi librium
    - Escape



Cancer and Immunity – Immune evasion

- Putative immune evasion mechanisms
  - Tumor-induced T cell anergy
  - Expression of negative costimulatory receptors on T cells (PD-1, TIM-3, CTLA-4)
  - Tregs
  - Suppressive myeloid-derived cells (MDSC, TAM)
  - Secretion of inhibitory cytokines (IL-10, TGF-β)
  - Antigen-loss variants (loss of MHC)
  - Production of enzymes which deplete essential amino acids (IDO, arginase)
  - Others
- Overcoming negative regulation in the tumor environment will be necessary to harness effective anti-tumor immunity



Cancer and Immunity – Immunotherapy: current approaches

- Cancer vaccines
  - Peptide-based
  - Cellular-based (i e. DC vaccines)
- Adoptive T cell therapy
  - Ex vivo expansion of tumor-infiltrating T cells and infusion into cancer-bearing hosts
  - Tumor Ag-specific TCR transduced T cell therapy
  - Chimeric antigen receptor (CAR) adoptive therapy (CD19)
- Immune checkpoint blockade
  - CTLA-4 blockade
  - PD-1 blockade
- Reversal of immune evasion
  - Treg depletion
  - IDO inhibition (1-methyl tryptophan and derivatives)
  - Prevention of tumor-induced T cell anergy (lymphodepleted host and adoptive T cell therapy)

Host Tumor Relationship

Augusto Ochoa, MD
Louisiana State University Health Sciences Center

Dr. Augusto Ochoa trained as an MD in his native Colombia (South America) and then went on to complete a residency in Pediatrics and a fellowship in Allergy and Immunology. He did postdoctoral work in basic immunology at the University of Minnesota. Later, he directed the Immunotherapy Laboratory at the Frederick Cancer Center of the National Cancer Institute in Frederick, MD. In 1997, he joined the Louisiana State University Health Sciences Center in New Orleans where he is Professor of Pediatrics, Allergy and Immunology, and the director of the Cancer Center. His research work has been primarily focused on cancer immunology and immunotherapy, with a special interest on the mechanisms down-regulating the immune response in patients. While at the National Institutes of Health, his group made the first observation that T cells from cancer patients had a decreased expression of various signaling molecules, including the T cell receptor, which made them unable to develop a protective response against tumors. His group later identified that these defects were induced by myeloid cell infiltrating the tumors and showed that the primary mediator of this effect was the depletion of the amino-acid L-arginine. These observations have been reproduced by many groups in patients with different types of tumors and remains today as one of the important mechanisms of tumor induced immune suppression.

Pre-Test Questions
Host Tumor Relationship

1. MDSC, Tumor associated macrophages (TAM), and T-regs abnormal immune cells are triggered only by the appearance of cancer.

A. True
B. False

2. CD4+,CD25+,FoxP3+ T cells are:

A. Highly immunosuppressive regulatory T cells
B. Potent inducers of anti-tumor responses
C. Mediators of Graft vs Host disease
D. Irrelevant in cancer because they are deleted in the fetal thymus

3. IL2 induces the activation of myeloid cells producing arginase and IDO.

A. True
B. False

4. What are the principal immunosuppressive mechanisms produced by myeloid-derived suppressor cells?

A. TGFb, IL10 and IL17
B. IFNg and IL2
C. Arginase, NO and H2O2
D. IDO

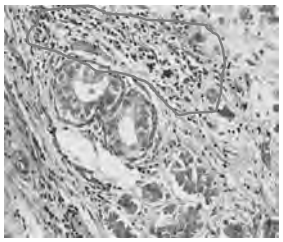
Suppressor Cells in Cancer: Mechanisms and Therapeutic Perspectives

Augusto C Ochoa M D
Stanley Scott Cancer Center
Louisiana Sate University
New Orleans

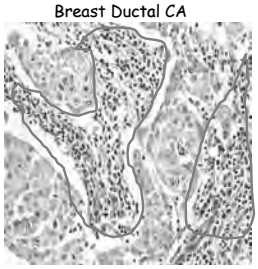
LSU Health Sciences Center

Inflammation and Cancer: What are the cells doing there?

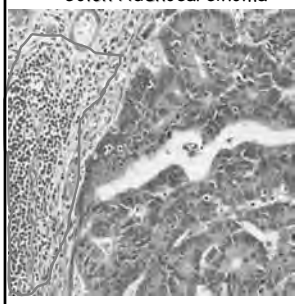
Breast Medullary CA



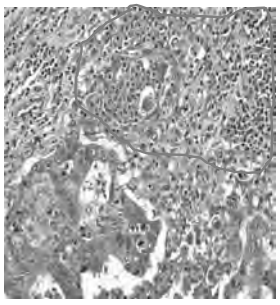
Breast Ductal CA

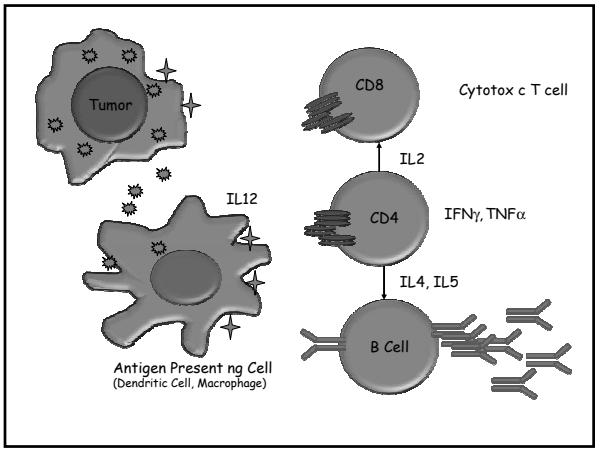


Colon Adenocarcinoma



Glioblastoma

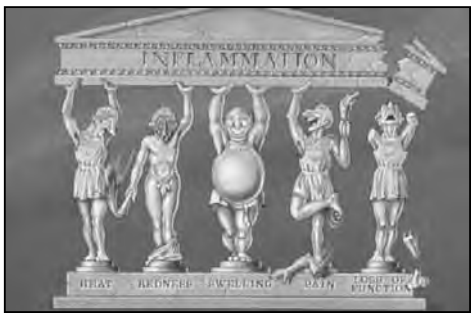


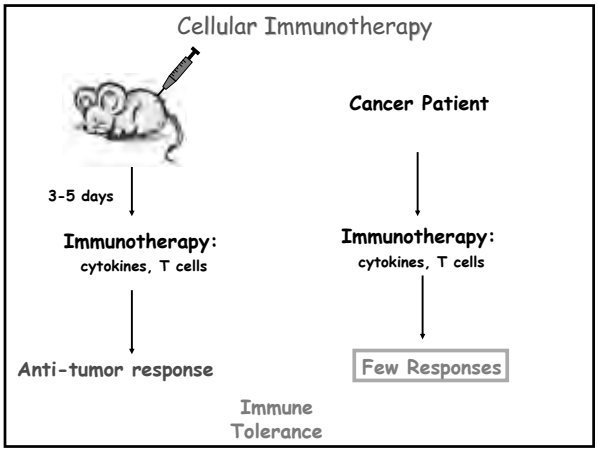


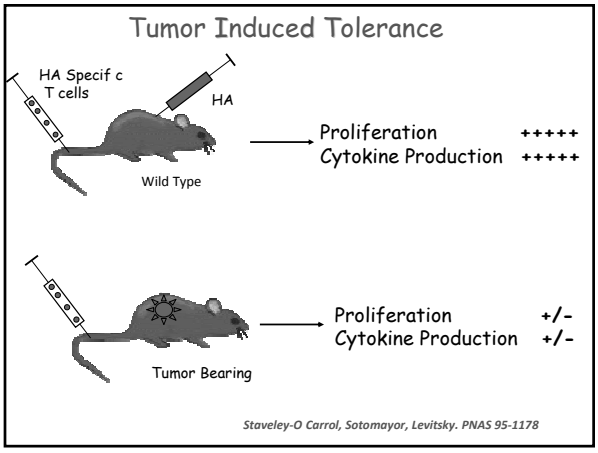
Inflammation (Immunity) and Cancer

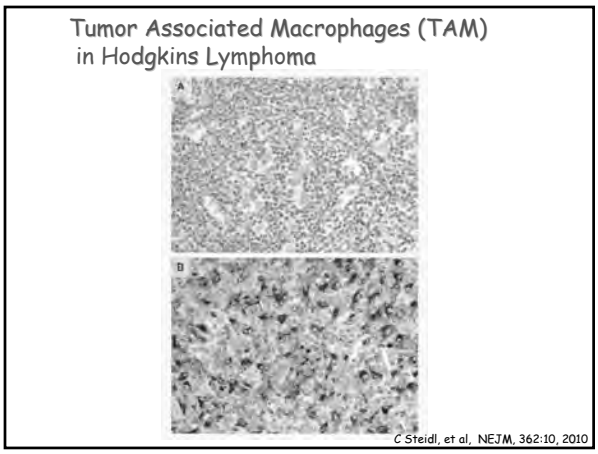
Prevent or Treat	Promote
• At least 20% of cancers have a preventable infectious component: HPV HBV H pylori • NK and T cells can eradicate established tumors (renal and melanoma)	• "Cancer originates in sites of chronic inflammation" Virchow 1863 • Hodgkins disease: Loss of DTH to DNCB and candida *(Hersch and Oppenheim – 1963)* • Melanoma: Decreased cellular response and increased antibody levels. *(Hellstrom and Hellstrom – 1968)* • NSAIDS prevent colon CA

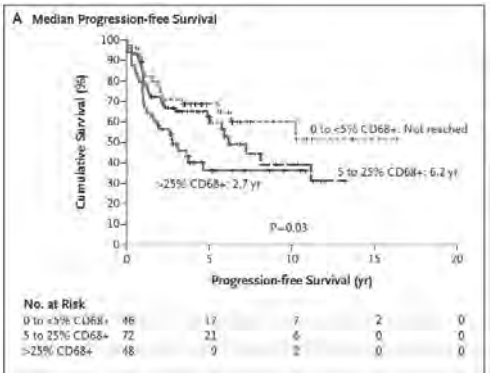
Chronic Inflammation : Turning Friend into Foe



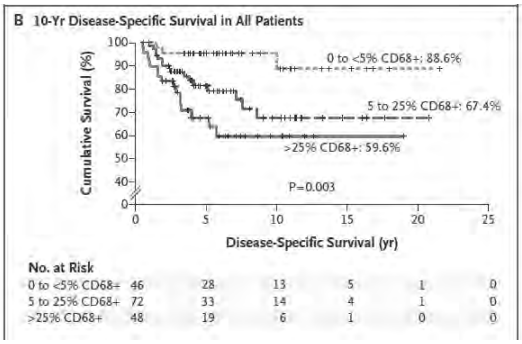

Cellular Immunotherapy
Cancer Patient
3-5 days
Immunotherapy:
cytokines, T cells
Immunotherapy:
cytokines, T cells
Anti-tumor response
Few Responses
Immune
Tolerance


Tumor Induced Tolerance
HA Specif c
T cells
HA
Wild Type
Proliferation +++++
Cytokine Production +++++
Tumor Bearing
Proliferation +/-
Cytokine Production +/-
Staveley-O Carrol, Sotomayor, Levitsky. PNAS 95-1178


Tumor Associated Macrophages (TAM)
in Hodgkins Lymphoma
A
B
C Steidl, et al, NEJM, 362:10, 2010

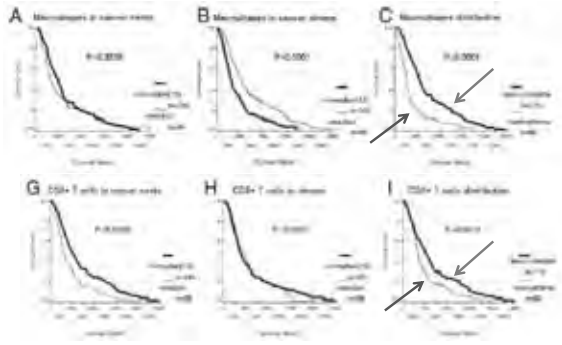


C Steidl, et al, NEJM, 362:10, 2010



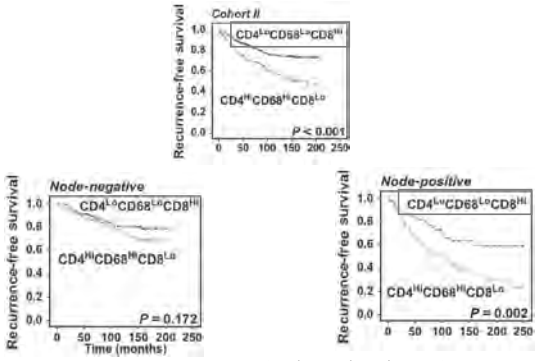
C Steidl, et al, NEJM, 362:10, 2010

Tumor Infiltrating Immune Cells in NSCLC



O. Kawai et al *Cancer* 113(6) 2008

Immune Infiltrates and RFS in Breast Cancer

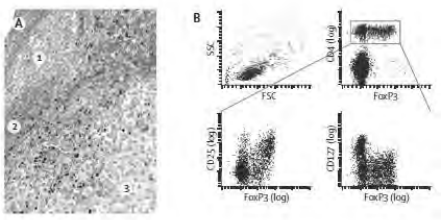


David DeNardo et al Cancer Discovery 2011

Mechanisms of Tumor Escape
1990-2010

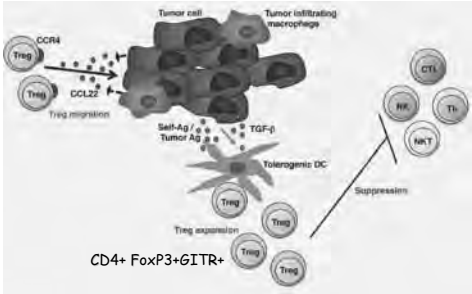
- Intrinsic changes in the tumor
  - Loss of MHC (immuno-editing)
  - Lack of co-stimulatory signals B7.1, B7.2
  - Expression of checkpoint signals B7H1, B7H4 (CTLA4)
- Factors produced by the tumors
  - TGFβ, IL10 ,IL17
- Immunosuppressive cells stimulated by tumors
  - Regulatory T cells (T-regs)
  - Myeloid-derived suppressor cells (MDSC)
  - Tumor Associated Macrophages (TAM)

Regulatory T cells (T-Regs) in Melanoma



Jacobs JFM et al, The Lancet 13, 2012

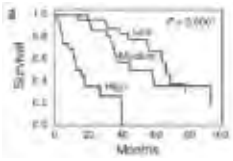
Regulatory T cells in Cancer



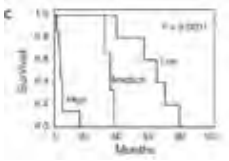
Nishikawa and Sakaguchi, Int. J. Cancer 127 (2010)

T-Reg Infiltration and Survival in Ovarian CA

Stages I – IV
N=70



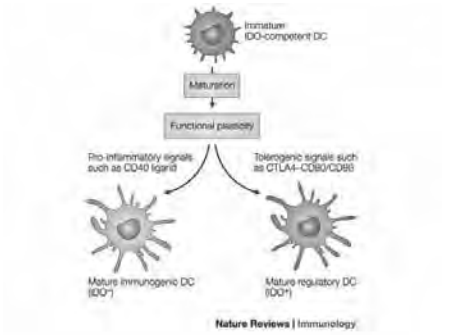
Stage IV

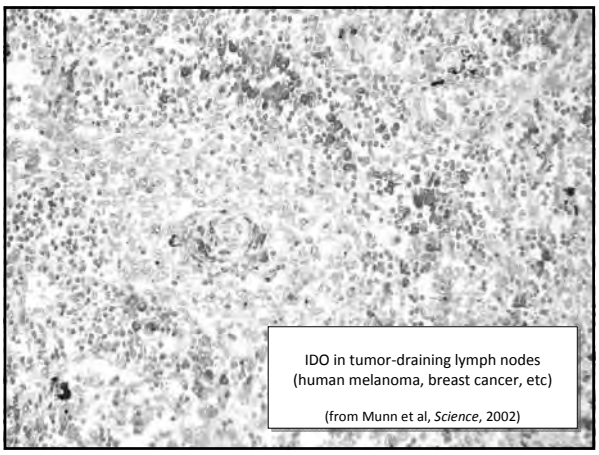


Curiel T, Nature Med 10(9), 2004

Immature DC

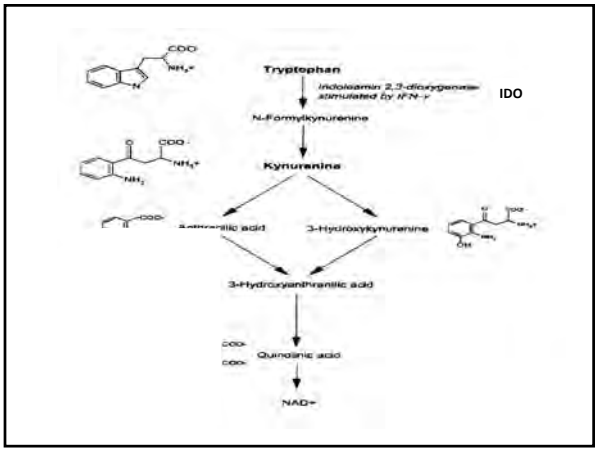
IDO (Indoleamine 2 3-dioxygenase)
David H. Munn and Andrew L. Mellor





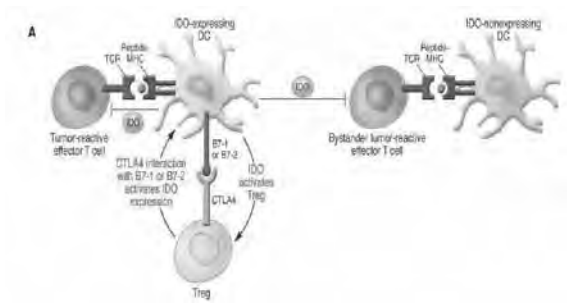
IDO in tumor-draining lymph nodes
(human melanoma, breast cancer, etc)

(from Munn et al, *Science*, 2002)



Indoleamine 2,3-dioxygenase (IDO)

- IDO is a natural endogenous molecular mechanism of immune suppression
- IDO can create acquired peripheral tolerance *de novo*
- IDO is counter-regulatory (induced by inflammation but suppresses immune responses)
- IDO regulates both innate and adaptive responses
  - control of local inflammation IL-6 etc
  - suppresses effector T cells activates Tregs



Myeloid-derived Suppressor Cells (MDSC) Tumor Associated Macrophages (TAM)

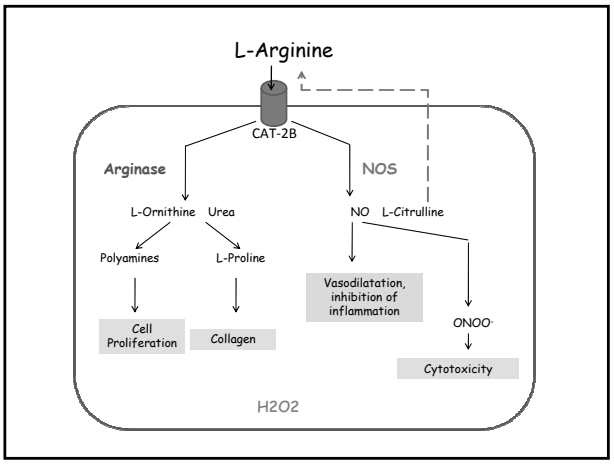
- CD11b+ GR1+ myeloid cells H&N pts (R Young) and tumor-bearing mice (D Gabrilovich)
- Immature DC to mature granulocytes
- Increased in Renal Cell Carcinoma (A Zea) and Pancreatic CA (O Finn)
- Produce Arginase 1 Nitric Oxide or H_2O_2 (Bronte E Ochoa A Ostrand-Rosenberg S)

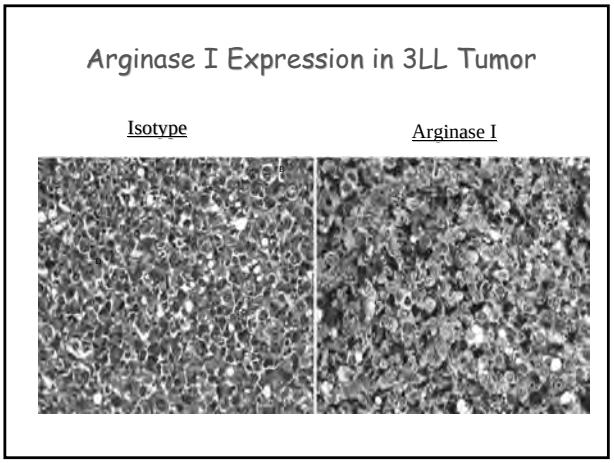
Effect of MDSC on OT-1/OT-2 T cells

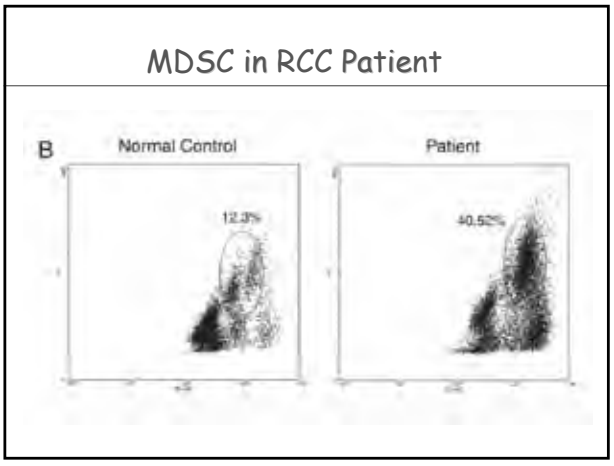
	No MDSC	Plus MDSC	Plus non-MDSC
No Stimuli	33	48.5	88.5
OVA 257-264	20713 →	217	23842
OVA 323-339	17073 →	164	15159

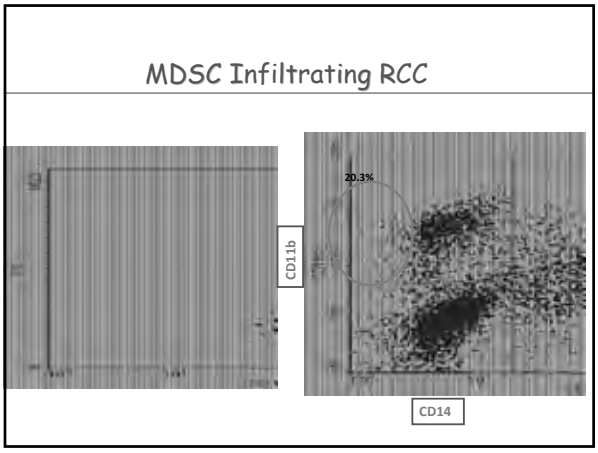
Inhibit IFNγ production and CD3ζ chain expression

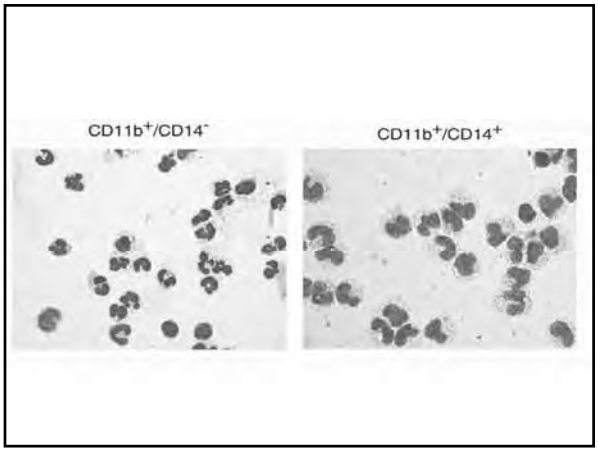
Decrease the therapeutic efficacy of radiation and chemotherapy

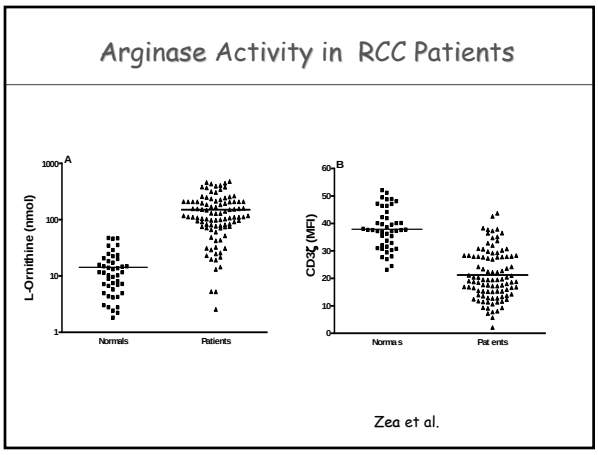

L-Arginine
CAT-2B
Arginase
NOS
L-Ornithine
Urea
NO
L-Citrulline
Polyamines
L-Proline
Vasodilatation, inhibition of inflammation
ONOO-
Cell Proliferation
Collagen
Cytotoxicity
H2O2


Arginase I Expression in 3LL Tumor
Isotype
Arginase I

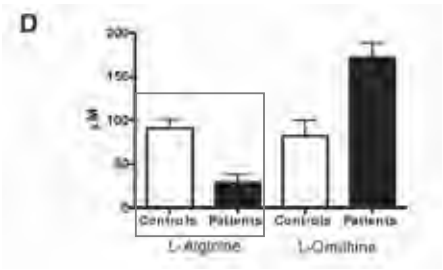

MDSC in RCC Patient
B
Normal Control
Patient
12.3%
40.52%


MDSC Infiltrating RCC
20.3%
CD11b
CD14

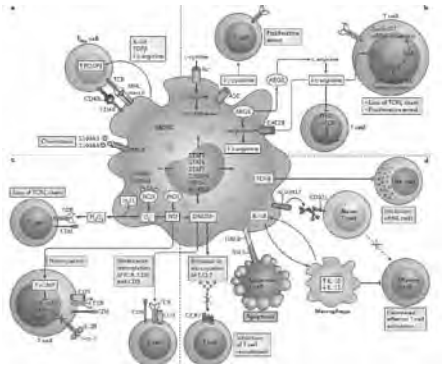

CD11b+/CD14-
CD11b+/CD14+


Arginase Activity in RCC Patients
A
L-Ornithine (nmol)
1000
100
10
1
Normals
Patients
B
CD3ζ (MFI)
60
50
40
30
20
10
0
Normals
Patients
Zea et al.

Arginine and Ornithine in Plasma of RCC Patients



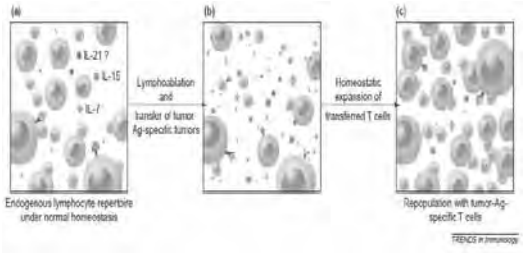
Zea et al.



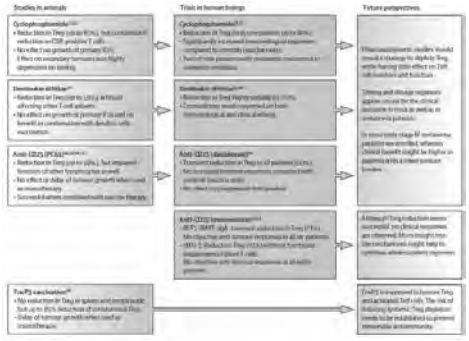
Blocking Cellular Immune suppression in Cancer

- Lymphodepletion
- Blocking T-regs
  - Anti-CD25
- Blocking IDO
  - 1-Methyl-tryptophan
- Blocking MDSC
  - Arginase inhbitors: Nor-NOHA, BEC and ABH - D. Christiansen
  - Tyros ne k nase nhibitors – Sunitin b – J. Finke
  - PDE5 inh bitors (Viagra and Cialis): I. Borrello
  - NOS2/Arginase inh bitors (nitroasp r n) – E. Bronte
  - All-Trans-ret noic acid (ATRA) D. Gabrilovich
  - Anti-CD11b – D. Denardo

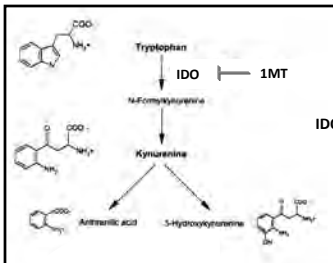
Lymphodepletion in Adoptive Cellular Therapy



Anti-T-reg Therapies

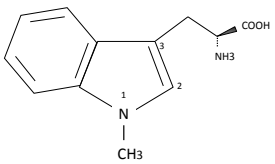


Jacobs JFM et al, The Lancet 13, 2012



IDO-inhibitor drug (NSC-721782):

1-methyl-[D]-tryptophan



ASCO American Society of Clinical Oncology www.asco.org

Abstract # 3004
2009

Phase I Trial of 1-methyl-D-tryptophan

MOFFITT

PI: Scott Antonia MD PhD
Co PI: Hatem Soliman MD
Dan Sullivan MD

Moffitt Cancer Center/Southeast Phase II Consortium

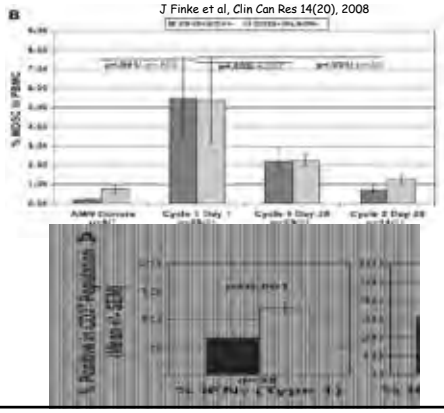
Chuck Link MD
Nick Vanahanian MD
William Ramsey MD PhD

NewLink Genetics Inc

- Hypophysitis seen in 3 patients
  this was a recall toxicity associated with prior ipilimumab therapy (anti-CTLA4 mAb)
- otherwise 1MT was well tolerated

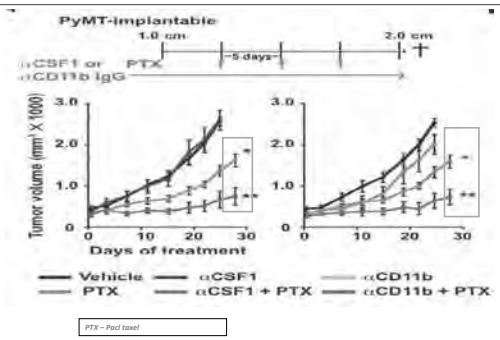
Sunitinib and MDSC in RCC

J Finke et al, *Clin Can Res* 14(20), 2008



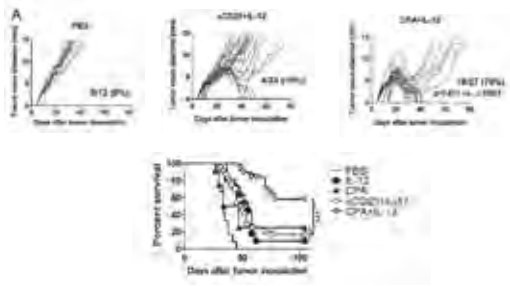
Myeloid Cell Inhibition and Chemotherapy

DeNardo et al. Cancer Discovery 2011

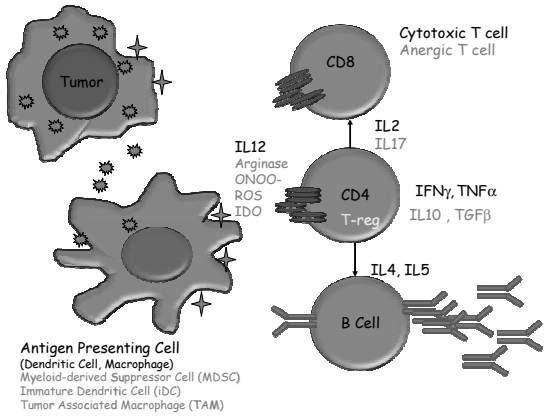


PTX – Paclitaxel

Chemo-Immunotherapy
(Cyclophosphamide + anti-CD25)



Medina-Echeverz J, J Immunol 186, 2011.



Research Question

- What is the hierarchy and/or sequence of these suppressor mechanisms?
- What are the tumor derived signals that select the type of suppressor cell?
- What is the combination of chemo and immuno therapeutic agents that will block the immunosuppressive cells and induce a therapeutic anti-tumor response?

Tumor Antigens and Cancer Vaccines

Leisha A. Emens, MD, PhD
Johns Hopkins University

Leisha A. Emens, MD, PhD, is an Associate Professor at the Sidney Kimmel Comprehensive Cancer Center at the Johns Hopkins University School of Medicine. She is a medical oncologist who specializes in breast cancer care, and is developing innovative immune-based therapies that incorporates cancer vaccines, standard cancer drugs, and immune checkpoint inhibitors for the treatment of breast cancer and ovarian cancer. Dr. Emens received her BA in Biochemistry and Cell Biology from the University of California at San Diego in 1984. In the Medical Scientist Training Program at Baylor College of Medicine, she received her PhD in Cell Biology in 1993, and her MD in 1995. She completed her internship and residency in internal medicine at the University of Texas at Southwestern Medical School in 1998 and completed fellowship training in medical oncology and hematology at Johns Hopkins University School of Medicine in 2001, when she joined the faculty. Dr. Emens is board-certified in internal medicine, medical oncology, and hematology by the American Board of Internal Medicine. She has received the Johns Hopkins University Clinician Scientist Award, the American Cancer Society Research Scholar Award, the YWCA President's Award, and the Maryland Governor's Citation for her work. She is a member of the American Society of Oncology, the American Association for Cancer Research, the American Society of Gene Therapy, and the Society for the Immunotherapy of Cancer. She is also a member of the editorial board of the *Journal of Clinical Oncology*, and the FDA Advisory Committee on Cellular, Tissue, and Gene Therapies.

Pre-Test Questions
Tumor Antigens and Cancer Vaccines

1. The following types of lymphocytes are associated with good prognosis when present in primary human cancers of distinct histologies EXCEPT:

A. B cells
B. CD3+CD8+ T cells
C. CD45RO+ T cells
D. T helper type 1 CD4+ T cells

2. The first therapeutic cancer vaccine approved for routine clinical use is based on a basic platform of:

A. Peptide antigen
B. Dendritic cells
C. Autologous tumor cells
D. Artifical scaffold

3. Challenges to the successful development of therapeutic cancer vaccines include:

A. Immune tolerance
B. Extent of disease burden
C. Suboptimal clinical trial design
D. All of the above

TUMOR ANTIGENS AND VACCINES

Leisha A. Emens MD, PhD
Associate Professor of Oncology
Cancer Immunology/Breast Cancer Research Programs
Johns Hopkins University School of Medicine

CONFLICT OF INTEREST

Biosante: Under a licensing agreement between Biosante and Johns Hopkins University, the University is entitled to milestone payments and royalty on sales of the GM-CSF-secreting cell-based vaccine product described in this presenation. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

Roche/Genentech: Xeloda Advisory Board, Breast Cancer Advisory Board, Research Funding

Bristol Myers Squibb: Breast Cancer PD-1 Advisory Board

"It is by no means inconceivable that small accumulations of tumour cells may develop, and because of their possession of new antigenic potentialities, provoke an effective immunological reaction with regression of the tumour and no clinical hint of its existence."

–Macfarlane Burnet
Immunologist, 1957

CANCER IMMUNOSURVEILLANCE IN HUMANS

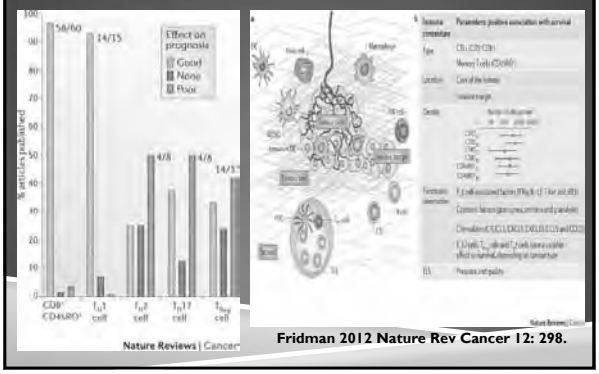
Site of Cancer*	Ratio of Cases Observed/Expected
Non-melanoma skin	24.7
Thyroid/Endocrine	14.3
Head and Neck	13.8
Cervix/Vulva/Vagina	10.8
Non-Hodgkn's lymphoma	10.3
Kidney/Ureter	9.1
Bladder	5.5
Colorectal	3.6
Lung	2.4
Brain	2.4
Prostate	2.1
Melanoma	1.7
Breast	1.3

Cancer incidence in immunosuppressed transplant patients
Adapted from Peto 2001 Nature 411: 390.

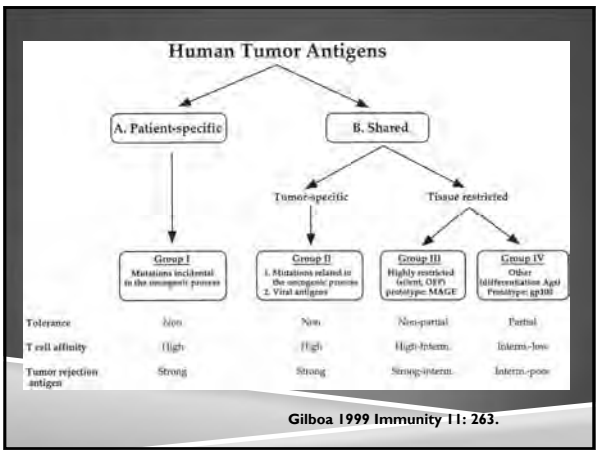
CANCER IMMUNOSURVEILLANCE IN IMMUNOCOMPROMISED HUMANS

- 400-500X increase in Kaposi's sarcoma (HHV-8)
- 28-49X increased in lymphoproliferative disease, inlcuding Hodgkin's disease (EBV)
- 100X increase in squamous cell vulvar and anal carcinomas (HPV)
- 20-38X increase in hepatocellular carcinoma (HBV and HCV)
- 14-16X increase in cervical cancer (HPV)

THE IMMUNE SYSTEM RECOGNIZES TUMORS



Fridman 2012 Nature Rev Cancer 12: 298.



Gilboa 1999 Immunity 11: 263.

EXAMPLES OF TUMOR ANTIGENS

Type of Cancer Antigen	Examples
Viral antigens	HPV E6/E7, EBV LMP, HBV, HCV
Novel cancer antigens	mutated k-ras (pancreas, lung cancers) p53 (many cancers) fusion proteins (bcr-abl in CML) many others
Overexpressed, nonmutated self proteins	HER-2 (breast and gastric cancers) hTERT (many cancers) Ganglioside GD3 (melanomas)
Embryonic/oncofetal proteins	NY-ESO-1, MAGE/BAGE/GAGE
Expression outside immunologically privileged site	Hu, Yo, GAD
Tissue-specific differentiation antigens	MART-1/melan-A, gp-100, tyrosinase, PSA

PREVENTIVE CANCER VACCINES

- Gardasil for the prevention of HPV-related cervical cancer
  - 2nd most common cause of cancer in women worldwide
  - HPV types 6, 11, 16, 18, quadrivalent vaccine of VLPs
  - Prevents 75% of cervical cancers, 70% of vaginal cancers, and 50% of vulvar cancers in girls and young women, and 90% of genital warts in young people
  - HPV-related head and neck cancer?
- HBV vaccines for the prevention of HBV-related liver cancer
  - 5th and 8th most common cancer in men and women respectively worldwide
  - Decreased incidence of HCC in children ages 6-9 years from 0.52-0.13 per 100,000

THERAPEUTIC CANCER VACCINE

Siipuleucel-T (Provenge^{R}) : First FDA-approved therapeutic cancer vaccine

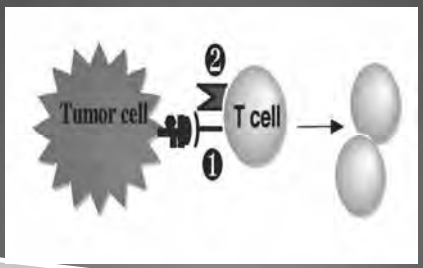
1. Composed of autologous dendritic cells loaded with prostatic acid phosphatase fused with granulocyte-macrophage colony-stimulating factor (PAP-GM-CSF)
2. Given as 3 treatments 2 weeks apart
3. Prolongs survival of men with metastatic hormone-refractory prostate cancer by 4.1 months
4. Retrospective data show that this is the greatest survival benefit demonstrated for this patient population to date
5. Minimal toxicity
6. Expensive ($100, 000)

SIPULEUCEL-T (PROVENGE^{R}): PHASE III IMPACT TRIAL

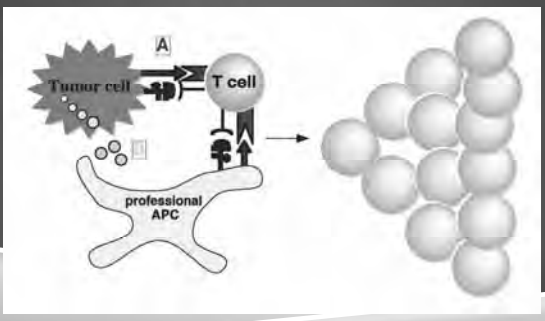
	Placebo (n=171)	Sipuleucel-T (n=341)	Delta or p-value
Overall survival	21.7 months	25.8 months	4.1 months
36-month survival	23.0%	31.7%	+8.7%
TTP	14.4 weeks	14.6 weeks	p=0.63
Relative reduction in risk of death	-------	22%	P=0.03
Relative reduction in risk of death from prostate cancer	-------	23%	P=0.04

Kantoff 2010 NEJM 363: 411.

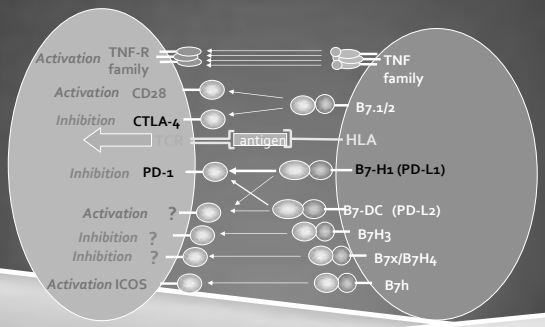
HOW ENDOGENOUS TUMOR ANTIGENS ARE RECOGNIZED



Two Signals Lead To Activation Rather Than Ignorance



SIGNAL TWO IS A COMPLEX RHEOSTAT



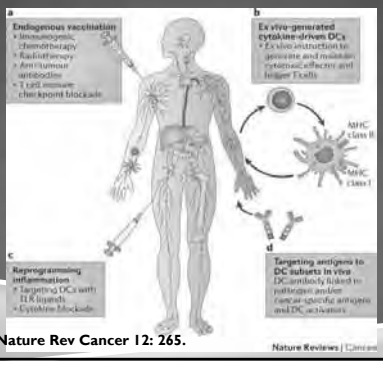
CANCER VACCINE PLATFORMS

Vaccine Platform	Rationale
Peptide	Sub-dominant/cryptic epitopes elicit immunity to self Ags, given with potent adjuvants to enhance immunogenicity
Protein	Given with potent adjuvants to enhance immunogenicity
Plasmid DNA	Stable transfection of skin/muscle allows Ag presentation
Dendritic cells	Potent antigen-presenting cells present tumor Ags
Viral or Bacterial Vectors	Initiate presentation through MHC Class I to stimulate T cells in the presence of a foreign stimulus/adjuvant
Whole Tumor Cells	Deliver multiple relevant tumor Ags
Engineered scaffold	Deliver optimal tumor Ags and co-stimuli

CANCER VACCINE PLATFORMS

Vaccine Platform	Immunogenicity	Toxicity	HLA Match Required
Dendritic cells	High	Low	yes
Peptide	Low	Low	yes
Protein	Moderate	Low	no
Plasmid DNA	Low	Low	no
Viral or Bacterial Vectors	High	High	no
Whole Tumor Cells	Moderate	Low	no
Heat Shock Proteins	High	Low	no
Engineered scaffold	High	Low	variable

DENDRITIC CELL VACCINES FOR CANCER TREATMENT



Palucka 2012 Nature Rev Cancer 12: 265.

PEPTIDE VACCINES FOR CANCER TREATMENT

Issues:

1. Typically weak immunogens and require adjuvants
2. Require MHC matching to the patient
3. Low toxicity
4. Ease and low cost of manufacture
5. Typically induce antigen-specific immunity, clinical responses rare
6. Need to activate $CD4^+$ and $CD8^+$ T cells—nested MHC Class I and II epitopes, add PADRE to mixture
7. Single or multiple, long or short, alone or in combination, best adjuvant

CANCER VACCINE ADJUVANTS

TLR Agonists	Non-specific Immunomodulators
microbial products	mineral salts, emulsions, microparticles, liposomes
BCG (TLR2, TLR4, NLR2)	Incomplete Freund's adjuvant
Poly I:C and Poly I:C12U (TLR3)	Montanide ISA 51 and 720
LPS (TLR2, TLR4)	Alum, MF59, QS21
Monophosphoryl lipid A (MPLA) (TLR4)	Keyhole Limpet Hemocyanin (KLH) protein
Imiquimod (TLR7, TLR8)	
CpG ODNs (TLR9)	

PHASE III MELANOMA PEPTIDE VACCINE TRIAL

- Stage IV or locally advanced Stage III cutaneous melanoma
- HLA-A2-positive
- suitability for IL-2 therapy

	IL-2 Alone (720KIU/kg) (n=94)	gp100 (210M)+ montanide ISA 51 followed by IL-2 (n=91)	p value
Clinical response	6%	16%	p=0.03
Progression-free survival	1.6 months	2.2 months	p=0.008
Overall survival	11.2 months	17.8 months	p=0.06

Schwartzentruber 2011 NEJM 364: 2119.

PHASE III PATIENT-SPECIFIC IDIOTYPE VACCINE TRIAL FOR FOLLICULAR LYMPHOMA

- chemotherapy-naïve follicular lymphoma in CR after primary chemotherapy
- bulky (>5 cm) Stage II, III, IV disease
- lymph node with surface IgG or IgM accessible for biopsy

	KLH+GM-CSF (n=41)	Id-KLH+GM-CSF (n=76)	p value
Progression-free survival	30.6 months	44.2 months	p=0.045
Overall survival	Not reached	Not reached	p=0.696

Schuster 2011 JCO 29: 2787.

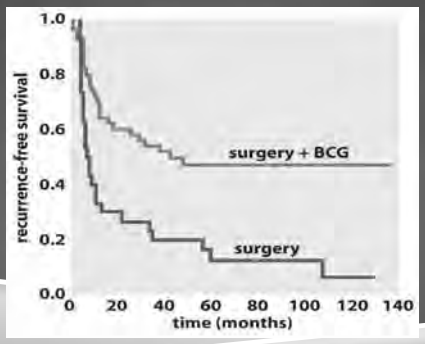
PHASE II POX VIRUS-PSA VACCINE TRIAL

- min symptomatic hormone-refractory metastatic prostate cancer
- vaccinia virus prime, followed by 6 fowlpox virus boosts
- PSA antigen+three immune costimulatory molecules (B7.1/ICAM-1/LFA-3)

	Empty vector+ saline (n=40)	PROSTVAC-VF+ GM-CSF (n=82)	p value
Progression-free survival	3.7 months	3.8 months	p=0.6
Median overall survival	16.6 months	25.1 months	p=0.0061

Kantoff 2010 JCO 28: 1099.

BACTERIA CAN TREAT SUPERFICIAL BLADDER CANCER

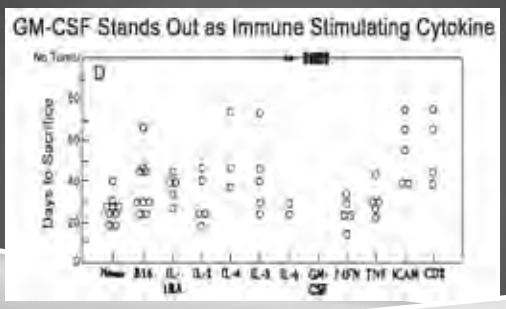


WHOLE TUMOR CELLS

Issues:

1. autologous vs. allogeneic vs. dendritic cell fusion
2. unmodified vs. modified
3. deliver multiple tumor antigens, both known and unknown
4. no requirement for HLA match
5. expensive, allogeneic not as expensive as patient-specific product
6. allogeneic vaccines are generalizable

SYSTEMATIC ANALYSIS OF MODIFIED TUMOR CELL VACCINES IN PRECLINICAL MODELS



Dranoff 1993 PNAS 90: 3539.

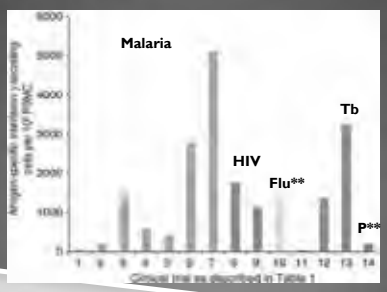
PHASE III TRIAL OF PROSTATE GVAX

- Taxane-naïve, symptomatic hormone-refractory prostate cancer
- Docetaxel 75 mg/m^2 every 3 weeks plus prednisone 10 mg/day vs. Docetaxel 75 mg/m^2 every 3 weeks plus GVAX 2 days later, then GVAX every 4 weeks (10 cycles chemotherapy given in each arm)

	Docetaxel 75 mg/m^2+Prednisone (n=204)	GVAX+Docetaxel 75 mg/m^2 (n=204)	p value
Deaths	47	67	p=0.03
Overall survival	14.1 months	12.2 months	p=0.0076

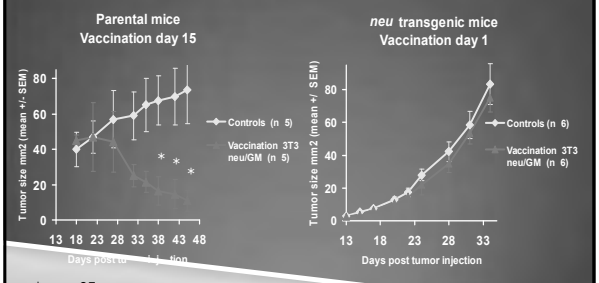
Small 2009 GU ASCO

AMPLITUDE OF T CELL IMMUNITY INDUCED BY VARIOUS VACCINES



Gilbert 2011 Immunology 135: 19.

IMMUNE TOLERANCE TO HER2 IN *NEU* TRANSGENIC MICE



* $p < .05$

Machiels 2001 Cancer Res 6: 3689.

IMMUNE TOLERANCE: A MAJOR BARRIER TO TUMOR IMMUNITY

- $CD4^+CD25^+$ regulatory T cells
- Myeloid-derived suppressor T cells
- Suboptimal T cell repertoire
- Inadequate positive co-stimulation
- Excessive negative counter-stimulation
- Ineffective T cell trafficking
- Suppressive tumor microenvironment

WHAT TO COMBINE WITH IMMUNOTHERAPY?

- Endocrine therapy
- Chemotherapy—dose and schedule key
- Therapeutic tumor-specific monoclonal antibodies
- Tyrosine kinase inhibitors
- Immune checkpoint modulators

CLINICAL TRIALS OF VACCINES AND TARGETED IMMUNOMODULATORS

Patient Population	Number of patients	Vaccine	Drug Regimen	Immunologic Outcome
metastatic melanoma	n=16	MART-1 pulsed autologous DC	Dose escalation tremelimumab (α-CTLA-4)	Low levels of MART-1 T cells
metastatic hormone-refractory prostate cancer	n=28	GM-CSF-secreting prostate tumor cells	Dose escalation ipilimumab (α-CTLA-4)	25% with ≥50% PSA decline; evidence of DC and T cell activation
refractory, unresectable melanoma	n=676	gp100	Ipilimumab (n=137) Ipilimumab+gp100 (n=403) gp100 (n=136)	Improved overall survival with ipilimumab

In early clinical development:
α-CD-40, α-PD-1, α-B7-H1

Ribas 2009 Clin Cancer Res 15: -6276.
van den Eertwegh 2012 Lancet Oncol 13: 509.
Hodi 2010 NEJM 363: 711.

"Hope is not a strategy—you have to follow the science"

THANK YOU!

Adoptive Immunotherapy with T Cells

Laurence J.N. Cooper, MD, PhD
MD Anderson Cancer Center

Dr. Laurence Cooper is a tenured Professor at The University of Texas MD Anderson Cancer Center (MDACC), with joint appointments in the Division of Pediatrics and Department of Immunology. He is Section Chief of Cell Therapy at the Children's Cancer Hospital (CCH) at MDACC and additionally serves as director of the institution's Immunology Laboratory of Physician-Scientists. Dr. Cooper earned his medical and doctorate degrees from Case Western Reserve University in Cleveland, Ohio and completed his fellowship in Pediatric Hematology/Oncology at the Fred Hutchinson Cancer Research Center at the University of Washington in Seattle. In 2006, he was recruited to join the CCH at MDACC, where he cares for children undergoing bone marrow transplantation (now known as Cell Therapy) and leads scientific efforts to develop new treatment approaches which pair gene engineering with immunotherapy. Dr. Cooper's research has resulted in him founding a company and in multiple patents. A former National Institutes of Health Research Center Scholar, Scholar of the Sidney Kimmel Foundation for Cancer Research, and Leukemia Society of America Fellow, Dr. Cooper is the principal investigator for numerous initiatives and trials. In 2007, he was elected to membership in the American Society for Clinical Investigation, which honors outstanding physician-scientists. Other tributes paid to Dr. Cooper include the 2010 "Best Boss" award MDACC, 2009 Faculty Scholar Awards MDACC, 2007 Induction into the American Society for Clinical Investigation, 2004 American Society of Gene Therapy Young Investigator Award, and 1999 American Society of Clinical Oncology Young Investigator Award. Dr. Cooper has coauthored dozens of peer-reviewed journal articles, abstracts and book chapters. Since 2006, he has initiated five trials under IND using T cells and NK cells. He is undertaking the first trials using a new approach to gene therapy based upon the *Sleeping Beauty* transposon system and has helped develop clinical-grade artificial antigen presenting cells for numerically expanding lymphocytes. He combines his clinical duties with research and mentoring to help translate immunology into immunotherapy.

Pre-Test Questions
Adoptive Immunotherapy with T Cells

1. Which one is the major concern in adoptive T cell therapy using TCR gene transduced T cells?

A. TCR gene transduction efficiency
B. Graft-versus-host-disease (autoimmune pathology) due to the mis-pairing of introduced with endogenous TCR chains
C. In vitro expansion of T cells
D. Detection of introduced TCR gene expressing T cells

2. What advantage(s) is/are there to infusing T cells that express a chimeric antigen receptor (CAR)?

A. CAR+ T cells recognize tumor-associated antigen independent of major histocompatibility complex (MHC)
B. CAR+ T cells exert an anti-tumor effect in clinical trials
C. CAR+ T cells do not distinguish between antigen on normal and malignant cells
D. A & B

3. Which of the following is true for T cells genetically modified to express CD19+ chimeric antigen receptor?

A. It targets CD19 on tumor cells only
B. It targets CD19 on normal B cells only
C. It targets CD19 irrespective of the cells on which it is expressed
D. None of the above

Faculty ~ Laurence J.N. Cooper, MD, PhD

The following relationships exist related to this presentation:

InCellerate, Inc. Founder

Adaptive Immunotherapy with T Cells

June 1, 2012
8:00 am – 1:00 pm
Hyatt Regency McCormick Place in Chicago, IL
Laurence J.N. Cooper
ljncooper@mdanderson.org

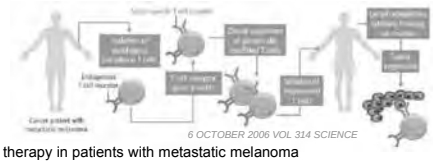
Bias of this presentation

- Combine gene therapy with T-cell therapy to over come issues of immune tolerance
- Use of mouse and humans to harvest desired immune receptors
- Common platforms for the development and release of T cells with redirected specificity

Tumor-specific T cells

- T cells that recognize tumor-associated antigen (TAA) through introduced chimeric antigen receptor independent of MHC
- T cells that recognize TAA though endogenous αβ T-cell receptor (TCR) in context of MHC

Harvesting and re-expressing melanoma-specific αβTCR

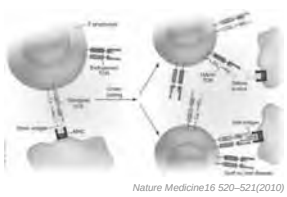


6 OCTOBER 2006 VOL 314 SCIENCE

TCR gene therapy in patients with metastatic melanoma

Target Antigen	TCRαβ	Patient	Response	CR	PR	Reference
MART-1/A2	human	31	4		4	*Science 2006 314 126-129 Blood. 2009 114 535-546*
MART-1/A2	human high avid ty	20	6		6 (3, 4, 9, 16+, 17+, 17+)	*Blood. 2009 114 535-546*
gp100/A2	mouse	16	3	1 (14+)	2 (4, 3)	*Blood. 2009 114 535-546*
NYESO1/A2	human	11	5	2 (22+, 20+)	3 (3, 8, 9+)	*J Clin Oncol 2011 29 917-924*

Toxicities from TCR transfer



Nature Medicine16 520–521(2010)

- GVHD due to the pairing of introduced and endogenous TCR chains in TCR gene-modified T cells in mouse model. *Nature Medicine16 565–570(2010)*
- On the contrary, there has been no report of GVHD in human clinical trial so far. *Molecular Therapy (2010)18 1744–1745*
- Further improvement of adoptive T cells therapy (e g. long-lived T cells) may increase the potential risk of GVHD.

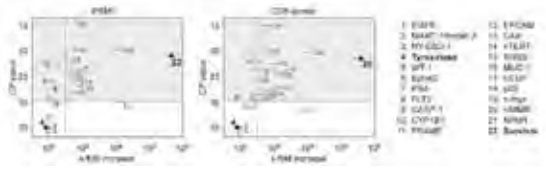
Potential Solutions

- Endogenous TCR can be disrupted by designed ZFN pairs to eliminate potential risk of GVHD *Nature Medic ne (2012) Published online 01 April 2012*
- Other approaches to avoid mis-pairing include
  i) use mouse derived TCRαβ constant region *Cancer Res 2006 66(17) 8878-86.*
  ii) Cys modification *Blood.2007 109 2331-2338. Cancer Res. 2007 67 3898-3903.*
  iii) siRNA to suppress endogenous TCRαβ *Cancer Res. 2009 69 9003-11.*
  iv) TCRαβ gene transfer to γδ T cells. *Cancer Res 2006 66(6) 3331-7.*

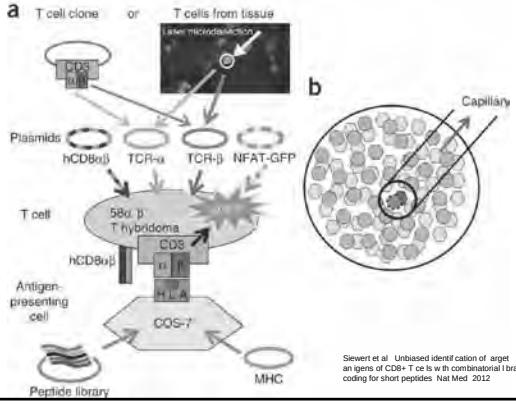
Fratricide

J Clin Invest. 2010;120(11):3869–3877.

MHC-restricted fratricide of human lymphocytes expressing survivin-specific transgenic T cell receptors

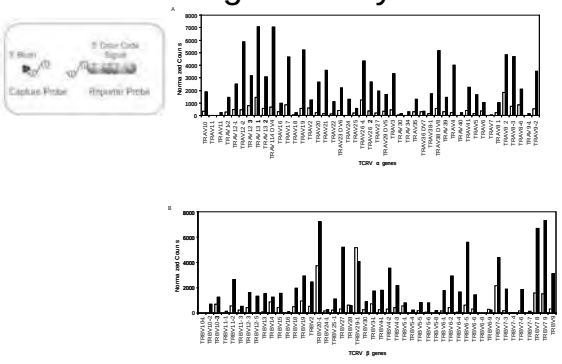


Identification of target antigens for CD8+ T cells



Siewert et al Unbiased identif cation of arget an igens of CD8+ T ce ls w th combinatorial l brar es coding for short peptides Nat Med 2012

Oligoclonality

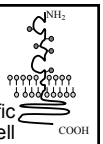


Tumor-specific T cells

- T cells that recognize tumor-associated antigen (TAA) through introduced chimeric antigen receptor independent of MHC
- T cells that recognize TAA though endogenous αβ T-cell receptor (TCR) in context of MHC

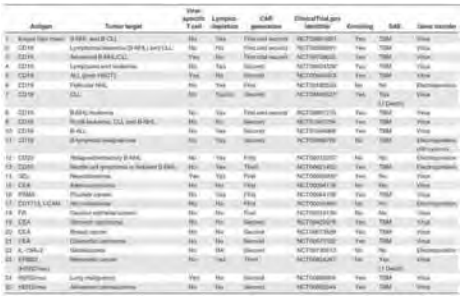
Rationale

Targeting CD19 determinant on B cells



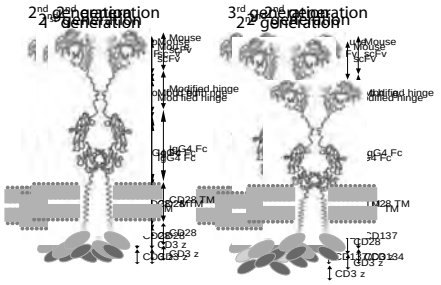
- CD19 antigen is a 95 kDa B lineage-specific membrane glycoprotein, found on >95% of B-cell lymphomas and B-ALL cells;
- CD19 is rarely lost during the process of neoplastic transformation, but disappears upon differentiation to mature plasma cells;
- CD19 is not expressed on hematopoietic stem cells, nor on normal tissues outside the B lineage;
- CD19 is not shed into the circulation.

Clinical trials in USA infusing CAR+ T cells



Blood. 2010 Aug 19;116(7):1035-44.

CAR design



Which T-cell sub-population to genetically modify?

Which T-cell sub-population to genetically modify?

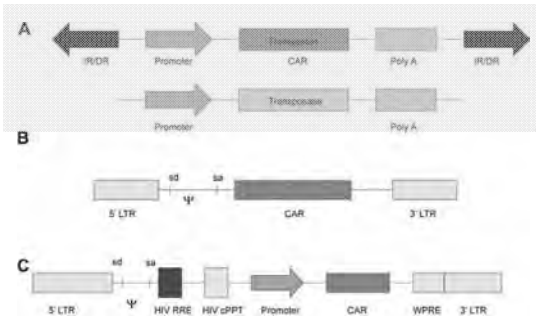
Which T-cell sub-population to genetically modify?

Published clinical data to date infusing CAR+ T cells targeting CD19

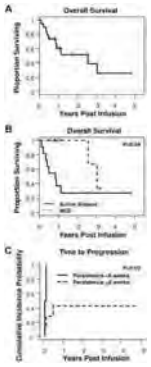
Inst tute	CD19+ Disease	Clinical Trial gov Identif ers	Chemotherapy prior to T-cell nfus on	IL-2 a ter T cell infusion	Gene transfer approach	CAR scaffold to append scFv	Mouse mAb clone used to derive scFv	CAR signal ng endodoma n(s)	Loss of normal B ce ls?
U Penn	CLL	NCT01029366	Yes	No	Lentiv rus	CD8alpha	FMC63	CD137 and CD3 zeta	Yes
NCI	Follicular Lymphoma and CLL	NCT00924326	Yes	Yes	Retrovirus	Truncated CD28	FMC63	CD28 and CD3 zeta	Yes
MSKCC	CLL and B ALL	NCT00466531 and NCT01044069	Yes	No	Retrovirus	Truncated CD28	SJ25C1	CD28 and CD3 zeta	Yes
BCM	B NHL or CLL	NCT00586391	No	No	Retrovirus	IgG1 CH_2CH_3 doma ns	FMC63	CD3 zeta vs CD28 and CD3 zeta	Yes
COH	Follicular Lymphoma	NCT00182650	Yes	Yes	Electroporat on	IgG4 hinge and CH_2 CH_3 doma ns	FMC63	CD3 zeta	No

Blood. 2012 Mar 22;119(12):2700-2.

Vector systems to express CAR transgenes used in clinical trials



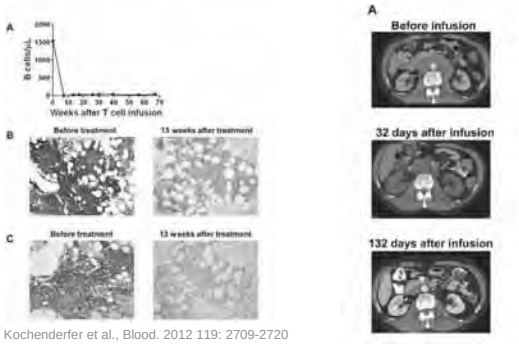
GD_2-targeted CAR+ T cells in patients with neuroblastoma



Antitumor activity and long-term fate of CAR+ T cells in patients with neuroblastoma

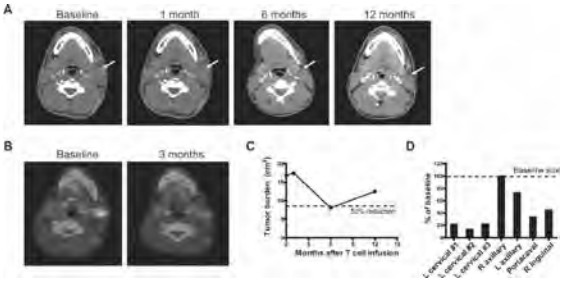
Louis C U et al. Blood 2011;118:6050-6056

CD19-targeted CAR+ T cells in patients with B-cell malignancies



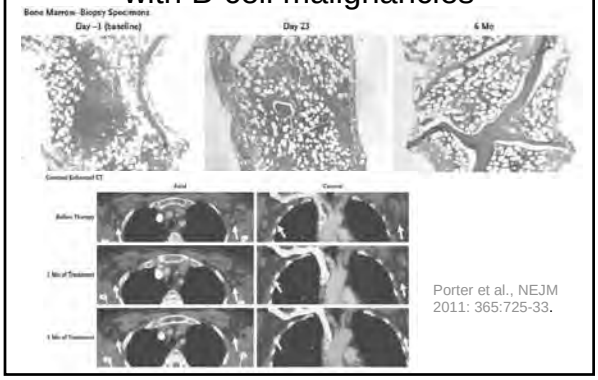
Kochenderfer et al., Blood. 2012 119: 2709-2720

CD20-targeted CAR+ T cells in patients with B-cell malignancies



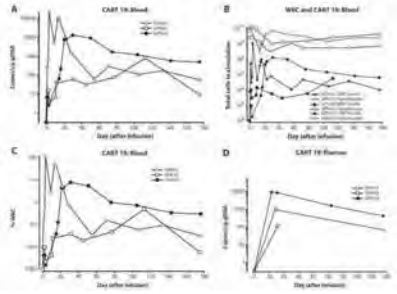
Till B G et al. Blood 2012;119:3940-3950

CD19-targeted CAR+ T cells in patients with B-cell malignancies



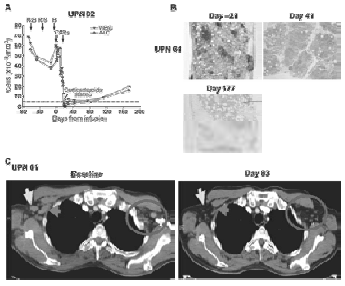
Porter et al., NEJM 2011: 365:725-33.

CD19-targeted CAR+ T cells in patients with B-cell malignancies



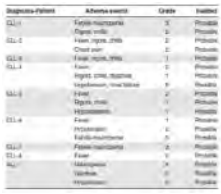
Sci Transl Med. 2011 Aug 10;3(95):95ra73.

CD19-targeted CAR+ T cells in patients with B-cell malignancies



Sci Transl Med. 2011 Aug 10;3(95):95ra73.

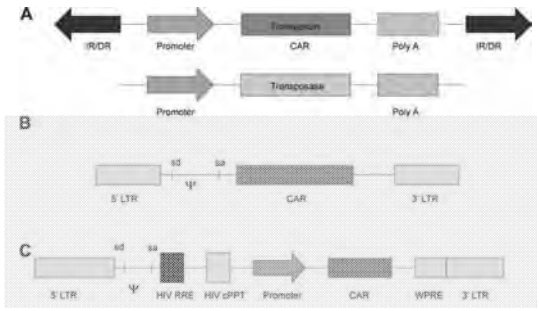
CD19-targeted CAR+ T cells in patients with B-cell malignancies



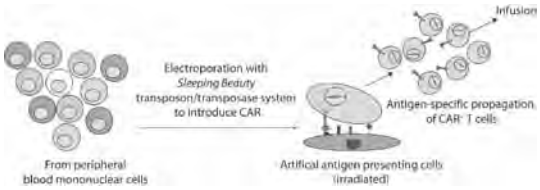
Brentjens et al 2011 Blood 118:4817 4828

Kochenderfer et al Blood 2012 119: 2709 2720

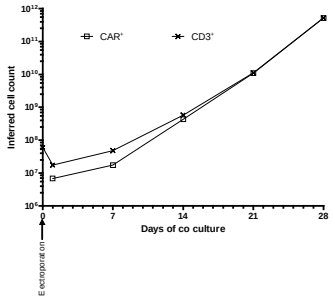
Vector systems to express CAR transgenes used in clinical trials



Genetic modification and propagation of T cells



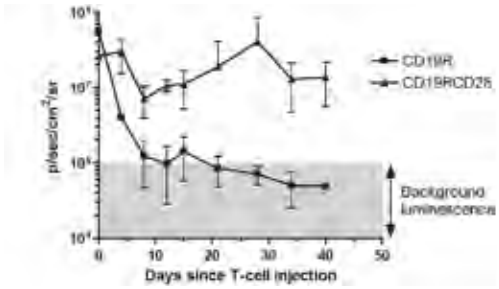
Selective outgrowth of CAR+ T cells on aAPC



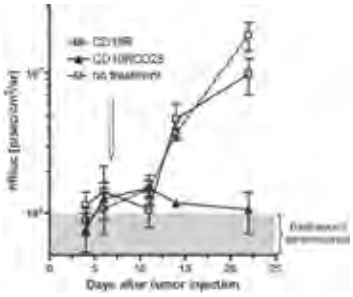
Mouse models – predictive?

- Infuse human CAR+ T cells into NOD/*Scid*/IL-2Rγ$^{-/-}$ (NSG) mice
- Does this predict for toxicity or potency?

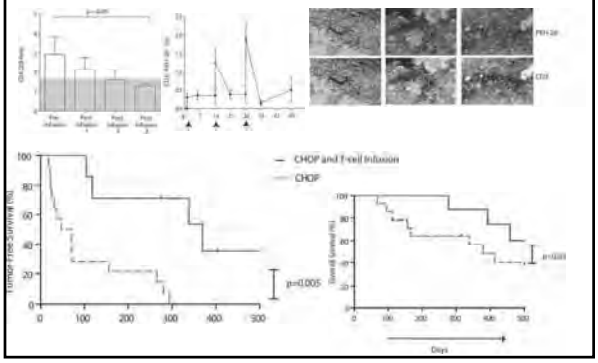
1st generation vs. 2nd generation CARs



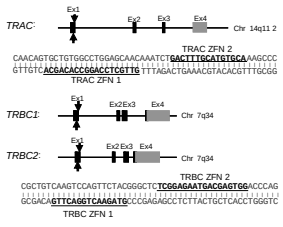
1st generation vs. 2nd generation CARs



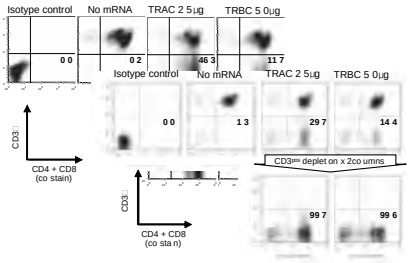
Companion canine NHL



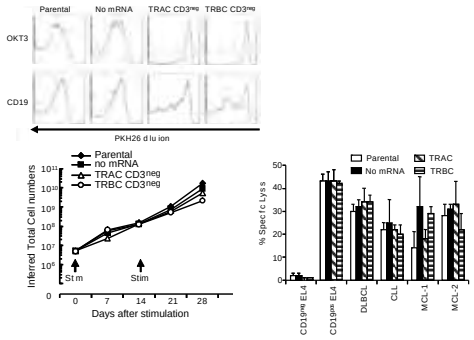
"Off-the-shelf" CAR+TCRneg T cells



"Off-the-shelf" CAR+TCRneg T cells



"Off-the-shelf" CAR+TCRneg T cells



Future approach to clinical trials

- Use of DNA vectors affords opportunity to change CAR design
- aAPC may be useful for propagating subsets of genetically modified T cells
- Off-the-shelf T cells
- Role of iCaspase9 co-expressed with CAR

Thanks

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SANGAMO BioSciences, Inc.

DANA-FARBER CANCER INSTITUTE

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Coinhibition and Costimulation

Drew M. Pardoll, MD, PhD
Johns Hopkins University School of Medicine

Dr. Pardoll is an Abeloff Professor of Oncology, Medicine, Pathology and Molecular Biology and Genetics at the Johns Hopkins University School of Medicine. He is Director of the Cancer Immunology and Hematopoiesis Program in the Sidney Kimmel Comprehensive Cancer Center. Dr. Pardoll completed his MD, PhD Medical Residency and Oncology Fellowship at Johns Hopkins University. Dr. Pardoll has published over 250 papers as well as over 20 book chapters on the subject of T cell immunology and cancer vaccines. He has served on the editorial boards of the *Journal of the National Cancer Institute* and *Cancer Cell*, and has served as a member of scientific advisory boards for the Cancer Research Institute, the University of Pennsylvania Human Gene Therapy Gene Institute, Biologic Resources Branch of the National Cancer Institute, Harvard-Dana Farber Cancer Center, Cerus Corporation, Global Medical Products Corporation, Genencor Corporation, CellGenesys Corporation, Mojave Therapeutics, the American Association of Clinical Oncology and the American Association of Cancer Research. Dr. Pardoll has made a number of basic advances in Cellular Immunology, including the discovery of gamma - delta T cells, NKT cells and interferon-producing killer dendritic cells. Over the past two decades, Dr. Pardoll has studied molecular aspects of dendritic cell biology and immune regulation, particularly related to mechanisms by which cancer cells evade elimination by the immune system. He is an inventor of a number of immunotherapies, including GVAX cancer vaccines and Listeria monocytogenes based cancer vaccines.

Slides and pre-test questions were not available at time of printing.

Notes

Antibody Based Immunotherapy

Robert J. Kreitman, MD
National Institutes of Health

Dr. Kreitman received his MD from Ohio State University in 1985 and trained in Internal Medicine at Duke University Medical Center from 1985 to 1988. He completed a fellowship in Medical Oncology at the National Institutes of Health, where he has remained working in the development of new recombinant biologic therapy for cancer. He now is Chief of the Clinical Immunotherapy Section of the Laboratory of Molecular Biology in the National Cancer Institute. He directs both clinical and laboratory research teams testing and developing recombinant immunotoxins for hematologic malignancies. He also studies the biology of hairy cell leukemia, which is particularly sensitive to immunotoxins, and directs clinical trials of these and other agents for hairy cell leukemia, in both newly diagnosed and relapsed disease.

Pre-Test Questions
Antibody Based Immunotherapy

1. Which unlabeled MAb is used to treat Paroxysmal nocturnal hemoglobinuria?:

A. Cetuximab
B. Alemtuzumab
C. Eculizumab
D. Denosumab

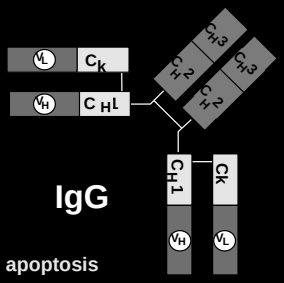
2. What is the mechanism of killing for protein toxins?:

A. Inhibition of protein synthesis
B. Induction of apoptosis
C. Thymidine kinase inhibition
D. Both A and B

Antibody Based Immunotherapy
Robert J. Kreitman, M.D.

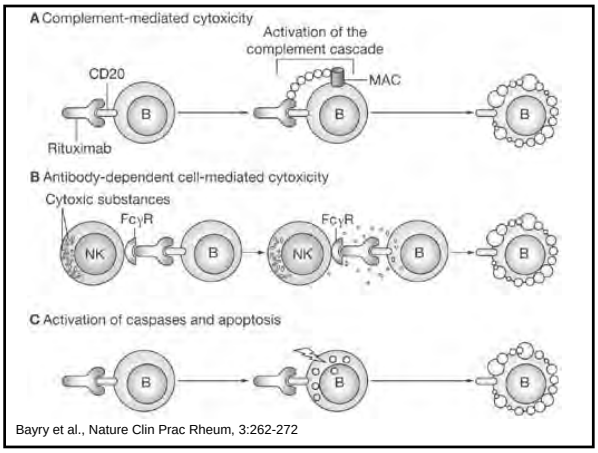
Disclosures: Co-inventor on NIH patent for moxetumomab pasudotox

Monoclonal antibodies



IgG

Induce apoptosis
CDC
ADCC



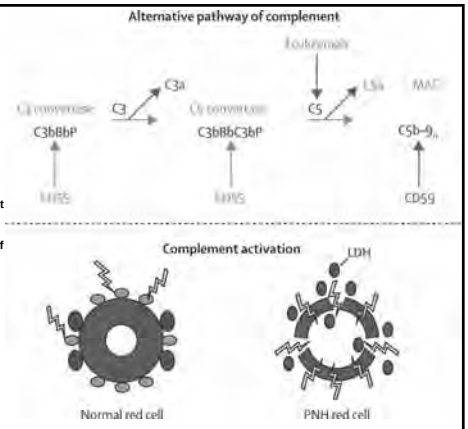
Bayry et al., Nature Clin Prac Rheum, 3:262-272

Unlabeled MAbs approved for malignancies

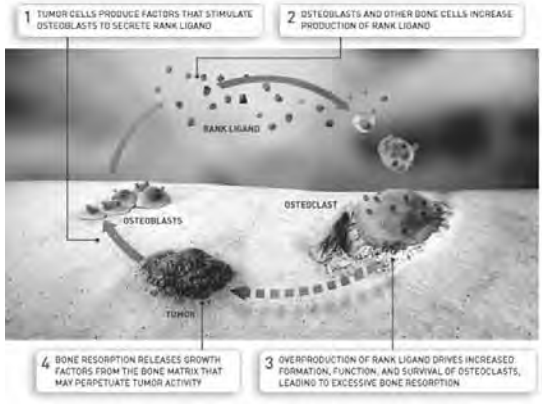
11/97	Rituximab	CD20	Non-Hodgkin's lymphoma (NHL)
10/98	Trastuzumab	Her2	Metastatic breast cancer (Gastric 10/10)
5/01	Alemtuzumab	CD52	B-cell chronic lymphocytic leukemia (CLL)
2/04	Bevacizumab	VEGF	Metastatic colorectal cancer (RCCA 7/09)
2/04	Cetuximab	EGFR	Metastatic colorectal cancer (EGFR+)
9/06	Panitumumab	EGFR	Colorectal cancer
3/07	Eculizumab	C5	Paroxysmal nocturnal hemoglobinuria (HUS 9/11)
10/09	Ofatumumab	CD20	CLL
11/10	Denosumab	RANKL	Prevention of SREs from bone metastases
3/11	Ipilimumab	CTLA4	Melanoma

Normal RBCs: glycosyl phosphatidylinositol (GPI)-anchored proteins decay accelerating factor [DAF or CD55] and membrane inhibitor of reactive lysis [MIRL or CD59]).

PNH cells express transmembrane proteins normally, but GPI-anchored proteins are not expressed because of mutant phosphatidyl-inositol glycan complementation class A (PIGA).

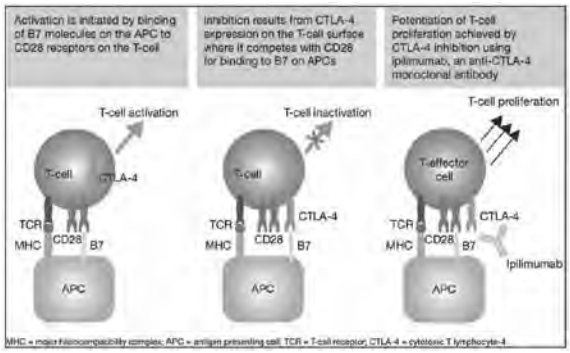


Parker, Lancet, 373:759, 2009



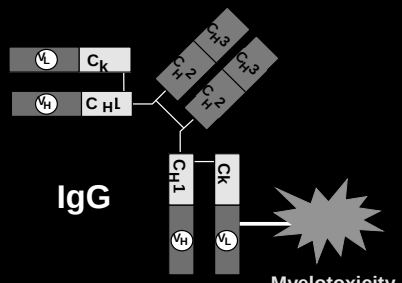
Mechanism of action of Bevacizumab

Mechanism of action of Ipilimumab



Tarhini et al., Cancer Biother Radiopharm, 25:601, 2010

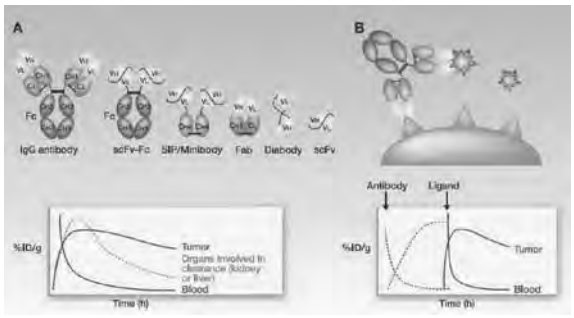
Radiolabeled Monoclonal antibodies



Targeted Radiotherapy

Myelotoxicity

Radioimmunotherapy



Approved (both anti-CD20)
^{90}Y-Ibritumomab tiuxetan (Zevalin)
^{131}I-Tositumomab (Bexxar)

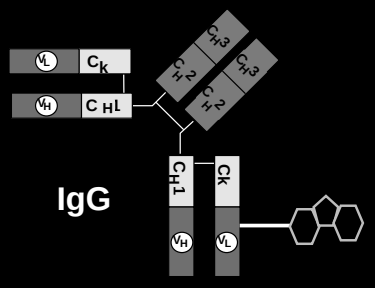
Steiner and Neri, CCR,17:6406, 2011

Radioimmunotherapy in development

^{131}I-chTNT-1/B	Tumor necrosis therapy	Brain tumors, solid tumors
^{131}I-BC8	Anti-CD45	Acute myeloid leukemia
^{177}Lu-J591	Anti-PSMA external domain	Prostate cancer
^{131}I-Metuximab	Anti-HAb18G/CD147	Hepatocellular cancer
^{177}Lu-DOTA-cG250	Anti-G250 antigen	Renal cancer
^{131}I-3F8	Anti-GD2 ganglioside	Medullo/neuroblastoma
^{131}I-L19	Fibronectin ED-B domain	NSCLC, heme tumors
^{131}I-F16	Tenascin-C A1 domain	Heme & solid tumors
^{90}Y-LL2	Anti-CD22	FL, NHL, ALL
^{90}Y-biotin/BC4-avidin	(Pretargeted)	Glioma, NHL
^{90}Y-hPAM4	anti-MUC1	Pancreatic cancer

Steiner and Neri, CCR,17:6406, 2011

Chemotherapy Immunoconjugates



Targeted Chemotherapy

Drug resistance Toxicity

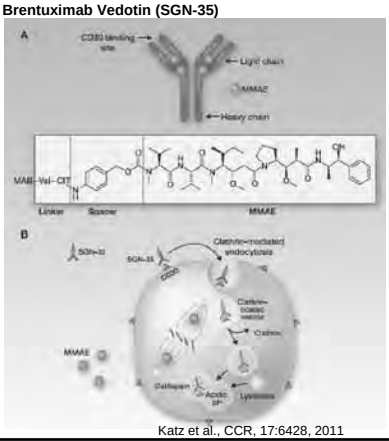
Brentuximab Vedotin (SGN-35)

Hodgkin's lymphoma
75% ORR (34% CR)

Anaplastic large cell lymphoma (ALCL)
87% ORR (57% CR)

Toxicity
Fatigue (36%)
Fever (33%)
Diarrhea, nausea (22%)
Neutropenia (22%)
Neuropathy (22%)

PML (progressive multifocal leukoencephalopathy, 2 cases)

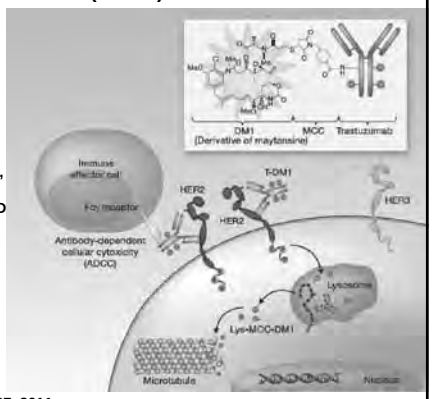


Katz et al., CCR, 17:6428, 2011

Trastuzumab Ematansine (T-DM1) for Her2+ breast cancer

- **2 phase II trials**
- **100 and 112 patients**
- **Prior chemo + anti-Her2 therapy**
- **ORR 26-35%**

Randomized trial
- **Recurrent, advanced, Metastatic,**
- **Arms: T-DM1 vs T + D (D=docetaxel)**
- **ORR 48 vs 41%**
- **CR = 5 vs 1%**
- **PFS data pending**

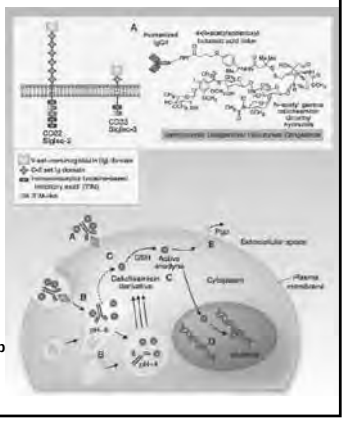


LoRusso, CCR, 17:6437, 2011

Calicheamicin targeted to CD33 and CD22

Gemtuzumab Ozogamicin (CD33)
- **Approved in 2000 for CD33+ AML**
- **Single-agent CR rates 10-27%**
- **Indicated for older AML patients**
- **Myelosuppression #1 toxicity**
- **Bilirubin (23%), ALT/AST (17%)**
- **Veno-occlusive disease (VOD)**
- **Addition Daunorubicin + ARAC failed to increase CRs or OS**
- **Withdrawn 2010, still in Japan**
- **Useful for APL**

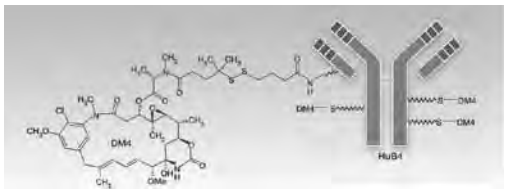
Inotuzumab Ozogamicin (CD22)
- **CMC-544 contains G544**
- **8 of 10 responses in NHL**
- **ORR 57% CR 18% in 49 ALL**
- **Toxicity: Fever, hypotension,**
- **Bilirubin, ALT/AST**
- **In randomized testing w/rituximab vs BenR or GemR**



Richart, CCR, 17:6417, 2011

Other DM1 and DM4 Conjugates in Development

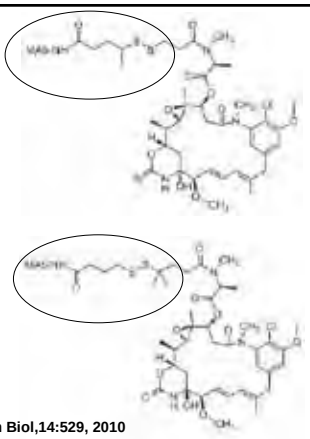
SAR3419	anti-CD19 + DM4	ALL, CLL, NHL, MM (ORR 17%, n=35)
AVE9633	anti-CD33 + DM4	AML, CML, MDS
IMGN901	anti-CD56 + DM1	MM, NK-, T-cell leuk, NHL (ORR 7%, n=28)
BT062	anti-CD138 + DM4	MM



Blanc et al., CCR, 17:6448, 2011 and FitzGerald et al., Cancer Res, 17:6300, 2011

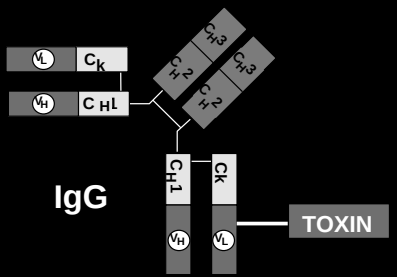
DM1

DM4



Alley et al., Curr Opin Chem Biol,14:529, 2010

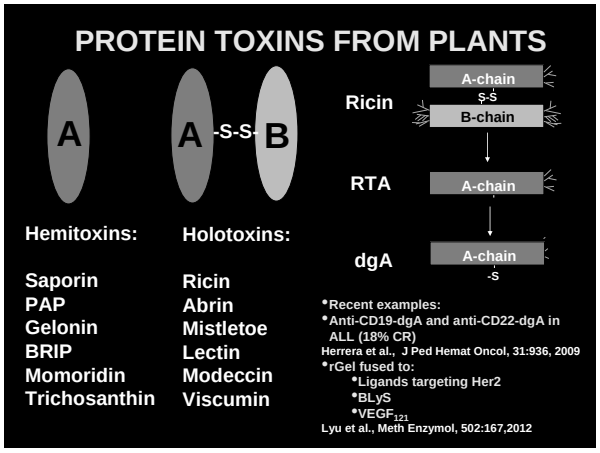
Immunotoxins

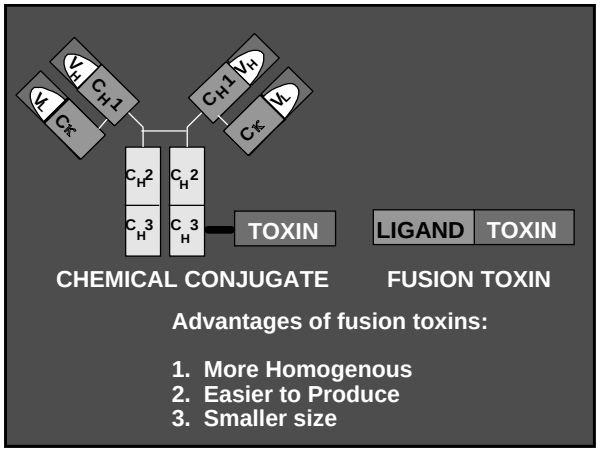


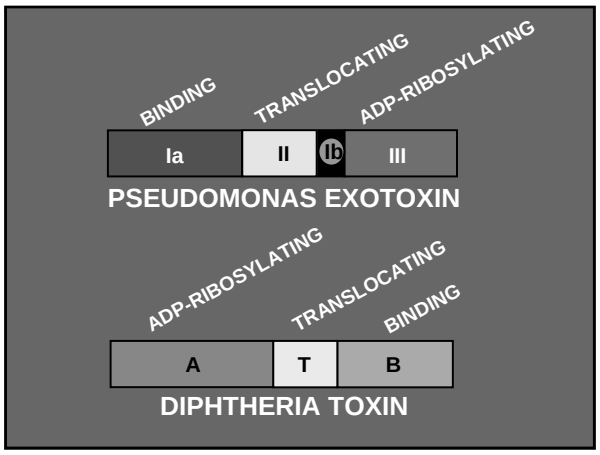
IgG

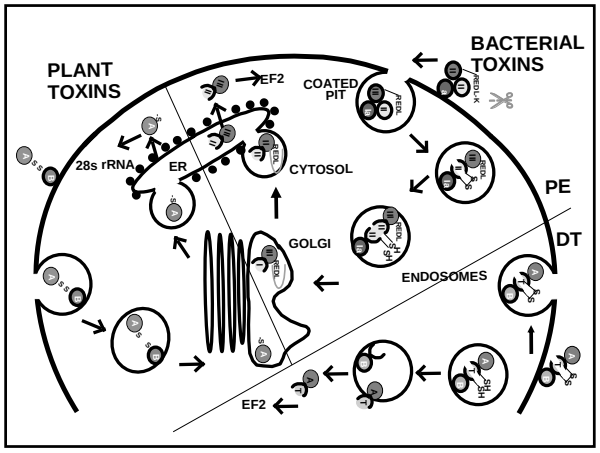
Inhibition of protein synthesis
Apoptosis

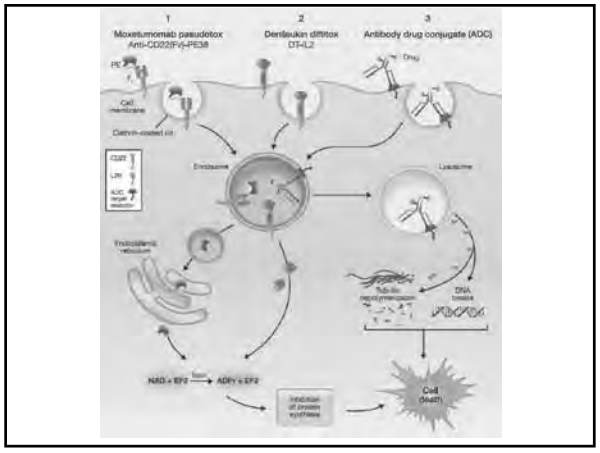
Immunogenicity


PROTEIN TOXINS FROM PLANTS
A
A -S-S- B
Hemitoxins:
Saporin
PAP
Gelonin
BRIP
Momoridin
Trichosanthin
Holotoxins:
Ricin
Abrin
Mistletoe
Lectin
Modeccin
Viscumin
Ricin
A-chain
S-S
B-chain
RTA
A-chain
dgA
A-chain
-S
•Recent examples:
•Anti-CD19-dgA and anti-CD22-dgA in ALL (18% CR)
Herrera et al., J Ped Hemat Oncol, 31:936, 2009
•rGel fused to:
•Ligands targeting Her2
•BLyS
•VEGF121
Lyu et al., Meth Enzymol, 502:167,2012


TOXIN
LIGAND
TOXIN
CHEMICAL CONJUGATE
FUSION TOXIN
Advantages of fusion toxins:
1. More Homogenous
2. Easier to Produce
3. Smaller size


BINDING
TRANSLOCATING
ADP-RIBOSYLATING
Ia
II
Ib
III
PSEUDOMONAS EXOTOXIN
ADP-RIBOSYLATING
TRANSLOCATING
BINDING
A
T
B
DIPHTHERIA TOXIN





SUMMARY OF PURIFICATION

Fermentation (*E. coli* BL21/λDE3)
↓ IPTG induction
E. coli cell paste
↓ Triton X-100 washes
Inclusion bodies
↓ Guanidine-DTE
Denatured-reduced recombinant protein
↓ 100x dilution into refolding buffer
Refolded protein
↓ Anion exchange sizing chromatography
Purified recombinant immunotoxin

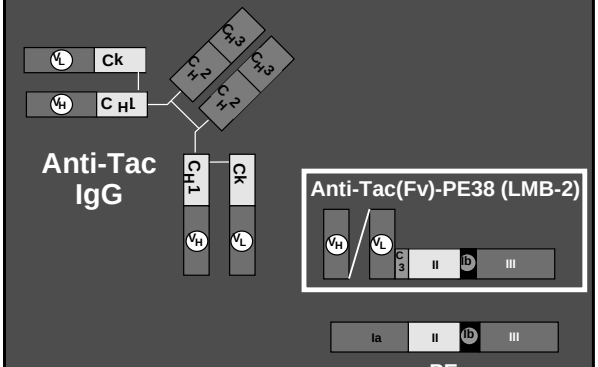
Denileukin diftitox
$DAB_{389}IL2$ (Ontak)

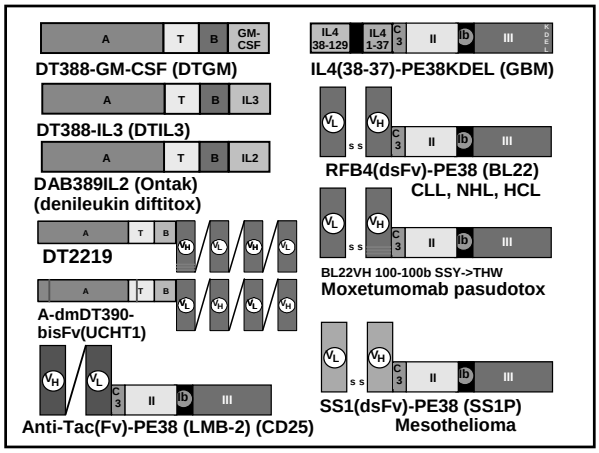
Phase I: 5 CRs, 8 PRs In 35 with CTCL, MTD 27ug/Kg qd x5
Phase III: 10% CRs, 30% ORR IN 71 With CTCL, @9-18 ug/Kg/d x5
Phase III: Placebo vs 9 vs 18 ug/Kg/d x5
- **ORR 16% vs 38% vs 49%**
- **CR 2% vs 11% vs 9%**
- **PFS 124d vs 794d vs >971d**

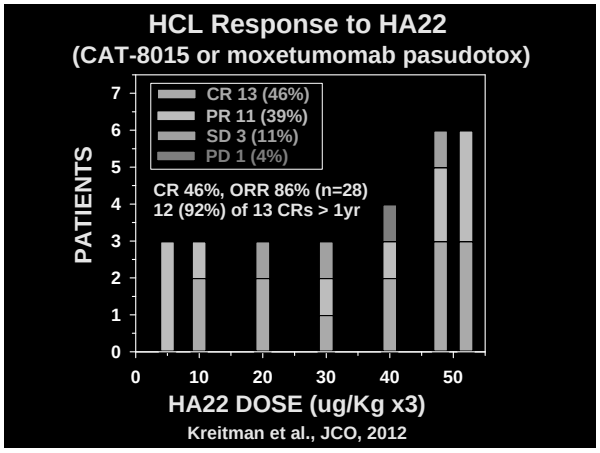
Only recombinant toxin approved by FDA
Toxicity: Fatigue, CLS, hypersensitivity (use steroids)
Other DXs: PTCL, psoriasis, CLL (4/18 pr), NHL (7% cr,18%pr)

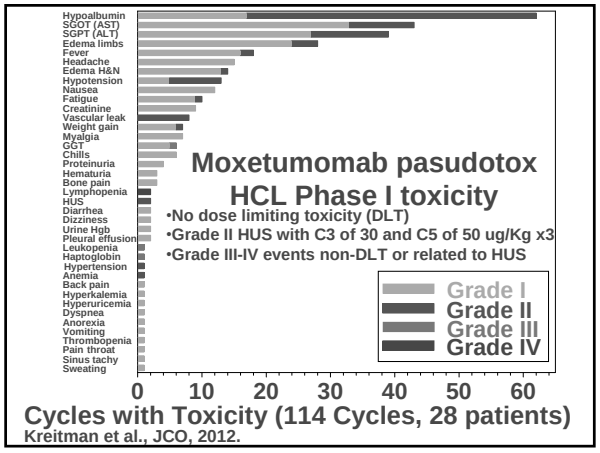
Prince et al., JCO, 28:1870, 2010, Lansigan et al., Cancer Manag Res 2:53, 2010.

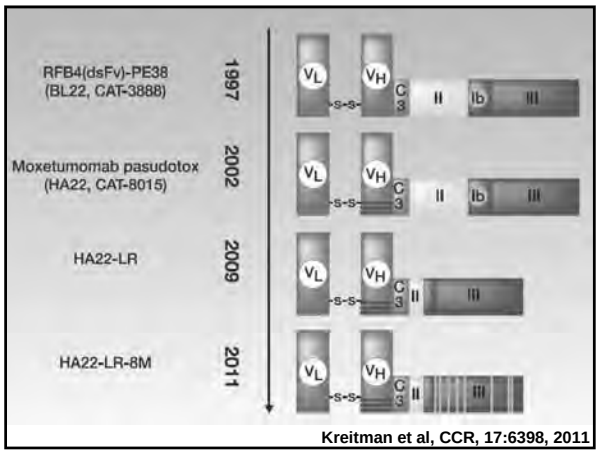
Recombinant Immunotoxins






HCL Response to HA22
(CAT-8015 or moxetumomab pasudotox)
CR 13 (46%)
PR 11 (39%)
SD 3 (11%)
PD 1 (4%)
CR 46%, ORR 86% (n=28)
12 (92%) of 13 CRs > 1yr
PATIENTS
0 1 2 3 4 5 6 7
0 10 20 30 40 50
HA22 DOSE (ug/Kg x3)
Kreitman et al., JCO, 2012

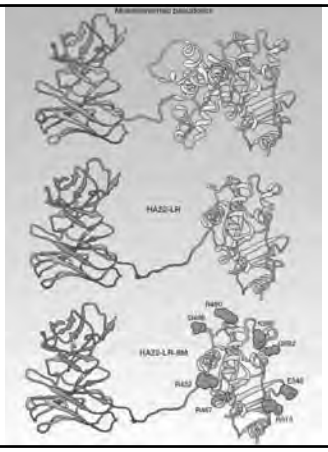

Hypoalbumin
SGOT (AST)
SGPT (ALT)
Edema limbs
Fever
Headache
Edema H&N
Hypotension
Nausea
Fatigue
Creatinine
Vascular leak
Weight gain
Myalgia
GGT
Chills
Proteinuria
Hematuria
Bone pain
Lymphopenia
HUS
Diarrhea
Dizziness
Urine Hgb
Pleural effusion
Leukopenia
Haptoglobin
Hypertension
Anemia
Back pain
Hyperkalemia
Hyperuricemia
Dyspnea
Anorexia
Vomiting
Thrombopenia
Pain throat
Sinus tachy
Sweating
Moxetumomab pasudotox
HCL Phase I toxicity
•No dose limiting toxicity (DLT)
•Grade II HUS with C3 of 30 and C5 of 50 ug/Kg x3
•Grade III-IV events non-DLT or related to HUS
Grade I
Grade II
Grade III
Grade IV
0 10 20 30 40 50 60
Cycles with Toxicity (114 Cycles, 28 patients)
Kreitman et al., JCO, 2012.


RFB4(dsFv)-PE38
(BL22, CAT-3888)
1997
Moxetumomab pasudotox
(HA22, CAT-8015)
2002
HA22-LR
2009
HA22-LR-8M
2011
VL
VH
C 3
II
Ib
III
Kreitman et al, CCR, 17:6398, 2011

Moxetumomab pasudotox: Median 8-fold more cytotoxic than BL22

HA22-LR: Median 16-fold more cytotoxic than moxetumomab pasudotox

HA22-LR-8M: Activity similar to HA22-LR



Kreitman et al, CCR, 17:6398, 2011

Conclusions

- The most common Mab-based therapies for cancer are unlabeled MAbs, but the 3 classic mechanisms of action, 1) apoptosis, 2) CDC and 3) ADCC only work for a minority of targets.
- Radioimmunotherapy is effective in CD20+ NHL (early and relapsed), but its use and development of new agents have been limited.
- Antibody-drug conjugates have used the high cytotoxicity of auristatins, maytansenoids, and calicheamicin to produced several clinically useful conjugates for both solid and hematologic tumors.
- Targeted protein toxins exploit the most powerful killing agents known, able to kill cells catalytically with as few as 1 molecule. Efficacy is limited by immunogenicity particularly in solid tumors.
- Denileukin diftitox is a targeted toxin fusion protein approved for CTCL, with some efficacy demonstrated in PTCL, CLL and NHL.
- Recombinant immunotoxins have demonstrated clinical efficacy, particularly moxetumomab pasudotox for HCL and SS1P for mesothelioma. Immunogenicity may be reduced by humanizing the toxin and by combination with chemotherapy.