Molecular & Cellular Immunology 3283

Cancer Immunity

Jonathan Irish, Ph.D.

Outline

Surveillance & immunoediting

Cancer immunotherapy

Hematological malignancies

Case study:
TLR9 KO lymphoma model

Example ‘Big Questions’ in Cancer Immunity

1. Are cancer cells ‘non-self’? If so, when do they become non-self and why does the immune system fail to kill them?

2. How do we therapeutically target cancer cells without harming immune system cells?

3. Can we vaccinate against cancer cells, break tolerance, transplant, or engineer anti-cancer cells without generating GVH or autoimmunity?

4. Do cancer cells modulate the immune system as part of initiation or progression?

5. Can we treat established, metastatic / disseminated cancer by activating a systemic immune response?

Timeline of Cancer Milestones – Nearly All Involve Immunology, the Immune System, or Hematological Cancers

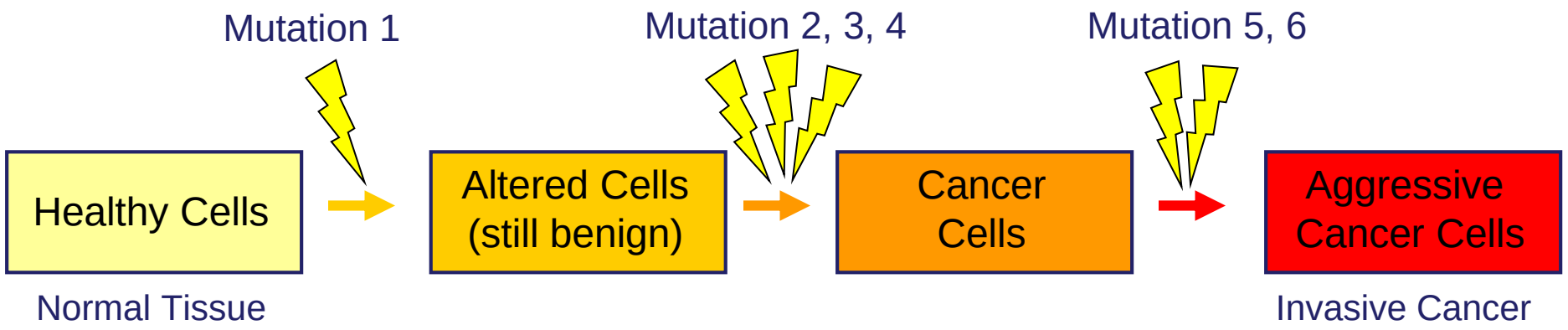
1889	1. Seed and soil hypothesis
1890	2. Cancer as a genetic disease
1909	3. Immune surveillance
1910	4. Viruses and cancer
1915	5. Hormones and cancer
1937	6. Cancer stem cells
1939	7. Angiogenesis
1950	8. Smoking and cancer
1953	9. Two-hit hypothesis
1960	10. Chromosome translocations
1971	11. Tumour suppressor genes
1972	12. Apoptosis and cancer
1975	13. Tumour microenvironment
1976	14. Clonal evolution & multistep tumourigenesis
	15. Cellular homologues of viral oncogenes
1978	16. Oncogenes encode proteins that regulate cell growth
1979	17. First human oncogene
1983	18. Oncogene co-operation
	19. Cancer epigenetics
1989	20. Cell cycle and DNA damage checkpoints
1990	21. Genetic basis for cancer predisposition
	22. Mechanisms of genetic instability in cancer
1999	23. Cancer profiling
2001	24. Targeted cancer therapy

Chemotherapy

Immune related topics we will discuss

http://www.nature.com/milestones/milecancer/timeline.html

Stepwise Model of Tumor Initiation and Progression



Cell, Vol. 87, 159–170, October 18, 1996, Copyright ©1996 by Cell Press

Lessons from Hereditary Colorectal Cancer

Kenneth W. Kinzler* and Bert Vogelstein*†
*The Johns Hopkins Oncology Center
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424 North Bond Street
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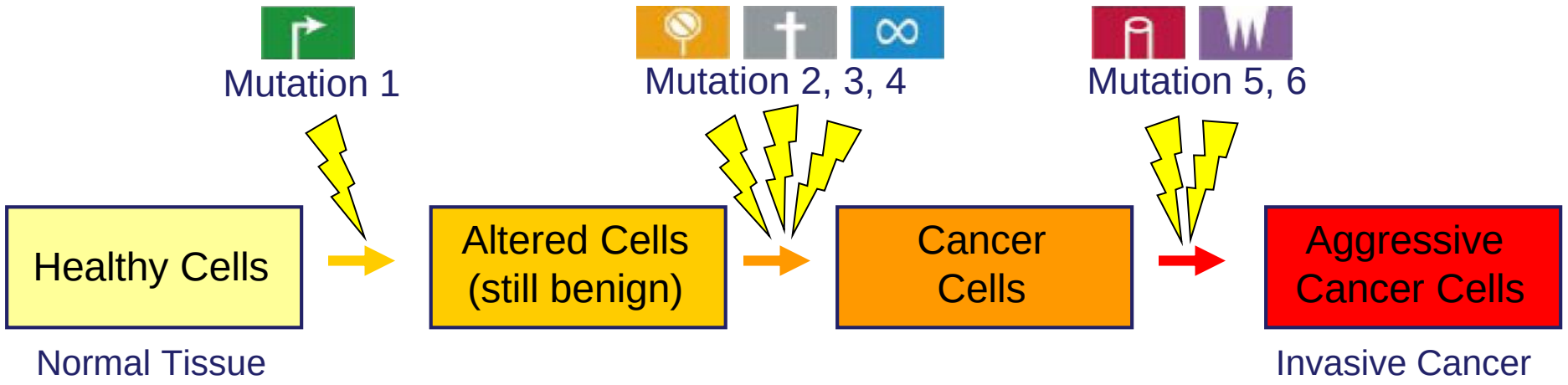
Acquired Capabilities of Cancer Cells



Acquired Capability

- Self-sufficient growth
- Insensitive to anti-growth
- Evading cell death
- Limitless replication potential
- Growing blood vessels
- Tissue invasion

Cell, Vol. 100, 57–70, January 7, 2000, Copyright ©2000 by Cell Press

The Hallmarks of Cancer

Douglas Hanahan* and Robert A. Weinberg†
*Department of Biochemistry and Biophysics and Hormone Research Institute
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San Francisco, California 94143
†Whitehead Institute for Biomedical Research and Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02142

Hallmarks of Cancer: The Next Generation

Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]

Hanahan and Weinberg, *Cell* 2000 & 2011

Changes to Cell Signaling Interactions in Cancer

INNOVATION

Mapping normal and cancer cell signalling networks: towards single-cell proteomics

Jonathan M. Irish, Nikesh Kotecha and Garry P. Nolan

146 | FEBRUARY 2006 | VOLUME 6

www.nature.com/reviews/cancer

Acquired Capability	Example Signaling Alteration
Self-sufficient growth	↑ RAS/RAF/ERK signaling
Insensitive to anti-growth	↓ STAT1, PTEN signaling
Evading cell death	↑ STAT5, ↓ p53 signaling
Limitless replication potential	↑ AKT signaling
Growing blood vessels	↑ VEGF signaling
Tissue invasion	↑ EGFR, WNT signaling

} Altered signaling supports cancer cell survival, aggressive behavior

Irish, Kotecha, & Nolan, *Nat Rev Cancer* 2006

Changes to Cell Signaling Interactions in Cancer

Table 2 | **Frequently altered signalling pathways and their role in cancer**

Cancer cell signalling alteration			References
Ligands and receptors	**Intracellular molecules**	**Acquired capability**[§]	
↑KIT, ↑PDGFR, ↑FLT3, ↑↓BCR, ↑↓TGFβ, ↑IGF1, ↑EGFR, ↑ERBB2	↑SFKs, ↑STAT5, ↑STAT3, ↓NF1, ↑Ras, ↑Raf, ↑ERK, ↑ZAP70, ↑MYC, ↑Smads, ↑PI3K, ↑AKT, ↑SHH, ↑GLI1	Self sufficiency in proliferation	67–85
↓Tumour-necrosis factor family*, ↑decoy receptor family, ↓interferon family[‡]	↓IκB, ↓NF-κB, ↑AKT, ↓p53, ↓caspases, ↓STAT1, ↑BCL2	Evasion of apoptosis, and evasion of killing by the immune system	20,78,79, 86–89
↑αvβ3 integrin, ↑β1 integrins, ↑EGFR, ↑WNT1, ↓E-cadherin	↑SFKs, ↑Ras, ↑Raf, ↑Erk, ↑Rho GTPases, ↑β-catenin, ↓ APC	Tissue invasion and metastasis	74,75,77, 88,90–92
↑↓TGFβ, ↓interferon family[‡]	↓ATM, ↓p53, ↓PTEN, ↓RB, ↓STAT1	Insensitivity to anti-proliferative cues	20,71, 77–79,84, 88,93
↑VEGF, ↑VEGFR1, ↑FGF, ↑αvβ3 integrin	↑Ras, ↑Raf, ↑Erk, ↑SFKs	Sustained angiogenesis	74,75,77,81, 90
↑IGF1	↑AKT	Limitless replicative potential	94

Irish, Kotecha, & Nolan, *Nat Rev Cancer* 2006

Cellular Evolution: Central to Cancer and Immunity

- Immune cells undergo programmatic somatic translocations and mutations, generating a diverse pool of cells for selection.

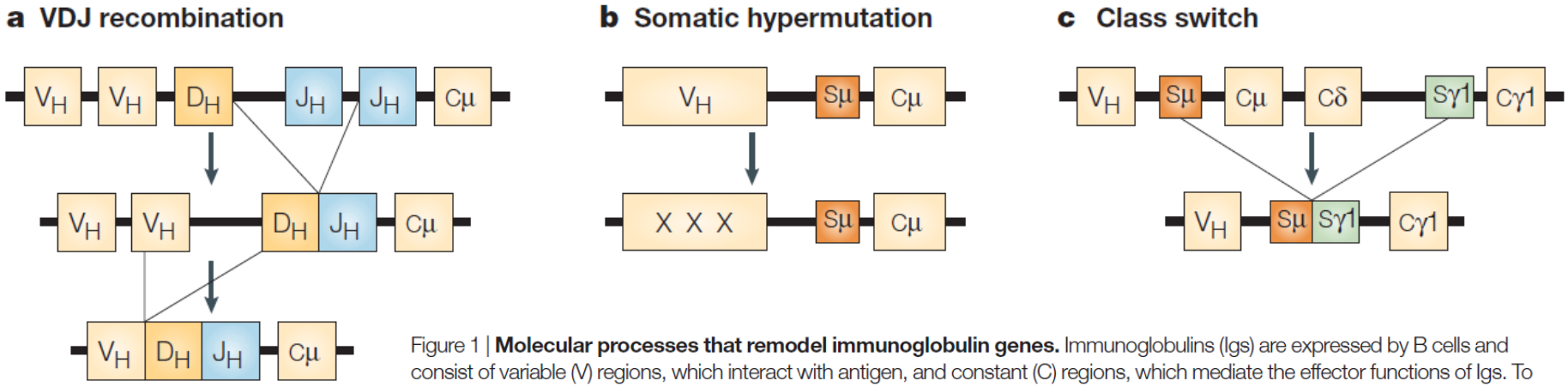


Figure 1 | **Molecular processes that remodel immunoglobulin genes.** Immunoglobulins (Igs) are expressed by B cells and consist of variable (V) regions, which interact with antigen, and constant (C) regions, which mediate the effector functions of Igs. To create a functional Ig, B cells must rearrange DNA segments that encode the heavy (H)- and light-chain (not shown) regions of the variable genes. **a** | First, through a process called 'V(D)J recombination', three gene segments, V_H, D_H and J_H, are joined to encode the H-chain variable region. The V regions of the κ- and λ-light chains, alternatively, are each encoded by two gene segments — the V_L and J_L genes (not shown). B-cell precursors first carry out D_H–J_H rearrangements in H-chain genes. These D_H–J_H rearrangements are followed by V_H–D_HJ_H rearrangements, resulting in the expression of a pre-B-cell receptor if the rearrangement is productive[3]. About 50 functional V_H gene segments, 27 D_H segments and 6 J_H segments are available in the germline, allowing the generation of a diverse repertoire of V_H gene rearrangements. The diversity is further increased by the addition or removal of nucleotides at the joining sites of the gene segments[3]. The cells then carry out rearrangements at their L-chain loci (not shown). The V-region of the Ig gene is ultimately connected to the C-region of the Ig gene (Cμ of IgM in diagram) **b** | The process of somatic hypermutation is activated when B cells reach the germinal centre (GC, shown in more details in FIG. 2). This process leads to the introduction of point mutations, deletions or duplications in the rearranged V-region of Ig genes (denoted by 'Xs' in the figure)[102]. These mutations occur in the V-region of Ig genes — not in the downstream Cμ region. **c** | Class switching results in the replacement of the originally expressed H-chain C-region gene with that of another Ig gene. In the diagram, the C-region for IgM (Cμ) and IgD (Cδ) are exchanged for the C-region of IgG (Cγ1) by recombination at the switch regions for these genes (Sμ and Sγ1, respectively). This results in an antibody with different effector functions but the same antigen-binding domain.

Küppers, *Nat Rev Cancer* 2005

Cellular Evolution: Central to Cancer and Immunity

- Immune cells undergo programmatic somatic translocation and mutation generating a diverse pool that undergoes selection.
- For both cancer and immunity, cells need to acquire new, heritable cellular features.
- When immune developmental checkpoints are dysregulated we see cancer, allergy, and autoimmunity.
- Examples of heritable cellular features
  - Genetic
    - Mutations (DNA basepair changes)
    - Amplifications / deletions (copy number changes)
    - Translocations (might include viruses, retrotransposons)
  - Epigenetic
    - Methylation / acetylation of DNA, histones
    - Prions
    - Infection by intracellular pathogens
    - Reprogramming, as with iPS cells (Oct4 + Sox2 + Nanog + Klf4 +/- Myc)

Cancer Immunoediting Model

Immunity, Vol. 21, 137–148, August, 2004, Copyright ©2004 by Cell Press

The Immunobiology of Cancer Immunosurveillance and Immunoediting

Gavin P. Dunn,[1] Lloyd J. Old,[2] and Robert D. Schreiber[1,*]

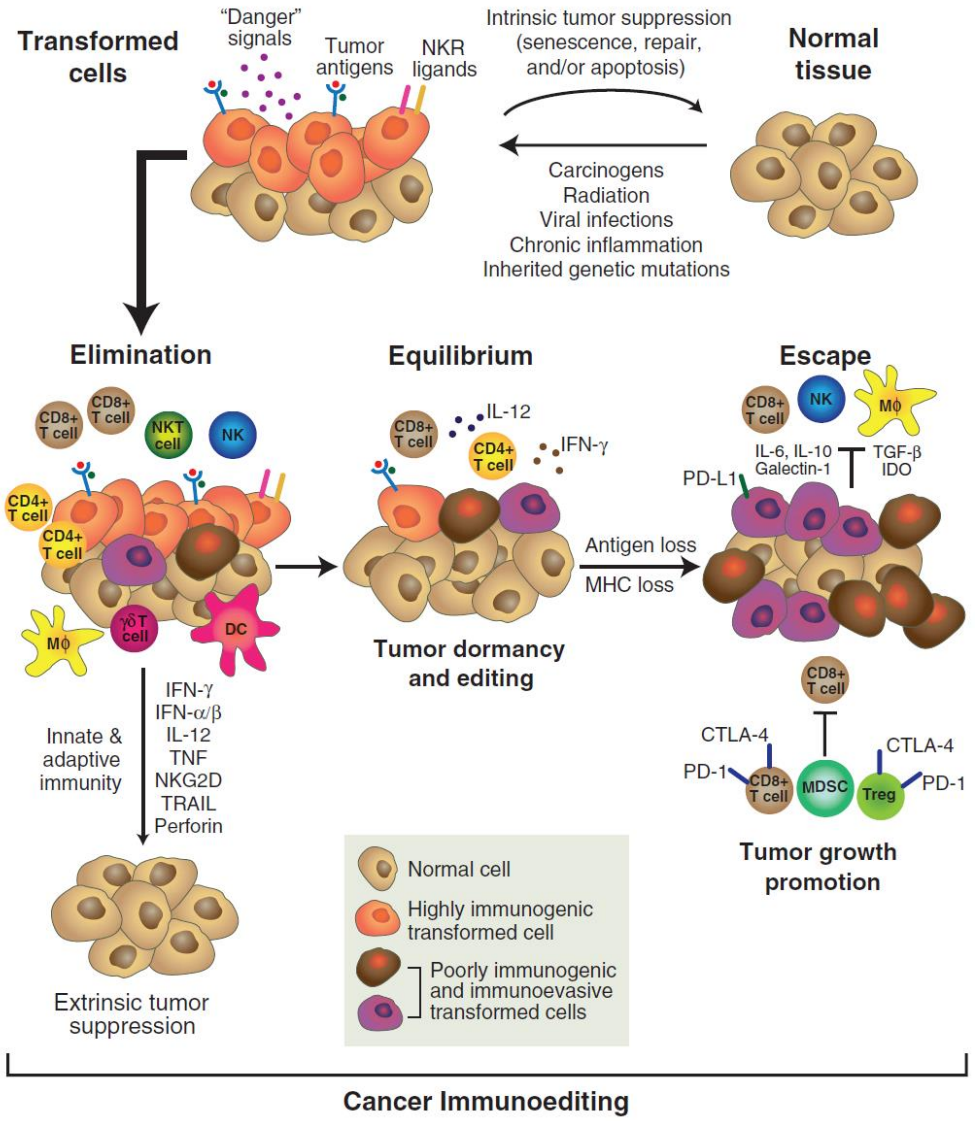
REVIEW

Cancer Immunoediting: Integrating Immunity's Roles in Cancer Suppression and Promotion

Robert D. Schreiber,[1*] Lloyd J. Old,[2] Mark J. Smyth[3,4]

Understanding how the immune system affects cancer development and progression has been one of the most challenging questions in immunology. Research over the past two decades has helped explain why the answer to this question has evaded us for so long. We now appreciate that the immune system plays a dual role in cancer: It can not only suppress tumor growth by destroying cancer cells or inhibiting their outgrowth but also promote tumor progression either by selecting for tumor cells that are more fit to survive in an immunocompetent host or by establishing conditions within the tumor microenvironment that facilitate tumor outgrowth. Here, we discuss a unifying conceptual framework called "cancer immunoediting," which integrates the immune system's dual host-protective and tumor-promoting roles.

www.sciencemag.org SCIENCE VOL 331 25 MARCH 2011



Schreiber et al., *Science* 2011

Landmarks in Tumor Immune Surveillance

1. 1909: Paul Ehrlich postulates a model where the immune system helps prevent the development of cancer.

2. 1957: Richmond Prehn and Joan Main observe differential rejection of chemically induced tumors (rejected) and spontaneous tumors (not rejected).

3. 1982: Aline van Pel and Thierry Boon demonstrate that mutagenized tumor cells generate specific immunity against spontaneous tumors.

4. 2001: Robert Schreiber et al. demonstrate 1) immunodeficient mice are susceptible to chemically induced and spontaneous tumors, and 2) the immune system selects for cancer escapees (or perhaps becomes tolerized to the tumor). Importance of IFNγ / STAT1 seen.

5. 2012: Tyler Jacks and Schreiber demonstrate T cell dependent immunoediting / cancer cell escape (as opposed to tolerization).

http://www.nature.com/milestones/milecancer/full/milecancer03.html

Immune Status Matters for Cancer

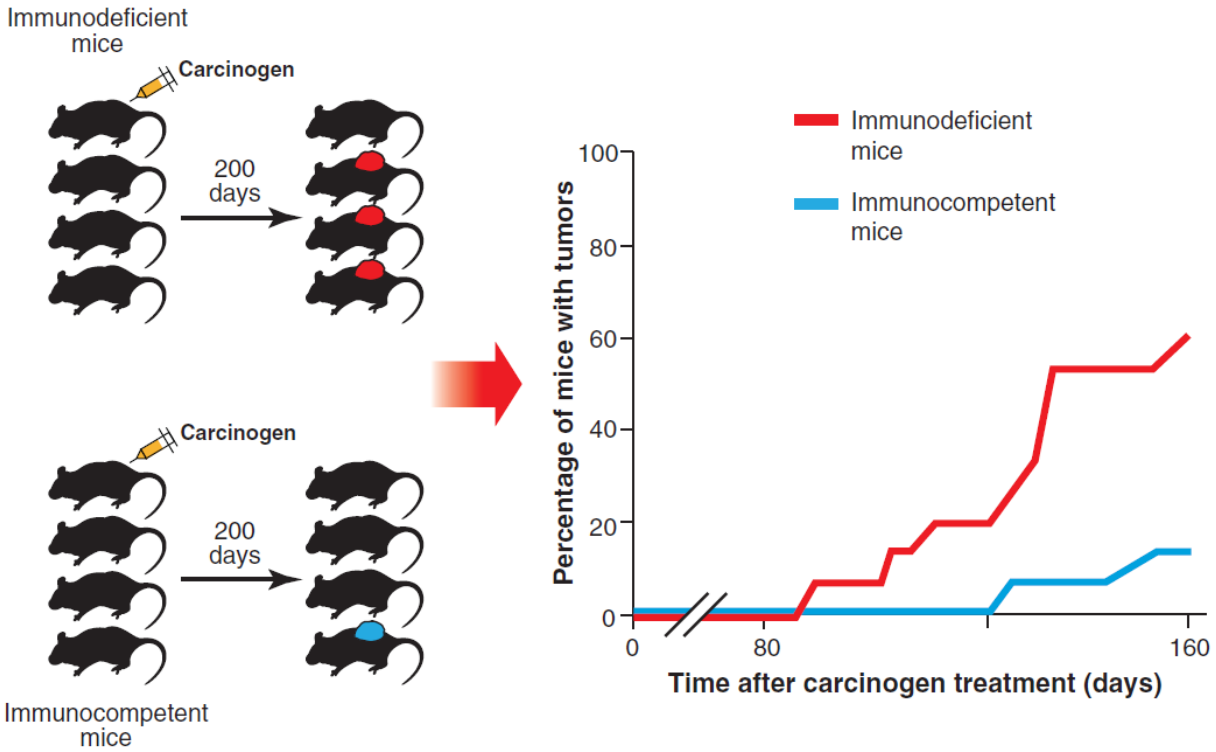


Fig. 1. The immune status of mice is a critical determinant of their susceptibility to tumors induced by chemical carcinogens. Over the past two decades, numerous studies have established that immunodeficient mice are more tumor prone than are immunocompetent mice after treatment with carcinogens such as MCA. The immunodeficient mice tested in such experiments include gene-targeted mice on pure genetic backgrounds with deficits of innate or adaptive immunity as well as wild-type mice rendered immunodeficient by chronic administration of monoclonal antibodies that, for example, deplete $CD4^+$ and $CD8^+$ T cells or interferon-γ. Immunodeficiency has also been found to increase the susceptibility of untreated mice to spontaneously arising tumors and to increase the incidence of tumor formation in mouse genetic models of cancer. Schematic is based on experiments described in (*13*).

Death Receptors and Cancer

TARGETING DEATH AND DECOY RECEPTORS OF THE TUMOUR-NECROSIS FACTOR SUPERFAMILY

Avi Ashkenazi

Cancer cells often develop resistance to chemotherapy or irradiation through mutations in the p53 tumour-suppressor gene, which prevent apoptosis induction in response to cellular damage. Death receptors — members of the tumour-necrosis factor receptor (TNFR) superfamily — signal apoptosis independently of p53. Decoy receptors, by contrast, are a non-signalling subset of the TNFR superfamily that attenuate death-receptor function. Agents that are designed to activate death receptors (or block decoy receptors) might therefore be used to kill tumour cells that are resistant to conventional cancer therapies.

Lpr and *Gld: an Informative Pair of Mutations*

Fas and Fas ligand: *lpr* and *gld* mutations

Shigekazu Nagata and Takashi Suda

Fas ligand (FasL) is a death factor that binds to its receptor, Fas, and induces apoptosis. Two mutations that accelerate autoimmune disease, lpr *and* gld, *are known to correspond to mutations within genes encoding Fas and FasL, respectively. Here, Shigekazu Nagata and Takashi Suda summarize current knowledge of Fas and FasL, and discuss the physiological role of the Fas system in T-cell development, cytotoxicity and cytotoxic T lymphocyte (CTL)-mediated autoimmune disease.*

Immunology Today 39 *Vol. 16 No. 1 1995*

While establishing a mouse MRI. strain, Andrews et al.12 discovered a mouse mutant that develops lymphadenopathy and splenomegaly. The autosomal recessive mutation responsible was located to mouse chromosome 19 (Ref. 13) and is referred to as *lpr (for* lymphoproliferation). Later, Roths et al.'" found a different mutant with a phenotype similar to *lpr, and this* mutation was designated *gld (for generalized lymphoproliferative* disease).

TNF and TNFR Superfamily

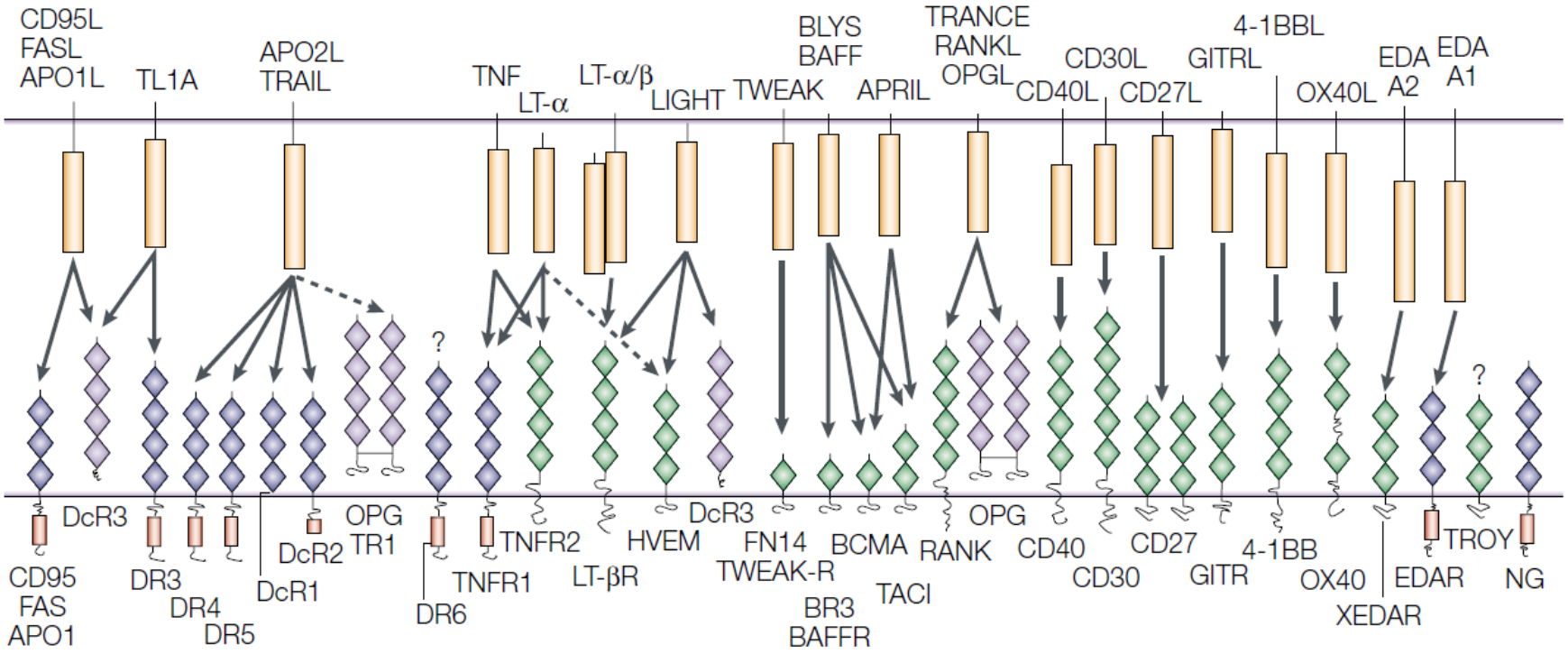
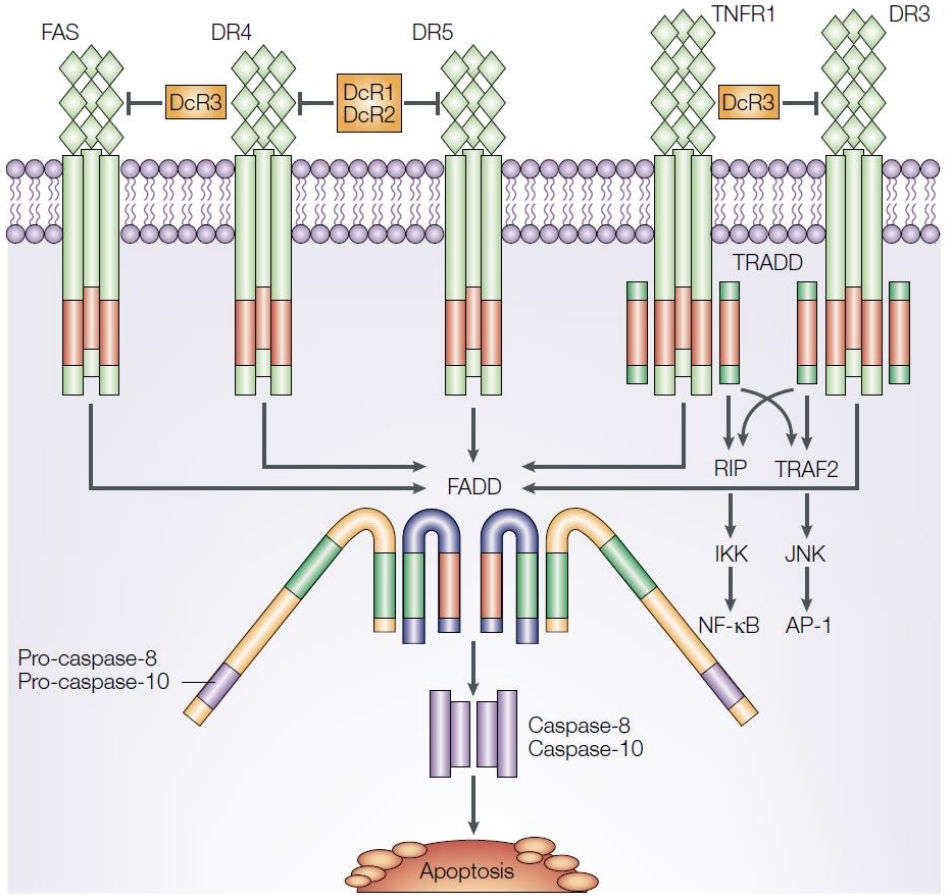
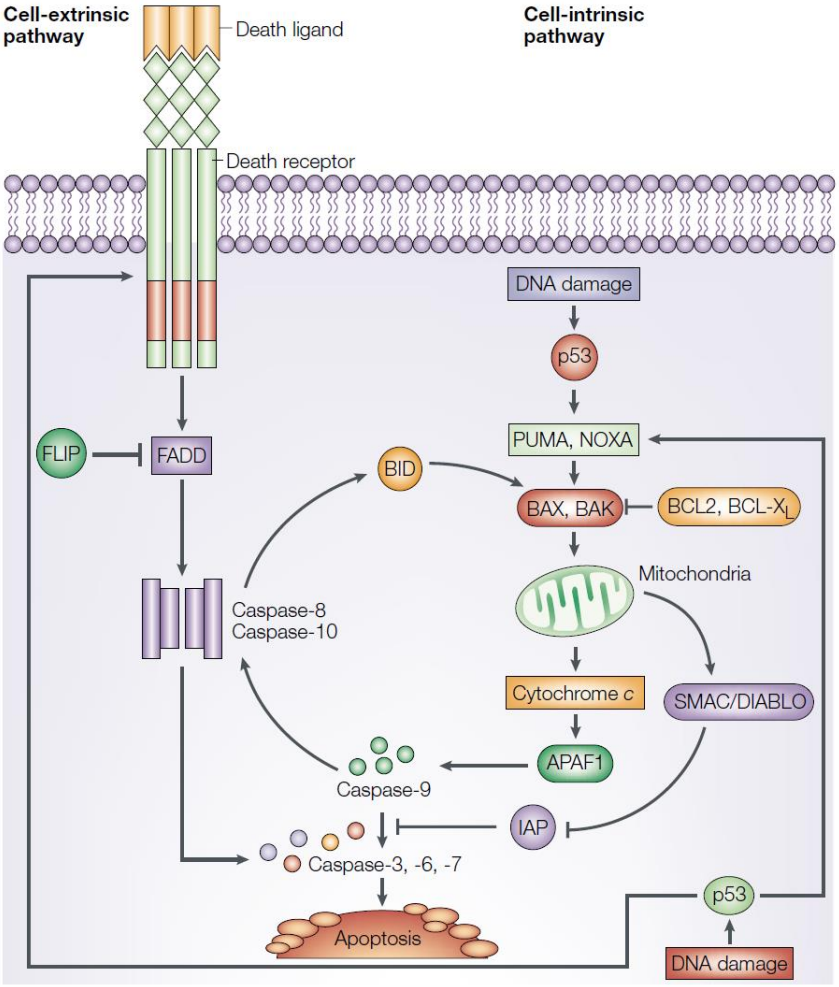


Figure 1 | **The TNF and TNFR superfamilies.** Ligands are shown in their schematic transmembrane form. Arrows indicate receptor interactions with solid lines for strong binding and dashed lines for low-affinity binding. Question marks indicate that cognate ligands have not yet been identified. Diamonds represent receptor cysteine-rich domains and red boxes denote receptor cytoplasmic death domains.

Cancer Cells Interfere with Death Receptor Signaling





Ashkenazi, *Nat Rev Cancer* 2002

Outline

Surveillance & immunoediting

Cancer immunotherapy

Hematological malignancies

Case study:
TLR9 KO lymphoma model

Tumor Immunotherapy Strategies

1. Cell based therapy (e.g. engineered T cells or DCs)

2. Antibody based targeted therapy that kills tumor cells (e.g. Rituximab α-CD20, Trastuzumab α-HER2)

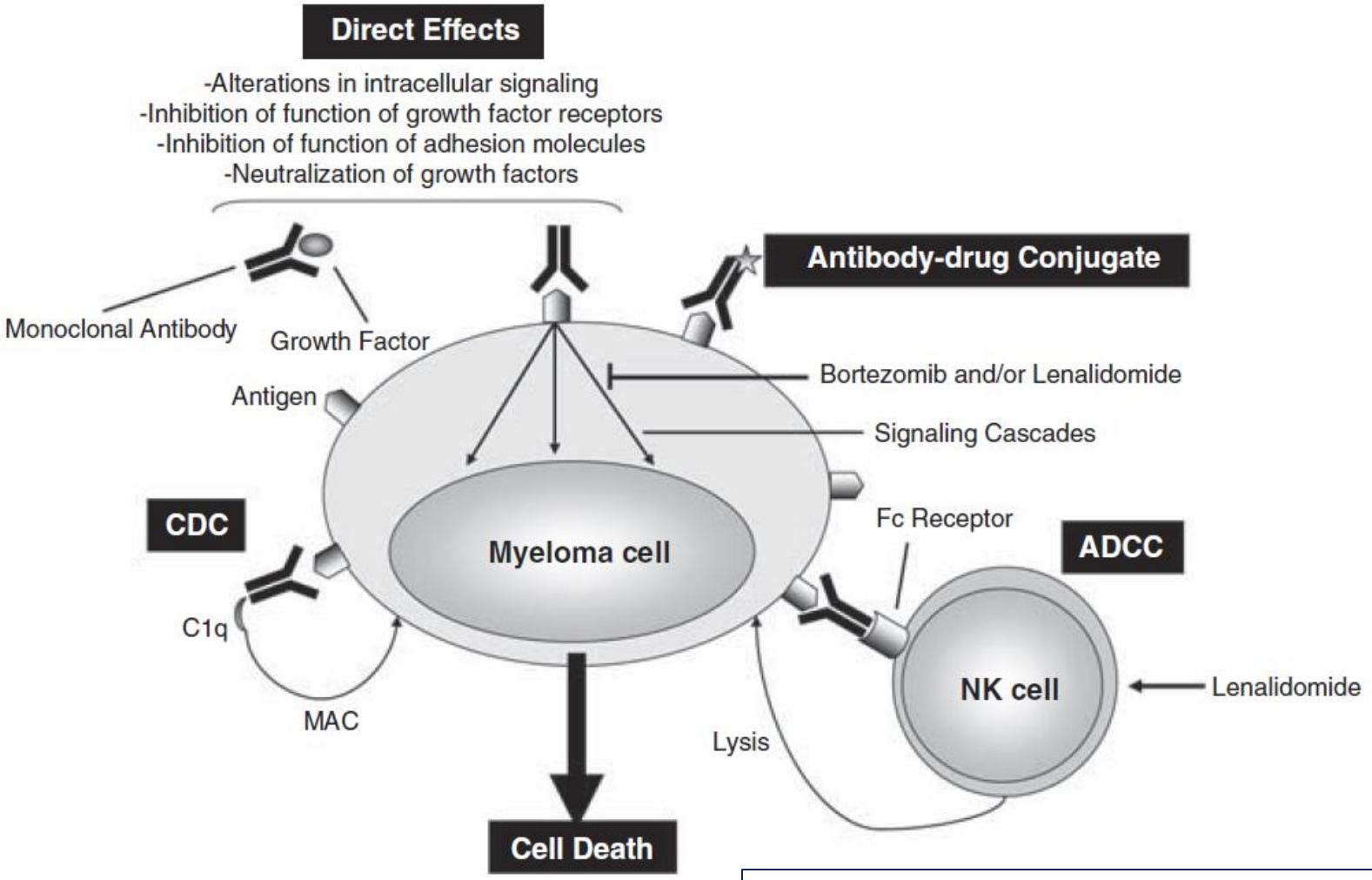
3. Vaccines (e.g. idiotype vaccination, vaccines vs. tumor causing microbes, DNA and other vaccines using tumor specific or associated antigens, dendritic cell vaccines)

4. Immunomodulation (e.g. ipilimumab α-CTLA4, α-PD-1, immunotransplant into lymphodepleted host, CD40 gene therapy, CpG DNA, depletion of T_{regs} or MDSCs)

DNA vaccines produced in bacteria can function like unmethylated CpG (innate immune signal, TLR9 pathway)

http://www.nature.com/milestones/milecancer/full/milecancer03.html

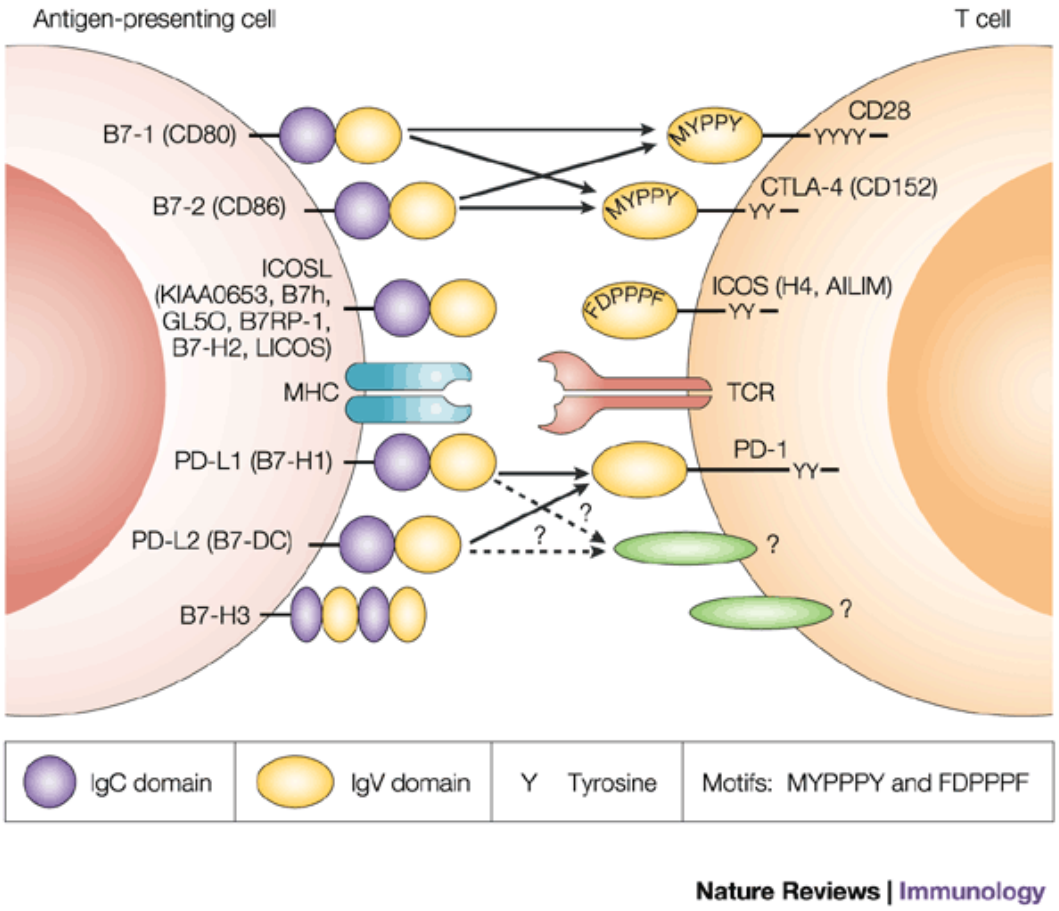
Potential Mechanisms of Antibody Based Therapy

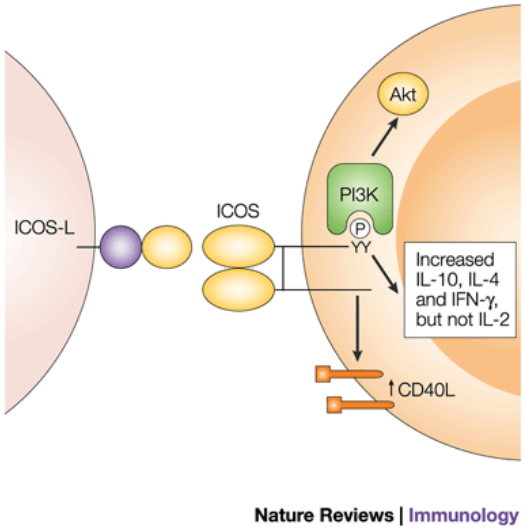


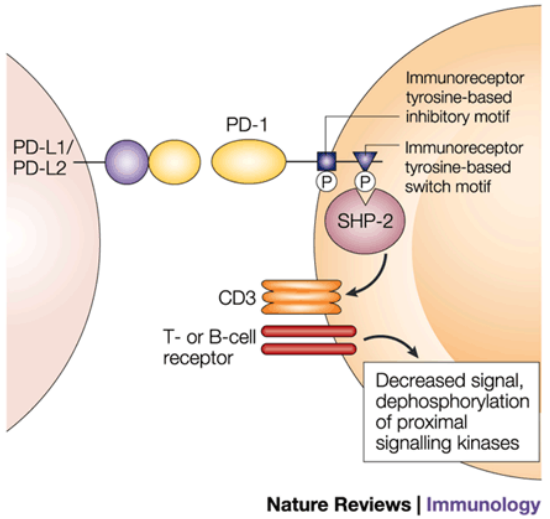
FcR polymorphisms govern α-CD20 therapy

Van de Donk et al., *Leukemia* 2012

B7-CD28 Family Members Regulate T Cells







Sharpe & Freeman, *Nat Rev Immunol* 2002

Anti-PD-1 Antibody Therapy in Melanoma

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JUNE 28, 2012 VOL. 366 NO. 26

Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer

Suzanne L. Topalian, M.D., F. Stephen Hodi, M.D., Julie R. Brahmer, M.D., Scott N. Gettinger, M.D., David C. Smith, M.D., David F. McDermott, M.D., John D. Powderly, M.D., Richard D. Carvajal, M.D., Jeffrey A. Sosman, M.D., Michael B. Atkins, M.D., Philip D. Leming, M.D., David R. Spigel, M.D., Scott J. Antonia, M.D., Ph.D., Leora Horn, M.D., Charles G. Drake, M.D., Ph.D., Drew M. Pardoll, M.D., Ph.D., Lieping Chen, M.D., Ph.D., William H. Sharfman, M.D., Robert A. Anders, M.D., Ph.D., Janis M. Taube, M.D., Tracee L. McMiller, M.S., Haiying Xu, B.A., Alan J. Korman, Ph.D., Maria Jure-Kunkel, Ph.D., Shruti Agrawal, Ph.D., Daniel McDonald, M.B.A., Georgia D. Kollia, Ph.D., Ashok Gupta, M.D., Ph.D., Jon M. Wigginton, M.D., and Mario Sznol, M.D.

BACKGROUND

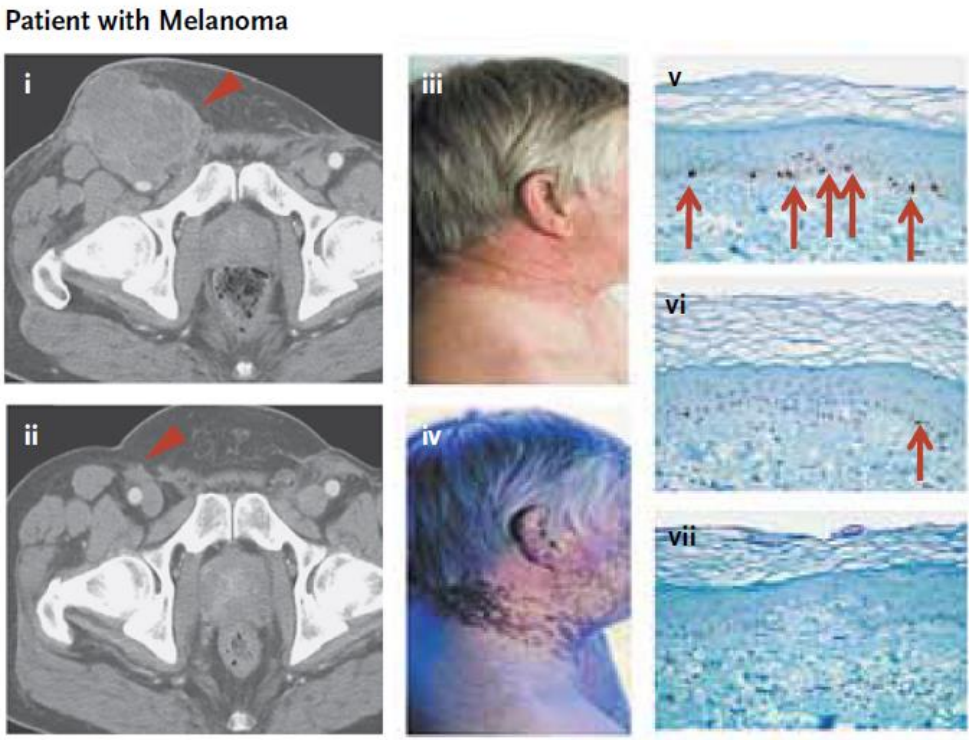
Blockade of programmed death 1 (PD-1), an inhibitory receptor expressed by T cells, can overcome immune resistance. We assessed the antitumor activity and safety of BMS-936558, an antibody that specifically blocks PD-1.

METHODS

We enrolled patients with advanced melanoma, non–small-cell lung cancer, castration-resistant prostate cancer, or renal-cell or colorectal cancer to receive anti–PD-1 antibody at a dose of 0.1 to 10.0 mg per kilogram of body weight every 2 weeks. Response was assessed after each 8-week treatment cycle. Patients received up to 12 cycles until disease progression or a complete response occurred.

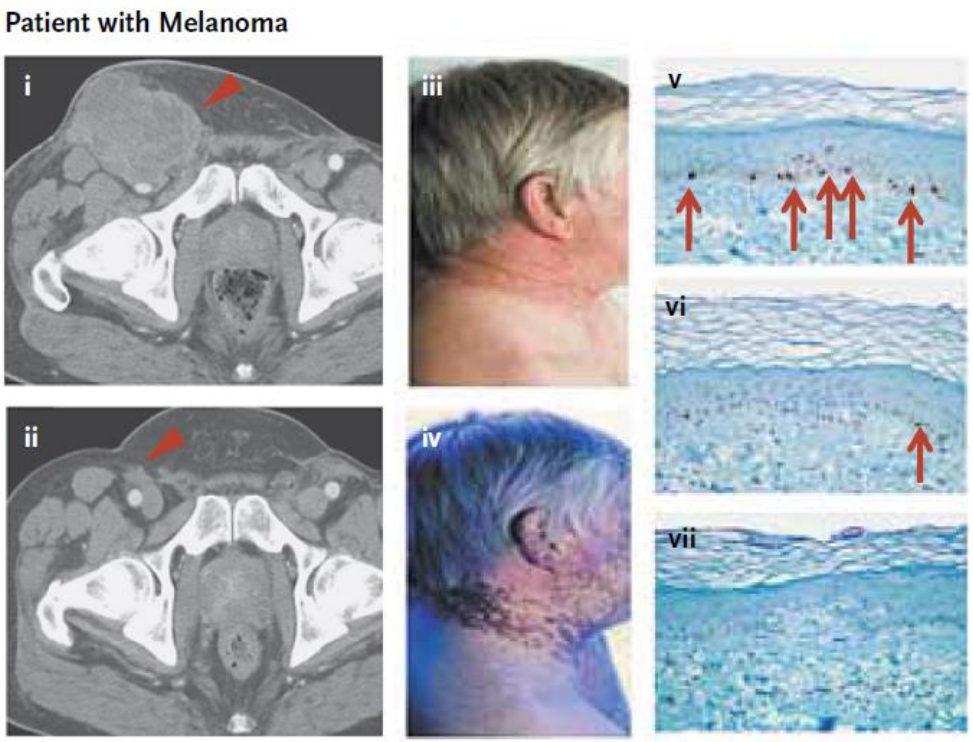
CONCLUSIONS

Anti–PD-1 antibody produced objective responses in approximately one in four to one in five patients with non–small-cell lung cancer, melanoma, or renal-cell cancer; the adverse-event profile does not appear to preclude its use. Preliminary data suggest a relationship between PD-L1 expression on tumor cells and objective response. (Funded by Bristol-Myers Squibb and others; ClinicalTrials.gov number, NCT00730639.)



Anti-PD-1 Antibody Therapy in Melanoma

operative field. Panel C shows a complete response in a 62-year-old patient with metastatic melanoma who received anti–PD-1 antibody at a dose of 3.0 mg per kilogram. Pretreatment computed tomographic scanning (i) revealed inguinal-lymph-node metastasis (arrowhead), which regressed completely after 13 months of treatment (ii). Numerous metastases in the subcutaneous tissue and retroperitoneum also regressed completely (not shown). Vitiligo, which developed after 6 months of treatment, is evident in photographs taken at 9 months under visible light (iii) and ultraviolet light (iv). Skin-biopsy specimens with immunohistochemical staining for micro-ophthalmia–associated transcription factor show that melanocytes (arrows) are abundant at the epidermal–dermal junction in normal skin (v), scarce in skin partially affected by vitiligo (vi), and absent in skin fully affected by vitiligo (vii). Panel D shows a par-



Using the Immune System to Treat Cancer

Adoptive immunotherapy for cancer: harnessing the T cell response

Nicholas P. Restifo, Mark E. Dudley and Steven A. Rosenberg

Abstract | Immunotherapy based on the adoptive transfer of naturally occurring or gene-engineered T cells can mediate tumour regression in patients with metastatic cancer. Here, we discuss progress in the use of adoptively transferred T cells, focusing on how they can mediate tumour cell eradication. Recent advances include more accurate targeting of antigens expressed by tumours and the associated vasculature, and the successful use of gene engineering to re-target T cells before their transfer into the patient. We also describe how new research has helped to identify the particular T cell subsets that can most effectively promote tumour eradication.

Cell Based Therapy: Adoptive Immunotherapy

Adoptive immunotherapy for cancer: harnessing the T cell response

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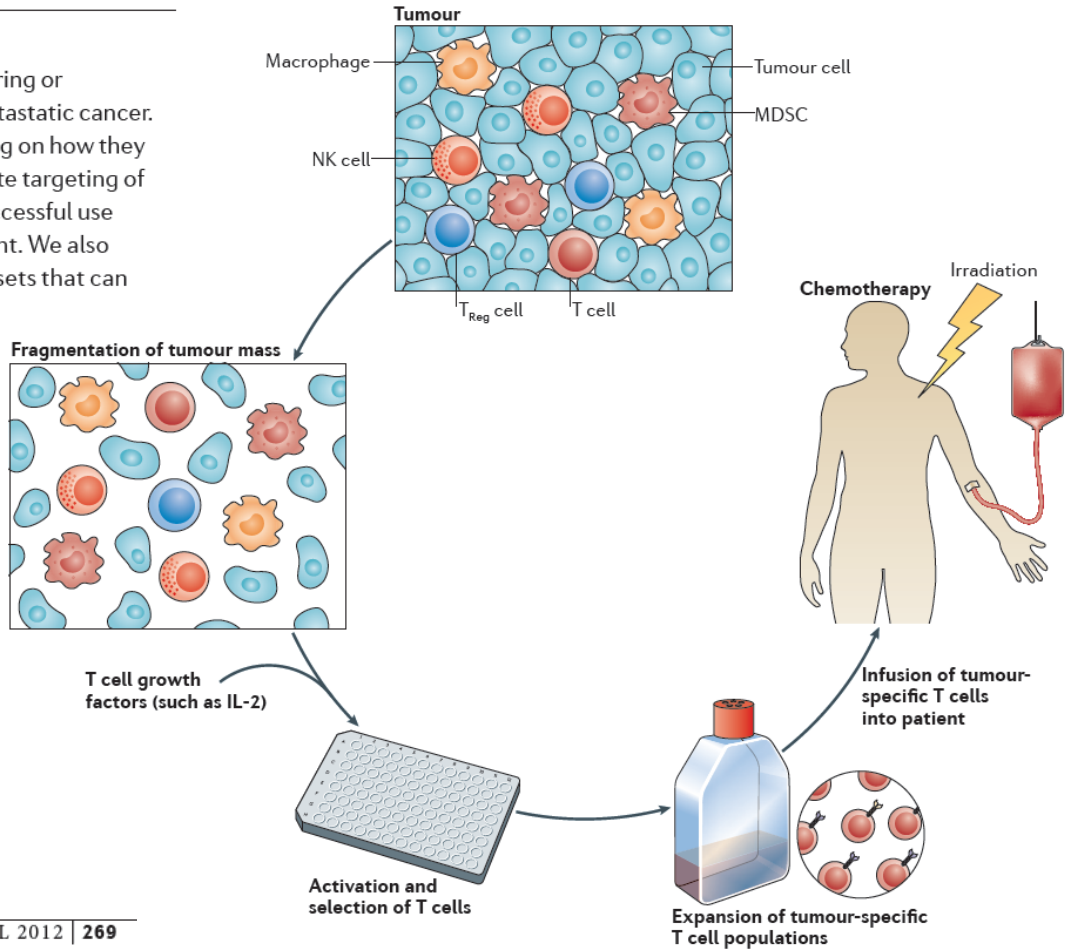
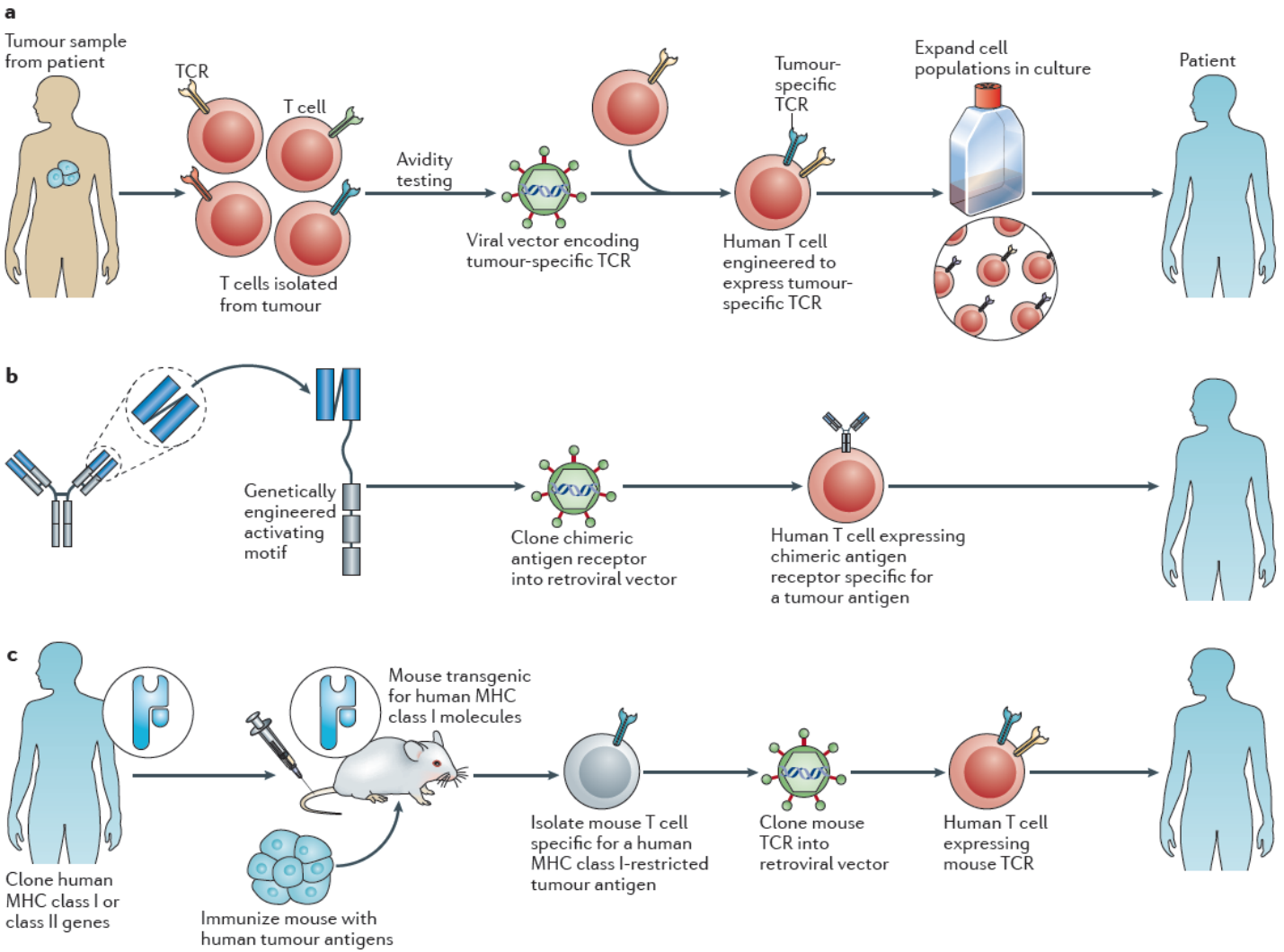


Restifo, Dudley, & Rosenberg, *Nat Rev Immunol* 2012

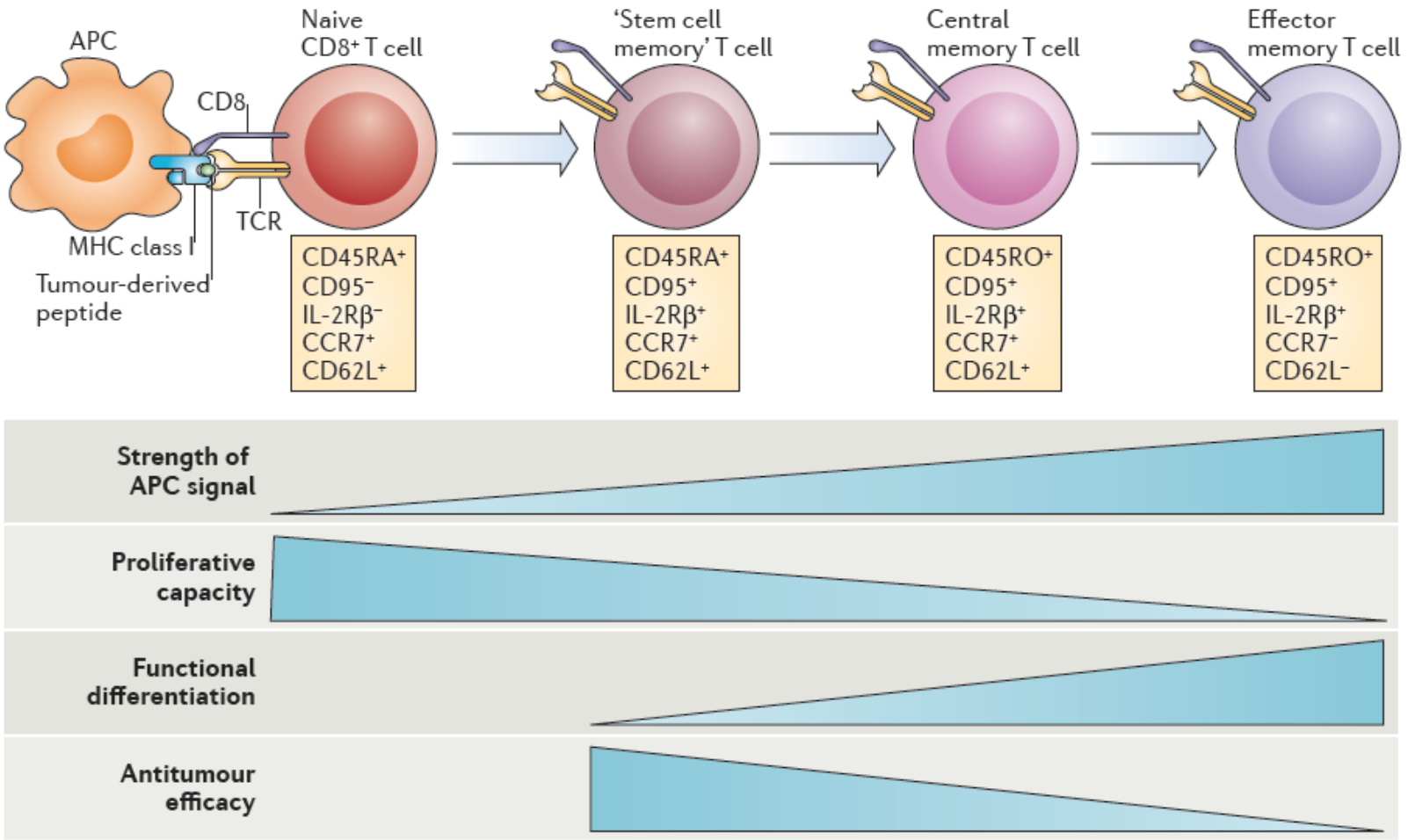
Strategies to Genetically Engineer T Cells



Cross-priming
The ability of certain antigen-presenting cells to load peptides that are derived from exogenous antigens onto MHC class I molecules. This property is atypical, because most cells exclusively present peptides from their endogenous proteins on MHC class I molecules.

Immunoediting
A process by which the immune system of a host may alter the gene expression of an emerging tumour, such that the most immunogenic epitopes are removed or 'edited', thereby facilitating tumour escape from immune recognition.

Challenges in Making Effective Anti-tumor T Cells



A Personalized Medicine Strategy

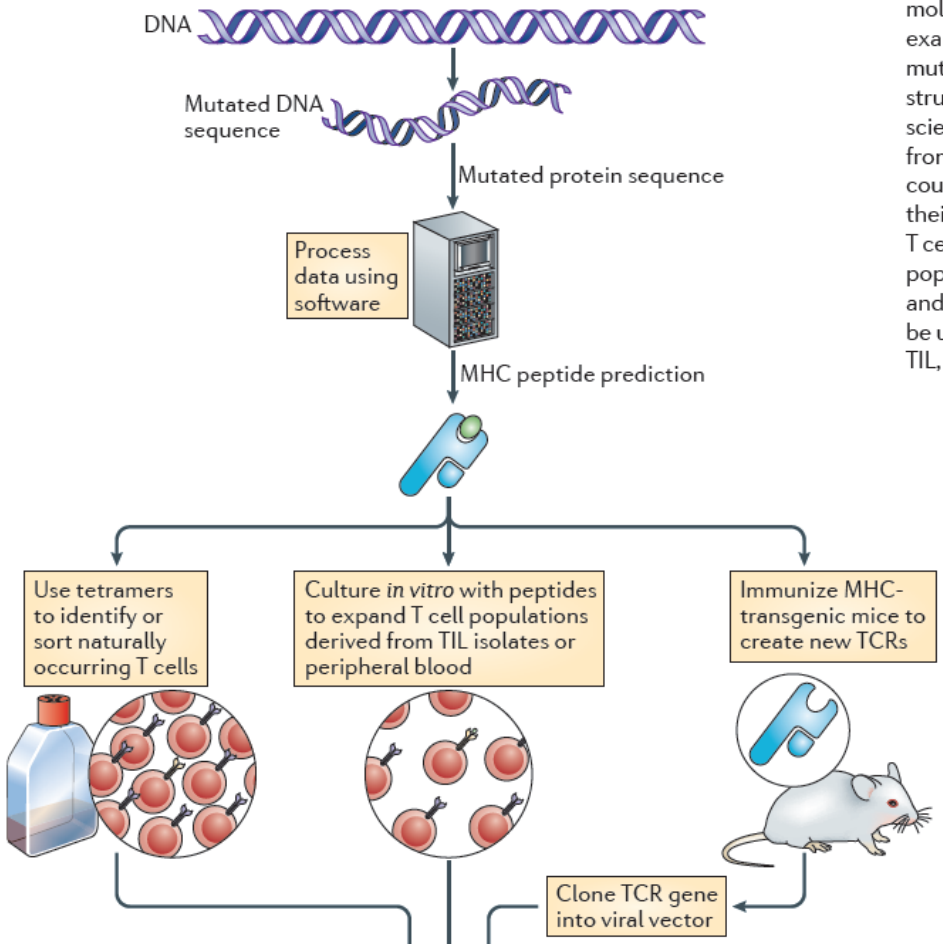
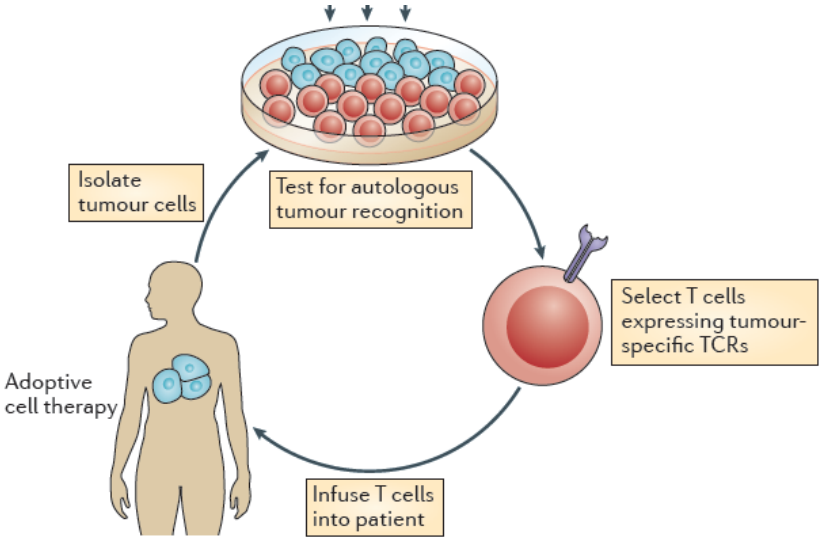


Figure 4 | **Highly personalized medicine.** Inexpensive and readily available DNA sequencing technology might revolutionize cancer immunotherapy, enabling a highly personalized approach to the identification of new tumour-associated antigens. The expressed genes from a patient's tumour can be sequenced to identify candidate mutant T cell epitopes. Relevant epitopes that could potentially bind to the MHC molecules of the patient could be predicted using peptide prediction algorithms (for example, see the HLA Peptide Binding Predictions website). If peptides derived from mutant proteins are found to be capable of forming new MHC-restricted target structures, the candidate peptides could be used in one of at least three ways. First, scientists can identify or sort cells that express relevant antigens (such as those derived from driver oncogenes) using tetramer-like reagents. Second, candidate peptides could be used to stimulate T cells that are already present in the patient's tumour or in their peripheral blood. Third, tumour antigens could be used to prime tumour-specific T cells in humanized mice that are transgenic for human MHC molecules. If the T cell populations generated are specific for the patient's tumour, they could be expanded and adoptively transferred if they are of human origin. Alternatively, mouse T cells can be used to identify suitable T cell receptors (TCRs) for gene-engineering approaches. TIL, tumour-infiltrating lymphocyte.



Restifo, Dudley, & Rosenberg, *Nat Rev Immunol* 2012

Targeting Cancer Cells and the Immune System

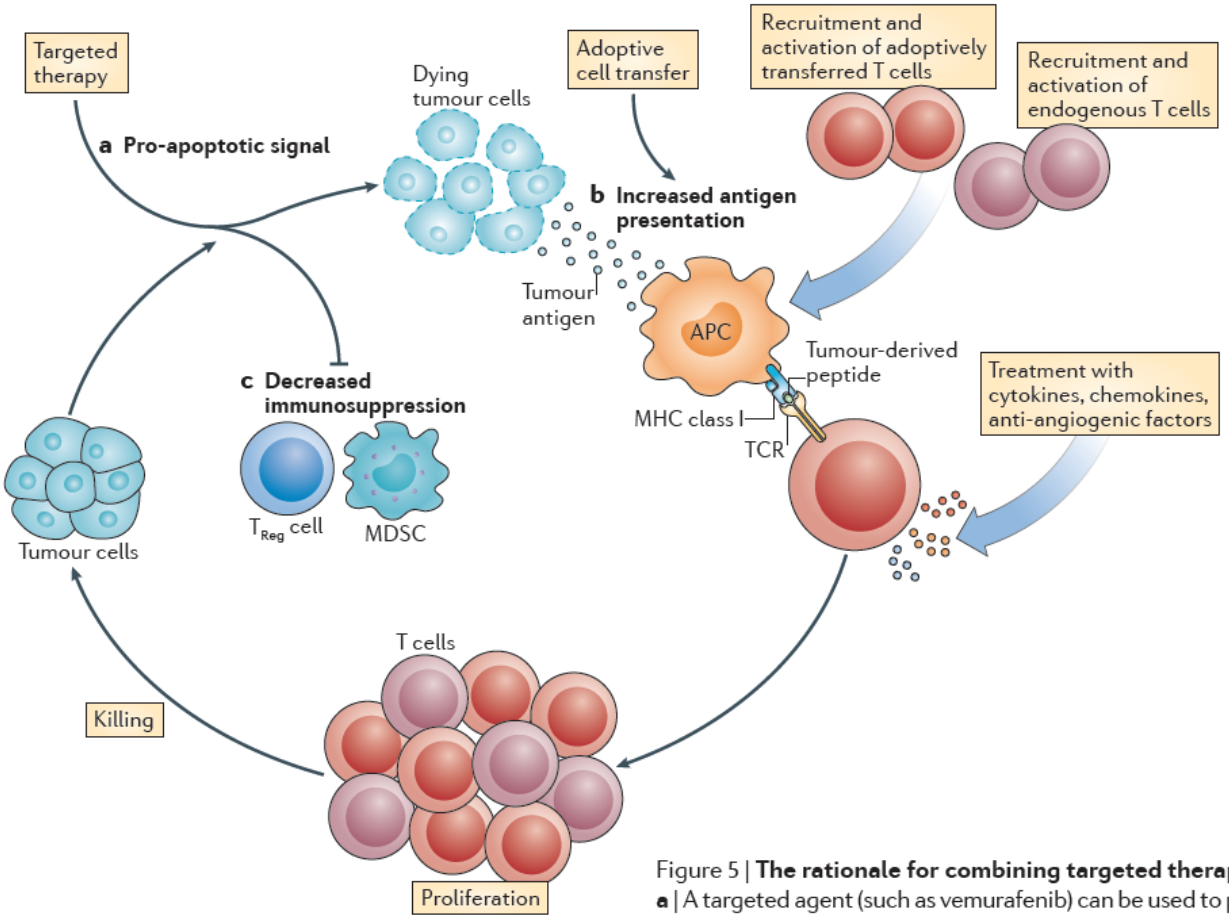


Figure 5 | **The rationale for combining targeted therapies with adoptive cell transfer-based immunotherapy.** **a** | A targeted agent (such as vemurafenib) can be used to promote apoptosis in tumour cells. **b** | Antigens released by dying tumour cells can then be acquired at an increased rate by antigen-presenting cells (APCs) that are present in the tissue or in local draining lymph nodes. These APCs process the tumour antigens and present tumour-derived peptides to T cells. This can lead to the priming of adoptively transferred tumour-specific T cells, as well as the activation of other endogenous tumour-specific T cell populations. Treatment with immunostimulatory cytokines and chemokines may increase the efficiency of tumour-specific T cell activation. **c** | Therapies that target immunosuppressive factors or cells present in the tumour microenvironment — such as regulatory T (T_{Reg}) cells and myeloid-derived suppressor cells (MDSCs) — may also promote increased activation of tumour-specific T cells. TCR, T cell receptor.

Cancer Vaccines

What is the difference between a tumor associated antigen and a tumor specific antigen?

IL-2 + Vaccination vs. gp100 Melanoma Antigen

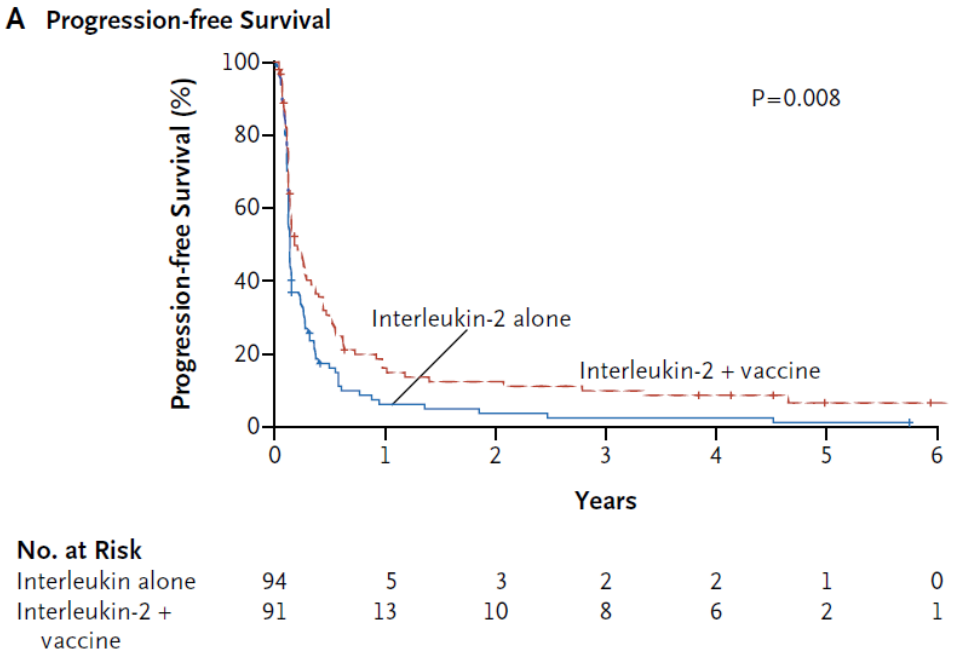
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

gp100 Peptide Vaccine and Interleukin-2 in Patients with Advanced Melanoma

Douglas J. Schwartzentruber, M.D., David H. Lawson, M.D., Jon M. Richards, M.D., Ph.D., Robert M. Conry, M.D., Donald M. Miller, M.D., Ph.D., Jonathan Treisman, M.D., Fawaz Gailani, M.D., Lee Riley, M.D., Ph.D., Kevin Conlon, M.D., Barbara Pockaj, M.D., Kari L. Kendra, M.D., Ph.D., Richard L. White, M.D., Rene Gonzalez, M.D., Timothy M. Kuzel, M.D., Brendan Curti, M.D., Phillip D. Leming, M.D., Eric D. Whitman, M.D., Jai Balkissoon, M.D., Douglas S. Reintgen, M.D., Howard Kaufman, M.D., Francesco M. Marincola, M.D., Maria J. Merino, M.D., Steven A. Rosenberg, M.D., Ph.D., Peter Choyke, M.D., Don Vena, B.S., and Patrick Hwu, M.D.

N ENGL J MED 364;22 NEJM.ORG JUNE 2, 2011



BACKGROUND

Stimulating an immune response against cancer with the use of vaccines remains a challenge. We hypothesized that combining a melanoma vaccine with interleukin-2, an immune activating agent, could improve outcomes. In a previous phase 2 study, patients with metastatic melanoma receiving high-dose interleukin-2 plus the gp100:209-217(210M) peptide vaccine had a higher rate of response than the rate that is expected among patients who are treated with interleukin-2 alone.

METHODS

We conducted a randomized, phase 3 trial involving 185 patients at 21 centers. Eligibility criteria included stage IV or locally advanced stage III cutaneous melanoma, expression of HLA*A0201, an absence of brain metastases, and suitability for high-dose interleukin-2 therapy. Patients were randomly assigned to receive interleukin-2 alone (720,000 IU per kilogram of body weight per dose) or gp100:209-217(210M) plus incomplete Freund's adjuvant (Montanide ISA-51) once per cycle, followed by interleukin-2. The primary end point was clinical response. Secondary end points included toxic effects and progression-free survival.

IL-2 + Vaccination vs. gp100 Melanoma Antigen

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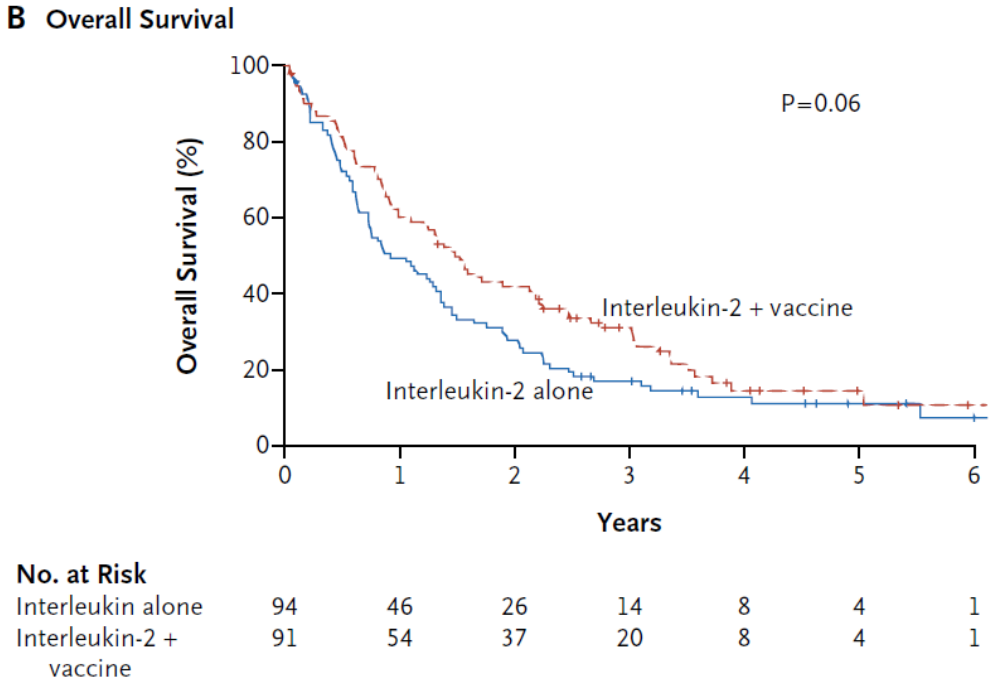

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Outline

Surveillance & immunoediting

Cancer immunotherapy

Hematological malignancies

Case study:
TLR9 KO lymphoma model

B Cell Lymphoma: Selection Failure

REVIEWS

MECHANISMS OF B-CELL LYMPHOMA PATHOGENESIS

Ralf Küppers

Abstract | Chromosomal translocations involving the immunoglobulin loci are a hallmark of many types of B-cell lymphoma. Other factors, however, also have important roles in the pathogenesis of B-cell malignancies. Most B-cell lymphomas depend on the expression of a B-cell receptor (BCR) for survival, and in several B-cell malignancies antigen activation of lymphoma cells through BCR signalling seems to be an important factor for lymphoma pathogenesis. Recent insights into the lymphomagenic role of factors supplied by the microenvironment also offer new therapeutic strategies.

Küppers, *Nat Rev Cancer* 2005

Mechanisms of Immunoglobulin Diversity

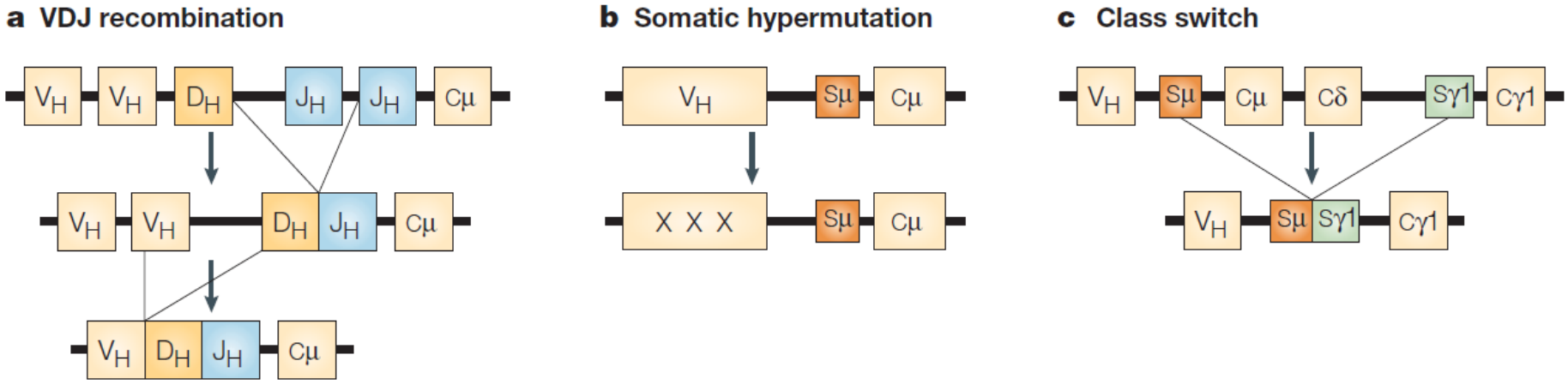
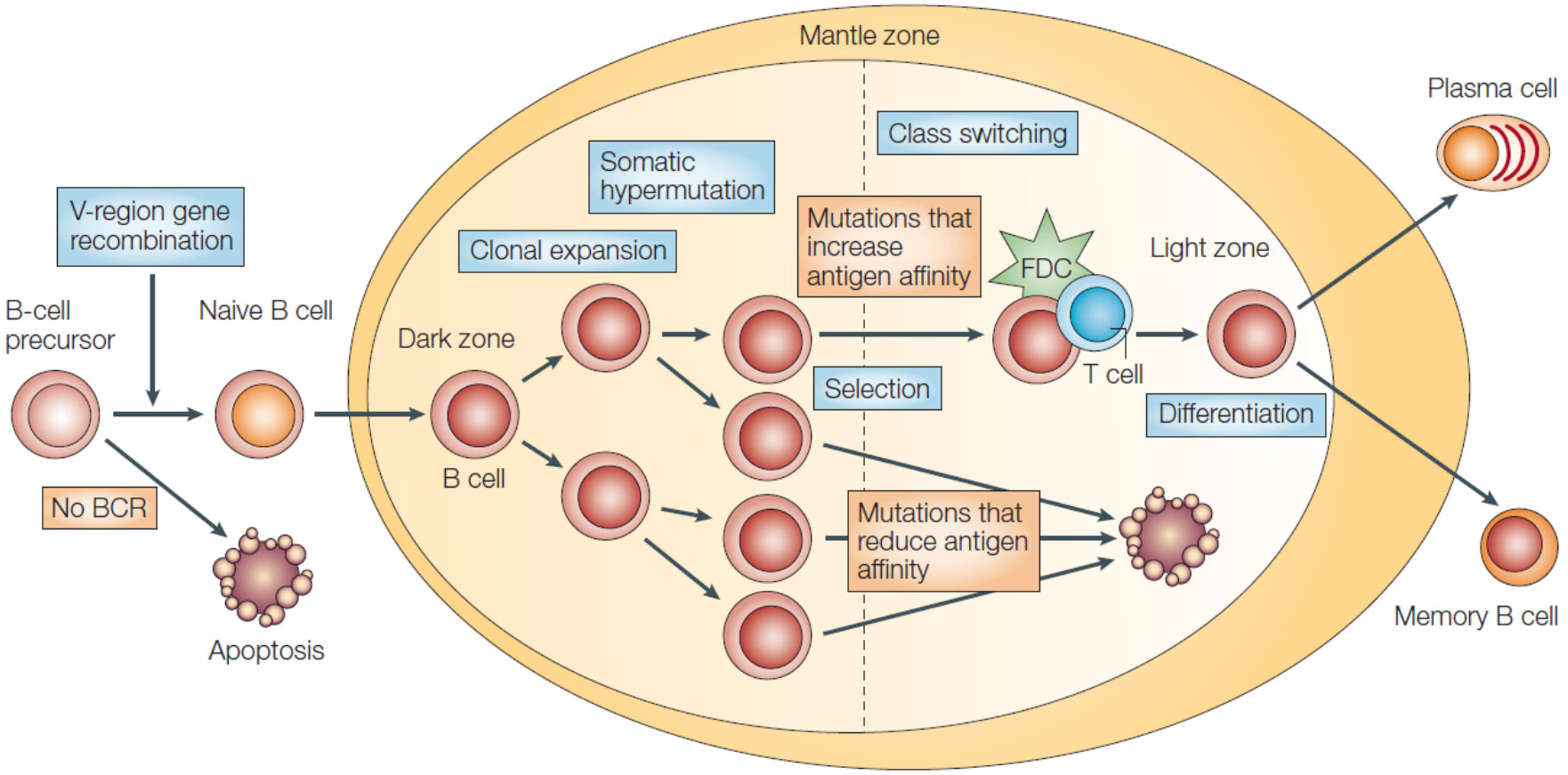


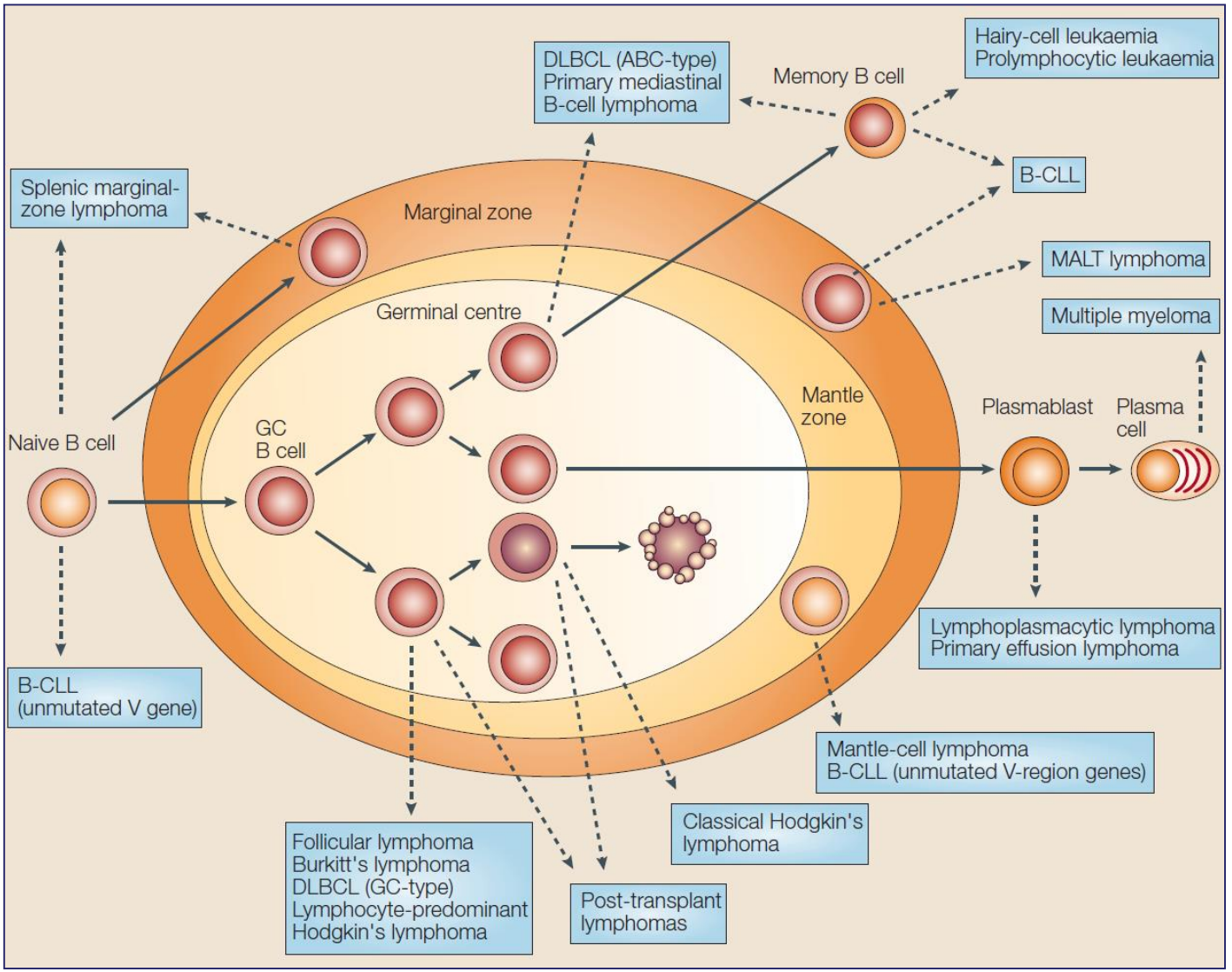
Figure 1 | **Molecular processes that remodel immunoglobulin genes.** Immunoglobulins (Igs) are expressed by B cells and consist of variable (V) regions, which interact with antigen, and constant (C) regions, which mediate the effector functions of Igs. To create a functional Ig, B cells must rearrange DNA segments that encode the heavy (H)- and light-chain (not shown) regions of the variable genes. **a** | First, through a process called 'V(D)J recombination', three gene segments, V_H, D_H and J_H, are joined to encode the H-chain variable region. The V regions of the κ- and λ-light chains, alternatively, are each encoded by two gene segments — the V_L and J_L genes (not shown). B-cell precursors first carry out D_H–J_H rearrangements in H-chain genes. These D_H–J_H rearrangements are followed by V_H–D_HJ_H rearrangements, resulting in the expression of a pre-B-cell receptor if the rearrangement is productive[3]. About 50 functional V_H gene segments, 27 D_H segments and 6 J_H segments are available in the germline, allowing the generation of a diverse repertoire of V_H gene rearrangements. The diversity is further increased by the addition or removal of nucleotides at the joining sites of the gene segments[3]. The cells then carry out rearrangements at their L-chain loci (not shown). The V-region of the Ig gene is ultimately connected to the C-region of the Ig gene (Cμ of IgM in diagram) **b** | The process of somatic hypermutation is activated when B cells reach the germinal centre (GC, shown in more details in FIG. 2). This process leads to the introduction of point mutations, deletions or duplications in the rearranged V-region of Ig genes (denoted by 'Xs' in the figure)[102]. These mutations occur in the V-region of Ig genes — not in the downstream Cμ region. **c** | Class switching results in the replacement of the originally expressed H-chain C-region gene with that of another Ig gene. In the diagram, the C-region for IgM (Cμ) and IgD (Cδ) are exchanged for the C-region of IgG (Cγ1) by recombination at the switch regions for these genes (Sμ and Sγ1, respectively). This results in an antibody with different effector functions but the same antigen-binding domain.

Küppers, *Nat Rev Cancer* 2005

Healthy B cell Development



Cellular Origin of B Cell Malignancies



Küppers, *Nat Rev Cancer* 2005

Hallmark IgH + Oncogene Translocations

Table 2 | **Mechanisms of B-cell lymphoma pathogenesis**

Lymphoma	Chromosomal translocations	Tumour-suppressor gene mutations	Viruses	Other alterations
Mantle-cell lymphoma	*CCND1–IgH* (95)[107]	*ATM* (40)[108,109]		Deletion on 13q14 (50–70)[110*]
B-cell chronic lymphocytic leukaemia	–	*ATM* (30)[111,112], *TP53* (15)[113]	–	Deletion on 13q14 (60)[114*]
Follicular lymphoma	*BCL2–IgH* (90)[12–14]	–	–	–
Diffuse large B-cell lymphoma	*BCL6*–various (35)[115,116], *BCL2–IgH* (15-30)[117], *MYC–IgH* or *MYC–IgL* (15)[118]	*CD95* (10–20)[25], *ATM* (15)[119], *TP53* (25)[120,121]	–	Aberrant hypermutation of multiple proto-oncogenes (50)[18]
Primary mediastinal B-cell lymphoma	–	*SOCS1* (40)[122]	–	Aberrant hypermutation of multiple proto-oncogenes (70)[123]
Burkitt's lymphoma	*MYC–IgH* or *MYC–IgL* (100)[124,125]	*TP53* (40)[113], *RB2* (20–80)[126]	EBV (endemic, 95; sporadic, 30)[28]	–
Post-transplant lymphomas	–	–	EBV (90)[28]	–
Classical Hodgkin's lymphoma	–	*IKBA* (10-20)[127–129], *IKBE* (10)[130], *CD95* (<10)[131]	EBV (40)[28]	*REL* amplifications (50)[132]
Lymphocyte-predominant Hodgkin's lymphoma	*BCL6*–various (48)[133]	–	–	–
Splenic marginal-zone lymphoma	–	–	–	Deletion on 7q22-36 (40)[134*]
MALT lymphoma	*API2–MALT1* (30)[135], *BCL10–IgH* (5)[136,137], *MALT1–IgH* (15-20)[138], *FOXP1–IgH* (10)[139]	*CD95* (5-80)[25,140,141‡]	Indirect role of *Helicobacter pylori* in gastric MALT lymphomas[85]	–
Lymphoplasmacytoid lymphoma	*PAX5–IgH* (50)[142]	–	–	–
Primary effusion lymphoma	–	–	HHV8 (95)[143], EBV (70)[28]	–
Multiple myeloma	*CCND1–IgH* (15-20)[144], *FGFR3–IgH* (10)[145], *MAF–IgH* (5-10)[146]	*CD95* (10)[147]	–	Various MYC alterations (40)[148], *RAS* mutations (40)[149], deletion on 13q14 (50)[150*]

Küppers, *Nat Rev Cancer* 2005

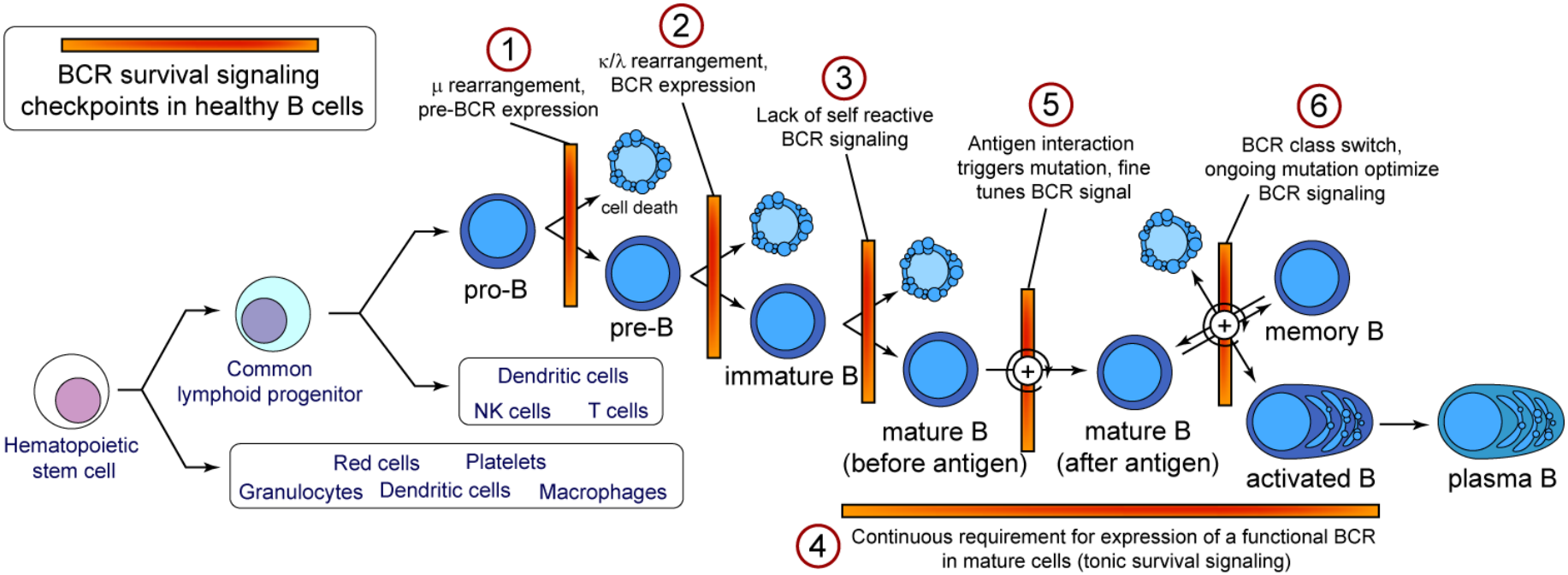
BCR as an 'Oncogene'

Lymphomas associated with BCR expression and indication for antigen activation

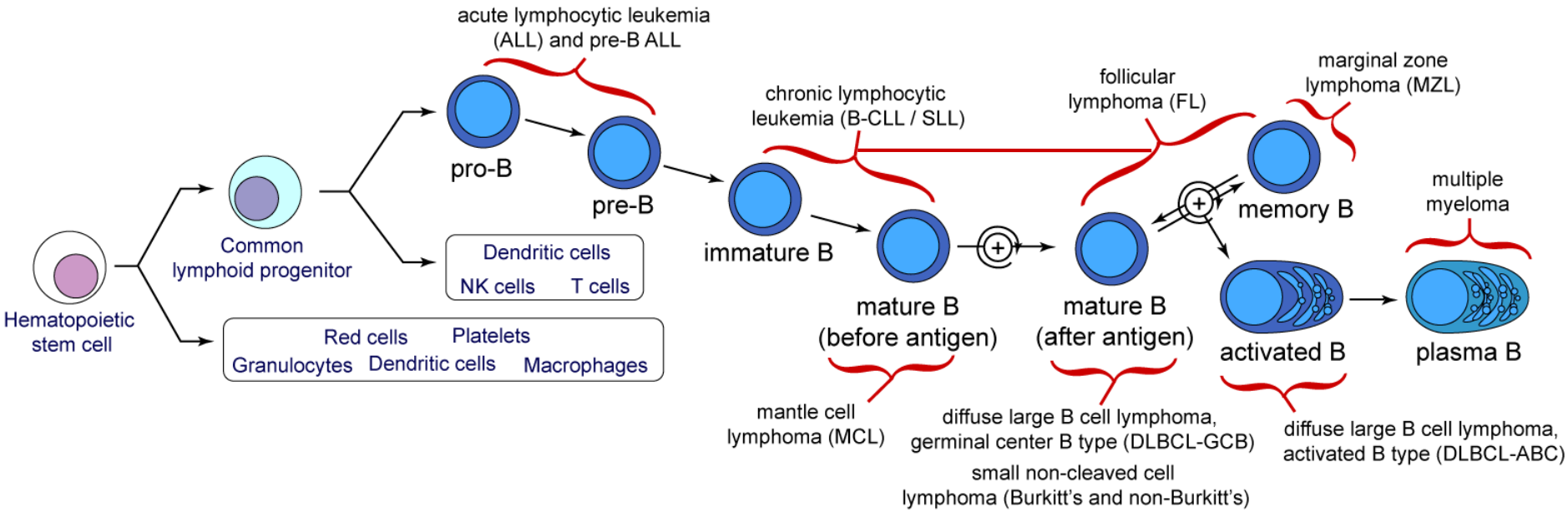
- Follicular lymphomas arise and grow in the germinal centre and in some patient samples the BCR is autoreactive. The BCR variable domain contains mutations that promote carbohydrate modification.
- Gastric mucosa-associated lymphoid tissue lymphomas are in many cases associated with autoreactive BCR, particularly with rheumatoid factors.
- B-cell chronic lymphocytic leukaemia has a restricted variable (V)-region gene repertoire and the BCR is often autoreactive. A BCR specific to human T-cell lymphotropic virus 1 has been identified in patients who are infected with this virus.
- In hepatitis C virus (HCV)-associated lymphomas, HCV-specificity of BCR has been reported in some cases. Disease regression occurs after antiviral therapy.
- In primary central nervous system lymphomas, about half the cases express the same heavy-chain (V_H) gene segment (VH4-34), whereas other genes of the BCR are diverse, indicating tumour-cell stimulation by superantigen binding to the BCR.

Küppers, *Nat Rev Cancer* 2005

BCR Signaling Checkpoints in Healthy Development

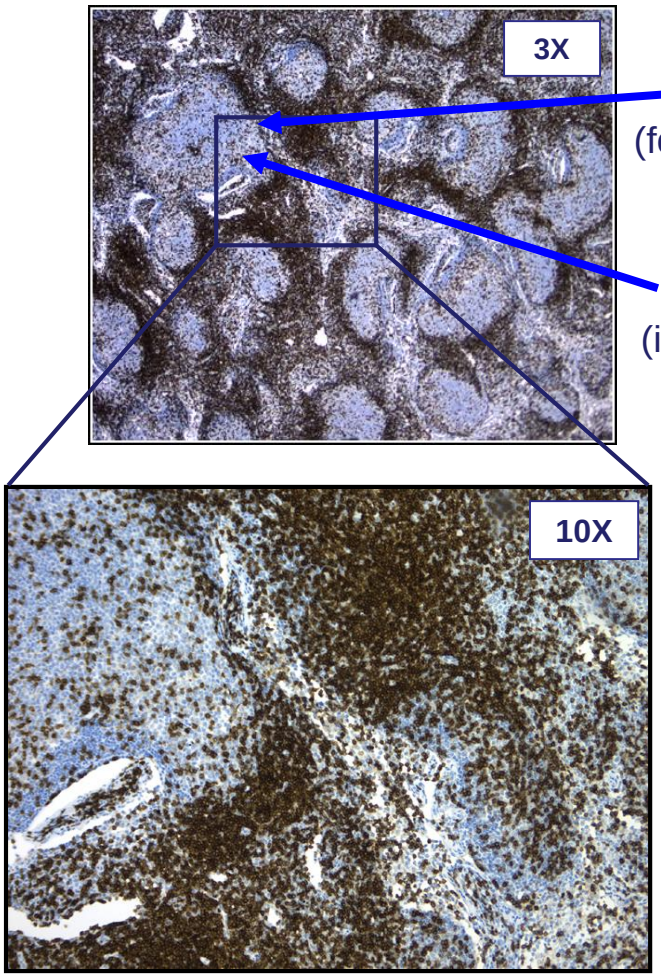


Failure to Control Ig Diversity Mechanisms



Follicular Lymphoma Tumors

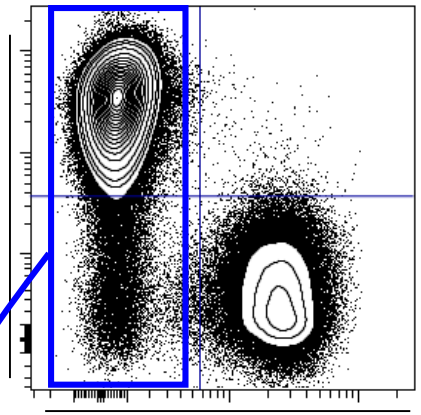
Follicular lymphoma histology
black stain = T Cells (CD3)



B Cells
(follicular structures)

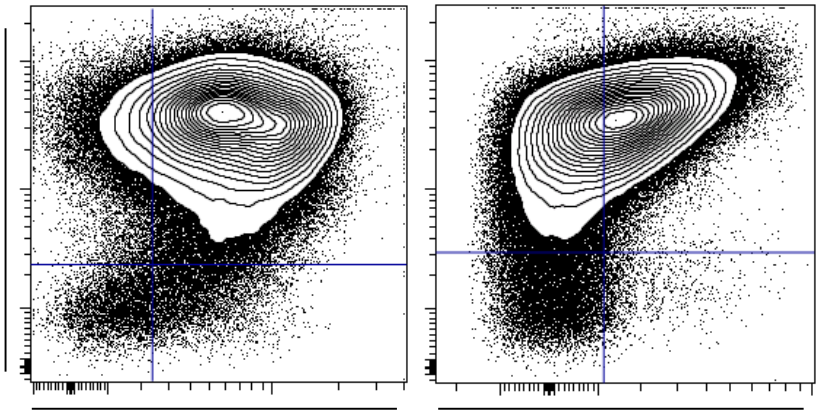
T Cells
(infiltrating tumor)

Follicular lymphoma, flow cytometry



Lymphoma
B cell receptor
Ig light chain (κ)

Tumor-infiltrating T cells (CD3)



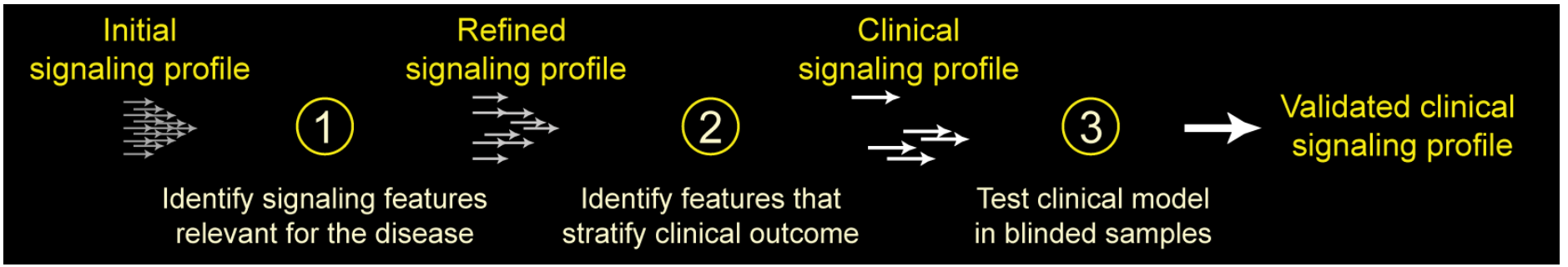
Lymphoma
B cell receptor
Ig light chain (κ)

BCL2

CD20

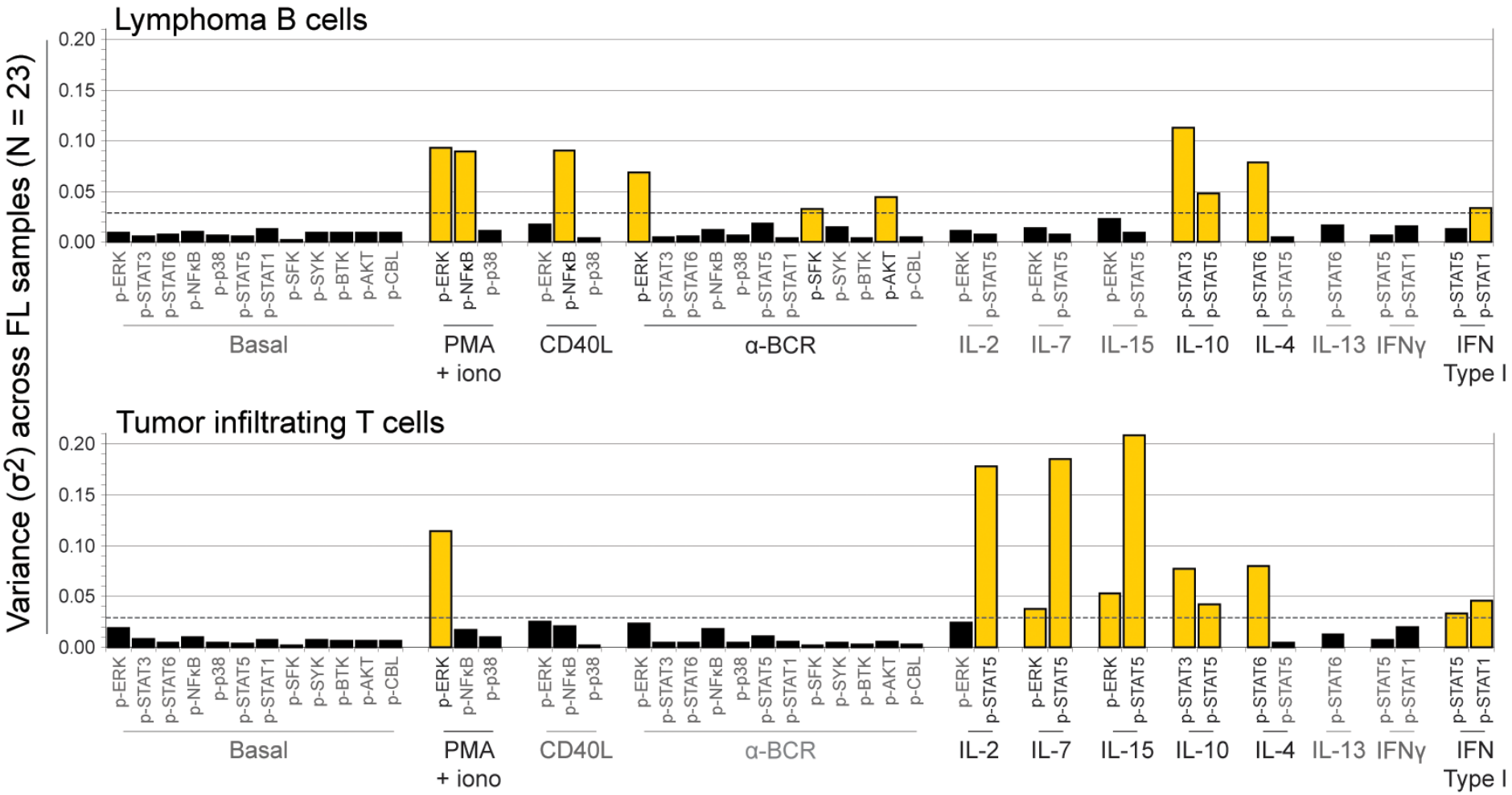
Irish et al., *PNAS* 2010

Validating a Cancer (Signaling) Profile



Irish et al., *PNAS* 2010

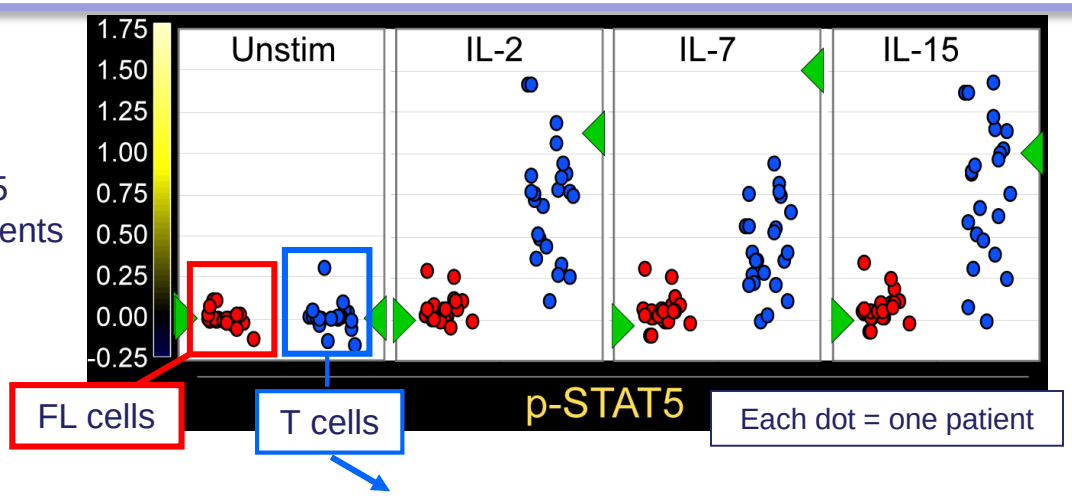
Key Signaling Events in Follicular Lymphoma



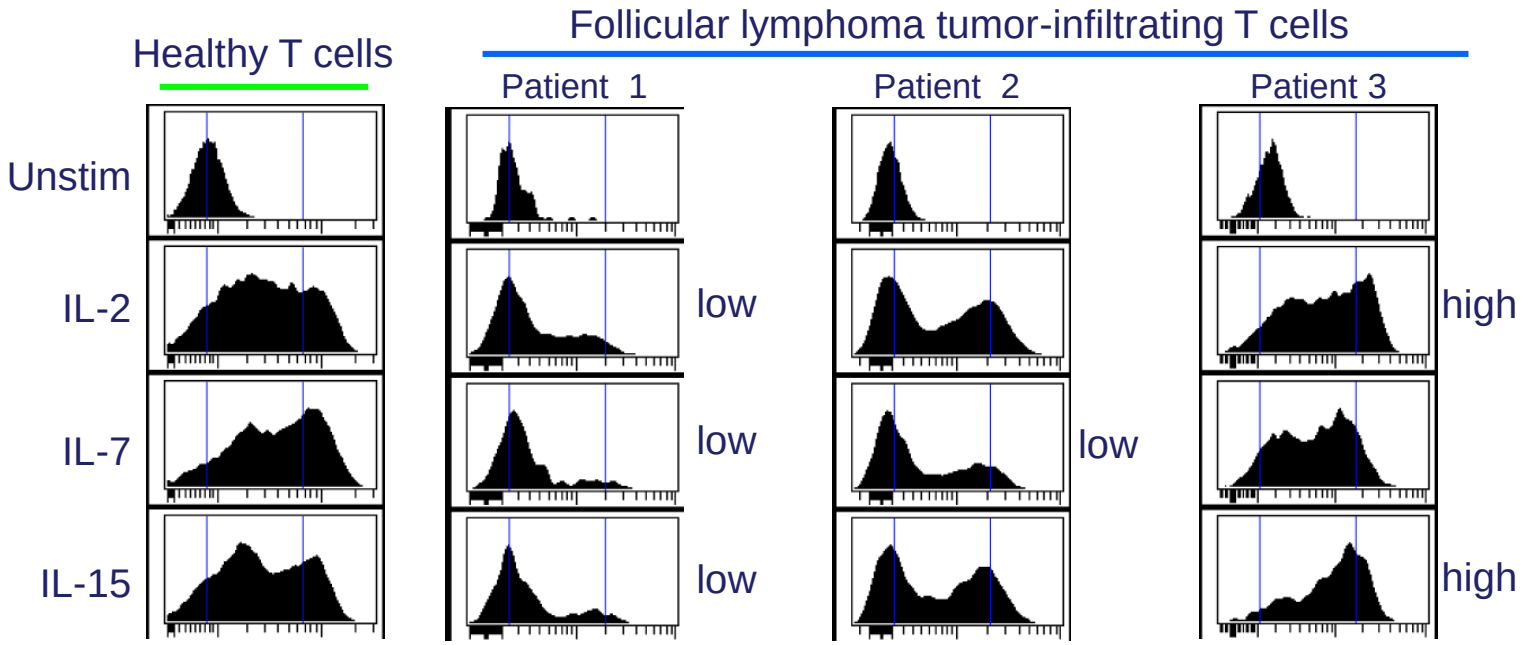
Irish et al., *PNAS* 2010

Abnormal Tumor Infiltrating T cell Signaling in FL

IL-2, IL-7, and IL-15 signaling for 23 FL patients



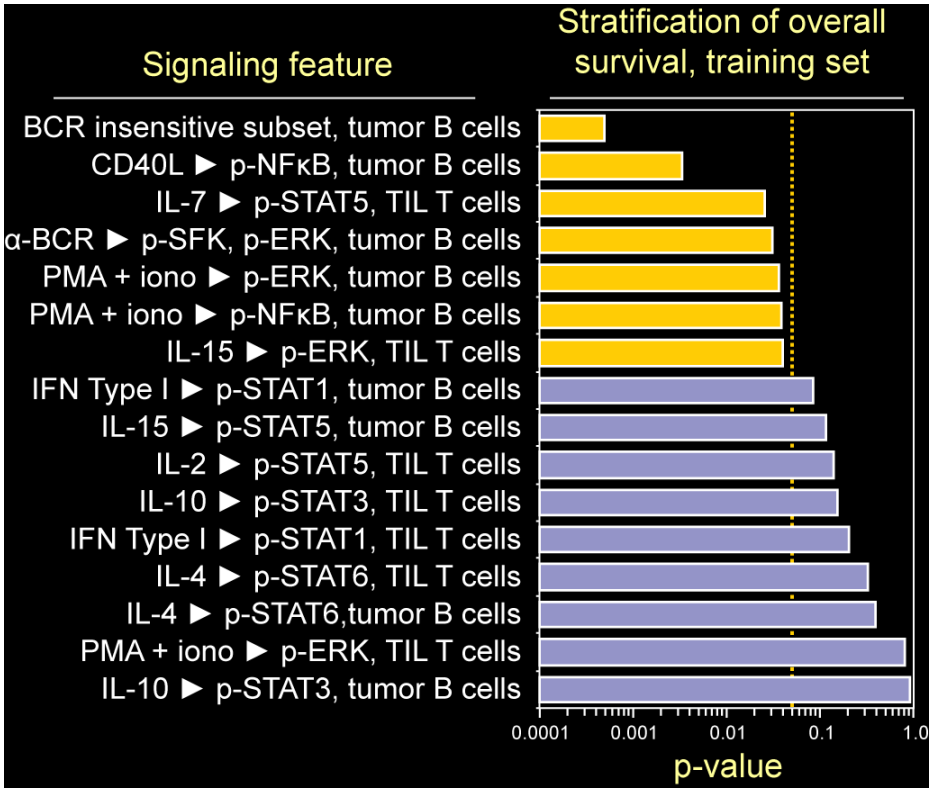
Green arrows indicate healthy PBMC average (N=6)

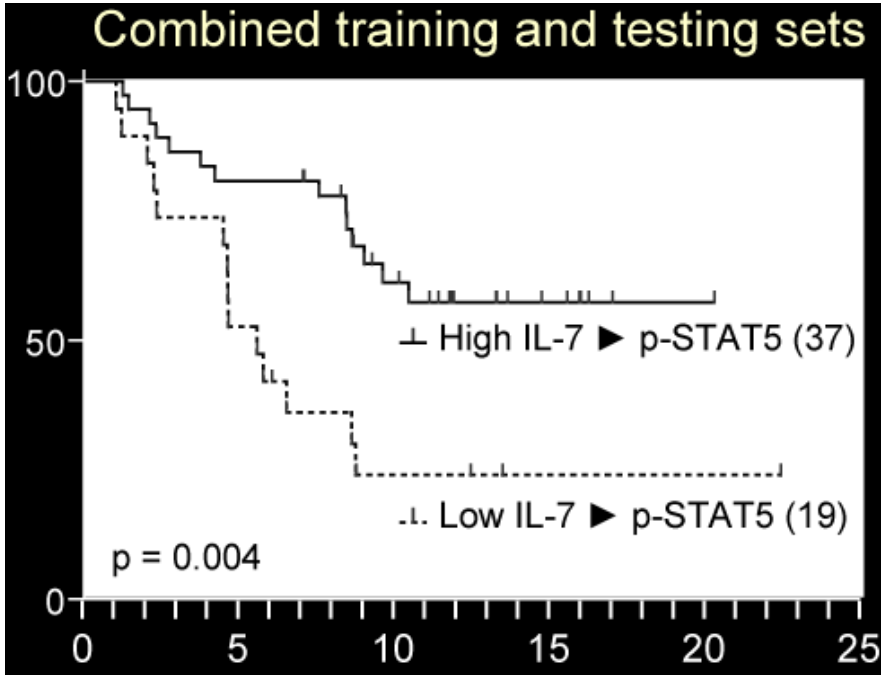


STAT5 phosphorylation

Irish et al., *PNAS* 2010

TIL T Cell Signaling Stratifies Overall Survival in FL





Irish et al., *PNAS* 2010

Outline

Surveillance & immunoediting

Cancer immunotherapy

Hematological malignancies

Case study:
TLR9 KO lymphoma model

Case Study: TLR9 KO Lymphoma Model

Your PhD project focuses on understanding the role of innate immune signaling in macrophages and dendritic cells in mouse models of lymphoma.

You have a mouse that is knock out for toll like receptor 9 (TLR9). TLR9 is expressed in antigen presenting cells (APCs) and increases innate immune function through NFkB and other signaling pathways that increase expression of surface molecules, such as CD80, CD86, and CD40.

Your PI suggests a series of experiments where you will study whether CpG synergizes with chemotherapy in a model of B cell lymphoma. In this model you will subcutaneously inject a clonal lymphoma B cell line into an immune competent mouse. Your PI would like you to treat with chemotherapy and CpG in TLR9 wild type and knockout mice.

- What tumor immunity issues exist in this therapy model?
- What would be good controls?
- Should you generate any more reagents or tools?

Case Study: TLR9 KO Lymphoma Model

1. Treatment of established tumors vs. rejection of tumor engraftment. Establish the tumor before treatment so that you are not just testing the ability of the immune system to reject a tumor, which isn't useful for human therapy.

2. Make sure there is "room to improve" on the gold standard. Treat with chemo +/- CpG and make sure that chemo alone does not cure the tumor.

3. Artificial immunity vs. the cell line. A TLR9 knockout host may resist a TLR9 expressing tumor since this is a foreign antigen. It would be useful to generate a TLR9 knockout tumor cell line so that mice +/- TLR9 will not different in their natural response to the tumor.

4. Mechanism of action may not be clear. In this case the tumor and host both can respond to the therapy (TLR9 is expressed in APCs, which include B cells, dendritic cells, and monocytes). This can be an opportunity as well.

5. Systemic vs. local immune response. Create two tumors, one on each flank. Treat one tumor locally with chemo + CpG and then assess the response at the flanking tumor. Systemic immunity will clear the flanking tumor.