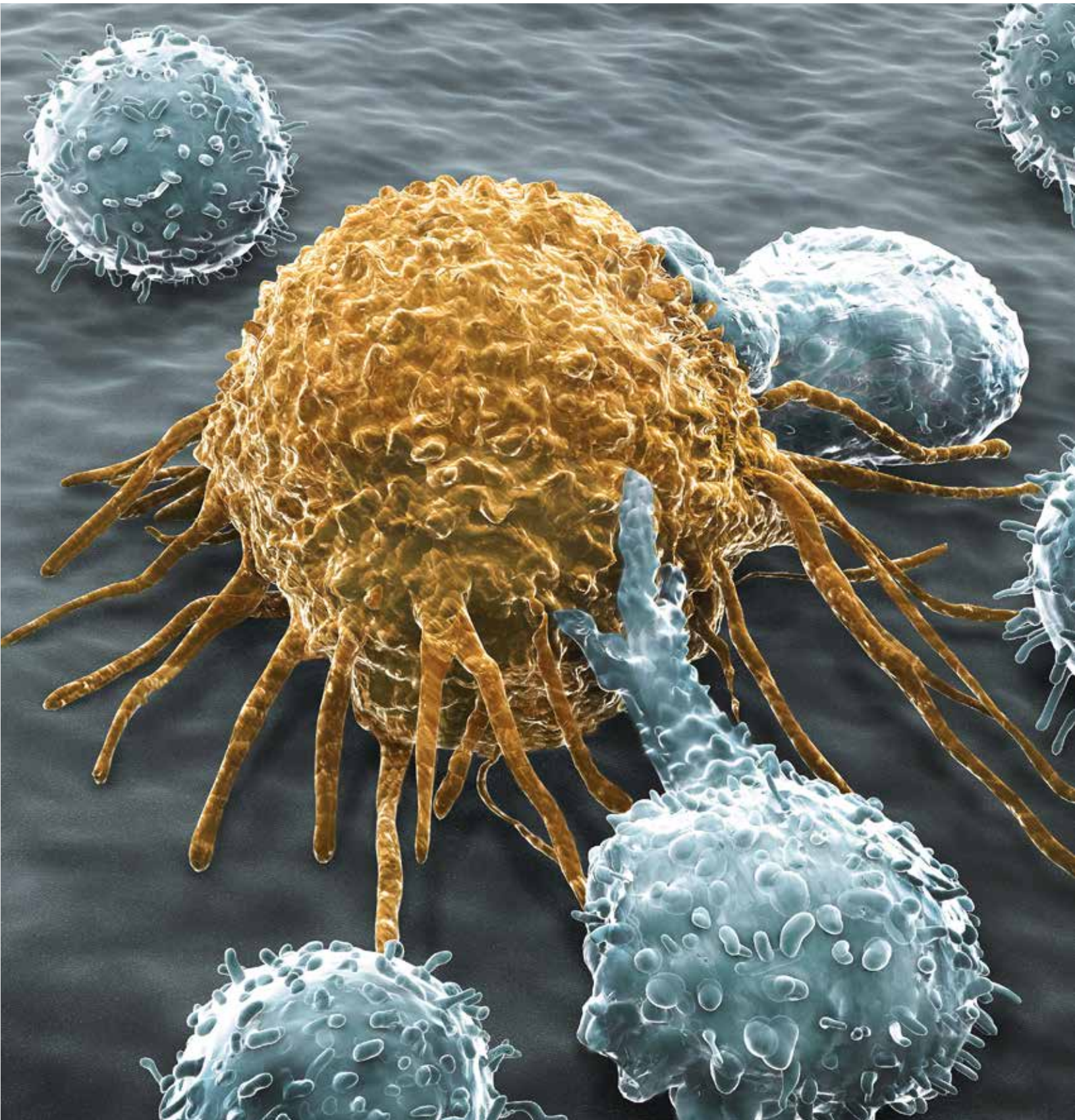
Cancer and the immune system

An overview of recent publications featuring Illumina® technology



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This document highlights recent publications that demonstrate the use of Illumina technologies in immunology research. To learn more about the platforms and assays cited, visit www.illumina.com.

INTRODUCTION

The outstanding results achieved with immune checkpoint inhibitors against melanoma[1] are the culmination of decades of basic research in mechanisms and control of the immune system. This research has created an armamentarium of tools, including mouse models that can accurately replicate checkpoint inhibitor responses, to address the challenge of cancer treatment.[2] Ironically, checkpoint inhibitors such as ipilimumab[3] and nivolumab[4] are themselves monoclonal antibodies. While the current treatments are focused on melanoma and do not benefit all patients equally, the solid foundation of scientific knowledge is allowing a relatively rapid and logical progression in the use of combinatorial and targeted therapies.[5]

High-throughput sequencing has shown remarkable utility in cancer and immunology research, as well as in the development of individualized immunotherapy. For example, high-throughput sequencing has dramatically improved our knowledge of the cancer genome and the intracellular mechanisms involved in tumor progression. In addition, careful analysis of the cancer genome can also reveal new epitopes that could be targeted by the immune system.[6] Sequencing can also be used to determine the immune repertoire as a real-time, highly sensitive monitor of clonal expansion and contraction of the cell populations in response to tumor growth or treatment.[7, 8, 9]

Reviews

Robinson W. H. (2015) Sequencing the functional antibody repertoire--diagnostic and therapeutic discovery. Nat Rev Rheumatol 11: 171-182

Chaudhary B., Abd Al Samid M., al-Ramadi B. K. and Elkord E. (2014) Phenotypic alterations, clinical impact and therapeutic potential of regulatory T cells in cancer. Expert Opin Biol Ther 14: 931-945

Giraldo N. A., Becht E., Remark R., Damotte D., Sautes-Fridman C., et al. (2014) The immune contexture of primary and metastatic human tumours. Curr Opin Immunol 27: 8-15

Linnemann C., Mezzadra R. and Schumacher T. N. (2014) TCR repertoires of intratumoral T-cell subsets. Immunol Rev 257: 72-82

Perez-Gracia J. L., Labiano S., Rodriguez-Ruiz M. E., Sanmamed M. F. and Melero I. (2014) Orchestrating immune check-point blockade for cancer immunotherapy in combinations. Curr Opin Immunol 27: 89-97

Rosenberg S. A. (2014) Finding suitable targets is the major obstacle to cancer gene therapy. Cancer Gene Ther 21: 45-47

1. Chapman P. B., D'Angelo S. P. and Wolchok J. D. (2015) Rapid eradication of a bulky melanoma mass with one dose of immunotherapy. N Engl J Med 372: 2073-2074
2. Spranger S., Bao R. and Gajewski T. F. (2015) Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 523: 231-235
3. Ribas A. (2012) Tumor immunotherapy directed at PD-1. N Engl J Med 366: 2517-2519
4. Topalian S. L., Hodi F. S., Brahmer J. R., Gettinger S. N., Smith D. C., et al. (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366: 2443-2454
5. Leavy O. (2015) Tumour immunology: A triple blow for cancer. Nat Rev Immunol 15: 265
6. Kreiter S., Vormehr M., van de Roemer N., Diken M., Lower M., et al. (2015) Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature 520: 692-696
7. Robinson W. H. (2015) Sequencing the functional antibody repertoire--diagnostic and therapeutic discovery. Nat Rev Rheumatol 11: 171-182
8. Ribas A. and Wolchok J. D. (2013) Combining cancer immunotherapy and targeted therapy. Curr Opin Immunol 25: 291-296
9. Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens. Curr Opin Immunol 25: 284-290
10. Pardoll D. M. (2012) The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12: 252-264
11. Chapman P. B., D'Angelo S. P. and Wolchok J. D. (2015) Rapid eradication of a bulky melanoma mass with one dose of immunotherapy. N Engl J Med 372: 2073-2074
12. Postow M. A., Chesney J., Pavlick A. C., Robert C., Grossmann K., et al. (2015) Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med 372: 2006-2017

IMMUNE CHECKPOINT INHIBITORS

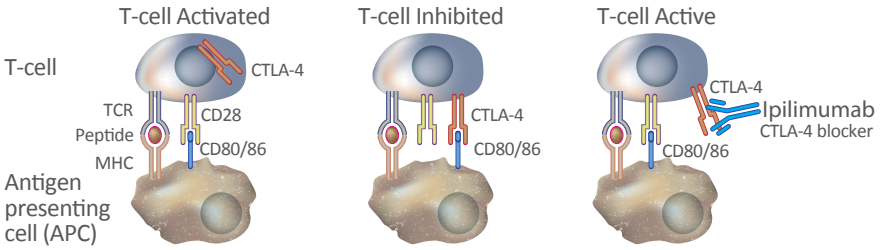
The use of effective strategies to block immune-inhibitory receptors is revolutionizing cancer therapy.[10] The use of monoclonal antibodies against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), programmed cell death 1 (PD-1), and programmed cell death 1 ligand (PD-L1) to inhibit the regulatory immune response has produced remarkable and durable responses.[11, 12, 13, 14] However, factors determining whether a patient will respond remain elusive.[15, 16] Several potential drug targets, such as the phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway[17, 18] and bromodomain-containing protein 4 (BRD4),[19] have also been explored to improve responses (Table 1).

> "It is ironic that we are now concerned about the possibility of overly vigorous antimelanoma responses" Chapman[20]

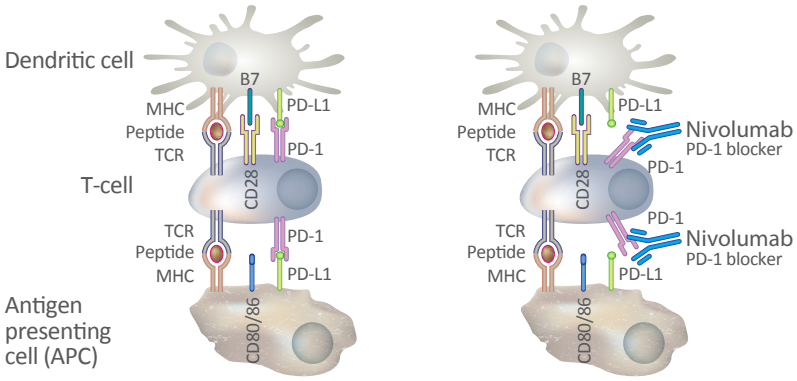
Table 1: Immune Checkpoint Inhibitors and Targets.

Name	Trade Name	Target	Sponsor	Reference
dabrafenib	Tafinlar	BRAF V600E mutation	GlaxoSmithKline (GSK)	21
vemurafenib	Zelboraf	BRAF V600E mutation	Daiichi-Sankyo	22
ipilimumab	Yervoy	CTLA-4	Bristol-Myers Squibb	23
tremelimumab	(not established)	CTLA-4	Pfizer	24
pembrolizumab	Keytruda	PD-1	Merck & Co	25
nivolumab	Opdivo	PD-1	Bristol-Myers Squibb	26

13. Topalian S. L., Hodi F. S., Brahmer J. R., Gettinger S. N., Smith D. C., et al. (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366: 2443-2454
14. Hodi F. S., O'Day S. J., McDermott D. F., Weber R. W., Sosman J. A., et al. (2010) Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363: 711-723
15. Delyon J., Mateus C., Lefeuvre D., Lanoy E., Zitvogel L., et al. (2013) Experience in daily practice with ipilimumab for the treatment of patients with metastatic melanoma: an early increase in lymphocyte and eosinophil counts is associated with improved survival. Ann Oncol 24: 1697-1703
16. Wolchok J. D., Kluger H., Callahan M. K., Postow M. A., Rizvi N. A., et al. (2013) Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 369: 122-133
17. Chen G., Chakravarti N., Aardalen K., Lazar A. J., Tetzlaff M. T., et al. (2014) Molecular profiling of patient-matched brain and extracranial melanoma metastases implicates the PI3K pathway as a therapeutic target. Clin Cancer Res 20: 5537-5546
18. Kim K., Skora A. D., Li Z., Liu Q., Tam A. J., et al. (2014) Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. Proc Natl Acad Sci U S A 111: 11774-11779
19. Segura M. F., Fontanals-Cirera B., Gaziel-Sovran A., Guijarro M. V., Hanniford D., et al. (2013) BRD4 sustains melanoma proliferation and represents a new target for epigenetic therapy. Cancer Res 73: 6264-6276
20. Chapman P. B., D'Angelo S. P. and Wolchok J. D. (2015) Rapid eradication of a bulky melanoma mass with one dose of immunotherapy. N Engl J Med 372: 2073-2074
21. Gibney G. T. and Zager J. S. (2013) Clinical development of dabrafenib in BRAF mutant melanoma and other malignancies. Expert Opin Drug Metab Toxicol 9: 893-899
22. Bollag G., Hirth P., Tsai J., Zhang J., Ibrahim P. N., et al. (2010) Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Nature 467: 596-599
23. Ribas A. (2012) Tumor immunotherapy directed at PD-1. N Engl J Med 366: 2517-2519
24. Reuben J. M., Lee B. N., Li C., Gomez-Navarro J., Bozon V. A., et al. (2006) Biologic and immunomodulatory events after CTLA-4 blockade with ticilimumab in patients with advanced malignant melanoma. Cancer 106: 2437-2444
25. Hamid O., Robert C., Daud A., Hodi F. S., Hwu W. J., et al. (2013) Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 369: 134-144
26. Topalian S. L., Hodi F. S., Brahmer J. R., Gettinger S. N., Smith D. C., et al. (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366: 2443-2454



Ipilimumab mechanism of action. Ipilimumab is a monoclonal antibody that binds to CTLA-4 and blocks the interaction of CTLA-4 with its ligands, CD80/CD86. Blockade of CTLA-4 augments T-cell activation and proliferation, including the activation and proliferation of tumor-infiltrating T-effector cells. (www.hcp.yervoy.com)



Nivolumab mechanism of action. Recognition of the tumor by the T cell through major histocompatibility complex (MHC)/antigen binding upregulates PD-L1 on the tumor cell surface. The PD-1/PD-L1 interaction inhibits T-cell–mediated tumor killing. Blockade of the PD-1/PD-L1 interaction reactivates T-cell–mediated tumor killing.

Reviews

Leavy O. (2015) Tumour immunology: A triple blow for cancer. Nat Rev Immunol 15: 265

Chaudhary B., Abd Al Samid M., al-Ramadi B. K. and Elkord E. (2014) Phenotypic alterations, clinical impact and therapeutic potential of regulatory T cells in cancer. Expert Opin Biol Ther 14: 931-945

Gubin M. M., Zhang X., Schuster H., Caron E., Ward J. P., et al. (2014) Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature 515: 577-581

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Chen G., Chakravarti N., Aardalen K., Lazar A. J., Tetzlaff M. T., et al. (2014) Molecular profiling of patient-matched brain and extracranial melanoma metastases implicates the PI3K pathway as a therapeutic target. Clin Cancer Res 20: 5537-5546

Ipilimumab and dabrafenib are two new therapies approved by the FDA for treating metastatic melanoma, but they have only modest activities in patients with brain metastases. The authors compared patient-matched brain and extracranial metastases and found they are quite similar. However, they found significant differences in the targetable pathways in brain metastases. In particular, the PI3K/AKT pathway appears to have a critical role for tumor activation.

Illumina Technology: HumanHT12 v4 BeadChip arrays

Kim K., Skora A. D., Li Z., Liu Q., Tam A. J., et al. (2014) Eradication of metastatic mouse cancers resistant to immune checkpoint blockade by suppression of myeloid-derived cells. Proc Natl Acad Sci U S A 111: 11774-11779

Poorly immunogenic cancers do not respond well to immunotherapy. The authors found that, for mice resistant to immune checkpoint modulators, co-treatment with epigenetic-modulating drugs and checkpoint inhibitors markedly improved treatment outcomes, curing more than 80% of tumor-bearing mice. The primary targets of the epigenetic modulators were myeloid-derived suppressor cells (MDSCs). A PI3K inhibitor that reduced circulating MDSCs also eradicated 4T1 tumors in 80% of the mice when combined with immune checkpoint inhibitors.

Illumina Technology: Genome Analyzer$_{IIx}$ and HiSeq

Robert L., Tsoi J., Wang X., Emerson R., Homet B., et al. (2014) CTLA4 blockade broadens the peripheral T-cell receptor repertoire. Clin Cancer Res 20: 2424-2432

The authors sequenced the complementarity-determining region 3 (CDR3) from the rearranged T-cell receptor (TCR) variable beta (V-beta) chain in peripheral blood mononuclear cells (PBMCs) of 21 patients, at baseline and 30–60 days after CTLA4 blockade with tremelimumab. They found an increase in diversity of the CDR3 sequences, but this diversity did not correlate with clinical responders and nonresponders.

Illumina Technology: Genome Analyzer

Snyder A., Makarov V., Merghoub T., Yuan J., Zaretsky J. M., et al. (2014) Genetic basis for clinical response to CTLA-4 blockade in melanoma. N Engl J Med 371: 2189-2199

The authors sequenced the malignant melanoma exomes from 64 patients treated with CTLA-4 blockade and developed a signature for tumors with a strong response to CTLA-4 blockade. They validated this signature in a second set of 39 patients with melanoma who were treated with anti–CTLA-4 antibodies and found that the predicted neoantigens activated T cells from patients treated with ipilimumab.

Illumina Technology: HiSeq 2000 to sequence exome libraries

Tumeh P. C., Harview C. L., Yearley J. H., Shintaku I. P., Taylor E. J., et al. (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515: 568-571

The authors showed that pre-existing CD81 T cells distinctly located at the invasive tumor margin are associated with expression of the PD-1/PD-L1 immune inhibitory axis and may predict response to therapy. They concluded that tumor regression after therapeutic PD-1 blockade requires pre-existing CD81 T cells that are negatively regulated by PD-1/PD-L1-mediated adaptive immune resistance.

Illumina Technology: HiSeq

Rochman Y., Yukawa M., Kartashov A. V. and Barski A. (2015) Functional characterization of human T cell hyporesponsiveness induced by CTLA4-Ig. PLoS One 10: e0122198

Spranger S., Bao R. and Gajewski T. F. (2015) Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 523: 231-235

Johnson D. B., Smalley K. S. and Sosman J. A. (2014) Molecular pathways: targeting NRAS in melanoma and acute myelogenous leukemia. Clin Cancer Res 20: 4186-4192

Sengsayadeth S., Wang T., Lee S. J., Haagenson M. D., Spellman S., et al. (2014) Cytotoxic T-lymphocyte antigen-4 single nucleotide polymorphisms are not associated with outcomes after unrelated donor transplantation: a center for international blood and marrow transplant research analysis. Biol Blood Marrow Transplant 20: 900-903

Westin J. R., Chu F., Zhang M., Fayad L. E., Kwak L. W., et al. (2014) Safety and activity of PD1 blockade by pidilizumab in combination with rituximab in patients with relapsed follicular lymphoma: a single group, open-label, phase 2 trial. Lancet Oncol 15: 69-77

T-CELL REPERTOIRE

The human immune system provides protection against an enormous variety of pathogens as well as tumors. This protection is mediated by a vast repertoire of receptors, on the surface of B and T cells, which bind to pathogenic or pathogen-derived antigens.[27] T cells mediate cellular immunity through the expression of heterodimeric (αβ or γδ) cell-surface receptors (TCRs), which engage heterologous cells presenting peptide antigens bound to the MHC.[28]

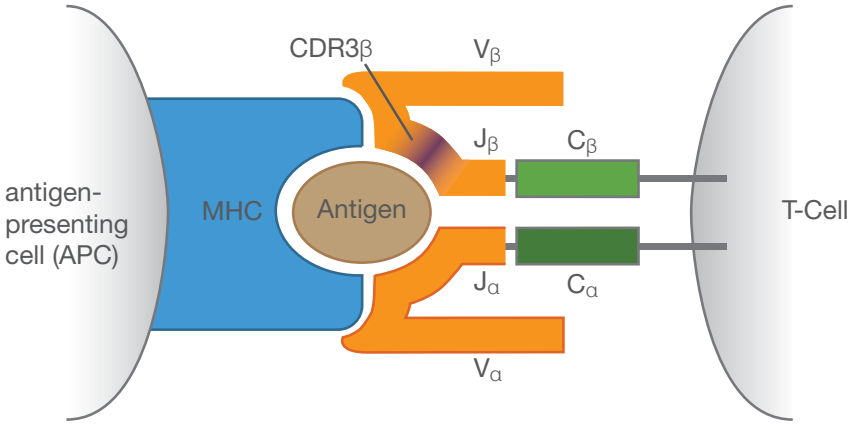
27. Robins H. (2013) Immunosequencing: applications of immune repertoire deep sequencing. Curr Opin Immunol 25: 646-652
28. Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease. Genome Med 5: 98

Reviews

Calis J. J. and Rosenberg B. R. (2014) Characterizing immune repertoires by high throughput sequencing: strategies and applications. Trends Immunol 35: 581-590

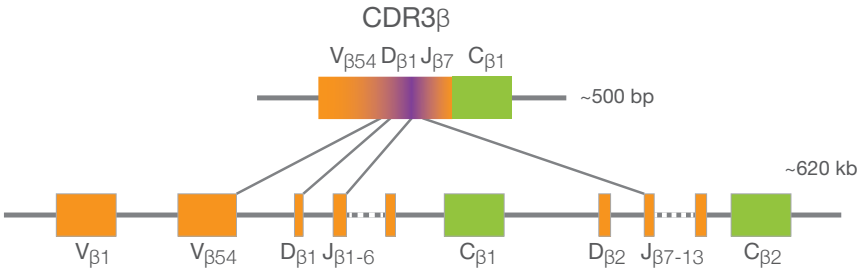
Linnemann C., Mezzadra R. and Schumacher T. N. (2014) TCR repertoires of intratumoral T-cell subsets. Immunol Rev 257: 72-82

Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease. Genome Med 5: 98



TCR-antigen-peptide-MHC interaction and TCR gene recombination. a) The antigen-presenting cell presents the peptide antigen bound to the MHC. The TCR (orange) binds to both the antigen and MHC. If the binding avidity is sufficiently high, the T cell is activated. The complementarity-determining region 3 (CDR3) domain is shown in purple.

The highly variable CDR3 regions in both the B-cell receptor (BCR) and TCR are short, between 15–60 nucleotides, making them particularly suitable for next-generation sequencing (NGS). NGS has been used extensively to determine T-cell populations.[29-32] In this process, the TCR β-chain is commonly used as a marker.[33]



Simplified representation of TCR-β chain variable (V), diversity (D), and joining (J) gene recombination resulting in TCR diversity. The TCR-β locus on chromosome 7 is approximately 620 kb in length. Initially one of the two D regions is joined with one of 13 J regions (both randomly selected), followed by joining of the DJ region to one of more than 50 V regions (also randomly selected), yielding a final VDJ region that is approximately 500 bp in length. The mechanism by which gene segments are joined also introduces base-pair variability which, together with the combinatorial selection of these segments, results in TCR diversity. A completely analogous process occurs for the TCR-α chain, without the D gene segment.

Traditional techniques, such as flow cytometry[34] or spectratyping,[35] have low resolution and cannot distinguish TCR clonotypes using the same TCR-Vβ segment or CDR3 with the same length.[36, 37] Fortunately, NGS techniques are able to determine the nucleotide sequences of all TCRβ CDR3 sequences present within a given T-cell population, even when they are present at very low frequency.[38] Due to the high diversity of the TCRβ CDR3 repertoire, the sequences obtained will, in most cases, represent individual TCR clonotypes.[39] NGS is an objective tool that can accurately determine T-cell populations for prognosis and monitor the response to treatment.[40]

Functional TCRs are heterodimeric proteins that comprise both α and β chains. Every T cell contains a unique combination of α and β chains and, for an accurate functional analysis, both subunits must be sequenced together. To avoid disrupting the α and β chain pairing through cell lysis,[41] several single-cell sequencing methods have been developed.[42] See *Single Cells and TCR Sequencing* for more information.

29. van Heijst J. W., Ceberio I., Lipuma L. B., Samilo D. W., Wasilewski G. D., et al. (2013) Quantitative assessment of T cell repertoire recovery after hematopoietic stem cell transplantation. Nat Med 19: 372-377
30. Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease. Genome Med 5: 98
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34. Langerak A. W., van Den Beemd R., Wolvers-Tettero I. L., Boor P. P., van Lochem E. G., et al. (2001) Molecular and flow cytometric analysis of the Vbeta repertoire for clonality assessment in mature TCRalphabeta T-cell proliferations. Blood 98: 165-173
35. Gorski J., Yassai M., Zhu X., Kissela B., Kissella B., et al. (1994) Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping. Correlation with immune status. J Immunol 152: 5109-5119
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37. Sherwood A. M., Emerson R. O., Scherer D., Habermann N., Buck K., et al. (2013) Tumor-infiltrating lymphocytes in colorectal tumors display a diversity of T cell receptor sequences that differ from the T cells in adjacent mucosal tissue. Cancer Immunol Immunother 62: 1453-1461
38. Robins H., Desmarais C., Matthis J., Livingston R., Andriesen J., et al. (2012) Ultra-sensitive detection of rare T cell clones. J Immunol Methods 375: 14-19
39. Robins H. S., Campregher P. V., Srivastava S. K., Wacher A., Turtle C. J., et al. (2009) Comprehensive assessment of T-cell receptor beta-chain diversity in alphabeta T cells. Blood 114: 4099-4107
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41. Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease. Genome Med 5: 98
42. Turchaninova M. A., Britanova O. V., Bolotin D. A., Shugay M., Putintseva E. V., et al. (2013) Pairing of T-cell receptor chains via emulsion PCR. Eur J Immunol 43: 2507-2515

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Qi Q., Liu Y., Cheng Y., Glanville J., Zhang D., et al. (2014) Diversity and clonal selection in the human T-cell repertoire. Proc Natl Acad Sci U S A 111: 13139-13144

The authors used NGS and nonparametric statistical analysis to estimate a lower bound for the total number of different TCR-β sequences in human repertoires. Their high minimal estimate is 100 million unique TCR-β sequences in naïve CD4 and CD8 T-cell repertoires of young adults.

Illumina Technology: MiSeq

Robert L., Tsoi J., Wang X., Emerson R., Homet B., et al. (2014) CTLA4 blockade broadens the peripheral T-cell receptor repertoire. Clin Cancer Res 20: 2424-2432

The authors sequenced the CDR3 regions from the rearranged TCR-Vβ chain in PBMCs of 21 patients, at baseline and 30–60 days after CTLA4 blockade with tremelimumab. They found an increase in diversity of the CDR3 sequences, but this diversity did not correlate with clinical responders and nonresponders.

Illumina Technology: Genome Analyzer

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The authors showed that pre-existing CD81 T cells distinctly located at the invasive tumor margin are associated with expression of the PD-1/PD-L1 immune inhibitory axis and may predict response to therapy. They concluded that tumor regression after therapeutic PD-1 blockade requires pre-existing CD81 T cells that are negatively regulated by PD-1/PD-L1-mediated adaptive immune resistance.

Illumina Technology: HiSeq

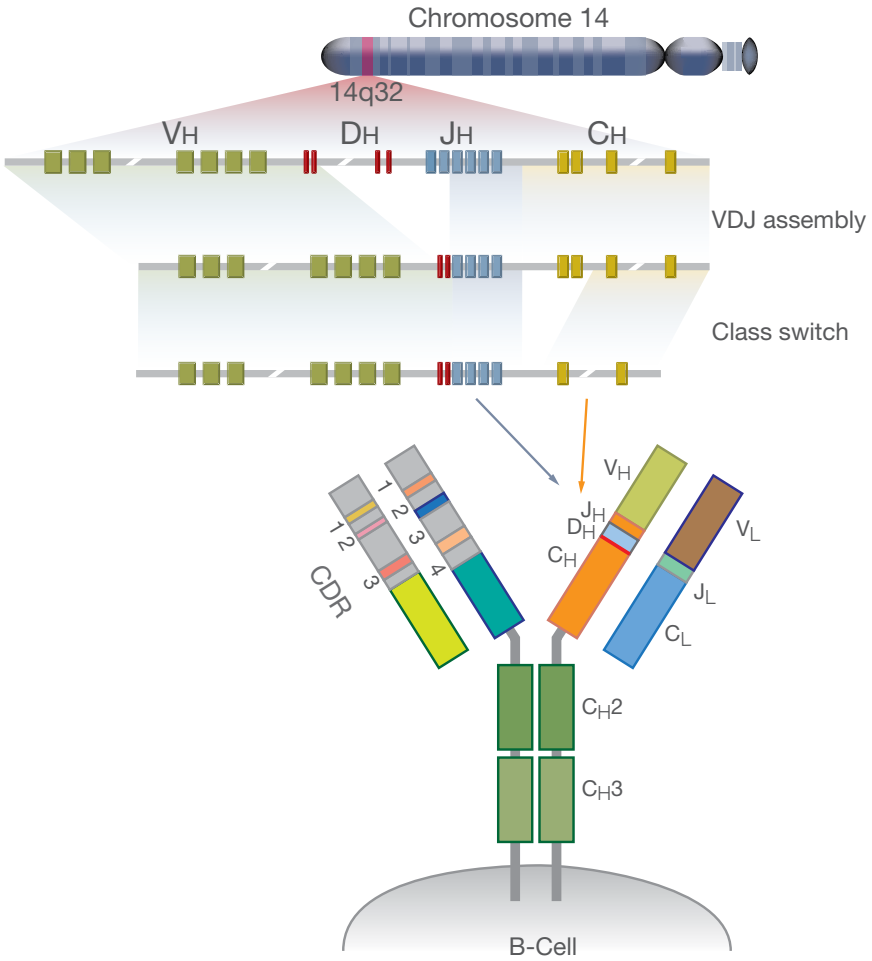
Bajor D. L., Xu X., Torigian D. A., Mick R., Garcia L. R., et al. (2014) Immune activation and a 9-year ongoing complete remission following CD40 antibody therapy and metastasectomy in a patient with metastatic melanoma. Cancer Immunol Res 2: 1051-1058

Madi A., Shifrut E., Reich-Zeliger S., Gal H., Best K., et al. (2014) T-cell receptor repertoires share a restricted set of public and abundant CDR3 sequences that are associated with self-related immunity. Genome Res 24: 1603-1612

ANTIBODY REPERTOIRE

Antibody repertoire sequencing is transforming our understanding of immune responses to autoimmunity, vaccination, infection, and cancer. This technique has the potential to provide next-generation biomarkers, diagnostic tools, and therapeutic antibodies for a spectrum of diseases, including rheumatic diseases.

43. Georgiou G., Ippolito G. C., Beausang J., Busse C. E., Wardemann H., et al. (2014) The promise and challenge of high-throughput sequencing of the antibody repertoire. Nat Biotechnol 32: 158-168



The primary antibody heavy-chain (IgH) repertoire is created predominantly by the somatic recombination of V, D, and J gene segments. Nontemplated nucleotides (indicated in red) can also be added. The antigen-binding site of a heavy chain is formed by the juxtaposition of the hypervariable complementarity-determining regions (CDR-H1, H2, and H3) and the framework 3 region (FR3). After productive IgH rearrangement, recombination of the light chain (IgL) ensues, and the heterodimeric pairing of H and L chains forms the complete antibody of the IgM isotype that is expressed on the surface of a newly formed immature B cell.[43]

Reviews

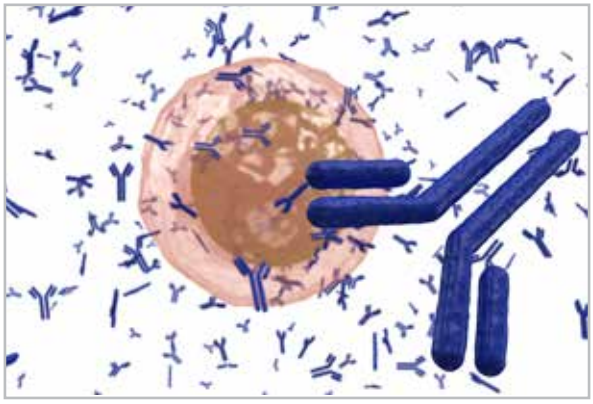
Robinson W. H. (2015) Sequencing the functional antibody repertoire--diagnostic and therapeutic discovery. Nat Rev Rheumatol 11: 171-182

Calis J. J. and Rosenberg B. R. (2014) Characterizing immune repertoires by high throughput sequencing: strategies and applications. Trends Immunol 35: 581-590

Dheilly N. M., Adema C., Raftos D. A., Gourbal B., Grunau C., et al. (2014) No more non-model species: the promise of next generation sequencing for comparative immunology. Dev Comp Immunol 45: 56-66

Georgiou G., Ippolito G. C., Beausang J., Busse C. E., Wardemann H., et al. (2014) The promise and challenge of high-throughput sequencing of the antibody repertoire. Nat Biotechnol 32: 158-168

Shugay M., Britanova O. V., Merzlyak E. M., Turchaninova M. A., Mamedov I. Z., et al. (2014) Towards error-free profiling of immune repertoires. Nat Methods 11: 653-655



Antibodies produced by B cells.

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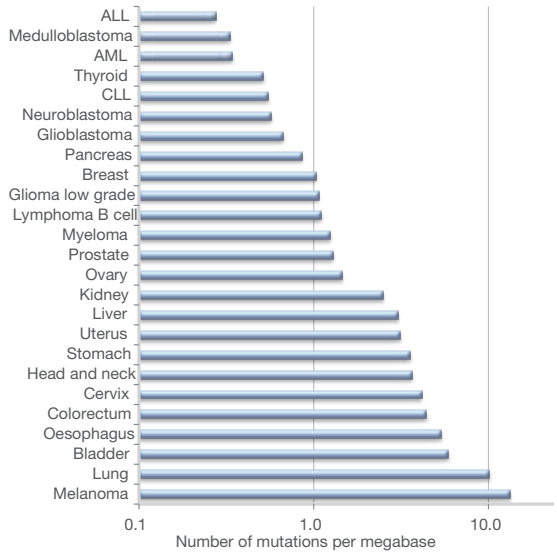
Birnbaum M. E., Mendoza J. L., Sethi D. K., Dong S., Glanville J., et al. (2014) Deconstructing the peptide-MHC specificity of T cell recognition. Cell 157: 1073-1087

Menzel U., Greiff V., Khan T. A., Haessler U., Hellmann I., et al. (2014) Comprehensive evaluation and optimization of amplicon library preparation methods for high-throughput antibody sequencing. PLoS One 9: e96727

CANCER EPITOPES

Due to extensive genetic and epigenetic alterations, tumor cells produce a vast array of proteins that are not present in normal cells. These proteins can lead to an altered repertoire of MHC class I-associated peptides. The spectrum of epitopes includes peptides from genes that are aberrantly expressed within tumor cells, but also the "neoantigens" that arise as a direct consequence of somatic mutations within tumor cells. These neoantigens are therefore specific to the tumor but also unique to the patient. T cells can recognize these antigens that are presented on the surface of human tumor cells and thereby mediate cancer regression.[44, 45]

The recent explosion in the use of NGS to characterize the cancer genome provides a unique opportunity to characterize the spectrum of potential tumor-specific antigens as well.[46, 47, 48] Exome sequencing data from animal models,[49, 50] as well as human cancers,[51, 52, 53] could predict T-cell reactivities against neoantigens formed by tumor-specific mutations. An understanding of which antigens form the prime targets in effective immunotherapies may ultimately lead to a more accurate prognosis and treatment.[54, 55]



The prevalence of somatic mutations across human cancer types.[56]

44. Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens. Curr Opin Immunol 25: 284-290
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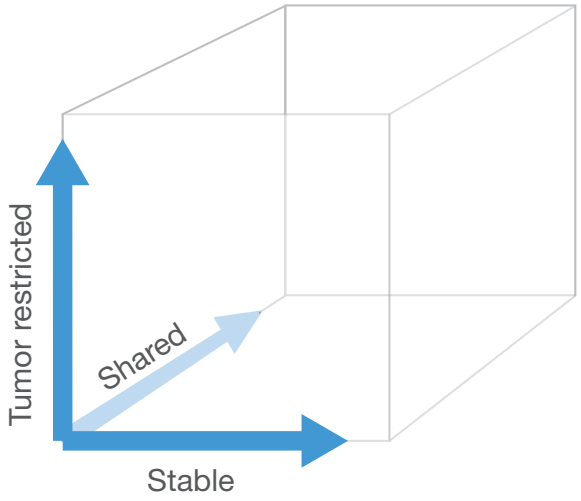
Reviews

Rosenberg S. A. (2014) Finding suitable targets is the major obstacle to cancer gene therapy. Cancer Gene Ther 21: 45-47

The development of lymphocytes with antitumor activity has become a major effort in studies of current cancer immunotherapy. However, the cancer cell-surface proteins that are targeted may still be expressed, albeit at a low level, in healthy tissue, leading to the risk of serious toxic effects from therapy. The major obstacle is the identification of suitable immunologic targets on cancer cells. An ideal source of antigens to target using genetically modified lymphocytes are shared mutations that are unique to each cancer type and are not found in normal tissues. For example, common mutations such as B-RAF in melanoma or K-RAS in pancreatic and other cancers would represent ideal targets for cell transfer immunotherapy, provided suitable antigen receptors can be identified. Gene editing of lymphocytes is opening up new potential for this area of gene therapy.

Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens. Curr Opin Immunol 25: 284-290

Cytotoxic T cells can recognize antigens that are presented on the surface of human tumor cells and thereby mediate cancer regression. To exploit this potential for cancer therapeutics, the challenge remains to identify tumor antigens that are: i) shared by patient groups; ii) expressed only in the tumor; and iii) have a low likelihood of antigen loss under selective pressure. With the development of NGS, it has become feasible to describe the repertoire of tumor-specific mutations within individual tumors with relative ease, offering the potential for predicting patient-specific mutated antigens meeting these criteria.



A 3D representation of human tumor-associated antigen characteristics. Stable: the likelihood of antigen retention upon T-cell pressure; tumor-restricted: the degree of uniqueness to the tumor, compared to normal; shared: the degree of sharing between patients.[57]

Heemskerk B., Kvistborg P. and Schumacher T. N. (2013) The cancer antigenome. EMBO J 32: 194-203

Haen S. P. and Rammensee H. G. (2013) The repertoire of human tumor-associated epitopes--identification and selection of antigens and their application in clinical trials. Curr Opin Immunol 25: 277-283

van Rooij N., van Buuren M. M., Philips D., Velds A., Toebes M., et al. (2013) Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 31: e439-442

57. Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens. Curr Opin Immunol 25: 284-290

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Green M. R., Kihira S., Liu C. L., Nair R. V., Salari R., et al. (2015) Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. Proc Natl Acad Sci U S A 112: E1116-1125

The authors used NGS to reconstruct the mutation hierarchy of enriched follicular lymphoma biopsies of 22 patients. They found that CREBBP mutant B cells stimulated less proliferation of T cells *in vitro* compared with wild-type B cells from the same tumor. Transcriptional signatures were indicative of a reduced number of tumor-infiltrating CD4 helper T cells and CD8 memory cytotoxic T cells.

Illumina Technology: HiSeq 2000

Kreiter S., Vormehr M., van de Roemer N., Diken M., Lower M., et al. (2015) Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature 520: 692-696

This study describes a process by which mutations identified by exome sequencing could be selected as vaccine targets, solely through bioinformatic prioritization on the basis of their expression levels and MHC class II-binding capacity. This information could be used for rapid production of synthetic polyneo-epitope messenger RNA (mRNA) vaccines. The authors show that vaccination with such "polytope" mRNA vaccines induces potent tumor control and complete rejection of established, aggressively growing tumors in mice.

Illumina Technology: HiSeq 2000 for exome and mRNA sequencing

Yadav M., Jhunjhunwala S., Phung Q. T., Lupardus P., Tanguay J., et al. (2014) Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 515: 572-576

The authors developed an approach that combines whole-exome and transcriptome sequencing analysis with mass spectrometry to identify neo-epitopes. Vaccination of mice confirmed the approach, with each predicted immunogenic peptide yielding therapeutically active T-cell responses. This approach could be used for the pharmacodynamic monitoring of T-cell responses, as well as for the development of personalized vaccines in cancer patients.

Illumina Technology: HiSeq 2000

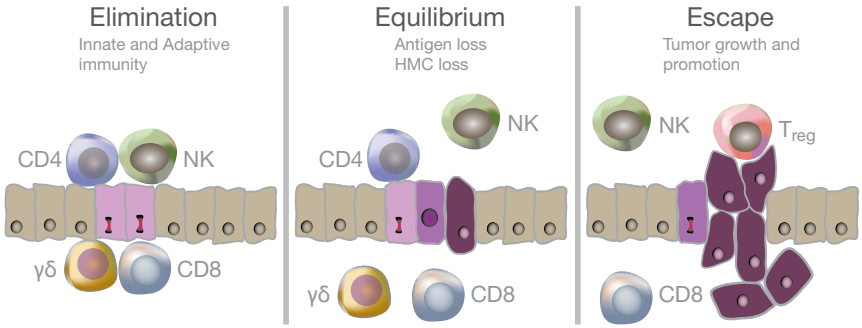
van Buuren M. M., Dijkgraaf F. E., Linnemann C., Toebes M., Chang C. X., et al. (2014) HLA micropolymorphisms strongly affect peptide-MHC multimer-based monitoring of antigen-specific CD8+ T cell responses. J Immunol 192: 641-648

Robbins P. F., Lu Y. C., El-Gamil M., Li Y. F., Gross C., et al. (2013) Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med 19: 747-752

van Rooij N., van Buuren M. M., Philips D., Velds A., Toebes M., et al. (2013) Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. J Clin Oncol 31: e439-442

CANCER IMMUNOEDITING

Cancer immunoediting is the process by which both the adaptive and the innate immune systems control tumor growth and shape tumor immunogenicity.[58, 59, 60, 61, 62] This process consists of three phases: elimination, equilibrium, and escape.[63] Elimination, or cancer immunosurveillance, is the process by which the adaptive and innate immune branches identify and destroy newly formed cancer cells. The longest phase, equilibrium, encompasses the state of balance between preventing tumor outgrowth and sculpting the immunogenicity of a small number of neoplastic cells. In the escape phase, the least immunogenic tumor cells progressively grow and spread as visible tumors.



Immunoediting both protects against and promotes tumor growth. The elimination phase describes the process in which the adaptive and innate immune response recognizes and eliminates tumors when they arise in tissues. The equilibrium phase encompasses the state of balance between the prevention of tumor outgrowth and the selection of cancer cells that are resistant to being killed. The outcome of this phase is the directional selection of neoplastic cells that no longer express foreign antigens or no longer express the MHC. The escape phase refers to the process of variant cancer cells escaping the immune's eradication mechanisms and/or recruiting regulatory cells to protect them.

Exome sequencing has enabled researchers to identify tumor epitopes experimentally, and to identify epitopes specifically from unedited tumors. This process has been demonstrated by a recent study that coupled massively parallel sequencing with a cDNA capture sequencing (cDNA CapSeq). The study revealed that T cell–dependent immunoediting is a mechanism underlying the proliferation of tumor cells that lack strong rejection antigens.[64]

58. Schreiber R. D., Old L. J. and Smyth M. J. (2011) Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 331: 1565-1570
59. Dunn G. P., Koebel C. M. and Schreiber R. D. (2006) Interferons, immunity and cancer immunoediting. Nat Rev Immunol 6: 836-848
60. Gajewski T. F., Woo S. R., Zha Y., Spaapen R., Zheng Y., et al. (2013) Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. Curr Opin Immunol 25: 268-276
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62. Shankaran V., Ikeda H., Bruce A. T., White J. M., Swanson P. E., et al. (2001) IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 410: 1107-1111
63. Mittal D., Gubin M. M., Schreiber R. D. and Smyth M. J. (2014) New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape. Curr Opin Immunol 27: 16-25
64. Matsushita H., Vesely M. D., Koboldt D. C., Rickert C. G., Uppaluri R., et al. (2012) Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 482: 400-404

Reviews

Mittal D., Gubin M. M., Schreiber R. D. and Smyth M. J. (2014) New insights into cancer immunoediting and its three component phases--elimination, equilibrium and escape. Curr Opin Immunol 27: 16-25

Vogelstein B., Papadopoulos N., Velculescu V. E., Zhou S., Diaz L. A., Jr., et al. (2013) Cancer genome landscapes. Science 339: 1546-1558

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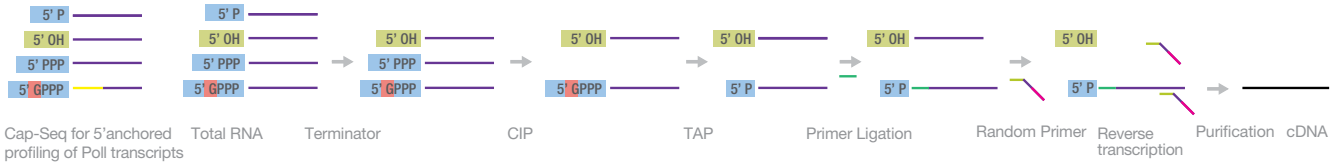
Rooney M. S., Shukla S. A., Wu C. J., Getz G. and Hacohen N. (2015) Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 160: 48-61

The authors developed an RNA-based metric of immune cytolytic activity and calculated it for thousands of The Cancer Genome Atlas (TCGA) solid tumor samples. They found that DNA amplifications were associated with high cytolytic activity, including immunosuppressive factors such as PDL1/2 and ALOX12B/15B. They also found evidence for immunoediting.

Illumina Technology: Genome Analyzer and HiSeq data

65. Illingworth R. S., Gruenewald-Schneider U., Webb S., Kerr A. R., James K. D., et al. (2010) Orphan CpG islands identify numerous conserved promoters in the mammalian genome. PLoS Genet 6: e1001134

Cap-Seq



CXXC affinity purification sequencing (CAP-Seq)[65] maps the 5' end of RNAs anchored to RNA polymerase II. In this method, RNA transcripts are treated with a terminator, calf intestine alkaline phosphatase (CIP), and then tobacco acid pyrophosphatase (TAP), followed by linker ligation and reverse transcription to cDNA. Deep sequencing of the cDNA provides high-resolution sequences of RNA polymerase II transcripts.

TUMOR MICROENVIRONMENT

The tumor microenvironment is defined as the cellular environment in which the tumor exists. This encompasses surrounding blood vessels, immune cells, fibroblasts, other cells, signaling molecules, and the extracellular matrix (ECM). Studies also report dynamic relationships between tumors and the microenvironment. These relationships include the extent to which the tumor can control the microenvironment by releasing extracellular signals (i.e. tumor angiogenesis), as well as the exertion of the microenvironment on cancerous cells that promote growth, such as in immunoediting (see *Cancer Immunoediting* for more details). The tumor microenvironment remains a major prognostic factor even in metastatic lesions, while being reproducible between the primary and metastatic tumor. Nevertheless, the prognostic impact of the Th1/cytotoxic T-cell infiltrate could vary according to the origin of the primary tumor.[66]

Emerging evidence suggests that distinct subsets of tumors may exist, reflecting distinct categories of immune escape. For example, the lack of chemokine-mediated trafficking, poor innate immune cell activation, and the presence of specific immune suppressive mechanisms characterize subsets of tumors.

Consolidated findings also demonstrate the different mechanisms by which chronic inflammation can create a pro-tumor microenvironment. Inflammatory responses can increase cellular response signals and accelerate the cell cycle, in turn increasing mutation rates and, ultimately, augmenting tumor growth.[67]

66. Giraldo N. A., Becht E., Remark R., Damotte D., Sautes-Fridman C., et al. (2014) The immune contexture of primary and metastatic human tumours. Curr Opin Immunol 27: 8-15

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Reviews

Perez-Gracia J. L., Labiano S., Rodriguez-Ruiz M. E., Sanmamed M. F. and Melero I. (2014) Orchestrating immune check-point blockade for cancer immunotherapy in combinations. Curr Opin Immunol 27: 89-97

Immunotherapy agents present new opportunities for developing cancer therapies. The inhibitory receptors on immune system cells (checkpoints) can be targeted with inhibitors to strengthen the immune response to cancer cells. Monoclonal antibodies (mAb) belong to this category of checkpoint inhibitors. Several mAbs are in clinical trials, and studies have demonstrated the potential for combination strategies of mAbs with chemotherapy and radiotherapy.

Giraldo N. A., Becht E., Remark R., Damotte D., Sautes-Fridman C., et al. (2014) The immune contexture of primary and metastatic human tumours. Curr Opin Immunol 27: 8-15

Gajewski T. F., Woo S. R., Zha Y., Spaapen R., Zheng Y., et al. (2013) Cancer immunotherapy strategies based on overcoming barriers within the tumor microenvironment. Curr Opin Immunol 25: 268-276

Galon J., Angell H. K., Bedognetti D. and Marincola F. M. (2013) The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity 39: 11-26

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Zhou P., Shaffer D. R., Alvarez Arias D. A., Nakazaki Y., Pos W., et al. (2014) *In vivo* discovery of immunotherapy targets in the tumour microenvironment. Nature 506: 52-57

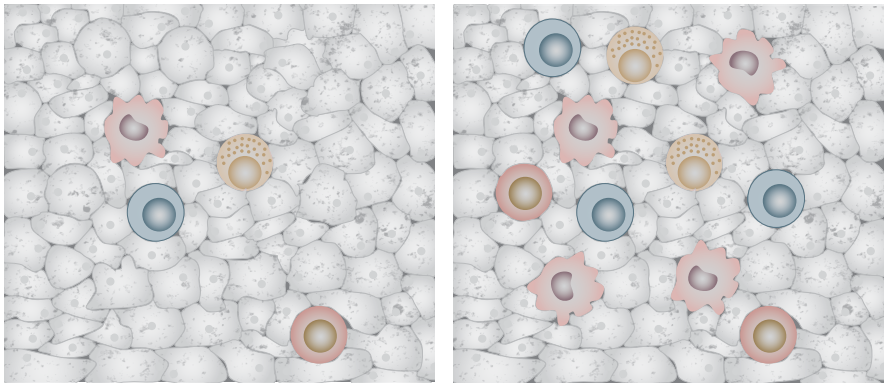
To discover regulators of immune function in tissue microenvironments, the authors developed an *in vivo* short hairpin RNA (shRNA) screen. The shRNAs targeting negative regulators became highly enriched in murine tumors upon tumor antigen recognition. The results showed that Ppp2r2d knockdown in tumors inhibited T-cell apoptosis, and enhanced T-cell proliferation and cytokine production.

Illumina Technology: Genome Analyzer

INTRATUMORAL T CELLS

The infiltration of human tumors by T cells is a common phenomenon. The extent of infiltration and the reactivity of the intratumoral T-cell populations can predict the course and the outcome of the disease.[68] To take advantage of this observation, autologous tumor-infiltrating lymphocytes (TILs) along with interleukin-2, following a lymphodepleting preparative regimen, have been used to treat patients with metastatic melanoma.[69] This approach can lead to durable cancer regressions in 20–40% of patients with metastatic melanoma, most of whom were refractory to established regimens.[70] Expansion of this approach requires identification, or even genetic modification, of the TILs as well as their targets.[71]

Initial experiments show that NGS can be used to quantify immune cell populations within the tumor.[72]



The infiltration of human tumors by T cells is a common phenomenon. The nature of such intratumoral T-cell populations can predict the course and the outcome of the disease.

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69. Rosenberg S. A. (1986) The adoptive immunotherapy of cancer using the transfer of activated lymphoid cells and interleukin-2. Semin Oncol 13: 200-206
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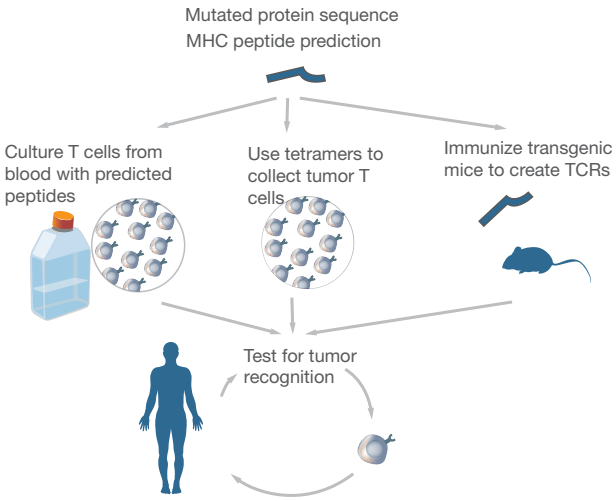
Ohnuki H., Jiang K., Wang D., Salvucci O., Kwak H., et al. (2014) Tumor-infiltrating myeloid cells activate Dll4/Notch/TGF-β signaling to drive malignant progression. Cancer research canres. 3118.2013

Emerson R. O., Sherwood A. M., Rieder M. J., Guenthoer J., Williamson D. W., et al. (2013) High-throughput sequencing of T-cell receptors reveals a homogeneous repertoire of tumour-infiltrating lymphocytes in ovarian cancer. J Pathol 231: 433-440

Sherwood A. M., Emerson R. O., Scherer D., Habermann N., Buck K., et al. (2013) Tumor-infiltrating lymphocytes in colorectal tumors display a diversity of T cell receptor sequences that differ from the T cells in adjacent mucosal tissue. Cancer Immunol Immunother 62: 1453-1461

CANCER IMMUNOTHERAPY

Intratumoral T cells have been utilized therapeutically in clinical studies of adoptive T-cell therapy.[73] For patients with metastatic melanoma, TIL-based adoptive transfer can result in tumor shrinkage of approximately 50%.[74] Conversely, the absence of TILs is considered to be a marker for poor efficacy of immunotherapies.[75] Two mechanisms may play a role in treatment resistance: lack of T cell migration due to low levels of inflammation and dominant immune suppression. Treatment with the cytokine Interleukin 2 (IL-2) to stimulate the growth and proliferation of T cells has produced durable responses in melanoma and renal cancer patients; unfortunately, this treatment is effective only in a fraction of patients.[76]



Highly personalized medicine. The expressed genes from a patient's tumor can be sequenced to identify candidate mutant T-cell epitopes. Peptides derived from mutant proteins could be used in one of at least three ways. First, cells that express relevant antigens can be sorted using tetramer-like reagents. Second, candidate peptides could be used to stimulate T cells that are already present in the patient's tumor or in their peripheral blood. Third, antigens could be used to prime tumor-specific T cells in humanized mice and adoptively transfer them if they are of human origin.[77]

Tumor immunogenicity results from mutations that generate tumor-specific antigens (TSAs). This is a common characteristic of most, but not all, cancers.[78] However, targeting TSAs offers the benefit of specificity for tumors, reducing the risk of inducing autoimmune reactions. It can also target driver mutations, precluding tumor escape by antigen loss. High-throughput sequencing offers the potential to identify mutations rapidly in individual tumors, predict peptides computationally that can best stimulate T-cell responses, and vaccinate patients against the unique TSAs in their tumors.[79, 80, 81]

73. Linnemann C., Mezzadra R. and Schumacher T. N. (2014) TCR repertoires of intratumoral T-cell subsets. Immunol Rev 257: 72-82
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79. Segal N. H., Parsons D. W., Peggs K. S., Velculescu V., Kinzler K. W., et al. (2008) Epitope landscape in breast and colorectal cancer. Cancer Res 68: 889-892
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81. Lu Y. C., Yao X., Li Y. F., El-Gamil M., Dudley M. E., et al. (2013) Mutated PPP1R3B is recognized by T cells used to treat a melanoma patient who experienced a durable complete tumor regression. J Immunol 190: 6034-6042

Dendritic cell (DC)-based cancer vaccines are well tolerated with few side effects. They can generate antitumor immune responses but, overall, they have been of limited benefit. Recent studies have demonstrated that CD141+ DCs play an important role in antitumor responses. Vaccines that directly target DCs *in vivo* are under development.[82]

82. Radford K. J., Tullett K. M. and Lahoud M. H. (2014) Dendritic cells and cancer immunotherapy. Curr Opin Immunol 27: 26-32

Reviews

Perez-Gracia J. L., Labiano S., Rodriguez-Ruiz M. E., Sanmamed M. F. and Melero I. (2014) Orchestrating immune check-point blockade for cancer immunotherapy in combinations. Curr Opin Immunol 27: 89-97

Immunotherapy agents present new opportunities for developing cancer therapies. The inhibitory receptors on immune system cells (checkpoints) can be targeted with inhibitors to strengthen the immune response to cancer cells. mAbs belong to this category of checkpoint inhibitors. Several mAbs are in clinical trials, and studies have demonstrated the potential for combination strategies of mAbs with chemotherapy and radiotherapy.

Choi W., Porten S., Kim S., Willis D., Plimack E. R., et al. (2014) Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell 25: 152-165

Darcy P. K., Neeson P., Yong C. S. and Kershaw M. H. (2014) Manipulating immune cells for adoptive immunotherapy of cancer. Curr Opin Immunol 27: 46-52

Hinrichs C. S. and Rosenberg S. A. (2014) Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol Rev 257: 56-71

Nishikawa H. and Sakaguchi S. (2014) Regulatory T cells in cancer immunotherapy. Curr Opin Immunol 27: 1-7

Couzin-Frankel J. (2013) Breakthrough of the year 2013. Cancer immunotherapy. Science 342: 1432-1433

Gajewski T. F. and Schumacher T. (2013) Cancer immunotherapy. Curr Opin Immunol 25: 259-260

Galon J., Angell H. K., Bedognetti D. and Marincola F. M. (2013) The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity 39: 11-26

Hutchinson E. (2014) Towards individualized cancer therapy: Challenges and prospects. Molecular Oncology 8: 1-8

Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens. Curr Opin Immunol 25: 284-290

Phan G. Q. and Rosenberg S. A. (2013) Adoptive cell transfer for patients with metastatic melanoma: the potential and promise of cancer immunotherapy. Cancer Control 20: 289-297

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Linnemann C., van Buuren M. M., Bies L., Verdegaal E. M., Schotte R., et al. (2015) High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. Nat Med 21: 81-85

To assess the occurrence of intratumoral CD4+ T-cell responses against nonsynonymous somatic mutations within these tumors, the authors used whole-exome-sequencing and RNA-sequencing data to identify the entire set of tumor-specific, nonsynonymous mutations within expressed genes. CD4+ neoantigen reactivity in this cancer is primarily directed toward private mutations, which would make them promising candidates for personalized immunotherapies.

Illumina Technology: HiSeq 2000

Garralda E., Paz K., Lopez-Casas P. P., Jones S., Katz A., et al. (2014) Integrated next-generation sequencing and avatar mouse models for personalized cancer treatment. Clin Cancer Res 20: 2476-2484

To identify putatively actionable tumor-specific genomic alterations, the authors performed whole-exome sequencing analysis of 25 patients with advanced solid tumors. From 14 patients, 10 successful mouse xenograph (Avatar) models were created. Prior testing of candidate treatments in these Avatar models correlated with clinical responses and helped to select empirical treatments in some patients with no actionable mutations.

Illumina Technology: HiSeq 2000

Yadav M., Jhunjhunwala S., Phung Q. T., Lupardus P., Tanguay J., et al. (2014) Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 515: 572-576

The authors developed an approach that combines whole-exome and transcriptome sequencing analysis with mass spectrometry to identify neo-epitopes. Vaccination of mice confirmed the approach, with each predicted immunogenic peptide yielding therapeutically active T-cell responses. This approach could be used for the pharmacodynamic monitoring of T-cell responses, as well as for the development of personalized vaccines in cancer patients.

Illumina Technology: HiSeq 2000

Zhou P., Shaffer D. R., Alvarez Arias D. A., Nakazaki Y., Pos W., et al. (2014) *In vivo* discovery of immunotherapy targets in the tumour microenvironment. Nature 506: 52-57

The authors show that *in vivo* discovery of therapeutic targets is possible by using short hairpin RNA (shRNA) screening. With this approach, they identified genes that modify the action of tumor-infiltrating CD8 T cells in tumor-bearing mice.

Illumina Technology: Genome Analyzer

Bajor D. L., Xu X., Torigian D. A., Mick R., Garcia L. R., et al. (2014) Immune activation and a 9-year ongoing complete remission following CD40 antibody therapy and metastasectomy in a patient with metastatic melanoma. Cancer Immunol Res 2: 1051-1058

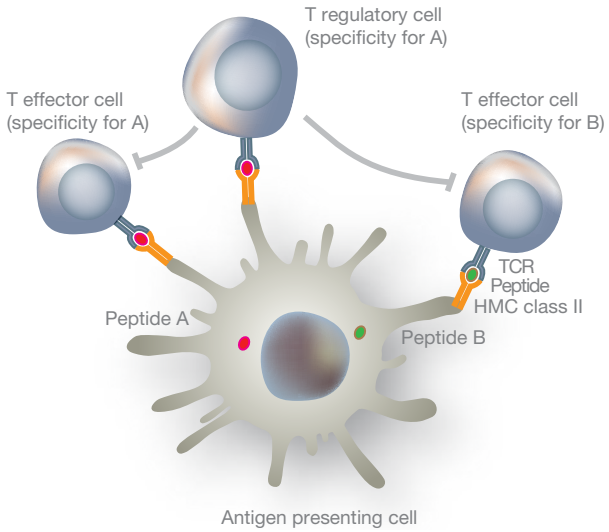
Robbins P. F., Lu Y. C., El-Gamil M., Li Y. F., Gross C., et al. (2013) Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat Med 19: 747-752

DENDRITIC CELLS

DCs regulate immune responses and play a role in the eradication of some cancers. They have been used as targets for vaccine development with limited success. It is now known that immature DCs generally induce tolerance rather than stimulate immunity, so most trials now incorporate Toll-like receptor (TLR) ligands and/or cytokines to activate DCs specifically. DC subsets can also vary in location, phenotype, and function. An improved understanding of these complexities ultimately may lead to more effective DC-based cancer vaccines.[83]

83. Radford K. J., Tullett K. M. and Lahoud M. H. (2014) Dendritic cells and cancer immunotherapy. Curr Opin Immunol 27: 26-32

DC and lymphocyte, colored scanning electron micrograph.



DCs are important antigen-presenting cells with the ability to present a broad range of antigens. They are especially potent T helper cell activators, but linked suppression represents a way in which regulatory T (TREG) cells support local self-tolerance. TREG cells inhibit antigen-presenting cells from presenting their cognate antigen. They can also inhibit bystander T cells, of the same and different antigen specificity, through soluble inhibitory factors.

Review

Darcy P. K., Neeson P., Yong C. S. and Kershaw M. H. (2014) Manipulating immune cells for adoptive immunotherapy of cancer. Curr Opin Immunol 27: 46-52

Radford K. J., Tullett K. M. and Lahoud M. H. (2014) Dendritic cells and cancer immunotherapy. Curr Opin Immunol 27: 26-32

Kawakami Y., Yaguchi T., Sumimoto H., Kudo-Saito C., Iwata-Kajihara T., et al. (2013) Improvement of cancer immunotherapy by combining molecular targeted therapy. Front Oncol 3: 136

Tesone A. J., Svoronos N., Allegrezza M. J. and Conejo-Garcia J. R. (2013) Pathological mobilization and activities of dendritic cells in tumor-bearing hosts: challenges and opportunities for immunotherapy of cancer. Front Immunol 4: 435

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Sundarasetty B. S., Chan L., Darling D., Giunti G., Farzaneh F., et al. (2015) Lentivirus-induced 'Smart' dendritic cells: Pharmacodynamics and GMP-compliant production for immunotherapy against TRP2-positive melanoma. Gene Ther in press:

Monocyte-derived conventional dendritic cells (ConvDCs) are hampered by difficulties in ConvDC manufacturing and low potency. In this study, the authors demonstrated higher potency of lentiviral vector (LV)-programmed DCs. Illumina MiSeq was used for quality inspection and integration site verification.

Illumina Technology: MiSeq

Ma Y., Mattarollo S. R., Adjemian S., Yang H., Aymeric L., et al. (2014) CCL2/CCR2-dependent recruitment of functional antigen-presenting cells into tumors upon chemotherapy. Cancer Res 74: 436-445

The therapeutic efficacy of anthracyclines as cancer chemotherapy relies on the induction of a DC and T-lymphocyte-dependent anticancer immune response. This study investigated the effects of anthracycline-based chemotherapy on the chemokine CCL2 and its receptor CCR2 in a mouse cancer model. The authors used Illumina Mouse BeadArray to characterize differential gene expression. They found that anthracycline-based chemotherapy promotes the intratumor accumulation of myeloid cells, including cells that mediate antigen presentation. These findings add to the understanding of the anticancer immune response elicited by immunogenic cell death.

Illumina Technology: Mouse WG-6 V.2 Expression Bead-Chips

Shalek A. K., Satija R., Adiconis X., Gertner R. S., Gaublomme J. T., et al. (2013) Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature 498: 236-240

Su X., Qian C., Zhang Q., Hou J., Gu Y., et al. (2013) miRNomes of haematopoietic stem cells and dendritic cells identify miR-30b as a regulator of Notch1. Nat Commun 4: 2903

HEMATOLOGICAL MALIGNANCIES

The development from a normal hematopoietic cell to a cancerous cell involves a multistep process of clonal evolution driven by a series of somatic mutations. These mutations progressively transform the cell from normal growth to a precancerous state and finally a cancerous state, where all checkpoints designed to regulate cell growth have been surmounted.

Induction of malignant transformations appears to involve at least two distinct phases: initiation and promotion. Initiation involves changes in the genome but does not, in itself, lead to malignant transformation. Malignant transformation requires a secondary step, termed promotion. Promotion can occur during the aggressive cell division that follows the initiation phase. It results from the accumulation of new DNA alterations—typically affecting proto-oncogenes, tumor-suppressor genes, or apoptotic genes—that result in unregulated cellular growth.

The ability of NGS to detect mutations in rare clonal types, or cells, through deep sequencing makes it possible to study the role of immune effector functions in the pathogenesis of hematological malignancies. A notable example has been the influx of reports that implicate autoreactive T cell clones in the pathogenesis of clonal stem cell disorders, such as myelodysplastic syndromes (MDS) and aplastic anemia (AA).[84] These studies have been supported by the understanding that impairment of antitumor immunity, which is physiologically mediated by T cells, can predispose the development of hematological malignancies. Collectively these T-cell repertoire studies and new reports that implicate immunoglobulin heavy chain rearrangements in clonal evolution of acute lymphoblastic leukemia (ALL) have quickly become one of the most exciting research areas in hematology.[85, 86, 87]

84. Fozza, C., and Longinotti, M. (2013) T-cell receptor repertoire usage in hematologic malignancies. Critical reviews in oncology/hematology 86: 201–211
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86. Gawad, C., Pepin, F., Carlton, V. E. H., Klinger, M., Logan, A. C., et al. (2012) Massive evolution of the immunoglobulin heavy chain locus in children with B precursor acute lymphoblastic leukemia. Blood 120: 4407–4417
87. Jan, M., Snyder, T. M., Corces-Zimmerman, M. R., Vyas, P., Weissman, I. L., et al. (2012) Clonal Evolution of Preleukemic Hematopoietic Stem Cells Precedes Human Acute Myeloid Leukemia. Science Translational Medicine 4: 149ra118–149ra118

High-throughput sequencing can be exploited to detect the rearranged CDR3 sequences carried in malignant B and T cells with unprecedented sensitivity and specificity. For example sequencing the *IGH* [88-94] and *TCR*β/γ [95] genes has been utilized at the time of diagnosis to identify malignant clones in patients with B- and T-lymphoid malignancies. This information was then used to track malignant clones during and after treatment. Serial sequencing of the *IGH* locus in pediatric B-ALL has revealed surprisingly dynamic evolution of the locus in some patients,[92] demonstrating that this technology may also generate valuable insights into the biology of B- and T-cell cancers.

Deep sequencing of antigen-receptor loci has multiple advantages for monitoring lymphoid malignancies. Compared to alternative approaches, deep sequencing demands less time, labor, has superior sensitivity,[92, 93, 95] and can simultaneously track all of the clones that comprise the malignant population. Its utility for monitoring disease burden in patients with chronic lymphocytic leukemia,[89, 90, 91] pediatric B-lineage ALL,[92, 93] and T-lineage ALL[95] has been demonstrated, and it will undoubtedly have utility for monitoring other lymphoid malignancies.

Reviews

Weaver W. M., Tseng P., Kunze A., Masaeli M., Chung A. J., et al. (2014) Advances in high-throughput single-cell microtechnologies. Curr Opin Biotechnol 25: 114-123

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Dose M., Emmanuel A. O., Chaumeil J., Zhang J., Sun T., et al. (2014) beta-Catenin induces T-cell transformation by promoting genomic instability. Proc Natl Acad Sci U S A 111: 391-396

Cancerous cells are characterized by dysfunction of the cell regulatory machinery, enabling uncontrolled growth. In some cancers, this regulatory dysfunction results in genomic instability, such as rogue recombination events. This study examined the connection between deregulation of β-catenin and genomic instability. The authors studied a mouse model with targeted activation of β-catenin and used chromatin immunoprecipitation sequencing (ChIP-Seq) to determine the link between transcription factor binding sites and translocation sites. They concluded that β-catenin promotes the genomic instability that leads to T-cell lymphomas.

Illumina Technology: Genome Analyzer$_{II}$

Joseph C. G., Darrah E., Shah A. A., Skora A. D., Casciola-Rosen L. A., et al. (2014) Association of the autoimmune disease scleroderma with an immunologic response to cancer. Science 343: 152-157

Scleroderma is an autoimmune connective tissue disease in which patients make antibodies to a limited group of autoantigens. Patients with scleroderma and antibodies against RPC1 are at increased risk for cancer. The authors sequenced the tumor and normal coding sequences of the *POLR3A*, *TOP1*, and *CENPB* genes in 16 patients. The results suggest that *POLR3A* mutations triggered cellular immunity and cross-reactive humoral immune responses.

Illumina Technology: Genome Analyzer$_{IIx}$

Palomero T., Couronne L., Khiabanian H., Kim M. Y., Ambesi-Impiombato A., et al. (2014) Recurrent mutations in epigenetic regulators, RHOA and FYN kinase in peripheral T cell lymphomas. Nat Genet 46: 166-170

Peripheral T cell lymphomas (PTCLs) are a heterogeneous and poorly understood group of non-Hodgkin lymphomas. In this study, whole-exome sequencing was used to analyze 12 tumor-normal DNA sample pairs, followed up with RNA sequencing using Illumina HiSeq 2000 and targeted deep resequencing on Illumina MiSeq to validate the identified genetic variants. The authors identified new and recurrent genetic defects, including mutations in *FYN*, *ATM*, *B2M* and *CD58*, implicating SRC signaling, impaired DNA damage response, and PTCLs escape from immune surveillance mechanisms.

Illumina Technology: HiSeq 2000, MiSeq

Papaemmanuil E., Rapado I., Li Y., Potter N. E., Wedge D. C., et al. (2014) RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. Nat Genet 46: 116-125

At least a quarter of ALL cases harbor the *ETV6-RUNX1* fusion gene. Although this gene fusion is characteristic for the disease, additional mutations are required for development of overt leukemia. This study used exome and low-coverage whole-genome sequencing to characterize secondary events associated with leukemic transformation. The authors found that *ATF7IP* and *MGA* are two new tumor-suppressor genes in ALL and described the parsimonious mutational process that transforms *ETV6-RUNX1*-positive lymphoblasts into leukemia.

Illumina Technology: Genome Analyzer

Sakata-Yanagimoto M., Enami T., Yoshida K., Shiraishi Y., Ishii R., et al. (2014) Somatic RHOA mutation in angioimmunoblastic T cell lymphoma. Nat Genet 46: 171-175

Angioimmunoblastic T cell lymphoma (AITL) is a distinct subtype of PTCL. This study investigated the molecular characteristics specific to this lymphoma subtype. Using Illumina HiSeq and MiSeq sequencing for whole-exome, targeted sequencing, and RNA sequencing, the authors identified somatic *RHOA* mutations specifically present in tumor cells. The authors suggest that impaired *RHOA* function, in cooperation with preceding loss of *TET2* function, contributes to AITL-specific pathogenesis.

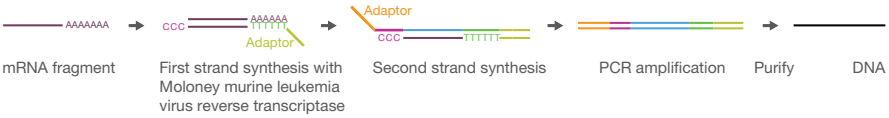
Illumina Technology: MiSeq and HiSeq 2000 for 100 bp reads

Sherwood A. M., Emerson R. O., Scherer D., Habermann N., Buck K., et al. (2013) Tumor-infiltrating lymphocytes in colorectal tumors display a diversity of T cell receptor sequences that differ from the T cells in adjacent mucosal tissue. Cancer Immunol Immunother 62: 1453-1461

SINGLE CELLS AND TCR SEQUENCING

Functional TCRs are heterodimeric proteins that comprise both an α and a β chain. Every T cell contains a unique combination of α and β chains and, for an accurate functional analysis, both subunits must be sequenced together. To avoid disrupting the α and β chain pairing through cell lysis,[96] several single-cell sequencing methods have been developed.[97]

Smart-Seq



Smart-Seq was developed as a single-cell sequencing protocol with improved read coverage across transcripts.[98] In this protocol, cells are lysed and the RNA hybridized to an oligo(dT)-containing primer. The first strand is then created with the addition of a few untemplated C nucleotides. An oligonucleotide primer is then hybridized to the poly(C) overhang and used to synthesize the second strand. Full-length cDNAs are PCR-amplified to obtain nanogram amounts of DNA. The PCR products are purified for sequencing.[99]

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TCR Chain Pairing

Cell-based emulsion RT-PCR technique for identifying TCR α–β chain pairing. Released TCR α and β mRNAs are reverse-transcribed, amplified, and overlap-extended within each droplet. Products are extracted from the emulsion, and fused molecules of interest are selectively amplified. Nonfused molecules are suppressed with blocking primers.[100]

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Ma Y., Mattarollo S. R., Adjemian S., Yang H., Aymeric L., et al. (2014) CCL2/CCR2-dependent recruitment of functional antigen-presenting cells into tumors upon chemotherapy. Cancer Res 74: 436-445

The therapeutic efficacy of anthracyclines as cancer chemotherapy relies on the induction of a DC and T-lymphocyte-dependent anticancer immune response. This study investigated the effects of anthracycline-based chemotherapy on the chemokine CCL2 and its receptor CCR2 in a mouse cancer model. The authors used Illumina Mouse BeadArray to characterize differential gene expression. They found that anthracycline-based chemotherapy promotes the intratumor accumulation of myeloid cells, including cells that mediate antigen presentation. These findings add to the understanding of the anticancer immune response elicited by immunogenic cell death.

Illumina Technology: Mouse (Gene Expression - BeadArray)

Papaemmanuil E., Rapado I., Li Y., Potter N. E., Wedge D. C., et al. (2014) RAG-mediated recombination is the predominant driver of oncogenic rearrangement in ETV6-RUNX1 acute lymphoblastic leukemia. Nat Genet 46: 116-125

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Illumina Technology: Genome Analyzer

Bolotin D. A., Shugay M., Mamedov I. Z., Putintseva E. V., Turchaninova M. A., et al. (2013) MiTCR: software for T-cell receptor sequencing data analysis. Nat Methods 10: 813-814

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Shalek A. K., Satija R., Adiconis X., Gertner R. S., Gaublomme J. T., et al. (2013) Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature 498: 236-240

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