Cancer and the Immune System

An Overview of Recent Publications Featuring Illumina® Technology

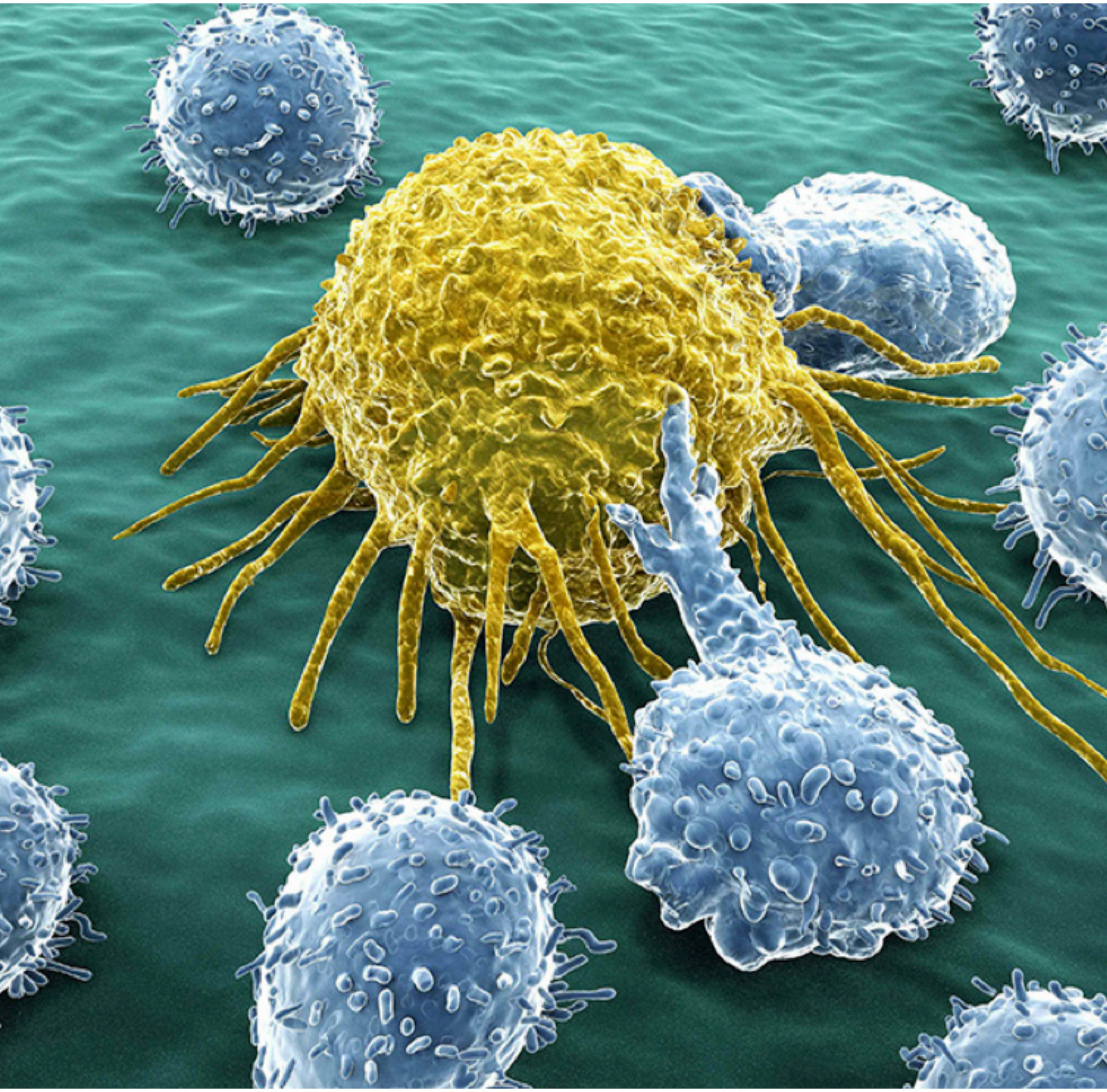


TABLE OF CONTENTS

INTRODUCTION

Advances in high-throughput sequencing have dramatically improved our knowledge of the cancer genome and the intracellular mechanisms involved in tumor progression and response to treatment. While the primary focus to date has been on the cancer cell, this technology can also be used to understand the interaction of the tumor cells and the cells in the surrounding tumor microenvironment. The tumor microenvironment is defined as the cellular environment in which the tumor exists. This includes surrounding blood vessels, immune cells, fibroblasts, other cells, signaling molecules, and the extracellular matrix. Expression analysis of the RNA levels can be used to determine the activation of pathways in the tumor microenvironment. Since common signaling pathways are involved in manifestation of several hallmarks of cancer, including cancer cell proliferation, survival, invasion, metastasis, and immunosuppression, targeting these shared signaling pathways in combination with immunotherapy may be a promising strategy for cancer treatment[1]. It is important to note that RNA-seq has the potential to track the activation of individual clones, which could ultimately lead to personalized treatment[2,3].

The human adaptive immune system provides protection against an enormous variety of pathogens and well as tumors. This protection is mediated by a vast repertoire of receptors on the surface of B and T cells that bind to pathogenic or pathogen derived antigens. With high throughput sequencing, millions of B or T cell receptor sequences can be read in parallel from a single sample. This provides a survey of the of the population of B or T cells already present, but also a real-time, highly-sensitive monitor of clonal expansion and contraction in these cell populations.

Reviews

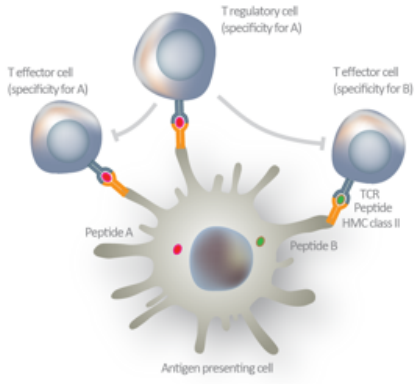
- Linnemann C., Mezzadra R. and Schumacher T. N. (2014) TCR repertoires of intratumoral T-cell subsets. Immunol Rev 257: 72-82
- Robins H. (2013) Immunosequencing: applications of immune repertoire deep sequencing. Curr Opin Immunol 25: 646-652
- Fridman W. H., Pages F., Sautes-Fridman C. and Galon J. (2012) The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12: 298-306

1. 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
2. Ribas A. and Wolchok J. D. (2013) Combining cancer immunotherapy and targeted therapy. Curr Opin Immunol 25: 291-296
3. Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens. Curr Opin Immunol 25: 284-290

DENDRITIC CELLS

Dendritic cells (DC) 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 DC generally induce tolerance rather than stimulate immunity, so most trials now incorporate Toll-like receptor (TLR) ligands and or cytokines to specifically activate DC. DC subsets can also vary in location, phenotype and function. An improved understanding of these complexities may ultimately lead to more effective DC-based cancer vaccines[4].

Dendritic cell and lymphocyte, colored scanning electron micrograph.



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

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

Reviews

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

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 the anthracycline-based chemotherapy promotes the intra-tumor 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

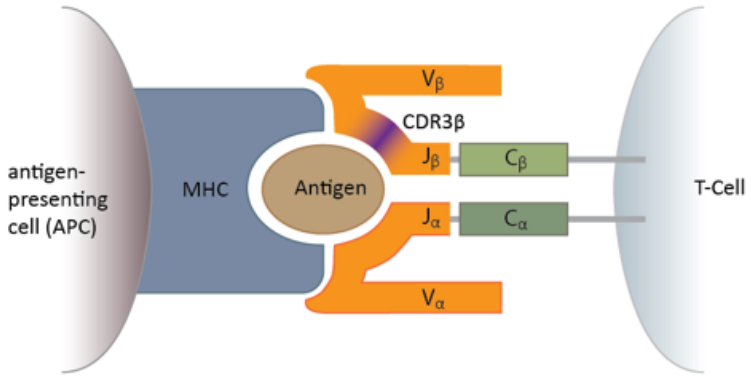
Shalek A. K., Satija R., Adiconis X., Gertner R. S., Gaublomme J. T., et al. (2013) Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature 498: 236-240

Individual cells can exhibit substantial differences in gene expression, and only recently have genome profiling methods been developed to monitor the expression of single cells. This study applied Smart-Seq single-cell RNA sequencing on Illumina HiSeq to investigate heterogeneity in the response of mouse bone marrow–derived dendritic cells (BMDCs) to lipopolysaccharide. The authors found extensive bimodal variation in messenger RNA abundance and splicing patterns, which was subsequently validated using RNA-fluorescence *in situ* hybridization for selected transcripts.

Illumina Technology: HiSeq

T-CELL REPERTOIRE

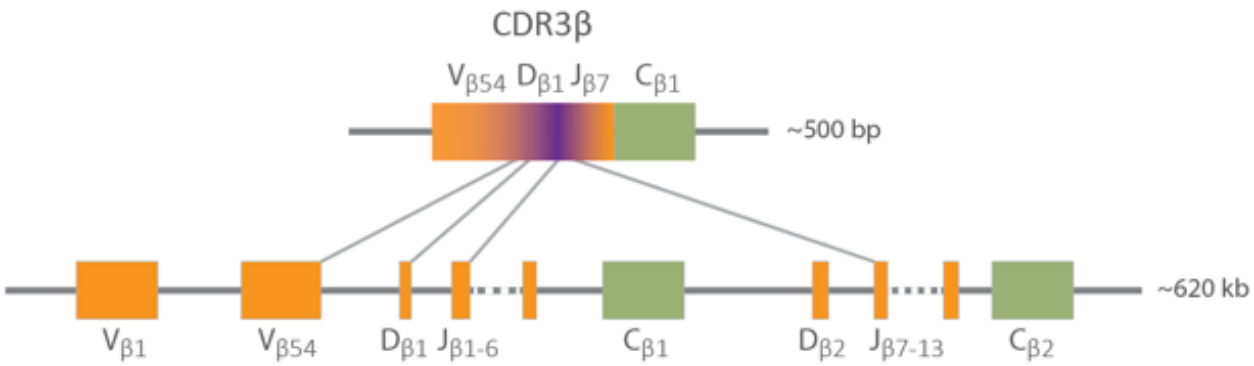
The human immune system provides protection against an enormous variety of pathogens and well as tumors. This protection is mediated by a vast repertoire of receptors, on the surface of B and T cells, that bind to pathogenic or pathogen derived antigens[5]. T cells mediate cellular immunity through the expression of heterodimeric (αβ or γδ) cell surface receptors (T-cell receptors, or TCRs), which engage heterologous cells presenting peptide antigens bound to major histocompatibility complex (MHC)[6].



T-cell receptor-antigen-peptide-MHC interaction and T-cell receptor (TCR) gene recombination. (a) The antigen-presenting cell presents the peptide antigen bound to the major histocompatibility complex (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 T cell receptor (TCR) are short, between 15 and 60 nucleotides, making them particularly suitable for next-generation sequencing. Next generation sequencing has been extensively used to determine the T-populations[7,8,9,10]. In this process the TCR β-chain is commonly used as a marker[11].

5. Robins H. (2013) Immunosequencing: applications of immune repertoire deep sequencing. Curr Opin Immunol 25: 646-652
6. Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease. Genome Med 5: 98
7. 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
8. Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease. Genome Med 5: 98
9. Meier J., Roberts C., Avent K., Hazlett A., Berrie J., et al. (2013) Fractal organization of the human T cell repertoire in health and after stem cell transplantation. Biol Blood Marrow Transplant 19: 366-377
10. La Gruta N. L. and Thomas P. G. (2013) Interrogating the relationship between naive and immune antiviral T cell repertoires. Curr Opin Virol 3: 447-451
11. Linnemann C., Heemskerk B., Kvistborg P., Kluin R. J., Bolotin D. A., et al. (2013) High-throughput identification of antigen-specific TCRs by TCR gene capture. Nat Med 19: 1534-1541



Simplified representation of TCR-β VDJ gene recombination resulting in TCR diversity. The TCR-β locus is located on chromosome 7 and 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 included.

Traditional techniques such as flow cytometry[12] or spectratyping[13] have low resolution and cannot distinguish TCR clonotypes using the same TCR Vβ-segment or CDR3 with the same length[14,15]. Fortunately next generation sequencing (NGS), techniques are able to determine the nucleotide sequences of all TCRβ CDR3 sequences present within a given T-cell population, even for when they are present at very low frequency[16]. Due to the high diversity of the TCRβ CDR3 repertoire, the sequences obtained will in most cases represent individual TCR clonotypes[17]. Next-generation sequencing is an objective tool that can accurately determine T-cell populations for prognosis and monitor response to treatment[18].

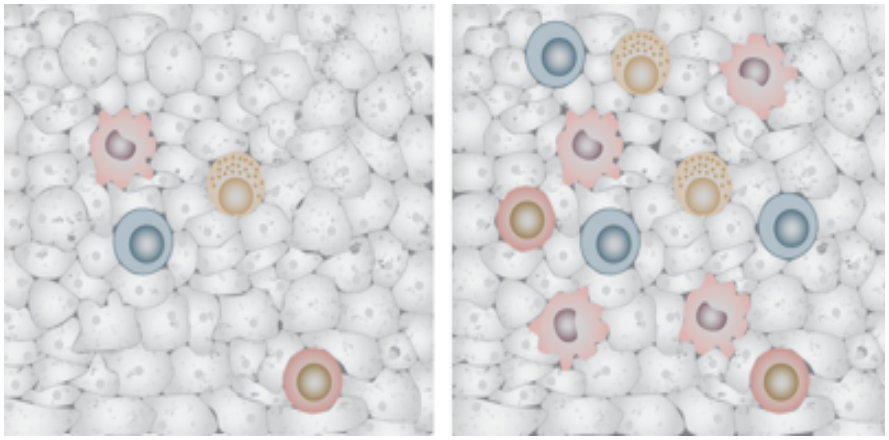
Functional TCRs are heterodimeric proteins that comprising both an α and a β chain. Every T cell contains a unique combination of α and β chains and, for an accurate functional analysis, both subunits must be sequenced together. To avoid disrupting the α and ß chain pairing through cell lysis[19], several single-cell sequencing methods have been developed[20]. See Single Cells and TCR Sequencing for more information.

12. 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
13. 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
14. Linnemann C., Heemskerk B., Kvistborg P., Kluin R. J., Bolotin D. A., et al. (2013) High-throughput identification of antigen-specific TCRs by TCR gene capture. Nat Med 19: 1534-1541
15. 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
16. 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
17. 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: 4099-4107Fridman W. H., Pages F., Sautes-Fridman C. and Galon J. (2012) The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12: 298-306
18. Fridman W. H., Pages F., Sautes-Fridman C. and Galon J. (2012) The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12: 298-306
19. Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease. Genome Med 5: 98
20. 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

INTRATUMORAL T-CELLS

The infiltration of human tumors by T cells is a common phenomenon. It has been observed that the extent of infiltration and the reactivity of the intratumoral T-cell populations can predict the course and the outcome of the disease[21]. 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[22]. This approach can lead to durable cancer regressions in 20–40% of patients with metastatic melanoma, most of whom were refractory to established regimens[23]. To expand this approach requires the identification, or even the genetic modification, of the TILs as well as their targets.

Initial experiments show that next-generation sequencing can be used to quantify immune cell populations within the tumor[25].



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

21. Fridman W. H., Pages F., Sautes-Fridman C. and Galon J. (2012) The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12: 298-306
22. Rosenberg S. A. (1986) The adoptive immunotherapy of cancer using the transfer of activated lymphoid cells and interleukin-2. Semin Oncol 13: 200-206
23. Rosenberg S. A., Yang J. C., Sherry R. M., Kammula U. S., Hughes M. S., et al. (2011) Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 17: 4550-4557
24. Rosenberg S. A. (2014) Finding suitable targets is the major obstacle to cancer gene therapy. Cancer Gene Ther 21: 45-47
25. Angell H. and Galon J. (2013) From the immune contexture to the Immunoscore: the role of prognostic and predictive immune markers in cancer. Curr Opin Immunol 25: 261-267

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

The cellular adaptive immune system mounts a response to many solid tumors mediated by TILs. This study employs the ImmunoSEQ platform (based on Illumina sequencing) for high-throughput molecular characterization of the TCR sequences of the TILs. With this approach, the authors were able to identify the exact clonality of TILs present at specific sampled sections of large tumors and compare them with peripheral blood. The authors showed that the TIL repertoires show strong similarity throughout each tumor and are distinct from the circulating T cell repertoire.

Illumina Technology: HiSeq

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

Tumors from colorectal cancer are generally immunogenic and commonly infiltrated with T lymphocytes. The authors used the ImmunoSEQ platform (based on Illumina sequencing) for high-throughput molecular characterization of the TCR sequences in TILs and compared them with TCRs from adjacent healthy mucosal tissue. The authors found the variation in diversity of the TIL repertoire was far wider than the variation of T-cell clones in the healthy mucosa, and the clonality was higher, on average, in the tumors. They determined that the immune response in the colorectal cancer tumors is different from that in the adjacent mucosal tissue.

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

This paper used a nested-primer PCR protocol to amplify nucleotide fragments before sequencing. T cell clones, each with one fixed productive TCR rearrangement, were doped into complex blood cell samples. Spiked-in clones of known TCRβ CDR3 sequence were then used to assess the accuracy. TCRs from a total of 11 samples were sequenced, with the doped T-cell clones ranging from 10% of the total sample to 0.001% (one cell in 100,000). The assay was able to detect even the rarest clones. The precision of the assay was demonstrated across five orders of magnitude.

Illumina Technology: Genome Analyzer

Reviews

- Ohnuki H., Jiang K., Wang D., Salvucci O., Kwak H., et al. (2014) Tumor-infiltrating myeloid cells activate Dll4/Notch/TGF-ß signaling to drive malignant progression. Cancer research canres. 74:2038-49, 2014

SINGLE CELLS AND TCR SEQUENCING

Functional TCRs are heterodimeric proteins that comprising 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[26] several single-cell sequencing methods have been developed[27].

Smart-Seq. Smart-Seq was developed as a single-cell sequencing protocol with improved read coverage across transcripts[28]. 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[29].

TCR chain pairing. Cell-based emulsion RT-PCR technique for identifying TCR alpha–beta chain pairing. Released TCR alpha and beta 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 30.

Gao C., Kozlowska A., Nechaev S., Li H., Zhang Q., et al. (2013) TLR9 signaling in the tumor microenvironment initiates cancer recurrence after radiotherapy. Cancer Res 73: 7211-7221

This study investigated the mechanism of the reported immunogenic potential of cancer radiotherapy and the response of nucleic acid receptors before and after local radiotherapy. The authors used Illumina HiSeq 2000 for RNA sequencing to characterize the differential expression patterns. The study suggests that combining localized tumor irradiation with myeloid cell-specific inhibition of TLR9/STAT3 signaling may help eliminate radioresistant cancers.

Illumina Technology: HiSeq 2000 for RNA-Seq

26. Woodsworth D. J., Castellarin M. and Holt R. A. (2013) Sequence analysis of T-cell repertoires in health and disease. Genome Med 5: 98
27. 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
28. Ramskold D., Luo S., Wang Y. C., Li R., Deng Q., et al. (2012) Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. Nat Biotechnol 30: 777-782
29. 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
30. 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

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 dendritic cell and T-lymphocyte-dependent anticancer immune responses. 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 intra-tumor 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)

IPapaemmanuil 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 acute lymphoblastic leukemia (ALL) cases have been found to harbor the ETV6-RUNX1 fusion gene. Although the 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

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

Individual cells can exhibit substantial differences in gene expression, and only recently have genome profiling methods been developed to monitor the expression of single cells. This study applied the Smart-seq single-cell RNA sequencing on Illumina HiSeq to investigate heterogeneity in the response of mouse bone-marrow-derived dendritic cells (BMDCs) to lipopolysaccharide. The authors found extensive bimodal variation in messenger RNA abundance and splicing patters, which was subsequently validated using RNA-fluorescence in situ hybridization for select transcripts.

Illumina Technology: HiSeq

Linnemann C., Heemskerk B., Kvistborg P., Kluin R. J., Bolotin D. A., et al. (2013) High-throughput identification of antigen-specific TCRs by TCR gene capture. Nat Med 19: 1534-1541

The transfer of TCR genes into patient T cells is a promising approach for the treatment of both viral infections and cancer. This study presents a new high-throughput assay for identifying TCR sequences by capture and sequencing of TCR genes on the Illumina HiSeq sequencing platform. The approach was validated by the assembly of a large library of cancer germline tumor antigen-reactive TCRs. The authors demonstrated the feasibility of identifying antigen-specific TCRs in oligoclonal T-cell populations from either human material or TCR-humanized mice.

Illumina Technology: TruSeq on Illumina HiSeq 2000

Mamedov I. Z., Britanova O. V., Zvyagin I. V., Turchaninova M. A., Bolotin D. A., et al. (2013) Preparing unbiased T-cell receptor and antibody cDNA libraries for the deep next generation sequencing profiling. Front Immunol 4: 456

This paper presents a detailed protocol, similar to SmartSeq, for the preparation of TCR and IgG cDNA libraries. The protocol can be performed in 1 to 2 days.

Illumina Technology: MiSeq and HiSeq 2000

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

The authors propose a single cell–based method to identify native pairs of alpha-beta TCR CDR3 chains within emulsion droplets by employing: 1) reverse transcription of alpha and beta chain mRNA; 2) PCR amplification; and 3) subsequent fusion via overlap extension. This PCR suppression technique resolves the issue of random overlap extension of gene pairs that may create a high level of noise after the emulsion stage. The authors propose that this methodology can be applied to the identification of native pairs of variable heavy-light antibody chains.

Illumina Technology: MiSeq 2 × 150 bp

Hou Y., Song L., Zhu P., Zhang B., Tao Y., et al. (2012) Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm. Cell 148: 873-885

This essential study establishes single-cell sequencing technology as a qualified means to explore genetic changes at a single-cell nucleotide level. To qualify the method, two single cells for a healthy individual were sequenced and compared for coverage and sequence quality. The assay was subsequently applied to a selection of 58 cells from a single myeloproliferative neoplasm patient to characterize the heterogeneity of the tumor. The false discovery rate of the mutational profiling was assessed by sequencing eight oral mucosal cells for comparison.

Illumina Technology: HiSeq 2000 with 100 bp reads and 1M genotyping

Reviews

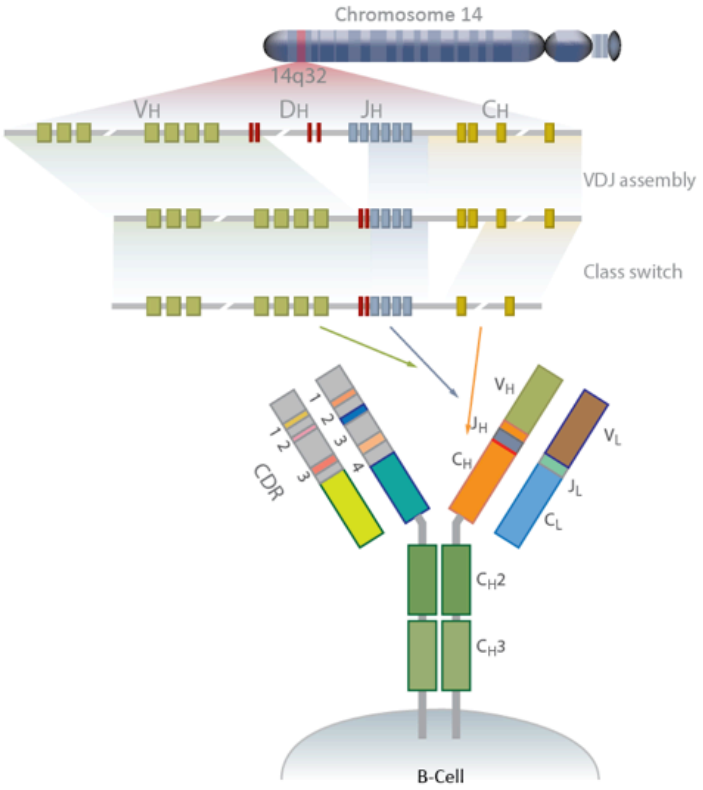
- Bolotin D. A., Shugay M., Mamedov I. Z., Putintseva E. V., Turchaninova M. A., et al. (2013) MiTCR: software for T-cell receptor sequencing data analysis. Nat Methods 10: 813-814
- Bolotin D. A., Mamedov I. Z., Britanova O. V., Zvyagin I. V., Shagin D., et al. (2012) Next generation sequencing for TCR repertoire profiling: platform-specific features and correction algorithms. Eur J Immunol 42: 3073-3083

CANCER ANTIGENS

Due to the extensive genetic and epigenetic alterations, tumor cells produce a vast array of proteins that are not present in normal cells and may 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 'neo-antigens' that arise as a direct consequence of somatic mutations within tumor cells. These neo antigens are therefore very 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[31,32].

The recent explosion in the use of next-generation sequencing to characterize the cancer genome provides a unique opportunity to also characterize the spectrum of potential tumor-specific antigens[33,34,35]. Exome sequencing data from animal models[36,37] as well as human cancers[38,39,40] could predict T-cell reactivities against neo-antigens 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[41,42].

31. Kvistborg P., van Buuren M. M. and Schumacher T. N. (2013) Human cancer regression antigens. Curr Opin Immunol 25: 284-290
32. Walter S., Weinschenk T., Stenzl A., Zdrojowy R., Pluzanska A., et al. (2012) Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 18: 1254-1261
33. Garraway L. A. and Lander E. S. (2013) Lessons from the cancer genome. Cell 153: 17-37
34. Yates L. R. and Campbell P. J. (2012) Evolution of the cancer genome. Nat Rev Genet 13: 795-80
35. Dong H. and Wang S. (2012) Exploring the cancer genome in the era of next-generation sequencing. Front Med 6: 48-55
36. 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
37. Castle J. C., Kreiter S., Diekmann J., Lower M., van de Roemer N., et al. (2012) Exploiting the mutanome for tumor vaccination. Cancer Res 72: 1081-1091
38. 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
39. 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
40. 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
41. Heemskerk B., Kvistborg P. and Schumacher T. N. (2013) The cancer antigenome. EMBO J 32: 194-203
42. 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



The primary antibody heavy chain repertoire is created predominantly by the somatic recombination of variable (V), diversity (D) and joining (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].

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

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

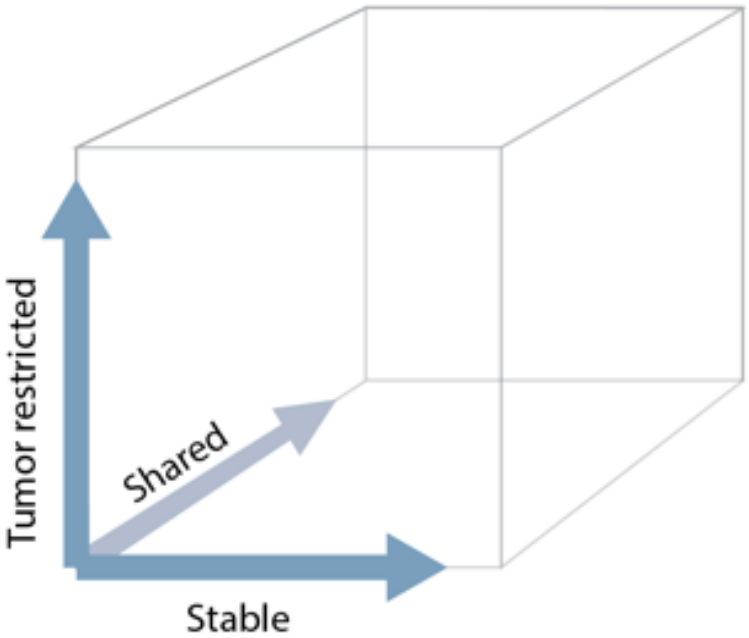
The development of lymphocytes with antitumor activity has become a major effort in studies of current cancer immunotherapy. However, the cancer cell surface proteins that are targeted may still be expressed, albeit at a low level, in healthy tissue with risk serious toxic effects of therapy. The major 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 on 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 new potential for this area of gene therapy.

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

In this review the authors describe two main classes of tumor-specific antigens. Neo-antigens may be newly displayed at the surface of tumor cells because a mutation increases the efficiency with which a peptide is presented by MHC molecules. Self-antigens are the display of epitopes from gene products that are normally only expressed as a consequence of the tissue-specific or transformation-induced gene expression profile of tumor cells.

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 both: 1) shared by patient groups, 2) expressed only in tumor and 3) have low likelihood of antigen loss under selective pressure. With the development of next-generation sequencing 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 3-D representation of human tumor-associated antigen characteristics. Stable: the likelihood of antigen retention upon T-cell pressure, Tumor restricted: degree of uniqueness to tumor compared to normal. Shared: degree of sharing between patients[44].

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

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

The authors used whole exome sequence data to identify the mutated proteins that were expressed in patient tumors. Candidate mutated T cell epitopes that were identified using an MHC binding algorithm were then synthesized and evaluated for recognition by tumor infiltrating lymphocytes (TIL). Using this approach, mutated antigens expressed on autologous tumor cells were identified as targets of three TIL that were associated with objective tumor regressions following adoptive transfer.

Illumina Technology: HiSeq 2000 and TruSeq library construction

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.

This paper is a case report of a patient with stage IV melanoma who exhibited a clinical response to ipilimumab treatment. Cancer exome–guided analysis of T-cell reactivity in this patient revealed reactivity against two neoantigens, including a dominant T-cell response against a mutant epitope of the ATR (ataxia telangiectasia and Rad3 related) gene product that increased strongly after ipilimumab treatment.

Illumina Technology: HiSeq2000 with 75 bp paired-end reads. Exome sequencing as well as mRNA-Seq

Castle J. C., Kreiter S., Diekmann J., Lower M., van de Roemer N., et al. (2012) Exploiting the mutanome for tumor vaccination. Cancer Res 72: 1081-1091

The authors show for the first time a correlation between tumor mutations and the epitope landscape by *in vivo* data, demonstrating that many nonsynonymous somatic mutations in tumors are immunogenic and confer antitumoral vaccine activity.

Illumina Technology: HiSeq 2000 DNA exome sequencing and mRNA-Seq

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

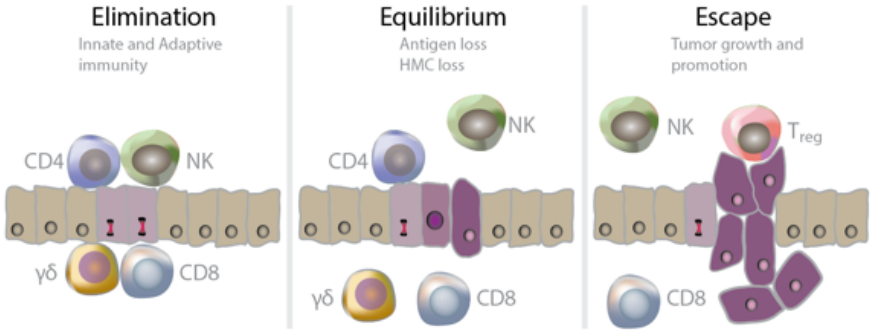
The authors use massively parallel sequencing to characterize expressed mutations in highly immunogenic methylcholanthrene-induced sarcomas derived from immunodeficient Rag22/2 mice. These results demonstrate that the strong immunogenicity of an unedited tumor can be ascribed to expression of highly antigenic mutant proteins and show that outgrowth of tumor cells that lack these strong antigens via a T-cell-dependent immunoselection process.

Immune Epitope Database and Analysis Resource (http://www.immuneepitope.org)

Illumina Technology: Genome Analyzer$_{IIx}$

CANCER IMMUNOEDITING

Cancer immunoediting is the process by which both the adaptive and the innate immune systems control tumor growth and shape the tumor immunogenicity[45-49]. This process consists of three phases: elimination, equilibrium and escape[50]. 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 major histocompatibility complex. 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 experimentally identify tumor epitopes, and to specifically identify epitopes from unedited tumors. This has been demonstrated by a recent study which coupled massively parallel sequencing with a cDNA capture sequencing (cDNA CapSeq) technique. The study revealed that T-cell dependent immunoediting is a mechanism underlying the proliferation of tumor cells that lack strong rejection antigens[51].

45. Schreiber R. D., Old L. J. and Smyth M. J. (2011) Cancer immunoediting: integrating mmunity's roles in cancer suppression and promotion. Science 331: 1565-1570
46. Dunn G. P., Koebel C. M. and Schreiber R. D. (2006) Interferons, immunity and cancer immunoediting. Nat Rev Immunol 6: 836-848
47. 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
48. Dunn G. P., Old L. J. and Schreiber R. D. (2004) The three Es of cancer immunoediting. Annu Rev Immunol 22: 329-360
49. 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
50. 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 27C: 16-25
51. 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

Cap-Seq. CXXC affinity purification sequencing (CAP-Seq)[52] 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

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 27C: 16-25
- 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
- Vogelstein B., Papadopoulos N., Velculescu V. E., Zhou S., Diaz L. A., Jr., et al. (2013) Cancer genome landscapes. Science 339: 1546-1558
- Greulich H., Kaplan B., Mertins P., Chen T. H., Tanaka K. E., et al. (2012) Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2. Proc Natl Acad Sci U S A 109: 14476-14481

52. 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

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

Cancer immunoediting is a process by which the immune system controls tumor outgrowth and modulates tumor immunogenicity. The authors used massively parallel sequencing to characterize expressed mutations in highly immunogenic methylcholanthrene-induced sarcomas derived from immunodeficient Rag2 -/- mice. They employed a modified form of exome sequencing involving complementary DNA (cDNA) capture by mouse exome probes and deep sequencing, termed cDNA capture sequencing (cDNA CapSeq). They identified mutant spectrin-β2 as a potential rejection antigen of the d42m1 sarcoma. They also demonstrated that T-cell-dependent immunoselection is a mechanism underlying the outgrowth of tumor cells that lack tong rejection antigens.

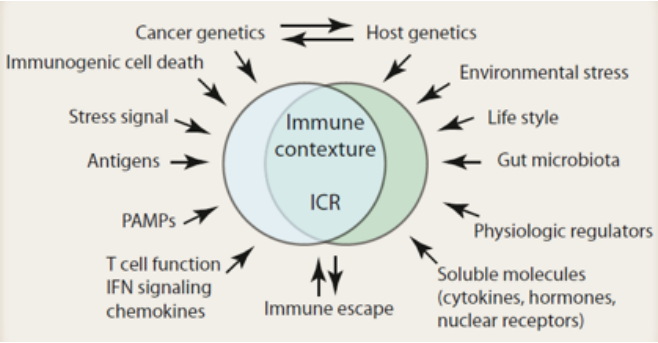
Illumina Technology: Genome Analyzer$_{IIx}$ for cDNA capture sequencing (cDNA CapSeq)

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 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 the metastatic lesions, while been reproducible between the primary and metastatic tumor. Nevertheless the prognostic impact of the Th1/cytotoxic T cell infiltrate could be different according to the origin of the primary tumor[53].

Emerging evidence suggests that distinct subsets of tumors may exist that reflect 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 can be found to characterize subsets of tumors.

There have also been consolidated findings that 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, which in turn increases mutation rates and, ultimately, augment tumor growth[54].



A schematic describing the potential cancer-centric and host-centric contributing factors, leading to the immune contexture and ICR. These are counter balanced by immune escape mechanisms. PAMPS, pathogen-associated molecular pattern[55].

Reviews

- 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 27C: 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

53. 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 27C: 8-15
54. Kundu J. K. and Surh Y. J. (2008) Inflammation: gearing the journey to cancer. Mutat Res 659: 15-30
55. 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

Reviews

- 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
- Hooper L. V., Littman D. R. and Macpherson A. J. (2012) Interactions between the microbiota and the immune system. Science 336: 1268-1273
- Klinke D. J., 2nd (2012) An evolutionary perspective on anti-tumor immunity. Front Oncol 2: 202
- Otranto M., Sarrazy V., Bonte F., Hinz B., Gabbiani G., et al. (2012) The role of the myofibroblast in tumor stroma remodeling. Cell Adh Migr 6: 203-219
- Gajewski T. F., Fuertes M., Spaapen R., Zheng Y. and Kline J. (2011) Molecular profiling to identify relevant immune resistance mechanisms in the tumor microenvironment. Curr Opin Immunol 23: 286-292

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 27C: 89-97

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

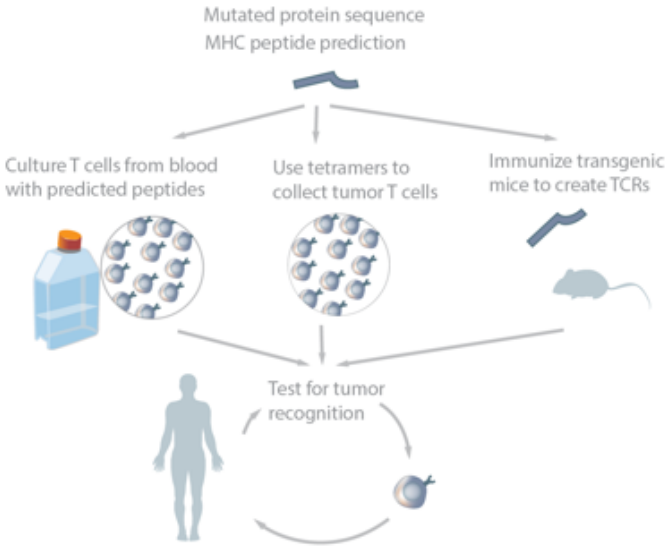
Arthur J. C., Perez-Chanona E., Mühlbauer M., Tomkovich S., Uronis J. M., et al. (2012) Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338: 120-123

Inflammation alters host physiology to promote cancer, as seen in colitis-associated colorectal cancer (CRC). Inflammatory cells and their associated mediators, including interleukin-6 (IL-6), tumor necrosis factor-alpha TNF-alpha), IL-23 and reactive oxygen species, have been implicated in creating a microenvironment conducive to the development of CRC. The authors sequenced the V6 region of 16S ribosomal RNA of carcinogen-treated $IL10^{-/-}$ and wild-type mice. They reveal that inflammation alters microbial richness. Subsequent mono-association of $IL10^{-/-}$ mice with polyketide synthase (pks)-deficient *E. coli* revealed that intestinal inflammation can alter microbial composition, which can promote tumorigenesis.

Illumina Technology: HiSeq 2000 for paired-end sequencing of 16S rRNA

CANCER IMMUNOTHERAPY

Intratumoral T cells have been utilized therapeutically in clinical studies of adoptive T-cell therapy[56]. For patients with metastatic melanoma, tumor-infiltrating lymphocyte (TIL)-based adoptive transfer can result in tumor shrinkage of approximately 50%[57]. Conversely, the absence of TILs is considered to be a marker for poor efficacy of immunotherapies[58]. 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, but unfortunately this is effective only in a fraction of patients[59].



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[60].

Tumor immunogenicity results from mutations that generate tumor-specific antigens (TSAs). This is a common characteristic of most, but not all, cancers[61]. 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 rapidly identify mutations in individual tumors, computationally predict peptides that can best stimulate T cell responses, and vaccinate patients against the unique TSAs in their tumors[62,63,64].

DC-based cancer vaccines are well tolerated with few side effects and can generate anti-tumor immune responses, but overall they have been of limited benefit. Recent studies have demonstrated that CD141+ DC play an important role in antitumor responses. Vaccines that directly target DC *in vivo* are under development[65].

56. Linnemann C., Mezzadra R. and Schumacher T. N. (2014) TCR repertoires of intratumoral T-cell subsets. Immunol Rev 257: 72-82
57. Darcy P. K., Neeson P., Yong C. S. and Kershaw M. H. (2014) Manipulating immune cells for adoptive immunotherapy of cancer. Curr Opin Immunol 27C: 46-52
58. Gajewski T. F. and Schumacher T. (2013) Cancer immunotherapy. Curr Opin Immunol 25: 259-260
59. Skrombolas D. and Frelinger J. G. (2014) Challenges and developing solutions for increasing the benefits of IL-2 treatment in tumor therapy. Expert Rev Clin Immunol 10: 207-217
60. Restifo N. P., Dudley M. E. and Rosenberg S. A. (2012) Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 12: 269-281
61. 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
62. 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
63. Castle J. C., Kreiter S., Diekmann J., Lower M., van de Roemer N., et al. (2012) Exploiting the mutanome for tumor vaccination. Cancer Res 72: 1081-1091
64. 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
65. Radford K. J., Tullett K. M. and Lahoud M. H. (2014) Dendritic cells and cancer immunotherapy. Curr Opin Immunol 27C: 26-32

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 27C: 89-97

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

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 to identify genes that modify the action of tumor-infiltrating CD8 T cells in tumor-bearing mice.

Illumina Technology: Genome Analyzer

Reviews

- Hinrichs C. S. and Rosenberg S. A. (2014) Exploiting the curative potential of adoptive T-cell therapy for cancer. Immunol Rev 257: 56-71
- Darcy P. K., Neeson P., Yong C. S. and Kershaw M. H. (2014) Manipulating immune cells for adoptive immunotherapy of cancer. Curr Opin Immunol 27C: 46-52
- Nishikawa H. and Sakaguchi S. (2014) Regulatory T cells in cancer immunotherapy. Curr Opin Immunol 27C: 1-7
- 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
- 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
- Restifo N. P., Dudley M. E. and Rosenberg S. A. (2012) Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 12: 269-281

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

This study is an investigation of immunoediting in cancer using Illumina Genome Analyzer. Targeted exome sequencing (cDNA capture) is used to characterize expressed mutations in highly immunogenic tumor cells in mice. A mutant spectrin-beta2 is predicted and validated as a rejection antigen, concluding that highly antigenic mutant proteins are the cause of the strong immunogenicity of unedited tumors.

Illumina Technology: Genome Analyzer

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

The authors describe a screening approach based on mining whole exome sequence data to identify the mutated proteins that were expressed in patient tumors. Candidate mutated T cell epitopes were identified using an MHC binding algorithm were synthesized and evaluated for recognition by tumor infiltrating lymphocytes. They found expressed, mutated antigens that were targeted by tumor infiltrating lymphocytes in three patients with objective tumor regressions following adoptive transfer.

Illumina Technology: HiSeq 2000

Castle J. C., Kreiter S., Diekmann J., Lower M., van de Roemer N., et al. (2012) Exploiting the mutanome for tumor vaccination. Cancer Res 72: 1081-1091

The immunogenicity and specificity of 50 validated mutations was determined by immunizing mice with long peptides encoding the mutated epitopes. The authors demonstrate the use of deep sequencing to systematically analyze immunogenicity mutations may pave the way for individualized immunotherapy of cancer patients.

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.

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

Reviews

- Greulich H., Kaplan B., Mertins P., Chen T. H., Tanaka K. E., et al. (2012) Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2. Proc Natl Acad Sci U S A 109: 14476-14481

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, and 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 next-generation sequencing 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, which implicate autoreactive T-cell clones in the pathogenesis of clonal stem cell disorders such as myelodysplastic syndromes (MDS) and aplastic anemia (AA)[66]. These studies have been supported by the widely consolidated understanding that impairment of anti-tumor 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 have quickly become one of the most exciting research areas in hematology[67,68,69].

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 25C: 114-123
- Fozza C. and Longinotti M. (2013) T-cell receptor repertoire usage in hematologic malignancies. Crit Rev Oncol Hematol 86: 201-211
- Warren E. H., Matsen F. A. t. and Chou J. (2013) High-throughput sequencing of B- and T-lymphocyte antigen receptors in hematology. Blood 122: 19-22
- Gottgens B. (2012) Genome-scale technology driven advances to research into normal and malignant haematopoiesis. Scientifica (Cairo) 2012: 437956

66. Fozza, C., and Longinotti, M. (2013) T-cell receptor repertoire usage in hematologic malignancies. Critical reviews in oncology/hematology 86: 201–211
67. Faham, M., Zheng, J., Moorhead, M., Carlton, V. E. H., Stow, P., et al. (2012) Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 120: 5173–5180
68. 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
69. 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

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: MiSeq and HiSeq 2000

Dose M., Emmanuel A. O., Chaumeil J., Zhang J., Sun T., et al. (2014) β-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 beta-catenin and genomic instability. The authors studied a mice model with targeted activation of beta-catenin and used ChIP-sequencing to determine the link between transcription factor binding sites and locations of translocation sites. The authors concluded the beta-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$_{IIx}$

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 acute lymphoblastic leukemia (ALL) cases have been found to harbor the ETV6-RUNX1 fusion gene. Although the 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 peripheral T cell lymphoma (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

In this study the authors used HTS to capture all potential V-J rearrangement combinations in the variable regions of TCRB and TCRG from pre-treatment and post-treatment samples of individuals with T-lineage acute lymphoblastic leukemia/lymphoma (T-ALL). This study identified the CDR3 repertoire from the pre-treatment samples of the individuals and subsequently paired these sequences to those in the post-treatment samples to assess minimal residual disease. The study reports that TCRB and TCRG HTS identified clonality at diagnosis in most cases (31 of 43 for TCRB and 27 of 43 for TCRG) and subsequent detected MRD. Given the greater germline diversity of TCRB, relative to TCRG, the authors' data supports TCRB sequencing for MRD assessment due to the greater specificity.

Illumina Technology: HiSeq for 54 bp reads

Gawad C., Pepin F., Carlton V. E., 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

B precursor acute lymphoblastic leukemia (B-ALL), is thought to result from malignant transformation and expansion of a single B cell. The authors sequenced the VH and JH segments to characterize the repertoire of IgH sequences in diagnostic samples of 51 children with B-ALL. The IgH rearrangement frequencies serve as a proxy for clonotype abundance. The authors report the presence of clonal IgH rearrangements in 43 of 51 cases with a wide-range of evolved IgH sequences (0 to 4024). They propose that this data indicates that VH replacement is the dominant molecular mechanism responsible for clonal evolution in B-ALL patients. They also suggest that IgH locus evolution in leukemic cell clones may be recapitulating some of the mechanisms of allelic exclusion that have been observed in normal lymphocytes, in which the V_H to D-J_H recombination is activated on one chromosome and inhibited on the other.

Illumina Technology: Genome Analyzer$_{IIx}$ for 115 bp reads from the J_H to V_H direction and 95 from V_H to J_H

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. Sci Transl Med 4: 149ra118

Acute myeloid leukemia (AML) is caused by the accumulation of multiple mutations in a single clone. This raises the question of how these mutations can accumulate in a single clone given the low rate of spontaneous mutation in hematopoietic cells and the lack of global genomic instability in leukemia. Here the authors used exome sequencing to identify coding mutations present in six AML patient samples with the FLT3-ITD, an internal tandem duplication mutation found in a high-risk AML subgroup. Subsequently, they sequenced fluorescent activated cell-sorted rare residual HSCs and detected leukemia-associated mutations including some mutations found in NPM1, TET2, and SMC1A genes. This study reveals a potential mechanism for AML clonal evolution and raises awareness regarding the importance of therapeutic targeting of pre-leukemic cells for more durable remission.

Illumina Technology: Genome Analyzer$_{IIx}$ (2 x 76 bp reads) and HiSeq 2000 (2 x 100 bp reads) for exome and transcriptome sequencing

Reviews

- Hou Y., Song L., Zhu P., Zhang B., Tao Y., et al. (2012) Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm. Cell 148: 873-885

TRACKING MALIGNANT LYMPHOCYTES

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[70-76] and TCRβ/α[77] has been utilized at the time of diagnosis to identify the malignant clones in patients with B- and T-lymphoid malignancies. This information was then used to track the malignant clones during and post-treatment. Serial sequencing of the IGH locus in pediatric B-ALL has revealed surprisingly dynamic evolution of the locus in some patients[74], demonstrating that this technology may also generate valuable insights into the biology of B- and T-cell cancers.

For the monitoring of lymphoid malignancies deep sequencing of antigen-receptor loci has multiple advantages. Compared to alternative approaches, deep sequencing demands less time, labor, has superior sensitivity[74,75,76], and can simultaneously track all of the clones that comprise the malignant population. Its utility for monitoring disease burden in patients with CLL[70-73], pediatric B-lineage ALL[74,75], and T-lineage ALL[77] has been demonstrated, and it will undoubtedly have utility for monitoring other lymphoid malignancies.

Logan, A. C., Zhang, B., Narasimhan, B., Carlton, V., Zheng, J., et al. (2013) Minimal residual disease quantification using consensus primers and high-throughput IGH sequencing predicts post-transplant relapse in chronic lymphocytic leukemia. Leukemia 1–7

In this study, the authors employed a HTS-based method to facilitate minimal residual disease quantification in chronic lymphocytic leukemia remitted patients who underwent hematopoietic cell transplantation. They used degenerate consensus primers to amplify all immunoglobulin heavy chain (IGH) genes in a mixture of polyclonal lymphoid cells. This IGH-HTS method has a validated detection limit of 10-6 and quantitative range above 10-5. They found that MRD doubling time <12 months with disease burden ≥10-5 was associated with relapse within 12 months of MRD assessment in 50% of patients, and within 24 months in 90% of patients. This analysis appears to have a similar predictive value as those published by other groups using ASO-PCR or multi parameter flow cytometry to quantify CLL MRD. These sensitive characteristics paired with the scalability features of this method offers significant benefits to the development of clinical trials testing therapeutic interventions in CLL based on MRD quantification.

Illumina Technology: Genome Analyzer for 115 bp for IGH-J to V reads and 95 bp reads from IGH–V to J.

70. Campbell, P. J., Pleasance, E. D., Stephens, P. J., Dicks, E., Rance, R., et al. (2008) Subclonal phylogenetic structures in cancer revealed by ultra-deep sequencing. Proceedings of the National Academy of Sciences of the United States of America 105: 13081–13086
71. Boyd, S. D., Marshall, E. L., Merker, J. D., Maniar, J. M., Zhang, L. N., et al. (2009) Measurement and clinical monitoring of human lymphocyte clonality by massively parallel VDJ pyrosequencing. Science Translational Medicine 1: 12ra23
72. Logan, A. C., Gao, H., Wang, C., Sahaf, B., Jones, C. D., et al. (2011) High-throughput VDJ sequencing for quantification of minimal residual disease in chronic lymphocytic leukemia and immune reconstitution assessment. Proceedings of the National Academy of Sciences 108: 21194–21199
73. Kalos, M., Levine, B. L., Porter, D. L., Katz, S., Grupp, S. A., et al. (2011) T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Science Translational Medicine 3: 95ra73
74. 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
75. Faham, M., Zheng, J., Moorhead, M., Carlton, V. E. H., Stow, P., et al. (2012) Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 120: 5173–5180
76. Brentjens, R. J., Davila, M. L., Riviere, I., Park, J., Wang, X., et al. (2013) CD19-Targeted T Cells Rapidly Induce Molecular Remissions in Adults with Chemotherapy-Refractory Acute Lymphoblastic Leukemia. Science Translational Medicine 5: 177ra38–177ra38
77. Wu, D., Sherwood, A., Fromm, J. R., Winter, S. S., Dunsmore, K. P., et al. (2012) High-Throughput Sequencing Detects Minimal Residual Disease in Acute T Lymphoblastic Leukemia. Science Translational Medicine 4: 134ra63–134ra63

Faham, M., Zheng, J., Moorhead, M., Carlton, V. E. H., Stow, P., et al. (2012) Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 120: 5173–5180

Quantification of minimal residual disease (MRD), the measurement of residual leukemia levels, during therapy has emerged as the most important predictor of outcome in ALL. This sequencing assay was used to detect rearrangements of all rearranged IgH and TCR gene segments present in leukemic clones: IgH@ complete (VHDJH), IGH@ incomplete (DJH), TRB@, TRD@, and TRG@, in the diagnostic bone marrow samples of 100 ALL patients known to have IgH rearrangements. The authors found that IgH@ complete VHDJH was most frequent gene rearrangement (n=96) and TRD@ was the second most frequent. This authors also reported that this sequencing assay detected minimal residual disease (MRD) in all 28 samples shown to be positive by flow cytometry, detected MRD in 35 of 36 shown to be positive by ASO-PCR and revealed MRD in 10 and 3 additional samples that were negative by flow cytometry and ASO-PCR, respectively.

Illumina Technology: Genome Analyzer for 115 base-pairs for IGH-J to V reads and 95 bp reads from IGH–V to J

Faham, M., Zheng, J., Moorhead, M., Carlton, V. E. H., Stow, P., et al. (2012) Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 120: 5173–5180

Quantification of minimal residual disease (MRD), the measurement of residual leukemia levels, during therapy has emerged as the most important predictor of outcome in ALL. This sequencing assay was used to detect rearrangements of all rearranged IgH and TCR gene segments present in leukemic clones: IgH@ complete (VHDJH), IGH@ incomplete (DJH), TRB@, TRD@, and TRG@, in the diagnostic bone marrow samples of 100 ALL patients known to have IgH.

Illumina Technology: Genome Analyzer for 115 base-pairs for IGH-J to V reads and 95 bp reads from IGH–V to J

Wei, Y., Chen, R., Dimicoli, S., Bueso-Ramos, C., Neuberg, D., et al. (2013) Global H3K4me3 genome mapping reveals alterations of innate immunity signaling and overexpression of JMJD3 in humanmyelodysplastic syndrome CD34. 1–10

The pathogenesis of myelodysplastic syndromes (MDS) has been attributed to genetic and epigenetic lesions, including alterations of DNA methylation and histone modifications. The authors used genome-wide H3Kme3 ChIP-Seq analysis of primary MDS bone marrow (BM) CD34+ cells to assess H3K4me3 genomic distribution in hematopoietic stem/early progenitor cells (HSPCs) of MDS. They identified 36 genes marked by differentially higher levels of promoter H3K4me3 in MDS; a majority of which were involved in innate immunity regulation and nuclear factor (NF)κ-B activation. Subsequent histone demethylase expression analysis of these promoter genes identified significant over expression of JMJD3, encoding a JmjC-domain with histone demethylase activity. Collectively, this data suggests that deregulation of H3K4me3 and newly identified role of JMJD3 in the transcription regulation of the genes identified by ChIP-Seq has a role in the pathogenesis of MDS.

Illumina Technology: HiSeq 2000 for 100 bp paired-end reads.

Reviews

- Warren E. H., Matsen F. A. t. and Chou J. (2013) High-throughput sequencing of B- and T-lymphocyte antigen receptors in hematology. Blood 122: 19-22
- Weng W. K., Armstrong R., Arai S., Desmarais C., Hoppe R., et al. (2013) Minimal residual disease monitoring with high-throughput sequencing of T cell receptors in cutaneous T cell lymphoma. Sci Transl Med 5: 214ra171
- Wu, D., Sherwood, A., Fromm, J. R., Winter, S. S., Dunsmore, K. P., et al. (2012) High-Throughput Sequencing Detects Minimal Residual Disease in Acute T Lymphoblastic Leukemia. Science Translational Medicine 4: 134ra63–134ra63

BIBLIOGRAPHY

Angell H. and Galon J. (2013) From the immune contexture to the Immunoscore: the role of prognostic and predictive immune markers in cancer. Curr Opin Immunol 25: 261-267

Arthur J. C., Perez-Chanona E., Mühlbauer M., Tomkovich S., Uronis J. M., et al. (2012) Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 338: 120-123

Bolotin D. A., Mamedov I. Z., Britanova O. V., Zvyagin I. V., Shagin D., et al. (2012) Next generation sequencing for TCR repertoire profiling: platform-specific features and correction algorithms. Eur J Immunol 42: 3073-3083 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

Boyd S. D., Marshall E. L., Merker J. D., Maniar J. M., Zhang L. N., et al. (2009) Measurement and clinical monitoring of human lymphocyte clonality by massively parallel VDJ pyrosequencing. Sci Transl Med 1: 12ra23

Brentjens R. J., Davila M. L., Riviere I., Park J., Wang X., et al. (2013) CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med 5: 177ra138

Campbell P. J., Pleasance E. D., Stephens P. J., Dicks E., Rance R., et al. (2008) Subclonal phylogenetic structures in cancer revealed by ultra-deep sequencing. Proc Natl Acad Sci U S A 105: 13081-13086

Castle J. C., Kreiter S., Diekmann J., Lower M., van de Roemer N., et al. (2012) Exploiting the mutanome for tumor vaccination. Cancer Res 72: 1081-1091

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

Dong H. and Wang S. (2012) Exploring the cancer genome in the era of next-generation sequencing. Front Med 6: 48-55

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

Dunn G. P., Old L. J. and Schreiber R. D. (2004) The three Es of cancer immunoediting. Annu Rev Immunol 22: 329-360

Dunn G. P., Koebel C. M. and Schreiber R. D. (2006) Interferons, immunity and cancer immunoediting. Nat Rev Immunol 6: 836-848

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

Faham M., Zheng J., Moorhead M., Carlton V. E., Stow P., et al. (2012) Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. Blood 120: 5173-5180

Fozza C. and Longinotti M. (2013) T-cell receptor repertoire usage in hematologic malignancies. Crit Rev Oncol Hematol 86: 201-211

Fridman W. H., Pages F., Sautes-Fridman C. and Galon J. (2012) The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12: 298-306

Gajewski T. F., Fuertes M., Spaapen R., Zheng Y. and Kline J. (2011) Molecular profiling to identify relevant immune resistance mechanisms in the tumor microenvironment. Curr Opin Immunol 23: 286-292

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

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

Gao C., Kozlowska A., Nechaev S., Li H., Zhang Q., et al. (2013) TLR9 signaling in the tumor microenvironment initiates cancer recurrence after radiotherapy. Cancer Res 73: 7211-7221

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

Garraway L. A. and Lander E. S. (2013) Lessons from the cancer genome. Cell 153: 17-37

Gawad C., Pepin F., Carlton V. E., 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

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

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 27C: 8-15

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

Gottgens B. (2012) Genome-scale technology driven advances to research into normal and malignant haematopoiesis. Scientifica (Cairo) 2012: 437956

Greulich H., Kaplan B., Mertins P., Chen T. H., Tanaka K. E., et al. (2012) Functional analysis of receptor tyrosine kinase mutations in lung cancer identifies oncogenic extracellular domain mutations of ERBB2. Proc Natl Acad Sci U S A 109: 14476-14481

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

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

Hooper L. V., Littman D. R. and Macpherson A. J. (2012) Interactions between the microbiota and the immune system. Science 336: 1268-1273

Hou Y., Song L., Zhu P., Zhang B., Tao Y., et al. (2012) Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm. Cell 148: 873-885

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

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. Sci Transl Med 4: 149ra118

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

Kalos M., Levine B. L., Porter D. L., Katz S., Grupp S. A., et al. (2011) T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 3: 95ra73

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

Klinke D. J., 2nd (2012) An evolutionary perspective on anti-tumor immunity. Front Oncol 2: 202

Kundu J. K. and Surh Y. J. (2008) Inflammation: gearing the journey to cancer. Mutat Res 659: 15-30

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

La Gruta N. L. and Thomas P. G. (2013) Interrogating the relationship between naive and immune antiviral T cell repertoires. Curr Opin Virol 3: 447-451

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

Linnemann C., Heemskerk B., Kvistborg P., Kluin R. J., Bolotin D. A., et al. (2013) High-throughput

identification of antigen-specific TCRs by TCR gene capture. Nat Med 19: 1534-1541

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

Logan A. C., Gao H., Wang C., Sahaf B., Jones C. D., et al. (2011) High-throughput VDJ sequencing for quantification of minimal residual disease in chronic lymphocytic leukemia and immune reconstitution assessment. Proc Natl Acad Sci U S A 108: 21194-21199

Logan A. C., Zhang B., Narasimhan B., Carlton V., Zheng J., et al. (2013) Minimal residual disease quantification using consensus primers and high-throughput IGH sequencing predicts post-transplant relapse in chronic lymphocytic leukemia. Leukemia 27: 1659-1665

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

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

Mamedov I. Z., Britanova O. V., Zvyagin I. V., Turchaninova M. A., Bolotin D. A., et al. (2013) Preparing unbiased T-cell receptor and antibody cDNA libraries for the deep next generation sequencing profiling. Front Immunol 4: 456

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

Meier J., Roberts C., Avent K., Hazlett A., Berrie J., et al. (2013) Fractal organization of the human T cell repertoire in health and after stem cell transplantation. Biol Blood Marrow Transplant 19: 366-377

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 27C: 16-25

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

Ohnuki H., Jiang K., Wang D., Salvucci O., Kwak H., et al. (2014) Tumor-infiltrating myeloid cells activate Dll4/Notch/TGF-ß signaling to drive malignant progression. Cancer research canres. 3118.2013

Otranto M., Sarrazy V., Bonte F., Hinz B., Gabbiani G., et al. (2012) The role of the myofibroblast in tumor stroma remodeling. Cell Adh Migr 6: 203-219

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

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

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 27C: 89-97

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

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

Ramskold D., Luo S., Wang Y. C., Li R., Deng Q., et al. (2012) Full-length mRNA-Seq from single-cell levels of RNA and individual circulating tumor cells. Nat Biotechnol 30: 777-782

Restifo N. P., Dudley M. E. and Rosenberg S. A. (2012) Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 12: 269-281

Ribas A. and Wolchok J. D. (2013) Combining cancer immunotherapy and targeted therapy. Curr Opin Immunol 25: 291-296

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

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

Robins H. (2013) Immunosequencing: applications of immune repertoire deep sequencing. Curr Opin Immunol 25: 646-652

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

Rosenberg S. A. (1986) The adoptive immunotherapy of cancer using the transfer of activated lymphoid cells and interleukin-2. Semin Oncol 13: 200-206

Rosenberg S. A., Yang J. C., Sherry R. M., Kammula U. S., Hughes M. S., et al. (2011) Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res 17: 4550-4557

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

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

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

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

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

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

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

Skrombolas D. and Frelinger J. G. (2014) Challenges and developing solutions for increasing the benefits of IL-2 treatment in tumor therapy. Expert Rev Clin Immunol 10: 207-217

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

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

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

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

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

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

Walter S., Weinschenk T., Stenzl A., Zdrojowy R., Pluzanska A., et al. (2012) Multipeptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. Nat Med 18: 1254-1261

Warren E. H., Matsen F. A. t. and Chou J. (2013) High-throughput sequencing of B- and T-lymphocyte antigen receptors in hematology. Blood 122: 19-22

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 25C: 114-123

Wei Y., Chen R., Dimicoli S., Bueso-Ramos C., Neuberg D., et al. (2013) Global H3K4me3 genome mapping reveals alterations of innate immunity signaling and overexpression of JMJD3 in human myelodysplastic syndrome CD34+ cells. Leukemia 27: 2177-2186

Weng W. K., Armstrong R., Arai S., Desmarais C., Hoppe R., et al. (2013) Minimal residual disease monitoring with high-throughput sequencing of T cell receptors in cutaneous T cell lymphoma. Sci Transl Med 5: 214ra171

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

Wu D., Sherwood A., Fromm J. R., Winter S. S., Dunsmore K. P., et al. (2012) High-throughput sequencing detects minimal residual disease in acute T lymphoblastic leukemia. Sci Transl Med 4: 134ra163

Yates L. R. and Campbell P. J. (2012) Evolution of the cancer genome. Nat Rev Genet 13: 795-806

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

Pub. No. 1170-2014-004 Current as of 29 May 2014

illumina®