

Understanding the Science behind Immuno-Oncology

Using the body's natural immune response to fight cancer

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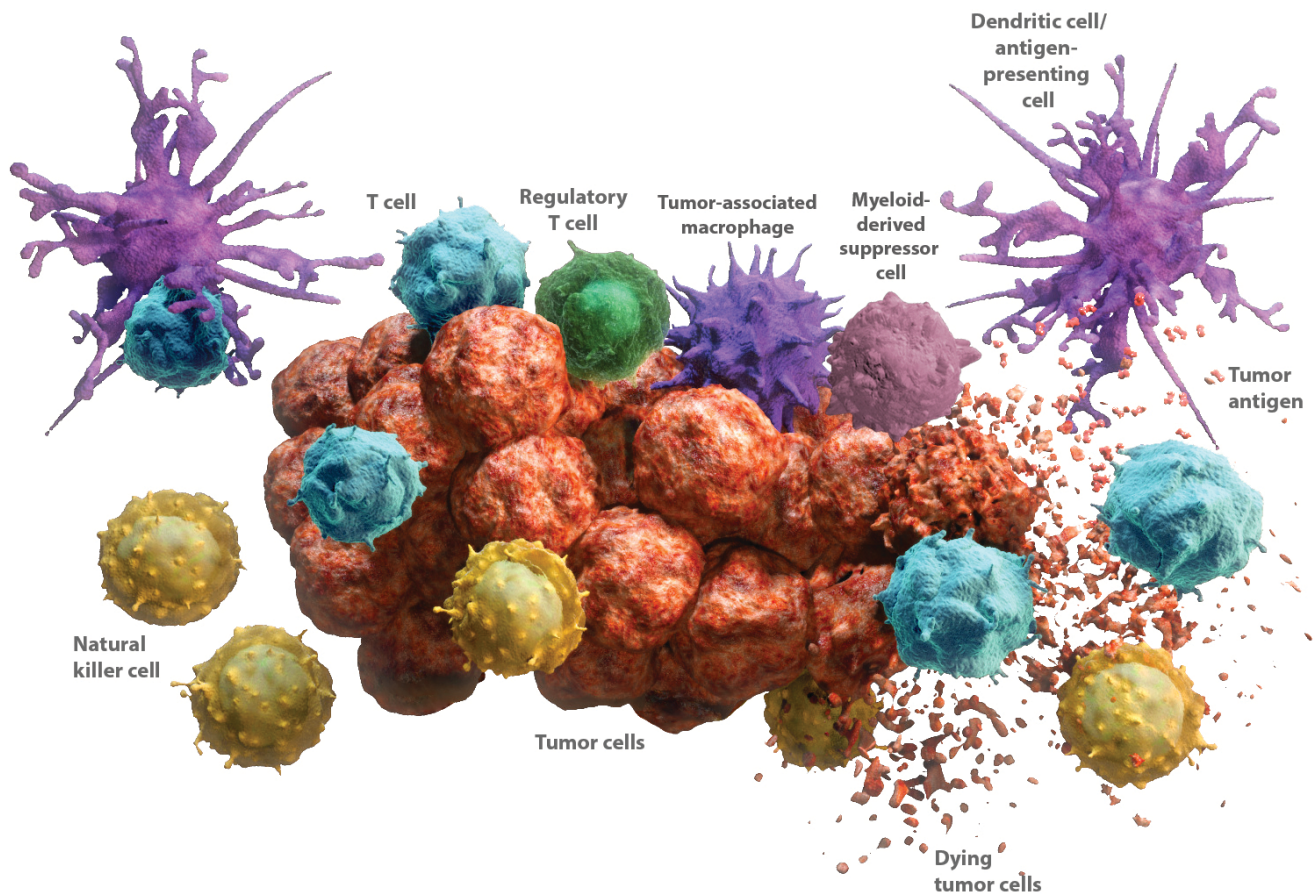
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Bristol-Myers Squibb – At the Forefront of Immuno-Oncology Research

Revealing the Potential of the Immune System in Cancer

Introduction to the tumor microenvironment and the immune response

The immune system is capable of **recognizing and eliminating tumor cells** in the tumor microenvironment. Innate and adaptive immunity act as a complementary network of self-defense against foreign threats.¹ This ability to recognize **foreign threats (nonself)** as distinct from **normal cells (self)**, is an essential feature of the immune system.²⁻⁴ Despite originating from normal cells, tumor cells can be recognized as nonself through production of tumor antigens.⁵

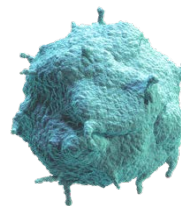


Antitumor activity of the innate and adaptive immune responses



Innate immune response

The first line of defense, it rapidly identifies and attacks tumor cells without antigen specificity.^{1,6-8} It recognizes activating and inhibitory signals from target cells to distinguish self from nonself.⁹⁻¹¹ **Natural Killer (NK) cells** are the main effector cells of innate immunity.¹



Adaptive immune response

An antigen-specific response that is activated by recognition of tumor antigens (nonself).^{1,8} Once activated, it can be sustained through a durable memory response.¹² **Cytotoxic T cells** are the main effector cells of adaptive immunity.¹

The antitumor activity of NK cells and cytotoxic T cells is regulated through a network of **activating** and **inhibitory** signaling pathways.^{4,13,14}



Activating Pathways:
Stimulating pathways trigger immune responses

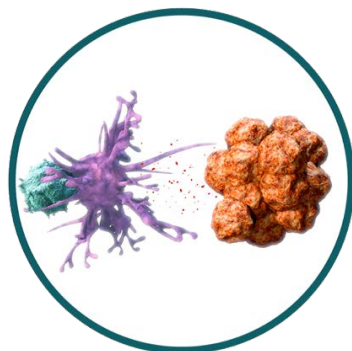


Inhibitory Pathways:
Pathways that counterbalance immune activation

The **balance between activating and inhibitory pathways** normally enables the immune system to attack tumor cells, while sparing healthy cells.¹³

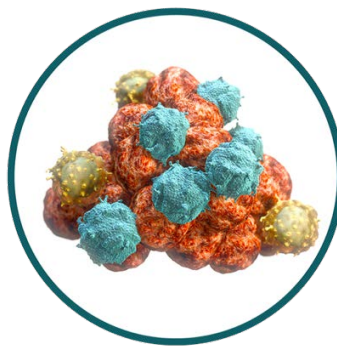
Key stages of the antitumor immune response

In both the innate and adaptive immune responses, immune cells have the potential to recognize and eliminate tumor cells. There are **three principal stages** in this process:



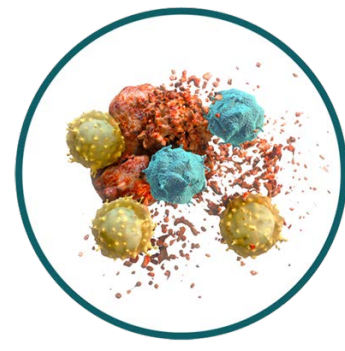
Presentation

The innate immune system rapidly identifies and attacks tumor cells. Tumor cell death releases tumor antigens, which can activate the cytotoxic T cells of the adaptive immune system.^{6,15}



Infiltration

Tumor antigens and other factors attract immune cells to the tumor site, where they invade and attack.¹⁵



Elimination

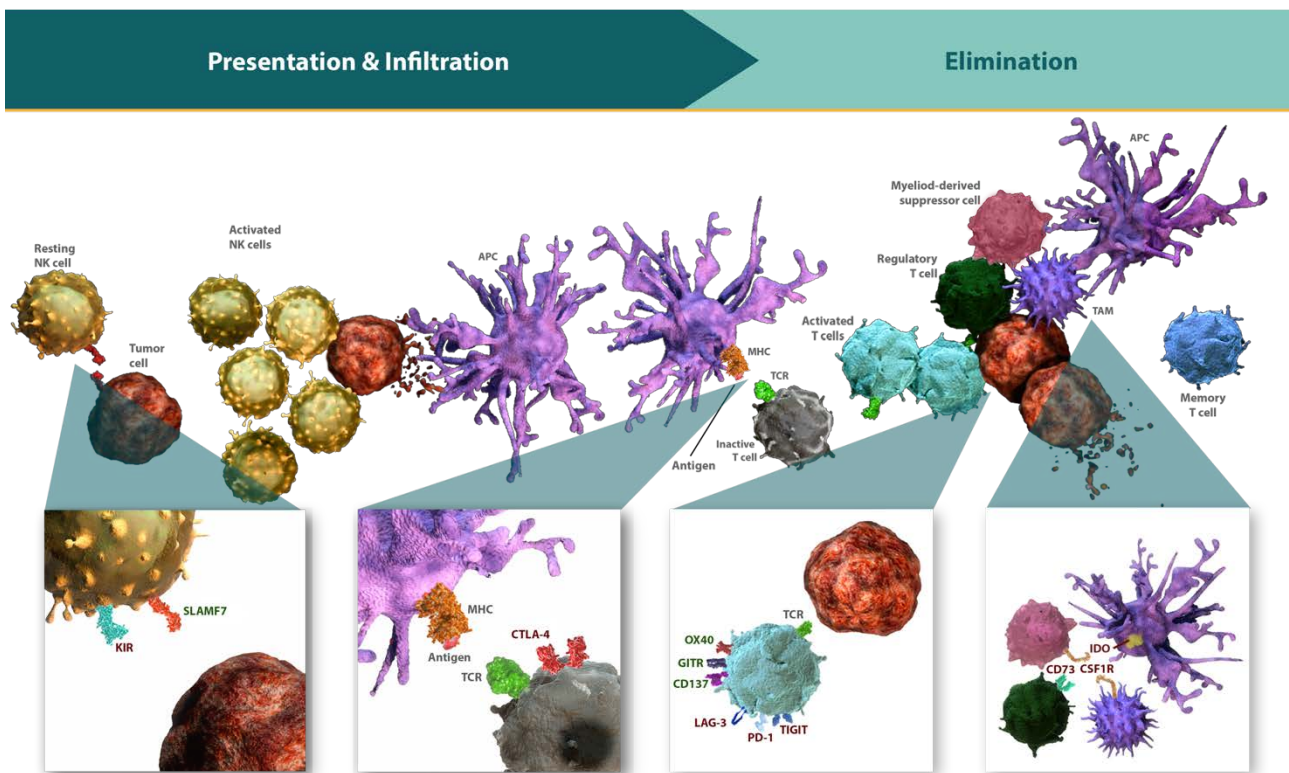
Activated cytotoxic T cells recognize tumor cells as the source of the antigen and target them for elimination.¹⁵

Tumor cells can evade and suppress immune activity

In order to survive and grow, tumor cells employ **different strategies** to outsmart the stages of the antitumor immune response. These mechanisms range from failing to **present** tumor antigens, to increasing expression of inhibitory proteins to prevent **elimination** by cytotoxic T cells.¹⁶⁻²⁰ The success of these strategies determines the ability of immune cells to react to the tumor.²¹

Immune pathways combine to refine response

The three stages of the immune response—presentation, infiltration, and elimination—are regulated through a network of **activating** and **inhibitory** signaling pathways that **combine to maintain immune balance**.^{4,14} Reestablishing the pathways that are impaired within tumors is a key focus of ongoing Immunology research.



Modulating immune pathways in combination may enhance the immune response, as suggested by preclinical data.²²⁻²⁵

Select pathways that modulate NK cell activity

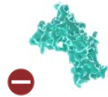
Current research is investigating the following **NK cell mechanisms** to understand how they can be modulated to restore the body's natural ability to fight cancer.

Activating pathway



SLAMF7 is an activating receptor on the surface of NK cells and other immune cells.²⁶ When engaged, SLAMF7 activates NK cells, the rapid responders of the immune system and the body's first line of defense against cancer.^{7,27}

Inhibitory pathway



KIR is an immune checkpoint receptor on the surface of NK cells that acts to stop NK cells from killing normal cells.¹⁰

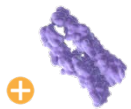
Select pathways that modulate effector T cell activity

Current research is investigating the following effector T cell mechanisms to understand how they can be modulated to restore the body's natural ability to fight cancer.

Activating pathways



CD137 is an activating receptor on the surface of NK cells and T cells that can stimulate them to reproduce and generate antitumor activity.^{28,29}

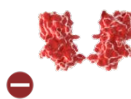


GITR is an activating receptor on the surface of T cells and other immune cells that helps to enhance cell reproduction and generate antitumor activity.³⁰⁻³²



OX40 is an activating receptor on the surface of activated cytotoxic T cells and Tregs.³³⁻³⁵ OX40 plays a dual role in the immune response, both activating and amplifying T-cell responses.³⁶⁻³⁹

Inhibitory pathways



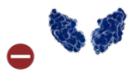
CTLA-4 is an immune checkpoint receptor on T cells that plays a key role in preventing T-cell overactivation.⁴⁰⁻⁴³ CTLA-4 signaling diminishes the ability of memory T cells to sustain an immune response.⁴⁴



PD-1 is an immune checkpoint receptor on cytotoxic T cells that plays a key role in T-cell exhaustion and prevention of autoimmunity.⁴⁵⁻⁴⁷



LAG-3 is an immune checkpoint receptor on the surface of both activated cytotoxic and regulatory T cells (Tregs).^{48,49} LAG-3 can negatively regulate T-cell proliferation and the development of lasting memory T cells.⁵⁰

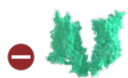


TIGIT is an immune checkpoint receptor expressed on the surface of cytotoxic, memory, and regulatory T cells (Tregs), as well as natural killer (NK) cells.^{51,52} On all of these cells, TIGIT can play a role in immune suppression.⁵¹⁻⁵³

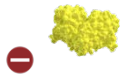
Select pathways that modulate non-effector cell activity

Current research is investigating the following non-effector cell mechanisms to understand how they can be modulated to restore the body's natural ability to fight cancer.

Inhibitory pathways



CD73 is a cell-surface enzyme on Tregs. CD73 is a critical checkpoint in the production of adenosine, which is a powerful inhibitor of the antitumor immune response, including proliferation and production of cytokines.⁵⁴



IDO is an intracellular enzyme that initiates the breakdown of tryptophan, an amino acid that is essential for T-cell survival.⁵⁵⁻⁵⁷



CSF1R is a receptor on the surface of macrophages and other cells of the myeloid lineage.⁵⁸ CSF1, the ligand for CSF1R, is a dominant regulator of macrophage differentiation and function.⁵⁹

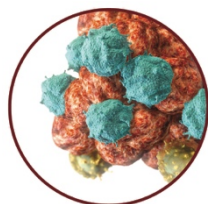
Ongoing Immuno-Oncology research focuses on these select signaling pathways, either alone or in combination, to understand how they can be modulated to restore the body's natural ability to fight cancer.

Exploring Predictors of Response: Immune-Biomarkers

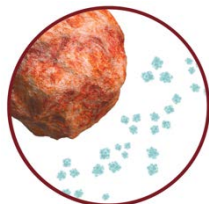
Immune-biomarkers are indicators of immune activity

Immune-biomarkers are measures of activity within the tumor microenvironment, differing from established gene mutation biomarkers, such as BRAF and EGFR.⁶⁰⁻⁶³

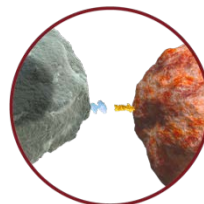
As components and regulators of the immune response, immune-biomarkers include:⁶⁰



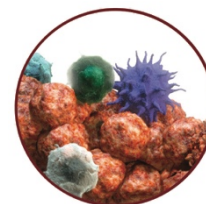
Tumor-infiltrating immune cells



Secreted peptides



Cell surface proteins

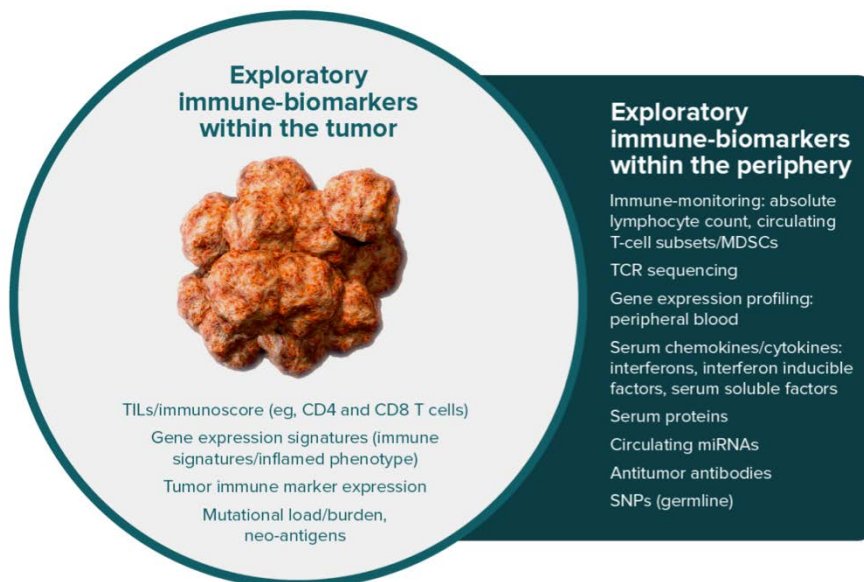


Immunosuppressive cells

Evaluating multiple immune-biomarkers may provide a more realistic representation of the tumor microenvironment, as well as a more accurate and comprehensive assessment of clinical relevance.^{63,64}

Exploratory immune-biomarkers

New immune-biomarkers are now being investigated across tumor types:⁶⁵⁻⁷⁶



The field of immune-biomarkers aims to characterize the ongoing interactions between the immune system and cancer.⁶⁰

Evolving Clinical Expectations in Immuno-Oncology

Immuno-Oncology is a different approach to fighting cancer

Immuno-Oncology seeks to activate the body's natural immune response to fight cancer. This is a fundamentally different approach to cancer treatment.

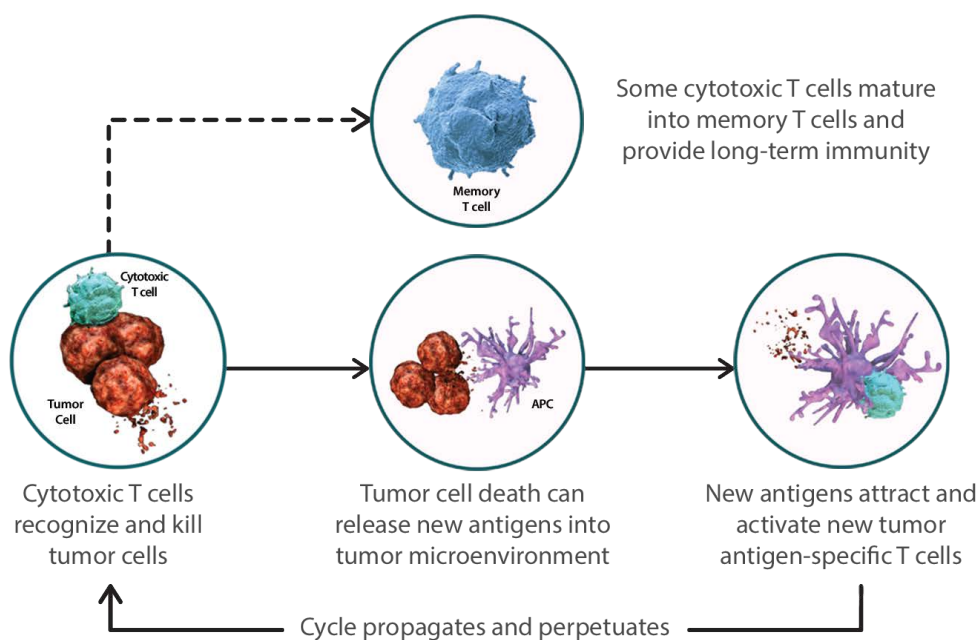
With this new approach comes unique considerations and distinctive characteristics that continue to be researched, such as:

- » Immune responses have the potential to deepen and sustain over time
- » Unique patterns of response, such as pseudo-progression
- » Unique endpoint considerations
- » Immune-mediated adverse reactions

Immune responses have the potential to deepen and sustain over time

The immune response evolves and expands over time by constantly recognizing and remembering tumor antigens. This ability—to propagate and perpetuate—suggests the intelligent nature of the immune response.¹⁵

Immune responses are dynamic and have the potential to improve and deepen over time.⁷⁷

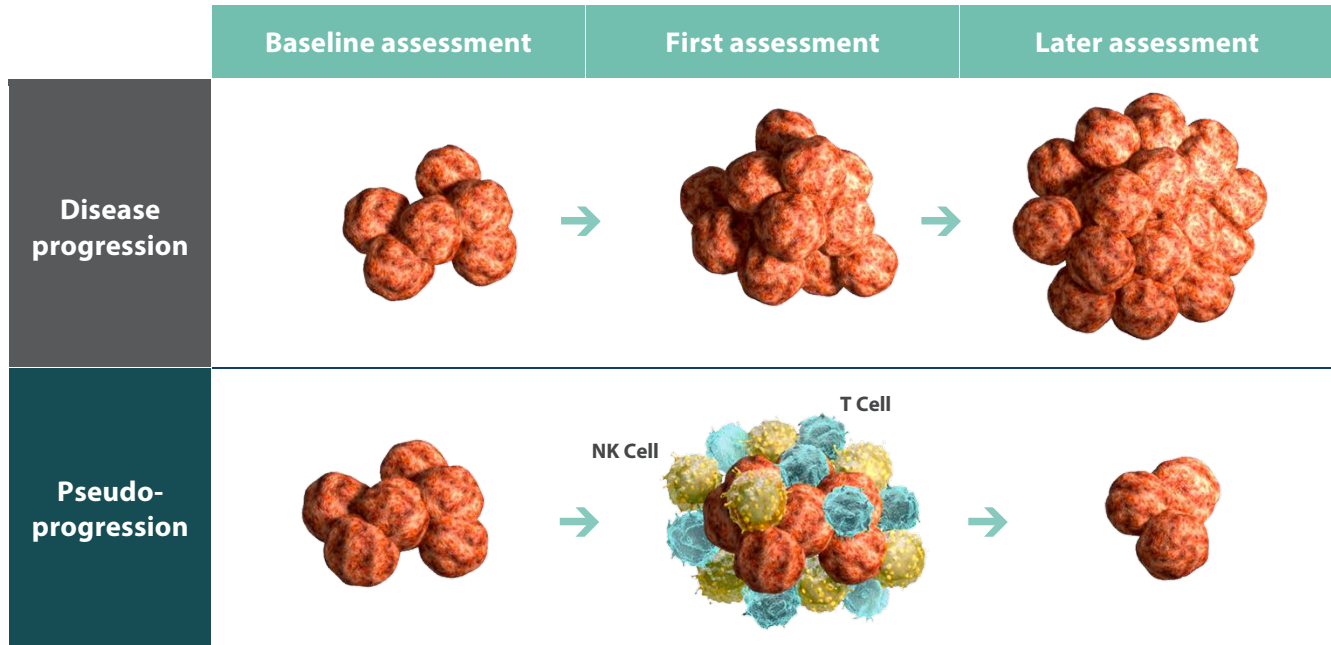


As the immune response continues to expand, some cytotoxic T cells mature into memory T cells that may provide long-term immune protection, even if the original stimulus is no longer present.^{12,78}

Pseudo-progression may reflect development of antitumor immunity

The nature of the antitumor immune response can create the appearance of disease progression, either as tumor growth or appearance of new lesions.⁷⁹ This is known as **pseudo-progression**. Pseudo-progression does not reflect tumor cell growth, but may be misclassified as disease progression.^{79,80}

Tumors may **appear to grow or new lesions may appear when immune cells infiltrate the tumor site**.⁷⁹ Due to the time required to mount an adaptive immune response, pseudo-progression may also reflect continued tumor growth until a sufficient response develops.^{79,81}



Pseudo-progression may be considered until disease progression can be confirmed

While uncommon, **pseudo-progression is an important consideration** when evaluating response to Immuno-Oncology therapies.⁸¹ Histologic confirmation is not always possible, but close monitoring of the following factors may help identify pseudo-progression:^{79,82}

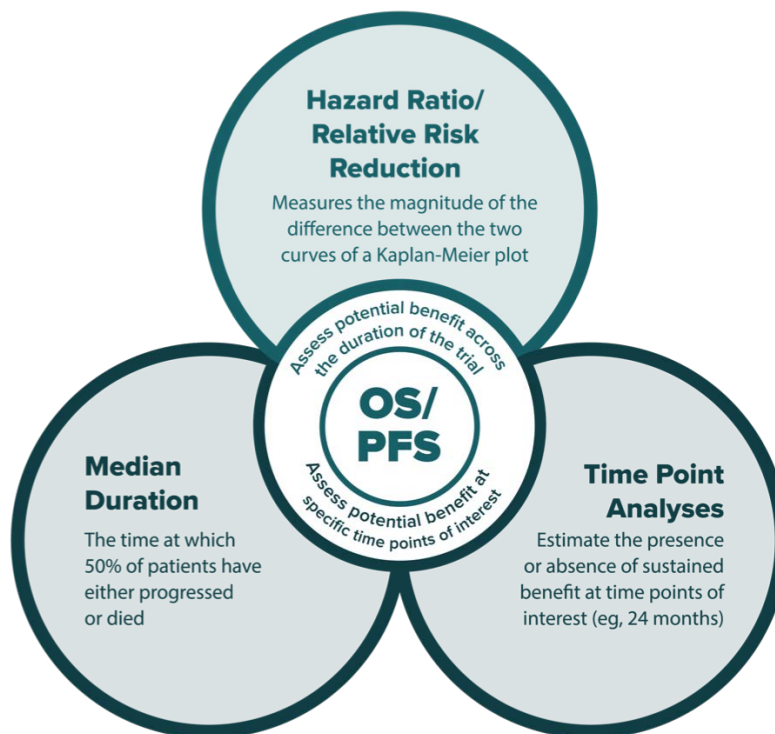
	Disease progression	Pseudo-progression
Performance status	Deterioration of performance	Remains stable or improves
Systemic symptoms	Worsen	May or may not improve
Symptoms of tumor enlargement	Present	May or may not be present
Tumor burden		
Baseline	Increase	Initial increase followed by response
New lesions	Appear and increase in size	Appear then remain stable and/or subsequently respond
Biopsy may reveal	Evidence of tumor growth	Evidence of immune-cell infiltration

Endpoint considerations for Immuno-Oncology research

The criteria currently used to assess potential benefit of cancer therapies are based on surgery, radiation therapy, and chemotherapy.⁸³ However, for **Immuno-Oncology**, a different way to fight cancer, a more comprehensive approach to endpoint assessment may be needed to recognize potential benefit.⁸⁴⁻⁸⁸

Overall survival (OS), **progression-free survival (PFS)**, and **response rate** are among endpoints used to measure outcomes in oncology research.^{89,90} OS is the **gold standard** to assess therapeutic benefit when possible.⁹⁰

In addition, key measures of response are **magnitude (size)**—measured as the proportion of patients with a predefined decrease in tumor burden, called the **Objective Response Rate (ORR)**—and **duration (time)**—assessed as the time from initial tumor response to disease progression, called the **Duration of Response (DoR)**.⁸⁹



Assessment of these measures **in combination** can provide a broad and comprehensive picture of the difference between the investigational arm and the control arm with respect to PFS and OS.^{85-87,91}

Applying multiple measures can illustrate the full scope of clinical benefit.

Immune-mediated adverse reactions

Immuno-Oncology therapies that modulate immune pathways **may enable the immune system to attack healthy cells** along with tumor cells. The effects are known as immune-mediated adverse reactions.⁹²

When managing complications of immune-mediated adverse reactions, please consider:

- » Patients, caregivers, and physicians should be educated to remain vigilant throughout and after Immuno-Oncology treatment to minimize complications, some of which may be life threatening⁹²
- » In addition, treatment algorithms are available for use by healthcare providers to assist them in managing immune-mediated adverse reactions⁹³

As research in immune system activation advances and more data are made available, understanding and appropriate management of immune-mediated adverse reactions will evolve.⁹⁴

Realizing the Potential of Immuno-Oncology Research

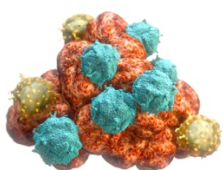
Depth of evidence for the immune response to cancer

Both solid tumors and hematologic malignancies are able to induce an immune response that can regulate their growth. This ability is known as **tumor immunogenicity**.^{95,96} The body is able to recognize and attack cancer through the following mechanisms:



Presentation

Traditionally immunogenic tumors are defined by a high rate of mutations.⁹⁷ These mutations create tumor antigens that can be recognized by the immune system, activating an antitumor immune response.⁹⁸



Infiltration

Tumor-infiltrating immune cells are present in the tumor microenvironment.⁹⁹⁻¹¹¹ Their presence demonstrates their capacity to identify and migrate to tumor cells.¹¹²



Elimination

Early in their development, some tumors display evidence of spontaneous regression.¹¹³ This suggests that the immune system is able to recognize and eliminate some tumor cells, and supports the concept that the body's own immune system has the ability to induce an antitumor response against cancer.^{113,114}

Broad potential of Immuno-Oncology research

Evidence for tumor immunogenicity across a wide range of solid tumors and hematologic malignancies provides the rationale for the breadth of Immuno-Oncology research across tumor types:¹¹⁵

Tumor Type	Evidence for tumor immunogenicity		
	PRESENTATION Presence of somatic mutations	INFILTRATION Evidence of immune-cell infiltration	ELIMINATION Evidence of spontaneous regression
Bladder ^{97,109}	● 97	● 109	
Breast ^{111,116}	● 116	● 111	
Colorectal ¹¹⁰	● 110	● 110	
Gastric/Esophageal ^{102,117}	● 117	● 102	
Glioblastoma ^{98,100}	● 98	● 100	
Head & Neck ^{103,118}	● 118	● 103	
Hepatocellular ¹⁰⁷	● 107	● 107	
Lung ^{97,102}	● 97	● 102	
Melanoma ^{97,102,113}	● 97	● 102	● 113
Ovarian ^{106,119}	● 119	● 106	
Pancreatic ¹¹⁰	● 110	● 110	
Prostate ^{104,120}	● 120	● 104	
Renal ^{97,105}	● 97	● 105	● 105
Non-Hodgkin Lymphoma ^{99,121}	● 121	● 99	
Hodgkin Lymphoma ^{108,122}	● 122	● 108	
Leukemia ¹²³	● 123		
Multiple Myeloma ^{124,101}	● 124	● 101	

Immuno-Oncology research is constantly evolving

Some of the ongoing research at Bristol-Myers Squibb focuses on:

- » Building an understanding of the dynamic mechanisms that govern the immune system's response to cancer
- » Understanding the role of immune signaling pathways, either alone or in combination, and how they can be modulated to restore the body's natural ability to fight cancer
- » Understanding predictors of response, such as immune-biomarkers, to help identify patients who are more likely to benefit from Immuno-Oncology therapies
- » Developing a more comprehensive approach to endpoint assessment, to better recognize the potential benefit of Immuno-Oncology research

The potential of Immuno-Oncology research continues to expand, driven by the many patients with advanced cancer who await the offer of renewed hope and the potential of a longer life.

For more detailed information on the science behind Immuno-Oncology, please visit www.iohcp.com.

References

1. Warrington R, Watson W, Kim HL, Antonetti FR. An introduction to immunology and immunopathology. *Allergy Asthma Clin Immunol.* 2011;7(suppl 1):S1-S8.
2. Van Parijs L, Abbas AK. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science.* 1998;280(5361):243-248.
3. Mapara MY, Sykes M. Tolerance and cancer: mechanisms of tumor evasion and strategies for breaking tolerance. *J Clin Oncol.* 2004;22(6):1136-1151.
4. Leung J, Suh W-K. The CD28-B7 family in anti-tumor immunity: emerging concepts in cancer immunotherapy. *Immune Netw.* 2014;14(6):265-276.
5. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science.* 2015;348(6230):69-74.
6. Liu C, Lou Y, Lizée G, et al. Plasmacytoid dendritic cells induce NK cell dependent, tumor antigen-specific T cell cross-priming and tumor regression in mice. *J Clin Invest.* 2008;118(3):1165-1175.
7. Cheng M, Chen Y, Xiao W, Sun R, Tian Z. NK cell-based immunotherapy for malignant diseases. *Cell Mol Immunol.* 2013;10:230-252.
8. Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer.* 2004;4(1):11-22.
9. Bryceson YT, Ljunggren HG, Long EO. Minimal requirement for induction of natural cytotoxicity and intersection of activation signals by inhibitory receptors. *Blood.* 2009;114(13):2657-2666.
10. Campbell KS, Purdy AK. Structure/function of human killer cell immunoglobulin-like receptors: lessons from polymorphisms, evolution, crystal structures and mutations. *Immunology.* 2011;132(3):315-325.
11. Martinet L, Smyth MJ. Balancing natural killer cell activation through paired receptors. *Nat Rev Immunol.* 2015;15:243-254.
12. Lau LL, Jamieson BD, Somasundaram T, Ahmed R. Cytotoxic T-cell memory without antigen. *Nature.* 1994;369(6482):648-652.
13. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer.* 2012;12(4):252-264.
14. Long EO, Kim HS, Liu D, Peterson ME, Rajagopalan S. Controlling natural killer cell responses: integration of signals for activation and inhibition. *Ann Rev Immunol.* 2013;31:227-258.
15. Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity.* 2013;39:1-10.
16. Harlin H, Meng Y, Peterson AC, et al. Chemokine expression in melanoma metastases associated with CD8+ T-cell recruitment. *Cancer Res.* 2009;69(7):3077-3085.
17. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β -catenin signalling prevents anti-tumour immunity. *Nature.* 2015;523(7559):231-235.
18. Gajewski TF. The next hurdle in cancer immunotherapy: overcoming the non-T-cell-inflamed tumor microenvironment. *Semin Oncol.* 2015;42(4):663-671.
19. Spranger S, Spaepen RM, Zha Y, et al. Up-regulation of PD-L1, IDO, and Tregs in the melanoma tumor microenvironment is driven by CD8+ T cells. *Sci Transl Med.* 2013;5:200ra116. doi:10.1126/scitranslmed.3006504
20. Ahmadzadeh M, Johnson LA, Heemskerck B, et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. *Blood.* 2009;114(8):1537-1544.
21. Hegde PS, Karanikas V, Evers S. The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. *Clin Cancer Res.* 2016;22(8):1865-1874.
22. Chen S, Lee LF, Fisher TS, et al. Combination of 4-1BB agonist and PD-1 antagonist promotes antitumor effector/memory CD8 T cells in a poorly immunogenic tumor model. *Cancer Immunol Res.* 2014;3(2):149-160.
23. Lu L, Xu X, Zhang B, Zhang R, Ji H, Wang X. Combined PD-1 blockade and GITR triggering induce a potent antitumor immunity in murine cancer models and synergizes with chemotherapeutic drugs. *J Transl Med.* 2014;12:36. doi: 10.1186/1479-5876-12-36.
24. Curran MA, Montalvo Welby, Yagita H, Allison JP. PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc Natl Acad Sci USA.* 2010;107(9):4275-4280.
25. Woo SR, Turnis ME, Goldberg MV, et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res.* 2011;72(4):917-927.
26. Bouchon A, Cella M, Grierson HL, Cohen JI, Colonna M. Cutting edge: activation of NK cell-mediated cytotoxicity by a SAP-independent receptor of the CD2 Family. *J Immunol.* 2001;167(10):5517-5521.
27. Cruz-Munoz ME, Dong Z, Shi X, Zhang S, Veillette A. Influence of CRACC, a SLAM family receptor coupled to the adaptor EAT-2, on natural killer cell function. *Nat Immunol.* 2009;10(3):297-305.
28. Pollok KE, Kim YJ, Zhou Z, et al. Inducible T cell antigen 4-1BB. Analysis of expression and function. *J Immunol.* 1993;150(3):771-781.
29. Melero I, Johnston JV, Shuffton WW, Mittler RS, Chen LI. NK1.1 cells express 4-1BB (CDw137) costimulatory molecule and are required for tumor immunity elicited by anti-4-1BB monoclonal antibodies. *Cell Immunol.* 1998;190(2):167-172.
30. Gurney AL, Marsters SA, Huang A, et al. Identification of a new member of the tumor necrosis factor family and its receptor, a human ortholog of mouse GITR. *Curr Biol.* 1999;9(4):215-218.
31. Hanabuchi S, Watanabe N, Wang YH, et al. Human plasmacytoid dendritic cells activate NK cells through glucocorticoid-induced tumor necrosis factor receptor-ligand (GITRL). *Blood.* 2006;107(9):3617-3623.
32. Tone M, Tone Y, Adams E, et al. Mouse glucocorticoid-induced tumor necrosis factor receptor ligand is costimulatory for T cells. *Proc Natl Acad Sci USA.* 2003;100(25): 15059-15064.
33. Evans DE, Prell RA, Thalhoffer CJ, Hurwitz AA, Weinberg AD. Engagement of OX40 enhances antigen-specific CD4 + T cell mobilization/memory development and humoral immunity: comparison of α OX-40 with α CTLA-4. *J Immunol.* 2001;167(12):6804-6811.
34. Ruby CE, Redmond WL, Haley D, Weinberg AD. Anti-OX40 stimulation in vivo enhances CD8+ memory T cell survival and significantly increases recall responses. *Eur J Immunol* 2007;37(1):157-166.
35. Tittle TV, Weinberg AD, Steinkeler CN, Maziarz RT. Expression of the T-cell activation antigen, OX-40, identifies alloreactive T cells in acute graft-versus-host disease. *Blood.* 1997;89(12):4652-4658.
36. Godfrey WR, Fagnoni FF, Harara MA, Buck D, Engleman EG. Identification of a human OX-40 ligand, a costimulator of CD4 + T cells with homology to tumor necrosis factor. *J Exp Med.* 1994;180(2):757-762.
37. Bansal-Pakala P, Halteman BS, Cheng MHY, Croft M. Costimulation of CD8 T cell responses by OX40. *J Immunol.* 2004;172(8):4821-4825.
38. Mousavi SF, Soroosh P, Takahashi T, et al. OX40 costimulatory signals potentiate the memory commitment of effector CD8+ T cells. *J Immunol.* 2008;181(9):5990-6001.
39. Vu MD, Xiao X, Gao W, et al. OX40 costimulation turns off Foxp3+ Tregs. *Blood.* 2007; 110(7):2501-2510.
40. Perkins D, Wang Z, Donovan C, et al. Regulation of CTLA-4 expression during T cell activation. *J Immunol.* 1996;156(11):4154-4159.
41. Le Mercier I, Lines JL, Noelle RJ. Beyond CTLA-4 and PD-1, the generation Z of negative checkpoint regulators. *Front Immunol.* 2015;6:418. doi: 10.3389/fimmu.2015.00418.
42. Walunas TL, Lenschow DJ, Bakker CY, et al. CTLA-4 can function as a negative regulator of T cell activation. *Immunity.* 1994;1(5):405-413.
43. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity.* 1995;3(5):541-547.
44. Chambers CA, Sullivan TJ, Truong T, Allison JP. Secondary but not primary T cell responses are enhanced in CTLA-4-deficient CD8+ T cells. *Eur J Immunol.* 1998;28(10):3137-3143.

45. Vibhakar R, Juan G, Traganos F, Darzynkiewicz Z, Finger LR. Activation-induced expression of human programmed death-1 gene in T-lymphocytes. *Exp Cell Res*. 1997;232(1):25-28.
46. Barber DL, Wherry EJ, Masopust D, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 2006;439(7077):682-687.
47. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor deficient mice. *Science*. 2001;291(5502):319-322.
48. Huang CT, Workman CJ, Flies D, et al. Role of LAG-3 in regulatory T cells. *Immunity*. 2004;21(4):503-513.
49. Baixeras E, Huard B, Mipssec C, et al. Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. *J Exp Med*. 1992;176(2):327-337.
50. Workman CJ, Cauley LS, Kim IJ, et al. Lymphocyte activation gene-3 (CD223) regulates the size of the expanding T cell population following antigen activation in vivo. *J Immunol*. 2004;172(9):5450-5455.
51. Yu X, Harden K, Gonzalez LC et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat Immunol*. 2009;10(1):48-57.
52. Stanitsky N, Simic H, Arapovic J et al. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Nat Acad Sci USA*. 2009;106(42):17858-17863.
53. Joller N, Lozano E, Burkett PR et al. Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. *Immunity*. 2014;40(4):569-581.
54. Kobie JJ, Shah PR, Yang L, Rebhahn JA, Fowell DJ, Mosmann TR. T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5O-adenosine monophosphate to adenosine. *J Immunol*. 2006;177(10): 6780-6786.
55. Mellor AL, Munn DH. Tryptophan catabolism and T-cell tolerance: immunosuppression by starvation? *Immunol Today*. 1999;20(10):469-473.
56. Munn DH, Sharma MD, Lee JR, et al. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science*. 2002;297(5588):1867-1870.
57. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. *Nat Rev Immunol*. 2004;4(10):762-774.
58. Stanley ER, Chitu V. CSF-1 receptor signaling in myeloid cells. *Cold Spring Harb Perspect Biol*. 2014;6:a021857.
59. Richardsen E, Uglehus RD, Johnsen SH, Busnd LT. Macrophage-colony stimulating factor (CSF1) predicts breast cancer progression and mortality. *Anticancer Res*. 2015;35(2):865-874.
60. Yuan J, Hegde PS, Clynes R, et al. Novel technologies and emerging biomarkers for personalized cancer immunotherapy. *J ImmunoTher Cancer*. 2016;4:3. doi: 10.1186/s40425-016-0107-3.
61. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949-954.
62. Mok TS. Personalized medicine in lung cancer: what we need to know. *Nat Rev Clin Oncol*. 2011;8(11):661-668.
63. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015; 348(6230):56-64.
64. Nelson D, Fisher S, Robinson B. The "trojan horse" approach to tumor immunotherapy: targeting the tumor microenvironment. *J Immunol Res*. 2014;2014:789069. doi: 10.1155/2014/789069.
65. Kerr KM, Tsao MS, Nicholson AG, Yatabe Y, Wistuba II, Hirsch FR. Programmed death-ligand 1 immunohistochemistry in lung cancer. In what state is this art? *J Thorac Oncol*. 2015;10(7):985-989.
66. Brown SD, Warren RL, Gibb EA, et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. *Genome Res*. 2014;24(5):743-750.
67. Anitei MG, Zeitoun G, Mlecnik B, et al. Prognostic and predictive values of the immunoscore in patients with rectal cancer. *Clin Cancer Res*. 2014;20(7):1891-1899.
68. Ascierto ML, Kmiecik M, Idowu MO, et al. A signature of immune function genes associated with recurrence-free survival in breast cancer patients. *Breast Cancer Res Treat*. 2012;131(3):871-880.
69. Han Y, Li H, Guan Y, Huang J. Immune repertoire: a potential biomarker and therapeutic for hepatocellular carcinoma. *Cancer Lett*. 20-15;pii:S0304-3835(15)00441-3.
70. Komatsu N, Matsueda S, Tashiro K, et al. Gene expression profiles in peripheral blood as a biomarker in cancer patients receiving peptide vaccination. *Cancer*. 2012;118(12): 3208-3221.
71. Luborsky J, Barua A, Shatavi SV, Kebede T, Abramowicz J, Rotmensch J. Anti-tumor antibodies in ovarian cancer. *Am J Reprod Immunol*. 2005;54(2):55-62.
72. Schneider BP, Shen F, Miller KD. Pharmacogenetic biomarkers for the prediction of response to antiangiogenic treatment. *Lancet Oncol*. 2012;13(10):e427-e436.
73. Whiteside TL. Immune responses to cancer: are they potential biomarkers of prognosis? *Front Oncol*. 2013;3:107. doi:10.3389/fonc.2013.00107.
74. Stanciu AE, Serdarevic N, Hurduc AE, Stanciu MM. IL-4, IL-10 and high sensitivity-CRP as potential serum biomarkers of persistent/recurrent disease in papillary thyroid carcinoma with/without Hashimoto's thyroiditis. *Scand J Clin Lab Invest*. 2014;75(7): 539-548.
75. Wang Z, Han J, Cui Y, Fan K, Zhou X. Circulating microRNA-21 as noninvasive predictive biomarker for response in cancer immunotherapy. *Med Hypotheses*. 2013; 81(1):41-43.
76. Xu B, Yuan L, Gao Q, et al. Circulating and tumor-infiltrating Tim-3 in patients with colorectal cancer. *Oncotarget*. 2014;6(24):20592-20603.
77. Markiewicz MA, Fallarino F, Ashikari A, Gajewski TF. Epitope spreading upon P815 tumor rejection triggered by vaccination with the single class I MHC-restricted peptide P1A. *Int Immunol*. 2001;13(5):625-632.
78. Kaech SM, Wherry EJ, Ahmed R, et al. Effector and memory T-cell differentiation: implications for vaccine development. *Nat Rev Immunol*. 2002;2:251-262.
79. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res*. 2009;15: 7412-7420.
80. Chiou VL, Burotto M. Pseudoprogression and immune-related response in solid tumors. *J Clin Oncol*. 2015;33:3541-3543.
81. Hales RK, Banchereau J, Ribas A, et al. Assessing oncologic benefit in clinical trials of immunotherapy agents. *Ann Oncol*. 2010;21:1944-1951.
82. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247.
83. Chen TT. Statistical issues and challenges in Immuno-Oncology. *J ImmunoTher Cancer*. 2013;1:18. doi: 10.1186/2051-1426-1-18.
84. Scagliotti GV, Bironzo P, Vansteenkiste JF. Addressing the unmet need in lung cancer: The potential. *Cancer Treat Rev*. 2015;41(6):465-475.
85. Friedman LM et al. Survival analysis. In: Friedman LM et al. Fundamentals of Clinical Trials, 4th ed. New York, NY: Springer; 2010:1-18.
86. Rich JT, Neely JG, Paniello RC, Voelker CCJ, Nussenbaum B, Wang EW. A practical guide to understanding Kaplan-Meier curves. *Otolaryngol Head Neck Surg*. 2010;143: 331-336.
87. Spruance SL, Reid JE, Grace M, Samore M. Hazard ratio in clinical trials. *Antimicrob Agents Chemother*. 2004;48:2787-2792.
88. Uno H, Claggett B, Tian L, et al. Moving beyond the hazard ratio in quantifying the between-group difference in survival analysis. *J Clin Oncol*. 2014;32:2380-2385.
89. Pazdur R. Endpoints for assessing drug activity in clinical trials. *Oncologist*. 2008;13(suppl 2):19-21.
90. Wilson MK, Karakasis K, Oza AM. Outcomes and endpoints in trials of cancer treatment: the past, present, and future. *Lancet Oncol*. 2015;16:e32-e42.
91. Brody T. Clinical Trials: Study Design, End points and Biomarkers, Drug Safety, and FDA and ICH Guidelines. London: Academic Press, 2012:165-190.
92. Amos S, Duong C, Westwood J, Ritchie DS, Junghans RP, Darcy PK, Kershaw MH: Autoimmunity associated with immunotherapy of cancer. *Blood*. 2011; 118: 499-509.

93. Gelaou L, Criscitiello C, Esposito A, Goldhirsch A, Curigliano G. Immune checkpoint blockade in cancer treatment: a double-edged sword cross-targeting the host as an “innocent bystander”. *Toxins (Basel)*. 2014;6(3):914-933.
94. Bertrand A, Kostine M, Barnette T, Truchetet ME, Schaevebeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. *BMC Med*. 2015;13:211-224.
95. Bachireddy P, Burkhardt UE, Rajasagi M, Wu CJ. Hematological malignancies: at the forefront of immunotherapeutic innovation. *Nat Rev Cancer*. 2015;15(4):201-215.
96. Blankenstein T, Coulie Pg, Gilboa E, Jaffee EM. The determinants of tumour immunogenicity. *Nat Rev Cancer*. 2012;12(4):307-313.
97. Lawrence MS, Stojanov P, Polak P, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. *Nature*. 2013;499(7457):214-218.
98. Schumacher T, Bunse L, Pusch S, et al. A vaccine targeting mutant IDH1 induced antitumor immunity. *Nature*. 2014;512(7514):324-327.
99. Ansell SM, Stenson M, Habermann TM, Jelinek DF, Witzig TE. CD41 T-cell immune response to large B-cell non-Hodgkin’s lymphoma predicts patient outcome. *J Clin Oncol*. 2001;19(3):720-726.
100. Berghoff AS, Kiesel B, Widhalm G, et al. Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neuro Oncol*. 2015;17(8):1064-1075.
101. Dhodapkar MV, Krasovsky J, Olson K. T cells from the tumor microenvironment of patients with progressive myeloma can generate strong, tumor-specific cytolytic responses to autologous, tumor-loaded dendritic cells. *Proc Natl Acad Sci USA*. 2002;99(20):13009-13013.
102. Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med*. 2015;21(8):938-945.
103. Heusinkveld M, Goedemans R, Briet RJP, et al. Systemic and local human papillomavirus 16-specific T-cell immunity in patients with head and neck cancer. *Int J Cancer*. 2012;131(2):E74-E85.
104. Hussein MR, AL-Assiori Mana, Musalan AO. Phenotypic characterization of the infiltrating immune cells in normal prostate, benign nodular prostatic hyperplasia and prostatic adenocarcinoma. *Exp Mol Pathol*. 2009;86(2):108-113.
105. Itsumi M, Tatsugami K. Immunotherapy for renal cell carcinoma. *Clin Dev Immunol*. 2010;2010:284581. doi: 10.1155/2010/284581.
106. Kandalaf LE, Motz GT, Duraiswamy J, Coukos G. Tumor immune surveillance and ovarian cancer: lessons in immune mediated tumor rejection or tolerance. *Cancer Metastasis Rev*. 2011;30:141-151.
107. Liang J, Ding T, Guo Z-W, et al. Expression pattern of tumour-associated antigens in hepatocellular carcinoma: association with immune infiltration and disease progression. *Br J Cancer*. 2013;109(4):1031-1039.
108. Schreck S, Friebel D, Buettner M, et al. Prognostic impact of tumour-infiltrating Th2 and regulatory T cells in classical Hodgkin lymphoma. *Hematol Oncol*. 2009;27(1):31-39.
109. Sharma P, Shen Y, Wen S, et al. CD8 tumor-infiltrating lymphocytes are predictive of survival in muscle-invasive urothelial carcinoma. *Proc Natl Acad Sci USA*. 2007; 104(10):3967-3972.
110. Tran E, Ahmadzadeh M, Lu Y-C, et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. *Science*. 2015;350(6266):1387-1390.
111. Whitford P, Mallon EA, George WD, Campbell AM. Flow cytometric analysis of tumour infiltrating lymphocytes in breast cancer. *Br J Cancer*. 1990;62(6):971-975.
112. Gajewski TF, Schreiber H, Fu Y-X. Innate and adaptive immune cells in the tumor microenvironment. *Nat Immunol*. 2013;14(10):1014-1022.
113. Kallialis LV, Drzewiecki KT, Klyvner H. Spontaneous regression of metastases from melanoma: review of the literature. *Melanoma Res*. 2009;19(5):275-282.
114. Maio M. Melanoma as a model tumour for Immuno-Oncology. *Ann Oncol*. 2012;23(suppl 8):viii10-viii14.
115. Antonia SJ, Larkin James, Ascierto PA. Immuno-oncology combinations: a review of clinical experience and future prospects. *Clin Cancer Res*. 2014;20(24):6258-6268.
116. Wood LD, Parsons DW, Jones S, et al. The genomic landscapes of human breast and colorectal cancers. *Science*. 2007;318(5853):1101-1113.
117. Matsueda S, Graham DY. Immunotherapy in gastric cancer. *World J Gastroenterol*. 2014;20(7):1657-1666.
118. Kass ES, Greinber JW, Kantor JA, et al. Carcinoembryonic antigen as a target for specific antitumor immunotherapy of head and neck cancer. *Cancer Res*. 2002;62(17): 5049-5057.
119. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian Carcinoma. *Nature*. 2011;474(7353):609-615.
120. Berger MF, Lawrence MS, Demichelis F, et al. The genomic complexity of primary human prostate cancer. *Nature*. 2011;470(7333):214-220.
121. Morin RD, Mendez-Lago M, Mungall AJ, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature*. 2011;476(7360):298-303.
122. Gunawardana J, Chan FC, Telenius A, et al. Recurrent somatic mutations of PTPN1 in primary mediastinal B cell lymphoma and Hodgkin lymphoma. *Nat Genet*. 2014;46(4): 329-335.
123. Rajasagi M, Shukla SA, Fritsch EF, et al. Systematic identification of personal tumor-specific neoantigens in chronic lymphocytic leukemia. *Blood*. 2014;124(3):453-462.
124. Walz S, Stickel JS, Kowalewski DJ, et al. The antigenic landscape of multiple myeloma: mass spectrometry (re)defines targets for T-cell-based immunotherapy. *Blood*. 2015; 126(10):1203-1213.