Releasing the Brakes in Cancer

Shweta Dubey* and Ankita Garg*

1 Amity Institute of Virology & Immunology, Amity University, Uttar Pradesh, India
2 University of California San Diego, La Jolla, California 92039-0672, USA

Abstract

Cancer is a heterogeneous group of diseases where abnormal cell growth with potential to invade other body parts takes control of normal homeostasis and becomes fatal if not timely and rightly treated. There are more than 100 types of cancers characterized so far and many yet to be identified. The World Health Organization estimates, that worldwide in 2012 there were 4 million new cancer cases and 8.2 million cancer related deaths. Amongst various treatment options available for cancer, immunotherapy offers an approach where the focus is on enhancing or even inducing an antitumor immune response. Induction or enhancement of anti-tumor immune response is a formidable challenge in cancer because tumor cells use multiple immune evasion strategies and avoid being detected or eliminated by immune cells. Immune checkpoints refer to a network of stimulatory or inhibitory signaling pathways in the immune system which are critical in maintaining self-tolerance, limiting tissue damage and modulating the quality of immune response. Substantial evidence indicates that up regulation of inhibitory signaling molecules (CTLA-4, PD-1) by tumor cells subvert activation of tumor antigen specific T effector cells. Therefore, blockade of inhibitory signaling pathways may be one potential way of revitalizing an exhausted immune response in tumors. Using this approach, antibodies directed against CTLA-4 and PD-1 have been shown an acceptable therapeutic benefit in preclinical models and cancer patients. This review will discuss the important immune checkpoints that have been identified critical to suppress anti-tumor immunity and have been exploited as drug targets.

Keywords: Cancer stem cells; Anti-tumor; Lymphatic

Tumors develop as a result of uncontrolled cell growth, avoiding programmed cell death and often bypassing the signals generated to restrict cell division [1]. During this uncontrolled growth, cancer cells undergo profound cellular and molecular changes forming a complex niche known as the Tumor Microenvironment (TME), which comprises of cells of tumor origin with genetic alterations and genetically unaltered non-malignant cells such as fibroblasts, endothelial cells (blood and lymphatic), mesenchymal cells and components of extracellular matrix [2]. It is now evident that the stromal structure is critical for tumor sustenance and it creates a pathway for infiltration of various immune cell types like natural killer cells (NK), macrophages, activated T-cells, tumor associated macrophages (TAM) and myeloid derived suppressor cells (MDSC). Since the formulation of “immune surveillance” hypothesis at the beginning of 20th century by Paul Ehrlich and later refined by Burnet and Thomas in 1950’s, immune cells particularly lymphocytes and NK cells have been established as critical for detection and destruction of tumor cells [3].

According to the concept of cancer ”immune editing”, immune selection favors the emergence of tumor variants that have accumulated antigenic alterations sufficient for the evasion of immune surveillance mechanisms leading to tumor progression [4]. Even though the down regulation of MHC 1 on tumor cells invoke a robust NK cell activity leading to tumor cell lysis, T-cells are critical to control tumor cell expansion. A concerted interaction between both innate and adaptive immune system is important for elimination of cancer cells [5]. Appropriate cues from a prior innate immune response influence T cell differentiation i.e. innate signals from cells like classically activated macrophages and dendritic cells induce activation of cytotoxic T cells (CTLs) and T helper (TH1) cells which is beneficial to the host in eliminating tumor cells [6]. On contrary, signals generated from alternatively activated macrophages (AAM) or myeloid derived suppressor cells (MDSC) [7,8] promote tumor metastasis and progression (Figure 1). Clinical manifestations of cancer may be visible once the immune response skews to promote tumor progression. Immunotherapy augments anti-tumor immune response by rewiring host immune response from tumor progression to tumor elimination (Figure 1).

Figure 1: The self-defense of tumors overweighs the anti-tumor immunity leading to clinical manifestations of cancer. The various immunotherapeutic approaches are adopted to reverse this imbalance.

Keywords:
Cancer stem cells; Anti-tumor; Lymphatic

Citation: Dubey S, Garg A (2016) Releasing the Brakes in Cancer. J Bioanal Biomed 8: 017-022. doi: 10.4172/1948-593X.1000147

Copyright: © 2016 Dubey S, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
or tumor associated (TA) antigens derived from oncogenic viruses (HPV, SV40), differentiation antigens (Tyrosinase, Carcinoembryonic antigens, Alpha-fetoprotein, prostate-specific antigen), epigenetically regulated antigens (cancer antigen-1, MAGE-antigens) and neoantigens allow T cells to distinguish between normal and transformed cell [9,10]. Induction of effector T cell response is sequential, antigen specific T cells are first primed in the secondary lymphoid organs through the interaction with antigen presenting cells (APC). APC particularly dendritic cells (DC) sample antigens from tumor cells and present antigens to CD4+ T cells via the MHC class-II pathway or to CD8+ T cells via cross presentation or cross priming [11,12]. This antigen recognition in association with MHC is insufficient to effectively activate T cells; APC provide additional costimuls that regulate the breadth of T cell activation. These multiple cosignals can be induced by stimulatory (CD80 / CD86: CD28) and inhibitory molecules also known as "immune checkpoints" [13,14]. The entire process of T cell activation and differentiation is finely regulated by a balance between multiple stimulatory or inhibitory receptors on T cells and their respective ligands present on APC (Table 1). APC also provide the additional costimulatory signals, which are mandatory for T cell priming. After the priming phase, several factors, including but not limited to defective T cell recruitment at tumor site, inactivation of effector functions of primed T cells or induction of T cell apoptosis contribute to the diminished response of antigen specific T cells at the site of tumor development ultimately causing reduced cancer elimination.

| T cells            | APCs / Tumor cells | Effect     |
|--------------------|--------------------|------------|
| Immunoglobulin family |                    |            |
| CD28               | B7.1 / B7.2        | +          |
| ICOS               | ICOS-L             | +          |
| CTLA-4             | B7.1 / B7.2        | -          |
| PD-1               | PDL-1 / PDL-2      | -          |
| BTLA               | HVEM               |            |
| LAG-3              | MHC-II             |            |
| CD160              | HVEM               |            |
| VISTA              | ?                  | -          |
| TNF-R family       |                    |            |
| CD4L / CD154       | CD40               | +          |
| CX-40              | CX-40L             | +          |
| 4-1BB              | 4-1BBL             | +          |
| GITR               | GITR-L             | +          |
| CD30               | CD30L              | +          |
| HVEM               | LIGHT              | +          |
| T cell immunoglobulin mucin family | | |
| TIM-1              | ?                  | +          |
| TIM-2              | ?                  | -          |
| TIM-3              | Galactin-9         | -          |
| Butyrophilin family |                    |            |
| BTN                | BTN-L              | -          |

* indicates stimulation and - indicates inhibition of T cell activation. ? Indicates receptor or ligand yet to be identified. ICOS: Inducible costimulator of T cells [67], CTLA-4: Cytotoxic T Lymphocyte Antigen 4 [68], PD-1: Programmed Death 1 [69], BTLA: B and T Lymphocyte Attenuator [70], LAG-3: Lymphocyte Activation Gene [71], VISTA: V-domain Ig Suppressor of T cell Activation [72], CX-40: CD134 [73], 4-1BB: CD137 [74], GITR: Glucocorticoid-Induced Tumour Necrosis factor Receptor [75], HVEM: Herpes Virus Entry Mediator [76], TIM: T cell Immunoglobulin Mucin family [77,78], BTN: Butyrophilin [79].

Table 1: Costimulatory molecules and their corresponding ligands expressed on T cells and APCs / tumor cells.

Of the various proteins that regulate T cell activation (Table 1), Cytotoxic T Lymphocyte Antigen-4 (CTLA-4), Programmed Death-1 (PD-1), B7 family members B7-H3, B7-H4, T cell Immunoglobulin and Mucin domain-containing protein 3 (Tim-3), and Lymphocyte Activation Gene-3 (LAG-3) block costimulation and abrogate the response of activated T cells (Figure 2). Abnormal expression of either of these inhibitory checkpoint molecules is a predominant immune evasion mechanism in cancers, chronic infections and autoimmune diseases. In this review, we will discuss the important immune checkpoints that have been identified critical to suppress anti-tumor immunity and have been exploited as drug targets. We will also discuss other immune check points and their antagonists in preclinical development for various cancers.

**CTLA-4 First Target Identified to Release Brakes**

Amongst all the therapies that have been used to potentiate immune response against cancer, immune checkpoint blockade has shown most promising results and has been appropriately heralded as a major scientific breakthrough in translational research. CTLA-4 was the first inhibitory immune checkpoint molecule to be clinically targeted to enhance T cell function. Like costimulatory CD28, CTLA-4 is also expressed on T cells; but unlike CD28, which is constitutively expressed on T cells, CTLA-4 expression is up regulated only after T cell activation and regulates early stages of T cell activation. Both CD28 and CTLA-4 bind to CD80 / CD86 on APCs but compared to CD28, CTLA-4 binds with much higher affinity. Therefore, expression of CTLA-4 on activated T cells induces a competitive inhibition of stimulatory CD28-CD80 / 86 signaling and inhibits T cell activation [15,16]. Functional studies on T cell activation suggest that crosslinking of CTLA-4 on TCR and CD28 stimulated T cells resulted in an anergic phenotype similar to that obtained when T cells are TCR stimulated in the absence of costimulatory signal. Specific pathways by which CTLA-4 suppresses T cell activation are still under investigation and it is suggested that activation of phosphatases downstream of CTLA-4 engagement with its ligands inhibits T cell activation. Critical role of CTLA-4 in T cell activation is best evident in cta-4-/- mice, which exhibit a fatal lymphoproliferative and immune hyperactivation phenotype [17,18]. This provided convincing confirmation for blocking CTLA-4 expression and restoring function of activated T cells. Subsequently, various studies in human and animal models suggested that blocking CTLA-4 inhibitory signaling or “taking the brakes off” the immune cells restored T cell homeostasis.

Due to lethal effects in cta-4-/- mice and absence of tumor specific CTLA-4 expression, CTLA-4 blockade did not originally appear to be a promising therapeutic strategy for cancer. However, Allison et al demonstrated that partial blockade with CTLA-4 blocking antibody was beneficial in elimination of tumor growth with low toxicity in mice [19]. In poorly immunogenic tumors, combination of CTLA-4 blockade with GM-CSF based tumor vaccine showed better results as compared to CTLA-4 monotherapy alone [20]. In general, combination of CTLA-4 blockade with any methods that enhanced tumor antigen presentation (DNA or peptide based vaccines) yielded better results in many preclinical studies [21,22]. These preclinical observations led to the development of anti-CTLA-4 antibodies for clinical use.

Two fully humanized CTLA-4 blocking antibodies: Ipilimumab (MDX-010) and Tremelimumab (CP-675,206) are presently under clinical investigation. Ipilimumab was approved in 2011 at a dose of 3 mg / kg for treatment of unresectable or metastatic melanoma by regulatory agencies in the United States and Europe [23]. Tremelimumab has been granted orphan drug status by FDA for treatment of malignant
Primary immune checkpoint molecules, such as CTLA-4 and PD-1/PD-L1, are crucial in the regulation of anti-tumor T cell responses. CTLA-4 and PD-1 molecules are negative regulators of T cell activation, with CTLA-4 being expressed on activated T cells and PD-1 on activated B, T, and myeloid cells.

The activated T cells express CTLA-4, which binds to CD80/86 on dendritic cells and macrophages, and PD-1 binds to PD-L1/L2 on tumor cells. This binding leads to a decrease in T cell proliferation, cytokine production, and cell adhesion, inhibiting the immune response.

In the context of mesothelioma, PD-1 and PD-L1 have been found to be highly expressed, indicating a possible therapeutic target. Antibodies against PD-1 or PD-L1, such as nivolumab (Opdivo) and pembrolizumab (Keytruda), have shown efficacy in clinical trials for mesothelioma, with complete response rates in some cases.

Beyond CTLA-4 and PD-1 Pathway

Beyond the traditional CTLA-4 and PD-1 blockade, there are emerging therapies targeting other immune checkpoints. These include PD-L2 and PD-L3, which are also expressed on tumors and could potentially be targeted for therapeutic benefit. Additionally, other immune checkpoints such as LAG-3, TIGIT, and TIM-3 are under investigation for their role in tumor immunity.

A comprehensive understanding of these immune checkpoints and their mechanisms is crucial for developing effective and personalized immunotherapies for cancer treatments. Further research is necessary to fully harness the potential of these therapies in the clinical setting.
Antibody | Molecule | Development Stage
---|---|---
Nivolumab | Fully Hu-IgG4 | US approved: Advanced Melanoma, Squamous NSCLC after CT
Pembrolizumab | Humanized IgG4 | US approved: Advanced Melanoma, Squamous NSCLC after CT
Pildilizumab | Humanized IgG4 | Ph II multiple tumors (Pancreatic, CRC, RCC, Prostate, CNS)
AMP-224 | Fc – PD-L2-Fusion | Ph I
MPDL3280A | Engineered Hu IgG1 | Ph III
MSB0010718C | Fully Hu IgG1 | Ph III

Table 2A: Clinical Development of Anti-PD-1 Checkpoint Inhibitors.

| Target | Antibody | Molecule | Development |
|---|---|---|---|
| CTLA-4 | Ipilimumab | Humanized IgG1 | Ph III (NSCLC, HNSCC) |
| | Tremelimunab | Fully Hu IgG2 | Ph I |
| | INCBO24360 | Small Molecule Inhibitor | Ph I |
| | NLG919 | Small Molecule Inhibitor | Ph I |
| | MDX-1105 | Fully Hu IgG4 | Ph I |
| | MED4736 | Engineered Hu IgG1 | Ph III |
| | MPDL3280A | Humanized IgG | Ph II; Ph III |
| | BMS-986016 | - | Ph I |
| | IMP321 | LAG3-Ig | Fusion protein |
| | B7-H3 | MGA271 | Humanized IgG1 | Ph I |
| | B7- H4 | - | - | Preclinical |
| | TIM-3 | - | - | Preclinical |

NSCLC - Non small cell lung cancer; RCC - Renal cell carcinoma; HNSCC - Hean and neck squamous cell carcinoma; GBM - Glioblastoma; CRC - Colon rectum cancer; SCLC - Small cell lung cancer

Table 2B: Clinical Development of Other Checkpoint Inhibitors Target Antibody.

[57]. Another inhibitory checkpoint molecule in the same category as CTLA-4 and PD-1 is LAG-3, which inhibits T cell proliferation, function [58-61] and contributes to the suppressive action of T regulatory cells (Tregs) [62]. Dual blockade of both PD-1 and LAG-3 has been shown to restore tumor specific immune response and enhance survival in murine models of tumor [58]. Currently clinical trials are underway to determine the safety and efficacy of combinatorial therapy with anti-LAG-3 antibody with or without PD-1 blockade in solid tumors (trial ID CA224-020, NLM Identifier NCT01968109). Apart from immune checkpoints, metabolic checkpoints such as inhibitor compounds for enzymes like indoleamine 2,3-dioxygenase (IDO), isocitrate dehydrogenase, adenosine signaling etc are also an emerging target for development of anti-cancer therapeutic molecules [63-65]. Tumor microenvironment presents many metabolic challenges which may contribute to a rewiring of anti-tumor T cell response. This new area of immunometabolism will certainly add new dimensions to manipulate T cell function; we are already noticing an exponential information explosion in this arena as well. This may open up entirely new avenues to treat immune mediated disorders. Combination of immune checkpoints which boost the immune response and metabolic checkpoints which provide a host friendly tumor microenvironment may also be one combinatorial approach in cancer therapy.

The targets for which biological or small molecule inhibitors are currently available are detailed in the Table 2, but the list is not comprehensive. Tumor immunotherapy has seen a dramatic transition from the era of Coley’s toxin [66] to immune checkpoints. Nevertheless, substantial data show that immunotherapy does not follow “one size fits all” approach and predictors of response to therapy need to be identified so that clinicians can selects patients for particular monotherapy or combination immunotherapy. Blocking a single molecule has not produced a completely curative response thus underscoring the importance of multiple, probably, redundant molecules working in tandem to promote immune escape of tumor cells. While it is possible that there are many other molecules still to be discovered there is substantial evidence to suggest that combinatorial therapy involving immune, molecular and metabolic checkpoints and not monotherapy alone might be the ideal way to develop completely curative and specific immune-therapeutic modalities.

Conclusion

Exploiting the immune system against tumor cells has been considered an attractive therapeutic option, successive failures or limitation of practical usage of various immune therapeutic approaches resulted in the loss of credibility of cancer immunotherapy. With the better understanding of T cell activation and regulation and its successful translation towards development of broad spectrum anti-cancer agents in form of immune checkpoint inhibitors has revived the immune therapy field. However, this novel treatment which engages patient’s immune response to target tumor cells needs to be integrated with conventional approaches as surgery, chemotherapy, radiation therapy and targeted therapy which directly attack cancer cells. Furthermore, achieving maximum clinical benefit from immunotherapeutic molecules may also require a careful investigation of extent of cooperatively between different immune checkpoints. It might also be important to contemplate combination treatments that can augment both innate (NK cells, γδ T cells etc) and adaptive arm of host immune system in tumor microenvironment for better clinical benefit.

References

1. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57-70.
2. Quail DF, Joyce JA (2013) Microenvironmental regulation of tumor progression and metastasis. Nat Med 19: 1423-1437.

3. BURNET M (1957) Cancer, a biological approach I The processes of control. Br Med J 1: 779-786.

4. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD (2002) Cancer immunoeediting: from immunosurveillance to tumor escape. Nat Immunol 3: 991-998.

5. Cohen S, Cohen MC (1978) Mechanisms of tumor immunity An overview. Am J Pathol 93: 449-458.

6. Hadrup S, Donia M, Thor Straten P (2013) EfectoR CDA and CD8 T cells and their role in the tumor microenvironment. Cancer Microenviron 6: 123-133.

7. Gabriolovich DI, Nagarji S (2009) Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9: 162-174.

8. Noy R,Pollard JW (2014) Tumor-associated macrophages: from mechanisms to immunity.41: 49-61.

9. Vigneron N (2015) Human Tumor Antigens and Cancer Immunotherapy. Biomed Res Int 2015: 948501.

10. Coulie PG, Van den Eynde BJ, van der Bruggen P2, Boon T3 (2014) Tumour antigens recognized by T lymphocytes at the core of cancer immunity. Nat Rev Cancer 14: 135-146.

11. Diamond MS, Kinder M, Matsushita H, Mashayekhi M, Dunn GP, et al. (2011) Type I interferon is selectively required by dendritic cells for immune rejection of tumors. J Exp Med 208: 1969-2003.

12. Steinman RM, Banchereau J (2007) Taking dendritic cells into medicine. Nature 449: 419-426.

13. Topalian SL, Drake CG2, Pardoll DM3 (2015) Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 27: 450-461.

14. Pardoll D (2015) Cancer and the Immune System: Basic Concepts and Targets for Intervention. Semin Oncol 42: 523-538.

15. Boulougouris G, McLeod JD, Patel YI, Ellwood CN, Walker LS, et al. (1998) Positive and negative regulation of human T cell activation mediated by the CTLA-4 / CD28 ligand CD80. J Immunol 161: 3919-3924.

16. Walker LS, Sansom DM (2011) The emerging role of CTLA4 as a cell-extrinsic regulator of T cell responses. Nat Rev Immunol 11: 852-863.

17. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Blustreale JA, et al. (1995) Loss of CTLA-4 leads to massive lymphoproliferation and fatal multorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunity 3: 541-547.

18. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, et al. (1995) Lymphoproliferative disorders with early lethality in mice deficient in Ctl-a. Science 270: 985-988.

19. Leach DR, Krummel MF, Allison JP (1996) Enhancement of antitumor immunity by CTLA-4 blockade. Science 273: 1734-1736.

20. Quezada SA, Peggs KS, Curran MA, Allison JP (2006) CTLA4 blockade and GM-CSF combination immunotherapy alters the intratumor balance of effector and regulatory T cells. J Clin Invest 116: 1935-1945.

21. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, et al. (2008) Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363: 711-723.

22. Hansel C, Yang JC, Sherry RM, Hwu P, Topalian SL, et al. (2003) Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A 100: 8372-8377.

23. Larsen CP, Pearson TC, Adams AB, Tso P, Shirasugi N, et al. (2005) Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties. Am J Transplant 5: 443-453.

24. Salomons B, Bluestone JA (2001) Complexities of CD28/BD: CTLA-4 costimulatory pathways in autoimmunity and transplantation. Annu Rev Immunol 19: 225-252.

25. Dall’Era M, Davis J (2004) CTLA4Ig: a novel inhibitor of costimulation. Lupus 13: 372-376.

26. Su VC, Harrison J, Rogers C, Ensom MH (2012) Belatacept: a new biologic and its role in kidney transplantation. Ann Pharmacother 46: 57-67.

27. Vélezou M, Pitt JM1, Daillère R1, Lepage P2, Waldschmidt N3, et al. (2015) Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota. Science 350: 1079-1084.

28. Klaenke, Blansfied JA, Tran KQ, Feldman AL, Hughes MS, et al. (2006) Enterococci in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol 24: 2283-2289.

29. Ishida Y, Agata Y, Shibahara K, Horio T (1992) Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J 11: 3887-3895.

30. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, et al. (2000) Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med 192: 1027-1034.

31. Dong H, Zhu G, Tamada K, Chen L (1999) B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin -10 secretion. Nat Med 5: 1365-1369.

32. Latchman Y, Wood CR, Chernova T, Chaudhary D, Borde M, et al. (2001) PD-L2 is a second ligand for PD-1 and inhibits T cell activation. Nat Immunol 2: 261-266.

33. Nishimura H, Nose M, Hiall H, Minato N, Horio T (1999) Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 11: 141-151.

34. Keir ME, Butte MJ, Freeman GJ, Sharpe AH (2008) PD-1 and its ligands in tolerance and immunity. Annu Rev Immunol 26: 677-704.

35. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, et al. (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439: 682-687.

36. Wherry EJ, Blattman JM, Murali-Krishna K, van der Most R, Ahmed R (2003) Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J Virol 77: 4911-4927.

37. Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, et al. (2009) Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 114: 1537-1544.

38. Zeng Z, Shi F, Zhou L, Zhang MN, Chen Y, et al. (2011) Upregulation of circulating PD-L1 / PD-1 is associated with poor post-cryoablation prognosis in patients with HBV-related hepatocellular carcinoma. PLoS one 6: e23621.

39. Shi F, Shi M, Zeng Z, Qi RZ, Liu ZW, et al. (2011) PD-1 and PD-L1 upregulation promotes CD8(+), T-cell apoptosis and postoperative recurrence in hepatocellular carcinoma patients. Int J Cancer 128: 887-896.

40. Jiang Y, Li Y, Zhu B (2015) T-cell exhaustion in the tumor microenvironment. Cell Death Dis 6: e1792.

41. Munetz S, Schaerli AR, Gao F, Däster S, Trella E, et al. (2014) Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat 146: 15-24.

42. Momtaz P, Postow MA (2014) Immunologic check points in cancer therapy: focus on the programmed death-1 (PD-1) receptor pathway. Pharmacogenomics Pers Med 7: 357-365.

43. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, et al. (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366: 2443-2454.

44. Berger R, Rotem Yehudar R, Slama G, Landes S, Kneller A, et al. (2008) Antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 114: 1537-1544.
Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. Clin Cancer Res 14: 3044-3051.

48. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, et al. (2013) Safety and tumor responses with lambrolizumab (anti-PD-0-1) in melanoma. N Engl J Med 369: 134-144.

49. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, et al. (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 366: 2455-2465.

50. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, et al. (2014) MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 515: 558-562.

51. Deal watch: GlaxoSmithKline and Amplimmune join forces on targeting PD-1 (2010) Nat Rev Drug Discov 9: 754.

52. Mahoney CJ, Freeman GJ, McDermott DF (2015) The Next Immune-Checkpoint Inhibitors: PD-1 / PD-L1 Blockade in Melanoma. Clin Ther 37: 764-782.

53. Lee L, Gupta M, Sahasranaman S (2016) Immune Checkpoint inhibitors: An introduction to the next-generation cancer immunotherapy. J Clin Pharmacol 56: 157-169.

54. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, et al. (2013) Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med 369: 122-133.

55. Duraiswamy J, Katuva KM, Freeman GJ, Coukos G (2013) Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. Cancer Res 73: 3591-3603.

56. Curran MA, Montalvo W, Yagita H, Allison JP (2010) 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 U S A 107: 4275-4280.

57. Fauci JM, Straughn JM Jr, Ferrone S, Buchsbaum DJ (2012) A review of CD8+ and CD4+ immune molecules and their role in ovarian cancer. Gynecol Oncol 127: 420-425.

58. Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, et al. (2012) Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Res 72: 917-927.

59. Goldberg MV, Drake CG (2011) LAG-3 in Cancer Immunotherapy. Curr Top Microbiol Immunol 344: 269-278.

60. Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, et al. (2009) Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nat Immunol 10: 29-37.

61. Workman CJ, Vignali DA (2003) The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells. Eur J Immunol 33: 970-979.

62. Huang CT, Workman CJ, Files D, Pan X, Marson AL, et al. (2004) Role of LAG-3 in regulatory T cells. Immunity 21: 503-513.

63. Buck MD, O’Sullivan D, Pearce EL (2015) T cell metabolism drives immunity. J Exp Med 212: 1345-1360.

64. O’Sullivan D, Pearce EL (2015) Targeting T cell metabolism for therapy. Trends Immunol 36: 71-80.

65. Leone RD, Lo YC, Powel JD (2015) A2aR antagonists: Next generation checkpoint blockade for cancer immunotherapy. Comput Struct Biotechnol J 13: 265-272.

66. Coley WB (1910) The Treatment of Inoperable Sarcoma by Bacterial Toxins (the Mixed Toxins of the Streptococcus erysipelas and the Bacillus prodigiosus). Proc R Soc Med 3: 1-48.

67. Hulot F, Dittrich AM, Beier KC, Eljaschewitsch B, Kraft R, et al. (1999) ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. Nature 397: 263-266.

68. Brunet JF, Denizol F, Luciani MF, Roux-Dosseto M, Suzan M, et al. (1987) A new member of the immunoglobulin superfamily—CTLA-4. Nature 328: 267-270.

69. Jin HT, Ahmed R, Okazaki T (2011) Role of PD-1 in regulating T-cell immunity. Curr Top Microbiol Immunol 350: 17-37.

70. Watanebe N, Gavrieli M, Sedy JR, Yang J, Fallerino F, et al. (2003) BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1. Nat Immunol 4: 670-679.

71. Nguyen LT, Ohashi PS1 (2015) Clinical blockade of PD1 and LAG3—potential mechanisms of action. Nat Rev Immunol 15: 45-56.

72. Lines JL, Sempere LF, Broughton T, Wang L, Noelle R (2014) VISTA is a novel broad- spectrum negative checkpoint regulator for cancer immunotherapy. Cancer Immunol Res 2: 510-7.

73. Curti BD, Kovacsovics Bankowski M, Morris N, Walker E, Chisholm L, et al. (2013) OX40 is a potent immune-stimulating target in late-stage cancer patients. Cancer Res 73: 7189-98.

74. Lin W, Voskens CJ, Zhang X, Schindler DG, Wood A, et al. (2008) Fc-dependent expression of CD137 on human NK cells: insights into “agonistic” effects of anti-CD137 monoclonal antibodies. Blood 112: 699-707.

75. Avogadri F, Yuan J, Yang A, Schaer D, Wolchok JD (2011) Modulation of CTLA-4 and GITR for cancer immunotherapy. Curr Top Microbiol Immunol 344: 211-244.

76. Montgomery RI, Warner MS, Lum BJ, Spear PG (1996) Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. Cell 87: 427-436.

77. Anderson AC (2014) Tim-3: an emerging target in the cancer immunotherapy landscape. Cancer Immunol Res 2: 134-144.

78. Fourcade J, Sun Z, Pagliano O, Chauvin JM, Sander C, et al. (2014) PD-1 and Tim-3 regulate the expansion of tumor antigen-specific CD8+ T cells induced by melanoma vaccines. Cancer Res 74: 1045-1055.

79. Arnett HA, Viney JL (2014) Immune modulation by butyrophilins. Nat Rev Immunol 14: 559-569.