REVIEW AND PERSPECTIVES

The immune response in cancer: from immunology to pathology to immunotherapy

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Abstract In the recent years, breakthrough advances in the characterization of the tumor-infiltrating immune cells and in the understanding of their influence on tumor invasion and metastasis have been accomplished. These studies have allowed the development of assays quantifying immune infiltrates to predict patient's clinical outcome. Increasing evidence supports their utility as prognostic and potentially teragnostic markers. The in-depth characterization of the tumor's immune profile and the standard histopathological criteria are becoming the optimal method of tumor classification in the era of personalized medicine. This review describes the major concepts in the anti-tumor immunity field, with particular focus on the tumor immune microenvironment and the delicate balance between inflammatory and anti-tumor immune responses, its importance as a prognostic tool, and its utility as a teragnostic marker for patients receiving new-generation immunotherapies.

Keywords Tumor microenvironment · Inflammation · Prognosis · Immunotherapies

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Introduction

The fact that tumors often grow in sites of chronic inflammation led Rudolf Virchow to propose a link between the immune system and cancer more than a century ago [1]. This hypothesis was proven accurate, and it is widely accepted that both inflammatory and immune cells can not only promote tumor development but also contribute to their elimination [2, 3]. At first, it could seem contradictory that the immune response could play a dual role in this process. Therefore, it is important to understand that the cellular and molecular mediators underlying both effects are different.

Inflammation and cancer

Several lines of evidence have established an association between chronic inflammation and cancer [2]. First, approximately 20 % of the tumors are linked to inflammation-inducing infectious organisms [4], including *Helicobacter pylori* and gastric cancer [5], hepatitis B and C viruses and hepatocellular carcinoma [6], and human papilloma virus and cervical and head/neck cancers [7, 8]. Second, chronic noxious stimuli or inflammatory diseases can favor neoplasia, such as cigarette smoke and asbestos/silica for lung carcinoma [9], gastroesophageal reflux for cancer of the esophagus [10], inflammatory bowel disease for colorectal cancer (CRC) [11], chronic pancreatitis for pancreatic cancer [12], and pelvic inflammatory disease for ovarian cancer [13]. Third, the chronic intake of nonsteroidal anti-inflammatory drugs inversely correlates with CRC incidence, and recent studies indicate a negative effect of aspirin consumption on tumor growth [14]. Finally, the neutralization of inflammatory mediators (e.g., cytokines and pro-inflammatory transcription factors) decreases the incidence and spreading of tumors both in mice

and in humans [15, 16]. Table 1 lists cancers where a chronic inflammation has been implicated in their pathophysiology.

The mechanisms directing inflammation-induced tumorigenesis are well known. DNA damage and extracellular matrix disruption by inflammatory mediators (e.g., through the production of reactive oxygen species [17] and matrix metalloproteinases [18], respectively), in addition to the stimulation of tumor cell growth by cytokines (e.g., IL-1B for gastric carcinoma [19] and IL-8 for melanoma [20]), are the main recognized tumor-promoting mechanisms.

Not surprisingly, once a tumor has emerged, an inflammatory microenvironment can also promote malignant cell growth, resulting in neo-angiogenesis, acquisition of new mutations, extracellular matrix disruption, tumor cell migration, and finally metastasis [21]. The main cellular mediators of this process are macrophages (and to a lesser extent neutrophils) that are, by far, the major immune cellular component of tumor infiltrates [22]. These cells produce high quantities of IL-1β, IL-6, IL-23, and TNF-α, the key cytokines

Table 1 Cancers associated with chronic inflammatory conditions

Inflammatory process	Associated neoplasia
Infectious etiology	
Human papilloma virus	Cervical cancer and head/neck cancer
Hepatitis B and C virus	Hepatocellular carcinoma
Epstein-Barr virus	Nasopharynx cancer and lymphoma
Human herpes virus type 8	Kaposi's sarcoma
Helicobacter pylori	Gastric cancer
Schistosoma haematobium	Bladder cancer
Opisthorchis viverrini and *Clonorchis sinensis*	Hepatocellular carcinoma
Chronic noxious stimuli	
Tobacco smoke	Lung cancer, esophageal cancer, etc.
Silica	Lung cancer
Asbestos	Mesothelioma
Alcohol intake	Esophageal cancer
Chronic pelvic inflammatory disease	Ovarian cancer
Aflatoxins	Hepatocellular carcinoma
Chronic inflammatory diseases	
Gastroesophageal reflux and Barret's esophagus	Esophageal cancer
Type A gastritis	Gastric cancer
Chronic pancreatitis	Pancreatic cancer
Inflammatory bowel disease	Colorectal cancer
Chronic osteomyelitis	Bone cancer
Hashimoto's thyroiditis	Thyroid lymphoma
Thyroiditis	Papillary thyroid cancer
NASH, Hemochromatosis	Hepatocellular carcinoma

mediating the inflammation-induced tumorigenesis (reviewed in [21] and [22]).

Immune control and tumor escape

In addition to the link between inflammation and tumorigenesis, other cellular and molecular mediators of the immune system can contribute to control of tumor growth and elimination. Several epidemiological observations support this fact, including scarce reports of spontaneous cancer regression [23], the increased incidence of cancer in immunosuppressed individuals [24] and the association between increased tumor-infiltrating T cells (TIL) and favorable clinical outcome [3, 25].

The fact that tumor cells express antigens encoded by mutated genes [26] often renders them targets of the immune cells. Indeed, autologous TIL can induce tumor cell lysis in vitro and in vivo [27], and tumor-specific lymphocytes are often detected in patients with cancer. This phenomenon has been well characterized in CRCs, where microsatellite instability (MSI, a genetic defect that impedes DNA mismatch repair) fosters the expression of thousands of new antigens on tumor cells. Characteristically, MSI+ tumors have a prominent CD8+ T cell infiltration and are associated with favorable clinical outcome [28].

The major cellular mediators of the anti-tumor immune response are the CD8+ T cells, in addition to the Th1-oriented CD4+ lymphocytes. The first are in charge of the elimination of tumor cells through the production of apoptosis-inducing molecules or cytotoxic granules (e.g., granzymes, perforin, and granulysin) [29], while the latter can provide help to the CD8+ T cells and foster the anti-tumor response by the secreting major cytokines, including IFN-γ [30]. Several lines of evidence suggest that mature dendritic cells (DC) orchestrate the T and B cell anti-tumor immune response. Characteristically, these cells are present in highly organized peritumor immune cellular aggregates, called tertiary lymphoid structures (TLS) [31] (discussed in the next section).

The major anti-tumor immune response cytokines and chemokines are IFN-γ, IL-12, CXCL9 and CXCL10, mainly involved in CD8+ T cell recruitment (CXCL9 and CXCL10) and activation (IL-12 and IFN-γ) [32, 33].

All these processes submit tumor cells to a significant selective pressure. In fact, tumor cells can develop mechanisms that modulate and/or inhibit the immune response, including as follows: first, the production of immunosuppressive molecules (e.g., IL-10 and TGF-B) that hamper the cytotoxic and proliferative capacity of T cells [34] and, second, the expression of ligands for inhibitory receptors expressed on the TIL [35]. Of particular relevance, PD-1 is a molecule expressed on activated and exhausted T cells that diminishes the strength of

the cellular immune response upon binding to its ligands (PD-L1 and PD-L2) [36]. Under physiological conditions, the expression of PD-L1 and PD-L2 is highly regulated, and it is limited to dendritic cells, macrophages, activated T cells (PD-L1 only) [37], and certain tissues where immunomodulation is required (e.g., syncytiotrophoblast in the placenta). Nevertheless, tumor cells can express these ligands and subsequently inhibit T cell activity. Similar mechanisms have been reported, including the expression of the ligands for TIM-3 and LAG-3, two additional inhibitory receptors expressed on T cells (reviewed in [35]). Ultimately, this microenvironment induces the development of suppressive immune cells, including CD4+ Tregs and myeloid-derived suppressor cells [34], that sustain self-tolerance against tumor antigens.

This complex interconnected network of myeloid and lymphoid cells, endothelial and lymphatic vessels, and stromal cells—named the tumor microenvironment [3, 38]—has been largely studied in the last decade. Its influence on patient's clinical outcome and tumor progression has been of particular interest: Patients with tumors that develop immunosuppressive mechanisms have the worst prognosis, and their tumors will often display a higher histological grade characterized by dedifferentiation, neo-vascularization, and an inflammatory infiltrate.

The immune microenvironment as a prognostic tool

Many studies have described the distribution of the inflammatory and immune infiltrate within different tumors. Overall, the macrophages, mast cells, and granulocytes are found infiltrating both the invasive margin (IM) and the center of the tumor. On the contrary, the lymphoid infiltration is more precisely distributed, and some locations are enriched in certain cell types: NK cells are mostly found in the stroma and are not in contact with tumor cells, B cells are mostly found in the IM of the tumors within lymphoid aggregates, and T cells, particularly CD8+ T cells, are mainly located in the IM but can also infiltrate the tumor center [3, 39].

The analysis of the immune microenvironment in retrospective cohorts across different tumors has established a clear correlation between the density of infiltrating immune cells and patient's clinical outcome. Overall, a high infiltration by CD8+ T lymphocytes and a cytotoxic signature are associated with good clinical outcome in many tumor types, including lung, liver, stomach, CRC, breast, esophageal, bladder, melanoma, ovarian, and prostate cancers (reviewed in [3]). However, there are exceptions to this rule, including diffuse large B cell lymphoma [40], Hodgkin lymphoma [41], and clear-cell renal cell carcinoma (ccRCC) [42], where high densities of tumor-infiltrating CD8+ T and/or Th1 cells have been associated with poor prognosis. Overall, Th1 CD4+ T cells show a similar clinical impact to that of CD8+ T cells, and the infiltration by other T cell subsets (Th2, Th17, and Treg) is less

clear and seems to be dependent on the cancer type [3]. Interestingly, several reports have established that the expression of cytotoxic associated molecules (e.g., TIA-1 or granzyme) improves the prognostic power of CD8 cell densities in some pathologies [43, 44].

Interestingly, lymphoid aggregates (TLS) can be detected in the invasive margin of most tumors. Some of them exhibit properties of active immune sites that resemble those arising in other tissues upon infection or secondary to autoimmune or chronic inflammatory diseases [31]. Characteristically, they exhibit a T cell zone with embedded mature DC, germinal centers with proliferating B cells, and they are surrounded by high endothelial venules. How these structures are induced is still unclear in human tumors. It has been hypothesized that they represent an active local anti-tumor immune response, where in situ antigen presentation and lymphocyte activation can occur under a protected environment [31]. Indeed, the density of TLS correlates with a memory Th1/cytotoxic tumor signature and a favorable clinical outcome in lung cancer [45, 46], RCC [42], melanoma [47], and breast cancer [48, 49].

In view of the clinical impact of infiltrating CD8+ T cells in cancer, sustained efforts are being made to validate and promote their quantification in the routine clinical setting; this approach has been called Immunoscore [50]. The development of automatized software that quantify the densities of immune cells after immunohistochemical staining is promoting the gradual change from semiquantitative approaches to quantitative and more powerful methods. Moreover, the quantification of multiple immune cell populations is being currently studied and standardized and probably will help to accurately assess the integral local anti-tumor immune response. A recent technology that has started to gain importance is multicolor immune histology: a method that simultaneously detects and quantifies multiple markers in the same tissue sample [51]. While this technique provides relevant information regarding the co-expression and spatial relationships among immune and other cells, it also highlights the need for novel and more sophisticated systems for analysis.

The immune microenvironment and other histopathological features

The link between the tumor immune infiltrate and other pathology/clinical parameters has been assessed in independent studies, and there is not yet a consensus on this matter. Overall, tumors poorly infiltrated with CD8+ T cells often display a higher histological grade, characterized by dedifferentiation, prominent vascularization, and inflammation. Two independent studies in large cohorts of melanoma lesions established a correlation between an increased lymphocyte infiltration (known as higher TIL grade) and thinner lesions (Clark level), smaller radial growth phase, lower stage, and negative sentinel lymph nodes [52–54]. A similar picture has

Fig. 1 Association between immune checkpoint expression, nuclear grade, and overall survival in ccRCC. IHC photomicrographs of PD-1 (*left panels*) and PD-L1 (*right panels*) staining in two ccRCC (*top and bottom panels*). The Furhman Grade (FG) and survival time of each patient are displayed. *ccRCC* clear-cell renal cell carcinoma
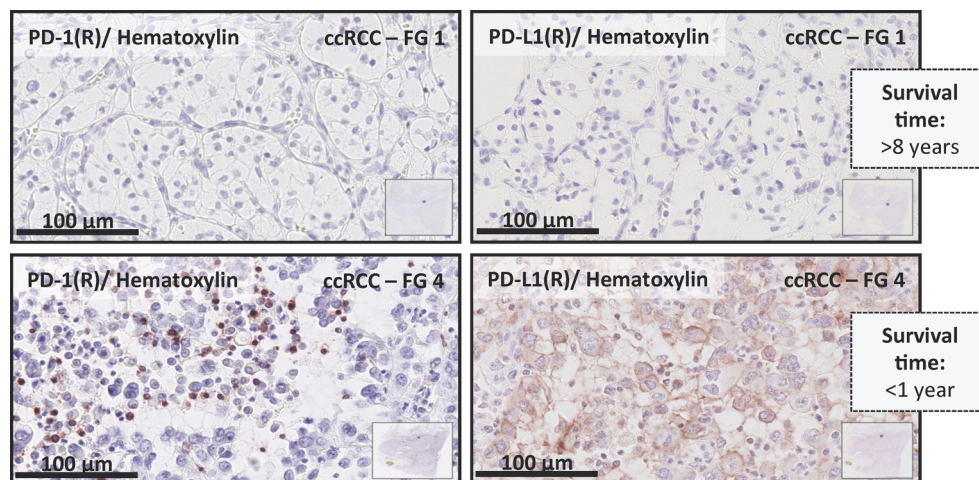

Table 2 Pathology parameters and CD8+ T cell infiltration in prevalent cancers

Cancer	Analyzed immune parameter	Associated pathology parameters	Reference
Melanoma	Not brisk or brisk infiltration (TIL grade 2 or 3) vs. TIL absence	Negative sentinel lymph nodes	[52]
		Lower mitotic rate	[53]
		Lower Clark level/tumor thickness	
		Lower tumor grade	[54]
Breast	Increased stromal/intraepithelial infiltration by CD8+ lymphocytes	Higher histological grade	[58]
			[62]
			[63]
		Lower stage	[64]
		ER-a (−) and/or PgR (−) tumor cells	[62]
			[58]
			[63]
		Basal phenotype	[62]
			[59]
		Her2+tumor cells	[63]
		Ki-67 (−) tumor cells	[64]
		Negative lymph node invasion	
		Smaller tumors (<2 cm)	[65]
Lung—NSCLC	Increased stromal infiltration by CD8+ lymphocytes	Moderate/Poor differentiation	[66]
			[67]
		Lower stage	[68]
		Negative angiolymphatic invasion	[69]
HCC	Increased stromal/intraepithelial infiltration by CD8+ lymphocytes	Lower stage (I–II vs. III–IV)	[70]
		Poorly differentiated tumors	[71]
Pancreas	Increased intratumor infiltration by CD8+ lymphocytes	Decreased tumor depth Lower stage	[72]
		Well-differentiated tumors	[73]
RCC	Invasive margin or intratumor CD8+ T cell infiltration	Higher nuclear grade	[42]
			[74]
Colorectal	Increased CD8+ and CD45RO+ cell densities	Negative perineural invasion	[52]
		Lower T stage	[51]
			[53]
		Negative lymph node invasion	[75]
			[76]
			[77]

NSCLC non-small-cell lung cancer, *HCC* hepatocellular carcinoma, *RCC* renal cell carcinoma, *ER-α* estrogen receptor-alpha, *PgR* progesterone receptor

been described in CRC, where there is an inverse correlation between the CD8+ and CD45RO+ (memory T lymphocytes) cell densities and the tumor stage [55] and perineural invasion [56]. Moreover, the density of innate cells increases, whereas that of most other T cell subsets decreases along with tumor progression in this pathology [57]. The association between checkpoint expression, nuclear grade, and overall survival in ccRCC is depicted in Fig. 1.

Nevertheless, this is not the case for all tumor types. Breast and RCC deserve particular attention because both the basal subtype in breast cancer and the inflammatory/inhibited subtype in RCC have been associated with an increased lympho- cytic infiltration in advanced stages [42, 58–60]. Evidence suggests that in these subtypes, the inflammatory microenvi- ronment largely impacts the beneficial effect of lymphocytes, while the latter exhibit a suppressed phenotype [38, 61]. The

link between pathology parameters and CD8+ T cell infiltra- tion in most prevalent cancers is summarized in Table 2.

Therapies that modulate the tumor microenvironment

In view of the important immune processes taking place with- in tumors, many therapies to boost the local immune response and diminish the inflammatory or suppressor molecules are being currently developed.

One of the first successful immunotherapies used in the clinical setting was recombinant IL-2, whose aim was to acti- vate and expand the intra-tumor T lymphocyte [78]. The treat- ment of thousands of patients in the late 1980s and 1990s established that only metastatic melanoma and metastatic RCC responded to this therapy, and complete response rate was limited to 10 % [78]. Because of the high rate of adverse

Fig. 2 Major immunopathological events in cancer natural history and prognosis. A cartoon depiction of the development and maintenance of a cancerous lesion. *Left panel*: Upon exposure to chronic inflammatory stimuli, normal cells can transform into neoplastic cells. Afterward, the immune selection can promote the prevalence of more malignant and less antigenic tumor cells. *Middle panel*: main immune and pathologic phenomena in cancer natural history. Early stages are generally characterized by immunogenic tumor cells and a subsequent CD8+ T cell infiltration; on the contrary, advanced tumor stages are characterized by increased densities of PD-1+/LAG-3+/TIM-3+ cells, tumor or immune expression of PD-L1/L2, high histological grade, and angiogenesis. *Right panel*: clinical relevance of the immunopathological tumor characterization. Advanced tumor stages are associated with poor prognosis, and if they are associated with particular immunologic patterns, they will more likely respond to immunotherapies
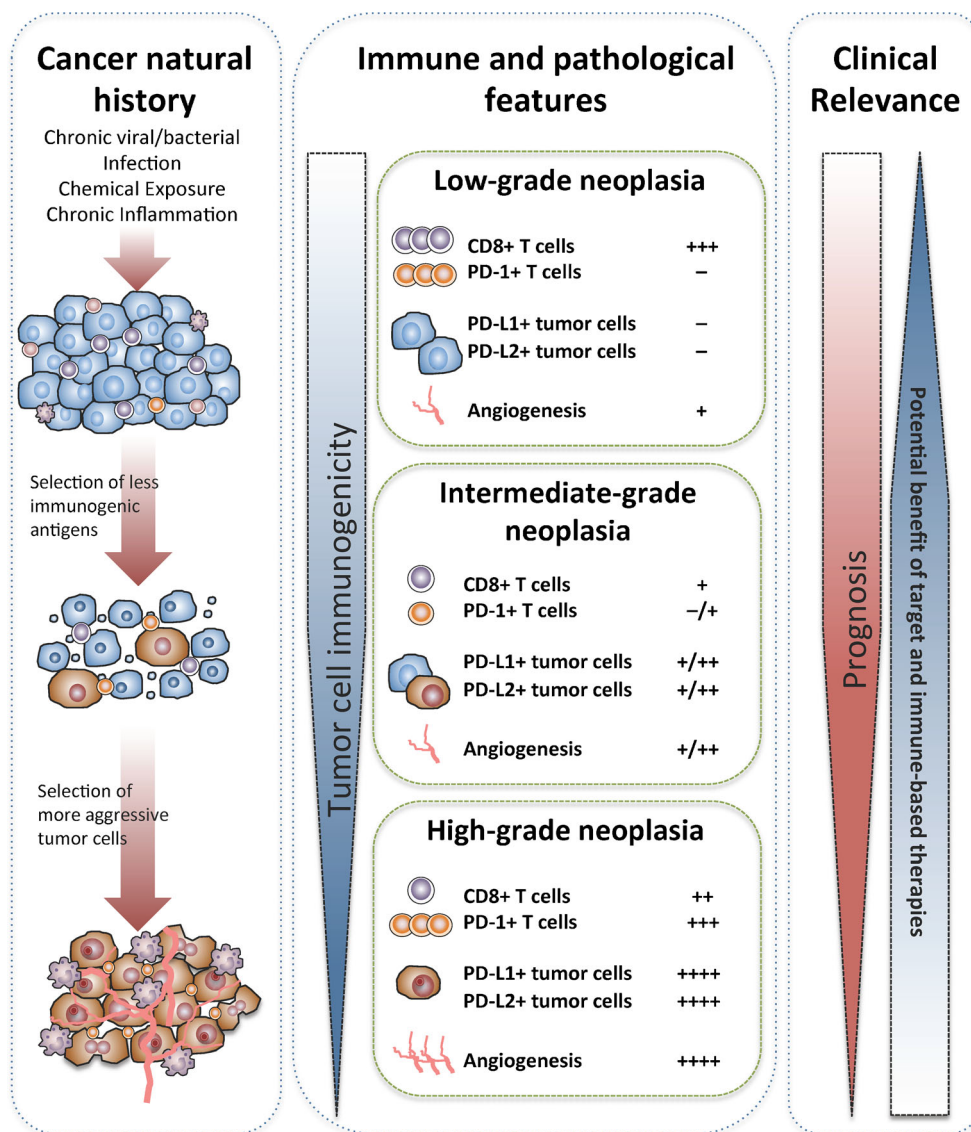
effects, this therapy was replaced over the years, but it set a precedent for the development of other immunotherapies: Boosting the T cell response could mediate complete destruction of large, vascularized, and invasive cancers in humans.

Other immunotherapies used in similar clinical scenarios are IFN-α and anti-angiogenic drugs (e.g., sunitinib and bevacizumab). In metastatic RCC, sunitinib as monotherapy has shown high objective response rates (up to 50 %) and currently is the first-line treatment option for metastatic RCC patients [79]. In addition to normalizing the tumor vascularization, this drug promotes anti-tumor immunity through different mechanisms [80].

New therapies based on the recent understanding of the immune-suppressive cells and T cell inhibitor pathways are being tested. The term checkpoint blockade describes the injection of monoclonal antibodies specific for inhibitory receptors expressed on the surface of lymphocytes (anti-PD-1, anti-CTLA-4, and anti-LAG-3) or their ligands on tumor or other suppressive immune cells (PD-L1 and PD-L2) [35, 81]. Several trials on increasing number of malignancies are ongoing; overall, they have shown exceptional results in some cancer including melanoma [82, 83], RCC [84–86], lung cancer [84], Hodgkin lymphoma [87], and bladder cancer [88].

Interestingly, the response rate of metastatic RCC to PD-1 blockade is approximately 25 % [84, 86]. Consistently, a recent work by our group showed that one third of the primary ccRCC displays a highly inflammatory/suppressive phenotype, characterized by high densities of PD-1+ and LAG-3+ T cells, in addition to the absence of TLS and PD-L1+/L2+ tumor cells [42]. Overall, these results suggest that this "suppressive immune profile" should guide the selection of suitable patients to receive immunotherapies.

Indeed, the analysis of the tumor microenvironment is becoming a powerful tool to predict the response to immunotherapies. Interestingly, preliminary data from clinical trials of PD-1 blockade suggest that the presence of (1) infiltrating CD8+ or PD-1+ T cells [89] and/or (2) PD-L1+ tumor [84, 87, 90] or immune cells [89, 91] is the more sensitive parameter to predict the patients' response to treatment [92].

Conclusions and perspectives

A significant amount of tumors develop under inflammatory stimuli coming from infectious organisms or diverse pathological processes. Once established, tumor cells express neo-antigens encoded by mutated genes that can induce a specific immune response. In this delicate balance, the selection pressure favors tumor cells that produce, firstly, inflammatory molecules that induce neo-vascularization, new mutations, tumor growth, and metastasis and, secondly, molecules that hamper the cellular immune response (Fig. 2). The characterization of the tumor infiltrates allows the identification of

tumors with the worst prognosis independently of tumor stage, and evidence suggests that it could soon become into a new pathological variable assessed in the routine clinical setting.

Sustained efforts are currently made to develop therapeutic agents to skew this tumor microenvironment toward an adaptive/anti-tumor immune response, and promising advances in cancer treatment have been recently made. The challenge toward personalized medicine is to effectively discriminate the potential responders for each specialized therapy. Recent evidence points that this analysis should largely rely on the pathological exploration of the tumor microenvironment, highlighting the central role of the pathologist in this new era.

References

1. Balkwill F, Mantovani A (2001) Inflammation and cancer: back to Virchow? Lancet 357:539–545
2. Mantovani A, Allavena P, Sica A, Balkwill F (2008) Cancer-related inflammation. Nature 454:436–444
3. Fridman WH, Pagès F, Sautès-Fridman C, Galon J (2012) The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer 12:298–306
4. de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M (2012) Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol 13: 607–615
5. Salama NR, Hartung ML, Müller A (2013) Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. Nat Rev Microbiol 11:385–399
6. Arzumanyan A, Reis HM, Feitelson MA (2013) Pathogenic mechanisms in HBV- and HCV-associated hepatocellular carcinoma. Nat Rev Cancer 13:123–135
7. Rothenberg SM, Ellisen LM (2012) The molecular pathogenesis of head and neck squamous cell carcinoma. J Clin Invest 122:1951–1957
8. Castle PE, Hillier SL, Rabe LK, Hildesheim A, Herrero R, Bratti MC, Sherman ME, Burk RD, Rodriguez AC, Alfaro M, Hutchinson ML, Morales J, Schiffman M (2001) An association of cervical inflammation with high-grade cervical neoplasia in women infected with oncogenic human papilloma virus (HPV). Cancer Epidemiol Biomark 10:1021–1027
9. Houghton AM (2013) Mechanistic links between COPD and lung cancer. Nat Rev Cancer 13:233–245
10. Pennathur A, Gibson MK, Jobe BA, Luketich JD (2013) Oesophageal carcinoma. Lancet 281:400–412

11. Dyson JK, Rutter MD (2012) Colorectal cancer in inflammatory bowel disease: what is the real magnitude of the risk? World J Gastroenterol 18:3839–3848

12. Pinho AV, Chantrill L, Rooman I (2014) Chronic pancreatitis: a path to pancreatic cancer. Cancer Lett 345:203–209

13. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA (2014) Ovarian cancer. Lancet 384:1376–1388

14. Chia WK, Ali R, Toh HC (2012) Aspirin as adjuvant therapy for colorectal cancer–reinterpreting paradigms. Nat Rev Clin Oncol 9: 561–570

15. Gus-Brautbar Y, Johnson D, Zhang L, Sun H, Wang P, Zhang S, Zhang L, Chen YH (2012) The anti-inflammatory TIPE2 is an inhibitor of the oncogenic Ras. Mol Cell 45:610–618

16. Sun B, Karin M (2014) The therapeutic value of targeting inflammation in gastrointestinal cancers. Trends Pharmacol Sci 35:349–357

17. Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ (2000) Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. Cancer Res 60:184–190

18. Shuman-Moss LA, Jensen-Taubman S, Stetler-Stevenson WG (2012) Matrix metalloproteinases: changing roles in tumor progression and metastasis. Am J Pathol 181:1895–1899

19. Xue H, Lin B, Ni P, Xu H (2010) Huang G Interleukin-1B and interleukin-1 RN polymorphisms and gastric carcinoma risk: a meta-analysis. J Gastroenterol Hepatol 25:1604–1617

20. Haghnegahdar H, Du J, Wang D, Strieter RM, Burdick MD, Nanney LB, Cardwell N, Luan J, Shattuck-Brandt R, Richmond A (2000) The tumorigenic and angiogenic effects of MGSA/GRO proteins in melanoma. J Leukoc Biol 67:53–62

21. Balkwill F (2004) Cancer and the chemokine network. Nat Rev Cancer 4:540–550

22. Qian BZ, Pollard JW (2010) Macrophage diversity enhances tumor progression and metastasis. Cell 141:39–51

23. Hoption-Cann SA, van Netten JP, van Netten C, Glover DW (2002) Spontaneous regression: a hidden treasure buried in time. Med Hypotheses 58:115–119

24. Bower M, Palmieri C, Dhillon T (2006) AIDS-related malignancies: changing epidemiology and the impact of highly active antiretroviral therapy. Curr Opin Infect Dis 19:14–19

25. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F (2006) Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313:1960–1964

26. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, Arthur CD, White JM, Chen YS, Shea LK, Hundal J, Wendl MC, Demeter R, Wylie T, Allison JP, Smyth MJ, Old LJ, Mardis ER, Schreiber RD (2012) Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 482: 400–404

27. Lennerz V, Fatho M, Gentilini C, Frye RA, Lifke A, Ferel D, Wölfel C, Huber C, Wölfel T (2005) The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. Proc Natl Acad Sci U S A 102:16013–16018

28. Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, Starling N (2010) Colorectal cancer. Lancet 375: 1030–1047

29. Restifo NP, Dudley ME, Rosenberg SA (2012) Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 12:269–281

30. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H (1998) The central role of CD4(+) T cells in the antitumor immune response. J Exp Med 188:2357–2368

31. Dieu-Nosjean MC, Goc J, Giraldo NA, Sautès-Fridman C, Fridman WH (2014) Tertiary Lymphoid Structures in cancer and beyond. Trends Immunol 35:571–580

32. Mlecnik B, Tosolini M, Charoentong P, Kirilovsky A, Bindea G, Berger A, Camus M, Gillard M, Bruneval P, Fridman WH, Pagès F, Trajanoski Z, Galon J (2010) Biomolecular network reconstruction identifies T-cell homing factors associated with survival in colorectal cancer. Gastroenterology 138:1429–1440

33. de Chaisemartin L, Goc J, Damotte D, Validire P, Magdeleinat P, Alifano M, Cremer I, Fridman WH, Sautès-Fridman C, Dieu-Nosjean MC (2011) Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer. Cancer Res 71:6391–6399

34. Zou W (2005) Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer 5:263–274

35. Topalian SL, Drake CG, Pardoll DM (2015) Immune checkpoint blockade: a common denominator approach to cancer therapy. Cancer Cell 27:450–461

36. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, Horton HF, Fouser L, Carter L, Ling V, Bowman MR, Carreno BM, Collins M, Wood CR, Honjo T (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

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

38. Giraldo NA, Becht E, Remark R, Damotte D, Sautès-Fridman C, Fridman WH (2014) The immune contexture of primary and metastatic human tumours. Curr Opin Immunol 27:8–15

39. Galon J, Angell HK, Bedognetti D, Marincola FM (2013) The continuum of cancer immunosurveillance: prognostic, predictive, and mechanistic signatures. Immunity 39:11–26

40. Muris JJ, Meijer CJ, Cillessen SA, Vos W, Kummer JA, Bladergroen BA, Bogman MJ, MacKenzie MA, Jiwa NM, Siegenbeek van Heukelom LH, Ossenkoppele GJ, Oudejans JJ (2004) Prognostic significance of activated cytotoxic T-lymphocytes in primary nodal diffuse large B-cell lymphomas. Leukemia 18:589–596

41. Scott DW, Chan FC, Hong F, Rogic S, Tan KL, Meissner B, Ben-Neriah S, Boyle M, Kridel R, Telenius A, Woolcock BW, Farinha P, Fisher RI, Rimsza LM, Bartlett NL, Cheson BD, Shepherd LE, Advani RH, Connors JM, Kahl BS, Gordon LI, Horning SJ, Steidl C, Gascoyne RD (2013) Gene expression-based model using formalin-fixed paraffin-embedded biopsies predicts overall survival in advanced-stage classical Hodgkin lymphoma. J Clin Oncol 31: 692–700

42. Giraldo NA, Becht E, Pages F, Skliris GP, Verkarre V, Vano Y, Mejean A, Saint-Aubert N, Lacroix L, Natario I, Lupo A, Alifano M, Damotte D, Cazes A, Triebel F, Freeman GJ, Dieu-Nosjean MC, Oudard SM, Fridman WH, Sautes-Fridman C (2015) Orchestration and prognostic significance of immune checkpoints in the microenvironment of primary and metastatic renal cell cancer. Clin Cancer Res. doi:10.1158/1078-0432.CCR-14-2926

43. Zlobec I, Karamitopoulou E, Terracciano L, Piscuoglio S, Iezzi G, Muraro MG, Spagnoli G, Baker K, Tzankov A, Lugli A (2010) TIA-1 cytotoxic granule-associated RNA binding protein improves the prognostic performance of CD8 in mismatch repair-proficient colorectal cancer. PLoS One 5, e14282

44. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N (2015) Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell 160:48–61

45. Goc J, Germain C, Vo-Bourgais TK, Lupo A, Klein C, Knockaert S, de Chaisemartin L, Ouakrim H, Becht E, Alifano M, Validire P, Remark R, Hammond SA, Cremer I, Damotte D, Fridman WH,

Sautès-Fridman C, Dieu-Nosjean MC (2014) Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. Clin Cancer Res 74:705–715

46. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V, Rabbe N, Laurans L, Tartour E, de Chaisemartin L, Lebecque S, Fridman WH, Cadranel J (2008) Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol 26:4410–4417

47. Cipponi A, Mercier M, Seremet T, Baurain JF, Théate I, van den Oord J, Stas M, Boon T, Coulie PG, van Baren N (2012) Neogenesis of lymphoid structures and antibody responses occur in human melanoma metastases. Cancer Res 72:3997–4007

48. Martinet L, Garrido I, Filleron T, Le Guellec S, Bellard E, Fournie JJ, Rochaix P, Girard JP (2011) Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. Cancer Res 71:5678–5687

49. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, de Wind A, Ravoet M, Le Buanec H, Sibille C, Manfouo-Foutsop G, Veys I, Haibe-Kains B, Singhal SK, Michiels S, Rothé F, Salgado R, Duvillier H, Ignatiadis M, Desmedt C, Bron D, Larsimont D, Piccart M, Sotiriou C, Willard-Gallo K (2013) CD4+ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest 123:2873–2892

50. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, Lugli A, Zlobec I, Hartmann A, Bifulco C, Nagtegaal ID, Palmqvist R, Masucci GV, Botti G, Tatangelo F, Delrio P, Maio M, Laghi L, Grizzi F, Asslaber M, D'Arrigo C, Vidal-Vanaclocha F, Zavadova E, Chouchane L, Ohashi PS, Hafezi-Bakhtiari S, Wouters BG, Roehrl M, Nguyen L, Kawakami Y, Hazama S, Okuno K, Ogino S, Gibbs P, Waring P, Sato N, Torigoe T, Itoh K, Patel PS, Shukla SN, Wang Y, Kopetz S, Sinicrope FA, Scripcariu V, Ascierto PA, Marincola FM, Fox BA, Pages F (2013) Towards the introduction of the Immunoscore in the classification of malignant tumors. J Pathol 232:199–209

51. Stack EC, Wang C, Roman KA, Hoyt CC (2014) Multiplexed immunohistochemistry, imaging, and quantitation: a review, with an assessment of Tyramide signal amplification, multispectral imaging and multiplex analysis. Methods 70:46–58

52. Taylor RC, Patel A, Panageas KS, Busam KJ, Brady MS (2007) Tumor-infiltrating lymphocytes predict sentinel lymph node positivity in patients with cutaneous melanoma. J Clin Oncol 25:869–875

53. Azimi F, Scolyer RA, Rumcheva P, Moncrieff M, Murali R, McCarthy SW, Saw RP, Thompson JF (2012) Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. J Clin Oncol 30:2678–2683

54. Thomas NE, Busam KJ, From L, Kricker A, Armstrong BK, Anton-Culver H, Gruber SB, Gallagher RP, Zanetti R, Rosso S, Dwyer T, Venn A, Kanetsky PA, Groben PA, Hao H, Orlow I, Reiner AS, Luo L, Paine S, Ollila DW, Wilcox H, Begg CB, Berwick M (2013) Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based genes, environment and melanoma study. J Clin Oncol 31:4252–4259

55. Mlecnik B, Tosolini M, Kirilovsky A, Berger A, Bindea G, Meatchi T, Bruneval P, Trajanoski Z, Fridman WH, Pagès F, Galon J (2011) Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. J Clin Oncol 29:610–618

56. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J (2005) Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 353:2654–2666

57. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Frederiksen T, Lafontaine L, Berger A, Bruneval P, Fridman WH, Becker C, Speicher MR, Trajanoski Z, Galon J (2013) Spatio-temporal dynamics of intratumoral cells reveal the immune landscape in human cancer. Immunity 39:782–795

58. Liu F, Lang R, Zhao J, Zhang X, Pringle GA, Fan Y, Yin D, Gu F, Yao Z, Fu L (2011) CD8+ cytotoxic T cell and FOXP3+ regulatory T cell infiltration in relation to breast cancer survival and molecular subtypes. Breast Cancer Res Treat 130:645–655

59. Liu S, Lachapelle J, Leung S, Gao D, Foulkes WD, Nielsen TO (2012) CD8+ lymphocyte infiltration is an independent favorable prognostic indicator in basal-like breast cancer. Breast Cancer Res 14:R48

60. Kowalczyk D, Skorupski W, Kwias Z, Nowak J (1997) Flow cytometric analysis of tumour-infiltrating lymphocytes in patients with renal cell carcinoma. Br J Urol 80:543–547

61. Becht E, Goc J, Germain C, Giraldo NA, Dieu-Nosjean MC, Sautès-Fridman C, Fridman WH (2014) Shaping of an effective immune microenvironment to and by cancer cells. Cancer Immunol Immunother 63:991–997

62. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH, Ellis IO, Green AR (2011) Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol 29:1949–1955

63. Baker K, Lachapelle J, Zlobec I, Bismar TA, Terracciano L, Foulkes WD (2011) Prognostic significance of CD8+ T lymphocytes in breast cancer depends upon botho estrogen receptor status and histological grade. Histopathology 58:1107–1116

64. Kim ST, Jeong H, Woo OH, Seo JH, Kim A, Lee ES, Shin SW, Kim YH, Kim JS, Park KH (2013) Tumor-infiltrating lymphocytes, tumor characteristics, and recurrence in patients with early breast cancer. Am J Clin Oncol 36:224–231

65. Chen Z, Chen X, Zhou E, Chen G, Qian K, Wu X, Miao X, Tang Z (2014) Intratumoral CD8+ cytotoxic lymphocyte is a favorable prognostic marker in node-negative breast cancer. PLoS One 17, e95475

66. Mori M, Ohtani H, Naito Y, Sagawa M, Sato M, Fujimura S, Nagura H (2000) Infiltration of CD8+ T cells in non-small cell lung cancer is associated with dedifferentiation of cancer cells, but not with prognosis. Tohoku J Exp Med 191:113–118

67. Wakabayashi O, Yamazaki K, Oizumi S, Hommura F, Kinoshita I, Ogura S, Dosaka-Akita H, Nishimura M (2003) CD4+ T cells in cancer stroma, not CD8+ T cells in cancer cell nests, are associated with favorable prognosis in human non-small cell lung cancers. Cancer Sci 94:1003–1009

68. Hiraoka K, Miyamoto M, Cho Y, Suzuoki M, Oshikiri T, Nakakubo Y, Itoh T, Ohbuchi T, Kondo S, Katoh H (2006) Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. Br J Cancer 94:275–280

69. Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM, Busund LT (2008) Prognostic effect of epithelial and stromal lymphocyte infiltration in non-small cell lung cancer. Clin Cancer Res 15:5220–5227

70. Ikeguchi M, Oi K, Hirooka Y, Kaibara N (2004) CD8+ lymphocyte infiltration and apoptosis in hepatocellular carcinoma. Eur J Surg Oncol 30:53–57

71. Kobayashi N, Hiraoka N, Yamagami W, Ojima H, Kanai Y, Kosuge T, Nakajima A, Hirohashi S (2007) FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. Clin Cancer Res 13:902–911

72. Fukunaga A, Miyamoto M, Cho Y, Murakami S, Kawarada Y, Oshikiri T, Kato K, Kurokawa T, Suzuoki M, Nakakubo Y, Hiraoka K, Itoh T, Morikawa T, Okushiba S, Kondo S, Katoh H

(2004) CD8+ tumor-infiltrating lymphocytes together with CD4+ tumor-infiltrating lymphocytes and dendritic cells improve the prognosis of patients with pancreatic adenocarcinoma. Pancreas 28:e26–e31

73. Tewari N, Zaitoun AM, Arora A, Madhusudan S, Ilyas M, Lobo DN (2013) The presence of tumour-associated lymphocytes confers a good prognosis in pancreatic ductal adenocarcinoma: an immunohistochemical study of tissue microarrays. BMC Cancer 24:436

74. Nakano O, Sato M, Naito Y, Suzuki K, Orikasa S, Aizawa M, Suzuki Y, Shintaku I, Nagura H, Ohtani H (2001) Proliferative activity of intratumoral CD8(+) T-lymphocytes as a prognostic factor in human renal cell carcinoma: clinicopathologic demonstration of antitumor immunity. Cancer Res 61:5132–5136

75. Zlobec I, Terracciano LM, Lugli A (2008) Local recurrence in mismatch repair-proficient colon cancer predicted by an infiltrative tumor border and lack of CD8+ tumor-infiltrating lymphocytes. Clin Cancer Res 14:3792–3797

76. Däster S, Eppenberger-Castori S, Hirt C, Zlobec I, Delko T, Nebiker CA, Soysal SD, Amicarella F, Iezzi G, Sconocchia G, Heberer M, Lugli A, Spagnoli GC, Kettelhack C, Terracciano L, Oertli D, von Holzen U, Tornillo L, Droeser RA (2014) High frequency of CD8 positive lymphocyte infiltration correlates with lack of lymph node involvement in early rectal cancer. Dis Markers 2014: 792183

77. Koelzer VH, Dawson H, Andersson E, Karamitopoulou E, Masucci GV, Lugli A, Zlobec I (2015) Active immunosurveillance in the tumor microenvironment of colorectal cancer is associated with low frequency tumor budding and improved outcome. Transl Res. doi:10.1016/j.trsl.2015.02.008

78. Rosenberg SA (2014) IL-2: the first effective immunotherapy for human cancer. J Immunol 192:5451–5458

79. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski 660 RM, Oudard S, Negrier S, Szczylik C, Pili R, Bjarnason GA, 661 Garcia-del-Muro X, Sosman JA, Solska E, Wilding G, Thompson 662 JA, Kim ST, Chen I, Huang X, Figlin RA (2009) Overall survival 663 and updated results for sunitinib compared with interferon alfa in 664 patients with metastatic renal cell carcinoma. J Clin Oncol 27: 665 3584–3590

80. Finke JH, Rayman PA, Ko JS, Bradley JM, Gendler SJ, Cohen PA (2013) Modification of the tumor microenvironment as a novel target of renal cell carcinoma therapeutics. Cancer J19:353–364

81. Speiser DE, Utzschneider DT, Oberle SG, Münz C, Romero P, Zehn D (2014) T cell differentiation in chronic infection and cancer: functional adaptation or exhaustion? Nat Rev Immunol 14:768–774

82. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbé C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ (2010) Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 363:711–723

83. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Wolchok JD, Hersey P, Joseph RW, Weber JS, Dronca R, Gangadhar TC, Patnaik A, Zarour H, Joshua AM, Gergich K, Elassaiss-Schaap J, Algazi A, Mateus C, Boasberg P, Tumeh PC, Chmielowski B, Ebbinghaus SW, Li XN, Kang SP, Ribas A (2013)

84. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. N Engl J Med 369:134–144

84. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 26:2443–2454

85. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 366:2455–2465

86. Motzer RJ, Rini BI, McDermott DF, Redman BG, Kuzel TM, Harrison MR, Vaishampayan UN, Drabkin HA, George S, Logan TF, Margolin KA, Plimack ER, Lambert AM, Waxman IM, Hammers HJ (2015) Nivolumab for metastatic renal cell carcinoma: results of a randomized phase II trial. J Clin Oncol 33:1430–1437

87. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattry D, Freeman GJ, Rodig SJ, Chapuy B, Ligon AH, Zhu L, Grosso JF, Kim SY, Timmerman JM, Shipp MA, Armand P (2015) PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med 372:311–319

88. Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA, Petrylak DP, Teng SL, Shen X, Boyd Z, Hegde PS, Chen DS, Vogelzang NJ (2014) MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. Nature 515:558–562

89. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, Chmielowski B, Spasic M, Henry G, Ciobanu V, West AN, Carmona M, Kivork C, Seja E, Cherry G, Gutierrez AJ, Grogan TR, Mateus C, Tomasic G, Glaspy JA, Emerson RO, Robins H, Pierce RH, Elashoff DA, Robert C, Ribas A (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515:568–571

90. Taube JM, Klein A, Brahmer JR, Xu H, Pan X, Kim JH, Chen L, Pardoll DM, Topalian SL, Anders RA (2014) Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy. Clin Cancer Res 20: 5064–5074

91. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN, Kohrt HE, Horn L, Lawrence DP, Rost S, Leabman M, Xiao Y, Mokatrin A, Koeppen H, Hegde PS, Mellman I, Chen DS, Hodi FS (2014) Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 515:563–567

92. Shin DS, Ribas A (2015) The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol 33C: 23–35