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Review Article

Is it Time to Reconsider and Re-Stratergise B Lymphocyte Based Immunotherapy?

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Introduction

The present scenario of pandemic era has witnessed milestone development in the field of immunotherapeutic and vaccine engineering. It has evoked a breech in the routine therapeutic strategies that was conventionally followed. The new outlook in the immunological aspect of major diseases as helped to refocus back on to the basic immune responses associated with innate response against disease prevention and progression. The current trend to re-strategize the treatment options for patients suffering from cancer in the light of immunotherapy has gained its importance. Recently, immune-therapeutic has gained a great interest in treatment of Oral Squamous Cell Carcinoma (OSCC). The T- cell based immune targeted approach of T-cell based therapy, CAR-T (chimeric antigen receptor) and its success has nailed its best attempt in immunotherapy of Head and Neck Cancer (HNSCC) [1,2]. However, the role of humoral response against the tumor especially of B lymphocytes has not gained its due importance for many reasons. The present situation on the other hand is more encouraging to attempt and utilize the vast potentiality of the B lymphocyte especially the antigen and antibody associated immune response.

Role of B Lymphocyte - Debatable

The role played by B lymphocyte in the tumor microenvironment is still at debate. This is mainly associated with the bidirectional response of B lymphocytes in the tumor microenvironment. Both the pro-tumorogenic and anti-tumorogenic response of B lymphocyte remains controversial, as the literature evidence to support both is limited. Generally, the pro-tumorogenic response of B lymphocytes include mainly the production of various cytokines and interleukins (ILs), especially IL-35, transforming growth factor-beta (TGF-β) and IL-10, that aid in tumor progression [3,4]. The B cell subtype of regulatory cells (Bregs) tends to promote metastasis. The B lymphocytes stimulate secretion of various angiogenic and pro-inflammatory factor especially, secretion of IL-8 and vascular endothelial growth factor that promote angiogenesis and tumor growth [5-7]. The presence of chemokine CXCL-13 is considered closely as a contributing factor for tumor progression along with various lymphotoxins such as STAT3 signalling pathway and nuclear factor-kappa B [8-10]. Mature B cells are divided into three main subsets: B1- B cells, mainly found in peritoneal and pleural cavities; B2 or follicular (FO) B cells, that is the most abundant and are located in the B cell areas of lymph nodes, Peyer's patches and spleen; Marginal Zone (MZ) B cells, located in the marginal sinus of the spleen [11]. B cells of different subsets vary in terms of their location and in the way they are activated in a T-dependent or a T-independent way. The studies conducted to evaluate the role played by the B lymphocytes in OSCC is gaining wide acceptance. For instance, the most recent study conducted by C. Phanthunane et al (2021) [12], study, using multiplex in situ immunofluorescence and computational image analyses of 138 patients with T1-T2 primary oral-tongue squamous cell carcinoma, observed a high density of CD20 cells that clustered together in the IM-S regions. Notably, it introduced the term of CD20 Cluster Score (a score that combines the number of CD20 cells within 20 μm radii of CD20 as well as CD4 cells, which probably yielded a significant association with OS (overall survival), DFS (disease free survival) and local recurrence rate. It is mostly pronounced in cases of low densities of CD4 cells at IM (invasive margin)-S (surface) regions. The study concluded that the presence of stromal clusters of B-cells together with CD4 T-cells at IM, which yields the so-called CD20 Cluster Score, acts as a strong independent prognostic factor in early-stage oral-tongue cancer. The other gathered data obtained from various studies Tsou P et al (2016), [13], Lao X M et al (2016),[14] and

Wirsing A M et al (2018) [15], study results suggested the anti-tumor response of B lymphocytes in the tumor microenvironment. This includes secretion of antibodies, presentation of tumor antigen to adjacent T-cells, and production of immune-potentiating cytokines, such as IFNγ and IL12. [16-20], B cells can associate with T cells and organize in Tertiary Lymphoid Structures (TLS) within the tumor, where it is believed that naïve T cells is usually activated, [21], which majorily highlights the potential role of B cells in modulating anti-tumor immunity. In the majority of cancer types, the infiltration of B cells is often associated with a good prognosis.

On the other hand, B lymphocytes, considered to mediate a pro-tumorogenic effect through induction of neovascularization, becoming regulatory B-cells (Bregs) is considered to cause progression of tumor to distant sites. This was suggested from the results of the study conducted by Zhou X, et al. (2016) [22]. The other studies conducted by de Viscer KE, et al. (2005) [23] and Ammirante, et al. (2010) [24], suggested the production of immune-suppressive cytokines, such as IL10, IL-35, and TGFβ would promote the pro-tumorogenic response in the tumor microenvironment. This tends to deregulate the tumor process. The study results of Wouters MCA, et al. (2018) [25] and Gentles A J, et al. (2015) [26], contradicting pointing out that tumor infiltrated B-cells and B-cells associated genes expressed in cancers tends to give a negative prognosis or even no effect on patients' survival. The other factor that often promote pro-tumorogenic response include Granzyme B that when transferred to T cells, tends to degrade the T cell receptor ξ chain without inducing T cell apoptosis. Lymphotoxin usually activates non-canonical and canonical NF-κB signalling and STAT3 usually provides inhibitory effect of B cells and survival signals to tumor cells.

B Cells and Tumor Microenvironment

To determine the role of B lymphocytes in tumor microenvironment, it requires to first evaluate the initiation of the tumor process from its inception. The pre-malignant state general transforms into a fully transformed tumor state through a steady or rapid escalation seen in both cellular and molecular elements. The role of B lymphocytes is critically expressed in pre-tumor environment or pre-malignancy state. However, there is only restricted data on the actual role played by B lymphocyte in the precancerous environment. The study results of Auclair and Ellis, et al. (1996) [27], done on the tongue lesions showed an increase in CD4 and B cells, but not in HLA/DR positive cells. Whereas, Ito, et al. (1999) [28], had no similar results of infiltrative cells. The increase in immune cell infiltration with progression of oral epithelium from hyperkeratosis to dysplasia and carcinoma is noted by G Gannot, et al. (2002) [29]. The results of the study however, revealed that in the tongue lesions, the changes in the epithelium from normal appearance to transformed were accompanied by a corresponding increase in the infiltration of CD4, CD8, CD14, CD19+20, and HLA/DR positive cells. The most significant change was an increase in B lymphocytes in tongue lesions,that was in accordance with the transformation level.

B Cell-Based Cancer Therapeutics

The future perspectives to introduce B lymphocytes into therapeutic protocol could aim for either suppressing or enhancing tumor specific response of B cells. The successful illustration contributed by Biagei, et al. (2005) [30] as the first clinical trial in the approach cancer vaccine, used CD40 cells as cellular adjuvant in cancer regression therapy. The vaccine composition included transduced autologous leukemic B cells isolated from patients diagnosed with Chronic Lymphocytic Leukaemia (CLL) combined with an adenoviral vector that contained human CD 40L gene. It was then administrated to 9 patient subjects. Out of the same, three patients demonstrated with positive results through 50% reduction in the size of the lymph node. Unfortunately, the drawback of the study was that the study induced T-cell response, which could not extend over the long-term tumor induced suppression. This study was the first-ever favourable proof in implementing B-cell-based immunotherapy and its role played in generating an antitumor response through the activation of T-cells directly.

The critical challenge that is usually faced would include in isolating and segregating each component of the tumor microenvironment and studying them separately. It has been described that B cells could help to predict response to some therapies, or Immune-Related Adverse Effect (irAE). Additionally, B cells can directly impact the efficiency of some cancer treatments. Efforts have been made in order to target these cells, either through their activation or, on the contrary, through their depletion/inhibition. Current therapies can influence B cells functions, leading to resistance to immunotherapy by the activation of Breg, or release of ATP which is converted into adenosine by B cells through extracellular vesicles, leading to the inhibition of T cells. Activation of antibody production contribute towards the adverse side effects of immunotherapy. On the other hand, other therapies activate anti-tumoral activities of B cells either by activating B cells with anti-tumorigenic functions or by inhibitions of Bregs.

However, the B Cells has an implicatory role in immune-suppression and resistance during cancer treatment. The experimental studies conducted in mice is of immunosuppressive B cells expressing IgA, IL-10 and PD-L1 in the Transgenic Adenocarcinoma Of The Mouse Prostate (TRAMP) model of metastatic Prostate Cancer (PC) in humans. In study subjects of patients, these cells are found to be enriched in therapy resistant patients. Affara N.I, et al. (2014) [31], study conducted on chemotherapy patients implied that the Squamous cell carcinomas (SCC) were highly enriched in CD20+ B cells, which are at least in part responsible for the resistance to platinum- (cisplatin and

carboplatin) and taxol-based (paclitaxel) chemotherapy as their removal using an anti-CD20 antibody prevents this resistance [32]. As opposed to chemotherapy, targeted therapy involves the modulation of specific pathways involved in oncogenic processes, such as signalling kinases like homolog B (BRAF) and MAPK (mitogen-activated protein kinase)/ERK kinase (MEK) inhibitors are widely used. Despite the high level of initial response rate, a very high percentage of patients tend to develop resistance [33]. Somasundaram R, et al. (2017) [34], demonstrated that tumor-associated B cells induce resistance through these inhibitors *in vitro* through the secretion of IGF-1 and confirmed an increase of CD20 and IGF-1 gene expression in tumor of resistant patients. Immunotherapy constitutes the reactivation, of the immune system which is mainly immunosuppressive in the tumor microenvironment. Despite the fact that immunotherapy has revolutionized the field of oncology, a high proportion of patients still don't benefit from this advanced modality of treatment.

B Cell-Based Cancer Vaccine A Possibility

The activation of B cells appears to be a promising approach, and several strategies have been developed in order to fully unleash the anti-tumor potential of B cells. B cell-based cancer vaccines consist in the stimulation of B cells in order to activate cytotoxic T cells against tumors. The use of CD40 stimulation has been widely studied the ligation of CD40 with its ligand CD40L induces the expression of co-stimulatory molecules and cytokines. CD40-activated B cells gain the potential to promote the activation of naïve and memory T cells [35]. The other greater advantage associated with CD40-activated B cells, that they are not sensitive to the immunosuppressive microenvironment [36]. They can still, efficiently reach secondary lymphoid organs when injected *in vivo*, where they can efficiently activate T cells [37]. The potential of these CD40-activated B cells has been tested and validated by Rossetti, R.A.M (2018) *in vivo* in models of Human papillomavirus 16 (HPV16) E6 and E7 expressing TC-1 tumor [38], which is most commonly linked with viral etiology of OSCC (Oral Squamous Cell Carcinoma). Other tumors include B16-F10 melanoma, E.G7 lymphoma [39], 4T1 breast tumor metastasis [40], sarcoma [41] and in spontaneous non-Hodgkin's lymphoma in dogs. [42]. In conclusion, CD40-activated B cells represent an interesting tool in cancer immunotherapy, and further studies, including clinical trials, should be performed to confirm this potential. The other important class of molecules that can also be strategized include Cytosine guanine dinucleotide-oligodeoxynucleotides (CpG-ODN), is a Toll-like receptor 9 (TLR9) ligand that can also be used to activate B cells. The other signalling pathway through "fusiokine" GIFT4, is a fusion between GM-CSF and IL-4 cytokines which cluster the respective receptors on B cells and leads to the activation of Janus kinase (JAK)/STAT pathway in the experimental study was reported by Deng. J, et al. (2014) [43]. This clustering triggers the proliferation of B cells and their differentiation from naïve B cells to activated helper B cells up-regulating CD19, CD25, CD27, CD40, CD69, MHC class I and II, CD80, CD83 and CD86 expression. These activated B cells act as APCs, secrete cytokines and express co-stimulatory markers, leading to the activation of T cells into cytotoxic T cells.

The other significant approach is through tumor-derived autophagosomes enriched in defective ribosomal products (DRibbles) that is usually captured and internalized by B cells. These DRibbles contain tumor specific antigens, and lead to the activation of B cells associated with increased expression of MHC class I and II molecules, CD86 and CD40. These cells can the stimulate tumor specific T cell response. The experimental evidences conducted in mice bearing lymphoma and hepatocellular carcinoma induce the control of tumor growth. Antibodies produced by B cells are also considered for therapeutic efficiency. Several examples of antibody-based therapies can include various clinical outcome, some of them targeting directly on tumor antigens. Anti-CD20 in B-cell related lymphoma/leukemia, anti-HER2 in breast cancer and other antibodies have immunomodulatory effects such as the one used for immune checkpoint blockade (anti-CTLA4, anti-PD-1/PDL-1, anti-LAG) [44,45].

Conclusion

B cells are usually, overlooked for their role in the anti-tumor immunity. However, they tend to play a fundamental role in the TME, where they can either enhance an efficient immune response by activating cytotoxic T cell response, producing anti-tumor antibodies and cytokines, or inhibit immunity and participating in cancer immune evasion [46-48]. These contradictory activities are achieved by different B cell populations, which can be induced or on the contrary inhibit in the TME. In this context, efforts should be done in order to either activate B cells with anti-tumor activities or inhibit the regulatory B cells. In fact, a better understanding of the B cell sub-populations appears to be essential in order to be able to develop new strategies to target them specifically for the targeted therapy. The identification of specific signalling pathways, expression of cell surface markers and immune checkpoint molecules, or dependency to cytokines could be useful in this approach. In this context, it is clear that more B cell markers should be included in order to distinguish it from other B cells populations. The recent arrival of novelties like mass cytometry or single-cell RNA-sequencing, has made the fine analysis of immune sub-populations possible. Unfortunately, the majority of the analyses have investigated T or myeloid cells; with very little focus on the B cell populations, though they represent an important fraction of the immune cells [47-55]. The use of these powerful tools should be applied to B lymphocytes, as they remain mysteries that need to be solved in order to fully develop their potential in the fight against cancer. The present circumstances, the recent bioengineering have revoked back to the potential of

natural immune response that is extensively used in vaccine engineering and other therapeutic methods to determine a better clinical outcome.

References

1. Kriegsmann K, Kriegsmann M, Cremer M, Schmitt M, Dreger P, et al. (2019) Cell-based immunotherapy approaches for multiple myeloma. Br J Cancer 20: 38-44.
2. Smith EL, Mailankody S, Ghosh A, Masakayan R, Staehr M, et al. (2017) Development and evaluation of a human single chain variable fragment (scFv) derived BCMA targeted CAR T cell vector leads to a high objective response rate in patients with advanced MM. Blood 1: 742.
3. Kemp TJ, Moore JM, Griffith TS (2010) Human B cells express functional TRAIL/Apo-2 ligand after CpG-containing oligodeoxynucleotide stimulation. J Immunol 173: 892-899.
4. Ammirante M, Luo JL, Grivennikov S, Nedospasov S, Karin M (2010) B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. Nature 464: 302-305.
5. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, et al. (2013) Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity 39: 782-795.
6. Bindea G, Mlecnik B, Angell HK, Galon J (2014) The immune landscape of human tumors: Implications for cancer immunotherapy. Oncoimmunology 3: e2745642.
7. Gu-Trantien C, Loi S, Garaud S, Equeter C, Libin M, et al. (2013) CD4+ follicular helper T cell infiltration predicts breast cancer survival. J Clin Invest 123: 2873-2892.
8. Teng MW, Galon J, Fridman WH, Smyth MJ (2015) From mice to humans: Developments in cancer immunoediting. J Clin Invest 125: 3338-3346.
9. Ammirante M, Luo JL, Grivennikov S, Nedospasov S, Karin M (2007) B-cellderived lymphotoxin promotes castration-resistant prostate cancer. Nature 464: 302-305.
10. Luo JL, Tan W, Ricono JM, Korchynskyi O, Zhang M, et al. (2010) Nuclear cytokine-activated IKKalpha con-trols prostate cancer metastasis by repressing Maspin. Nature 446: 690-694.
11. Woo JR, Liss MA, Muldong MT, Palazzi K, Strasner A, et al. (2014) Tumor infiltrating B-cells are increased in prostate cancer tissue. J Transl Med 12: 30-47.
12. Allman D, Pillai S (2008) Peripheral B cell subsets. Curr. Opin. Immunol 20: 149-157.
13. Phanthunane C, Wijers R, de Herdt M, Langeveld TP, Koljenovic S, et al. (2021) B-cell clusters at the invasive margin associate with longer survival in early-stage oral-tongue cancer patients. Oncoimmunology 10: 1882743.
14. Tsou P, Katayama H, Ostrin EJ, Hanash SM (2016) The emerging role of b cells in tumor immunity. Cancer Res 76: 5597-5601.
15. Lao XM, Liang YJ, Su YX, Zhang SE, Zhou XI, et al. (2016) Distribution and significance of interstitial fibrosis and stroma-infiltrating B cells in tongue squamous cell carcinoma. Oncol Lett 11: 2027-2034.
16. Wirsing AM, Ervik IK, Seppola M, Uhlin-Hansen L, Steigen SE, et al. (2018) Presence of high-endothelial venules correlates with a favorable immune microenvironment in oral squamous cell carcinoma. Mod Pathol 31: 910-922.
17. Hamanaka Y, Suehiro Y, Fukui M, Shikichi K, Imai K, et al. (2003) Circulating anti-MUC1 IgG antibodies as a favorable prognostic factor for pancreatic cancer. Int J Cancer 103: 97-100.
18. Kurtenkov O, Klaamas K, Mensdorff-Pouilly S, Miljukhina L, Shljapnikova L, et al. (2007) Humoral immune response to MUC1 and to the Thomsen-Friedenreich (TF) glycotope in patients with gastric cancer: relation to survival. Acta Oncol 46: 316-323.
19. Hirasawa Y, Kohno N, Yokoyama A, Kondo K, Hiwada K, et al. (2000) Natural autoantibody to MUC1 is a prognostic indicator for non-small cell lung cancer. Am J Respir Crit Care Med 161: 589-594.
20. Bruno TC, Ebner PJ, Moore BL, Squalls OG, Waugh KA, et al. (2017) Antigen-presenting intratumoral b cells affect cd4(+) til phenotypes in non-small cell lung cancer patients. Cancer Immunol Res 5: 898-907.
21. Deola S, Panelli MC, Maric D, Selleri S, Dmitrieva NI, et al. (2008) Helper B cells promote cytotoxic T cell survival and proliferation independently of antigen presentation through CD27/CD70 interactions. J Immunol 180: 1362-1372.
22. Dieu-Nosjean MC, Goc J, Giraldo NA, Sautes-Fridman C, Fridman WH (2014) Tertiary lymphoid structures in cancer and beyond. Trends Immunol 35: 571-580.
23. Zhou X, Su YX, Lao XM, Liang YJ, Liao GQ (2016) CD19(+) IL-10(+) regulatory B cells affect survival of tongue squamous cell carcinoma patients and induce resting CD4(+) T cells to CD4(+) Foxp3 (+) regulatory T cells. Oral Oncol 53: 27-35.
24. de Visser KE, Korets LV, Coussens LM (2005) De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell 7: 411-423.
25. Ammirante M, Luo J-L, Grivennikov S, Nedospasov S, Karin M (2010) B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. Nature 464: 302-305.
26. Phanthunane C, Wijers R, de Herdt M, Langeveld TP, Koljenovic S, et al. (2021) B-cell clusters at the invasive margin associate with longer survival in early-stage oral-tongue cancer patients. Oncoimmunology 10: 1882743.
27. Wouters MCA, Nelson BH (2018) Prognostic significance of tumor-infiltrating b cells and plasma cells in human cancer. Clin Cancer Res 24: 6125-6135.
28. Foss RD, Ellis GL, Auclair PL (1996) Salivary gland cystadenocarcinomas: a clinicopathologic study of 57 cases. The American journal of surgical pathology 20: 1440-1447.
29. Kurihara Y, Hatori M, Ando Y, Ito D, Toyoshima T, et al. (2009) Inhibition of cyclooxygenase-2 suppresses the invasiveness of oral squamous cell carcinoma cell lines via down-regulation of matrix metalloproteinase-2 production and activation. Clinical & experimental metastasis 26: 425-432.
30. Gannot G, Gannot I, Vered H, Buchner A, Keisari Y (2002) Increase in immune cell infiltration with progression of oral epithelium from hyperkeratosis to dysplasia and carcinoma. British journal of cancer 86: 1444-1448.

31. Biagi E, Rousseau R, Yvon E, Schwartz M, Dotti G, et al. (2005) Responses to human CD40 ligand/human interleukin-2 autologous cell vaccine in patients with B-cell chronic lymphocytic leukemia. Clinical Cancer Research 11: 6916-6923.

32. Shalapour S, Font-Burgada J, Di Caro G, Zhong Z, Sanchez-Lopez E, et al. (2015) Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. Nature 521: 94-98.

33. Affara NI, Ruffell B, Medler TR, Gunderson AJ, Johansson M, et al. (2014) B cells regulate macrophage phenotype and response to chemotherapy in squamous carcinomas. Cancer cell 25: 809-821.

34. Griffin M, Scotto D, Josephs DH, Mele S, Crescioli S, et al. (2017) BRAF inhibitors: resistance and the promise of combination treatments for melanoma. Oncotarget 8: 78174.

35. Somasundaram R, Zhang G, Fukunaga-Kalabis M, Perego M, Krepler C, et al. (2017) Tumor-associated B-cells induce tumor heterogeneity and therapy resistance. Nat. Commun 8: 607.

36. Wierz M, Pierson S, Guyonnet L, Viry E, Lequeux A, et al. (2018) Dual PD1/LAG3 immune checkpoint blockade limits tumor development in a murine modelof chronic lymphocytic leukemia. Blood 131: 1617-1621.

37. Shimabukuro-Vornhagen A, Draube A, Liebig TM, Rothe A, Kochanek M, et al. (2012) The immunosuppressive factors IL-10, TGF-beta, and VEGF do not act the antigen-presenting function of CD40-activated B cells. J. Exp. Clin. Cancer Res 2012.

38. Gonzalez NK, Wennhold K, Balkow S, Kondo E, Bolck B, et al. (2015) *In vitro* and *in vivo* imaging of initial B-T-cell interactions in the setting of B-cell based cancer immunotherapy. Oncoimmunology 2015.

39. Rossetti RAM, Lorenzi NPC, Yokochi K, Rosa M, Benevides L, et al. (2018) B lymphocytes can be activated to act as antigen presenting cells to promote anti-tumor responses. PLoS ONE 2018.

40. Wennhold K, Weber TM, Klein-Gonzalez N, Thelen M, Garcia-Marquez M, et al. (2017) CD40-activated B cells induce anti-tumor immunity *in vivo*. Oncotarget 8: 27740-27753.

41. Li Q, Lao X, Pan Q, Ning N, Yet J, et al. (2011) Adoptive transfer of tumor reactive B cells confers host T-cell immunity and tumor regression. Clin. Cancer Res 17: 498-4995.

42. Deng W, Han Q, Liao L, You S, Deng H, et al. (2005) Effects of allogeneic bone marrow-derived mesenchymal stem cells on T and B lymphocytes from BXSB mice. DNA and cell biology 24: 458-463.

43. Li W, Zhou M, Ren H, Hu HM, Lu L, et al. (2013) Tumor-derived autophagosomes (DRibbles) induce B cell activation in a TLR2-MyD88 dependent manner. PLoS ONE 2013.

44. Ren H, Zhao S, Li W, Dong H, Zhou M, et al. (2014) Therapeutic antitumor efficacy of B cells loaded with tumor-derived autophagasomes vaccine (DRibbles). J. Immunother 37: 383-393.

45. Largeot A, Pagano G, Gonder S, Moussay E, Paggetti J (2019) The B-side of cancer immunity: the underrated tune. Cells 8: 449.

46. Azizi E, Carr AJ, Plitas G, Cornish AE, Konopacki C, et al. (2018) Single-Cell Map of Diverse Immune Phenotypes in the Breast Tumor Microenvironment. Cell 174: 1293-1308.

47. Chung W, Eum HH, Lee HO, Lee KM, Lee HB, et al. (2017) Single-cell RNA-seq enables comprehensive tumour and immune cell profiling in primary breast cancer.Nat. Commun 8: 15081.

48. Milne K, Kobel M, Kalloger SE, Barnes RO, Gao D, et al. (2009) Systematic analysis of immune infiltrates in high-grade serous ovarian cancer reveals CD20, FoxP3 and TIA-1 as positive prognostic factors. PLoS ONE 4: e6412.

49. Tirosh I, Izar B, Prakadan SM, Wadsworth MH, Treacy D, et al. (2016) Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science 352: 189-196.

50. Lambrechts D,Wauters E, Boeckx B, Aibar S, Nittner D, et al. (2018) Phenotype molding of stromal cells in the lung tumor microenvironment. Nat. Med 24: 1277-1289.

51. Lavin Y, Kobayashi S, Leader A, Amir ED, Elefant N, et al. (2017) Innate Immune Landscape in Early Lung Adenocarcinoma by Paired Single-Cell Analyses. Cell 169: 750-765.

52. Chevrier S, Levine JH, Zanotelli VRT, Silina K, Schulz D, et al. (2017) An Immune Atlas of Clear Cell Renal Cell Carcinoma. Cell 169: 736-749.

53. Sade-Feldman M, Yizhak K, Bjorgaard SL, Ray JP, de Boer CG, et al. (2018) Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. Cell 2018.

54. Frederick DT, Barzily-Rokni M, Bjorgaard SL, Ray JP, de Boer CG, et al. (2018) Defining T Cell States Associated with Response to Checkpoint Immunotherapy in Melanoma. Cell 998: 1020-1013.