Evaluation of TTV replication as a biomarker of immune checkpoint inhibitors efficacy in melanoma patients

Rémi Pescarmona, William Mouton, Thierry Walzer, Stéphane Dalle, Anaïs Eberhardt, Karen Brengel-Pesce, Marine Villard, Sophie Trouillet-Assant, Sébastien Viel

1 Centre International de Recherche en Infectiologie (CIRI), INserm U1111, CNRS UMR5308, ENS Lyon, Université Claude Bernard Lyon 1, Lyon, France, 2 Laboratoire d’immunologie, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, Pierre-Bénite, France, 3 Laboratoire Commun de Recherche, Hospices Civils de Lyon, bioMérieux, Centre Hospitalier Lyon Sud, Pierre-Bénite, France, 4 Virologie et Pathologie Humaine—Virpath Team, Centre International de Recherche en Infectiologie (CIRI), INserm U1111, CNRS UMR5308, ENS Lyon, Université Claude Bernard Lyon 1, Lyon, France, 5 Université Lyon 1, Lyon, France, 6 Service de dermatologie, Centre Hospitalier Lyon Sud, Hospices Civils de Lyon, Pierre-Bénite, France

☯ These authors contributed equally to this work.
‡ STA and SV also contributed equally to this work and share last authorship.
* remi.pescarmona@chu-lyon.fr

Abstract

Torque Teno Virus (TTV) is a small, non-enveloped, single-stranded and circular DNA virus that infects the majority of the population worldwide. Increased levels of plasma TTV viral load have been observed in various situations of immune deficiency or dysregulation, and several studies have suggested that TTV levels may be inversely correlated with immune competence. The measurement of TTV viremia by qPCR has been proposed as a potential biomarker for the follow-up of functional immune competence in immunosuppressed individuals, particularly hematopoietic stem cell transplant recipients. We hypothesized that TTV viral load could be used as a prognostic marker of immune checkpoint inhibitor (ICI) efficacy, and therefore investigated the TTV viral load in melanoma patients treated with nivolumab or pembrolizumab before and after 6 months of treatment. In the present study, TTV viral load was not different in melanoma patients before anti-PD-1 introduction compared to healthy volunteers, was not modified by ICI treatment and did not allow to distinguish patients with treatment-sensitive tumor from patients with treatment-resistant tumor.

1. Introduction

Torque Tenuis Virus (TTV), formerly called Transfusion Transmitted Virus was first described in 1997 [1]. It belongs to the Anelloviridae family that includes Alphatorquevirus genus and represents about 70% of the human virome [2]. It is a small, non-enveloped, single-stranded and circular DNA virus that infects human very early in life [3]. Probably more than 65% of the population worldwide is chronically infected by this virus [4], even if the immune system can control virus replication, which results in low or undetectable viral load.
Although no clinical diseases have been related to TTV infection [3], increased levels of plasma TTV viral load have been observed in various situations of immune deficiency or dysregulation (sepsis, HIV infection, cancer, and autologous or allogeneic hematopoietic stem cell transplantation) [5–8]. Several studies have suggested that TTV levels may be inversely correlated with immune competence [9], as TTV replication is under the control of T lymphocytes [10]. Based on this observation, the measurement of TTV viremia by qPCR has been proposed as a biomarker for the follow-up of functional immune competence in immunosuppressed individuals, particularly hematopoietic stem cell transplant recipients [11–16]. TTV levels were described as relevant biomarker to predict the risk of microbial infections or graft versus host disease, and guide the administration of immunosuppressive therapy or antimicrobial prophylaxis after hematopoietic stem cell transplantation.

T lymphocytes are essential players of cancer immunosurveillance. However, they may present an exhausted phenotype characterized by poor functionality and high expression of “immune checkpoint” receptors such as PD-1 and CTLA-4 when infiltrating tumors. PD-1 and CTLA-4 binding to their ligands, respectively PD-L1 and CD80/CD86, expressed by tumor and other cells, leads to functional exhaustion [17] and impairs anti-tumor immunity. Immune checkpoint inhibitors [ICI] are monoclonal antibodies that were developed to block these ligand/receptor interactions and thus restore T lymphocyte-based immunity and improve clinical outcomes. They were first approved for melanoma, non-small-cell lung cancer and kidney cancer and they have now been shown to provide a survival advantage in many other types of cancer. However, despite promising results in different studies [18–21], only a fraction of patients treated by ICI have clinical benefit whereas many patients experience adverse events or become resistant [22, 23]. In recent years, significant efforts have been undertaken to identify predictive markers of response/toxicity, exploring many immunological and genetic markers. The most widely studied is the expression of PD-L1 on tumor cells or in tumor infiltrating lymphocytes in patients with lung cancer [24–26], and it appears that patients with high PD-L1 expression have a better response rate to PD-1/PD-L1 therapy [27, 28]. Genomic biomarkers, such as tumor mutation burden, could also be good predictive biomarkers of efficacy [29–31]; patients with high burden of tumor mutations deriving greater benefits of the treatment. Similarly, transcriptomic analysis has found a tumor transcriptional signature of poor prognosis [32] or good prognosis; the latter includes high expression of TH1 genes including those coding for IFNγ and PD-L1 [33]. Furthermore, an evaluation of the tumor micro-environment particularly a description of the major phenotype of tumor infiltrating lymphocytes, seems to be very useful to guide ICI treatment [34]. However, almost all biomarkers that have been used to assess ICI biotherapy response suffer from a lack of sensitivity or specificity. There is therefore still a great need for new sensitive and specific predictive biomarkers of ICI efficacy.

In the present study, we hypothesized that TTV viral load could be used as a prognostic marker of ICI efficacy. We investigated this biomarker in a population of melanoma patients, which was described as a condition of immunosuppression [35, 36]: TTV viral load was measured in patients with treatment-sensitive tumor and patients with treatment-resistant tumor before and after 6 months of treatment with anti-PD-1 monoclonal antibodies [nivolumab or pembrolizumab. It was found that TTV viral load was not different in melanoma patients compared to healthy volunteers (HV) before anti-PD-1 introduction. In addition, TTV viral load was neither modified by ICI treatment or allowed to distinguish patients with treatment-sensitive tumor from patients with treatment-resistant tumor.
2. Materials and methods

Study population

This retrospective single-center study included 43 patients [Table 1] treated with anti-PD-1 (nivolumab or pembrolizumab) for metastatic melanoma in the dermatology department of the university hospital (Lyon Sud, Hospices Civils de Lyon, Lyon, France) between December 2013 and April 2019. Written informed consent was obtained from participants and the study was approved by a regional review board (Comité de Protection des Personnes Île de France XI, Saint-Germain-en-Laye, France, number 12027) and is registered in ClinicalTrial.gov (MelBase, NCT02828202). Each patient was examined prior to the first injection of anti-PD-1 (V1), which was the first line of treatment, and after 6 months of treatment (V2) after which they were classified as either patients with treatment-sensitive tumor (complete or partial response) or patients with treatment-resistant tumor. Concomitantly, 43 age/sex-matched HV from donors to the national blood service (Etablissement Français du Sang, EFS) were recruited. According to the EFS standardized procedures for blood donation, and in accordance with the articles R.1243–49 and following ones of the French public health code, written non-opposition for the use of donated blood for research purposes was obtained from healthy individuals. The age and sex of blood donors were forwarded anonymously to the research laboratory. Regulatory authorizations for the handling and conservation of these samples were obtained from the regional ethics committee (Comité de Protection des Personnes Sud-Est II, Bron, France) and the French ministry for research (Ministère de l’Enseignement supérieur de la Recherche et de l’Innovation, Paris, France).

Plasma collection

Whole blood was collected in EDTA tubes at V1 and V2 for each patient and centrifuged at 2000g for 10 minutes at room temperature. Plasma was then frozen at -80°C until DNA extraction.

Table 1. Patient characteristics.

|                         | All patients | Treatment-sensitive tumor | Treatment-resistant tumor | Healthy volunteers |
|-------------------------|--------------|----------------------------|----------------------------|--------------------|
| Patients (n)            | 43           | 18                         | 25                         | 43                 |
| Sex (n (%))             |              |                            |                            |                    |
| Male                    | 27 (62.8)    | 9 (50.0)                   | 18 (72.0)                  | 30 (69.8)          |
| Female                  | 16 (37.2)    | 9 (50.0)                   | 7 (28.0)                   | 13 (30.2)          |
| Age (median years (range)) | 66 (25–84)   | 62 (39–81)                 | 69 (25–84)                 | 52 (28–67)         |
| Treatment (n (%))       |              |                            |                            |                    |
| Nivolumab               | 16 (37.2)    | 6 (33.3)                   | 10 (40.0)                  | -                  |
| Pembrolizumab           | 27 (62.8)    | 12 (66.7)                  | 15 (60.0)                  | -                  |
| Stage of disease (n (%))|              |                            |                            |                    |
| IV                      | 37 (86.0)    | 16 (88.9)                  | 21 (84.0)                  | -                  |
| IIIC                    | 1 (2.3)      | -                          | 1 (4.0)                    | -                  |
| IIIB                    | 4 (9.3)      | 2 (11.1)                   | 2 (8.0)                    | -                  |
| NR                      | 1 (2.3)      | -                          | 1 (4.0)                    | -                  |
| Mutations (n (%))       |              |                            |                            |                    |
| BRAF                    | 14 (32.6)    | 6 (33.3)                   | 8 (32.0)                   | -                  |
| KRAS                    | 6 (14.0)     | 2 (11.1)                   | 4 (16.0)                   | -                  |
| Undetermined            | 2 (4.7)      | -                          | 2 (8.0)                    | -                  |
| Brain meets (n (%))     | 8 (18.6)     | 4 (22.2)                   | 4 (16.0)                   | -                  |

https://doi.org/10.1371/journal.pone.0255972.t001
TTV DNA extraction and load quantification

Total nucleic acids (elution volume 50μL) were extracted from 200μL of plasma sample using an easyMag extractor (bioMérieux, Marcy-l’Etoile, France) following the manufacturer’s instructions. The presence and viral load of TTV DNA were then determined using the TTV R-GENE® kit (available for research use only, not for diagnostic procedure; Ref#69–030, bio-Mérieux) as previously described [37]. Real-time PCR amplification was performed according to manufacturer’s instructions on a Stratagene® Mx3005P™ platform (Stratagene, La Jolla, CA, USA).

Statistical analysis

Log_{10} transformed TTV load format was used for analysis (log_{10} copy/mL). A Mann-Whitney test was used to compare TTV viral load levels between groups and a p-value < 0.05 was considered to indicate statistical significance. Data were analyzed and plotted using GraphPad Prism software (version 5; GraphPad software, La Jolla, CA, USA) and R (version 3.5.1).

3. Results

On the 43 patients included in the study, 30 (69.8%) had a detectable TTV viral load prior to the initiation of anti-PD-1 therapy (V1). Similarly, 31/43 (72.1%) HV had a quantifiable TTV viral load (Table 1).

The median TTV viral load was not significantly different in melanoma patients (2.20 log_{10} copy/mL) compared to HV (1.38 log_{10} copy/mL, p = 0.468; Fig 1A).

There were 18 patients whose tumor responded to treatment and 25 who did not. Before anti-PD-1 initiation (V1), 11 (61.1%) patients with treatment-sensitive tumor and 19 (76.0%) patients with treatment-resistant tumor had a detectable TTV viral load. There was no significant difference in the median TTV viral load between patients with treatment-sensitive tumor (2.26 log_{10} copy/mL) and patients with treatment-resistant tumor (2.13 log_{10} copy/mL; Fig 1B).

After 6 months of treatment (V2), 26/43 (60.4%) of patients had a detectable TTV viral load; 11 of whom were in the treatment-sensitive tumor group and 15 were in the treatment-resistant tumor group. In patients with treatment-sensitive tumor, the TTV load became undetectable for one patient between V1 and V2, while for 6 patients the TTV load remained undetectable, and for one patient the TTV load was detected only at V2. In patients with treatment-resistant tumor, the TTV load became undetectable for 6 patients between V1 and V2, while for 4 patients it remained undetectable, and for 2 patients the TTV load was detected only at V2. In all patients, median viral loads were not significantly different between patients with treatment-sensitive tumor (1.92 log_{10} copy/mL) and patients with treatment-resistant tumor (1.06 log_{10} copy/mL, p > 0.999) after 6 months of treatment (Fig 1C).

Finally, there was no significant difference between the median TTV viral load before (2.20 log_{10} copy/mL) and after 6 months of treatment (1.88 log_{10} copy/mL) indicating that the treatment has no impact on TTV replication whether tumor is sensitive or resistant to ICI treatment (Fig 1D).

4. Discussion

In the present study, median TTV viral load was not different in untreated melanoma patients (V1) compared to HV, contrary to what it was reported for other immunosuppressive situations [7, 8] and other types of cancer [38].
Moreover, TTV DNA viral load was not different in patients with treatment-sensitive tumor compared to patients with treatment-resistant tumor before anti-PD-1 treatment, and thus did not allow to identify patients’ candidates to ICI treatment. Strikingly, TTV viral load was also not changed after treatment, irrespective of response status. However, ICI, including anti-PD-1, are now well known to unleash T cell responses [39] and a decrease of viral load was therefore expected following immune checkpoint inhibition. The maximum T lymphocyte activity probably occurs during the first weeks after ICI treatment and may be normalized after 6 months, thus it would be interesting to monitor TTV replication earlier than 6 months after anti-PD-1 treatment in future studies, for example after 2 or 4 weeks.

It is of note that the results found in melanoma patients contrast with primary lung cancer patients for which TTV DNA viral load is significantly higher compared to HV [40]. This suggests that overall, melanoma patients retain a good T cell function and that the prognostic value of this marker may be greater in cancers where T cell function is more affected.

Finally, about 30% of TTV DNA viral loads were below the limit of detection for patients and HV. If patients with an undetectable viral load were excluded from statistical analysis, the increase of TTV DNA viral load in melanoma patients would have been significant. The development of a more sensitive technique would allow better monitoring of the TTV viral load and its kinetics following ICI treatment.

This study is a proof of concept study and results have to be confirmed on a larger cohort.
5. Conclusions
In conclusion, a 6 months treatment with nivolumab or pembrolizumab had no significant impact on TTV replication whether patient tumor was sensitive or resistant to ICI treatment. Consequently, TTV viral load at melanoma diagnosis is not a reliable predictive biomarker of anti-PD-1 efficacy. Before ruling out TTV monitoring as a biomarker of efficacy of ICI treatment, these results have to be confirmed in other types of cancer and with other ICI (ipilimumab, durvalumab, cemiplimab, atezolizumab, avelumab). Moreover, TTV viral load should be monitored earlier than 6 months after treatment initiation.

Acknowledgments
We thank Philip Robinson for constructive criticism of the manuscript. We also thank the Centre de Ressources Biologiques at the Saint-Louis Hospital in Paris for plasma collection.

Author Contributions
Conceptualization: Thierry Walzer, Stéphane Dalle, Sophie Trouillet-Assant.
Data curation: William Mouton, Anaïs Eberhardt, Sophie Trouillet-Assant.
Funding acquisition: Stéphane Dalle, Sophie Trouillet-Assant.
Investigation: William Mouton.
Supervision: Thierry Walzer, Sophie Trouillet-Assant, Sébastien Viel.
Visualization: Rémi Pescarmona, Sébastien Viel.
Writing – original draft: Rémi Pescarmona.
Writing – review & editing: Rémi Pescarmona, William Mouton, Thierry Walzer, Stéphane Dalle, Anaïs Eberhardt, Karen Brengel-Pesce, Marine Villard, Christine Lombard, Sophie Trouillet-Assant, Sébastien Viel.

References
1. Kapoor A, Kumar A, Simmonds P, Bhuva N, Singh Chauhan L, Lee B, et al. Virome Analysis of Transfusion Recipients Reveals a Novel Human Virus That Shares Genomic Features with Hepaciviruses and Pegiviruses. Katze MG, editor. mBio. 2015 Sep 22; 6(5):e01466–15. https://doi.org/10.1128/mBio.01466-15 PMID: 26396247
2. Biagini P. Classification of TTV and related viruses (anelloviruses). Curr Top Microbiol Immunol. 2009; 331:21–33. https://doi.org/10.1007/978-3-540-70972-5_2 PMID: 19230555
3. Focosi D, Antonelli G, Pistello M, Maggi F. Torque tenovirus: the human virome from bench to bedside. Clin Microbiol Infect. 2016 Jul; 22(7):589–93. https://doi.org/10.1016/j.cmi.2016.04.007 PMID: 27093875
4. Focosi D, Spezia PG, Macera L, Salvadori S, Navarro D, Lanza M, et al. Assessment of prevalence and load of torque tenovirus viremia in a large cohort of healthy blood donors. Clin Microbiol Infect (Internet). 2020 Jan (cited 2020 Feb 17); Available from: https://linkinghub.elsevier.com/retrieve/pii/S1198743X20300367
5. Fogli M, Torti C, Malacarne F, Fiorentini S, Albani M, Izzo I, et al. Emergence of Exhausted B Cells in Asymptomatic HIV-1-Infected Patients Naive for HAART is Related to Reduced Immune Surveillance. Clin Dev Immunol. 2012; 2012:1–10. https://doi.org/10.1155/2012/829584 PMID: 22474482
6. Solis M, Velay A, Gantner P, Baussjon J, Filipputtu A, Freitag R, et al. Torque tenovirus viremia for early prediction of graft rejection after kidney transplantation. J Infect. 2019 Jul; 79(1):56–60. https://doi.org/10.1016/j.jinf.2019.05.010 PMID: 31100359
7. Strassi R, Schiemann M, Doberer K, Görzer I, Puchhammer-Stöckl E, Eskandary F, et al. Quantification of Torque Tenovirus Viremia as a Prospective Biomarker for Infectious Disease in Kidney Allograft Recipients. J Infect Dis. 2018 Sep 8; 218(8):1191–9. https://doi.org/10.1093/infdis/jiy306 PMID: 30007341
8. MiPrea group, REALISM group, Mallet F, Perret M, Tran T, Meunier B, et al. Early herpes and TTV DNAemia in septic shock patients: a pilot study. Intensive Care Med Exp. 2019 Dec; 7(1):28. https://doi.org/10.1186/s40635-019-0256-z PMID: 31104220

9. Mitchell AB, Glanville AR. Kinetics of TTV-DNA Plasma Load: A Global Measure of Immune Suppression? Transplantation. 2019 Apr; 103(4):660–1. https://doi.org/10.1097/TP.0000000000002437 PMID: 30907854

10. Focosi D, Macera L, Boggi U, Nelli LC, Maggi F. Short-term kinetics of torque teno virus viremia after induction immunosuppression confirm T lymphocytes as the main replication-competent cells. J Gen Virol. 2015 Jan 1; 96(1):115–7. https://doi.org/10.1099/vir.0.70094-0 PMID: 25304651

11. Albert E, Solano C, Giménez E, Focosi D, Pérez A, Macera L, et al. Kinetics of Alphatorquevirus plasma DNAemia at late times after allogeneic hematopoietic stem cell transplantation. Med Microbiol Immunol (Berl). 2019 Apr; 208(2):253–8. https://doi.org/10.1007/s00430-019-00586-w PMID: 30852649

12. Gilles R, Herling M, Hollick U, Heger E, Awerkiew S, Fish I, et al. Dynamics of Torque Teno virus viremia could predict risk of complications after allogeneic hematopoietic stem cell transplantation. Med Microbiol Immunol (Berl). 2017 Oct; 206(5):355–62. https://doi.org/10.1007/s00430-017-0511-4 PMID: 28702856

13. Schmitz J, Krobe G, Kondakci M, Schuler E, Magorsch M, Adams O. The Value of Torque Teno Virus (TTV) as a Marker for the Degree of Immunosuppression in Adult Patients after Hematopoietic Stem Cell Transplantation (HSCT). Biolog Blood Marrow Transplant (Internet). 2019 Nov (cited 2020 Feb 17); Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083879119307426 https://doi.org/10.1016/j.bbmt.2019.11.002 PMID: 31712192

14. Masouridi-Levrat S, Pradier A, Simonetta F, Kaiser L, Chalandon Y, Roosnek E. Torque teno virus in patients undergoing allogeneic hematopoietic stem cell transplantation for hematological malignancies. Bone Marrow Transplant. 2016 Mar; 51(3):440–2. https://doi.org/10.1038/bmt.2015.266 PMID: 26551776

15. Wohlfarth P, Leiner M, Schoenhofer C, Hopfinger G, Goerzer I, Puchhammer-Stoeckl E, et al. Torquevirus Dynamics and Immune Marker Properties in Patients Following Allogeneic Hematopoietic Stem Cell Transplantation: A Prospective Longitudinal Study. Biolog Blood Marrow Transplant. 2018 Jan; 24(1):194–9. https://doi.org/10.1016/j.bbmt.2017.09.020 PMID: 29032273

16. Focosi D, Maggi F, Albani M, Macera L, Ricci V, Gragnani S, et al. Torquevirus viremia kinetics after autologous stem cell transplantation are predictable and may serve as a surrogate marker of functional immune reconstitution. J Clin Virol. 2010 Feb; 47(2):189–92. https://doi.org/10.1016/j.jcv.2009.11.027 PMID: 20034850

17. Arasanz H, Gato-Cañas M, Zuazo M, Ibáñez-Vea M, Breckpot K, Kochan G, et al. PD1 signal transduction pathways in T cells. Oncotarget. 2017 Aug 1; 8(31):51936–45. https://doi.org/10.18632/oncotarget.17232 PMID: 28881701

18. Prieto PA, Yang JC, Sherry RM, Hughes MS, Kammu US, White DE, et al. CTLA-4 blockade with ipilimumab: long-term follow-up of 177 patients with metastatic melanoma. Clin Cancer Res Off J Am Assoc Cancer Res. 2012 Apr 1; 18(7):2039–47. https://doi.org/10.1158/1078-0432.CCR-11-1823 PMID: 22271879

19. Wolchok JD, Weber JS, Maio M, Neyns B, Harmankaya K, Chin K, et al. Four-year survival rates for patients with metastatic melanoma who received ipilimumab in phase II clinical trials. Ann Oncol Off J Eur Soc Med Oncol. 2013 Aug; 24(8):2174–80. https://doi.org/10.1093/annonc/mdt161 PMID: 23666915

20. Schadendorf D, Hodi FS, Robert C, Weber JS, Margolin K, Hamid O, et al. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. J Clin Oncol Off J Am Soc Clin Oncol. 2015 Jun 10; 33(17):1889–94.

21. Topalian SL, Szolnai M, McDermott DF, Kluger HM, Carvajal RD, Sharfman WH, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol Off J Am Soc Clin Oncol. 2014 Apr 1; 32(10):1020–30.

22. Restifo NP, Smyth MJ, Snyder A. Acquired resistance to immunotherapy and future challenges. Nat Rev Cancer. 2016; 16(2):121–8. https://doi.org/10.1038/nrc.2016.2 PMID: 26822578

23. Michot JM, Bigenwald C, Champiat S, Collins M, Carbonnel F, Postel-Vinay S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. Eur J Cancer Oxf Engl 1990. 2016 Feb; 54:139–48. https://doi.org/10.1016/j.ejca.2015.11.016 PMID: 26765102

24. Taube JM, Anders RA, Young GD, Xu H, Sharma R, McMillar TL, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med. 2012 Mar 28; 4(127):127ra37. https://doi.org/10.1126/scitranslmed.3003689 PMID: 22461641
25. Lyford-Pike S, Peng S, Young GD, Taube JM, Westra WH, Akpeng B, et al. Evidence for a role of the PD-1:PD-L1 pathway in immune resistance of HPV-associated head and neck squamous cell carcinoma. Cancer Res. 2013 Mar 15; 73(6):1733–41. https://doi.org/10.1158/0008-5472.CAN-12-2384 PMID: 23288508

26. Herbst RS, Soria J-C, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014 Nov 27; 515 (7528):563–7. https://doi.org/10.1038/nature14011 PMID: 25428504

27. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012 Jun 28; 366(26):2443–54. https://doi.org/10.1056/NEJMoa1200690 PMID: 22658127

28. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. N Engl J Med. 2015 May 21; 372(21):2018–28. https://doi.org/10.1056/NEJMoa1501824 PMID: 25891174

29. Lawrence MS, Stojanov P, Polak P, Kryukov GV, Cibulskis K, Sivachenko A, et al. Mutational heterogeneity in cancer and the search for new cancer-associated genes. Nature. 2013 Jul 11; 499(7457):214–8. https://doi.org/10.1038/nature12213 PMID: 23770567

30. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science. 2015 Apr 3; 348(6230):124–8. https://doi.org/10.1126/science.aaa1348 PMID: 25765070

31. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015 Jun 25; 372(26):2509–20. https://doi.org/10.1056/NEJMoa1500596 PMID: 26028255

32. Hugo W, Zaretsky JM, Sun L, Song C, Moreno BH, Hu-Lieskovan S, et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell. 2016 Mar 24; 165(1):35–44. https://doi.org/10.1016/j.cell.2016.02.065 PMID: 26997480

33. Taube JM, Young GD, McMillen TL, Chen S, Salas JT, Pritchard TS, et al. Differential Expression of Immune-Regulatory Genes Associated with PD-L1 Display in Melanoma: Implications for PD-1 Pathway Blockade. Clin Cancer Res Off J Am Assoc Cancer Res. 2015 Sep 1; 21(17):3969–76. https://doi.org/10.1158/1078-0432.CCR-15-0244 PMID: 25944800

34. Gajewski TF, Schreiber H, Fu Y-X. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013 Oct; 14(10):1014–22. https://doi.org/10.1038/ni.2703 PMID: 24048123

35. Kamran N, Li Y, Sierra M, Alghamri MS, Kadiyala P, Appelman HD, et al. Melanoma induced immunosuppression is mediated by hematopoietic dysregulation. OncoImmunology. 2018 Mar 4; 7(3): e1408750. https://doi.org/10.1080/2162402X.2017.1408750 PMID: 29399415

36. Umansky V, Sevko A. Melanoma-induced immunosuppression and its neutralization. Semin Cancer Biol. 2012 Aug; 22(4):319–26. https://doi.org/10.1016/j.semcancer.2012.02.003 PMID: 22349515

37. Kulifaj D, Durgueil-Lariviere B, Meynier F, Munteanu E, Pichon N, Dubé M, et al. Development of a standardized real time PCR for Torque teno viruses (TTV) viral load detection and quantification: A new tool for immune monitoring. J Clin Virol. 2018 Aug; 105:118–27. https://doi.org/10.1016/j.jcv.2018.06.010 PMID: 29957546

38. Camci C, Guney C, Balkan A, Buyukberber N, Buyukberber S, Kadayifci A, et al. The prevalence of TT virus in cancer patients. New Microbiol. 2002 Oct; 25(4):463–8. PMID: 12437226

39. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nat Rev Immunol. 2020 May 20;

40. Sawata T, Bando M, Nakayama M, Mato N, Yamashawa H, Takahashi M, et al. Clinical significance of changes in Torque teno virus DNA titer after chemotherapy in patients with primary lung cancer. Respir Investig. 2018 Mar; 56(2):173–8. https://doi.org/10.1016/j.resinv.2017.12.004 PMID: 29548656