

Neoantigen heterogeneity: a key driver of immune response and sensitivity to immune checkpoint blockade?

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Body of article

Modulation of co-inhibitory and co-stimulatory immune checkpoint pathway activity with antibody-based therapies has emerged as a promising anti-cancer strategy. Although responses to such agents are limited to a modest fraction of treated patients, those deriving benefit have the potential for durable remissions and possibly even cure [1-8]. The identification of biomarkers predictive of response and resistance to such therapies therefore remains an area of high scientific priority.

In humans, adoptive transfer of tumour-infiltrating lymphocytes (TILs) with concomitant administration of interleukin-2 (IL-2) mediates tumour regression in 34-40% of patients with advanced melanoma [9]. Efforts have been focused for some time on the characterisation of antigens recognised by TILs. Melanoma TILs have been demonstrated to recognise shared antigens on melanoma cell lines established from different patients, in a class I major-histocompatibility complex (MHC)-restricted manner *in vitro* [10,11]. The first gene identified to code for an antigen recognised on human tumours by autologous TILs was MAGE-1, silent in normal tissues except in testes, and expressed by a number of other solid tumour subtypes [12]. Subsequently, three further self-proteins, all melanoma/melanocyte lineage-specific, encoded by MART-1, tyrosinase and gp100, were identified [13-15]. Although these 'public' tumour-associated antigens appeared attractive targets for both adoptive cell-based and vaccination strategies, neither approach was observed to yield particularly promising activity in the clinical setting [reviewed

in 16].

The identification of cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a co-inhibitory immune checkpoint molecule, followed by the demonstration of efficient rejection of established murine tumours with CTLA-4 'blockade', highlighted immune regulation as a potential contributor to the limited clinical activity of therapeutic strategies directed against these tumour-associated antigens [17,18]. In a pooled analysis of patients with advanced melanoma treated with ipilimumab, an antibody directed against CTLA-4, three-year survival was found to range between 20 and 26% [19]. Amongst a cohort of 107 patients with advanced melanoma, one of the earliest to be treated with nivolumab, an anti-PD-1 antibody, 5-year survival was recently reported as 34% (Hodi S., AACR, 2016). Whilst this is remarkable in a solid tumour subtype many deemed 'untreatable', it is important to acknowledge that the majority of patients do not respond to these forms of immune checkpoint blockade, at least as monotherapy. The search for predictive biomarkers in this setting has been challenging, however the identification of an inextricable relationship between the genomic landscape and anti-tumour immunity has served to re-highlight the importance of identifying the most relevant substrates for T cell recognition, whilst simultaneously addressing regulation at the tumour site.

The search for clinically relevant targets of immune response has shifted focus more recently. Tumour-specific mutations may serve as 'private' neoantigens, eliciting anti-tumour T cell responses [20-23]. In contrast to non-mutated self antigens, these are thought to be of particular relevance in tumour control, since the quality of the T cell pool available for these antigens is not affected by central T cell tolerance [24]. Antibody-mediated blockade of co-inhibitory immune checkpoint molecules serves to remove the regulation limiting the activity of neoantigen-reactive tumour-infiltrating T cells. Patients with advanced melanoma and non-small cell lung cancer (NSCLC) deriving benefit from CTLA-4 and PD-1 blockade respectively appear to have tumours enriched with putative neoantigens [25-27]. The relationship between genomics and anti-tumour immunity is, however, complex. Analysis of 110

patients with advanced melanoma undergoing CTLA-4 blockade demonstrated an association between neoantigen burden and clinical benefit, however no recurrent neoantigen peptide sequences predicted responder patient populations [28].

Although neoantigens arise from tumour-specific mutations and genetic heterogeneity within single tumours is well described, the impact of intra-tumour heterogeneity (ITH) upon the neoantigen landscape and anti-tumour immunity has remained unclear [29-30]. Analysis of 139 patients with predominantly early-stage adenocarcinoma of the lung, derived from the The Cancer Genome Atlas (TCGA) database, demonstrated that a high burden of clonal neoantigens, present in every cancer cell, combined with a low relative fraction of subclonal neoantigens (low neoantigen ITH) was associated with improved overall survival [31]. The prognostic value of combining these two metrics appeared greater than considering either total neoantigen burden or neoantigen ITH alone. Importantly, even in the presence of a high burden of clonal neoantigens, a high relative fraction of subclonal neoantigens impacted negatively on outcome. Analysis of differentially expressed immune-related genes between patients with high and low clonal neoantigen burden demonstrated that tumours with a high burden of clonal neoantigens displayed an inflamed phenotype, with observed high levels of CD8A, IFN- γ , GzmB, STAT-1, PD-1, LAG-3 and PD-L1/2 gene expression. In keeping with these findings, sensitivity to CTLA-4 and PD-1 blockade in patients with advanced melanoma (n=135) and NSCLC (n=31) appeared enhanced in tumours enriched for clonal neoantigens. Once again, even in the presence of high clonal neoantigen burden, a high relative fraction of subclonal neoantigens was observed to impact negatively on response to therapy.

Tumour-specific neoantigens therefore influence anti-tumour immune responses. Tumours enriched with clonal neoantigens, shared by all tumour cells, display an inflamed phenotype and appear sensitive to immune checkpoint blockade, provided they are accompanied by a low relative fraction of subclonal neoantigens. The mechanism underlying the negative contribution of subclonal neoantigens remains to be elucidated, however

these findings highlight the importance of determining whether existing strategies, including cytotoxic chemotherapy and radiation, induce subclonal neoantigens, potentially impacting negatively on immunosurveillance and subsequent response to immune checkpoint blockade. Such observations will need to be balanced against the potential for the same therapies to have a vaccination effect through induction of immunogenic cell death [32]. Adoptive transfer of high numbers of effector T cells reactive to clonal neoantigens and/or vaccination against multiple clonal neo-epitopes, combined with appropriate checkpoint blockade, may serve to overcome the significant challenge posed by ITH. Such hypotheses, however, require validation. In an era of personalised medicine, these findings serve, at the very least, to help better identify those most likely to derive benefit from checkpoint blockade, allowing improved patient stratification. Importantly, they also move the tumour immunology field a step closer to the ultimate goal of achieving durable remissions for the majority, rather than a select few.

References

1. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010 Aug 19;363(8):711–23.
2. Robert C, Thomas L, Bondarenko I, O'Day S, M D JW, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011 Jun 30;364(26):2517–26.
3. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol*. 2015 Apr;16(4):375–84.
4. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med*. 2015 Jan 22;372(4):320–30.
5. Robert C, Ribas A, Wolchok JD, Hodi FS, Hamid O, Kefford R, et al. Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet*. 2014 Sep 20;384(9948):1109–17.

6. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. *Lancet Oncol.* 2015 Aug;16(8):908–18.
7. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med.* 2013 Jul 11;369(2):122–33.
8. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med.* 2015 Jul 2;373(1):23–34.
9. Rosenberg SA, Packard BS, Aebbersold PM, Solomon D, Topalian SL, Toy ST, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. A preliminary report. *N Engl J Med.* 1988 Dec 22;319(25):1676–80.
10. Kawakami Y, Zakut R, Topalian SL, Stötter H, Rosenberg SA. Shared human melanoma antigens. Recognition by tumor-infiltrating lymphocytes in HLA-A2.1-transfected melanomas. *J Immunol Baltim Md 1950.* 1992 Jan 15;148(2):638–43.
11. Hom SS, Schwartzentruber DJ, Rosenberg SA, Topalian SL. Specific release of cytokines by lymphocytes infiltrating human melanomas in response to shared melanoma antigens. *J Immunother Emphas Tumor Immunol Off J Soc Biol Ther.* 1993 Jan;13(1):18–30.
12. Van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science.* 1991 Dec 13;254(5038):1643–7.
13. Coulie PG, Brichard V, Van Pel A, Wölfel T, Schneider J, Traversari C, et al. A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med.* 1994 Jul 1;180(1):35–42.
14. Brichard V, Van Pel A, Wölfel T, Wölfel C, De Plaen E, Lethé B, et al. The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. *J Exp Med.* 1993 Aug 1;178(2):489–95.
15. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Sakaguchi K, Appella E, et al. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor rejection. *Proc Natl Acad Sci U S A.* 1994 Jul 5;91(14):6458–62.
16. June CH. Adoptive T cell therapy for cancer in the clinic. *J Clin Invest.* 2007 Jun;117(6):1466–76.

17. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, et al. A new member of the immunoglobulin superfamily--CTLA-4. *Nature*. 1987 Jul 16;328(6127):267-70.
18. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 1996 Mar 22;271(5256):1734-6.
19. 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.
20. Segal NH, Parsons DW, Peggs KS, Velculescu V, Kinzler KW, Vogelstein B, et al. Epitope landscape in breast and colorectal cancer. *Cancer Res*. 2008 Feb 1;68(3):889-92.
21. Castle JC, Kreiter S, Diekmann J, Löwer M, van de Roemer N, de Graaf J, et al. Exploiting the mutanome for tumor vaccination. *Cancer Res*. 2012 Mar 1;72(5):1081-91.
22. Robbins PF, Lu Y-C, El-Gamil M, Li YF, Gross C, Gartner J, et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat Med*. 2013 Jun;19(6):747-52.
23. Lu Y-C, Yao X, Li YF, El-Gamil M, Dudley ME, Yang JC, et al. Mutated PPP1R3B is recognized by T cells used to treat a melanoma patient who experienced a durable complete tumor regression. *J Immunol Baltim Md 1950*. 2013 Jun 15;190(12):6034-42.
24. Gilboa E. The makings of a tumor rejection antigen. *Immunity*. 1999 Sep;11(3):263-70.
25. Van Rooij N, van Buuren MM, Philips D, Velds A, Toebes M, Heemskerk B, et al. Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J Clin Oncol Off J Am Soc Clin Oncol*. 2013 Nov 10;31(32):e439-442.
26. Snyder A, Makarov V, Merghoub T, Yuan J, Zaretsky JM, Desrichard A, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014 Dec 4;371(23):2189-99.
27. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015 Apr 3;348(6230):124-8.
28. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science*. 2015 Oct 9;350(6257):207-11.

29. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. *N Engl J Med*. 2012 Mar 8;366(10):883–92.
30. De Bruin EC, McGranahan N, Mitter R, Salm M, Wedge DC, Yates L, et al. Spatial and temporal diversity in genomic instability processes defines lung cancer evolution. *Science*. 2014 Oct 10;346(6206):251–6.
31. McGranahan N, Furness AJS, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* [Internet]. 2016 Mar 3 [cited 2016 Mar 17]; Available from: <http://www.sciencemag.org/cgi/doi/10.1126/science.aaf1490>
32. Pfirschke C, Engblom C, Rickelt S, Cortez-Retamozo V, Garris C, Pucci F, et al. Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy. *Immunity*. 2016 Feb 16;44(2):343–54.

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