Cancer Immune therapy: Prognostic significance and implications for therapy of PD-1 in BCG-relapsing bladder cancer

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BCG immune therapy is used for (1) preventing bladder cancer relapse following complete tumor excision of Ta and T1 papillary tumors and for (2) eradication of carcinoma in-situ (CIS) which is not usually completely removed due to its diffuse and often multifocal involvement of the bladder. Tumors that relapse despite adequate BCG are often aggressive and life threatening if not managed appropriately. Thus, radical cystectomy is the gold standard for BCG failures. Alternatives to cystectomy include intravesical valrubicin, which is FDA-approved for BCG unresponsive CIS and off-label approaches including repeat instillation of BCG with or without interferon-α or intravesical instillation of other immune or chemotherapeutic agents. Currently, a major obstacle in this field is lack of understanding of immune regulatory pathways driving BCG responsiveness, making it difficult to understand why many patients fail therapy. This work by Kikuchi and colleagues is significant because it seeks to elucidate characteristics of BCG unresponsive tumors and their prognostic significance. Using a cohort of patients deemed BCG unresponsive, the authors found that tumor PD-1 staining was significantly increased in BCG unresponsive tumors compared to pre-treatment tumors from the same patient and that increased PD-1 staining in post-BCG treated tumors was associated with a worse outcome.

PD-1 (programmed death-1 or CD279) was discovered in 1992 as a gene responsible for programmed cell death[1]. Yet, its potential role in regulating immune tolerance did not emerge for many years when phenotypes of mice deficient in PD-1 were characterized [2]. Prior elucidation of the potential of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) blockade in treating cancer[3] facilitated identification of PD-1 ligands and the role of PD-1 in cancer. Because PD-1 is structurally similar to CTLA-4, the ligand to PD-1 was identified by identifying ligands that were similar to CTLA-4's binding ligands B7-1 and B7-2[4, 5]. We now know that expression of PD-1 on naive T cells is induced by TCR activation and it is one of the earliest markers of T cell activation [6]. However, constitutive PD-1 expression resulting from chronic stimulation results in an exhausted T cell phenotype, thereby putting the brakes on T cell activation to limit autoimmune tissue damage. Binding of PD-1 by its ligand PD-L1 induces apoptosis in T cells [7] and by expressing PD-L1, tumors exploit this phenomenon to favor their growth and progression [8-10]. Thus, an increase in the

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frequency PD-1 expressing cells in the tumor microenvironment might be expected after failure of therapy. Indeed others have shown increased PD-1 expressing tissue following failed therapy in leukemia [11] and lymphoma [12].

Several shortcomings to this work are notable. Given the importance of PD-1's ligand binding, the lack of PD-L1 staining in this study is unfortunate. Previously, Inman and colleagues showed that tumor PD-L1 was a key determinant of stage progression among a cohort of patients treated with BCG [13]. Further, most patients with CIS that failed BCG immunotherapy showed 15–20 higher fold of PD-L1 expression especially, within BCG granulomas. Also, level of PD-L1 is relatively low in CIS tumors before BCG treatment but increased after therapy showing that PD-L1 expression is not a static property but a dynamic feature that changes in response to therapy. PD-1 could also be engaged by PD-L2, which is also detectable on tumors cells [14] but was not analyzed in this cohort. Finally, since PD-1 is expressed both on tumor cells and immune cells [15], distinguishing tumor from immune cell PD-1 staining is important[16].

Are there other limitations of the study design that could bias the results or limit its generalizability? One issue is that the pre-treatment tumor blocks are older than post-treatment blocks. Antigen decay in paraffin-embedded tissue sections for immunohistochemistry is a common occurrence [17] which may have affected PD-1 staining in this study. It is possible that there is loss of PD-1 expression over time and this would systemically favor increased PD-1 expression in newer (BCG unresponsive) tissue samples. Second, the importance of BCG maintenance has been proven in randomized controlled trials and is recommended in bladder cancer guidelines. Nevertheless, in this study BCG maintenance was not given. This is becoming more of an issue as trials in the BCG unresponsive setting are emerging. Patients are not eligible for BCG unresponsive clinical trials unless they have received an adequate amount of BCG, which includes at least 5 of 6 induction instillations and at least 2 of 3 maintenance [18]. In this population, no patients received maintenance BCG as the standard approach in Japan is to give 6-8 weeks of induction only. Thus, it is difficult to generalize these findings towards what we consider as BCG unresponsive phenotypes.

In conclusion, this study has important prognostic relevance and validates earlier findings by Inman's group supporting a role for PD-1/PD-L1 axis signaling in BCG unresponsive tumors. Although the conclusions drawn are subject to certain limitations, it moves the field forward by working to characterize BCG unresponsive tumors and suggests that PD-1/PD-L1 blockade could be a therapeutic strategy in the BCG unresponsive patients.

References

1. Ishida Y , et al., Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J, 199211(11): p.3887–95. [PubMed: 1396582]

2. Nishimura H , et al., Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity, 199911(2): p. 141–51. [PubMed: 10485649]

3. Leach DR , Krummel MF , and Allison JP , Enhancement of antitumor immunity by CTLA-4 blockade. Science, 1996 271(5256): p. 1734–6. [PubMed: 8596936]

4. Freeman GJ , et al., Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med, 2000192(7): p. 1027–34. [PubMed: 11015443]

5. Dong H , et al., B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nature medicine, 1999 5(12): p. 1365–9.

6. Chikuma S , et al., PD-1-mediated suppression of IL-2 production induces CD8+ T cell anergy in vivo. J Immunol, 2009 182(11): p. 6682–9. [PubMed: 19454662]

7. Dong H , et al., Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med, 2002 8(8): p. 793–800. [PubMed: 12091876]

8. Yokosuka T , et al., Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. J Exp Med, 2012 209(6): p. 1201–17. [PubMed: 22641383]

9. Hui E , et al., T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. Science, 2017 355(6332): p. 1428–1433. [PubMed: 28280247]

10. Ramsay AG , Immune checkpoint blockade immunotherapy to activate antitumour T-cell immunity. Br J Haematol, 2013 162(3): p. 313–25. [PubMed: 23691926]

11. Schnorfeil FM , et al., T cells are functionally not impaired in AML: increased PD-1 expression is only seen at time of relapse and correlates with a shift towards the memory T cell compartment. J Hematol Oncol, 2015 8: p. 93. [PubMed: 26219463]

12. Xu-Monette ZY , Zhou J , and Young KH , PD-1 expression and clinical PD-1 blockade in B-cell lymphomas. Blood, 2018 131(1): p. 68–83. [PubMed: 29118007]

13. Inman BA , et al., PD-L1 (B7-H1) expression by urothelial carcinoma of the bladder and BCG-induced granulomata: associations with localized stage progression. Cancer, 2007 109(8): p. 1499–505. [PubMed: 17340590]

14. Yearley JH , et al., PD-L2 Expression in Human Tumors: Relevance to Anti-PD-1 Therapy in Cancer. Clin Cancer Res, 2017 23(12): p. 3158–3167. [PubMed: 28619999]

15. He Y , et al., PD-1, PD-L1 Protein Expression in Non-Small Cell Lung Cancer and Their Relationship with Tumor-Infiltrating Lymphocytes. Med Sci Monit, 2017 23: p. 1208–1216. [PubMed: 28275222]

16. Massard C , et al., Safety and Efficacy of Durvalumab (MEDI4736), an Anti-Programmed Cell Death Ligand-1 Immune Checkpoint Inhibitor, in Patients With Advanced Urothelial Bladder Cancer. J Clin Oncol, 2016 34(26): p. 3119–25. [PubMed: 27269937]

17. Grillo F , et al., Factors affecting immunoreactivity in long-term storage of formalin-fixed paraffin-embedded tissue sections. Histochem Cell Biol, 2015 144(1): p. 93–9. [PubMed: 25757745]

18. Kamat AM , et al., Once BCG Unresponsive, Always BCG Unresponsive: An Open Letter to the FDA to Enhance Recruitment into Clinical Trials in Bladder Cancer. Bladder Cancer, 2017 3(3): p. 145–146. [PubMed: 28824940]