Journal of Mucosal Immunology Research

Rudd and Taylor, J Mucosal Immunol Res 2019, 3:1

Short Communication Open Access

Small Molecule Inhibitors as an Alternative to Antibody Blockade in Immunotherapy

Christopher E Rudd [1,2] **and Alison Taylor**[3*]

[1]*Division of Immunology and Oncology Research Center, Maisonneuve, Rosemont Hospital, Montreal, Canada*
[2]*Département of Medicine, University of Montreal, Montreal, Canada*

[3]*Leeds Institute of Cancer & Pathology, Wellcome Trust Brenner Building, St James's University Hospital, United Kingdom*

[*]**Corresponding author:** Dr. Alison taylor, Leeds Institute of Cancer & Pathology, Wellcome Trust Brenner Building, St James's University Hospital, United Kingdom, E-mail: a.taylor1@leeds.ac.uk

Received date: October 26, 2019; **Accepted date:** November 11, 2019; **Published date:** November 18, 2019

Abstract

The application of immune checkpoint blockade for the treatment of cancer has revolutionized immunotherapy regimes over the past few years. This approach has seen much success using antibody blockade of programmed cell death-1 (PD-1) or its ligand, PD-L1. However, there are many limitations to antibody blockade, including cost, tumour penetration and autoimmune complications. Patients may suffer from adverse side effects and many remain uncured. Combination of therapies with antibodies can improve response rates, but may also increase serious side effects. Here, we look at the use of small molecule inhibitors as an alternative to antibodies in targeting intracellular pathways for co-receptor blockade and synergies in immunotherapy.

Keywords: Immunology; T-Lymphocyte; Immunotherapy; Antibody

Introduction

Immune Checkpoint Blockade (ICB) is at the forefront of immunotherapy regimes in the treatment of cancer [1-3]. Antibody blockade of programmed cell death-1 (PD-1) or its ligand, PD-L1 has played a prominent role in this immunotherapeutic approach with Nivolumab, Pembrolizumab and Atezolizumab [4-6] being the first FDA approved anti-PD1/PD-L1 antibodies alongside more recently approved Cemiplimab, Avelumab and Durvalumab [7,8]. Over 1300 studies involving combinations of PD-1 or PD-L1 antibodies are listed on the Clinicaltrials.gov registry.

Practical Approach

The use of PD-1 mAb prevents T cells from recognising the PD-1 ligand (PDL-1) on tumour cells. As part of the body's natural defence, T cells patrol the body for foreign cells and mount an immune response against them in order to destroy them. This recognition is carried out through PD-1-PDL-1/2 interaction; cells expressing PD1-L1/2 are recognised by the T cell and inhibitory signals are sent preventing effector cytotoxic responses. However, cancer cells may also express PDL-1/2 and this can lead to evasion of the immune response and formation of a tumour [9].

PD-1 mAbs have been used alone [10] or in combination with mAbs against other checkpoint molecules, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) [11]. Both mono and combination therapies have shown much success, however not all patients are cured, resistance may develop and there is a correlation with increased immune-related adverse events (irAEs) which include colitis, hepatitis, pneumonitis, cardiotoxicity, nephritis and vitiligo [12-16]. Around 10% of patients receiving anti-PD-1/PD-L1 antibodies suffer from grade 3-4 irAEs. These are serious side effects which need to be addressed in the development of new/improved treatments as well as improving efficacy.

A major advance would be to develop small molecules that modulate co-receptors or their signalling pathways for enhanced anti-tumour activity. The use of Small Molecule Inhibitors (SMIs) would provide several advantages over antibodies including, a short pharmacokinetic profile allowing flexible dosing and rapid withdrawal should signs of irAEs develop, the ability to cross membranes leading to better distribution and tumour penetration, and oral bioavailability, which will have a positive impact on the patient`s quality of life.

One approach for enhanced anti-tumour immunity is to inhibit pathways that control the expression of inhibitory co-receptors such as PD-1. SMIs have been used to impair PD-1/PD-L1 interaction by recognizing the PDL-1 binding pockets at the interface of PD-1 and blocking PD-1/PDL-1 binding directly and/or by inducing dimerization of PDL-1 (i.e. BMS-1001 and BMS-1166 Bristols-Myers-Squibb) [17]. Other SMIs can act simultaneously against two checkpoint inhibitor pathways due to their recognition of binding pockets with high sequence similarity [18,19]. Our recent work has highlighted the serine/threonine kinase glycogen synthase kinase-3 (GSK-3) as an alternative target. There are two ubiquitously expressed and highly conserved isoforms of GSK-3, GSK-3α and GSK-3β, which have shared and distinct substrates as well as functional effects. Both forms have been implicated in processes ranging from glycogen metabolism to gene transcription, apoptosis and microtubule stability.

GSK-3 is constitutively active in resting T cells [20,21] and is inhibited by receptor induced activation signals [22]. During T cell activation, the co-receptor CD28 binds to phosphoinositide 3-kinase, activating Akt, which phosphorylates Ser-21 and Ser-9 on GSK-3α and GSK-3, respectively [23], inhibiting GSK-3 activity. Inactivation of GSK-3 occurs by serine phosphorylation (Ser9:, Ser21: α) which allows its own phospho-serine tail to bind and block the active site [24,25]. This is a highly dynamic event whereby the serine tail switches rapidly between phosphorylated and dephosphorylated states causing a

fluctuation of binding and release from the active site. This allows "primed" substrates that have accumulated in high levels to compete for the active site and become phosphorylated by GSK-3.

We have previously shown that inhibition of GSK-3 resulted in a down-regulation of *Pdcd1* (PD-1) transcription *via* upregulation of the transcription factor Tbet [26]. This led to enhanced cytotoxic functionality of CD8+ T cells and increased levels of IFN-γ and Granzyme B expression, promoting viral clearance [26]. Further to this our current work shows that inhibition of GSK-3 can control B16 and EL4 tumour growth and is as effective as PD-1 blockade [27].

We have shown *in vitro* inhibition of GSK-3 by SMIs or siRNA to act primarily in CD8+ T cells reducing PD-1 expression. This inhibition has been shown further using SMIs *in vivo* in comparison to anti-PD-1 mAb treatment. T cells from GSK-3-/- mice also showed a reduction in PD-1 expression and B16 pulmonary metastasis was reduced to a similar extent in both Pdcd-/- and GSK-3-/- mice. Both models revealed a decrease in *Pdcd1* transcription, with an increase in *Tbx21* (Tbet) transcription and elevated numbers of CD8+ TILs expressing CD107a+ (LAMP1) and granzyme B (GZMB). Down-regulation of Tbet with siRNA resulted in increased PD-1 expression indicating that Tbet inhibits PD-1 transcription, a finding consistent with that of another lab [28]. Inhibition of GSK-3 in T cells with down-regulated Tbet had no effect on PD-1 expression indicating GSK-3 to operate upstream of and dependent on Tbet which in turn inhibits PD-1 expression.

Despite this, it is important to note that GSK-3 is likely to affect other aspects of T cell function in a PD-1 independent fashion. GSK-3 SMIs may eventually be found to alter the expression of other receptors and mediators and provide a potential advantage over anti-PD-1 blockade. However, in the context of the models examined to date, the down-regulatory effect on PD-1 plays a central role in generating anti-tumour immunity.

Overall, there are potential advantages and disadvantages to the use of GSK-3 SMIs versus anti-PD-1 antibody therapies.

Anti-PD-1 immunotherapy is associated with irAEs such as fatigue, rash and possible autoimmune complications such as colitis and although we cannot exclude these effects with GSK-3 SMIs, to date, we have seen no evidence of autoimmunity in the GSK-3-/- mice. However, there is the potential for GSK-3 inactivation to effect the function of other host cells or the tumour itself. We have not seen any direct effect of GSK-3 SMI on the growth of B16 melanoma cells, but GSK-3 inhibition has been reported to directly inhibit the growth of multiple myeloma, neuroblastoma, hepatoma and prostate tumours [29-33]. This may however be of added benefit whereby GSK-3 inhibitors can directly inhibit the growth of some tumours in addition to an enhancing effect on the immune system. However, in our studies, the major effect of GSK-3 SMIs was the amplification of the immune system. This was shown by the effects on *ex vivo* T cells, adoptive transfer experiments and by the elimination of tumours in mice with GSK-3 specifically deleted in their T cells.

With regard to patient benefit, several inhibitors are now moving forward into clinical trials. Lithium chloride is a classical inhibitor of GSK-3 which has been used for decades for the treatment of bipolar disease. Tideglusib has been investigated in a phase 2 oral study to treat progressive supranuclear palsy [34] and will be used in a new clinical trial in congenital Myotonic Dystrophy (ClinicalTrials.gov Identifier: NCT03692312). More recently, 9-ING-41, a potent GSK-3β inhibitor is being used in a phase 1/2 study to evaluate its safety and efficacy, as a single agent and in combination with cytotoxic agents, in patients with refractory cancers (ClinicalTrials.gov Identifier: NCT03678883).

Conclusion

Overall this shows numerous possibilities for GSK-3 SMIs in clinical applications and as research progresses, it is likely that developments in immunotherapy will move beyond the targeting of immune checkpoint blockade pathways such as CTLA-4 and PD-1 and focus will move to other approaches such as SMIs. Further work is needed to uncover the full range of down-stream effects that may be regulated by GSK-3 regulation in anti-tumour immunity, but overall these findings identify a potential alternate approach in the treatment of cancer.

References

1. Tellier J, Nutt SL (2019) Plasma cells: The programming of an antibody-secreting machine. Eur J Immunol 49: 30-37.
2. Willis SN, Nutt SL (2019) New players in the gene regulatory network controlling late B cell differentiation. Curr Opin Immunol 58: 68-74.
3. Martincic K, Alkan SA, Cheatle A, Borghesi L, Milcarek C (2009) Transcription elongation factor ELL2 directs immunoglobulin secretion in plasma cells by stimulating altered RNA processing. Nat Immunol 10: 1102-1109.
4. Park KS, Bayles I, Szlachta-McGinn A, Paul J, Boiko J, et al. (2014) Transcription elongation factor ELL2 drives immunoglobulin secretory specific mRNA production and the unfolded protein response. J Immunol 193: 4663-4674.
5. Smith SM, Carew NT, Milcarek C (2015) RNA polymerases in plasma cells trav-ELL2 the beat of a different drum. World J Immunol 5: 99-112.
6. Bayles I, Milcarek C (2014) Plasma cell formation, secretion, and persistence: The short and the long of it. Crit Rev Imm 34: 481-499.
7. Milcarek C, Albring M, Langer C, Park KS (2011) The Eleven-Nineteen Lysine-rich Leukemia gene (ELL2) influences the histone H3 modifications accompanying the shift to secretory Immunoglobulin heavy chain mRNA production. J Biol Chem 286: 33795-33803.
8. Nelson AM, Carew NT, Smith SM, Milcarek C (2018) RNA splicing in the transition from B cells to antibody secreting cells: The influences of ELL2, snRNA and ER stress. J Immunol. 201: 3073-3083.
9. Carew NT, Nelson AM, Liang Z, Smith SM, Milcarek C (2018) Linking Endoplasmic Reticular Stress and Alternative Splicing. Int J Mol Sci 19: 3919.
10. Shen X, Klarić L, Sharapov S, Mangino M, Ning Z, et al. (2017) Multivariate discovery and replication of five novel loci associated with Immunoglobulin G N-glycosylation. Nat Commun 8: 447.
11. Nelson AM, Milcarek C (2017) Transcription elongation factor ELL2 in antibody secreting cells, myeloma, and HIV infection: A full measure of activity. Curr Trends Immunol 18: 1-11.
12. Xu L, Tang H, Chen DW, El-Naggar AK, Wei P, et al. (2015) Genome-wide association study identifies common genetic variants associated with salivary gland carcinoma and its subtypes. Cancer 121: 2367-2374.
13. Wu Y, Graff RE, Passarelli MN, Hoffman JD, Ziv E, et al. (2018) Identification of pleiotropic cancer susceptibility variants from genome-wide association studies reveals functional characteristics. Cancer Epidem Bio & Amp Prev 27: 75-85.
14. Michels TC, Petersen KE (2017) Multiple Myeloma: Diagnosis and treatment. Am Fam Physician 95: 373-383.
15. Ali M, Ajore R, Wihlborg AK, Niroula A, Swaminathan B, et al. (2018) The multiple myeloma risk allele at 5q15 lowers ELL2 expression and increases ribosomal gene expression. Nat Commun 9: 1649-1649.
16. Swaminathan B, Thorleifsson G, Jöud M, Ali M, Johnsson E, et al. (2015) Variants in ELL2 influencing immunoglobulin levels associate with multiple myeloma. Nat Commun 6: 7213.

17. Chen Y, Zhou C, Ji W, Mei Z, Hu B, et al. (2016) ELL targets c-Myc for proteasomal degradation and suppresses tumour growth. Nat Commun 7: 11057.
18. Gao J, Ward JF, Pettaway CA, Shi LZ, Subudhi SK, et al. (2017) VISTA is an inhibitory immune checkpoint that is increased after ipilimumab therapy in patients with prostate cancer. Nat Med 23: 551-555.
19. Koyama S, Akbay EA, Li YY, Herter-Sprie GS, Buczkowski KA, et al. (2016) Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun 7: 10501.
20. Embi N, Rylatt DB, Cohen P (1980) Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. Eur J Biochem 107: 519-527.
21. Woodgett JR (1990) Molecular cloning and expression of glycogen synthase kinase-3/factor A. Embo J 9(8):2431-2438.
22. Woodgett JR (2001) Judging a protein by more than its name: GSK-3. Sci Signal 2001:re12.
23. Cross DAE, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378: 785-789.
24. Doble BW, Woodgett JR (2003) GSK-3: Tricks of the trade for a multi-tasking kinase. J Cell Sci 116: 1175-1186.
25. Rayasam GV, Tulasi VK, Sodhi R, Davis JA, Ray A (2009) Glycogen synthase kinase 3: More than a namesake. Br J Pharmacol 156: 885-898.
26. Taylor A, Harker JA, Chanthong K, Stevenson PG, Zuniga EI, et al. (2016) Glycogen Synthase Kinase 3 Inactivation drives T-bet-Mediated Downregulation of Co-receptor PD-1 to Enhance CD8(+) Cytolytic T Cell Responses. Immunity 44: 274-286.
27. Taylor A, Rothstein D, Rudd CE (2018) Small molecule inhibition of PD-1 transcription is an effective alternative to antibody blockade in cancer therapy. Can Res 78: 706-717.
28. Kao C, Oestreich KJ, Paley MA, Crawford A, Angelosanto JM, et al. (2011) Transcription factor T-bet represses expression of the inhibitory receptor PD-1 and sustains virus-specific CD8+ T cell responses during chronic infection. Nat Immunol 12: 663-671.
29. Zhu Q, Yang J, Han S, Liu J, Holzbeierlein J, et al. (2010) Suppression of glycogen synthase kinase 3 activity reduces tumor growth of prostate cancer in vivo. Prostate 71: 835-845.
30. Klein PS, Melton DA (1996) A molecular mechanism for the effect of lithium on development. Proc Natl Acad Sci U S A 93: 8455-8459.
31. Piazza F, Manni S, Tubi LQ, Montini B, Pavan L, et al. (2010) Glycogen Synthase Kinase-3 regulates multiple myeloma cell growth and bortezomib-induced cell death. BMC Cancer 10: 526.
32. Dickey A, Schleicher S, Leahy K, Hu R, Hallahan D, et al.(2011) GSK-3beta inhibition promotes cell death, apoptosis, and in vivo tumor growth delay in neuroblastoma Neuro-2A cell line. J Neuro Oncol 104: 145-153.
33. Beurel E, Eggelpoel MJBV, Kornprobst M, Moritz S, Delelo R, et al. (2009) Glycogen synthase kinase-3 inhibitors augment TRAIL-induced apoptotic death in human hepatoma cells. Biochem Pharmacol 77: 54-65.
34. Tolosa E, Litvan I, Hoglinger GU, Burn D, Lees A, et al. (2014) A phase 2 trial of the GSK-3 inhibitor tideglusib in progressive supranuclear palsy. Mov Disord 29: 470-478.