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Targeting Tumor-Related Immunosuppression using Metronomic Cyclophosphamide in Combination with Lenalidomide -New Mechanisms for Old Drugs

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Standard or high-dose chemotherapy regimens have achieved significant success in cancer therapy; however, these gains reached a plateau over the past two decades in part due to drug resistance and toxicity. One approach to overcoming this has been the use of metronomic chemotherapy, which refers to the frequent administration of chemotherapy, often daily, with no prolonged drug-free breaks, at doses significantly lower than the maximum tolerated dose [1,2]. One of the most frequently used drugs in such protocols is a relatively safe and inexpensive agent- Cyclophosphamide (CTX). Since its approval in 1959 by the Food and Drug Administration, CTX, has been one of the most widely used alkylating agents in the treatment of hematological and solid malignancies. At high doses it has potent cytotoxic and lymphoablative activity; however, at low doses using a metronomic protocol it has immunostimulatory and anti-angiogenic activity while retaining antitumor activity, providing an alternative therapeutic strategy for select patients [1]. The effectiveness of metronomic chemotherapy regimens, at least in animal models, can be improved when combined with anti-angiogenic agents resulting in sustained tumor regressions [3]. Herein we discuss the immunomodulating effect of a novel metronomic chemotherapy combination and its potential application to patients with metastatic Castration-Resistant Prostate Cancer (CRPC).

Limitations of Current Prostate Cancer Therapy

The American Cancer Society estimates that 240,890 men in the United States will be diagnosed in 2012 with prostate cancer, resulting in approximately 33,720 deaths [4]. Those patients who have recurrent or metastatic prostate cancer will inevitably develop castration-resistant disease after an initial period of hormone responsiveness. Since chemotherapy has a limited impact on overall patient survival and is accompanied by treatment-related toxicity, immunotherapy has become an attractive approach to treat elderly patients with prostate cancer. This is supported by the recent phase III trial of the sipuleucel-T vaccine in patients with CRPC demonstrated a statistically significant improvement in Overall Survival (OS) [5]. However, in patients with advanced prostate cancer, tumor related immune suppression and tolerance can both facilitate tumor progression and impede active specific immunotherapy.

Targeting Tumor Related Immunosuppression

A patient's immune response can have an important role in preventing the development of malignancy and immunotherapy has promise in treating neoplasia. However, patients with cancer, especially those with advanced disease, are known to be immunologically compromised [6], facilitating tumor progression and limiting the efficacy of immune intervention. Immunity in cancer patients is negatively regulated by a number of tumor induced cellular mechanisms, including regulatory T-cells (Tregs) and Myeloid-Derived Suppressor Cells (MDSCs). It is our hope that increasing our understanding of the mechanisms that hinder immune cell number, function, induction, and expansion will lead to the development of new therapeutic approaches.

Tregs are a subset of human lymphocytes that have attracted significant interest recently due of their role in controlling T-cell responses in neoplasia [7,8]. Various methods to isolate this T-cell subset are based on the expression of cellular surface markers; however, none is specific for Tregs, and their identity is best measured by their suppressor function. The classical Treg phenotype, $CD4^{+}CD25^{br}Foxp3^{+}$, has shown immunosuppressive function *in vitro,* and increased levels of Tregs have been found in the tumors and peripheral blood of prostate cancer patients [9,10]. Consistent with these observations, and with the increased frequency of $CD4^{+}CD25^{high}FoxP3^{+}$ Tregs in the Peripheral Blood (PB) of prostate cancer patients, is a direct correlation between Tregs and tumor stage and grade [9,11]. Together these findings suggest that Tregs have a role in prostate cancer progression, suggesting that therapeutic strategies aimed at their inhibition and/or depletion may improve outcomes and response to immunotherapy for prostate cancer patients [9]. Currently, a variety of Treg-depleting agents are being examined, and a few have been approved for routine clinical use. Further, a few, small clinical studies in melanoma and prostate cancer have combined these drugs with vaccines resulting in the demonstration of efficacy [12,13].

MDSCs, another cellular population with immune suppressive activity, encompass a heterogeneous population of immature cells that expand during pathological conditions including cancer [14]. MDSCs are immature myeloid cells that include precursors for Dendritic Cells (DCs), macrophages, and granulocytes with multiple immunosuppressive mechanisms including arginase, inducible nitric oxide synthase, and reactive oxygen species. MDSCs are major contributors to the tumor associated immune dysfunction in association with their accumulation in the blood, bone marrow, and tumor and can inhibit both adaptive and innate immunity [15]. MDSCs expand in response to tumor-secreted growth factors supporting the hypothesis that inflammation promotes MDSC accumulation in association with the down-regulation of immune surveillance and antitumor immunity and facilitation of tumor growth [16]. This hypothesis has been supported in several studies in cancer patients,

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where MDSC expansion has been associated with tumor angiogenesis, growth, and impaired responses to immunotherapy [17,18]. This mechanism of tumor progression is supported by preclinical studies demonstrating an increase in MDSC frequency during tumor growth [19-22], increased number of myeloid precursors that directly correlate with tumor burden [23] and suppressed T-cell numbers and function [14,20,24]. Clinically, MDSC frequency has correlated with tumor size and number, as well as, with cancer pathological grade and clinical stage [25,26].

Chemotherapy Agents that Target Tregs and MDSCs

Since the observation by Maguire [27] that CTX could increase T-cell responses, numerous studies have suggested that some anticancer agents, in addition to their direct cytotoxic effects on tumor cells, can promote immunity, resulting in enhanced antitumor responses [28-31]. Metronomic CTX chemotherapy has been shown to selectively reduce the frequency of circulating Tregs with little influence on the number of lymphocytes and NK cells [32]. Numerous mechanisms have been proposed for CTX-induced immunomodulatory effects including inducing a Th2/Th1 shift in cytokine production [33], reducing tumor-induced suppressor T-cell frequencies [32], a reduction in various soluble mediators [34], and an enhanced long-term survival and proliferation of lymphocytes [35]. Moreover, CTX has intrinsic "pro-immunogenic" activities on tumor cells, inducing the apoptosis of a variety of tumor types [36]. However, CTX has also been shown to transiently increase MDSCs in non-tumor-bearing animals [37], and a higher level of MDSCs has been reported in patients with advanced cancer who received combination doxorubicin and CTX therapy [26]. This increase in MDSCs may be protocol dependent with a resulting MDSC decrease when administered using a metronomic protocol [38].

Emerging data has demonstrated that lenalidomide has significant clinical activity in patients with metastatic CRPC [39], and it has shown immunomodulatory activity [40,41] including a reduction in the number of immunosuppressive Tregs [42]. Lenalidomide has also been shown to modulation of tumor cell microenvironment [43] and to inhibit angiogenesis [44]. Thus, treatment with lenalidomide *in vivo* has also been shown to. Little information exists regarding lenalidomide's effect on MDSCs. However, since MDSCs are expanded by tumor-secreted growth factors, such as Vascular Endothelial Growth Factor (VEGF) [44] whose expression is inhibited by lenalidomide that could potentially inhibit MDSC recruitment and expansion. Further, lenalidomide inhibits inflammation [45] and; therefore, is likely to limit MDSC expansion. Consistent with this mechanism of action, a recent study demonstrated that lenalidomide administration could augment the T-cell response to pneumococcal (PCV) vaccination in multiple myeloma patients [46].

Rational of Dual Inhibition of MDSCs and Tregs

As Tregs and MDSCs are immunosuppressive, therapeutic strategies that reduce these cells may augment host anti-tumor immunity to endogenous and exogenous antigens and result in therapeutic benefit. However, given the propensity of tumors for multiple and varied mechanisms of immune evasion, targeting a single cellular mediator may not be sufficient to control tumor growth. To overcome this limitation, one possible approach would be to target multiple suppressive elements (MDSCs and Tregs), which we posit will improve therapeutic activity. Both lenalidomide and CTX have demonstrated clinical immunoregulatory and therapeutic activity for patients with metastatic prostate cancer. Further, CTX can decrease the proportion of Tregs in cancer patients; while lenalidomide can potentially blunt the surge of MDSCs from CTX by inhibiting MDSC recruitment and expansion through cytokine modulation and down regulation. We are currently investigating the therapeutic and immunoregulatory activity of this novel combination of metronomic CTX and lenalidomide in a phase I/II investigator-initiated study in patients with metastatic CRPC who have failed prior docetaxel therapy [47]. The longitudinal impact of chemotherapy on the frequency and function of MDSCs and Tregs is under analysis, and the potential correlation of these changes with treatment outcome will be investigated. It is hoped that this study will lead to the development of new biomarkers to monitor biological activity of this novel chemotherapy in patients with CRPC.

References

1. Kerbel RS, Klement G, Pritchard KI, Kamen B (2002) Continuous low-dose anti-angiogenic/metronomic chemotherapy: from the research laboratory into the oncology clinic. Ann Oncol 13: 12-15.
2. Kerbel RS, Kamen BA (2004) The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 4: 423-436.
3. Daenen LG, Shaked Y, Man S, Xu P, Voest EE, et al. (2009) Low-dose metronomic cyclophosphamide combined with vascular disrupting therapy induces potent antitumor activity in preclinical human tumor xenograft models. Mol Cancer Ther 8: 2872-2881.
4. Siegel R, Ward E, Brawley O, Jemal A (2011) Cancer statistics, 2011. CA Cancer J Clin 61: 212-236.
5. Kantoff PW, Schuetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, et al. (2010) Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol 28: 1099-1105.
6. Whiteside TL (2006) Immune suppression in cancer: effects on immune cells, mechanisms and future therapeutic intervention. Semin Cancer Biol 16: 3-15.
7. Sakaguchi S (2000) Regulatory T cells: key controllers of immunologic self-tolerance. Cell 101: 455-458.
8. Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133: 775-787.
9. Miller AM, Lundberg K, Ozenci V, Banham AH, Hellstrom M, et al. (2006) CD4+CD25high T cells are enriched in the tumor and peripheral blood of prostate cancer patients. J Immunol 177: 7398-7405.
10. Sfanos KS, Bruno TC, Maris CH, Xu L, Thoburn CJ, et al. (2008) Phenotypic analysis of prostate-infiltrating lymphocytes reveals TH17 and Treg skewing. Clin Cancer Res 14: 3254-3261.
11. Yokokawa J, Cereda V, Remondo C, Gulley JL, Arlen PM, et al. (2008) Enhanced functionality of CD4+CD25(high)FoxP3+ regulatory T cells in the peripheral blood of patients with prostate cancer. Clin Cancer Res 14: 1032-1040.
12. Curiel TJ (2008) Regulatory T cells and treatment of cancer. Curr Opin Immunol 20: 241-246.
13. Barnett BG, Rüter J, Kryczek I, Brumlik MJ, Cheng PJ, et al. (2008) Regulatory T cells: a new frontier in cancer immunotherapy. Adv Exp Med Biol 622: 255-260.
14. Talmadge JE (2007) Pathways mediating the expansion and immunosuppressive activity of myeloid-derived suppressor cells and their relevance to cancer therapy. Clin Cancer Res 13: 5243-5248.
15. Vuk-Pavlović S, Bulur PA, Lin Y, Qin R, Szumlanski CL, et al. (2010) Immunosuppressive CD14+HLA-DRlow/- monocytes in prostate cancer. Prostate 70: 443-455.
16. Gabrilovich DI, Nagaraj S (2009) Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9:162-174.
17. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, et al. (2006) Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol 24: 3089-3094.
18. Antonia SJ, Mirza N, Fricke I, Chiappori A, Thompson P, et al. (2006) Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. Clin Cancer Res 12: 878.

19. Gabrilovich DI, Velders MP, Sotomayor EM, Kast WM (2001) Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. J Immunol 166: 5398-5406.

20. Ostrand-Rosenberg S (2010) Myeloid-derived suppressor cells: more mechanisms for inhibiting antitumor immunity. Cancer Immunol Immunother 59: 1593-1600.

21. Almand B, Clark JI, Nikitina E, van Beynen J, English NR, et al. (2001) Increased production of immature myeloid cells in cancer patients: a mechanism of immunosuppression in cancer. J Immunol 166: 678-689.

22. Younos I, Donkor M, Hoke T, Dafferner A, Samson H, et al. (2011)Tumor- and organ-dependent infiltration by myeloid-derived suppressor cells. Int Immunopharmacol 11: 816-826.

23. Ribechini E, Greifenberg V, Sandwick S, Lutz MB (2010) Subsets, expansion and activation of myeloid-derived suppressor cells. Med Microbiol Immunol 199: 273-281.

24. Brandau S, Trellakis S, Bruderek K, Schmaltz D, Sheller G, et al. (2011) Myeloid-derived suppressor cells in the peripheral blood of cancer patients contain a subset of immature neutrophils with impaired migratory properties. J Leukoc Biol 89: 311-317.

25. Yuan XK, Zhao XK, Xia YC, Zhu X, Xiao P (2011) Increased circulating immunosuppressive CD14(+)HLA-DR(-/low) cells correlate with clinical cancer stage and pathological grade in patients with bladder carcinoma. J Int Med Res 39: 1381-1391.

26. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, et al. (2009) Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. Cancer Immunol Immunother 58: 49-59.

27. Maguire HC Jr, Ettore VL (1967) Enhancement of dinitrochlorobenzene (DNCB) contact sensitization by cyclophosphamide in the guinea pig. J Invest Dermatol 48: 39-43.

28. North RJ (1982) Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. J Exp Med 155: 1063-1074.

29. Berd D, Mastrangelo MJ, Engstrom PF, Paul A, Maguire H (1982) Augmentation of the human immune response by cyclophosphamide. Cancer Res 42: 4862.

30. Berd D, Maguire HC Jr, Mastrangelo MJ (1986) Induction of cell-mediated immunity to autologous melanoma cells and regression of metastases after treatment with a melanoma cell vaccine preceded by cyclophosphamide. Cancer Res 46: 2572-2577.

31. Berd D, Mastrangelo MJ (1988) Effect of low dose cyclophosphamide on the immune system of cancer patients: depletion of CD4+, 2H4+ suppressor-inducer T-cells. Cancer Res 48:1671-1675.

32. Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, et al. (2007) Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother 56: 641-648.

33. Matar P, Rozados VR, Gervasoni SI, Scharovsky GO (2002) Th2/Th1 switch induced by a single low dose of cyclophosphamide in a rat metastatic lymphoma model. Cancer Immunol Immunother 50: 588-596.

34. Bracci L, Moschella F, Sestili P, La Sorsa V, Valentini M, et al. (2007) Cyclophosphamide enhances the antitumor efficacy of adoptively transferred immune cells through the induction of cytokine expression, B-cell and T-cell homeostatic proliferation, and specific tumor infiltration. Clin Cancer Res 13: 644-653.

35. Schiavoni G, Mattei F, Di Pucchio T, Santini SM, Bracci L, et al. (2000) Cyclophosphamide induces type I interferon and augments the number of CD44(hi) T lymphocytes in mice: implications for strategies of chemoimmunotherapy of cancer. Blood 95: 2024-2030.

36. Berd D, Sato T, Maguire HC Jr, Kairys J, Mastrangelo MJ (2004) Immunopharmacologic analysis of an autologous, hapten-modified human melanoma vaccine. J Clin Oncol 22: 403-415.

37. Angulo I, de las Heras FG, García-Bustos JF, Gargallo D, Muñoz-Fernández MA, et al. (2000) Nitric oxide-producing CD11b(+)Ly-6G(Gr-1)(+)CD31(ER-MP12)(+) cells in the spleen of cyclophosphamide-treated mice: implications for T-cell responses in immunosuppressed mice. Blood 95: 212-220.

38. Bertolini F, Paul S, Mancuso P, Monestiroli S, Gobbi A, et al. (2003) Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. Cancer Res 63: 4342-4346.

39. Dahut WL, Aragon-Ching JB, Woo S, Tohnya TM, Gulley JL, et al. (2009) Phase I study of oral lenalidomide in patients with refractory metastatic cancer. J Clin Pharmacol 49: 650-660.

40. McDaniel JM, Zou JX, Fulp W, Chen DT, List AF, et al. (2011) Reversal of T-cell tolerance in myelodysplastic syndrome through lenalidomide immune modulation. Leukemia.

41. Zhu D, Corral LG, Fleming YW, Stein B (2008) Immunomodulatory drugs Revlimid (lenalidomide) and CC-4047 induce apoptosis of both hematological and solid tumor cells through NK cell activation. Cancer Immunol Immunother 57: 1849-1859.

42. Idler I, Giannopoulos K, Zenz T, Bhattacharya N, Nothing M, et al. (2010) Lenalidomide treatment of chronic lymphocytic leukaemia patients reduces regulatory T cells and induces Th17 T helper cells. Br J Haematol 148: 948-950.

43. Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, et al. (1999) Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. J Immunol 163: 380-386.

44. Lu L, Payvandi F, Wu L, Zhang LH, Hariri RJ, et al. (2009) The anti-cancer drug lenalidomide inhibits angiogenesis and metastasis via multiple inhibitory effects on endothelial cell function in normoxic and hypoxic conditions. Microvasc Res 77: 78-86.

45. Gorgun G, Ramsay AG, Holderried TA, Zahrieh D, Le Dieu R, et al. (2009) E(mu)-TCL1 mice represent a model for immunotherapeutic reversal of chronic lymphocytic leukemia-induced T-cell dysfunction. Proc Natl Acad Sci U S A 106: 6250-6255.

46. Noonan K, Rudraraju L, Ferguson A, Emerling A, Pasetti MF, et al. (2012) Lenalidomide-induced immunomodulation in multiple myeloma: impact on vaccines and antitumor responses. Clin Cancer Res 18: 1426-1434.

47. Wang J, McGuire TR, Schwarz JK, Meza JL, Talmadge JEE (2012) Phase I trial of metronomic cyclophosphamide (CTX) and lenalidomide (LEN) in patients with castration-resistant prostate cancer (CRPC). J Clin Oncol 30: 164.