Immunotherapy: Open Access

Harada, Immunother Open Acc 2016, 2:3
DOI: 10.4172/2471-9552.1000124

Commentary OMICS International

Immunogenic Chemotherapy Using Cyclophosphamide and Gemcitabine

Mamoru Harada*

Department of Immunology, Faculty of Medicine, Shimane University,, Shimane, Japan

***Corresponding author:** Mamoru Harada MD, PhD, Department of Immunology, Faculty of Medicine, Shimane University,, Izumo, Shimane 693-8501, Japan, Tel: +81-(0)853-20-2150; Fax: 81-(0)853-20-2150; E-mail: haramamo@med.shimane-u.ac.jp

Received date: August 19, 2016, **Accepted date:** September 06, 2016, **Published date:** September 11, 2016

Commentary

Chemotherapeutic drugs are designed to kill cancer cells, and some enhance anti-tumor T cell immunity. We recently reported immune-generating chemotherapy using cyclophosphamide (CTX) and gemcitabine (GEM) in a murine model [1]. We utilized these drugs because they decrease the numbers of regulatory T (Treg) cells and myeloid-derived suppressor cells (MDSCs), both of which are increased in tumor-bearing hosts. This study describes immunogenic chemotherapy using CTX and GEM. Chemotherapy is the most frequently used treatment modality for patients with cancer. The *in vivo* anti-tumour effects of chemotherapy are generally dose-dependent, and the clinically admissible dosage is the maximum dose at which patients can tolerate the adverse effects. Remarkable tumor regression can be induced when chemotherapeutic drugs are administered at high doses; however, myelosuppressive side effects and immunosuppression are inevitable. Some reports suggest that anti-tumor immunity plays a crucial role controlling tumor growth after chemotherapy [2]. Chemotherapeutic drugs can suppress tumor growth in immune-competent hosts, but this effect is diminished in immune-incompetent hosts. Chemotherapeutic drugs strongly affect the "subsequent" anti-tumor T cell response *in vivo* (Figure 1). Immunological competence can be maintained when chemotherapeutic drugs are administered at low doses, but cancer cells still survive. Additionally, many immunosuppressive cells remain in tumor-bearing hosts. As a result, it is difficult to elicit anti-tumor T cell immunity with these drugs. In contrast, a significant number of cancer cells die when chemotherapeutic drugs are administered at high doses, but immunological competence is lost. Even if high-dose chemotherapy decreases the number of immunosuppressive cells in tumor-bearing hosts, anti-tumor T cell immunity cannot be generated in cancer-bearing hosts. However, when chemotherapeutic drugs are administered at moderate doses, immunological competence can be maintained, and death of cancer and immunosuppressive cells is induced considerably. Subsequently, "endogenous" anti-tumor T cell immunity is generated in tumor-bearing hosts. Some chemotherapeutic drugs induce "immunogenic" cancer cell death, resulting in anti-tumor T cell immunity in tumor-bearing hosts. The Zitvogel and Kroemer laboratories have revealed the detailed mechanisms by which anti-tumor T cell immunity can be induced after administration of certain chemotherapeutic agents, such as anthracycline [2]. Calreticulin is constitutively expressed in the endoplasmic reticulum of anthracycline-treated dying tumor cells, and it migrates to the cell surface to provide phagocytic signals to dendritic cells (DCs), consequently promoting their uptake [3]. Simultaneously, dying tumor cells secrete high-mobility-group box 1 protein as a "danger" signal to DCs, resulting in efficient processing and cross-presentation of tumor antigens by DCs [4]. These studies further revealed that dying cancer cells release ATP and stimulate purinergic receptors on DCs, leading to the formation of inflammasomes and release of interleukin (IL)-1β [5]. Thereafter, DCs prime tumor antigen-specific CD4+ T cells and, subsequently, CD8+ T cells. Importantly, CTX has the potential to induce immunogenic cancer cell death [2]. Anti-tumor T cells are the most potent effector cells against tumor cells, but several barriers inhibit their effector function in tumor-bearing hosts. Specifically, the tumor-bearing state is usually associated with immunosuppression by immune-suppressive cells, including CD4+ CD25+ Treg cells and MDSCs [6,7]. Treg cells possess immunosuppressive activity via immunosuppressive cytokines and cell-contact mechanisms. MDSCs consist of monocytic and granulocytic MDSCs [8], which play crucial roles in tumor-associated immunosuppression [9,10]. MDSCs exert immunosuppressive effects on anti-tumor T cells through arginase-1, reactive oxygen species, IL-6, and IL-10. Immunosuppression mediated by these cells must be overcome to successfully induce the anti-tumor T cell response. Interestingly, several chemotherapeutic drugs have the potential to mitigate immunosuppression by Treg cells and MDSCs. Many studies have shown that low-dose CTX increases anti-tumor immune responses in tumor-bearing hosts by mitigating Treg cell-mediated immunosuppression [11-16]. CTX has multifaceted effects on immunity. In addition to its effect on Treg cells, CTX influences DC homeostasis, secretion of type I interferon (IFN), and polarization of CD4+ T cells into Th1 and/or Th17 cells [11]. Low-dose CTX decreases IL-10 levels, thereby altering the Th1/Th2 balance in favor of Th1 [17,18]. We also reported that low-dose CTX relieves Treg-mediated immunosuppression and restores T cell proliferation and IFN- production in murine colon tumor-bearing mice [19]. Similarly, several chemotherapeutic drugs decrease the number of MDSCs. GEM decreased the number of MDSCs and improved the anti-tumor activity of cytotoxic T lymphocytes and natural killer (NK) cells in a murine model [20]. GEM decreased the number of MDSCs in a murine mammary carcinoma model [21]. In addition, both 5-fluorouracil and docetaxel decreased the number of splenic and intratumoral MDSCs without impairing immunity [22,23].

Metronomic chemotherapy refers to the administration of chemotherapeutic agents at relatively low, minimally toxic doses, without a prolonged drug-free period. This type of chemotherapy has been suggested to be more effective and to result in fewer toxic side effects compared with those of conventional, maximum-tolerated dose chemotherapy [24-27]. In fact, metronomic chemotherapy has been used in patients with several types of cancers, and clinical responses have been observed [28-30]. Metronomic chemotherapy primarily targets circulating endothelial progenitor cells and inhibits angiogenesis via production of thrombospondin-1 [31]. However, some preclinical and clinical studies suggest that anti-tumor immunity is involved in the anti-tumor effects following metronomic chemotherapy. Metronomic CTX therapy reduces the number of circulating Treg cells as well as their immunosuppressive function and restores NK cell activity and T cell proliferation [28]. Importantly, this

effect was observed only with low-dose CTX, as higher doses resulted in depletion of all lymphocyte subpopulations. In addition, metronomic low-dose CTX transiently reduces the number of Treg cells but induces stable tumor-specific T cell responses in patients with metastasized breast cancer [30].

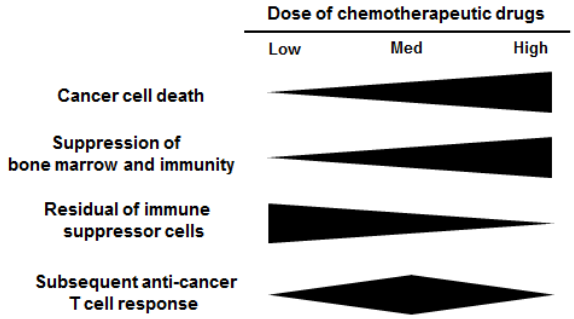

Figure 1: Dose-dependent effects of chemotherapeutic drugs on the anti-cancer T cell response.

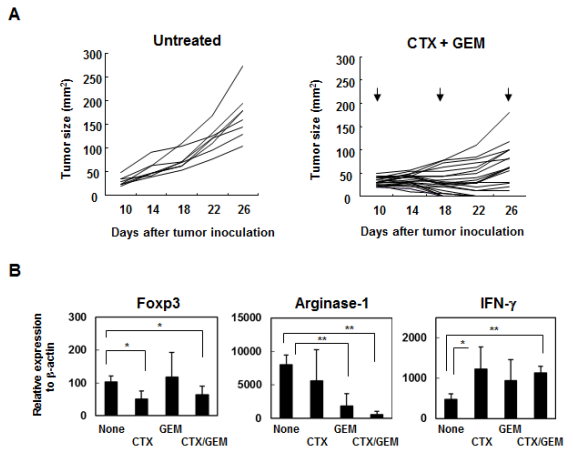

Figure 2: Anti-tumor effects of immunogenic chemotherapy using cyclophosphamide (CTX) and gemcitabine (GEM). (A) CT26-bearing BALB/c mice were treated with low-dose CTX and GEM on days 10, 18, and 26 after tumor inoculation. Arrows indicate drug injection times. Lines represent tumor growth in individual mice. (B) The tumor-bearing mice were injected intraperitoneally with low-dose CTX and/or GEM on day 10 after tumor inoculation. Two days later, tumor tissues were collected, and mRNA expression was evaluated by real-time polymerase chain reaction. *P<0.05, **P<0.01 [1].

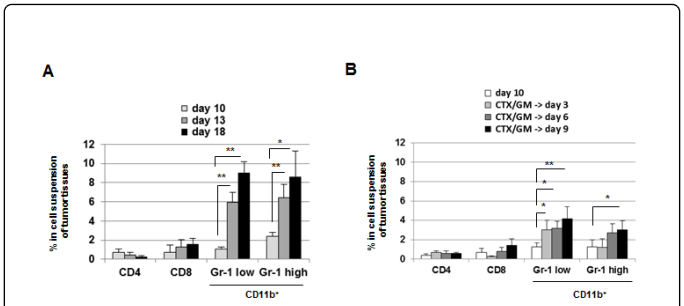

Figure 3: Kinetic analysis of tumor-infiltrating immune cells after chemotherapy with cyclophosphamide (CTX) and gemcitabine (GEM). (A) BALB/c mice were injected subcutaneously with CT26 cells. Tumor tissues were harvested on the indicated days, and tumor-infiltrating immune cells were analyzed by flow cytometry. (B) Low-dose CTX and GEM were injected intraperitoneally 10 days after inoculating the CT26 cells. Tumor-infiltrating immune cells were analyzed by flow cytometry on days 3, 6, and 9 after administering CTX and GEM. *P<0.05, **P<0.01 [33].

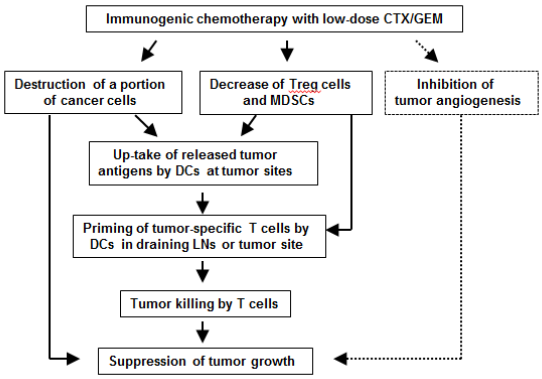

Figure 4: Mechanisms of the anti-tumor effects of immunogenic chemotherapy using cyclophosphamide (CTX) and gemcitabine (GEM).

This anti-tumor effect was significantly attenuated in CT26-bearing nude mice, suggesting that the anti-tumor effect depends on T cells. Expectedly, low-dose CTX and GEM decreased Foxp3 and arginase-1 mRNA expression levels, which are markers of Treg cells and MDSCs, respectively (Figure 2B) [1]. The CTX and GEM combination increased secretion of IFN-γ, which is a marker of cellular immunity. Subsequently, we examined the kinetics of tumor-infiltrating immune cells in CT26-bearing mice and found that the numbers of two types of MDSCs, monocytic CD11b+ Gr-1 low and granulocytic CD11b+ Gr-1 high cells, increased remarkably from days 10 to 13 after tumor inoculation (Figure 3A) [33].

The percentages of monocytic and granulocytic MDSCs in tumor tissues on day 10 after tumor inoculation were approximately 1% and 2.2%, respectively, whereas that on day 13 was approximately 6%. In contrast, the percentage of CD4+ T cells, which include conventional

CD4+ T cells and Treg cells, was <1% in tumor tissues. We also examined the kinetic recovery of tumor-infiltrating cells after one administration of low-dose CTX and GEM on day 10 after CT26 inoculation. Importantly, administering low-dose CTX and GEM suppressed the subsequent increase in the number of MDSCs to almost half that in untreated mice (Figure 3B). Repeated administration of low-dose CTX and GEM at 8-day intervals did not suppress T cells in the spleen [1].

Based on this information, we previously reported the effects of immunogenic chemotherapy using low-dose CTX and GEM [1]. In that study, we administered low-dose chemotherapeutic drugs over 8-day intervals. This protocol cannot be termed metronomic, because metronomic refers to the administration of chemotherapeutic agents without a prolonged drug-free period [26,32]. Therefore, this protocol should be called "intermittent" immunogenic chemotherapy. We used CTX and GEM, because they are representative chemotherapeutic drugs that diminish the numbers of Treg cells and MDSCs, as described above. As shown in (Figure 2A), immunogenic chemotherapy using low-dose (50 mg/kg) CTX and (50 mg/kg) GEM at 8-day intervals induced drastic anti-tumor effects on subcutaneously established CT26 colon carcinoma [1].

These results indicate that immunogenic chemotherapy using low-dose CTX and GEM evoked anti-tumor T cell immunity in vivo without impairing T cell immunity in cancer-bearing hosts. (Figure 4) summarizes the mechanisms by which immunogenic chemotherapy using low-dose CTX and GEM suppresses tumor growth. These chemotherapeutic drugs destroy a portion of cancer cells. Simultaneously, CTX and GEM mitigate immunosuppression by Treg cells and MDSCs, respectively, leading to an increase in DC antigen-presenting activity. Tumor-infiltrating DCs take up tumor antigens from dying tumor cells. After these DCs migrate to draining lymph nodes and prime tumor-specific T cells, the primed and activated T cells travel to tumor sites where they lyse tumor cells. Additionally, CTX can inhibit tumor angiogenesis. Thus, immune cell-mediated cytolysis of tumor cells and inhibition of tumor angiogenesis synergistically exert anti-tumor effects. Taken together, these data suggest that intermittent immunogenic chemotherapy using low-dose CTX and GEM mitigates Treg- and MDSC-mediated immunosuppression, resulting in *in vivo* induction of anti-tumor T cell immunity. As both drugs have been widely used to treat various types of malignant cancers, this type of chemotherapy could be safely applied clinically.

References

1. Tongu M, Harashima N, Monma H, Inao T, Yamada T, et al. (2013) Metronomic chemotherapy with low-dose cyclophosphamide plus gemcitabine can induce anti-tumor T cell immunity in vivo. Cancer Immunol Immunother 62: 383-391.
2. Kroemer G, Galluzzi L, Kepp O, Zitvogel L (2013) Immunogenic cell death in cancer therapy. Annu Rev Immunol 31: 51-72.
3. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, et al. (2007) Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 13: 54-61.
4. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, et al. (2007) Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 13: 1050-1059.
5. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, et al. (2009) Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat Med 15: 1170-1178.
6. Yamaguchi T, Sakaguchi S (2006) Regulatory T cells in immune surveillance and treatment of cancer. Semin Cancer Biol 16: 115-123.
7. Ostrand-Rosenberg S, Sinha P (2009) Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol 182: 4499-4506.
8. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI (2008) Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol 181: 5791-5802.
9. Young M, Wright MA, Pandit R (1997) Myeloid differentiation treatment to diminish the presence of immune-suppressive CD34+ cells within human head and neck squamous cell carcinomas. J Immunol 159: 990-996.
10. Almand B, Clark J, 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.
11. Sistigu A, Viaud S, Chaput N, Bracci L, Proietti E, et al. (2011) Immunomodulatory effects of cyclophosphamide and implementations for vaccine design. Semin Immunopathol 33: 369-383.
12. Le DT, Jaffee EM (2012) Regulatory T-cell modulation using cyclophosphamide in vaccine approaches: a current perspective. Cancer Res 72: 3439-3444.
13. Ghiringhelli F, Larmonier N, Schmitt E, Parcellier A, Cathelin D, et al. (2004) CD4+ CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. Eur J Immunol 34: 336-344.
14. Lutsiak ME, Semnani RT, De Pascalis R, Kashmiri SV, Schlom J, et al. (2005) Inhibition of CD4(+)25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. Blood 105: 2862-2868.
15. Liu JY, Wu Y, Zhang XS, Yang JL, Li HL, et al. (2007) Single administration of low dose cyclophosphamide augments the antitumor effects of dendritic cell vaccine. Cancer Immunol Immunother 56: 1597-1604.
16. Wada S, Yoshimura K, Hipkiss EL, Harris TJ, Yen HR, et al. (2009) Cyclophosphamide augments antitumor immunity: Studies in an autochthonous prostate cancer model. Cancer Res 69: 4309-4318.
17. Matar P, Rozados VR, Gervasoni SI, Scharovsky OG (2001) Down regulationof T-cell-derived production by low-dose cyclophosphamide treatment in tumor-bearing rats restores in vitro normal lymphoproliferative response. Int Immunopharmacol 1: 307-319.
18. 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.
19. Tongu M, Harashima N, Yamada T, Harada T, Harada M (2010) Immunogenic chemotherapy with cyclophophamide and doxorubicin against established murine carcinoma. Cancer Immunol Immunother 59: 769-777.
20. Suzuki E, Kapoor V, Jassar AS, Kaiser LR, Albelda SM (2005) Gemcitabine selectively eliminate splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhance antitumor immune activity. Clin Cancer Res 11: 6713-6721.
21. Le H, Graham L, Cha E, Morales JK, Manjili MH, et al. (2009) Gemcitabine directlyinhibits myeloid derived suppressor cells in BALB/c mice bearing 4T1 mammary carcinoma and augments expansion of T cells from tumor-bearing mice. Int Immunopharmacol 9: 900-909.
22. Kodumudi KN, Woan K, Gilvary DL, Sahakian E, Wei S, et al. (2010) A novel chemoimmunomodulating property of docetaxel: suppression of myeloid-derived suppressor cells in tumor bearers. Clin Cancer Res 16: 4583-4594.
23. Vincent J, Mignot G, Chalmin F, Ladoire S, Brichard M, et al. (2010) 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in nehhanced T cell-dependent antitumor immunity. Cancer Res 70: 3052-3061.
24. Hanahan D, Bergers G, Bergsland E (2000) Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumor angiogenesis in mice. J Clin Invest 105: 1045-1047.

25. Man S, Bocci G, Francia G, Green SK, Jothy S, et al. (2002) Antitumor effects in mice of low-dose (metronomic) cyclophosphamide administered continuously through the draining water. Cancer Res 15: 2731-2735.
26. Pasquier E, Kavallaris M, André N (2010) Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol 7: 455-465.
27. Penel N, Adenis A, Bocci G (2012) Cyclophosphamide-based metronomic chemotherapy: after 10 years of experience, where do we stand and where are we going? Crit Rev Oncol Hematol 82: 40-50.
28. 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.
29. Lord R, Nair S, Schache A, Spicer J, Somaihah N, et al. (2007) Low dose metronomic oral cyclophosphamide for hormone resistant prostate cancer: a phase II study. J Urol 177: 2136-2140.
30. Ge Y, Domschke C, Stoiber N, Schott S, Heil J, et al. (2012) Metronomic cyclophosphamide treatment in metastasized breast cancer patients: immunological effects and clinical outcome. Cancer Immunol Immunother 61: 353-362.
31. Bocci G, Francia G, Man S, Lawler J, Kerbel RS (2003) Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. Proc Natl Acad Sci USA 100: 12917-12922.
32. Kerbel RS, Kamen BA (2004) The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 4: 423-436.
33. Tongu M, Harashima N, Tamada K, Chen L, Harada M (2015) Intermittent chemotherapy can retain the therapeutic potential of anti-CD137 antibody during the late tumor-bearing state. Cancer Sci 106: 9-17.