Preface

Cancer is the leading cause of death worldwide, and is responsible for nearly 10 million deaths in 2020 as per the GLOBOCAN 2020 estimates of cancer incidence and mortality produced by the International Agency for Research on Cancer. An estimated number of 19.3 million new cancer and nearly 10.0 million cancer deaths occurred in 2020, worldwide. The global cancer burden is expected to be rise by 47% from 2020 with 28.4 million cases in 2040. The majority of cancers upto 90–95% of cases are due to genetic mutations from environmental and lifestyle factors and the remaining 5–10% are due to genetic inheritance. The biological capabilities of cancer that are acquired during their multistep development are called hallmarks of cancer. They include sustaining proliferative signaling, resisting cell death, evading growth suppressors, inducing angiogenesis, enabling replicative immortality, activating invasion and metastasis, reprogramming energy metabolism and evading immune destruction.

Infiltration of T lymphocytes into the tumor is a major challenge because the tumor cells regulate lymphocytes infiltration through multiple mechanisms. Even after infiltration, TILs are still challenged by multiple immunosuppressive pathways orchestrated by tumor cells. One of the major immunosuppressive pathways is the adenosine-A2A receptor pathway. During hypoxic conditions of solid tumors, adenosine concentration is increased locally. Following its release, adenosine acts on A2A receptors expressed on stromal cells and downregulates adhesion proteins leading to decreased infiltration of TILs. In addition, adenosine inhibits the proliferation of cytotoxic T cells and increases the proliferation of Tregs. It has been reported that adenosine enhances the PD-1 expression on TILs via activation of A2A receptors. In a recent study, Hadar et al. reported that caffeine enhances the release of proinflammatory cytokines. However, it is unclear whether caffeine promotes anti-tumor immune response through infiltration of T lymphocytes or decreased expression of PD-1 receptor on T lymphocytes.

Our results demonstrate that percentage of cytotoxic T cells were significantly more in tumors of caffeine-treated mice than water-drinking mice. Conversely, percentages of regulatory T cells were significantly less in tumors of caffeine-treated mice than water-drinking mice. The decrease in regulatory T cells in turn enhances the proliferation and function of cytotoxic T cells. Further, our results indicated that caffeine-treated mice have

higher intra-tumoral levels of TNF-α and IFN-γ than water-drinking mice. The expression of PD-1 on the cytotoxic T cells and regulatory T cells correlates with a worse survival of various cancers. Our results demonstrate that caffeine treatment decreases the expression of PD-1 on cytotoxic T cells and regulatory T cells. Therefore, we suggest that caffeine-enhanced anti-tumor activity involves blockade of immunosuppression induced by PD-1 expression. The possible mechanism behind anti-tumor immune response of caffeine treatment relates to the antagonism of adenosine A2A receptor. Activation of A2A receptors by adenosine on T cells leads to decreased proliferation of cytotoxic T cells, decreased production of cytokines (TNF-α and IFN-γ), and decreased expression of PD-1. The Ki of caffeine against mice A2A receptor is 44 µM. In the present study, lower levels of caffeine 0.02 % w/v (equivalent to 40 mg/kg/day), 0.04 % w/v (equivalent to 80 mg/kg/day), and 0.08 % w/v (equivalent to 160 mg/kg/day) were used. In an earlier study, a serum concentration of 40 µM was observed in mice following a single injection of 20 mg/kg caffeine. Therefore, the doses of caffeine used in the present study would have antagonized the A2A receptors. It is also interesting that in humans consuming moderate to heavy caffeine a plasma concentration of 10-50 µM was observed. The observed caffeine-enhanced anti-tumor immune response in the present study at 0.04% and 0.08% suggests that consuming 4 and 8 cups of coffee per day such effects can be achieved in humans.

Immunotherapy represented by immune checkpoint blockers (ICBs) such as anti–CTLA4 and anti–PD1 monoclonal antibodies (mAbs) has emerged as a promising treatment option for cancer patients in recent years. However, the clinical response rate of ICBs is limited to a subset of the patient population (15-30%), while the majority of patients are primarily resistant to PD1 blockade. The tumor biopsies of patients treated with anti–PD1 mAb revealed that patients who did not respond to the therapy lacked CD8+ T cells inside tumor lesions. There is accumulating evidence indicating that resistant to anti–PD1 therapy is largely dependent on tumor microenvironment where tumor cells utilize multiple and non-overlapping immunosuppressive mechanisms to facilitate immune escape. It can be hypothesized that a combination immunotherapy designed to attract CD8+ T cells into tumor microenvironment and to block non-overlapping immunosuppressive mechanisms may improve the antitumor activity of anti–PD1 mAb in resistant patients. One of the immunosuppressive pathways involved in tumor immune escape is adenosine-A2A receptor pathway. Because activation of both A2A receptor and

PD1 on activated T cells suppresses T cell function, co-targeted blockade of both A2A receptor and PD1 may enhance the anti-tumor activity of anti-PD1 mAb. In the present study, we aimed to investigate whether caffeine can enhance the therapeutic activity of anti-PD1 mAb against carcinogen- and cell line-induced tumor models in mice. Another approach to attain this goal involves the induction of immunogenic conditions in the tumor microenvironment. For example, some chemotherapies can stimulate T cell immunosurveillance by influencing tumor-host interactions. Oxaliplatin is one of the chemotherapeutic agent that can effectively augment antitumor immune response by inducing immunogenic cell death and also facilitates the activation of dendritic cells. In the present study, we also aimed to investigate the therapeutic role oxaliplatin in murine model of melanoma and evaluate the therapeutic efficacy of oxaliplatin and anti-PD1 monoclonal antibody co-administration.

In the present study, we investigated the anti-tumor effect of caffeine and anti-PD1 mAb combination therapy against 3-MCA-induced tumors in mice. We found that the combination therapy showed synergistic anti-tumor activity than caffeine or anti-PD1 mAb monotherapy. In this study, the combination therapy caused a significant prolongation in the overall survival period of mice. We further investigated the therapeutic activity and the possible mechanism of action of combination therapy against B16F10 melanoma tumors. Our results revealed that the combination therapy possesses a significant anti-tumor activity against B16F10 melanoma tumors. To identify the possible mechanism of action, we isolated TILs from the harvested B16F10 melanoma tumors and subjected them to flow cytometric analysis. The results revealed a lower T lymphocyte infiltration into the tumors of control mice, indicating tumor cells ability to avoid immune destruction. However, the possible blockade of adenosine-A2A receptor pathway by caffeine and blockade of PD1 pathway by anti-PD1 mAb increased the infiltration of total T lymphocyte population into the melanoma tumors. The combination therapy showed synergistic increase in infiltration of T lymphocytes into the tumors, possibly due to combined blockade of both the pathways. Our results demonstrate that the combination therapy synergistically increased the frequency of CD4+ and CD8+ T cells. Conversely, the percentage of CD4+CD25+ T regulatory cells was decreased by the combination. In our study, we found that caffeine and anti-PD1 mAb combination therapy significantly increased intra-tumoral TNF-α and IFN-γ levels leading to cytotoxic effect on tumor cells. Our work suggests that administration of caffeine and anti-PD1 mAb harness the

therapeutic potential of effector T cells in vivo possibly due to combined blockade of PD1 and adenosine-A2A receptor pathway. Our study provides the scientific basis for testing combination regimens of caffeine and anti-PD1 mAbs for sustained tumor control in cancer patients.

In the present study, we also investigated the anti-tumor effect of oxaliplatin and anti-PD1 mAb combination therapy against 3-MCA-induced tumors in mice. We found that the combination therapy showed synergistic anti-tumor activity than oxaliplatin or anti-PD1 mAb monotherapy. In the present study, the combination therapy of oxaliplatin and anti-PD1 caused a significant prolongation in the overall survival period of mice with carcinogen-induced tumors. We further investigated the effect of combination terapy against B16F10 melanoma tumors. Our results revealed that the combination therapy of oxaliplatin and anti-PD1 caused a significant anti-tumor activity against B16F10 melanoma tumors. In the present study, the observed lower CD4 and CD8 T lymphocyte infiltration into the tumors of control mice, indicates the ability of tumor cells in avoiding immune destruction. Our results demonstrate that the combination therapy synergistically increased the frequency of CD4+ and CD8+ T cells. In the present study, it has been also observed that combination therapy demonstrated significant increase in the release of DAMPs like calreticulin and HMGB1 and cytokines like TNF-α, and IFN-γ. Based on the observed results, the possible mechanism behind the increased antitumor activity of the combination therapy might be due to the release of DAMPs by oxaliplatin and simultaneous inhibition of PD1 blockade by anti-PD1 antibody. In our study, we found that oxaliplatin and anti-PD1 mAb combination therapy significantly increased intra-tumoral TNF-α and IFN-γ levels leading to cytotoxic effect on tumor cells. Our study provides the scientific basis for testing combination regimens of oxaliplatin and anti-PD1 mAbs for sustained tumor control in cancer patients.

Caffeine-anti-PD1 and oxaliplatin-anti-PD1 combination therapies could be a potential strategy to increase the overall response rate of anti-PD1 monoclonal antibody.

Key words: Caffeine; oxaliplatin; anti-PD1; anti-tumor immune response; adenosine-A2A receptor pathway; immunogenic cell death