



## Re-Thinking the Interplay between Tumorigenesis and Immunity

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### Introduction

Immune therapy of cancer has finally reached maturation with multiple finalized or currently ongoing successful clinical trials in several different cancers investigating the administration of checkpoint inhibitor blockers. Checkpoint inhibitors such as CTLA-4 (cytotoxic T lymphocyte-associated protein 4) and PD-1 (programmed death-1) are in healthy tissue central for the avoidance of autoimmunity by inhibiting effector T cells once these have completed killing of infected cells. In the cancer setting, blockers of CTLA-4 and PD-1 have shown significant increase in overall survival in phase III trials and subsequently been approved for marketing as Yervoy® (anti-CTLA-4, Bristol-Myers Squibb), Opdivo® (anti-PD-1, Bristol-Myers Squibb) and Keytruda® (anti-PD-1, Merck) [1-4]. Along with these are promising clinical data from combination regimens of CTLA-4 and PD-1 inhibition [5,6], and convincing pre-clinical data from the combination of anti-PD-1 with immune modulation of several other checkpoint inhibiting or stimulating receptors in mouse studies, while early phase clinical studies are on-going [7]. Even though the clinical results are very convincing compared to previous treatments of metastatic cancer, we are still far from a complete understanding of the complex interplay between carcinogenesis and immunity, and importantly, only a small fraction of the treated patients with metastatic cancer obtain survival benefit in the longer run [8,9].

Carcinogenesis occurs in interplay between tumor cells, epigenetic mechanisms, immunoediting, inflammation and anti-tumor immune responses. As an extra layer could be added the treatment we provide – being chemotherapeutics with an immune-stimulating or –inhibiting impact, antibodies against checkpoint molecules, cultured cells or vaccination. Often only one or two aspects are focused on, but we need to see the whole picture in order to understand. The best strategy for enhancing clinical efficacy may be combining the checkpoint inhibitor blockers with other immune treatments in a smart fashion. Thus, the promising clinical development stresses the importance of doing proper immune monitoring and further exploratory studies to elucidate the mechanisms are warranted. The adaptive immune system is active against cancer, but over time the most immunogenic cancer cell clones are eradicated leaving the less immunogenic ones with better growth conditions. This immune editing process may eventually lead to a very suppressive tumor microenvironment and the cancer cells escaping the immune system [10]. Further, the chronic inflammation state often orchestrated by the tumor is known to be tumor-promoting by suppressing the adaptive immune effector populations by secretion of cytokines, reactive oxygen and nitrogen species amongst other substances [11,12]. The last pillar in this framework of immunity and cancer is the epigenetic mechanisms, turning on or off the transcription of genes involved in tumor suppression of promotion, including tumor suppressor genes as TP53 and BRCA1 and 2.

In order to design future anti-cancer immune treatments it seems important to take into account all of these pillars, as I elaborated on in a recent review in Danish Medical Journal [13]. In addition 'hidden' immune effects of chemotherapeutics have been shown previously, thus complicating the cause-and-effect relation between a certain treatment and the clinical outcome even further. These includes the inhibiting effect on regulatory T cells by cyclophosphamide and gemcitabine [14,15]. We investigated the immunological effects of cyclic administration of 5-Azacytidine to patients with myelodysplastic syndrome or acute myeloid leukemia. By separating T cells and tumor cells ex vivo we were able to measure specifically the in vivo drug-related impact on the tumor cells and on the T cells. We found in vivo treatment with the drug to exert the tumor cells more prone to T cell-mediated cytokine secretion, possibly due to 5-Azacytidine's epigenetic effect as a demethylating agent, and the subsequently increased production and surface-expression of T cell targets [16].

Many tumors are infiltrated by lymphocytes, and although these do include tumor-specific T cells often the majority of cells do not recognize the tumor [17]. Thus the idea of in vivo specifically stimulating or ex vivo selecting the population of tumor-specific T cells is tempting, possibly in combination with administration of checkpoint inhibitor blockers to release the brake on the effector T cells. These approaches have, however, been restricted by the lack of appropriate methods to identify the tumor-specific cells. The patient population is very diverse in terms of human leukocyte antigen (HLA) expression and in addition within each tumor the protein expression is heterogenous [18], complicating the task of doing specific immune monitoring. In order to perform direct ex vivo monitoring perhaps the best approach is to stain cells with a library of HLA multimers and antibodies targeting appropriate surface markers for flow cytometry, enabling the exact enumeration of specific T cells together with their phenotype. To do so, knowledge of the relevant T cell epitopes and a high-throughput production platform for a broad library of HLA monomers is needed, also further elaborated on in my recent review [13]. Recently, however, two interesting studies indicated that the intratumoral tumor-specific CD8+ T cells are always co-expressing PD-1 [19,20], suggesting the PD-1 receptor to be a pan-specific marker and opening up the possibility of enumerating and possibly activating the full population of tumor-specific T cells without knowing their exact targets. Further studies in exploring this possibility are warranted.

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