RESEARCH ARTICLE

Targeting Tumor-Induced Immunosuppression Using Conventional Cancer Therapeutics

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Conflict of interest:
Dr Loskog and Dr Eriksson are employees of Lokon Pharma AB. Dr Loskog is a board member of Lokon Pharma, Vivolux, Repos Pharma, Bioimics, Tanea Medical, and Aros Biotech. She is an advisor to NEXTTBE AB and the inventor of a patent granted to Lokon Pharma.

Abstract

Immunosuppression remains a challenge in the immunotherapy field and needs to be combated to increase survival of patients suffering from cancer. There have been no phase III trials that have in an organized, statistically reliable setting compared immunotherapy outcome with or without so called preconditioning, or metronomic conditioning, in randomized settings. The current view on preconditioning is that it may be used both to reduce tumor load and to reduce suppressive immune cells prior to immunotherapy. For combination treatments, immunotherapy such as checkpoint blockade has shown benefit in combination with chemotherapy even if the major goals of those studies may not have been to condition the patient for a better immune response due to reduced immunosuppression. Nevertheless, there is a need to further enhance the promising effect of cancer immunotherapy. We argue herein that there are several interesting conventional cancer therapeutics to explore for combinational use with immunotherapy to enhance response rates and achieve a longer overall survival of patients. This review will discuss mechanisms of conventional cancer therapeutics of interest for combination therapy. For example, gemcitabine and several tyrosine kinase inhibitors have profound effect on myeloid-derived suppressor cells while tyrosine kinase inhibitors can enhance T cell infiltration into tumors likely due to increased chemokine signaling. Further, cyclophosphamide is well known for its capacity to reduce Tregs and is used to precondition patients prior T cell therapy. How to combine these agents with immunotherapy to further increase patient survival is an important next step in the immunotherapeutic field.

1. Introduction

Cancer immunotherapy has come to stay. However, it has been a long and winding road leading to the toolbox of approved immunotherapeutics that we use today. The first observation that immune cells were gathering in the tumor bed was done in 1863 by Virchow[1] and about 30 years thereafter, William Coley used mixtures of live and inactivated bacteria as a treatment for sarcoma.[2,3] Unfortunately, this crude bacterial soup also put patients at risk for infection and further progress was on hold. Nevertheless, the idea lived on and the first approved modern immunotherapeutics was a development of Coley's concept. In 1990, Bacillus Calmette-Guérin (BCG) was approved for early-stage bladder cancer. It had been tested for a variety of advanced malignancies but initially failed as reviewed by Lobo et al.[4] The attenuated bacteria triggers release of an array of cytokines that ultimately evoke Th2 type CD4 T cells and NK cells as major effectors as elegantly shown in publications from the group of Brandau and Boehle.[5,6] However, CD8 T cells may play a role as well as reviewed by Lim et al. Like most other *in vivo* activation-dependent immunotherapies, BCG is not strong enough to overcome tumor-mediated immunosuppression in full bloom. In fact, many early immunotherapies failed when being tested for advanced cancer and the medical community lost faith in the cancer immunotherapy concept. Back then, tumor-induced immunosuppression was not yet understood but a handful of believers refused to give up and like Don Quijote de La Mancha fighting windmills, they continued to develop novel drugs that would perhaps work better in patients. There are a couple of events that are

important to recognize as they lay the groundwork for where we stand today in the successful era of cancer immunotherapy. The first event was the acknowledgement of cancer-induced immunosuppression. Suppressive immune cells were demonstrated almost 50 years ago but remained a controversial finding for long.[8] The spell was broken by the evidence presented by Sakagushi et al showing that a subpopulation of FoxP3+ T cells indeed had a specific suppressive phenotype and function, so called T regulatory cells (Tregs) (Sakagushi)[9] and the subsequent publications demonstrating that these cells were increased in most types of cancer.[10-14] This first event leads us directly to the next: the understanding that it will be necessary to combat immunosuppression, such as Tregs, to facilitate cancer immunotherapy. The third event was the realization of pseudoprogression, a phenomenon that can be triggered by immunotherapeutics as they may induce inflammation leading to an initial swelling of the tumor before a response is noted.[15] This last event may be the reason for many early trial failures as they may have regarded this early sign of response as progressive disease removing the patient from further treatment and follow-up. As we now understand these concepts, success with immunotherapy is finally here to stay. Old failures become new possibilities. How to combine *in vivo* activating immunotherapeutics with other drugs to override immunosuppression is a current hot debate in the community.

In this mini-review, the use of conventional cancer treatments to target tumor-induced immunosuppression as a combination treatment to immunotherapy will be discussed.

2. Tumor-driven immunosuppression

Tumor cells are in general rather similar to normal healthy cells, from which they derive. Thus, tolerance to self is a major initial factor of immunosuppression in the early development of a malignancy. As the tumor gathers mutations, the risk of being killed by the immune system increases. As reviewed elsewhere, tumors surviving immune surveillance, are those that gained capacity to produce immunosuppressive substances, reduced lymphocyte recognition receptors, reduced their sensitivity to apoptosis and reprogramed the surrounding stroma to act as a barrier to infiltrating tumor-targeting lymphocytes.[16] There are two major groups of suppressive immune cells that can be targeted as a mean of reducing immunosuppression in cancer, the Tregs and a heterogenous group of immature myeloid cells collectively called myeloid-derived suppressor cells (MDSCs).[17,18] Tregs are divided into many classes but in general, they are commonly CD4+ T cells that either differentiated into suppressive cells from a naïve status under TGF-β and IL2 cytokine pressure or become suppressive already during development in the thymus (natural Tregs).[17] As tumor cells and its stroma produce TGF-β, they can recruite Tregs to the tumor bed by differentiation of circulating naïve CD4+ T cells. Tregs can suppress both lymphocytes including T, B and NK cells but also myeloid cells like macrophages and dendritic cells (DCs). Tregs may express a variety of suppressive molecules including TGF-β that can directly suppress T effector cells or induce even more Tregs, IL10 that suppress DC maturation leading to poor T effector cell responses, TIGIT that binds to DCs to increase their IL10 production while reducing IL12, CTLA-4 that binds to costimulatory molecules CD80/86 which increases indoleamine 2,3-dioxygenase (IDO) expression that in turn decrease tryptophan concentration that is otherwise needed for T effector cells during proliferation, and IL35 that can suppress T effector cell proliferation

and effector functions.[17,19,20] MDSCs are also divided into two classes, the ones with monocytic or granulocytic character. They originate from stem cells in the bone marrow during hematopoiesis but are prematurely released into the blood and home to the tumor bed due to for example tumor-induced granulocyte, macrophage-colony stimulating factor (GM-CSF) and IL6.[18] Hence, they can be either myeloid progenitor cells or immature myeloid cells. The latter lack suppressive function in a healthy individual but gains it via the growth factors and cytokines released in the tumor microenvironment such as prostaglandin E2 and TGF-β. MDSCs express arginase I and inducible nitric oxide synthase (iNOS) that both have suppressive functions including reducing the CD3 complex which in turns reduce antigen recognition capacity. Further, they can release IDO that stimulates apoptosis in T cells and can also reduce NK cell-mediated killing mechanisms or reactive oxygen species (ROS) which also targets T effector cell functions.[21-23]

As reviewed elsewhere, there are also other immune cells that play an important role to mediate the tumor immunosuppression. For example, monocytes are recruited to the tumor bed due to the release of VEGF, GM-CSF, IL6, IL10 and TGF-β which also differentiate the monocytes to M2 macrophages that participate in promoting angiogenesis and immunosuppression in general. The dysregulated blood vessels formed in tumors during angiogenesis are poorly expressing receptors necessary for T cell attachment, rolling and transmigration which prevents effective lymphocyte infiltration into the tumor microenvironment. The M2 macrophages produce many molecules that promotes tumor progression but also the suppressors Arginase I, IL10 and ROS.[16] In cancer, these immunosuppressive cells accumulate in tumor tissue and suppress immune reactions against the tumor. At later stages, the immunosuppression becomes systemic with high levels of suppressive cells in the blood. At this stage, the tumor-induced immunosuppression to block anti-tumor responses is also a major concern for combating infectious disease.

3. Preconditioning or conditioning patients

4.1 Preconditioning with cyclophosphamide and fludarabine

What can we do to reduce the immunosuppressive cells in cancer to increase the response rate and survival to activating immunotherapeutics? The most refined systematic development can be exemplified by the work of Dr Rosenberg during the development of a T cell medicinal product to treat malignant melanoma. The product is based on so called tumor-infiltrating lymphocytes (TIL) that are cultured *ex vivo* from tumor biopsies in high quantities of IL2 until a sufficient TIL population has expanded and can be reintroduced to the patient by intravenous administration. TIL therapy did not show sustained efficacy in patients before they realized that they had to precondition the patients with lymphodepleting agents such as cyclophosphamide and fludarabine a few days prior to the TIL infusion.[24] Later, full body irradiation was also added prior to TIL infusion. Because of this preconditioning treatment, long-lasting objective responses were seen in many patients.[25] Some patients even had a complete remission of their cancer that were still sustained many years later causing the now famous immunotherapy tail in survival curves.[26,27] The lymphodepleting strategy decreased the number of suppressive immune cells with focus on the Treg.[28] It is also believed that the preconditioning resulted in space for the developing immune response to expand and to a burst of cytokines that promotes TIL *in vivo* expansion and

function. The preconditioning concept was the key for success also for chimeric antigen receptor (CAR) T cell therapy. The early publications on CAR T cell trials demonstrated safety of gene engineering but modest responses in patients.[29] The first successful study demonstrated that second generation CAR T cells could induce complete responses in patients with B cell leukemia when infused after preconditioning.[30] Since then, the field of CAR T cells exploded. Multiple clinical trials have proven their remarkable capacity to eradicate even high tumor burden in patients but thus far mainly in B cell derived tumors. Today, CAR T cells are commercially available for B cell leukemia, lymphoma and multiple myeloma. Of note, all protocols use a preconditioning strategy. Nevertheless, it has also been noted that before failure to CAR T cell therapy, myeloid suppressive cells increased in patient blood.[31] Hence, it is appealing to consider a metronomic conditioning following the initial preconditioning step to keep regulatory cells at bay and perhaps favor a sustained effect.

In mice, preconditioning treatment has been directly compared to metronomic conditioning, but the results demonstrated that preconditioning is likely more beneficial than metronomic conditioning since the latter may interfere with immune activation.[32] Preconditioning led to better survival of infused T cells, better infiltration in different organs and a significantly better treatment outcome compared to either preconditioning or T cell therapy alone. Hence, for metronomic conditioning, dose and type of conditioning must be carefully selected. For solid malignancies, it may not be sufficient with a short pre-conditioning regiment for sustained effect. Further, as they already receive standard-of-care cancer therapy that may act on suppressive immune cells, it may be beneficial to combine immunotherapy with the standard-of-care regimen. At least if it is not toxic to T effector cells while acting on the myeloid cell population like gemcitabine or tyrosine kinase inhibitors (TKIs).

4.2 Gemcitabine

Gemcitabine is a nucleoside analog and therefore arrests cell proliferation. Interestingly, gemcitabine treatment has been shown in many studies to affect the immune profile of patients with a focus on the reduction of MDSCs. Myelosuppression is a well-known side effect of chemotherapies.[33] In our own studies, gemcitabine treatment of patients with pancreatic cancer could reduce granulocytic MDSCs already during the first treatment cycle. The effect does not seem lasting as the reduction was reversed during the cycle resting period.[34] In patients with non-small cell lung cancer, treatment with gemcitabine plus cisplatin significantly reduced serum TGF-β1 levels in patients who had a complete or partial response to chemotherapy.[35] TGF-β1 was significantly reduced in our treated pancreatic cancer patients as well[34]. The decrease in both MDSCs and TGF-β1 may be due to gemcitabine's ability to affect STAT3 which is an important signaling pathway in MDSC expansion and function.[21] Gemcitabine has been shown to regulate several genes under the control of STAT3.[36] We have previously shown that gemcitabine may prevent STAT3 phosphorylation in myeloid cells but in pancreatic cancer cell lines STAT3 phosphorylation was not affected by gemcitabine.[34] As reviewed elsewhere, PD-L1 expression is one of the mechanisms of T cell suppression mediated by MDSCs.[16] In pancreatic cancer, gemcitabine treatment did not affect PD-L1 expression. PD-L1 was instead increased subsequently to the first gemcitabine.[34] In general, gemcitabine is considered to induce myelosuppression rather than decreasing the lymphocyte population. Gemcitabine reduced Treg levels modestly

after two treatments of patients with pancreatic cancer, but the function of effector T cells was not affected.[34] Correspondingly, upon gemcitabine treatment, Plate et al. detected an increase in the $CD4^+$ T cell population and an enhanced T cell function in pancreatic cancer patients.[37] Further, T cell function was not impaired in pancreatic cancer patients treated with gemcitabine in combination with cisplatin.[38]

Hence, the body of data suggests that gemcitabine should be given as a metronomic rather than preconditioning strategy as the inhibiting effect on suppressive cells and molecules seem reversible even during a two-weeks treatment free period. Further, combinations with PD1/PDL1 checkpoint blockade may be of high interest.

4.3 Tyrosine kinase inhibitors

Tyrosine kinase inhibitors (TKI) are small molecules that block tyrosine kinase signaling which can affect multiple intracellular signaling pathways in both healthy and transformed cells. The TKI sunitinib was primarily used to treat renal cell carcinoma due to its capacity to block VEGF signaling but later, it was shown that sunitinib could hamper MDSCs as part of the mechanisms-of-action.[39] Another molecule affected by TKI inhibition is STAT-3.[40-42] STAT-3 signaling is driving MDSCs, PDL1 upregulation and fibrosis formation which makes it a very interesting target in cancer.[43-45] There are many studies published that show how TKIs can modulate the immune system both *in vitro* and *in vivo.*[46-51] For example, in patients with chronic myeloid leukemia (CML) continuous TKI treatment with dasatinib or imatinib leads to a decreased level of MDSCs, Arginase I, myeloperoxidase and IL10, while IL12 and activated lymphocytes including both experienced T cells and NK cells expanded.[52] Further, plasma proteomics revealed general tilt towards Th1 immunity and loss of angiogenic stimuli.[53] Recently, it was shown that the TKI imatinib can reduce Tregs as one of the mechanisms behind the positive immune effect using TKIs in cancer.[54] Interestingly, VEGF has a dual role in cancer promoting not only angiogenesis but also suppresses T cell infiltration into the tumor. In an animal study, sunitinib treatment resulted in increased presence of chemokines that attracts T cells (CXCL10 and CXCL11) with subsequent influx of infiltrating T cells.[55] TKIs are commonly well tolerated in patients as they can be administered for long periods of time in patients suffering from CML. Hence, TKI treatment during immunotherapy is an interesting combination option.

4. Concluding remarks

Immunotherapy has revolutionized cancer treatment climbing to first line option for many cancer indications. Nevertheless, there are several hurdles to overcome to increase the response rate and more importantly, to further increase survival of patients. As discussed herein, immunosuppression is a major factor determining the success of immunotherapy. As many conventional cancer therapeutics including chemotherapy and tyrosine kinase inhibitors exerts effect on immunosuppressive cells and their effector molecules, they should be considered as available options for immunotherapy combinations. Some trials are already exploring interesting combinations using chemotherapy as preconditioning or metronomic treatments, but randomized combination studies are warranted to fully understand the mechanism-of-action and combination capacity.

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