REPROGRAMMING IMMUNITY IN RENAL CELL CARCINOMA

RETHINKING TYROSINE KINASE INHIBITORS

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Angiogenesis, which is regulated by a fine balance between pro- and antiangiogenic signals, represent a key event in the development of tumors.

Immune dysfunction has been well documented in cancer patients, including those affected by renal cell carcinoma.

RCC patients a shift from a type-1 mediated CD4+ T cell response producing IFN gamma to a type-2 cytokine response involving IL.

Type-1 mediated CD4+ T cell response is critical for the development of effective antitumor immunity, while type-2 cytokine response tipically mediates humoral immunity.

• Antiangiogenic molecules can inhibit many immunosuppressive mechanisms, such as:
  
  ▪ **myeloid-derived suppressor cells (MDSC)**
  ▪ regulatory T cells (Treg)
  ▪ immunosuppressive cytokines
  ▪ and others.

• Besides, they play a crucial role in induce an efficient immunostimulatory response.

• In this respect, emerging evidence indicates that tyrosine TKIs modulate hematopoiesis and immune functions.

• The effect on myelopoiesis depends on their different selectivity for FLT3 and c-Kit receptors, expressed on hematopoietic stem cells and precursosr cells.

CANCER IMMUNE RESPONSE CYCLE

**STEP 1**
Cancer antigens

**STEP 2**
Dendritic cell

**STEP 3**
Blood vessel

**STEP 4**
Active cytotoxic T cell

**STEP 5**
Tumor microenvironment

**STEP 6**
Tumor cell

**STEP 7**
Tumor apoptosis
The cancer-immunity cycle and immunotherapy: targeting several rate-limiting steps in a dynamic equilibrium
Formation of neoantigens

Frequently

Regularly

Occasionally

Science. 2015;348(6230):69-74
FACTORS THAT INFLUENCE THE CANCER-IMMUNE SET POINT

Characterization of T cells and TAMs in the ccRCC TME Using Mass Cytometry
Experimental approach used in this study.

Chevrier et al. Cell 2017
**Characterization** of T cells and TAMs in the ccRCC TME Using Mass Cytometry:

- Markers used to characterize TAM phenotypes.
- Markers used to characterize T cell phenotypes.

Ag, antigen; DC, dendritic cells; pDC, plasmacytoid dendritic cells; NK, natural killer; R, receptor; SR, scavenger receptor; TCR, T cell receptor; TLR, toll-like receptor.

Chevrier et al. Cell 2017
Identification of the Main Immune Components of the ccRCC TME
• The immune system regulates angiogenesis in cancer by way of both pro- and antiangiogenic activities.

• A bidirectional link between angiogenesis and the immune system has been clearly demonstrated.

• Antiangiogenic molecules do not inhibit only VEGF signaling pathways but also other pathways which affect immune system.

• Understanding of the role of these pathways in the regulation of immunosuppressive mechanisms by way of specific inhibitors is growing.
• MDSC myeloid-derived suppressor cells
• IMC immature cells
• APC antigen presenting cells
• DCs dendritic cells
• Treg T regulatory cells
• **MDSCs are immature myeloid cells** that, in chronic inflammatory conditions, fail to eventually differentiate into granulocytes (G), macrophages (M) or dendritic cells (DCs).

• **MDSCs comprise a heterogeneous population** that can present widely distinct phenotypical characteristics, although they always exhibit remarkable immunosuppressive and tumorigenic activities.

• **MDSC tumorigenic activity** includes the secretion of factors promoting neoangiogenesis, the production of growth factors (GF), matrix metalloproteinases (MP) and cytokines that activate Th2 type and Treg cells.

THE ORIGIN OF MDSCs

Tumor micro-environment

HSC

Immature myeloid cells

Basophil
Eosinophil
Neutrophil

PMN MDSC

M-MDSC

Monocyte

Macrophage

DC

Suppressive DC

Antigen-specific T cell tolerance and nonspecific suppression

- Nonspecific suppression
- Support tumor angiogenesis and metastasis
THE ORIGIN OF MDSCs
In peripheral lymphoid organs, immune suppression by MDSC is mainly antigen-specific, contact-dependent and utilizes several major pathways, including the production of reactive nitrogen and oxygen species (NO, ROS and PNT), elimination of key nutrition factors for T cells from the microenvironment (L-arginine, L-tryptophan and L-cysteine), disruption of homing of T cells (through the expression of ADAM17), production of immunosuppressive cytokines (TGF-β, IL-10), and induction of T regulatory (Treg) cells. After migration to the tumor, MDSC are exposed to inflammatory and hypoxic tumor microenvironment. This results in significant HIF-1α-mediated elevation of Arg1 and iNOS and downregulation of ROS production, upregulation of inhibitory PD-L1 on MDSC surface, and production of CCL4 and CCL5 chemokines attracting Tregs to the tumor. Overall, these alterations result in more potent non-specific immunosuppressive activity of MDSCs inside the tumor.
Mon/M-MDSC are produced in the BM from hematopoietic progenitor cells and recruited to the tumor by several chemokines. Hypoxic conditions, including HIF1α, prevalent in the TME induces the downregulation of pSTAT3, which results in M-MDSC differentiation to TAM. Other factors known to induce TAM differentiation are activation of CD45 phosphatase, LIF, IL-6, and thrombin. In the spleen, Mon/M-MDSC have a high level of pSTAT3 due to the lack of hypoxic conditions. High STAT3 activity prevents differentiation of Mon/M-MDSC to TAM in the spleen.
Lactic acid produced by tumor cells and IL-4 produced by Th2 cells in the TME can drive the metabolism of TAM and TADC towards oxidative phosphorylation (oxphos) while inhibiting glycolysis. Lipids are known to have a role in negative regulation of TADC function. MDSC in peripheral tissue have decreased rates of oxphos and glycolysis compared to tumor-infiltrating MDSC (T-MDSC). Fatty acids derived from the TME drive the metabolism of T-MDSC towards fatty acid oxidation (FAO) and oxphos. Glycolytic rates are also increased in T-MDSC but how the TME influences this process, and the role AMPK plays in this process is unclear.
Schema of MDSC expansion and activation. Hematopoietic progenitor cells (HPC) proliferate, differentiate, and commit to various hematopoietic lineages including committed myeloid progenitors (CMP). Under conditions of myelosuppression or increased levels of growth factors (GF), significant increases in MDSCs occur in the peripheral blood (PB), spleen, and tumors. During expansion, MDSCs are mobilized into the circulation, lymphoid organs, and tumor microenvironments.
IMMUNE RECOVERY AFTER ANTI-ANGIOGENIC TKIs
TKIs depletes myeloid-derived suppressor cells and synergizes with a cancer vaccine to enhance antigen-specific immune responses and tumor eradication. Draghiciu et al Oncoimmunology 2015

- Effect of TKIs on intratumoral and intrasplenic levels of CD8+ T cells.
- Combined effect of immunization and TKIs on intratumoral and intrasplenic levels of total and E7-specific CD8+ T cells, MDSC and Treg.
- Intrinsic immunosuppressive activity of MDSCs after TKIs treatment.
- In vivo antitumor response of combinatorial treatment and effect on blood immune cells.
• Elevated MDSC in mRCC patients decline in response to TKIs

• Declines in MDSC are associated with decreases in IFN-\(\gamma\)-producing T cells after TKIs therapy

• In vitro depletion of patient MDSC partially restores patient T-cell production of IFN-\(\gamma\)

• In vitro effect of TKIs on MDSC-mediated T-cell suppression and MDSC viability and differentiation.

• Changes in patients MDSC and Treg in response to TKIs are directly associated.

• **TKIs reverses immune suppression** and decreases T-regulatory cells in RCC patients.
  Finke Clin Cancer Res 2008

• TKIs-induced myeloid lineage redistribution in RCC patients:CD1c dendritic cell frequency predicts progression-free survival.
  Van Cruijsen Clin Cancer Res 2008

• The novel role of TKI in the reversal of immune suppression and modulation of tumor microenvironment for immune-based cancer therapies.
  Ozao-Choy Cancer Res 2009

• **TKIs inhibiton of STAT2 induces RCC tumor cell accumulation in renal cell patients carcinoma.**
  Xin Cancer Res 2009

• **TKIs mediates reversal of myeloid-derived suppressor cell accumulation in renal cell patients carcinoma.**
  Ko Clin Cancer Res 2009

• **Anti-angiogenic therapy increases PD-L1 expression in human RCC.**
  Liu Cancer Immunol Res 2015
ANTIANGIOGENIC THERAPY INCREASES PD-L1 EXPRESSION IN HUMAN RCC.

Human placenta tissues and TMAs from untreated RCC controls or RCCs treated with sunitinib or bevacizumab were IHC stained with anti–PD-L1 antibody.

SHIFTING THE BALANCE TOWARD ANTI-CANCER IMMUNITY WITH COMBINED VEGF/PD-L1 BLOCKADE

PD-L1, programmed death-ligand 1; VEGF, vascular endothelial growth factor.
TRANSCRIPTOME MAP OF ANGIOGENESIS AND IMMUNE-ASSOCIATED GENES IN RCC TUMORS

Angiogenesis
(e.g., CD34, KDR, VEGFA)

Immune, Antigen Presentation
(e.g., CD8A, IFNG, PSMB8)

Myeloid Inflammation
(e.g., IL6, PTGS2, IL8)

PD-L1 IHC

TRANSCRIPTOME MAP OF ANGIOGENESIS AND IMMUNE-ASSOCIATED GENES IN RCC TUMORS

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MOLECULAR CORRELATES OF DIFFERENTIAL RESPONSE TO IMMUNOTHERAPY ± ANTIANGIOGENIC THERAPY

TKIs

IMMUNOTHERAPY

TKIs + IMMUNOTHERAPY

McDermott D, et al. IMmotion150 biomarkers: AACR 2017
• TKIs *mediates reversal of MDSC accumulation* in RCC patients and thereby restores patient T cell function.

• TKIs has a *toxic, rather than DC-differentiating effect* on RCC patients MDSC in vitro, which may account for its partial inhibition of MDSC suppressive effect in vitro.

• TKIs-mediated MDSC declines in RCC patients were not correlated with changes in tumour volume.
• Similar to the human studies, TKIs treatment reduces MDSC levels and restored T cell response in several mouse tumour models.

• TKIs inhibits the pathological expansion in the spleen of proliferative M-MDSC.

• TKIs has apoptotic, rather than DC-differentiating effect on M-MDSC.

• TKIs-mediated MDSC decline may not be attributed to single target.
• The TKIs not only inhibit angiogenesis and tumor growth, but also have the potential of interacting with the function of the immune system

• Presently available data seem to suggest that may exert immune in the vast majority—but not all—the studies reported

• Trials of combination of these TKIs with different types of immune manipulation should be rationally designed taking into account all these complex effects, which ultimately deserve further insights