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INVEST IN CANCER RESEARCH WORLDWIDE

SWISS BRIDGE Award for Cancer Research 2014
Summaries of the two supported research projects

Professor Laurence Zitvogel, MD, PhD, Gustave Roussy Comprehensive Cancer Centre, France, receives 250 000 Swiss francs for the project entitled:

Impact of gut microbiota in the efficacy and toxicity of immune checkpoint blockers in oncology

Summary

Monoclonal antibodies targeting immune checkpoint blockers (CTLA4, PD-1) have come of age in the treatment of metastatic melanoma, advanced lung, renal cancers and other hematological and solid malignancies. However, the immune-related side effects and the costs of these therapies prompt the search for their precise mode of action and for biomarker discovery.

We recently highlighted the crucial role of gut microbiota in modulating the efficacy and/or toxicity of distinct anticancer agents. Hence, we reported that chemotherapeutic agents, by compromising, to some extent, the intestinal integrity, facilitate the gut permeability and selective translocation of distinct species of bacteria in secondary lymphoid organs. There, anti-commensal pathogenic TH17 cell responses are primed, facilitating the accumulation of TH1 helper cells in tumor beds post-chemotherapy as well as tumor regression. Importantly, the redox equilibrium of myeloid cells contained in the tumor microenvironment is also influenced by the intestinal microflora, contributing to tumor responses. Hence, the anticancer efficacy of alkylating agents (such as cyclophosphamide (CTX)) and platinum salts (oxaliplatine, cis-platine) is compromised in germ-free mice or animals treated with broad spectrum or vancomycine antibiotics.
The cytotoxic T lymphocyte antigen-4 (CTLA-4)-blocking antibody ipilimumab is a FDA and EMEA-approved treatment for metastatic melanoma despite frequent grade II-III colitis. To uncouple efficacy and toxicity, we analysed the changes and role of gut microbiota during CTLA4 blockade. Here, we show that a broad spectrum antibiotic cocktail (ATBx), imipenem and colistine severely compromised the efficacy of CTLA4 blockade while vancomycine ameliorated it. A loss of efficacy of CTLA4 blockade was also observed in germ-free mice. ATBx or germ-free environment both reduced splenic and intratumoral IFNγproducing $CD8^+$ T cell proliferation and differentiation, impaired the upregulation of ICOS on splenic T cells, both hallmarks of anti-CTLA4 blockade. In parallel, anti-CTLA4 mAb induced a loss of epithelial barrier integrity, increased goblet cell numbers and the production of the antimicrobial peptide lipocalin-2 as well as upregulated RORγt and IFNγ in colonic lamina propria-residing T cells. Efficacy and toxicity of CTLA4 blockade were both amplified by anti-IL-10 Ab or in IL-10 deficient mice while high levels of lipocalin-2 correlated with complete responses to CTLA4 blockade. Pyrosequencing analyses of 16SrRNA of feces nucleic acids as well as culture of feces in specific conditions revealed a dramatic enrichment of *Bacteroidales* and more specifically of *B.distasonis* and *B.thetaiotaomicron*, and to a lesser extent of *E. Coli*. We conclude from these unpublished data that i) gut microbiota (more specifically Gram negative species) is involved in the clinical efficacy and toxicity of CTLA4 blockade, ii) *Bacteroidales* and E*. Coli* may be the actors of such clinical outcomes. To uncouple efficacy from toxicity, we now hypothesize, based on the litterature, that zwitterionic polysaccharides (harbored by *Bacteroidales*) could mediate the immunogenicity and adjuvanticity of ipilimumab while *E. coli* could drive the graft-versus-host disease-like colitogenic effects.

This program project presented to the Swiss foundation consists in **four workpackages**:

WP 1. Demonstrate the cause-effect relationship between distinct Gram negative bacterial species (identified in pyrosequencing and culture) and the antitumor effects and/or toxicity (colitis) of anti-CTLA4 Ab (mono-association of germ free or ATBx-treated mice with *E. Coli* and/or *Bacteroidales*)
WP 2. Investigate the role of TLR4, TLR2 or NOD1-2 or IL-10 in the efficacy and/or toxicity of CTLA4 blockade as well as the regulation of CTLA4 expression in the gut according to TLR or NOD signaling
WP. 3. Molecular bases of the immunogenicity of Bacteroidales and therapeutic exploitation (vaccines and T cell transfer based on Bacteroidales-derived zwitterionic polysaccharides)
WP 4. Translational research: validate the relevance of such findings (immunogenicity of *Bacteroidales*- derived zwitterionic polysaccharides and colitogenic effects of *E. Coli)* in melanoma patients treated with Ipilimumab and experiencing clinical benefit and/or colitis (investigational protocol at Gustave Roussy, IRB approval by Gustave Roussy 2011).

Professor Adrian Ochsenbein, MD, Department of Medical Oncology, Inselspital, University Hospital and University of Berne, Switzerland, receives 250 000 Swiss francs for the project entitled:

Targeting TNF receptor TNIK signaling to eliminate cancer stem cells

Summary

The concept that cancer develops in a hierarchical tree from disease-originating cancer stem cells (CSCs) that self-renew and give rise to more differentiated, non-cancer-initiating cells by asymmetric division was first documented in leukemia and is now widely accepted for several solid tumors. From a clinical point of view, CSCs are of fundamental interest since they are resistant against most of our current cancer treatments such as irradiation and chemotherapy and probably also against more targeted therapies such as tyrosine kinase inhibitors (TKIs) and immunotherapy. Resistance of CSCs to treatment is mediated by cell intrinsic characteristics such as expression of drug efflux proteins but also by the interactions of CSCs with their microenvironment. This is best documented for leukemia stem cells (LSCs) that depend on signals from their surrounding niche to maintain stem cell characteristics such as quiescence and self-renewal. The immune system is an important part of the tumor microenvironment in solid tumors and leukemia and may contribute to tumor control in some situations. However, it is well documented that especially cells of the innate immune system contribute to tumor progression.
Over the last years, our lab investigated the mechanisms by which the adaptive immune system, especially T cells, contributes to the progression of solid tumors and leukemia. However, the molecular interactions of the immune system with CSCs remain ill-defined. We recently documented that signaling via CD27, a tumor necrosis factor receptor (TNFR) superfamily member, induces expansion of chronic myeloid leukemia (CML) stem cells by activating the Wnt pathway via TNFR-associated factor 2 (TRAF2) and TRAF2- and NCK-interacting kinase (TNIK). The Wnt pathway is crucially involved in leukemogenesis of CML and acute myeloid leukemia (AML) and also in tumorigenesis of different solid tumors including colon cancer. Importantly, Wnt pathway activation is a hallmark of CSCs and is necessary to maintain several important stem cell characteristics. This leads to the possibility that immune cells expressing TNFR ligands, such as the CD27 ligand CD70, expand CSCs by signaling via the TNFR/TRAF2/TNIK/Wnt pathway. A central tool to analyze the TNFR/TRAF2/TNIK/Wnt-pathway in CSCs will be the conditional TNIK loss-of-function mouse ($TNIK^{fl/fl}$) crossed to an taxomifen-inducible Cre background ($Cre\text{-}ER^{TM}$:$TNIK^{fl/fl}$). This mouse was recently established in our lab and is now crossed on a tamoxifen inducible ubi-Cre background. Built on our

previous work and the new (Cre-ER^{TM}:TNIK$^{fl/fl}$) mouse we will analyze the following defined questions:

Aim 1: TNFR/TNIK/Wnt signaling in LSCs: We will address if CD27/TRAF2/TNIK/Wnt signaling influences LSCs in retrovirally induced AML in mice. The use of our inducible (Cre-ER^{TM}:TNIK$^{fl/fl}$). mouse model will allow to deplete TNIK at different stages of the disease and to analyze the effect on LSCs. In addition, we built up an AML biobank that currently contains frozen peripheral blood mononuclear cells (PBMCs) from approximately 100 patients at diagnosis that will be used to analyze TRAF2/TNIK/Wnt signaling in human AML stem cells. We will then analyze if other members of the TNFR family such as the lymphotoxin β receptor (LTβR) and glucocorticoid-induced TNFR family related protein (GITR) similarly expand AML stem cells via TNIK.

Aim 2: TNFR/TNIK/Wnt signaling in colorectal CSCs: We will cross APC^{min} x Kras mice to (Cre-ER^{TM}:TNIK$^{fl/fl}$) mice and delete TNIK before and after development of colorectal tumors. Tumor formation and survival will be analyzed. TNIK-proficient and TNIK-deficient CSCs will be compared in serial spheroid forming assays *in vitro* and serial re-transplantation experiments *in vivo*. The expression of TRAF2-binding TNFRs will be stained and the functional relevance will be analyzed using blocking antibodies in spheroid formation assays. In addition, the immune cell subsets expressing TNFR-ligands will be explored.

Together, these experiments will help to investigate the possibility of manipulating the TRAF2/TNIK/Wnt signaling pathway in order to target CSCs.