

How to escape the immune response: what tumours have to teach to transplant immunologists?

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LIST OF ABBREVIATIONS

CAR, Chimeric Antigen Receptor

Tregs, Regulatory T Cell

NKT, Natural Killer T cell

TAAAs, Tumor Associated Antigens

DC, Dendritic Cell

mAbs, monoclonal Antibodies

BiTEs, Bi-specific T Cell Engagers

PTLD, Posttransplant Lymphoproliferative Disorder

NMSC, Non-Melanoma Skin Cancer

SIR, Standardized Incidence Ratio

SMR, Standardized Mortality Ratio

ABSTRACT

Recent progress in deciphering the mechanisms underlying the concepts of tumor immunosurveillance and immunoevasion has opened new opportunities for the development of effective anti-tumor therapies. Transplant physicians and immunologists have much to learn from those direct clinical translations of basic science. The 2016 Beaune Seminar in Transplant research brought together researchers from both fields to explore and discuss significant advances in cancer biology, immunotherapies and their potential impacts for the management of cancer in transplant recipients.

Oncology is a field of medical research that has been particularly productive over the last decade, from both a basic science and a clinical standpoint. From the perspective of transplant immunologists and physicians, oncology should certainly merit special attention. Besides the fact that cancer is still a frequent and devastating complication of most immunosuppressive drugs used in transplantation, the main reason why the transplant community could benefit from further interaction with oncologists is because of the extraordinarily successful development of many anti-tumor immunotherapies. These are based on the principle of reactivating the immune system to eliminate the tumor and target so-called checkpoint inhibitors. More generally, similarities between the concepts of immune evasion and immune tolerance are obvious (figure 1) and should stimulate dialogue between researchers in both fields.

With those considerations as a backdrop, the 2016 Beaune Seminar in Transplant Research (www.beaune-seminar.fr) brought together oncologists, transplant physicians and immunologists from both fields, to explore recent and significant advances in the understanding of cancer biology, the development of innovative therapies and the management of cancer in transplant recipients.

1. CANCER BIOLOGY

Cancer immunoediting: from surveillance to evasion

While innate and adaptive immunities are known to work in concert to eliminate or keep in check tumor cells, they can also be involved in tumor progression. This Janus-like action of the immune system is referred to as cancer immunoediting and this concept was brought to the attention of the Beaune audience by Christophe Caux from Lyon, France. Operational immunosurveillance results from the interplay, at the

tumor site, between various innate and adaptative immune cells types, soluble cytokines and chemokines that together create a sophisticated immunologic microenvironment. The ability of this so-called “immune contexture” in controlling tumor progression depends critically upon the type (T lymphocytes, NKT cells, B cells, macrophages, dendritic cells, etc.), cell density, the functional polarization (Th1, Th2, Th17, etc.) and localization of immune cells within the regions of the tumor (core vs margin). Tumor associated antigens (TAAs) recognized by T cells act as a driving force and not surprisingly CD8⁺ T cells have been shown to be a key component of a fully effective anti-tumor immune response. Additionally, tertiary lymphoid structures that develop within the tumor microenvironment, similar to those that develop in other situations of chronic stimulation of the immune system, are suggested to amplify anti-tumor reactivity locally. Immunosurveillance fails when the immune system is no longer able to contain tumors growth. This process referred to as tumor evasion remains however intimately modulated by the immune response. Tumor cells pervert immunosurveillance mechanisms in order to resist, avoid and suppress anti-tumor immune response. Besides the increased expression of oncogenes that confers resistance to cytotoxic effector cells, tumors escape by impairing TAAs recognition and subsequent T cells stimulation and by literally hijacking physiological immune regulatory responses, thereby shutting down the productive anti-tumor response (figure 1).

Clinical translation: The Immunoscore

Given the importance of the host immune response in controlling cancer progression, immunological characteristics of the tumor microenvironment have naturally been seen as potential tool to predict tumor and patient outcome. The ‘immunoscore’ is a

scoring system based on the study of the immune microenvironment that ultimately aims at stratifying the risk of tumor progression. By enumerating CD3+ and CD8+ subpopulations in colorectal cancer, the Immunoscore proved to be superior than conventional staging classification systems^{1,2}. Additionally, other cell types (M2 macrophages, PD-L1+ cells, etc.) together with other phenotypes (Th1, effector memory T cells, etc.) have been associated with clinical outcome. Interestingly, these immunological biomarkers could also help with selecting the best therapeutic option according to tumor phenotype (eg anti-PD1 immunotherapy for PD-L1 expressing tumor)³⁻⁵.

2. IMMUNOTHERAPY IN ONCOLOGY

Recent progress in deciphering the mechanisms underlying tumor immunoevasion has opened new opportunities for the development of ingenious anti-tumor immunotherapies. François Ghiringhelli (Dijon, France) and Angus Thompson (Pittsburgh, USA) reviewed the expanding spectrum of those innovative therapeutic strategies (figure 2).

Immunotherapy: an overview

Active immunotherapies are procedures that either directly activate or restimulate the host immune system in order to enable it to mount or reinstate an effective tumor-specific immune response

Therapeutic cancer vaccines based either on the direct administration of TAAs or through the use of Dendritic Cells (DC) of various types (usually ex vivo manipulated DC loaded with TAAs) have been and are still being evaluated in clinical trials. Those approaches have so far turned out to be less effective than initially anticipated. This may be due to a variable expression of TAAs by the tumor over time as well as to the neutralization of vaccine-induced T cells at the tumor site by the mechanisms alluded to above. In this regard, blockade of T cell checkpoints inhibitors has emerged as the most promising active immunotherapy with currently different monoclonal Antibodies (mAbs) targeting CTLA4 and the PD pathway (PD1 and PD-L1). Interestingly, while anti-CTLA4 therapy acts in the periphery of tumor by both strengthening CD8⁺ T cell activation and harnessing the suppressive function of Treg, anti-PD therapy may exert a more intra-tumor-specific action. Indeed, most normal human tissues do not express PD-L1. In contrast, PD-L1 is abundantly expressed on the tumor cell surface (either constitutionally or induced by INF γ) and constitutes a “molecular shield” that disarms *in situ* anti-tumor T cells³. Thus, anti-PD therapy has the potential to directly restore tumor immunogenicity. T cell checkpoint inhibitors blocking immunotherapies have been approved for the treatment of melanoma and are being actively evaluated in other cancers (non-small cell lung cancer, renal cell carcinoma, etc.).

Besides active immunotherapy, strategies using agents with intrinsic anti-tumor activity have also been developed (passive immunotherapy, figure 2). CAR (chimeric antigen receptor) are synthetic receptors transduced on T cells. The extracellular domain comprises a single-chain variable fragment (scVF) of an antibody specific for a tumor antigen or a tumor cell type. The intracellular domain contains signaling molecules (ζ -chains of the CD3 complex) and a co-stimulatory receptor (eg CD28). Thus, CAR technology permits turning, ex vivo, T cells of any specificity into tumor-

specific cells that are secondarily adoptively transferred to the patients. Anti-CD19 CARs have shown impressive results in patients with refractory B cell malignancies⁶. Bi-specific T Cell Engagers (BiTEs) are fusion protein linking scFV of 2 different antibodies specific on 1 side for CD3 and on the other side for a TAA. They act by recruiting and redirecting bystander T cells toward an anti-tumor response. Similar to CAR-T cells, BiTES have been evaluated with some success in B cell malignancy.

Determinants of response to immunotherapy

Phenotype of the immune tumor microenvironment can help predict the tumor response to immunotherapy. Stratification of the tumor microenvironment according to the expression of PD1 on tumor infiltrating lymphocytes and PD-L1 expression on tumor cells is predictive of the response to anti-PD1⁴. Similarly, study of T cell clonality analyzed in tumor biopsies might be informative. Restricted TCR repertoire of CD8+ T cells, that might be suggestive of an enriched tumor-specific T cell population, has been shown to correlate with sensitivity to anti-PD1 in patients with melanoma⁵. This suggests that patients responding to anti-PD1 immunotherapy are those who have already mounted a specific anti-tumor T cell response that is locally neutralized through the PD1-PDL-1 interaction.

The nature of gut microbiota may also influence the response to immunotherapy. Bertrand Routy (Paris, France) reported data on the loss of efficacy of CTLA4 blockade in “sterilized-gut” mice. In this model, gavage with gut bacteria (*Bacteroides fragilis*) as well as adoptive transfer of *B. fragilis* specific T cells restored therapeutic activity of CTLA4 blockade, suggesting T cell cross reactivity between tumor-associated and microbial antigens. Alternatively, in a model of fecal

microbial transplantation, feces collected from patients with metastatic melanoma were transplanted to germ-free tumor-bearing mice. Those mice were then treated with anti-CTLA4 blocking Ab. Only mice that were transplanted with feces enriched in *Bacteroides* species showed sensitivity to CTLA4 blockade. Similar evidences have been recently reported regarding anti PD-L1 efficacy and commensal bacteria (*Bifidobacterium*)⁷.

3. CANCER AND TRANSPLANTATION

The burden of cancer in organ transplantation

Overall, the standardized incidence ratio for all tumors in transplant patients is 4.3, with the highest relative risk for Posttransplant Lymphoproliferative Disorder (PTLD), Kaposi sarcoma, renal carcinoma and nonmelanoma skin cancer (NMSC). Specific mechanisms can promote carcinogenesis in transplantation. As for lymphoproliferative disease, Laurent Genestier (Lyon, France) reviewed the classical EBV-induced B cell transformation that operates in the majority of transplant patients with PTLD and discussed alternative indirect transformation mechanisms involving chronic infection with non B cell invading pathogens (eg *Helicobacter Pylori*) or chronic stimulation of B cells by allo-antigens. Indirect transformation is not a specific feature of B cell malignancies since Dr Genestier's group recently shown that NKT cell lymphoma could be generated in mice chronically challenged with bacterial antigens. More generally, the negative impact of immunosuppressive drugs is largely accepted. However, as reminded by Paul Harden (Oxford, UK), the influence of immunosuppression has been clearly documented principally for NMSC. Many cohort studies have reported an increased incidence of NMSC with immunosuppression exposure, but definitive evidence of the causal relationship between

immunosuppressive load and NMSC came from an interventional trial unambiguously demonstrating the beneficial impact of reducing immunosuppression⁸.

The nature of immunosuppression matters as well. In this respect, mTOR inhibitors have attracted great interest from the transplant community and have been suggested to reduce the risk of NMSC recurrence^{9,10}. The impact of mTOR inhibitors in transplant patients with tumors other than NMSC is more controversial. Guillaume Canaud (Paris, France) underlined the fact that clinical use of mTOR inhibitors could be optimized. For instance, genome sequencing approaches have determined specific gene mutation associated with mTOR sensitivity or resistance and thus could identify patients most likely to respond¹¹.

Screening the transplant patient for cancer

Cancer screening in the general population follows guidelines based on a strong level of evidence and that are regularly updated. While the increased-risk for cancer is well documented for transplant patients, screening policies are less formalized. Jacques Dantal (Nantes, France) proposed an integrative approach taking into account both the standardized incidence ratio (SIR) and standardized mortality ratio (SMR) derived from different transplant registries¹². This approach allows identification of moderate and high-risk patients that would need more intensive screening. For instance, as for colorectal cancer, guidelines in the general population are not adapted to the high SIR and SMR reported for transplant patients. Systematic colonoscopy in transplant recipients after the age of 50 could be discussed. On the other hand, no increased SIR and SMR have been reported for prostate cancer in transplant patients. Using the same recommendations as for the general population screening should be followed in

this case. The screening strategy described by Dr Dantal is mainly based on epidemiological data and as such needs to be discussed and adjusted on a case-by-case basis.

Other tools have been suggested to improve the screening of transplant patients: Immune profiling of different circulating cell subtypes (CD4⁺ cells, Tregs, NK cells, $\gamma\delta$ T cells), longitudinal monitoring of EBV or Teno Torque viral load (as a global marker for over-immunosuppression), identification of genetic factors conferring increased susceptibility to cancer. Along the same lines, Paul Harden (Oxford, UK) reported the Oxford experience with the monitoring of CD57⁺ cells. CD57 is a marker of immune senescence and can be used to measure functional immune deficiency. In a study performed at the Oxford Transplant Centre, patients with the highest proportion of circulating CD57^{hi}CD8⁺ cells were found to be at higher risk for developing squamous cell carcinoma¹³.

However, albeit promising, none of these screening strategies has demonstrated clinical benefits as yet.

CONCLUDING REMARKS – What tumors have to teach to transplant physicians/immunologists?

By developing clinical strategies that integrate genetic and proteomic tumor profiling, Oncology has paved the way for precision medicine. The ultimate goal of precision medicine is to personalize patient's treatment based on a panel of informative biomarkers. For instance, overexpression of certain neo- antigens helps in shaping the immunogenicity of melanoma tumors and can be used as a genetic signature to select

patients who are the most likely to respond to immune checkpoints blockade. Similar initiatives of precision medicine are being developed in the field of transplantation such as the development of molecular classifiers^{14,15} or others integrative omics approaches (eg the BIOMARGIN study- NCT02832661-). While those initiatives are substantially improving our understanding of specific processes involved in graft injury, they have not translated into therapeutic personalization yet. Oncology achievement in that matter reminds us that the journey is not the destination, and should stimulate us to advance the field of personalized medicine in transplantation.

The closing lecture of the 16th Beaune Seminar was given by Alberto Sanchez-Fueyo (London, UK) who reflected on the evident parallel between immune evasion and immune tolerance as illustrated in figure 1. Cancer successfully evades the immune system by establishing a network of immunosuppressive mechanisms acting both remotely at the site of initiation of the response (in the secondary lymphoid organs) and locally within the tumour. While accumulating evidence suggests that the allogeneic response is - at least partly - regulated within the graft itself, current models employed to understand transplantation tolerance in humans do not take sufficiently into account the role of the allograft. In this respect, extensive evaluation of biopsy specimens obtained from the so-called operationally tolerant liver and kidney recipients is likely to be the most direct way to significantly advance the field of clinical transplant tolerance. This opportunity has been so far unfairly overlooked.

In addition, given, on the one hand, the importance of the tumor microenvironment in shaping immunoediting and, on the other hand, the high prevalence of cancer in transplant patients, there is a need to better understand the effect of immunosuppressive drugs and regimens upon the mechanisms governing tumor immune surveillance.

Finally, several aspects of anti-tumor immunotherapy are important to consider for the transplant physician. Most molecules currently used in the clinics aim at strengthening the cytolytic effector arm of the immune response and are, in this respect, potentially deleterious for transplant recipients. Cases of serious immune-related adverse effects, including severe acute graft rejection, are being reported in the literature with the use of checkpoint inhibitors blocking agents^{16,17}. This should, however, not preclude the transplant physician from exploring the potential of certain promising approaches developed in Oncology especially those based on genetically-engineered T cells. For instance, CAR technology has been recently advantageously exported in the transplantation field for the generation of HLA- allo-specific Tregs¹⁸. While this is certainly true for most organ transplant recipients, those anti-tumor immunotherapies might be helpful in the context of allogeneic hematopoietic stem cell transplantation. This was recently suggested by the use of ipilimumab, a blocking anti-CTLA4 mAb, that proved to restore antitumor reactivity (graft versus tumor effect) in patients with relapsing hematologic cancer¹⁹.

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FIGURES LEGENDS

Figure 1. Analogy between main pathways of tumor escape and common mechanisms of immune tolerance.

Tumor escape is based on (A-) impairment of immune recognition and stimulation and (B-) the establishment of an immunosuppressive microenvironment. These mechanisms are common with the classical mechanisms of immune tolerance: IGNORANCE, ANERGY and REGULATION.

DC, Dendritic Cells; MHC, Major Histocompatibility Complex; TCR, T Cell receptor; T Reg, Regulatory T Cell ; TAA, Tumor Associated antigens.

Figure 2. Different types of Cancer Immunotherapy.

DC, Dendritic Cells; MHC, Major Histocompatibility Complex; TCR, T Cell receptor ; T Reg, Regulatory T Cell ; Ab, Antibody; mAb, monoclonal Antibody;; CAR T Cell, Chimeric Antigen Receptor T-Cell.

Figure 1

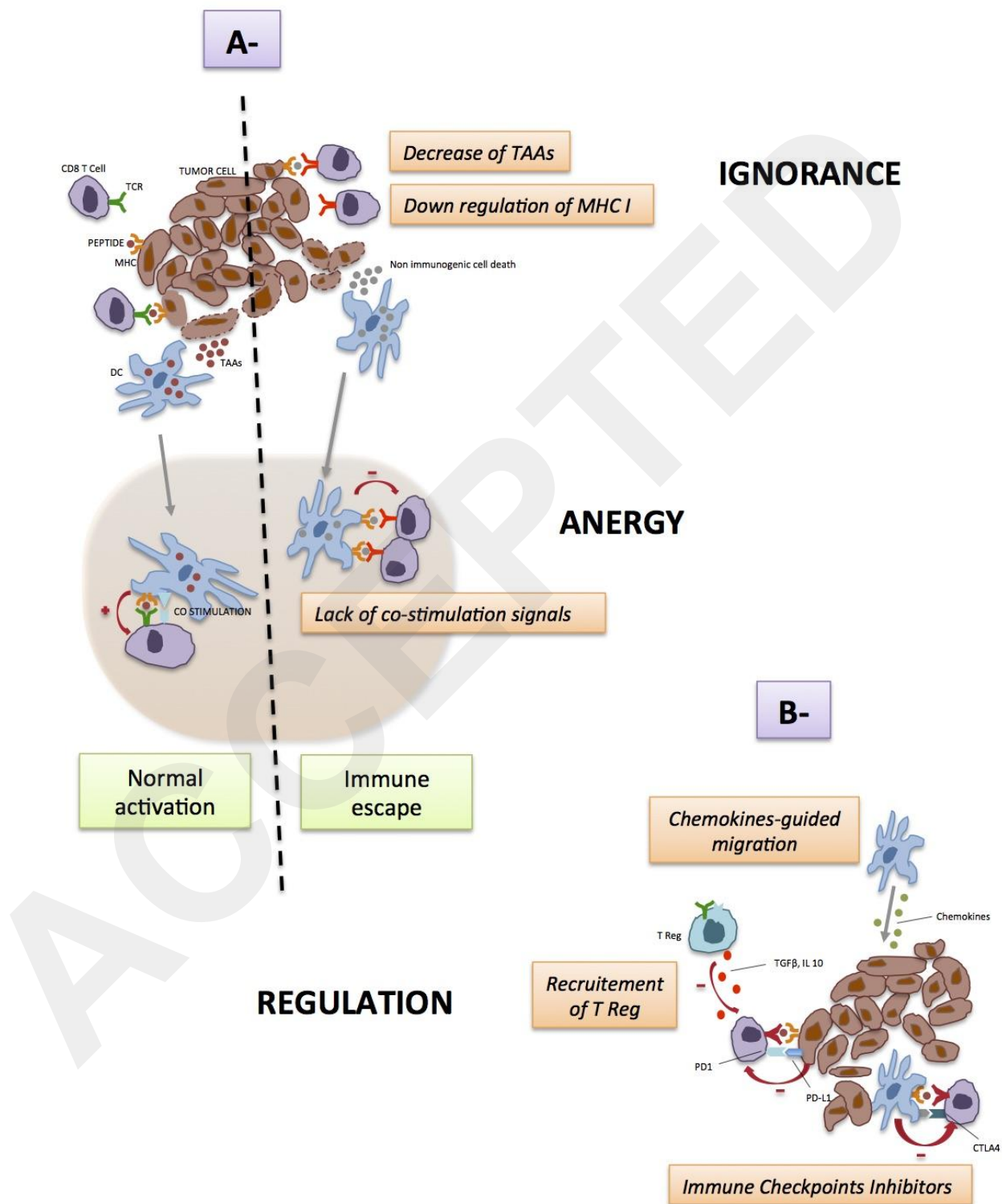
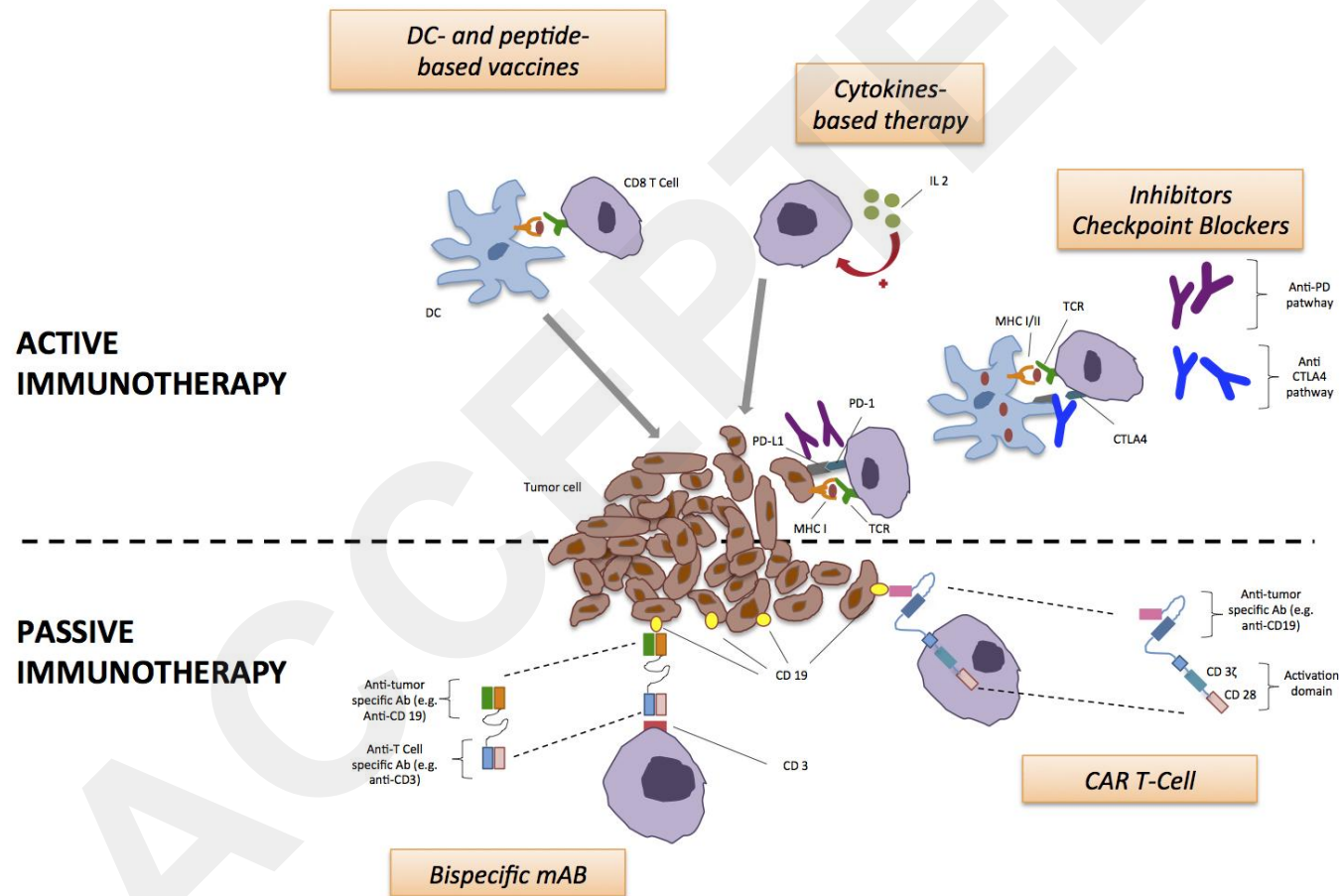


Figure 2



Adapted from François Ghiringhelli