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Prédiction de la réponse aux inhibiteurs de points de contrôle immunitaire dans le traitement des tumeurs solides : une revue systématique de cinq biomarqueurs sanguins émergents

Quentin Mangon

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UNIVERSITÉ CLERMONT AUVERGNE
UFR DE MÉDECINE ET DES PROFESSIONS PARAMÉDICALES

THÈSE D'EXERCICE
pour le
DIPLOME D'ÉTAT DE DOCTEUR EN MÉDECINE
par

MANGON, Quentin Dimitri

Présentée et soutenue publiquement le Mercredi 20 Octobre 2021

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LISTE DES ABREVIATIONS

b-TMB : Blood Tumor Mutational Burden
B2M : Beta-2-Microglobulin
CCRCC : Clear Cell Renal Cell Carcinoma
CRC : Colorectal Cancer
ctDNA : circulating tumor DNA
DFS : Disease-Free Survival
ELISA : Enzyme-Linked Immunosorbent Assay
HBV : Hepatitis B Virus
HCC : Hepatocellular Carcinoma
HLA : Human Leukocyte Antigen
HR : Hazard Ratio
ICI : Immune Checkpoint Inhibitors
KIR : Killer Immunoglobulin-like Receptor
LOH : Loss Of Heterozygosity
MMRd : Mismatch Repair deficient
MSI : Microsatellite Instability
Mut/Mb : Mutation Per Megabase
Ng/mL : Nanogram Per Milliliter
NGS : Next Generation Sequencing
NK : Natural Killer
NSCLC : Non Small Cell Lung Cancer
OS : Overall Survival
ORR : Objective Response Rate
PD-1 : Programmed Cell Death Protein-1
PD-L1 : Programmed Death-Ligand 1
PD-L2 : Programmed Death-Ligand 2
PFS : Progression Free Survival
RFS : Recurrence Free Survival
sPD-L1 : soluble Programmed Cell Death Protein-1
TIL : Tumor Infiltrating Lymphocyte
TMB : Tumor Mutational Burden
TPS : Tumor Proportion Score
t-TMB: tissue Tumor Mutational Burden
TTF : Time to Treatment Failure
WES : Whole Exome Sequencing

INTRODUCTION

Les cancers sont la deuxième cause de mortalité dans le monde, derrière les maladies cardiovasculaires, avec environ 1 décès prématuré sur 6 (1). En France, ils représentent même la première cause de mortalité depuis 2004, malgré un taux de mortalité en constante diminution depuis 25 ans, favorisée par une majoration de l'incidence chaque année depuis 30 ans (2).
Devant ce problème majeur de santé publique, de nombreux progrès et découvertes ont pu être réalisés, permettant une amélioration de la prise en charge, tant au niveau du diagnostic et du dépistage qu'au niveau thérapeutique.

Les immunothérapies anti-cancéreuses sont une des avancées majeures sur le plan thérapeutique. Basées sur un principe de réactivation du système immunitaire contre les cellules tumorales, contrairement aux chimiothérapies conventionnelles qui favorisent l'apoptose cellulaire, elles ont permis de révolutionner le pronostic de nombreux cancers, notamment pulmonaires, urinaires et cutanés, avec très souvent une bien meilleure tolérance et une durée d'efficacité allongée comparativement aux chimiothérapies.

La classe thérapeutique la plus utilisée parmi les immunothérapies anti-cancéreuses est celle des inhibiteurs de points de contrôle immunitaire (ICI, *immune checkpoint inhibitors*). Les points de contrôle immunitaire sont des récepteurs membranaires présents à la surface des cellules immunitaires, qui participent physiologiquement à la modulation de leur activation afin de limiter l'intensité et la durée de la réaction immunitaire. Afin d'échapper à la surveillance immunitaire antitumorale, les cellules tumorales peuvent exprimer (et/ou sécréter) des ligands de ces récepteurs (CTLA4, PD-L1 entre autres). Les ICI vont alors cibler ces différents points de contrôle afin de lever le frein immunitaire engendré par la tumeur.

Malgré l'efficacité globale des ICI, une proportion non négligeable de patients ne répondra pas au traitement. De plus, leur délai d'action est souvent plus tardif que les chimiothérapies, avec parfois un délai nécessaire de 2 à 3 mois de traitement avant d'obtenir une efficacité sur la maladie, rendant difficile leur utilisation chez des patients avec une maladie très symptomatique ou rapidement évolutive. Dans ce contexte, il est indispensable d'identifier des biomarqueurs, facilement dosables, qui pourraient prédire la réponse aux ICI, dans le but de sélectionner les patients ayant le plus de probabilité de répondre favorablement.

L'objectif de ce travail de thèse était d'identifier dans la littérature récente de potentiels biomarqueurs sanguins prédictifs d'une réponse favorable aux ICI dans les tumeurs solides, du fait de leur rôle clé dans la réponse immunitaire antitumorale. Nous avons ainsi identifié cinq biomarqueurs intéressants et avons rédigé un article de synthèse de la littérature dont une première version constitue la première partie de ce manuscrit. A partir des données disponibles, nous avons ensuite initié un travail de méta-analyse sur les trois biomarqueurs les plus étudiés. Nous avons contacté un certain nombre d'auteurs afin d'obtenir des données manquantes, et étant à ce jour dans l'attente de leur réponse, nous présentons donc un travail statistique préliminaire dans la deuxième partie du manuscrit. Si l'analyse statistique finale est satisfaisante, elle sera ainsi intégrée à la revue de la littérature lors de sa soumission. Enfin, nous présentons dans la dernière partie du manuscrit un projet de protocole de recherche clinique visant à évaluer de manière prospective et concomitante les cinq biomarqueurs identifiés, dans le but d'établir un potentiel score composite pronostique et prédictif de la réponse aux ICI dans les tumeurs solides.

1. REVUE DE LA LITTÉRATURE

1.1 Introduction

Despite improvement in diagnosis and treatment, cancers remain the second cause of mortality in the world. Indeed, cancer kills 10 million of people each year, which represents about one in six deaths, according to the World Health Organization. Among treatments that have considerably improved the survival of patients with cancer, especially in metastatic stage, immunotherapy is the main one. Moreover, this therapeutic often offers a much better tolerance than chemotherapy and, in case of success, a frequently much longer effective period. Immune Checkpoint Inhibitors (ICI) represent the main class of immunotherapy applied in solid tumors. The molecules mostly used are anti-PD-1 antibodies (nivolumab, pembrolizumab, cemiplimab ...), anti-PD-L1 antibodies (atezolizumab, durvalumab, avelumab ...) and anti-CTLA-4 antibodies (ipilimumab, tremelimumab ...).

The principle of these treatments is not to have a direct anti-tumor activity but to block specific immune checkpoints present between tumor cells and T cells. Indeed, these checkpoints inhibit immune anti-tumor response through several mechanisms (immune cells apoptosis, inhibition of cytotoxicity, induction of immune tolerance ...), and thus, ICI allow effective T cells activity recovery (3,4).

For now, ICI are applied in several solid tumors and only in a few hematological malignancies. In locally advanced/metastatic non-small cell lung cancers (NSCLC), first-line monotherapy by pembrolizumab significantly improved overall survival (OS) in patients with a PD-L1 TPS (Tumor Proportion Score) $\geq 50\%$ (3 years-OS rate 31.3% versus 18.4% in chemotherapy group) (5). The combination of pembrolizumab with chemotherapy is now the standard of treatment in patients with PD-L1 TPS $< 50\%$, since the addition of pembrolizumab proved survival benefit

in Keynote-189 and Keynote-407 studies (6,7). In advanced melanomas, where chemotherapy never found success nor acceptable tolerance, ICI allowed durable survival (5 years-OS rate 52% in the nivolumab-plus-ipilimumab group in the Checkmate-067 study), with a favorable tolerance in patients who had a 6-months life expectancy under chemotherapy (8). ICI give also encouraging results in cutaneous epidermoid carcinomas (9). In advanced renal cell carcinomas, ICI are recently applied in first line treatment, regardless of PD-L1 tissue expression (10). The combination of pembrolizumab and axitinib significantly improved survival with a median OS of 45.7 months versus 40.1 months in sunitinib group in the Keynote-426 study (11). Nivolumab associated to ipilimumab offered also superior survival compared to sunitinib in Checkmate-267 study (OS Hazard Ratio (HR) 0.69 ; IC95% [0.59-0.81]) (12).

Unfortunately, not all patients will respond to ICI, about 40 to 60 % depending on the type of cancer (13–15). Moreover, considering their expensive cost and frequent lengthy delay until effectiveness, there is a need to find biomarkers that can predict if the patient would really benefit from this treatment. For now, only a few biomarkers proved their significance. The tissue PD-L1 expression is the main one, used essentially in lung cancers to determine treatment algorithm. However, it is not uncommon that patients with a high PD-L1 tissue expression do not respond to ICI therapies (16). Some other biomarkers proved their prognosis value but are not used in clinical practice yet. For example, a high CD8 T+ cells infiltration in tumor or a high tumor mutational burden (TMB) are correlated to a better response to ICI (17), whereas a decreased infiltration of myeloid cells or macrophages (18), or a low expression of ligands or receptors from immune checkpoints are associated to a worse survival (19–21).

Considering the physiopathology of tumor cells development, the fundamental role of immune system and the mechanism of action of ICI therapies, we took interest in five potential immune biomarkers that seem to play an important role in ICI success.

1.2. Results

1.2.1. Soluble PD-L1

PD-1 (programmed cell death protein-1) receptors are a family of transmembrane immunoglobulins located at the surface of many immune system cells, including T and B cells, natural killer (NK) and NKT cells. Two types of ligands can bind it, PD-L1 and PD-L2 (programmed death-ligand 1 and 2). PD-L1 is the main one, expressed by antigen presenting cells in healthy patients, and by various cancer cells. When PD-L1 is bound to its receptor, it triggers an inhibitory signal that sets off several immune evasion mechanisms, including apoptosis and inhibition of cytotoxicity, cytokine release and T-cells proliferation. Thus, the PD-1/PD-L1 is a fundamental way of modulation of the adaptive response against tumor cells, explaining the recent development of PD-1 and PD-L1 inhibitors, which have revolutionized the prognosis of many neoplasia (22).

The tumor tissue PD-L1 level is already well studied in numerous cancers. It corresponds to the percentage of tumor cells expressing PD-L1 and is measured in immunohistochemistry. It has a prognostic and a decisional role to determine the adequate treatment in several cancers, mainly in lung ones. For example, patients with advanced non-small-cell lung cancer with a PD-L1 TPS of 50% or greater will have a benefit to be treated by ICI alone, while they will preferably be treated by a combination of ICI and chemotherapy if PD-L1 TPS is under 50%. Nevertheless, measure of tumoral PD-L1 expression has some limitations. Frequently, PD-L1 levels do not predict ICI therapy response. In case of NSCLC, even if PD-L1 TPS is high (50% or greater),

40 to 60 percent of patients will not respond to ICI (5–7). This rate is approximately the same in advanced renal cell carcinoma (10,11) and advanced melanoma (8), regardless of PD-L1 expression. PD-L1 tissue levels can also be altered by chemotherapy (23). Finally, there is a significant spatial heterogeneity within the same tumor, and an invasive biopsy is required with sometimes a too small sized sample with not enough tumor cells to get an optimal result (24).

The circulating PD-L1 level, or soluble PD-L1 (sPD-L1), could be an effective substitute to the tissue PD-L1. sPD-L1 is measured by ELISA (enzyme-linked immunosorbent assay) in a simple blood sample (plasma). Its origin is not totally certified yet, but it may come from the proteolytic cleavage of membrane-bound PD-L1 tumor cells and macrophages. It is usually undetectable in healthy subjects but have been studied in some auto-immune diseases like rheumatoid polyarthritis (25,26). Considering it can bind to the PD-1 receptor of circulating T cells and tumor infiltrating lymphocytes (TIL), leading to the suppression of their anti-tumor activity before they reach tumor cells, it may be of major significance for the success of ICI treatments.

Several studies have investigated the role of soluble PD-L1 in prognosis of solid tumors. A meta-analysis by Wei et al., including 1040 patients with solid tumors (hepatocellular, renal, lung, gastric, and biliary tract cancers) from 8 eligible studies, demonstrated that a high soluble PD-L1 level was associated with a shorter OS (HR 2.26, 95%IC 1.83-2.80, $p < 0.001$). It is important to note that the cut-off value picked to determine if a patient is in the « high sPD-L1 level » group was not the same between studies (from 0.0183ng/mL to 7.32ng/mL) (27). Zhang et al demonstrated that the level of circulating PD-L1 was higher in advanced NSCLC (n=109), compared to control healthy patients (n=65), and was significantly correlated to abdominal organ metastasis. Patients with high sPD-L1 expression had also a worst prognosis than patients

with low expression (OS 26 months vs 18,7 months, $p < 0.001$) (28). Okuma et al described the same results in a study including 96 patients with advanced lung cancer, with a significant reduction of OS in patients with high plasma sPD-L1 levels (13 months vs 24 months in patients with low sPD-L1 levels, $p = 0.037$) (29). In Clear Cell Renal Cell Carcinoma (CCRCC), Larrinaga et al. found, in a cohort of 89 patients (including 23 metastatic), that the baseline sPD-L1 level was higher in metastatic patients compared to non-metastatic patients, especially in case of synchronous metastasis, and was associated with a worse survival. They did not find a significant correlation between circulating levels and tissue levels and, oddly, showed that baseline levels were higher in healthy patients than in CCRCC (30). In colorectal cancers (CRC), Wang et al. reported that CRC patients with higher circulating PD-L1 level (> 4.41 pg/mL) had a worse prognosis ($p = 0.004$) (31). In hepatocellular carcinoma (HCC), results were similar, with Han et al. proving, in a cohort of 81 HBV-related HCC, that patients with lower baseline sPD-L1 levels had a better prognosis with a longer OS (32).

Several teams also studied the predictive value of sPD-L1 level for response to ICI treatments. NSCLC patients with low plasma sPD-L1 levels before nivolumab had better response (59% vs 25%), longer time to treatment failure (TTF 5.36 months vs 1.48 months, $p = 0.032$) and longer OS compared to patients with high sPD-L1 in Okuma et al. study (33). Constantini et al. showed that increase of sPD-L1 at 2 months after nivolumab introduction for NSCLC was associated to a worse response (objective response rate ORR 17% in case of increase of sPD-L1 versus 68% in case of decrease or stability), and a worse PFS (progression-free survival) and OS (34). Ando et al reported that the decrease of sPD-L1 level during treatment was significantly correlated with a better response (HR 0.731, $p = 0.027$) after 4 cycles of ICI in their cohort of 21 patients (11 NSCLC, 9 gastric cancers and 1 bladder cancer) (35). In mesothelioma, a significant increase in sPD-L1 levels was detected after the first cycle of ICI and maintained

during treatment. Patients with sPD-L1 concentrations below the optimal cut-offs at baseline, at day 1 of cycle 2 and at day 1 of cycle 5 had a significantly longer OS (36). In melanoma, Zhou et al reported that patients with a high pre-treatment level of sPD-L1 were more at risk to progress under ICI (CTLA-4 or PD-1 blockade). These patients also displayed either decrease or no change in cytokine production after treatment, and the authors suggested that high pre-treatment concentrations of sPD-L1 may suppress effective antitumor immunity (37). Results are slightly different in Incorvaia et al study, which included 56 patients with metastatic CCRCC treated by nivolumab. They showed that patients with a high baseline level of sPD-L1 (>0,66 ng/mL) had a longer PFS. In the group of long-responder patients (PFS > 18 months), after 2 cycles of nivolumab administration, plasma levels of sPD-L1 were lower than baseline (38).

1.2.2. Blood Tumor Mutational Burden

The Tumor Mutational Burden (TMB) corresponds to the total number of somatic mutations in coding sequence in a tumor specimen. Measured by Next Generation Sequencing (NGS)-based approaches, TMB is classically defined by all non-synonymous coding mutations in a tumor genome and is reported in mutations per mega base of DNA (Mut/Mb). It is hypothesized that a highly mutated tumor leads to an enhanced production of the load and the variety of tumor neoantigens not typically found in the normal human genome. These neoantigens can be thus recognized as « non self » and activate cytotoxic T-cells. Genomic instability increasing immunogenicity and T-cell reactivity against tumor, it also may increase odds of response to ICI treatments. TMB can be viewed as a probabilistic indirect measure of immunogenicity, even if a high TMB does not necessarily equal to a high number of neoantigens on the surface of tumor cells, and neoantigens quality is probably more important than quantity (39,40).

TMB was first studied in its tissue form (t-TMB), which requires a tumor biopsy, and showed promising results in prediction of benefit from ICI treatments. Several teams studied the correlation between t-TMB and response to ICI. Most of them demonstrated that a high t-TMB was correlated with a better response to ICI and longer PFS and OS in several types of cancer (41,42). For example, Kim et al. showed in a cohort of 501 patients with advanced gastrointestinal, genitourinary, or rare cancers, that high t-TMB (observed in 11.6% of patients) was correlated with a better ORR to ICI than low t-TMB (ORR 75% vs 25%) (43). These results were also observed in a meta-analysis with 27 tumor types or subtypes treated by anti-PD1 or anti-PD-L1, which found a significant correlation between t-TMB and ORR ($p < 0.001$), with a correlation coefficient of 0.74 (44). Another meta-analysis, including 8 studies and 2661 patients, found also a better PFS for ICI-treated patients with a high t-TMB compared to low t-TMB (HR = 0.47, 95%CI 0.35-0.63, $p = 0.0001$) (45). The analysis of pan-tumor genomic biomarkers from four Keynote clinical trials (including more than 300 patients samples across 22 tumor types) showed that t-TMB was associated with best overall response ($p < 0.001$) (46). However, obtaining t-TMB from a tumor requires an invasive test, and it is rather common (up to 30%) that the sample does not supply sufficient tissue to adequately determine TMB, especially in patients with advanced disease. Furthermore, even with a large enough sample, t-TMB can vary according to the area of the sample (due to intra-tumoral heterogeneity), making t-TMB sometimes not enough representative (42).

The blood tumor mutational burden (b-TMB), calculated through analysis of mutations in circulating tumor DNA (ctDNA), is a promising non-invasive method to supplement or replace t-TMB. ctDNA can be released by tumor cells through apoptosis, necrosis, and active secretion from extracellular vesicle and circulating tumor cells. Commonly known as liquid biopsy, ctDNA is already applied to track targetable mutations and longitudinal tumor evolution in real

time. Its analysis seems to be more representative of the primary and metastatic tumor than tissue biopsy. Moreover, some studies have demonstrated that there is high concordance for genomic hotspot mutations between paired tissues and ctDNA biopsies in multiple tumors (47,48).

At this time, b-TMB was mainly studied in NSCLC under ICI. Lung cancer is indeed one of the highest mutated cancer (mutation frequencies from 0.1 to 100 Mut/Mb), particularly in smokers (49). In most of these studies, higher is the b-TMB, better is the response to ICI treatments. The main difference lies in the threshold picked to determine whether b-TMB is high or low. Gandara et al, in a retrospective analysis of two large cohorts of NSCLC treated by atezolizumab (samples from POPLAR and OAK trials), showed with a threshold of 16 Mut/Mb, that a high b-TMB was correlated to a better PFS (HR 0.57; CI95% 0.33-0.99) (50). Wang et al demonstrated, in two cohorts of 48 and 50 patients, that a superior PFS and a better ORR were found in patients with b-TMB \geq 6 Mut/Mb (PFS: HR 0.39; 95%CI 0.18-0.84, p=0.01 – ORR: 39.3% if b-TMB \geq 6 vs 9.1% if b-TMB < 6) (51). Chen et al, in a cohort of 42 NSCLC patients under ICI, proved that patients with b-TMB \geq 11 Mut/Mb had a longer PFS than those with b-TMB < 11 Mut/Mb (6.1 months vs 1.8 month, HR 0.39, 95%IC 0.13-1.17, p=0.011) (41). Si et al, in 2019, studied b-TMB in 809 advanced NSCLC patients from the phase III MYSTIC study (first line durvalumab +/- tremelimumab vs chemotherapy). They showed that patients with a b-TMB \geq 20 Mut/Mb had a better PFS (D+T vs CT: HR 0.53; 95%CI 0.34-0.81 - D vs CT: HR 0.77; 95%CI 0.52-1.13) and a better OS (D+T vs CT: HR 0.49 ; 95%CI 0.32-0.74 - D vs CT: HR 0.72 ; 95%CI 0.50-1.05) (52). These studies were also able to demonstrate that t-TMB and b-TMB were correlated. In a small cohort of 13 NSCLC treated by ICI, patients with a low b-TMB (<6 Mut/Mb) had a shorter PFS (HR 3.96, 95%CI 1.083-14.48, p < 0.01) and a lower ORR (14,2% vs 83,3%, p=0.02) than patients with high b-TMB. Non responders

patients also had significantly lower b-TMB levels than responders (median 4 vs 11.5 Mut/mb; $p < 0.01$) (53).

At the opposite, Chae et al, in a cohort of 136 NSCLC patients treated by ICI, found that patients with higher b-TMB had a shorter PFS (45 vs 335 days; HR 5.3; 95%CI 1.3-24.6; $p < 0.01$) and a shorter OS (106 days vs NR; HR 6.0; 95%CI 1.3-27.1; $p < 0.01$) (47). In the same way, OS benefit was similar regardless of b-TMB (cut off 10 Mut/Mb) in the Checkmate-227 study (nivolumab and ipilimumab in advanced NSCLC) (54). Considering these contradicting results, Nie et al hypothesized that the correlation between b-TMB and ICI response rate might be not linear. Hence, they analyzed samples from patients randomly assigned to receive atezolizumab in POLAR and OAK trials (as Gandara et al) and chose this time a 7 and a 14 Mut/Mb cut-offs. They showed that PFS and OS were statistically better in both low (≤ 7) and high (≥ 14) b-TMB populations than in medium (8-13) b-TMB population (55).

There were only few studies in other types of cancers. Georgiadis et al demonstrated the feasibility of pan-cancer b-TMB estimation through a hybrid-capture-based 98-kb gene panel in metastatic patients enrolled in a pembrolizumab clinical trial. They showed that high TMB in pretreatment plasma predicted progression-free survival (HRs 0.23; $p = 0.003$) (56). Kim et al analyzed ctDNA from 61 patients with metastatic gastric cancer treated by pembrolizumab. They showed that patients with higher b-TMB had an ORR of 83% whereas patients with lower b-TMB obtained an ORR of 7.7% ($p = 0.0014$) and a trend toward improved PFS (median NR vs 87 days; $p = 0.054$). Interestingly, patients with decreasing ctDNA after 6 weeks of pembrolizumab beginning had a significant improvement in ORR and PFS (57). The study of Araujo et al., including 38 patients with different types of tumors, showed that b-TMB and t-TMB were correlated with a median b-TMB of 5 Mut/Mb and 6 Mut/Mb for t-TMB. In this

study, high b-TMB was not associated with better survival, but all responder patients to ICI (n=3) had a decrease in the variant allele frequency of mutations detected in ctDNA (58).

1.2.3. HLA typing

The success of an ICI treatment is based on the recognition of presented tumor antigens by T cells and their activation. The Major Histocompatibility Complex (MHC), or Human Leukocyte Antigen (HLA) in humans, is a crucial actor of this process. Its gene complex is located on the short arm of the sixth chromosome and encodes for the expression of cell surface molecules devoted to antigen presentation to the T-cell receptors (TCR), which recognize the combination “MHC molecule-peptide”. Two types of HLA molecules are described: type I, expressed on almost every nuclear cell, and type II which expression is mostly restricted to the surface of antigen-presenting cells.

When a TCR binds to a complex HLA molecule/peptide, it triggers a series of activator or inhibitor signals from the T-cell, depending on the nature of the presented peptide: self-molecule, infectious peptide, tumoral neoantigen. Hence, when a tumor cell harbors at its surface a neoantigen presented by a class-I HLA molecule, it can be recognized by the TCR of a specific CD8⁺ T-cell, leading to its cytotoxic activity and the destruction of the modified cell (59).

HLA-class I molecules consist of a single alpha chain, bound to a beta-2-microglobulin (B2M) molecule. The HLA-class I gene complex consists of 3 “classic” genes (HLA-A, B and C), each one dramatically polymorphic (more than 20 000 class-I alleles are currently described) (60), and its expression is codominant. The peculiarity of these genes lies in the allelic differences: number shifts of nucleotides (mostly located in the alpha 1 and 2 domains) between two alleles explain the diversity of HLA molecules, which is directly related to the range of peptides that can be presented to CD8⁺ T-cells (61).

The disruption of antigen presentation is a well-known mechanism of immune escape from cancer cells, either by downregulating HLA-I expression, by the loss of HLA gene heterozygosity by tumor cells, or by the homozygosity of patient germline HLA-I genes. All these situations are today described playing a part in the response to ICI treatment.

The more diverse the HLA-I molecules on a cell surface are the wider the peptide repertoire that can be presented to CD8+ T-cells is. Thus, one can postulate that any homozygosity from the HLA-I gene complex could lead to a less effective ICI response.

Chowell et al. first confirmed this hypothesis in a cohort of 269 melanomas and 100 NSCLC patients, reporting in *Science* in 2018 that patients who presented a genomic homozygosity in at least one of the HLA-I loci had a shorter OS (especially if the homozygosity was in the B and C loci), compared to the one with a maximal heterozygosity at HLA-I loci (HR 1.31, 95%CI 1.03-1.70, p=0.028). They could not reach significance when studying only the NSCLC patients. Those results were then confirmed in a validation cohort of 1166 patients (62).

Abed et al. upheld this first demonstration by reporting, in a cohort of 161 advanced NSCLC patients, that genomic homozygosity in one or more HLA-I loci was associated with a shorter OS (HR=2.17, 95%CI 1.3-4.17, p=0.02) and trends toward a shorter PFS (HR=1.62 95% CI 0.92-2.86, p=0.093) especially with older patients and those treated with anti-PD1 rather than anti-PD-L1 immunotherapy. It is of interest to notice that tumor PD-L1 expression was assessed in these patients, and more than 50% had a PD-L1 TPS \geq 50%. The effect of HLA-I homozygosity was more pronounced in patients with strong PD-L1 expression (>50%) (63).

On the contrary, Negrao et al. did not find a correlation between HLA class-I zygosity and PFS nor OS in 200 advanced NSCLC treated with ICI, suggesting that the impact of HLA genotype could depend on the tumor type. However, they found a tendency to a better PFS with a heterozygosity in B or C loci. Furthermore, comparing with the study of Abed et al. (63), they

defined PD-L1 expression as positive above 1% and ICI were often introduced in second or third lines therapy (64). Moreover, Correale et al. demonstrated, in 119 metastatic NSCLC patients receiving anti-PD1 therapy, that heterozygosity in the A locus was associated to a worse OS (20.8 months vs more than 40 months, $p=0.03$) (65).

Finally, Goodman et al used the “Patient Harmonic-mean Best Rank” or PHBR score described by Marty et al. in 2017 (66), which characterize the sub-peptidome that can be effectively presented by the MHC-I molecules of an individual. Thus, the PHBR score reflects the ability of MHC-I to efficiently present a mutated driver neoantigen, with a lower score indicating a more efficient presentation. Goodman et al established that the lower the PHBR is, the better the ORR to ICI is (78% if $PHBR < 0.5$ vs 43% if $PHBR \geq 0.5$, $p=0.049$). Median OS and PFS were also higher in the lower PHBR score group (67).

In addition to germline homozygosity, loss of HLA heterozygosity (LOH) in tumors seems to occur in numbers of patient, as demonstrated by McGranahan et al., describing a computational tool to assess the LOH of HLA in tumor cells, and who reported LOH in 40% of NSCLCs (68).

The HLA genetic complex is polygenic (containing 6 classic genes) and highly polymorphic (huge numbers of alleles being described for each gene or HLA locus) (60). HLA molecules with similar peptide binding features have been grouped into one supertype (69,70). If a peptide is able to bind to one allele within a supertype, it can also bind to all other alleles in this supertype. Thus, it has been hypothesized that the presence of a certain supertype can influence the success of immunotherapies. In their previously reported study, Abed et al. also described that, among eight superotypes evaluated, HLA-A02 had a statistically significant association with improved survival (63), whereas Correale et al reported, in lung cancers, that the absence

of supertypes HLA-A01 and HLA-A02 was correlated to a worst PFS (7.5 vs 15.9 months, $p=0,01$) (65). In advanced renal cell carcinoma, the presence of some HLA types (A*01:01, A*03:01, B*40:02, B*57:01, C*06:02) was associated to a better PFS (71). In the study of Chowell et al. in ICI-treated melanoma, patients with HLA-B44 supertype had extended survival, whereas the HLA-B62 supertype was associated with poorer outcome (62). Ishida et al. on their side reported that, in a small cohort of 69 melanomas treated by nivolumab, the HLA-A26 allele was associated with a better response to treatment, but this allele is only found in 1-3% of population outside of Japan (72).

The downregulation of HLA-I antigen expression is another struggling point in the response to ICI treatment, easily explorable by immunohistochemical staining. Ladanyi et al. demonstrated that HLA-I expression level in lymph node metastasis (but interestingly not in cutaneous or subcutaneous metastasis) of melanoma was correlated to clinical response to ipilimumab. This clinical effect (demonstrated as longer survival) was, in case of higher expression of HLA-I antigens in lymph node, associated with a stronger intra-tumoral infiltrate of CD8+ and CD45+RO T cells, and of lymphocytes expressing PD-1 (73). Rodig et al. studied metastatic melanoma patients who benefited from ipilimumab-nivolumab combination and examined tumor cells MHC expression. They elegantly demonstrated that the loss of MHC-I expression (due to a transcriptional repression of *HLA-A*, *HLA-B*, *HLA-C* and *B2M*) is predicting of a lower response to anti-CTLA4, but not anti-PD-1 therapy. More precisely, in CheckMate-069, the loss of MHC-I was associated to the lack of clinical response to anti-CTLA4 (progressive disease or stable disease, negative predictive value 100%). In the opposite way, MHC-II expression in tumor (associated with the presence of IFN- γ , and IFN- γ -mediated gene signatures) seemed in favor of good response to anti-PD-1 treatments (and not to anti-CTLA4 ones). If needed, these data highlighted the benefits of the combination of these two ICI,

requiring distinct antigen presentation pathways (74). Kwak et al. also reported in their study that the loss of HLA-I molecules was significantly correlated to low tumor infiltrating lymphocytes (TIL) density, leading to a worst response to ICI and a poorer prognosis, in microsatellite instability (MSI) gastric cancers (75). In immunotherapy-naïve colorectal cancers, Young Na et al demonstrated that reduced HLA A/B/C expression was frequent in MSI-high patients. In MSS patients, patients without HLA downregulation had a better DFS (disease-free survival) and OS (HR 0,518, IC95% 0.283-0.946, p=0,032 in multivariate analysis). These results were then confirmed in a validation cohort (76). HLA-I expression was also studied in other cancers usually not treated with ICI. Imai et al. studied 36 pancreatic ductal adenocarcinomas, and showed that a low HLA-I expression was correlated to a poorer survival, and that high HLA-I expression was significantly associated with a higher recurrence free survival (RFS) ($p < 0.001$) and OS ($p = 0.0027$) (77).

1.2.4. Beta-2 microglobulin

Beta-2 microglobulin (B2M) protein is a major component of MHC class I molecules (a part of the invariant light chain). This protein is present on the membrane of almost all nucleated cells, and its free soluble form can be detected in several body fluids (blood, urine, cerebrospinal fluid). In clinical practice, serum and urine B2M levels are applied to monitor some nephropathies and these concentrations are increased in some hematologic malignancies, particularly in multiple myeloma and lymphoma. This raise may be a reflect of increased cell production or cell destruction. Serum B2M levels are also measured to evaluate the prognosis of some chronic inflammatory diseases and some infection such as HIV (78,79).

It has been described that serum B2M levels can also increase in case of solid tumors. In breast cancer, high level of B2M in serum is associated with distant metastasis and with a poor survival outcome (80). There is also a significant increase in serum B2M levels in patients with

urothelial cancer, and tumor surgery results in normalization of B2M concentration (81). High levels of urine B2M is associated with distant metastasis in prostate cancer and with a shortened survival in patients with bone metastasis (82).

Gene encoding B2M is located on the fifteenth chromosome. Any mutation or deletion that could lead to its downregulation can result to B2M structural modification and to alteration of its connection to the alpha heavy chain of MHC. This disruption can thus impair HLA-I molecules ability of efficient antigen presentation to T cells. ICI therapy being based on the recognition of cancer-specific antigens presented by HLA-1 complexes, B2M integrity is a crucial part of success. Moreover, in opposition to HLA mutations that would influence a specific MHC-I molecule, a *B2M* mutation would affect all MHC-I molecules (83). In this context, several studies, of small sizes for most of them, have investigated the correlation between membrane B2M expression and its link to ICI therapy success.

In melanoma, Restifo et al. realized an *in vitro* analysis of five independently established melanoma cell cultures (from patients with metastatic melanoma), with a complete loss of cell-surface HLA class I molecules. In these cells, expression of *B2M* mRNA was variable and lower than seen in control cell lines, and no immunoreactive B2M protein was detected by western blot analysis. Genetic insertion of functional *B2M* (by recombinant vaccinia virus) fully restored the antigen-processing capabilities of cell lines, and restored the ability of CD8+ T cells to recognize the cell lines in the tumor (84). In 2016, Zaretsky performed WES on samples from 4 metastatic melanoma patients, who initially responded to pembrolizumab then progressed (months to years later). Samples were collected before treatment and at the time of relapse. One patient acquired a truncating mutation of *B2M*. Immunohistochemical analysis confirmed the loss of surface expression of MHC class I, even if diffuse intracellular staining

indicated continued production of MHC class I molecules, confirming that B2M has a role in their transport to the cell surface (85). Sade-Felman et al performed a whole exome sequencing (WES) on tumor samples from 17 patients with metastatic melanoma under ICI, including 7 non-responders and 5 patients who responded initially then progressed. They found that 29,4% of patients with progressive disease had a *B2M* mutation (point mutations, deletion or LOH). Then, using 2 cohorts of melanoma patients under ipilimumab (n = 105) and pembrolizumab (n = 38), they showed that there was three times more LOH of *B2M* in non-responders compared to responders (30% vs 10%). *B2M* LOH was significantly correlated to a shorter OS, and loss of both copies of *B2M* was only present in non-responder patients (86). In lung cancers, Gettinger et al investigated samples from 14 ICI refractory patients and matched them with patient-derived xenografts (PDXs). One of the 14 patients had an acquired homozygous loss of *B2M*, and *B2M* downregulation (by loss of heterozygosity) was found in two more PDXs. Interestingly, CRISPR-mediated knock-out of *B2m* in an immunocompetent lung cancer mouse model conferred resistance to PD-1 blockade *in vivo*, suggesting that *B2M* mutation has a significant role in ICI resistance (87). Castro et al showed that *B2M* mutations were highly enriched in tumors with microsatellite instability, and that somatic *B2M* mutations in microsatellite stable tumors were associated with higher overall mutation burden and a larger fraction of HLA-binding neoantigens (compared to wild type tumors) (83). Germano et al analyzed Mismatch Repair deficient (MMRd) colorectal tumor cells, which frequently present a homozygous loss of *B2M* but still respond to ICI therapy. They depicted MMRd *B2M null* cells that responded to ICI by recruiting preferentially CD4+ T cells, and that did not require CD8+ T cells (88). In gastric cancers (stage I and II), where nivolumab is now the standard treatment in combination with chemotherapy, Park et al showed that 65.3% of stage II tumors and 59.5% of stage III tumors had a negative immunohistochemistry expression of B2M (and HLA-A, B and C). This negative expression was inversely correlated with CD8+ T cells

infiltration and PD-L1 expression, and correlated with unfavorable prognosis (89). *B2M* somatic mutations seem to usually appear at the early stage of tumor development, compared to HLA mutations which may occurred at early or late stage (83,90).

1.2.5. Killer-cell Immunoglobulin-like Receptors

NK cells are cytotoxic lymphocytes, dedicated to the elimination of virus infected and tumor cells. Their activation is regulated by a complex integration of inhibitory and activator signals. Such signals are sensed notably by Killer-cell Immunoglobulin-like Receptors (KIRs), an array of either inhibitory or activator receptors (91,92). These receptors are encoded by 14 highly polymorphic genes. Their nomenclature depends on their number of extracellular domains (2 (KIR2D) or 3 (KIR3D)), and on the length of their intracytoplasmic tail (long (L) or short (S)) (93). KIR-ligands are mainly identified as HLA-I proteins, expressed by all nucleated cells (92), also encoded by a hugely polymorphic gene system (60). Although normally expressed in a ubiquitous way, HLA class-I molecules are frequently downregulated in cancer cells, as shown earlier, to facilitate their escape from T cytotoxic cells. In a normal cell, the binding of HLA-I molecules to KIR triggers an inhibitory signal, preventing the NK cells from releasing its cytotoxic granules. When modified cells lose their self-molecules, they become abnormally sensitive to NK cells, a process described as the “missing self” theory (94).

As KIR and KIR-ligands are independently inherited, their vast allelic diversity gives rise to the notions of NK cell repertoire, and diversity in NK cell subset responsiveness. It has even been demonstrated, in autologous stem cell transplantation, that KIR and HLA genotypes association could be predictive of host immune profile (95).

In highly immunogenic bladder cancer, Guillamon et al. obtained the KIR and HLA-ligands genotypes of 132 newly diagnosed patients, and compared them to patients with other cancers,

plasma cell disorders and healthy controls. They were able to sort out the combination of KIR2DL5⁺/HLA-C*16⁺ as a marker of dramatically lower progression-free rate (7.1%, p=0.00001) than KIR2DL5⁻/HLA-C*16⁺ (60.0%) or HLA-C*16⁻ (71.7%) genotypes (96). These data pave the way to explore KIR and KIR-ligands as potential biomarker of ICI success. However, reports are still scarce in this area. One of the largest cohort was reported by Ishida et al. who analysed the KIRotype of 112 Japanese melanoma patients receiving nivolumab, and demonstrated that there was no KIR gene nor KIR/KIR ligands association that was associated to a better (nor worse) response to treatment. Moreover, nor KIR gene was predictive of suffering an adverse event of grade 2 or more (97).

Just like HLA-I molecules, the rate of expression of inhibitory KIR is an important factor of ICI success and could theoretically be linked to the prognosis. Considering this hypothesis, He et al. in 2016, inspected the KIR2D family and KIR3DL1 expression on tumor cells and tumor infiltrating lymphocytes of 62 NSCLC patients, mostly advanced adenocarcinoma. They reported that patients with a negative expression of KIR2D, on tumour cells or TIL, had a significantly longer OS than patients who were KIR2D positive (p<0.001). Likewise, the positive expression of KIR3DL1 on both type of cell was significantly correlated to a shorter OS (p<0.001). Neither of KIR2D and KIR3DL1 expression was correlated to a clinical characteristic (98). In another study, He et al. investigated the possible link between PD1 and inhibitory KIR expression on TIL and tumour cells. They found out that PD-1 expression was correlated with KIR 2D (L1, L2, L3, L4) on both type of cells and hypothesized that a part of the resistance to anti-PD-1 therapies might be related to the negative tumour immunity regulation of HLA/KIR (99).

1.2.6. Summary of the physiological mechanism of each biomarker

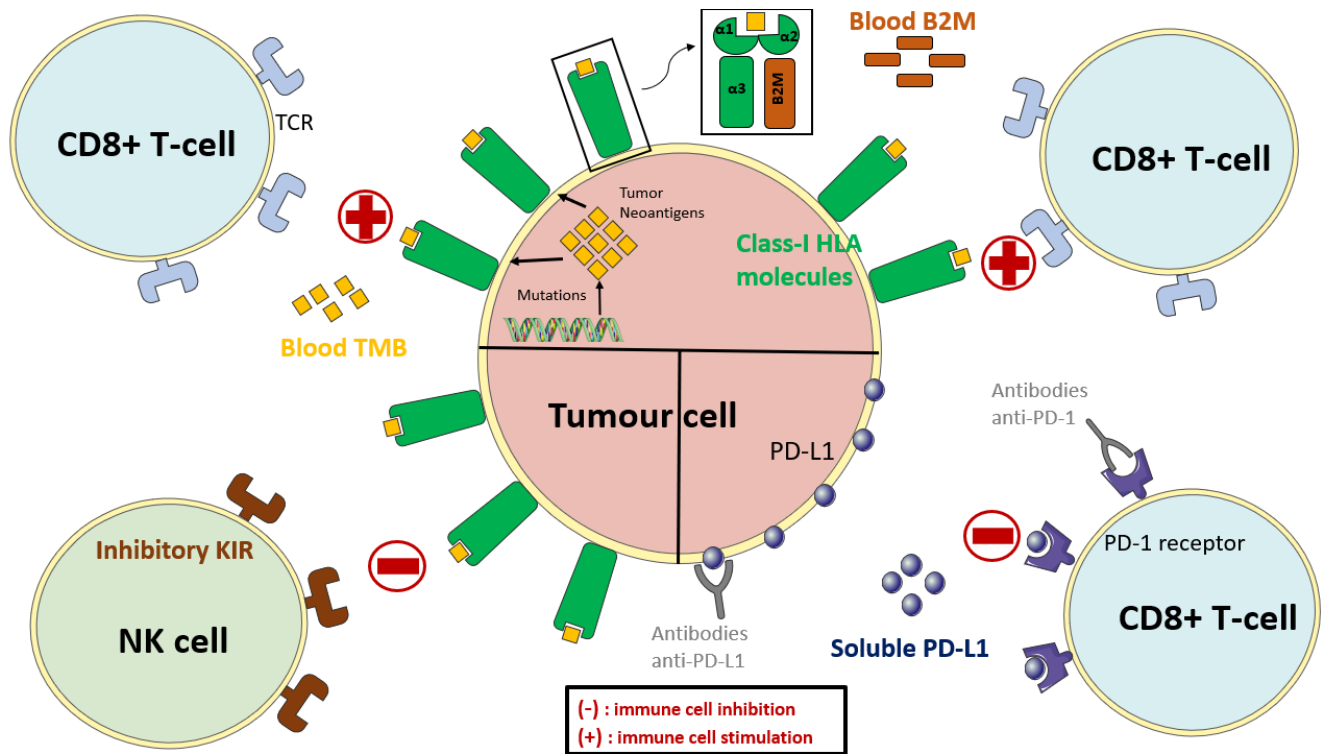


Figure 1. Physiological mechanism of each biomarker

1.3. Discussion

Immune check point inhibitors are a key part of the therapeutic arsenal against cancer. They have literally revolutionized the prognosis of several cancers, especially melanoma and lung ones, with an often much better tolerance than chemotherapy. However, not all patients will respond to immunotherapy in a durable way, and the need to find efficient and reproductive biomarkers that can predict ICI response become more and more important. In this context, we described in this article five potential immune biomarkers that could be predictive of a favorable response to ICI treatment.

Soluble PD-L1 showed promising results in different cancer subtypes. Several studies have demonstrated that a high sPD-L1 level is correlated to a worse prognosis, and that its baseline

level is correlated to the success of ICI treatment. In most of studies, patients with a low sPD-L1 level before starting ICI have a better response and a better survival than patients with a high sPD-L1 rate (27–29,31–33). sPD-L1 dynamic under ICI treatment may also be predictive of success or disease progression since an increase of sPD-L1 level during treatment is correlated to a worse response and a worse survival in some of studies (34–38).

However, there is a need for more investigations before sPD-L1 use in clinical practice. The optimal threshold to decide if patients is in the high or the low sPD-L1 group must be determinate. Indeed, chosen cut-offs are very different between studies, and it is difficult to identify if the difference is due to technical reasons, or if there is a real variability between cancer subtypes or cancer stages. The monitoring of sPD-L1 dynamic under treatment also needs to be more explored. First results are encouraging, but on a limited scale. If results are confirmed, sPD-L1 could be applied as an early biomarker of treatment failure such as, for example, CA-19.9 or ACE in digestive cancers. Moreover, sPD-L1 detection by ELISA is relatively easy and low cost. The correlation between tissue PD-L1 and soluble PD-L1 has not been well studied yet despite sPD-L1 potential to be an adequate tPD-L1 substitute. Further investigations should focus on this point. Finally, it should be interesting to investigate relationship between soluble PD-L1 levels and clinical or other biological characteristics.

Based on the pending questions, sPD-L1 is a promising biomarker but needs to be further investigated before its use in clinical practice.

The blood tumor mutational burden, measured in the circulating tumor DNA, is considering as an accurate representation of the overall tumor antigen production. Numerous studies showed that a high tissue TMB is correlated to a better ICI response and a better prognosis (41–46). However, the collection of an adequate tissue sample to determinate t-TMB is often not easy. In this context, b-TMB seems to be a promising non-invasive substitute of t-TMB to predict the

success of an ICI treatment. Most of studies taking interest in b-TMB were in the field of lung cancers since they are one of the highest mutated neoplasia. There are only a few studies in other cancers than lung, especially in renal carcinoma and melanoma which are however classically treated by ICI. Even if results are sometimes conflicting, the general trend of these studies is that a high baseline b-TMB level is associated to a better response to ICI and a better survival (41,49–53,56–58). One author hypothesized that the correlation between b-TMB and ICI response rate might be not linear (55). Interestingly, b-TMB and t-TMB are often correlated in these studies (41,49–52).

Nevertheless, there are still several limitations and unanswered questions. The threshold to decide whether b-TMB has a good prognostic value needs to be specified, since this rate is very different according to studies. The potential non-linear correlation suggested by Nie et al must also be confirmed. Moreover, the follow-up of b-TMB evolution under treatment has rarely been evaluated yet but could be of major significance and may be an early biomarker of upcoming disease progression. On a technical point, NGS is the best and adequate technique to measure b-TMB, but it is expensive and sequencing results can vary between laboratories (panel size, composition and bioinformatics pipelines). NGS also has a long turn-around time. Importantly, there is a need of a minimal amount of ctDNA detected in blood to calculate b-TMB, and only a subset of cancers shed significant number of circulating tumor cells in circulation (48).

Based on the challenges and the contradictory results concerning b-TMB as a biomarker, further understanding is warranted before the integration of this factor into clinical practice.

Human Leukocyte Antigen (HLA) molecules present, in their groove, peptides to T lymphocytes. Expressed on almost all nucleated cells, HLA-I molecules allow either to healthy cells to present self-peptides being so protected, or infected or modified cells to present

infectious or modified peptides and to be lysed by CD8⁺ T cells. This key role in T cell activation makes HLA as an excellent candidate as biomarker to ICI success.

A simple postulate has been confirmed: the more diverse the HLA-I molecules on a cell surface are, the widest the peptide repertoire that can be presented to CD8⁺ T cells is. Thus any homozygosity in HLA-I genes, or LOH in tumors cells, reduces the ability of MHC presentation and thus the efficacy of ICI. This has been demonstrated in both pulmonary cancers and melanoma in several studies (62,63,66–68), although two other studies did not reach those same results (64,65). Furthermore, the ability of HLA molecules to present peptides relies on the characteristics of its groove. It has thus been reported that some HLA molecules are associated with better response to ICI than other ones (62,63,71,72). Lastly, the rate of expression of HLA-I molecules, evaluated by immunohistochemistry, is also of interest. Indeed, downregulation of HLA-I is frequently observed in tumor cells, leading to a lower T cell activation. Two significant studies in melanoma showed that ICI-treated patients with a reduced expression of HLA-I molecules had a worse survival compared to those without downregulation in HLA-I molecules (73,74).

Considering all these results, HLA-I molecules play a major role in the success of ICI therapies. Further large-scale studies (both genotyping and expression rate) are now mandatory to strengthen those primary results and position HLA as an emergent biomarker of ICI success.

Beta-2-microglobulin is an essential component of the HLA class-I molecules structure and plays thus a fundamental role in the anti-tumor immune response. Only a few studies explored the prognosis value of the soluble form of B2M in solid tumors. High levels of B2M in patients' serum or urine are often associated with a more advanced disease, and a worse prognosis in these small studies (80–82). Some other teams rather studied the impact of *B2M* gene mutations on the ICI success, but usually in small cohorts. These studies showed that a *B2M* loss of

heterozygosity in melanomas, a *B2M* downregulation in several cancers including melanomas and colorectal MSI cancers, and a *B2M* mutation or deletion, were correlated to a worse prognosis and a lesser response to ICI therapies (83–90). Considering the small size of these studies, more investigations are needed to confirm those primarily but encouraging results, especially in solid cancers like pulmonary and melanomas, where ICI are an essential component of the therapeutic arsenal. Systematical NGS sequencing of *B2M* gene should be interesting to detect a potential loss of heterozygosity or the existence of dysfunctional mutations or deletions and could be therefore an emerging biomarker of ICI therapy success. Moreover, the correlation between the soluble form of B2M (that can be measured in a simple blood sample by biochemical analysis) and the molecular expression of *B2M* needs to be explored, to determine if a *B2M* mutation can be detected or at less suspected by a non-invasive and low-cost blood sample analysis.

Killer-cell Immunoglobulin-like Receptors (KIRs) consist of an array of inhibitory and activator receptors, mainly expressed by NK cells. Most of them recognize HLA-I molecules, which triggers inhibitory signals to the NK cells, and theoretically protects all healthy nucleated cells from lysis from these innate cytotoxic lymphocytes. On the contrary, the decrease of the expression of HLA-I molecules on the surface of some modified cells allows the elimination of these cells by NK cells, according to a theory called “missing self.

KIR highly polymorphic genes lead to the notion of NK cell repertoire, and the fact that their genes are independently inherited of their ligands (HLA-I) genes explains the diversity of NK cell subset responsiveness. Exploring the KIR genotypes, associated to the HLA-I genotype, could thus seem of interest as potential biomarker of ICI success. However, to date, only few studies report conflicting results. Few studies have reported the correlation between expression by tumor cells or tumor-infiltrating lymphocytes of specific KIRs and response to ICIs,

especially KIR2D family (96–99). At the end, KIR and KIR-ligands genotypes raise attention as potential biomarker of response to ICI, but more studies are needed to assess their efficacy before potentially introducing these biomarkers in clinical practice.

Outcome of each biomarker on ICI response

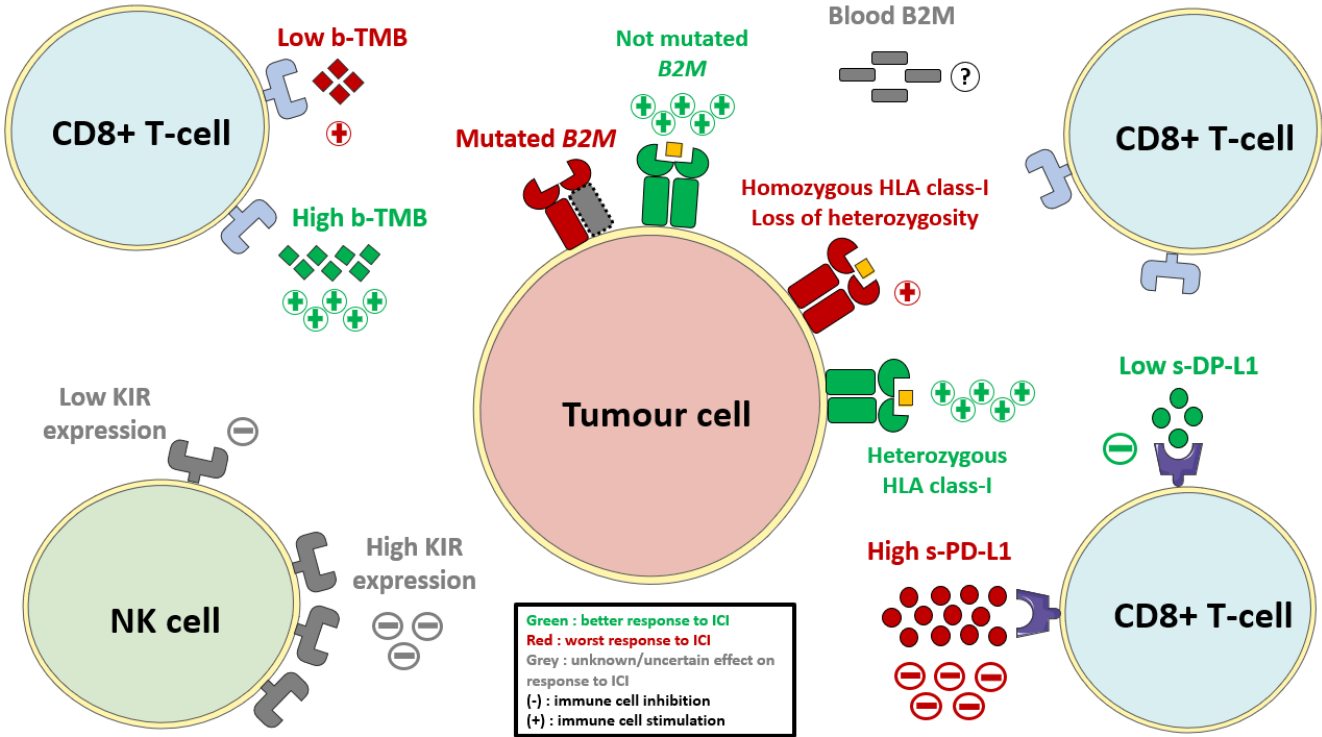


Figure 2 : Outcome of each biomarker on ICI response

1.4. Conclusion

According to the results of this systematic review, five biomarkers are interesting due to their potential role in the success of ICI treatment: the soluble PD-L1, the blood TMB, the HLA typing, the beta-2-microglobulin and the Killer-cell Immunoglobulin-like Receptors. Each of them may be an effective predictive biomarkers of ICI success. However, further studies are needed to strengthen those primary results before they can be used in clinical practice.

2. ANALYSES STATISTIQUES : premiers résultats

A partir des données disponibles dans les études rapportées, nous avons débuté un travail statistique de méta-analyse des 3 biomarqueurs les plus étudiés, à savoir le PD-L1 soluble, la charge tumorale mutationnelle sanguine et le système HLA.

Ce travail a consisté en une comparaison des taux de survie globale (OS) selon les taux de PD-L1 soluble ou de b-TMB, ou en fonction du typage HLA ou de son niveau d'expression. Certaines données n'ayant pas été publiées par les auteurs dans les articles étudiés, nous les avons contactés afin d'obtenir les résultats manquants et sommes, à ce jour, en attente de leur réponse. Nous partagerons donc dans ce manuscrit un travail statistique préliminaire réalisé en collaboration avec Bruno Pereira, qui sera complété puis intégré dans notre article lors de sa soumission.

PD-L1 soluble

La figure 3 ci-dessous rapporte l'impact d'un taux de sPD-L1 supérieur au seuil désigné dans chaque étude sur le taux de survie globale (OS) exprimé en Hazard Ratio. Cette analyse statistique montre qu'un taux de s-PD-L1 élevé (supérieur au seuil défini) a un impact pronostique péjoratif sur la survie globale, avec un Hazard Ratio à 2,32 ([1,878 – 2,866], $p < 0,001$).

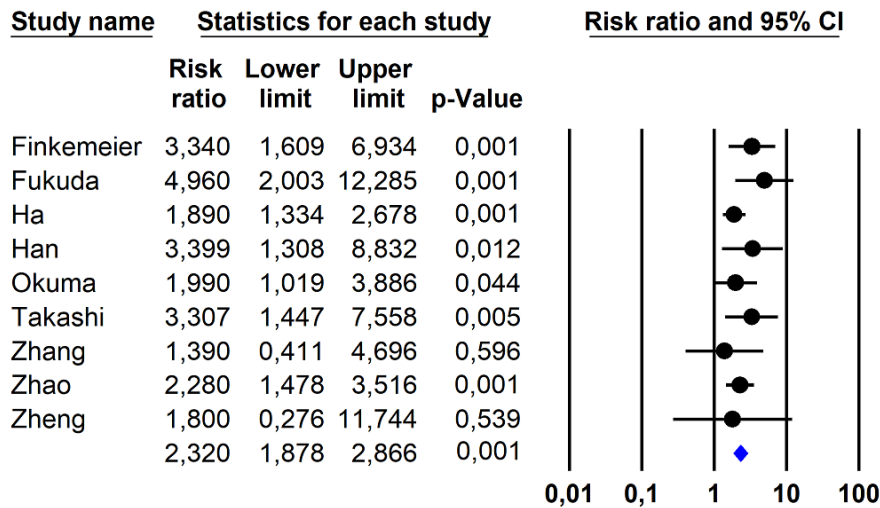


Figure 3 : méta-analyse des données de survie globale en cas de taux élevé de s-PD-L1

Charge mutationnelle tumorale sanguine

L'analyse statistique des différentes études portant sur le b-TMB (Figure 4) révèle que les patients présentant une charge mutationnelle tumorale sanguine supérieure au seuil défini dans chaque étude ont une tendance non significative à une meilleure survie globale comparativement aux patients ayant un b-TMB inférieur au seuil défini, avec un HR à 0,776 ([0,488-1,233], $p = 0,283$).

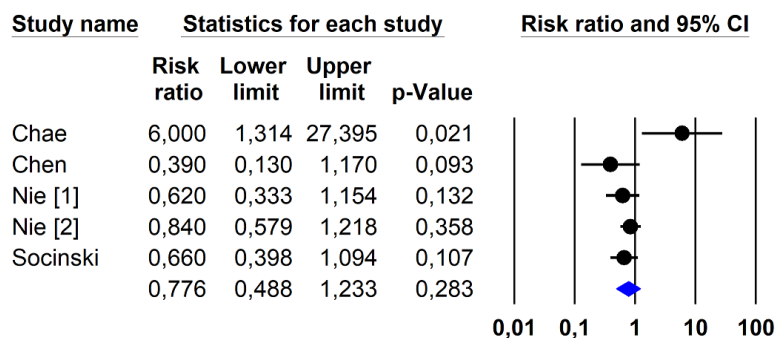


Figure 4 : méta-analyse des données de survie globale en cas de taux élevé de b-TMB

A noter que l'HR de l'étude de Chae et al concerne les patients du groupe b-TMB inférieur au seuil défini dans l'étude, alors que les autres études rapportent le HR des patients du groupe b-

TMB élevé, justifiant un HR supérieur à 1 dans cette seule étude (la figure sera donc modifiée avec le HR des patients ayant un b-TMB élevé dans l'étude de Chae et al.).

Système HLA

Nous avons réalisé à ce jour deux analyses statistiques sur ce biomarqueur.

La première analyse (Figure 5) porte sur l'impact sur la survie globale de la présence d'une homozygotie dans au moins un des locus des molécules HLA de classe I. Les résultats montrent une tendance non significative de réduction de la survie globale en cas de non-hétérozygotie dans au moins 1 des locus, avec un HR à 1,16 ([0,88-1,529), p=0,292).

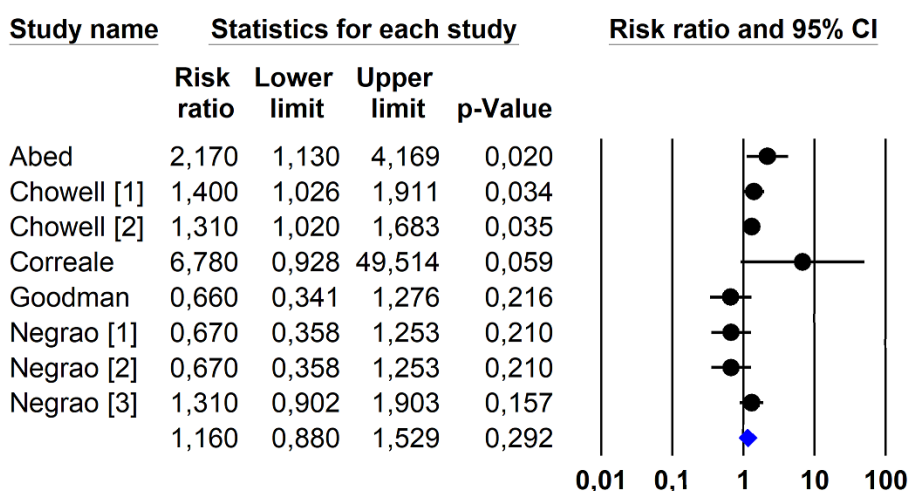


Figure 5 : méta-analyse des données de survie globale selon la présence d'une homozygotie en classe I du système HLA.

La seconde analyse (Figure 6) porte sur l'impact sur la survie globale de la perte d'expression des molécules HLA de classe-I tumorales. Les résultats montrent une tendance non significative à une amélioration de la survie globale lors de l'absence de diminution d'expression avec un HR à 0,715 ([0,502,1,02], p=0,064).

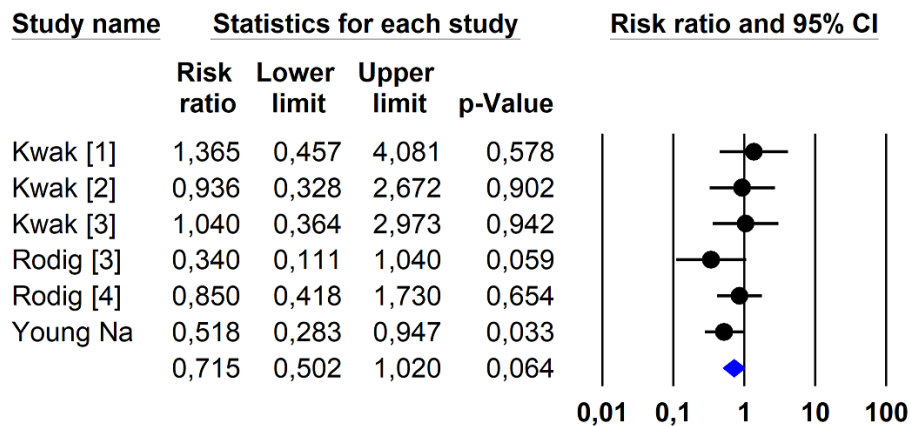


Figure 6 : méta-analyse des données de survie globale selon la présence d'une perte d'expression de molécule HLA de classe-I tumorales

Notons que ces résultats concernent l'impact pronostique de ces biomarqueurs sur la survie globale, mais que nous souhaitons également réaliser une analyse statistique sur le potentiel prédictif de ces biomarqueurs sur la réponse aux ICI (données en attente).

3. PROJET DE PROTOCOLE

Les résultats des études analysées dans le cadre de notre revue de littérature orientent donc vers un potentiel caractère prédictif de ces cinq biomarqueurs vis-à-vis de la réponse aux ICI dans les tumeurs solides. Cependant, l'ensemble de ces données reste pour l'instant insuffisant pour envisager une utilisation de ces biomarqueurs en pratique clinique, mais nous paraît suffisamment pertinent pour développer à court terme un protocole de recherche clinique prospectif visant à les évaluer.

Ce protocole serait multicentrique, incluant des patients atteints d'un cancer métastatique et éligibles à un traitement par ICI en première ligne thérapeutique (principalement cancers du poumon, du rein, et mélanome).

Les analyses réalisées seraient :

- Au diagnostic du patient :
 - Dosage de PD-L1 soluble (et analyse du PD-L1 tumoral pour analyse de corrélation) ;
 - Dosage du b-TMB (et réalisation du t-TMB pour analyse de corrélation) ;
 - Dosage de la B2M sérique et séquençage du gène *B2M* ;
 - Typage HLA du patient, mesure d'expression des protéines HLA de classe I, recherche de LOH sur la tumeur ;
 - Génotypage KIR.
- Suivi en cours de traitement pour évaluation de la cinétique :
 - Du PD-L1 soluble ;
 - Du b-TMB ;
 - De la B2M sérique ;
 - De l'expression des protéines HLA de classe I, recherche de LOH sur la tumeur

Les objectifs seraient multiples :

- Evaluer/confirmer l'impact pronostique des cinq biomarqueurs sanguins.
- Evaluer/confirmer leur capacité à prédire la réponse aux ICI.
- Déterminer les cut-offs de PD-L1 soluble, B2M, et b-TMB permettant de classer les patients entre bons et mauvais répondeurs, et déterminer si ces cut-offs sont les mêmes quel que soit le type histologique traité, ou influencés par certaines caractéristiques cliniques et biologiques
- Etudier la présence ou l'absence de relation entre ces cinq biomarqueurs.
- Etablir la faisabilité d'un score sanguin, pronostique et prédictif de la réponse aux ICI, qui serait composé de ces cinq biomarqueurs.

En cas de validation de la faisabilité et de la bonne valeur prédictive de ce score composite sanguin, il pourrait ainsi être facilement calculé au diagnostic des patients présentant un cancer métastatique avec indication à un traitement par ICI. Voici un exemple de grille (Tableau 1) qui pourrait être proposée. Ainsi, les patients avec un score élevé auraient plus de chance de répondre à un traitement par ICI, alors que le bénéfice à réaliser ce type de traitement chez les patients avec un score bas serait probablement minime.

	0	1	Techniques	*
PD-L1 soluble *	Elevé	Bas	ELISA	<i>Cut-off à déterminer</i>
b-TMB *	Bas	Elevé	NGS (biopsie liquide)	<i>Cut-off à déterminer</i>
B2M sérique *	?	?	Biochimie	<i>Impact à déterminer</i>
Mutation B2M	Présente	Absente	NGS (biopsie liquide)	
Typage HLA patient	Homozygotie classe I (dans ≥1 locus)	Hétérozygotie classe I	NGS	
Expression HLA classe I	Diminuée	Normale	Immunohistochimie	
Génotypage KIR *	?	?	NGS	<i>Impact à déterminer</i>

Tableau 1 : proposition de grille de score composite

CONCLUSION DE LA THESE

L'immunothérapie par inhibiteurs de points de contrôle immunitaire a révolutionné le pronostic de nombreux cancers, mais son délai d'action et son inefficacité chez une proportion non négligeable de patients rendent primordial l'identification de facteurs prédictifs de bonne réponse, afin de mieux sélectionner les patients pouvant véritablement en bénéficier.

Nous avons identifié dans la littérature cinq biomarqueurs sanguins émergents, à savoir le taux de PD-L1 soluble, la charge mutationnelle tumorale sanguine, le typage HLA, la bêta-2-microglobuline et le répertoire KIR. Nous avons pu mettre en évidence dans notre revue qu'au moins trois de ces biomarqueurs (PD-L1 soluble, charge mutationnelle tumorale et typage HLA), largement étudiés pour leur impact pronostic, semblent avoir également un caractère prédictif d'une réponse et d'un bénéfice à un traitement par ICI. Les données concernant le répertoire KIR et la bêta-2-microglobuline sont encore peu nombreuses mais encourageantes.

Ces données restent, pour l'instant, insuffisantes pour permettre l'utilisation de ces biomarqueurs en clinique, mais nous semblent suffisamment pertinentes pour développer un protocole de recherche clinique à large échelle, visant à établir un « score » composite sanguin. Ce score pourrait ainsi être réalisé à partir d'une analyse sanguine préthérapeutique et pourrait guider le clinicien dans le choix de la thérapeutique pour le patient.

Clermont-Ferrand, le
Pierre CLAVELOU
Doyen - Directeur



Clermont-Ferrand, le 08 OCT 2011
Le Président du Jury

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(Conseil national de l'ordre des médecins)

SERMENT D'HIPPOCRATE

Au moment d'être admis(e) à exercer la médecine, je promets et je jure d'être fidèle aux lois de l'honneur et de la probité.

Mon premier souci sera de rétablir, de préserver ou de promouvoir la santé dans tous ses éléments, physiques et mentaux, individuels et sociaux.

Je respecterai toutes les personnes, leur autonomie et leur volonté, sans aucune discrimination selon leur état ou leurs convictions. J'interviendrai pour les protéger si elles sont affaiblies, vulnérables ou menacées dans leur intégrité ou leur dignité.

Même sous la contrainte, je ne ferai pas usage de mes connaissances contre les lois de l'humanité.

J'informerai les patients des décisions envisagées, de leurs raisons et de leurs conséquences.

Je ne tromperai jamais leur confiance et n'exploiterai pas le pouvoir hérité des circonstances pour forcer les consciences.

Je donnerai mes soins à l'indigent et à quiconque me les demandera. Je ne me laisserai pas influencer par la soif du gain ou la recherche de la gloire.

Admis(e) dans l'intimité des personnes, je tairai les secrets qui me seront confiés.

Reçu(e) à l'intérieur des maisons, je respecterai les secrets des foyers et ma conduite ne servira pas à corrompre les mœurs.

Je ferai tout pour soulager les souffrances. Je ne prolongerai pas abusivement les agonies. Je ne provoquerai jamais la mort délibérément.

Je préserverai l'indépendance nécessaire à l'accomplissement de ma mission. Je n'entreprendrai rien qui dépasse mes compétences. Je les entretiendrai et les perfectionnerai pour assurer au mieux les services qui me seront demandés.

J'apporterai mon aide à mes confrères ainsi qu'à leurs familles dans l'adversité.

Que les hommes et mes confrères m'accordent leur estime si je suis fidèle à mes promesses ; que je sois déshonoré(e) et méprisé(e) si j'y manque.

SERMENT D'HIPPOCRATE

En présence des Maîtres de cette FACULTE et de mes chers CONDISCIPLES, je promets et je jure d'être fidèle aux lois de l'Honneur et de la Probité dans l'exercice de la Médecine.

Je donnerai mes soins gratuits à l'indigent et je n'exigerai jamais un salaire au-dessus de mon travail. Admis dans l'intérieur des maisons, mes yeux ne verront pas ce qui s'y passe, ma langue taira les secrets qui me seront confiés et mon état ne servira pas à corrompre les mœurs ni à favoriser le crime.

Respectueux et reconnaissant envers mes MAÎTRES, je rendrai à leurs enfants l'instruction que j'ai reçue de leurs pères.

Que les HOMMES m'accordent leur estime si je suis fidèle à mes promesses. Que je sois couvert d'OPPROBRE et méprisé de mes confrères si j'y manque.

Prédiction de la réponse aux inhibiteurs de points de contrôle immunitaire dans le traitement des tumeurs solides : une revue systématique de cinq biomarqueurs sanguins émergents

CONTEXTE : Les cancers restent la deuxième cause de mortalité, malgré des progrès diagnostiques et thérapeutiques majeurs ces dernières années. Les traitements d'immunothérapie par inhibiteurs de points de contrôle immunitaire (ICI, *Immune Checkpoint Inhibitors*) ont ainsi révolutionné le pronostic d'un certain nombre de tumeurs solides. Cependant, une proportion non négligeable de patients ne répondra pas à ce type de traitement, rendant indispensable l'identification de facteurs prédictifs de cette réponse afin d'adapter au mieux la thérapeutique choisie. Or à ce jour, aucun biomarqueur prédictif fiable et reproductible n'a été clairement mis en évidence.

OBJECTIF : rédiger une revue de la littérature concernant cinq biomarqueurs intéressants du fait de leur rôle clé dans la réponse immunitaire antitumorale, et pouvant ainsi potentiellement prédire la réponse aux ICI. Ces cinq biomarqueurs sont le PDL1 soluble, la charge mutationnelle tumorale sanguine, la bêta 2 microglobuline, le système HLA et le génotypage KIR. La rédaction de cette revue avait pour objectif d'établir un rationnel bibliographique pertinent dans le but de développer secondairement un protocole de recherche clinique dans ce domaine.

RESULTATS : les données récentes de la littérature sur ces cinq biomarqueurs concernent essentiellement leur impact pronostique et dans une moindre mesure leur capacité à prédire le bénéfice des traitements d'immunothérapie. Les biomarqueurs les plus étudiés sont le taux de PD-L1 soluble, la charge mutationnelle tumorale sanguine et le système HLA. Même si les résultats sont parfois discordants, il semblerait qu'une meilleure réponse aux ICI soit observée en cas de taux de PD-L1 soluble bas au diagnostic, de charge mutationnelle tumorale sanguine élevée au diagnostic, de typage HLA avec une hétérozygotie maximale en classe I, ainsi qu'en cas d'absence de perte d'hétérozygotie en classe I par les cellules tumorales. Les données concernant le génotypage KIR et la bêta-2-microglobuline sont moins étayées mais semblent tout de même présenter un lien avec la réponse à l'immunothérapie.

CONCLUSION : les données de cette revue nous semblent pertinentes mais insuffisantes à ce jour pour une intégration de ces biomarqueurs en routine. Nous proposons donc de développer un protocole de recherche multicentrique visant à les évaluer de manière prospective et concomitante, afin de pouvoir établir un « score » composite sanguin, pronostique et prédictif de la réponse aux ICI, qui pourrait être facilement appliqué en clinique.

Mots-clés :

- Tumeurs solides
- Inhibiteurs de points de contrôle immunitaire
- Charge mutationnelle tumorale sanguine
- Génotypage KIR
- Biomarqueurs
- PD-L1 soluble
- Système HLA
- Bêta-2-microglobuline