



**HAL**  
open science

# 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

► **To cite this version:**

Quentin Mangon. 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. Human health and pathology. 2021. dumas-03559978

**HAL Id: dumas-03559978**

<https://dumas.ccsd.cnrs.fr/dumas-03559978v1>

Submitted on 7 Feb 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



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

**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**

**Directeur de thèse :** Madame DOUGE Aurore, Docteur, CHU Clermont-Ferrand, Service d'Oncologie Médicale

**Président du jury :** Monsieur BAY Jacques-Olivier, Professeur, UFR de Médecine et des Professions paramédicales de Clermont-Ferrand

**Membres du jury :**

Monsieur RUIVARD Marc, Professeur, UFR de Médecine et des professions paramédicales de Clermont-Ferrand

Monsieur ROUZAIRE Paul-Olivier, Maître de Conférences des Universités, UFR de Pharmacie de Clermont-Ferrand





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

**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**

**Directeur de thèse** : Madame DOUGE Aurore, Docteur, CHU Clermont-Ferrand, Service d'Oncologie Médicale

**Président du jury** : Monsieur BAY Jacques-Olivier, Professeur, UFR de Médecine et des Professions paramédicales de Clermont-Ferrand

**Membres du jury** :

Monsieur RUIVARD Marc, Professeur, UFR de Médecine et des professions paramédicales de Clermont-Ferrand

Monsieur ROUZAIRE Paul-Olivier, Maître de Conférences des Universités, UFR de Pharmacie de Clermont-Ferrand

# UNIVERSITE CLERMONT AUVERGNE

---

PRESIDENTS HONORAIRES  
UNIVERSITE D'AUVERGNE

: **JOYON** Louis  
: **DOLY** Michel  
: **TURPIN** Dominique  
: **VEYRE** Annie  
: **DULBECCO** Philippe  
: **ESCHALIER** Alain

PRESIDENTS HONORAIRES  
UNIVERSITE BLAISE PASCAL

: **CABANES** Pierre  
: **FONTAINE** Jacques  
: **BOUTIN** Christian  
: **MONTEIL** Jean-Marc  
: **ODOUARD** Albert  
: **LAVIGNOTTE** Nadine

PRESIDENT DE L'UNIVERSITE et  
PRESIDENT DU CONSEIL ACADEMIQUE PLENIER  
PRESIDENT DU CONSEIL ACADEMIQUE RESTREINT  
VICE-PRESIDENTE DU CONSEIL D'ADMINISTRATION  
VICE-PRESIDENT DE LA COMMISSION DE LA RECHERCHE  
VICE PRESIDENTE DE LA COMMISSION DE LA  
FORMATION ET DE LA VIE UNIVERSITAIRE  
DIRECTEUR GENERAL DES SERVICES

: **BERNARD** Mathias  
: **DEQUIEDT** Vianney  
: **FOGLI** Anne  
: **HENRARD** Pierre  
  
: **PEYRARD** Françoise  
: **PAQUIS** François



## UFR DE MEDECINE ET DES PROFESSIONS PARAMEDICALES

DOYENS HONORAIRES

: **DETEIX** Patrice  
: **CHAZAL** Jean

DOYEN  
RESPONSABLE ADMINISTRATIVE

: **CLAVELOU** Pierre  
: **ROBERT** Gaëlle

## LISTE DU PERSONNEL ENSEIGNANT

### **PROFESSEURS HONORAIRES :**

MM. BACIN Franck - BEGUE René-Jean - BEYTOUT Jean - BOMMELAER Gilles - BOUCHER Daniel - BUSSIERE Jean-Louis - CANO Noël - CASSAGNES Jean - CATILINA Pierre - CHABANNES Jacques - CHAZAL Jean - CHIPPONI Jacques - CHOLLET Philippe - CITRON Bernard - COUDERT Jean - DASTUGUE Bernard - DAUPLAT Jacques - DECHELOTTE Pierre - DEMEOCQ François - DE RIBEROLLES Charles - DETEIX Patrice - ESCANDE Georges - Mme FONCK Yvette - MM. GENTOU Claude - Mme GLANDDIER Phyllis - MM. IRTIUM Bernard - JACQUETIN Bernard - Mme LAVARENNE Jeanine - MM. LAVERAN Henri - LESOURD Bruno - LEVAI Jean-Paul - MAGE Gérard - MARCHEIX Jean- Claude - MICHEL Jean-Luc - MONDIE Jean-Michel - PHILIPPE Pierre - PLAGNE Robert - PLANCHE Roger - PONSONNAILLE Jean - REY Michel - Mme RIGAL Danièle - MM. ROZAN Raymond - SCHOEFFLER Pierre - SIROT Jacques - RIBAL Jean-Pierre - SOUTEYRAND Pierre - TANGUY Alain - TERVER Sylvain - THIEBLOT Philippe - TOURNILHAC Michel - VANNEUVILLE Guy - VIALLET Jean-François - Mlle VEYRE Annie

### **PROFESSEURS EMERITES :**

MM. AUMAITRE Olivier - BOITEUX Jean-Paul - CHAMOIX Alain - DUBRAY Claude - ESCHALIER Alain - KEMENY Jean-Louis - LABBE André - Mme LAFEUILLE Hélène - MM. LEMERY Didier - LUSSON Jean-René

## **PROFESSEURS DES UNIVERSITES-PRATICIENS HOSPITALIERS**

### **CLASSE EXCEPTIONNELLE**

M. VAGO Philippe	Histologie-Embryologie Cytogénétique
M. AVAN Paul	Biophysique et Traitement de l'Image
M. DURIF Franck	Neurologie
M. BOIRE Jean-Yves	Biostatistiques, Informatique Médicale et Technologies de Communication
M. BOYER Louis	Radiologie et Imagerie Médicale option Clinique
M. POULY Jean-Luc	Gynécologie et Obstétrique
M. CANIS Michel	Gynécologie-Obstétrique
Mme PENAULT-LLORCA Frédérique	Anatomie et Cytologie Pathologiques
M. BAZIN Jean-Etienne	Anesthésiologie et Réanimation Chirurgicale
M. BIGNON Yves Jean	Cancérologie option Biologique
M. BOIRIE Yves	Nutrition Humaine
M. CLAVELOU Pierre	Neurologie
M. GILAIN Laurent	O.R.L.
M. LEMAIRE Jean-Jacques	Neurochirurgie
M. CAMILLERI Lionel	Chirurgie Thoracique et Cardio-Vasculaire
M. DAPOIGNY Michel	Gastro-Entérologie
M. LLORCA Pierre-Michel	Psychiatrie d'Adultes

M.	PEZET Denis	Chirurgie Digestive
M.	SOUWEINE Bertrand	Réanimation Médicale
M.	BOISGARD Stéphane	Chirurgie Orthopédique et Traumatologie
Mme	DUCLOS Martine	Physiologie
M.	SCHMIDT Jeannot	Médecine d'Urgence
M.	BERGER Marc	Hématologie
M.	GARCIER Jean-Marc	Anatomie-Radiologie et Imagerie Médicale
M.	ROSSET Eugénio	Chirurgie Vasculaire
M.	SOUBRIER Martin	Rhumatologie
M.	ABERGEL Armando	Hépatologie
Mle	BARTHELEMY Isabelle	Chirurgie Maxillo-Faciale
M.	RUIVARD Marc	Médecine Interne

### *1ère CLASSE*

M.	CAILLAUD Denis	Pneumo-physiologie
M.	VERRELLE Pierre	Radiothérapie option Clinique
M.	D'INCAN Michel	Dermatologie -Vénérologie
Mme	JALENQUES Isabelle	Psychiatrie d'Adultes
M.	GERBAUD Laurent	Epidémiologie, Economie de la Santé et Prévention
M.	TAUVERON Igor	Endocrinologie et Maladies Métaboliques
M.	MOM Thierry	Oto-Rhino-Laryngologie
M.	RICHARD Ruddy	Physiologie
M.	SAPIN-DEFOUR Vincent	Biochimie et Biologie Moléculaire
M.	BAY Jacques-Olivier	Cancérologie
M.	COUDEYRE Emmanuel	Médecine Physique et de Réadaptation
Mme	GODFRAIND Catherine	Anatomie et Cytologie Pathologiques
M.	LAURICHESSE Henri	Maladies Infectieuses et Tropicales
M.	TOURNILHAC Olivier	Hématologie
M.	CHIAMBARETTA Frédéric	Ophthalmologie
M.	FILAIRE Marc	Anatomie – Chirurgie Thoracique et Cardio-Vasculaire
M.	GALLOT Denis	Gynécologie-Obstétrique
M.	GUY Laurent	Urologie
M.	TRAORE Ousmane	Hygiène Hospitalière
M.	ANDRE Marc	Médecine Interne
M.	BONNET Richard	Bactériologie, Virologie
M.	CACHIN Florent	Biophysique et Médecine Nucléaire
M.	COSTES Frédéric	Physiologie
M.	FUTIER Emmanuel	Anesthésiologie-Réanimation
Mme	HENG Anne-Elisabeth	Néphrologie
M.	MOTREFF Pascal	Cardiologie
Mme	PICKERING Gisèle	Pharmacologie Clinique
M.	RABISCHONG Benoît	Gynécologie Obstétrique
M.	CHABROT Pascal	Radiologie et Imagerie Médicale
M.	DESCAMPS Stéphane	Chirurgie Orthopédique et Traumatologique
Mme	HENQUELL Cécile	Bactériologie Virologie
M.	POMEL Christophe	Cancérologie – Chirurgie Générale

## **2ème CLASSE**

Mme CREVEAUX Isabelle	Biochimie et Biologie Moléculaire
M. FAICT Thierry	Médecine Légale et Droit de la Santé
Mme KANOLD LASTAWIECKA Justyna	Pédiatrie
M. TCHIRKOV Andréï	Cytologie et Histologie
M. CORNELIS François	Génétique
M. LESENS Olivier	Maladies Infectieuses et Tropicales
M. AUTHIER Nicolas	Pharmacologie Médicale
M. BROUSSE Georges	Psychiatrie Adultes/Addictologie
M. BUC Emmanuel	Chirurgie Digestive
M. LAUTRETTE Alexandre	Néphrologie Réanimation Médicale
Mme BRUGNON Florence	Biologie et Médecine du Développement et de la Reproduction
M. ESCHALIER Romain	Cardiologie
M. MERLIN Etienne	Pédiatrie
Mme TOURNADRE Anne	Rhumatologie
M. DURANDO Xavier	Cancérologie
M. DUTHEIL Frédéric	Médecine et Santé au Travail
Mme FANTINI Maria Livia	Neurologie
M. SAKKA Laurent	Anatomie – Neurochirurgie
M. BOURDEL Nicolas	Gynécologie-Obstétrique
M. GUIEZE Romain	Hématologie
M. POINCLOUX Laurent	Gastroentérologie
M. SOUTEYRAND Géraud	Cardiologie
M. EVRARD Bertrand	Immunologie
M. POIRIER Philippe	Parasitologie et Mycologie
Mme PHAM DANG Nathalie	Chirurgie Maxillo-Faciale et Stomatologie
Mme SARRET Catherine	Pédiatrie

## **PROFESSEURS DES UNIVERSITES DE MEDECINE GENERALE**

### **1ère CLASSE**

M. CLEMENT Gilles	Médecine Générale
M. VORILHON Philippe	Médecine Générale

## **PROFESSEURS DES UNIVERSITES**

### **2ème CLASSE**

Mme MALPUECH-BRUGERE Corinne	Nutrition Humaine
------------------------------	-------------------



## PROFESSEURS ASSOCIES DES UNIVERSITES

Mme BOTTET-MAULOUBIER Anne	Médecine Générale
M. CAMBON Benoît	Médecine Générale
M. TANGUY Gilles	Médecine Générale

## MAITRES DE CONFERENCES DES UNIVERSITES - PRATICIENS HOSPITALIERS

### *HORS CLASSE*

Mme CHAMBON Martine	Bactériologie Virologie
Mme BOUTELOUP Corinne	Nutrition
Mme FOGLI Anne	Biochimie Biologie Moléculaire
Mle GOUAS Laetitia	Cytologie et Histologie, Cytogénétique

### *1ère CLASSE*

M. MORVAN Daniel	Biophysique et Traitement de l'Image
Mle GOUMY Carole	Cytologie et Histologie, Cytogénétique
M. MARCEAU Geoffroy	Biochimie Biologie Moléculaire
Mme MINET-QUINARD Régine	Biochimie Biologie Moléculaire
M. ROBIN Frédéric	Bactériologie
Mle VERONESE Lauren	Cytologie et Histologie, Cytogénétique
M. DELMAS Julien	Bactériologie
Mle MIRAND Audrey	Bactériologie Virologie
M. OUCHCHANE Lemlih	Biostatistiques, Informatique Médicale et Technologies de Communication
M. LIBERT Frédéric	Pharmacologie Médicale
Mle COSTE Karen	Pédiatrie
Mle AUMERAN Claire	Hygiène Hospitalière
Mme CASSAGNES Lucie	Radiologie et Imagerie Médicale
M. LEBRETON Aurélien	Hématologie
M. BUISSON Anthony	Gastroentérologie
M. BOUVIER Damien	Biochimie et Biologie Moléculaire
M. MAQDASY Salwan	Endocrinologie, Diabète et Maladies Métaboliques
Mme NOURRISSON Céline	Parasitologie - Mycologie
Mme PONS Hanaë	Biologie et Médecine du Développement et de la Reproduction

**2ème CLASSE**

M.	JABAUDON-GANDET Matthieu	Anesthésiologie – Réanimation Chirurgicale
M.	COLL Guillaume	Neurochirurgie
M.	GODET Thomas	Anesthésiologie-Réanimation et Médecine Péri-Opératoire
M.	LACHAL Jonathan	Pédopsychiatrie
M.	MOUSTAFA Farès	Médecine d'Urgence

**MAITRES DE CONFERENCES DES UNIVERSITES  
DE MEDECINE GENERALE**

**1ère CLASSE**

Mme	VAILLANT-ROUSSEL Hélène	Médecine Générale
-----	-------------------------	-------------------

**2ème CLASSE**

Mme	LAPORTE Catherine	Médecine Générale
-----	-------------------	-------------------

**MAITRES DE CONFERENCES DES UNIVERSITES**

**HORS CLASSE**

M.	BLANCHON Loïc	Biochimie Biologie Moléculaire
M.	MARCHAND Fabien	Pharmacologie Médicale
Mme	VAURS-BARRIERE Catherine	Biochimie Biologie Moléculaire

**CLASSE NORMALE**

M.	BAILLY Jean-Luc	Bactériologie Virologie
Mle	AUBEL Corinne	Oncologie Moléculaire
Mle	GUILLET Christelle	Nutrition Humaine
M.	BIDET Yannick	Oncogénétique
M.	DALMASSO Guillaume	Bactériologie
M.	PIZON Frank	Santé Publique
M.	SOLER Cédric	Biochimie Biologie Moléculaire
M.	GIRAUDET Fabrice	Biophysique et Traitement de l'Image
M.	LOLIGNIER Stéphane	Neurosciences – Neuropharmacologie

Mme MARTEIL Gaëlle  
M. PINEL Alexandre

Biologie de la Reproduction  
Nutrition Humaine

**MAITRES DE CONFERENCES ASSOCIES DES UNIVERSITES**

M. BERNARD Pierre  
Mme ESCHALIER Bénédicte  
Mme RICHARD Amélie  
M. TESSIERES Frédéric  
Mme ROUGE Laure

Médecine Générale  
Médecine Générale  
Médecine Générale  
Médecine Générale  
Médecine Générale

## REMERCIEMENTS

**Monsieur le Professeur Jacques-Olivier BAY**, je vous remercie de me faire l'honneur de présider ce jury. Merci également pour l'incalculable opportunité que vous m'avez donné de pouvoir travailler dans l'Oncologie, s'orienter vers une nouvelle spécialité n'est pas un choix facile mais savoir que ce serait sous votre direction n'a fait que me conforter dans ma décision. Votre empathie et bienveillance envers les patients et le personnel, votre constante accessibilité quand j'en avais besoin et votre immense savoir médical sont des exemples que je m'efforcerai d'atteindre tout au long de ma carrière.

**Madame le Docteur Aurore DOUGE**, il m'est impossible de te remercier suffisamment pour toute ton aide et ton accompagnement tout au long de cette thèse. Tu as toujours été présente pour m'aider, toujours eu les mots justes lorsqu'il fallait, avec une gentillesse et une patience qui n'ont jamais fait défaut. Je pense sincèrement que je n'aurai pu avoir de meilleure directrice de thèse, et si j'arrive à te rendre aussi fière de ce travail que je le suis d'avoir pu le réaliser à tes côtés, alors j'aurai réussi ma thèse. Les réunions de thèse avec une bière me manqueront tout de même. C'est particulièrement intimidant d'envisager prendre ta suite en tant que CCA en Oncologie vu l'excellent travail que tu réalises, mais grâce à toi j'aurai toujours un modèle envers qui me référer.

**Monsieur le Docteur Paul-Olivier ROUZAIRE**, je te suis grandement reconnaissant d'avoir accepté de participer à ce travail. Ton implication tout au long de cette thèse était bien au-delà de ce que je pouvais espérer, avec une bonne humeur, une bienveillance et une rigueur sans lesquels rien de tout ça n'aurait été possible. Ton dynamisme, ta pédagogie et ta bienveillance m'ont permis de retrouver un profond intérêt pour l'immunologie, et je suis certain que mon futur stage dans ton service ne pourra que le confirmer.

**Monsieur le Professeur Marc RUIVARD**, je vous suis extrêmement reconnaissant d'avoir accepté de faire partie de mon jury. Je n'ai malheureusement pas encore eu l'occasion de travailler dans votre service, mais je suis convaincu que ce semestre sera le plus intéressant et formateur de mon internat, ayant pu constater vos immenses connaissances et votre passion pour la Médecine Interne.

# TABLE DES MATIERES

LISTE DES TABLEAUX ET FIGURES .....	10
LISTE DES ABREVIATIONS .....	11
INTRODUCTION .....	12
1. REVUE DE LA LITTERATURE .....	14
1.1. Introduction.....	14
1.2. Results.....	16
1.2.1. <i>Soluble PD-L1</i> .....	16
1.2.2. <i>Blood tumor mutational burden</i> .....	19
1.2.3. <i>HLA typing</i> .....	23
1.2.4. <i>Beta-2-microglobulin</i> .....	27
1.2.5. <i>Killer Immunoglobulin-like Receptors</i> .....	30
1.2.6. <i>Summary of the physiological mechanism of each biomarker</i> .....	32
1.3. Discussion.....	32
1.4. Conclusion.....	37
2. ANALYSE STATISTIQUE : premiers résultats.....	38
3. PROJET DE PROTOCOLE.....	41
CONCLUSION .....	44
REFERENCES BIBLIOGRAPHIQUES .....	45

# LISTE DES TABLEAUX ET FIGURES

## **Tableaux**

Tableau 1 : Proposition de grille de score composite (page 43)

## **Figures**

Figure 1 : Mécanismes physiologiques des biomarqueurs étudiés (page 32)

Figure 2 : Effet de chaque biomarqueur sur la réponse aux ICI (page 37)

Figure 3 : Méta-analyse des données de survie globale en cas de taux élevé de s-PD-L1 (page 39)

Figure 4 : Méta-analyse des données de survie globale en cas de taux élevé de b-TMB (page 39)

Figure 5 : Méta-analyse des données de survie globale selon la présence d'une homozygotie en classe I du système HLA (page 40)

Figure 6 : Méta-analyse des données de survie globale selon l'absence d'une perte d'expression de molécule HLA de classe-I tumorales (page 41)

# 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/mg;  $p < 0.01$ ) (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 ( $\leq 7$ ) and high ( $\geq 14$ ) 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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- $\gamma$ , and IFN- $\gamma$ -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).

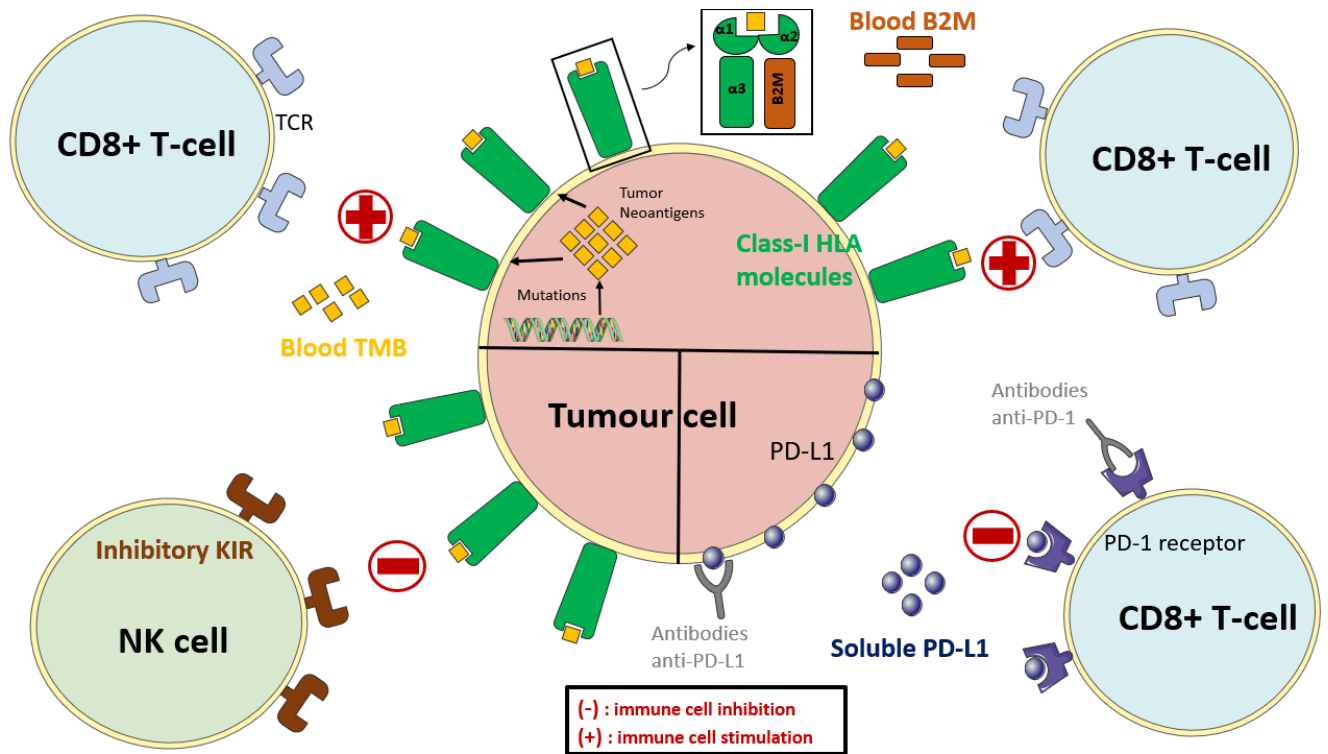
As KIR and KIR-ligands are independently inherited, their vast allelic diversity gives rise to the notions of NK cell repertoire, and diversity in NK cell subset responsiveness. It has even been demonstrated, in autologous stem cell transplantation, that KIR and HLA genotypes association could be predictive 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<sup>+</sup>/HLA-C\*16<sup>+</sup> as a marker of dramatically lower progression-free rate (7.1%, p=0.00001) than KIR2DL5<sup>-</sup>/HLA-C\*16<sup>+</sup> (60.0%) or HLA-C\*16<sup>-</sup> (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<sup>+</sup> 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<sup>+</sup> 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.

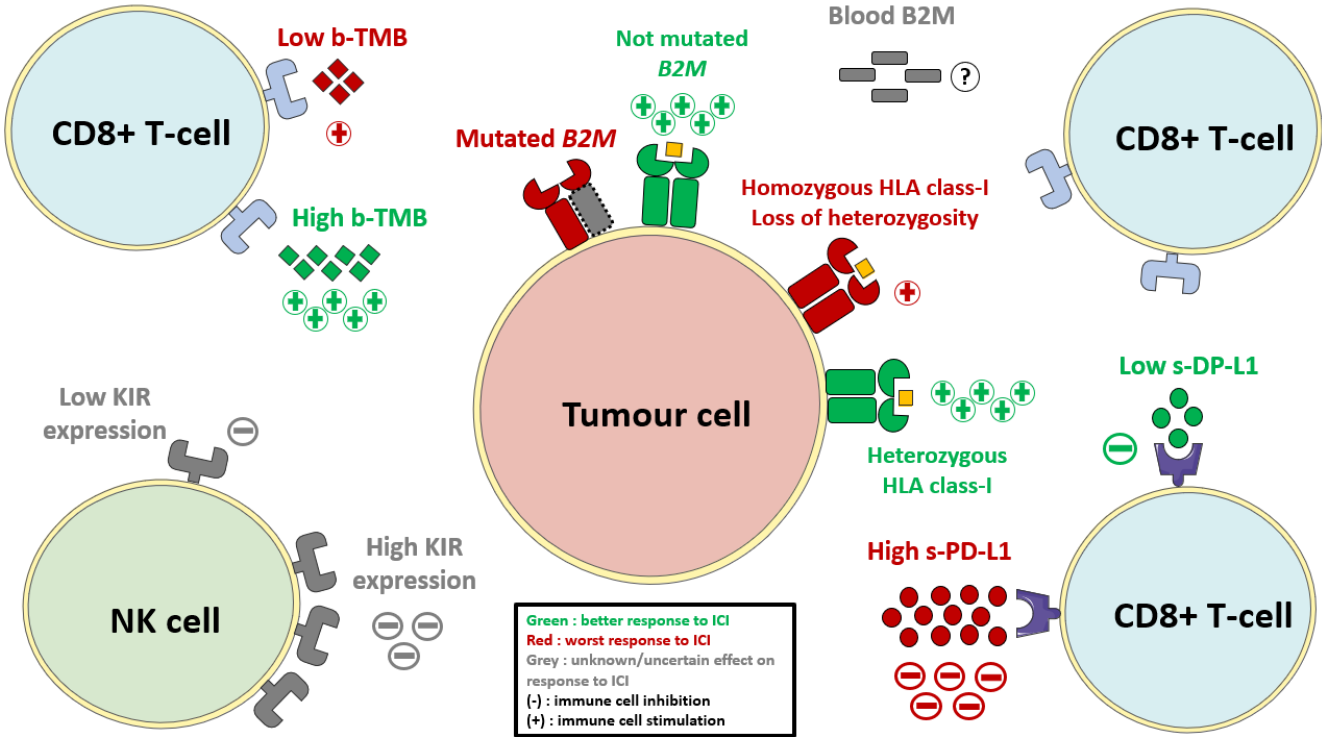
**Killer-cell Immunoglobulin-like Receptors (KIRs)** consist of an array of inhibitory and activator receptors, mainly expressed by NK cells. Most of them recognize HLA-I molecules, which triggers inhibitory signals to the NK cells, and theoretically protects all healthy nucleated cells from lysis from 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.

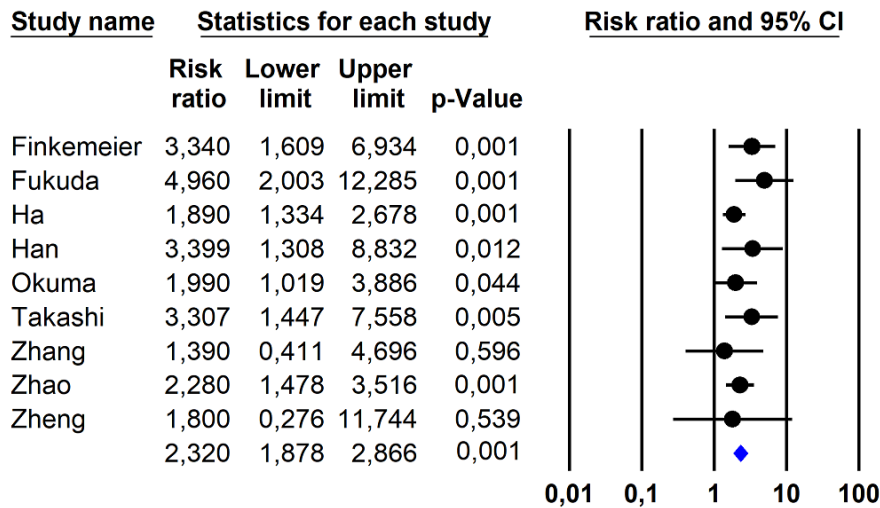
## 2. ANALYSES STATISTIQUES : premiers résultats

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**

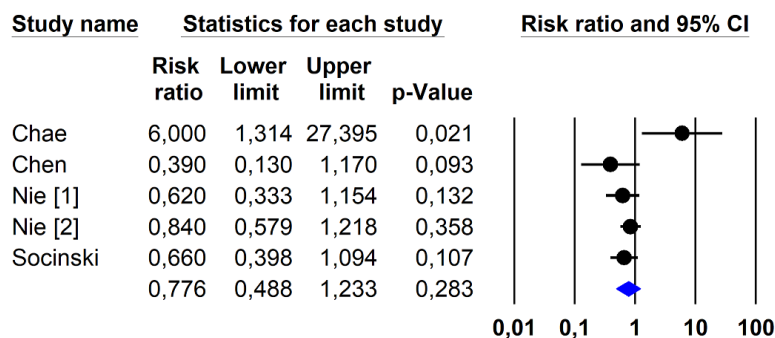
La figure 3 ci-dessous rapporte l'impact d'un taux de sPD-L1 supérieur au seuil désigné dans chaque étude sur le taux de survie globale (OS) exprimé en Hazard Ratio. Cette analyse statistique montre qu'un taux de s-PD-L1 élevé (supérieur au seuil défini) a un impact pronostique péjoratif sur la survie 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**

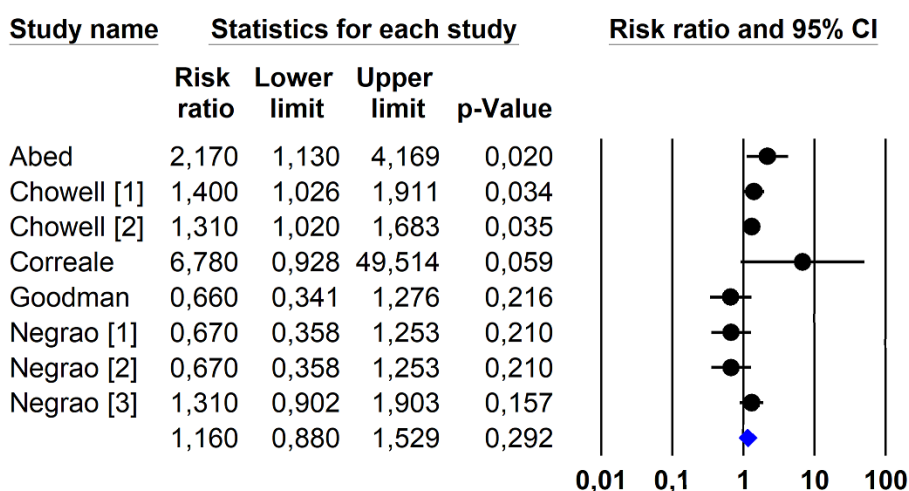
A noter que l'HR de l'étude de Chae et al concerne les patients du groupe b-TMB inférieur au seuil défini dans l'étude, alors que les autres études rapportent le HR des patients du groupe b-

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

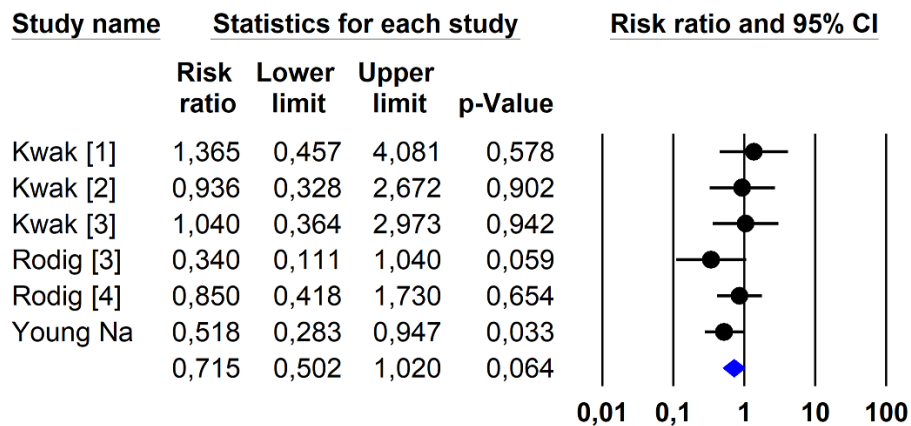
Nous avons réalisé à ce jour deux analyses statistiques sur ce biomarqueur.

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 $\geq 1$ 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

CHU ESTAING - CLERMONT-FERRAND  
Service d'Hématologie Clinique Adultes  
et de Thérapie Cellulaire  
Professeur Jacques-Olivier BAY  
Chef de Service  
N° A.D.E.L.I. : 63 10 4253 8  
N° RPPS : 10003167912  
Tél. : 04.73.750.074 - Fax : 04.73.750.081

UFR de Médecine et des Professions Paramédicales  
Service de la formation – Pôle 4  
TSA 50400  
28, Place Henri-Dunant  
63001 Clermont-Ferrand Cedex 1



## REFERENCES BIBLIOGRAPHIQUES

1. Mattiuzzi C, Lippi G. Current Cancer Epidemiology. *J Epidemiol Glob Health*. déc 2019;9(4):217-22.
2. Cowppli-Bony A, Colonna M, Ligier K, Jooste V, Defossez G, Monnereau A, et al. [Descriptive epidemiology of cancer in metropolitan France: Incidence, survival and prevalence]. *Bull Cancer (Paris)*. août 2019;106(7-8):617-34.
3. Kuzume A, Chi S, Yamauchi N, Minami Y. Immune-Checkpoint Blockade Therapy in Lymphoma. *Int J Mol Sci*. 30 juill 2020;21(15):E5456.
4. Singh S, Hassan D, Aldawsari HM, Molugulu N, Shukla R, Kesharwani P. Immune checkpoint inhibitors: a promising anticancer therapy. *Drug Discov Today*. janv 2020;25(1):223-9.
5. Mok TSK, Wu Y-L, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet Lond Engl*. 4 mai 2019;393(10183):1819-30.
6. Garassino MC, Gadgeel S, Esteban E, Felip E, Speranza G, Domine M, et al. Patient-reported outcomes following pembrolizumab or placebo plus pemetrexed and platinum in patients with previously untreated, metastatic, non-squamous non-small-cell lung cancer (KEYNOTE-189): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol*. mars 2020;21(3):387-97.
7. Paz-Ares L, Vicente D, Tafreshi A, Robinson A, Soto Parra H, Mazières J, et al. A Randomized, Placebo-Controlled Trial of Pembrolizumab Plus Chemotherapy in Patients With Metastatic Squamous NSCLC: Protocol-Specified Final Analysis of KEYNOTE-407. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer*. oct 2020;15(10):1657-69.
8. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Cowey CL, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol*. nov 2018;19(11):1480-92.
9. Migden MR, Rischin D, Schmults CD, Guminski A, Hauschild A, Lewis KD, et al. PD-1 Blockade with Cemiplimab in Advanced Cutaneous Squamous-Cell Carcinoma. *N Engl J Med*. 26 juill 2018;379(4):341-51.
10. Albiges L, Tannir NM, Burotto M, McDermott D, Plimack ER, Barthélémy P, et al. Nivolumab plus ipilimumab versus sunitinib for first-line treatment of advanced renal cell carcinoma: extended 4-year follow-up of the phase III CheckMate 214 trial. *ESMO Open*. nov 2020;5(6):e001079.
11. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. *N Engl J Med*. 21 mars 2019;380(12):1116-27.
12. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob J-J, Cowey CL, et al. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. *N Engl J Med*. 5 oct 2017;377(14):1345-56.
13. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N Engl J Med*. 10 nov 2016;375(19):1823-33.

14. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med.* 22 oct 2015;373(17):1627-39.
15. Yi M, Yu S, Qin S, Liu Q, Xu H, Zhao W, et al. Gut microbiome modulates efficacy of immune checkpoint inhibitors. *J Hematol Oncol* *J Hematol Oncol.* 27 mars 2018;11(1):47.
16. Ready N, Farago AF, de Braud F, Atmaca A, Hellmann MD, Schneider JG, et al. Third-Line Nivolumab Monotherapy in Recurrent SCLC: CheckMate 032. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer.* févr 2019;14(2):237-44.
17. Durgeau A, Virk Y, Corgnac S, Mami-Chouaib F. Recent Advances in Targeting CD8 T-Cell Immunity for More Effective Cancer Immunotherapy. *Front Immunol.* 2018;9:14.
18. Peranzoni E, Ingangi V, Masetto E, Pinton L, Marigo I. Myeloid Cells as Clinical Biomarkers for Immune Checkpoint Blockade. *Front Immunol.* 2020;11:1590.
19. Goldberg MV, Drake CG. LAG-3 in Cancer Immunotherapy. *Curr Top Microbiol Immunol.* 2011;344:269-78.
20. Harjunpää H, Guillerey C. TIGIT as an emerging immune checkpoint. *Clin Exp Immunol.* mai 2020;200(2):108-19.
21. Solinas C, De Silva P, Bron D, Willard-Gallo K, Sangiolo D. Significance of TIM3 expression in cancer: From biology to the clinic. *Semin Oncol.* oct 2019;46(4-5):372-9.
22. Khan M, Zhao Z, Arooj S, Fu Y, Liao G. Soluble PD-1: Predictive, Prognostic, and Therapeutic Value for Cancer Immunotherapy. *Front Immunol.* 2020;11:587460.
23. Guo L, Song P, Xue X, Guo C, Han L, Fang Q, et al. Variation of Programmed Death Ligand 1 Expression After Platinum-based Neoadjuvant Chemotherapy in Lung Cancer. *J Immunother Hagerstown Md* 1997. août 2019;42(6):215-20.
24. Munari E, Zamboni G, Lunardi G, Marchionni L, Marconi M, Sommaggio M, et al. PD-L1 Expression Heterogeneity in Non-Small Cell Lung Cancer: Defining Criteria for Harmonization between Biopsy Specimens and Whole Sections. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer.* août 2018;13(8):1113-20.
25. Dai S, Jia R, Zhang X, Fang Q, Huang L. The PD-1/PD-Ls pathway and autoimmune diseases. *Cell Immunol.* juill 2014;290(1):72-9.
26. Wasén C, Erlandsson MC, Bossios A, Ekerljung L, Malmhäll C, Töyrä Silfverswärd S, et al. *Front Immunol.* 2018;9:1677.
27. Wei W, Xu B, Wang Y, Wu C, Jiang J, Wu C. Prognostic significance of circulating soluble programmed death ligand-1 in patients with solid tumors: A meta-analysis. *Medicine (Baltimore).* janv 2018;97(3):e9617.
28. Zhang J, Gao J, Li Y, Nie J, Dai L, Hu W, et al. Circulating PD-L1 in NSCLC patients and the correlation between the level of PD-L1 expression and the clinical characteristics. *Thorac Cancer.* juill 2015;6(4):534-8.
29. Okuma Y, Hosomi Y, Nakahara Y, Watanabe K, Sagawa Y, Homma S. High plasma levels of soluble programmed cell death ligand 1 are prognostic for reduced survival in advanced lung cancer. *Lung Cancer Amst Neth.* févr 2017;104:1-6.
30. Larrinaga G, Solano-Iturri JD, Errarte P, Unda M, Loizaga-Iriarte A, Pérez-Fernández A, et al. Soluble PD-L1 Is an Independent Prognostic Factor in Clear Cell Renal Cell Carcinoma. *Cancers.* 7 févr 2021;13(4):667.
31. Wang C, Weng M, Xia S, Zhang M, Chen C, Tang J, et al. Distinct roles of programmed death ligand 1 alternative splicing isoforms in colorectal cancer. *Cancer Sci.* janv 2021;112(1):178-93.

32. Han X, Gu Y-K, Li S-L, Chen H, Chen M-S, Cai Q-Q, et al. Pre-treatment serum levels of soluble programmed cell death-ligand 1 predict prognosis in patients with hepatitis B-related hepatocellular carcinoma. *J Cancer Res Clin Oncol*. févr 2019;145(2):303-12.
33. Okuma Y, Wakui H, Utsumi H, Sagawa Y, Hosomi Y, Kuwano K, et al. Soluble Programmed Cell Death Ligand 1 as a Novel Biomarker for Nivolumab Therapy for Non-Small-cell Lung Cancer. *Clin Lung Cancer*. sept 2018;19(5):410-417.e1.
34. Costantini A, Julie C, Dumenil C, Hélias-Rodzewicz Z, Tisserand J, Dumoulin J, et al. Predictive role of plasmatic biomarkers in advanced non-small cell lung cancer treated by nivolumab. *Oncoimmunology*. 2018;7(8):e1452581.
35. Ando K, Hamada K, Watanabe M, Ohkuma R, Shida M, Onoue R, et al. Plasma Levels of Soluble PD-L1 Correlate With Tumor Regression in Patients With Lung and Gastric Cancer Treated With Immune Checkpoint Inhibitors. *Anticancer Res*. sept 2019;39(9):5195-201.
36. Chiarucci C, Cannito S, Daffinà MG, Amato G, Giacobini G, Cutaia O, et al. Circulating Levels of PD-L1 in Mesothelioma Patients from the NIBIT-MESO-1 Study: Correlation with Survival. *Cancers*. 5 févr 2020;12(2):E361.
37. Zhou J, Mahoney KM, Giobbie-Hurder A, Zhao F, Lee S, Liao X, et al. Soluble PD-L1 as a Biomarker in Malignant Melanoma Treated with Checkpoint Blockade. *Cancer Immunol Res*. juin 2017;5(6):480-92.
38. Incorvaia L, Fanale D, Badalamenti G, Porta C, Olive D, De Luca I, et al. Baseline plasma levels of soluble PD-1, PD-L1, and BTN3A1 predict response to nivolumab treatment in patients with metastatic renal cell carcinoma: a step toward a biomarker for therapeutic decisions. *Oncoimmunology*. 27 oct 2020;9(1):1832348.
39. Romero D. Immunotherapy: Relying on quality over quantity. *Nat Rev Clin Oncol*. janv 2018;15(1):6-7.
40. Neoantigen Quality Predicts Immune Response, Survival. *Cancer Discov*. janv 2018;8(1):6.
41. Chen X, Fang L, Zhu Y, Bao Z, Wang Q, Liu R, et al. Blood tumor mutation burden can predict the clinical response to immune checkpoint inhibitors in advanced non-small cell lung cancer patients. *Cancer Immunol Immunother* CII. 25 avr 2021;
42. McNamara MG, Jacobs T, Lamarca A, Hubner RA, Valle JW, Amir E. Impact of high tumor mutational burden in solid tumors and challenges for biomarker application. *Cancer Treat Rev*. sept 2020;89:102084.
43. Fridland S, Choi J, Nam M, Schellenberg SJ, Kim E, Lee G, et al. Assessing tumor heterogeneity: integrating tissue and circulating tumor DNA (ctDNA) analysis in the era of immuno-oncology - blood TMB is not the same as tissue TMB. *J Immunother Cancer*. août 2021;9(8):e002551.
44. Yarchoan M, Hopkins A, Jaffee EM. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. *N Engl J Med*. 21 déc 2017;377(25):2500-1.
45. Zhu J, Zhang T, Li J, Lin J, Liang W, Huang W, et al. Association Between Tumor Mutation Burden (TMB) and Outcomes of Cancer Patients Treated With PD-1/PD-L1 Inhibitions: A Meta-Analysis. *Front Pharmacol*. 2019;10:673.
46. Cristescu R, Mogg R, Ayers M, Albright A, Murphy E, Yearley J, et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. *Science*. 12 oct 2018;362(6411):eaar3593.
47. Chae YK, Davis AA, Agte S, Pan A, Simon NI, Iams WT, et al. Clinical Implications of Circulating Tumor DNA Tumor Mutational Burden (ctDNA TMB) in Non-Small Cell Lung Cancer. *The Oncologist*. juin 2019;24(6):820-8.

48. Siravegna G, Mussolin B, Venesio T, Marsoni S, Seoane J, Dive C, et al. How liquid biopsies can change clinical practice in oncology. *Ann Oncol Off J Eur Soc Med Oncol*. 1 oct 2019;30(10):1580-90.
49. Augustus E, Zwaenepoel K, Siozopoulou V, Raskin J, Jordaens S, Baggerman G, et al. Prognostic and Predictive Biomarkers in Non-Small Cell Lung Cancer Patients on Immunotherapy-The Role of Liquid Biopsy in Unraveling the Puzzle. *Cancers*. 2 avr 2021;13(7):1675.
50. Gandara DR, Paul SM, Kowanetz M, Schleifman E, Zou W, Li Y, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med*. sept 2018;24(9):1441-8.
51. Wang Z, Duan J, Cai S, Han M, Dong H, Zhao J, et al. Assessment of Blood Tumor Mutational Burden as a Potential Biomarker for Immunotherapy in Patients With Non-Small Cell Lung Cancer With Use of a Next-Generation Sequencing Cancer Gene Panel. *JAMA Oncol*. 1 mai 2019;5(5):696-702.
52. Si H, Kuziora M, Quinn KJ, Helman E, Ye J, Liu F, et al. A Blood-based Assay for Assessment of Tumor Mutational Burden in First-line Metastatic NSCLC Treatment: Results from the MYSTIC Study. *Clin Cancer Res Off J Am Assoc Cancer Res*. 15 mars 2021;27(6):1631-40.
53. Ma Y, Li Q, Du Y, Cai J, Chen W, Zhao G, et al. Blood Tumor Mutational Burden as a Predictive Biomarker in Patients With Advanced Non-Small Cell Lung Cancer (NSCLC). *Front Oncol*. 2021;11:640761.
54. Hellmann MD, Paz-Ares L, Bernabe Caro R, Zurawski B, Kim S-W, Carcereny Costa E, et al. Nivolumab plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer. *N Engl J Med*. 21 nov 2019;381(21):2020-31.
55. Nie W, Qian J, Xu M-D, Gu K, Qian F-F, Hu M-J, et al. A non-linear association between blood tumor mutation burden and prognosis in NSCLC patients receiving atezolizumab. *Oncoimmunology*. 2020;9(1):1731072.
56. Georgiadis A, Durham JN, Keefer LA, Bartlett BR, Zielonka M, Murphy D, et al. Noninvasive Detection of Microsatellite Instability and High Tumor Mutation Burden in Cancer Patients Treated with PD-1 Blockade. *Clin Cancer Res Off J Am Assoc Cancer Res*. 1 déc 2019;25(23):7024-34.
57. Kim ST, Cristescu R, Bass AJ, Kim K-M, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med*. sept 2018;24(9):1449-58.
58. Araujo DV, Wang A, Torti D, Leon A, Marsh K, McCarthy A, et al. Applications of Circulating Tumor DNA in a Cohort of Phase I Solid Tumor Patients Treated With Immunotherapy. *JNCI Cancer Spectr*. juin 2021;5(3):pkaa122.
59. Garrido F. HLA Class-I Expression and Cancer Immunotherapy. *Adv Exp Med Biol*. 2019;1151:79-90.
60. Voorter CEM, Gerritsen KEH, Groeneweg M, Wieten L, Tilanus MGJ. The role of gene polymorphism in HLA class I splicing. *Int J Immunogenet*. avr 2016;43(2):65-78.
61. Buhler S, Nunes JM, Sanchez-Mazas A. HLA class I molecular variation and peptide-binding properties suggest a model of joint divergent asymmetric selection. *Immunogenetics*. juill 2016;68(6-7):401-16.
62. Chowell D, Morris LGT, Grigg CM, Weber JK, Samstein RM, Makarov V, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science*. 2 févr 2018;359(6375):582-7.

63. Abed A, Calapre L, Lo J, Correia S, Bowyer S, Chopra A, et al. Prognostic value of HLA-I homozygosity in patients with non-small cell lung cancer treated with single agent immunotherapy. *J Immunother Cancer*. nov 2020;8(2):e001620.
64. Negrao MV, Lam VK, Reuben A, Rubin ML, Landry LL, Roarty EB, et al. PD-L1 Expression, Tumor Mutational Burden, and Cancer Gene Mutations Are Stronger Predictors of Benefit from Immune Checkpoint Blockade than HLA Class I Genotype in Non-Small Cell Lung Cancer. *J Thorac Oncol Off Publ Int Assoc Study Lung Cancer*. juin 2019;14(6):1021-31.
65. Correale P, Saladino RE, Giannarelli D, Giannicola R, Agostino R, Staropoli N, et al. Distinctive germline expression of class I human leukocyte antigen (HLA) alleles and DRB1 heterozygosity predict the outcome of patients with non-small cell lung cancer receiving PD-1/PD-L1 immune checkpoint blockade. *J Immunother Cancer*. juin 2020;8(1):e000733.
66. Marty R, Kaabinejadian S, Rossell D, Slifker MJ, van de Haar J, Engin HB, et al. MHC-I Genotype Restricts the Oncogenic Mutational Landscape. *Cell*. 30 nov 2017;171(6):1272-1283.e15.
67. Goodman AM, Castro A, Pyke RM, Okamura R, Kato S, Riviere P, et al. MHC-I genotype and tumor mutational burden predict response to immunotherapy. *Genome Med*. 19 mai 2020;12(1):45.
68. McGranahan N, Rosenthal R, Hiley CT, Rowan AJ, Watkins TBK, Wilson GA, et al. Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. *Cell*. 30 nov 2017;171(6):1259-1271.e11.
69. Sette A, Sidney J. Nine major HLA class I supertypes account for the vast preponderance of HLA-A and -B polymorphism. *Immunogenetics*. nov 1999;50(3-4):201-12.
70. Kanguane P, Sakharkar MK, Rajaseger G, Bolisetty S, Sivasekari B, Zhao B, et al. A framework to sub-type HLA supertypes. *Front Biosci J Virtual Libr*. 1 janv 2005;10:879-86.
71. Motzer RJ, Robbins PB, Powles T, Albiges L, Haanen JB, Larkin J, et al. Avelumab plus axitinib versus sunitinib in advanced renal cell carcinoma: biomarker analysis of the phase 3 JAVELIN Renal 101 trial. *Nat Med*. nov 2020;26(11):1733-41.
72. Ishida Y, Otsuka A, Tanaka H, Levesque MP, Dummer R, Kabashima K. HLA-A\*26 Is Correlated With Response to Nivolumab in Japanese Melanoma Patients. *J Invest Dermatol*. nov 2017;137(11):2443-4.
73. Ladányi A, Papp E, Mohos A, Balatoni T, Liskay G, Oláh J, et al. Role of the anatomic site in the association of HLA class I antigen expression level in metastases with clinical response to ipilimumab therapy in patients with melanoma. *J Immunother Cancer*. juin 2020;8(1):e000209.
74. Rodig SJ, Gusenleitner D, Jackson DG, Gjini E, Giobbie-Hurder A, Jin C, et al. MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma. *Sci Transl Med*. 18 juill 2018;10(450):eaar3342.
75. Kwak Y, Koh J, Park Y, Hong YJ, Park KU, Kim H-H, et al. Differential prognostic impact of CD8+ T cells based on human leukocyte antigen I and PD-L1 expression in microsatellite-unstable gastric cancer. *Br J Cancer*. avr 2020;122(9):1399-408.
76. Na HY, Park Y, Nam SK, Lee KS, Oh H-K, Kim D-W, et al. Expression of human leukocyte antigen class I and  $\beta$ 2-microglobulin in colorectal cancer and its prognostic impact. *Cancer Sci*. janv 2021;112(1):91-100.
77. Imai D, Yoshizumi T, Okano S, Uchiyama H, Ikegami T, Harimoto N, et al. The prognostic impact of programmed cell death ligand 1 and human leukocyte antigen class I in pancreatic cancer. *Cancer Med*. juill 2017;6(7):1614-26.

78. Bethea M, Forman DT. Beta 2-microglobulin: its significance and clinical usefulness. *Ann Clin Lab Sci.* juin 1990;20(3):163-8.
79. Lifson AR, Hessel NA, Buchbinder SP, O'Malley PM, Barnhart L, Segal M, et al. Serum beta 2-microglobulin and prediction of progression to AIDS in HIV infection. *Lancet Lond Engl.* 13 juin 1992;339(8807):1436-40.
80. Jongvilaikasem S, Sampao S, Kanjanapradit K, Saetang J, Sangkhathat S, Mahattanobon S. Serum  $\beta$ -2 microglobulin levels are associated with distant metastasis in patients with breast cancer. *Mol Clin Oncol.* juin 2021;14(6):118.
81. Stefanovic V, Mitic-Zlatkovic M, Ignjatovic I, Vlahovic P, Hadzi-Djokic J. beta2-microglobulin in patients with urothelial cancer. *World J Urol.* oct 1999;17(5):319-23.
82. Abdul null, Hoosein null. Changes in beta-2 microglobulin expression in prostate cancer. *Urol Oncol.* 1 juill 2000;5(4):168-72.
83. Castro A, Ozturk K, Pyke RM, Xian S, Zanetti M, Carter H. Elevated neoantigen levels in tumors with somatic mutations in the HLA-A, HLA-B, HLA-C and B2M genes. *BMC Med Genomics.* 25 juill 2019;12(Suppl 6):107.
84. Restifo NP, Marincola FM, Kawakami Y, Taubenberger J, Yannelli JR, Rosenberg SA. Loss of functional beta 2-microglobulin in metastatic melanomas from five patients receiving immunotherapy. *J Natl Cancer Inst.* 17 janv 1996;88(2):100-8.
85. Zaretsky JM, Garcia-Diaz A, Shin DS, Escuin-Ordinas H, Hugo W, Hu-Lieskovan S, et al. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. *N Engl J Med.* 1 sept 2016;375(9):819-29.
86. Sade-Feldman M, Jiao YJ, Chen JH, Rooney MS, Barzily-Rokni M, Eliane J-P, et al. Resistance to checkpoint blockade therapy through inactivation of antigen presentation. *Nat Commun.* 26 oct 2017;8(1):1136.
87. Gettinger S, Choi J, Hastings K, Truini A, Datar I, Sowell R, et al. Impaired HLA Class I Antigen Processing and Presentation as a Mechanism of Acquired Resistance to Immune Checkpoint Inhibitors in Lung Cancer. *Cancer Discov.* déc 2017;7(12):1420-35.
88. Germano G, Lu S, Rospo G, Lamba S, Rousseau B, Fanelli S, et al. CD4 T Cell-Dependent Rejection of Beta-2 Microglobulin Null Mismatch Repair-Deficient Tumors. *Cancer Discov.* juill 2021;11(7):1844-59.
89. Park Y, Koh J, Kwak Y, Ahn S-H, Park DJ, Kim H-H, et al. Clinicopathologic significance of human leukocyte antigen class I expression in patients with stage II and III gastric cancer. *Cancer Immunol Immunother CII.* nov 2019;68(11):1779-90.
90. Olbryt M, Rajczykowski M, Widłak W. Biological Factors behind Melanoma Response to Immune Checkpoint Inhibitors. *Int J Mol Sci.* 6 juin 2020;21(11):E4071.
91. Sivori S, Vacca P, Del Zotto G, Munari E, Mingari MC, Moretta L. Human NK cells: surface receptors, inhibitory checkpoints, and translational applications. *Cell Mol Immunol.* mai 2019;16(5):430-41.
92. Falco M, Moretta L, Moretta A, Bottino C. KIR and KIR ligand polymorphism: a new area for clinical applications? *Tissue Antigens.* déc 2013;82(6):363-73.
93. Moretta L, Moretta A. Killer immunoglobulin-like receptors. *Curr Opin Immunol.* oct 2004;16(5):626-33.
94. Kärre K. Natural killer cell recognition of missing self. *Nat Immunol.* mai 2008;9(5):477-80.
95. Marra J, Greene J, Hwang J, Du J, Damon L, Martin T, et al. KIR and HLA genotypes predictive of low-affinity interactions are associated with lower relapse in autologous

hematopoietic cell transplantation for acute myeloid leukemia. *J Immunol Baltim Md* 1950. 1 mai 2015;194(9):4222-30.

96. Guillaumon CF, Gimeno L, Server G, Martínez-Sánchez MV, Escudero JF, López-Cubillana P, et al. Immunological Risk Stratification of Bladder Cancer Based on Peripheral Blood Natural Killer Cell Biomarkers. *Eur Urol Oncol*. avr 2021;4(2):246-55.

97. Ishida Y, Nakashima C, Kojima H, Tanaka H, Fujimura T, Matsushita S, et al. Killer immunoglobulin-like receptor genotype did not correlate with response to anti-PD-1 antibody treatment in a Japanese cohort. *Sci Rep*. 29 oct 2018;8(1):15962.

98. He Y, Bunn PA, Zhou C, Chan D. KIR 2D (L1, L3, L4, S4) and KIR 3DL1 protein expression in non-small cell lung cancer. *Oncotarget*. 13 déc 2016;7(50):82104-11.

99. He Y, Liu S, Mattei J, Bunn PA, Zhou C, Chan D. The combination of anti-KIR monoclonal antibodies with anti-PD-1/PD-L1 monoclonal antibodies could be a critical breakthrough in overcoming tumor immune escape in NSCLC. *Drug Des Devel Ther*. 2018;12:981-6.

## ***(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