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Mechanisms of immune pressure and immune escape in hematological diseases

Simona Pagliuca

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Ecole Doctorale BioSE (Biologie-Santé-Environnement)

**Mechanisms of immune pressure and immune escape in
hematological diseases**

**Mémoire pour l'obtention du diplôme
D'HABILITATION A DIRIGER DES RECHERCHES**

**Par Simona PAGLIUCA
CNU sous-section 47-03**

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*A lei,
luce sempre accesa*

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Abbreviations

HLA: Human leukocyte antigen*

MHC: Major histocompatibility complex*

TCR: T-cell receptor

APC: Antigen presenting cells

CDR: Complementary determining region

HED: HLA evolutionary divergence

HCT: Hematopoietic cell transplantation

GvHD: Graft versus host disease

GvL: Graft versus leukemia

GvH: Graft versus host

CMV: Cytomegalovirus

EBV: Epstein barr virus

MBP: Myelin basic protein

PDB: Protein database

Chr: Chromosome

IMGT: ImMunoGeneTics information system

UTR: Untranslated region

BMF: Bone marrow failure

IAA: Idiopathic aplastic anemia

PNH: Paroxysmal nocturnal hemoglobinuria

AML: Acute myeloid leukemia

MDS: Myelodysplastic syndrome

HC: Healthy controls

TET2: Ten-eleven translocation 2

DNMT: DNA methyl transferase

IDH: Isocitrate dehydrogenase

ASXL1: ASXL Transcriptional Regulator 1

KMT2: histone-lysine N-methyltransferase

EZH2: Enhancer of zeste homolog 2

KDM6A: Lysine Demethylase 6A

PHF6: PHD finger protein 6

EP300: E1A Binding Protein P300

SF3B1: Splicing Factor 3b Subunit 1
SRSF2: Serine/arginine-rich splicing factor 2
U2F1/2: U2 Small Nuclear RNA Auxiliary Factor1/2
ZRSR2: Zinc Finger CCCH-Type, RNA Binding Motif And Serine/Arginine Rich 2
SF1: Splicing Factor 1
PRPF8: Pre-mRNA Processing Factor 8
LUC7L2: LUC7 Like 2, Pre-mRNA Splicing Factor
STAG2: Stromal Antigen 2
SMC3: Structural Maintenance Of Chromosomes 3
RUNX1: RUNX Family Transcription Factor 1
ETV6: ETS Variant Transcription Factor 6
GATA2: GATA Binding Protein 2
CEBPA: CCAAT Enhancer Binding Protein Alpha
IRF1: Interferon Regulatory Factor 1
BCOR: BCL6 Corepressor
JAK2: Janus Kinase 2 MPL: MPL proto-oncogene
CALR: Calreticulin
CSF3R: Colony Stimulating Factor 3 Receptor
NF1: Neurofibromin 1
CBL: Cbl Proto-Oncogene
ATM: ataxia telangiectasia mutated
NPM1: Nucleophosmin 1
SETBP1: SET Binding Protein 1
WT1: Wilms' tumor *gene*
DDX41: DEAD-Box Helicase 41
FLT3: FMS-like tyrosine kinase 3
PD-1: Programmed cell death 1
PD-L1: Programmed cell death-ligand 1
CTLA4: cytotoxic T-lymphocyte-associated protein 4

*Those two terms are used as synonyms

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Summary

The present work aims at presenting my cursus from the earliest phases of my clinical training to the last, more mature stages of my career.

This is a long and intertwined (ongoing) road that is leading me to develop a unique transversal research expertise that finds its application in many fields of immunology and hematology.

Through this essay I will describe my personal and professional path focusing on the clinical training, the research axes and the pedagogic activities developed during these years and the future perspectives. Specifically, I will address all the principal topics of my research background and how I am currently applying them to the ongoing, and future early and long-terms projects. The natural propensity of this research converges towards questions related to the understanding of the immune control in hematological diseases and to the implementation of adapted immunotherapeutic strategies for our patients.

An annex, gathering some introductory concepts of my research work, is available to help the Reader going through the flow of this manuscript.

During these years of intense labor, I had the privilege to know and work closely with several worldwide renewed experts in hematology and immune oncology, who opened up my horizons, learn from the best physician-scientists in the field and progressively develop my expertise.

This journey, through several Countries and Institutions, led me in Nancy, where I am finding a prolific, stimulating and open-mind research and clinical environment able to satisfy the future years of my professional life.

Résumé en Français

Le présent manuscrit vise à présenter mon cursus professionnel depuis les premières phases de ma formation clinique jusqu'aux étapes courantes, plus matures de ma carrière.

C'est un chemin long et entrelacé (en cours) qui me conduit à développer une expertise de recherche transversale unique qui trouve son application dans des nombreux domaines de l'immunologie et de l'hématologie, avec une constellation de travaux dont la propension naturelle converge vers des questions liées à la compréhension du contrôle immunitaire dans les maladies hématologiques et à la mise en place de stratégies immunothérapeutiques adaptées pour les patients.

A travers cet essai je décrirai mon parcours personnel et professionnel clinique, les axes de recherche et les activités pédagogiques développées au cours de ces années et les perspectives futures. Plus précisément, j'aborderai tous les principaux sujets de mon parcours de recherche et comment je les applique actuellement aux projets en cours et futurs à court et à long terme. Une annexe, rassemblant quelques concepts introductifs de mon travail de recherche, est disponible pour aider le Lecteur à parcourir le flux de ce manuscrit.

Au cours de ces années de travail intense, j'ai eu le privilège de connaître et de travailler en étroite collaboration avec plusieurs experts mondiaux, reconnus en hématologie et en immunoncologie, qui m'ont permis d'ouvrir mes horizons, d'apprendre et de développer progressivement mon expertise.

Ce parcours, à travers plusieurs Pays et Institutions, m'a conduit à Nancy, où je trouve un environnement de recherche et clinique prolifique, stimulant et ouvert d'esprit à même de satisfaire les années futures de ma vie professionnelle.

Parcours personnel, intégration dans l'équipe de recherche et résumé des projets

Hématologue italienne à la base, j'ai travaillé pendant quatre ans au sein du service d'hématologie greffe de l'Hôpital Saint Louis à Paris, d'abord en tant qu'interne en mobilité étrangère (2014-2015) et puis comme chef de clinique assistant (Nov 2016-Avr 2019). C'est ainsi que mon grand intérêt pour la recherche dans le domaine de la greffe et des insuffisances médullaires a pu être cultivé. Cet intérêt m'a amené à poursuivre une thèse à l'Université de Paris et à organiser une mobilité aux États-Unis, où j'ai finalisé mes études doctorales qui portent (entre les autres) sur des projets de recherche dans le domaine de l'immunogénétique des rechutes leucémiques post-allogreffe. En raison de ces affinités scientifiques et cliniques, je suis rentrée en contact avec le Professeur Rubio et le Prof. Feugier personnalités très renommées au niveau nationale et internationale, et j'ai commencé à réfléchir sur la possibilité d'être intégrée dans leur superbe équipe. C'est ainsi que j'ai débuté en Novembre 2021 un parcours clinique au sein du Service d'Hématologie en tant que praticien hospitalier contractuel. Malgré mon engagement intensif dans l'activité clinique qui me permet de continuer à cultiver mon expertise dans le domaine de la greffe de cellules souches et des thérapie cellulaires, j'ai commencé, à m'intégrer dans les projets de recherche en cours et à déposer les bases pour des nouveaux projets au sein de l'équipe de recherche CNRS IMOPA.

Tout en gardant les nombreuses collaborations de recherche avec l'établissement américain, je commence donc un parcours de recherche à l'Université de Lorraine focalisée sur les mêmes thématiques immunohématologiques.

Pendant ma période aux USA j'ai pu développer dans le cadre de mes projets de recherches doctorales une expertise dans l'immunogenetique de l'échappement immunitaire dans les pathologies hématologiques exposées à une pression immunitaire (description des travaux de thèse <https://www.theses.fr/2021UNIP7013>). Ces travaux ont permis la soutenance d'une thèse de science en octobre 2021, la publication des nombreux articles scientifiques : (PMID : 34236054, 34748628, 36131910, 34845315, 36499220...) et la présentation de ces projets à des nombreux congrès internationaux avec l'obtention de plusieurs prix scientifiques (voir prochaines sections).

Plus dans le détail voici-dessous un résumé de mes travaux scientifiques pendant les études doctorales.

La variabilité structurelle et fonctionnelle du système HLA constitue la base moléculaire permettant l'élaboration de réponses immunitaires adaptatives efficaces. Cette diversité permet

à une cellule de présenter aux effecteurs immunitaires un répertoire antigénique étendu, et garantit une activité lymphocytaire de type T diversifiée.

Cette variabilité génétique est maintenue par l'action de pressions immunitaires évolutives qui ont historiquement contribué à structurer l'immunité adaptative humaine. En effet une exposition permanente à des pathogènes variés, suite à la nécessité de répondre à un grand nombre d'antigènes, a favorisé l'accumulation d'évènements mutationnels et l'émergence d'un système HLA hyper-polymorphe, théoriquement capable de produire des réponses immunes, efficaces et multiples.³

Tout évènement entraînant une dysfonction de cette variabilité peut être à la base de processus pathologiques, altérant les capacités de présentation antigénique et la réactivité des effecteurs lymphocytaires de type T. Il est bien connu que les configurations germinales des génotypes HLA peuvent influencer le risque de développement de certaines maladies auto-immunes, ou modifier la réponse anti-tumorale ; il est donc aussi envisageable que des anomalies somatiques puissent réduire cette diversité, pouvant ainsi être à la base de mécanismes d'échappement immunologique.^{4,5,6}

L'objectif de ce travail est d'analyser le rôle de la dysfonction moléculaire du complexe majeur d'histocompatibilité ainsi que son impact sur la fonctionnalité du répertoire lymphocytaire T dans un contexte d'hémopathies, en suggérant un rôle central de ce mécanisme dans l'évolution clonale des pathologies bénignes et malignes.

A partir d'une approche multi-omique, nous avons caractérisé le paysage moléculaire des structures engagées dans la présentation antigénique et dans l'activation de l'immunité adaptative au sein de multiples cohortes de patients, ayant comme diagnostic une insuffisance médullaire ou une hémopathie maligne. Nous avons décortiqué au sein de maladies différentes un mécanisme d'évasion immunologique commun associé à des signatures génomiques et transcriptomiques similaires permettant l'échappement immunitaire en réaction à la pression exercée par l'immunité adaptative.

Ces mécanismes ont été explorés dans le détail chez les patients atteints d'aplasie médullaire, paradigme de l'hématopoïèse clonale. Nous avons eu recours à l'échelle de la cellule souche hématopoïétique aux techniques de séquençage haut débit ciblé et du génome entier, ainsi qu'à l'analyse transcriptomique à cellule unique (*single cell RNA sequencing*). Nous avons ainsi pu mettre en évidence le rôle de certaines molécules HLA de classe II dans l'initiation du dysfonctionnement immunologique ainsi que l'implication d'autres modulateurs du remaniement de l'antigène et de la régulation des gènes HLA. Ce travail met en évidence le rôle des mutations somatiques ainsi que la perte d'allèles HLA spécifiques dans le mécanisme

d'évasion des cellules souches hématopoïétiques dans un contexte d'attaque immunologique. Par ailleurs, nous avons pu montrer que ces aberrations génomiques responsables de l'échappement immunitaire ne semblent pas favoriser l'évolution clonale maligne dans le contexte de l'aplasie médullaire.

Dans cette maladie nous avons aussi étudié les structures immunogénétiques possiblement responsables de la susceptibilité auto-immune et de la dysfonction lymphocytaire T. Ce travail a notamment permis d'identifier une signature d'acides aminés spécifiques dans le site de présentation antigénique des gènes HLA DRB1 et DQB1 possiblement impliqués dans la présentation d'auto-antigènes. La caractérisation en parallèle du répertoire T, avec l'utilisation de nouvelles techniques bio-informatiques pouvant suivre la spécificité des clones T, nous a aussi permis de mettre en évidence des clonotypes auto-réactifs et potentiellement cross-réactifs chez ces patients. Ces résultats permettent d'envisager l'identification d'antigènes autologues ainsi que celle des récepteurs lymphocytaire T associés permettant d'expliquer au moins en partie le caractère auto-immune de cette maladie.

Tous ces aspects immunogénétiques ont aussi été étudiés chez des patients atteints d'une hémopathie maligne avec un possible rôle oncogénique des mutations somatiques ou de la perte d'allèle dans le système HLA.

Pour une analyse moléculaire précise des aberrations génomiques dans la région HLA, j'ai personnellement développé, avec la collaboration d'informaticiens et bioinformaticiens, une plateforme bio-informatique capable de détecter des mutations punctiformes, des insertions, des délétions et des altérations du nombre de copies dans ces gènes hyperpolymorphes, à partir de données de séquençage à haut débit ciblé ou du génome entier.

Nous nous sommes aussi intéressées aux patients allogreffés pour des pathologies myéloïdes, chez lesquels les profils de reconstitution du répertoire lymphocytaire T et les mécanismes d'échappement HLA-médiés ont été étudié dans le détail. Dans cette optique nous avons ainsi exploré de manière systématique la dynamique du répertoire T du receveur en la comparant avec celle du donneur à partir de données de séquençage à haut débit. Les échantillons ont été analysés en prégreffe, à 1 mois, 3 mois et 6 mois après la greffe et au moment de la maladie aiguë du greffon contre l'hôte. Cette étude a permis d'analyser les caractéristiques du nouveau répertoire lymphocytaire T après allogreffe, sa diversité intrinsèque, sa spécificité, la superposition et la divergence de ce répertoire vis-à-vis du répertoire du donneur, dans des contextes cliniques particuliers comme la maladie du greffon contre l'hôte ou l'infection à cytomégalovirus. Les analyses que j'ai réalisé au cours de ces études ont permis de confirmer que la diversité du répertoire T diminue au cours du suivi post-greffe dans la majorité des cas,

indépendamment du type de greffe envisagée. La réduction de la diversité du système HLA du receveur et du donneur peut impacter sur l'étendue du répertoire T avec comme conséquence une diminution de la capacité de présentation antigénique. La spécificité antigénique du répertoire du donneur varie aussi avec une tendance à reconnaître les antigènes du soi plus importante dans la période précoce post-allogreffe et dans le contexte de GvHD aiguë, puis dans une phase plus tardive les antigènes tumoraux. Ces aspects illustrent la dynamique des réponses alloréactives après allogreffe de cellules souches hématopoïétiques (CSH).

Nous avons ensuite analysé les mécanismes d'échappement immunitaire et ses implications dans les rechutes des néoplasies myéloïdes après allogreffe avec des techniques de séquençage haut débit, du séquençage RNA total et à cellule unique appliquées sur une large cohorte d'échantillons du diagnostic de la maladie jusqu'à la rechute post- allogreffe. Les données issues de cette étude montrent que les mutations somatiques des gènes HLA sont particulièrement fréquentes dans les rechutes post-allogreffes par rapport aux rechutes post-chimiothérapie ou aux leucémies au moment du diagnostic. Ces événements arrivent souvent dans des situations de rechutes plus tardives possiblement consécutives à la pression immunologique de l'effet « réaction de la greffe contre la leucémie » (*graft-versus-leukemia*) et l'échappement à celui-ci. Il s'y associe aussi une génomique différente par rapport aux patients qui ne sont pas HLA mutés (moins de mutations dans des gènes *drivers* et plus d'évènements dans les gènes associés avec la régulation de l'épigénome).

Ces résultats illustrent comment la dysfonction germinale ou somatique de la diversité HLA peut constituer un mécanisme physiopathologique essentiel à la genèse du dysfonctionnement de l'immunité adaptative, de l'échappement immunitaire, et de la prédisposition à l'auto-immunité. L'une des perspectives cliniques de ce travail est la révision des stratégies immuno-modulatrices dans les désordres hématologiques.

Ces travaux ont un axe de recherche commun : la dysfonction multimodale de la synapse HLA-TCR-antigène et ses implications dans les hémopathies bénignes et malignes. Ce domaine de recherche, initié par la découverte du complexe majeur d'histocompatibilité dans les années 70, est essentiel à la compréhension de la majorité des maladies humaines, qu'elles soient infectieuses, auto-immunes ou néoplasiques. De nombreuses applications thérapeutiques en découlent que ce soit sur le plan vaccinal mais aussi de l'immunothérapie ou de l'immuno-modulation après allogreffe.

Plusieurs articles scientifiques ont été finalisés (voir grille et liste ci-dessous) et plusieurs études collaboratives multicentriques internationales ont été initiées (avec la *European Blood and*

Marrow Transplant Society (EBMT) et le Center for International Blood and Marrow Transplant Research (CIBMTR).

Cette recherche a aussi été l'occasion d'une collaboration scientifique avec une société bio-informatique qui a développé un outil capable de fournir un typage HLA à partir de données de séquençage (ciblé, génome entier et transcriptomique), avec des applications potentielles en clinique. A partir de cet outil, nous avons développé une approche bio-informatique capable de disséquer le paysage mutationnel de la région HLA à partir de multiples sources de données (*Pagliuca et al, Leukemia 2022, Pagliuca et al, Nature Comm 2023*)

Toutes ces thématiques s'intègrent parfaitement dans le programme de recherche conduit au sein de l'équipe 6 de l'IMoPA où j'ai déjà commencé à mettre en place, grâce à l'aide des premiers financements un programme de recherche visant à mieux comprendre les mécanismes immunopathologiques des rechutes post-allogreffe, les déterminants moléculaires des réponses aux thérapies cellulaires, les mécanismes d'auto-réactivité dans les insuffisances médullaires idiopathiques. Ces programmes de recherche seront valorisés avec la participation d'étudiants en M1, M2 ou en thèse de science, en cours de recrutement.

Mon expertise s'intègre au sein de projets en cours dans l'équipe de recherche susmentionnée, avec contribution d'une capacité analytique de type immuno-informatique en participant aux projets de reconstitution immunologique post-allogreffe (M.T. Rubio), de l'impact des polymorphismes HLA dans les greffes non compatibles (A. Aarnick), des signatures immunosuppressives des cellules myéloïdes suppressives (MDSC, M. D'Aveni), des signatures de la sénescence cellulaires des cellules souches mésenchymateuses (CSM, N. De Isla). Tous ces projets sont dans des phases avancée de développement (manuscripts en préparation ou soumis).

1. CURRICULUM VITAE

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WORK EXPERIENCE

- Dates (from – to) **November 2021-to date**
Physician-scientist Hematology Department, CHRU de Nancy (Director Pierre Feugier). Praticien Hospitalier Contractuel (Nov 2021-Oct 2022) with protected research time. Chef de Clinique assistant (3rd year, Nov 2022-Sept 2023). Audition PH le 5/04/2023, PHU since Sept 1st 2023.

Current position : Praticien Hospitalier Universitaire (Associate Professor)

since Sept 1 2023).

Referent for Competence Center for Rare Diseases: Idiopathic and inherited bone marrow failure disorders, CHRU de Nancy

Audition pre-CNU section 47-03 on June 15th 2023.

Full Professorship (PU-PH) nomination : June 2024.

November 2019 to October 2021:

Research Scholar/Post-doctoral fellow in the Translational Hematology and Oncology Research Department, Lerner Research Institute, Cleveland Clinic Foundation. Chair Department: Jaroslaw Maciejewski.

November 2018 to October 2021:

PhD program 'Ecole Doctorale “Hématologie – Oncogénèse – Biothérapies” University Paris Diderot of Paris. Thesis Director: Régis Peffault de Latour, Co-director: Jaroslaw Maciejewski Program Director Raphael Itzykson.

October 2021: Thesis defense at the University of Paris (Saint Louis Research Institute): «Genomic dysfunction of major histocompatibility complex in hematological disorders exposed to an immunological pressure»

July 2016 to Mai 2019

“Chef de Clinique assistant” in Bone Marrow Transplantation Unit at Saint Louis Hospital, Paris, France. Head of Department Gérard Socié.

June 2011 to June 2016:

Resident in Hematology at University Federico II of Naples. (Graduation 50/50 cum laude). Head of department: Fabrizio Pane.

Sept 2015 to June 2016: Clinical internship in acute leukemia and bone marrow transplantation inpatient and outpatient unit at Federico II University of Naples.

Sept 2014 to Sept 2015: Clinical internship in Hematology and Transplantation unit at Saint Louis Hospital, Paris, France.

March 2014 to Aug 2014: Clinical Internship in Myeloproliferative disorders outpatient Unit at Federico II University of Naples.

Sept 2012 to March 2014: Clinical Internship in Bone marrow Transplantation Unit of Federico II University, with acquisition of specific expertise in management of Bone Marrow Failure disorders.

March 2011 to Aug 2012: Clinical Internship in Lymphoproliferative disorders outpatient Unit at Federico II University

Sept 2009 to Sept 2010: Research Internship in molecular Hematology Laboratory of CEINGE institute of Federico II University of Naples.

Sept 2008 to July 2011: Intern student in Hematology Department of Federico II University of Naples. Training in Acute Leukemia.

2011 - 2016:

**EDUCATION AND
TRAINING**

Clinical Board in Hematology in Hematology at University Federico II of Naples.

Residency Thesis: “*Evaluation of novel endpoints as treatment outcome in allogeneic hematopoietic stem cell transplantation: a retrospective joint Naples-Paris study*”. (Graduation 50/50 cum laude).

2014 - 2015:

Master in Allogeneic Hematopoietic stem cell transplantation (Diplome Interuniversitaire: “*Allogreffe de cellule souches hématopoïétiques*”) at Pierre et Marie Curie University of Paris

2010-2015

Complementary Training in hematological cytopathology.

2010 - 2016

Obtention of Italian (2010) and French (2016) medical board.

2004 – 2010:

MD Degree (Graduation 110/110 cum laude) at Federico II University of Naples.

Graduation Thesis: New molecular strategies in lymphoproliferative disorders.

2008 – 2009:

Erasmus project at Université Libre de Bruxelles.

- Clinical and experimental stage in Hematology, Institut Jules Bordet (Bruxelles)
- Clinical stage in Oncologic Surgery, Institut Jules Bordet (Bruxelles)
- Clinical stage in Internal Medicine, Hopital d’Ixelles (Bruxelles)
- Clinical stage in Gynecology, Hopital d’Ixelles. (Bruxelles)

1999 - 2004

High School Diploma, Liceo Classico, sperimentazione linguistica G.B. Vico, 100/100 summa cum laude.

Other:

Basic life support and pre-hospital trauma care competences (BLS-D and PTC). Good Clinical Practice (GCP) training, certified by Novartis Company on may 2014, july 2017, January 2019, February 2021

**PERSONAL SKILLS
AND COMPETENCES**

MOTHER TONGUE

ITALIAN

OTHER LANGUAGES

- Reading skills
- Writing skills
- Verbal skills

ENGLISH
EXCELLENT
EXCELLENT
EXCELLENT

- Reading skills
- Writing skills
- Verbal skills

FRENCH
EXCELLENT
EXCELLENT
EXCELLENT

- Reading skills
- Writing skills
- Verbal skills

SPANISH
GOOD
GOOD
GOOD

SOCIAL SKILLS
AND COMPETENCES

Good social skills. Ability to adapt to very different situations and to team work. Speaker at national and international scientific meetings.

ORGANISATIONAL SKILLS
AND COMPETENCES

Supervision of undergraduated medical students. Proficient in teamwork. Principal investigator and project leader in several national and international clinical and translational studies.

TECHNICAL SKILLS
AND COMPETENCES

Clinical skills:

Management of patients diagnosed with malignant and non-malignant hematological diseases. Expertise in bone marrow failure disorders, autoimmune cytopenias, hematopoietic stem cell transplantation and cellular therapies. Management of patients both in in-patient and out-patient units. Management of acute and late-onset post-transplant complications. Immunomodulatory strategies of patients with refractory hematological disorders.

Research skills:

Solid training in biostatistics and bioinformatics. Excellent knowledge of most used statistical programs: R, SPSS, GraphPad. Knowledge of principal R-bioconductor packages and pipelines for genomic, transcriptomic and methylation analyses. Daily use of R-studio. Excellent knowledge of most used linux-based bioinformatics platforms for genomic studies, with training in NGS sequencing data-preprocessing and variant calling. User of GATK best-practices for variant discovery. Good knowledge of R, Java and Python programming. Excellent knowledge of all the relevant statistical methodologies related to survival and time-to-event outcome analysis with specificity to transplant studies.

ARTISTIC SKILLS
AND COMPETENCES
Music, writing, design, etc.

Diploma in (music) theory and solfeggio at age of 15. Four years Classic Piano studying (1996-2000). Two years experience in choral music (2012-2014).

1.1. PUBLICATIONS

The name of each (co)supervised student/fellow appears underlined>

Selective strong synergism of Ruxolitinib and second generation tyrosine kinase inhibitors to overcome bone marrow stroma related drug resistance in chronic myelogenous leukemia

Concetta Quintarelli, Biagio De Angelis, Santa Errichiello, Simona Caruso, Nicola Esposito, Irene Colavita, Maddalena Raia, Simona Pagliuca, Novella Pugliese, Antonio M. Risitano, Marco Picardi, Luigia Luciano, Giuseppe Saglio, Giovanni Martinelli, Fabrizio Pane
Leuk Res. 2014 Feb;38(2):236-42. doi: 10.1016/j.leukres.2013.11.006. Epub 2013 Nov 15.

Excellent Outcome of Concomitant Intensive Immunosuppression and Eculizumab in Aplastic Anemia /Paroxysmal Nocturnal Hemoglobinuria Syndrome

Serena Marotta, Giovanna Giagnuolo, Santina Basile, Simona Pagliuca, Francesco Grimaldi, Fabrizio Pane and Antonio Maria Risitano
J Hematol Thromb Dis 2014, 2:1 <http://dx.doi.org/10.4172/2329-8790.1000128>

Early ultrasonographic finding of septic thrombophlebitis is the main indicator of central venous catheter removal to reduce infection-related mortality in neutropenic patients with bloodstream infection

M. Picardi, S. Pagliuca, F. Chiurazzi, D. Iula, M. Catania, F. Rossano and F. Pane
Ann Oncol. 2012 Aug;23(8):2122-8. doi: 10.1093/annonc/mdr588. Epub 2012 Jan 6.

Hematopoietic stem cell transplantation for aplastic anemia and paroxysmal nocturnal hemoglobinuria: current evidence and recommendations.

Marotta S, Pagliuca S, Risitano AM.

Expert Rev Hematol. 2014 Dec;7(6):775-89. doi: 10.1586/17474086.2014.967678. Epub 2014 Oct 21. Review.

B-cell non-Hodgkin lymphoma and pseudo-Gaucher cells in a lymph node fine needle aspiration.

Cozzolino I, Picardi M, **Pagliuca S**, Ciancia G, Luigia L, Pettinato G, Vetrani A. *Cytopathology*. 2015 May 28. doi: 10.1111/cyt.12254.

Characteristics and outcomes of Aplastic Anemia in HIV patients: a brief report from Severe Aplastic Anemia Working Party of the European Society of Blood and Bone marrow Transplantation (EBMT)

Simona Pagliuca, Laurence Gérard, Austin Kulasekararaj, Mary Eapen, David Boutboul, Hans Martin, Marco Aurelio Salvino, Cora Knol-Bout' Carlo Dufour' Régis Peffault de Latour and Judith Marsh. *Bone Marrow Transplant*. 2015 Oct 19. doi: 10.1038/bmt.2015.252.

Success of haploidentical hematopoietic stem cells transplantation in the treatment of graft failure.

Pagliuca S, Legendre H, Morin S, Rabian F, Naveau L, Zine M, Wang L, Marchand T, Coman T, Xhaard A, Ferry C, Robin M, De Latour RP, Socié G, de Fontbrune FS. *Ann Hematol*. 2015 Oct 16. (Corresponding author)

Endothelial complications after hematopoietic stem cell transplantation

Simona Pagliuca, Serena Marotta, Antonio M. Risitano. *DCTH - 4•2015 - 197-217*

Long-Term Outcomes of Cord Blood Transplantation from an HLA-Identical Sibling for Patients with Bone Marrow Failure Syndromes: A Report From Eurocord, Cord Blood Committee and Severe Aplastic Anemia Working Party of the European Society for Blood and Marrow Transplantation.

Pagliuca S, Peffault de Latour R, Volt F, Locatelli F, Zecca M, Dalle JH, Comoli P, Vettenranta K, Diaz MA, Reuven O, Bertrand Y, Diaz de Heredia C, Nagler A, Ghavamzadeh A, Sufliarska S, Lawson S, Kenzey C, Rocha V, Dufour C, Gluckman E, Passweg J, Ruggeri A. *Biol Blood Marrow Transplant*. 2017 Nov;23(11):1939-1948. doi: 10.1016/j.bbmt.2017.08.004. Epub 2017 Aug 7.

Combined intensive immunosuppression and eculizumab for aplastic anemia in the context of hemolytic paroxysmal nocturnal hemoglobinuria: a retrospective analysis.

Pagliuca S, Risitano AM, De Fontbrune FS, Robin M, Iori AP, Marotta S, Michonneau D, Villate A, Desmier D, Socié G, De Latour RP. *Bone Marrow Transplant*. 2018 Jan;53(1):105-107. doi: 10.1038/bmt.2017.220. Epub 2017 Nov 13. No abstract available. (Corresponding author)

Utility and Safety of Liver Biopsy in Patients with Undetermined Liver Blood Test Anomalies after Allogeneic Hematopoietic Stem Cell Transplantation: A Monocentric Retrospective Cohort Study.

Ruggiu M, Bedossa P, Rautou PE, Bertheau P, Plessier A, Peffault de Latour R, Robin M, Sicre de Fontbrune F, **Pagliuca S**, Villate A, Xhaard A, Socié G, Michonneau D. *Biol Blood Marrow Transplant*. 2018 Jul 30. pii: S1083-8791(18)30426-9. doi: 10.1016/j.bbmt.2018.07.037. [Epub ahead of print]

Aplastic Anemia & MDS International Foundation (AA&MDSIF): Bone Marrow Failure Disease Scientific Symposium 2018.

Becktell K, Berlyne D, **Pagliuca S**, Pommert L, Prata PH, Margolis D, de Latour RP, Dufour C, Pierri F. *Leuk Res*. 2019 Mar 20;80:19-25. doi: 10.1016/j.leukres.2019.03.003.

Allogeneic reactivity-mediated endothelial cell complications after HSCT: a plea for consensual definitions.

Pagliuca S, Michonneau D, Sicre de Fontbrune F, Sutra Del Galy A, Xhaard A, Robin M, Peffault de Latour R, Socié G.
Blood Adv. 2019 Aug 13;3(15):2424-2435. doi: 10.1182/bloodadvances.2019000143. Review.

EBV-associated post-transplant lymphoproliferative disease in patients who received anti-CD 20 after hematopoietic stem cell transplantation.

Pagliuca S, Bommier C, Michonneau D, Meignin V, Salmona M, Robin M, Prata PH, Xhaard A, de Fontbrune FS, Feghoul L, Dhedin N, de Latour RP, Caillat-Zucman S, Goff JL, Socié G.
Biol Blood Marrow Transplant. 2019 Aug 14. pii: S1083-8791(19)30520-8. doi: 10.1016/j.bbmt.2019.08.006.

A monocentric study of steroid-refractory acute graft-versus-host disease treatment with tacrolimus and mTOR inhibitor.

Xhaard A, Launay M, Sicre de Fontbrune F, Michonneau D, Sutra Del Galy A, Coman T, **Pagliuca S**, Dhedin N, Robin M, Peffault de Latour R, Socié G.
Bone Marrow Transplant. 2019 Aug 14. doi: 10.1038/s41409-019-0633-y.

Cord blood transplantation for bone marrow failure syndromes: state of art.

Pagliuca S, Ruggeri A, Peffault de Latour R.
Stem Cell Investig. 2019 Dec 5;6:39. doi: 10.21037/sci.2019.10.04. eCollection 2019.
(Corresponding author)

Elastography improves accuracy of early hepato-biliary complications diagnosis after allogeneic stem cell transplantation.

Debureau PE, Bourrier P, Rautou PE, Zagdanski AM, De Boutiny M, **Pagliuca S**, Sutra Del Galy A, Robin M, Peffault de Latour R, Plessier A, Sicre de Fontbrune F, Xhaard A, de Lima Prata PH, Valla D, Socié G, Michonneau D.
Haematologica. 2020 Jul 30;haematol.2019.245407. doi: 10.3324/haematol.2019.245407.

Thrombocytapheresis and sequential chemotherapy for extreme symptomatic thrombocytosis secondary to myelofibrosis: a case report.

Debureau PE, Brignier A, Soret-Dulphy J, Réa D, Michonneau D, Peffault de Latour R, Sicre de Fontbrune F, **Pagliuca S**, Parquet N, Prata HP, Marouf A, Xhaard A, Kiladjian JJ, Socié G, Robin M.
Ann Hematol. 2020 Apr;99(4):897-898. doi: 10.1007/s00277-020-03948-0. Epub 2020 Feb 7.

Human-Derived α 1-Antitrypsin is Still Efficacious in Heavily Pretreated Patients with Steroid-Resistant Gastrointestinal Graft-versus-Host Disease.

Giannoni L, Morin F, Robin M, Peyneau M, Schlageter MH, Desmier D, **Pagliuca S**, Sutra Del Galy A, Sicre de Fontbrune F, Xhaard A, Dhedin N, Moins-Teisserenc H, Peffault de Latour R, Socié G, Michonneau D.
Biol Blood Marrow Transplant. 2020 May 25:S1083-8791(20)30315-3. doi: 10.1016/j.bbmt.2020.05.014. Online ahead of print.

Long-term outcomes and risk factor analysis of steroid-refractory graft versus host disease after hematopoietic stem cell transplantation.

Pagliuca S, Prata PH, Xhaard A, Frieri C, Giannoni L, Sutra Del Galy A, Brignier A, Sicre de Fontbrune F, Michonneau D, Dhedin N, Peffault de Latour R, Socié G, Robin M.

Bone Marrow Transplant. 2020 Jun 25. doi: 10.1038/s41409-020-0977-3. Online ahead of print.
PMID: 32587336

Deciphering the Therapeutic Resistance in Acute Myeloid Leukemia.

Gurnari C, **Pagliuca S**, Visconte V.

Int J Mol Sci. 2020 Nov 12;21(22):8505. doi: 10.3390/ijms21228505.

PMID: 33198085 Free PMC article. Review.

Frequency and perturbations of various peripheral blood cell populations before and after eculizumab treatment in paroxysmal nocturnal hemoglobinuria.

Gurnari C, Graham AC, Efanov A, **Pagliuca S**, Durrani J, Awada H, Patel BJ, Lichtin AE, Visconte V, Sekeres MA, Maciejewski JP.

Blood Cells Mol Dis. 2021 Mar;87:102528. doi: 10.1016/j.bcmed.2020.102528. Epub 2020 Dec 8.

PMID: 33341510

Novel invariant features of Good syndrome.

Gurnari C, Durrani J, Pagliuca S, Kishtagari A, Awada H, Kerr CM, Adema V, Kongkiatkamon S, Lichtin AE, Patel BJ, Visconte V, Sekeres MA, Cook JR, Maciejewski JP.

Leukemia. 2021 Jan 7. doi: 10.1038/s41375-020-01114-z. Online ahead of print.

PMID: 33414481

Point-of-care ultrasound with handheld devices in hematology: a monocentric single-stage phase II study.

Pagliuca S, Bailly C, Talbot A, Bertinchamp R, Peyrony O, Elezi A, Bourrier P, Lengline E.

Leuk Lymphoma. 2021 Jan 8:1-9. doi: 10.1080/10428194.2020.1869958.

PMID: 33416408

Implication of PIGA genotype on erythrocytes phenotype in Paroxysmal Nocturnal Hemoglobinuria.

Gurnari C, Pagliuca S, Patel BJ, Awada H, Kongkiatkamon S, Terkawi L, Zawit M, Corey S, Lichtin AE, Carraway HE, Wahida A, Visconte V, Maciejewski JP.

Leukemia. 2021 Jan 22. doi: 10.1038/s41375-020-01113-0.

PMID: 33483614 No abstract available.

Clonal trajectories and cellular dynamics of myeloid neoplasms with SF3B1 mutations.

Awada H, Kerr CM, Durmaz A, Adema V, Gurnari C, Pagliuca S, Zawit M, Kongkiatkamon S, Rogers HJ, Sauntharajah Y, Sekeres MA, Carraway H, Maciejewski JP, Visconte V.

Leukemia. 2021 Feb 18. doi: 10.1038/s41375-021-01176-7.

PMID: 33603144

Friend or foe? The case of Wilms' Tumor 1 (WT1) mutations in acute myeloid leukemia.

Awada H, Durmaz A, Gurnari C, Kishtagari A, Zawit M, Pagliuca S, Visconte V.

Blood Cells Mol Dis. 2021 Feb 20;88:102549. doi: 10.1016/j.bcmed.2021.102549. Online ahead of print.

PMID: 33636567

Molecular Targeted Therapy in Myelodysplastic Syndromes: New Options for Tailored Treatments

Pagliuca S, Gurnari C, Visconte V.

Cancers 2021, 13(4), 784; <https://doi.org/10.3390/cancers13040784>

The Interactome between Metabolism and Gene Mutations in Myeloid Malignancies.

Gurnari C, **Pagliuca S**, Visconte V. *Int J Mol Sci.* 2021 Mar 19;22(6):3135. doi: 10.3390/ijms22063135. PMID: 33808599

Vacuolization of hematopoietic precursors: an enigma with multiple etiologies.

Gurnari C, **Pagliuca S**, Durkin L, Terkawi L, Awada H, Kongkiatkamon S, Zawit M, Hsi ED, Carraway HE, Rogers HJ, Visconte V, Maciejewski JP. *Blood.* 2021 Mar 9;blood.2021010811. doi: 10.1182/blood.2021010811. PMID: 33690844

A Therapeutic Strategy for Preferential Targeting of TET2 Mutant and TET-dioxygenase Deficient Cells in Myeloid Neoplasms.

Guan Y, Tiwari AD, Phillips JG, Hasipek M, Grabowski DR, **Pagliuca S**, Gopal P, Kerr CM, Adema V, Radivoyevitch T, Parker Y, Lindner DJ, Meggendorfer M, Abazeed M, Sekeres MA, Mian OY, Haferlach T, Maciejewski JP, Jha BK. *Blood Cancer Discov.* 2021 Mar;2(2):146-161. doi: 10.1158/2643-3230.BCD-20-0173. Epub 2020 Dec 7. PMID: 33681816

A non-cytotoxic regimen of decitabine to treat refractory T-cell large granular lymphocytic leukemia.

Zawit M, Gurnari C, **Pagliuca S**, Awada H, Maciejewski JP, Sauntharajah Y. *Clin Case Rep.* 2021 Sep 15;9(9):e04533. doi: 10.1002/ccr3.4533. eCollection 2021 Sep. PMID: 34552731 Free PMC article.

Monoclonal IgM gammopathy in adult acquired pure red cell aplasia: culprit or innocent bystander?

Gurnari C, **Pagliuca S**, Awada H, Zawit M, Patel BJ, Visconte V, Valent J, Rogers HJ, Maciejewski JP. *Blood Cells Mol Dis.* 2021 Nov;91:102595. doi: 10.1016/j.bcmed.2021.102595. Epub 2021 Jul 14. PMID: 34280665 No abstract available.

Clinical and basic implications of dynamic T cell receptor clonotyping in hematopoietic cell transplantation.

Pagliuca S, Gurnari C, Hong S, Zhao R, Kongkiatkamon S, Terkawi L, Zawit M, Guan Y, Awada H, Kishtagari A, Kerr CM, LaFramboise T, Patel BJ, Jha BK, Carraway HE, Visconte V, Majhail NS, Hamilton BK, Maciejewski JP. *JCI Insight.* 2021 Jul 8;6(13):e149080. doi: 10.1172/jci.insight.149080. PMID: 34236054 Free PMC article.

The Similarity of Class II HLA Genotypes Defines Patterns of Autoreactivity in Idiopathic Bone Marrow Failure Disorders.

Pagliuca S, Gurnari C, Awada H, Kishtagari A, Kongkiatkamon S, Terkawi L, Zawit M, Guan Y, LaFramboise T, Jha BK, Patel BJ, Hamilton BK, Majhail NS, Lundgren S, Mustjoki S, Sauntharajah Y, Visconte V, Chan T, Yang CY, Lenz T, Maciejewski JP. *Blood.* 2021 Nov 5;blood.2021012900. doi: 10.1182/blood.2021012900. Online ahead of print. PMID: 34748628

Pegcetacoplan versus Eculizumab in PNH.

Gurnari C, **Pagliuca S**, Maciejewski JP.
N Engl J Med. 2021 Oct 28;385(18):1725. doi: 10.1056/NEJMc2106424.
PMID: 34706184 No abstract available.

Molecular characterization of the histone acetyltransferase CREBBP/EP300 genes in myeloid neoplasia.

Kongkiatkamon S, **Pagliuca S**, Adema V, Nagata Y, Kerr CM, Walter W, Awada H, Hutter S, Gurnari C, Rogers HJ, Meggendorfer M, Sauntharajah Y, Haferlach T, Visconte V, Maciejewski JP.
Leukemia. 2021 Nov 29. doi: 10.1038/s41375-021-01479-9. Online ahead of print.
PMID: 34845315 No abstract available.

Current Opinions on the Clinical Utility of Ravulizumab for the Treatment of Paroxysmal Nocturnal Hemoglobinuria.

Gurnari C, Nautiyal I, **Pagliuca S**.
Ther Clin Risk Manag. 2021 Dec 14;17:1343-1351. doi: 10.2147/TCRM.S273360. eCollection 2021.
PMID: 34934322 Free PMC article. Review.

Alternative Splicing in Myeloid Malignancies.

Gurnari C, **Pagliuca S**, Visconte V.
Biomedicines. 2021 Dec 6;9(12):1844. doi: 10.3390/biomedicines9121844.
PMID: 34944660 Review.

Eltrombopag inhibits TET dioxygenase to contribute to hematopoietic stem cell expansion in aplastic anemia. Guan Y, Hasipek M, Jiang D, Tiwari AD, Grabowski DR, **Pagliuca S**, Kongkiatkamon S, Patel B, Singh S, Parker Y, LaFramboise T, Lindner D, Sekeres MA, Mian OY, Sauntharajah Y, Maciejewski JP, Jha BK. J Clin Invest. 2022 Feb 15;132(4):e149856. doi: 10.1172/JCI149856. PMID: 35085104 Free PMC article.

Is nature truly healing itself? Spontaneous remissions in Paroxysmal Nocturnal Hemoglobinuria. Gurnari C, **Pagliuca S**, Kewan T, Bahaj W, Mori M, Patel BJ, Visconte V, Maciejewski JP. Blood Cancer J. 2021 Nov 27;11(11):187. doi: 10.1038/s41408-021-00582-5. PMID: 34839350 Free PMC article. No abstract available.

TET2 mutations as a part of DNA dioxygenase deficiency in myelodysplastic syndromes.

Gurnari C, **Pagliuca S**, Guan Y, Adema V, Hershberger CE, Ni Y, Awada H, Kongkiatkamon S, Zawit M, Coutinho DF, Zalberg IR, Ahn JS, Kim HJ, Kim DDH, Minden MD, Jansen JH, Meggendorfer M, Haferlach C, Jha BK, Haferlach T, Maciejewski JP, Visconte V. Blood Adv. 2022 Jan 11;6(1):100-107. doi: 10.1182/bloodadvances.2021005418. PMID: 34768283 Free PMC article.

Personalized Risk Schemes and Machine Learning to Empower Genomic Prognostication Models in Myelodysplastic Syndromes.

Awada H, Gurnari C, Durmaz A, Awada H, **Pagliuca S**, Visconte V. Int J Mol Sci. 2022 Mar 3;23(5):2802. doi: 10.3390/ijms23052802. PMID: 35269943 Free PMC article. Review.

Rare germline alterations of myeloperoxidase predispose to myeloid neoplasms.

Kongkiatkamon S, Terkawi L, Guan Y, Adema V, Hasipek M, Dombrowski T, Co M, Walter W, Awada H, Parker Y, Hutter S, **Pagliuca S**, Gurnari C, Rogers HJ, Meggendorfer M, Lindner DJ, Haferlach T, Visconte V, LaFramboise T, Jha BK, Maciejewski JP.
Leukemia. 2022 Jun 27. doi: 10.1038/s41375-022-01630-0. PMID: 35761024

Clinical and Molecular Determinants of Clonal Evolution in Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria.

Gurnari C#, **Pagliuca S**#, Prata PH#, Galimard JE, Catto LFB, Larcher L, Sebert M, Allain V, Patel BJ, Durmaz A, Pinto AL, Inacio MCB, Hernandez L, Dhedin N, Caillat-Zucman S, Clappier E, Sicre de Fontbrune F, Voso MT, Visconte V, Peffault de Latour R, Soulier J, Calado RT, Socié G, Maciejewski JP. *J Clin Oncol.* 2022 Sep 2;JCO2200710. doi: 10.1200/JCO.22.00710. PMID: 36054881

#First authors.

Pattern of somatic mutation changes after allogeneic hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes.

Hong S, Rybicki L, Gurnari C, **Pagliuca S**, Zhang A, Thomas D, Visconte V, Durrani J, Sobecks RM, Kalaycio M, Gerds AT, Carraway HE, Mukherjee S, Sekeres MA, Advani AS, Majhail NS, Hamilton BK, Patel BJ, Maciejewski JP. *Bone Marrow Transplant.* 2022 Jul 27. doi: 10.1038/s41409-022-01762-y. Online ahead of print. PMID: 35896698

Individual HLA heterogeneity and its implications for cellular immune evasion in cancer and beyond

Simona Pagliuca*, Carmelo Gurnari, Marie Thérèse Rubio, Valeria Visconte and Tobias L. Lenz *Front. Immunol.*, 05 September 2022 <https://doi.org/10.3389/fimmu.2022.944872> (Corresponding author)

UBA1 Screening in Sweet Syndrome With Hematological Neoplasms Reveals a Novel Association Between VEXAS and Chronic Myelomonocytic Leukemia.

Gurnari C, Mannion P, Pandit I, **Pagliuca S**, Voso MT, Maciejewski JP, Visconte V, Rogers HJ. *Hemasphere.* 2022 Sep 27;6(10):e775. doi: 10.1097/HS9.0000000000000775. eCollection 2022 Oct. PMID: 36187875

Molecular landscape of immune pressure and escape in aplastic anemia.

Pagliuca S, Gurnari C, Hercus C, Hergalant S, Nadarajah N, Wahida A, Terkawi L, Mori M, Zhou W, Visconte V, Spellman S, Gadalla SM, Zhu C, Zhu P, Haferlach T, Maciejewski JP. *Leukemia.* 2022 Oct 17. doi: 10.1038/s41375-022-01723-w. Online ahead of print. PMID: 36253429

Digging into the HLA pockets: A new association with acute leukaemias.

Gurnari C, **Pagliuca S**. *Br J Haematol.* 2022 Nov 7. doi: 10.1111/bjh.18529. Online ahead of print. PMID: 36342469

Filling the Gap: The Immune Therapeutic Armamentarium for Relapsed/Refractory Hodgkin Lymphoma.

Hazane Leroyer E, Ziegler C, Moulin C, Campidelli A, Jacquet C, Rubio MT, Feugier P, **Pagliuca S**. *J Clin Med.* 2022 Nov 6;11(21):6574. doi: 10.3390/jcm11216574. PMID: 36362802 (Corresponding author)

Comprehensive Transcriptomic Analysis of VISTA in Acute Myeloid Leukemia: Insights into Its Prognostic Value.

Pagliuca S, Gurnari C, Zhang K, Kewan T, Bahaj W, Mori M, Nautiyal I, Rubio MT, Ferraro F, Maciejewski JP, Wang L, Visconte V.
Int J Mol Sci. 2022 Nov 28;23(23):14885. doi: 10.3390/ijms232314885.
PMID: 36499220

Outcome prediction in myelodysplastic neoplasm undergoing hematopoietic cell transplant in the molecular era of IPSS-M.

Gurnari C, Gagelmann N, Badbaran A, Awada H, Dima D, **Pagliuca S**, D'Aveni-Piney M, Attardi E, Voso MT, Cerretti R, Wolschke C, Rubio MT, Maciejewski JP, Kröger N.
Leukemia. 2023 Jan 28. doi: 10.1038/s41375-023-01820-4. Online ahead of print.
PMID: 36709353

Editorial: Endocrinological sequelae of hematopoietic stem cell transplantation.

Giudice V, Gurnari C, **Pagliuca S**, Castaldi MA, Selleri C. Front Endocrinol (Lausanne). 2023 Feb 14;14:1151213. doi: 10.3389/fendo.2023.1151213. eCollection 2023.PMID: 36864835

Impact of TP53 on outcome of patients with myelofibrosis undergoing hematopoietic stem cell transplantation.

Gagelmann N, Badbaran A, Salit RB, Schroeder T, Gurnari C, **Pagliuca S**, Panagiota V, Rautenberg C, Cassinat B, Thol FR, Wolschke C, Robin M, Heuser M, Rubio MT, Maciejewski JP, Reinhardt HC, Scott BL, Kröger N. Blood. 2023 Mar 20;blood.2023019630. doi: 10.1182/blood.2023019630. Online ahead of print.PMID: 36940410

A multimodal analysis of genomic and RNA splicing features in myeloid malignancies.

Durmaz A, Gurnari C, Hershberger CE, **Pagliuca S**, Daniels N, Awada H, Awada H, Adema V, Mori M, Ponvilawan B, Kubota Y, Kewan T, Bahaj WS, Barnard J, Scott J, Padgett RA, Haferlach T, Maciejewski JP, Visconte V. iScience. 2023 Feb 18;26(3):106238. doi: 10.1016/j.isci.2023.106238. eCollection 2023 Mar 17.PMID: 36926651

Efficacy of haematopoietic stem cell boost as a rescue for poor graft function after haematopoietic stem cell transplantation: A multicentre retrospective study on behalf of the Francophone Society for Bone Marrow Transplantation and Cell Therapy (SFGM-TC).

Gaffet M, Wiedemann A, Dalle JH, Bilger K, Forcade E, Robin M, Cornillon J, Labussière-Wallet H, Ceballos P, Bulabois CE, Loschi M, Orvain C, Rubio MT, Neven B, **Pagliuca S**,* Pochon C*. Br J Haematol. 2023 Mar 27. doi: 10.1111/bjh.18744. Online ahead of print.PMID: 36974355 (*Co-last)

IPSS-M in myelodysplastic neoplasms arising from aplastic anemia and paroxysmal nocturnal hemoglobinuria.

Gurnari C, Prata PH, Catto LFB, Durmaz A, Larcher L, Sébert M, Allain V, Kewan T, **Pagliuca S**, Pinto AL, B Inacio MC, Hernandez L, Dhedin N, Caillat-Zucman S, Clappier E, Sicre de Fontbrune F, Voso MT, Visconte V, Peffault de Latour R, Soulier J, Socie G, Calado RT, Maciejewski JP. Blood. 2023 Apr 13;blood.2023020108. doi: 10.1182/blood.2023020108. Online ahead of print.PMID: 37053552

Age-related immune cell dynamics influence outcomes after allogeneic haematopoietic cell transplantation.

Jandin A, Pochon C, Campidelli A, D'Aveni M, Kicki C, Notarantonio AB, Roth Guepin G, Mbuyi TA, Feugier P, Chastagner P, Schweitzer C, de Carvalho Bittencourt M, Rubio MT, **Pagliuca S**. Br J Haematol. 2023 Apr 24. doi: 10.1111/bjh.18822. Online ahead of print.PMID: 37092504
(Corresponding author)

Leukemia relapse via genetic immune escape after allogeneic hematopoietic cell transplantation.

Pagliuca S, Gurnari C, Hercus C, Hergalant S, Hong S, Dhuyser A, D'Aveni M, Aarnink A, Rubio MT, Feugier P, Ferraro F, Carraway HE, Sobecks R, Hamilton BK, Majhail NS, Visconte V, Maciejewski JP. *Nat Commun.* 2023 May 31;14(1):3153. doi: 10.1038/s41467-023-38113-4. PMID: 3725854

Current use of androgens in bone marrow failure disorders: a report from the Severe Aplastic Anemia Working Party of the European Society of Blood and Marrow Transplantation.

Pagliuca S, Kulasekararaj AG, Eikema DJ, Piepenbroek B, Iftikhar R, Satti TM, Griffin M, Laurino M, Kupesiz A, Bertrand Y, Fattizzo B, Yakoub-Agha I, Aljurf M, Corti P, Massacesi E, Lioure B, Calabuig M, Klammer M, Unal E, Wu D, Chevallier P, Forcade E, Snowden JA, Ozdogu H, Risitano A, De Latour RP. *Haematologica.* 2023 May 18. doi: 10.3324/haematol.2023.282935. Online ahead of print. PMID: 37199126 (**Corresponding author**)

Molecular patterns identify distinct subclasses of myeloid neoplasia.

Kewan T, Durmaz A, Bahaj W, Gurnari C, Terkawi L, Awada H, Ogbue OD, Ahmed R, **Pagliuca S**, Awada H, Kutoba Y, Mori M, Ponvilawan B, Al-Share B, Patel BJ, Carraway HE, Scott J, Balasubramanian SK, Bat T, Madanat Y, Sekeres MA, Haferlach T, Visconte V, Maciejewski JP. *Nat Commun.* 2023 May 30;14(1):3136. doi: 10.1038/s41467-023-38515-4. PMID: 37253784

ARTICLES SUBMITTED AND UNDER REVIEW

Systemic complement activation influences outcomes after allogeneic hematopoietic cell transplantation: a prospective French multicenter trial

Anne Béatrice Notarantonio^{1*}, Maud D'aveni-Piney^{1,2*°}, **Simona Pagliuca¹**, Yayha Ashraf³, Jacques-Emanuel Galimard⁴, Aliénor Xhaard⁵, Ambroise Marçais⁶, Felipe Suarez⁶, Eolia Brissot⁷, Pierre Feugier¹, Saik Urien⁸, Naim Bouazza⁸, Sébastien Jacquelin², Tchao Meatchi⁹, Patrick Bruneval⁹, Véronique Frémeaux-Bacchi³, Régis Peffault De Latour⁵, Olivier Hermine^{6,2*}, Marie Agnès Durey-Dragon^{3*}, Marie-Thérèse Rubio^{1,2*°} (under 2nd round of review in *American Journal of Hematology*) **IF: 13.265**

Clonal evolution in aplastic anemia: failed tumor surveillance or maladaptive recovery?

Carmelo Gurnari^{1,2*}, Simona Pagliuca^{3,4*}, Jaroslaw P. Maciejewski (Accepted *Leukemia and Lymphoma*) **IF 2.996**

THE MANY FACETS OF IMMUNE-MEDIATED THROMBOCYTOPENIA: PRINCIPLES OF IMMUNOBIOLOGY AND IMMUNOTHERAPY.

Thomas Moulinet, Anthony Moussu, Ludovic Pierson, Simona Pagliuca Review article (Submitted upon invitation in *Blood Reviews*, under review) **IF 10.8 (Corresponding author)**

1.2. SELECTED ABSTRACTS (S. Pagliuca as first, last or presenting author)

AMD3100 SYNERGIZES WITH FLUDARABINE INCREASING THE APOPTOTIC RATE IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

Pagliuca S, Rinaldi P, Alagia A, Pane F, Rinaldi CR

University of Naples Federico II/CEINGE-Biotecnologie Avanzate, Italy.

VEBEP PLUS RADIOTHERAPY REGIMEN IN NEWLY DIAGNOSED EARLY-UNFAVORABLE STAGE HODGKIN LYMPHOMA

S Pagliuca, M Raimondo, S Basile, M Di Perna, N Pugliese, F Pane, M Picardi,*
70 | haematologica | 2013; 98(s1) Poster abstract 44° CONGRESSO SIE

IRON COMPARTIMENTALISATION IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA (PNH) PATIENTS DURING ANTI-C5 TREATMENT

Pagliuca S, Basile S, Cangiano A, Marotta S, Cardano F, Ricci P, Iori AP, Notaro R, Pane F, Imbraco M, Matarazzo M, Risitano AM
haematologica | 2013; 98(s3) | 15 Poster abstract 44° CONGRESSO SIE

OUTCOMES OF CORD BLOOD TRANSPLANTATION FROM AN HLA-IDENTICAL SIBLING FOR PATIENTS WITH INHERITED OR ACQUIRED BONE MARROW FAILURE DISORDERS: A REPORT FROM EUROCORD, CORD BLOOD COMMITTEE (CBC-CTIWP) AND SEVERE APLASTIC ANEMIA WORKING PARTY (SAAWP) OF THE EUROPEAN GROUP OF BLOOD AND BONE MARROW TRANSPLANTATION (EBMT).

Simona Pagliuca, Régis Peffault De Latour, Franco Locatelli, Prof, Jean-Hugues Dalle, Kim Vettenranta, Miguel Angel Diaz, Or Reuven, Cristina Diaz De Heredia, Arnon Nagler, Ardeshir Ghavamzadeh, Sabina Sufliarska, Sarah Lawson, Chantal Kenzey, Vanderson Rocha, Carlo Dufour, Eliane Gluckman, Jakob Passweg, Annalisa Ruggeri.
ORAL ABSTRACT 57TH ASH MEETING

FEASIBILITY AND SAFETY OF BONE MARROW HARVEST IN SUBARACHNOID ANAESTHESIA: A SINGLE CENTER EXPERIENCE OF 140 HEALTHY DONORS

S. Pagliuca, S. Marotta, F. Grimaldi², C. Maglione, M. Di Fraia, A. Raiola, I. Pezzullo, B. Serio, P. Ricci, F. Pane, C. Selleri, G. De rosa, A. Risitano. BMT: 2016; Issue P427, page S341 Poster abstract 42nd EBMT Meeting

LONG-TERM FOLLOW UP OF PATIENTS WITH IMMUNE-MEDIATED BONE MARROW FAILURE SYNDROMES TREATED WITH ALEMTUZUMAB-BASED IMMUNOSUPPRESSION

S. Pagliuca, S. Marotta, F. Grimaldi, P. Ricci, L. Luciano, A. Risitano
BMT: 2016; Issue P709, page S494 Poster abstract 42nd EBMT Meeting

EVALUATION OF GRAFT VERSUS HOST DISEASE AND RELAPSE FREE SURVIVAL AS NOVEL ENDPOINT IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: A RETROSPECTIVE JOINT NAPLES-PARIS STUDY

Simona Pagliuca, Antonio M Risitano, Sylvie Chevret, Flore Sicre de Fontbrune, Alienor Xhaard, Tereza Coman, David Michonneau, Serena Marotta, Mathilde Ruggiu, Swann Bredin, Régis Peffault de Latour, Gérard Socié and Marie Robin
Blood 2016 128:2285; POSTER ABSTRACT 58TH ASH MEETING (Abstract Achievement award)

OUTCOMES OF HEMATOPOIETIC STEM CELL TRANSPLANTATION AFTER CHRONIC GRAFT VERSUS HOST DISEASE AND EVALUATION OF REVISED

FAILURE FREE SURVIVAL IN A RETROSPECTIVE ANALYSIS FROM TWO REFERRAL CENTERS.

Simona Pagliuca, Flore Sicre De Fontbrune, Antonio M Risitano, David Michonneau, Alban Villate, Rudy Birsen, Camille Rouzaud, Aurelien Sutra del Galy, Nabergoj Mitja, Regis Peffault De Latour, Gérard Socié and Marie Robin

Blood 2017 130:4578; POSTER ABSTRACT 59TH ASH MEETING (Abstract Achievement award)

EVALUATION OF NOVEL ENDPOINTS AS TREATMENT OUTCOME IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: A COMPARISON OF "CLASSICAL" AND "REFINED" DEFINITION OF GRAFT-VERSUS HOST DISEASE FREE AND RELAPSE FREE SURVIVAL FOR REGISTRY-BASED STUDIES

Simona Pagliuca, Antonio M Risitano, Flore Sicre De Fontbrune, David Michonneau, Alban Villate, Rudy Birsen, Camille Rouzaud, Aurelien Sutra del Galy, Nabergoj Mitja, Regis Peffault De Latour, Marie Robin and Gérard Socié

Blood 2017 130:3311; POSTER ABSTRACT 59TH ASH MEETING

IMMUNOGENOMICS OF APLASTIC ANEMIA: IMMUNE-ESCAPE MEDIATED BY CLASS I/II HLA SOMATIC MUTATIONS. S. Pagliuca Oral presentation. NDDK Bioinformatic exploration in hematology September 2020

EVOLUTIONARY DIVERGENCE OF CLASS I AND II HLA GENES AS PREDICTOR OF SEVERITY AND RESPONSE TO IMMUNE-SUPPRESSION IN PATIENTS DIAGNOSED WITH APLASTIC ANEMIA/PAROXYSMAL NOCTURNAL HEMOGLOBINURIA. Poster presentation. AAMDS Symposium July 2020.

IMPACT OF HLA EVOLUTIONARY DIVERGENCE ON CLINICAL FEATURES OF PATIENTS WITH APLASTIC ANEMIA AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA.

Simona Pagliuca, Carmelo Gurnari, Diego Chowell, Chirag Krishna, Bhumika J. Patel, Hassan Awada, Cassandra M Kerr, Sunisa Kongkiatkamon, Ashwin Kishtagari, Thomas Laframboise, Yogenthiran Sauntharajah, Babal K. Jha, Valeria Visconte, Timothy Chan, and Jaroslaw Maciejewski. ORAL ABSTRACT 62nd ASH MEETING

LEUKEMIA RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: FROM RECAPITULATION/ ACQUISITION OF LEUKEMOGENIC HITS TO IMMUNE ESCAPE DUE TO SOMATIC CLASS I/ II HLA MUTATIONS.

Simona Pagliuca, Carmelo Gurnari, Sanghee Hong, , Cassandra M Kerr, Sunisa Kongkiatkamon, Hassan Awada, Ashwin Kishtagari, Misam Zawit, Laila Terkawi, Valeria Visconte, Eric D. Hsi, Betty K. Hamilton, Hetty E. Carraway, Navneet S. Majhail, and Jaroslaw P. Maciejewski. POSTER ABSTRACT 62nd ASH MEETING

IMMUNOGENOMICS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: A MODEL OF IMMUNE ESCAPE. Carmelo Gurnari, **Simona Pagliuca**, Cassandra M Kerr, Hassan Awada, Sunisa Kongkiatkamon, Laila Terkawi, Neetha Parameswaran, Misam Zawit, Adam Wahida, Valeria Visconte, and Jaroslaw P. Maciejewski. POSTER ABSTRACT 62nd ASH MEETING

IMPLICATION OF PIGA GENOTYPE ON CLINICAL FEATURES OF PNH.

Carmelo Gurnari, **Simona Pagliuca**, Bhumika J. Patel, Hassan Awada, Cassandra M Kerr, Sunisa Kongkiatkamon, Laila Terkawi^{1*}, Misam Zawit, Valeria Visconte, Adam Wahida and Jaroslaw P. Maciejewski, POSTER ABSTRACT 62nd ASH MEETING

INHIBITION OF CRITICAL DNA DIOXYGENASE ACTIVITY IN IDH1/2 MUTANT MYELOID NEOPLASMS.

Simona Pagliuca, Yihong Guan, Anand D. Tiwari, Dale Grabowski, Carmelo Gurnari, Cassandra M Kerr, Sunisa Kongkiatkamon, Metis Hasipek, Laila Terkawi, Misam Zawit, Hassan Awada, Valeria Visconte, PYogenthiran Saunthararajah, James G Phillips, Jaroslaw P. Maciejewski, and Babal K. Jha. POSTER ABSTRACT 62nd ASH MEETING

IMMUNOGENOMICS OF APLASTIC ANEMIA: THE ROLE OF HLA SOMATIC MUTATIONS AND THE HLA EVOLUTIONARY DIVERGENCE. **Simona Pagliuca**, Carmelo Gurnari, Hassan Awada, Cassandra M Kerr, Bhumika J. Patel, Laila Terkawi, Misam Zawit, Sunisa Kongkiatkamon, Pedro H Prata, Ashwin Kishtagari, Adam Wahida, Thomas Laframboise, Valeria Visconte, Regis Peffault De Latour, and Jaroslaw P. Maciejewski. POSTER ABSTRACT 62nd ASH MEETING

TCR REPERTOIRE DYNAMICS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MYELOID MALIGNANCIES

Simona Pagliuca, Gurnari, S. Hong, R. Zhao, S. Kongkiatkamon, L. Terkawi, M. Zawit, Y. Guan, A. Kishtagari, H. Awada, C. M. Kerr, T. LaFramboise, B. J. Patel, B. K. Jha, H. E. Carraway, V. Visconte, N. S. Majhail, B. K. Hamilton, and J. P. Maciejewski ORAL Abstract 47th EBMT meeting

HLA EVOLUTIONARY DIVERGENCE INFLUENCES CHARACTERISTICS AND OUTCOMES OF PATIENTS WITH APLASTIC ANEMIA AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Simona Pagliuca, MD Carmelo Gurnari, Bhumika Patel, Hassan Awada, Ashwin Kisthagari, Cassandra Kerr, Sunisa Kongkiatkamon, Laila Terkawi, Misam Zawit, Thomas LaFramboise, Valeria Visconte, Jaroslaw Maciejewski. ORAL Abstract 47th EBMT meeting (EBMT best young abstract award)

IMMUNE ESCAPE DRIVEN BY SOMATIC MUTATIONS IN CLASS I/ II HLA ALLELES IN AML RELAPSING AFTER ALLOGENEIC HSCT: A PIECE OF THE PUZZLE

Simona Pagliuca, MD Carmelo Gurnari MD, Sanghee Hong MD, Cassandra M. Kerr MS, Sunisa Kongkiatkamon MD, Misam Zawit MBBS, Laila Terkawi MS, Valeria Visconte PhD, Betty Hamilton MD, Eric D. Hsi MD, Hetty E. Carraway, MD, Navneet S. Majhail MD, and Jaroslaw P. Maciejewski, MD, PhD. ORAL Abstract 47th EBMT meeting (EBMT best young abstract award and presidential session)

IMMUNE ESCAPE VIA CLASS I AND II HLA SOMATIC MUTATIONS IN MYELOYDYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA RELAPSING AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

Simona Pagliuca, MD Carmelo Gurnari MD, Sanghee Hong MD, Cassandra M. Kerr MS, Sunisa Kongkiatkamon MD, Misam Zawit MBBS, Laila Terkawi MS, Valeria Visconte PhD, Betty Hamilton MD, Eric D. Hsi MD, Hetty E. Carraway, MD, Navneet S. Majhail MD, and Jaroslaw P. Maciejewski, MD, PhD. ORAL ABSTRACT MDS FOUNDATION MEETING 2021 (Tito Bastianello AWARD)

LO STUDIO DELLE DISFUNZIONI DI TET2 SVELA IMPLICAZIONI CLINICO-PROGNOSTICHE IN PAZIENTI AFFETTI DA MDS. Simona Pagliuca ORAL 48° SIE Meeting

TRAIETTORIE DI EVOLUZIONE CLONALE DOPO ANEMIA APLASTICA ED EMOGLOBINURIA PAROSSISTICA NOTTURNA. Simona Pagliuca ORAL 48° SIE Meeting

LO STUDIO DELLE DISFUNZIONI DI TET2 SVELA IMPLICAZIONI CLINICO-PROGNOSTICHE IN PAZIENTI AFFETTI DA MDS. Simona Pagliuca ORAL 48° SIE Meeting

SPECTRUM OF MOLECULAR MODES OF IMMUNE ESCAPE IN IDIOPATHIC APLASTIC ANEMIA AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

Simona Pagliuca, Carmelo Gurnari Colin Hercus, Niroshan Nadarajah, Adam Wahida, Laila Terkawi, Sunisa Kongkiatkamon, Misam Zawit, Tariq Kewan, Waled Bahaj, Babal K Jha, Yihong Guan, Minako Mori, Caiying Zhu, Valeria Visconte, Ping Zhu, Torsten Haferlach, Jaroslaw Maciejewski ORAL ABSTRACT 63nd ASH MEETING (ASH IPIG-AWARD)

MOLECULAR SIGNATURES OF IMMUNE PRESSURE AND IMMUNE ESCAPE IN HEMATOLOGICAL MALIGNANCIES Simona Pagliuca, Carmelo Gurnari, Tariq Kewan1, Waled Bahaj, Minako Mori, Laila Terkawi, Ishani Nautiyal, Valeria Visconte, Jaroslaw Maciejewski. POSTER ABSTRACT 63nd ASH MEETING

IMMUNOGENETIC DRIVERS IN POST-TRANSPLANT ACUTE MYELOID LEUKEMIA RECURRENCE: GENOMIC DYSFUNCTION OF HUMAN LEUKOCYTE ANTIGEN DIVERSITY Simona Pagliuca, Carmelo Gurnari, Laila Terkawi, Ishani Pandit, Lisa Rybicki, Tariq Kewan, Waled Bahaj, Minako Mori, Suresh Balasubramanian, Ashwin Kishtagari, Hetty E. Carraway, Betty K. Hamilton, Navneet S. Majhail, Valeria Visconte and Jaroslaw P. Maciejewski POSTER ABSTRACT 63nd ASH MEETING

THE IMMUNOLOGICAL SYNOPSIS OF ACUTE MYELOID LEUKEMIA RELAPSING AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION: PLEIOTROPY OF IMMUNE ESCAPE –

Simona Pagliuca Oral 48th EBMT meeting

GENOMIC DYSFUNCTION OF HUMAN LEUKOCYTE ANTIGEN DIVERSITY AS DRIVER OF POST-TRANSPLANT ACUTE MYELOID LEUKEMIA RECURRENCE – Oral 48th EBMT meeting

Simona Pagliuca Oral 48th EBMT meeting

A TRANSCRIPTOMIC-BASED RATIONAL APPROACH FOR CONSIDERING VISTA AS A NEW MOLECULAR TARGET IN ACUTE MYELOID LEUKEMIA– Simona Pagliuca Oral 48th EBMT meeting

Current Use of Androgens in Bone Marrow Failure Disorders: A Report from the Severe Aplastic Anemia Working Party (SAAWP) of the European Society of Blood and Marrow Transplantation (EBMT)

Simona Pagliuca, Austin Kulasekararaj, Dirk-Jan Eikema, Brian Piepenbroek, Raheel Iftikhar, Tariq Mahmood Satti, Morag Griffin, Marica Laurino, O. Alphan Kupesiz, Yives Bertrand, Bruno Fattizzo,

Ibrahim Yakoub-Agha, Mahmoud Aljurf, Paola Corti, Erika Massaccesi, Bruno Lioure, Marisa Calabuig, Matthias Klammer, Emel Unal, Depei Wu, Patrice Chevallier, Edouard Forcade, John A. Snowden, Hakan Ozdogu, Antonio Risitano, Régis Peffault de Latour. POSTER ABSTRACT 64th ASH MEETING

GENOMIC LANDSCAPE OF MYELOID SARCOMA: A PLEA FOR BETTER MOLECULAR CHARACTERIZATION AND A ROAD MAP FOR TARGETED THERAPIES

C. Collignon¹, A.-B. Notarantonio¹, G. Roth Guepin¹, C. Bonmati¹, M. D'Aveni¹, A. Campidelli¹, C. Moulin¹, C. Jacquet¹, C. Pochon¹, M. Muller¹, M. Divoux¹, C. Gurnari², V. Visconte³, T. Kewan³, F. Ferraro⁴, P. Feugier¹, M.T. Rubio¹, F. Grimaldi⁵, **S. Pagliuca**¹
POSTER PRESENTATION 49th EBMT MEETING

AGE-RELATED DYNAMICS OF IMMUNE CELL RECONSTITUTION INFLUENCE OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION

Jandin¹, C. Pochon¹, A. Campidelli¹, A.-B. Notarantonio¹, M. D'Aveni¹, C. Kicki¹, G. Roth-Guepin¹, T. Anita Mbuyi¹, M. De Carvalho Bittencourt¹, C. Schweitzer¹, P. Feugier¹, P. Chastagner¹, M.-T. Rubio¹, **S. Pagliuca**¹
POSTER PRESENTATION 49th EBMT MEETING,
POSTER PRESENTATION ASH MEETING 2022,
ORAL PRESENTATION SFGM-TC 2022

Current Use of Androgens in Bone Marrow Failure Disorders: A Report from the Severe Aplastic Anemia Working Party (SAAWP) of the European Society of Blood and Marrow Transplantation (EBMT)

Simona Pagliuca, Austin Kulasekararaj, Dirk-Jan Eikema, Brian Piepenbroek, Raheel Iftikhar, Tariq Mahmood Satti, Morag Griffin, Marica Laurino, O. Alphan Kupesiz, Yives Bertrand, Bruno Fattizzo, Ibrahim Yakoub-Agha, Mahmoud Aljurf, Paola Corti, Erika Massaccesi, Bruno Lioure, Marisa Calabuig, Matthias Klammer, Emel Unal, Depei Wu, Patrice Chevallier, Edouard Forcade, John A. Snowden, Hakan Ozdogu, Antonio Risitano, Régis Peffault de Latour. ORAL Presentation 49th EBMT MEETING

1.3. COLLABORATIONS

Prechemotherapy risk factors for invasive fungal diseases: prospective analysis of 1,192 patients with newlydiagnosed acute myeloid leukemia (SEIFEM 2010-a multicenter study).

Haematologica. 2015 Feb;100(2):284-92. doi: 10.3324/haematol.2014.113399.

Stem cell transplantation in severe congenital neutropenia: an analysis from the European Society for Blood and Marrow Transplantation.

Blood. 2015 Oct 15;126(16):1885-92; quiz 1970. doi: 10.1182/blood-2015-02-628859. Epub 2015 Jul 16.

Evaluation of the practice of antifungal prophylaxis use in patients with newly diagnosed acute myeloid leukemia: results from the SEIFEM 2010-B registry. PMID: 22955439, DOI: 10.1093/cid/cis773

Nocardia Infections in Hematopoietic Cell Transplant Recipients: A Multicenter International Retrospective Study of the Infectious Diseases Working Party of the European Society for Blood and Marrow Transplantation
34596213 DOI: 10.1093/CID/CIAB866

1.4. SIGAPS SCORE
(In bold contribution as a PI or co-PI)

PMID	Nb Aut.	Revue	Année	IF	Catég.	Coeff. Cat.	Pos.	Coeff. Pos.	Score	Score Frac.	Type
37258544	17	Nat Commun	2023	17.694	A	8	1	4	32	1.1428572	Journal Article
37253784	24	Nat Commun	2023	17.694	A	8	k	1	8	0.2285714	Journal Article
37199126	26	Haematologica	2023	11.047	A	8	1	4	32	0.8648649	Journal Article
37092504	14	Br J Haematol	2023	8.615	B	6	DA	4	24	0.96	Journal Article
37066269	17	Res Sq	2023		NC	1	1	4	4	0.1428571	Other
37053552	23	Blood	2023	25.476	A	8	k	1	8	0.2352941	Journal Article
36974355	16	Br J Haematol	2023	8.615	B	6	ADA	3	18	0.6666667	Journal Article
36940410	18	Blood	2023	25.476	A	8	k	1	8	0.2758621	Journal Article
36926651	19	iScience	2023	6.107	B	6	k	1	6	0.2	Journal Article
36864835	5	Front Endocrinol (Lausanne)	2023	6.055	B	6	3	2	12	0.8571429	Editorial
36709353	14	Leukemia	2023	12.883	A	8	k	1	8	0.32	Letter
36499220	12	Int J Mol Sci	2022	6.208	B	6	1	4	24	1.0434783	Journal Article
36362802	8	J Clin Med	2022	4.964	C	4	DA	4	16	0.8421053	Review
36342469	2	Br J Haematol	2023	8.615	B	6	DA	4	24	3	Comment
36253429	16	Leukemia	2023	12.883	A	8	1	4	32	1.1851852	Journal Article
36187875	8	Hemasphere	2022	8.3	B	6	k	1	6	0.3157895	Journal Article
36131910	5	Front Immunol	2022	8.786	B	6	1	4	24	1.7142858	Review
36054881	24	J Clin Oncol	2023	50.717	A	8	2	3	24	0.6857143	Journal Article
35896698	19	Bone Marrow Transplant	2022	5.174	C	4	k	1	4	0.1333333	Letter
35761024	21	Leukemia	2022	12.883	A	8	k	1	8	0.25	Journal Article
35269943	6	Int J Mol Sci	2022	6.208	B	6	ADA	3	18	1.0588236	Review
35106810	8	Hematol Oncol	2022	4.85	C	4	3	2	8	0.4210526	Letter
35085104	17	J Clin Invest	2022	19.456	A	8	k	1	8	0.2857143	Journal Article
34944660	3	Biomedicines	2021	4.757	C	4	2	3	12	1.0909091	Review
34934322	3	Ther Clin Risk Manag	2021	2.755	D	3	DA	4	12	1.0909091	Review
34845315	15	Leukemia	2022	12.883	A	8	2	3	24	0.9230769	Journal Article
34839350	8	Blood Cancer J	2021	9.812	B	6	2	3	18	0.9473684	Letter
34768283	22	Blood Adv	2022	7.637	B	6	2	3	18	0.5454546	Journal Article
34748628	21	Blood	2021	25.476	A	8	1	4	32	1	Journal Article

34706184	3 N Engl J Med	2021	176.08	A+	14	2	3	42	3.8181819	Letter
34552731	6 Clin Case Rep	2021		NC	1	3	2	2	0.1176471	Other
34280665	9 Blood Cells Mol Dis	2021	2.372	E	2	2	3	6	0.3	Letter
34236054	19 JCI Insight	2021	9.484	B	6	1	4	24	0.8	Journal Article
33808599	3 Int J Mol Sci	2021	6.208	B	6	2	3	18	1.6363637	Review
33690844	12 Blood	2021	25.476	A	8	2	3	24	1.0434783	Journal Article
33681816	Blood Cancer 19 Discov	2021		NC	1	k	1	1	0.0333333	Journal Article
33668555	3 Cancers (Basel)	2021	6.575	B	6	1	4	24	2.1818182	Review
33636567	7 Blood Cells Mol Dis	2021	2.372	E	2	ADA	3	6	0.3333333	Journal Article
33603144	14 Leukemia	2021	12.883	A	8	k	1	8	0.32	Journal Article
33483614	13 Leukemia	2021	12.883	A	8	2	3	24	1	Letter
33416408	8 Leuk Lymphoma	2021	2.996	D	3	1	4	12	0.631579	Journal Article
33414481	14 Leukemia	2021	12.883	A	8	3	2	16	0.64	Letter
33341510	11 Blood Cells Mol Dis	2021	2.372	E	2	k	1	2	0.0909091	Journal Article
33198085	3 Int J Mol Sci	2020	5.923	B	6	2	3	18	1.6363637	Review
32732366	16 Haematologica	2021	11.047	A	8	k	1	8	0.2962963	Journal Article
32587336	Bone Marrow 13 Transplant	2021	5.174	C	4	1	4	16	0.6666667	Journal Article
32454215	Biol Blood Marrow 15 Transplant	2020	5.742	B	6	k	1	6	0.2307692	Journal Article
32039261	3 Stem Cell Investig	2019		NC	1	1	4	4	0.3636364	Review
32030449	15 Ann Hematol	2020	3.673	C	4	k	1	4	0.1538462	Letter
31421238	Biol Blood Marrow 15 Transplant	2019	3.853	C	4	1	4	16	0.6153846	Journal Article
31413313	Bone Marrow 11 Transplant	2020	5.483	B	6	k	1	6	0.2727273	Journal Article
31409584	8 Blood Adv	2019	4.584	B	6	1	4	24	1.2631579	Review
30908982	9 Leuk Res	2019	2.214	D	3	3	2	6	0.3	Journal Article
30071321	Biol Blood Marrow 13 Transplant	2018	3.599	C	4	k	1	4	0.1666667	Journal Article
29131151	Bone Marrow 11 Transplant	2018	4.674	B	6	1	4	24	1.0909091	Letter
28797779	Biol Blood Marrow 22 Transplant	2017	4.484	B	6	1	4	24	0.7272727	Journal Article
26479981	Bone Marrow 11 Transplant	2016	3.874	C	4	1	4	16	0.7272727	Letter
26471035	15 Ann Hematol	2016	3.083	C	4	1	4	16	0.6153846	Letter
26033037	7 Cytopathology	2016	2.38	D	3	3	2	6	0.3333333	Letter
25331515	3 Expert Rev Hematol	2014	2.07	D	3	2	3	9	0.8181818	Review
24280282	15 Leuk Res	2014	2.351	D	3	k	1	3	0.1153846	Journal Article
22228450	7 Ann Oncol	2012	7.384	A	8	2	3	24	1.3333334	Journal Article
Total								891		

Rank SIGAPS: The quality of the publication is defined according to the impact factor of the journal of the same specialty: A if the journal is part of the top 10%, otherwise B if it remains in the first quarter, otherwise C if it stays in the first half, etc. Each category is assigned a weighting: 8 points for A journals, 6 points for B journals, 4 points for C journals, 3 points for D journals, 2 points for E journals, one point for NC journals,

The position among the co-authors is specified in the column of the year of publication. “k” means: other position than first (1), second (2), third (3), second to last (ADA) or last (DA).

1.5. FUNDINGS (S. Pagliuca PI/project leader)

- APLASTIC ANEMIA AND MDS INTERNATIONAL FOUNDATION RESEARCH GRANT: “*Impact of eltrombopag treatment in combination with immunosuppression on immune derangements of aplastic anemia: a research translational from the randomized EBMT race study.*” (May 2016)
- Philippe Foundation Inc. Grant (Dec 2018) pour le projet: *Genomewide Association Study and Assessment of risk disease in patients with Idiopathic Aplastic Anemia.*
- Scholarship F. Lo Coco, (Oct 2019) Società Italiana di Ematologia pour le projet: *Assessment of molecular dysfunctions in HLA machinery in myeloid malignancies relapsing after allogeneic hematopoietic stem cell transplantation*
- Mobility grant Fondation pour la Recherche Contre le Cancer pour le projet (March 2020): *Etude du dysfonctionnement moléculaire de la machinerie HLA dans les néoplasies myéloïdes en rechute après greffe allogénique de cellules souches hématopoïétiques*
- Fondation Maladies Rares-HPN (Mars 2021) pour le projet: “Immunogenomics of idiopathic bone marrow failure disorders: immune-escape mediated by class I/II HLA somatic mutations.”
- JANSSEN-HORIZON research grant : “Immunomics of tumor-surveillance in CAR-T cell therapies: molecular determinants of success and failure”
- SIGN’IT ARC “Immunomics of tumor-surveillance in CAR-T cell therapies: molecular determinants of success and failure”

1.6. AWARDS

- Abstract Achievement award 57th ASH Meeting
- Abstract Achievement award 58th ASH Meeting
- Abstract Achievement award 62th ASH Meeting
- EBMT Best Young Abstract 2021 (x2)
- TITO BASTIANELLO AWARD – MDS FOUNDATION 2021
- ASH-IPIG AWARD and Abstract Achievement award 63th ASH Meeting
- Prix Capucine – SFGM-TC 2022: “Déterminants moléculaires de l’alloréactivité dans la greffe des cellules souches hématopoïétiques chez les patients atteints d’hémopathie myéloïde”

Grant application/award	Project	Funding	Period
APLASTIC ANEMIA AND MDS INTERNATIONAL FOUNDATION RESEARCH GRANT	<i>Impact of eltrombopag treatment in combination with immunosuppression on immune derangements of aplastic anemia</i>	60 000 \$	2015-2017
Philippe Foundation Inc	<i>Genomewide Association Study and Assessment of risk disease in patients with Idiopathic Aplastic Anemia</i>	12000 \$	2019-2020

Scholarship F. Lo Coco (Società Italiana di Ematologia)	<i>Assessment of molecular dysfunctions in HLA machinery in myeloid malignancies relapsing after allogeneic hematopoietic stem cell transplantation</i>	30,000 €	2020-2021
Mobility grant Fondation pour la Recherche Contre le Cancer	<i>Etude du dysfonctionnement moléculaire de la machinerie HLA dans les néoplasies myéloïdes en rechute après greffe allogénique de cellules souches hématopoïétiques</i>	30,000 €	2020-2021
Fondation Maladies Rares-HPN grant	<i>Immunogenomics of idiopathic bone marrow failure disorders: immune-escape mediated by class I/II HLA somatic mutations</i>	15,000 €	2021
ASH abstract achievement award (x4)		2000 \$	2015, 2017, 2021
EBMT Best Young Abstract award (x2)		1,000 €	2021
TITO BASTIANELLO AWARD – MDS FOUNDATION		10,000 €	2021
ASH-IPIG AWARD		2000 \$	2021
JANSSEN-HORIZON Research grant	<i>Immunomics of tumor-surveillance in CAR-T cell therapies: molecular determinants of success and failure</i>	150,000 €	2023-2025
Prix Capucine	<i>Déterminants moléculaires de l'alloréactivité dans la greffe des cellules souches hématopoïétiques chez les patients atteints d'hémopathie myéloïde</i>	15,000 €	2023-2024
SIGN'IT 2023– SIGN'IT 2023 : Signatures en immunothérapie - ARC	<i>Immunomics of tumor-surveillance in CAR-T cell therapies: molecular determinants of success and failure</i>	600000 €	2023-2026

1.7. SCIENTIFIC SOCIETIES

Professional Memberships	
2022	Société francophone de greffe de moelle et de thérapie cellulaire (SFGM-TC)
2020	MDS Foundation
2019	Società Italiana di Ematologia (SIE)
2019	Società Italiana di Ematologia Sperimentale (SIES)
2019	American Society of Hematology (ASH)
2019	European Society of Hematology (EHA)
2015	European Society for Blood and Marrow Transplantation (EBMT)
2015	Aplastic Anemia and MDS International Foundation (AAMDS-IF)

Active member and project leader in:

- Cellular Therapy and Immunobiology Working Party (CTIWP) of the EBMT (Immunotolerance Subcommittee leader)
- Severe Aplastic Anemia Working Party (SAAWP) of the EBMT.
- Société Francophone de Greffe de moelle et thérapie cellulaire (Ateliers d'harmonisation 2023)

1.8. INVITED CONFERENCES (FACULTY MEMBER)

- European School of Hematology – Bone Marrow Failure session (Paris, November 2022). Lecture “*Human leucocyte antigen in aplastic anemia: cause or consequence?*”
- EBMT 47th, 48th, 49th, 50th editions
- Invitation as “international expert” to the University of Zurich, Markus Manz lab, Experimental Hematology. Lecture on “*Mechanisms of immunoediting in hematological disorders*” (March 2023)
- Société Francophone de Médecine Interne (Paris December 2023)

1.9. REVIEWER INVITATIONS

Journals: Blood, Haematologica, British Journal of Haematology, Frontiers in Medicine, Frontiers in Immunology, Annals in Hematology, Cancers, Cells, Journal of Clinical Medicine, International Journal of Molecular Sciences, Hemato, biomolecules, Current Medical Research & Opinion, Bone marrow transplant, Transplantation and Cellular Therapy, Therapeutic Advances in Medical Oncology, Therapeutic Advances in Hematology, Blood Reviews

Grants : Agence National de la Recherche (ANR) Appel à projet générique (AAPG2023)

Other reviewing activities : Prix de thèse de l'AIH 2023

2. PEDAGOGICAL ACTIVITIES

2.1. PERSONAL PEDAGOGICAL TRAINING

- “Chef de clinique des universités-assistant des hôpitaux” training seminar, Faculty of Medicine, University of Paris (October 2016)
- SIDES certification in 2017
- Participation in the Common Welcome Days for “Chef de clinique des universités-assistant des hôpitaux” (JACASM), "the chef's camp" 2022.
- “Diplome universitaire” in pedagogy planned for the year 2023/2024.

2.2. PEDAGOGICAL CONTRIBUTIONS

- Implementation of a training course in the application of echostetoscopy in hematology. (in Hôpital Saint Louis, Paris, 2019-2020, PI E. Lengline) Publication: “*Point-of-care ultrasound with handheld devices in hematology: a monocentric single-stage phase II study.* Pagliuca S, Bailly C, Talbot A, Bertinchamp R, Peyrony O, Elezi A, Bourrier P, Lengline E. *Leuk Lymphoma.* 2021 Jun;62(6):1379-1385. doi: 10.1080/10428194.2020.1869958”
- Training of MD students to the Observed Structured Clinical Examination (OSCE) using simulated patients (SPs) – University of Lorraine (from 2022)
- Writing and proofreading of exam questions for 1st and 2nd cycle of MD curricula
- Writing and proofreading of OSCE scenarios – University of Lorraine (from 2022)

2.3. TEACHING

- Faculty of Medicine University of Lorraine
 - 1^{er} cycle UE « Tissu Sanguin » /FGSM3 (Director : Pr P. FEUGIER)
 - 1^{er} cycle UE « immunopathologie et immunointervention »/ FGSM3 (Director : Pr. M. de CARVALHO)
 - International Master in Biomédecine (IMBM), UE «Therapeutic Potential of Stem cells»
 - Master 1 UE 8.311 « Immunopathologie et immunothérapies
- International PhD programs:
 - «Immunology, Molecular Medicine and Applied Biotechnology » University of Rome Tor Vergata, Rome, Italy
 - «Translational Medicine » University of Campania Luigi Vanvitelli, Naples, Italy
- Teaching in Hematology department of CHRU de Nancy:
 - General semiology MD students 1 and 2nd cycles.
 - Preparation for the French National Board Exam: bedside training, courses and clinical cases, and Exam questions and OSCE writing and proofreading
 - Residents: classes, journal clubs
 - Nurses: specific training in the management of patients receiving hematopoietic stem cell transplantation and other cellular therapies.

2.4. SIAPS SCORE

Nom PAGLIUCA	page 9/9
Prénom Simona	
Discipline (sous-section CNU) 4701 - Hématologie ; transfusion (2 options)	
UFR Nancy	

	2021-22	2020-21	2019-20
TOTAL HEURES D'ENSEIGNEMENT	34	0	0
Points de jury heures d'enseignement	20	0	0
Total retenu heures d'enseignement (Max = 200)	54	0	0
TOTAL RESPONSABILITES PEDAGOGIQUES	80	0	0
Points de jury Responsabilités pédagogiques	40	0	0
Total retenu Responsabilités pédagogiques (Max = 400)	120	0	0
TOTAL INNOVATIONS PEDAGOGIQUES	80	60	0
Points de jury Innovations pédagogiques	40	0	0
Total retenu Innovations pédagogiques (Max = 400)	120	60	0
SCORE SIAPS RETENU (Max = 1000)	294	60	0

Certifié le _____

Signature du Candidat

Signature du Doyen



Individual Pedagogical Ability Score in Health and Life Sciences (Score Individuel d'Aptitude Pédagogique en Santé)

This score has been approved by the Dean of the Faculty of Medicine of the University of Lorraine, Pr. Marc Braun, on April 5th 2023.

Of note is that more than 20 hours of magistral classes have been realized since.

2.5. SUPERVISION OF PEDAGOGICAL WORKS

- MD Thesis :

- Alizée JANDIN (2022), Thesis Director for the project : *Age-related immune cell dynamics influences outcomes after allogeneic hematopoietic cell transplantation*. (Last author, Published British Journal of Haematology, IF 8.6)
- Maud GAFFET (2022), Thesis co-supervision, Project : *Efficacy of hematopoietic stem cell boost as a rescue for poor graft function after hematopoietic stem cell transplantation: a multicenter retrospective study on behalf of the Francophone Society of Bone Marrow Transplantation and Cellular Therapy (SFGM-TC)*. (Co-last author, Published British Journal of Haematology, IF 8.6).
- Anne-Béatrice NOTARANTONIO (2022) help in supervision for the project: *Systemic complement activation influences outcomes after allogeneic*

*hematopoietic cell transplantation: a prospective French multicenter trial.
(Second author, Submitted American Journal of Hematology, IF 13.2)*

- Thesis « diplômes inter-universitaires »

- Bilal MAKKI (2022) « DIU Allogreffe de cellules souches hématopoïétiques », Université Pierre et Marie Curie. Supervision for the project : « *Impact de la prophylaxie de la GvHD par PT-Cy seul ou PT-Cy et ATG sur la reconstitution immunitaire après allogreffe de cellules souches hématopoïétiques.* »

- Master 2 (ongoing at the University de Lorraine):

- Esther HAZANE LEROYER (2023/2024). Project: « *Immunogénétique de l'hématopoïèse clonale : rôle des gènes HLA dans l'hématopoïèse clonale de signification indéterminée (CHIP) et leur implication dans le risque de néoplasie myéloïde. Étude à large échelle à partir de la banque de données UK Biobank.* »
- Clément COLLIGNON (2023/2024). Project : « *Molecular and functional landscape of extramedullary acute myeloid leukemia (collaboration avec Washington University, Missouri, St. Louis, USA).* »

- PhD Thesis:

- Carmelo GURNARI (2020-). Ph.D in Immunology, Molecular Medicine and Applied Biotechnology (Department of Biomedicine and Prevention, University of Rome Tor Vergata, Italy. Project: *T Cell Control and Immune Escape in MDS: Foundation for Targeted Immunotherapies.* Co-supervision. Co-last author, manuscript in progress.

Other projects carried out in collaboration with Dr. GURNARI during his PhD thesis (help in supervision of statistical methodology, bioinformatic analysis and result interpretation):

1- *Clinical and Molecular Determinants of Clonal Evolution in Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria. Co-first author.* Published. JCO 2023 IF 50.7

2- *Digging into the HLA pockets: A new association with acute leukaemias.* Editorial, **Last author.** Published. Br J Haematol. 2023 IF 8.6

3- *Current Opinions on the Clinical Utility of Ravulizumab for the Treatment of Paroxysmal Nocturnal Hemoglobinuria. Last author.* Published. Ther Clin Risk Manag. 2021 Dec IF 2.5

4- *Is nature truly healing itself? Spontaneous remissions in Paroxysmal Nocturnal Hemoglobinuria. Second author.* Blood Cancer J. 2021 Nov IF 9.8

5- *Monoclonal IgM gammopathy in adult acquired pure red cell aplasia: culprit or innocent bystander? Second author.* Published. Blood Cells Mol Dis. 2021 Nov IF 2.3

6- *Personalized Risk Schemes and Machine Learning to Empower Genomic Prognostication Models in Myelodysplastic Syndromes. Second to the last author.* Published. Int J Mol Sci. 2022 Mar IF 6.2

- Sunisa Kongkiatkamon (2019-2022) Department of Medicine, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Project: *Molecular characterization of the histone acetyltransferase CREBBP/EP300 genes in myeloid neoplasia*. **Co-supervision** in statistical analysis and methodology. **Second author**. Leukemia 2022, IF 12.8

Summary table of (co)supervised trainees

* Official supervision as thesis director, training/master supervisor.

**Other supervisions (project (co)supervisor, statistical/methodology supervision)

Student/ trainee	Type of supervision	Level of training	Period	Productivity	Author contribution
Côme Bommier**	Research Project	Resident in Hematology Saint Louis Hospital	2019	Publication: PMID: 31421238	S. Pagliuca first author and C. Bommier second author
Victoria Frattolillo*	Mobility stage (Tutor/supervisor)	MD/PhD student at the Vanvitelli University, Naples	2022	Stage validation Ongoing project (The role of BCLAF1 in hematological disorders)	S. Pagliuca last author
Sunisa Kongkiatkamon**	Research Project	PhD student, Chulalongkorn University Bangkok, Thailand and Cleveland Clinic	2021-2022	Publication: PMID: 34845315	KongkiatkamonS first author, S. Pagliuca second author
Carmelo Gurnari**	Research Projects (data analysis and interpretation)	Resident (Hematology) PhD student, Tor Vergata University and Cleveland Clinic	2021-to date	Large series of projects on immune escape in hematological disorders and clonal evolution. Publications : PMIDs 34839350, 33483614, 34934322, 33668555, 33414481, 33808599, 34706184, 34944660, 33690844, 34280665, 34236054, 33636567, 34748628 and several ongoing projects (most of	C. Gurnari first or second author; S. Pagliuca first, second or last author

				these projects were submitted to international congresses and were selected as oral or poster communications)	
Arda Dumaz**	Research Project (data analysis and interpretation)	Post-doc Cleveland Clinic	2022	Immunogenetics of LGL leukemia (HLA/TCR and beyond); ongoing project	
Bilal Makki*	DIU allo-HCT project (tutor)	Resident (Hematology) CHRU de Nancy	2022	Immune reconstitution after sequential conditioning regimens followed by PT-Cy or PT-Cy + ATG (manuscript in preparation)	
Anne Béatrice Notarantonio**	MD thesis (rapporteur)/Research Project (data analysis, interpretation, manuscript writing)	Resident (Hematology) CHRU de Nancy	2022	Role of complement activation in allo-HCT (under review in Am. Journal of Hematology)	AB. Notarantonio first author; S. Pagliuca second author
Alizée Jandin*	MD thesis (Thesis Director)	Resident (Hematology) CHRU de Nancy	2022	Age related immune dynamics after allo-HCT (POSTER PRESENTATION 49th EBMT MEETING, POSTER PRESENTATION ASH MEETING 2022, ORAL PRESENTATION SFGM-TC 2022); PMID: 37092504	A. Jandin first author; M. Rubio and S. Pagliuca last authors
Maude Gaffet*	MD thesis /Research Project (data analysis, interpretation, manuscript writing)	Resident (Hematology) CHRU de Nancy	2022	HSC injection in poor graft function (PMID: 36974355)	M.Gaffet first author; S. Pagliuca and C. Pochon co-last authors
Anthony Moussu**	Research project/Hematology stage	Resident (Internal medicine) CHRU de Nancy	2023	Immune mediated thrombocytopenia and beyond. Review of literature (submitted)	A.Moussu and L. Pierson second and third author; S. Pagliuca last author.
Ludovic Pierson**					

Esther Hazan Leroyer*	Research Project/Master 2 ongoing	Resident (Hematology) CHRU de Nancy	2023/2024	Immune escape and immunotherapy in hodgkin lymphoma; Review of literature PMID: 36362802	E. Hazane Leroyer first name; S. Pagliuca last author
				Immunogenetics of clonal hematopoiesis (Project master)	
Clément Collignon*	Research Project/Master 2 ongoing	Resident (Hematology) CHRU de Nancy	2023/2024	Molecular landscape of myeloid sarcoma (49th EBMT poster presentation; project master)	C. Collignon first author; S. Pagliuca and F. Ferraro last authors

3. RESEARCH ACTIVITIES

The following table summarizes my research activities/involvement during my training and professional path. All the projects/studies where I was, or I am involved as a PI are marked with (*)

3.1. CHRONOLOGICAL SYNOPSIS

2008-2010
· Experimental studies on new therapeutic target in lymphoproliferative disorders (Inhibition of CXCR4/SDF-1 axis as chemosensibilization in chronic lymphocytic leukemia models)*
2011-2012
Lymphoproliferative disorders (in particular Hodgkin Lymphoma)
Infection diseases in hematologic patients
Interventional Ultrasonography in hematologic patients
· Participation to ongoing National studies from GITIL/FIL group (HD0607, HD0802) and GIMEMA and SEIFEM group
· Observational studies on patient ongoing to high dose chemotherapy for lymphoproliferative disorders
· Observational studies on microbiological surveillance in hematological patients from local Hematology Division
· Diagnostic work-up in lymphoproliferative disorders (with particular attention to radiologic imaging and cytology aspects)
2012 to 2019
Allogeneic hematopoietic stem cell transplantation
· Observational studies on the outcome of patients undergoing SCT
· Participation to EBMT data entry (Promise)
· Participation as local co-investigator to ongoing National and International multicentre studies in Italy and France, including:

o Haplo-identical SCT using high-dose cyclophosphamide
o Bu-Cy vs Bu flu conditioning regimen
o Dose Busulfan in Fludarabine-Busulfan-Thymoglobuline conditioning regimen for AML patients
o EPO in the setting of refractory anemia after allogeneic stem cell transplantation
o Romiplostim in persistent thrombocytopenia after allogeneic stem cell transplantation
o Anti-FLT3 treatment as post-transplant maintenance in AML FLT3-ITD mutated
o Role of allogeneic SCT in idiopathic myelofibrosis
o Role of allogeneic SCT in T cutaneous lymphoma
o Role of allogeneic SCT in Adult T cell Leukemia/lymphoma HTLV1+
o Low-dose steroids vs observation as early intervention for grade I aGvHD
o Mabcampath as salvage treatment for steroid-refractory aGvHD
o TK inhibitors for the treatment of refractory chronic GvHD
o Srk inhibitors for the treatment of refractory chronic GvHD
o Ruxolitinib for the treatment of refractory acute and chronic GvHD
o Ibrutinib for the treatment of refractory acute GVHD
o Marabivir for the treatment of post-transplant resistant CMV infection
o Letermovir for patients with post-transplant resistant CMV infection
o Alternative donors (Haplo vs MUD 9/10) for elderly patients (>65 years) with AML
o Alternative donors (Haplo vs 9/10) for adult patients (55 years) with AML CR1.
o Role of echography+elastasonography in the detection of precocious signs of VOD/SOS.
o Role of allogeneic stem cell transplantation in MDS
o Second allogeneic transplantation for treatment of graft failure
o Pre-transplant antiPD-1 treatment in relapse/refractory Hodgkin Lymphoma
o Cord blood transplantation in Severe Aplastic Anemia
o Role of allogeneic stem cell transplantation in myelofibrosis
· Participation as principal investigator to European retrospective Studies from Eurocord and EBMT, including: *
o Cord Blood Transplantation in Bone marrow failure Syndromes (SAAWP and Eurocord retrospective study) *
o HSCT for Severe Aplastic Anemia in HIV infected patients (SAAWP retrospective study) *
Myelodysplastic syndrome
· Hypomethylating agents and Bcl2-inhibitor for treatment of high-risk MDS
· Lenalidomide for 5q- low risk MDS
· Immunosuppressive treatment for selected MDS patients
· Role of allogeneic SCT for MDS patients
· Iron overload in MDS patients
· Risk adapted treatment strategy for MDS
Other cytopenias:
· LGL associated cytopenia: immunosuppressive treatment by methotrexate, cyclosporine, or mabcampath
· Immune thrombocytopenia
· Chronic immune neutropenia

Myeloproliferative Neoplasms and Chronic Myeloid Leukemia
· Observational studies on the outcome of II generation TKI treatment in patients with CML
· First Line Therapy with II generation TKI
· Second Line therapy with II generation TKI
· Ponatinib Compassionate Use in patients with CML after I and II generation TKI treatment failure
· Bosutinib in I and II line CML patients
· Observational studies in patients with PH negative Myeloproliferative Neoplasms
· Splenectomy in MF patients undergoing hematopoietic stem cell transplantation
Bone marrow failure syndromes
Aplastic anemia
· Mabcampath as salvage IST for AA patients
· Abnormalities of the immune system in AA patients
· Horse ATG+CSA vs Horse ATG+CSA+Eltrombopag in first line treatment for SAA (RACE Study. Co-Investigator at Saint Louis Hospital and Federico II University).
· Androgens for patients with BMF (retrospective study on behalf of SAAWP of EBMT) *
Paroxysmal nocturnal hemoglobinuria
· Observational studies on the outcome of eculizumab treatment
· Iron overload during eculizumab treatment for PNH (including possible iron-chelating therapy)
· Long-term complication of PNH, with and without anti-complement treatment
· C3-mediated extravascular hemolysis in PNH patients on eculizumab
· Clinical development of novel anti-complement agents
· Participation to the Phase 1 trial with TT30 for PNH patients
· Participation to in vitro studies on compstatin analogs and other molecules
· ALXN1210 for naïve adult patients with PNH (ALXN1210-301)
· ALXN1210 for PNH patients treated with Eculizumab (ALXN1210-302)
· Subcutaneous versus intravenous Ravulizumab in PNH patients treated with Eculizumab (ALXN1210-303)
Other lineage-restricted bone marrow failures
· Pure red cell aplasia: role of IST by mabcampath
· Pure white cell aplasia: role of IST by mabcampath
Fanconi anemia
· Long-term outcome of FA patients, with and without SCT
Other inherited bone marrow failures
· Cord Blood Transplantation for Bone marrow failure syndromes (SAAWP/Eurocord retrospective Study) *
2019 to date, ongoing projects:
Immunogenomics and immune-transcriptomic of myeloid diseases and bone marrow failures
· Study of the immuno-transcriptomic signatures in AML and MDS patients*
· Validation of a new bioinformatic platform for the study of class I and class II HLA somatic mutations. *
· HLA genomic dysfunction in BMF *
· HLA genomic dysfunction in AML relapsing after allogeneic SCT *
· Study of the TCR repertoire in patients receiving allogeneic SCT*

HLA evolutionary divergence (HED) in BMF (Evaluation of HED in AA and PNH predisposition, outcomes and clinical spectrum). *
HED in AML patients relapsing after allogeneic HSCT*
Role of donor/recipient HED in hematological malignancies undergoing allogeneic HSCT (collaborative study with the CTIWP of EBMT) *
Immunomics, HLA and TCR repertoires in MDS patients*
Genomic and immune landscape of patients with extramedullary acute myeloid leukemia*
Post-transplant alloreactivity and molecular immunobiology in cellular therapies
CAR-T cell immunomics: molecular determinants of success and failure*
Molecular determinants of GvH responses*
Genomics of myeloid disorders and BMF
Molecular landscape of evolved BMF syndromes*
Role of p53 in AML and MDS (comprehensive analysis of > 6000 patients from public and restricted datasets)
Role of EP300 haploinsufficiency in myeloid neoplasms.
Molecular signatures of TET2 and IDH mutated leukemia. *
Role of VISTA in AML*
Drug discovery:
TET2 inhibition in TET2 loss of function myeloid neoplasm and in IDH mutated cancers.
Role of eltrombopag and other TPO synthetic derivatives in TET2 inhibition

3.2. RESEARCH AXES

3.2.1 Clinical research axis I: Poorly codified situations in allogeneic hematopoietic cell transplantation.

Allogeneic hematopoietic cell transplantation (allo-HCT) holds a pivotal place in the setting of therapeutic algorithms for hematological disorders. Its therapeutic index is principally related to the immunogenic graft-versus-leukemia (GvL) effect that, together with an efficient immune reconstitution ensures the success of the procedure, contributing to disease control and immune competence against infections.¹⁻⁴ However, the therapeutic efficacy of this procedure can be hampered by the occurrence of significant morbidity, because of specific transplant and disease-related events including, graft versus host disease (GvHD), endothelial syndromes (ES), graft failure, underlying disease relapse, and altered immune recovery, driving important infectious risks.

Since early phases of my career, during the years of my bone marrow transplant fellowship, first in Naples and later in Paris, my scientific interest has been captured by the clinical landscape of these complications, seeking to understand (and possibly control) challenges and vulnerabilities of complex post-transplant situations. Indeed, such an interest drove several works in the setting of poorly codified scenarios, including primary graft failure, endothelial complications, post-

transplant lymphoproliferative disorders and steroid-refractory GvHD, with major implications for the current research activities.

3.2.1.a Primary graft failure and poor graft function

Primary graft failure (PGF) is a life-threatening situation characterized by rejection of the hematopoietic stem cell (HSC) graft after an allo-HCT procedure, with or without recipient hematopoietic recovery.^{5,6} During my first year as a trainee in Saint Louis Hospital (2014-2015) we managed a patient, initially diagnosed with a myelodysplastic syndrome, experiencing a repetitive graft failure, after two consecutive allo-HCT (from a matched unrelated donor and a cord blood unit). Given the good clinical conditions of the patient, despite the persistence of bone marrow failure, we decided to perform a third allogeneic procedure using a haploidentical donor, able to finally drive a hematopoietic recovery. This case (Pagliuca et al. *Annals of Hematology* 2015, the first in literature), prompted the whole team in Saint Louis Hospital to conduct a nationwide, multicenter, clinical trial investigating the role of haploidentical donor HCT in the context of PGF, currently ongoing in France (NCT05126186, PI: Pr. Régis Peffault De Latour). A similar complication called “poor graft function”, denotes a status of insufficient hematological recovery after allo-HCT, despite of a full donor chimerism. Also, in this case, no consensual recommendations exist to drive medical decision. Reinjection of HSCs from the same donor, has been implemented as a strategy to improve the altered hematopoiesis and to overcome infectious risk. However, factors impacting recovery remain unknown and modalities of reinjection are usually at the discretion of the practicing physician. In recent years, we conducted a study on behalf of SFGM-TC to inform on epidemiology, outcomes and prognostic factors of this procedure. Specifically, we included 55 patients across multiple centers in France and demonstrated response rates of nearly 40% and 2-year survival of >60% in the context of an otherwise deadly complication. Furthermore, we observed that the timing of injection and the degree of cytopenia were strongly associated with outcomes.

This study, represented the MD thesis work of Maude GAFFET, finalized in October 2022 and published in February 2023 in the *British Journal of Haematology* (M. GAFFET et al. *BJH* 2023, co-last author S. Pagliuca). For this work, I assured the statistical and methodological management, co-supervising with Dr. Cécile Pochon the MD thesis candidate.

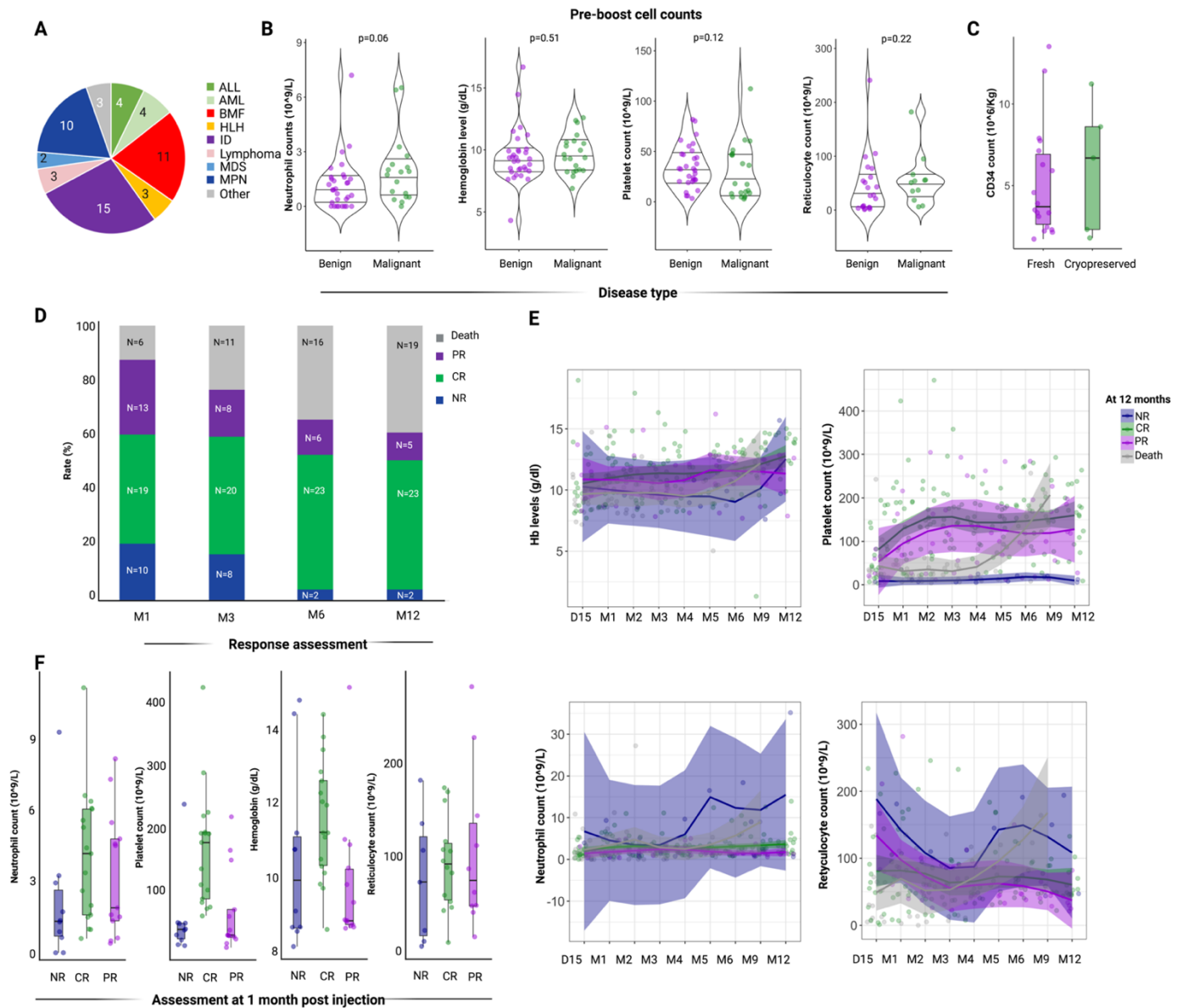


Figure 1. Clinical and biological characteristics of patients receiving a CD34+ boost (From M. GAFFET et al, *BJH* 2023). A) Pie chart showing the distribution of diagnosis with the number of patients for each category (ALL: acute lymphoblastic leukemia, AML acute myeloid leukemia, BMF: bone marrow failure, HLH: Hemophagocytic lymphohistiocytosis, ID: immune deficiency, MDS: myelodysplastic syndrome, MPN: myeloproliferative neoplasm). B) Violin plots displaying the pre-boost (day 0) counts distributed according to disease category. C) Box plot representing the distribution of CD34+ cell count according to the status of the cellular product (fresh or cryopreserved). D) Bar plot showing the rate of response and deaths at 1, 3, 6 and 12 months respectively (PR: partial response, CR: complete response, NR: non-response). E) Scatter plots with smoothed conditional means (smoothed lines) representing each basal (pre-boost) value dynamic across time (from day +15 to 12 months after injection) and for each category of patients as reported as 12 months. F) Box plots reporting the distribution of each value at 1-month post-boost according to the response status (NR: non-response, CR: complete response, PR: partial response).

3.2.1.b Endothelial complications and post-transplant complement activation

Endothelial syndromes (ES) represent a group of rare and dismal complications that can occur after allo-HCT and are characterized by systemic endothelial damage. Veno-occlusive disease, capillary leak syndrome, engraftment syndrome, transplant-associated microangiopathy, diffuse alveolar hemorrhage and idiopathic pneumonia syndrome are the main nosological entities (**Figure 2**). These entities can be triggered by chemotherapy, infections and calcineurin inhibitors, but allogeneic reactivity is claimed to be the common denominator. Endothelial damages are thought to activate several deleterious pathways (pro-apoptotic, pro-coagulant, pro-inflammatory) and can lead to multi-organ failure. Complement and inflammation but also cellular-mediated responses play a pivotal role in their pathophysiology. However, clinical manifestations of each syndrome may overlap, and relationship with graft-versus-host disease could be tiny. The lack of well-defined diagnostic criteria does not allow clear-cut comparisons in the current literature. Therapeutic efforts have been made to intercept the pathogenic mechanisms leading to endothelial dysfunction, but remission rates and survival remain mostly unsatisfactory. In this extremely poor codified context whereby consensus definitions were largely outdated, when in Saint Louis Hospital under the guidance of Pr. Gérard Socié, I systematically reviewed the incidence, clinical features, and treatment approaches of the ES, pleading for the development of internationally accepted standard definitions. This review of literature was organized in a perspective that was published on Blood Advances in 2019 (Pagliuca S. First author).

Given the prominent role of complement in endothelial activation, especially after high intensity conditioning regimens, and given the overlapping nature of these disorders with acute GvHD, the question whether complement activation could be of interest in pathophysiology of GvHD itself and other post-transplant complications remains to date unanswered.

Between 2010 and 2012 a prospective protocol has been conducted in several hospitals of the Assistance Publique des Hôpitaux de Paris (PI. Pr. Marie Thérèse Rubio) to understand the complement role in post-transplant outcomes. I had the opportunity to work on this project at my arrival in Nancy Hospital, helping the PI with the application of a statistical methodology taking into account time-dependent covariates in longitudinal data, modeling complex complement-activation metrics in univariable and multivariable setting and defining their role in impacting outcomes. Specifically, in this work we tracked the activation of complement cascade, performing serial measurements of complement proteins (C3, C4, Factor B) and regulators (C1 esterase inhibitor, CD46, CD55, CD59, CD88, Factor H, Factor I) along with CH50 activity, for 12 weeks after allo-HCT in 85 patients receiving a myeloablative conditioning (MAC) regimen for various hematological malignancies. Based on the kinetics of C3, C4 plasma levels and CH50 activity, 26

patients showed an “activated” complement profile. Complement activation was a major predictive factor of impaired post-transplant overall survival, progression free survival, acute GVHD and non-relapse mortality and was strongly associated with the occurrence of acute gastrointestinal (GI) GVHD (**Figure 3**). Through specific immune staining, we showed the deposition of C1q, C3d, C4d and of C5b9 components on endothelial cells in GI GVHD lesions in a way quantitatively correlated with the histological grade of GVHD. Complement activation was associated also with higher incidence of infections, capillary leak syndrome and transplant-associated thrombotic microangiopathy, in line with increased endothelial toxicity. This study has been finalized this year, represented the MD thesis work of Dr. Anne-Béatrice Notarantonio (Pagliuca S second author, last author Marie Thérèse Rubio) and has been currently accepted in the American Journal of Hematology. These findings not only represent the proof of concept of the importance of the dynamic monitoring of complement activity but provide also a rationale for the use of complement inhibitory therapeutics in post-allo-HCT setting. Discussions with a company involved in the development of proximal complement inhibitors are ongoing for a future clinical trial in this perspective.

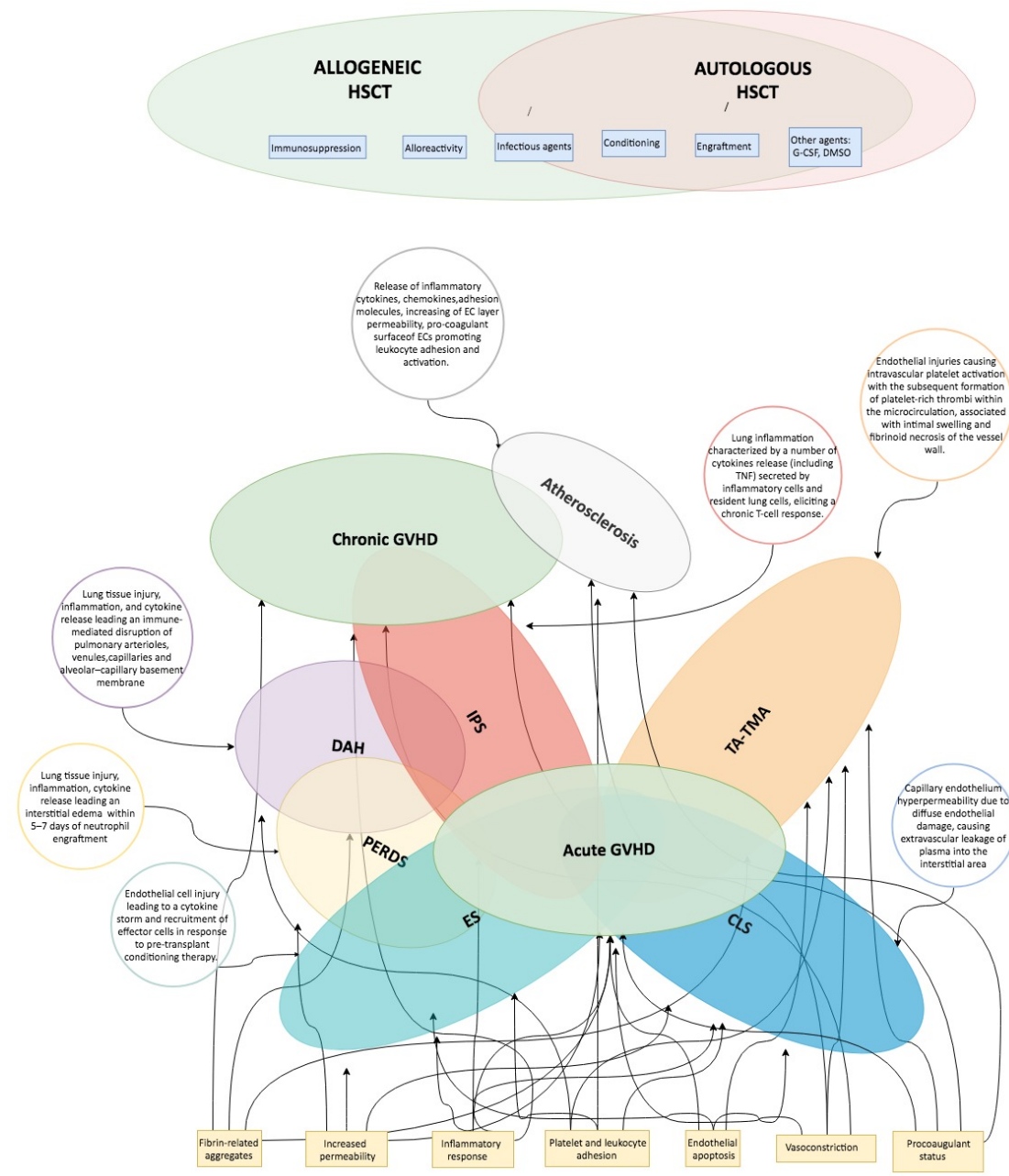


Figure 2. Overlapping pathophysiology of endothelial syndromes. Adapted from Pagliuca et al. *Blood Adv* 2019.

The Venn diagram highlights the overlap of endothelial syndromes, both diagnostically and pathophysiologically, with GVHD. Many clinical and pathophysiological features of GVHD parallel those of other endothelial cell activation syndromes in which a single organ is targeted. Atherosclerosis and several delayed forms of IPS have here been proposed as late endothelial dysfunction syndromes overlapping with chronic GVHD.

Abbreviations: GVHD: Graft-versus-host disease; CLS: capillary leak syndrome; ES: engraftment syndrome; TA-TMA: transplant associated thrombotic microangiopathy; DAH: diffuse alveolar hemorrhage; IPS: idiopathic pneumonia syndrome; PERDS: peri-engraftment respiratory distress syndrome; HSCT: hematopoietic stem cell transplantation.

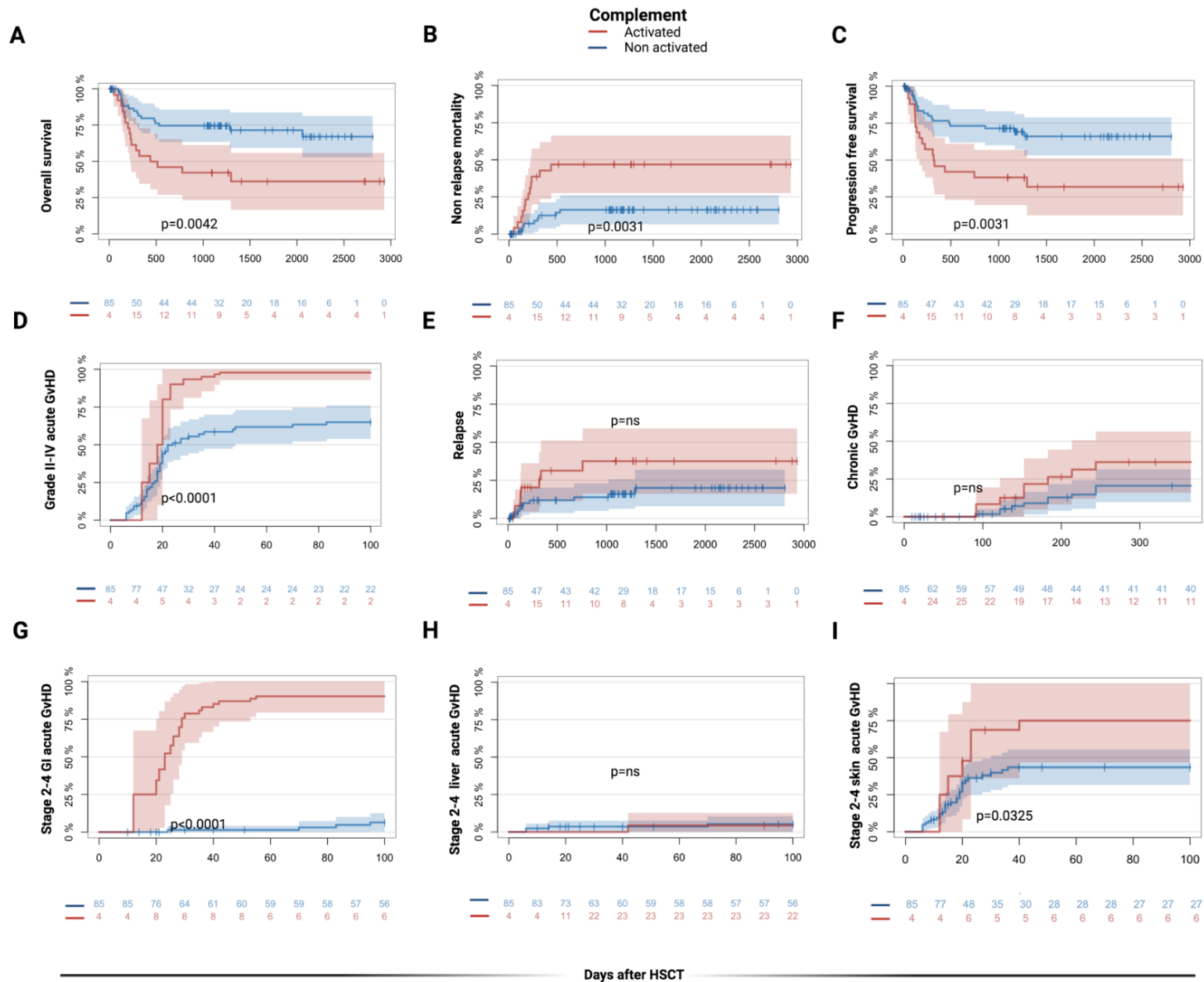


Figure 3: Impact of complement activation profile on post-transplant outcomes. *Adapted from Notarantonio et al. AJH 2023.*

Simon-Makuch plots representing the time-dependent impact of complement activation on overall survival (A), Non relapse mortality (B), Progression free survival (C), Acute grade II-IV GvHD (D), relapse (E), chronic GvHD (F).

3.2.1.c Post-transplant lymphoproliferative disorders

Post-transplantation lymphoproliferative disease (PTLD) is another post-allo-HCT serious complication associated with Epstein-Barr virus (EBV) infection. Anti-CD-20 therapy is currently used as a preemptive strategy to control EBV reactivation, however, PTLD may still occur in some patients and many questions have been risen concerning the epidemiology of this complication and possible risk factors associated with rituximab refractoriness. I had the opportunity when in Saint Louis Hospital to conduct a monocentric study on behalf of the group, together with a young hematologist in training, Dr. Côme Bommier at that time at the 3rd year of the residency. Indeed, we analyzed outcomes and risk factors associated with PTLD transformation in 208 HCT recipients diagnosed with EBV-DNAemia and receiving at least 1 course of rituximab. We were able to show a 2-year cumulative incidence of PTLD of 6.3% (95% CI, 3.5% to 10.1%), for a median follow-up of patients diagnosed with PTLD of 37.85 months. Through multivariable analysis we identified 4 risk factors associated with PTLD: HCT from an unrelated donor, recipient HLA-DRB1*11:01, fever at diagnosis of EBV infection, and donor-recipient sex-mismatched HCT, possibly in line with higher doses of T-depleting agents. The presence of more than 2 of these risk factors was associated with an increased risk of developing PTLD (**Figure 4**). These findings identified risk factors associated with PTLD in EBV-infected patients after HCT and defined patient subgroups that may benefit from intensified preemptive strategies. The study has been published in the journal *Transplantation and Cellular Therapy* in 2019 (S. Pagliuca First Author, Côme Bommier second author).

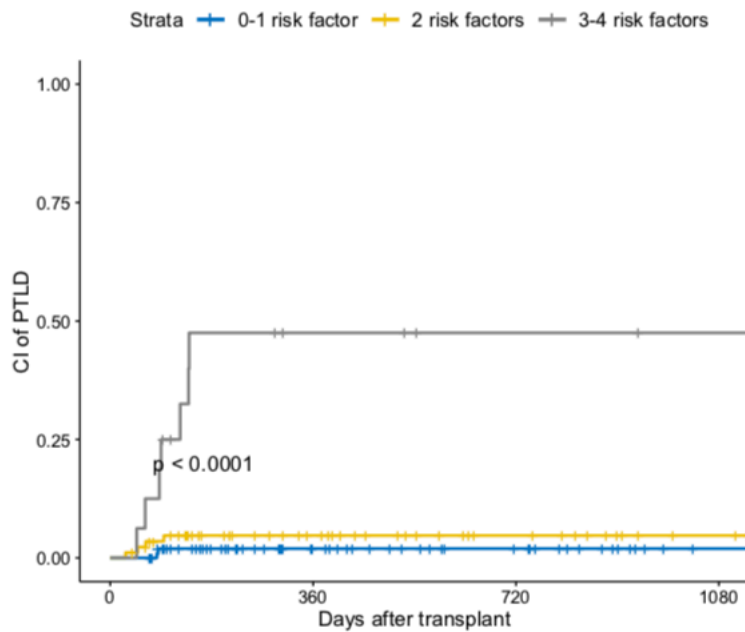


Figure 4. Cumulative incidence of PTLD in transplant recipients with significant EBV-DNAemia receiving rituximab preemptive therapy according to number of risk factors: (1) HSCT from an unrelated donor; (2) recipient HLA- DRB1*11:01; (3) fever at diagnosis of EBV infection; (4) sex-mismatched HSCT (*Adapted from Pagliuca S et al. TCT 2019*)

3.2.1.d Steroid-refractory GvHD

Steroid-refractory GvHD constitutes another challenging post-HCT complication. It is characterized by GvHD unresponsiveness to first line of treatment (steroid-based therapies) and associated with a status of profound immunodepression, increased infectious risk and highly impaired quality of life.^{7,8,1} Patients often need the administration of heavy immunosuppressive treatments, and a proper therapeutic algorithm is not codified after the second line, with center and investigator experience mostly guiding the flux of therapeutic agents prescribed in these situations.^{9,10} Given the difficulties to analyze such a dynamic scenario often characterized by more or less quick clinical and therapeutic changes, scarce real-life data are available to track dynamic outcomes and quality of life after steroid refractoriness. With in mind these limitations, we characterized, thanks to a data collection work that I conducted on more than one thousand patients undergone allo-HCT at Saint Louis Hospital, the dynamic changes of patients diagnosed with acute or chronic GvHD and the factors associated with steroid refractoriness, tracking at the same time the failure free survival of patients responding to immunosuppressive treatments. We conducted thus a retrospective study on outcomes and risk factors associated with acute and chronic steroid-refractory GVHD in a large cohort of 1207 patients receiving HCT in Saint Louis Hospital between 2007 and 2017. Among patients who developed an acute and/or a chronic GVHD, the cumulative incidences of acute and chronic steroid-refractory disease, were 31 and 48% respectively at day+100 and one-year post-HCT. Through a multivariable analysis we were able to identify several risk factors associated with the development of a steroid-

refractory disease. For acute GVHD steroid-refractoriness we identified I) a very high disease-risk-index, II) an unrelated donor, III) the absence of in vivo T-depletion as GVHD prophylaxis, IV) a reduced intensity conditioning regimen. For chronic GVHD, I) the use of peripheral blood stem cells, II) unrelated donors and III) absence of in vivo T-depletion were more likely associated with a steroid-refractory disease. After the construction of a multistate dynamic model, we observed that the probability of being alive without relapse after the resolution of all GVHD episodes, was about 36% in the long-term (**Figure 5**). This study has been published in 2019 in *Bone Marrow Transplant* journal (S. Pagliuca first author)¹¹ and inform on the epidemiology of this complication, still construing an unmet medical need in transplant science.

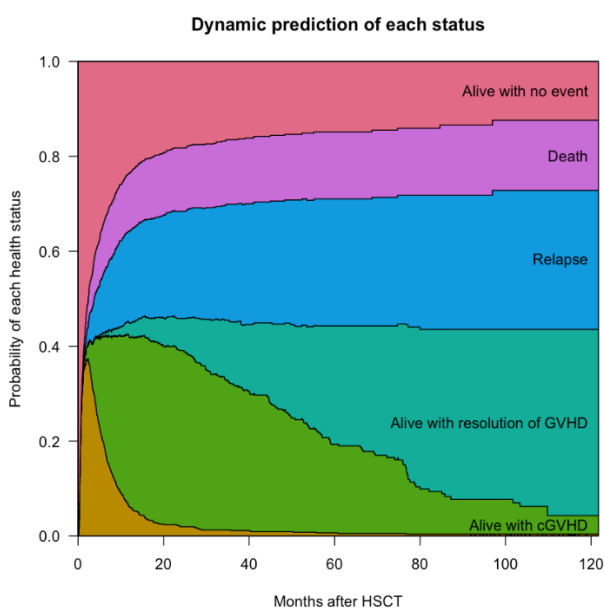


Figure 5: Multistate model of patients receiving allo-HCT in Saint Louis Hospital. The model indicates the dynamic prediction of each status (no event, acute GVHD, chronic GVHD, resolution of GVHD, relapse, death) Adapted from Pagliuca et al. *BMT* 2020

3.2.2 Clinical research axis II: Specific situations in bone marrow failure disorders

Since the very beginning of my career, because of the specific expertise of the Institutions I used to work in, while approaching transplant related topics, my training has been particularly biased towards the management of patients with bone marrow failure (BMF) disorders (inherited and idiopathic). This interest prompt me to develop a research axis around, first, clinical and, later on, translational topics in this spectrum of disorders (the latter better developed in further sections of this manuscript). Idiopathic BMF constitutes a group of disorders, mainly characterized by an immune-mediated destruction of HSCs and progenitors in bone marrow, featuring a clinico-molecular phenotype including cytopenia, clonal hematopoiesis, a certain degree of hemolysis, and eventual propensity to myeloid transformation. This spectrum of disorders includes two main entities: aplastic anemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH) that may be more or less present in overlap. Other phenotypes may range from pure red cell aplasia (PRCA) and pure white cell aplasia (PWCA) that

may share some molecular features with AA, other forms of autoimmune cytopenias and with large granular lymphocytes (LGL) proliferations.

AA is a paradigmatic autoimmune BMF disorders, characterized by aberrant T cell responses against immunodominant (unknown) antigens presented via human leukocyte antigen (HLA) molecules on the surface of HSCs. Given the deep contribution on a translational level offered with our studies, pathophysiology will be detailed in further translational research sections. From a clinical and therapeutic point view, its cure, based on disease severity, age and donor availability, may require allo-HCT or immunosuppressive regimens. As a non-malignant disorder, particular attention in current transplant strategies is focused on reducing as much as possible the risk of GvHD and graft failure. For this reason, transplant approaches are classically reserved to young patients having a matched related or unrelated donor, while, in absence of a suitable donor, patients with severe AA (SAA) will be preferably treated with standard immune suppression (classically anti-thymoglobulin and cyclosporine) accessing transplant approaches from alternative donors only in case of failure.

The last few years have seen a profound breakthrough of the therapeutic scenario of SAA patients with the integration of Eltrombopag in standard immunosuppression schemes for front line treatment (RACE trial).¹² Indeed, during the years of my clinical training in Naples and Saint Louis Hospital, I had the privilege to be involved as a co-investigator and directly follow patients included in this trial, actively participating in this revolution. The proximity with the trial headquarters gave me the opportunity to obtain my first research funding (while still being a resident) for translational researches in AA (AAMDSFI research grant). Part of this funding was exploited during my years in Cleveland Clinic.

Several research works conducted in the field of aplastic anemia at that time concerned again specific poorly codified situations including HLA identical cord blood transplant for patients with BMF, transplant and immune suppression within rare high-risk cohorts such as HIV infected patients or patients with concomitant florid hemolytic clones.

3.2.2.a Cord blood transplantation in BMF

Patients with idiopathic or inherited BMF (Fanconi anemia (FA), Diamond-Blackfan anemia (DBA), dyskeratosis congenita (DC), and others), lacking a suitable donor or failing immunosuppression, still have a poor prognosis. Cord blood transplantation (CBT) has extended the possibility of HCT for many patients in case of the absence of an eligible donor, and although in the last years, this procedure is less used in several hematological diseases, it remains an option for the treatment of patients with BMF syndromes (**Figure 6**). We have reviewed the state of the art of the use of CBT for BMF in an

invited special issue of a specialized transplant journal (*Pagliuca et al. Stem Cell Investigation 2019*) and I had the honor to conduct on behalf of Eurocord and the Severe Aplastic Anemia Working party (SAAWP) of the EBMT a retrospective study on the use of HLA identical cord blood units (CBU) in patients with inherited and acquired BMF. This cohort has been since then the largest one ever published with these specificities. As a matter of fact, CBT from HLA-identical siblings is an attractive option for patients with BMF syndromes because of the low risk of GvHD and the absence of risk to the donor. In this study we analyzed the outcomes of 117 patients (97 with inherited and 20 with acquired BMF) who received an HLA identical CBU. This analysis showed excellent cumulative incidence of engraftment (almost 90% at +100 days post-transplant) and a very low number of events in terms of acute and chronic GvHD (100-day cumulative incidence of grades II to IV acute GVHD was 15.2%, and 7-year CIF of chronic GVHD was 14.5%). This study pointed out the importance, whenever possible, of collecting a CBU at birth of a new sibling especially in presence of inherited BMF.

Of note on the personal level this was the first project that initiated me to multicenter registry studies and their methodology, starting from the conceptualization of a study synopsis, passing through all the steps of data collection, queries organization, data management and analysis, interpretation, presentation to a consortia meeting and paper writing. Importantly, this study, started in 2015, introduced me to the statistical methodology related to survival and competitive risk analysis, stimulating my interest in SPSS and R programming.

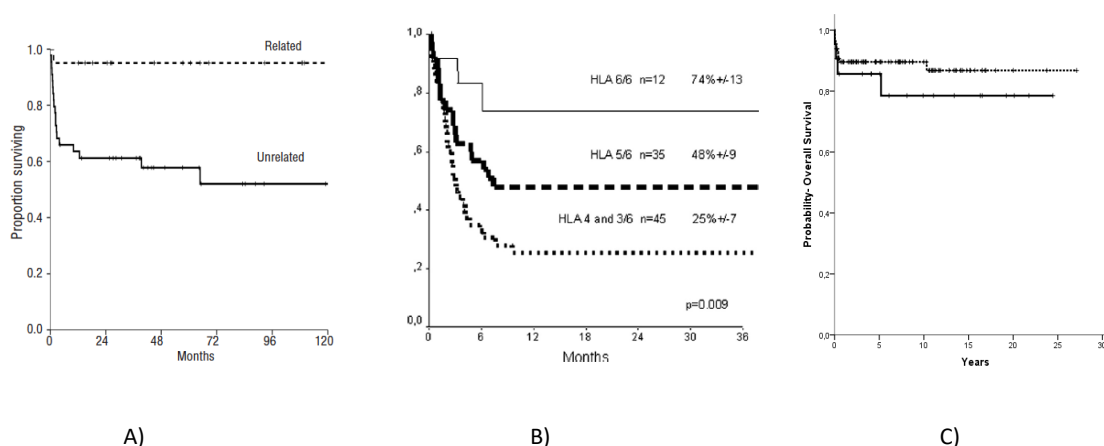


Figure 6: Results of CBT approaches in BMF patients (adapted from Pagliuca et al. Stem Cell Investigation 2019).
 A) Overall survival of patients with inherited BMF other than Fanconi Anemia receiving a related or an unrelated CBT¹³.
 B) Overall survival of Patients with Fanconi Anemia receiving an unrelated CBT¹⁴. C) Overall survival of Patients with inherited (dotted line) and acquired (solid line) BMF receiving a related CBT¹⁵.

3.2.2.b Combined treatment approaches for patients with AA/PNH syndromes

PNH is characterized by complement-mediated intravascular hemolysis, thromboembolic complications and BMF, which may range from mild cytopenia to SAA.^{16,17} Even if the clinical manifestations may change over time,¹⁸ most, if not all PNH patients, have a degree of AA with or without significant hemolysis that may require a treatment according to its severity. While classical hemolytic PNH patients without significant other cytopenia than anemia may respond well to anti-complement therapies,^{19,20} concomitant treatment for patients who associated severe thrombocytopenia or neutropenia is still unknown. Thus, in a small proportion of patients AA and hemolysis may be present at the same time, eventually representing a concomitant indication for both immunosuppressive therapy and eculizumab.

We identified in Saint Louis Hospital and Federico II University several cases undergone a simultaneous treatment and we sought to analyze the outcomes. At that time, beyond some single case experience,^{21,22} no data were available in the literature concerning the management of this rare condition. We therefore retrospectively assessed characteristics and outcomes of patients who received intensive immunosuppressive in combination with eculizumab (concomitantly or sequentially administered), from a cohort of PNH patients seen in the two institutions between 2007 and 2016. We identified among 145 PNH patients 9 patients with an overlapping AA/PNH syndrome. These patients received all immunosuppressive regimens before or after the starting of anti-complement therapy (eculizumab was the only therapeutic agent available at that time). None of the patients was required to discontinue eculizumab and most of the patients were in response (partial or complete), with an overall response rate of 66% (6/9 patients), in the range of responses seen for patients with SAA undergoing standard immunosuppression.²³ Intriguingly two cases of clonal myeloid evolution (a very rare event) were seen in this small cohort, highlighting the clonal instability (possibly under the immune pressure) of situations of overt hemolysis and hematopoietic failure. This study has been published in 2017 (*Pagliuca et al. Bone Marrow Transplantation 2017*).

This experience showed for the first time that immunosuppression can be delivered even concomitantly to eculizumab treatment (and vice versa, eculizumab can follow immunosuppression), without any unexpected severe side effect or pharmacological interferences.

In the era of new anti-complement inhibitors, the question as to immunosuppression should administered concomitantly to new anti-hemolytic treatments remains pending, but this study represents a benchmark for future experiences in this view.

3.2.2.c Aplastic anemia in the context of human immunodeficiency virus infection

Decreasing in the incidence of several acquired immunodeficiency syndrome (AIDS)-defining diseases, and so, longer-life expectancy of HIV infected patients, after the introduction of highly active anti-retroviral therapy (HAART) in 1995, run parallel with an increase in other non-commonly AIDS-related pathologies, such as autoimmune disorders²⁴. While hematological abnormalities are frequent in patients infected with HIV and may have a complex origin, there have been only anecdotal case reports of SAA in the setting of HIV infection.²⁵⁻²⁸ Although advances in HCT and immunosuppressive therapy have improved survival in SAA and at least 80% of patients are alive at 5 years post diagnosis, the management of SAA in the setting of HIV infection remains a challenge. Indeed, prompted by common cases discussed internationally, we conducted a study on behalf of SAAWP retrospectively assessing the characteristics of eight HIV-infected patients worldwide who presented with SAA and describe outcomes after both transplant and non-transplant strategies. This study has been published in 2016 (*Pagliuca et al BMT 2016*) and shows the importance of considering allo-HCT for SAA developed in this context, in situations of controlled HIV viral load in the era of HAART.²⁹ In fact, while non-transplanted group was associated with worse outcomes (3/4 patients died of complications, while non-responding to immunosuppressive therapy), most of the patients undergoing allo-HCT (3/4 who received reduced intensity conditioning regimens) were alive at the last follow-up (median follow-up of 59 months at the end of the study). Despite the small number of patients and the retrospective nature, this study represents to date the proof of concept (the first and last one published to date) that HCT should not be contraindicated in HIV-infected patients presenting with SAA.

3.2.2.d Androgens in bone marrow failure disorders

On the wave of these reports on behalf of the SAAWP, another study carried out since 2018 concerned the use of an historical treatment in the context of idiopathic and inherited BMF: androgenic compounds.

Anabolic steroids have been in use for several decades as a class of therapeutics in BMF, due to their pleiotropic effects on erythropoiesis, telomere regulation and immune homeostasis.³⁰ The potential role of androgens in this condition was initially based on the observation, back to the beginning of 20th century, of some sporadic cases of spontaneous remission in young boys with AA at the onset of puberty.^{31,32} These spontaneous hematological recoveries, together with some reports describing the occurrence of myeloid hyperplasia in breast cancer patients receiving testosterone,³³ set the stage for the use of androgen-based protocols alone or in association with various immune suppressive regimens for the treatment of acquired or inherited BMF,^{34,35,36,37} with data generated from randomized

trials since early 80ies.^{38,39} However the discordant results produced by these historical trials failed to prompt androgens as a front line treatment in idiopathic setting. Danazol, 3-alpha-etiocholanolone, nandrolone, oxymetholone, oxandrolone are some of the anabolic-androgenic compounds used in BMF setting, showing variable response and toxicity profiles.^{35,37,40-45} Although integrated in the therapeutic armamentarium for acquired and inherited BMF, many outstanding questions, as to the ideal molecule, optimal dose, timing, disease subgroup, place in the treatment algorithm, factors influencing response, remain unanswered. Moreover, lack of systematic analysis of long-term outcomes of patients receiving androgens, preclude clear positions on their safety profile, especially in terms of oncogenic potential and toxicity.

In this study we assembled the largest international cohort of patients receiving androgens for acquired or inherited BMF on behalf of the SAAWP of the EBMT in order to track their current real-life use, indications, efficacy, toxicity and long-term effects.

We identified 274 patients across 82 EBMT affiliated centers, 193 with acquired (median age of 32) and 81 with inherited BMF (median age of 8 years) **Figure 7**. With a median duration of androgen treatment of 5.6 and 20 months respectively, complete/partial remission rates at 3 months were of 6%/29% in acquired and 8%/29% in inherited disorders. Five-year overall survival and failure free survival (FFS) were respectively 63% and 23% in acquired and 78% and 14% in inherited contexts. Androgen initiation after second line treatments for acquired, and after > 12 months post-diagnosis for inherited group were identified as factors associated with improved FFS in multivariable analysis. Androgen use was associated with a manageable incidence of organ-specific toxicity and low rates of solid and hematological malignancies. Sub-analysis of transplant-related outcomes after exposure to these compounds showed probabilities of survival and complications similar to other transplanted BMF cohorts.

This study has just been published (Pagliuca S. et al. *Haematologica* May 2023) and represents to date the largest series informing on BMF outcomes after androgen use. In particular it delivers a unique opportunity to define general recommendations regarding the place of these compounds in patient treatment.

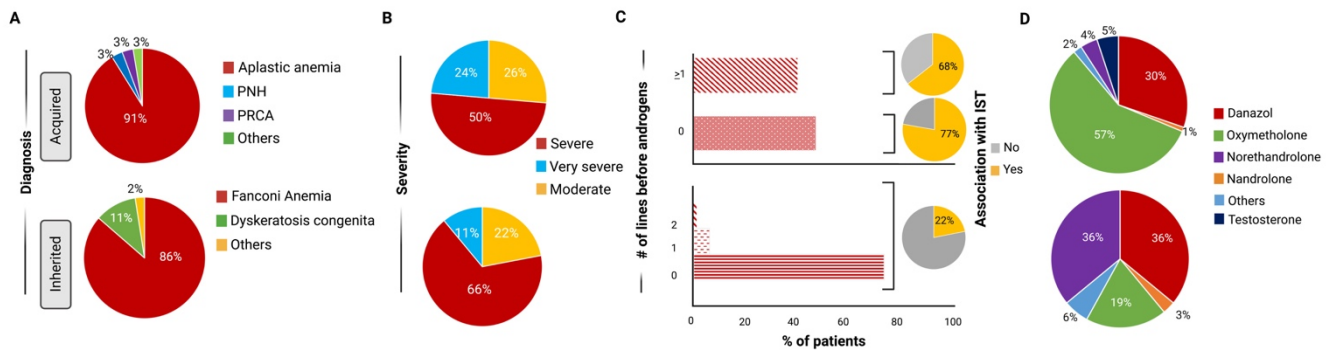


Figure 7: Clinical background of acquired and inherited BMF cohorts (Adapted from Pagliuca et al. *Haematologica* 2023). **A)** Pie charts showing the distributions of diagnosis as identified into the registry in inherited and acquired disorders. **B)** Pie charts depicting the distribution of severity of BMF disorders (upper: acquired, lower: inherited). **C)** Bar charts showing the distribution of patients according the number of lines received before androgen therapy (upper: acquired, lower: inherited). Pie charts showing the associations with IST (gray: No; yellow: Yes; upper: acquired, lower: inherited). **D)** Pie charts showing the distribution of androgenic compounds used for acquired (upper) and inherited (lower) disorders.

3.2.3 Major translational research axis: Genomic dysfunction of human leucocyte antigen diversity in hematological disorders exposed to an immune pressure

3.2.3.a Personal premise

Given the studies conducted in the context of allo-HCT and BMF between 2015 and 2019 that initiated me to these research fields, paralleling years of intensive clinical training, I decided to devote the following years of my career to develop the translational aspects missing with my clinically focused background. Therefore, I enrolled in a PhD program at the University of Paris, and I started to develop my research project in partnership with the Translational Hematology and Oncology Research Department of Cleveland Clinic under the guidance of Pr. Jaroslaw Maciejewski, a renewed BMF world expert. Without knowing the outcomes of this decision, and with many people discouraging me from this choice, requiring many sacrifices on the personal and family level, I left my comfort zone and started this new adventure. Therefore, my son and I went to Cleveland while my husband remained in Paris for work. It was November 2019, just 2 months before COVID-19 spread. My son was 2 years old. COVID-19 arrived soon. Everything closed, included the international boundaries. I did not know at that time, but I would have seen my husband again only ten months later.

And I started to work on my projects. And I started to study, to try and to fail. And I started to try again and fail again. And I tried until I succeed. I remember that I was sometimes scared, sometimes reassured that the hard work would have payed back all the difficulties.

As a disclosure, I have to acknowledge that that period would have been harder if Carmelo and Valeria, my Cleveland colleagues and friends, were not there and if the working environment would not have been so funny, cozy and worm. This helped us to go through COVID-19, to work productively and to learn together from our research.

3.2.3.b Development of a transversal research thematic

Indeed, I started to refine my knowledge of HLA, T cell receptor (TCR) repertoires, adaptive immune system and immune peptidomes and how all these components mix together to induce immune competence or immune failure in pathological conditions. When you study immune system and the evolution of ideas around the concept of immune competence, you likely start to be interested on how these ideas came up historically and how they were supported by research. It appears almost incredible to me that all the proofs of immune responses in the context of tumor, infectious and autoimmune control, have always been merely indirect, with to date many questions remaining to be answered on how a competent adaptive immune system elaborates its responses to control aberrant cellular status. Of note, most of our understanding of pathophysiological mechanisms involved in immune control and failure derive from transplant studies, and more recently from the development and application of adoptive transfer cellular therapies.

I started to approach this thematic starting from aplastic anemia and allo-HCT, my passionate topics. And I started to go deeper into the molecular details of HLA molecules and TCR repertoires as proxies for adaptive immune responses trying to define approaches to individualize the specificities of these reactions. While studying the molecular components of T cell adaptive responses I was interested in understanding the mechanisms of failure in hematological diseases and I soon realized that these mechanisms were not different from those operating in solid tumors, despite specificities related to context, targeted tissue and microenvironment, with the same patterns of immune evasion transversally found across diseases.

On a molecular point of view, one of the foundations for a competent adaptive immune system is the structural and functional variability of HLA molecules which ensures responses against infectious and tumor antigens through the breadth of the immune peptidome presented, sustaining in physiological conditions an efficient diversification of TCR repertoires.^{46,47,48} This variability is supposed to be maintained by evolutionary forces that have historically shaped the human adaptive

immune system due to pathogen exposure, favoring, via the accumulation of mutational events, the assortment of a hyper-polymorphic HLA system able to elaborate efficient immune responses.^{49,50,51} Any dysfunctional event impacting this diversity may be at the basis of pathological processes, disturbing antigen presentation capabilities and T-cell reactivity. If, on one side, particular germline configurations of HLA genotypes can convey an immunogenetic risk for autoimmune and complex disorders or predispose to a better response to anti-cancer treatments, on the other side, somatic rearrangements of HLA loci (e.g., HLA genomic losses and mutations), decreasing such variability, can modulate mechanisms of immune escape well assessed in cancer biology.

A concept that started to capture our (my) attention while studying the impact of HLA in hematological disorders was the fact that we could mathematically measure HLA heterogeneity (structural and genomic variability) at a particular locus. This measure, called “HLA evolutionary divergence” (HED) recently developed by an evolutionary biologist, Pr. Tobias Lenz, and firstly applied to quantitate the number of infectious antigens that HLA complexes could present, seems of particular interest for many aspects of immune competence from autoimmune disorders, to anti-tumor control and immune reconstitution after allo-HCT (see Annex I for further explanations).

Collaborations with Pr. Lenz regarding many aspects of our work allowed us to explore this immunogenetic aspect under an evolutionary perspective providing further instruments to understand the pathophysiology of immune dysregulation in BMF and after transplant.

Indeed, my PhD thesis work (<https://www.theses.fr/2021UNIP7013>) was devoted to the investigation across multiple cohorts of hematological patients, of the multifaced molecular dysfunction of HLA machinery and its impact on TCR functionality, demonstrating how this mechanism may shape clonal evolution, construing a common pathogenic lynchpin encompassing benign and malignant pathophysiology.

These concepts are well summarized in a review published last-year from our group (*Pagliuca et al. T. Lenz last author, Frontiers in Immunology 2022*), where we reported our perspective on the concept of HLA heterogeneity and how it may influence tumor biology and autoimmune disorders.⁵² For the Reader’s convenience, most of these biological aspects are summarized in the Annex 1 of this thesis manuscript.

Indeed, through a multiomic approach we characterized the molecular landscape of the antigen presentation and T-cell activation structures in multiple cohorts of patients diagnosed with idiopathic BMF disorders and hematological malignancies. This aspect was also largely studied in the context

of allogeneic HCT for myeloid neoplasms, where we were able to dissect the patterns of TCR reconstitution and the molecular mechanisms of HLA-mediated immune escape.

It is noteworthy to mention that on a methodological point of view, given the absence of analytical tools to identify somatic genomic alterations in the hyperpolymorphic HLA loci as well as the difficulties to represent TCR data and specificities, out of next generation sequencing (NGS) data, a great part of my work was also consecrated to develop analytical bioinformatic utilities to solve the current methodological issues and answer our biological questions. I thus collaborated with IT and bioinformaticians to optimize the genomic analysis of HLA region and the analysis of TCR repertoires. Among the other things, this research led to a no-profit scientific collaboration with a bioinformatic company (Novocraft Technologies) that has now developed a tool providing high-resolution HLA genotyping from all kinds of sequencing data (targeted, whole genome, whole exome, and transcriptomic), with potential applicability in clinical setting. We therefore developed a bioinformatic approach able to dissect the mutational somatic landscape of the hyperpolymorphic HLA region from multiple sources of genomic data (deposited into the freely available repository: <https://github.com/SMNPAG/HLA-mutations> under the DOI: <https://doi.org/10.5281/zenodo.7651570>).

The institution where this research has been conducted (Cleveland Clinic) represents one of the cardinal centers for the genomics of BMF disorders and myeloid malignancies and has contributed over the years to the discovery of new genes involved in leukemia and myelodysplastic syndrome pathogenesis. A chapter opened with the present research was the integration of a perspective of immunoediting as a process shaping the malignant clonal evolution in multiple contexts. Taken together this scientific literature may help in understanding the immunogenetic basis of clonal hematopoietic disorders and the genomic dysfunction beyond the "adaptive" and "maladaptive" immune evasion.

3.2.3.c Immunogenetics and clonal evolution in idiopathic BMF

On the wake of our immunogenetic studies we focused a lot on idiopathic AA, a disease whereby many pathophysiological and etiological instances remain so far unknown (namely, the identification of antigenic challenges involved and their influence on T cell activation).

In the following sections, while providing a brief overview of the multifaceted and complex immune pathophysiology of AA and PNH, I will present the principal results of the studies that I had the privilege to conduct while in Cleveland either as a project leader or contributing to supervising younger colleagues.

3.2.3.c.1 Idiopathic BMF disorders: concepts of immune pathophysiology and clonal evolution

AA is a prototypic immune mediated bone marrow failure disorder, characterized by an HLA-restricted T cell autoreactivity against unknown antigens presented on the surface of HSCs.⁵³

The multimodal immunological derangement at the basis of this disease encompasses all the arms of the adaptive and innate immune system and may drive clonal selection mechanisms (**Figure 8**). CD8+ T cell oligoexpansion together with CD4+ aberrant activation and interferon-gamma (IFN γ) inflammatory response are pledges of disease pathogenesis and may be theoretically reverted by immunosuppressive therapeutic regimens.^{54,55,56,57}

AA-related immunological abnormalities concern numbers and functions of different subsets of immune cells, including CD8+ cytotoxic lymphocytes, CD4+ helper, and T regulatory cells (Tregs), described in patients with AA with or without PNH.^{58,59} Particularly, restricted CD8+ TCR are detectable at disease onset and have been related to treatment outcomes and relapse.^{60,61} These oligoclonal cells produce an inflammatory cytokine milieu enriched in IFN γ and act as late effectors of HSC compartment reduction, inducing FAS-mediated apoptosis.^{62,53,63} CD4+ T cells subsets dysregulation also plays an essential role in AA pathogenesis, with an imbalance in T helper (Th) CD4+ cells subsets and redistribution of Th1, Th2 and Th17 responses. Increased Th17 populations are found in peripheral blood and bone marrow in AA and their number is indirectly correlated with Treg cell counts.⁵⁷ Tregs usually contribute to suppressing autoreactivity of T cell subclones, constraining the possible development of autoimmunity. Two distinct Treg subpopulations (A and B subtypes) characterized by specific phenotypes, gene expression signature and function, have been identified in AA patients and associated with variable response to immune suppression.⁶⁴ This skewing of T cell subgroups causes (and is at the same time consequences of) an overshooting of inflammatory cytokines, particularly IFN γ , and tumor necrosis factor α (TNF α), whose levels have been reported to be higher in bone marrow of patients with AA compared to healthy controls.⁶⁵ IFN- γ plays a significant role in suppressing HSCs, as suggested by in vitro inhibition of cell cycle progression and induction of apoptosis of hematopoietic progenitors.^{11,67,68} This cytokine may also exert its inhibitory effect on HSC impairing the homeostatic survival signal delivered by thrombopoietin through its cognate receptor c-MPL.⁶⁹ Single nucleotide polymorphisms of the IFN- γ gene have been shown in a high percentage of AA patients, resulting in higher levels of IFN- γ expression compared with normal controls.^{70,71,72,73} Among other effects, IFN- γ contributes to upregulating the expression of HLA complexes, with a pivotal impact on class II transcriptomic regulation and, specifically, on DR molecules.^{74,75,76} This aspect is of particular interest giving that

HLA risk allele profiles identified in historical AA cohorts belong to this locus (e.g., DRB1*15:01).^{77,78,79,80,81,82,83} In this context, it is not excluded that this IFN- γ overproduction elicits the presentation of immunodominant peptides via the upregulated targets.

PNH, on the other hand, is considered an acquired clonal disorder of HSCs, linked to AA, clinically characterized by cytopenia, hemolysis, and thrombophilia. The presence of acquired loss-of-function genomic aberrations in phosphatidylinositol glycan anchor biosynthesis class A (PIGA) gene, (chr. Xq22.2) represents the etiopathological hallmark of this disease.^{84,85} At least 20% of patients bear more than 1 mutation and subsequently more than 1 clone (PIGA mosaicism), rendering this disease particularly puzzled in terms of clinical pictures and molecular features.^{86,87} PNH cells lack all glycosylphosphatidylinositol (GPI) anchor proteins, including CD55 and CD59, which, among other functions, work as complement regulators, especially abundant on the surface of red blood cells. This feature renders PNH erythrocytes particularly vulnerable to complement-mediated lysis.⁸⁸ Giving the prominent role of complement system in disease pathogenesis, many efforts in the last decades have been spent in the identification of pharmacological targets able to control PNH clinical manifestations.^{19,16,89,90,89,91} Thus eculizumab and ravulizumab, two humanized monoclonal antibodies inhibiting the terminal steps of complement cascade, are effective in preventing hemolysis, improving anemia and reducing the risk of thrombosis.^{20,92,93} While historically, great attention in terms of therapeutical approaches has been invested in terminal complement pathway blockade, recent advances now show the efficacy of proximal complement inhibition in containing disease symptoms.^{94,95,96,97}

AA and PNH, both rare diseases, often overlap and the detection of PNH clones in patients with AA either at diagnosis or during disease evolution has been widely described. It is also not uncommon that clinical pictures characterizing both disorders intertwine switching over the time and after immune suppression.^{98,99,100,101} Among different types of mutations causing clonal hematopoiesis within AA, PIGA somatic hits are very frequent as up to 70% of patients with AA may harbor a detectable PNH clone.^{102,103,104,105}

In a historical insert published on Cell in 1997, Lucio Luzzatto, Monica Blesser and Bruno Rotoli beautifully gave form to one of the most quoted and fascinating theories about the expansion of PNH clones, the "escape hypothesis", defining the arising of PIGA mutation within the context of AA a "blessing in disguise".^{103,106} Since then, it was suggested that BMF in PNH is related to a T-cell-mediated autoimmune mechanism targeting GPI-positive but not GPI-negative HSCs. Thus the GPI-negative HSCs do not significantly sustain the hematopoiesis unless a T-cell-mediated autoimmune noxa targets selectively GPI-positive HSCs, favoring the expansion and differentiation of GPI-negative HSCs themselves, responsible for the classic hemolytic PNH phenotype.^{103,107,108} Compelling

evidence shows that the immunological pressure deriving from the T-cell mediated HSC selection configures an environment suitable for clonal selection processes at the basis of clonal evolution.^{109,110} Generally speaking clonal hematopoiesis has been shown in almost half of AA patients, most frequently as acquired mutations in DNMT3A, ASXL1, BCOR, BCORL1, and the same PIGA gene.^{106,110} Those mutations not only correlate with a different pattern of response to immunosuppressive therapy and generally different outcomes but had also a variable dynamics in the setting of clonal pressure, within a process of Darwinian fitness of the clone.^{110,111}

The emergence of immune privileged PNH clones represents thus now a well-established mechanism of non-malignant recovery, leading to secondary PNH expansion. In contrast, the occurrence of somatic leukemogenic mutations may enable selective expansion of immune-resistant malignant clones initiating progression to MDS or AML.^{112,113,114,105} Malignant evolution rate approaches 15%-20% at 10 years from diagnosis and is often associated with the acquisition of high-risk cytogenetic aberrations such as monosomy 7 or the deletion 7q.^{115,116,117}

More recently further evidence has supported the direct role of class I HLA molecules in the ontogeny and clonal complications of AA.^{118,119,120} Loss of expression of HLA molecules on the surface of HSC cells via genetic lesions selected by immune pressure has been shown as able to sustain a hematopoiesis resistant to immune destruction, in a way potentially alternative to PNH.^{120,118,121,122} Specifically, genetic loss of HLA loci (as either copy neutral loss of heterozygosity of chromosome arm 6p (6pLOH) or loss-of-function somatic mutations), translating in a loss of presentation of immunodominant antigens, can be present in AA and hypoplastic MDS patients as marker of immune selection, in the context of clonal hematopoiesis.^{119,118,120,112} The presence of multiple mutations in the coding region of class I HLA genes has been demonstrated in 20-30% of AA patients and the hypothesis that few risk alleles targeted by those hits may be closely involved in the self-antigenic presentation in AA remains more than captivating.^{120,121,119} More uncertain is the role of somatic aberrations in HLA region in the context of malignant clonal evolution, that represented one of our principal research questions. Recent evidence at the time of our studies were produced by Prof. Nakao's group, showing that the presence of HLA class I negative granulocytes was not associated with clonal expansion to MDS/AML in Japanese AA patients.¹²³

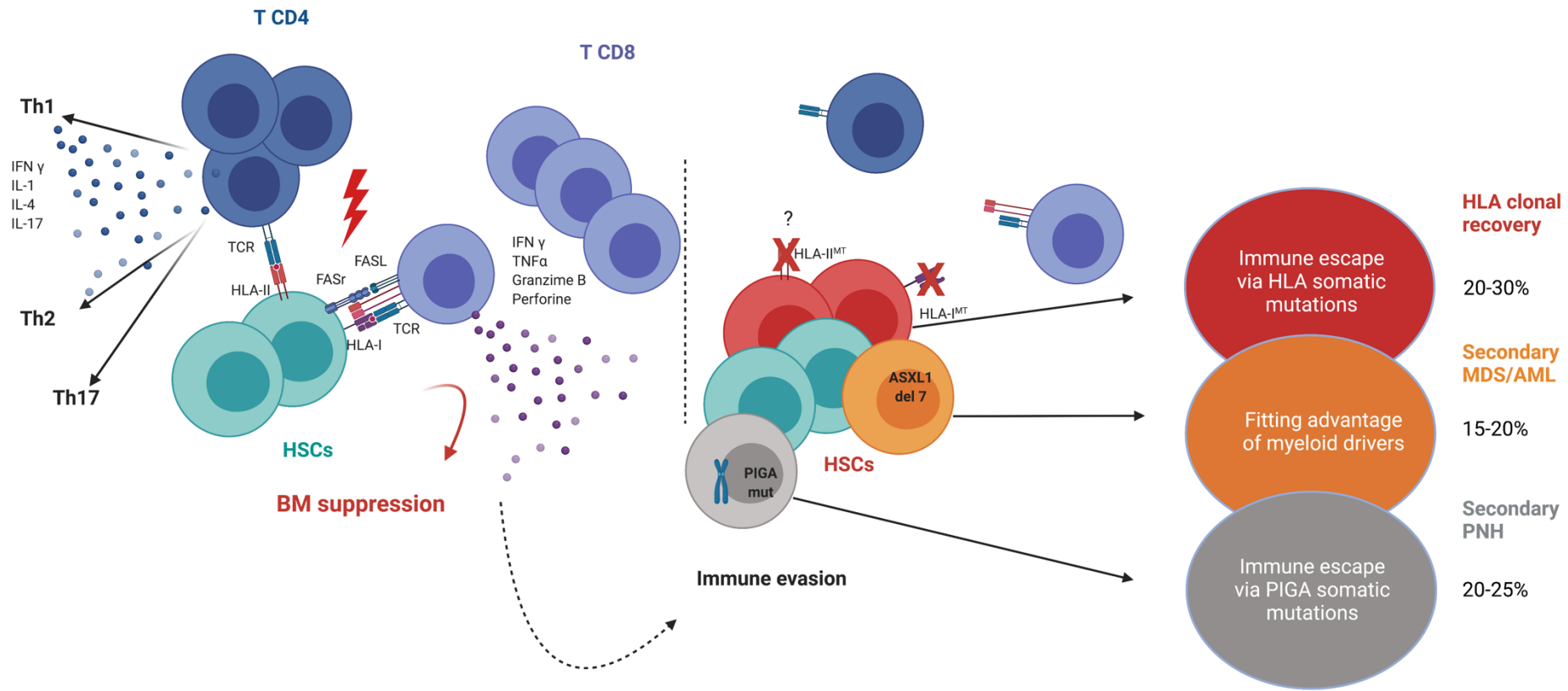


Figure 8: Immune pathophysiology of idiopathic aplastic anemia

AA is thought to be initiated by the recognition and destruction of HSCs by cytotoxic T lymphocytes, which recognize some unknown antigen presented on HSCs via their HLA class I molecules. Evidence supporting this hypothesis is the limited usage of T-cell receptor beta subsets at diagnosis, suggestive of the presence of a clonal expansion of CD8+ T cells, which diminish or disappear with successful IST. Overproduction of inflammatory cytokines creates a chain reaction able to induce a derangement of the arms of the adaptive immunity including Th1, Th2 and Th17 responses, which all contribute to establishing the bone marrow failure, associated with the apoptosis of HSCs. Hematopoietic recovery either after a successful immune suppression or in the context of the chronic immune selection process, may harbor clonal abnormalities and resultant expanded cell populations will be characterized by a fitting survival advantage, increasing their resistance to immune attack. This way different mechanisms of immune escape or cell proliferation may lead to “adaptive” (HLA and PNH expansion) and “maladaptive” (myeloid progression) HSC clonal expansion. Frequencies of these modes of clonal recovery are reported in the figure.

3.2.3.c.2 Deep characterization of HLA-Antigen-TCR synopsis in idiopathic bone marrow failure disorders

Inspired by the emerging role of HED as an immunogenetic determinant of a competent adaptive immune system, our research question was to understand whether the functional and structural variability of HLA molecules could influence the proclivity to autoimmunity. To that end, we performed a large case-control study in a cohort of 300 AA patients and almost 3000 healthy and disease controls, by deeply dissecting their genotypic configurations, functional divergence, self-antigen binding capabilities and TCR repertoire specificities. We first performed HLA allele frequency estimation that helped in the identification and confirmation of the alleles more likely associated with the disease.

We then examined the impact of the global genotypic and molecular HLA diversity for both class I and II loci on either disease predisposition or characteristics at diagnosis and clinical outcomes.

We observed that class II HLA molecules of AA patients were characterized by a high level of structural homology, only partially explained by specific risk allele profiles. This analysis was followed by a recursive study of peptide binding sites within the loci more frequently interested by skewed allelic frequencies (DRB1 and DQB1) in order to identify specific amino acid patterns potentially associated with self-antigen presentation in AA patients. We were able this way to characterize specific residues enriched in the peptide binding cleft of those class II HLA molecules affecting the interface HLA-antigen-TCR β and potentially constituting the basis of T-cell dysfunction and autoreactivity (**Figure 9**). When analyzing associations with clinical outcomes, low HED was associated with risk of malignant progression and worse survival, underlying reduced tumor surveillance in clearing potential neoantigens derived from mechanisms of clonal hematopoiesis.

Finally, we studied how risk and divergent allelic profiles influenced the presentation of HSC immunopeptidome and the TCR repertoire characteristics.

An important part of this work relates to antigen modeling within the peptide binding cleft of DRB1*15:01, the strongest risk allele identified in our cohort, proceeding via analogies with antigenic structures already identified for this allele in other autoimmune disorders (e.g., myelin basic protein [MBP] in multiple sclerosis).

This work has been published at the end of 2021 (Pagliuca et al. Blood 2021) and among others comported a tight collaboration with evolutionary biologists, geneticists, immuno-oncologists, protein engineers and computational biologists who helped me in defining strategies to analyze complex immunogenetic and immunopeptidome data.

This study received several recognitions on the international levels (ASH award achievement award and EBMT best young abstract) and was featured in the program of the last European school of hematology (ESH) meeting on BMF that was held in Paris in November 2022.

As a future perspective, this work has opened a new line of translational research in Maciejewski lab, investigating the efficacy of antigen-based therapeutics that, similarly to a first in class HLA-DRB1 agonist, PV-267, under investigation in multiple sclerosis (see ANNEX I), can adapt into the binding groove of DRB*15:01 specifically modeling on the residues identified as enriched in AA patients.

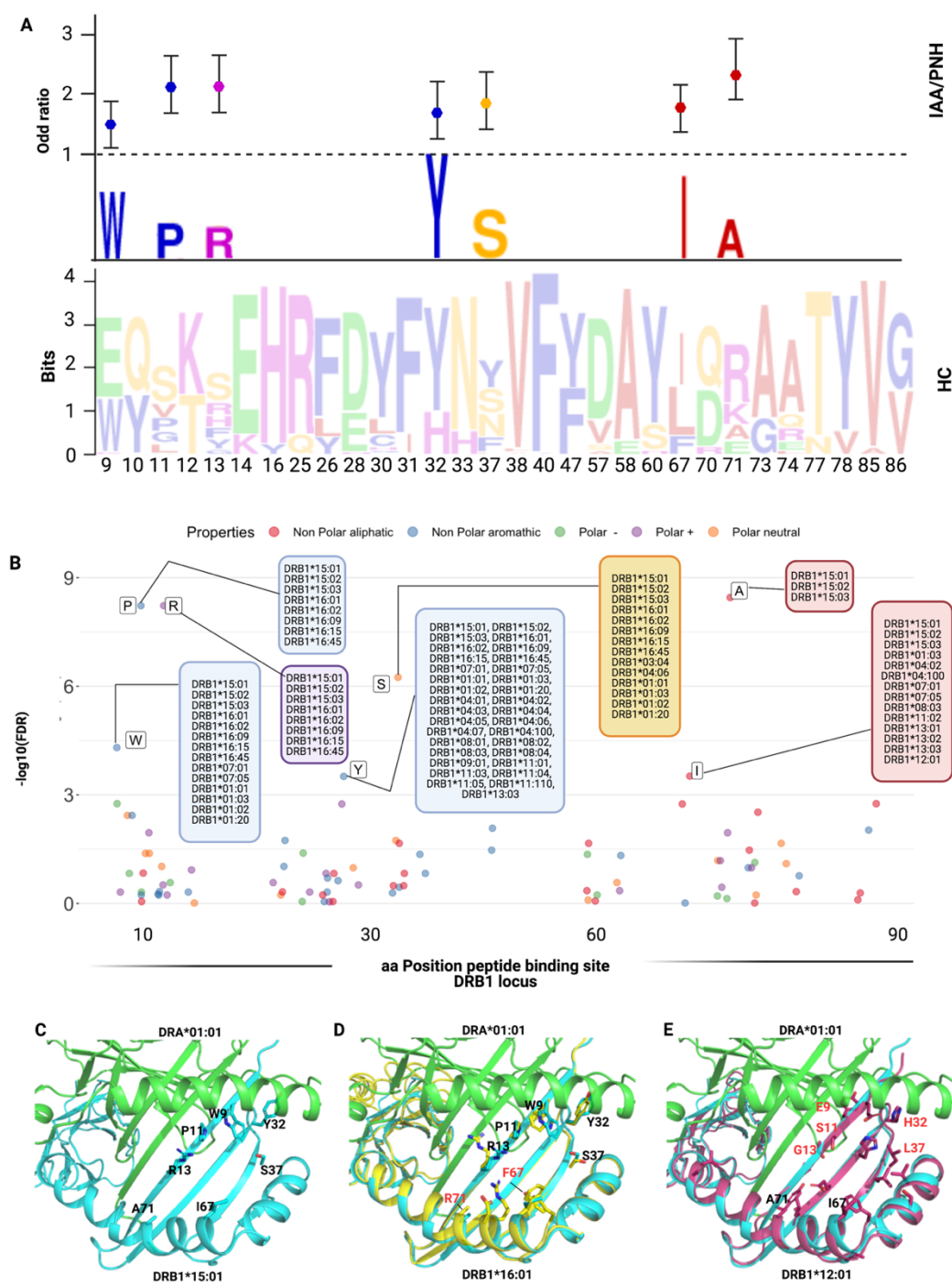


Figure 9: Recursive analysis of the amino acid sequence within the peptide binding site of DRB1 locus of idiopathic aplastic anemia and PNH patients (adapted from Pagliuca et al. Blood 2021).

Abbreviations: IAA: Idiopathic aplastic anemia; PNH: paroxysmal nocturnal hemoglobinuria; HC: healthy controls; aa: amino acid

A) Lower panel: WebLogo visualization representing the contribution of single aminoacids within the variable portion of the peptide binding site of DRB1 locus. The x-axis indicates each variable position (as per IPD-IMGT-HLA

reference). Letters represent each possible amino acid at each given position; Letters' height illustrates the frequency of each amino acid in healthy control population. Colors indicate the chemico-physical properties as per legend in B.

Upper panel: stylized visualization of amino acids differentially distributed between HC and IAA/PNH cohorts. Letters' height illustrates the frequency of each amino acid in disease population. Markers indicate the odd ratios resulting from the logistic regression analysis studying each aminoacidic contribution in determining the phenotype.

B) Scatter plot showing the significance level of each variable amino acid in the peptide binding site of DRB1 locus found enriched in IAA/PNH population compared to HC. Each dot represents the negative logarithm base 10 for the adjusted p-value (q-value) referring to each amino acid. The position on x-axis indicate the position within the peptide binding site according to IPD-IMGT-HLA reference). Only the amino acids presenting a q-value < 10⁻⁴ are considered significant for this analysis and labelled in the figure. Alleles presenting the indicated amino acid at the given position are indicated in the boxes. Colors represent the chemico-physical properties as per legend.

C) Crystallographic structure showing the position of the 7 amino acids significantly enriched in the peptide binding groove of DR molecules of IAA/PNH patients. This 3D structure has been visualized with the PyMOL program based on the structure of DRB1*15:01-DRA*01:01-myelin binding protein (PDB:1BX2) and HLA sequences retrieved from IPD-IMGT/HLA database v. 3.40. Only the 7 amino acids identified in the previous analysis have been highlighted.

D) Binding site of DRB1*16:01-DRA*01:01 based on the homology model of DRB1*16:01. The structure of DRB1*16:01 has been superimposed to DRB1*15:01. Residues differing from the risk pattern seen in DRB1*15:01 are colored in red.

E) Binding site of DRB1*12:01-DRA*01:01 based on the homology model of DRB1*12:01. The structure of DRB1*12:01 has been superimposed to the structure of DRB1*15:01. Residues differing from the risk pattern seen in DRB1*15:01 are colored in red.

3.2.3.c.3 Immune pressure and immune escape in aplastic anemia

The immune attack inevitably generates a depauperated HSC compartment in the bone marrow, resulting in peripheral cytopenias of various degrees which are responsible for high morbidity and mortality if the disease is left untreated. In this context, the occurrence of *PIGA*- and class I HLA mutant clonal expansions have been invoked as mechanisms of HSCs immune evasion from T-cell pressure. While PNH evolution is a well-recognized mode of immune escape and is encountered in up to 20% of IAA patients during the disease course, genomic dysfunction of class I HLA molecules has been more recently (but less) characterized. This mechanism has been shown able to reduce T-cytotoxic induced HSCs destruction by decreasing the presentation threshold of putative autoantigens, however its possible role in determining myeloid neoplasia transformation in the context of this disease remains one of the outstanding open questions.

Immune evasion is a range of molecular events well assessed in cancer biology where multiple mechanisms may converge in determining a similar phenotype of decreased immunogenic clones and reduced T-cell activation as a result of the initial immune pressure (*i.e.*, induced by tumor surveillance or immunotherapy). In our work we provocatively suggested the presence of similar forces of

immunoediting able to sculpt, shape and imprint (as in the context of tumor progression) clonal hematopoiesis and trajectories of clonal evolution. Indeed, in a large cohort of patients with AA and PNH we studied the genetic and transcriptomic make-up of the antigen presentation complexes by using single-cell RNA, high throughput DNA sequencing and SNP-array platforms. We observed that at disease onset HSPCs displayed activation of selected HLA class I and II restricted mechanisms, without extensive inhibition of immune checkpoint apparatus. Using a newly implemented bioinformatic framework we found that not only class I but also class II genes were often impaired by acquisition of genetic aberrations. We also demonstrated the presence of novel somatic alterations in immune genes possibly contributing to the evasion from the autoimmune T-cells. In contrast, these hits were absent in myeloid neoplasia. These aberrations were not mutually exclusive with PNH and did not correlate with the accumulation of myeloid-driver hits. Our findings shed light on the mechanisms of immune activation and escape in AA and define alternative modes of clonal hematopoiesis.

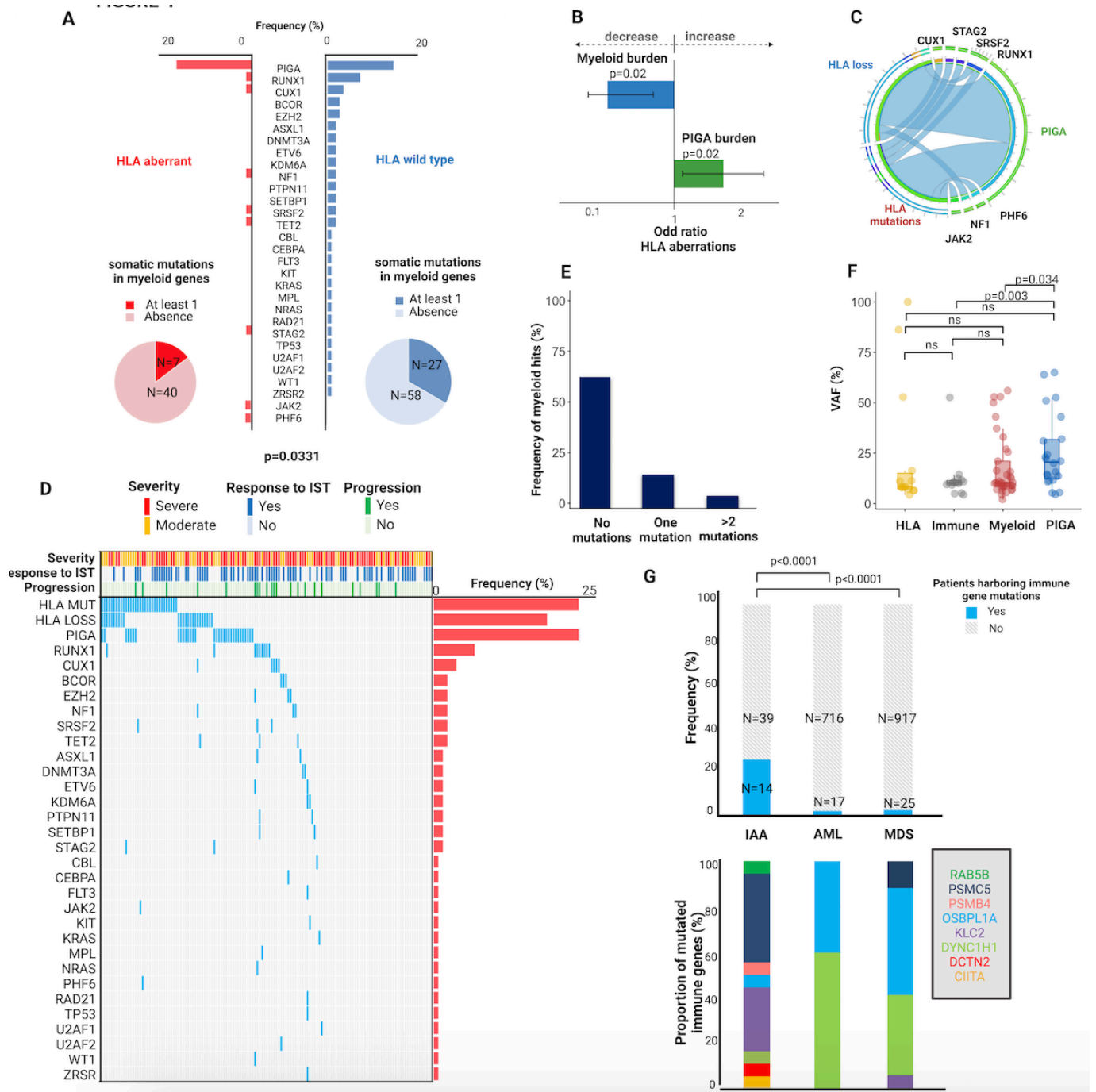


Figure 9: Landscape of HLA aberrations and myeloid molecular lesions. **A)** Bar plot representing the frequency of myeloid alterations within HLA-mutated and non-mutated group, with pie charts depicting the distribution and the total number of myeloid mutations. p-value calculated using chi-square test. **B)** Bar plot indicating the odd ratio (position of the box on x-axis) and the 95% confident interval (positions of error bars) for associations with HLA aberrations and respectively the myeloid burden (number of mutations in myeloid genes, blue box) or PIGA burden (number of mutations in PIGA gene, green box). Odd ratio calculated with a general linear regression model using Poisson distribution **C)** Circle plot showing the number and reciprocal distribution of the pattern of co-mutations within HLA aberrant samples. **D)** Waterfall plot showing the distribution of HLA and myeloid lesions (clustered for HLA mutations). Each colored spot indicates the presence of the lesion while gray spots its absence. The bar plot on the right captures the frequency of each aberrant entity. **E)** Barplots showing the rank of myeloid somatic hits in patients undergone a targeted myeloid and PIGA

gene panel sequencing (N=132). **F)** Boxplots showing the distribution of VAF in HLA, myeloid, PIGA and non-HLA immune genes. **G)** Barplots representing the distribution of non-HLA mutated immune genes across different cohorts of patients identified through WGS (IAA: idiopathic aplastic anemia; AML: acute myeloid leukemia; MDS: myelodysplastic syndromes) Numbers indicate number of patients in each group (light blue: mutated; gray: non mutated).

This is a concept completely new in the immunobiology of an autoimmune disease and in our view, it is not unlikely that similar immune evasion mechanisms may be present in tissues targets of immune processes in other contexts of autoreactivity. This study was featured in Leukemia journal last year (Pagliuca et al 2022) and the bioanalytical platform that I have implemented for the study of somatic changes in HLA region, as described in the original work, has been applied for many other works from our group and remains in use by our members of our lab for ongoing projects.

Specifically, I comanaged with Dr. Carmelo Gurnari, a young brilliant hematologist, in training at that time, a number of further studies on immunobiology and clonal evolution in AA and PNH, including projects conducted in the setting of international collaborations and multi-institutional partnerships.

[Clinical and Molecular Determinants of Clonal Evolution in Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria.](#)

Gurnari C, **Pagliuca S**, Prata PH, Galimard JE, Catto LFB, Larcher L, Sebert M, Allain V, Patel BJ, Durmaz A, Pinto AL, Inacio MCB, Hernandez L, Dhedin N, Caillat-Zucman S, Clappier E, Sicre de Fontbrune F, Voso MT, Visconte V, Peffault de Latour R, Soulier J, Calado RT, Socié G, Maciejewski JP. *J Clin Oncol.* 2023 Jan 1;41(1):132-142. doi: 10.1200/JCO.22.00710. Epub 2022 Sep 2. PMID: 36054881

[Is nature truly healing itself? Spontaneous remissions in Paroxysmal Nocturnal Hemoglobinuria.](#)

Gurnari C, **Pagliuca S**, Kewan T, Bahaj W, Mori M, Patel BJ, Visconte V, Maciejewski JP. *Blood Cancer J.* 2021 Nov 27;11(11):187. doi: 10.1038/s41408-021-00582-5. PMID: 34839350

[Implication of PIGA genotype on erythrocytes phenotype in Paroxysmal Nocturnal Hemoglobinuria.](#)

Gurnari C, **Pagliuca S**, Patel BJ, Awada H, Kongkiatkamon S, Terkawi L, Zawit M, Corey S, Lichtin AE, Carraway HE, Wahida A, Visconte V, Maciejewski JP. *Leukemia.* 2021 Aug;35(8):2431-2434. doi: 10.1038/s41375-020-01113-0. Epub 2021 Jan 22. PMID: 33483614

[Monoclonal IgM gammopathy in adult acquired pure red cell aplasia: culprit or innocent bystander?](#)

Gurnari C, **Pagliuca S**, Awada H, Zawit M, Patel BJ, Visconte V, Valent J, Rogers HJ,

Maciejewski JP. *Blood Cells Mol Dis.* 2021 Nov;91:102595. doi: 10.1016/j.bcmed.2021.102595. Epub 2021 Jul 14. PMID: 34280665

[Current Opinions on the Clinical Utility of Ravulizumab for the Treatment of Paroxysmal Nocturnal Hemoglobinuria.](#)
Gurnari C, Nautiyal I, **Pagliuca S.** *Ther Clin Risk Manag.* 2021 Dec 14;17:1343-1351. doi: 10.2147/TCRM.S273360. eCollection 2021. PMID: 34934322

3.2.3.d Immunogenetics of leukemia relapse after transplant

Allo-HCT holds a pivotal place in the therapeutic algorithm of myeloid neoplasms (MN).^{124,125} Either as an upfront treatment in diseases with low blast burden, after disease control in myelodysplastic syndromes (MDS) or following consolidation therapy in intermediate and high-risk acute myeloid leukemia (AML), its therapeutic index is principally related to the immunogenic GvL effect, exerted by donor-derived immune effectors on leukemic cells.¹²⁶ Relapse following allo-HCT may be mechanistically multifactorial and includes re-expansion of the residual leukemia with or without acquisition of novel driver lesions along with the development of various immune escape mechanisms contributing to the evasion from GvL effect.^{127,128}

Post-transplant alloreactivity ideally acts via class I and II HLA-restricted mechanisms. Therefore, one of the biological modes of relapse after allo-HCT is the loss of expression of disparate immunodominant HLA alleles via loss of heterozygosity (LOH) or epigenetic down-regulation, which have been demonstrated in haploidentical, mismatched unrelated and matched related settings.^{129,130,131} While large genomic aberrations including the loss or the duplication of an entire haplotype or of a single allele have been noted across various studies,^{129,132,133,134} more granular lesions of the HLA region including microdeletions and point mutations, remain underestimated in allo-HCT. Such lesions, ultimately resulting in phenotypes of immune resistance, have been reported in the context of clonal adaptive recovery in immune-mediated AA or as immune escape drivers in solid tumors, together with up-modulation of negative immune checkpoint regulators.^{135,136,137}

HLA heterogeneity and the type of HLA polymorphisms in certain loci have also been found as factors influencing the direction of alloreactive responses.^{138,139,140,141,142} HED (discussed above) a metric reflecting the immunopeptidome diversity, has been recently linked to post-immunotherapy outcomes in solid tumors.¹⁴³ Furthermore, we and others demonstrated that recipient HED correlates with the efficiency of immune reconstitution after allo-HCT.^{144,145} Since this score mirrors the antigenic spectrum capacity, it may be considered a surrogate of the individuals' ability to present leukemia associated antigens (LAA) in transplant recipients.^{143,146,147}

Again, in analogy with solid cancer immunotherapy, we hypothesized that, also in the context of allo-HCT, a reduced variability of HLA genotypes as well as genomic mechanisms of escape can converge in overlapping immune-escape phenotypes.

In this work we investigate relapsed leukemia in allo-HCT recipients for the presence of non-driving genomic lesions in immune elements as part of the mutational landscape of recurrent leukemia. To unravel the genomic dysfunctions leading to immune-escape, we studied HED and both genetic and transcriptomic inactivation of HLA via somatic mutations or down-modulation as determinants of leukemic relapse in the setting of allo-HCT.

We demonstrated how both germline and somatic dysfunction of HLA heterogeneity provides an immunogenetic framework prone to post-transplant leukemia immune escape and relapse.

Particularly we showed that preexistent germline-encoded low HED of class II HLA genotypes (en in particular DQB1 and DPB1) constitutes an independent factor associated with disease relapse.

To link immune control dysfunction with abnormal HLA heterogeneity, we performed deep targeted NGS for TCR Vbeta CDR3 after 3-6 months following allo-HCT and characterized the specificity of TCR repertoire using our previously described bioinformatic framework.¹⁴⁵ Overall, patients with higher class I and II HED were characterized by a less expanded repertoire together with increased cancer-related clonotypes and less clonally-polarized features compared to those with lower divergence. We then assessed the distribution and the frequency of HLA mutations in a subset of longitudinally genotyped patients relapsing after allo-HCT. We compared specimens at diagnosis, at post-chemotherapy and post-allo-HCT relapse sequenced for both HLA mutations and myeloid genes. Whereas somatic HLA hits were present in 22% of patients at diagnosis and in 38% of patients at post allo-HCT relapse, in cases with relapse following chemotherapy no HLA hits were found underlying the possible non-immune mediated mechanisms driving post-chemotherapy relapse (**Figure 10**). In addition to somatic HLA hits, we also investigated the transcriptional changes of HLA and immune genes occurring from diagnosis to post allo-HCT relapses. In post-transplant specimens, HLA genes were often down-regulated, specifically class II loci HLA-DRB1, -DQB1 and -DPB1. In those with low HLA expression, somatic HLA aberrations were virtually absent, except for 1 case with a mutation in HLA-DRB1. Overall, expression of classical immune checkpoint genes was not significantly altered at relapse, whereas mRNA levels of interferon-gamma response pathway and antigen presentation machinery genes were predominantly decreased. In addition, we also observed a down-modulation of Th1 and Th2 cytokine-induced signaling pathways, toll like receptor signaling, neutrophil degranulation, TP53-mediated apoptosis and caspase activation in post-transplant relapses.

In sum with our work, through a combination of NGS-based HLA and myeloid genotyping and immune-transcriptomic analyses, we defined granular molecular of immune-resistance relapsing after transplant. With our previously validated bioanalytic framework,^{148,149} we were able to identify somatic single nucleotide mutations and small indels in HLA genes along with specific allelic losses. We show that somatic HLA mutations can constitute a potential escape mechanism in patients with MN relapsing after allo-HSC in both matched related and unrelated settings. Indeed, both mutations and deletions of one of the HLA alleles could be detected in fully matched allo-HCT, whereas it is remarkable that in the mismatched setting HLA mutations affected both matched and mismatched alleles. We therefore conclude that the immune selection process leading to clonal escape targets the allele presenting the most immunodominant antigenic peptide, likely not limited to the mismatched ones.¹²⁹ Indeed, HLA LOH events often involve large genomic portions of the short arm of chromosome 6, thereby affecting a whole haplotype and not only the mismatched allele and resulting in a global impairment of antigen presentation.^{127,150,151,152} The finding of HLA mutations in relapsed allo-HCT is further supported by the low response rate to DLI. The analysis of the available cohort showed that patients with HLA mutations (including non-UTR alterations) had decreased likelihood of responsiveness to this salvage strategy.

In contrast to tumor control post-immunotherapy, class II HLA genes more than class I were the major drivers of immunologic escape. This result underscores the role of tumor-surveillance of CD4 T-cell specificities^{153,154,155} and antigen-presenting cells, including leukemia-derived dendritic cells^{156,157,158,159} in GvL effect. These findings define key molecular modes of post-transplant leukemia escape contributing to relapse.

This work has been just published on Nature Communication (Pagliuca & Gurnari et al. Nat Comm 2023).

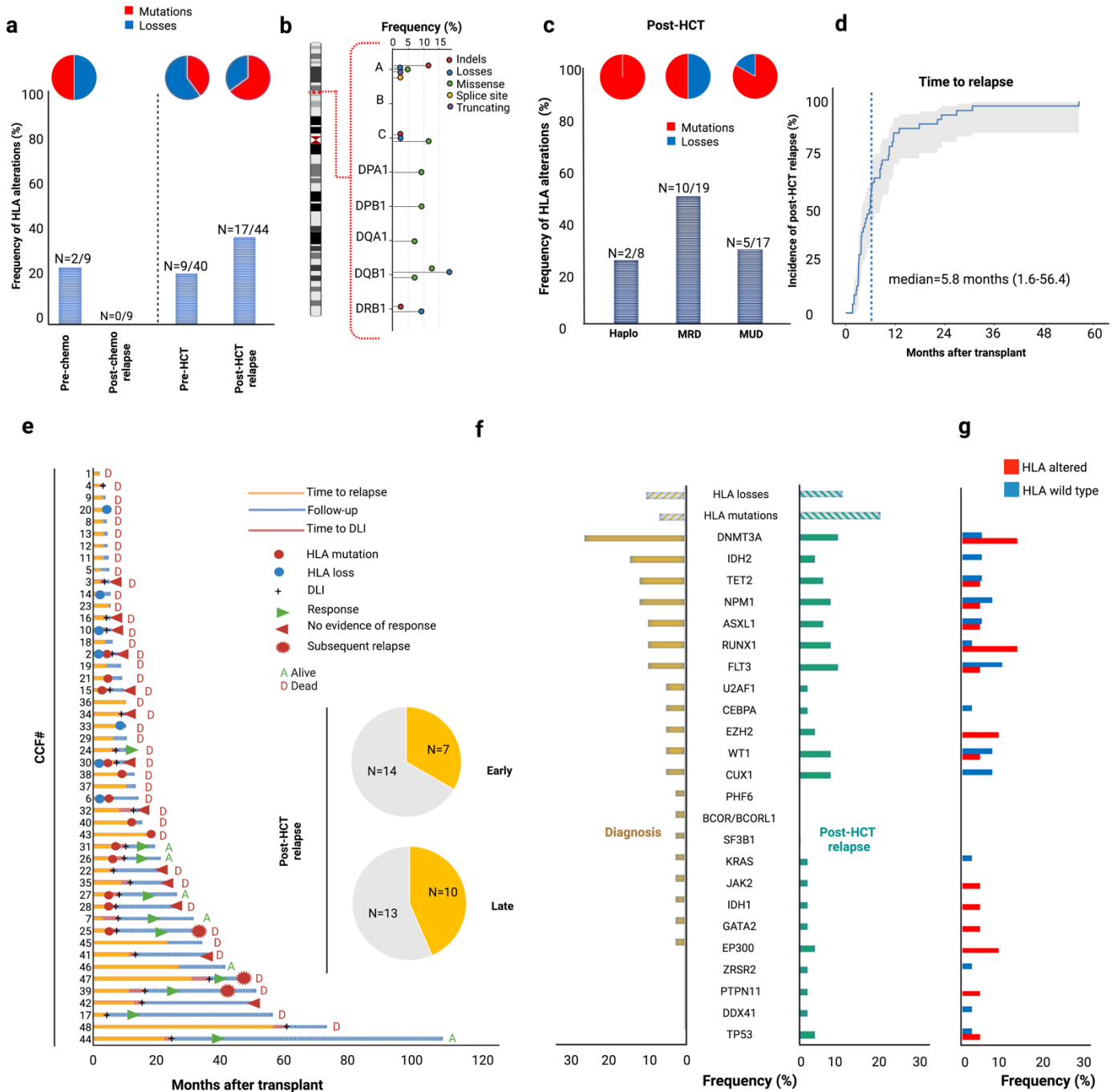


Figure 11. **A)** Barplot indicating the frequency of HLA mutations in each studied group. Numbers of patients harboring HLA aberrations are reported on the top of each bar. Pie charts capture the frequency of mutations and losses in each group. **B)** Lollipop chart showing the frequency of each HLA aberration. The height of the line denotes the frequency, while the color of each dot indicates the type of aberration. **C)** Frequency of HLA alterations according to donor group (Haplo: haploidentical donor); MRD (matched related donor); MUD (matched unrelated donor). Pie charts represent the distribution of mutations or losses in each group. **D)** Time dependent curve indicating the incidence of relapse of the sequenced group (median time to relapse was 5.8 months). **E)** Swimming plot capturing main clinico-biological information for each patient of the sequenced cohort. The yellow barplot indicates the frequency of HLA alterations in late (after 6 months) vs early (before 6 months) post-HCT relapses. **F)** Barplots showing the frequency and distribution of HLA mutations, losses and

mutations in main myeloid driver genes in AML/MDS at diagnosis (yellow) and at post-HCT relapses (green). **G)** Frequency of mutations in myeloid driver genes in HLA-altered vs HLA wild type post-HCT specimens.

3.2.3.e Genomics and epigenetics of myelodysplastic syndromes.

Another domain of intensive research during the years in Cleveland Clinic has concerned a series of studies to which I actively contributed providing methodology inputs, data analysis substrate or wet lab support.

Indeed, at the end of 2020, when a new adult-onset inflammatory syndrome called VEXAS (vacuoles, E1 enzyme, X linked, autoinflammatory, somatic) was described for the first time by the National Institute of Health (NIH), our lab was the first one to confirm these data in an independent cohort of patients. This syndrome is genetically associated with somatic mutations affecting methionine 41 of the X-linked gene UBA1. We started from the cases with overt presence of vacuoles as pathognomonic cytopathological finding and through chart review of patients enrolled at The Cleveland Clinic between 2005 and 2020, we identified 24 cases with this feature among 11 772 bone marrow specimens. All patients underwent BM evaluation as part of the initial workup and/or follow-up for an MN or to define an unexplained cytopenia. Among these patients 2 tested positive for UBA mutational screening with classical hotspot mutations. These patients had concomitant Sweet syndrome with one case having a previous diagnosis of rheumatoid arthritis. Both patients had low risk MDS characterized by normal cytogenetics and presence of a DNMT3A mutation. This study served as a first reservoir of epidemiologic and histopathological information when VEXAS was still an underestimated disorder. This work was published on *Blood* at the beginning of 2021 (Gurnari et al. *Blood* 2021) and the histopathological images were featured on the cover of the journal.

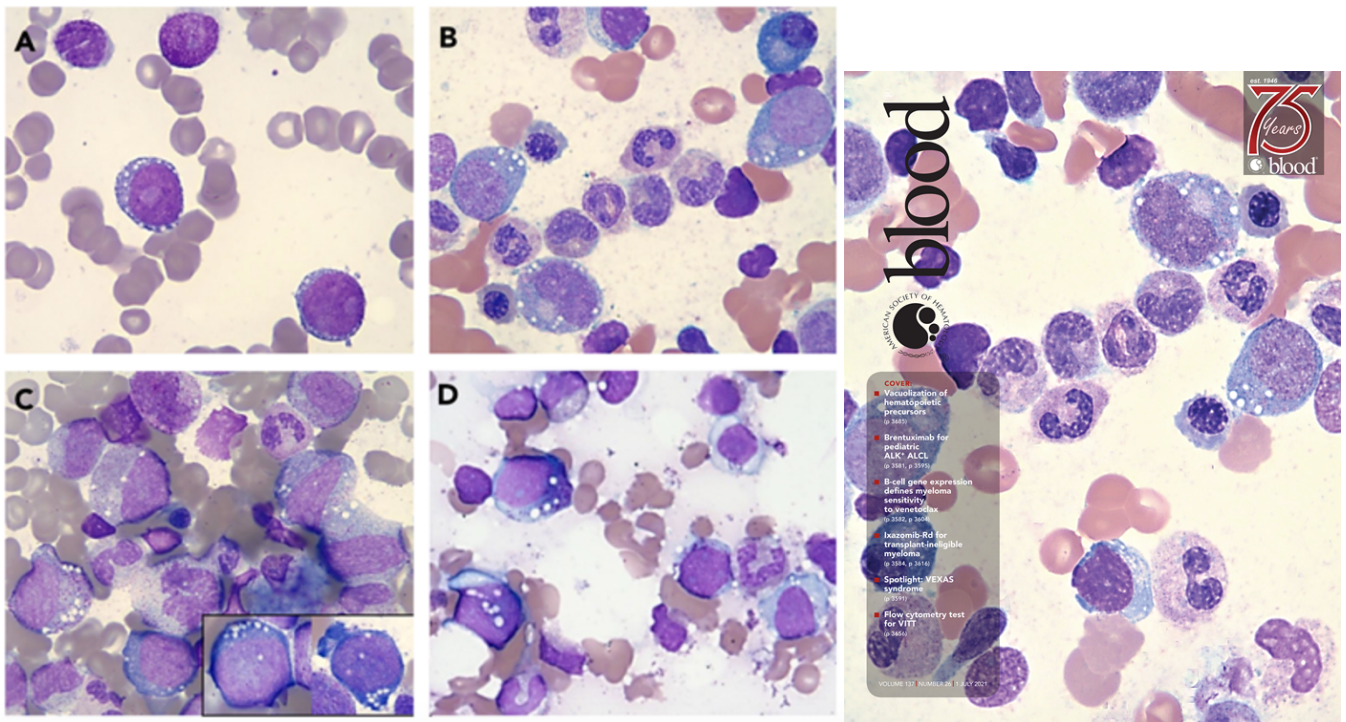


Figure 12. Vacuolization of hematopoietic progenitors in various conditions. Wright-Giemsa stains (magnification $\times 1000$) showing a representative example of a "blast-only" pattern of vacuolization in a case with high-risk MDS (vacuoles in 2 blasts) (A) and vacuolization of immature erythroid and myeloid cells in 2 patients with a *UBA1* mutation and VEXAS syndrome (B-C) and in a patient with copper deficiency (D). Right panel: Blood journal cover featuring our study.

While actively working on the study of immune escape in AA and allo-HCT, an active topic of our lab was to develop small molecules targeting mutated hematopoietic stem cells at preleukemic or earlier stages (i.e. in clonal hematopoiesis of indetermined potential -CHIP- or in MDS). One of these strategies preferentially targeted and eliminate leukemia cells with *TET2* mutations, one of the most common mutations found acute myeloid leukemia. In fact, our research team designed *TETi76*, a TET enzyme inhibitor able to replicate and amplify the effects of a natural molecule called 2HG (2-hydroxyglutarate), which inhibits the enzymatic activity of TET genes. The TET DNA dioxygenase gene family codes for enzymes that remove chemical groups from DNA molecules, which ultimately changes what genes are expressed and can contribute to the development and spread of disease. While all members of the TET family are dioxygenases, the most powerful enzymatic activity belongs to *TET2*. Even when *TET2* is mutated, however, its related genes *TET1* and *TET3* provide residual enzymatic activity. While significantly less, this activity is

still enough to facilitate the spread of mutated cancer cells. This new pharmacologic strategy allowed to selectively eliminate *TET2* mutant leukemia cells centers on targeting their reliance on this residual DNA dioxygenase activity. This molecule has been shown to promote the synthetic lethality of *TET2* mutated cells, at the same time stimulating the proliferation of hematopoietic stem cells, without affecting their oncogenic capacity featuring important applications in hematology. On the same wave we were able to demonstrate also that, Eltrombopag, an approved thromboietin receptor agonist, in use in AA and immune thrombocytopenic purpura, was able to phenocopy *TET2* loss, creating a cellular status very similar to *TET2* inhibition and therefore stimulating HSC proliferation. These findings opened several doors in terms of application: from new biological insights concerning the use of Eltrombopag in AA to the application of *TET2* inhibitory strategies in graft failure prevention after allo-HCT and in *TET2* mutated neoplasms.

The works issued from this research axis in which I provided contribution on several levels are listed below:

[A Therapeutic Strategy for Preferential Targeting of **TET2** Mutant and TET-dioxygenase Deficient Cells in Myeloid Neoplasms.](#)

Guan Y, Tiwari AD, Phillips JG, Hasipek M, Grabowski DR, **Pagliuca S**, Gopal P, Kerr CM, Adema V, Radivoyevitch T, Parker Y, Lindner DJ, Meggendorfer M, Abazeed M, Sekeres MA, Mian OY, Haferlach T, Maciejewski JP, Jha BK. *Blood Cancer Discov.* 2021 Mar;2(2):146-161. doi: 10.1158/2643-3230.BCD-20-0173. Epub 2020 Dec 7. PMID: 33681816

[The Interactome between Metabolism and Gene Mutations in Myeloid Malignancies.](#)

Gurnari C, **Pagliuca S**, Visconte V. *Int J Mol Sci.* 2021 Mar 19;22(6):3135. doi: 10.3390/ijms22063135. PMID: 33808599

[**TET2** mutations as a part of DNA dioxygenase deficiency in myelodysplastic syndromes.](#)

Gurnari C, **Pagliuca S**, Guan Y, Adema V, Hershberger CE, Ni Y, Awada H, Kongkiatkamon S, Zawit M, Coutinho DF, Zalcborg IR, Ahn JS, Kim HJ, Kim DDH, Minden MD, Jansen JH, Meggendorfer M, Haferlach C, Jha BK, Haferlach T, Maciejewski JP, Visconte V. *Blood Adv.* 2022 Jan 11;6(1):100-107. doi: 10.1182/bloodadvances.2021005418. PMID: 34768283

[Eltrombopag inhibits TET dioxygenase to contribute to hematopoietic stem cell expansion in aplastic anemia.](#)

Guan Y, Hasipek M, Jiang D, Tiwari AD, Grabowski DR, **Pagliuca S**, Kongkiatkamon S, Patel B, Singh S, Parker Y, LaFramboise T, Lindner D, Sekeres MA, Mian OY, Sauntharajah Y, Maciejewski JP, Jha BK. *J Clin Invest.* 2022 Feb 15;132(4):e149856. doi: 10.1172/JCI149856.PMID: 35085104

[Inhibition of Critical DNA Dioxygenase Activity in IDH1/2 Mutant Myeloid Neoplasms](#)

Simona Pagliuca, MD, Yihong Guan, PhD, Anand D. Tiwari, PhD, Dale Grabowski, BS, Carmelo Gurnari, MD, Cassandra M Kerr, MS, Sunisa Kongkiatkamon, MD, Metis Hasipek, PhD, Laila Terkawi, Misam Zawit, MBBS, Hassan Awada, MD, Valeria Visconte, PhD, Yogenthiran Sauntharajah, MD, James G Phillips, PhD, Jaroslaw P. Maciejewski, MD PhD, Babal K. Jha, PhD. *Blood* (2020) 136 (Supplement 1): 28. <https://doi.org/10.1182/blood-2020-143094>

3.2.3.f Post-allo-HCT immune reconstitution

When approaching immunogenetics of HLA, it was natural to start investigating the features of TCR repertoires in the diseases and the cohorts of patients I was studying. Indeed, one of the lines of research of our lab was to address the architecture and the dynamics of recovery of the T cell repertoire after allo-HCT.

As said, an effective TCR repertoire diversification contributes to the success of allo-HCT. Although notable efforts have been invested in understanding the dynamics of TCR repertoire reconstruction after transplant, many aspects concerning the forces that shape its complexity remain controversial. Indeed, since my years in Cleveland, an important part of my work has been consecrated to the molecular study of alloreactive response after allo-HCT. We prospectively profiled the TCR repertoire of 135 serial specimens derived from a cohort of 35 first HCT recipients diagnosed with myeloid malignancies, along with a comparative cohort of relative donors and unrelated healthy individuals. Specifically, we analyzed the superstructure of TCR clonality, the spectrum of antigenic specificities, the structural diversity, and the overlap and divergence with donor repertoire in early and late post-transplant phases (**Figure 13**). For basic concepts regarding the TCR genomic structure and composition please see Annex 1.

A progressive reduction of repertoire's diversity was common to all transplant platforms due to a rarefaction of the number of unique clonotypes with a parallel increase in the number and size of expanded TCRs. This pattern was particularly pronounced in patients developing GvHD. While more than 95% of recipients' repertoire was represented by "de novo" clonotypes, not arisen from donors,

for overlapping specificities, recipient and donor clonal sizes were directly correlated, highlighting how the propensity to clonal expansion could go beyond single shared sequences. Donor and pre-transplant TCR diversity, as well as divergence of class I HLA genotypes were major predictors of recipient TCR repertoire recovery. CDR3-based specificity spectrum analysis indicated predominant expansion of pathogen- and tumor-associated clonotypes in the late post-allo-HCT phase, while autoreactive clones were more expanded in case of GvHD occurrence. The presence of a higher proportion of pathological expanded clonotypes negatively impacted survival and on the risk of relapse, while a higher overlap with donor repertoire was associated with an increased risk of chronic GvHD.

Our results shed light on the global architecture of early and late TCR reconstitution and on the recognition spectra of the new T cell compartment, through the use of clonotypes as markers of immunological responses. This work has been published on the journal JCI insight in July 2021 (Pagliuca et al. Gurnari second author).

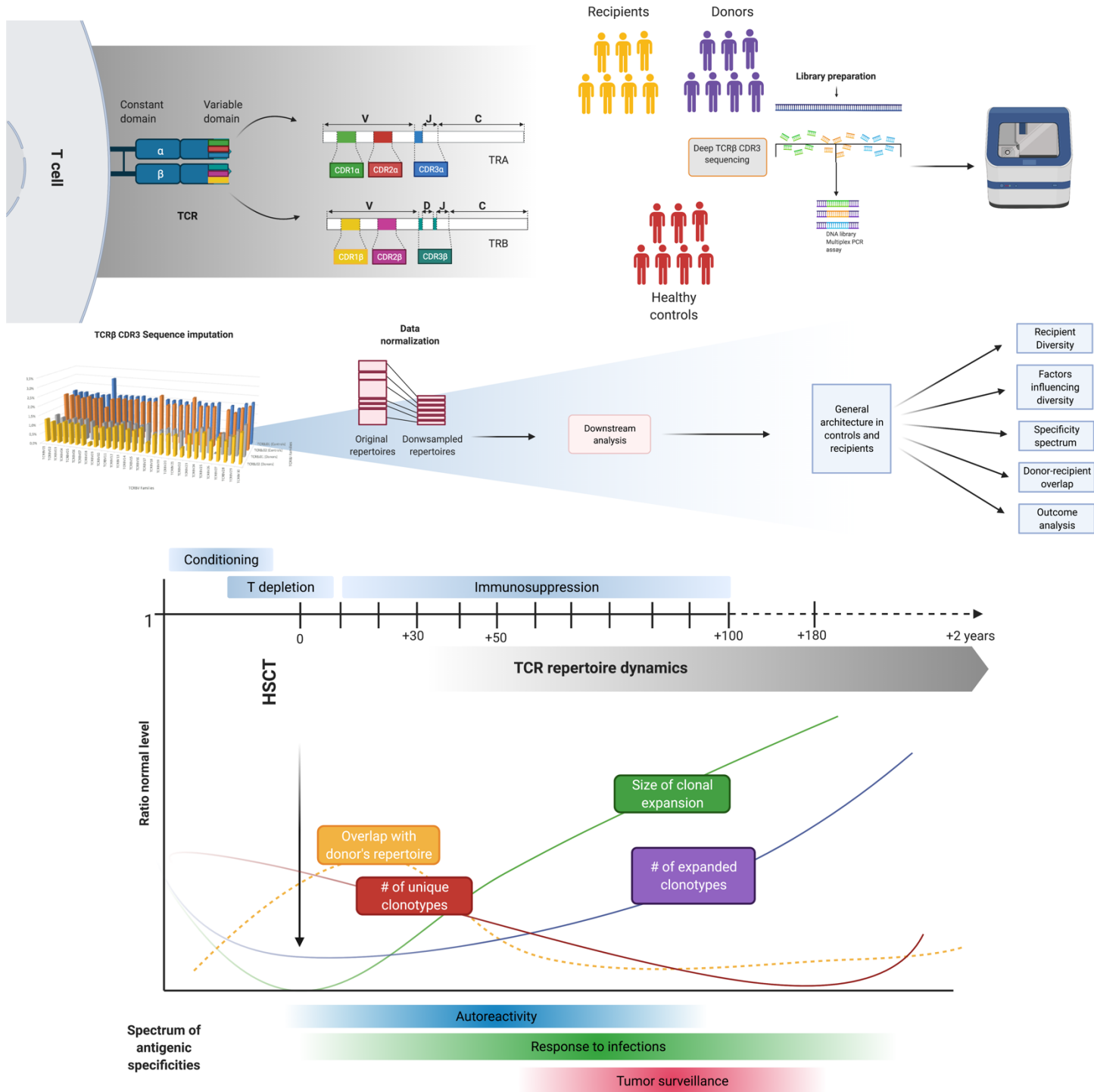


Figure 13: Visual abstract - Clinical and basic implications of dynamic T cell receptor clonotyping in hematopoietic cell transplantation

This work has been the praeludium of a series of ongoing projects since the start of my clinical and research activity in Nancy University Hospital and Rubio Lab, deepening the study of post-transplant immune reconstitution and the interest towards the molecular underpinnings behind specific situations such as steroid-refractory GvHD or relapse.

4. CURRENT AND FUTURE PROJECTS

4.1. Clinical and research work in Nancy University Hospital/University of Lorraine

I am currently serving as an attending physician in the Clinical Hematology Department of the CHRU of Nancy, and I am building a position as a senior investigator at the University of Lorraine, working within the Cellular Engineering, Cellular Immunotherapy and Translational Approaches (CECITA) program in the research unit Molecular Engineering and Articular Pathophysiology (IMoPA), under the direction of Prof. Rubio.

A major focus of Rubio's laboratory is the development of new cellular therapies that can contribute to the improvement of alloreactivity and immunosuppression protocols. This teamwork is dedicated to developing new cell therapies in different pathological contexts: infections, allogeneic hematopoietic stem cell transplantation (allo-HCT) or other dysimmune diseases and cancers. The team is focused on the development of new therapeutic strategies from i) the identification of cell candidates by exploring immune responses in human translational studies, ii) deep characterization of the candidate cells at the phenotypic, functional, metabolic, and transcriptomic/genomic levels, iii) clinical grade cell production and transfer into clinic.

In November 2021 once back to France, I joined the hematology department of the CHRU of Nancy and the UMR 7365 IMoPA team of the University of Lorraine where my work and my expertise have been harmoniously integrated in a stimulating and dynamic research environment. Thus, while maintaining numerous research collaborations with the USA institution, I began a research career at the University of Lorraine focused on the same immune-hematological themes.

I had the honor to be involved in a series of ongoing projects led by Pr. Rubio, in charge of the UMR 7365 of the IMoPA team and I am progressively acquiring funding for my own projects, integrated in the research thematic of the team.

Specifically, three projects on the topic of hematological or immune reconstitution and immune derangement after allo-HCT have been finalized so far in the context of this cooperation. Two of

them have been discussed so far in the previous sections and have served as thesis works of two MD students (Maud GAFFET and Anne Béatrice Notarantonio)

- [Efficacy of haematopoietic stem cell boost as a rescue for poor graft function after haematopoietic stem cell transplantation: A multicentre retrospective study on behalf of the Francophone Society of Bone Marrow Transplantation and Cellular Therapy \(SFGM-TC\).](#)

Gaffet M, Wiedemann A, Dalle JH, Bilger K, Forcade E, Robin M, Cornillon J, Labussière-Wallet H, Ceballos P, Bulabois CE, Loschi M, Orvain C, Rubio MT, Neven B, **Pagliuca S**,* Pochon C.* *Br J Haematol.* 2023 Mar 27. doi: 10.1111/bjh.18744. Online ahead of print.PMID: 36974355

*Co-last authors

- [Systemic complement activation influences outcomes after allogeneic hematopoietic cell transplantation: a prospective French multicenter trial](#)

Anne Béatrice Notarantonio^{1*}, Maud D'aveni-Piney^{1,2*}, **Simona Pagliuca**¹, Yayha Ashraf³, Jacques-Emanuel Galimard⁴, Aliénor Xhaard⁵, Ambroise Marçais⁶, Felipe Suarez⁶, Eolia Brissot⁷, Pierre Feugier¹, Saik Urien⁸, Naim Bouazza⁸, Sébastien Jacquelin², Tchao Meatchi⁹, Patrick Bruneval⁹, Véronique Frémeaux-Bacchi³, Régis Peffault De Latour⁵, Olivier Hermine^{6,2*}, Marie Agnès Durey-Dragon^{3*}, Marie-Thérèse Rubio^{1,2*}. Submitted to American journal of Haematology

*Co first authors

The third one, concerns another MD thesis project brilliantly conducted by Alzée JANDIN, already valorized as an oral and poster communication at several national and international meetings (ASH, EBMT, SFGM-TC) and published in British Journal of Haematology this year (figures from this paper have been also selected for the cover of the current journal issue).

- [Age-related immune cell dynamics influence outcomes after allogeneic haematopoietic cell transplantation.](#)

Jandin A, Pochon C, Campidelli A, D'Aveni M, Kicki C, Notarantonio AB, Roth Guepin G, Mbuyi TA, Feugier P, Chastagner P, Schweitzer C, de Carvalho Bittencourt M, Rubio MT, **Pagliuca S**. *Br J Haematol.* 2023 Apr 24. doi: 10.1111/bjh.18822. Online ahead of print.PMID: 37092504

This last study was focused on the investigation of factors related to an efficient post-transplant immune reconstitution in adults and children with hematological disorders. As a matter of fact, differences in outcomes in adult and pediatric populations suggest an age-related contribution to post-transplant immune reconstitution; however, it is unclear how recipient and donor age may affect the dynamics of single immune cells. In this study, we retrospectively collected and analyzed immunological data of 174 patients (58 children and 116 adults) consecutively transplanted for hematological disorders in our center. We showed that trajectories of specific immune cells were strictly dependent on recipient age and pretransplant virus exposure, with the strongest effect seen on T CD4+ and B-cell counterparts, while donor age and transplant platforms had a minimal impact. This mirrored different kinetics of immune reconstitution in adult and pediatric patients, with major divergences in immune cell composition in late post-transplant phases, featuring better survival, relapse-free survival and cumulative incidence of pathogen-specific infections in younger patients.

These findings underpin the importance of recipient age on post-transplant immune cell recovery and define the basic dynamics of the immune reconstitution in pediatric and adult populations.

Several projects in collaboration with other institutions have been recently initiated. Two of them will serve as Master 2 works for two residents affiliated at our Hematology department

- GENOMIC LANDSCAPE OF MYELOID SARCOMA: A PLEA FOR BETTER MOLECULAR CHARACTERIZATION AND A ROAD MAP FOR TARGETED THERAPIES (Master student: Clément Collignon).

This project is the product of an ongoing international partnership with the Washington University (WashU, St. Louis, Missouri, USA) and the Federico II University (Naples, Italy). Preliminary data have been presented at the 49th EBMT meeting (Collignon C. first author, Pagliuca S. last author, **Figure 14**).

Of note for this project the Master candidate will spend 9 months in the laboratory of immunohematology of WashU, under the guidance of Pr. Francesca Ferraro, MD, PhD, being in constant contact with me to secure the correct conduct and the finalization of his master (and of the project) in due time.

To give some insights to this project: myeloid sarcoma (MS) constitutes a rare, high-risk form of acute myeloid leukemia AML due to extramedullary localization of leukemic blasts. This entity could occur de novo or at leukemia relapse, after chemotherapy or allogeneic hematopoietic cell transplantation, and may be isolated or concomitant to bone marrow (BM) infiltration. Small case series exploiting paired high-throughput sequencing of solid lesions vs BM show the presence of complex clonal architectures, sometimes divergent from the genetic structure of BM leukemia and, although some recurrent somatic lesions have been identified, clear genomic correlations are only putative and not investigated in larger cohorts with comparative studies.

To date, little is known about the genetic events that are associated with the localization of AML cells in extramedullary organs, and whether these MS cells have different molecular and functional properties from bone marrow AML cells. In this proposal, we will define the mechanisms that drive the establishment of MS as well as the functional consequences as this entity relates to potential treatment resistance and relapse. Currently, the evaluation, treatment or monitoring of MS is not part of the standard diagnostic assessment for AML patients; however, our study has the potential to establish the evaluation of extramedullary AML cells as a potential adjunct biomarker and therapeutic target to prevent leukemic relapse and improve survival– thus, transforming clinical practice. In the era of sophisticated risk stratification and ultra-sensitive molecular disease assessment, understanding

the contribution of subclinical extramedullary AML cells to AML relapse is therefore urgently needed.

Our proposal has multiple conceptual and technical innovations:

1. Through *paired* BM/MS whole exome sequencing, we will determine if clonal selection or divergence occurs in MS sites while simultaneously exploring additional determinants of extramedullary localization.
2. By applying spatial transcriptomic techniques, we will gain unprecedented insights on both the spatial localization of cells within tumor environments and the gene expression patterns in the context of cellular and immunological niches. This will provide functional information on mechanisms that may facilitate the establishment of genetic subtypes of AML cells in certain tissues and provide biomarkers predictive of MS risk.
3. In addition to that we have developed in collaboration with WashU a MS skin transplant model and used it as a proof-of-concept to show that small amount of subclinical AML cells residing in the dermis can contribute to systemic AML relapse. We plan to use this tool to test different genetic subtypes of AML to gain information on the molecular mechanisms driving MS mediated relapse. This project has been recently funded by a Leukemia Research Foundation grant (PIs: Francesca Ferraro, Simona Pagliuca, David Chen).

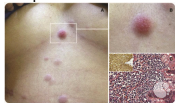


Genomic landscape of myeloid sarcoma: a plea for a better molecular characterization and a road map for targeted therapies

Clément Collignon¹, Anne-Béatrice Notarantonio¹, Gabrielle Roth Guepin¹, Caroline Bonmati¹, Maud D'Aveni¹, Arnaud Campidelli¹, Charline Moulin¹, Caroline Jacquet¹, Cécile Pochon¹, Marc Muller¹, Marion Divoux¹, Francesco Grimaldi³, Pierre Feugier¹, Marie Thérèse Rubio¹, Francesca Ferraro⁴ & Simona Pagliuca¹

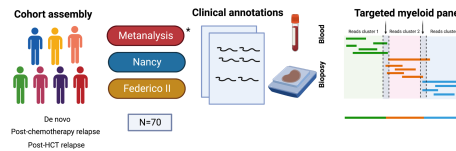
INTRODUCTION

Myeloid sarcoma (MS) constitutes a rare, high-risk form of acute myeloid leukemia (AML), characterized by infiltration and destruction of otherwise normal tissues by leukemic blasts. This entity could occur de novo or at leukemia relapse, after chemotherapy or allogeneic hematopoietic cell transplantation, and may be isolated or concomitant to bone marrow (BM) infiltration. Small case series exploiting paired high-throughput sequencing of solid lesions vs BM show the presence of complex clonal architectures, sometimes divergent from the genetic structure of BM leukemia and, although some recurrent somatic lesions have been identified, clear genomic correlations are only putative and not investigated in larger cohorts with comparative studies.



A-B: Dermal nodules on the scalp, abdomen, breast, and genitalia in a 27-yo woman. C: Skin biopsy showing a population of immature cells (original magnification $\times 100$; hematoxylin and eosin stain) positive for CD45/LCA, CD43, CD68 (panel C inset; original magnification $\times 100$; immunoperoxidase stain), CD163, myeloperoxidase (focal), and Ki-67 (~60%). Adapted from ASH Image Bank.

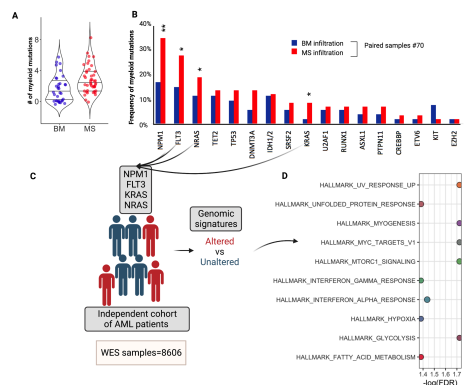
METHODS



Here we assembled a large metanalytic cohort of 70 patients with paired MS/BM molecularly profiled samples, recruited from our institutions (Nancy University Hospital and Federico II of Naples) and from 10 public studies.¹⁻¹⁰

RESULTS

- In patients with MS, solid lesions, compared to BM, displayed a higher complexity due to an increased mutational burden (average mutational rate of 2.75 vs 1.9, $p=0.011$, A), and a higher variant allele frequency (Median 33 vs 19%, $p=0.008$).
- When paralleled with BM, mutational landscape of MS was characterized by a different configuration of myeloid lesions with an enrichment in NPM1 (36 vs 17%), FLT3 (29 vs 15%), NRAS (19 vs 12%) and KRAS (9 vs 2%) mutations (B).
- The acquisition of some of these lesions in extramedullary localizations, initially not present in BM, occurred in 53% of the patients and concerned particularly NPM1 and FLT3 mutations.



- When comparing this mutational background with an assembled non-MS multi-study AML cohort,¹¹ the enrichment in NPM1 mutations in MS lesions was more evident ($p=0.003$). We also noticed a slight increment of co-occurrence of mutations in NPM1 and RAS genes (20% in MS vs 14% in non-MS AML vs 11% in BM of MS patients), while lesions in FLT3 and RAS genes were in all cases mutually exclusives, likely for overlapping deleterious effects on cellular functions.

- Since leukemia sanctuaries in solid organs are supposed to exist because of failure of immune control, we reasoned that altered NPM1/FLT3/RAS pathways could share some molecular features prone to tissue invasion and immune escape. We explored this possibility at genomic level, by investigating the patterns of co-mutations in patients with myeloid neoplasms harboring lesions in these genes and profiled with whole exome sequencing (N=8606) (C).¹²
- Gene enrichment analysis of the "altered" vs "unaltered" group showed an increased occurrence of mutations in genes involved in mTOR signaling, interferon alpha and gamma response, hypoxia and unfolded protein response that could play a role in the proclivity to tissue invasion of these molecular subsets of AML (D).

CONCLUSIONS

This comparative study sheds light into the unique molecular features of MS, pleading for the need to molecularly characterize all solid lesions occurring in AML context, and to explore targeted therapies in this high-risk category of patients.

REFERENCES

- Engel, N. W. et al. Newly diagnosed isolated myeloid sarcoma-paired NGS panel analysis of extramedullary tumor and bone marrow. *Ann Hematol* **100**, 499–503 (2021).
- Pastoret, C. et al. Detection of clonal heterogeneity and targetable mutations in myeloid sarcoma by high-throughput sequencing. *Leuk Lymphoma* **58**, 1008–1012 (2017).
- Wernstein, B. et al. Molecular Discordance between Myeloid Sarcomas and Concurrent Bone Marrows Occurs in Actionable Genes and is Associated with Worse Overall Survival. *J Mol Diagn* **22**, 338–345 (2020).
- Abbas, H. A. et al. Clinical and molecular characterization of myeloid sarcoma without medullary leukaemia. *Leuk Lymphoma* **62**, 3402–3410 (2021).
- Fang, L. et al. Somatic ASXL1 p.R693X mutation identified by next generation sequencing in isolated myeloid sarcoma involving the mediastinum. *Curr Med Res Opin* **36**, 1003–1007 (2020).
- Greenland, N. Y. et al. Genomic analysis in myeloid sarcoma and comparison with paired acute myeloid leukemia. *Hum Pathol* **108**, 76–83 (2021).
- Stölzel, F. et al. Clonal evolution including partial loss of human leukocyte antigen genes favoring extramedullary acute myeloid leukemia relapse after matched related allogeneic hematopoietic stem cell transplantation. *Transplantation* **93**, 744–749 (2012).
- Taminsh-Katsuragawa, Y. et al. Gastric Myeloid Sarcoma Mimicking a Scirrhous Gastric Cancer. *Intern Med* **61**, 1231–1235 (2022).
- Tyrer, J. W. et al. Functional genomic landscape of acute myeloid leukaemia. *Nature* **562**, 526–531 (2018).
- Fouillet, L. et al. A complex mutational profile and a distinct clonal evolution during NPM1 myeloid sarcoma. *Leuk Lymphoma* **60**, 2328–2330 (2019).
- <https://bit.ly/3hAncy>
- <https://bit.ly/3W8CC8B>

AFFILIATIONS and CONTACTS

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www.ebmt.org

Figure 14: EBMT poster presentation of the project “Genomic Landscape of Myeloid sarcoma.” Preliminary data of the MS project, presented at 49th EBMT meeting by C. Collignon, master student.

- IMMUNOGENETICS OF CLONAL HEMATOPOIESIS: ROLE of HLA ALLELES IN CHIPs AND THEIR IMPLICATION IN THE RISK OF LEUKEMIC TRANSFORMATION.

(Master student: Esther Hazane)

This study has been conceptualized as a new line of research in our lab, in collaboration with the department Inserm UMRS-1256 “Nutrition-Génétique et Exposition aux Risques Environnementaux (N-GERE)” of Lorraine University (Collaborator: master co-director, Sébastien Hergalant) and the Center for Precision Medicine of the Vanderbilt University Medical Center, Nashville, Tennessee USA, collaborator: Assistant professor Alexander Bick). This project seeks to investigate how HLA configurations influence clonal hematopoiesis, topic that represents an outstanding open question to date and seems to be prominent to understand pathognomonic mechanisms of clonal evolution and leukemic transformation. We will address this question exploiting samples from the UK biobank (to which we recently granted access), representing the largest biologic and genomic resource currently existing (blood and other tissue samples from more than 400000 individuals). We will apply the HLA pipelines that we have developed in our previous researches together with the expertise of Bick Lab, a world leader group in the context of the study clonal hematopoiesis (<https://www.vumc.org/cpm/person/alexander-g-bick-md-phd>).

Of note this group has recently observed the flagrant role of HLA in influencing the mutational burden in hematopoietic cells (Weinstock et al. Science Advances 2023, Bick A. Last author).¹⁶⁰

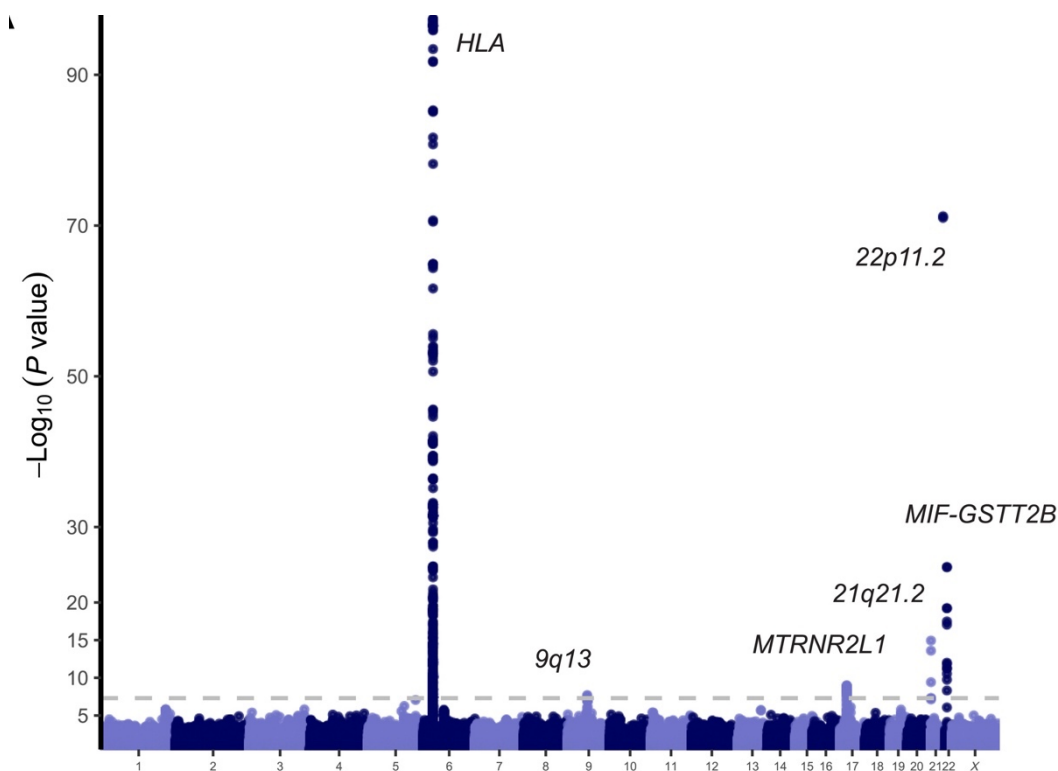


Figure 15. Manhattan plot of germline determinants of recurrent non-missense somatic mutations as a feature of clonal hematopoiesis in > 40000 genomes (Adapted from Weinstock et al. Science Advances 2023). The figure shows the prominent role of germlines polymorphisms of HLA loci in subjects with clonal hematopoiesis compared to individuals without hematopoietic-related somatic mutations.

This project has been submitted to the University of Lorraine to obtain funding to secure Esther Hazane's "year of research", to be enrolled in the Master 2 program "Biologie Moléculaire et Cellulaire - Parcours: Immunologie et ImmunoPathologie" at the University of Paris Sorbonne, in collaboration with the Institut Pasteur.

Other two projects currently in the phase of funding acquisition and patient/sample recruitment, consist in the definition of molecular determinants of the immune reactions in the context of cellular therapies.

IMMUNOMICS OF TUMOR-SURVEILLANCE IN CAR-T CELL THERAPIES: MOLECULAR DETERMINANTS OF SUCCESS AND FAILURE

This project rises from research questions still remained unanswered in the context of scientific literature about the mechanisms of expansion of anti-tumoral and toxicity related CAR-T cell clones in the context of these cellular therapies.

To introduce the concept, as a matter of fact, CAR-T cell therapies have revolutionized the immunotherapy field for hematological cancers and their use is now widespread. While representing a new therapeutic option for many patients formerly ineligible for intensive chemotherapy treatments, their efficacy and toxicity rates may incredibly vary across the disease spectrum and the molecular basis of this variability is far from being fully elucidated.

In this view, based on previous knowledge generated in post-transplant and aplastic anemia field by our works, our intention is to delineate the qualitative and quantitative characteristics of the TCR repertoire expansion and dynamics together with the somatic genomic spectrum of CAR and non-CAR-T cell fractions, by profiling a prospective cohort of patients receiving FDA and EMA-approved CAR-T products at our Institution. We hypothesize in fact that the individual specificity and expansion of T-cell clones enhanced with the CAR-T cell stimulation may dictate the patterns of response and the toxicity profile of these cellular therapies. To that end, we want to deploy cutting-edge sequencing and computational platforms to delve into the TCR repertoire, the genomic integration sites of the chimeric constructs, and the genomic alterations at the basis of lymphocyte activation and proliferation. We will perform multimodal immunological monitoring based on multiparametric flow cytometry, TCR-V β sequencing, whole-genome sequencing, and lentiviral

integration analysis. We expect to identify the specificity of the most expanded CAR and non-CAR clones in each patient, advancing the characterization of the determinants able to drive an anti-tumoral effect or a toxic event. This research has the potential to be transformative with bench-to-bedside applications translating into the possibility to optimize the delivered cellular products and consequently the outcomes of patients receiving these therapies.

This project is ongoing, more than 50 patients have been already included at our institution, issuing more than 300 specimens ready to be analyzed. Of note for this study, we obtained funding from JANSSEN-HORIZON and ARC for three years. A GANTT diagram and study design of the project is available below (**Figure 16**).

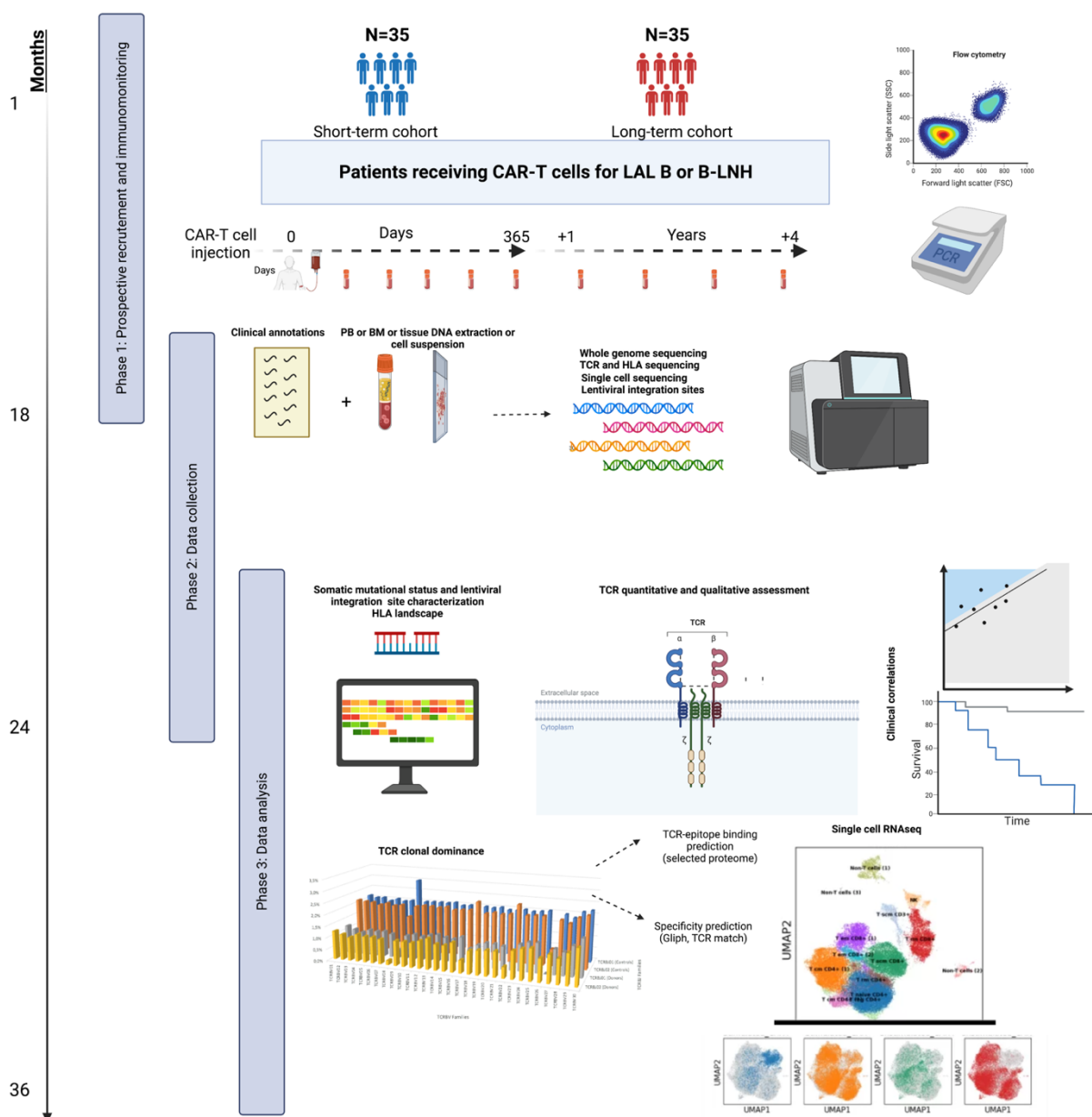


Figure 16: GANTT diagram and study design of the project: IMMUNOMICS OF TUMOR-SURVEILLANCE IN CAR-T CELL THERAPIES.

MOLECULAR DETERMINANTS OF ALLOREACTIVITY IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR MYELOID MALIGNANCIES

As stated in the previous sections GvL effect is the alloreactive response ensuring disease control via the recognition of leukemia associated antigens by donor-derived T-cell effectors and guarantees the success of allo-HCT procedures.¹⁶¹ However, its discrimination from GvHD, mainly related to a deleterious attack of the recipient's healthy tissues by the donor's adaptive immune system, remains elusive.^{161,11} GvL and GvHD are two forms of graft versus host (GvH) responses that remain to date molecularly indistinguishable each other. NGS-based TCR analysis may allow the characterization CD4⁺ and CD8⁺ cell specificity towards given antigens.^{144,162,163} We and others previously demonstrated that the oligoclonal expansion of specific T effectors represents a hallmark of GvHD and TCR β sequences directed against cancer-related epitopes may be identified in the late post-transplant period especially in patients who did not experience relapse.^{144,164} In our previous work we have focused on understanding the role of HLA structural variability in the immunogenetic predisposition of relapse and post-transplant immune escape in the context of MDS and AML. In this new project, we seek to explore the "beating heart" of alloreactivity, postulating that the most interesting setting to study GvL responses is given by patients who further develop immunological tolerance together with long-lasting disease control (patients never developing relapse nor GvHD, therefore potentially harboring the most specific GvL effect, tolerating non-tumor-derived recipient antigens). Therefore, our intention is to characterize the basis of this T cell antitumor control by directly distilling the identity of the cellular effectors harboring this property.

The principal aim of this study will be:

- To characterize HLA-restricted T cell specificities driving GvL and to isolate dominant T cell clones responsible of GvHD

As secondary aims we plan:

- To identify immunodominant antigens targeted by T cell effectors both in non-relapsing and GvHD samples characterizing the molecular targets of both GvH responses.
- To investigate immune activation signatures associated with immunological tolerance and disease control of long post-transplant non-relapsed/non-GvHD survivors vs immune-suppressive patterns possibly existing in patients who eventually relapse.

We will apply TCR β sequencing, HLA genotyping, and single cell transcriptomic analyses to post-transplant peripheral blood specimens from patients with MDS and AML receiving a matched related donor allo-HCT. To reduce the bias of the great variability of transplant procedures we will restrict

the sampling to a homogeneous category of patients (receiving a reduced intensity, fludarabine-based conditioning regimen together with a cyclosporine-mycophenolate- and anti-thymocyte globulin-based GvHD prophylaxis). Specifically, we will consider post-transplant samples at day +100 and +1 year, from four different subgroups: I) patients who a posteriori never developed acute/chronic GvHD nor relapse (index group); II) Patients with grade II-IV acute GvHD around day +90-100 sampled before any treatment (control group 1); III) Patients who developed an early relapse within the first year after transplant (control group 2), IV) Patients who developed a de novo chronic GvHD around 1 year post-HCT. We plan to retrospectively recruit at least 100 patients with a minimum required per group sample size of N=25 (**Figure 17**).

A multistep bioanalytical pipeline, which we have developed in the course of previous studies, will be devised to:

- o Identify the HLA-restricted T cell specificities (dominating in CD4+ and CD8+ fractions) by means of sequence-based TCR matching. Parallelisms with single cell RNA transcriptomics will be used to track the dominant immune signatures in each alloreactive group.
- o Model T cell epitopes based on dominant TCR sequences (starting from neoantigen analysis on AML/MDS cells and self-antigen expressed by healthy tissues targeted by GvHD), to identify the molecular targets of GvL and GvHD.
- o Compute differential expression analyses on single cell CD3+ subsets devoted to distinguishing the immune signatures between a) GvL vs acute GvHD (day +100); b) GvL vs chronic GvHD (1 year); c) GvL vs Relapse (+ 100 days and 1 year)

The ultimate purpose of this research will be to identify new molecular targets able to overcome T cell exhaustion, enhance disease control and limiting devastating GvHD responses, to finally improve outcomes of MDS and AML patients receiving an allo-HCT.

We will exploit for our research purposes samples issued from the largest GvHD biorepository to date generated (Cryostem multicenter project France, <https://www.cryostem.org/en/>) collecting to date more than 5000 samples from patients undergoing allo-HCT.

Several funding requests have been submitted during these last months. For the moment we have obtained a funding from the ASSOCIATION CAPUCINE, under the scientific leadership of SFGM-TC (15000 €), and other grant applications are ongoing.

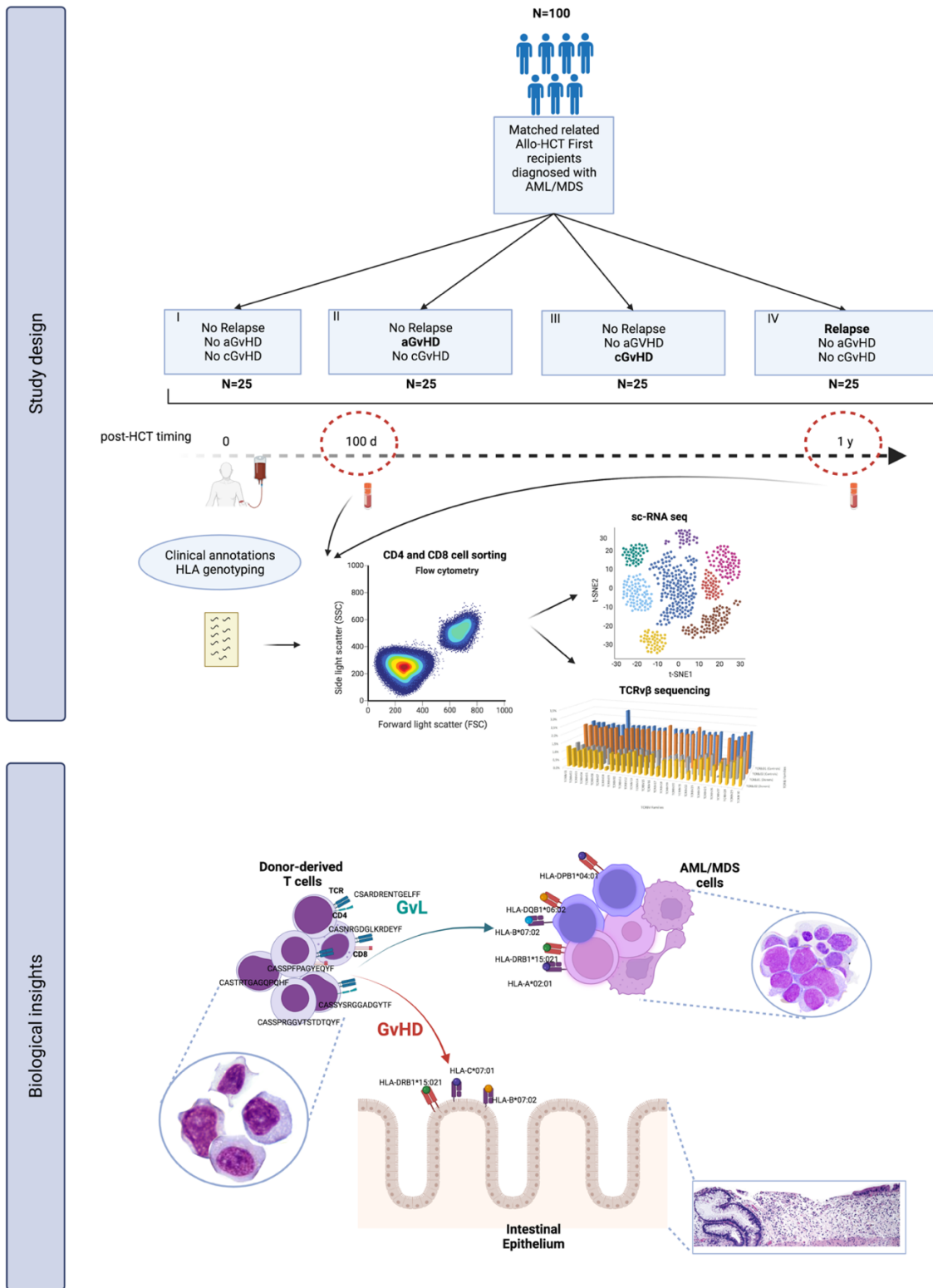


Figure 17. Study design of the project: MOLECULAR DETERMINANTS OF ALLOREACTIVITY IN ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR MYELOID MALIGNANCIES

Of note is that the biological and the computational resources related to the projects described in this section will be centralized and held by the UMR 7365 of IMoPA team of the University of Lorraine.

4.2. Current clinical activity and long-term clinical research projects

Given the progressive clinical and translational expertise acquired during the last years of my career, my current activities at Nancy University Hospital delve into:

- Immunotherapy and cellular therapies in the context of hematological malignancies.
- LGL leukemias
- Molecular diagnostics
- Immunomonitoring of post-cellular therapies
- Allogeneic stem cell transplantation in acute myelodysplasia and leukemia.
- Complications of allograft patients with expertise in the field of endothelial and alloreactivity syndromes
 - Post-transplant leukemia relapses (patient-adapted immunomodulatory strategies)
 - Immunomediated and inherited bone marrow failure disorders

For this last expertise, our center is granting the labelling as a regional competence center for idiopathic and inherited BMF. In this view I have the honor to have been appointed as a regional referent in our Institution for adult and pediatric patients with aplastic anemia.

The pathologies that belong to this rare disease group and for the management of which our center is implicated in are:

- Acquired aplastic anemia
- Paroxysmal nocturnal hemoglobinuria
- Fanconi anemia (disease)
- Congenital dyskeratosis (telomeropathies)
- Blackfan-Diamond anemia
- GATA2 syndrome
- Shwachman Diamond Syndrome
- Congenital amegakaryocytosis
- Refractory idiopathic sideroblastic anemia
- Other constitutional aplastic anemia

Indeed, in cooperation with the other members of the transplant and cell therapy team and conventional hematology of our institution, I am contributing to the expansion in terms of expertise and cellular therapy indications and I am helping in the implementation of useful follow-up tools for post-HCT and post-CAR-T cell immune monitoring, in collaboration with the molecular biology laboratory and the cytometry laboratory of the University Hospital of Nancy. (Dr Marc Muller and Pr Marcelo de Carvalho).

The need to expand the medical team in the cell therapy sector is more than evident: the activity of this field is exponentially increased in respect of:

I) allo-HCT with the significant increase of transplant indications, the possibility to access alternative donors, the introduction of less intensive conditioning regimens, the optimization of immunosuppression procedures, and the accessibility of this procedure to patients who were previously excluded

II) CAR-T cells with also a significant increase of indications and the application of these procedures in early therapeutic phases (several products have been recently approved as second line treatments). Moreover, the advent of allogeneic CAR-T cells in the context of clinical trials and in the context of compassionate use seem very close, with their use in clinics foreseen in the next coming months.

Future clinical research projects (under ongoing feasibility discussion/ funding negotiation) in direct extension with previous translational studies, discussed in the previous sections, concern several fields of my expertise.

- A Phase II study in patients with refractory idiopathic aplastic anemia (Funding negotiation)
Under the aegis of the National Reference Center of Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria in Saint Louis Hospital, we have submitted to the attention of Swedish Orphan Biovitrum (Sobi) Pharmaceutical company, a proposal for conducting a pilot, open label, single arm multicentric phase II Study study regarding the use of Emapalumab (Gamifant®) for patients with refractory and relapsed idiopathic aplastic anemia. This proposal is in phase of evaluation and of funding acquisition (PIs: Simona Pagliuca, Régis Peffault De Latour and Jaroslaw Maciejewski). This trial has been designed to improve the response rate of patients diagnosed with idiopathic aplastic anemia who fail to achieve a complete hematological remission, by using Emapalumab, an intravenous Interferon-gamma (IFN- γ) blocking agent. As a rational, IFN- γ is well known to be implicated in the immune pathophysiology of IAA and other bone marrow failure syndromes (BMF).^{66,67,68,165} The effects of IFN- γ on HSCs can influence their development, homeostasis, and

fate, leading to irreversible HSC depletion.^{68,166,167} In particular in the setting of idiopathic AA, IFN- γ , induced by the aberrant T-helper 1 (Th1) T-cell response, is responsible of a FAS-mediated apoptosis of hematopoietic stem cells (HSC). In animal models of aplastic anemia, it has been shown that injection of alloreactive lymphocytes results in bone marrow failure, but pancytopenia can be prevented with anti-IFN- γ and anti-TNF monoclonal antibody.¹⁶⁸

Emapalumab is a monoclonal antibody which binds and neutralizes IFN- γ , approved by Food and Drug Administration (FDA) for adult and pediatric (newborn and older) patients with primary hemophagocytic lymphohistiocytosis (HLH).^{169,170} Based on the well-known role of IFN- γ in the pathogenesis of idiopathic aplastic anemia and considering the safety of the use of maximal doses of the current commercial product, Gamifant®) we assume that this agent may be used as a rescue therapy in the context of refractory and poor responsive AA.

- Use of anti-complement inhibitors to prevent deleterious complement activation in the context of allo-HCT (feasibility discussion).
- Use of PD1 inhibitors in the context of extra-medullary AML (feasibility discussion).
- Use of other immunotherapy strategies in the context of post-transplant AML/MDS relapses (conceptualization).

In view of the expertise developed, other projects not discussed in detail in the previous sections, are ongoing with large multicenter consortia including CIBMTR and EBMT in which I am involved as PI.

- Proteogenomic landscape of the major histocompatibility complex in idiopathic aplastic anemia (CIBMTR)
- Impact of donor-recipient HLA evolutionary divergence on outcomes of bone marrow transplant from unrelated donors in patients with idiopathic aplastic anemia
- State of the art of immune monitoring procedures after chimeric antigen receptor T-cell therapies in EBMT centers
- Role of HLA Evolutionary Divergence in Mismatched Unrelated Donor Hematopoietic Cell Transplantation for Malignant Hematological Disorders

CONCLUSIONS

In sum, on the wake of an international background that helped me in nurturing an expertise in this complex field of the immune-hematology, and after a complex professional path, I have the honor to be integrated in a dynamic and stimulating clinical and research environment that is prompting all of us to be featured among the top French and European Centers in terms of patient care and management in the context of cellular and immunotherapies. In such a great environment I will continue to develop all the aspects of this eclectic activity and to supervise the work of students and trainees in order to maintain a high quality of care, research and teaching.

REFERENCES

1. Passweg JR, Baldomero H, Peters C, et al. Hematopoietic SCT in Europe: data and trends in 2012 with special consideration of pediatric transplantation. *Bone Marrow Transplant*. 2014;49(6):744–750.
2. Passweg JR, Baldomero H, Chabannon C, et al. Hematopoietic cell transplantation and cellular therapy survey of the EBMT: monitoring of activities and trends over 30 years. *Bone Marrow Transplant*. 2021;
3. Passweg JR, Baldomero H, Basak GW, et al. The EBMT activity survey report 2017: a focus on allogeneic HCT for nonmalignant indications and on the use of non-HCT cell therapies. *Bone Marrow Transplant*. 2019;54(10):1575–1585.
4. Jandin A, Pochon C, Campidelli A, et al. Age-related immune cell dynamics influence outcomes after allogeneic haematopoietic cell transplantation. *Br J Haematol*. 2023;bjh.18822.
5. Ferrà C, Sanz J, Díaz-Pérez M-A, et al. Outcome of graft failure after allogeneic stem cell transplant: study of 89 patients. *Leukemia & Lymphoma*. 2015;56(3):656–662.
6. Storb R, Prentice RL, Thomas ED, et al. Factors associated with graft rejection after HLA-identical marrow transplantation for aplastic anaemia. *Br J Haematol*. 1983;55(4):573–585.
7. Appelbaum FR. Hematopoietic-cell transplantation at 50. *N. Engl. J. Med*. 2007;357(15):1472–1475.
8. Pasquini MC, Wang Z, Horowitz MM, Gale RP. 2010 report from the Center for International Blood and Marrow Transplant Research (CIBMTR): current uses and outcomes of hematopoietic cell transplants for blood and bone marrow disorders. *Clin Transpl*. 2010;87–105.
9. Xhaard A, Rocha V, Bueno B, et al. Steroid-Refractory Acute GVHD: Lack of Long-Term Improved Survival Using New Generation Anticytokine Treatment. *Biology of Blood and Marrow Transplantation*. 2012;18(3):406–413.
10. Martin PJ, Rizzo JD, Wingard JR, et al. First- and Second-Line Systemic Treatment of Acute Graft-versus-Host Disease: Recommendations of the American Society of Blood and Marrow Transplantation. *Biology of Blood and Marrow Transplantation*. 2012;18(8):1150–1163.
11. Pagliuca S, Prata PH, Xhaard A, et al. Long-term outcomes and risk factor analysis of steroid-refractory graft versus host disease after hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2020;
12. Peffault de Latour R, Kulasekararaj A, Iacobelli S, et al. Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia. *N Engl J Med*. 2022;386(1):11–23.
13. Bizzetto R, Bonfim C, Rocha V, et al. Outcomes after related and unrelated umbilical cord blood transplantation for hereditary bone marrow failure syndromes other than Fanconi anemia. *Haematologica*. 2011;96(1):134–141.
14. Gluckman E, Rocha V, Ionescu I, et al. Results of Unrelated Cord Blood Transplant in Fanconi Anemia Patients: Risk Factor Analysis for Engraftment and Survival. *Biology of Blood and Marrow Transplantation*. 2007;13(9):1073–1082.
15. Pagliuca S, Peffault de Latour R, Volt F, et al. Long-Term Outcomes of Cord Blood Transplantation from an HLA-Identical Sibling for Patients with Bone Marrow Failure Syndromes: A Report From Eurocord, Cord Blood Committee and Severe Aplastic Anemia Working Party of the European Society for Blood and Marrow Transplantation. *Biology of Blood and Marrow Transplantation*. 2017;23(11):1939–1948.
16. Risitano AM. Paroxysmal nocturnal hemoglobinuria and other complement-mediated hematological disorders. *Immunobiology*. 2012;217(11):1080–1087.
17. Young NS. Current concepts in the pathophysiology and treatment of aplastic anemia. *Hematology Am Soc Hematol Educ Program*. 2013;2013:76–81.

18. de Latour RP, Mary JY, Salanoubat C, et al. Paroxysmal nocturnal hemoglobinuria: natural history of disease subcategories. *Blood*. 2008;112(8):3099–3106.
19. Brodsky RA, Young NS, Antonioli E, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2008;111(4):1840–1847.
20. Hillmen P, Young NS, Schubert J, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N. Engl. J. Med.* 2006;355(12):1233–1243.
21. Alashkar F, Dührsen U, Röth A. Horse anti-thymocyte globulin and eculizumab as concomitant therapeutic approach in an aplastic paroxysmal nocturnal hemoglobinuria patient: go or no-go? *Eur. J. Haematol.* 2016;97(4):403–405.
22. Asano J, Ueda R, Tanaka Y, Shinzato I, Takafuta T. Effects of immunosuppressive therapy in a patient with aplastic anemia-paroxysmal nocturnal hemoglobinuria (AA-PNH) syndrome during ongoing eculizumab treatment. *Intern. Med.* 2014;53(2):125–128.
23. Scheinberg P, Nunez O, Weinstein B, et al. Horse versus rabbit antithymocyte globulin in acquired aplastic anemia. *N. Engl. J. Med.* 2011;365(5):430–438.
24. Iordache L, Launay O, Bouchaud O, et al. Autoimmune diseases in HIV-infected patients: 52 cases and literature review. *Autoimmunity Reviews.* 2014;13(8):850–857.
25. Hapgood G, Hoy JF, Morrissey CO, Jane SM. Immune-mediated cytopenias in human immunodeficiency virus: the first reported case of idiopathic aplastic anaemia successfully treated with immunosuppression: Brief Communication. *Intern Med J.* 2013;43(4):452–455.
26. Bentwich Z, Kalinkovich A, Weisman Z, Grossman Z. Immune activation in the context of HIV infection. *Clinical and Experimental Immunology.* 2001;111(1):1–2.
27. Besson C, Goubar A, Gabarre J, et al. Changes in AIDS-related lymphoma since the era of highly active antiretroviral therapy. *Blood.* 2001;98(8):2339–2344.
28. Moses A, Nelson J, Bagby GC. The influence of human immunodeficiency virus-1 on hematopoiesis. *Blood.* 1998;91(5):1479–1495.
29. Pagliuca S, Gérard L, Kulasekararaj A, et al. Characteristics and outcomes of aplastic anemia in HIV patients: a brief report from the severe aplastic anemia working party of the European Society of Blood and Bone Marrow Transplantation. *Bone Marrow Transplantation.* 2016;51(2):313–315.
30. Eder IE, Culig Z, Putz T, et al. Molecular Biology of the Androgen Receptor: From Molecular Understanding to the Clinic. *Eur Urol.* 2001;40(3):241–251.
31. Shahidi NT, Diamond LK. Testosterone-induced remission in aplastic anemia. *AMA J Dis Child.* 1959;98:293–302.
32. Shahidi NT. Androgens and erythropoiesis. *N Engl J Med.* 1973;289(2):72–80.
33. Kennedy BJ. EFFECTS OF INTENSIVE SEX STEROID HORMONE THERAPY IN ADVANCED BREAST CANCER. *JAMA.* 1953;152(12):1135.
34. Najean Y, Haguenaer O. Long-term (5 to 20 years) Evolution of nongrafted aplastic anemias. The Cooperative Group for the Study of Aplastic and Refractory Anemias. *Blood.* 1990;76(11):2222–2228.
35. Townsley DM, Dumitriu B, Liu D, et al. Danazol Treatment for Telomere Diseases. *New England Journal of Medicine.* 2016;374(20):1922–1931.
36. Kirschner M, Vieri M, Kricheldorf K, et al. Androgen derivatives improve blood counts and elongate telomere length in adult cryptic dyskeratosis congenita. *Br J Haematol.* 2021;193(3):669–673.
37. Vieri M, Kirschner M, Tometten M, et al. Comparable Effects of the Androgen Derivatives Danazol, Oxymetholone and Nandrolone on Telomerase Activity in Human Primary Hematopoietic Cells from Patients with Dyskeratosis Congenita. *Int J Mol Sci.* 2020;21(19):E7196.
38. Champlin RE, Ho WG, Feig SA, et al. Do androgens enhance the response to antithymocyte globulin in patients with aplastic anemia? A prospective randomized trial. *Blood.* 1985;66(1):184–188.
39. Bacigalupo A, Chaple M, Hows J, et al. Treatment of aplastic anaemia (AA) with antilymphocyte globulin (ALG) and methylprednisolone (MPred) with or without androgens: a randomized trial from the EBMT SAA working party. *Br. J. Haematol.* 1993;83(1):145–151.
40. Kirschner M, Vieri M, Kricheldorf K, et al. Androgen derivatives improve blood counts and elongate telomere length in adult cryptic dyskeratosis congenita. *Br J Haematol.* 2021;193(3):669–673.
41. Seewald TR, Zeigler ZR, Gardner FH. Successful treatment of severe refractory aplastic anemia with 3-beta etiocholanolone and nandrolone decanoate. *Am. J. Hematol.* 1989;31(3):216–218.
42. Gardner FH, Juneja HS. Androstane therapy to treat aplastic anaemia in adults: an uncontrolled pilot study. *Br. J. Haematol.* 1987;65(3):295–300.
43. Androgen therapy in aplastic anaemia: a comparative study of high and low-doses and of 4 different androgens. French Cooperative Group for the Study of Aplastic and Refractory Anemias. *Scand J Haematol.* 1986;36(4):346–352.
44. Jaime-Pérez JC, Colunga-Pedraza PR, Gómez-Ramírez CD, et al. Danazol as first-line therapy for aplastic anemia. *Annals of Hematology.* 2011;90(5):523–527.
45. Rose SR, Kim M-O, Korbee L, et al. Oxandrolone for the treatment of bone marrow failure in Fanconi anemia: Oxandrolone Use in FA Bone Marrow Failure. *Pediatric Blood & Cancer.* 2014;61(1):11–19.

46. Saunders PM, Vivian JP, O'Connor GM, et al. A bird's eye view of NK cell receptor interactions with their MHC class I ligands. *Immunol Rev.* 2015;267(1):148–166.
47. Flajnik MF, Kasahara M. Comparative Genomics of the MHC. *Immunity.* 2001;15(3):351–362.
48. Römisch K. How antigenic peptides are made to fit their groove. *Trends Biotechnol.* 2001;19(10):378.
49. Kaufman J. Unfinished Business: Evolution of the MHC and the Adaptive Immune System of Jawed Vertebrates. *Annu. Rev. Immunol.* 2018;36(1):383–409.
50. Flajnik MF, Kasahara M. Origin and evolution of the adaptive immune system: genetic events and selective pressures. *Nat Rev Genet.* 2010;11(1):47–59.
51. Kaufman J, Volk H, Wallny H-J. A “Minimal Essential Mhc” and an “Unrecognized Mhc”: Two Extremes in Selection for Polymorphism. *Immunol Rev.* 1995;143(1):63–88.
52. Pagliuca S, Gurnari C, Rubio MT, Visconte V, Lenz TL. Individual HLA heterogeneity and its implications for cellular immune evasion in cancer and beyond. *Front. Immunol.* 2022;13:944872.
53. Young NS. Current concepts in the pathophysiology and treatment of aplastic anemia. *Hematology.* 2013;2013(1):76–81.
54. Risitano AM. Oligoclonal and polyclonal CD4 and CD8 lymphocytes in aplastic anemia and paroxysmal nocturnal hemoglobinuria measured by Vbeta CDR3 spectratyping and flow cytometry. *Blood.* 2002;100(1):178–183.
55. Sloand E. Intracellular interferon-gamma in circulating and marrow T cells detected by flow cytometry and the response to immunosuppressive therapy in patients with aplastic anemia. *Blood.* 2002;100(4):1185–1191.
56. Kordasti S, Marsh J, Al-Khan S, et al. Functional characterization of CD4+ T cells in aplastic anemia. *Blood.* 2012;119(9):2033–2043.
57. de Latour RP, Visconte V, Takaku T, et al. Th17 immune responses contribute to the pathophysiology of aplastic anemia. *Blood.* 2010;116(20):4175–4184.
58. Kordasti S, Marsh J, Al-Khan S, et al. Functional characterization of CD4+ T cells in aplastic anemia. *Blood.* 2012;119(9):2033–2043.
59. Solomou EE, Rezvani K, Mielke S, et al. Deficient CD4+ CD25+ FOXP3+ T regulatory cells in acquired aplastic anemia. *Blood.* 2007;110(5):1603–1606.
60. Risitano AM, Maciejewski JP, Green S, et al. In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR β -CDR3 sequencing. *The Lancet.* 2004;364(9431):355–364.
61. Wlodarski MW, Gondek LP, Nearman ZP, et al. Molecular strategies for detection and quantitation of clonal cytotoxic T-cell responses in aplastic anemia and myelodysplastic syndrome. *Blood.* 2006;108(8):2632–2641.
62. Jerez A, Clemente MJ, Makishima H, et al. STAT3 mutations indicate the presence of subclinical T-cell clones in a subset of aplastic anemia and myelodysplastic syndrome patients. *Blood.* 2013;122(14):2453–2459.
63. Maciejewski J, Selleri C, Anderson S, Young NS. Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro. *Blood.* 1995;85(11):3183–3190.
64. Kordasti S, Costantini B, Seidl T, et al. Deep phenotyping of Tregs identifies an immune signature for idiopathic aplastic anemia and predicts response to treatment. *Blood.* 2016;128(9):1193–1205.
65. Dubey S, Shukla P, Nityanand S. Expression of interferon-gamma and tumor necrosis factor-alpha in bone marrow T cells and their levels in bone marrow plasma in patients with aplastic anemia. *Ann. Hematol.* 2005;84(9):572–577.
66. Zombos NC, Gascon P, Djeu JY, Young NS. Interferon is a mediator of hematopoietic suppression in aplastic anemia in vitro and possibly in vivo. *Proceedings of the National Academy of Sciences.* 1985;82(1):188–192.
67. Sloand E, Kim S, Maciejewski JP, et al. Intracellular interferon- γ in circulating and marrow T cells detected by flow cytometry and the response to immunosuppressive therapy in patients with aplastic anemia. *Blood.* 2002;100(4):1185–1191.
68. Selleri C, Maciejewski JP, Sato T, Young NS. Interferon-gamma constitutively expressed in the stromal microenvironment of human marrow cultures mediates potent hematopoietic inhibition. *Blood.* 1996;87(10):4149–4157.
69. Alvarado LJ, Huntsman HD, Cheng H, et al. Eltrombopag maintains human hematopoietic stem and progenitor cells under inflammatory conditions mediated by IFN- γ . *Blood.* 2019;133(19):2043–2055.
70. Bestach Y, Sieza Y, Attie M, et al. Polymorphisms in TNF and IFNG are associated with clinical characteristics of aplastic anemia in Argentinean population. *Leuk. Lymphoma.* 2015;56(6):1793–1798.
71. El Mahgoub IR, Afify RAA, Botros SKA, Fawzy R. Immunoregulatory cytokines gene polymorphisms in Egyptian patients affected with acquired aplastic anemia. *Ann. Hematol.* 2014;93(6):923–929.
72. Lee Y-G, Kim I, Kim JH, et al. Impact of cytokine gene polymorphisms on risk and treatment outcomes of aplastic anemia. *Ann. Hematol.* 2011;90(5):515–521.

73. Dufour C, Capasso M, Svahn J, et al. Homozygosity for (12) CA repeats in the first intron of the human IFN-gamma gene is significantly associated with the risk of aplastic anaemia in Caucasian population. *Br. J. Haematol.* 2004;126(5):682–685.
74. Basham TY, Merigan TC. Recombinant interferon-gamma increases HLA-DR synthesis and expression. *J Immunol.* 1983;130(4):1492–1494.
75. Aglietta M, Piacibello W, Stacchini A, et al. Effect of interferon-gamma on HLA class II antigen expression and sensitivity to prostaglandin E1 by normal and leukemic myeloid progenitors. *Leuk Res.* 1988;12(4):299–303.
76. Amatruda TT, Bohman R, Ranyard J, Koeffler HP. Pattern of expression of HLA-DR and HLA-DQ antigens and mRNA in myeloid differentiation. *Blood.* 1987;69(4):1225–1236.
77. Sauntharajah Y, Nakamura R, Nam J-M, et al. HLA-DR15 (DR2) is overrepresented in myelodysplastic syndrome and aplastic anemia and predicts a response to immunosuppression in myelodysplastic syndrome. *Blood.* 2002;100(5):1570–1574.
78. Maciejewski JP, Follmann D, Nakamura R, et al. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and the PNH/aplastic anemia syndrome. *Blood.* 2001;98(13):3513–3519.
79. Dhaliwal JS, Wong L, Kamaluddin MA, Yin LY, Murad S. Susceptibility to aplastic anemia is associated with HLA-DRB1*1501 in an aboriginal population in Sabah, Malaysia. *Hum Immunol.* 2011;72(10):889–892.
80. Oguz FS, Yalman N, Diler AS, et al. HLA-DRB1*15 and pediatric aplastic anemia. *Haematologica.* 2002;87(7):772–774.
81. Song EY, Park S, Lee DS, Cho HI, Park MH. Association of human leukocyte antigen-DRB1 alleles with disease susceptibility and severity of aplastic anemia in Korean patients. *Hum Immunol.* 2008;69(6):354–359.
82. Yari F, Sobhani M, Vaziri MZ, et al. Association of aplastic anaemia and Fanconi's disease with HLA-DRB1 alleles. *Int J Immunogenet.* 2008;35(6):453–456.
83. Nakao S, Takami A, Sugimori N, et al. Response to immunosuppressive therapy and an HLA-DRB1 allele in patients with aplastic anaemia: HLA-DRB1*1501 does not predict response to antithymocyte globulin. *Br J Haematol.* 1996;92(1):155–158.
84. Takahashi M, Takeda J, Hirose S, et al. Deficient biosynthesis of N-acetylglucosaminyl-phosphatidylinositol, the first intermediate of glycosyl phosphatidylinositol anchor biosynthesis, in cell lines established from patients with paroxysmal nocturnal hemoglobinuria. *J. Exp. Med.* 1993;177(2):517–521.
85. Araten DJ, Luzzatto L. The mutation rate in PIG-A is normal in patients with paroxysmal nocturnal hemoglobinuria (PNH). *Blood.* 2006;108(2):734–736.
86. Nishimura J, Inoue N, Wada H, et al. A patient with paroxysmal nocturnal hemoglobinuria bearing four independent PIG-A mutant clones. *Blood.* 1997;89(9):3470–3476.
87. Clemente MJ, Przychodzen B, Hirsch CM, et al. Clonal PIGA mosaicism and dynamics in paroxysmal nocturnal hemoglobinuria. *Leukemia.* 2018;32(11):2507–2511.
88. Mahoney JF, Urakaze M, Hall S, et al. Defective glycosylphosphatidylinositol anchor synthesis in paroxysmal nocturnal hemoglobinuria granulocytes. *Blood.* 1992;79(6):1400–1403.
89. Schubart A, Anderson K, Mainolfi N, et al. Small-molecule factor B inhibitor for the treatment of complement-mediated diseases. *Proc. Natl. Acad. Sci. U.S.A.* 2019;116(16):7926–7931.
90. Risitano AM. Therapeutic complement modulation for hematological diseases: Where we stand and where we are going. *Semin. Hematol.* 2018;55(3):113–117.
91. Risitano AM, Marotta S, Ricci P, et al. Anti-complement Treatment for Paroxysmal Nocturnal Hemoglobinuria: Time for Proximal Complement Inhibition? A Position Paper From the SAAWP of the EBMT. *Front Immunol.* 2019;10:1157.
92. Kulasekararaj AG, Hill A, Rottinghaus ST, et al. Ravulizumab (ALXN1210) vs eculizumab in C5-inhibitor-experienced adult patients with PNH: the 302 study. *Blood.* 2019;133(6):540–549.
93. Lee JW, Sicre de Fontbrune F, Wong Lee L, et al. Ravulizumab (ALXN1210) vs eculizumab in adult patients with PNH naive to complement inhibitors: the 301 study. *Blood.* 2019;133(6):530–539.
94. Risitano AM, Marotta S, Ricci P, et al. Anti-complement Treatment for Paroxysmal Nocturnal Hemoglobinuria: Time for Proximal Complement Inhibition? A Position Paper From the SAAWP of the EBMT. *Front Immunol.* 2019;10:1157.
95. Risitano AM, Kulasekararaj AG, Lee JW, et al. Danicopan: an oral complement factor D inhibitor for paroxysmal nocturnal hemoglobinuria. *Haematologica.* 2020; Online ahead of print.
96. Risitano AM, Peffault de Latour R. How we (II) treat paroxysmal nocturnal haemoglobinuria: diving into the future. *Br J Haematol.* 2021;
97. Hillmen P, Szer J, Weitz I, et al. Pegcetacoplan versus Eculizumab in Paroxysmal Nocturnal Hemoglobinuria. *N Engl J Med.* 2021;384(11):1028–1037.
98. Dacie JV, Lewis SM. Paroxysmal Nocturnal Haemoglobinuria: Variation in Clinical Severity and Association with Bone-Marrow Hypoplasia. *Br J Haematol.* 1961;7(4):442–457.

99. Lewis SM, Dacie JV. The Aplastic Anaemia–Paroxysmal Nocturnal Haemoglobinuria Syndrome. *Br J Haematol.* 1967;13(2):236–251.
100. Tichelli A, Gratwohl A, Nissen C, Speck B. Late Clonal Complications in Severe Aplastic Anemia. *Leukemia & Lymphoma.* 1994;12(3–4):167–175.
101. Luzzatto L, Risitano AM. Advances in understanding the pathogenesis of acquired aplastic anaemia. *Br J Haematol.* 2018;182(6):758–776.
102. Dingli D, Luzzatto L, Pacheco JM. Neutral evolution in paroxysmal nocturnal hemoglobinuria. *Proceedings of the National Academy of Sciences.* 2008;105(47):18496–18500.
103. Luzzatto L, Bessler M, Rotoli B. Somatic Mutations in Paroxysmal Nocturnal Hemoglobinuria: A Blessing in Disguise? *Cell.* 1997;88(1):1–4.
104. Pu JJ, Mukhina G, Wang H, Savage WJ, Brodsky RA. Natural history of paroxysmal nocturnal hemoglobinuria clones in patients presenting as aplastic anemia. *Eur. J. Haematol.* 2011;87(1):37–45.
105. Young NS, Maciejewski JP. Genetic and environmental effects in paroxysmal nocturnal hemoglobinuria: this little PIG-A goes “Why? Why? Why?” *J Clin Invest.* 2000;106(5):637–641.
106. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia. *New England Journal of Medicine.* 2015;373(1):35–47.
107. Young NS, Maciejewski JP, Sloand E, et al. The relationship of aplastic anemia and PNH. *International Journal of Hematology.* 2002;76(S2):168–172.
108. Luzzatto L, Bessler M. The dual pathogenesis of paroxysmal nocturnal hemoglobinuria: *Current Opinion in Hematology.* 1996;3(2):101–110.
109. Yoshizato T, Dumitriu B, Hosokawa K, et al. Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia. *New England Journal of Medicine.* 2015;373(1):35–47.
110. Kulasekararaj AG, Jiang J, Smith AE, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. *Blood.* 2014;124(17):2698–2704.
111. Caporale LH, Doyle J. In Darwinian evolution, feedback from natural selection leads to biased mutations. *Ann. N. Y. Acad. Sci.* 2013;1305:18–28.
112. Babushok DV. A brief, but comprehensive, guide to clonal evolution in aplastic anemia. *Hematology Am Soc Hematol Educ Program.* 2018;2018(1):457–466.
113. Sun L, Babushok DV. Secondary myelodysplastic syndrome and leukemia in acquired aplastic anemia and paroxysmal nocturnal hemoglobinuria. *Blood.* 2020;136(1):36–49.
114. Tiu R, Gondek L, O’Keefe C, Maciejewski JP. Clonality of the stem cell compartment during evolution of myelodysplastic syndromes and other bone marrow failure syndromes. *Leukemia.* 2007;21(8):1648–1657.
115. Maciejewski JP, Selleri C. Evolution Of Clonal Cytogenetic Abnormalities in Aplastic Anemia. *Leukemia & Lymphoma.* 2004;45(3):433–440.
116. Maciejewski JP, Risitano A, Sloand EM, Nunez O, Young NS. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. *Blood.* 2002;99(9):3129–3135.
117. Socié G, Rosenfeld S, Frickhofen N, Gluckman E, Tichelli A. Late clonal diseases of treated aplastic anemia. *Semin Hematol.* 2000;37(1):91–101.
118. Katagiri T, Sato-Otsubo A, Kashiwase K, et al. Frequent loss of HLA alleles associated with copy number-neutral 6pLOH in acquired aplastic anemia. *Blood.* 2011;118(25):6601–6609.
119. Zaimoku Y, Takamatsu H, Hosomichi K, et al. Identification of an HLA class I allele closely involved in the autoantigen presentation in acquired aplastic anemia. *Blood.* 2017;129(21):2908–2916.
120. Babushok DV, Duke JL, Xie HM, et al. Somatic HLA Mutations Expose the Role of Class I-Mediated Autoimmunity in Aplastic Anemia and its Clonal Complications. *Blood Adv.* 2017;1(22):1900–1910.
121. Mizumaki H, Hosomichi K, Hosokawa K, et al. A frequent nonsense mutation in exon 1 across certain HLA-A and -B alleles in leukocytes of patients with acquired aplastic anemia. *Haematologica.* 2020;
122. Imi T, Katagiri T, Hosomichi K, et al. Sustained clonal hematopoiesis by HLA-lacking hematopoietic stem cells without driver mutations in aplastic anemia. *Blood Adv.* 2018;2(9):1000–1012.
123. Hosokawa K, Mizumaki H, Yoroidaka T, et al. HLA class I allele-lacking leukocytes predict rare clonal evolution to MDS/AML in patients with acquired aplastic anemia. *Blood.* 2021;137(25):3576–3580.
124. Passweg JR, Baldomero H, Chabannon C, et al. Hematopoietic cell transplantation and cellular therapy survey of the EBMT: monitoring of activities and trends over 30 years. *Bone Marrow Transplant.* 2021;56(7):1651–1664.
125. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood.* 2017;129(4):424–447.
126. O’Neill AT, Chakraverty R. Graft Versus Leukemia: Current Status and Future Perspectives. *JCO.* 2021;39(5):361–372.
127. Vago L, Gojo I. Immune escape and immunotherapy of acute myeloid leukemia. *J Clin Invest.* 2020;130(4):1552–1564.
128. Hong S, Rybicki L, Gurnari C, et al. Pattern of somatic mutation changes after allogeneic hematopoietic cell transplantation for acute myeloid leukemia and myelodysplastic syndromes. *Bone Marrow Transplant.* 2022;57(10):1615–1619.

129. Vago L, Perna SK, Zanussi M, et al. Loss of Mismatched HLA in Leukemia after Stem-Cell Transplantation. *New England Journal of Medicine*. 2009;361(5):478–488.
130. Toffalori C, Zito L, Gambacorta V, et al. Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation. *Nature Medicine*. 2019;25(4):603–611.
131. Stölzel F, Hackmann K, Kuithan F, et al. Clonal evolution including partial loss of human leukocyte antigen genes favoring extramedullary acute myeloid leukemia relapse after matched related allogeneic hematopoietic stem cell transplantation. *Transplantation*. 2012;93(7):744–749.
132. Vago L, Toffalori C, Ciceri F, Fleischhauer K. Genomic loss of mismatched human leukocyte antigen and leukemia immune escape from haploidentical graft-versus-leukemia. *Semin. Oncol*. 2012;39(6):707–715.
133. Dermime S, Mavroudis D, Jiang YZ, et al. Immune escape from a graft-versus-leukemia effect may play a role in the relapse of myeloid leukemias following allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 1997;19(10):989–999.
134. Shyr DC, Zhang BM, Saini G, et al. HLA-haplotype loss after TCR $\alpha\beta$ /CD19-depleted haploidentical HSCT. *Bone Marrow Transplant*. 2021;56(3):733–737.
135. Ahmad M, Rees Robert C, Ali Selman A. Escape from immunotherapy: possible mechanisms that influence tumor regression/progression. *Cancer Immunology, Immunotherapy*. 2004;53(10):.
136. Ritter C, Fan K, Paschen A, et al. Epigenetic priming restores the HLA class-I antigen processing machinery expression in Merkel cell carcinoma. *Scientific Reports*. 2017;7(1):.
137. Shukla SA, Rooney MS, Rajasagi M, et al. Comprehensive analysis of cancer-associated somatic mutations in class I HLA genes. *Nat. Biotechnol*. 2015;33(11):1152–1158.
138. Fuchs EJ, McCurdy SR, Solomon SR, et al. HLA informs risk predictions after haploidentical stem cell transplantation with posttransplantation cyclophosphamide. *Blood*. 2022;139(10):1452–1468.
139. Sajulga R, Bolon Y-T, Maiers MJ, Petersdorf EW. Assessment of HLA-B genetic variation with an HLA-B leader tool and implications in clinical transplantation. *Blood Adv*. 2022;6(1):270–280.
140. Petersdorf EW, Bengtsson M, De Santis D, et al. Role of HLA-DP Expression in Graft-Versus-Host Disease After Unrelated Donor Transplantation. *J Clin Oncol*. 2020;38(24):2712–2718.
141. Arrieta-Bolaños E, Crivello P, He M, et al. A core group of structurally similar HLA-DPB1 alleles drives permissiveness after hematopoietic cell transplantation. *Blood*. 2022;blood.2022015708.
142. Ruggeri A, De Wreede LC, Müller CR, et al. Integrating biological HLA-DPB1 mismatch models to predict survival after unrelated hematopoietic cell transplantation. *Haematologica*. 2022;
143. Chowell D, Krishna C, Pierini F, et al. Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy. *Nature Medicine*. 2019;25(11):1715–1720.
144. Pagliuca S, Gurnari C, Hong S, et al. Clinical and basic implications of dynamic T cell receptor clonotyping in hematopoietic cell transplantation. *JCI Insight*. 2021;6(13):e149080.
145. Daull A-M, Dubois V, Labussière-Wallet H, et al. Class I/Class II HLA Evolutionary Divergence Ratio Is an Independent Marker Associated With Disease-Free and Overall Survival After Allogeneic Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia. *Front Immunol*. 2022;13:841470.
146. Roerden M, Nelde A, Heitmann JS, et al. HLA Evolutionary Divergence as a Prognostic Marker for AML Patients Undergoing Allogeneic Stem Cell Transplantation. *Cancers (Basel)*. 2020;12(7):1835.
147. Merli P, Crivello P, Strocchio L, et al. Human leukocyte antigen evolutionary divergence influences outcomes of paediatric patients and young adults affected by malignant disorders given allogeneic haematopoietic stem cell transplantation from unrelated donors. *Br J Haematol*. 2022;
148. Pagliuca S, Gurnari C, Hercus C, et al. Molecular landscape of immune pressure and escape in aplastic anemia. *Leukemia*. 2022;
149. Gurnari C, Pagliuca S, Prata PH, et al. Clinical and Molecular Determinants of Clonal Evolution in Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria. *JCO*. 2022;JCO.22.00710.
150. Afable MG, Wlodarski M, Makishima H, et al. SNP array–based karyotyping: differences and similarities between aplastic anemia and hypocellular myelodysplastic syndromes. *Blood*. 2011;117(25):6876–6884.
151. Savage SA, Viard M, O’Hugin C, et al. Genome-wide Association Study Identifies HLA-DPB1 as a Significant Risk Factor for Severe Aplastic Anemia. *Am J Hum Genet*. 2020;106(2):264–271.
152. Montesion M, Murugesan K, Jin DX, et al. Somatic HLA Class I Loss Is a Widespread Mechanism of Immune Evasion Which Refines the Use of Tumor Mutational Burden as a Biomarker of Checkpoint Inhibitor Response. *Cancer Discov*. 2021;11(2):282–292.
153. Ferraro F, Miller CA, Christensen KA, et al. Immunosuppression and outcomes in adult patients with de novo acute myeloid leukemia with normal karyotypes. *Proc Natl Acad Sci USA*. 2021;118(49):e2116427118.
154. Stevanović S, van Schie MLJ, Griffioen M, Falkenburg JH. HLA-class II disparity is necessary for effective T cell mediated Graft-versus-Leukemia effects in NOD/scid mice engrafted with human acute lymphoblastic leukemia. *Leukemia*. 2013;27(4):985–987.

155. Matte-Martone C, Liu J, Zhou M, et al. Differential requirements for myeloid leukemia IFN- γ conditioning determine graft-versus-leukemia resistance and sensitivity. *Journal of Clinical Investigation*. 2017;127(7):2765–2776.
156. Woiciechowsky A, Regn S, Kolb H-J, Roskrow M. Leukemic dendritic cells generated in the presence of FLT3 ligand have the capacity to stimulate an autologous leukemia-specific cytotoxic T cell response from patients with acute myeloid leukemia. *Leukemia*. 2001;15(2):246–255.
157. Dickinson AM, Norden J, Li S, et al. Graft-versus-Leukemia Effect Following Hematopoietic Stem Cell Transplantation for Leukemia. *Front Immunol*. 2017;8:496.
158. Schroeder MA, Choi J, Staser K, DiPersio JF. The Role of Janus Kinase Signaling in Graft-Versus-Host Disease and Graft Versus Leukemia. *Biol Blood Marrow Transplant*. 2018;24(6):1125–1134.
159. Li N, Chen Y, He W, et al. Anti-CD3 preconditioning separates GVL from GVHD via modulating host dendritic cell and donor T-cell migration in recipients conditioned with TBI. *Blood*. 2009;113(4):953–962.
160. Weinstock JS, Laurie CA, Broome JG, et al. The genetic determinants of recurrent somatic mutations in 43,693 blood genomes. *Sci. Adv*. 2023;9(17):eabm4945.
161. Negrin RS. Graft-versus-host disease versus graft-versus-leukemia. *Hematology*. 2015;2015(1):225–230.
162. Kaplinsky J, Arnaout R. Robust estimates of overall immune-repertoire diversity from high-throughput measurements on samples. *Nature Communications*. 2016;7(1):.
163. Birnbaum ME, Mendoza JL, Sethi DK, et al. Deconstructing the peptide-MHC specificity of T cell recognition. *Cell*. 2014;157(5):1073–1087.
164. Pagliuca S, Gurnari C, Terkawi L, et al. Immunogenetic Drivers in Post-Transplant Acute Myeloid Leukemia Recurrence: Genomic Dysfunction of Human Leukocyte Antigen Diversity. *Blood*. 2021;138(Supplement 1):3921–3921.
165. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T Cells and Immune Tolerance. *Cell*. 2008;133(5):775–787.
166. Takizawa H, Boettcher S, Manz MG. Demand-adapted regulation of early hematopoiesis in infection and inflammation. *Blood*. 2012;119(13):2991–3002.
167. de Bruin AM, Voermans C, Nolte MA. Impact of interferon- γ on hematopoiesis. *Blood*. 2014;124(16):2479–2486.
168. Bloom ML, Wolk AG, Simon-Stoos KL, et al. A mouse model of lymphocyte infusion-induced bone marrow failure. *Experimental Hematology*. 2004;32(12):1163–1172.
169. Al-Salama ZT. Emapalumab: First Global Approval. *Drugs*. 2019;79(1):99–103.
170. Vallurupalli M, Berliner N. Emapalumab for the treatment of relapsed/refractory hemophagocytic lymphohistiocytosis. *Blood*. 2019;134(21):1783–1786.

ANNEX 1

1. Major histocompatibility complex: history, structure, evolution and diversity

1.1. Brief historical background

Antigen presentation via major histocompatibility complex (MHC) glycoproteins to T and, in part, NK cells constitutes a foundation for the processes of immune surveillance and central tolerance in most mammalian species.

The discovery of MHC genes dates back to the first half of the last century, when the role of the immune system started to be clear in transplantation and rejection of tissue allografts, first in animal models then in humans. Pioneer of those researches was Peter Medawar whose work was followed by Peter Gorer and George Snell's studies.^{1,2} The historical momentum in which the existence of HLA was formally stated was set up only a decade later when the French immunologist Jean Dausset discovered the first HLA antigen (HLA-A2) in 1958 while studying antibody formation against donor leukocytes and platelets in polytransfused patients.^{3,4} Later on, two independent teams, led by Jon van Rood (Netherlands) and Rose Payne (US), showed the presence of anti-leukocyte antibodies in the sera of pregnant women, characterizing respectively for the first time, the antigens Bw4 and Bw6, and A2 and A3.^{5,6} The landmark importance of those researches was renowned through the conferment of the Nobel Prizes in Physiology and Medicine to Medawar, Snell, and Dausset between 1960 and 1980.

After that, an international effort led to the establishment in 1964 of the International Histocompatibility Workshops (IHW), which enabled the progressive characterization of MHC gene cluster and the identification of serologically defined alleles.

The gradual advancement of molecular genomics allowed the identification of an incredibly high number of polymorphisms responsible for the huge variability of this gene cluster, such that, to date, more than 31000 different alleles can be identified for classical and non-classical HLA genes (IPD-IMGT/HLA database, v3.44, June 2021, **Figure 1S**).

Intensive researches in the last decades have contributed to defining how genetic variation within the MHC region may determine predisposition to human diseases and the study of the functional consequences of this immunogenetic diversity is now paradigmatic for human and evolutionary genomics.

Figure 1S. Number of classical and non-classical HLA alleles identified to date in IPD/IMGT-HLA database

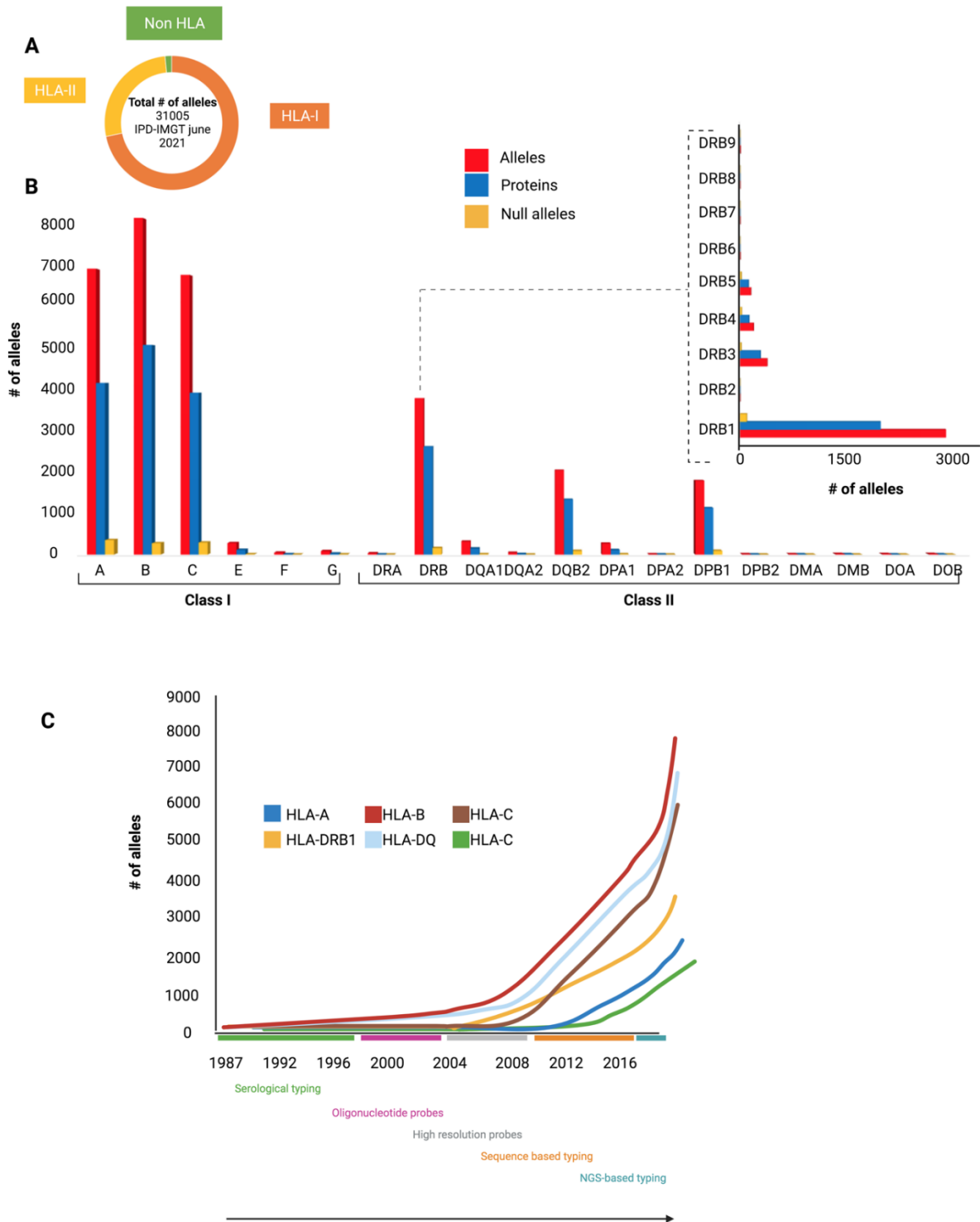


Figure 1S. A) Circle graph capturing the distribution of the number of HLA class I, class II and non-HLA alleles to date known within the MHC region. Data extracted from the IPD-IMGT/HLA database v.3.44 downloaded in June 2021. **B)** Bar graphs depicting the distribution of the number of alleles, proteins and sequences leading to null alleles (characterized by the presence of germline truncating mutations, evolutionary selected), per locus among classical and non-classical HLA genes. Of note is that DRB1 is responsible of the greatest variability within the DRB locus. **C)** Line chart representing the progressive growth in the number of discovered class I and class II alleles across different typing and sequencing technologies,

Data for A and B were extracted from the IPD-IMGT/HLA database v.3.44, downloaded in June 2021. Panel C was adapted from *Robinson et al.*^{7,8}

1.2. Structure and evolution of MHC gene cluster

MHC genomic cluster includes more than 280 genes and encompasses a region of approximately 4 Mb, embedded in the short arm of chromosome 6 (embracing the cytobands 6p22.1-21.3 – location according to GRCh37 coordinates: chr6:28,477,797-33,448,354 – Genome reference consortium: <https://www.ncbi.nlm.nih.gov/grc/human/regions/MHC>). Three distinctive regions can be conventionally identified within this cluster, based on the topographical and functional homology of the genes involved: class I, II and III (**Figure 2S**). Class I region contains classical (HLA-A, B, C) and non-classical (HLA-E to Z) class I loci, MHC I invariant chain genes (MICA, MICB, MICC, MICE) together with other pseudogenes and genes not related to immunity. Class II comprises loci encoding DQ, DR, DP α and β chains and other genes associated with antigen degradation, transportation and assembly (including the IFN- γ inducible immunoproteasome components LMP2 and LMP7 and the transporters TAP1 and TAP2). An extended class II region incorporates few genes with an immune function, including TAP binding protein (TAPBP or tapasin) and Retinoid X Receptor Beta (RXRB), associated respectively with MHC-class I post-translational processing and transcriptional regulation. Class III region is densely populated with genes important for innate and adaptive immune functions, including among others, loci for the complement components C2, C4A-B and CBF, tumor necrosis factor (TNF) and heat shock protein family A (HSPA1).

Figure 2S. Schematic structure of human MHC region

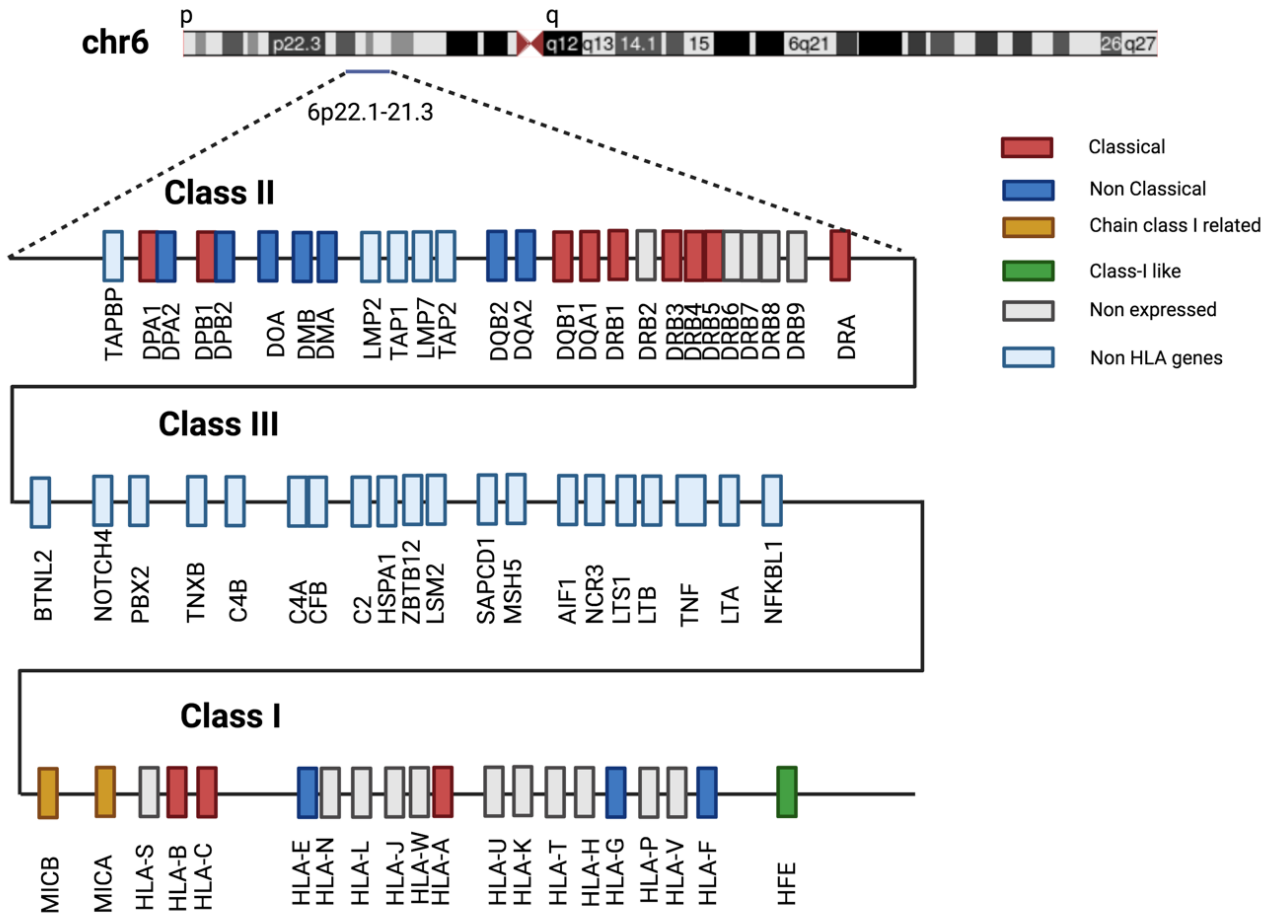


Figure 2S. Selection of class I, II and III HLA and non-HLA genes located in 6p22.1-21.3 region. Gene-locus classification reported in legend follows IPD-IMGT/HLA database description (Vignette modified from: <http://hla.alleles.org/alleles/index.html>)

Patterns of inter-species conservation of those genomic regions uncover some interesting aspects associated with the evolution of the adaptive immune system. In non-mammalian vertebrates, characterized by a rudimentary adaptive immune system in which only one MHC of each class is dominantly expressed, genes encoding proteins for antigen processing machinery (TAP and TAPBP) are very polymorphic and pinpointed next to class I MHC loci, underlining that the coevolution of those two clusters is of crucial importance for their function, likely defining their specificities for immune responses and resistance to pathogens.^{9,10} In humans and in most mammalian species instead, characterized by a fully developed adaptive immune system, specificities against pathogens are essentially based on the hyper-polymorphic variable regions within class I and II MHC loci, while genes encoding transporters and other members of antigen presenting complex are characterized by a low, if not null, variability and located far from class I region, highlighting their capabilities to work and interact with the products of multiple alleles of class I MHC multi-loci family.⁹ Those differences in the genomic architecture of MHC cluster across the phylogenetic tree, represent a product of the

evolutionary forces that have shaped vertebrate immune system, conducting, via mechanisms of genome wide duplication, rearrangements, genetic drift and balancing selection, to the complex hyper-polymorphic mammalian and human MHC system, whose main feature is the capability to adapt to a wide range of pathogens and develop complex immune responses.^{10,11,12}

Introducing this evolutionary perspective is of crucial importance in understanding the immunogenetic risk related to certain immune-related or infectious disorders and may help in tracking patterns of MHC dysfunction at the basis of cancer evolution or susceptibility to complex diseases.

1.3.Variability of antigen presenting structures and divergent allele advantage model

Class I and II MHC complexes have the ability to present to immune effectors a broad assortment of protein-derived antigens on the surface of cells and their structural variability assures the extension of this immune peptidome. The wide range of HLA polymorphisms currently present in human populations, has been selected by evolutionary forces as the result of a process of balancing selection, advantaging alleles or allelic configurations able to sustain the pathogen-derived antigenic burden to which human populations have been exposed throughout their history.^{13,14, 15,16} This sequence variation, consisting in an increased rate of point mutations, inframe or frameshift indels, is extremely high within the site of contact with the antigen (**Figure 3S**), underlying a Darwinian selection favoring the diversification of peptide-binding capabilities in order to adapt to a wide range of pathogen-derived peptides.⁸ Nevertheless, pathogen immune peptidome repertoires are rarely overlapping and certain rare HLA variants can show signs of specializations against restricted sets of peptides, advantaging their maintenance in the population.¹⁷

Different non-mutually exclusive mechanisms have been proposed over the last decades to explain the pathogen-derived balancing selection of HLA genotypes.^{18,19,20} Among those, the heterozygous advantage hypothesis, first formulated by Doherty and Zinkernagel in 1975, assumes that individuals with an heterozygous MHC genotype are able to present a wider range of peptides and subsequently to support immune responses against a larger range of pathogens, compared to homozygous genotypes.²¹

This assumption is further expanded by the divergent allele advantage model stipulating that homologous HLA alleles with higher sequence divergence have the capability to bind more diverse and less overlapping peptide repertoires than lower divergent genotypes.^{22,23,14} The validity of this model in sustaining allelic diversity is now supported by a number of studies approaching this topic through computational tools exploring the binding capabilities of different sets of HLA genotypes towards known pathogen-derived proteomic references.^{23,14,24,25}

This quantitative approach investigating the immune recognition power beyond HLA polymorphisms has been elegantly shown as a milestone for the immune control of a number of infections from viral and microbial pathogens^{26,17,27,28,29} and is of crucial importance for rational vaccine development.³⁰

Figure 3S: Example of sequence variation in HLA – A locus

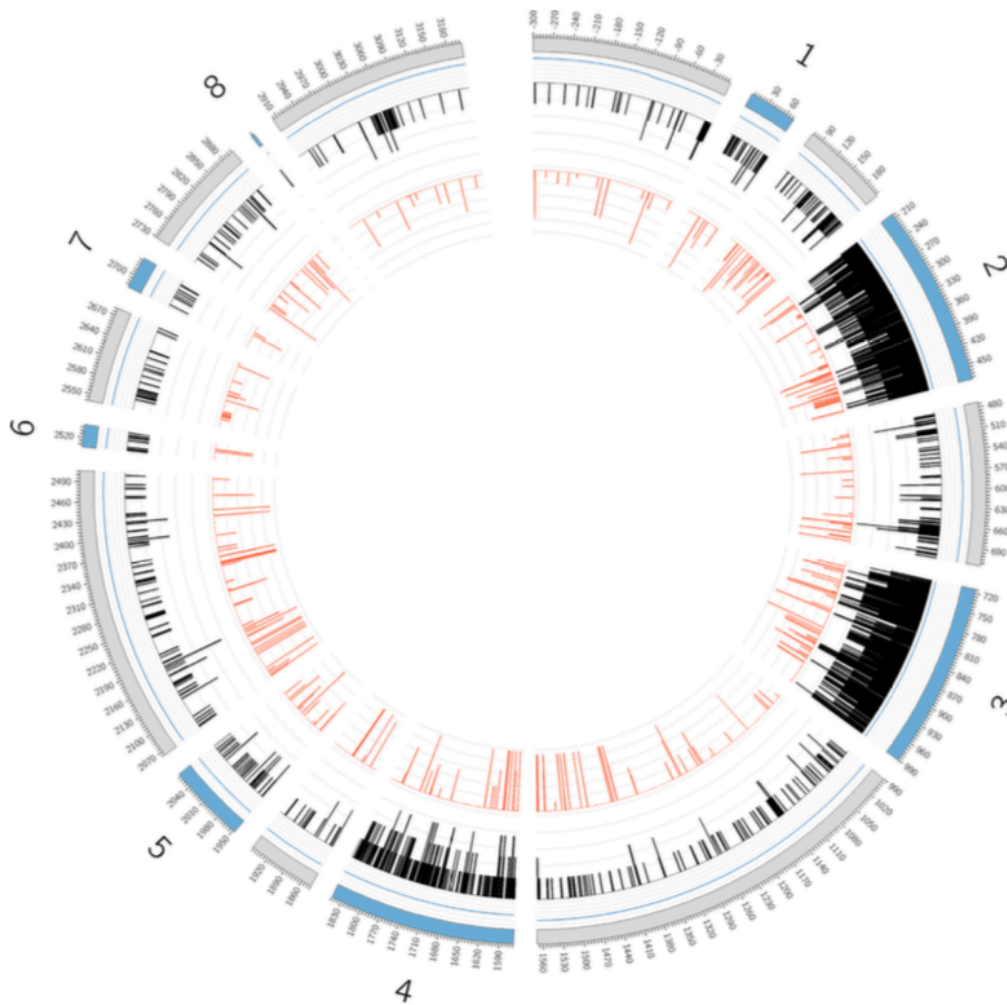


Figure 3S. Adapted from Robinson *et al.*⁸ Circle plot showing the sequence variation within HLA-A locus. The external layer represents the different genomic regions. Exons are filled in blue and numbered, introns are colored in gray. The next layer (blue line) represents the percentage of sequences present in the database. The black histogram ring represents the numbers of polymorphisms seen at each position. The inner ring (in red), the frequency of the second most common polymorphism at each position. A high variation can be seen in exons 2 and 3, and clear regions of conserved variation are present throughout the locus.

1.4.HLA evolutionary divergence and its clinical implications

HLA evolutionary divergence (HED)²⁶ is a metric recently implemented to quantitatively assess the structural variability of MHC complexes within their antigen binding site. Its calculation is based on the physio-chemical composition of the amino acid sequence forming the peptide groove, computed applying the Grantham distance, a score predicting the evolutionary distance between two residues, largely used for the prediction of damaging or deleterious amino acid substitutions.³¹ As higher

Grantham scores reflect higher amino acid distance, higher HED values define more divergent HLA sequences and vice versa (**Figure 4S**).

This quantitative parameter has rapidly found applicability in many contexts due to its capability to capture the extent of the immune peptidome presented by two heterozygous HLA alleles and, *in extenso*, by an individual with a given genotype.

A recent landmark line of studies in the field of immunotherapy shows that cancer patients homozygous for at least one class I HLA locus are able to present a restricted and less diversified repertoire of tumor-derived neoantigens to CD8⁺ T-cells compared to fully heterozygous individuals, defining an immunogenetic configuration that may reduce the chances to respond to checkpoint inhibitors.³² Interestingly, somatic loss of heterozygosity within class I HLA loci may simulate this configuration, and in the same study this was shown to be an independent predictor of poor survival and poor response to immunomodulating agents, indicating how selective pressures derived from the presentation of immunodominant peptides may link to certain HLA genotypic patterns.³² In continuum with this work, HED was shown to add a further layer of stratification to better characterize genotypes of heterozygous patients, demonstrating how advanced-stage melanoma patients with higher divergent class I alleles could respond better to immunotherapy, despite their tumor burden.³³ Moreover, compelling evidence shows that class I HLA genotypes may sculpt tumor genomes by deleting subclones harboring somatic mutations able to affect the immune peptidome presented by tumor cells.^{34,35} This way, the oncogenic mutational profile of a tumor may depend on the probability of derived neo-antigen to be presented by given HLA supertypes.³⁶ It is thus understandable how individual variation of HLA genotypes may contribute to shaping the dynamics of the anti-tumor adaptive immune response, restraining tumor oncogenic potential, and/or inducing mechanisms of immune escape.

Figure 4S: HLA evolutionary divergence

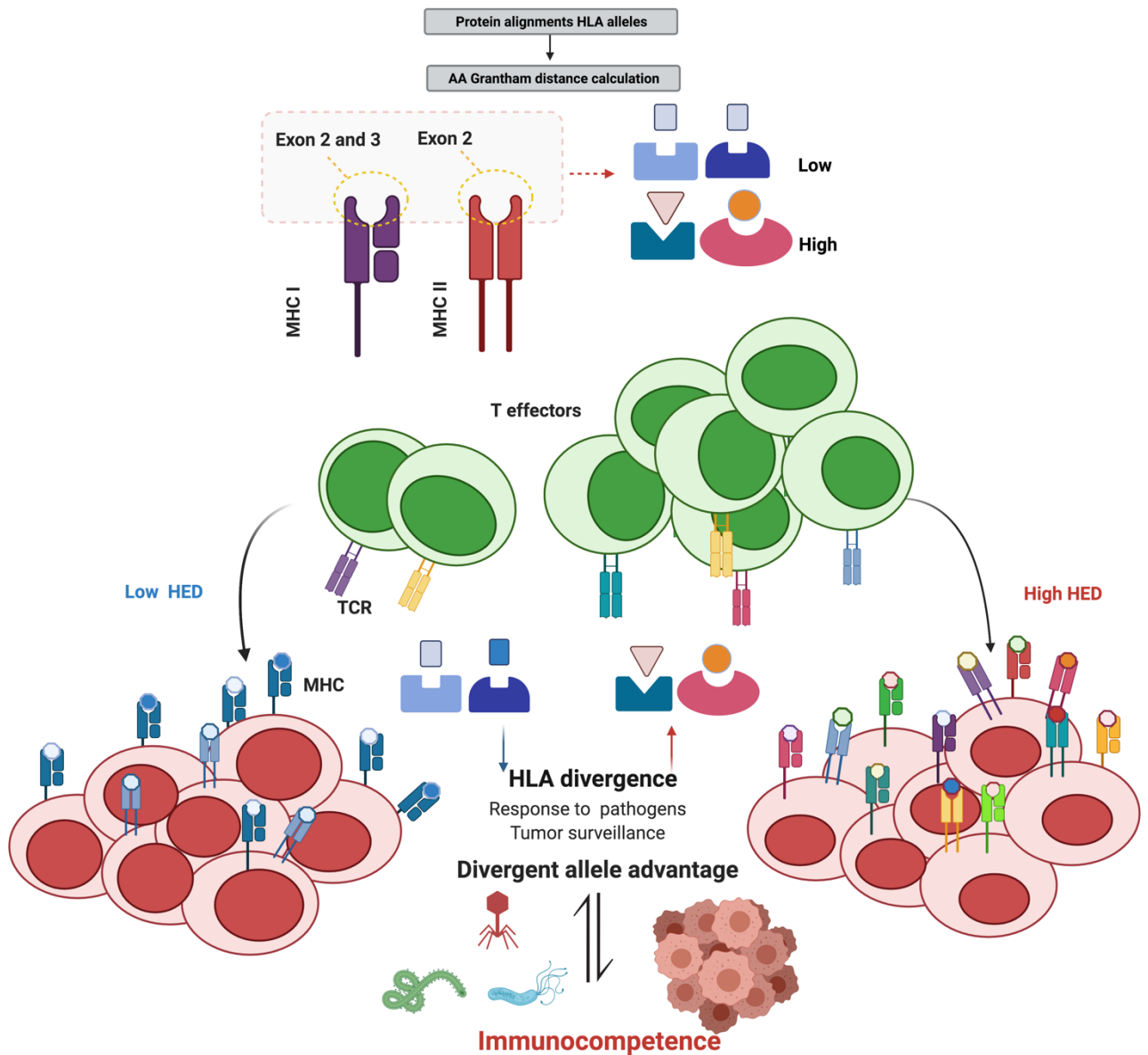


Figure 4S: Sketch of HED computation. Briefly, Grantham distance scores are applied to define the distance between two amino acids in a homologous position within the peptide binding site of 2 heterozygous alleles. This computation is made using a dictionary including all the protein sequences of exons 2 and 3 for class I alleles and exon 2 for class II alleles, assembled from the IPD-IMGT/HLA database.⁴⁵ On a pathophysiological point of view, the divergence of HLA binding sites can influence patterns of T-cell expansion in different processes, from response to infections to tumor surveillance, being higher HLA divergence associated with stronger immunocompetence and vice versa.

2. T-cell receptor repertoire: origin, diversification and evolution

2.1. Germline organization and V(D)J recombination

Diversification of fully competent T cell receptor repertoires (TCR) is a process in continuous evolution, shaped throughout life, whose maintenance is assured by the interaction with self and non-self-antigens presented by HLA complexes. Generally speaking, a large repertoire of unique TCRs accounts for the ability of the adaptive immune system to respond to a wide variety of antigens. TCR diversity is the result of a stochastic process involving rearrangements of the variable (V), diversity (D) and junctional (J) segments within the TRA, TRB, TRD and TRG genes,³⁷ which along with junctional hypermutability, have a theoretical potential to generate up to 10^{18} different complementary determining region (CDR) sequences. While TRA and TRD loci are localized embedded together along the long arm of chromosome 14 (14q11.2), TRB and TRG are in two separate regions on chromosome 7 (respectively 7q35 and 7p15-p14). This organization of TR genes is highly preserved across species, with an astonishing degree of germline sequence conservation within the constant domains.^{38,39}

The genomic configuration of each TR gene consists of a variable number of segments in each V, J and D region. As an example, in an individual it is possible to find 42 TRB-V, 2 TRB-D, 12 TRB-J, 43 TRA-V, 58 TRA-J, 8 TRD-V, 3 TRD-D, 4 TRD-J functional gene segments.^{40,41,42,43,44} This number may vary from person to person based on the number of alleles inherited.³⁹ To date, 863 V, 745 D, 816 J polymorphic sequences have been reported only for TRB locus, each accounting for a specific TRB gene family (**Figure 5S**).

In T lymphocytes at an early stage of maturation, the V(D)J recombinase complex, involving a constellation of enzymes (including the recombination activating genes 1 and 2 -RAG1 and RAG2-, the terminal deoxynucleotidyl transferase -TdT-, the Artemis nuclease and DNA repair enzymes) is responsible for the somatic recombination events in V(D)J regions. First, the recombination signal sequences (RSSs) surrounding V(D)J segments are recognized: those repeated regions are composed as following: a conserved heptamer (CACAGTG), a non-conserved "spacer" of 12 or 23 nucleotides and a conserved nonamer (ACAAAACC).⁴⁰ The "spacer" represents a key element for the regulation of the recombination process marking the genomic points to recombine since the segments within the V(D)J regions are precisely separated either by 12 or 23 base pairs (rule of 12/23).^{40,45} RAG1 and RAG2 bind an RSS close to a coding segment and create a synapsis between two proximal regions, introduce a single-strand nick ("untwisted" DNA portion, without phosphodiester bonds between nucleotides) in the sequence, which alters the electrostatic landscape of the double helix and forms a hairpin loop including the

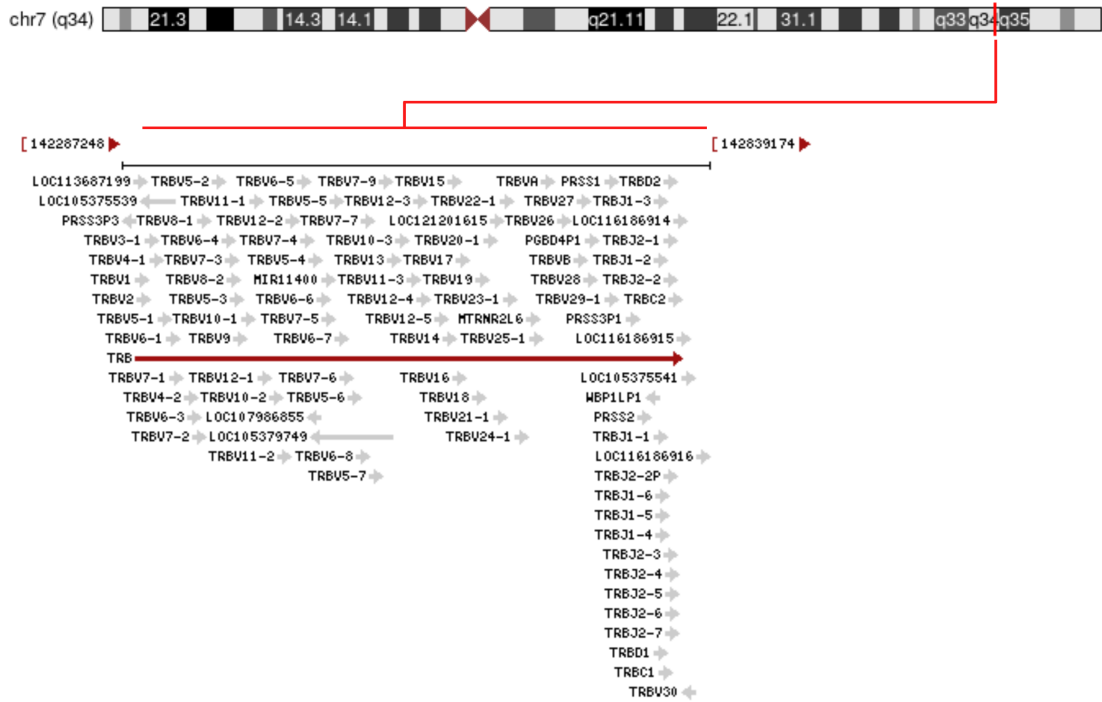
coding elements. This is then repaired through the intervention of Artemis and DNA repair enzymes. Finally, DNA polymerase and TdT trim and add nucleotides to the incomplete new sequences, joined together after this process, contributing to the junctional diversity of the recombined V(D)J sequence (**Figure 6S**).

Within the $\alpha\beta$ TCR repertoire, the CDR3 of the β -chain (encoded by TRB) is the principal site of antigen contact and its variety exceeds that of α -chains.⁴⁶ CDR3 amino acid sequences can unambiguously mark TCR β clonotypes as natural barcodes of individual T cell clones and, by extension, can be associated with a recognized antigenic specificity.¹⁰ CDR3-encoded clonal specificities evolve *de novo* in the thymus or from the pool of clonotypes existent within the memory T cell compartment and regress with age-related thymus involution.³⁷

TCR behavior during thymic selection is of crucial importance for the generation of a self-tolerant repertoire. Immature T cells characterized by TCRs that interact too weakly or too strongly with self-antigens presented by specialized thymus presenting cells are eliminated (negative selection).^{47,48,49,50} Only TCRs surviving to thymic selection reach the periphery and are able to induce specific adaptive immune responses (positive selection). Every T cell has its TCR specificity toward a particular antigen and a given MHC molecule presenting the peptide in question. This feature, known as MHC restriction, is at the basis of the T cell recognition properties. An intriguing intuition is that TCR genomic sequences have been skewed during their evolution toward the recognition of their cognate MHC molecules. CDR1 and CDR2 sequences have been exposed to evolutionary pressures to bind HLA molecules and hence are less polymorphic compared to the CDR3 region (point of antigen contact). Structural modeling highlights loose correspondences between MHC and residues in those two regions, supporting the hypothesis of an intrinsic TCR-bias for MHC at species level.^{51,52}

Figure 5S: Organization of TRB locus and its polymorphisms

A



B

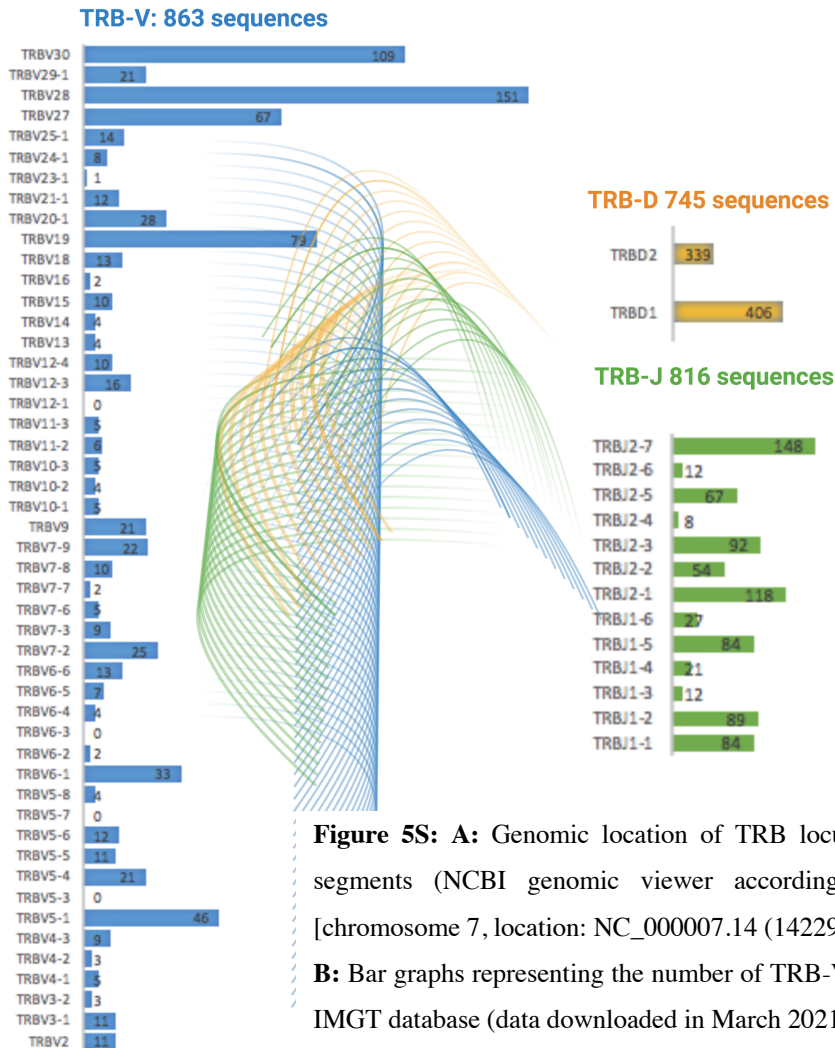


Figure 5S: A: Genomic location of TRB locus and organization of its gene segments (NCBI genomic viewer according to GRCh38.p13 coordinates [chromosome 7, location: NC_000007.14 (142299011-142813287)]).

B: Bar graphs representing the number of TRB-V, D and J sequences reported in IMGT database (data downloaded in March 2021).

Figure 6S: TCR $\alpha\beta$ germline configuration and somatic rearrangement

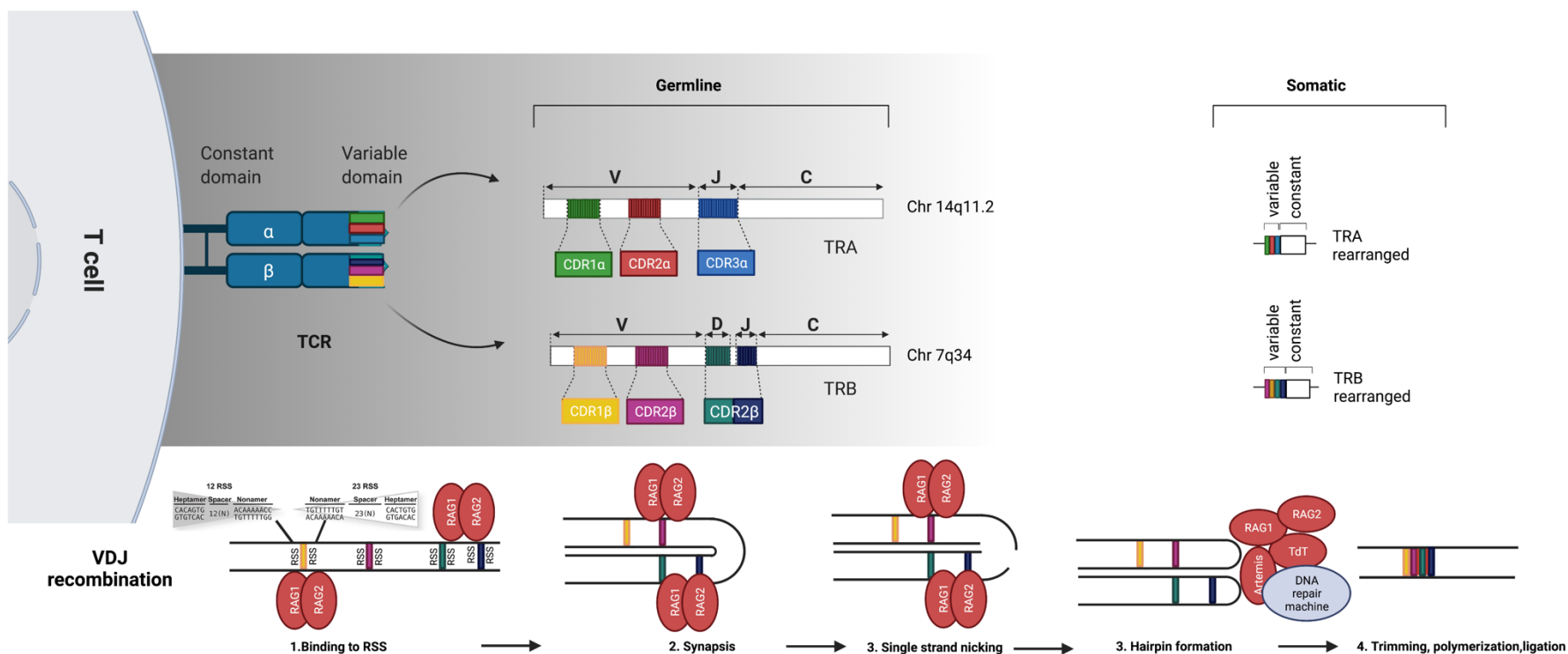


Figure 6S: Graphical representation of TCR $\alpha\beta$ origin and somatic recombination. V and J genetic segments of the TRA gene (on Chr 14q11.2) and V D and J of TRB (Chr 7q34) are spliced together to the constant segment by the recombination-activating genes (RAG1 and RAG2) and several other enzymes included in the V(D)J recombinase complex. After RAG intervention, provoking single-strand nicks in the segments involved in the recombination and inducing a DNA hairpin loop formation, the terminal deoxynucleotidyl transferase (TdT), and a DNA polymerase repair the breaks by adding non-template nucleotides. The immense variability of TCR β templates is assured by the stochastic recombination and rearrangements of each of the V-D and J genomic segments and by the infidelity of this reparation process, enabling the affinity of each mature TCR β toward a specific antigen and a given MHC complex.

One of the most informative features of TCR repertoire analysis is the quantification of its diversity and the characterization of factors that may influence it. The number (richness) and the relative abundance (evenness) of clonotypes carried by an individual may be altered with age, viral infections, vaccinal response.^{53,54,54,55,56} A number of metrics, in general borrowed from ecology, are used to define the diversity of TCR repertoires (i.e., Simpson Index, Chao-1 estimator, Shannon index, etc.).^{57,58,59} One of the major challenges associated with the quantification of TCR diversity is that sampling procedures are inadequate to fully characterize the diversity of a complete T cell repertoire because the number of T cells possibly contained in a blood sample is finite and cells are likely randomly captured. Nevertheless, simplification is needed to explain complex models and, at the moment, those metrics remain useful parameters to assess inter-sample variability.

Immunogenetic variation has been shown to contribute to the assortment of this immense diversity of TCR repertoire in healthy individuals. In a recent study including 666 healthy subjects sequenced for their HLA genotypes and TCR repertoires, TCR diversity positively correlated with structural variability of class I HLA alleles and decreased with age and cytomegalovirus (CMV) infection.⁶⁰

Figure 7S: 3D structure of a TCR-MHC class I complex

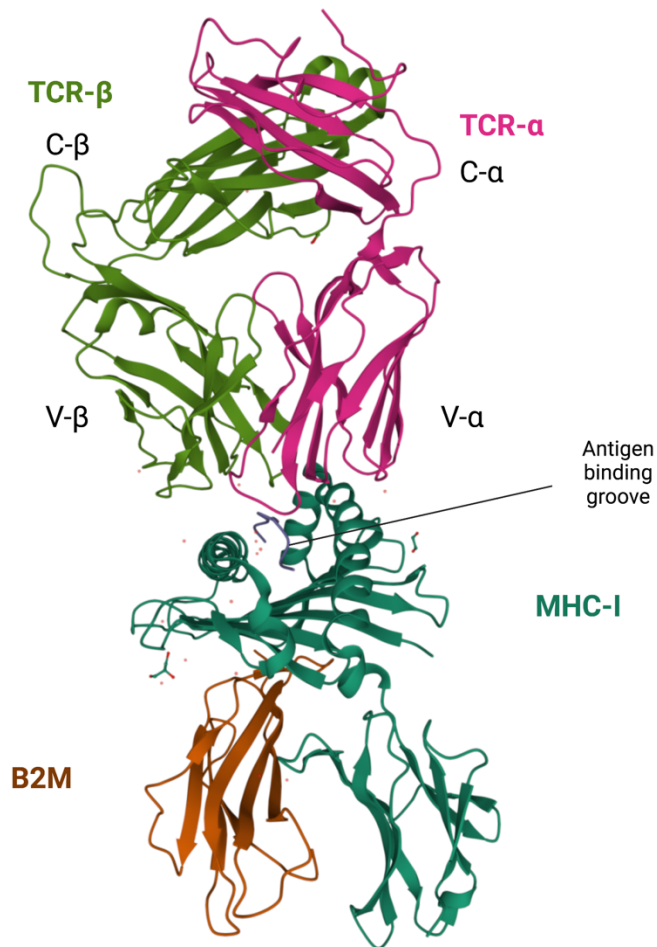


Figure 7S: 3D Crystallographic structure of TCR $\alpha\beta$ chains interacting with an MHC class I molecule (Allele A*02:01 and β 2 microglobulin – B2M). Those structures were extracted from the Protein data bank (PDB) website and reoriented in order to show MHC-TCR synapsis (ref PDB:4MNQ) ⁶¹.

Figure 8S: TCR structural bias for MHC

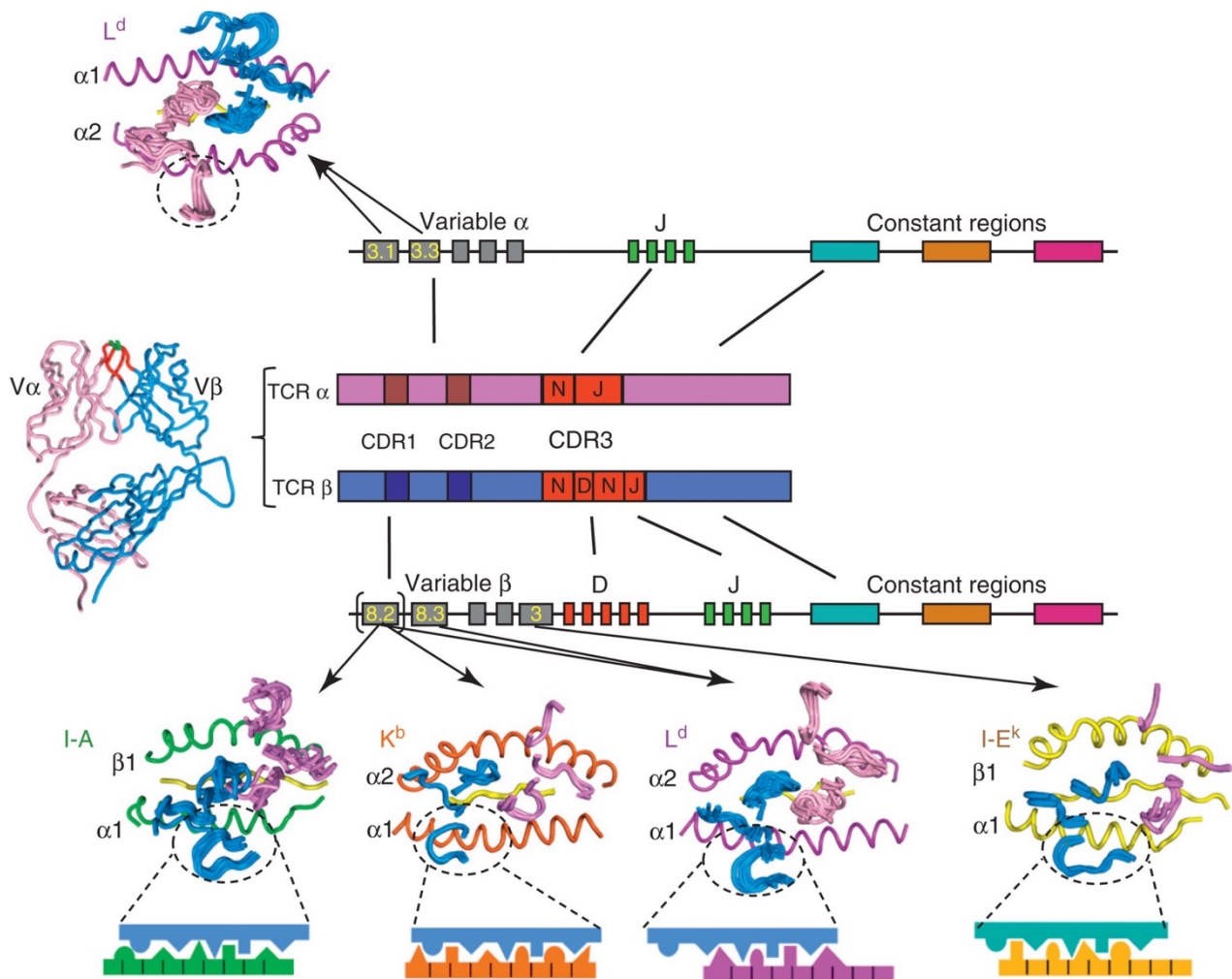


Figure 8S. Adapted from Garcia *et al.*⁵² Different TCR-MHC synapses, based on diverse TCR germline CDR 1 and 2 loops (in dotted circles). The electrostatic landscape of those interactions may induce different specificities for MHC (illustrated by cartoons at the bottom).

3. Immune peptidome: origin and regulation

The understanding of the biology beyond antigen presentation and the mechanistic interactions between TCRs and MHC molecules opens the way to the definition of the immune peptidome origin.

Literally, this entity characterizes the ensemble of peptides derived from the degradation of intracellular or extracellular proteins that are then presented to T-cell effectors via class I and class II HLA molecules. While class I complexes allow the presentation to CD8⁺ T cells, class II present

peptides to CD4+ T lymphocytes. Despite the similarity in terms of mechanics of presentation, different routes of peptide generation and processing are necessary for the two types of MHC restriction (**Figure 9S**).⁶²

Figure 9S: MHC class I and II antigen processing and presentation pathways

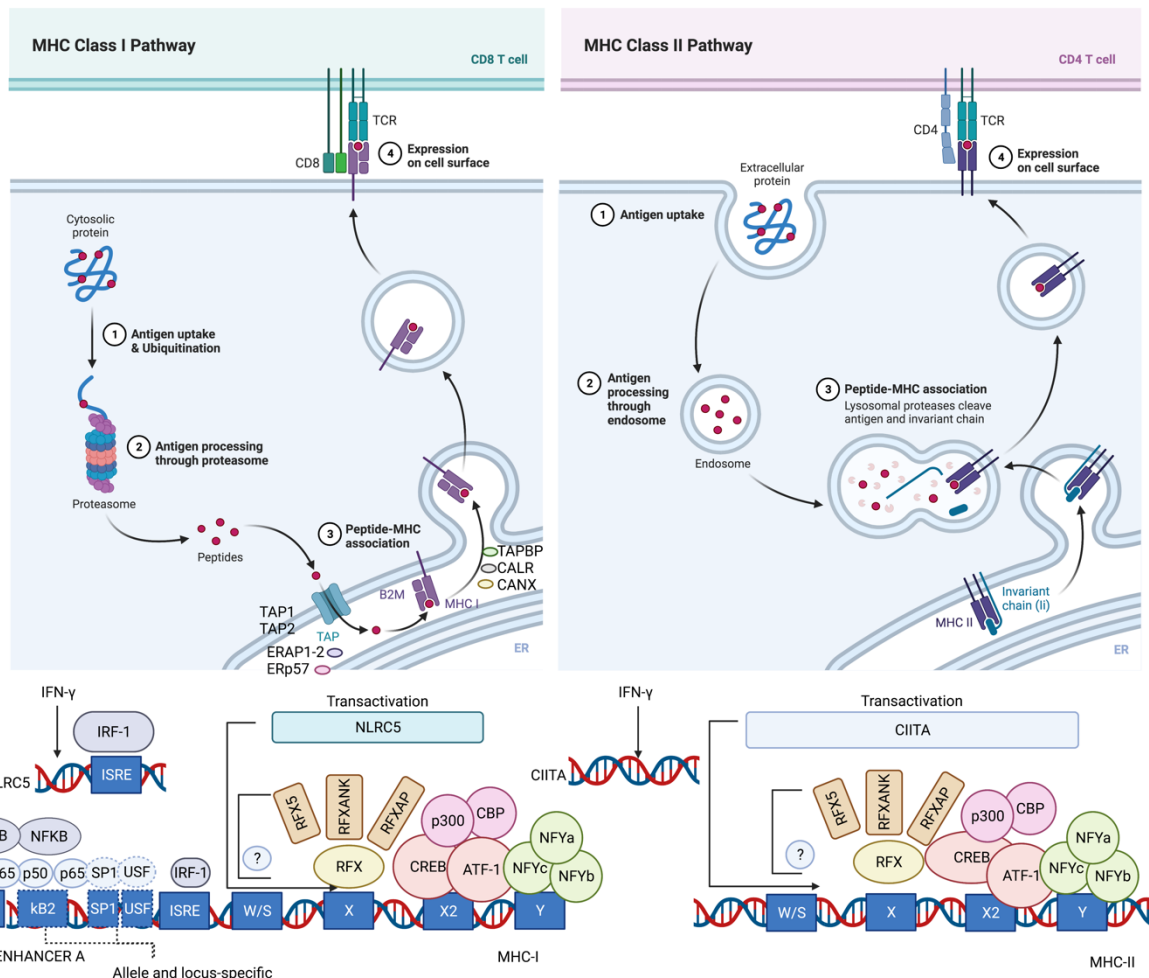


Figure 9S: Steps of antigen degradation and presentation via MHC class-I (left) and class II (right) pathways. Bottom figures depict the transcriptional regulation of genes in class I and class II MHC genomic cluster. *Figure created using a BioRender template.*

3.1. Class-I MHC processing and presentation

Class I HLA complexes allow the presentation of peptides derived from intracellular proteins, degraded via the proteasome pathway,⁶² a cytosolic multi-enzyme complex that fragments proteins into peptides after a process of tagging via an enzymatic cascade involving ubiquitination enzymes (E1, E2 and E3).⁶³

It is estimated that after ubiquitination and protein breakdown (encompassing almost 80% of the intracellular proteome), only <0.1% of the peptides are loaded into the binding cleft of class I

molecules.⁶⁴ The human proteasome is composed of a central catalytic 20S core and constantly in activity, shaped in a barrel conformation and formed by four heptameric rings, arranged according to a palindromic pattern α_{1-7} , β_{1-7} , β_{1-7} , α_{1-7} . This protein complex can exist in multiple conformations based on its basal activity and the participation of proteasome regulators. The 20S constitutive proteasome is ubiquitously expressed and incorporates the catalytic subunits β_1 (PSMB6), β_2 (PSMB7) and β_5 (PSMB5). The immunoproteasome, which is constitutively expressed only in immune cells and induced under inflammatory conditions, especially after interferon- γ (IFN- γ) and tumor necrosis factor- α (TNF- α) stimulation, in non-immune cells, harbors instead three specific catalytic subunits: low molecular mass protein (LMP2, or PSMB9), LMP7 (PSMB8), and LMP10 (PSMB10).^{65,66,67} The binding of five regulators to 20S proteasome facilitates substrate entry: the multisubunit 19S regulator (mediating the ubiquitin-dependent fragmentation of substrates), PA28 α/β (IFN- γ inducible with a possible role in immunoproteasome) and PA28 γ (with an intranuclear location, possibly implicated in cell cycle regulation); the function of the remaining two regulators PA200 and PI31 remains to be characterized (**Figure 10S**). One important characteristic of the immune peptide generation is that those oligosequences are not randomly mounted on the class I MHC binding cleft but rather accurately selected based on their length and composition by peptidase and other enzymatic activities able to edit the pool of protein fragments. Proteins involved in this pathway are cytosolic proteins, including regulatory, misfolded and damaged proteins, defective ribosomal products, viral proteins in infected cells or mutated proteins in neoplastic cells.^{68,69,70,71} Interestingly, the particular peptide repertoire generated can quantitatively and qualitatively vary based on which proteasome subunit is involved (constitutive vs. immune). Both constitutive and immunoproteasome are able to produce class I associated epitopes; however, the specific peptide repertoire generated can quantitatively and qualitatively vary between the two complexes.⁷¹

Figure 10S: Proteasome regulation and composition

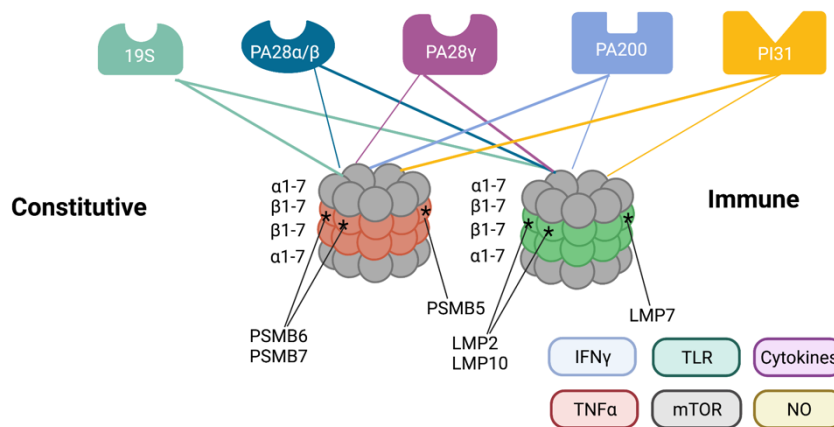


Figure 10S: Inspired to *Kammerl et al.*⁷² Composition and regulator contribution to standard and immunoproteasome. PA, proteasome activator mTOR, mammalian target of rapamycin PI, proteasome inhibitor NO, nitric oxide RA, retinoic acid TLR, Toll-like receptor agonist (Those last are other potential triggers of the immunoproteasome). The thickness of the lines indicates the specificity of the enzymatic regulator for either the constitutive or the immunoproteasome.

It has been shown that those differences, basically associated with different cleavage motifs, can affect T-cell specificity and activation and are possibly associated with mechanisms of tolerance and autoimmunity.⁷³ Of note is that immunodominant epitopes derived from infectious organisms have been shown to be preferentially associated with the immunoproteasome pathway.⁷⁴ Interestingly, knocked-out mouse models for the three immunoproteasome subunits, showed meaningful differences in terms of epitope repertoire from wild-type mice, affecting antigen presentation and efficiency of T cell responses.⁷⁵

Peptide fragments consequently generated are then transported into the endoplasmic reticulum (ER) by specialized Transporters 1 and 2 ATP Binding Cassette Subfamily B Member (TAP1 and TAP2), able to bind and transport peptides of 8-12 amino acids preferentially and with a hydrophobic or basic C-terminus, with an affinity for class I MHC.^{62,76,77} The assembly takes place via the activity of several chaperone proteins: calnexin, the thiol oxidoreductase (ERp57), calreticulin, and tapasin forming along with TAP1 and TAP2 the class I MHC loading complex.^{78,79,80} After antigen internalization, processing and binding to the peptide cleft, and opportune folding and oxidation processes, the heavy chain finally associates with β_2 microglobulin (β_2M), stabilizing the heterodimer and optimizing the peptide load.^{81,82} This process is followed by the release of the MHC class I-antigen complexes into vesicles that navigate

through the Golgi apparatus, drift to the cell membrane and are exposed at extracellular level for the interaction with CD8+ expressing cells.

3.2. Class-II MHC processing and presentation

Class II MHC has the capability to present peptides generated through the endosomal-lysosomal antigen-processing. These peptides derive from a variety of proteins that gain access to those compartments through the endocytic pathway or via mechanisms of autophagy. After their synthesis, class II MHC binds into the ER a protein known as invariant chain (Ii). This contains specific amino acid motifs that convey, once complexed with them, class II MHC α and β chains to the endosomal-lysosomal antigen-processing compartment.⁸³ The complex Ii-class II MHC transits through the Golgi network to reach the plasma membrane, where targeting motifs of invariant chains facilitate the assembly of clathrin drift consenting the endocytosis.^{84,85} After that, Ii is then degraded by proteolytic instances habilitating the antigen recognition capabilities of class II molecules.⁸⁶ This last step is obtained when a small fragment of Ii, termed class II-associated invariant chain peptide (CLIP), is released from the peptide binding groove. This process is regulated by two enzymes, recognized as class II-like molecules, HLA-DM (enabling CLIP release) and HLA-DO (inhibiting HLA-DM activity) according to pH of endosome-lysosome antigen-processing compartments.⁸⁷ Antigen is then loaded within the peptide-binding cleft and the new operative MHC molecule is transported through the multivesicular bodies to the cell surface for the interaction with T CD4+ cells. Specialized antigen presenting cells (APC including dendritic cells -DCs, macrophages and B cells) may have a variable and complex system of multivesicular bodies and endosome-lysosome compartments, according to their antigen uptake capabilities as well as the characteristics of antigens internalized. DCs for instance, have the capacity to interiorize extracellular material through a process called macropinocytosis, a non-specific mechanism of extracellular material acquisition (fluid, degraded proteins, solutes), operating through large endocytic vesicles (macropinosomes). B cell antigen uptake is instead more defined, associated with B cell receptor (BCR) binding specificity.

Phagocytosis is another main process of antigen internalization and is specific of DCs and macrophages. This process is also involved in peripheral tolerance through the presentation of self-antigens when DCs phagocyte apoptotic cells. Structural analysis of phagosome membrane showed that ER represents a possible origin of such a structure.⁸⁸ This characteristic is particularly important since it explains the mechanics of class I cross-antigen presentation to CD8+ by DCs.^{88,89} Phagosomes can then be fused with lysosomes to generate phagolysosomes, with a highly

proteolytic potential, finalizing antigen formation and consenting the complete peptide loading on class II molecules.^{90,90}

APC activation generates efficient antigen–class II MHC complexes, increasing their expression on cell surface. Many external triggers can operate, facilitating this pathway; for instance, Toll-like receptor activation can enhance transcription translation of class II genes and enhance phagosome maturation and trafficking.^{91,92} An interesting aspect of class II-related presentation is that it can also originate from mechanisms of autophagocytosis, involving almost essentially intracellular proteins. Autophagosomes in fact, can intertwine with endosomal-lysosomal antigen presenting compartments forming autophagolysosomes. This process is also supposed to be associated with the regulation of peripheral tolerance.⁹³

3.3. Transcriptional regulation of MHC genes

Class I MHC genes undergo complex processes of transcriptional regulation, partially responsible for their variable expression on the surface of cells from different tissue and different stage of maturation/senescence. Several conserved regulatory promoter elements (enhancer A, interferon-stimulated response element (ISRE) and the SXY-module, **Figure 11**, left-bottom panel) are responsible for either a constitutive or an inducible gene-transcription activation.⁹⁴ Very interestingly, the same elements can also regulate the transcriptional activation of the $\beta 2M$ promoter as an example of how sharing biological functions can dictate patterns of genetic coevolution.⁹⁴ While the enhancer A represents a binding site for the nuclear factor (NF)- κB , ISRE is bound by interferon regulatory factors (IRF) such as IRF1-9, activated respectively by TNF α and IFN γ via the Janus-family kinase/signal transducer and activator of transcription, (JAK/STAT pathway). Those routes can be over-induced in inflammatory situations, generally characterized by an upregulation of both mediators, enhancing the upregulation of class I HLA molecules on the surface of cells in certain conditions. Other transcriptional regulators, for which binding sites can be found within the 5' untranslated region (UTR) of class I HLA genes, are represented by the upstream stimulatory factor (USF)1 and 2 and by the specificity protein 1 (SP1). Variable penetrance of those regulatory regions may add another layer of heterogeneity of class I gene expression operating in allele- and locus-specific manner.^{94,95} For instance, locus A constitutively present 2 NF κB sites within 5' UTR region, while HLA-B alleles have only one binding site for this transcriptional factor and are instead characterized by the presence of an SP1 module (NF κB and SP1 are known to interact in certain pathological conditions), that is instead absent within the promoter region of HLA-A alleles.⁹⁶

A network of transcription factors regulates the tissue expression of class I molecules. NOD-like receptor (NLR) family and acid domain containing 5 (NLRC5) is the principal class I master regulator that, translocating from the cytosol to the nucleus, despite the lack of a DNA binding site, can directly orchestrate the assembly of the enhanceosome on the 4-boxes DNA domain of the SXY module (X1, X2, Y, S boxes, Figure 10). This complex of regulators includes i) the Regulatory Factor X (RFX) complex formed by the subunits RFX5, RFX-Associated Protein (RFXAP) and RFX Associated Ankyrin Containing Protein (RFXANK), that binds X1 box ii) X2 Binding Protein (X2BP) including CAMP Responsive Element Binding Protein 1 (CREB1), CAMP Responsive Element (CREM) and the *Activating Transcription Factor 1 (ATF1)*, that bind X2 box iii) NFY complex constituted by NFYa, NFYb and NFYc subunits iv) non-identified proteins binding S box.^{97,98,99,100}

Interestingly, the structure of the enhanceosome, but not its mechanisms of regulation, is common for class I and II genes. Class II transactivator (CIITA) is the master regulator for class II enhanceosome, exerting a role very similar to NLRC5. Interestingly while NLRC5 coordinates the transcription of other genes involved in class I presentation (β 2M, TAP1 and LMP2), CIITA activates the assembly of this complex on class II-like molecules implicated in antigen trafficking and processing (HLA-DM and DO).¹⁰¹

3.4. (Re)Modeling peptide immunogenicity

Triggering an effective immune response is one of the characteristics that distinguish the peptidome from the immune peptidome, as well as an attribute researched for most vaccinal strategies. The degree of activation of T or B-cell responses may strongly depend on the physiochemical characteristics of the antigenic structures recognized by TCR and BCR, based on their specificity. In recent years we have been assisting to a flourishing of pipelines developed with the purpose to identify, predict or quantify this particular feature characterizing immune peptides.^{102,103,104,105,106,107,108}

An interesting approach recently used to capture qualitative characteristics able to track epitopes possibly associated with CD8+ responses resides in the identification of amino acid signatures enriched in known immunogenic vs. non-immunogenic self-antigens.¹⁰⁴ In this seminal work, three major biochemical properties were studied: hydrophobicity, polarity, and side-chain bulkiness (enrichment in long carbon chains or carbon rings), by means of three quantitative metrics: respectively Kyte–Doolittle, Grantham, Zimmerman.^{109,31,110} Results of the statistical model incorporated those quantitative parameters and identified the enrichment of hydrophobic amino

acids within the sites of contact with TCR. Hydrophobicity of particular residues has been suggested as a hallmark of immunogenicity not only for class I-presented epitopes, but also for B-cell recognized antigens.¹⁰⁴

The knowledge of biochemical properties governing antigen presentation can have important implications. With the explosion of the sequencing platforms and high throughput methods for characterization of DNA and protein sequences, as well as the possibility to build aggregated and highly curated datasets of such multi-omics data, we are assisting to a revolution in the design of protein-based therapeutics (e.g., vaccines or recombinant enzymes), becoming now more and more data-driven. Computational models in fact may now be used for protein engineering approaches to modulate (either increase or decrease) the immunogenicity of a given protein product. An example of applicability in cancer therapy is represented by a data-driven attempt of reducing the adverse allergic and anaphylactic responses of *Erwinia chrysanthemi* L-Asparaginase (Erwinase, an anti-cancer treatment frequently used in anti-lymphoblastic leukemia regimens), by performing high throughput characterization of the mutational impact in residues supposed to be implicated in such reactions.¹¹¹ This approach implies a deep mutational scanning of therapeutically-related proteins and the integration of the mutated amino acids in structural models that can be further *in vivo* tested and refined.¹¹²

Studying the immunogenic potential of peptides can be of particular interest also in autoimmune diseases, where the identification of patterns eliciting the autoreactive instances can be used for precision medicine approaches. Existing examples are found in recent therapeutic developments in the setting of Type 1 Diabetes (T1D), multiple sclerosis (MS) and rheumatoid arthritis (RA).¹¹³ For instance, the antigen-based vaccine Diamyd® is an immunotherapy recently entered in phase III of investigation that exploits the immunization against a known T1D-associated self-antigen, the glutamic acid decarboxylases 65 (GAD65), to induce tolerance in children carrying the risk haplotype DRB1*01:03-DQB1*02:01.¹¹⁴

Another very intriguing approach based on antigen-remodeling is to target the peptide binding site of specific HLA molecules involved in the pathogenesis of immune disorders. In RA, interestingly, peptides built on the backbone of type II collagen-associated epitope and containing an amino acid substitution in the site of interaction with the binding pocket 1 (P1, within the groove of class II molecules) have been shown particularly effective in blocking the binding of this self-antigen, inhibiting at the same time interaction with autoreactive TCRs.¹¹⁵ As other interesting example, a highly selective inhibitor, now in preclinical development, has also been identified for DRB1*15:01 risk allele, with potential applicability in MS and other autoimmune disorders (PV-267, **Figure 11S**).^{116,117,118}

Figure 11S: Examples of antigen-based therapeutics

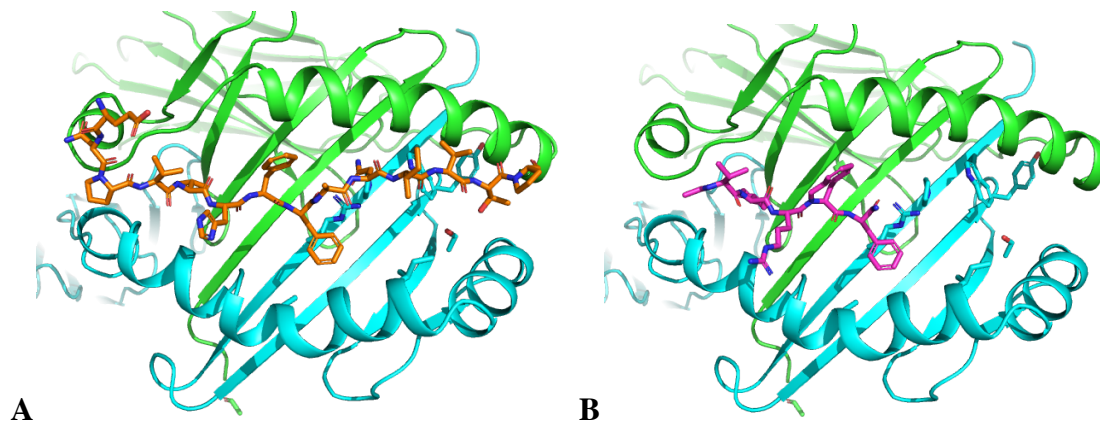


Figure 13: A) Structural model of myelin basic protein bound to the peptide groove of the DR2 molecule (formed by DRA*01:01 -green - and DRB1*15:01 - light blue -). B) Structural model of PV-267, a small molecule binding the cleft of DR2, inhibiting the binding of MBP and the interaction with the TCR. Structural modeled created with I-TASSER and pyMol, based on the known crystal structure of the complex DR2-MBP¹¹⁹ (sequences retrieved from IPD/IMGT-HLA database and UniProt). PV-267 structure: PUBCHEMID: 72715806

References – Annex 1

1. Medawar PB. The behaviour and fate of skin autografts and skin homografts in rabbits: A report to the War Wounds Committee of the Medical Research Council. *J Anat.* 1944;78(Pt 5):176–199.
2. Gorer PA, Lyman S, Snell GD, Haldane JBS. Studies on the genetic and antigenic basis of tumour transplantation Linkage between a histocompatibility gene and “fused” in mice. *Proc. R. Soc. B.* 1948;135(881):499–505.
3. Dausset J. Les Thrombo-Anticorps. *Acta Haematol.* 1958;20(1–4):185–194.
4. Dausset J. Iso-leuco-anticorps. *Acta Haematol.* 1958;20(1–4):156–166.
5. Payne R, Rolfs MR. Fetomaternal Leukocyte Incompatibility. *J. Clin. Invest.* 1958;37(12):1756–1763.
6. Van Rood JJ, Eernisse JG, Van Leeuwen A. Leucocyte Antibodies in Sera from Pregnant Women. *Nature.* 1958;181(4625):1735–1736.
7. Robinson J, Guethlein LA, Cereb N, et al. Distinguishing functional polymorphism from random variation in the sequences of >10,000 HLA-A, -B and -C alleles. *PLoS Genet.* 2017;13(6):e1006862.
8. Robinson J, Barker DJ, Georgiou X, et al. IPD-IMGT/HLA Database. *Nucleic Acids Research.* 2019;gkz950.
9. Kaufman J. Antigen processing and presentation: evolution from a bird’s eye view. *Mol Immunol.* 2013;55(2):159–161.
10. Kaufman J. Unfinished Business: Evolution of the MHC and the Adaptive Immune System of Jawed Vertebrates. *Annu. Rev. Immunol.* 2018;36(1):383–409.
11. Flajnik MF, Kasahara M. Origin and evolution of the adaptive immune system: genetic events and selective pressures. *Nat Rev Genet.* 2010;11(1):47–59.
12. Kaufman J, Volk H, Wallny H-J. A “Minimal Essential Mhc” and an “Unrecognized Mhc”: Two Extremes in Selection for Polymorphism. *Immunol Rev.* 1995;143(1):63–88.
13. Fumagalli M, Sironi M, Pozzoli U, et al. Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. *PLoS Genet.* 2011;7(11):e1002355.
14. Pierini F, Lenz TL. Divergent Allele Advantage at Human MHC Genes: Signatures of Past and Ongoing Selection. *Molecular Biology and Evolution.* 2018;35(9):2145–2158.
15. Hedrick PW, Thomson G. Evidence for balancing selection at HLA. *Genetics.* 1983;104(3):449–456.
16. Brandt DYC, César J, Goudet J, Meyer D. The Effect of Balancing Selection on Population Differentiation: A Study with HLA Genes. *G3 Genes/Genomes/Genetics.* 2018;8(8):2805–2815.
17. Özer O, Lenz TL. Unique pathogen peptidomes facilitate pathogen-specific selection and specialization of MHC alleles. *Mol Biol Evol.* 2021;msab176.

18. Apanius V, Penn D, Slev PR, Ruff LR, Potts WK. The Nature of Selection on the Major Histocompatibility Complex. *Crit Rev Immunol*. 1997;17(2):179–224.
19. Spurgin LG, Richardson DS. How pathogens drive genetic diversity: MHC, mechanisms and misunderstandings. *Proc Biol Sci*. 2010;277(1684):979–988.
20. Radwan J, Babik W, Kaufman J, Lenz TL, Winternitz J. Advances in the Evolutionary Understanding of MHC Polymorphism. *Trends in Genetics*. 2020;36(4):298–311.
21. Doherty PC, Zinkernagel RM. Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature*. 1975;256(5512):50–52.
22. Wakeland EK, Boehme S, She JX, et al. Ancestral polymorphisms of MHC class II genes: divergent allele advantage. *Immunol Res*. 1990;9(2):115–122.
23. Lenz TL. COMPUTATIONAL PREDICTION OF MHC II-ANTIGEN BINDING SUPPORTS DIVERGENT ALLELE ADVANTAGE AND EXPLAINS TRANS-SPECIES POLYMORPHISM: MHC-ANTIGEN BINDING AND DIVERGENT ALLELE ADVANTAGE. *Evolution*. 2011;65(8):2380–2390.
24. Arora J, McLaren PJ, Chaturvedi N, et al. HIV peptidome-wide association study reveals patient-specific epitope repertoires associated with HIV control. *Proc Natl Acad Sci U S A*. 2019;116(3):944–949.
25. Manczinger M, Boross G, Kemény L, et al. Pathogen diversity drives the evolution of generalist MHC-II alleles in human populations. *PLoS Biol*. 2019;17(1):e3000131.
26. Pierini F, Lenz TL. Divergent Allele Advantage at Human MHC Genes: Signatures of Past and Ongoing Selection. *Molecular Biology and Evolution*. 2018;35(9):2145–2158.
27. Abidi SH, Shahid A, Lakhani LS, et al. Population-specific evolution of HIV Gag epitopes in genetically diverged patients. *Infect Genet Evol*. 2013;16:78–86.
28. Arora J, Pierini F, McLaren PJ, et al. HLA Heterozygote Advantage against HIV-1 Is Driven by Quantitative and Qualitative Differences in HLA Allele-Specific Peptide Presentation. *Mol Biol Evol*. 2020;37(3):639–650.
29. Norström MM, Veras NM, Huang W, et al. Baseline CD4+ T cell counts correlates with HIV-1 synonymous rate in HLA-B*5701 subjects with different risk of disease progression. *PLoS Comput Biol*. 2014;10(9):e1003830.
30. Eckels DD. MHC: function and implication on vaccine development. *Vox Sang*. 2000;78 Suppl 2:265–267.
31. Grantham R. Amino Acid Difference Formula to Help Explain Protein Evolution. *Science*. 1974;185(4154):862–864.
32. Chowell D, Morris LGT, Grigg CM, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science*. 2018;359(6375):582–587.
33. Chowell D, Krishna C, Pierini F, et al. Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy. *Nature Medicine*. 2019;25(11):1715–1720.
34. DuPage M, Mazumdar C, Schmidt LM, Cheung AF, Jacks T. Expression of tumour-specific antigens underlies cancer immunoediting. *Nature*. 2012;482(7385):405–409.
35. Matsushita H, Vesely MD, Koboldt DC, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. *Nature*. 2012;482(7385):400–404.
36. Marty R, Kaabinejadian S, Rossell D, et al. MHC-I Genotype Restricts the Oncogenic Mutational Landscape. *Cell*. 2017;171(6):1272–1283.e15.
37. Jung D, Alt FW. Unraveling V(D)J Recombination. *Cell*. 2004;116(2):299–311.
38. Parra ZE, Lillie M, Miller RD. A model for the evolution of the mammalian t-cell receptor α/δ and μ loci based on evidence from the duckbill Platypus. *Mol Biol Evol*. 2012;29(10):3205–3214.
39. Mahe E, Pugh T, Kamel-Reid S. T cell clonality assessment: past, present and future. *J Clin Pathol*. 2018;71(3):195–200.
40. Turner SJ, Doherty PC, McCluskey J, Rossjohn J. Structural determinants of T-cell receptor bias in immunity. *Nat Rev Immunol*. 2006;6(12):883–894.
41. Lefranc MP. TRA (T cell receptor alpha). *Atlas Genet Cytogenet Oncol Haematol* 2003;7:245–8. TRA (T cell receptor alpha).
42. Lefranc MP. TRD (T cell receptor delta). *Atlas Genet Cytogenet Oncol Haematol* 2003;7:252–4. TRD (T cell receptor delta).
43. Lefranc MP. TRG (T cell receptor gamma). *Atlas Genet Cytogenet Oncol Haematol* 2003;7:255–6. TRG (T cell receptor gamma).
44. Lefranc MP. TRB (T cell receptor beta). *Atlas Genet Cytogenet Oncol Haematol* 2003;7:249–51. TRB (T cell receptor beta).
45. Roth DB. V(D)J Recombination: Mechanism, Errors, and Fidelity. *Microbiol Spectr*. 2014;2(6):.
46. Woodsworth DJ, Castellarin M, Holt RA. Sequence analysis of T-cell repertoires in health and disease. *Genome Med*. 2013;5(10):98.
47. Jerne NK. The somatic generation of immune recognition. *Eur. J. Immunol*. 1971;1(1):1–9.
48. Goldrath AW, Bevan MJ. Selecting and maintaining a diverse T-cell repertoire. *Nature*. 1999;402(6759):255–262.

49. Kosmrlj A, Jha AK, Huseby ES, Kardar M, Chakraborty AK. How the thymus designs antigen-specific and self-tolerant T cell receptor sequences. *Proc Natl Acad Sci U S A*. 2008;105(43):16671–16676.
50. Kappler JW, Roehm N, Marrack P. T cell tolerance by clonal elimination in the thymus. *Cell*. 1987;49(2):273–280.
51. Marrack P, Scott-Browne JP, Dai S, Gapin L, Kappler JW. Evolutionarily conserved amino acids that control TCR-MHC interaction. *Annu Rev Immunol*. 2008;26:171–203.
52. Garcia KC. Reconciling views on T cell receptor germline bias for MHC. *Trends in Immunology*. 2012;33(9):429–436.
53. Naylor K, Li G, Vallejo AN, et al. The Influence of Age on T Cell Generation and TCR Diversity. *J Immunol*. 2005;174(11):7446–7452.
54. Boyd SD, Liu Y, Wang C, Martin V, Dunn-Walters DK. Human lymphocyte repertoires in ageing. *Current Opinion in Immunology*. 2013;25(4):511–515.
55. Meyer-Olson D, Shoukry NH, Brady KW, et al. Limited T Cell Receptor Diversity of HCV-specific T Cell Responses Is Associated with CTL Escape. *Journal of Experimental Medicine*. 2004;200(3):307–319.
56. Qi Q, Liu Y, Cheng Y, et al. Diversity and clonal selection in the human T-cell repertoire. *Proceedings of the National Academy of Sciences*. 2014;111(36):13139–13144.
57. Arstila TP. A Direct Estimate of the Human T Cell Receptor Diversity. *Science*. 1999;286(5441):958–961.
58. Simpson EH. Measurement of Diversity. *Nature*. 1949;163(4148):688–688.
59. Hill MO. Diversity and Evenness: A Unifying Notation and Its Consequences. *Ecology*. 1973;54(2):427–432.
60. Krishna C, Chowell D, Gönen M, Elhanati Y, Chan TA. Genetic and environmental determinants of human TCR repertoire diversity. *Immun Ageing*. 2020;17(1):26.
61. Cole DK, Miles KM, Madura F, et al. T-cell Receptor (TCR)-Peptide Specificity Overrides Affinity-enhancing TCR-Major Histocompatibility Complex Interactions. *Journal of Biological Chemistry*. 2014;289(2):628–638.
62. Neeffjes J, Jongsma MLM, Paul P, Bakke O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol*. 2011;11(12):823–836.
63. Ben-Nissan G, Sharon M. Regulating the 20S Proteasome Ubiquitin-Independent Degradation Pathway. *Biomolecules*. 2014;4(3):862–884.
64. Yewdell JW, Reits E, Neeffjes J. Making sense of mass destruction: quantitating MHC class I antigen presentation. *Nat Rev Immunol*. 2003;3(12):952–961.
65. Sijts EJ a. M, Kloetzel PM. The role of the proteasome in the generation of MHC class I ligands and immune responses. *Cell Mol Life Sci*. 2011;68(9):1491–1502.
66. Aki M, Shimbara N, Takashina M, et al. Interferon-gamma induces different subunit organizations and functional diversity of proteasomes. *J Biochem*. 1994;115(2):257–269.
67. Hallermalm K, Seki K, Wei C, et al. Tumor necrosis factor- α induces coordinated changes in major histocompatibility class I presentation pathway, resulting in increased stability of class I complexes at the cell surface. *Blood*. 2001;98(4):1108–1115.
68. Glickman MH, Ciechanover A. The Ubiquitin-Proteasome Proteolytic Pathway: Destruction for the Sake of Construction. *Physiological Reviews*. 2002;82(2):373–428.
69. Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. *Nature*. 2003;426(6968):895–899.
70. Yewdell JW, Schubert U, Bennink JR. At the crossroads of cell biology and immunology: DRiPs and other sources of peptide ligands for MHC class I molecules. *J Cell Sci*. 2001;114(Pt 5):845–851.
71. Leone P, Shin E-C, Perosa F, et al. MHC Class I Antigen Processing and Presenting Machinery: Organization, Function, and Defects in Tumor Cells. *JNCI Journal of the National Cancer Institute*. 2013;105(16):1172–1187.
72. Kammerl IE, Meiners S. Proteasome function shapes innate and adaptive immune responses. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2016;311(2):L328–L336.
73. Toes RE, Nussbaum AK, Degermann S, et al. Discrete cleavage motifs of constitutive and immunoproteasomes revealed by quantitative analysis of cleavage products. *J Exp Med*. 2001;194(1):1–12.
74. Sijts AJ, Ruppert T, Rehermann B, et al. Efficient generation of a hepatitis B virus cytotoxic T lymphocyte epitope requires the structural features of immunoproteasomes. *J Exp Med*. 2000;191(3):503–514.
75. Kincaid EZ, Che JW, York I, et al. Mice completely lacking immunoproteasomes show major changes in antigen presentation. *Nat Immunol*. 2011;13(2):129–135.
76. Trowsdale J, Hanson I, Mockridge I, et al. Sequences encoded in the class II region of the MHC related to the “ABC” superfamily of transporters. *Nature*. 1990;348(6303):741–744.
77. Deverson EV, Gow IR, Coadwell WJ, et al. MHC class II region encoding proteins related to the muKidrug resistance family of transmembrane transporters. *Nature*. 1990;348(6303):738–741.
78. Diedrich G, Bangia N, Pan M, Cresswell P. A role for calnexin in the assembly of the MHC class I loading complex in the endoplasmic reticulum. *J Immunol*. 2001;166(3):1703–1709.

79. Morrice NA, Powis SJ. A role for the thiol-dependent reductase ERp57 in the assembly of MHC class I molecules. *Curr Biol*. 1998;8(12):713–716.
80. Sadasivan B, Lehner PJ, Ortmann B, Spies T, Cresswell P. Roles for Calreticulin and a Novel Glycoprotein, Tapasin, in the Interaction of MHC Class I Molecules with TAP. *Immunity*. 1996;5(2):103–114.
81. Degen E, Cohen-Doyle MF, Williams DB. Efficient dissociation of the p88 chaperone from major histocompatibility complex class I molecules requires both beta 2-microglobulin and peptide. *J Exp Med*. 1992;175(6):1653–1661.
82. Williams AP, Peh CA, Purcell AW, McCluskey J, Elliott T. Optimization of the MHC class I peptide cargo is dependent on tapasin. *Immunity*. 2002;16(4):509–520.
83. Cresswell P. Invariant Chain Structure and MHC Class II Function. *Cell*. 1996;84(4):505–507.
84. Dugast M, Toussaint H, Dousset C, Benaroch P. AP2 clathrin adaptor complex, but not AP1, controls the access of the major histocompatibility complex (MHC) class II to endosomes. *J Biol Chem*. 2005;280(20):19656–19664.
85. McCormick PJ, Martina JA, Bonifacino JS. Involvement of clathrin and AP-2 in the trafficking of MHC class II molecules to antigen-processing compartments. *Proc Natl Acad Sci U S A*. 2005;102(22):7910–7915.
86. Castellino F, Germain RN. Extensive trafficking of MHC class II-invariant chain complexes in the endocytic pathway and appearance of peptide-loaded class II in multiple compartments. *Immunity*. 1995;2(1):73–88.
87. Busch R, Rinderknecht CH, Roh S, et al. Achieving stability through editing and chaperoning: regulation of MHC class II peptide binding and expression. *Immunol Rev*. 2005;207:242–260.
88. Houde M, Bertholet S, Gagnon E, et al. Phagosomes are competent organelles for antigen cross-presentation. *Nature*. 2003;425(6956):402–406.
89. Guermonprez P, Saveanu L, Kleijmeer M, et al. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature*. 2003;425(6956):397–402.
90. Levin R, Grinstein S, Canton J. The life cycle of phagosomes: formation, maturation, and resolution. *Immunol Rev*. 2016;273(1):156–179.
91. Blander JM, Medzhitov R. Toll-dependent selection of microbial antigens for presentation by dendritic cells. *Nature*. 2006;440(7085):808–812.
92. Mantegazza AR, Guttentag SH, El-Benna J, et al. Adaptor protein-3 in dendritic cells facilitates phagosomal toll-like receptor signaling and antigen presentation to CD4(+) T cells. *Immunity*. 2012;36(5):782–794.
93. Harlé G, Kowalski C, Dubrot J, et al. Macroautophagy in lymphatic endothelial cells inhibits T cell-mediated autoimmunity. *J Exp Med*. 2021;218(6):e20201776.
94. van den Elsen PJ. Expression Regulation of Major Histocompatibility Complex Class I and Class II Encoding Genes. *Front Immun*. 2011;2:.
95. Gobin SJ, Keijsers V, van Zutphen M, van den Elsen PJ. The role of enhancer A in the locus-specific transactivation of classical and nonclassical HLA class I genes by nuclear factor kappa B. *J Immunol*. 1998;161(5):2276–2283.
96. Perkins ND, Edwards NL, Duckett CS, et al. A cooperative interaction between NF-kappa B and Sp1 is required for HIV-1 enhancer activation. *EMBO J*. 1993;12(9):3551–3558.
97. Meissner TB, Li A, Biswas A, et al. NLR family member NLRC5 is a transcriptional regulator of MHC class I genes. *Proceedings of the National Academy of Sciences*. 2010;107(31):13794–13799.
98. Masternak K, Barras E, Zufferey M, et al. A gene encoding a novel RFX-associated transactivator is mutated in the majority of MHC class II deficiency patients. *Nat Genet*. 1998;20(3):273–277.
99. Moreno CS, Emery P, West JE, et al. Purified X2 binding protein (X2BP) cooperatively binds the class II MHC X box region in the presence of purified RFX, the X box factor deficient in the bare lymphocyte syndrome. *J Immunol*. 1995;155(9):4313–4321.
100. Jongsma MLM, Guarda G, Spaapen RM. The regulatory network behind MHC class I expression. *Molecular Immunology*. 2019;113:16–21.
101. Ludigs K, Seguí-Estévez Q, Lemeille S, et al. NLRC5 exclusively transactivates MHC class I and related genes through a distinctive SXY module. *PLoS Genet*. 2015;11(3):e1005088.
102. Silver ML, Guo H-C, Strominger JL, Wiley DC. Atomic structure of a human MHC molecule presenting an influenza virus peptide. *Nature*. 1992;360(6402):367–369.
103. Stewart-Jones GBE, McMichael AJ, Bell JI, Stuart DI, Jones EY. A structural basis for immunodominant human T cell receptor recognition. *Nat Immunol*. 2003;4(7):657–663.
104. Chowell D, Krishna S, Becker PD, et al. TCR contact residue hydrophobicity is a hallmark of immunogenic CD8+ T cell epitopes. *Proc Natl Acad Sci U S A*. 2015;112(14):E1754–1762.
105. van der Merwe PA, Dushek O. Mechanisms for T cell receptor triggering. *Nat Rev Immunol*. 2011;11(1):47–55.
106. Moutaftsi M, Peters B, Pasquetto V, et al. A consensus epitope prediction approach identifies the breadth of murine TCD8+ cell responses to vaccinia virus. *Nat Biotechnol*. 2006;24(7):817–819.

107. Karosiene E, Rasmussen M, Blicher T, et al. NetMHCIIpan-3.0, a common pan-specific MHC class II prediction method including all three human MHC class II isotypes, HLA-DR, HLA-DP and HLA-DQ. *Immunogenetics*. 2013;65(10):711–724.
108. Tenzer S, Peters B, Bulik S, et al. Modeling the MHC class I pathway by combining predictions of proteasomal cleavage, TAP transport and MHC class I binding. *CMLS, Cell. Mol. Life Sci.* 2005;62(9):1025–1037.
109. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. *Journal of Molecular Biology*. 1982;157(1):105–132.
110. Zimmerman JM, Eliezer N, Simha R. The characterization of amino acid sequences in proteins by statistical methods. *Journal of Theoretical Biology*. 1968;21(2):170–201.
111. Yari M, Eslami M, Ghoshoon MB, Nezafat N, Ghasemi Y. Decreasing the immunogenicity of *Erwinia chrysanthemi* asparaginase via protein engineering: computational approach. *Mol Biol Rep*. 2019;46(5):4751–4761.
112. Faber MS, Whitehead TA. Data-driven engineering of protein therapeutics. *Curr Opin Biotechnol*. 2019;60:104–110.
113. Ponda P, Hirsch D. Antigen-based immunotherapy for autoimmune disease: current status. *ITT*. 2014;1.
114. Wherrett DK, Bundy B, Becker DJ, et al. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. *Lancet*. 2011;378(9788):319–327.
115. Fridkis-Hareli M, Rosloniec EF, Fugger L, Strominger JL. Synthetic peptides that inhibit binding of the collagen type II 261–273 epitope to rheumatoid arthritis-associated HLA-DR1 and -DR4 molecules and collagen-specific T-cell responses. *Human Immunology*. 2000;61(7):640–650.
116. Moutsianas L, Jostins L, Beecham AH, et al. Class II HLA interactions modulate genetic risk for multiple sclerosis. *Nat Genet*. 2015;47(10):1107–1113.
117. Ji N, Somanaboeina A, Dixit A, et al. Small molecule inhibitor of antigen binding and presentation by HLA-DR2b as a therapeutic strategy for the treatment of multiple sclerosis. *J Immunol*. 2013;191(10):5074–5084.
118. Huynh M, Eggenhuizen PJ, Olson GL, et al. HLA-DR15-specific inhibition attenuates autoreactivity to the Goodpasture antigen. *J Autoimmun*. 2019;103:102276.
119. Smith KJ, Pyrdol J, Gauthier L, Wiley DC, Wucherpfennig KW. Crystal Structure of HLA-DR2 (DRA*0101, DRB1*1501) Complexed with a Peptide from Human Myelin Basic Protein. *Journal of Experimental Medicine*. 1998;188(8):1511–1520.