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FCGR3A F158V alleles frequency differs in multiple myeloma patients from healthy population

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ABSTRACT

FCGR3A presents a single nucleotide polymorphism at location 158 (V/F), which affects its binding to the fragment crystallizable (Fc) of antibodies (Abs). FcγRIIIa-158 V allotype has the highest affinity and is associated with a better clinical response to IgG1 monoclonal Abs (mAb) treatment. We compared the allele frequency of *FCGR3A*-F158V polymorphism in cohorts of patients with B-cell lymphoproliferative disorders, including multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS), non-Hodgkin lymphoma (NHL), and B-cell chronic leukemia (B-CLL). *FCGR3A*-158F homozygous were enriched and tended to be in MM and MGUS patients, respectively; but neither in B-CLL nor in NHL patients. We identified a significantly lower concentration of CD8 T-cells and resting memory CD4 T-cells in MM patients bone marrow with the F/F genotype, associated with an increase in the macrophage percentage. In contrast, natural killer cells increased in V/V homozygous patients. This suggests a deregulation of the immune microenvironment in *FCGR3A*-F/F homozygous patients. However, we did not observe difference in response following treatment combining chemotherapy associated or not with daratumumab, an IgG1 mAb direct against CD38. Our findings suggest that *FCGR3A* F158V polymorphism can regulate the immune environment and affect the development of tumor plasma cells.

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Introduction

The low affinity receptor for immunoglobulin (Ig) Fc, FcγRIIIa/CD16a, is mainly expressed by mast cells, macrophages and natural killer (NK) cells, allowing these cells to recognize targets opsonized by IgG.[1] Its best described role is in NK cells- and macrophages-mediated antibody-dependent cell-mediated cytotoxicity.[1] This receptor is encoded by FCGR3A, a gene located on chromosome 1 and presenting few variants, such as the F158V functional polymorphism,[2–6] which due to a single nucleotide change results in nucleotidic triplets coding for a phenylalanine (F) or a Valine (V). The FcγRIIIa 158 V variant binds the Fc from IgG1 and IgG2 with better affinity than the 158F variant,[7–9] This polymorphism is known to have an impact on clinical response to mAb treatment. For example, non-Hodgkin lymphoma (NHL) patients homozygous for the high affinity allele (V) are better responders to anti-CD20 mAb such as rituximab.[3–10] Similar results were also found in systemic lupus erythematosus and in rheumatoid arthritis.[6,11] This is also the case for solid cancer patients treated with cetuximab, trastuzumab[12,13] and anti-CTLA-4 mAb;[14] although for cetuximab observations have shown contradictory results.[2,15,16] Finally, V homozygous patients show worse transplant survival in allografted renal patients than patients bearing the F allele. This could be related to an increased interaction between FcγRIIIa 158 V and endogenous antibodies directed to the graft.[17] The allelic frequency has been described in several studies obtaining values between 35 to 39% of F/F donors[4,5,17] and a review study[13] combined results from 21 studies and 6961 subjects found frequencies of about 43% F/F, 45% V/F and 12% V/V. Of note, different human populations can also present different frequency distribution.[18] This polymorphism has been associated to the severity of Guillain-Barré syndrome with an increase of the F/F allotype,[19] which also increases in bullous pemphigoid (BP) disease.[20]

We evaluate here the frequency of this polymorphism in several B-cell lymphoproliferative disorders. We found that FCGR3A F/F proportion increases in MM patients and tended to increase in MGUS, which is often described as a pre-myeloma state.[21,22] We hypothesize that expression of two low affinity FCGR3A 158F alleles is a risk factor regarding MM.

Materials/subjects and methods

Ethical statement

Samples were obtained from patients enrolled in the HEMODIAG_2020 (ID-RCB: 2013-A00260-45) clinical program approved by the "Comité de Protection des Personnes

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Sud Méditerrannée I" with the reference 1324. Samples were collected at diagnosis or relapse Written informed consent was obtained from each patient following French regulation. Umbilical cord blood units (UCBs) were provided by the Biological Resource Center of University Hospital of Montpellier (E Tuaillon, J de Vos, BIOBANQUES Identifier – BB-0033–00031).

Patients

We analyzed the F158V genotype from 121 donors of our regional UCB bank, 46 NHL or B cell chronic leukemia (B-CLL) patients, 224 MM patients, and 77 donors presenting the MGUS condition. Baseline demographics and clinical characteristics related to the MM patients can be found in Table 1.

MM patients were treated with the standard CHU Montpellier treatment for the moment they were diagnosed. These include i) VTD : (Velcade/thalidomide/dexametasone) + Melphalan + maintenance revlimide (lénalidomide); ii) VRD : (Velcade/revlimide/dexametasone; iii) MPV : (Melphalan + Pred + Velcade); iv) Rev Dex (Revlimide + dexametasone); v) autologous graft + consolidation VRD + maintenance revlimide. For those treated with daratumumab, they were also treated with several cotreatments such as dexametasone or revlimide or pomalidomide or velcade.

PCLI was analyzed as previously reported.[24] Briefly, whole bone marrow was incubated with or without BrdU (10 µM), (Flow Kit APC,Biosciences, San Jose, CA, USA) at 37°C for 2 h. After red blood cell lysis, leukocytes were stained with anti-CD19, anti-CD20, anti-CD27, anti-CD38, anti-CD56, anti-CD200, and anti-CD45 mAbs, for 20 min, washed twice in PBS/10% goat serum, fixed and permeabilized (BrdU Flow Kit from BD Biosciences, BDBiosciences). The cells were incubated for 1 h at 37°C in PBS containing DNAse I (300 µg/mL) and stained with anti-Kappa light chain, anti-Lambda light chain, and anti-BrdU-APC mAb (BrdUFlow Kit from BD Biosciences) for 20 min. Nuclei were stained in PermWash containing DAPI (2 µg/mL) (4',6 Diamido-2-phenylindole, dilactate from Invitrogen, Life Technologies) for 20 min. Singlets were plotted on FSC-A versus FSC-H and SSC-A versus SSC-H plots to remove debris and to select the total leukocyte population. PC and B cells were selected on CD45/CD38 and CD20/CD19 plots, respectively. Abnormal PC were selected based on the CD19, CD27, CD56, CD117, and CD200 signals and PCLI analyzed as previously described.[24,25] Five million events were acquired per tube and the minimum number of abnormal plasma cells needed was 20. The maximum sensitivity of the method is 0.0004%. The analyses were done using FlowJo software (FlowJo, LLC).

The MM RNA-seq based risk score was computed as previously described[26] using 267 genes. The RNA-seq-based risk score is defined by the sum of the beta coefficient derived from the Cox model for each prognostic gene weighted by – 1 or + 1 according to the MMC gene expression above or below the Maxstat defined cutpoint.[26] Affymetrix U133P chips were also used, as previously described,[27] to analyze GEP and to calculate previously published UAMS HRS risk score[28] as previously described.[26]

Table 1. Baseline demographics and clinical characteristics related to MM patients.

Characteristics	N = 224
Age at diagnosis, yr, %	
median, years	62 years
<65 years, %	59%
Sexe, %	
Male	51%
ISS disease stage at diagnosis, %	
I	37%
II	32%
III	31%
Newly diagnosed patients, %	89.8%
Patients at relapse, %	10.2%
Plasma cell leukemia at diagnosis, %	3.5%
Bone lesions at diagnosis, %	63%
Anaemia at diagnosis (Hb <10 g/dl), %	23%
Hypercalcemia at diagnosis (calcemia ≥2,6 mmol/L), %	20.5%
Type of multiple myeloma, %	
Ig G	55%
Ig A	24%
Light chain myeloma	21%
Molecular classification [23]	
CD1	6%
CD2	20%
HY	24%
LB	13%
MF	4%
MS	14%
PR	19%
Plasma cell labeling index (PCLI) ≥ 1% at diagnosis, %	27%

Gene expression profiling of bone marrow microenvironment

MM cells were purified using anti-CD138 MACS microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) as previously described.[26,29] Purified MM cells from patients and normal bone marrow environment fraction RNA sequencing was done as previously described.[26,29] The RNA sequencing (RNA-seq) library preparation was done with 150 ng of input RNA using the Illumina TruSeq Stranded mRNA Library Prep Kit. Paired-end RNA-seq were performed with Illumina NextSeq sequencing instrument (Helixio, Clermont-Ferrand, France). RNA-seq read pairs were mapped to the reference human GRCh37 genome using the STAR aligner. All statistical analyses were performed with the statistics software R, and R packages developed by Bioconductor project (https://www.bioconductor.org).[30] The expression level of each gene was summarized and normalized using the DESeq2 R/Bioconductor package.[31] CIBERSORTx suite was used to estimate the immune cell type abundance in bulk RNAseq from MM bone marrow samples from MM patients at diagnosis ($N = 110$) with paired RNA-seq data of purified MM cells as described.[32,33] Of note, the pairwise correlation between flow cytometry data and corresponding CIBERSORTx deconvolution cell signature was tested and analyzed in a data set of 255 samples.[34] Seventy-eight per cent of the tested cell type signatures showed significant correlation between the flow cytometry results and CIBERSORTx deconvolution results.

DNA extraction

Five hundred microliters of UCB or patient's blood were collected, and DNA extracted using NucleoSpin®Blood column kit (Macherey-Nagel), following the provider's instructions. DNA concentration and purity were assessed using a spectrophotometer (ThermoFisher scientific, NanoDrop Microvolume Spectrophotometer).

Multiplexed polymerase chain reaction (PCR)

Fifteen nanograms of DNA from each sample were used with PCR Qiagen AllTaq PCR core kit (Qiagen) in a multiplexed PCR assay to identify the *FCGRIIIA* F158V polymorphism (V allele 70bp; F allele 117bp), as detailed in Figure S1 and described previously.[35]

Quantitative PCR (qPCR) Taqman

We used 10 nanograms of DNA (3 µL at 3.33ng/µL) from all UCB donors, 47 MM samples with sufficient material for analysis, and all 77 MGUS samples. TaqMan® SNP Genotyping Assays (SNP ID: rs396991, PN 4,351,379 40×) and qPCR TaqMan MasterMix (Thermo Fisher Scientific) were employed, following the method described by Burchard.[35] Samples were analyzed using a LightCycler® 480 (Roche). The qPCR program included a Pre-PCR at 60°C for 1 min, initial denaturation 92°C for 10 min and 40 PCR cycles (92°C, 15 sec and 60°C, 60 sec) with a temperature change rate of 4.4°C/sec. The genotype end-point analysis mode is described in Figure S2.

Statistical analysis

Statistics were performed using Prism V7.04. Statistical tests are detailed in each figure legend. CIBERSORTx suite was used to estimate the immune cell type abundance in bulk RNAseq from MM bone marrow samples from MM patients ($n = 112$) with paired RNA-seq data of purified MM cells as described.[32,33]

Results

MM patients are more frequently FCGR3A-158F homozygous

We analyzed the F158V genotype of donors from our regional UCB bank by multiplexed polymerase chain reaction (PCR) (Figure S1) and confirmed it by TaqMan quantitative PCR (qPCR) (Figure S2). Allelic frequencies in healthy population were: 40.50% F/F, 44.63% V/F, and 14.88% V/V (Figure S3A), hence 60% of the population bearing a V allele (Figure 1). This distribution closely matched frequencies reported in previous studies.[4,5,13,17]

We next analyzed samples from 46 B-CLL or NHL patients and found a similar repartition as in the healthy cohort (Figure 1 and Figure S3). This cohort contained 37 NHL with a similar repartition. In contrast, the short number of B-CLL patients precluded any interpretation. In a cohort of 77 MGUS patients, we observed an allele frequency that tended ($p = 0.0695$) to diverge from the healthy population with an increase in the F/F genotype (50.65%; Figure 1 and Figure S3).

Finally, we analyzed a cohort of 224 MM patients and found a repartition significantly different compared to the healthy donor, MGUS and NHL/CLL cohorts (Figure 1 and Figure S3). This difference was mainly due to an increased proportion of the F/F homozygous population (57.59%) in the MM cohort (Figure S3). Of note, SNP analysis was performed by multiplexed polymerase chain reaction (PCR) and confirmed by qPCR Taqman and/or RNAseq (Supplemental Figure S1 and S2). All three technologies gave identical results.

Our results agree with those described by Niels et al.[36] that found an increase for F/F (46.9%) and a decrease for V/V (10.4%) populations. However, this study lacks a comparison with a HD population, which precludes any statistical comparison.

FCGR3A genotype is associated with deregulation of bone marrow immune microenvironment

To test if *FCGR3A* genotype was associated with intrinsic characteristics of tumor plasma cells, we tested plasma cells for proliferation index, gene expression profile and RNA seq. We found no significant difference in plasma cell labeling index (PCLI – percentage of MM cells in S phase)[24] between F/F and F/V-V/V MM patients (Figure S4A). No difference in the percentage of high-risk patients based on GEP-based UAMS HRS score[28] or RNA-seq based risk score[26] was found between F/F and F/V-V/V MM patients (Figures S4B, C).

We then used RNA-seq data of the non-tumor bone marrow fraction from our patients when available (110 patients (all samples collected at diagnosis) out of the 224 investigated), to explore the correlation between *FCGR3A* genotype and the marrow immune cell subpopulations. In order to assess the association between FCGR3A genotype and the composition of the tumor microenvironment, CIBERSORTx was used to deconvolute the intratumor immune cell composition using bulk RNA-seq data. CIBERSORTx[32] can leverage single cell RNAseq derived reference profiles of 22 different immune cell subpopulations to bulk bone marrow tissue immune cell dissection[32,33] (Supplementary Table S1). Using the CIBERSORTx suite, we estimated the immune cell type frequency in bulk RNA-seq from MM bone marrow samples (Figure 2). Of interest, we identified a significant lower concentration of CD8 T-cells and resting memory CD4 T-cells in the bone marrow of MM patients with the F/F genotype together with an increased percentage of macrophages M0. Resting M0 macrophages are derived from the bone marrow and are considered as precursors of polarized macrophages.[37] We identified a significantly lower percentage of M0 macrophages within the bone marrow of MM patients with F/V or V/V genotype. Regarding NK-cells, we did not find statistical differences (Figure 2); however, we observed an increased concentration of this lymphocyte lineage in patients with the V/V genotype compared to the other two genotypes (Figure 2). Altogether, these data suggest a significant deregulation of immune microenvironment in *FCGR3A*-FF homozygous patients.

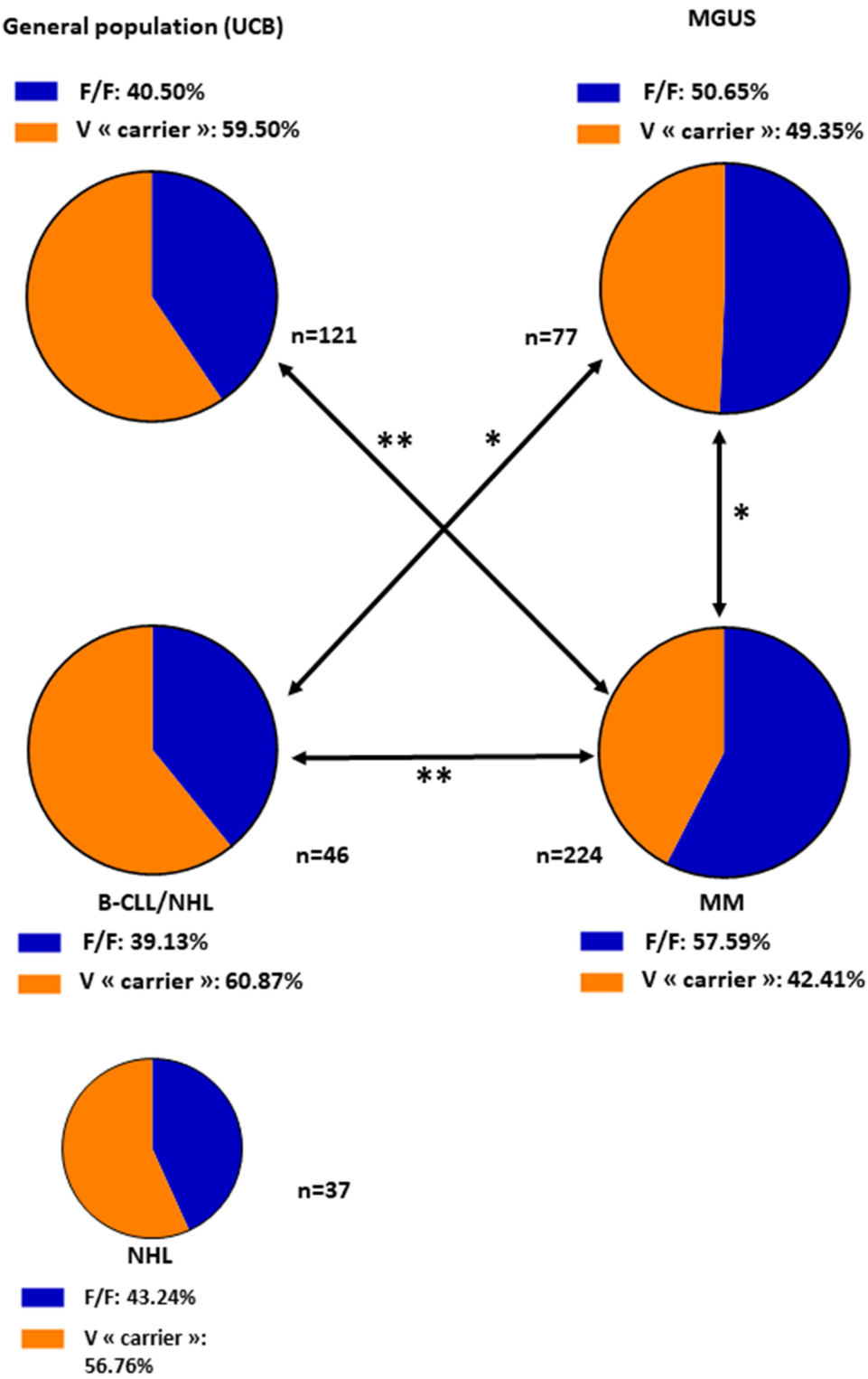

Figure 1. FcγRIIIA F158V genotype repartition on the different cohorts. Graphics depict the percentage of the different FCγRIIIA genotypes and the number of donors in each group. Statistical analysis was performed using the Chi^2 test and comparing the different cohorts; *$p < 0.05$; **$p < 0.0001$.

FCGR3A F158V genotype does not impact progression free survival (PFS) or overall survival (OS) of MM patients

As previously described, the *FCGR3A* F158V polymorphism impacts the response to mAb treatment in several onco-hematological diseases.[3–10] We investigated the link between *FCGR3A* genotype and the outcome of MM patients including patients at diagnosis treated by high dose therapy (HDT) followed by autologous hematopoietic stem cell transplantation (ASCT, $n = 76$) and patients at relapse treated by the anti-CD38 mAb daratumumab ($n = 30$).[38] MM patients were separated in V "carrier" or non-V "carrier" (Figure 3) or in three groups according to their genotypes (Figure S4). No significant

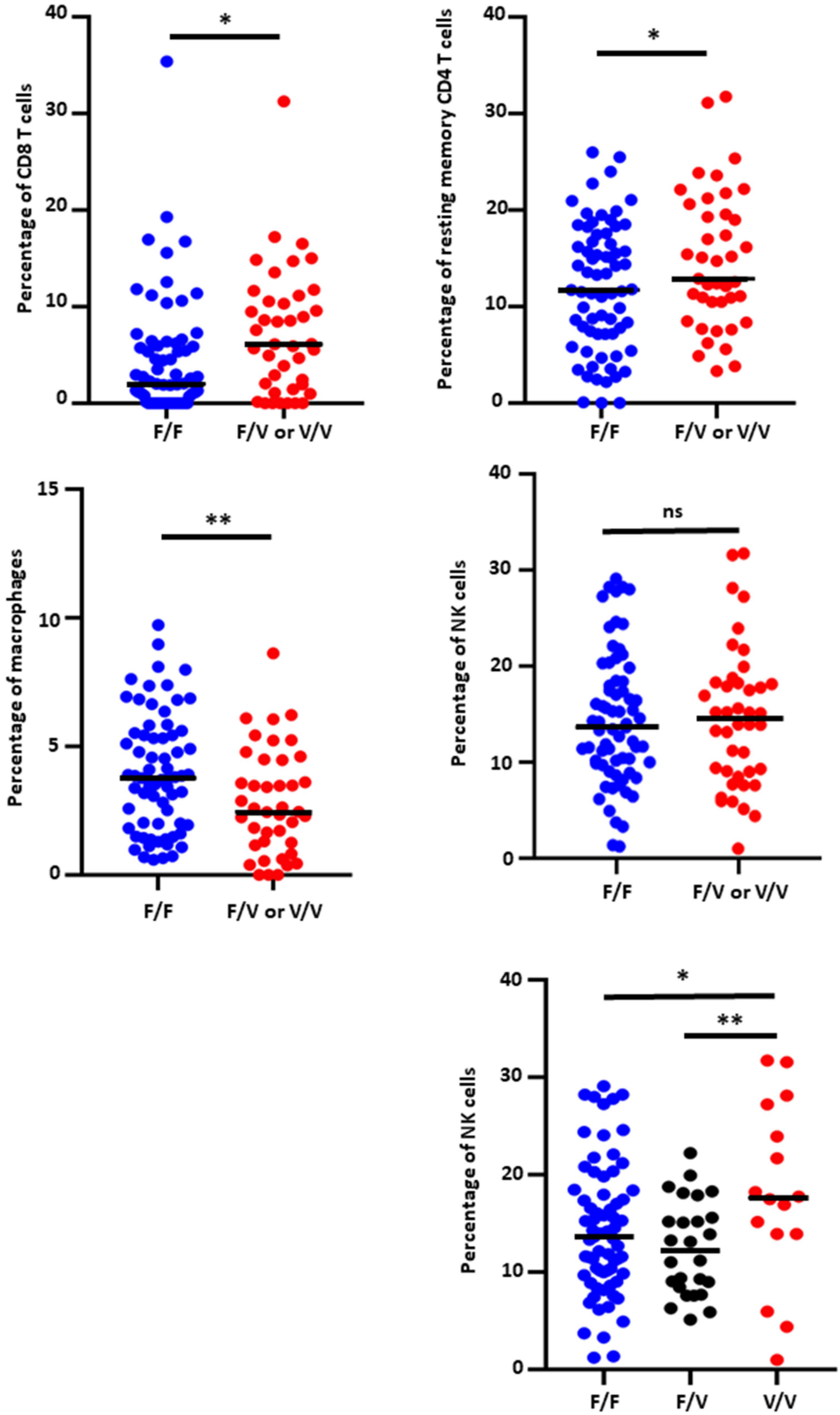

Figure 2. Impact of FCγRIIIA F158V genotype on bone marrow in immune cell populations. Using CIBERSORTx suite, we estimated the immune cell type abundance in bulk RNAseq from the non-tumor bone marrow samples from multiple myeloma (MM) patients ($N = 110$) at diagnosis. Patients were stratified accordingly to their genotype and the percentage of the different immune cell populations was depicted in the graphs. Statistical analysis was performed using Student t-test; *$p < 0.05$; **$p < 0.01$.

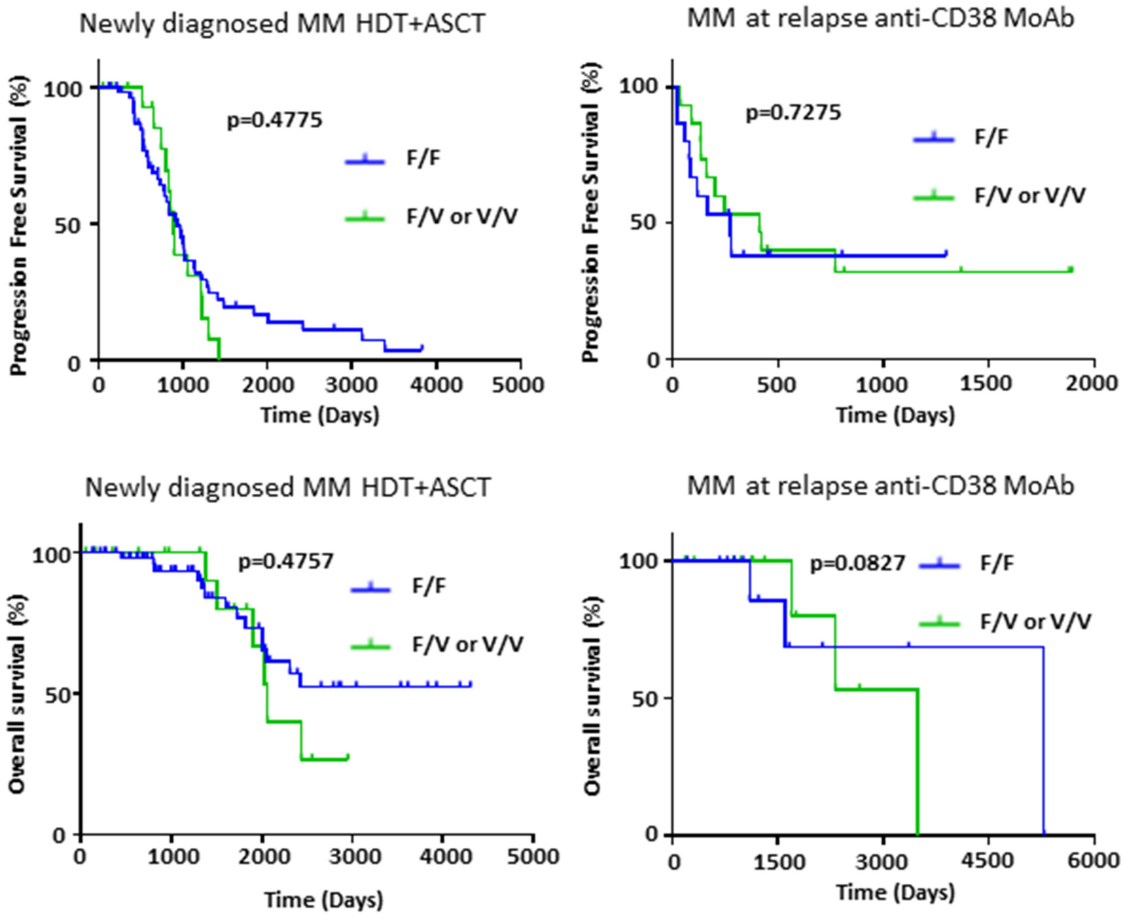

Figure 3. Impact of FCγRIIIA F158V genotype on progression-free survival (PFS) and overall survival (OS) after chemotherapy regimens or after daratumumab. Graphs depict the outcome of MM patients stratified by their FCγRIIIA genotype, who were at diagnosis treated by high dose therapy (HDT) and autologous hematopoietic stem cell transplantation (ASCT) ($n = 76$) or at relapse treated by the anti-CD38 mAb daratumumab ($n = 30$). Graphs show the Kaplan-Meier curves of PFS and OS. Statistical differences were analyzed using the Mantel-Cox test.

association between *FCGR3A* genotype and clinical outcome, *i.e.* progression-free survival (PFS) and overall survival (OS), could be identified in newly diagnosed patients treated using first line treatment regimen comprising HDT followed by ASCT or patients at relapse treated with daratumumab. However, non-V "carriers" tended to better response to treatment. Of note, a previous work shows that F/F patients better respond to daratumumab than other groups.[36] Hence, taken all results together show that V "carriers" do not exhibit better response to different treatments, including daratumumab.

Discussion

We found that the *FCGR3A* 158 F/F genotype is more frequent in MM patients and tended to be in the MGUS cohort. Hence, our findings suggest that having two FCGRIIIA low affinity F alleles could increase the risk to develop MGUS and, later on, MM. It is possible that the MGUS patients with a F/F genotype will evolve more frequently toward an MM, thereby specifically decreasing the proportion of MGUS patients with this genotype. This could be an interesting matter for future clinical studies. But, at the same time, our MGUS cohort was small ($n = 77$), which probably precluded our results to reach statistical signification. It is uncertain why F/F genotype is enriched in MM patients. We hypothesize that, as previously reported in renal graft context,[39] this could be linked to the interaction between immune cell effectors and endogen antibodies recognizing tumor-associated antigens expressed by plasma cells, thereby rendering immunosurveillance more efficient in V "carriers." Interestingly we did not find the same enrichment in B-CLL and NHL malignancies, hence the tumor microenvironment could play a role in tumor progression. We estimated the marrow immune cells frequency in MM patients by RNA-seq data[32] and identified a significant higher concentrations of NK cells in homozygous V/V MM patients compared to F/F or V/F patients. We also observed lower CD8 T cells and resting memory CD4 T cells and higher macrophages in homozygous F/F MM patients compared to the homozygous V/V patients. Hence, F homozygosis could lead to MM progression through deregulation of bone marrow immune microenvironment. Quantitative and functional immune changes during myelomagenesis have been reported,[40] and functional studies have demonstrated that NK cells appear functional in MGUS patients but lose cytotoxicity during disease progression.[41,42] Interestingly, FCER1G, a gene coding for

Fc epsilon1gamma, a subunit of FcεRI, which is another crystallizable fragment (Fc) receptor, has been linked to MM patient survival.[43] In particular, patients expressing higher FCER1G levels show improved overall survival.[43] All this suggests that Fc receptors can affect tumor initiation and tumor progression by modulating immune cell recruitment in the tumor microenvironment.

In contrast to patients treated with mAb in B malignancies,[3–5,10] or solid tumors,[2,13,14,17] we found that *FCGR3A* F158V polymorphism does not impact the response to daratumumab treatment in MM patients. We although observed a tendency for V "carriers" to respond worse to treatment, which is in agreement with other groups.[36] Perhaps if our cohort were larger, we would have observed statistical differences between the different groups. Several possibilities could explain the impaired response of V "carriers." First, our patients have been treated with a number of therapies before daratumumab, which could largely affect the immune cells in the tumor environment, e.g. FCGRIIIA expressing cells. Second, daratumumab induces NK-cell fratricide reducing NK cell numbers in MM patients, although the higher CD38 expression in MM cells than in NK cells should favor MM cell, mAb mediated, elimination.[44] In any case, it would be clinically relevant to investigate how *FCGR3A158VF* polymorphism impacts MM patient outcome especially for those treated with mAb targeting antigens absent on NK cells, as BCMA. Moreover, the increase of CD8 T cells and memory CD4 T cells in the bone marrow of MM patients with a FCGR3A V/F or V/V genotype could improve their response to bispecific antibodies targeting a MM expressing antigen, e. g., BCMA, and a T cell antigen such as CD3. At least two anti-BCMA x anti-CD3 bispecific antibodies are currently being tested for MM treatment, i.e. linvoseltamab (NCT05137054) and elranatamab (NCT04649359). It would be interesting to investigate whether patients carrying the FCGR3A V/F or V/V genotype respond better to these mAbs due to the increase in T cell populations. Unfortunately, deconvolution analysis didn't allow to investigate activation or exhaustion phenotypes of T cells.

During tumor progression, cancer cells are immune sculpted.[45] Hence, it is possible that the tumor cells that have already escaped to the NK cell immune surveillance are not any more their targets[46] explaining the lack of *FCGR3A* F158V polymorphism impact during progression or after treatment. Moreover, NK cells are dysfunctional in MM patients,[41,42,47] and they cannot therefore any longer mediate a proper anti-tumor response. In summary, the reason why this polymorphism plays a role in MM incidence and not in tumor progression or in the response to treatment could be due to multiple factors and/or a combination of them.

To conclude, the *FCGR3A* F158V polymorphism can impact the development of MM by modulating the immune cells in the tumor microenvironment.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Data-sharing statement

Data are available on demand to the corresponding author.

Author contributions

M.V., G.C., and J.M. supervised the project and obtained funding to realize it. M.V., J.M., M.C., and N.R. designed experiments. M.C., N.R., L.C., C.M., M.C.M., C.J., F.G., S.Z., and J.P. collected samples, performed experiments, and collected data. M.C. and J.M. analyzed data and performed statistical analysis. M.V., J.M., and M.C. interpreted data. M.C., J.M., and M.V. wrote the manuscript. G.C. and J.M. gave access to patient's samples. J.M. performed patient follow-up analysis. All authors have read and agreed with the manuscript.

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