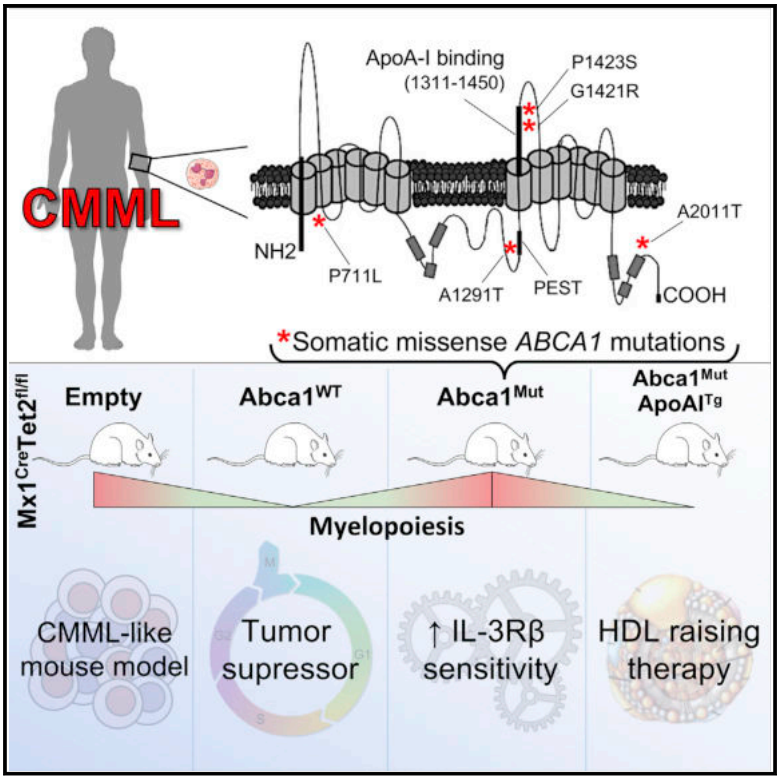
Article

Cell Reports

ABCA1 Exerts Tumor-Suppressor Function in Myeloproliferative Neoplasms

Graphical Abstract



Highlights

- *ABCA1* somatic mutations were identified in CMML patients
- *ABCA1* mutations fail to repress myeloproliferative neoplasms in *Tet2*-deficient mice
- *ABCA1* mutations sustain IL-3Rβ signaling-driven myelopoiesis in Tet2-deficient HSPCs
- Overexpression of apoA-1 overcomes *ABCA1*/*TET2* co-mutant myeloproliferative neoplasms

Authors

Manon Viaud, Omar Abdel-Wahab, Julie Gall, ..., Ross L. Levine, Alan R. Tall, Laurent Yvan-Charvet

Correspondence

yvancharvet@unice.fr

In Brief

Viaud et al. show that *ABCA1* mutants identified in CMML patients diminish the tumor-suppressor functions of *ABCA1* and cooperate with Tet2 loss to confer the hypersensitivity of myeloid progenitors to IL-3 receptor β canonical signaling, which can be prevented by raising HDL levels.

Viaud et al., 2020, Cell Reports *30*, 3397–3410
March 10, 2020
https://doi.org/10.1016/j.celrep.2020.02.056

CellPress

Cell Reports
Article

ABCA1 Exerts Tumor-Suppressor Function in Myeloproliferative Neoplasms

Manon Viaud,[1,4] Omar Abdel-Wahab,[2,4] Julie Gall,[1,4] Stoyan Ivanov,[1] Rodolphe Guinamard,[1] Sophie Sore,[1] Johanna Merlin,[1] Marion Ayrault,[1] Emma Guilbaud,[1] Arnaud Jacquel,[1] Patrick Auberger,[1] Nan Wang,[3] Ross L. Levine,[2] Alan R. Tall,[3] and Laurent Yvan-Charvet[1,5,*]

[1]Institut National de la Santé et de la Recherche Médicale (INSERM) U1065, Université Côte d'Azur, Centre Méditerranéen de Médecine Moléculaire (C3M), Atip-Avenir, Fédération Hospitalo-Universitaire (FHU) Oncoage, 06204 Nice, France
[2]Human Oncology and Pathogenesis Program and Leukemia Service, Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA
[3]Division of Molecular Medicine, Department of Medicine, Columbia University, New York, NY 10032, USA
[4]These authors contributed equally
[5]Lead Contact
*Correspondence: yvancharvet@unice.fr
https://doi.org/10.1016/j.celrep.2020.02.056

SUMMARY

Defective cholesterol efflux pathways in mice promote the expansion of hematopoietic stem and progenitor cells and a bias toward the myeloid lineage, as observed in chronic myelomonocytic leukemia (CMML). Here, we identify 5 somatic missense mutations in *ABCA1* in 26 patients with CMML. These mutations confer a proliferative advantage to monocytic leukemia cell lines *in vitro*. *In vivo* inactivation of *ABCA1* or expression of *ABCA1* mutants in hematopoietic cells in the setting of Tet2 loss demonstrates a myelosuppressive function of *ABCA1*. Mechanistically, *ABCA1* mutations impair the tumor-suppressor functions of WT *ABCA1* in myeloproliferative neoplasms by increasing the IL-3Rβ signaling via MAPK and JAK2 and subsequent metabolic reprogramming. Overexpression of a human apolipoprotein A-1 transgene dampens myeloproliferation. These findings identify somatic mutations in *ABCA1* that subvert its anti-proliferative and cholesterol efflux functions and permit the progression of myeloid neoplasms. Therapeutic increases in HDL bypass these defects and restore normal hematopoiesis.

INTRODUCTION

Most human adult cancers develop through a multistep acquisition of a wide range of somatic mutations that initiate or maintain self-renewal of the malignant clone. The elucidation of the somatic mutational landscape of many solid tumors and hematologic malignancies has occurred in the last decade (Sjöblom et al., 2006; Ley et al., 2013). These mutations are referenced in the Catalog of Somatic Mutations In Cancer (COSMIC) and The Cancer Genome Atlas (TCGA) and may provide potential insights into the mechanisms underlying cancer. In myeloid malignancies, hematopoietic stem and progenitor cells (HSPCs) acquire specific combinations of leukemia disease alleles required to promote hematopoietic transformation (Shih et al., 2015; Kunimoto et al., 2018). Recent studies have shown that mutations in a small number of genes, including loss-of-function mutations in ten-eleven translocation 2 (TET2) are common in the elderly and provide a proliferative advantage to hematopoietic stem cells (HSCs), giving rise to clonal hematopoiesis (Busque et al., 2012). Clonal hematopoiesis mutations are associated with an ~10-fold increase in the risk of developing a hematological malignancy, including myeloproliferative disorders and leukemias and a 2- to 3-fold risk of developing atherosclerotic cardiovascular disease (CVD).

Increased high-density lipoprotein (HDL) levels are well known to be associated with a reduced risk of CVD. A recent meta-analysis of randomized controlled trials of lipid-altering therapies revealed that for every 10-mg/dL increase in the plasma HDL-cholesterol level among trial participants, there was a 36% lower risk of cancer incidence during >625,000 person-years of follow-up and >8,000 incident cancers (Jafri et al., 2010). While not establishing causation, this association suggests that HDL may be linked to tumor cell biology in humans. The ability of HDL and its apolipoproteins to promote the efflux of cholesterol from cells depends in part on the ATP-binding cassette transporters ABCA1 and ABCG1, but it can also be mediated by scavenger receptor B1 and passive efflux pathways (Tall and Yvan-Charvet, 2015). The reduced expression of cholesterol efflux mediators and the increased levels of cellular cholesterol have been associated with different solid tumors; however, it is unclear whether these are secondary changes or have a role in promoting cell proliferation (Bovenga et al., 2015; Clendening and Penn, 2012; Dang, 2012; Lin and Gustafsson, 2015; Mullen et al., 2016). Mice with defective cholesterol efflux in hematopoietic cells develop progressive myeloid expansion with an underlying dramatic HSPC expansion in the bone marrow (BM), an enhanced interleukin-3-granulocyte-macrophage colony-stimulating factor (IL-3-GM-CSF) signaling pathway, and marked extramedullary hematopoiesis (Murphy et al., 2011; Wang et al., 2014; Westerterp et al., 2012; Yvan-Charvet et al., 2010). We also demonstrated that HDL-raising therapies could limit Mpl-W515L and Flt3-ITD-driven myeloproliferative disorders (Gautier et al., 2013).

While these findings suggest a potential role for cholesterol efflux pathways in modulating the development of myeloid

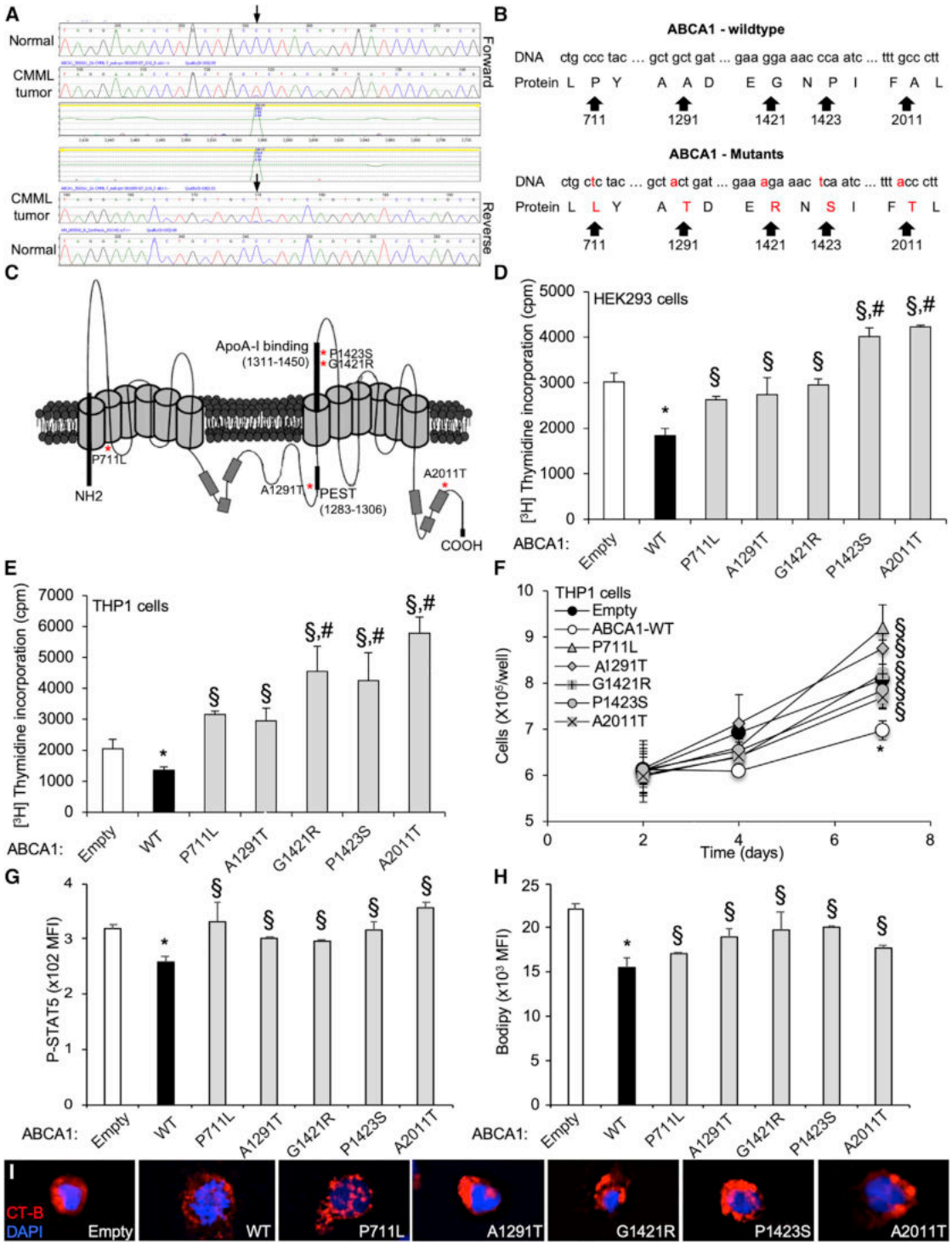


Figure 1. Identification of Loss of Function *ABCA1* Mutants in CMML

Forward (upper trace) and reverse (lower trace) sequence traces of ABCA1 gene demonstrating a heterozygous cytosine-to-thymine substitution (arrows) present in myeloid cell DNA from patients with chronic myelomonocytic leukemia (CMML). The mutation is not present in buccal DNA from the same patient (upper trace).

(A and B) DNA sequence (A) and protein translation (B) for both the wild-type (WT) and mutant *ABCA1* alleles. The mutations result in amino acid substitution at codons 711, 1,291, 1,421, 1,423, and 2,011.

(C) Representative 3D structure of ABCA1 transporter. The asterisks represent localizations of *ABCA1* mutants.

(legend continued on next page)

malignancies and leukemias, they have not established a causative role of specific somatic mutations in cholesterol efflux genes in these disorders. Chronic myelomonocytic leukemia (CMML) is typically a disease of the elderly with few treatment options. Recent studies in CMML patients have shown changes reminiscent of those observed in mice with defective cholesterol efflux in hematopoietic cells, including: (1) frequently mutated tumor-suppressor genes encoding regulators of GM-CSF signaling (RAS, CBL), (2) hypersensitivity of myeloid progenitors to GM-CSF, and (3) a proportion of "classical" $CD14^{+}CD16^{-}$ monocytes >94% (Itzykson et al., 2017). In addition, these patients often have mutations in genes associated with clonal hematopoiesis, including TET2 and ASXL1. In the present study, we have used high-throughput sequencing to identify mutations in *ABCA1* in CMML patient samples. Further studies in a mouse model of myeloproliferative neoplasms driven by hematopoietic Tet2 deficiency have shown that these somatic mutations abrogate the tumor-suppressor function of WT *ABCA1*, resulting in the failure to suppress canonical IL3-receptor β (IL-3Rβ) signaling-driven myelopoiesis. The loss of the myelosuppressive function of *ABCA1* mutants can be overcome by raising HDL levels through the overexpression of the human apolipoprotein A-1 (apoA-1) transgene.

RESULTS

Identification of *ABCA1* Somatic Mutations in CMML

Sequencing of full-length *ABCA1*, *ABCG1*, and *NR1H2/3* (liver X receptors [LXRs]) in 26 CMML samples revealed a somatic mutational frequency of 19% of samples for *ABCA1* (n = 5) and 0% for *ABCG1* and *NR1H2/3*. All of the mutations were somatic missense mutations, with only 1 mutation observed in each patient sample (Figures 1A and S1A; Table S1). The identity of the paired samples was verified by Sequenom single-nucleotide polymorphism (SNP) genotyping, demonstrating that the likelihood of a match occurring by chance was $<1 \times 10^{-13}$ (data not shown). These *ABCA1* mutations occur in evolutionarily conserved regions (Figures 1B and 1C). The *ABCA1* mutations have not been previously described, even though different *ABCA1* mutations have been identified in Tangier disease (Brunham et al., 2006; Sjöblom et al., 2006). Sequencing of other genes implicated in the pathogenesis of CMML in the same samples revealed that *ABCA1* mutations co-existed with known oncogenic mutations in JAK2, Flt3, and N-Ras (Emanuel, 2008). We noted that (1) of the 4 genes sequenced, somatic non-synonymous mutations were found in only 2 of the 4 genes and (2) the somatic nonsynonymous mutation rate for *ABCA1* was higher than the expected background silent mutation rate and higher than expected by chance alone by binomial tests ($p = 3.6 \times 10^{-10}$ for *ABCA1*), suggesting that mutations in *ABCA1* do not represent passenger gene effects.

Functional Analysis of *ABCA1* Mutations *In Vitro*

Given the key role of the ABCA1-dependent cholesterol efflux pathway in controlling myeloid expansion (Tall and Yvan-Charvet, 2015), we sought to test whether *ABCA1* CMML mutations affect cellular proliferation. We used site-directed mutagenesis to introduce each of these 5 somatic mutations individually into the *ABCA1* cDNA. To compare the ability of *ABCA1* mutants to control proliferation, we transiently transfected HEK293 cells with the *ABCA1* cDNAs. Overexpression of wild-type (WT)-*ABCA1* resulted in an ~1.7-fold decrease in cell proliferation compared with empty vector-transfected cells (Figure 1D). All of the mutants located in either the N- and C-terminal regions (P711L and A2011T), the PEST sequence (A1291T), or the apoA-1 binding region (G1421R and P1423S) exhibited a significant reduction in anti-proliferative activity (Figures 1C and 1D). We also tested the relevance of these *ABCA1* mutations in human THP-1 monocytic leukemia cell lines that express endogenous ABCA1 (Figure S1B). *ABCA1-P711L*, *ABCA1-A1291T*, *ABCA1-G1421R*, *ABCA1-P1423S*, and *ABCA1-A2011T* displayed reductions in anti-proliferative activity, compared to *ABCA1*-WT in lentivirus-transduced cells (Figure 1E), which is consistent with observations in HEK293 cells. Although the proliferation rate of THP1 cells was slowed down by transient transfection, we showed a growth advantage of all mutations over a culture period of 1 week compared to WT-*ABCA1* expression (Figure 1F). Stable cell lines expressing *ABCA1-G1421R* and *ABCA1-A2011T* (Figure S1C) with a normal proliferation rate showed growth and proliferative advantages compared to *ABCA1*-WT (Figures S1D and S1E). We also observed an activation of the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway in *ABCA1* mutant-transduced cells compared to WT-*ABCA1*, as illustrated by the higher levels of pSTAT5 quantified by flow cytometry (Figure 1G). Because the assessment of cholesterol efflux capacity is not practical in suspension cells, we next tested the dependence of ABCA1 mutations on their efflux capacity in differentiated THP-1 macrophages. In this setting, 3 of the 5 ABCA1 mutations (*ABCA1-A1291T*, *ABCA1-G1421R*, and *ABCA1-P1423S*) showed a decrease in the ability of apoA-1 to promote cholesterol efflux compared with WT-*ABCA1* (Figure S1F). Nevertheless, quantification of BODIPY (bore-dipyrromethene)-neutral lipid staining revealed higher neutral lipid accumulation in *ABCA1* mutant-transduced cells compared to WT-*ABCA1* (Figure 1H). The quantification of cellular cholesterol content confirmed these findings (Figure S1G). The increased proliferation of these cells was also associated with increased cholera

(D and E) [^{3}H]-Thymidine proliferation assays (pulsed for 2 h) were performed in HEK293 cells transiently transfected with plasmid constructs expressing *ABCA1-WT* and *ABCA1* mutants or empty vector (D), or in THP-1 monocytic leukemia cells transduced for 72 h with lentiviral particles expressing *ABCA1-WT*, *ABCA1* mutants or empty vector (E).

(F) THP-1 cells transduced for 72 h with *ABCA1* mutants exhibit growth advantage over a 7-day period compared with *ABCA1-WT*.

(G and H) Expression of phosphoSTAT5 (G) and BODIPY staining (H) determined by flow cytometry in these cells.

(I) Confocal images of lipid raft staining in THP-1 cells transduced for 72 h with empty, *ABCA1-WT*, and *ABCA1* mutants.

Values are means ± SEMs of at least 3 experiments performed in triplicate. *$p < 0.05$ *ABCA1-WT* versus empty control. #$p < 0.05$ *ABCA1* mutants versus empty control. §$p < 0.05$ versus *ABCA1-WT*.

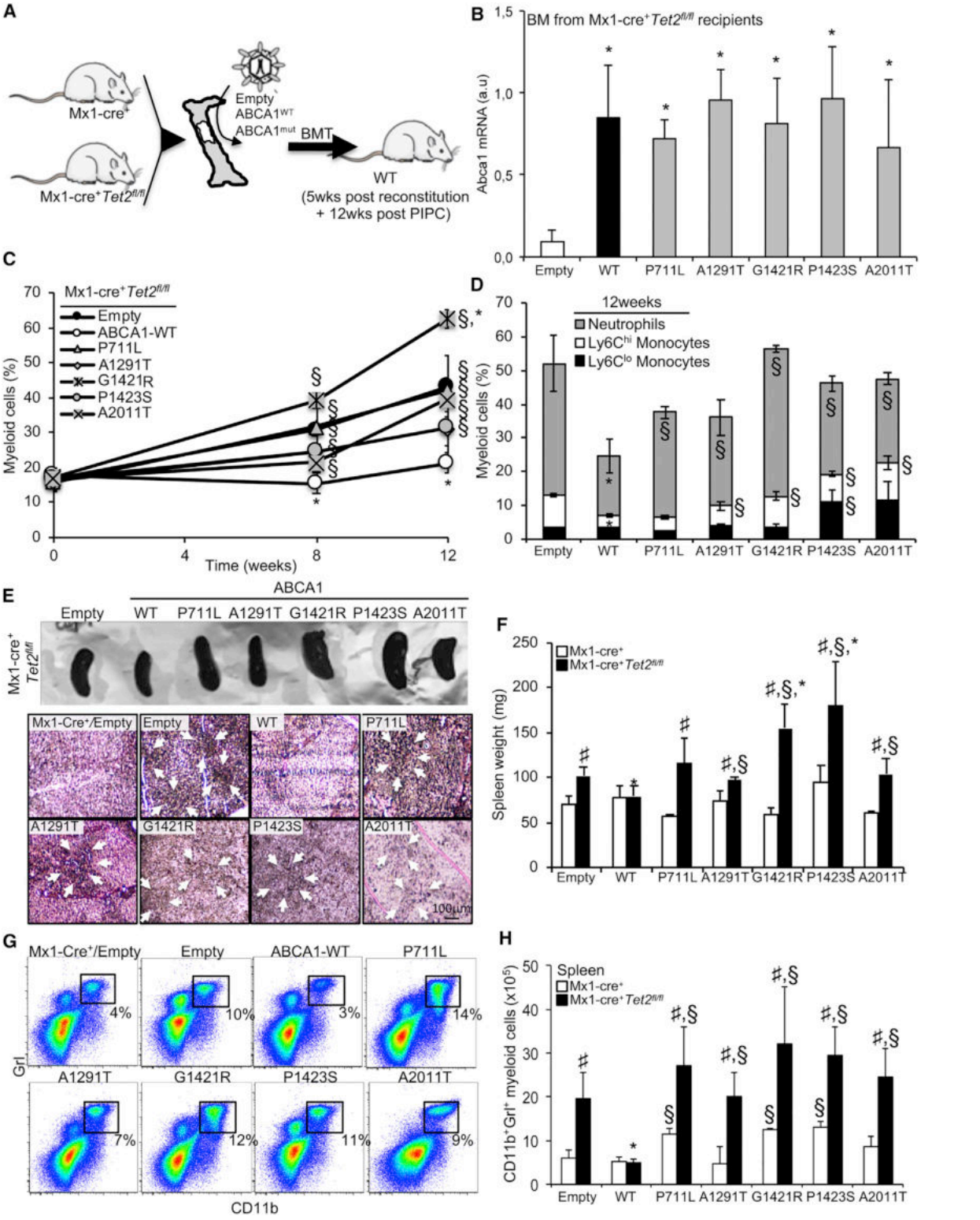


Figure 2. Loss of Functional *ABCA1* Reduces Tumor Suppression in Myelomonocytic Leukemia Induced by Tet2 Loss

(A) Experimental overview. BM from Mx1-Cre^{+} or Mx1-Cre^{+}Tet2$^{fl/fl}$ mice were transduced with lentiviral particles expressing *ABCA1-WT*, *ABCA*1 mutants, or empty vector before bone marrow transplantation (BMT) into lethally irradiated WT mice, and after a 5-week recovery period, the mice were injected with poly(I:C) and analyzed over a 12-week-period.

(legend continued on next page)

toxin subunit B (CTx-B) staining of *ABCA1* mutant-transduced cells compared to WT-*ABCA1* at the cell surface (*ABCA1-A1291T*, *ABCA1-G1421R*, and *ABCA1-P1423S*) or in intracellular endosomal-like structure (*ABCA1-P711L* and *ABCA1-A2011T*) (Figure 1I), suggesting the increased formation of cholesterol-rich lipid raft or perturbed intracellular cholesterol trafficking (Dietrich et al., 2001).

ABCA1 Mutants Associated with CMML Fail to Suppress Myelopoiesis *In Vivo*

Previous studies have suggested that the loss of *ABCA1* function alone is insufficient to promote prominent myelopoiesis in hypercholesterolemic mice (Yvan-Charvet et al., 2010). We hypothesized that the proliferative effects of *ABCA1* mutants observed in CMML may become more evident when combined with other CMML mutant alleles. Tet2 inactivation through loss-of-function mutation is commonly found in CMML (Bowman and Levine, 2017; Solary et al., 2014). Therefore, to assess the *in vivo* effects of *ABCA1* mutants, BM cells from WT or Mx1-Cre^{+}Tet2$^{fl/fl}$ mice (i.e., mice bearing the conditional Tet2 allele and the interferon-inducible Cre transgene) were transduced with pLKO-Puro-GFP lentiviral vectors containing WT-*ABCA1* or *ABCA1* mutants and transplanted into lethally irradiated C57BL/6J mice (Figure 2A). Animals were analyzed 5 weeks after BM reconstitution (T0) and at the indicated time point following polyinosinic:polycytidylic acid (PIPC) injection (Figure 2A). Consistent with earlier works (Moran-Crusio et al., 2011; Quivoron et al., 2011), we observed the loss of Tet2 expression in the BM of WT recipient mice transplanted with Mx1-Cre^{+}Tet2$^{fl/fl}$ BM compared to Mx1-Cre^{+} BM (Figure S2A), and ablation of the gene was paralleled by a significant reduction in hydroxylation of 5-methylcytosine (5hmC) in a pool of peripheral blood cells, which reflect the enzymatic activity of TET2 (Figure S2B). Quantification of Abca1 mRNA expression confirmed similar levels of overexpression of *ABCA1*-WT and mutants in the BM of Mx1-Cre^{+}Tet2$^{fl/fl}$ recipients (Figure 2B) and controls (data not shown). Despite similar leukocyte counts (Figure S2C), the overexpression of WT-*ABCA1* on a Tet2-deficient background caused a marked reduction in myeloid cells (Gr-1^{high}CD11b^{high}) in blood over time (Figure 2C), reflecting mainly lower inflammatory Ly6C^{hi} monocyte and neutrophil counts (Figures 2D and S2D). In contrast, *ABCA1* mutant-expressing animals on a Tet2-deficient background exhibited higher peripheral myeloid cells (both monocytes and neutrophils) compared to *ABCA1-WT*-transduced animals (Figures 2C, 2D, and S2D). These effects were not observed when *ABCA1-WT* or mutants were transduced on a WT background (Figures S2E and S2F). T and B cell numbers and hematocrit and platelet counts were normal on both backgrounds (data not shown). These data indicate that *ABCA1* mutants impede the protective effect of *ABCA1*-WT in preventing myeloid expansion on a Tet2-deficient background. The 5 *ABCA1* mutations identified in CMML patients were found to be loss-of-function mutations, as demonstrated by their failure to suppress blood leukocyte counts in the setting of Tet2 deficiency.

ABCA1 Mutants Fail to Prevent CMML-Associated Extramedullary Hematopoiesis and Splenomegaly

The overexpression of *ABCA1-WT* suppressed the splenomegaly of animals transplanted with Tet2-deficient BM (Figures 2E and 2F). Despite a variability within the groups, this was statistically not observed in *ABCA1* mutant-transduced animals on a Tet2-deficient background (Figure 2F). Although the median survival of Tet2-deficient mice was estimated to be 560 days (Kunimoto et al., 2018), the Kaplan-Meier survival curve indicated that the survival of ABCA1-WT-transduced animals on a Tet2-deficient background is significantly better compared to empty vector-transduced animals and ABCA1 mutant-transduced animals over a 140-day period (Figure S2G). Pathological examination revealed a robust infiltration of myeloid cells, including significant destruction of normal spleen architecture in *ABCA1* mutant-transduced animals on a Tet2-deficient background compared to *ABCA1-WT* transduced animals, similar to what was observed in empty vector-transduced animals (Figure 2E). Flow analysis of the spleen confirmed an increased proportion and number of CD11b^{+}Grl^{+} myeloid cells in *ABCA1* mutant-transduced animals on a Tet2-deficient background, and to some extent, on a WT background, compared to *ABCA1-WT*-transduced animals (Figures 2G, 2H, and S2H). An increased percentage of HSPCs (LSK cells [Lineage^{-} Sca1^{+} c-Kit^{+}]) was also observed in the spleens of *ABCA1* mutant-transduced animals compared to *ABCA1-WT*-transduced animals (Figure S2I). Thus, unlike WT ABCA1, specific *ABCA1* mutants associated with human CMML were unable to limit the increased extramedullary hematopoiesis and splenomegaly that are classical features of CMML.

ABCA1 Mutants Fail to Suppress Expansion and Myeloid Bias of Tet2-Deficient HSPCs

Tet2 loss or defective cholesterol efflux pathways leads to BM HSPCs and differentiation toward a myeloid lineage fate *in vivo*

(B) Modulation of *Abca1* mRNA expression levels in the BM of the aforementioned mouse models.
(C) Quantification of the percentage of peripheral blood myeloid cells determined by hematology cell counter over the course of 12 weeks after poly(I:C) injection in recipient mice transplanted with empty, *ABCA1-WT*, or *ABCA1* mutants expressing Mx1-Cre^{+}Tet2$^{fl/fl}$ BM.
(D) Peripheral blood myeloid subsets (CD115^{+}Ly6C^{hi} and CD115^{+}Ly6C^{lo} monocytes and CD115^{-}Ly6C^{hi} neutrophils) were also quantified in these mice at the indicated time point.
(E) Representative spleen (upper panel) and hematoxylin and eosin (H&E) staining of paraffin-embedded spleen sections from recipient mice transplanted with control or Mx1-Cre^{+}Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT*, or *ABCA1* mutants (lower panel). Original magnification × 200. Arrows indicate extensive cellular infiltrate.
(F) Quantification of spleen weight of these mice.
(G and H) Representative dot plot (G) and quantification (H) of CD11b^{+} Grl^{+} myeloid cells determined by flow cytometry in the spleens of recipient mice transplanted with control or Mx1-Cre^{+}Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT*, or *ABCA1* mutants.
The results are means ± SEMs of 5–9 animals per group. ND, not detectable. *$p < 0.05$ versus empty control on a Tet2-deficient background. §$p < 0.05$ versus *ABCA1-WT*. #$p < 0.05$ and ##$p < 0.001$ versus Mx1-Cre^{+} controls.

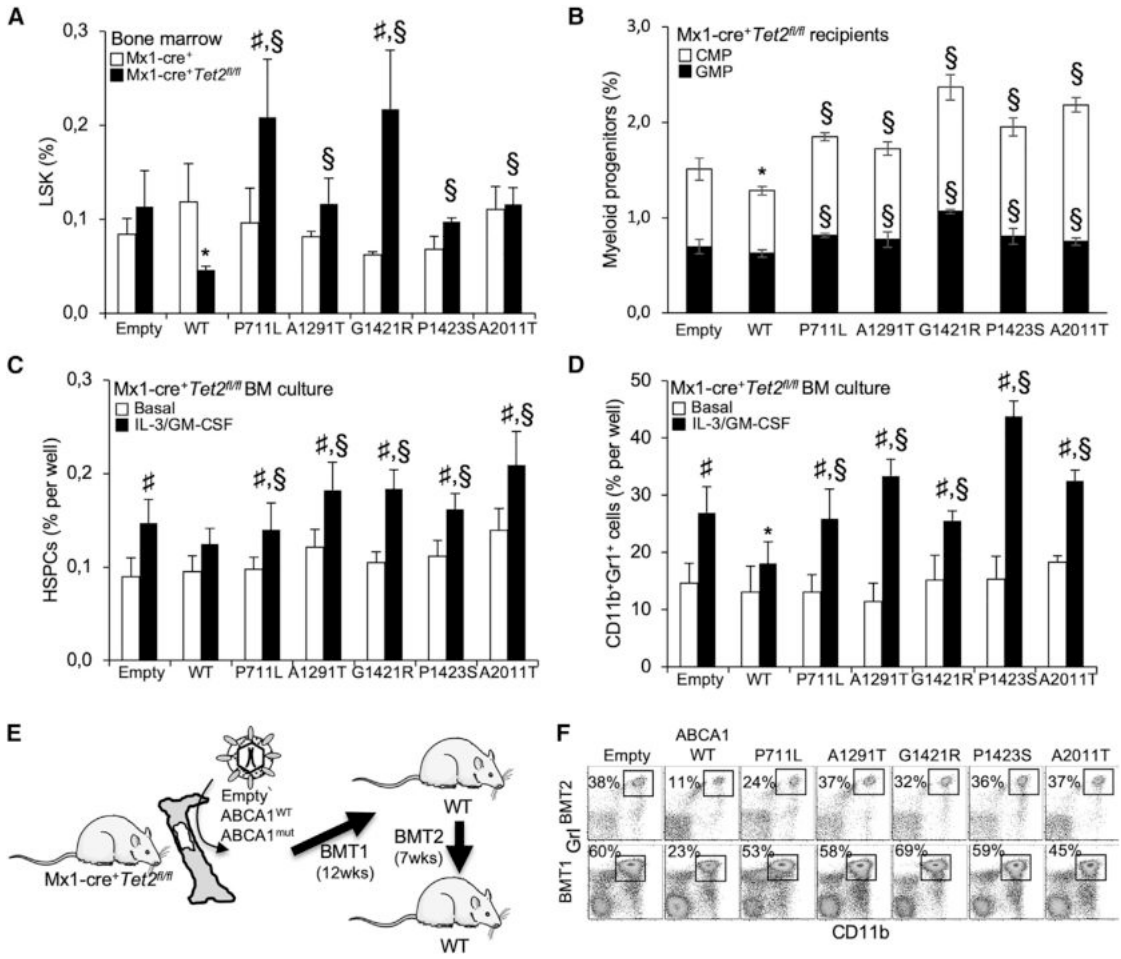


Figure 3. *ABCA1* Mutants Support Tet2-Deficient HSPC Expansion and Myeloid Lineage Commitment and Spreads CMML-like Disease in Serial BM Transplantation

(A and B) Quantification of hematopoietic stem (A) and progenitor (B) cells in the BM of recipient mice transplanted with control or Mx1-Cre^{+}Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT*, or *ABCA1* mutants. Lineage(Lin)$^{-}$ Sca1^{-}c-Kit^{+} LSK cells are hematopoietic stem and progenitor cells (HSPCs); Lin^{-}Sca1^{-}c-Kit^{+}CD34^{hi}FcγR^{hi} are granulocyte-monocyte progenitors (GMPs); and Lin^{-}Sca1^{-}c-Kit^{+}CD34^{hi}FcγR^{low} are common myeloid progenitors (CMPs). The results are the means ± SEMs of 5–9 animals per group.

(C and D) The quantification of hematopoietic progenitors (C) and myeloid cells (D) in BM cultures isolated from Mx1-Cre^{+}Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT*, or *ABCA1* mutants and grown *ex vivo* for 72 h in liquid culture in the presence or absence of 6 ng/mL IL-3 and 2 ng/mL GM-CSF. The results are the means ± SEMs of experiments performed in triplicate.

(E) Experimental overview. BM from WT and *ABCA1* mutant-transduced animals on a Tet2-deficient background were serially transplanted into lethally irradiated WT mice and analyzed 7 weeks later.

(F) Quantification of the percentage of peripheral blood CD11b^{hi}Gr-1^{hi} myeloid cells was determined by flow cytometry at the end of the study period. The results are means ± SEMs.

*p < 0.05 versus empty control transduced animals on a Tet2-deficient background. §p < 0.05 versus *ABCA1-WT*. #p < 0.05 and ##p < 0.001 versus Mx1-Cre^{+} controls.

(Moran-Crusio et al., 2011; Quivoron et al., 2011; Yvan-Charvet et al., 2010). Analysis of the BM HSPCs showed a reduction in the LSK cells in *ABCA1-WT*-transduced animals on a Tet2-deficient background compared to empty control-transduced animals. This effect was lost in *ABCA1* mutant-transduced animals (Figure 3A). Although *ABCA1* mutants barely altered the percentage of BM megakaryocyte-erythrocyte progenitors (MEPs; Lineage^{-} Sca1^{-} c-Kit^{+}CD34^{low}FcγR^{low}) (Figures S3A and S3B), the granulocyte-monocyte progenitors (GMPs; Lineage^{-}Sca1^{-} c-Kit^{+}CD34^{hi}FcγR^{hi}) and the common myeloid progenitors (CMPs; Lineage^{-} Sca1^{-} c-Kit^{+}CD34^{hi}FcγR^{low}) were significantly increased in *ABCA1* mutant-transduced BM compared to *ABCA1-WT*-transduced BM both on WT (Figures S3A and S3B) and Tet2-deficient backgrounds (Figure 3B). To determine whether *ABCA1* mutants engage HSPCs to differentiate into the myelomonocytic lineages, BM cells from *ABCA1* mutant-transduced animals were cultured *ex vivo* in the presence of IL-3 and GM-CSF. BM cultures showed that *ABCA1* mutants disengaged the suppressive effects of *ABCA1* on HSPC expansion (Figure 3C) and myeloid lineage expansion (Figure 3D)

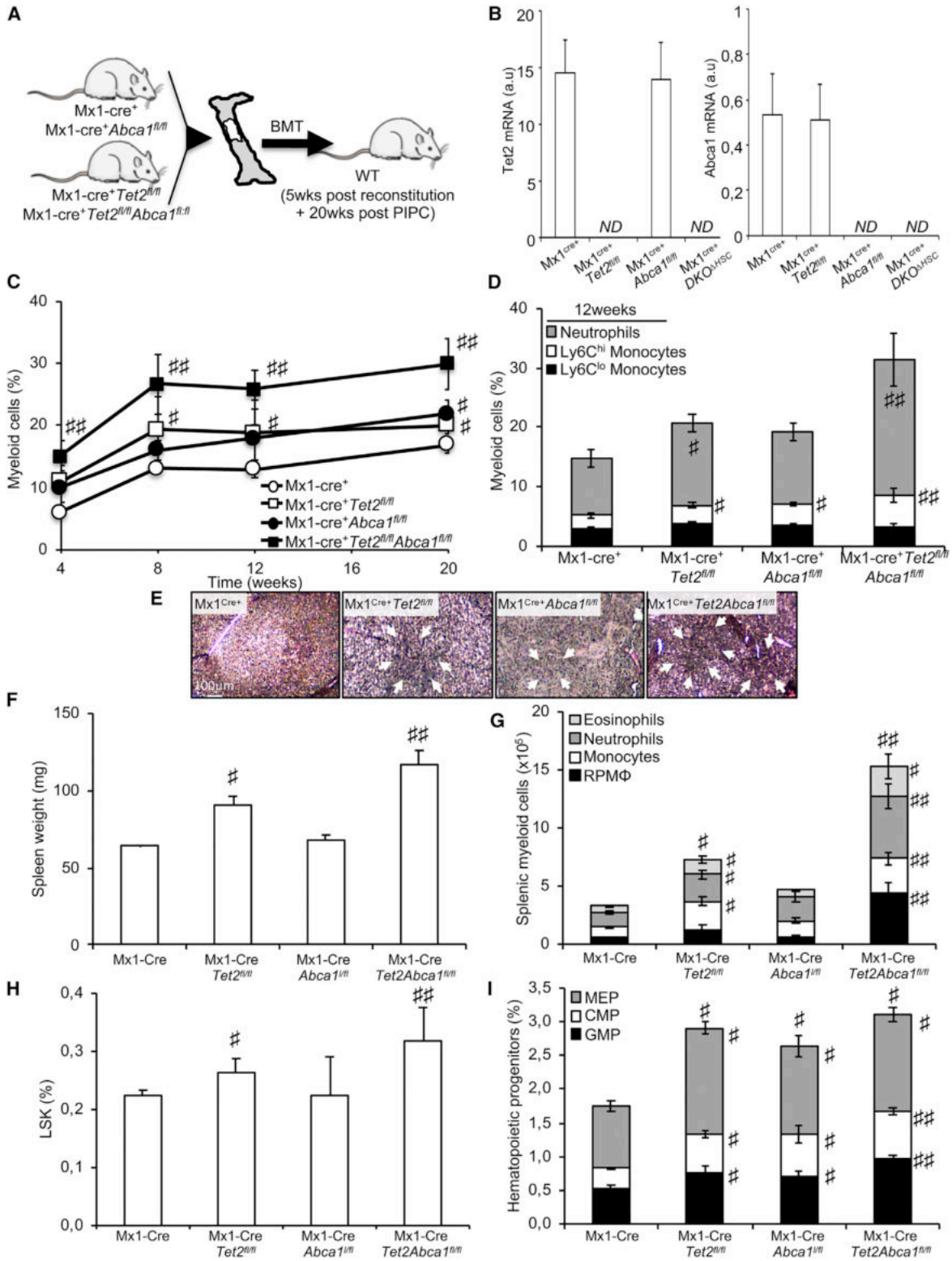


Figure 4. *ABCA1* Invalidation Propagates Myelopoiesis and Accelerates Extramedullary Hematopoiesis on a Tet2-Deficient Background

(A) Experimental overview. BM from Mx1-Cre^{+}, Mx1-Cre^{+}Abca1$^{fl/fl}$, Mx1-Cre^{+}Tet2$^{fl/fl}$, and Mx1-Cre^{+}Tet2$^{fl/fl}$Abca1$^{fl/fl}$ mice were transplanted into lethally irradiated WT mice, and after a 5-week recovery period, the mice were injected with poly(I:C) and analyzed over a 20-week period.

(B) Modulation of *Abca1* and Tet2 mRNA expression levels in the BM of the aforementioned mouse models.

(legend continued on next page)

in the Tet2-deficient background. These findings suggest that in contrast to *ABCA1-WT*, *ABCA1* mutants allow Tet2-deficient HSPCs to proliferate and to favor myelopoiesis as a result of increased IL-3-GM-CSF signaling. Of note, in secondary transplants (Figure 3E), *ABCA1* mutant-transduced animals had a significantly higher percentage of peripheral Gr-1^{high}CD11b^{high} myeloid cells compared to *ABCA1-WT*-transduced animals (Figure 3F) and failed to suppress splenomegaly (Figure S3C) or splenic cholesterol accumulation (Figure S3D). These findings confirm that ABCA1 inactivation not only confers a significant competitive advantage to Tet2-deficient HSPCs *in vivo* but also enhances a TET2-induced transplantable myeloproliferative disorder.

ABCA1 Deficiency Cooperates with Tet2 Loss to Propagate Myeloid Transformation

In parallel, we crossed Mx1-Cre^{+}Tet2$^{fl/fl}$ mice (Moran-Crusio et al., 2011; Quivoron et al., 2011) to Abca1$^{fl/fl}$ mice (Yvan-Charvet et al., 2010) to generate Mx1-Cre^{+}Tet2$^{fl/fl}$Abca1$^{fl/fl}$ (referred to subsequently as DKO$^{\Delta HSC}$ [double knockout, DKO]) mice. BM cells from these mice were subsequently transplanted into lethally irradiated C57BL/6J mice (Figure 4A). Animals were analyzed 5 weeks after BM reconstitution (T0) and at the indicated time point following PIPC injection (Figure 4A). We confirmed the excision of both Tet2 and Abca1 mRNA expression in the BM of Mx1-Cre^{+}Tet2$^{fl/fl}$ mice and Mx1-Cre^{+}Abca1$^{fl/fl}$ mice, respectively (Figure 4B). DKO$^{\Delta HSC}$ mice also underwent a marked increase in peripheral myeloid cells compared to single Abca1 or Tet2-deficient mice (Figures 4C, 4D, and S4A). These findings show a cooperative effect between *ABCA1* and *Tet2* mutations on myeloid expansion. In addition, Abca1 deficiency exacerbated the splenomegaly of Tet2-deficient animals, while having no effect on its own (Figures 4E and 4F). DKO$^{\Delta HSC}$ mice also exhibited higher myeloid cell infiltration compared to single-KO mice after pathological examination (Figure 4E), with an increased percentage and number of eosinophil, neutrophil, monocyte, and red pulp macrophages (RPMs) determined by flow cytometry (Figures 4G, S4B, and S4C). Thus, the complete deletion of *ABCA1* in Tet2-deficient mice increased spleen size and enhanced extramedullary myelopoiesis. Consistently, DKO$^{\Delta HSC}$ mice showed an increased frequency of LSK cells compared with WT or Tet2-deficient mice (Figure 4H). There was also an additive effect of *ABCA1* and *TET2* deficiency in promoting BM myeloid progenitor expansion (Figure 4I). These data indicate that concurrent ABCA1 deficiency and Tet2 loss in hematopoietic cells exacerbated myeloid transformation, supporting the finding that *ABCA1* mutations identified in CMML patients were loss-of-function mutations.

Cholesterol Accumulation Links *ABCA1* Mutants and Tet2 Loss to IL3-Rβ Signaling Hypersensitivity

We next sought to identify the mechanisms responsible for the lack of tumor-suppressor function of *ABCA1* mutants in Tet2-deficient BM cells. Increased cholesterol accumulation and reduced expression of ABCA1 have been repeatedly observed in cancer cells (Bovenga et al., 2015; Lin and Gustafsson, 2015). Thus, taking advantage of publicly available gene expression datasets (Kunimoto et al., 2018), we interrogated whether Tet2-deficient LSK, CMP, and GMPs cells could transcriptionally regulate cholesterol metabolic pathways. We did not observe a major transcriptional regulation of the genes involved in cholesterol metabolism (<10% overall changes), including LXR target genes and *ABCA1* in Tet2-deficient hematopoietic progenitors (Figure S5A). The functional behavior of these cells was next assessed by quantifying the neutral lipid accumulation in single-KO and DKO$^{\Delta HSC}$ HSPCs by flow cytometry using BODIPY staining. An increase in cellular neutral lipid content in single-KO and DKO$^{\Delta HSC}$ HSPCs and committed myeloid progenitors (i.e., GMP and CMP) was observed compared to controls (Figures 5A and 5B). The combined deficiency of Tet2 and Abca1 also led to a cumulative BODIPY-neutral lipid staining per cell (expressed as mean fluorescence intensity [MFI]) in myeloid progenitors compared to single-KO cells (Figure 5B) but not in HSPCs (Figure 5A). Consistently, the overexpression of WT-*ABCA1* reduced BODIPY staining in Tet2-deficient CMP progenitors, but not in Tet2-deficient HSPCs (Figures 5C and 5D). However, *ABCA1* mutants failed to reduce BODIPY staining on a Tet2-deficient background (Figures 5C and 5D) and to some extent on a control background (i.e., *ABCA1-A1291T*, *G1421R*, and *P1423S*) (Figure S5B). The anti-tumor activity of ABCA1 could be linked to the inhibition of sterol regulatory element-binding protein-2 (SREBP-2) and cholesterol biosynthesis target genes, which has recently been linked to the reduced expression of cell growth-facilitating mevalonate products in a solid tumor (Moon et al., 2019). However, the accumulation of cholesterol in *ABCA1* mutant-transduced Tet2-deficient BM cells rather was associated with the reduced expression of genes in the mevalonate pathway (Figure 5E), which is a well-known feedback regulatory mechanism by which sterols accumulating in the endoplasmic reticulum (ER) limit the processing and expression of the target genes of SREBP-2 (Brown and Goldstein, 2009; Spann and Glass, 2013).

Given the activation of the IL3-Rβ canonical pathway in myeloid cells with a defective cholesterol efflux pathway (Yvan-Charvet et al., 2010) and the hypersensitivity of Tet2-deficient myeloid cells to GM-CSF (Kunimoto et al., 2018), we next assessed whether the myelosuppressive function of *ABCA1* on

(C) Quantification of the percentage of peripheral blood myeloid cells determined by hematology cell counter over the course of 20 weeks after poly(I:C) injection in recipient mice transplanted with the BM from Mx1-Cre^{+}, Mx1-Cre^{+}Abca1$^{fl/fl}$, Mx1-Cre^{+}Tet2$^{fl/fl}$, and Mx1-Cre^{+}Tet2$^{fl/fl}$Abca1$^{fl/fl}$ mice.
(D) Peripheral blood myeloid subsets (CD115^{+}Ly6C^{hi} and CD115^{+}Ly6C^{lo} monocytes and CD115^{-}Ly6C^{hi} neutrophils) were also quantified in these mice at the indicated time point.
(E) Representative H&E staining of paraffin-embedded spleen sections from these mice. Original magnification × 200. Arrows indicate extensive cellular infiltrate.
(F and G) Quantification of spleen weight (F) and myeloid subsets (eosinophils, neutrophils, monocytes, and red pulp macrophages [RPMs]) in the spleens (G) of recipient mice transplanted with the BM from Mx1-Cre^{+}, Mx1-Cre^{+}Abca1$^{fl/fl}$, Mx1-Cre^{+}Tet2$^{fl/fl}$, and Mx1-Cre^{+}Tet2$^{fl/fl}$Abca1$^{fl/fl}$ mice.
(H and I) Quantification of hematopoietic stem (H) and progenitor (I) cells in the BM of these mice. Lin^{-}Sca1^{-}c-Kit^{+} LSK cells are HSPCs; Lin^{-}Sca1^{-} cKit^{+} CD34^{low}FcγR^{low} are megakaryocyte-erythrocyte progenitors (MEPs); Lin^{-}Sca1^{-}c-Kit^{+}CD34^{hi}FcγR^{hi} are GMP; and Lin^{-}Sca1^{-}c-Kit^{+}CD34^{hi}FcγR^{low} are CMPs. The results are the means ± SEMs of 5–7 animals per group. ND, not detectable. #$p < 0.05$ and ##$p < 0.001$ versus Mx1-Cre^{+} controls.

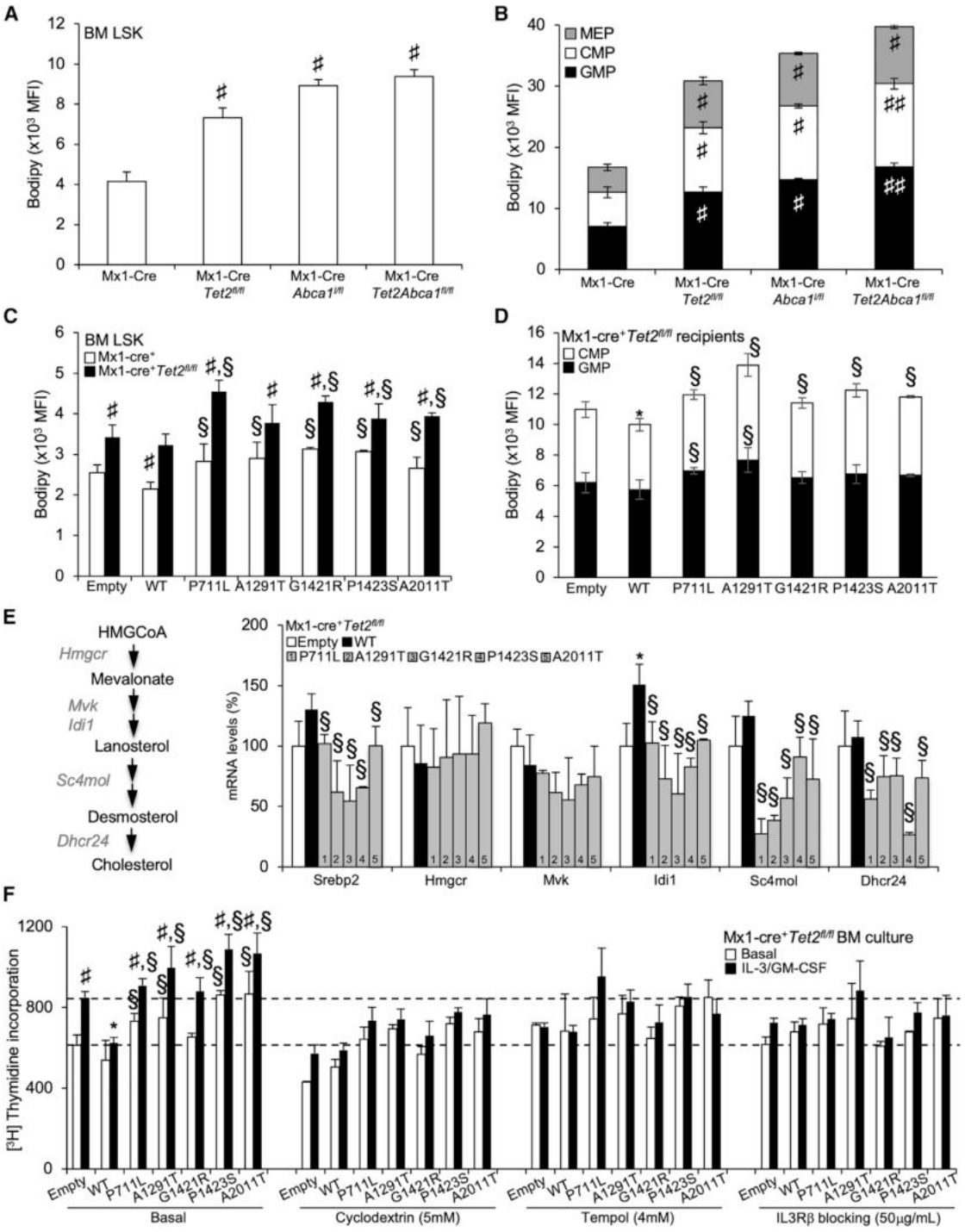


Figure 5. Cholesterol Accumulation Couples *ABCA1* Invalidation and Tet2 Deficiency to IL-3 Receptor β Signaling Hypersensitivity

(A–D) Quantification of BODIPY staining by flow cytometry expressed as mean fluorescence intensity (MFI) as a surrogate of cellular cholesterol neutral lipid per cell (A–D) in BM hematopoietic stem (A and C) and progenitor (B and D) cells (i.e., LSKs, MEPs, CMPs, and GMPs) of recipient mice transplanted with Mx1-Cre^{+}, Mx1-Cre^{+}Abca1$^{fl/fl}$, Mx1-Cre^{+}Tet2$^{fl/fl}$, and Mx1-Cre^{+}Tet2$^{fl/fl}$Abca1$^{fl/fl}$ BM (A and B) or control and Mx1-Cre^{+}Tet2$^{fl/fl}$ BM expressing empty, *ABCA1-WT*, or *ABCA1* mutants (C and D). Results are means ± SEMs of 5–9 animals per group.

(E) mRNA expression of SREBP-2 and cholesterol biosynthesis target genes (Hmgcr, Mvk, Idi1, Sc4mol, and Dhcr24) from empty, WT, and *ABCA1* mutant-transduced BM on a Tet2-deficient background isolated at the end of the study period. The expression of mRNA was normalized to m36B4. mRNA levels were expressed as percentage over WT whole BM cells.

(legend continued on next page)

a Tet2-deficient background could be attributed to its role in removing excess cellular cholesterol in committed myeloid progenitors. Excess cellular cholesterol was removed by treatment with cyclodextrin in *ABCA1* mutant-transduced Tet2-deficient BM cells cultured in the presence or absence of IL-3 and GM-CSF. We validated our *ex vivo* BM culture proliferation assay by showing that inhibition of the IL-3Rβ signaling pathway using the IL-3Rβ blocking antibody prevented BM proliferation in IL-3 and GM-CSF treatment (Figure 5F). We next showed that both the IL-3Rβ blocking antibody and cyclodextrin prevented the proliferation of *ABCA1* mutant-transduced Tet2-deficient BM cells, similar to the effect of *ABCA1*-WT overexpression (Figure 5F), without affecting cell viability (data not shown). Consistently, western blot analysis showed higher levels of pErk1/2 or pJak2 in ABCA1 mutant-transduced Tet2-deficient BM cells compared to *ABCA1-WT*-transduced cells (Figure S5C). mRNA expression analysis also revealed higher cyclin D1 and PU.1 gene expression in *ABCA1* mutant-transduced Tet2-deficient BM cells (Figure S5D). These genes are IL-3Rβ signaling targets involved in proliferation and myeloid lineage commitment. In contrast, we did not observe changes in the expression of the key transcription factor CCAAT/enhancer-binding protein alpha (C/EBPα) or in the negative regulators of RAS signaling, including dual-specificity phosphatase 1 (DUSP1) or sprouty-related, EEVH1 domain-containing protein 1 (Spred1) (Kunimoto et al., 2018). These findings suggest that the tumor-suppressor function of *ABCA1* relies on its capacity to modulate cholesterol-dependent IL-3Rβ signaling and downstream mitogen-activated protein kinase (MAPK) and JAK2 signaling. We and others recently showed that reduced autophagy and enhanced mitochondrial reactive oxygen species (ROS) production were 2 major metabolic events promoting HSPC expansion and myeloid commitment downstream of the IL-3Rβ signaling pathway (Lum et al., 2005; Sarrazy et al., 2016). Thus, we estimated autophagic activity using the Cyto-ID probe by flow cytometry. Reduced autophagic activity was observed in *Tet2*- and *Abca1*-deficient myeloid progenitors (Figure S5E) and in *ABCA1* mutant-transduced CMP and GMP cells compared to *ABCA1-WT*-transduced BM both on WT and Tet2-deficient cells (Figure S5F). Finally, suppression of mitochondrial ROS with tempol prevented the basal proliferation and IL-3-GM-CSF hypersensitivity of *ABCA1* mutant-transduced Tet2-deficient BM cells (Figure 5F). These data confirm that *ABCA1* mutations impair the myelosuppressive function of *ABCA1* by increasing IL-3-GM-CSF hypersensitivity and downstream metabolic sequelae in Tet2-deficient myeloid progenitors.

Increased HDL Reverses Increased Myelopoiesis and Splenomegaly Caused by *ABCA1* Mutants

Given the ability of increased HDL to suppress HSPC myeloid lineage commitment and rescue the myeloproliferative disorder of mice with defective cholesterol efflux (Yvan-Charvet et al., 2010), we hypothesized that raising HDL would alleviate some of the myeloproliferative phenotypes of *ABCA1* mutant-transduced Tet2-deficient animals. First, HDL treatment *ex vivo* reduced the proliferation rates of *ABCA1* mutant-transduced Tet2-deficient BM cells (Figure 6A). This was not the case with low-density lipoprotein (LDL) treatment (data not shown). HDL treatment also reduced BODIPY cholesterol staining (Figure 6B) of *ABCA1* mutant-transduced Tet2-deficient BM cells. We next explored the efficacy of the apoA-1 transgene to increase HDL levels *in vivo* (Figures 6C and 6D). Transduction of *ABCA1-A1291T*, *G1421R*, and A2011T was selected as the proof of concept and compared to BM transduced with empty vector or *ABCA1*-WT. Similar to ABCA1-WT-transduced BM cells, the human apoA-1 transgene prevented the peripheral myeloid expansion of mice transplanted with *ABCA1* mutant-transduced Tet2-deficient BM (Figure 6E) and also abolished their splenomegaly (Figure 6F). These data demonstrate that increased apoA-1 production and increased HDL show robust preclinical efficacy in myeloproliferative neoplasms driven by *ABCA1* and *TET2* mutants.

DISCUSSION

Here, we identified recurrent somatic mutations in the cholesterol exporter *ABCA1* in CMML patients. The expression of *ABCA1* mutants *in vitro* confers a growth and proliferative advantage *in vitro* compared to the expression of WT-*ABCA1*. The relevance of these mutations is underscored by the loss of the tumor-suppressor function of WT-*ABCA1* in the context of Tet2 deficiency. *ABCA1* deficiency also accelerated the myeloproliferative disorder of Tet2-deficient BM transplanted mice, as illustrated by increased myelopoiesis and extramedullary hematopoiesis. The myelosuppressive function of ABCA1 was further illustrated by the failure of *ABCA1* mutants to limit a fully penetrant myeloproliferative disorder after serial transplantation. The underlying mechanism involves the accumulation of cellular cholesterol in myeloid progenitors, resulting in IL-3Rβ signaling hypersensitivity with sustained ERK1/2 and JAK2 signaling and subsequent metabolic perturbations (e.g., reduced autophagy, enhanced mitochondrial ROS formation). In addition, the overexpression of the WT human apoA-1 transgene rescued the loss of the myelosuppressive function of *ABCA1* mutants. These findings provide further evidence that these effects were caused by defective cholesterol efflux and suggest a therapeutic application of HDL-raising therapies for CMML patients bearing *ABCA1* mutants.

The classical hallmarks of cancer are intimately intertwined with an assortment of metabolic processes that a tumor cell effectively hijacks to facilitate malignant transformation (Boroughs and DeBerardinis, 2015; Hanahan and Weinberg, 2011; Kroemer and Pouyssegur, 2008). Several studies, largely performed *in vitro*, suggest that altered cholesterol metabolism

(F) Proliferation rates were determined after 2 h [^{3}H]-thymidine pulse labeling in BM cells from empty, *ABCA1-WT*, and *ABCA1* mutant-transduced animals on a Tet2-deficient background that were grown for 72 h in liquid culture in the presence or absence of 6 ng/mL IL-3 and 2 ng/mL GM-CSF and the indicated chemical compounds. Results are means ± SEMs of cultures from at least 3 independent mice.

*$p < 0.05$ versus empty control-transduced animals on a Tet2-deficient background. §$p < 0.05$ versus *ABCA1-WT*. #$p < 0.05$ and ##$p < 0.001$ versus Mx1-Cre$^+$ controls.

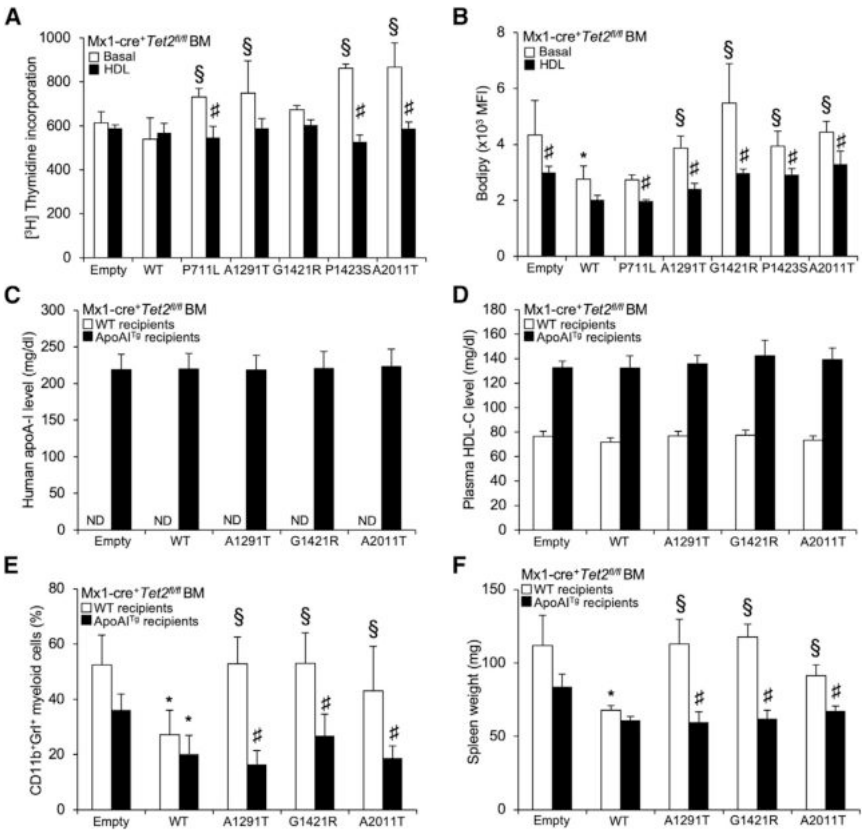


Figure 6. HDL Overcome Loss-of-Function *ABCA1* Mutants and Limit the Myeloproliferative Disorder Induced by These Mutants in Tet2-Deficient Mice

(A) Proliferation rates were determined after 2 h [^{3}H]-thymidine pulse labeling in BM cells from empty, *ABCA1-WT*, and *ABCA1* mutant-transduced animals on a Tet2-deficient background that were grown for 72 h in liquid culture in the presence or absence of 50 μg/mL polyethylene glycol (PEG)-HDL.

(B) The quantification of BODIPY staining was determined by flow cytometry in these cells and expressed as the MFI. The results are means ± SEMs of cultures from at least 3 independent mice.

(C and D) Levels of human apoA-1 (hApoA-1) (C) and plasma HDL-cholesterol levels (D) were determined in WT or apoA-1 transgenic recipient mice transplanted with Mx1-Cre$^+$Tet2$^{fl/fl}$ BM transduced with lentiviral particles expressing *ABCA1-WT* or *ABCA1* mutants or empty vector.

(E and F) Peripheral blood CD11b$^+$Gr1$^+$ myeloid subsets (E) and spleen weight (F) were quantified at the end of the study period (i.e., 7 weeks post-poly(I:C) injection that followed a 5-week recovery period post-BMT) in WT or apoA-1 transgenic recipient mice transplanted with Mx1-Cre$^+$Tet2$^{fl/fl}$ BM transduced with lentiviral particles expressing *ABCA1-WT* or *ABCA1* mutants or empty vector. Results are means ± SEMs of 4–5 animals per group.

*$p < 0.05$ versus empty control-transduced animals on a Tet2-deficient background. §$p < 0.05$ versus *ABCA1-WT*. #$p < 0.05$ versus non-HDL-treated conditions or transduced animals on a non-ApoA1^{Tg} background.

(i.e., enhanced cholesterol synthesis and reduced cholesterol efflux) is linked to the cellular transformation that is most likely to provide the essential building blocks required to maintain their aberrant survival and growth (Bovenga et al., 2015; Clendening and Penn, 2012; Dang, 2012; Lin and Gustafsson, 2015; Mullen et al., 2016). This synergistic metabolic regulation is a highly cooperative process that is thought to involve a transcriptional response of non-mutant genes (i.e., "cooperation response genes") induced by oncogenic mutations, including the downregulation of the cholesterol exporter ABCA1 (Hirsch et al., 2010; McMurray et al., 2008). Conversely, short amphipathic α-helical peptides, the presumed mimetics of apoA-1 (the major apolipoprotein constitutive of HDL), inhibited ovarian tumor development (Su et al., 2010), synthetic HDL nanoparticles inhibited B cell lymphoma xenografts (Yang et al., 2013), and injection of human apoA-1 promoted tumor and metastases regression in mice inoculated with malignant melanoma cells (Zamanian-Daryoush et al., 2013). These findings indicate decreased ABCA1 efflux activity in a variety of solid tumors that may have pathophysiological importance. However, efforts to identify causal somatic mutations in genes governing cholesterol efflux pathways have been limited so far to human colon cancer samples (Sjöblom et al., 2006; Smith and Land, 2012). Similar to solid tumors, early studies have associated hematological malignancy, especially chronic myelocytic leukemia, with perturbed cholesterol metabolism (Dessì et al., 1995; Gilbert and Ginsberg, 1983). Hypercholesterolemia and defective hematopoietic cholesterol efflux pathways control many aspects of HSPC homeostasis in mice, including their proliferation and expansion, their myeloid lineage commitment, and their mobilization from the BM (Lacy et al., 2019; Soehnlein and Swirski, 2013; Tall and Yvan-Charvet, 2015). We also demonstrated that the overexpression of the human apoA-1 transgene prevented the myeloproliferation of mice bearing the Mpl-W515L or Flt3-ITD oncogenes (Gautier et al., 2013). The discovery of *ABCA1* mutants in CMML samples with loss of myelosuppressive activity in cell culture and *in vivo* provides causal evidence of the role of ABCA1 efflux activity in myeloproliferative disorders.

Functional studies have suggested co-occurrence of specific combinations of leukemia disease alleles to drive myeloid transformation (Kunimoto et al., 2018; Shih et al., 2015). These mutations include genes that control signal transduction (e.g., somatic RAS mutations) and metabolic pathways (e.g., somatic isocitrate dehydrogenase [IDH] mutations) and those that regulate epigenetic modifications. One of the epigenetic modifiers most commonly mutated in myeloid malignancies is TET2 (Itzykson et al., 2017), which catalyzes the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, leading to DNA demethylation (Tahiliani et al., 2009). These mutations are thought to largely arise spontaneously as a result of replicative stress in

aging HSCs providing the first-step acquisition of clonal expansion and a bias toward the myeloid lineage (Moran-Crusio et al., 2011; Quivoron et al., 2011), but they are not sufficient to induce myeloid transformation and IL-3Rβ hypersensitivity (Itzykson et al., 2017). Our findings suggest that these *ABCA1* mutants, due to their loss of myelosuppressive functions, could play a pivotal role in providing a second hit, which cooperates with CH alleles to drive myeloid transformation.

Somatic mutations in *ABCA1* have not previously been described in leukemia; however, loss-of-function mutations in the *ABCA1* gene have previously been characterized in Tangier disease, a rare autosomal-recessive disorder (Kang et al., 2010). Apart from mutations located in the apoA-1 binding pocket, loss-of-function mutations have been located in the N- and C-terminal regions or the PEST sequence, involving several mechanisms such as mislocalization of ABCA1 away from the cell surface, impaired dimerization, ER exit failure, or protein interaction-dependent destabilization and degradation, all leading to impaired cholesterol efflux (Kang et al., 2010). Thus, the proliferative advantage of some ABCA1 mutants (*ABCA1-A1291T*, *ABCA1-G1421R*, and *ABCA1-P1423S*) over empty control could be attributed to a change in the dimerization with endogenous ABCA1, as previously observed in Tangier disease (i.e., dominant-negative effect) (Brunham et al., 2006; Kang et al., 2010). Engineered mutations in ABCA1 have also revealed the additional regulation of receptor tyrosine kinase signaling by lipid-dependent and -independent export activity (Vaughan et al., 2009; Tang et al., 2009). Thus, future studies are necessary to pinpoint the molecular mechanism at the origin of the impaired ABCA1 efflux activity or signal transduction and subsequent myelosuppressive functions of the newly identified ABCA1 mutants.

Monocytosis and IL-3Rβ hypersensitivity are hallmarks of CMML (Itzykson et al., 2017), and we previously showed that the accumulation of plasma membrane cholesterol in myeloid progenitors with defective cholesterol efflux pathways is the culprit of enhanced IL-3Rβ signaling (Yvan-Charvet et al., 2010). However, *ABCA1* anti-tumor activity in murine hepatocellular carcinomas driven by p53 loss has recently been attributed to a distinct mechanism involving the inhibition of SREBP-2 maturation with the consequent reduced expression of cell growth-facilitating mevalonate products, which are intermediates in the cholesterol biosynthesis pathway (Moon et al., 2019). This mechanism is inconsistent with our data in the hematopoietic context, since reduced expression of SREBP-2 and cholesterol biosynthesis target genes were observed in *ABCA1* mutant-transduced leukemic cells, and the loss of the anti-proliferative activity of *ABCA1* mutants was overcome by HDL or cyclodextrin-mediated cholesterol efflux in cell culture and by increased HDL levels *in vivo*. HDL and cyclodextrin promote cholesterol efflux by non-ABCA1 pathways, which should deplete cellular and ER cholesterol (Tall and Yvan-Charvet, 2015), leading to increased SREBP2 processing and increased cholesterol biosynthesis (Brown and Goldstein, 2009; Spann and Glass, 2013). Moreover, the overexpression of cholesterol efflux-promoting genes leads to the increased expression of SREBP-2 target genes (Wang et al., 2006). The reason for the apparent discrepancy between these studies is unknown, but it could be related to the specific mutant alleles and tissue context being evaluated (Lee et al., 2013). The present study rather shows that the IL-3Rβ hypersensitivity observed in the presence of *ABCA1* mutants metabolically reprograms myeloid cells downstream of receptor tyrosine kinase signaling activation, which is the culprit of mitochondrial ROS production, known to drive hyperproliferation and leukemic hematopoiesis (Vander Heiden et al., 2009; Abdel-Wahab and Levine, 2010).

Treatment for CMML remains challenging, since there are few validated therapeutic targets, and conventional cytotoxic drugs have limited activity. The only curative option to date is allogenic stem cell transplantation. However, most patients are diagnosed at an advanced age and are not candidates for stem cell transplantation (Itzykson et al., 2017). Our data indicate that cooperative epigenetic remodeling by *TET2* loss and *ABCA1* mutants supports myeloid transformation, which may provide a rationale for mechanism-based therapy. Given the emerging association between HDL and cancer (Pirro et al., 2018), the identification of *ABCA1* mutations in CMML provides a potential target for therapeutic intervention with HDL-raising therapies such as infusions of reconstituted HDL, which are in phase III clinical trials for acute coronary syndrome. This could be repurposed for the treatment of myeloid malignancies with genetic inactivation of ABCA1 function.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- LEAD CONTACT AND MATERIALS AVAILABILITY
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Primary CMML patient samples
  - Mice
  - Cell Line
- METHOD DETAILS
  - Genetic analysis of primary patient samples
  - Plasmids
  - Mice and treatments
  - Lentiviral BM transplantation
  - White blood cell counts-
  - Histopathology
  - HEK293 cell transfection and culture
  - Human THP-1 monocytic leukemia cells and treatments
  - BM harvest and treatment
  - [^{3}H]-Thymidine proliferation assay
  - Isotopic cholesterol efflux assay
  - Cellular and tissue cholesterol content
  - Flow cytometry analysis
  - Cholera Toxin staining
  - Antibodies
  - RNA analysis
  - Analysis of DNA hydroxymethylation
  - Western Blotting
- QUANTIFICATION AND STATISTICAL ANALYSIS
- DATA AND CODE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2020.02.056.

ACKNOWLEDGMENTS

We thank Dr. Fréderic Larbret for assistance with flow cytometry and Dr. Véronique Corcelle for assistance in animal facilities. A PACA region PhD fellowship and the La Ligue Nationale Contre le Cancer support M.V. This work was supported by the Inserm Atip-Avenir program, the European Marie Curie Career Integration Grant (CIG-630926), and the Fondation ARC (ARC-R14027AA-RAC14007AAA) to L.Y.-C. This work was supported in part by NIH grants HL107653 and HL137663 and the Leducq Foundation to A.R.T., and by MSKCC Support Grant/Core Grant P30 CA008748.

AUTHOR CONTRIBUTIONS

R.L.L., A.R.T., and L.Y.-C. conceived the project, designed the experiments, and wrote the manuscript. M.V., O.A.-W., and J.G. performed most of the molecular, histological, and *in vivo* experiments. S.I., R.G., S.S., J.M., M.A., and A.J. helped with the experimental design and assisted with the data analysis. P.A. and N.W. provided scientific advice and helped with the experimental design. L.Y.-C. also designed and supervised the study and obtained funding. All of the authors read, edited, and approved the manuscript.

DECLARATION OF INTERESTS

R.L.L. is on the supervisory board of QIAGEN and is a scientific advisor to Loxo, Imago, C4 Therapeutics, and Isoplexis, each of which include an equity interest. He receives research support from and has consulted for Celgene and Roche, has received research support from Prelude Therapeutics, and has consulted for Astellas, Incyte, Janssen, Morphosys, and Novartis. He has received honoraria from Lilly and Amgen for invited lectures and from Gilead for grant reviews. The authors have filed a patent, EB19024, on the use of HDL-raising therapies in the treatment of myeloproliferative neoplasms.

Received: May 2, 2019
Revised: November 23, 2019
Accepted: February 12, 2020
Published: March 10, 2020

REFERENCES

Abdel-Wahab, O., and Levine, R.L. (2010). Metabolism and the leukemic stem cell. J. Exp. Med. *207*, 677–680.

Boroughs, L.K., and DeBerardinis, R.J. (2015). Metabolic pathways promoting cancer cell survival and growth. Nat. Cell Biol. *17*, 351–359.

Bovenga, F., Sabbà, C., and Moschetta, A. (2015). Uncoupling nuclear receptor LXR and cholesterol metabolism in cancer. Cell Metab. *21*, 517–526.

Bowman, R.L., and Levine, R.L. (2017). TET2 in Normal and Malignant Hematopoiesis. Cold Spring Harb. Perspect. Med. *7*, a026518.

Brown, M.S., and Goldstein, J.L. (2009). Cholesterol feedback: from Schoenheimer's bottle to Scap's MELADL. J. Lipid Res. *50* (*Suppl*), S15–S27.

Brunham, L.R., Singaraja, R.R., and Hayden, M.R. (2006). Variations on a gene: rare and common variants in ABCA1 and their impact on HDL cholesterol levels and atherosclerosis. Annu. Rev. Nutr. *26*, 105–129.

Busque, L., Patel, J.P., Figueroa, M.E., Vasanthakumar, A., Provost, S., Hamilou, Z., Mollica, L., Li, J., Viale, A., Heguy, A., et al. (2012). Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. Nat. Genet. *44*, 1179–1181.

Clendening, J.W., and Penn, L.Z. (2012). Targeting tumor cell metabolism with statins. Oncogene *31*, 4967–4978.

Dang, C.V. (2012). Links between metabolism and cancer. Genes Dev. *26*, 877–890.

Dessì, S., Batetta, B., Spano, O., Sanna, F., Tonello, M., Giacchino, M., Tessitore, L., Costelli, P., Baccino, F.M., Madon, E., et al. (1995). Clinical remission is associated with restoration of normal high-density lipoprotein cholesterol levels in children with malignancies. Clin. Sci. (Lond.) *89*, 505–510.

Dietrich, C., Volovyk, Z.N., Levi, M., Thompson, N.L., and Jacobson, K. (2001). Partitioning of Thy-1, GM1, and cross-linked phospholipid analogs into lipid rafts reconstituted in supported model membrane monolayers. Proc. Natl. Acad. Sci. USA *98*, 10642–10647.

Emanuel, P.D. (2008). Juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. Leukemia *22*, 1335–1342.

Gautier, E.L., Westerterp, M., Bhagwat, N., Cremers, S., Shih, A., Abdel-Wahab, O., Lütjohann, D., Randolph, G.J., Levine, R.L., Tall, A.R., and Yvan-Charvet, L. (2013). HDL and Glut1 inhibition reverse a hypermetabolic state in mouse models of myeloproliferative disorders. J. Exp. Med. *210*, 339–353.

Gilbert, H.S., and Ginsberg, H. (1983). Hypocholesterolemia as a manifestation of disease activity in chronic myelocytic leukemia. Cancer *51*, 1428–1433.

Gough, P.J., and Raines, E.W. (2003). Gene therapy of apolipoprotein E-deficient mice using a novel macrophage-specific retroviral vector. Blood *101*, 485–491.

Hanahan, D., and Weinberg, R.A. (2011). Hallmarks of cancer: the next generation. Cell *144*, 646–674.

Hirsch, H.A., Iliopoulos, D., Joshi, A., Zhang, Y., Jaeger, S.A., Bulyk, M., Tsichlis, P.N., Shirley Liu, X., and Struhl, K. (2010). A transcriptional signature and common gene networks link cancer with lipid metabolism and diverse human diseases. Cancer Cell *17*, 348–361.

Itzykson, R., Duchmann, M., Lucas, N., and Solary, E. (2017). CMML: clinical and molecular aspects. Int. J. Hematol. *105*, 711–719.

Jafri, H., Alsheikh-Ali, A.A., and Karas, R.H. (2010). Baseline and on-treatment high-density lipoprotein cholesterol and the risk of cancer in randomized controlled trials of lipid-altering therapy. J. Am. Coll. Cardiol. *55*, 2846–2854.

Kang, M.H., Singaraja, R., and Hayden, M.R. (2010). Adenosine-triphosphate-binding cassette transporter-1 trafficking and function. Trends Cardiovasc. Med. *20*, 41–49.

Kroemer, G., and Pouyssegur, J. (2008). Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell *13*, 472–482.

Kunimoto, H., Meydan, C., Nazir, A., Whitfield, J., Shank, K., Rapaport, F., Maher, R., Pronier, E., Meyer, S.C., Garrett-Bakelman, F.E., et al. (2018). Cooperative Epigenetic Remodeling by TET2 Loss and NRAS Mutation Drives Myeloid Transformation and MEK Inhibitor Sensitivity. Cancer Cell *33*, 44–59.e8.

Lacy, M., Atzler, D., Liu, R., de Winther, M., Weber, C., and Lutgens, E. (2019). Interactions between dyslipidemia and the immune system and their relevance as putative therapeutic targets in atherosclerosis. Pharmacol. Ther. *193*, 50–62.

Lee, B.H., Taylor, M.G., Robinet, P., Smith, J.D., Schweitzer, J., Sehayek, E., Falzarano, S.M., Magi-Galluzzi, C., Klein, E.A., and Ting, A.H. (2013). Dysregulation of cholesterol homeostasis in human prostate cancer through loss of ABCA1. Cancer Res. *73*, 1211–1218.

Ley, T.J., Miller, C., Ding, L., Raphael, B.J., Mungall, A.J., Robertson, A., Hoadley, K., Triche, T.J., Jr., Laird, P.W., Baty, J.D., et al.; Cancer Genome Atlas Research Network (2013). Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N. Engl. J. Med. *368*, 2059–2074.

Lin, C.-Y., and Gustafsson, J.-Å. (2015). Targeting liver X receptors in cancer therapeutics. Nat. Rev. Cancer *15*, 216–224.

Lum, J.J., Bauer, D.E., Kong, M., Harris, M.H., Li, C., Lindsten, T., and Thompson, C.B. (2005). Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell *120*, 237–248.

McMurray, H.R., Sampson, E.R., Compitello, G., Kinsey, C., Newman, L., Smith, B., Chen, S.-R., Klebanov, L., Salzman, P., Yakovlev, A., and Land, H. (2008). Synergistic response to oncogenic mutations defines gene class critical to cancer phenotype. Nature *453*, 1112–1116.

Moon, S.-H., Huang, C.-H., Houlihan, S.L., Regunath, K., Freed-Pastor, W.A., Morris, J.P., Tschaharganeh, D.F., Kastenhuber, E.R., Barsotti, A.M., Culp-Hill,

R., et al. (2019). p53 Represses the Mevalonate Pathway to Mediate Tumor Suppression. Cell *176*, 564–580.e19.

Moran-Crusio, K., Reavie, L., Shih, A., Abdel-Wahab, O., Ndiaye-Lobry, D., Lobry, C., Figueroa, M.E., Vasanthakumar, A., Patel, J., Zhao, X., et al. (2011). Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. Cancer Cell *20*, 11–24.

Mullen, P.J., Yu, R., Longo, J., Archer, M.C., and Penn, L.Z. (2016). The interplay between cell signalling and the mevalonate pathway in cancer. Nat. Rev. Cancer *16*, 718–731.

Murphy, A.J., Akhtari, M., Tolani, S., Pagler, T., Bijl, N., Kuo, C.-L., Wang, M., Sanson, M., Abramowicz, S., Welch, C., et al. (2011). ApoE regulates hematopoietic stem cell proliferation, monocytosis, and monocyte accumulation in atherosclerotic lesions in mice. J. Clin. Invest. *121*, 4138–4149.

Pikman, Y., Lee, B.H., Mercher, T., McDowell, E., Ebert, B.L., Gozo, M., Cuker, A., Wernig, G., Moore, S., Galinsky, I., et al. (2006). MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. *3*, e270.

Pirro, M., Ricciuti, B., Rader, D.J., Catapano, A.L., Sahebkar, A., and Banach, M. (2018). High density lipoprotein cholesterol and cancer: marker or causative? Prog. Lipid Res. *71*, 54–69.

Quivoron, C., Couronné, L., Della Valle, V., Lopez, C.K., Plo, I., Wagner-Ballon, O., Do Cruzeiro, M., Delhommeau, F., Arnulf, B., Stern, M.-H., et al. (2011). TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. Cancer Cell *20*, 25–38.

Rubin, E.M., Krauss, R.M., Spangler, E.A., Verstuyft, J.G., and Clift, S.M. (1991). Inhibition of early atherogenesis in transgenic mice by human apolipoprotein AI. Nature *353*, 265–267.

Sarrazy, V., Viaud, M., Westerterp, M., Ivanov, S., Giorgetti-Peraldi, S., Guinamard, R., Gautier, E.L., Thorp, E.B., De Vivo, D.C., and Yvan-Charvet, L. (2016). Disruption of Glut1 in Hematopoietic Stem Cells Prevents Myelopoiesis and Enhanced Glucose Flux in Atheromatous Plaques of ApoE(-/-) Mice. Circ. Res. *118*, 1062–1077.

Shih, A.H., Jiang, Y., Meydan, C., Shank, K., Pandey, S., Barreyro, L., Antony-Debre, I., Viale, A., Socci, N., Sun, Y., et al. (2015). Mutational cooperativity linked to combinatorial epigenetic gain of function in acute myeloid leukemia. Cancer Cell *27*, 502–515.

Sjöblom, T., Jones, S., Wood, L.D., Parsons, D.W., Lin, J., Barber, T.D., Mandelker, D., Leary, R.J., Ptak, J., Silliman, N., et al. (2006). The consensus coding sequences of human breast and colorectal cancers. Science *314*, 268–274.

Smith, B., and Land, H. (2012). Anticancer activity of the cholesterol exporter ABCA1 gene. Cell Rep. *2*, 580–590.

Soehnlein, O., and Swirski, F.K. (2013). Hypercholesterolemia links hematopoiesis with atherosclerosis. Trends Endocrinol. Metab. *24*, 129–136.

Solary, E., Bernard, O.A., Tefferi, A., Fuks, F., and Vainchenker, W. (2014). The Ten-Eleven Translocation-2 (TET2) gene in hematopoiesis and hematopoietic diseases. Leukemia *28*, 485–496.

Spann, N.J., and Glass, C.K. (2013). Sterols and oxysterols in immune cell function. Nat. Immunol. *14*, 893–900.

Su, F., Kozak, K.R., Imaizumi, S., Gao, F., Amneus, M.W., Grijalva, V., Ng, C., Wagner, A., Hough, G., Farias-Eisner, G., et al. (2010). Apolipoprotein A-I (apoA-I) and apoA-I mimetic peptides inhibit tumor development in a mouse model of ovarian cancer. Proc. Natl. Acad. Sci. USA *107*, 19997–20002.

Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., and Rao, A. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science *324*, 930–935.

Tall, A.R., and Yvan-Charvet, L. (2015). Cholesterol, inflammation and innate immunity. Nat. Rev. Immunol. *15*, 104–116.

Tang, C., Liu, Y., Kessler, P.S., Vaughan, A.M., and Oram, J.F. (2009). The macrophage cholesterol exporter ABCA1 functions as an anti-inflammatory receptor. J. Biol. Chem. *284*, 32336–32343.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science *324*, 1029–1033.

Vaughan, A.M., Tang, C., and Oram, J.F. (2009). ABCA1 mutants reveal an interdependency between lipid export function, apoA-I binding activity, and Janus kinase 2 activation. J. Lipid Res. *50*, 285–292.

Wang, N., Ranalletta, M., Matsuura, F., Peng, F., and Tall, A.R. (2006). LXR-induced redistribution of ABCG1 to plasma membrane in macrophages enhances cholesterol mass efflux to HDL. Arterioscler. Thromb. Vasc. Biol. *26*, 1310–1316.

Wang, M., Subramanian, M., Abramowicz, S., Murphy, A.J., Gonen, A., Witztum, J., Welch, C., Tabas, I., Westerterp, M., and Tall, A.R. (2014). Interleukin-3/granulocyte macrophage colony-stimulating factor receptor promotes stem cell expansion, monocytosis, and atheroma macrophage burden in mice with hematopoietic ApoE deficiency. Arterioscler. Thromb. Vasc. Biol. *34*, 976–984.

Westerterp, M., Gourion-Arsiquaud, S., Murphy, A.J., Shih, A., Cremers, S., Levine, R.L., Tall, A.R., and Yvan-Charvet, L. (2012). Regulation of hematopoietic stem and progenitor cell mobilization by cholesterol efflux pathways. Cell Stem Cell *11*, 195–206.

Yang, S., Damiano, M.G., Zhang, H., Tripathy, S., Luthi, A.J., Rink, J.S., Ugolkov, A.V., Singh, A.T.K., Dave, S.S., Gordon, L.I., and Thaxton, C.S. (2013). Biomimetic, synthetic HDL nanostructures for lymphoma. Proc. Natl. Acad. Sci. USA *110*, 2511–2516.

Yvan-Charvet, L., Pagler, T., Gautier, E.L., Avagyan, S., Siry, R.L., Han, S., Welch, C.L., Wang, N., Randolph, G.J., Snoeck, H.W., and Tall, A.R. (2010). ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. Science *328*, 1689–1693.

Zamanian-Daryoush, M., Lindner, D., Tallant, T.C., Wang, Z., Buffa, J., Klipfell, E., Parker, Y., Hatala, D., Parsons-Wingerter, P., Rayman, P., et al. (2013). The cardioprotective protein apolipoprotein A1 promotes potent anti-tumorigenic effects. J. Biol. Chem. *288*, 21237–21252.