REGULAR ARTICLE

blood advances

The *CADM1* tumor suppressor gene is a major candidate gene in MDS with deletion of the long arm of chromosome 11

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Key Points

- We detail at clinical, cytological, cytogenetic, and molecular levels 113 cases of MDS and MDS/MPN with del(11q), a rare recurrent event.
- *CADM1,* a tumor suppressor gene identified initially in solid tumors, *ATM*, *CBL,* and *KMT2A* are deleted and/or mutated in del(11q).

Myelodysplastic syndromes (MDS) represent a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis leading to peripheral cytopenias and in a substantial proportion of cases to acute myeloid leukemia. The deletion of the long arm of chromosome 11, del(11q), is a rare but recurrent clonal event in MDS. Here, we detail the largest series of 113 cases of MDS and myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN) harboring a del(11q) analyzed at clinical, cytological, cytogenetic, and molecular levels. Female predominance, a survival prognosis similar to other MDS, a low monocyte count, and dysmegakaryopoiesis were the specific clinical and cytological features of del(11q) MDS. In most cases, del(11q) was isolated, primary and interstitial encompassing the 11q22-23 region containing *ATM*, *KMT2A*, and *CBL* genes. The common deleted region at 11q23.2 is centered on an intergenic region between *CADM1* (also known as *Tumor Suppressor in Lung Cancer 1*) and *NXPE2*. *CADM1* was expressed in all myeloid cells analyzed in contrast to *NXPE2*. At the functional level, the deletion of Cadm1 in murine

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Requests for data sharing may be submitted to Eric Delabesse (delabesse.eric@iuct-oncopole.fr).

The full-text version of this article contains a data supplement.

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Lineage$^-$Sca1$^+$Kit$^+$ cells modifies the lymphoid-to-myeloid ratio in bone marrow, although not altering their multilineage hematopoietic reconstitution potential after syngenic transplantation. Together with the frequent simultaneous deletions of *KMT2A*, *ATM*, and *CBL* and mutations of *ASXL1*, *SF3B1*, and *CBL*, we show that *CADM1* may be important in the physiopathology of the del(11q) MDS, extending its role as tumor-suppressor gene from solid tumors to hematopoietic malignancies.

Introduction

Myelodysplastic syndromes (MDS) are defined according to the World Health Organization (WHO) classification by cytopenias, bone marrow (BM) dysplasia, and recurrent cytogenetic abnormalities.[1,2] They represent a heterogeneous group of clonal hematopoietic stem cell disorders characterized by ineffective hematopoiesis leading to peripheral cytopenias and, in a substantial proportion of cases, to acute myeloid leukemia (AML).[3,4] According to the revised International Prognostic Scoring System (IPSS-R), their prognosis is mainly based on the degree of cytopenias, the percentage of blasts in the BM, and the presence of karyotypic abnormalities.[5]

Recurrent driver chromosomal abnormalities and mutations are found in MDS.[6] More specifically, copy-number abnormalities are a hallmark of MDS with complete or partial deletions of chromosomes 5 (-5/5q), 7 (-7/7q), 11 (11q), 12 (12p), 13 (13q), 17 (17p), 18 (18q), and 20 (20q). The most frequent among these chromosomal deletions are −7/7q (del(7q)) and −5/5q (del(5q)) deletions.[6] MDS with isolated del(5q), recognized as a specific entity by the WHO classification, is associated with unique features, such as anemia; frequent, increased platelet counts; low blast percentage in bone marrow; the presence of characteristic small, nonlobated megakaryocytes; and a low rate of leukemic transformation.[6] Del(5q) leads to haploinsufficiency of numerous genes, including *RPS14*, miR-145, and *CSNK1A1* within the 5q32-33 critical deleted region underlying the necessary combination of various events in the development of MDS consequently to this deletion.[6]

Deletion of the long arm of chromosome 11 (del(11q)) is a rare clonal abnormality found in 0.6% to 3% of MDS and myelodysplastic/myeloproliferative neoplasms (MDS/MPN).[2,7,8] According to the WHO 2008 classification, it represents a hallmark of MDS in a context of unexplained cytopenia.[9] Since its first description in 1992,[10] few studies have investigated this deletion in MDS.[7,10-14] The first substantial study analyzed 19 cases,[12] leading to assign a very good prognosis to del(11q) as a sole abnormality in the IPSS-R classification.[5,13] The largest cohort published so far included 56 cases demonstrating an association of del(11q) with *SF3B1* mutations without delineating a common deleted region (CDR).[8]

Here, we explore the clinical, cytological, cytogenetic, and molecular features of 113 MDS and MDS/MPN cases harboring a del(11q), representing the largest series of patients so far. Cytogenetic and molecular analyses showed that *ATM* and/or *KMT2A* were deleted in all tested cases, and that *CBL* was deleted or mutated in most of the cases. Our approach allowed to define a CDR located within the 11q23.2 band centered on an intergenic region between *CADM1* and *NXPE2*, the former gene being the only 1 consistently expressed in myeloid cells. Finally, *CADM1* deletion by genomic editing leads to hematopoietic features reminiscent of MDS. Therefore, del(11q) MDS as del(5q) MDS could be the result of multiple combined haploinsufficiencies of genes located in the 11q22-q23 region, such as *ATM, CADM1, KMT2A,* or *CBL* genes.

Methods

Patients and inclusion criteria

In this retrospective study, 113 patients (103 MDS and 10 MDS/MPN) were included over an 11-year period by 24 French and Belgian laboratories affiliated to the Groupe Francophone de Cytogénétique Hématologique. Inclusion criteria were the presence of a clonal del(11q) at karyotype from MDS or MDS/MPN patients defined according to the WHO 2016 classification and the availability of remaining cytogenetic pellet for fluorescence in situ hybridization (FISH) analysis (supplemental Table 1). The research was approved by the Toulouse University ethical committee and all human participants gave written informed consent. Karyotypes were established on a median of 20 metaphases per patient (range 5-33). All analyses were performed on the cytogenetic pellet used for diagnosis. Karyotypes were reviewed at 2 successive workshops and written according to International System for Human Cytogenomic Nomenclature 2016.[15] Scoring of clonal chromosomal abnormalities was established according to IPSS-R.[13,16] Morphological classification was done locally and validated centrally using the WHO 2016 classification.[2] Cytopenias were defined according to WHO 2016.[2] Each case was assigned a risk score according to the IPSS-R for MDS[5] and therapy-related MDS (t-MDS).[17] A cohort of 88 MDS without del(11q) enrolled by Groupe Français des Myélodysplasies clinical centers in the PHRC MDS-04 was included as control (Other MDS group, Table 1).

FISH analyses

FISH analyses were performed for all patients, either locally or centrally, using the following chromosome 11 probes from centromere to 11q telomere (supplemental Figure 1A): D11Z1 (centromeric probe, Vysis), *CCND1* (Vysis), *ATM* (Vysis), *ZBTB16* (Kreatech), *CADM1* (RP11-713B9), *ZPR1* (RP11-87O6 and RP11-4N9), *KMT2A* (Vysis), *CBL* (RP11-1105H9 and RP11-122H4), *MIR125B1* (RP11-382J20), *FLI1* (RP11-432L13 and RP11-744N12), and D11S1037 (subtelomeric probe, Vysis).

Mutational screening

A specific *CBL* mutational screening was performed for 41 MDS with del(11q) targeting the *CBL* mutations hotspots (exons 7 to 9) by high resolution melting PCR (detailed in supplemental Methods). Positive signals were confirmed by Sanger sequencing.

Specific resequencing detailed in supplemental Methods was performed for 36 del(11q) samples by next generation sequencing of 28 genes (*ASXL1, CBL, CSF3R, DNMT3A, ETV6, EZH2, GATA1, GATA2, IDH1, IDH2, JAK2, KDM6A, KIT, KRAS, NPM1, NRAS,*

Table 1. Summary of clinical and cytological data of del(11q) MDS and MDS/MPN compared with other MDS

Characteristics	del(11q) MDS and MDS/MPN	del(11q) MDS	Other MDS	*P* value MDS
Patients	113	103	88	
Age, years, median (range)	74 (31-94)	74 (31-94)	71 (44-94)	0.24
Sex ratio	0.74	0.74	1.26	0.020
Blood count				
Hemoglobin (Hb), g/dL, median (range)	9.9 (4.5-14.8)	9.9 (5.5-14.8)	9.4 (6.4-14.9)	0.14
Anemia (Hb <10 g/dL)	52%	52%	58%	0.43
Mean corpuscular volume, fL, median (range)	101 (77-139)	101 (77-139)	99.5 (76-129)	0.035
Platelets (plt), G/L, median (range)	173 (17-1234)	173 (17-1234)	183 (5-494)	0.71
Thrombocytopenia (plt <100 G/L)	32%	32%	27%	0.44
White blood count (WBC), G/L, median (range)	4.0 (1.0-94)	4.0 (1.0-18.4)	4.2 (1.0-18.4)	0.85
Neutrophil Count (ANC), G/L median (range)	2.3 (0.2-52.6)	2.0 (0.2-13.4)	2.2 (0.3-15.8)	0.61
Neutropenia (ANC <1.8 G/L)	36%	36%	37%	0.96
Monocyte count, G/L, median (range)	0.3 (0-15)	0.2 (0-0.9)	0.4 (0-0.9)	0.0004
Lymphocyte count, G/L, median (range)	1.2 (0-5.0)	1.1 (0.1-3.5)	1.3 (0.3-3.7)	0.57
Bone marrow				
% BM blasts, median (range)	4 (0-14)	4 (0-13)	3 (0-19)	0.086
Dyserythropoiesis	73%	75%	71%	0.58
Dysgranulopoiesis	51%	51%	69%	0.025
Dysmegakaryopoiesis	78%	80%	57%	0.001

PHF6, PTPN11, RAD21, RUNX1, SF3B1, SMC1A, SMC3, STAG2, TET2, TP53, U2AF1, and *WT1*). Additional resequencing for *CSMD1, ETNK1, PRPF8, SRSF2,* and *ZRSR2* was performed for 18 del(11q) samples. Specific resequencing of the complete coding regions of *CADM1* and *NXPE2* was performed for 89 samples, 19 MDS with del(11q), 3 MDS, 2 MDS/MPN, and 60 AML without del(11q).

Genomic microarray analysis

Twelve samples with a deletion boundary located between *ATM* and *KMT2A* were analyzed for copy number changes at a very high resolution using Affymetrix Cytoscan HD arrays (Affymetrix) and detailed in supplemental Methods.

NXPE2 and CADM1 expression

cDNA was synthesized using 1 μg of ribonucleic acid (RNA) and the SuperScript Vilo kit (Invitrogen) according to the manufacturer's instructions in a final volume of 25 μL. Quantitative PCR was performed as detailed in supplemental Methods in 21 MDS, 10 MDS/MPN and 55 AML samples (supplemental Table 2).

CADM1 genomic editing

Single-guide RNAs (sgRNAs) targeting the *Cadm1* exon 2 were subcloned in pLKO5-sgRNA-EFS-GFP.[18] Editing was performed using *Rosa26-Cas9* mice. Lineage negative (Lin^-) BM cells were purified using a magnetic microbeads Lineage Cell Depletion Kit (Miltenyi Biotech) from 6-8-week-old *Rosa26-Cas9*-expressing ($CD45.2^+$) mice and analyzed using antibodies from Pharmingen (BD Biosciences) detailed in supplemental Methods.

Purified $Lin^-Sca1^+kit^+$ (LSK) cells were plated in suspension in StemSpan medium (StemCell Technologies) with 50 ng/mL murine thrombopoietin (TPO) and 50 ng/mL murine stem cell factor (SCF). After 24 hours of prestimulation, LSK cells were transduced by lentiviruses at a multiplicity of infection of 10 (MOI10) and 2 μg/mL polybrene (Sigma Aldrich). Efficiency of the genomic editing was analyzed using next generation sequencing of the targeted *Cadm1* exon 2 and the CRISPResso software.[19]

Clonogenic and transplantation assays

Transduced LSK cells were plated in triplicate in multipotential methylcellulose (MethoCult 3434, StemCell Technologies). Colonies were numbered and characterized 8 days later. Transduced LSK ($CD45.2^+$) were transplanted into 6-8 week-old recipient mice ($CD45.1^+$) pretreated with 30 mg/kg of Busulfan (Busilvex, Pierre Fabre) 24h before transplantation. Chimerism of engrafted and transduced cells was analyzed by flow cytometry (FACS) in BM and thymus 2 months after transplantation. Donor-derived cells ($CD45.2^+GFP^+$) from primary BM were transplanted into secondary recipients pretreated with Busulfan (30 mg/kg). Kinetics of engraftment of transduced cells (% $CD45.2^+GFP^+$) was assessed by BM aspirations 2, 8, and 21 months after transplantations.

BM and thymus of recipient mice ($CD45.1^+$) 8 weeks after transplantation were analyzed using antibodies obtained from Pharmingen (BD Biosciences, detailed in supplemental Methods). Cytological analysis was performed on at least 200 purified donor-derived cells (% $CD45.2^+GFP^+$) from each sgCTL and sgCadm1 #2 transplanted mice.

Statistics

Statistical analyses were performed using Stata 15.1 as detailed in supplemental Methods.

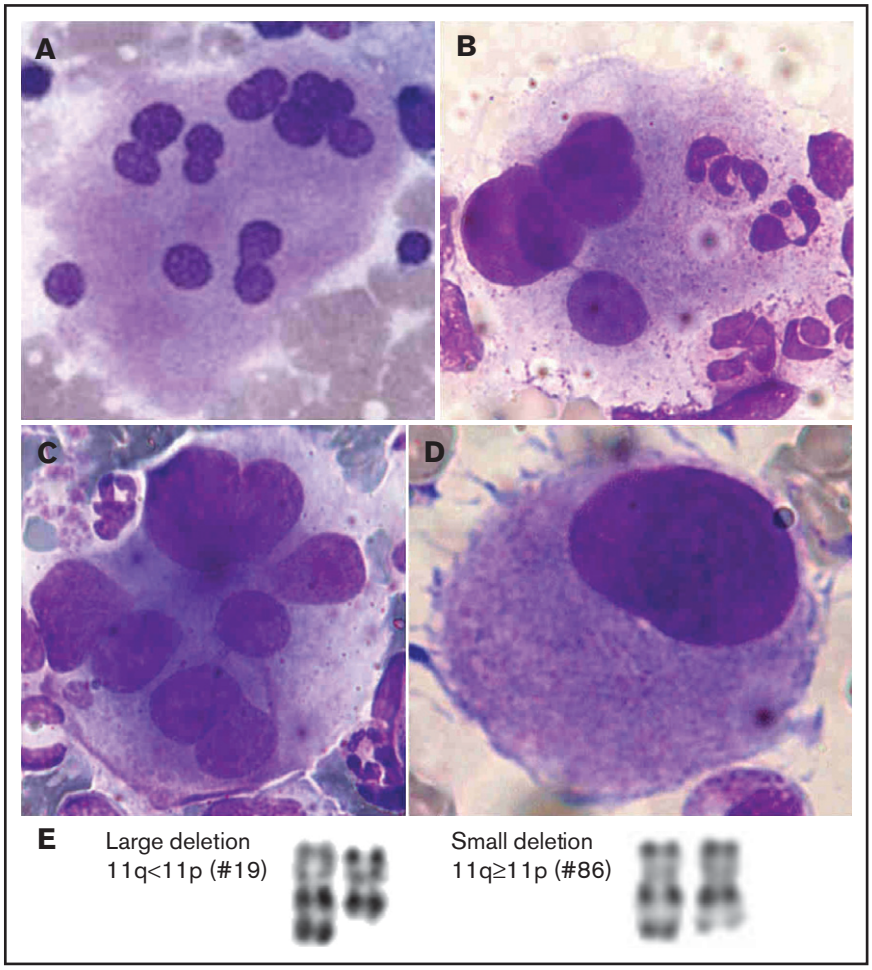

Figure 1. Cytological and cytogenetic presentations of del(11q) MDS. (A-D) May-Grünwald Giemsa staining of del(11q) bone marrow. Megakaryocytes were often large and plurinucleated (A, B, and C, original magnification x×50) giving an aspect of ball bags (C) and, when associated to 5q deletion, presence of small and nonlobated megakaryocytes ([D], original magnification ×100). (E) Cytogenetic presentations of del(11q). The 2 main types of del(11q) on *R*-banded partial karyotypes from 2 representative cases, from left to right: large deletion, del(11)(q14q24), in case #19 and small deletion, del(11)(q22q24), in case #86.

Results

Patients with del(11q) are preferentially female, have a lower monocyte blood count and bone marrow dysmegakaryopoiesis

A series of 113 MDS or MDS/MPN with del(11q) was collected through the Groupe Francophone de Cytogénétique network, MDS being more prevalent than MDS/MPN (103 vs 10 cases, respectively). Clinical and biological data were compared with a cohort of 88 MDS patients without del(11q) ("other MDS") established by the Groupe Français des Myélodysplasies clinical network (Table 1). Patients with del(11q) patients had a median age of 74 years (range 31-94), similar to other MDS (P = .24), and were preferentially female with a sex ratio of 0.74, in contrast to 1.26 in other MDS (P = .020, Table 1).

Anemia was the more prevalent cytopenia in del(11q) patients with a median hemoglobin concentration of 9.9 g/dL, similar to other MDS (P = .14, Table 1; supplemental Figure 2). The mean corpuscular volume was slightly but significantly higher in del(11q) patients (median of 101 fL vs 99.5 fL, P = .035, Table 1; supplemental Figure 2). A significant lower monocyte count was found in del(11q) patients with a median of 0.2×10^9/L compared with 0.4×10^9/L in other MDS (P = .0004; Table 1; supplemental Figure 2). The white blood cell, neutrophil, and lymphocyte counts and the rate of neutropenia, the platelet count, and the rate of thrombocytopenia were similar to other MDS (Table 1; supplemental Figure 2).

The BM smears were reviewed centrally under the coordination of the Groupe Francophone d'Hématologie Cellulaire. The medullar blast count and dyserythropoiesis were similar in del(11q) patients compared with other MDS. Significantly, dysgranulopoiesis was less frequent and dysmegakaryopoiesis increased in del(11q) MDS patients (Table 1) with frequent presence of multinucleated megakaryocytes (Figures 1A-D). It is noteworthy that small and nonlobated megakaryocytes were present when del(11q) was associated to del(5q) (Figure 1D).

Chromosome 11q deletion is mainly interstitial and primary event

The del(11q) appeared mainly as an interstitial deletion extending from 11q13 to 11q24 in 102/113 cases (Figure 1E), either as a

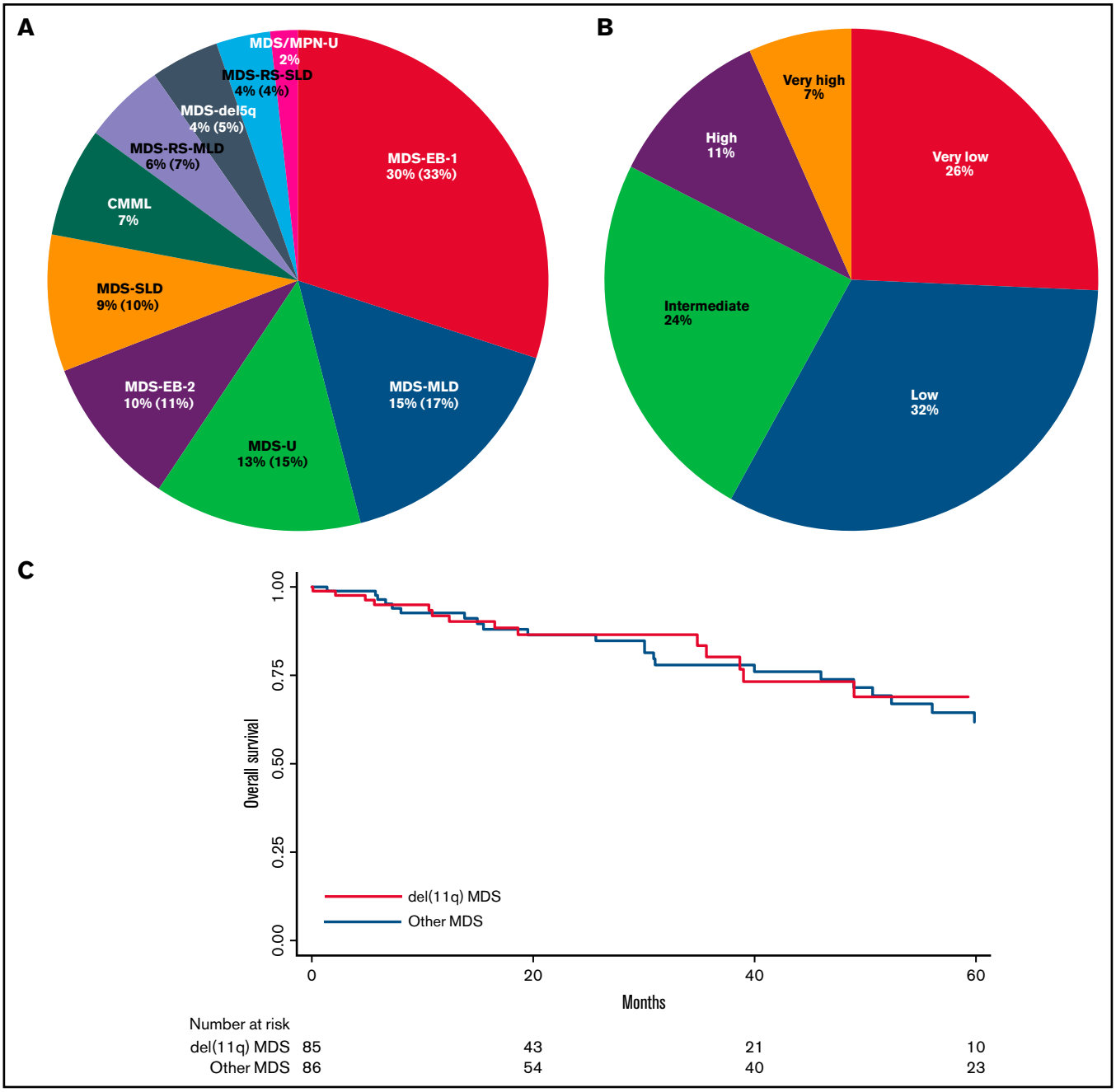

Figure 2. WHO 2016, IPSS-R classifications and overall survival of del(11q) MDS. (A) WHO 2016 classification of del(11q) MDS and MDS/MPN. (B) IPSS-R applied to the primary del(11q) MDS by risk category: very low = risk score ≤1.5; low = risk score >1.5-3; intermediate = risk score >3-4.5; high = risk score >4.5-6; very high = risk score >6. (C) Overall survival of del(11q) MDS compared with other MDS.

large deletion leading to a short del(11q) chromosome (11q shorter than 11p, 49 cases) or as a smaller deletion (11q appearing equal in size or longer than 11p, 53 cases). In 11 cases, the deletion of chromosome 11q was more complex, presenting cytogenetically as an unbalanced translocation, a di- or tri-centric chromosome 11, or a ring chromosome 11.

In approximately half of cases (48%, 54/113), del(11q) was present as an isolated clonal abnormality ([CA]; supplemental Table 1). Additional chromosomal abnormalities (ACA) were present in the remaining 59 cases, mainly as a single ACA (38%) thus leading to a double clonal abnormality karyotype as defined by IPSS-R or less frequently as multiple ACA, thus leading to a complex karyotype (14%). Only 3 cases have more than 3 CA (very complex karyotype as defined by IPSS-R[5]). The del(5q) abnormality was the most prevalent ACA (20%) followed by trisomy 8 (10%) and loss of chromosome Y (14% of male patients, supplemental Table 1). No monosomy 7, del(7q), or 3q26 abnormalities were identified in the 113 del(11q) cases. The del(11q) was mainly primary (59%, 67/113 cases); only 8 cases had del(11q) as a secondary cytogenetic

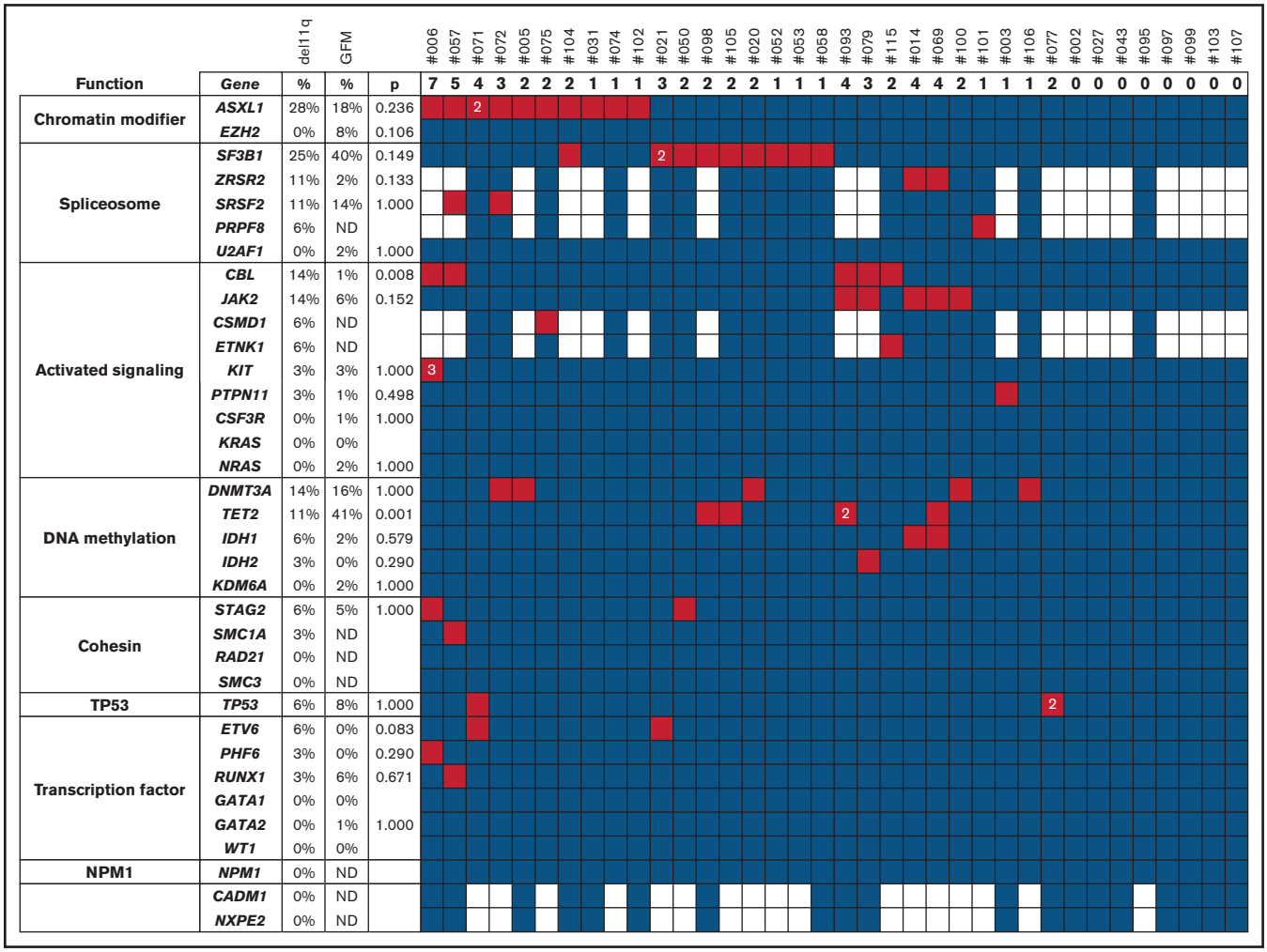

Figure 3. Mutations in del(11q) MDS. Mutation screening of 36 del(11q) MDS by next generation sequencing. Genes were classified according to their function (2 first columns). Comparison with other MDS is indicated in the 3 central columns (del[11q] MDS mutation frequency, other MDS mutation frequency, and *P* value comparing the frequencies between these 2 groups). Mutations are indicated by red squares containing the number of mutations per sample if higher than 1, the absence of mutations by green squares, and the absence of analysis by white squares. Patients are labeled at the top.

abnormality, including 2 cases with a del(5q) as a primary abnormality. Two cases presented with 2 independent clones (supplemental Table 1).

Patients with del(11q) have a prognosis similar to other MDS

Del(11q) cases were classified according to WHO 2016,[2] the most frequent being MDS with excess blasts type 1 (MDS-EB-1, 30%) followed by MDS with multilineage dysplasia (MDS-MLD, 15%), unclassifiable MDS (MDS-U, 13%), and MDS with excess blasts type 2 (MDS-EB-2, 10%; Figure 2A). The IPSS-R risk scoring[5,13] combining cytological and cytogenetic data were defined in 74 primary MDS cases with del(11q) as very low in 26%, low in 32%, intermediate in 24%, high in 11%, and very high in 7% (vs 16%, 52%, 16%, 9%, and 6% in other MDS, respectively), with a significant lower frequency of low IPSS-R risk group in del(11q) (32% vs 52%, $P = .015$; supplemental Table 1; Figure 2B). Recently, the IPSS-R scoring had been shown to apply also to *t*-MDS cases,[17] revealing also in our series a majority of low or very low risk MDS in del(11q) *t*-MDS (7/18 and 6/18 cases, respectively; supplemental Table 1).

The clinical follow-up was 22 months for del(11q) patients and 39 months for other MDS, on average. Transformation to AML was seen in 6 del(11q) patients out of 87 (7%) and 11 out of 72 patients for other MDS (15%, $P = .089$), keeping in mind than the follow-up was longer in the other MDS group. Overall survival was similar between del(11q) MDS and other MDS patients ($P = .84$, Figure 2C).

CBL mutation rate is significantly increased in del(11q) MDS

The *CBL* gene located in the 11q23.3 chromosomal band is recurrently mutated in MDS.[6,14,20] *CBL* is significantly more frequently mutated in del(11q) compared with other MDS (14% vs 1%, respectively, $P = .008$, Figure 3; supplemental Figure 3;

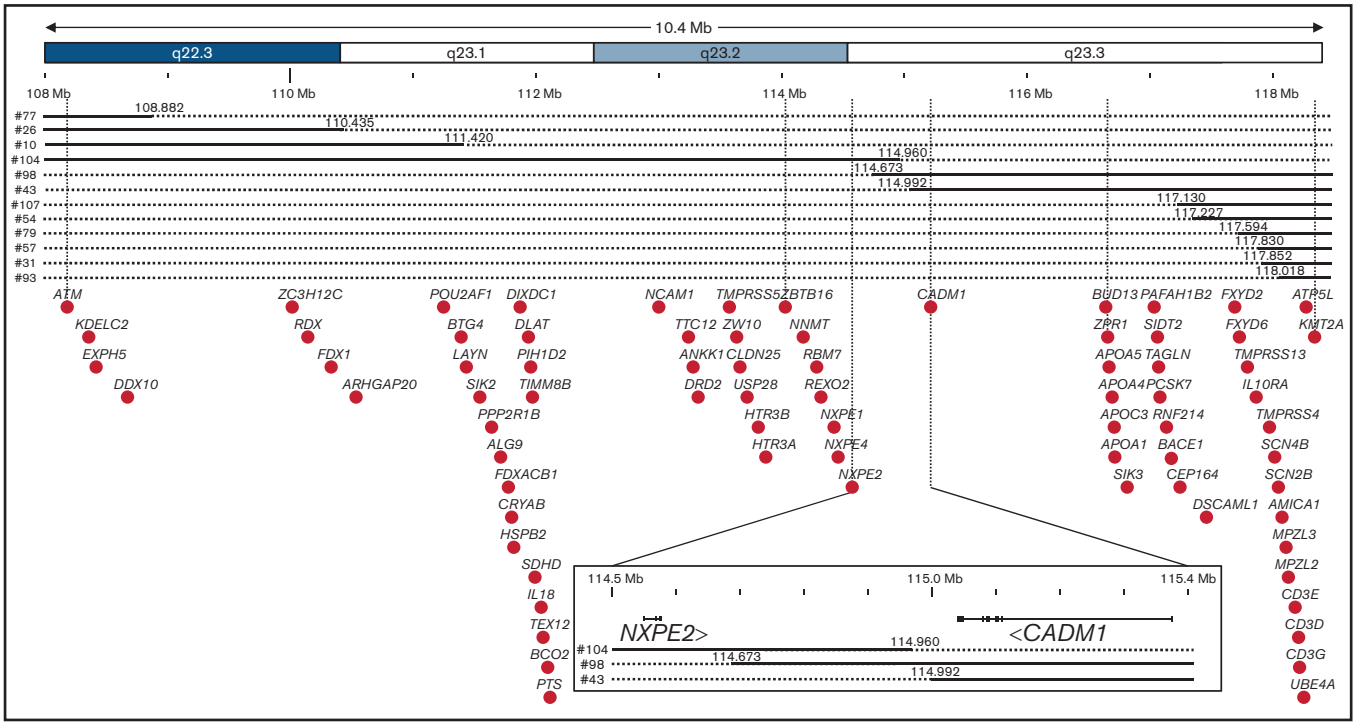

Figure 4. CDR of del(11q) MDS defined by high resolution microarray analysis. Detailed map of the chromosome 11 summarizing microarray data. Chromosomal bands are indicated at the top with the genomic location indicated below according to *hg19* assembly human genome. Continuous line: presence of the 2 alleles; dashed line: deletion of 1 of the 2 alleles. Genes located in the region are indicated by black dots. The common deleted region was indicated by vertical lines with a magnification of it at the bottom, indicating location of exons of *NXPE2* and *CADM1*, their orientation, and the 3 samples defining the del(11q) MDS CDR.

supplemental Table 3). In addition to mutations, the *CBL* gene was deleted in 84% of del(11q) (49/58; supplemental Figure 3). *CBL* deletions without *CBL* mutations were detected in 74% (43 cases), *CBL* mutations without *CBL* deletions in 5% (3 cases), and both deletions and mutations of *CBL* occurred in only 6 cases (10%, supplemental Figure 3). Overall, *CBL* abnormalities were very frequent but not constant in del(11q) MDS.

In addition to *CBL* mutations, a targeted resequencing of 36 del(11q) cases revealed frequent mutations in *ASXL1* (28%), *SF3B1* (25%), *DNMT3A* (14%), *JAK2* (14%), *SRSF2* (11%), *TET2* (11%), and *ZRSR2* (11%). These mutations were present at a similar rate compared with other MDS except for *TET2*, which was much less frequently mutated in del(11q) compared with other MDS (11% vs 41% respectively, $P = .001$; Figure 3; supplemental Table 3). *SF3B1* mutations were significantly associated within del(11q) MDS with the presence of ring sideroblasts (4/4, $P = .002$), similar to other MDS (27/30, $P < .001$, Figure 3).

CADM1 is a candidate gene for the del(11q) CDR

The del(11q) CDR was first investigated by FISH analysis using proximal (*CCND1*, 11q13.3) and distal probes (D11S1037, subtelomeric, 11q25; supplemental Figure 1B). Probes were retained on both copies of chromosome 11 in 37/37 cases tested for *CCND1* and 51/54 cases for D11S1037. The 3 cases with an 11q25 telomeric terminal deletion were the cases with a complex mechanism of del(11q) described above. Thus, FISH analysis confirms that all tested cases with a simple del(11q) are interstitial deletions (supplemental Figure 1B; supplemental Table 1). Inside this large 11q region, *ATM* and/or *KMT2A* were deleted in all cases, being both deleted in 86% of the cases (95/110). Fifteen cases lost only 1 of these 2 genes, either *ATM* but not *KMT2A* in 11 cases ($ATM^-/KMT2A^+$) or *KMT2A* but not *ATM* in 4 cases ($ATM^+/KMT2A^-$). Consequently, the CDR by FISH analysis is located between these 2 genes, in a 10 Mb region (supplemental Figure 1B; supplemental Table 1).

To delineate the CDR at a much higher resolution, 12 cases that deleted only *ATM* (8 $ATM^-/KMT2A^+$) or *KMT2A* (4 $ATM^+/KMT2A^-$) were analyzed by comparative genomic hybridization analysis allowing to refine the CDR to a 0.4 Mb intergenic region between *CADM1* and *NXPE2* (Figure 4; supplemental Figure 4). *CADM1* (cell adhesion molecule 1), also known as *TSLC1* (tumor suppressor in lung cancer 1) or *IGSF4* (immunoglobulin superfamily member 4), encodes a protein of 471 amino acids involved in cell-cell adhesion[21] and was initially identified as a tumor-suppressor gene in human non–small cell lung[22] and nasopharyngeal carcinoma.[23] *NXPE2* (neurexophilin and PC-esterase domain family member 2) encodes a protein of 559 amino acids without defined function. The reading frames of these 2 genes were deleted in all patients but 2 cases for *CADM1* (#43 and #98) and 1 case for *NXPE2* (#104; Figure 4). This intergenic region deleted between *NXPE2* and *CADM1* in those 3 cases contain 4 regulatory elements (e35780, e35781, e35783, and e35784) binding to the *Cadm1* promoter according to Encode 3,[24] whose deletion could impair its expression (supplemental Figure 5).

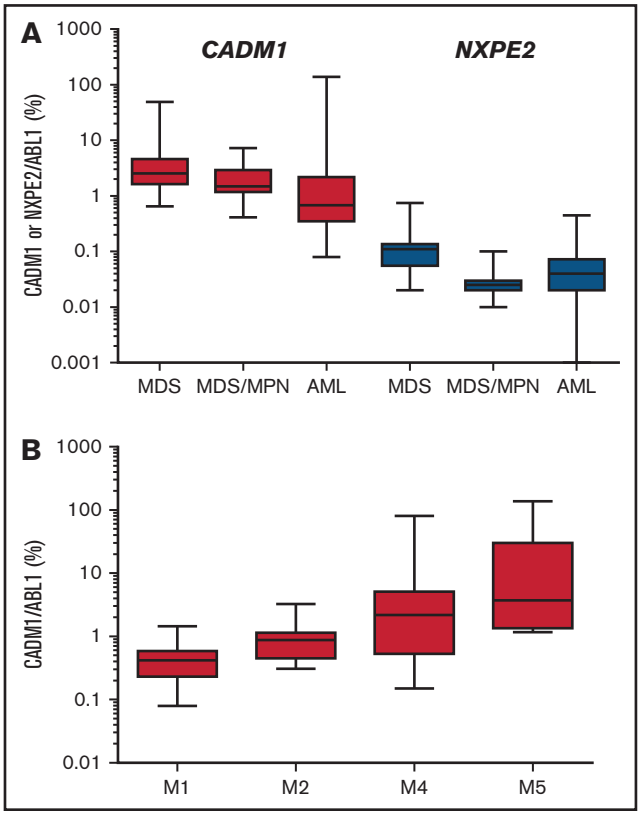

Figure 5. Expression of CADM1 and NXPE2 in myeloid cells. (A) Expression of *CADM1* and *NXPE2* in 21 MDS, 10 chronic myelo-monocytic leukemias, and 55 AML samples presented as a box with whiskers. The box extends from the 25th to the 75th percentiles of expression. The line in the middle of the box was the median. The whiskers indicate the 10th and the 90th percentiles. (B) Expression of *CADM1* in AML according to their FAB classification.

None of these 2 genes was mutated in a cohort of 89 myeloid malignancies, including 20 MDS with del(11q) (Figure 3). Expression of these 2 genes reveals that *CADM1* was expressed 71-fold higher (mean expression relative to *ABL1*: 5.9%, range 0.08% to 138%) than *NXPE2* (mean 0.08%, range 0% to 0.75%). *NXPE2* expression was not detected in 6 cases (Figure 5A; supplemental Table 2). Expression of *CADM1* was higher in acute monoblastic/monocytic (M5a/M5b) and myelomonocytic (M4) leukemias compared with acute myeloblastic leukemias (M1/M2), suggesting a higher expression of *CADM1* in monocytic progenitors (Figure 5B; supplemental Table 2). Although we were unable to evaluate the expression of these genes in our del(11q) MDS cases, the high expression of *CADM1* in myeloid malignancies and its almost constant deletion in del(11q) strongly suggest that it represents a candidate gene of the del(11q) CDR in MDS.

Genomic editing of Cadm1 perturbs myeloid development

To investigate the effect of *Cadm1* loss on hematopoietic development in vivo, we edited *Cadm1* gene by clustered regularly interspaced short palindromic repeats-*Cas9* (CRISPR-*Cas9*) strategy. First, we designed 3 sgRNAs targeting the genomic region encoding the exon 2 of *Cadm1*. To select the sgRNA exhibiting the most editing efficiency of *Cadm1* locus, we transduced Lin$^-$ cells from the BM of Cas9-expressing mice with lentiviral vectors containing the sgCadm1 #1, #2, #3, or a nontargeted control sgRNA-encoding sequence (sgCTL) (supplemental Figures 6A-B). The sgCadm1 #2 exhibited the highest rate of insertions/deletions (InDels) (supplemental Figures 6C-D). Therefore, sgCadm1 #2 was selected to perform the following in vitro and in vivo functional experiments. Purified Cas9-expressing LSK cells, a population enriched in hematopoietic stem/progenitor cells (HSPCs),[25] were transduced with sgCTL or sgCadm1 #2 lentiviral vector, both expressing concomitantly the GFP (Figure 6A). The sgCadm1 #2 allowed for an efficient transduction (87.7%), as revealed by the percentage of GFP$^+$ cells 8 days after the in vitro expansion in methylcellulose (supplemental Figure 7A) and an efficient genomic editing, as revealed by the presence of 89% of InDels in the *Cadm1* locus (supplemental Figures 7B-C). The numbers of GFP$^+$ colonies in methylcellulose (total, CFU-GEMM, CFU-GM, and BFU-E) were similar between sgCTL and sgCadm1 #2 (Figure 6B), indicating that *Cadm1* deletion did not affect the clonogenic activity of hematopoietic progenitors. In parallel, CD45.2$^+$ *Cas9*-expressing LSK cells transduced with sgCTL or sgCadm1 #2 were transplanted into CD45.1$^+$ syngenic recipient mice (Figure 6A). The analysis of engrafted donor-derived cells (CD45.2$^+$GFP$^+$) revealed that the proportions of the hematopoietic stem/progenitor compartments (LSK, LK, MEP/CMP, and GMP cells) were not altered by *Cadm1* deletion (supplemental Figures 8A-B), corroborating our previous observations in clonogenic assay (Figure 6B). In addition, thymic reconstitution of donor-derived cells was not modified by *Cadm1* deletion (supplemental Figures 8C-D), indicating that *Cadm1* deletion did not alter the multipotent activity of HSPCs. However, we observed that *Cadm1* deletion modified the relative proportions of myeloid and B-cells in the BM of recipient mice (Figures 6C-D). Thus, we purified donor-derived cells (CD45.2$^+$GFP$^+$) from sgCTL and sgCadm1 #2 transplanted mice. *Cadm1* was edited in almost all transduced CD45.2$^+$ cells in transplanted mice (Figure 6E; supplemental Figure 9), with the presence of only a few distinct InDels in engrafted cells underlying the oligoclonal dynamic of hematopoietic reconstitution from HSPCs.[26] In accordance with the immunophenotype of donor-derived cells (Figures 6C-D), *Cadm1* deletion perturbed the balance between myeloid and lymphoid cells (Figure 6F). Finally, donor-derived cells (CD45.2$^+$GFP$^+$) from primary BM were transplanted into secondary recipients to assess the impact of *Cadm1* deletion on the long-term hematopoietic reconstitution. While secondary recipients did not develop hematopoietic neoplasms even after a period of 21 months (supplemental Figure 10A), the balance between myeloid and lymphoid cells was still perturbed in engrafted sgCadm1 donor-derived cells (CD45.2$^+$GFP$^+$) 2 and 8 months after transplantation (supplemental Figures 10B-C), suggesting an intrinsic differentiation bias in those cells. Together, this functional in vivo approach suggests that *Cadm1* loss-of-function perturbs the terminal myeloid cell production.

Discussion

Deletion of chromosome 11 is recurrently found in MDS and other hematological malignancies, such as chronic lymphocytic leukemia (CLL),[27,28] mantle cell lymphoma,[29] and in solid tumors such as lung cancer,[22] nasopharyngeal carcinomas,[23] gliomas,[30] and neuroblastoma.[31] Our analysis of del(11q) MDS represents the largest study of this very rare but recurrent chromosomal deletion. In our

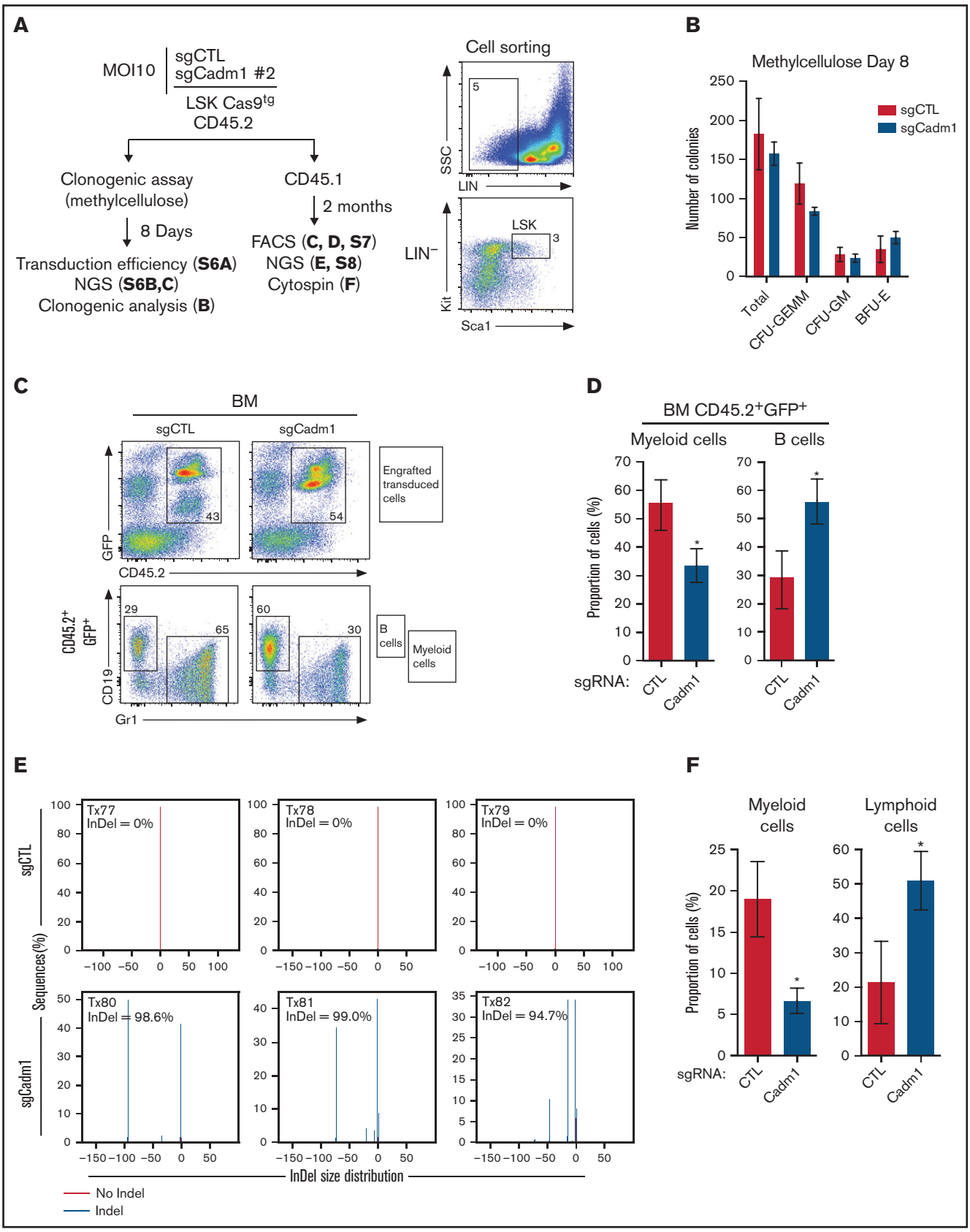
A
MOI10
sgCTL
sgCadm1 #2
LSK Cas9tg
CD45.2
Clonogenic assay
(methylcellulose)
8 Days
Transduction efficiency (S6A)
NGS (S6B,C)
Clonogenic analysis (B)
CD45.1
2 months
FACS (C, D, S7)
NGS (E, S8)
Cytospin (F)
Cell sorting
SSC
LIN
LIN−
Kit
Sca1
LSK
B
Methylcellulose Day 8
Number of colonies
sgCTL
sgCadm1
Total
CFU-GEMM
CFU-GM
BFU-E
C
BM
sgCTL
sgCadm1
GFP
CD45.2
Engrafted transduced cells
CD45.2+ GFP+
CD19
Gr1
B cells
Myeloid cells
D
BM CD45.2+GFP+
Myeloid cells
B cells
Proportion of cells (%)
sgRNA:
CTL
Cadm1
E
Tx77
InDel = 0%
Tx78
InDel = 0%
Tx79
InDel = 0%
Tx80
InDel = 98.6%
Tx81
InDel = 99.0%
Tx82
InDel = 94.7%
Sequences (%)
InDel size distribution
No Indel
Indel
F
Myeloid cells
Lymphoid cells
Proportion of cells (%)
sgRNA:
CTL
Cadm1

Figure 6.

MDS cohort, del(11q) was mainly a primary clonal abnormality found predominantly in women presenting with mild cytopenias, a low monocyte blood count, and the frequent presence of multinucleated megakaryocytes. MDS with del(11q) were enriched in *CBL* gain-of-function mutations in a similar way to acquired uniparental disomy of chromosome 11 in MDS and MDS/MPN.[32] *ASXL1* and *SF3B1* mutations were also frequent in del(11q) MDS, although at a similar rate compared with other MDS.[8,33] *TET2* mutations, in contrast, were less frequent than in other MDS.[20]

Our extended analysis of del(11q) MDS identified a unique interstitial CDR between *ATM* (11q22.3) and CBL (11q23.3), leading to a hemizygous deletions of *CADM1* (deleted in 98% of del(11q) MDS), *ATM* (deleted in 96%), *KMT2A* (deleted in 90%), and *CBL* genes (deleted in 84%). The del(11q) MDS CDR is different from the 1 identified in CLL where *ATM* has been defined as the critical gene.[27,28] As shown in other deletions in MDS, the effect of del(11q) might be due to concomitant deletions of *ATM*, *CBL,* and *KMT2A,* in addition to *CADM1* leading to MDS and MDS/MPN development. *ATM* encodes a serine/threonine kinase which is critical to the phosphorylation of numerous proteins during the cell cycle, including p53, Brca1, Chk2, Rad17, and Rad9. Germline mutations in *ATM* are associated with ataxia telangiectasia, an autosomal recessive disorder.[34] *CBL* encodes an E3 ubiquitin ligase that ubiquitinates signaling proteins, such as the tyrosine kinases Flt3, PDGFRA, Kit, or Jak2. In adult patients, *CBL* mutations are found in ~5% of myeloid neoplasms,[35] mainly in chronic myelo-monocytic leukemias,[36] but are very rare in MDS.[37] With a frequency of mutations of 14% in our series and 8% in the Stengel series,[8] *CBL* was more frequently mutated in del(11q) MDS than in other MDS.

The unique minimal CDR seen in del(11q) MDS is centered in an intergenic region between *NXPE2* and *CADM1*. Expression and functional analysis of *CADM1* suggest a specific function in hematopoiesis. *CADM1* encodes a glycoprotein of 471 amino acids composed of 3 immunoglobulin-like domains involved in cell adhesion: a transmembrane domain and a short cytoplasmic domain with a binding motif homologous to that of glycophorin C.[38] It forms homodimers at the cell membrane[22] with homophilic[39] and heterophilic[40] cell-cell adhesion activity. Few publications investigated the mechanism of action of *Cadm1* in the development of tumors, rendering it difficult to distinguish a direct effect by haploinsufficiency to an obligatory 2-hit transformation. Non–small cell lung cancer cells present with a frequent loss of heterozygosity of *CADM1* associated with a frequent inactivation of the second *CADM1* allele by promoter methylation.[22] The recurrent deletion of *CADM1* is also found in other solid tumors, such as liver, pancreatic, and prostate cancers, and especially in metastatic tumors.[38,41] In acute lymphoblastic leukemias, *CADM1* promoter is frequently methylated and inactivated.[42] Its homozygous inactivation in mice is viable but associated with a male infertility.[43-45] The heterozygous mutant mice have no reported abnormalities. *Cadm1*-null mice die significantly faster than wildtype mice due to spontaneous tumor development.[46] Van der Weiden et al challenged *Cadm1*-null mice by irradiation leading to the development of lymphomas and leukemias.[46] Recently, the extracellular domain of CADM1 has been shown to restrict tumor growth and metastases by interacting with HER2 and integrin $\alpha 6\beta 4$ at the cell surface and reducing STAT3 activation.[47] The inhibition of this signaling pathway using the JAK1/2 inhibitor ruxolitinib replicates these effects specifically in *CADM1*-null cells.[47] This drug, which is mainly used in myelofibrosis, has been tested in only 1 MDS clinical trial, but none of the 11 patients investigated displayed a del(11q).[48] Consequently, ruxolitinib could be an interesting targeted therapy to be specifically evaluated in del(11q) MDS patients.

Here, we used CRISPR strategy to address the effect of *Cadm1* deletion on hematopoietic development at the functional level. Although not altering their multilineage hematopoietic reconstitution potential, the genomic editing of *Cadm1* of murine LSK cells diminished the myeloid-to-lymphoid ratio in BM after primary and secondary transplantations. However, this phenotype was not associated with the long-term development of a myeloid neoplasm. This observation suggests that *Cadm1* deletion is not sufficient by itself to induce clonal transformation in mice and requires collaborative oncogenic events, such as *CBL* or *ASXL1* mutations and additional deletions in the del(11q) region, as observed in del(11q) MDS patients. Nevertheless, the perturbation of myeloid cell production and/or terminal differentiation observed in mice could mimic a preneoplastic state before malignant transformation.

In summary, our report identifies unique features of del(11q) MDS with various combinations of deletions of 4 genes, *ATM*, *CBL*, *KMT2A,* and *CADM1,* a tumor suppressor gene identified initially in solid tumors.

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Figure 6. Functional inactivation of Cadm1 in mice. (A) Experimental procedure to study the effect of *Cadm1* edition on murine hematopoietic development (left panel). LSK cells from *Cas9*-expressing mice were purified by cell sorting (right panel) and transduced with either sgCadm1#2 or control sgRNA (sgCTL) lentiviral vectors. Transduced *Cas9*-expressing LSK cells (CD45.2$^+$) were either plated in clonogenic assay or transplanted into syngenic recipient mice (CD45.1$^+$, n = 3 for each condition). (B) Number of total CFU-GEMM, CFU-GM, and BFU-E colonies in clonogenic assay. (C) BM chimerism, analyzed by FACS 2 months after transplantation, illustrated by the percentage of transduced donor cells (% CD45.2$^+$GFP$^+$) found in the recipient BM (upper panel). Immunophenotype of engrafted donor-derived cells (CD45.2$^+$GFP$^+$) was analyzed using the Gr1 (Myeloid cells, My) and CD19 (B-cells) markers. FACS profiles and gating strategy were shown for 1 representative mouse of each condition (lower panel). (D) Proportion of myeloid and B cells (%) within total donor-derived CD45.2$^+$GFP$^+$ cells from the BM of recipient mice. (E-F) Donor-derived cells (CD45.2$^+$GFP$^+$) from each sgCTL and sgCadm1 transplanted mouse purified by cell sorting. Genome editing efficiency of targeted *Cadm1* region performed on purified donor-derived cells (CD45.2$^+$GFP$^+$) for each condition (E). Cytological analysis was performed on purified CD45.2$^+$GFP$^+$ cells from each condition, and the proportion of myeloid and lymphoid cells was calculated (F).

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A full list of study group members appears in "Appendix."

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References

1. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: International Agency for Research on Cancer; 2008.
2. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: International Agency for Research on Cancer; 2017.
3. Adès L, Itzykson R, Fenaux P. Myelodysplastic syndromes. *Lancet.* 2014;383(9936):2239-2252.

4. Hellström-Lindberg E, Tobiasson M, Greenberg P. Myelodysplastic syndromes: moving towards personalized management. *Haematologica.* 2020; 105(7):1765-1779.

5. Greenberg PL, Tuechler H, Schanz J, et al. Revised international prognostic scoring system for myelodysplastic syndromes. *Blood.* 2012;120(12): 2454-2465.

6. Ogawa S. Genetics of MDS. *Blood.* 2019;133(10):1049-1059.

7. Wang SA, Abruzzo LV, Hasserjian RP, et al. Myelodysplastic syndromes with deletions of chromosome 11q lack cryptic MLL rearrangement and exhibit characteristic clinicopathologic features. *Leuk Res.* 2011;35(3):351-357.

8. Stengel A, Kern W, Meggendorfer M, Haferlach T, Haferlach C. MDS with deletions in the long arm of chromosome 11 are associated with a high frequency of SF3B1 mutations. *Leukemia.* 2017;31(9):1995-1997.

9. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood.* 2009;114(5):937-951.

10. Solé F, Prieto F, Badia L, et al. Cytogenetic studies in 112 cases of untreated myelodysplastic syndromes. *Cancer Genet Cytogenet.* 1992;64(1): 12-20.

11. Kobayashi H, Espinosa R III, Fernald AA, et al. Analysis of deletions of the long arm of chromosome 11 in hematologic malignancies with fluorescence in situ hybridization. *Genes Chromosomes Cancer.* 1993;8(4):246-252.

12. Haase D, Germing U, Schanz J, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. *Blood.* 2007;110(13):4385-4395.

13. Schanz J, Tüchler H, Solé F, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. *J Clin Oncol.* 2012;30(8):820-829.

14. Klampfl T, Milosevic JD, Puda A, et al. Complex patterns of chromosome 11 aberrations in myeloid malignancies target CBL, MLL, DDB1 and LMO2. *PLoS One.* 2013;8(10):e77819.

15. McGowan-Jordan J, Simons A, Schmid M, eds. ISCN 2016: An International System for Human Cytogenomic Nomenclature. New York, NY: Karger Medical Scientific; 2016.

16. Chun K, Hagemeijer A, Iqbal A, Slovak ML. Implementation of standardized international karyotype scoring practices is needed to provide uniform and systematic evaluation for patients with myelodysplastic syndrome using IPSS criteria: An International Working Group on MDS Cytogenetics Study. *Leuk Res.* 2010;34(2):160-165.

17. Kuendgen A, Nomdedeu M, Tuechler H, et al. Therapy-related myelodysplastic syndromes deserve specific diagnostic sub-classification and risk-stratification-an approach to classification of patients with t-MDS. *Leukemia.* 2021;35(3):835-849.

18. Heckl D, Kowalczyk MS, Yudovich D, et al. Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. *Nat Biotechnol.* 2014;32(9):941-946.

19. Pinello L, Canver MC, Hoban MD, et al. Analyzing CRISPR genome-editing experiments with CRISPResso. *Nat Biotechnol.* 2016;34(7):695-697.

20. Bersanelli M, Travaglino E, Meggendorfer M, et al. Classification and Personalized Prognostic Assessment on the Basis of Clinical and Genomic Features in Myelodysplastic Syndromes. *J Clin Oncol.* 2021;39(11):1223-1233.

21. Masuda M, Yageta M, Fukuhara H, et al. The tumor suppressor protein TSLC1 is involved in cell-cell adhesion. *J Biol Chem.* 2002;277(34): 31014-31019.

22. Kuramochi M, Fukuhara H, Nobukuni T, et al. TSLC1 is a tumor-suppressor gene in human non-small-cell lung cancer. *Nat Genet.* 2001;27(4): 427-430.

23. Lung HL, Cheng Y, Kumaran MK, et al. Fine mapping of the 11q22-23 tumor suppressive region and involvement of TSLC1 in nasopharyngeal carcinoma. *Int J Cancer.* 2004;112(4):628-635.

24. Gorkin DU, Barozzi I, Zhao Y, et al. An atlas of dynamic chromatin landscapes in mouse fetal development [published corrections appear in Nature. 2020;586(7831):E31 and 2021;589(7842):E4]. *Nature.* 2020;583(7818):744-751.

25. Miller CL, Dykstra B, Eaves CJ. Characterization of mouse hematopoietic stem and progenitor cells. Curr Protoc Immunol. 2008; 22B.22.21-22B.22.31.

26. Jordan CT, Lemischka IR. Clonal and systemic analysis of long-term hematopoiesis in the mouse. *Genes Dev.* 1990;4(2):220-232.

27. Stilgenbauer S, Liebisch P, James MR, et al. Molecular cytogenetic delineation of a novel critical genomic region in chromosome bands 11q22.3-923.1 in lymphoproliferative disorders. *Proc Natl Acad Sci USA.* 1996;93(21):11837-11841.

28. Stankovic T, Kwok M. Genetics in the era of targeted CLL therapy. *Blood.* 2020;135(26):2333-2334.

29. Schaffner C, Idler I, Stilgenbauer S, Döhner H, Lichter P. Mantle cell lymphoma is characterized by inactivation of the ATM gene. *Proc Natl Acad Sci USA.* 2000;97(6):2773-2778.

30. Houshmandi SS, Surace EI, Zhang HB, Fuller GN, Gutmann DH. Tumor suppressor in lung cancer-1 (TSLC1) functions as a glioma tumor suppressor. *Neurology.* 2006;67(10):1863-1866.

31. Mlakar V, Jurkovic Mlakar S, Lopez G, Maris JM, Ansari M, Gumy-Pause F. 11q deletion in neuroblastoma: a review of biological and clinical implications. *Mol Cancer.* 2017;16(1):114.

32. Sanada M, Suzuki T, Shih LY, et al. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature.* 2009;460(7257): 904-908.

33. Tefferi A, Idossa D, Lasho TL, et al. Mutations and karyotype in myelodysplastic syndromes: TP53 clusters with monosomal karyotype, RUNX1 with trisomy 21, and SF3B1 with inv(3)(q21q26.2) and del(11q). *Blood Cancer J.* 2017;7(12):658.

34. Stankovic T, Skowronska A. The role of ATM mutations and 11q deletions in disease progression in chronic lymphocytic leukemia. *Leuk Lymphoma.* 2014;55(6):1227-1239.

35. Kales SC, Ryan PE, Nau MM, Lipkowitz S. Cbl and human myeloid neoplasms: the Cbl oncogene comes of age. *Cancer Res.* 2010;70(12): 4789-4794.

36. Patnaik MM, Tefferi A. Chronic Myelomonocytic leukemia: 2020 update on diagnosis, risk stratification and management. *Am J Hematol.* 2020; 95(1):97-115.

37. Makishima H, Cazzolli H, Szpurka H, et al. Mutations of e3 ubiquitin ligase cbl family members constitute a novel common pathogenic lesion in myeloid malignancies. *J Clin Oncol.* 2009;27(36):6109-6116.

38. Murakami Y. Involvement of a cell adhesion molecule, TSLC1/IGSF4, in human oncogenesis. *Cancer Sci.* 2005;96(9):543-552.

39. Biederer T, Sara Y, Mozhayeva M, et al. SynCAM, a synaptic adhesion molecule that drives synapse assembly. *Science.* 2002;297(5586): 1525-1531.

40. Shingai T, Ikeda W, Kakunaga S, et al. Implications of nectin-like molecule-2/IGSF4/RA175/SgIGSF/TSLC1/SynCAM1 in cell-cell adhesion and transmembrane protein localization in epithelial cells. *J Biol Chem.* 2003;278(37):35421-35427.

41. Faraji F, Pang Y, Walker RC, Nieves Borges R, Yang L, Hunter KW. Cadm1 is a metastasis susceptibility gene that suppresses metastasis by modifying tumor interaction with the cell-mediated immunity. *PLoS Genet.* 2012;8(9):e1002926.

42. Paulsson K, An Q, Moorman AV, et al. Methylation of tumour suppressor gene promoters in the presence and absence of transcriptional silencing in high hyperdiploid acute lymphoblastic leukaemia. *Br J Haematol.* 2009;144(6):838-847.

43. Fujita E, Kouroku Y, Ozeki S, et al. Oligo-astheno-teratozoospermia in mice lacking RA175/TSLC1/SynCAM/IGSF4A, a cell adhesion molecule in the immunoglobulin superfamily. *Mol Cell Biol.* 2006;26(2):718-726.

44. van der Weyden L, Arends MJ, Chausiaux OE, et al. Loss of TSLC1 causes male infertility due to a defect at the spermatid stage of spermatogenesis. *Mol Cell Biol.* 2006;26(9):3595-3609.

45. Yamada D, Yoshida M, Williams YN, et al. Disruption of spermatogenic cell adhesion and male infertility in mice lacking TSLC1/IGSF4, an immunoglobulin superfamily cell adhesion molecule. *Mol Cell Biol.* 2006;26(9):3610-3624.

46. van der Weyden L, Arends MJ, Rust AG, Poulogiannis G, McIntyre RE, Adams DJ. Increased tumorigenesis associated with loss of the tumor suppressor gene Cadm1. *Mol Cancer.* 2012;11(1):29.

47. Vallath S, Sage EK, Kolluri KK, et al. CADM1 inhibits squamous cell carcinoma progression by reducing STAT3 activity. *Sci Rep.* 2016;6(1): 24006.

48. Abaza Y, Hidalgo-Lopez JE, Verstovsek S, et al. Phase I study of ruxolitinib in previously treated patients with low or intermediate-1 risk myelodysplastic syndrome with evidence of NF-kB activation. *Leuk Res.* 2018;73:78-85.