5 Lengfelder E, Hofmann WK, Nowak D. Impact of arsenic trioxide in the treatment of acute promyelocytic leukemia. *Leukemia* 2012; **26**: 433–442.
6 Sanz MA, Grimwade D, Tallman MS, Lowenberg B, Fenaux P, Estey EH *et al.* Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood* 2009; **113**: 1875–1891.
7 Creutzig U, Zimmermann M, Dworzak M, Urban C, Henze G, Kremens B *et al.* Favourable outcome of patients with childhood acute promyelocytic leukaemia after treatment with reduced cumulative anthracycline doses. *Br J Haematol* 2010; **149**: 399–409.
8 Zhang XW, Yan XJ, Zhou ZR, Yang FF, Wu ZY, Sun HB *et al.* Arsenic trioxide controls the fate of the PML-RARalpha oncoprotein by directly binding PML. *Science* 2010; **328**: 240–243.
9 Arnould C, Philippe C, Bourdon V, Gregoire MJ, Berger R, Jonveaux P. The signal transducer and activator of transcription STAT5b gene is a new partner of retinoic acid receptor alpha in acute promyelocytic-like leukaemia. *Hum Mol Genet* 1999; **8**: 1741–1749.
10 Gallagher RE, Mak S, Paietta E, Cooper B, Ehmann WC, Tallman MS. Identification of a second acute promyelocytic leukemia (APL) patient with the STAT5b-RAR fusion gene among PML-RAR -negative Eastern Cooperative Oncology Group (ECOG) APL Protocol Registrants. *Blood* 2004; **104**: 821a.
11 Kusakabe M, Suzukawa K, Nanmoku T, Obara N, Okoshi Y, Mukai HY *et al.* Detection of the STAT5B-RARA fusion transcript in acute promyelocytic leukemia with the normal chromosome 17 on G-banding. *Eur J Haematol* 2008; **80**: 444–447.
12 Iwanaga E, Nakamura M, Nanri T, Kawakita T, Horikawa K, Mitsuya H *et al.* Acute promyelocytic leukemia harboring a STAT5B-RARA fusion gene and a G596V missense mutation in the STAT5B SH2 domain of the STAT5B-RARA. *Eur J Haematol* 2009; **83**: 499–501.
13 Qiao C, Zhang SJ, Chen LJ, Miao KR, Zhang JF, Wu YJ *et al.* Identification of the STAT5B-RARalpha fusion transcript in an acute promyelocytic leukemia patient without FLT3, NPM1, c-Kit and C/EBPalpha mutation. *Eur J Haematol* 2011; **86**: 442–446.
14 Jovanovic JV, Rennie K, Culligan D, Peniket A, Lennard A, Harrison J *et al.* Development of real-time quantitative polymerase chain reaction assays to track treatment response in retinoid resistant acute promyelocytic leukemia. *Front Oncol* 2011; **1**: 35.
15 Chen H, Pan J, Yao L, Wu L, Zhu J, Wang W *et al.* Acute promyelocytic leukemia with a STAT5b-RARalpha fusion transcript defined by array-CGH, FISH, and RT-PCR. *Cancer Genet* 2012; **205**: 327–331.
16 Schnittger S, Bacher U, Haferlach C, Kern W, Alpermann T, Haferlach T. Clinical impact of FLT3 mutation load in acute promyelocytic leukemia with t(15;17)/PML-RARA. *Haematologica* 2011; **96**: 1799–1807.

Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)

A G polymorphism in the *CRBN* gene acts as a biomarker of response to treatment with lenalidomide in low/int-1 risk MDS without del(5q)

Leukemia (2013) **27**, 1610–1613; doi:10.1038/leu.2013.59

E3 ubiquitin ligase protein Cereblon (CRBN) has been identified as a direct molecular target for the teratogenicity of thalidomide and cytotoxicity of immunomodulatory drugs (IMiDs) such as lenalidomide.[1,2] *CRBN* gene is highly conserved and its related protein of 442 AA is ubiquitously expressed in humans. CRBN is part of a functional E3 ubiquitin ligase complex together with the DNA damage-binding protein-1 (DDB1), Cullin 4A and Roc1, and acts as a substrate receptor for client proteins to be ubiquitinated and degraded by proteasome.[1] CRBN itself undergoes auto-ubiquitination, and thalidomide is described as an inhibitory molecule for CRBN auto-ubiquitination activity.

Thalidomide or lenalidomide have demonstrated their efficacy in the treatment of various hematological disorders including myeloma[3] and myelodysplastic syndromes (MDS), either with or without deletion of 5q chromosome.[4–6] It has been recently shown that changes in gene expression patterns induced by lenalidomide are abrogated by the depletion of *CRBN* gene in myeloma cell lines.[7] Furthermore, $>85\%$ patients with low expression of *CRBN* are resistant to treatment with thalidomide, and, conversely, high *CRBN* gene levels correlate with better survival in myeloma patients treated with maintenance thalidomide.[7,8] In low/int-1 risk MDS, the erythroid response rate to thalidomide is quite low (16–30%) and responses are usually short,[9] whereas patients with low/int-1 risk MDS with del5q have a high response rate (50–67%) to lenalidomide.[5,10] By contrast, $>30\%$ of low/int-1 MDS patients without del(5q) respond to this treatment.[6,11] Altogether, these data provided us with a rationale to investigate the role of *CRBN* gene in the response to lenalidomide in low/int-1 MDS patients without del(5q).

Sixty-six MDS patients from five hematological centers with low/int-1 risk MDS without del5q were included in this study between January 2010 and December 2012. All patients were anemic and resistant to erythropoiesis-stimulating agents either epoetin alpha/beta (60 000 U/week) or darbopoetin (300 μg/week) during 12 weeks, and remained transfusion dependent ($\geqslant$4U of Red Blood Cells during the previous 8 weeks). They received a dose of 10 mg/day of lenalidomide for 21 days every 28 days, and the erythroid response rate, hematological improvement-erythroid was evaluated according to the IWG 2006 criteria after at least 3 months of treatment. Fifteen patients were not evaluable due to treatment toxicity or early death. The characteristics of the 51 remaining patients who completed 3 months of treatment (26 RARS, 8 RCMD-RS, 7 RCMD, 4 RA, 5 RAEB-1 and 1 CMML) are described in Table 1. Forty-one (80%) patients had a normal karyotype, five presented with trisomy 8, two presented del11q and one had del20q. Karyotype was unavailable for one patient. Comparisons using Pearson χ^2 or Fischer's exact test for qualitative data, and Wilcoxon test for quantitative data were performed on Stata MP 12.1 software (Ritme, Paris, France). All tests were two-sided and a $P<0.05$ were considered to have statistical significance.

Fourteen (27%) patients were responders to lenalidomide, according to IWG 2006 with 8 (57%) major responses and 6 (43%) minor responses according to IWG 2000, and 37 (73%) were nonresponders. No difference of age ($P=0.901$), sex ($P=0.334$) or International Prognostic Scoring System (IPSS; $P=0.324$) at treatment onset was observed between the two groups. The overall response rate was equivalent to that reported in the literature.[6,11]

The *CRBN* gene located in 3p26.2 contains 11 predicted exons. To address the putative role of *CRBN* in the response to lenalidomide in MDS patients, we conducted genetic studies

Accepted article preview online 26 February 2013; advance online publication, 26 April 2013

Table 1. Characteristics of 51 low/int-1 MDS patients without del(5)q and treated by lenalidomide

UPN	*Age (years)*	*WHO*	*Karyotype*	*IPSS*	*HI-E*	*CRBN (rs1672753)*
2	76	RARS	46,XX	low	No	A/A
4	75	RARS	46,XY	low	No	A/G
5	70	RCMD-RS	46,XY	low	No	A/A
15	62	RARS	46,XY	int-1	No	A/G
16	73	RARS	46,XY	int-1	No	A/A
21	66	RARS	46,XY	low	No	A/A
22	68	RCMD-RS	46,XX	int-1	No	A/A
24	55	RARS	46,XX	low	No	A/A
25	76	RARS	46,XX	low	No	A/G
27	63	CMML	46,XY	int-1	No	A/A
34	73	RARS	46,XX	low	No	A/A
36	63	RARS	46,XY	int-1	No	A/G
38	76	RAEB-1	46,XX	int-1	No	A/A
42	74	RARS	46,XX	int-1	No	A/A
45	64	RARS	46,XX	low	No	A/A
46	70	RAEB-1	46,XX,del(11)q	int-1	No	A/G
48	70	RA	46,XY	low	No	A/A
56	77	RCMD-RS	46,XY	low	No	A/A
57	77	RA	46,XX	low	No	A/A
61	67	RCMD	46,XY	low	No	A/A
62	79	RARS	45,X,-Y	low	No	A/G
63	76	RAEB-1	46,XY,del(12)(q21;q23-24)	int-1	No	A/G
67	62	RCMD	46,XY	low	No	A/A
69	75	RA	46,XX	low	No	A/A
70	80	RARS	NA	NA	No	A/A
73	62	RAEB-1	46,XY,del(20)(q11)	int-1	No	A/A
74	63	RARS	46,XY	int-1	No	A/A
75	75	RCMD	46,XY	low	No	A/A
83	77	RCMD-RS	46,XY	low	No	A/A
86	74	RCMD-RS	46,XX	low	No	A/A
87	71	RAEB-1	46,XY	int-1	No	A/A
99	65	RARS	46,XY	int-1	No	A/A
106	79	RARS	46,XX	low	No	A/A
111	72	RCMD-RS	46,XY	low	No	A/A
118	54	RARS	46,XX	int-1	No	G/G
126	77	RARS	46,XY	low	No	A/A
127	74	RARS	47,XY, +8	int-1	No	A/G
7	74	RA	46,XX	low	Yes	A/G
13	62	RCMD-RS	46,XY	int-1	Yes	A/G
39	62	RARS	46,XY	int-1	Yes	A/G
54	80	RCMD	47,XY, +8	int-1	Yes	A/A
93	77	RCMD	47,XX,del(11)q, +8	int-1	Yes	A/G
114	77	RCMD	46,XY	low	Yes	A/A
121	89	RCMD-RS	46,XY	low	Yes	A/G
132	73	RARS	46,XY	int-1	Yes	A/A
33	72	RCMD	46,XY	int-1	Yes	A/G
103	68	RARS	47,XY, +8	int-1	Yes	A/G
110	69	RARS	47,XY, +8	int-1	Yes	A/A
116	54	RARS	46,XX	low	Yes	A/A
123	74	RARS	46,XY	low	Yes	A/A
130	74	RARS	46,XY	low	Yes	A/G

Abbreviations: CMML, chronic myelomonocytic leukemia; HI-E, hematological improvement-erythroid; IPSS, International Prognostic Scoring System; int-1, intermediate-1; NA, not available; RA, refractory anemia; RAEB-1, refractory anemia with excess blasts type 1; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RCMD-RS, RCMD with ringed sideroblasts; UPN, unique patient number. The response to lenalidomide was evaluated according to IWG 2006 (HI-E: yes/no), after four cycles ($n = 46$) or after three cycles of treatment (underlined UPN, patient $n = 5$).

including comparative genomic hybridization (CGH) and Sanger sequencing to search for deletion or mutation of *CRBN* gene. Genomic DNA from six responders (two RARS, two RCMD, two RCMD-RS) and six nonresponders (three RARS, two RCMD, one RCMD-RS) were analyzed by CGH. Copy number variations have been detected in six patients, gains in four patients at chr2q33.1, 6q26, 7q34 and 17q25.3, a deletion of chr11 in one patient (also detected by the karyotype analysis) and a micro-deletion at chr10q21 in one patient. No deletion/gain of the 3p26.2 region has been identified. Furthermore, the regions encoding for *CRBN* putative partners (*DDB1, DDB2, CUL4A, CUL4B, RBX1, DCAF4L1, DCAF4L2, DCAF5, DCAF6, DCAF7, DCAF8, DCAF8L1, DCAF10, DCAF11, DCAF12, DCAF12L1, DCAF12L2, DCAF13, DCAF15, DCAF16, DCAF17, DDA1* and *RP11-574F21.3*) were normal.

CRBN gene entire coding sequence and part of the promoter region upstream the 5′-untranslated region were sequenced in 12 patients including 6 responders and 6 nonresponders. We found no nucleotide changes in the coding sequence except for a synonymous change at nucleotide 3197918 in exon 6 corresponding to tyrosine 244 in one case. Then, we analyzed the distribution of the A/G polymorphism located at − 29 nt of the 5′-untranslated region (located on chr3 at nt 3221430 and referenced as rs1672753). According to public databases (ucsc.genome.edu), the A allele is described as the ancestral allele from shrimp to human, although

the G allele is notified as the reference allele. This GC-rich region was amplified as a 448-pb PCR product using the following primers: CRBN1F: 5′-GCAGGCCTGTAATTGTCCCT-3′ and CRBN1R: 5′-GCAACAGAGCAGCGAAGAAA-3′ in a GC-rich buffer as PCR mix. The distribution of A and G alleles in our cohort (67% and 33%, respectively) was not statistically different ($P = 0.095$) from that of the general population as described (75.3% and 24.7%, respectively) in ucsc.genome.edu.

We then analyzed the impact of this *CRBN* A/G polymorphism according to the response to lenalidomide in low/int-1 MDS patients without del(5q). We found that 8 (57%) of the 14 responders had the G allele (all A/G) versus 9 (24%) of the 37 nonresponders (one G/G and 8 A/G). The crude odds ratio for response to treatment in the G allele group with reference to the A allele group was equal to 4.14 (95% confidence interval: 1.17–14.76), thus significantly different from 1 ($P = 0.045$). In order to investigate potential confusion bias, differences for clinical and hematological characteristics between the G and the A allele groups were studied. No sex ($P = 0.674$) or age ($P = 0.624$) difference was found, and a trend ($P = 0.057$) for an increased proportion of int-1 IPSS status in the G allele group ($11/17 = 64\%$) compared with the A allele group ($12/33 = 36\%$) was observed. This suggests that the best response rate in the group of patients with G allele is not linked to a more favorable IPSS.

Among the 51 patients of our cohort, bone marrow mononuclear cell RNA was available before treatment for 36 patients (22 nonresponders and 14 responders). *CRBN* gene expression level was analyzed by using an reverse transcriptase quantitative PCR strategy. The primers (CRBN_F in exon 7: 5′-CAAGAAACAGCTACGT GAATGG-3′, CRBN_R in exon 8: 5′-GAAGTCGCTGGATAGCACTG)-3′ allowed the detection of the full-length transcript (NM_016302.3) and two other transcripts including CRBN-002, whose upregulation is predictive of the response to thalidomide in myeloma.[12] Normalized relative quantities were calculated by using *ACTIN, HPRT1* and *UBC* as reference genes. No statistical difference in the *CRBN* gene expression level was observed between responders and nonresponders. Although the rs1672753 polymorphism was located within a target region for transcription factors, the G allele ($n = 12$) was not associated with a different level of *CRBN* mRNA in comparison to the A allele ($n = 24$). These preliminary data indicate that, unlike current observation in myeloma, the *CRBN* expression level does not seem to be involved in the mechanism of response to lenalidomide in low/int-1 MDS without del(5)q.

In this study, we have identified a relevant molecular biomarker that could predict the response to lenalidomide in low/int-1 MDS without del(5q). Previous studies showed that a favorable karyotype or a molecular signature consisting of a set of downregulated erythroid-specific genes is predictive of response in these patients.[13,14] Although IMiDs directly inhibit CRBN, we could not exclude that these drugs may target other molecules, notably in MDS with del(5q). Indeed, secondary resistance to lenalidomide in MDS with del(5q) was found to be associated with overexpression of CDC25C.[15] Further studies are required in larger cohorts to confirm our findings and to investigate the biological consequences of *CRBN* polymorphism.

CONFLICT OF INTEREST

VS is consultant for Celgene, Janssen and Novartis. PF is consultant for Celgene, Ortho Biotech, Roche, Novartis, Cephalon, Epicept, Amgen, and Merck. FD is consultant for Novartis. MF is consultant for Celgene

ACKNOWLEDGEMENTS

This work was supported by the Direction régionale de la recherche clinique Assistance-Publique Hôpitaux de Paris (PHRC MDS-04). We thank Raphael Petit and Rosa Sapena for collecting clinical data, and Morgane Bourmaud and Audrey Gauthier for technical assistance.

AUTHORS CONTRIBUTIONS

VS, SK, VC and CL performed the experiments; AR performed all statistical analysis; LA, VS, SP, AT, PF, FD provided samples; PF, MF, OK wrote the paper; OK conceived and was responsible for the study design.

V Sardnal[1], A Rouquette[2], S Kaltenbach[3], C Bally[4], V Chesnais[5,6,7,8], C Leschi[5,6,7,8], L Ades[4], V Santini[9], S Park[10], A Toma[11], P Fenaux[4], F Dreyfus[10], M Fontenay[1,5,6,7,8] and O Kosmider[1,5,6,7,8]
[1]*Assistance Publique-Hôpitaux de Paris, Service d'Hématologie Biologique, Hôpitaux Universitaires Paris Centre, Hôpital Cochin, Paris, France;*
[2]*Unité fonctionnelle d'épidémiologie et de biostatistiques, Hôpitaux Universitaires Paris Centre, Hôtel-Dieu, Paris, France;*
[3]*Laboratoire de cytogénétique, Hôpital Necker, Paris, France;*
[4]*Service d'hématologie clinique, Hôpital Avicenne, Bobigny, France;*
[5]*Département d'Immunologie et Hématologie, Institut Cochin, Paris, France;*
[6]*INSERM U1016, Paris, France;*
[7]*CNRS UMR8104, Paris, France;*
[8]*Université Paris Descartes, Paris, France;*
[9]*Functional Unit of Hematology, University of Florence, Florence, Italy;*
[10]*Assistance Publique-Hôpitaux de Paris, Unité fonctionnelle d'hématologie clinique, Hôpitaux Universitaires Paris Centre, Hôpital Cochin, Paris, France and*
[11]*Service d'hématologie clinique, Hôpital Henri-Mondor, Créteil, France*
E-mail: olivier.kosmider@cch.aphp.fr

REFERENCES

1 Ito T, Ando H, Suzuki T, Ogura T, Hotta K, Imamura Y *et al.* Identification of a primary target of thalidomide teratogenicity. *Science* 2010; **327**: 1345–1350.
2 Lopez-Girona A, Mendy D, Ito T, Miller K, Gandhi AK, Kang J *et al.* Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. *Leukemia* 2012; **26**: 2326–2335.
3 Andhavarapu S, Roy V. Immunomodulatory drugs in multiple myeloma. *Expert Rev Hematol* 2013; **6**: 69–82.
4 Adès L, Fenaux P. Immunomodulating drugs in myelodysplastic syndromes. *Am Soc Hematol Educ Program* 2011; **2011**: 556–560.
5 List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E *et al.* Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. *N Engl J Med* 2006; **355**: 1456–1465.
6 Raza A, Reeves JA, Feldman EJ, Dewald GW, Bennett JM, Deeg HJ *et al.* Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. *Blood* 2008; **111**: 86–93.
7 Zhu YX, Braggio E, Shi CX, Bruins LA, Schmidt JE, Van Wier S *et al.* Cereblon expression is required for the antimyeloma activity of lenalidomide and pomalidomide. *Blood* 2011; **118**: 4771–4779.
8 Broyl A, Kuiper R, van Duin M, van der Holt B, El Jarari L, Bertsch U *et al.* High cereblon expression is associated with better survival in patients with newly diagnosed multiple myeloma treated with thalidomide maintenance. *Blood* 2013; **121**: 624–627.
9 Raza A, Meyer P, Dutt D, Zorat F, Lisak L, Nascimben F *et al.* Thalidomide produces transfusion independence in long-standing refractory anemias of patients with myelodysplastic syndromes. *Blood* 2001; **98**: 958–965.
10 Fenaux P, Giagounidis A, Selleslag D, Beyne-Rauzy O, Mufti G, Mittelman M *et al.* A randomized phase 3 study of lenalidomide versus placebo in RBC transfusion-dependent patients with Low-/Intermediate-1-risk myelodysplastic syndromes with del5q. *Blood* 2011; **118**: 3765–3776.
11 Komrokji RS, Lancet JE, Swern AS, Chen N, Paleveda J, Lush R *et al.* Combined treatment with lenalidomide and epoetin alfa in lower-risk patients with myelodysplastic syndrome. *Blood* 2012; **120**: 3419–3424.
12 Gandhi AK, Avet-Loiseau H, Waldman M, Thakurta A, Aukerman SL, Chen G *et al.* Detection and quantification of cereblon protein and mRNA in multiple myeloma cell lines and primary $CD138^+$ multiple myeloma cells. *ASH* 2012, (abstract #4043).

13 Ebert BL, Galili N, Tamayo P, Bosco J, Mak R, Pretz J *et al.* An erythroid differentiation signature predicts response to lenalidomide in myelodysplastic syndrome. *PLoS Med* 2008; **5**: e35.

14 Sugimoto Y, Sekeres MA, Makishima H, Traina F, Visconte V, Jankowska A *et al.* Cytogenetic and molecular predictors of response in patients with myeloid malignancies without del[5q] treated with lenalidomide. *J Hematol Oncol* 2012; **5**: 4.

15 Wei S, Chen X, McGraw K, Zhang L, Komrokji R, Clark J *et al.* Lenalidomide promotes p53 degradation by inhibiting MDM2 auto-ubiquitination in myelodysplastic syndrome with chromosome 5q deletion. *Oncogene* 2013; **32**: 1110–1120.

Loss of Tcf7 diminishes hematopoietic stem/progenitor cell function

Leukemia (2013) **27**, 1613–1614; doi:10.1038/leu.2012.354

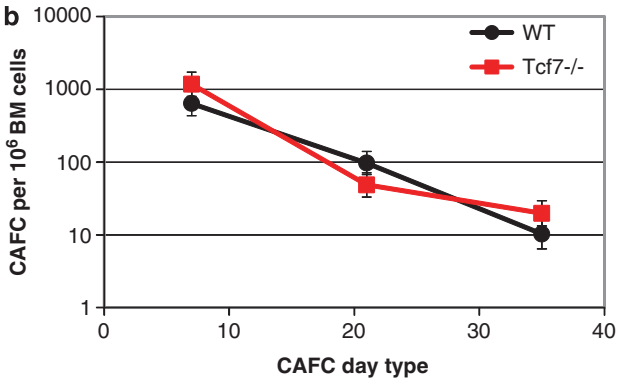
The canonical Wnt-β-catenin pathway is an evolutionarily conserved and tightly regulated pathway in development. Activation of this pathway occurs upon binding of a soluble Wnt protein to a membrane-associated receptor, and leads to the disruption and inhibition of a protein complex responsible for the phosphorylation and breakdown of β-catenin. Inhibition of this so-called destruction complex, composed of the tumor suppressor Apc, the Ser-Thr kinases Gsk-3β and CK-I, and the scaffold and tumor suppressor protein Axin, results in stabilization and (nuclear) accumulation of β-catenin. Stabilized β-catenin forms a bipartite transcription factor with the Tcf-Lef family of transcription factors (including Tcf7, Tcf7l1, Tcf7l2 and Lef1) to activate a Wnt-controlled gene expression program.[1]

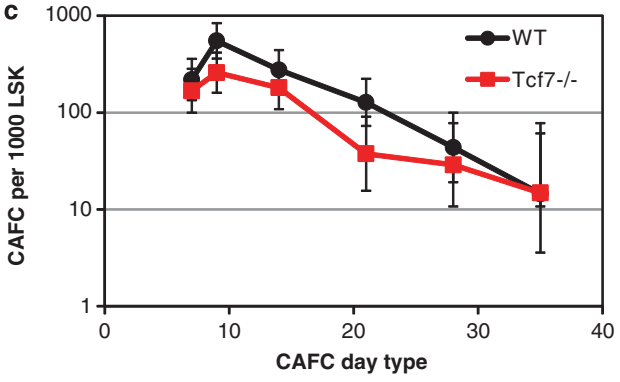
In the hematopoietic system, a role for Wnt signaling was first demonstrated during T-cell development in the thymus.[2] Subsequently, opposing effects of Wnt signaling on hematopoiesis have been reported. For example, Mx-Cre-mediated deletion of β- or β- and γ-catenin did not effect hematopoiesis, but stabilized forms of β-catenin resulted in either enhancement of hematopoietic stem cell (HSC) function and maintenance of an immature phenotype or exhaustion of the HSC pool.[3–8] These differences might be explained by different levels of Wnt pathway activation. By using various targeted hypomorphic Apc alleles and a conditional deletion allele of Apc, which generates different Wnt signaling levels, it has been shown that different, lineage-specific Wnt dosages regulate HSCs, myeloid precursors and T lymphoid precursors during hematopoiesis.[9]

The *Tcf7* gene is a complex gene of which several different isoforms, including isoforms that lack the β-catenin binding domain, have been found. *Tcf7* has been reported to be a β-catenin-Tcf7l2 target gene and to act as a feedback repressor of β-catenin-Tcf7l2 target genes. In this way, Tcf7 may assist Apc to suppress malignant transformation of epithelial cells.[10] Tcf7 expression is most abundantly expressed in T-lymphocytes, but is also expressed in HSCs.[11,12] B6-Tcf7-deficient mice develop a progressive block in the early thymocyte development, but are fully immunocompetent and live for over 1 year.[2,13] Here, we report the impact of loss of Tcf7 on HSC and progenitor cells *in vitro* and *in vivo*.

a

	BMC/HL	%LSK
WT	$5.0x10^7$	0.24
$Tcf7^{-/-}$	$4.9x10^7$	0.25





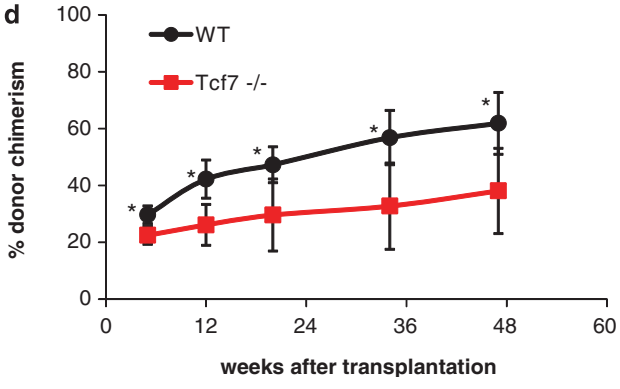


Figure 1. Comparison of LSK frequency and functionality *in vitro* an *in vivo* between WT and B6Tcf7−/− mice. (**a**) BM cellularity and LSK frequency (pooled from three mice). BM cells (**b**) and purified LSK (**c**) were plated in CAFC cultures, and CAFC frequency was determined at different culture times. (**d**) *In vivo* functionality of unseparated BM cells (2 million) transplanted in competition with normal BM cells (1 million). The percentage of donor chimerism was determined in blood on nucleated blood cells by flow cytometry and the % donor chimerism was calculated (% donor/(% donor + % competitor)).

Accepted article preview online 5 December 2012; advance online publication, 4 January 2013