

SUPPLEMENTARY INFORMATION

In vitro Genotoxicity Studies

Table 1: Different cumulative MOIs (c.MOI) used in the experiments

Sample	Vector	#100818 - c.MOI	#101222 - c.MOI	#110727 - c.MOI
1	MOCK	-	-	-
2	RV.SF	10	10	20
3	LV.SF	20	20	40
4	G1XCGD	20	5	5
5	G1XCGD	20	10	10
6	G1XCGD	20	10	20
7	G1XCGD	40	20	20
8	G1XCGD	40	20	40
9	G1XCGD	-	40	-

Table 2: Vector copy numbers as determined by qPCR

Sample	Vector	#100818 - c.MOI	#101222 - c.MOI	#110727 - c.MOI
1	MOCK	-	-	-
2	RV.SF	1.4	3.6	5.6
3	LV.SF	6.7	6.1	8.1
4	G1XCGD	0.3	10.0	7.2
5	G1XCGD	0.5	22.5	10.5
6	G1XCGD	0.5	16.5	13.8
7	G1XCGD	4.0	23.2	14.4
8	G1XCGD	2.9	22.0	14.9
9	G1XCGD	-	25.3	-

Cell numbers in the first 8 days of the IVIM assay

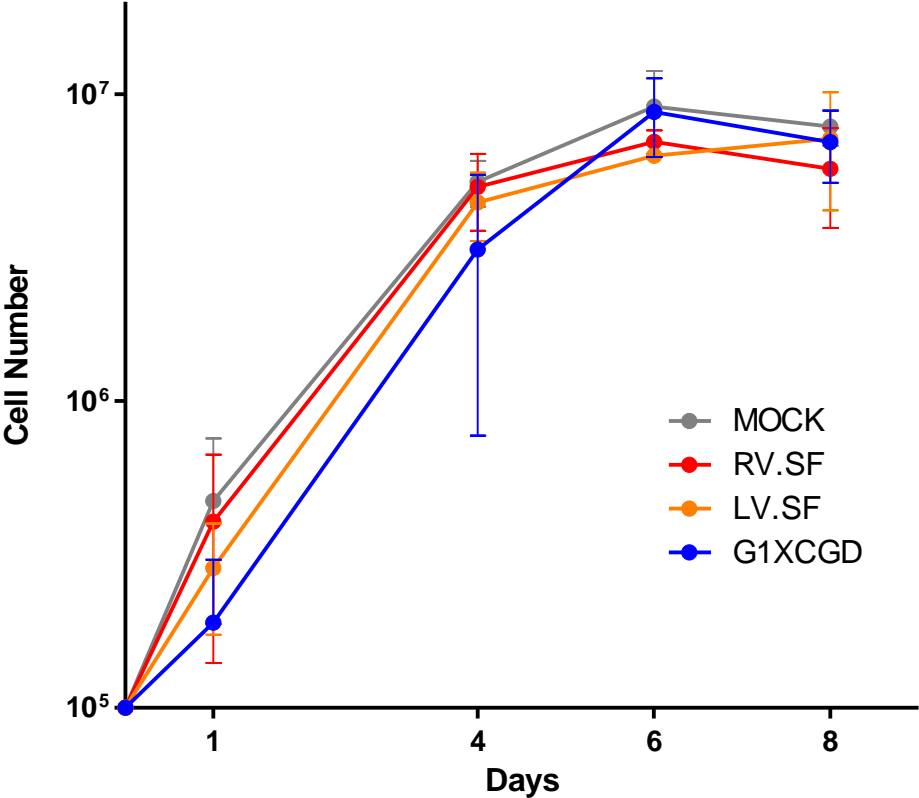


Figure 1: Cell number in the first 8 days of the IVIM assay. The test vector G1XCGD (blue) showed normal proliferation behaviour and was statistically not different from the MOCK (grey) treated control or the positive control vectors RV.SF (red) or LV.SF (orange).

***In vivo* Genotoxicity Studies**

Table 3. List of animals in different groups

Group	Mouse
R-A	animal #1
	animal #2
	animal #3
	animal #4
	animal #5
	animal #6
	animal #7
RV-SF (STF)	MOI 50

Group	Mouse
R-B	animal #37
	animal #38
	animal #39
	animal #40
	animal #41
	animal #42
e.f.	
RV-SF (S3F11)	MOI 50

Group	Mouse
L-1	animal #8
	animal #9
	animal #10
	animal #11
	animal #12
	animal #13
	e.f.
LV-SF (STF)	MOI 30

Group	Mouse
L-2	animal #21
	animal #22
	animal #23
	animal #24
	animal #25
	animal #26
animal #27	
LV-SF (STF)	MOI 71

Table 3 cont.

Group	Mouse
G-1	animal #14
	animal #15
	animal #16
	animal #17
	animal #18
	animal #19
	animal #20
G1XCGD (STF)	MOI 100

Group	Mouse
G-2	animal #28
	animal #29
	animal #30
	animal #31
	animal #32
	animal #33
	e.f.
G1XCGD (STF)	MOI 205

Group	Mouse
M	animal #34
	animal #35
	animal #36
	animal #43
	animal #44
	-
	-
Mock	NA

Group	Mouse
B	animal #45
	animal #46
	animal #47
	-
	-
	-
	-
Leukemia	NA

Mice transplanted with G1XCGD transduced cells survive with normal health parameters

Three animals had to be killed shortly after transplantation due to engraftment failure. Apart from that, all other 44 mice receiving transduced or mock treated cells survived with either stable or increased body weight and normal spleen weight (Figure 2a and 2b). Red and white blood cell counts and platelet numbers were normal for all gene therapy groups (Figure 3 a-c). In contrast, the leukaemia control animals continuously lost weight already one week after transplantation. A critical health status was reached after four weeks so that the mice had to be sacrificed. The spleen was significantly enlarged (ca. 6.5-fold compared to MOCK) and the blood parameters indicated a leucocytosis accompanied by anaemia and thrombocytopenia. Histopathologic analysis of the spleen and bone marrow confirmed the diagnosis of leukaemia. After 26 weeks, we sacrificed the mice and performed a blinded, good laboratory practice (GLP) grade histopathology of the bone marrow (sternum) and spleen. We also included the samples of the leukaemia control group to ensure a reliable identification of malignant abnormalities by the pathologist who were unaware of the group assignments. The blinded examination of the H&E and Giemsa stained slides positively identified the malignant lymphoma in the three animals having received the leukemic cells. In nearly all gene therapy treated animals, the cellularity in the BM was normal. Apart from certain exceptions, most of the observed slight abnormalities were also present in the Mock group and only represented incidental findings (Table 4 summarizes the findings). Only in the group receiving RV-SF transduced cells (culture medium S3F11) one mouse with an early lymphoma was identified. Interestingly, lymphoid hyperplasia (LH) in the spleen was observed in 9 out of 13 animals of both lentiviral gene therapy treated groups and 3 of 7 RV-SF treated mice but not in Mock treated animals. However, a certain level of LH in the spleen of mice is normal as long as the architectural structures and cellularity are preserved (Suttie et al., 2006).

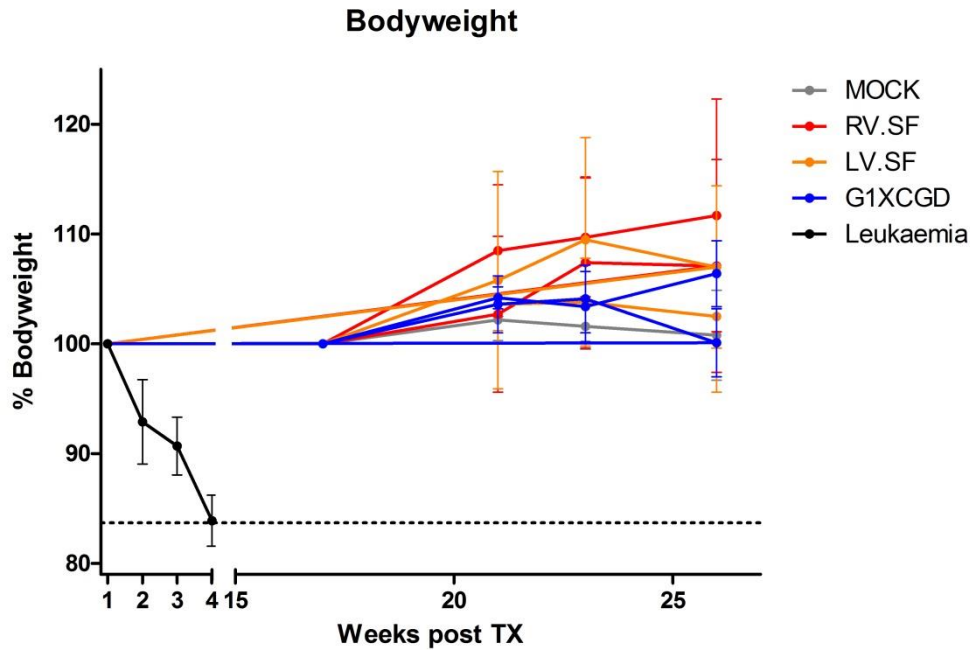


Figure 2a. Body weight of transplanted animals. The body weight of animals in groups R, L, G and M (A) and group B (B) in percent over time. Mice receiving cells modified with the test vectors appeared normal. The red dashed line indicates the critical body weight when animals of group B.

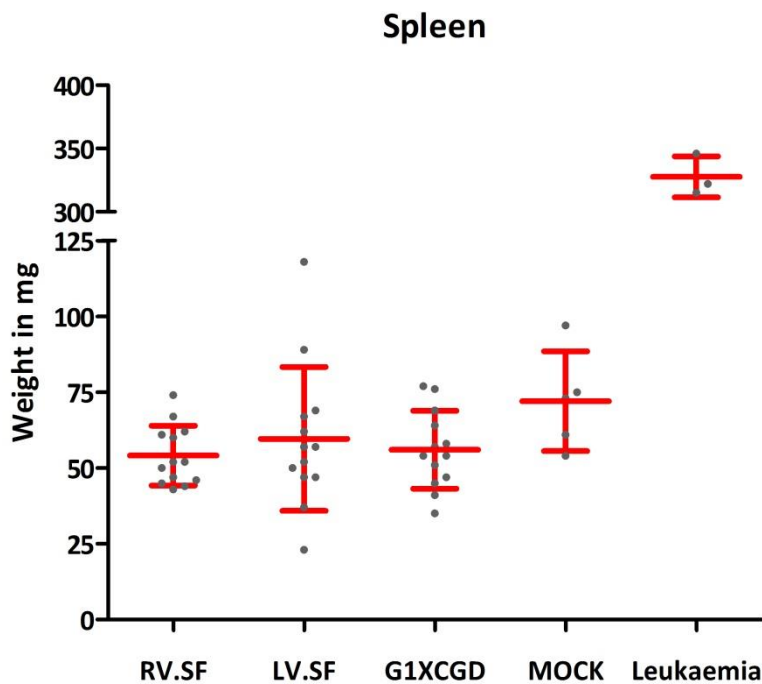


Figure 2b. Spleen weight of transplanted animals. At the time of end analysis, the spleen of the transplanted animals was prepared and weighed. The organ of the leukemic animals had a significantly higher weight ($p < 0.0001$, one-way ANOVA) compared to all other animals. There was no statistical difference animals receiving MOCK treated cells and all other gene therapy groups.

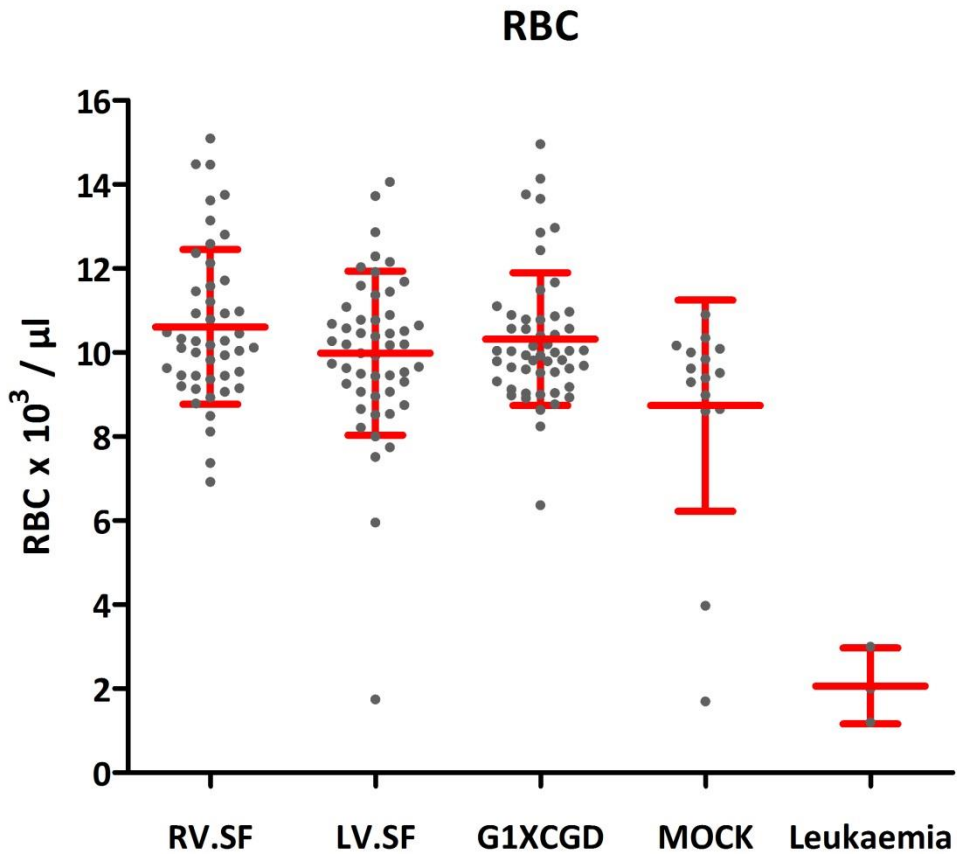


Figure 3a. Red blood cell count (RBC). Leukaemia animals had a very low RBC and had short breath at the time of sacrifice. Overall, gene therapy groups survived with normal blood parameters. For LV.SF we observed two animals (#21 and #22 – both time of end analysis = tEA), for G1XCGD one mouse (#17 - tEA) and for MOCK two animals (#35 – 10 weeks post transplantation; #44 - tEA with a lower RBC level). Dots represent measurements of individual mice at the different bleeding time points over whole experiment. Bars represent means \pm standard deviation.

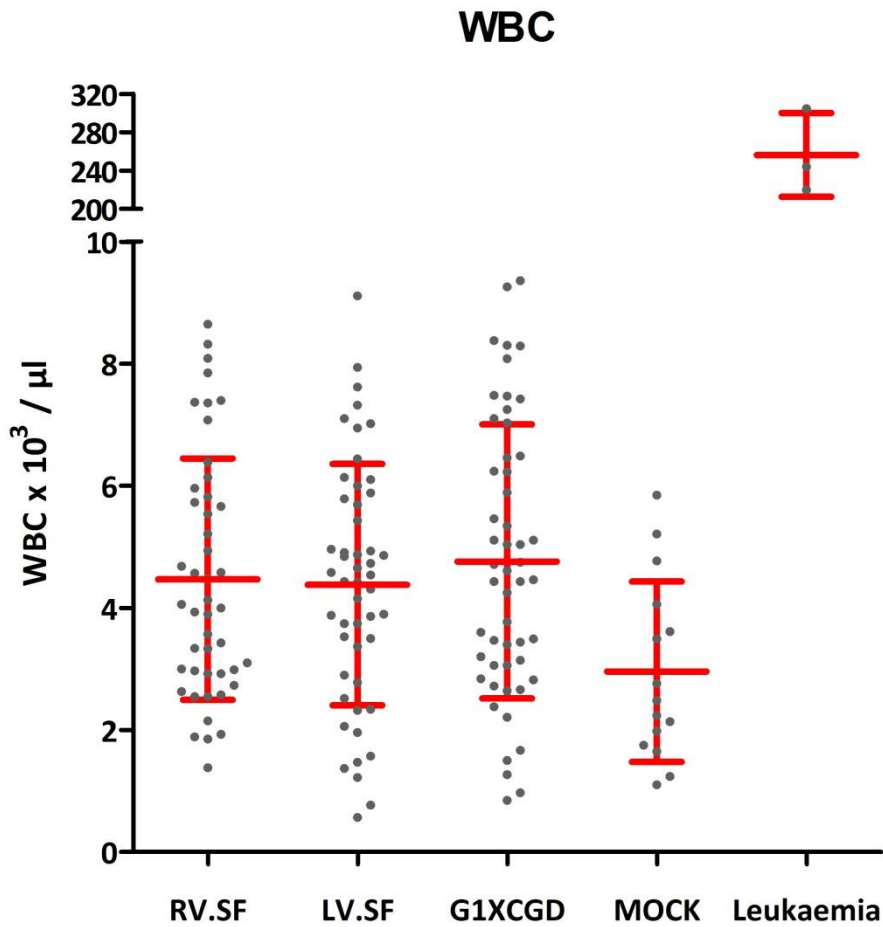


Figure 3b. White blood cell count (WBC). Leukaemia animals had a very high WBC at the time of sacrifice. Overall, gene therapy groups survived with normal WBC levels. Dots represent measurements of individual mice at the different bleeding time points over whole experiment. Bars represent means \pm standard deviation.

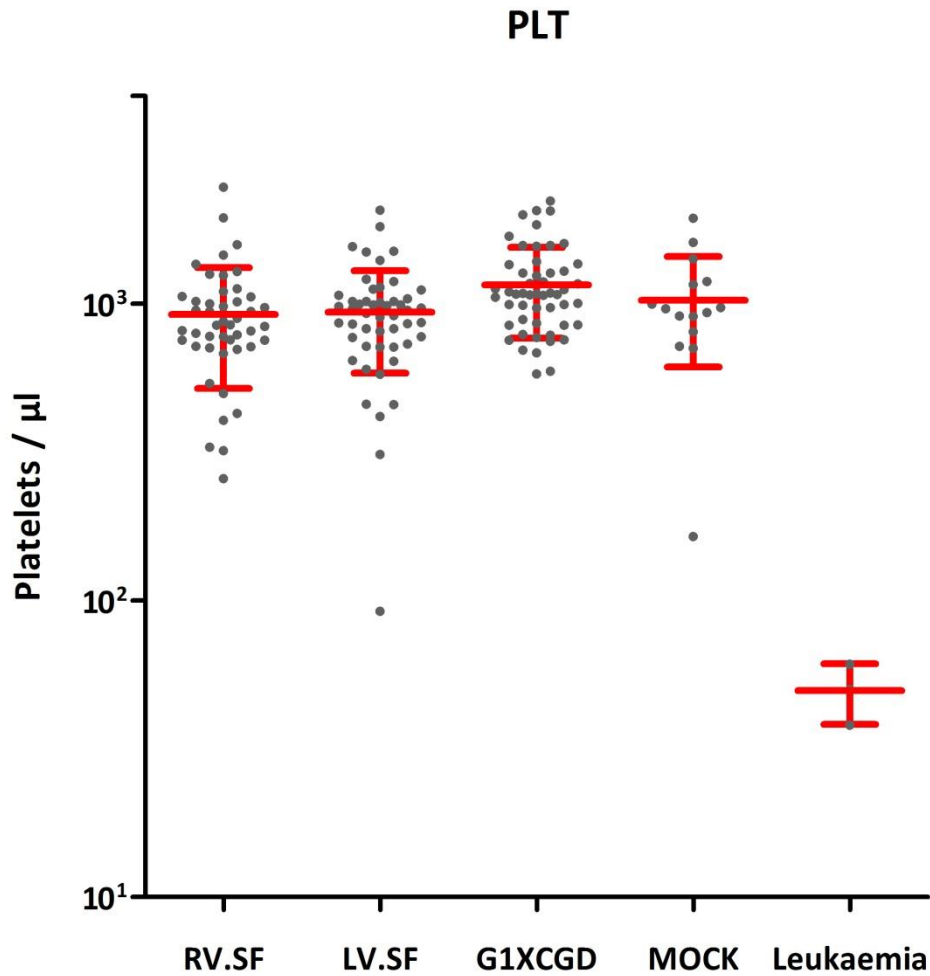


Figure 3c. Platelet count (PLT). Leukaemia animals had a very low PLT at the time of sacrifice. Overall, gene therapy groups survived with normal PLT levels. For LV.SF we observed one animal (#22 time of end analysis = tEA) and one animal for MOCK (#44 – tEA) with low PLT. The same mice also had low WBC and RBC levels, so that a technical problem during measurement cannot be excluded. Dots represent measurements of individual mice at the different bleeding time points over whole experiment. Bars represent means \pm standard deviation.

Mouse #	Group	BM	BM	BM	BM	BM	SP	SP	SP
		Cellularity	Megakaryocytes	Hemosiderin	Foci MMC	MC Infiltrates	ALH	LH	EMH
1	RV-SF (SFT)								S
2	RV-SF (SFT)								S
3	RV-SF (SFT)								
4	RV-SF (SFT)								S
5	RV-SF (SFT)	A							S
6	RV-SF (SFT)								S
7	RV-SF (SFT)								S
37	RV-SF (S3F11)	C							
38	RV-SF (S3F11)								S
39	RV-SF (S3F11)								S
40	RV-SF (S3F11)								S
41	RV-SF (S3F11)	EL							S
42	RV-SF (S3F11)								
45	Leukemia	ML	ML	ML	ML	ML	ML	ML	S
46	Leukemia	ML	ML	ML	ML	ML	ML	ML	S
47	Leukemia	ML	ML	ML	ML	ML	ML	ML	

Legend:

	Normal or not applicable
	Not explicitly stated in the report as normal or abnormal
ML / EL	ML = Malignant Lymphoma; EL = Early Lymphoma
A	Very slight change of the Myeloid:Erythroid (M:E) ratio
B	Very slight decrease of all hematopoietic cell series leading to a lower cellularity replaced by a slightly increased number of adipocytes
C	Slightly increased number of adipocytes in association with a slight decrease of myeloid cells and very slight decrease of megakaryocytes
	Increase of megakaryocytes
	Increase hemosiderin storage
	Foci of monomorphic cells (MMC)
	Mononuclear cell (MC) infiltration
	Atypical lymphoid hyperplasia (ALH)
VS / S	Very slight (VS) or slight (S) extramedullary hematopoiesis

BM = Bone marrow; SP = Spleen

Description of findings:

The histopathologic analysis by the Fraunhofer Institute for Experimental Toxicology in Hannover (ITEM) was performed in single blinded fashion. The bone marrow and spleen samples were labelled with the individual mouse numbers, which were not known to the scientists at ITEM. The animals #45-47 (group B – leukemia mice) reliably were identified to suffer from a malignant lymphoma. Only one mouse of group R-B (#41) was diagnosed with an early lymphoma. All other mice showed a normal bone marrow cellularity and did not differ from the scoring of the Mock animals.

139 glass slides from the bone marrow (sternum) and the spleen of 47 mice (1 H&E [hematoxylin-eosin]- and 1 Giemsa-stained paraffin section of the bone marrow and 1 H&E-stained paraffin section of the spleen) were examined. Some observations did not comply with normal cellularity as determined by Dr. Kolling and Dr. Ernst from the ITEM. Since these deviations were also seen in animals transplanted with non-transduced cells, this observation cannot be related to the vectors used in this study. For clarification, the animal numbers of the Mock group (**#34,35,36,43,44**) are highlighted in bold blue. Histopathological findings are summarized below.

35 mice (Nos. 1-4, 6-10, 12, 14, 15, 18, 19, 22-29, 31-33, **34-36**, 38-42, **43, 44**) showed **normal cellularity of the bone marrow (sternum)** that means marrow spaces were occupied by intermingled hematopoietic cells. All stages of the erythroid and myeloid series were present as well as megakaryocytes and pigment containing macrophages. Within the limits of subjective histological evaluation the relative proportions of the hematopoietic marrow components were consistent with published descriptions of rodents.⁵⁸⁻⁶⁰

3 Mice (45-47, group B) were detected with **malignant lymphoma** in the spleen, which also replaced the whole bone marrow thus leading to a severe decrease of all (severe, multifocal) blood lineages. The spleen of these animals showed a slight/moderate extramedullary hematopoiesis in addition. An **early lymphoma** was diagnosed in the spleen of mouse 41 (group R-B) with slight extramedullary hematopoiesis (mainly erythropoiesis).

Bone Marrow

Slight **decrease of myeloid** cells associated with very slight increase of erythroid cells leading to a change of the Myeloid:Erythroid (M:E) ratio -which generally has a mild myeloid predominance (Elmore, 2006)- was observed in mouse 5.⁵⁹ Slight **increase of the erythroid cell** series led to a focal higher cellularity and also to a change of the M:E ratio in mouse 20. Very slight **decrease of all hematopoietic** cell series leading to a lower cellularity replaced by a slightly increased number of adipocytes was seen in animal 30. Animal 37 also showed a slightly increased number of adipocytes in association with a slight decrease of myeloid cells and very slight decrease of megakaryocytes.

Slight/moderate **increase of megakaryocytes** was found in 5 mice (11, 13, 16, 21, and 42.)

Slight increase of **hemosiderin storage** due to physiological turnover of erythrocytes was observed in 7 mice (13, 18, 21, 24, 33, 39, and 44).

Small **foci of monomorphic cells** in the bone marrow were detected in 9 mice (10, 21, 22, 26, 29, 31, 33, 36, and 44)

Focal/multifocal very slight/slight **mononuclear cell infiltration** in the attached muscle or fat tissue of the sternum was detected in 11 mice (3, 4, 7, 9, 12, 13, 21, 22, 29, 37, and 43).

The bone marrow of mouse 17 was missing.

Spleen

10 mice (4, 6, 10, 14, 23, 28, 29, 33, 36 and 43) showed **atypical lymphoid hyperplasia** in the spleen with partly pleomorphic and abnormal appearing lymphoid

cells. It can not be excluded that this lesion is to be seen as precursor lesion to malignant lymphoma. Since similar effects were also seen in animals transplanted with non-transduced cells (bold blue), this observation cannot be related to the vectors used in this study

Lymphoid hyperplasia was observed in 21 mice (2, 3, 5, 9, 11, 12, 13, 15-20, 22, 24-27, 30-32).

Very slight (16, 19, 24, 25, 26, 31, 32, **36, 44**)/slight (1, 2, 4-11, 14, 15, 22, 29, 30, **34, 38, 39, 41, 43, 45, 46**) **extramedullary hematopoiesis** was found in 22 mice. Some degree of extramedullary hematopoiesis is present in normal rodents especially mice.⁶¹ Moderate increased extramedullary hematopoiesis was detected in 6 mice (12, 13, 33, 37, **42** (left shift), and 47) and severe in one mouse (21).

Atypical moderate extramedullary hematopoiesis was found in one mouse (23) with increased number of immature and abnormal cells of all hematopoietic cell lines.

Slight increase of **hemosiderin storage** was noted in one mouse (13).

One focal **mononuclear cell infiltration in the mesenterial fat** was diagnosed in 2 mice (2, 12).