Supplemental Data

SUPPLEMENTAL METHODS

Samples and patients

MSCs were isolated from total marrow aspirations of CMML patients and patients with normal marrows (healthy donors) (10 ml of bone marrow in heparin lithium tubes). All patients signed an informed consent (CRB cession: 2019-02-07-C, BRIF: BB-0033-00069, Agreement: AC 2014-2094). CMML patients had not received any antitumor treatment at the time of marrow aspiration. Mononuclear cells were isolated through a density gradient (Ficoll, Eurobio) and magnetically sorted according to their expression of CD34 (AutoMACS Pro, Miltenyi Biotec). CD34 negative fraction was cultured and MSCs were isolated thanks to their adherence to plastic. Cells obtained after this process responded to MSCs definition, as >95% of cells expressed the markers CD90, CD73, CD105 but not hematopoietic markers (CD45), they adhered to plastic and had a trilineage differentiation ability into chondrocytes, osteoblasts and adipocytes (Krampera, Cytotherapy 2013). These different characteristics had previously been demonstrated by our team with this protocol of isolation and culture, and have not been tested again for this study (Meunier et al, Oncotarget 2018).

Monocytes were isolated from blood of CMML patients, patients with a chronic non-clonal monocytosis (20 ml of blood in heparine lithium tubes), and from Buffy coats of healthy blood donors. The classification in CMML or non-clonal monocytosis (post-inflammatory) was based on dysplastic features on bone marrow (for CMML) and on the threshold of 94,4% MO1 in the peripheral blood (Selimoglu-Buet et al, Blood 2015). Accumulation of classical monocytes defines a subgroup of MDS that frequently evolves into CMML (Selimoglu-Buet et al, Blood. 2017). Anonymous blood donors

(Regional Blood Bank, EFS Auvergne-Rhone-Alpes) who also volunteered to provide blood for research purposes signed a form indicating that they do not preclude the use of their sample for medical research (this procedure is in accordance with the regulation in force in France ("Arrêté du 6 novembre 2006"; L. 1223-3 article of the Public Health). CMML patients had not received any antitumoral treatment at the time of blood uptake. Mononuclear cells were isolated through a density gradient (Ficoll, Eurobio) and magnetically sorted according to their expression of CD14 (AutoMACS Pro, Miltenyi Biotec). A flow cytometry analysis (FACSCanto II, Beckton Dickinson) was performed after each cell sorting with CD3, CD14, CD16 markers (CD3-APC, CD14-PE, Beckman Coulter; CD16-BV421, BD Biosciences). We obtained a cell population with > 98% of cells positive for CD14, < 1% positive for CD3. Expression of CD16 was variable according to Selimoglu-Buet D et al. (Blood 2015).

Cytologic analysis was performed by cytospin and May Grünwald Giemsa staining.

$CD14^+$ fraction was cultured (cf below).

Normal hematopoietic stem cells were isolated from total marrow aspirations of healthy donors (10 ml of bone marrow in heparine lithium tubes). Bone marrow from healthy donors were collected by bone marrow aspiration before cardiac surgery after signed consent and approval by our local ethical committee. CMML hematopoietic stem cells were isolated from bone marrow of CMML patients. Mononuclear cells were isolated through a density gradient (Ficoll, Eurobio) and magnetically sorted according to their expression of CD34 (AutoMACS Pro, Miltenyi Biotec). $CD34^+$ fraction was extracted and frozen at -80°C in albumin + 20% DMSO. When needed, cells were thawed following our local protocol with a viability > 90% after thawing.

Cell culture

All cells were cultured at 37°C with CO2 5%.

Culture medium for HL60 cells contained RPMI, Foetal Bovine Serum (FBS) 10%, Glutamine 1%, penicillin/streptomycin 1% (Life Technologies, Paisley, UK).

Culture medium for MSCs contained DMEM, FBS 10%, Glutamine 1%, penicillin/streptomycin 1%. Cells were expanded until 90-100% of confluence. EVs were extracted when cells reached a confluence of 90-100% at the second passage. Seventy-two hours before EVs extraction, MSCs were incubated in Exofree medium, i.e. their usual culture medium deprived of FBS EVs through an overnight 100000g ultracentrifuge. This process also participated to cell stimulation by depriving them of some of FBS nutritive elements.

Monocytes were cultured in a serum-free medium containing RPMI and Glutamine 1%. Serum deprivation was intentional to stimulate cell activation and vesiculation. Culture flasks were coated with polyhydroxyethylmethacrylate (pHEMA, Sigma Aldrich, Saint Louis, USA) to prevent adherence to plastic so that cells could be analyzed at the end of the culture. At the end of the culture (H37-H40), a new cytospin was performed as well as a flow cytometry quantification of cell viability with AnnexinV-FITC and 7-AAD (BD Biosciences) markers.

CD34+ cells (hereafter HSCs) were cultured in a medium containing Myelocult™ H5100 (Stemcell technologies) with 100 ng/mL of FLT3-ligand (Peprotech), 100 ng/mL of Stem Cell Factor (SCF, Peprotech) and 50 ng/mL of Thrombopoietin (TPO, Peprotech).

EVs extraction

EVs derived from MSCs were extracted after a 72-hours incubation in exosome-free medium (Exofree medium). EVs derived from monocytes were extracted after 37 to 44 hours of incubation in serum-free medium.

Culture supernatant was collected and centrifuged successively (Supplemental Figure 1A): 800g for 10 minutes, 2 000g for 10 minutes, 10 000g for 30 minutes, and 100 000g for 1 hour and 10 minutes in a fixed-angle rotor (70ti, Beckman Coulter) using Optima L-60 Ultracentrifuge (Beckman Coulter). This is an adaptation of C. Théry's protocol (Théry et al, Curr Protoc Cell Biol, 2006). The second 100 000g ultracentrifugation was not done to increase the amount of EVs collected even though it probably implies a higher protein contamination.

10 000g and 100 000g pellets were resuspended in PBS filtered at 0.22µm and corresponded respectively to the collects of medium EVs (mEVs) and small EVs (sEVs). NTA characterization and hemostasis experiments were performed on fresh EVs.

EVs characterization

Nanoparticle Tracking Analysis

All EVs samples were analyzed by *Nanoparticle Tracking Analysis* on Nanosight NS300 (Malvern, UK). This technique calculates the particles size and concentration based both on light scattering and Brownian motion but does not provide any functional data.

The same acquisition parameters (camera level 11, detection threshold 6) were used all along the different experimentations and the focus was adapted to each sample to improve the EVs discrimination. PBS used for rinses and dilutions had been filtered at 0.22µm to prevent small impurities to modify the results.

Two dilutions were tested for each sample EVs derived from MSCs and monocytes, and repeated recordings were realized. For each sample dilution, the software records a number of events and calculate the concentration according to the dilution and the volume. Then we calculated final concentration as the mean of the concentrations of each of the two dilutions, pondered on the number of events recorded by the instrument.

Western Blot

EVs isolated and MSCs were washed in cold TBS (Bio-Rad, Hercules, CA, USA). They were resuspended in reducing Laemmli Buffer (Laemmli Sample Buffer with β-mercaptoethanol) for Alix and Flotillin, or non-reducing Laemmli Buffer for CD63 (Laemmli Sample Buffer only). Samples were heated at 60°C for 5 minutes and separated on a SDS-PAGE gel (Any kD Precast Gels, Bio-Rad, CA, USA). Proteins were transferred with Trans-Blot system (Bio-rad, Hercules, CA, USA) on nitrocellulose membrane. Membrane was blocked in TBST (Bio-rad, Hercules, CA, USA) containing 3% of BSA (Merck) and incubated overnight at 4°C with primary antibody (Rabbit anti-ALIX, ab76608 1/1000e, AbCam; Rabbit anti-CD63, ab216130 1/500, Abcam; Mouse anti-Flotillin, 610820 1/1000e, BD, Mouse anti-Tissue Factor (anti human-CD142, clone HTF-1), 16-1429-82 1/1000, eBioscience). After incubation, membrane was washed three times in TBST for 15 minutes at RT. Membrane was incubated with the HRP-conjugated secondary antibody (Goat Anti-Rabbit IgG-HRP, Jackson ImmunoResearch, Goat Anti-mouse IgG-HRP, Jackson ImmunoResearch) during 1 hour at AT. After incubation, membrane was washed three time in TBST for 15 minutes and imaged with ChemiDoc+ (Bio-rad, Hercules, CA, USA).

Hemostasis

Plasma preparation

Due to small amounts of thrombin generated with the commercial normal poor platelet plasma initially used (CCN-10, Cryopep, Montpellier, France) and also to prevent a systematic bias due to a single plasma lot, we switched to a single healthy donor plasma prepared following guidelines (Lacroix R et al, JTH 2013) and then to a pool of 4 healthy donors plasmas (2 women and 2 men). Within less than two hours after blood sampling, two successive centrifugations (2500g, 15 minutes, room temperature) were performed, plasma was aliquoted in 1 ml fractions.

Thrombin generation assay (TGA)

Thrombin generation was measured using the Calibrated Automated Thrombogram (CAT) initiated by automatically dispensing fluorogenic substrate (FluCa, Stago), calibrated against the Thrombin Calibrator commercialized by Diagnostica Stago. Data were analyzed with Thrombinoscope software (V5.0, Thrombinoscope BV), according to Hemker's method (Hemker et al, 2003).

For each TGA, a positive control containing 0.5 pM of tissue factor and 2 µM of phospholipids (PPP Reagent Low half diluted in PBS, Stago, France), and a negative control containing 2 µM of phospholipids only (MP-Reagent half diluted in PBS, Stago) were used.

For each MSCs culture we measured the thrombin generated by 0.2 million of cells, 115 million of mEVs half diluted in MP-Reagent and 115 million of sEVs half diluted in MP-Reagent. EVs quantification was obtained thanks to NTA data.

For each monocytes culture, we measured the thrombin generated by 10 million of mEVs or sEVs half diluted in MP-Reagent. The number of cells and EVs tested was chosen based on the poorer sample.

20 µl of controls (FT 0.5pM, MPR) or samples (cells, mEvs or sEvs) were mixed with 80 µl of plasma in a 96-well plate (Immulon 2HB, Thermo Scientific).

For each test, we analyzed statistically the lag time (minutes) and the peak of thrombin generated (nM).

Some thrombin generation assays were conducted with a normal plasma compared with a factor VII-deficient lyophilized plasma (defVII-PPP, Diagnostica Stago, France). Other thrombin generation assay on EVs were performed with or without an inhibiting anti-tissue factor antibody at a final concentration of 8 µg/ml (anti human-CD142, clone HTF-1, eBioscience).

In order to overcome the plasma change variability, we normalized the lag time and the thrombin peak on the positive control (TF 0.5 pM). Results are thus presented as fold changes between samples and controls.

Fibrinography

This new approach of coagulation enables an analysis of the coagulation phase but also the fibrinolysis phase when adding tissue Plasminogen Activator (t-PA) along with tissue factor at the reaction onset (Yeromonahos et al, Biophys J 2010; Yeromonahos et al, Arterioscler Thromb Vasc Biol 2012; Dassi et al, Hemasphere 2019 *In press*).

The model was developed from fibrin physical properties of light diffusion to determine the internal nanostructure of fibrin fibers during the clot formation. This structural analysis enables the calculation of the mean number of protofibrils per fibrin fiber according the time.

With Fibrinography, in a normal plasma, we can see an increase of the number of protofibrils per fibrin fiber during the clot formation and a decreasing number during fibrinolysis.

Fibrinography data were recorded as triplicates on a spectrophotometer (SPECTROstar Omega, BMG Labtech, Champigny sur Marne, France).

80 µl of normal plasma (CCN-10, Cryopep, Montpellier, France) were added to 20 µl of controls or samples in a 96-well plate (Immulon 2HB, Thermo Scientific). Fibrinography test was triggered by an automatic injection of 20µL of Fluobuffer (calcium chlorure solution 100 mM, Stago, France). Optic density evolution was followed every 20 to 22 seconds.

Calculation of fibrin nanostructure was realized based on Yeromonahos equation (Yeromonahos, Biophys J 2010; Dassi et al, Hemasphere 2019 *In press*). For each test the “clot lysis time” was extracted. It corresponds to the delay between the 50% peak time and the second value of 50% of the peak during the fibrinolysis phase (Figure 3A). The coagulation time and the T_{Np97} were also analysed. They correspond respectively to the time of the beginning of coagulation and the time at 97% of clot formation.

For each experiment, a positive coagulation control containing 0.5 pM of tissue factor and 2 µM of phospholipids (PPP Reagent Low half diluted in PBS, Stago, France), a positive fibrinolysis control containing 0.5 pM of tissue factor, 2 µM of phospholipids and 80 ng/mL of Actilyse (t-PA, Boehringer Mannheim, Germany) were tested.

For each MSCs, we measured the impact on coagulation and fibrinolysis of 115 million of mEVs or sEVs half diluted in TF, MP-Reagent and t-PA. The number of cells and

EVs tested was normalized based on the poorer sample and is identical to the TGA experiments. EVs quantification was obtained thanks to NTA data.

Transmission Electron Microscopy: evidence of TF on sEVs membranes

sEVs were used fresh (i.e directly after extraction) for electron microscopy assay and resuspended in PBS. Grids were coated with carbon the day before the assay. Vesicles were adsorbed at room temperature during 5 minutes on carbon grids. Grids were put, on their coated side, on a 10 µL drop containing sEVs previously filled on a parafilm sheet. sEVs were fixed 5 minutes with a PBS+PFA 4% fixing solution. After washing and blocking steps by transferring the grids from one drop to another, sEVs were incubated with the anti-TF rabbit polyclonal primary antibody (1/10, ab104513, AbCam) during 30 minutes. Then, they were washed in 1mg/mL PBS/BSA solution to saturate non-specific sites and incubated with the gold labelled anti-protein A rabbit monoclonal secondary antibody (PAG15 1/50, Utrecht Medical School) for 1 hour. Then, grids were successively washed in PBS for 5 minutes and in water for 10 minutes. Finally, grids were transferred onto drops of methylcellulose 2%/uranyl acetate 5% solution (in 9/1 proportions), incubated on ice and in dark for 10 minutes. Grids were collected with a loop and dried on Whatman paper and stored for at least 2 hours before reading on the microscope. Grids have been observed with a transmission electron microscope (JEOL1200 EX) with a digital camera (VELETA).

MSCs and monocytes supernatant co-culture

CMML monocytes were cultured during 48 hours in RPMI medium only complemented with Glut 1%. Afterwards, monocytes were pelleted and supernatant recovered to

eliminate cells and others debris by serial centrifugations (800g, 10 minutes; 800g, 10 minutes; 2000g, 10minutes).

Firstly, 0.2 million MSCs issued from healthy donor were cultured with monocytes supernatant and 0.2 million MSCs of the same patient were cultured with a medium containing RPMI and Glut 1% during 48 hours. Afterwards, hemostasis assays were performed on MSCs.

In a second time, five culture conditions were tested. For each, 0.2 million MSCs issued from healthy donor were cultured with different types of supernatant during 48 hours: 1) Medium alone 2) Monocytes supernatant 3) Monocytes supernatant ExoFree (ultracentrifuged twice to eliminate EVs) 4) The pellet after first ultracentrifugation corresponding to mEVs resuspended in Medium 5) The pellet after the second ultracentrifugation corresponding to sEVs resuspended in Medium 6) mEVS and sEVs isolated from CMML monocytes SN in the same time. Both mEVs and sEVs were characterized with Nanoparticle Tracking Analysis and counted, so that the same amount of mEVs and sEVs were putted in culture medium (fixed to 10 million EVs as for previous experiments). Afterwards, hemostasis assays were performed on MSCs. For each condition, TGA was performed on approximately 0.025 million MSCs, in replicates with or without an inhibiting anti-tissue factor antibody (anti human-CD142, clone HTF-1, eBioscience) at a final concentration of 8 µg/ml.

HSCs and sEVs co-culture

Incorporation of CMML sEVs into HD HSCs

To demonstrate the transfer of sEVs produced by CMML MSCs into HSCs, we labelled sEVs with 2 µM of Vybrant DiD cell-labeling solution (Molecular Probes, Thermo Fisher Scientific France) during 1 hour at 37°C. Labelled sEVs were then washed with PBS

and recovered by 100 000 g ultracentrifugation. They were then applied on HSCs. For this experiment, HSCs were thawed, according to our protocol, only few hours before co-culture with sEVs. Cells were counted and seeded on chambered cell culture Slides equivalent to 96 wells culture plate (Nunc Lab-Tek, Sigma, France) to allow direct microscope observation and to optimize cells viability. The medium used was Myelocult H5100 (StemCell technologies) with 100 ng/mL of FLT3-ligand (Peprotech), 100 ng/mL of Stem Cell Factor (SCF, Peprotech) and 50 ng/mL of Thrombopoietin (TPO, Peprotech) and 100 ng/mL of IL-3 (Miltenyi). sEVs were applied on cells at the rate of 10^5 sEVs per HSC (we have set this concentration based on previous assays to optimize the visualization of the penetration of Evs in HSCs). After 18 hours, 1µl of anti-CD34-FITC was added to wells containing HSCs for imaging on live cells by confocal microscopy (63x/1.4 oil immersion objective, Zeiss Dynascope LSM710 NLO from Institute for Advanced Biosciences in Grenoble). Images were taken at the level of the equatorial planes of the cells.

Thrombin generation assay (TGA) of HSCs

CMML or HD HSCs were thawed according to our protocol. After thawing, HSCs were counted and cultured for 48h in 96 wells plate. Thereafter, they were counted again and co-cultured without or with sEVs (derived from CMML or HD MSCs) at the rate of 1.10^5 sEVs per HSCs during 48h. They were cultured in a medium containing Myelocult™ H5100 (Stemcell technologies) with 100 ng/mL of FLT3-ligand (Peprotech), 100 ng/mL of Stem Cell Factor (SCF, Peprotech) and 50 ng/mL of Thrombopoietin (TPO, Peprotech).

Afterwards, cells were harvested and hemostasis assays were performed with or without an inhibiting anti-tissue factor antibody at a final concentration of 8 µg/ml (anti human-CD142, clone HTF-1, eBioscience).

Proliferation-Thymidine incorporation

CMML or HD HSCs were thawed and counted according to our protocol. They were co-cultured with or without sEVs (derived from HD or CMML MSCs) at the rate of 1.10^5 sEVs per HSCs. For condition conditions with HTF-1, sEVs were previously incubated with the antibody during 1hour before to be added on HSCs. During co-culture, HSCs were cultured in a medium containing Myelocult^{TM} H5100 (Stemcell technologies) with 100 ng/mL of FLT3-ligand (Peprotech), 100 ng/mL of Stem Cell Factor (SCF, Peprotech) and 50 ng/mL of Thrombopoietin (TPO, Peprotech), in triplicates in 96 round-bottom wells plate during 6 days.

After 6 days of culture, 1µCi/well ^{3}H-[methylthymidine] was added for the last 16-18 hours. Cells were collected with a Filtermate 196 multiple harvester (Packard Inc, Prospect, Ct) and thymidine incorporation was measured in a TopCount liquid scintillation counter (Packard Inc).

Statistics

Statistics were made with bilateral unpaired or paired t-test on Graphpad Prism5 software (http://www.graphpad.com/scientific-software/prism/). Results are expressed as mean ± SEM. Spearman test was performed to study the correlation between CMML biological characteristics and EVs PCA. Statistical significance was determined by a p-value ≤ 0.05. Significance of the test: * ≤0.05, ** ≤0.005, *** ≤0.0005, **** ≤0.0001.

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SUPPLEMENTAL FIGURES

Supplemental Table 1– Patients and samples

CMML: chronic myelomonocytic leukemia, HD: healthy donor, MSCs: mesenchymal stromal cells, HSCs: hematopoietic stem cells, TGA: Thrombin Generation Assay. EVs: extracellular vesicles

Supplemental Table 2 – Correlation between CMML biological characteristics and EVs PCA

CMML: chronic myelomonocytic leukemia, HD: healthy donor, HSCs: hematopoietic stem cells, SN: supernatant, mEVs: medium extracellular vesicles, sEVs: small extracellular vesicles.

Spearman test was performed. Statistical significance was determined by a p-value ≤0.05.

Supplemental Figure 1 – Characterization of sEVs

CMML: chronic myelomonocytic leukemia, HD: healthy donor, MSCs: mesenchymal stromal cells, SN: supernatant, mEVs: medium extracellular vesicles, sEVs: small extracellular vesicles

A – sEVs extraction in culture supernatant (SN) with an adaptation of C. Théry's protocol.

B – sEVs visualization by electron microscopy. Two small EVs of 67 and 80nm respectively with the typical cup shape appearance.

Observation was made on transmission electron microscope (JEOL1200 EX) with a digital camera (VELETA), G=100 000, at room temperature.

C – Western blot analysis of HD or CMML MSCs, mEVs and sEVs showing the expression of Flotillin (48 kDa), Alix (100 kDa for the monomeric form and 200 kDa for dimeric form) and CD63 (26-50 kDa).

MSCs were used as positive control and Exofree medium as negative control.

CD63 is a glycoprotein and an EVs tetraspanin well-known marker. It shows variable aspect on depending on the cell and EVs because of its different N-linked glycosylation sites. Therefore, it can have the aspect of a smear spanning from 26kDa to 50kDA according to its glycosylation status.

Supplemental Figure 2 – Monocytes isolation and culture (CMML patient)

CMML: chronic myelomonocytic leukemia

A – Cytospin of $CD14^+$ fraction after cell sorting.

Optic microscopy, May Grünwald Giemsa staining, G=40, at room temperature.

B – Flow cytometry characterization of $CD14^+$ fraction after cell sorting.

C – Cytospin of $CD14^+$ fraction after 40h of incubation with serum deprivation.

Optic microscopy, May Grünwald Giemsa staining, G=40, at room temperature.

D – Flow cytometry characterization of apoptosis and necrosis after 40h of incubation with serum deprivation.

Supplemental Figure 3 – Co-culture HSCs with sEVs from HD and CMML HSCs without or with anti-HTF-1 antibody.

CMML: chronic myelomonocytic leukemia, HD: healthy donor, HSCs: hematopoietic stem cells, SN: supernatant, mEVs: medium extracellular vesicles, sEVs: small extracellular vesicles, TF: Tissue Factor, TGA: Thrombin Generation Assay.

Lag times are expressed as fold changes of the lag time obtained with positive control (TF 0.5 pM).

The negative control used was MPR 0.5. Any thrombin generated after corresponded to contact phase activation.

Experiments were performed without or with a blocking anti TF antibody HTF-1 at the final concentration of 8µg/ml.

Significance of the test: * ≤0.05, ** ≤0.005, *** ≤0.0005, **** ≤0.0001

A – TGA results performed with or without HTF-1 and after HD HSCs and sEVs co-culture.

B – HSCs proliferation assay results performed with or without HTF-1 and after HSCs co-culture with sEVs.

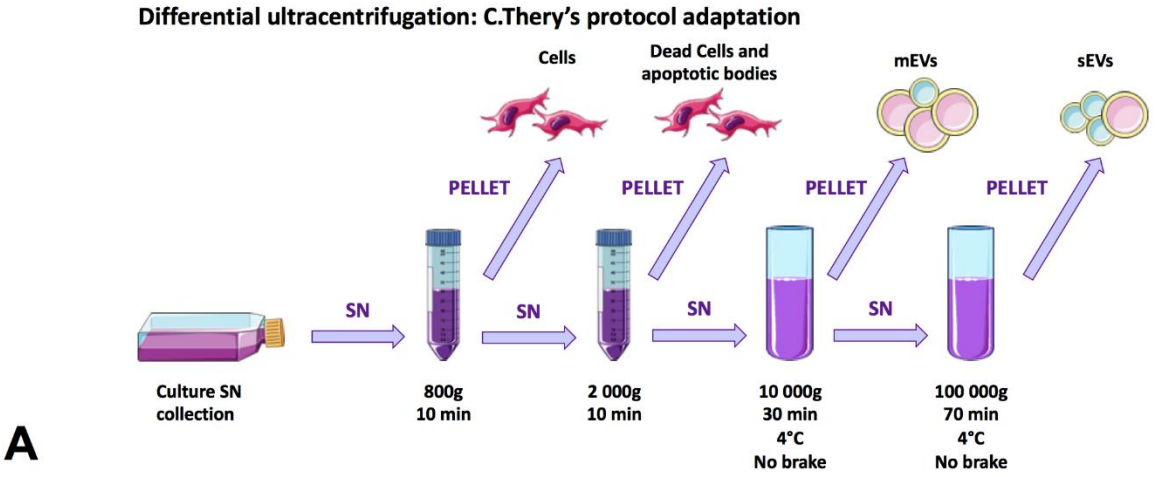
Supplemental Table 1

		demography		disease characteristics							Cells utilisation	EVs procoagulant activity		
		age	sex	bone marrow karyotype	molecular biology	blood %MO-1	Marrow myelobasts (%)	Marrow monocytes (%)	peripheral monocytes (G/L)	CMML type (0-1-2)		Fold change Lagtime mEVs	Fold change Lagtime sEVs	Fold change Lagtime Evs
CMML MSCs (n=5)	patient 1	76	M	46 XY del (13) (q13q21)(3)/ 46 XY (17)	ND	ND	1,5	6	3.2	1	TGA on MSCs and EVs collect	0,5	0,3	0,4
	patient 2	66	F	46 XX (20)	TET2	99.4	8	14	3.5	2		0,4	0,4	0,4
	patient 3	68	M	46 XY (20)	ND	ND	5,5	2	13.7	0		0,5	0,3	0,4
	patient 4	90	F	46 XX (20)	ND	90	1,5	14,5	1.5	2		0,7	0,5	0,6
	patient 5	92	F	46 XX (20)	ND	87	5,5	4	1.4	0		0,3	0,5	0,4
HD MSCs (n=8)	patient 6	61	F	ND	ND	ND			0.3					
	patient 7	74	M	ND	ND	ND			0.5					
	patient 8	68	M	46 XY (20)	ND	ND			1.1					
	patient 9	53	M	ND	ND	72			0.6					
	patient 10	40	M	ND	ND	ND			0.4					
	patient 11	64	M	cardiac surgery patient							EVs collect and co-culture with CMML monocytes supernatant			
	patient 12	57	F											
	patient 13	61	M											
CMML monocytes (n=13)	patient 14	74	M	47, XY, +14(18)/46, XY(2)	ND	82	2	9,5	1.3	2	TGA on EVs monocytes	0,1	0,2	0,2
	patient 15	84	F	46 XX (20)	ND	91	4,5	8	1.1	1		0,3	0,3	0,3
	patient 16	78	M	46 XY (20)	ASXL1, JAK2, TET2	94.4	2,5	14	3,2	2		0,2	0,3	0,2
	patient 17	84	M	46 XY (20)	SRSF2, TET2	97,5	1	16	2.3	2		0,3	0,5	0,4
	patient 18	74	M	46 XY (20)	ND	75.6	1,5	7,5	1.4	1		0,6	0,7	0,7
	patient 19	85	F	46 XX (20)	CBL, SRSF2, TET2	81.5	1,5	10,5	1.3	2		0,7	1,0	0,8
	patient 20	74	M	47, XY, +14(18)/46, XY(2)	ND	82	2	9,5	1.3	2		0,2	0,2	0,2
	patient 21	67	F	46 XX (20)	TET2	72	8	16,5	3.5	2		0,2	0,4	0,3
	patient 22	79	F	45,X,-X(20)	ND	94,6			1.91		Co-culture with HD MSCs			
	patient 23	81	M	46 XY (20)	ND	93.1			1.8					
	patient 24	84	M	45,X,-Y(20)	ND	96.7			1.8					
	patient 25	58	F	46 XX (20) t(X;2) (q21;q23) (20)	ND	89			2.8					
	patient 26	85	M	45, X, -Y (20)	ND	67			3.9					
Reactionnal monocytes (n=4)	patient 27	82	F	46 XX (20)	ND	55.1			0.89		TGA on EVs monocytes			
	patient 28	68	M	ND	ND	65.7			0.8					
	patient 29	52	F	ND	ND	78			1					
	patient 30	87	M	45,X,-Y(6)/46 XY (14)	ND	40.8			0.81					
HD monocytes (n=1)	patient 31	unknow (anonymous blood donor patient)									TGA on EVs monocytes			
CMML HSCs (n=5)	patient 32	77	M	46 XY (20)	ND	84			0.3		Co-culture with sEVs			
	patient 33	75	M	46 XY (20)	ND	85			1.4					
	patient 34	93	F	46 XX (20)	ND	80			1.3					
	patient 35	67	F	46 XX (20)	TET2	72			3.5					
	patient 36	58	F	46 XX (20) t(X;2) (q21;q23) (20)	ND	89			2.8					
HD HSCs (n=6)	patient 37	75	M	ND	ND	88			1.1		Co-culture with sEVs			
	patient 38	81	M	45,X,-Y(7)/46 XY (13)	ND	91			0.3					
	patient 39	77	F	ND	ND	73			0.5					
	patient 40	61	M	cardiac surgery patient										
	patient 41	53	M	ND	ND	62			0.4					
	patient 40	83	F	ND	ND	62			9.4					

Supplemental Table 2

		Statistical analysis			
		Marrow myelobasts (%)	**Marrow monocytes (%)**	**Blood monocytes (G/L)**	**CMML type (0-1-2)**
CMML MSCs Evs (Fold change lagtime)	**mEVs**	r=-0,703	r=0,4104	r=-0,0513	r=0,4056
		p=0,2333	p=0,5000	p=>0.9999	p=0,6000
	sEVs	r=0	r=0,4743	r=-0,9487	r=0,25
		p=>0.9999	p=0,4667	p=0,0667	p=0,8667
	EVs	r=-0,559	r=0,7071	r=-0,7071	r=0,559
		p=0,6000	p=0,4000	p=0,4000	p=0,8000
CMML Monocytes EVs (Fold change lagtime)	**mEVs**	r=-0,5138	r=-0,3273	r=-0,3119	r=-0,4025
		p=0,2413	p=0,4778	p=0,4976	p=0,4762
	sEVs	r=-0,5455	r=0,01802	r=0,09091	r=-0,07977
		p=0,2099	p=0,9889	p=0,8468	p=0,9524
	EVs	r=-0,5688	r=-0,1637	r=-0,1743	r=-0,2415
		p=0,1968	p=0,7254	p=0,7016	p=0,6667

Supplemental Figure 1



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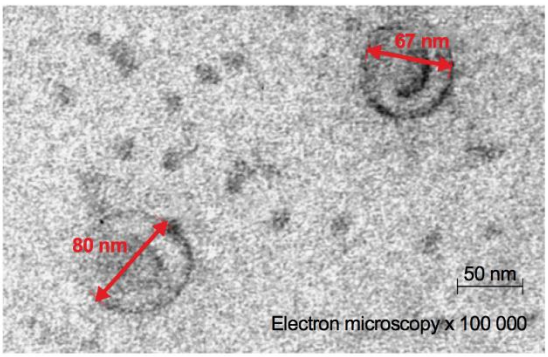

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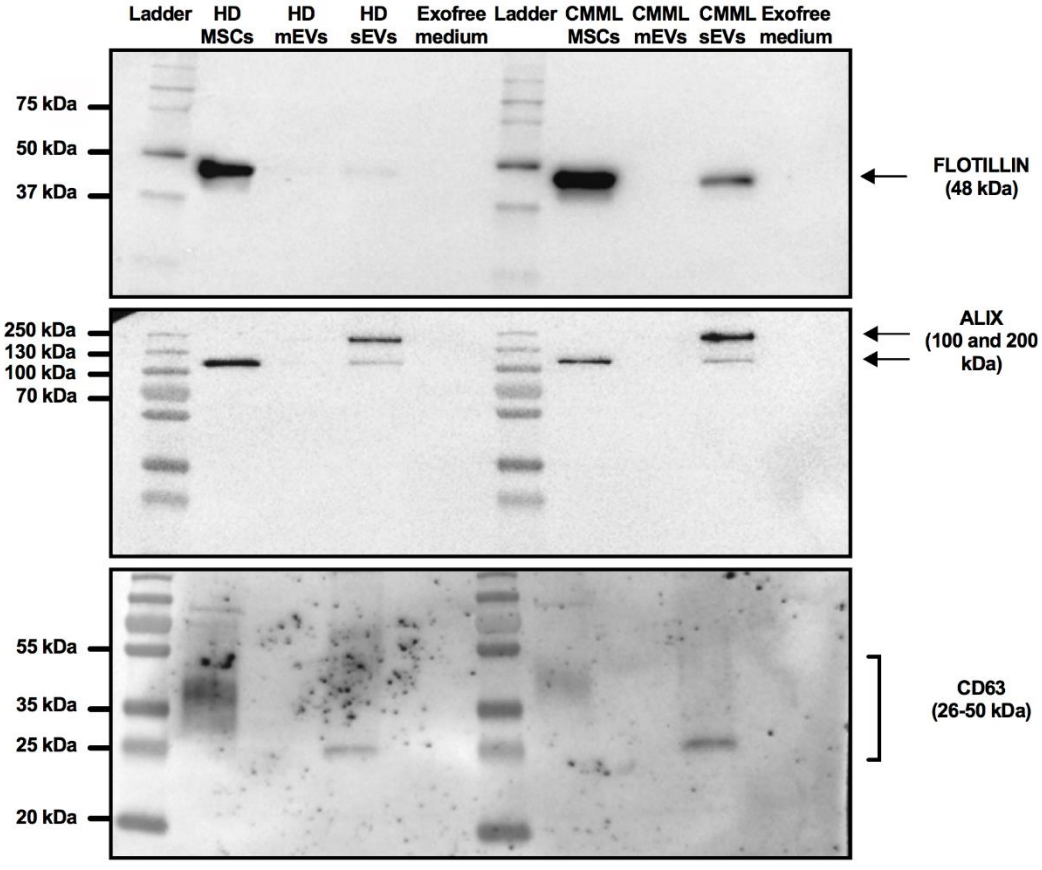

C

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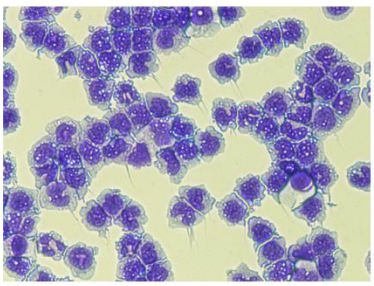
Supplemental Figure 2



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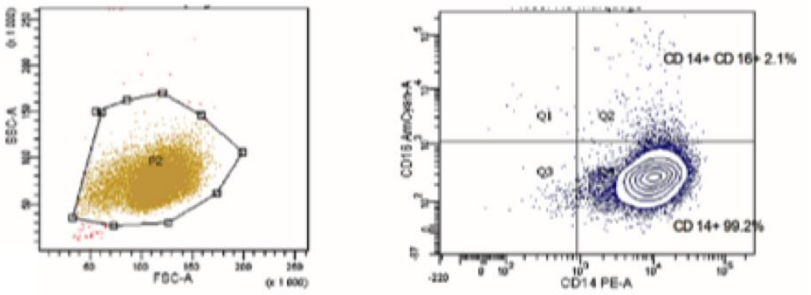

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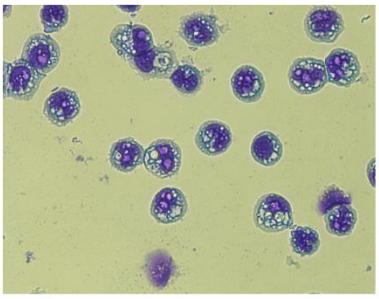

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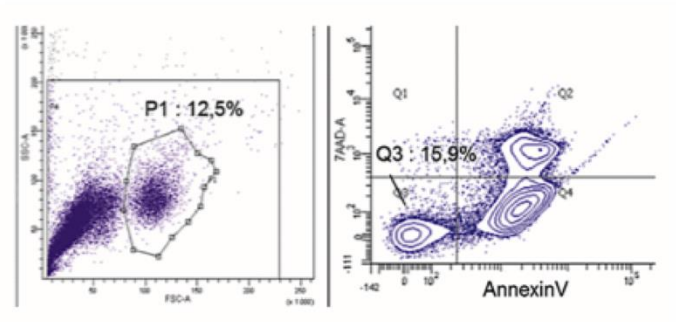

D

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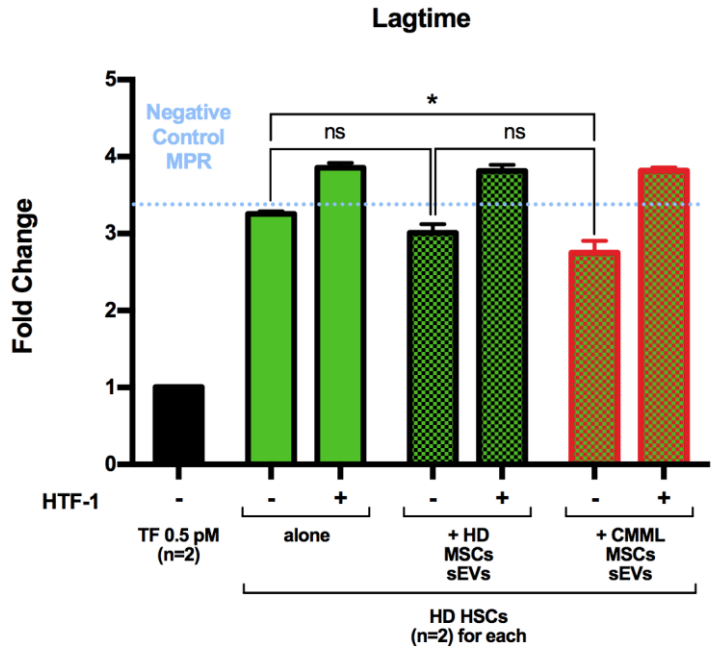
Supplemental Figure 3



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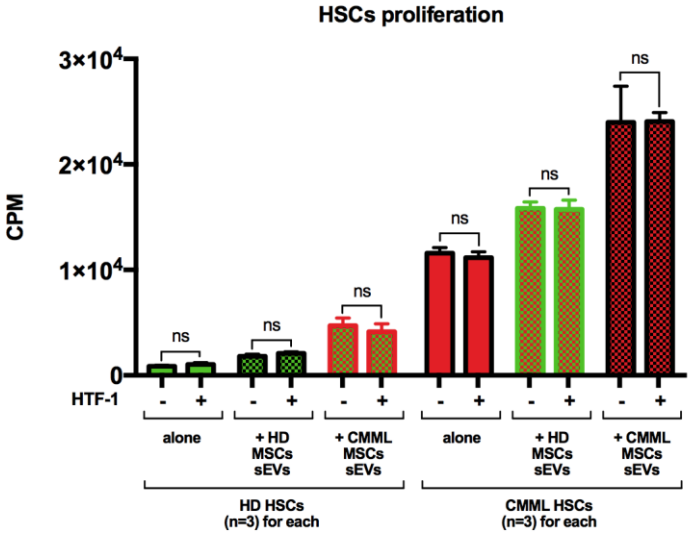

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