Plasma-activated medium as an innovative anticancer strategy: Insight into its cellular and molecular impact on in vitro leukemia cells

Supporting Information for Plasma Processes and Polymers

Plasma activated medium as an innovative anticancer strategy: insight into its cellular and molecular impact on in *vitro* leukemia cells.

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Preliminary results on PAM cytotoxic effects on blasts from leukemic patients

The present study was approved by the Comitato Etico e Sperimentazione del Farmaco dell'Azienda Ospedaliero-Universitaria Pisana, Area Vasta Nord-Ovest Toscana. Written informed consent was obtained from patients. The study was conducted according to the principles expressed in the Declaration of Helsinki (1996) and its amendments (Fortaleza, 2013).

Patients characteristics are presented on Table S1. Leukemia diagnosis was established by a combined morphological, immunological, cytogenetic and molecular analyses, which were performed to peripheral or bone marrow blood samples. Samples were collected in tubes containing preservative-free heparin. Leukemic cells were isolated by Ficoll-Histopaque density gradient centrifugation, washed with PBS 1x solution and then suspended in proper medium (RPMI 1640 added with 15% FBS, 1% L-glu and 1 % antibiotics solution).

Table S1. Clinical features of patients

Diagnosis	Sex	Age	Karyotype/Molecular biology	Timing	Previous therapy	Risk (European Leukemia Network)	FAB
AML1	F	69	/		None	Intermediate-I	M1
AML2	F	69	FLT3 positive/ITD[a]		None	Intermediate-I	M0
AML3	M	73	/		None	Intermediate-I	M0
AML4	M	65	FLT3 positive/ITD		None	Intermediate-I	M1
AML5	F	41	+8, del20		None	Intermediate-I	M0
AML6	M	59	FLT3 positive/ITD		None	Intermediate-I	M1
AML7	F	49	FLT3 positive/ITD	Relapse	3+7[b] 3+5+3[c] 2[d]	Intermediate-I	M0
AML8	M	65	Monosomy 7	Non-responder	3+7	Adverse prognosis	M1

[a] internal tandem duplication.

[b] combination drug protocol used as induction chemotherapy and consisting of three days of anthracyclines and seven days of cytarabine; [c] consolidation chemotherapy consisting of three days anthracyclines, five days cytarabine and three days etoposide; [d] consolidation therapy two days cytarabine at high dosage.

Blast treatment was performed as previously described in Material and Methods section (2.3 Treatment conditions) and cell viability was assessed using Guava Viacount reagent (2.4 Analysis of cell viability).

Results from eight AML samples showed a different cytotoxic activity of PAM according to patient's characteristics, such as FLT3/ITD mutations. On FLT3 positive/ITD patients (AML2, AML4 and AML6), we did not observe any cytotoxic effect both after 24 (Figure S1a) and 48 h (data not shown) from PAM exposure, whereas on FLT3 negative patients (AML3, AML5 and AML8) PAM decreased cell viability (Figure S1b). Interestingly, the highest cytotoxic effect of PAM was observed in blasts of one relapsing (AML7) and one refractory patient (AML8) (**Figure S1**). At PAM 180 s, the viability of AML7 blasts was 56.0% (Figure S1a) and the viability of AML8 blasts was around 30% (**Figure S1b**), compared to 100% of untreated blasts (dashed line). For both relapsing and refractory samples, it was not possible to test all two PAM treatment conditions, due to the insufficient number of blasts. Blast viability was checked also after 48 h from PAM' exposure, but any significant difference was recorded in PAM cytotoxicity compared to 24 h, except for a general decrease in blast viability, that includes untreated cells (data not shown).

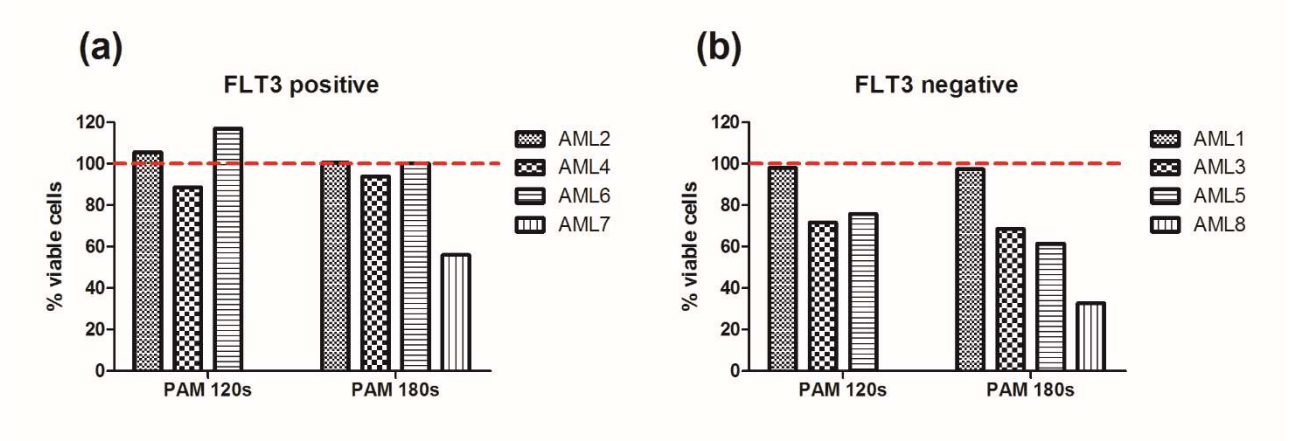

Figure S1: Percentage of viable blasts after 24 h from PAM exposure. Patients were classified according to FLT3 mutations (a, positive; b, negative).