Ematologia – Policlinico Universitario di Palermo

Cryopreserved platelets : results from *in vitro* and *in vivo* studies

Mariasanta Napolitano, MD, PhD, Lucio Lo Coco*, Giorgia Saccullo MD ,Piera Stefania Arfò, PhD, Claudia Di Gesù, PhD Ivan Noel Sciortino,PhD Giovanni De Francisci, MD, Amalia Reina, PhD, Giuseppe Tarantino, MD, Rosalia Agliastro, MD3*, and Sergio Siragusa, MD.

Background: Cryopreserved PLTs (CRY-PLTs) are reported to have a greater in vivo hemostatic effect than liquid-stored PLTs. Aims of this study were: i.to evaluate the thrombin generation potential of buffy coat derived cryopreserved PLTs (CRY BC-PLT) in comparison with fresh buffy coat derived platelets concentrates; ii.to determine the efficacy and safety of CRY-PLTs transfusion in hematological patients with severe thrombocytopenia

Materials and methods: Buffy coat derived platelets were obtained from 5 buffy coats and pooled. The final PLTs concentrates were leukoreduced and transferred to a 650 mL patented cryopreservation kit which allowed mixing with dimethyl sulfoxide (DMSO 25%) in a closed system and following removal of supernatant without further manipulations. BC-PLTs were washed prior freezing, suspended in homologous plasma from 1 of the 5 donors to a final concentration of 200 mL and frozen at – 80°.CRY- BC PLTs were preserved at -80°C with 6% DMSO In vitro assays were performed before freezing and at 3,6 and 9 months after thawing . Thrombin generation (TGA) was tested in CRY BC-PLTs and compared to TG potential of fresh BC PLTs. Endogenous thrombin potential (ETP) and peak height (PH) were determined. Flow Cytometry assays for PLTs activation markers and thromboelastography were also determined.CRY-BC PLTs , were infused in five hematological patients with acute leukemia (AL) and severe thrombocytopenia (PLTs <10× 10^9/L) participating to the trial NCT02032134. Patients were monitored up to 7 days after infusion. Plasma from patients transfused with CRY-BC PLTs was tested for TGA pre-treatment and 24 hours after treatment

Results Fourty nine BC-PLTs from 245 healthy volunteer donors (145 males and 100 females, mean age: 48.16.±18.91) were prepared, cryopreserved and analyzed up to 9 months after storage. Cryopreserved PLTs show a good thrombin generation potential that is stably maintained up to 9 months after cryopreservation. Thrombin generation of CRY-BC PLTs is comparable to fresh BC-PLTs, even if slightly decreased. Infusion of CRY-BC PLT (1U) was effective in controlling mucosal bleeding (epistaxis) in two patients with AL and severe thrombocytopenia. CRY-PLT were also effective for prophylaxis in 3 patients with very low platelets count secondary to chemotherapy. CRY-BC PLTs were safe and they did not determine any thrombotic event .At flow-cytometry, CRY-BC PLTs expressed higher activation markers (CD62P,CD63) than fresh BC PLTs.CRY-BC PLTs activation/deterioration is accompanied by an effective hemostatic *in vivo* function. CRY-BC PLTs are effective and safe in preventing and controlling bleeding.

	Pre-freezing. (n= 49)	CRY-BC PLT At 3 m	CRY-BC PLT At 6 m	CRY-BC PLT At 9 m
Platelet count (x10^9/L)	1427 +/- 150	1335 +/- 99.34	1290 +/- 88.4	1200 +/- 78.4
GPIb/CD42b (%)	92.7 +/- 4.29	23.6 +/- 27.5*	16.38+/- 12.54*	17.3 +/- 9.6*
GP53/CD62p (%)	59.0 +/- 11.02	71.1 +/- 14.6	76.89 +/- 8.65	70.9 +/- 7.4
PAC-1 (%)	1.9 +/- 1.34	0.62 +/- 0.4*	0.63 +/- 0.83 *	0.49 +/- 0.48*
ETP (nM min)	529.25+/-98.64	558.82+/-114.67*	548.57+/-93.38*	533.04+/-10.3.15*
PH (nM)	132.77+/-44.9	103.4+/-44.9*	108.0+/-36.7*	132.0+/-44.6*

Table legend: CRY BC PLT: Cryopreserved buffy coat derived platelets; ETP: Endogenous Thrombin Potential,determined at thrombin generation assay (TGA) ; m= Months, PH: Peak Height at TGA

The study was funded by Sicilia PO Regione Sicilia CUP: G33F11000030004