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CELL FACTORY

Extracellular vesicles as graft biomarkers to address lung transplantation outcome

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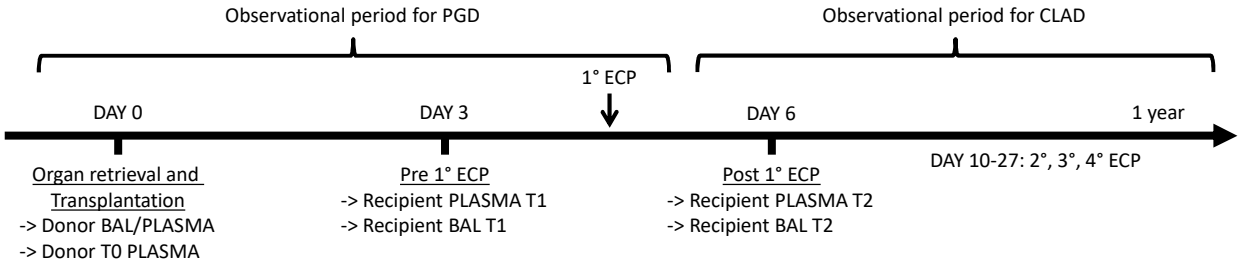
- Lung transplantation is the last therapeutic option for end-stage pulmonary failure. Yet, clinical complications may rise after transplantation, such as **primary grafts dysfunction** (PGD) or **chronic lung allograft dysfunction** (CLAD). Current clinical parameters have failed to assess the quality of the graft and to predict transplantation outcome.

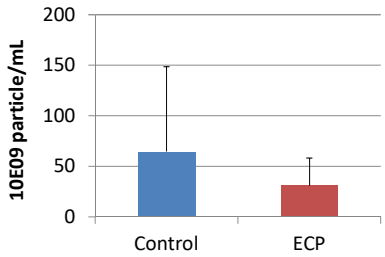
- **Extracorporeal photopheresis** (ECP) is a treatment for graft-versus-host disease. Peripheral blood white blood cells (WBC) are isolated, exposed to 8-methoxypsoralen photosensitizing agent, and subsequently treated with ultraviolet radiation before reinfusion into the patient, causing massive WBC apoptosis.

Our working hypothesis is that **extracellular vesicles (EV)** produced by either the **pre-transplantation organ** (donor) or **host** (recipient) could be non-invasive biomarkers to evaluate tissue damage at the cellular level and to **monitor organ engraftment**. Two cohorts of patients receiving (n=10) or not (n=10) ECP are currently being enrolled in the study. Study schematic:

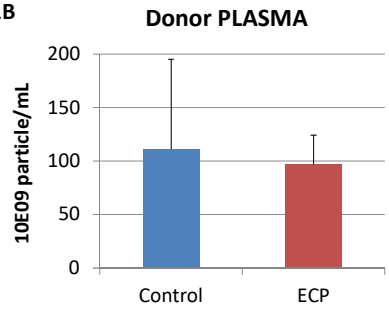


Preliminary results showed presence of particles of different sizes in **bronchoalveolar lavage** (BAL) and **plasma** of both donors (n=3) and recipients (n=3), as analyzed by nanoparticle tracking analysis (Nanosight NS300, Malvern). The particles showed highly polydispersed size distributions in a 50-1000 nm range. Different kinetics of particle production were observed in the recipients: both BAL and plasma samples showed lower particle concentration after ECP (T2). At the end of the study normalization on pre-ECP values will be carefully evaluated to remove any recipient-related bias.

1A



1B

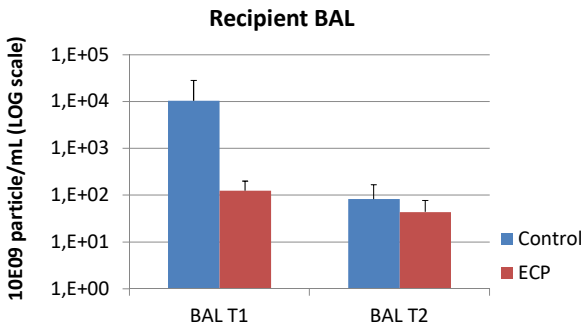


2A

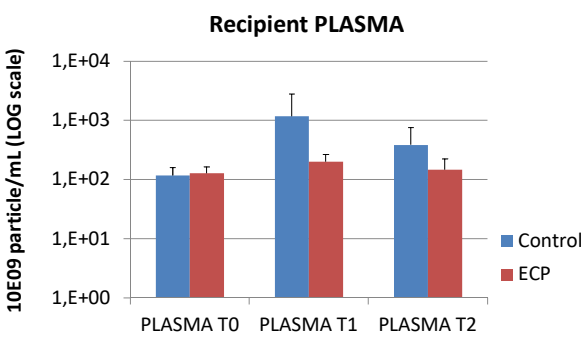
Mean particle size (nm)	ECP	Control
BAL T1 recipient	224 ± 25	226 ± 53
BAL T2 recipient	220 ± 60	251 ± 37
PLASMA T1 recipient	128 ± 36	106 ± 33
PLASMA T2 recipient	159 ± 43	153 ± 39

Figure 1: Histograms showing particle concentration in donor BAL (**A**) and plasma (**B**) before organ transplantation.
Figure 2: Table reporting mean size of detected particles in recipients at T1 and T2 (**A**). Histograms showing particle concentration in recipient BAL (**B**) and plasma (**C**) before (T1) and after (T2) ECP.
All data are presented as mean ± standard deviation.

2B



2C



Particle samples will be analyzed for **RNA content and antigen expression to define EV identity**, upon correlation with lung transplantation outcome that will be evaluated at the conclusion of the study. For the time being, no PGD or CLAD were observed. The identification of specific EV kinetics patterns and RNA signatures represents a promising approach to define biomarkers useful for thoracic surgeons who want to manage in advance complications associated to lung transplantation.

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