# PLATELET LYSATE BOOSTS TRANSGENE LEVELS AND MAINTAINS UNDIFFERENTIATED T CELL SUBTYPES FOLLOWING LENTIVIRAL DELIVERY TO HUMAN PRIMARY T CELLS

Christina Dann, Cook Regentec, USA
Christina.Dann@Cookregentec.com
Emanuele Canestrari, Cook Regentec, USA
Brianna McSwain, Cook Regentec, USA
Steve Charlebois, Cook Regentec, USA

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Adoptive immunotherapy with T lymphocytes (T cells) modified *ex vivo* has emerged as a promising therapeutic strategy to treat various cancer and autoimmune diseases. T cells engineered to express chimeric antigen receptors (CARs) have shown high rates of clinical response in patients with hematological malignancies and even early indications of clinical activity in solid tumors. Key steps in the manufacture of CAR T cell therapies are the *ex vivo* transduction of autologous cells with a viral vector and expansion of the genetically modified cells. Patient T cells respond inconsistently when cultured in chemically defined media; therefore, basal media are typically supplemented with serum. AB serum (ABS) has supply limitations and may not be sufficient to meet the expected demand for immunotherapies while fetal bovine serum (FBS) carries a risk of pathogen transmission as well as xeno-immunization against bovine antigens. Human platelet lysate (hPL) obtained from transfusable donor platelets is widely recognized as a valuable alternative to both FBS and ABS for production of clinical cellular therapies. The goal of the present study was to evaluate the feasibility of using a new pathogen-reduced human platelet lysate during lentiviral transduction of primary T cells. PR hPL is produced with a method that has been demonstrated to reduce pathogens, including enveloped and non-enveloped viruses. Cryopreserved T cells enriched from peripheral blood mononuclear cells of normal donors were thawed, activated and cultured in media containing interleukin-2 and different concentrations of FBS, ABS or PR hPL. After four days of culture, cells were transduced with a lentiviral vector to deliver a green fluorescent protein (GFP) reporter transgene. We found that two days after transduction with CMV-GFP lentivirus the percentage of GFP positive cells was generally comparable among the various conditions suggesting that the transduction process itself was similarly efficient. Interestingly, cells transduced/cultured in PR hPL consistently exhibited brighter GFP compared to cells in ABS with a mean fluorescence intensity (MFI) on average 2.5-fold higher (range 1.9 – 3.5-fold, depending on donor). PGK-GFP and EF1A-GFP lentiviruses were similarly tested and expression of the PGK-GFP reporter was also found to be higher with PR hPL. We additionally examined the phenotype of cells transduced and cultured in PR hPL using CCR7 and CD62L, markers for less differentiated T cells including $T_{naive}$ ($T_n$) and $T_{central\ memory}$ ($T_{cm}$). Cells in PR hPL exhibited a higher fraction of $T_n/T_{cm}$ T cells than cells in ABS. Emerging *in vivo* and clinical data in the CAR T field predict that the presence of less differentiated T cells is associated with improved persistence following transfer. Our study demonstrates the feasibility of using PR hPL for primary T cell modification and expansion. Furthermore, the data predict that one may be able to use less lentivirus for a given therapeutic dose of transgene expression, thereby allowing a reduction in cost of producing the therapy.

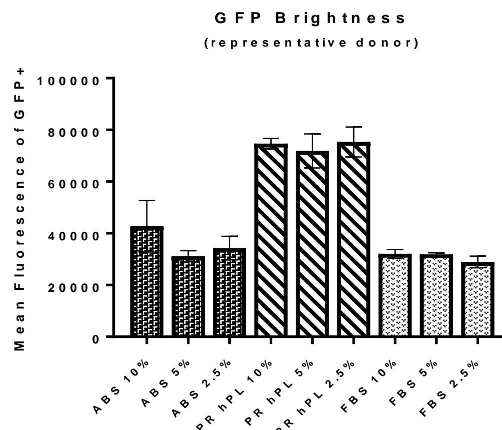


*Figure 1 – GFP brightness 2 days*