3020

Evaluation of engineering strategies allowing efficient adoptive transfer of CAR T-cells in an allogeneic setting

Alan Williams[1], Julien Valton[1], Mohit Sachdeva[1], Brian Busser[1], Sonal Temburni[1], Laurent Poirot[2] and Philippe Duchateau[2]

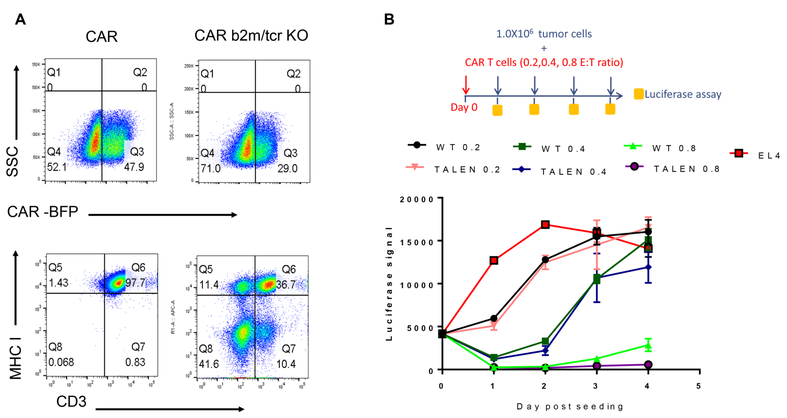
[1]Cellectis, Inc. 430 East 29th Street New York, 10016, NY - [2]Cellectis SA 8 rue de la Croix Jarry 75013 Paris

Abstract

The adoptive transfer of CAR T-cells represents a highly promising strategy to fight against multiple cancers. The clinical outcome of such therapies is intimately linked to the ability of effector cells to engraft, proliferate and specifically kill tumor cells within patients. When allogeneic CAR T-cell infusion is considered, host versus graft and graft versus host reactions must be avoided to prevent rejection of adoptively transferred cells, to minimize host tissue damages and to elicit significant antitumoral outcomes.
This work proposes a cell-engineering strategy to address the aforementioned considerations. We report the successful generation of murine B2M deficient- TCRαβ- deficient CAR T-cells and their use to develop a syngeneic mouse model of CAR T cell persistence. In addition, we describe an *in vitro* platform to mimic and prevent T cell dependent and NK cell dependent rejection of human CAR T cells. Finally, our *in vitro* platform will permit the efficient screening of inhibitors of NK cell activity. The combined *in vivo* and *in vitro* systems will be a valuable tool for advancing the persistence of CAR T therapies in immunocompetent settings.

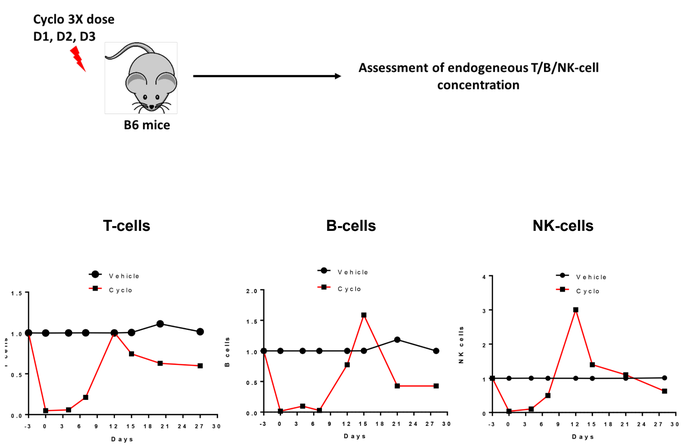
Murine cells

Generation and function of TCRαß/B2M deficient murine CAR T-cells



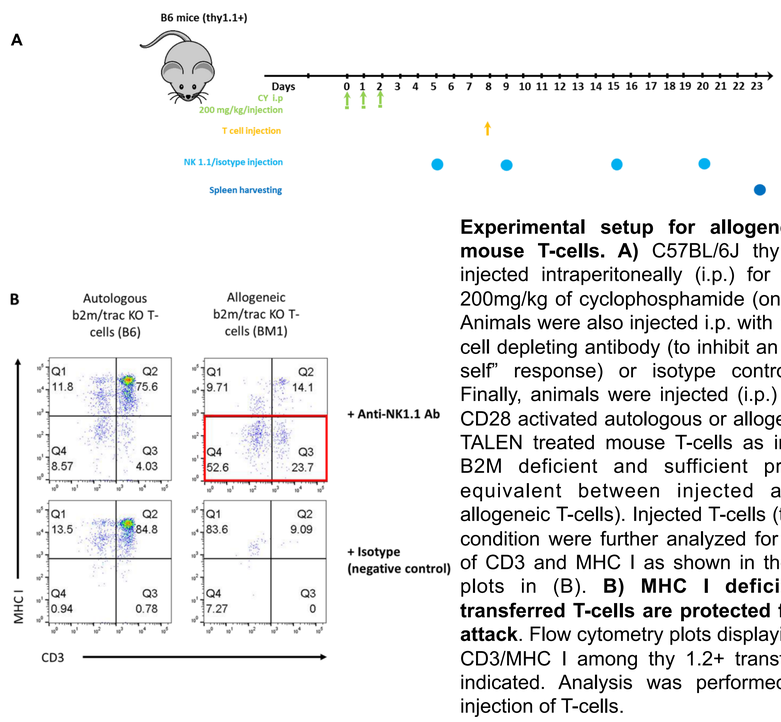
A) TCRαß and B2M deficient murine CAR T-cells can be generated by TALEN® treatment. Representative flow cytometry plots of CAR expression and CD3/MHC I expression in the presence or absence of CAR retroviral infection and TRAC/B2M TALEN treatment. **B) Engineered murine CAR T-cells display cytolytic activity toward tumor cells.** WT or TRAC/B2M TALEN® treated (41% TCRαß and B2M deficient) primary murine T-cells expressing similar levels of CAR were co-cultured at 0.2, 0.4, and 0.8 E:T ratios with 1E6 luciferase + tumor targets at Day 0. Every 24 hours, a sample of culture was analyzed for luciferase signal before re-challenge with 1E6 tumor targets supplied in fresh culture medium.

Kinetic of murine lymphocyte depletion and



Regeneration of mouse lymphocytes post cyclophosphamide treatment. Mice were treated with either vehicle or 200mg/kg of cyclophosphamide for three consecutive days (Day -3 to -1 with one dose per day). Blood samples were collected at Day 0 and after as indicated where the number of T, B and NK cells were analyzed by flow cytometry. Data are normalized for the volume of blood collected and plotted relative to the frequency of cells observed in vehicle treated mice. (Vehicle N=4; Cyclophosphamide N=4).

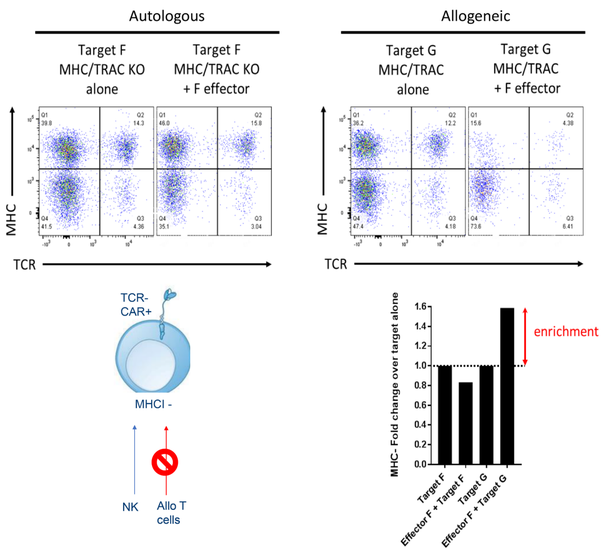
Allogeneic transfer of murine T-cells



Experimental setup for allogeneic transfer of mouse T-cells. A) C57BL/6J thy1.1+ mice were injected intraperitoneally (i.p.) for three days with 200mg/kg of cyclophosphamide (one dose per day). Animals were also injected i.p. with 100µg of anti-NK cell depleting antibody (to inhibit an NK cell "missing self" response) or isotype control as indicated. Finally, animals were injected (i.p.) with 25E6 CD3/CD28 activated autologous or allogeneic TRAC/B2M TALEN treated mouse T-cells as indicated (TRAC/B2M deficient and sufficient proportions were equivalent between injected autologous and allogeneic T-cells). Injected T-cells (thy 1.2+) of each condition were further analyzed for their expression of CD3 and MHC I as shown in the flow cytometry plots in (B). **B) MHC I deficient allogeneic transferred T-cells are protected from host T-cell attack**. Flow cytometry plots displaying expression of CD3/MHC I among thy 1.2+ transferred T-cells as indicated. Analysis was performed 15 days post injection of T-cells.

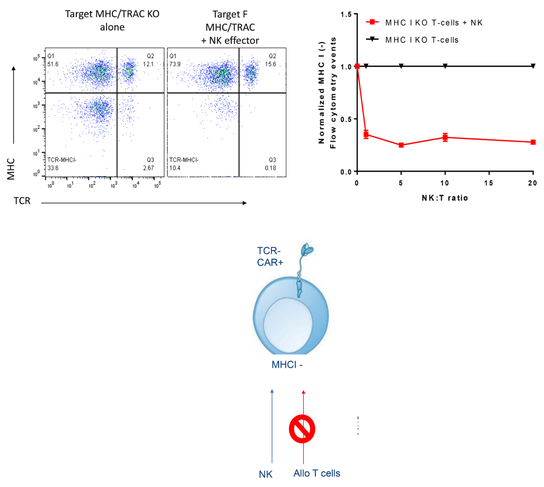
Human cells

In vitro-Mixed Lymphocyte Reaction (MLR)



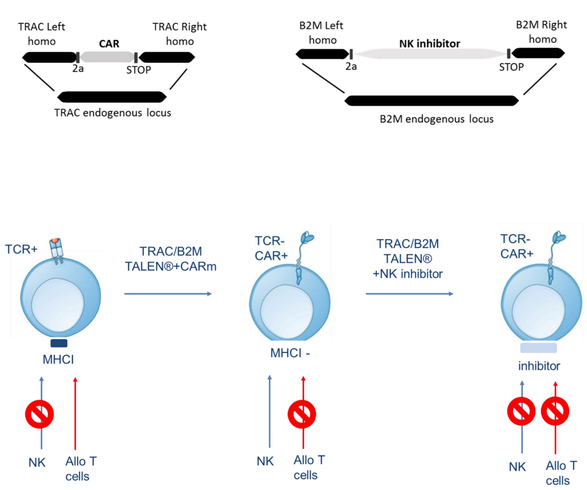
Allogeneic MHC I negative human T-cells are protected from allo-responsive T-cell attack during mixed lymphocyte reaction (MLR). T-cells from Donor F were previously enriched by priming them against irradiated target PBMCs from Donor G. Effector T-cells were then co-cultured with CFSE labeled TRAC/B2M TALEN treated targets for the autologous (effector F + target F) or allogeneic (effector F+ target G) condition in triplicate at a 1:1 E:T ratio for 24 hours. While no enrichment of MHC I - targets was observed in the autologous condition, an increased frequency of MHC I - targets from Donor G was observed in the presence of effector F T-cells. These data indicated that a cytotoxic allogeneic response against MHC I + targets can be initiated within 24 hours post co-culture.

In vitro human NK cell cytotoxicity



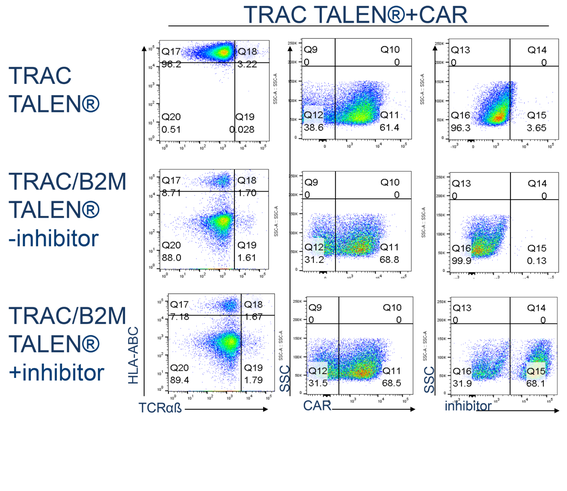
MHC I negative human T-cells can be targeted for NK cell attack. MHC I – T-cells were cultured in the presence or absence of CD2/NKp46 activated NK cells at the indicated E:T ratios. The data demonstrate greater than 50% depletion of MHC I negative T-cells at all E:T ratios tested.

CAR and NK inhibitor targeted integration



Schematic of targeted integration constructs. Diagrams showing constructs for double targeted integration of CAR and NK inhibitors at the human TRAC and B2M loci respectively. The engineered CAR T-cells products would be resistant to both NK and allogeneic T-cell cytolytic activity.

CAR and NK inhibitor expression



Double targeted integration of CAR and NK inhibitor constructs in TRAC/B2M deficient human T-cells. Flow cytometry analysis of engineered CAR T-cells treated with TALEN® and targeted integration constructions as indicated. NK inhibitor expression is documented within TRAC/B2M deficient CAR + T-cells.

Conclusions

We have developed a platform for the study of the persistence of CAR T-cells in allogeneic settings. We provide data demonstrating that allogeneic transfer of T-cells into MHC I mismatched hosts could be made possible by ablating MHC I expression from the surface of the transferred T-cells. We also show that MHC I negative T-cells could be potentially depleted by endogenous NK cells. To overcome this depletion of T-cells in vivo, we propose a screening platform to identify relevant genetically encoded NK inhibitors.