Improved ex vivo expansion of tumor-infiltrating lymphocytes from endometrial and ovarian cancer biopsies using dendritic cells derived from the leukemic cell line DCOne

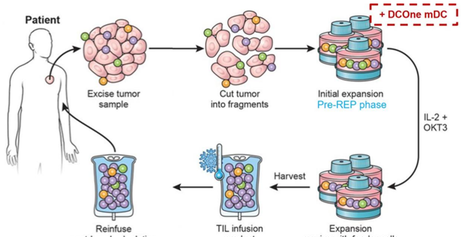
Haoxiao Zuo[1], Ziyu Wang[1] Jyoti Naik[1], Willemiek Iwema[2], Annechien Plat[2], Nienke van Rooij[2], Hans Nijman[2], Tariq Mughal[1, 3], Marco de Bruyn[2] and Alex Karlsson-Para[1] and Satwinder Kaur Singh[1]

[1] Mendus AB, Stockholm, Sweden; [2] University of Groningen, University Medical Center Groningen, Groningen, The Netherlands; [3] Tufts University Medical School, Boston, USA

satwinder.singh@mendus.com

Introduction

Adoptive cell therapy using tumor-infiltrating lymphocytes (TILs) has shown remarkable and durable anti-tumor responses in metastatic melanoma patients, even when prior therapies like checkpoint inhibitors have failed[1,2]. Beyond melanoma, TIL therapy is being explored for a wide range of solid tumors, including breast, lung, and ovarian cancers[3,4,5]. However, translating TIL therapy to other cancer types remains challenging, primarily due to the difficulty in producing sufficient quantities of TILs with the current laborious and lengthy manufacturing processes. We have recently shown successful induction of tumor-directed immune responses in high grade serous ovarian cancer patients after primary treatment using an off-the-shelf leukemic derived dendritic cell vaccination from DCOne cell line[6] (phase I ALISON trial, NCT04739527).



Schematic overview of the current TIL production process and potential DCOne platform-based improvements. Adapted from Turcotte et al. J Immunother Cancer 2025

Methods

In this study we describe a novel and robust method by repurposing the leukemic-derived dendritic cells from DCOne cell line (DCOne mDC) to expand TILs from tumor samples from ovarian and endometrial cancer patients. TILs obtained from fresh tumor biopsies were stimulated with either high dose of IL-2 or with DCOne mDC in the presence of IL-2 up to maximum of 28 days to obtain TILs. TILs were further expanded for 14 days using the standard rapid expansion protocol (REP) with irradiated allogeneic PBMC as feeder cells in the presence of anti-CD3 antibody and IL-2. Purity, viability, phenotype and functionality of expanded TILs was assessed pre- and post-REP expansion.

1) Rohaan MW, et al. N. Engl. J. Med. 2022;387:2113–2125, 2) Zacharakis N, et al. Nat Med. 2018;24:724–30, 3) Ben-Avi R, et al. Cancer Immunol Immunother. 67(8):1221–1230, 4) Pedersen M, et al. Oncoimmunology 2018;7, 5) Kazemi MH, et al. Front Immunol. 2022:13:1018962, 6) Vledder A et al. J Clin Oncol 2025;43: 5566-5566

DCOne Platform Enhances Early TIL Expansion and CD8+ Phenotype

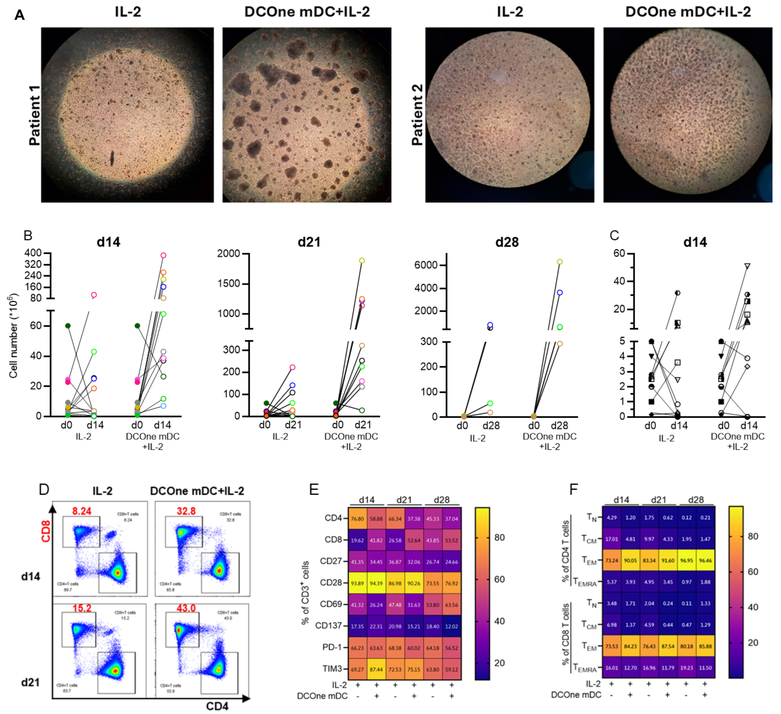


Figure 1: (A) Morphological changes of TILs on day7 of pre-REP induced by DCOne mDC stimulation from an ovarian cancer patient. (B) DCOne mDC together with IL-2 induced higher TIL proliferation on day14, day 21, and day 28, compared to IL-2 alone from ovarian cancer biopsies and (C) endometrial cancer biopsies. (D) Representative flow cytometry analysis of CD4 and CD8 T cells from one ovarian cancer patient followed by 14- or 21-day expansion with or without DCOne mDC in the presence of IL-2. (E) Heatmaps illustrating changes in the frequencies of CD4, CD8 T cells as well as changes in expression of different activation and exhaustion markers. (F) Heatmaps illustrating changes in the frequencies of naïve T cells (T_N), central memory T cells (T_{CM}), effector memory T cells (T_{EM}), and terminally differentiated effector memory T cells (T_{EMRA}) from CD4 T cells and CD8 T cells was quantified using flow cytometry.

DCOne mDC Stimulation Amplifies TIL Yield After REP with Increased Tumor-Specific Activity

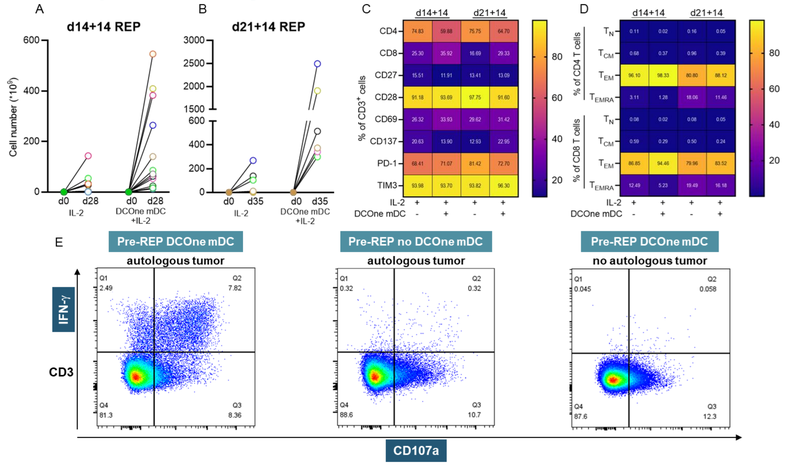


Figure 2: TILs generated with or without DCOne mDC were further expanded for 14 days rapid expansion protocol (REP) using irradiated allogeneic PBMC as feeder cells in the presence of anti-CD3 antibody and IL-2. (A, B) The TILs stimulated by DCOne mDC and IL-2 showed higher total expansion in comparison to IL-2 alone after 14 (pre-REP)+14 (REP) days (A) or 21 (pre-REP)+14 (REP) days (B). (C) Heatmaps illustrating changes in the frequencies of CD4, CD8 T cells as well as changes in expression of different activation and exhaustion markers and T cell differentiation in CD4 and CD8 subsets (D). (E) The frequency of IFN-γ+ and CD107a+ T cells post autologous tumor stimulation.

Conclusions

- Addition of DCOne mDC to standard IL-2 cultures during the pre-REP phase leads to more robust expansion of TILs up to day 28 compared to IL-2 cultures alone
- The young TILs expanded in the presence of DCOne mDC during the pre-REP phase contain a larger proportion of effector memory CD8+ T cells
- DCOne mDC stimulation during the pre-REP phase, in addition to IL-2, followed by standard REP expansion leads to superior total expansion of functional TILs with enhanced tumor-specific activity
- Leukemic-derived DC manufactured using the DCOne cell line can be used to develop more robust TIL expansion protocols to treat gynecological cancers and potentially other solid tumors