Abstract

Background
For more than a decade, allogeneic peripheral blood mononuclear cells (PBMC) have been used as accessory feeder cells that provide "costimulatory signals" necessary for the expansion of tumor-infiltrating lymphocytes (TILs) in the presence of IL-2 and CD3 stimulation (Rapid Expansion Protocol [REP]). The intrinsic heterogeneity of allogeneic PBMC is an important variable when considering the expansion and resulting phenotype of Post-REP TILs prepared for transplantation. The procurement of allo-PBMC in large numbers is also challenging and expensive. Our objective was to evaluate artificial antigen presenting cells (aAPC) as a potential substitute for PBMC. We developed a novel aAPC, CD64+ MOLM14 human leukemia cell line, genetically engineered to express recombinant CD86 (B7-2) & CD137-L (4-1BBL) (MOLM14-86/137 or aMOLM14).

Methods

The MOLM14-86/137 cell line was generated via transduction of wild type MOLM-14 with lentiviral vectors encoding genomic DNA of CD86 or CD137 downstream of the U3 promoter from MSCV. aMOLM14 were γ-irradiated at 100 Gy and frozen. aMOLM14 were cocultured with TILs in media containing OKT3 (30 ng/ml) and IL-2 (3000 IU/ml) for 11 or 14 days in G-Rex 24 well plates. We calculated their expansion (D11 or 14) and examined their differentiation/activation (flow cytometry), metabolic rate, and function.

Results

Compared to TILs cocultured with PBMC, we obtained 95-100% TILs via coculture with aMOLM14 at low ratio. This is within the range expected via coculture with PBMC. Conversely, aMOLM14 cocultured at higher ratio enhanced TIL expansion more than PBMC feeders. aMOLM14 reproducibly expanded TILs, with less variability in expansion rate than PBMC. Both artificial APC and PBMC demonstrate similar OXPHOS, glycolysis, and cytotoxicity profiles. TIL cultured with aAPC secreted similar IFN-γ and Granzyme B when compared with PBMC feeders.

Figure 1. Phenotypic characterization of parental and engineered (aMOLM14) cell lines

A) Summary of the costimulatory molecules expressed endogenously on wild type MOLM-14. B) Flow cytometry contour plot showing the expression of CD137 and CD86 on engineered MOLM-14.

Table 1. Summary of TIL expansion with artificial APC’s

Conclusions

- Coculture of TILs with aMOLM14 resulted in expansion that is similar to or better than that obtained by PBMC, and metabolic and cytotoxocity profiles that are similar to that obtained with PBMC.
- Investigation of aMOLM14 based REP protocol in a clinical setting is warranted.
- Future work will involve characterizing other immunologic molecules on aMOLM14, including release of HMBG1, cytokines, and chemokines and complete testing for adventitious viruses.

Disclosure and Funding Statement

This study was funded by Lion Biotechnologies, Inc. AV, AG, AS, JS, MAB, DW, IF, SW, JP, KR, MA, LS, BR, MF, JU, and MTL are employees of Lion Biotechnologies, Inc. and have stock options.

Acknowledgment

Outstanding support from Eden Frazier, Charlene Catalane, Christopher Mosychuk, Marcus Machin, and Alexis Hutchison, is greatly appreciated. All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors. Graphic services were provided by AOIC, LLC, and were funded by Lion Biotechnologies, Inc.