POSTER # 47

Artificial Antigen Presenting Cells Promote Expansion of Tumor-Infiltrating Lymphocytes (TILs)

Anand Veerapathran, Aishwarya Gokuldass, Amanda Stramer, Jyothi Sethuraman, Michelle A Blaskovich, Doris Wiener, Ian Frank, Seth Wardell, Joy Prewitt, Krit Ritthipichai, Michelle Abelson, Laurelis Santiago, Brian Rabinovich, Maria Fardis, James Bender, Michael T Lotze

Lion Biotechnologies, Inc. 999 Skyway Road, Suite 150, San Carlos, CA 94070

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+ MOLM-14 human leukemia cell line, genetically engineered to express recombinant CD86 (B7-2) & CD137-L (4-1BBL) (MOLM14-86/137 or aMOLM14).

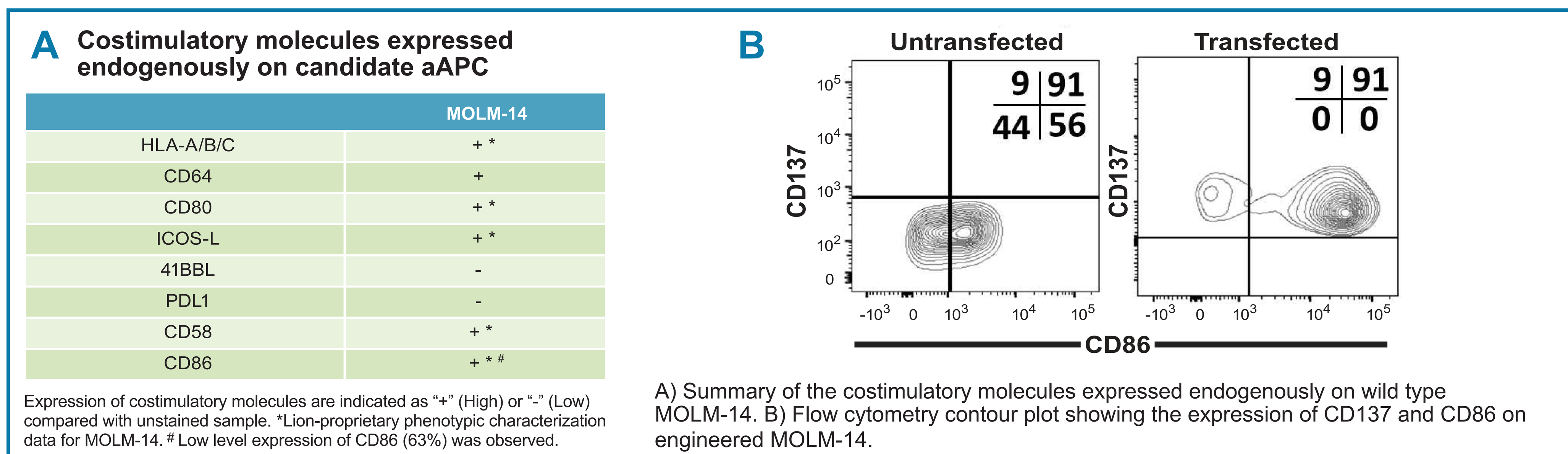
Methods

The MOLM14-86/137 (aMOLM14) cell line was generated via transduction of wild type MOLM-14 with lentiviral virions encoding genomic DNA of CD86 or CD137 downstream of the U3 promoter from MSCV. aMOLM14 were γ-irradiated at 100Gy 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 expand TIL, 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



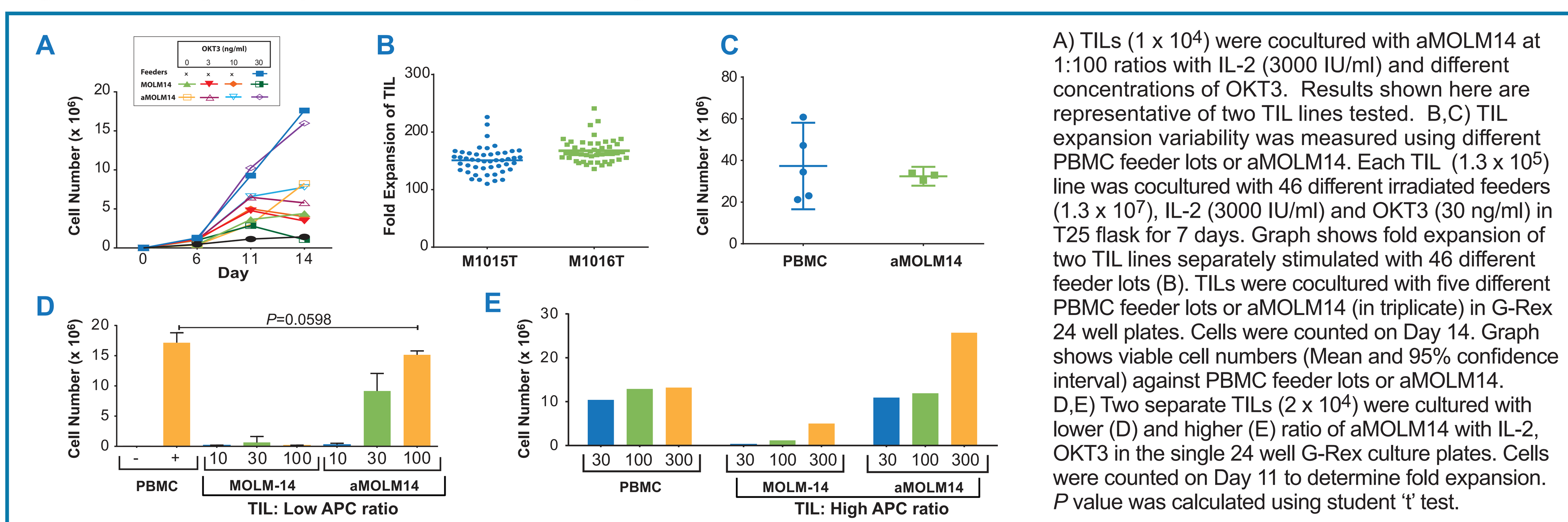
A Costimulatory molecules expressed endogenously on candidate aAPC

	MOLM-14
HLA-A/B/C	+ *
CD64	+
CD80	+ *
ICOS-L	+ *
41BBL	-
PDL1	-
CD58	+ *
CD86	+ * #

Expression of costimulatory molecules are indicated as “+” (High) or “-” (Low) compared with unstained sample. *Lion-proprietary phenotypic characterization data for MOLM-14. # Low level expression of CD86 (63%) was observed.

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.

Figure 2. Rapid expansion of TILs using aMOLM14 or PBMC feeders



A) TILs (1 x 10^4) were cocultured with aMOLM14 at 1:100 ratios with IL-2 (3000 IU/ml) and different concentrations of OKT3. Results shown here are representative of two TIL lines tested. B,C) TIL expansion variability was measured using different PBMC feeder lots or aMOLM14. Each TIL (1.3 x 10^5) line was cocultured with 46 different irradiated feeders (1.3 x 10^7), IL-2 (3000 IU/ml) and OKT3 (30 ng/ml) in T25 flask for 7 days. Graph shows fold expansion of two TIL lines separately stimulated with 46 different feeder lots (B). TILs were cocultured with five different PBMC feeder lots or aMOLM14 (in triplicate) in G-Rex 24 well plates. Cells were counted on Day 14. Graph shows viable cell numbers (Mean and 95% confidence interval) against PBMC feeder lots or aMOLM14. D,E) Two separate TILs (2 x 10^4) were cultured with lower (D) and higher (E) ratio of aMOLM14 with IL-2, OKT3 in the single 24 well G-Rex culture plates. Cells were counted on Day 11 to determine fold expansion. *P* value was calculated using student ‘t’ test.

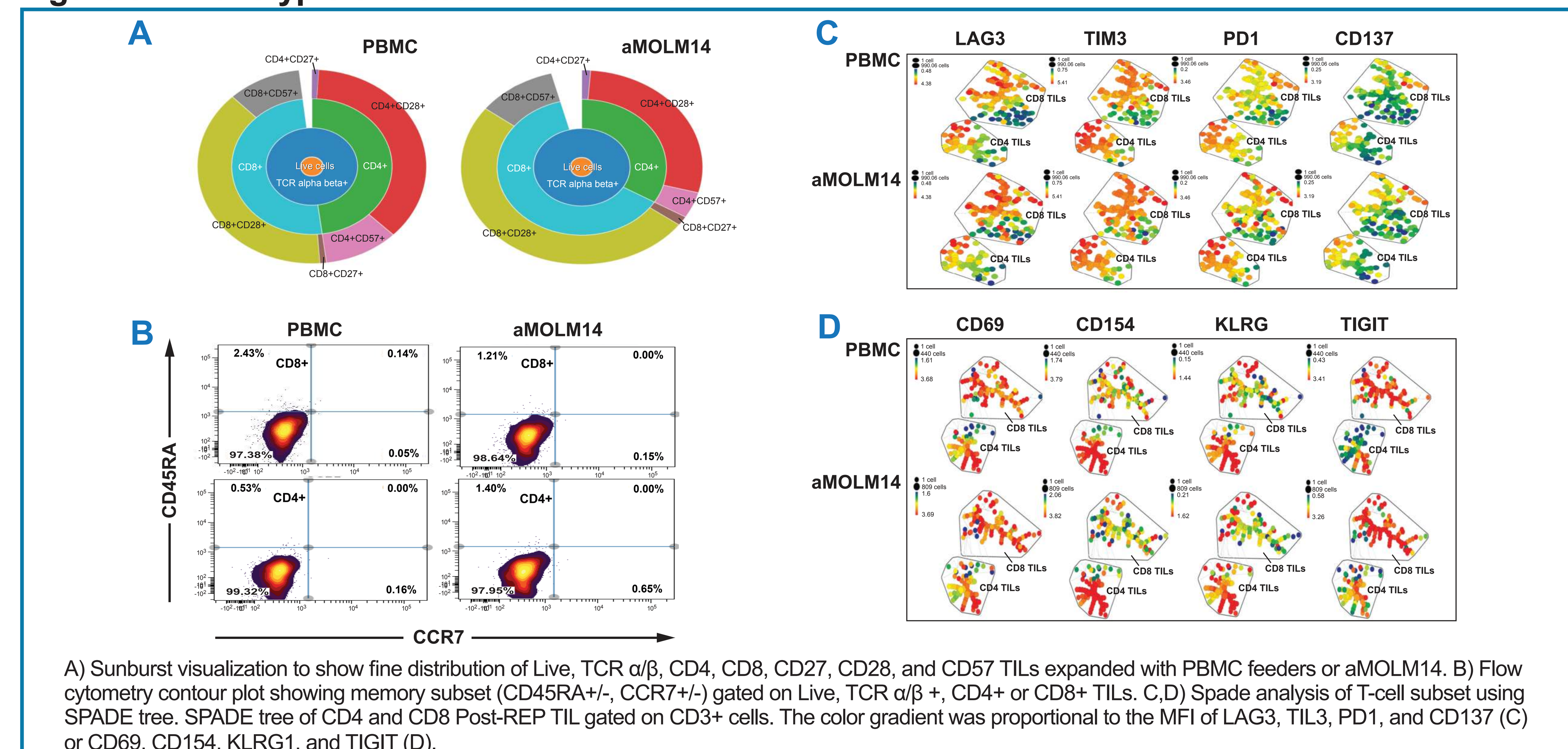
Disclosure and Funding Statement

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

Acknowledgment

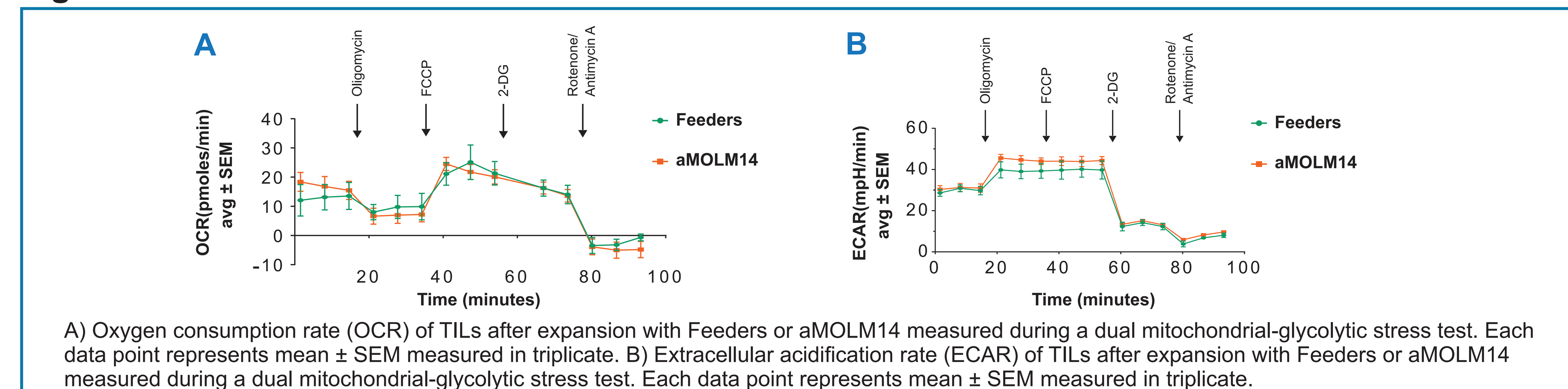
Outstanding support from Eden Frazier, Charlene Catalane, Christopher Mosychuk, Marcus Machin, and Alexis Hutchison, is greatly appreciated. All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors. Graphic services were provided by AOIC,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

Figure 3. Phenotypic characterization of Post-REP TILs



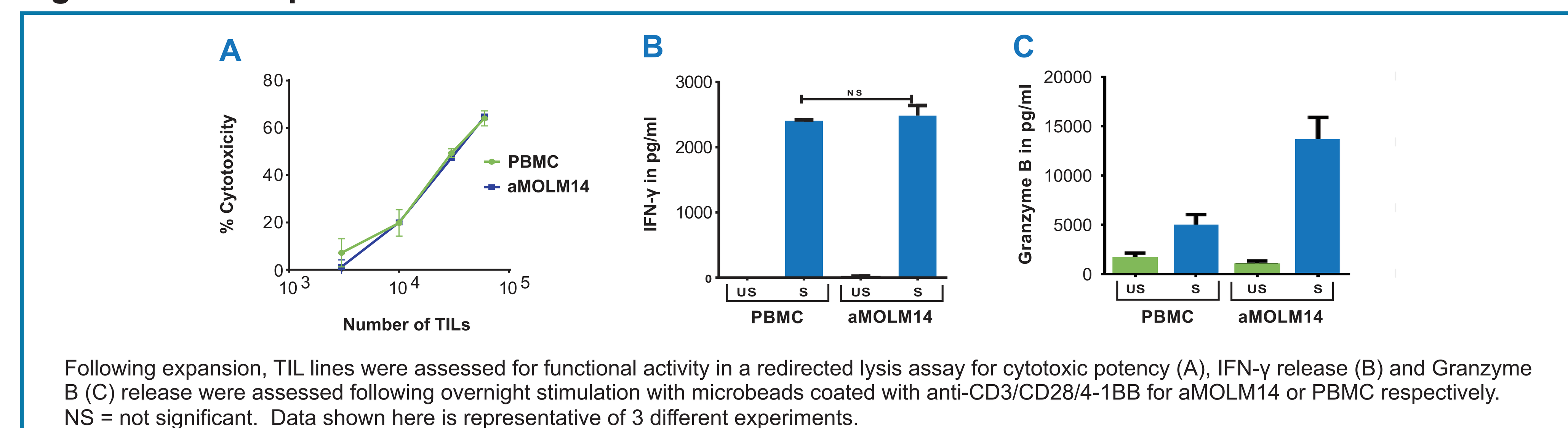
A) Sunburst visualization to show fine distribution of Live, TCR α/β, CD4, CD8, CD27, CD28, and CD57 TILs expanded with PBMC feeders or aMOLM14. B) Flow cytometry contour plot showing memory subset (CD45RA+/-, CCR7+/-) gated on Live, TCR α/β +, CD4+ or CD8+ TILs. C,D) Spade analysis of T-cell subset using SPADE tree. SPADE tree of CD4 and CD8 Post-REP TIL gated on CD3+ cells. The color gradient was proportional to the MFI of LAG3, TIL3, PD1, and CD137 (C) or CD69, CD154, KLRG1, and TIGIT (D).

Figure 4. Metabolism of Post-REP TILs



A) Oxygen consumption rate (OCR) of TILs after expansion with Feeders or aMOLM14 measured during a dual mitochondrial-glycolytic stress test. Each data point represents mean ± SEM measured in triplicate. B) Extracellular acidification rate (ECAR) of TILs after expansion with Feeders or aMOLM14 measured during a dual mitochondrial-glycolytic stress test. Each data point represents mean ± SEM measured in triplicate.

Figure 5. TILs expanded with artificial APC are functional



Following expansion, TIL lines were assessed for functional activity in a redirected lysis assay for cytotoxic potency (A), IFN-γ release (B) and Granzyme B (C) release were assessed following overnight stimulation with microbeads coated with anti-CD3/CD28/4-1BB for aMOLM14 or PBMC respectively. NS = not significant. Data shown here is representative of 3 different experiments.

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

	TIL#	Fold Expansion		Relative Expansion	CD8 (%)		CD4 (%)		Relative CD8	Relative CD4
		PBMC	aAPC		PBMC	aAPC	PBMC	aAPC		
aMOLM14	M1032-T2	2112	1936	0.92	53	65	44	27	1.226	0.614
	M1033-T6	1761	1598	0.91	50	57	36	40	1.140	1.111
	M1021T-5	2053	2024	0.99	91	82	8	17	0.901	2.125
	M1030T-4	860	853	0.99	46	78	51	12	1.696	0.235
	M1045	858*	758*	0.88	-	-	-	-	-	-
	M1021T-1	1866	1620	0.87	-	-	-	-	-	-
	M1032T-1	2423	2049	0.85	-	-	-	-	-	-
	M1042	1278	1704	1.33	8	8	88	89	0.919	1.015
	M1043	1601	1587	0.99	90	87	5	5	0.968	0.947

Fold expansions were determined on Day 11* or Day 14

Conclusions

- Coculture of TILs with aMOLM14 resulted in expansion that is similar to or better than that obtained by PBMC, and metabolic and cytotoxicity profiles that are similar to that obtained with PBMC.
- Investigation of a aMOLM14 based REP protocol in a clinical setting is warranted.
- Future work will involve characterizing other immunologic molecules on aMOLM14, including release of HMGB1, cytokines, and chemokines and complete testing for adventitial viruses.

Presented at Society for Immunotherapy of Cancer in National Harbor, Maryland, USA, November 9-13, 2016