Iovance Peripheral Blood Lymphocytes (PBL): A Potential Cell Therapy Strategy For The Treatment of Chronic Lymphocytic Leukemia

Lavakumar Karyampudi[1], Michelle Blaskovich*[1], Ian Frank*[1], Nermin Gerges[1], Amanda Stramer[1], Arvind Natarajan[1], Meixiao Long[2], Cecile Chartier[1], John Byrd[2], Maria Fardis[1]

[1]Iovance Biotherapeutics, San Carlos, CA, USA; [2]The Ohio State University, Columbus, OH, USA
*authors contributed equally to this work

IOVANCE BIOTHERAPEUTICS
ADVANCING IMMUNO-ONCOLOGY

Point of contact: Lavakumar Karyampudi
Email: kumar.karyampudi@iovance.com

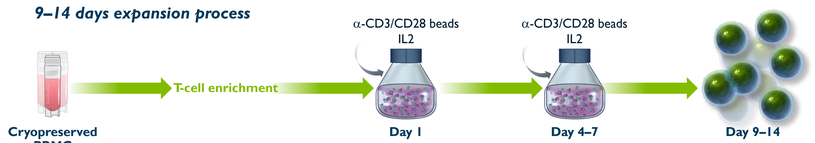
BACKGROUND

- Complete remissions in chronic lymphocytic leukemia (CLL) are rare and patients relapsing after treatment with ibrutinib are in need of novel improved therapeutic strategies.
- Adoptive cell therapies (ACT), including chimeric antigen receptor (CAR) T cells are under development for the treatment of CLL, however, these therapies are typically genetically modified products and are monoclonal. ACT using a polyclonal, non-genetically altered product, may provide a more favorable benefit/risk profile.
- Generating T cell product for ACT is a complex manufacturing process as a high percentage of T cells in common adult hematologic malignancies including CLL or small lymphocytic lymphoma (SLL) are in an exhausted/dysfunctional state.
- Ibrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, is known to improve proliferative and effector functions of T cells by inhibiting IL-2 inducible T cell kinase (ITK).
- This study presents preliminary data on successful generation of peripheral blood lymphocytes (PBL) as bulk T cell product (product name: IOV-2001) from ibrutinib-treated patients with CLL.
- Clinically relevant doses of IOV-2001 can be produced with 50 mL blood, with no need for leukapheresis.
- First-in-patient testing of IOV-2001 is planned.

STUDY OBJECTIVES

- To develop a short and efficient method for the generation of PBL from peripheral blood mononuclear cells (PBMC) of ibrutinib-treated patients with CLL.
- To demonstrate autologous tumor-killing capability in the expanded PBLs.

Figure 1. IOV-2001 Manufacturing Process



Cryopreserved PBMC obtained from peripheral blood of CLL patients were enriched for T cells. Enriched fractions were expanded for a duration of 9–14 days in the presence of αCD3/αCD28 beads and interleukin-2 (IL-2) to obtain IOV-2001 product.

MATERIALS AND METHODS

- **Patients samples:** Cryopreserved peripheral blood mononuclear cells (PBMC) were obtained from three different groups of chronic lymphocytic leukemia (CLL) patients (i.e. treatment-naïve, ibrutinib-naïve (or pre-ibrutinib), post-ibrutinib). Clinical samples were provided by The Ohio State University.
- **Flow cytometry:** PBL were analyzed for memory subsets using flow cytometry.
- **ELIspot:** IFNγ production by PBL in response to non-specific TCR engagement was measured following stimulation with mAb–coated Dynabeads (αCD3/αCD28/αCD137). IFNγ secretion was assessed by ELIspot (Immunspot CTL) and IFNγ+ cells were enumerated using Immunospot S6 entry analyzer.
- **Autologous tumor killing assay:** Cytotoxicity of PBL was measured by flow cytometry based method. Briefly, effector (E) cells (PBL) were labeled with carboxyfluorescein succinyl ester (CFSE) and Target (T) cells (autologous CD19+ cells/Leukemia cells) were labeled with CellTrace violet (CTV). E and T cells were mixed at different ratios and incubated for 24 hours. Cells were harvested following co-culture and stained with annexin-V and propidium iodide (PI). Target cell killing was assessed by calculating percent CTV+ Annexin-V+ PI+ cells from coculture wells.
- **Gene expression analysis using nanoString nCounter® system:** nCounter CAR T characterization panel (nanoString, Seattle) was used. Data were normalized by scaling with geometric mean of the built-in control gene probes for each sample.

RESULTS

IOV-2001: PBL obtained from post-ibrutinib PBMC expanded successfully

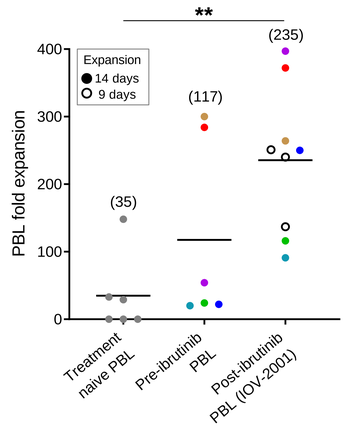


Figure 2. Cryopreserved PBMC were obtained from three different groups of CLL patients: treatment naïve, pre-ibrutinib (ibrutinib-naïve) and post-ibrutinib (minimum 2 cycles of ibrutinib treatment). PBL were expanded as described in Figure 1. Fold expansion is representative of total number of T cells in final PBL product over number of T cells in enriched fraction. Mean fold expansion of each group is shown in parentheses. Paired pre- and post-ibrutinib patients samples are color matched. Statistical significance was assessed by a Mann-Whitney t-test **p≤0.01

PBL (IOV-2001) expanded from post-ibrutinib PBMC showed higher-fold expansion compared to those obtained from pre-ibrutinib PBMC and treatment naïve PBMC

IOV-2001 is comprised of >97% T cells and the majority of T cells are effector memory phenotype

		SAMPLE TYPE			
		14 days expansion		9 days expansion	
VARIABLE		Treatment naïve PBL (n=6)	Pre-ibrutinib PBL (n=6)	Post-ibrutinib PBL (IOV-2001) (n=6)	Post-ibrutinib PBL (IOV-2001) (n=3)
TCRαβ+ cells (%)		86	95	97	98
T cell subsets (% viable cells)	CD4+	81	80	81	69
	CD8+	1	13	13	27
Memory T cell subsets (% CD4+ T cells)	T_N	4	0.3	0.2	0.8
	T_{CM}	30	17	17	27
	T_{EM}	61	82	81	72
	T_{EMRA}	5	1	2	0.6
Memory T cell subsets (% CD8+ T cells)	T_N	1	0.8	0.4	2
	T_{CM}	28	16	14	32
	T_{EM}	66	78	82	64
	T_{EMRA}	4	5	4	2

Table. 1 Phenotyping of PBL product was performed to identify percent of viable T cells and their subsets. Using flow cytometry, samples were evaluated for the presence of CD4+ and CD8+ T cell lineages, and memory T cell subsets.

IOV-2001 consisted of 97-98% TCRαβ+ cells and majority of T cell subsets (Range 64-82%) are effector memory subsets (T_{EM} CD45RA$^-$CCR7$^-$)

IOV-2001: Beneficial effect of prior ibrutinib exposure on IFNγ secretion

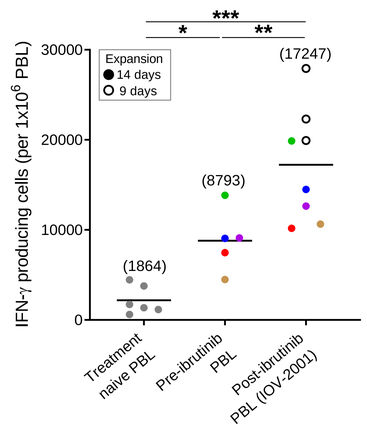


Figure 3. IFNγ secretion by different groups of PBL in response to non-specific TCR engagement was assessed by Enzyme-Linked Immunosorbent Spot (ELISpot) assay. Data is shown as IFNγ+ T cells per million PBL. Mean number of IFNγ+ T cells per million PBL in each group is shown in parentheses. Paired patients samples are color matched. Statistical significance was assessed by a Mann-Whitney t-test *p<0.05, ** p≤0.01, *** p≤0.001

IOV-2001 showed significantly higher increase in IFNγ release in response to non-specific TCR engagement compared to PBL derived from pre-ibrutinib PBMC or from treatment naïve PBMC

Potent cytotoxic activity of IOV-2001 against autologous leukemia cells

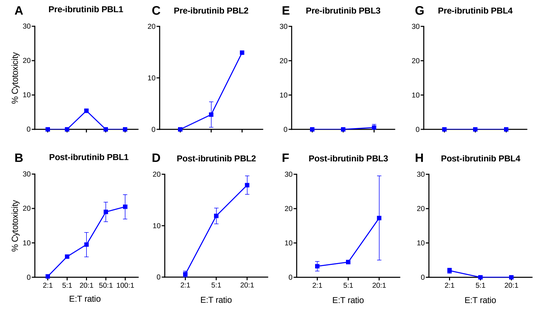


Figure 4. Cytotoxicity of PBL against autologous CD19+ cells (Leukemia cells) was measured using flow cytometry-based cell-killing assay. Lytic activity representative of four pre-ibrutinib PBL **(upper panel)** and four IOV-2001 products **(lower panel)** is shown. Panels A & B, C & D, E & F, G & H represent paired samples.

Anti-tumor activity of IOV-2001 is antigen-specific and HLA (class I/II) dependent

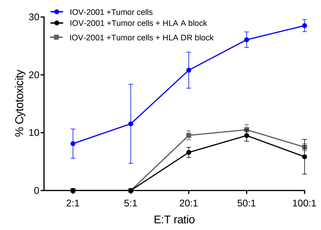


Figure 5. Target specificity (autologous CD19+/leukemia cells) of IOV-2001 was determined by HLA blockade experiments.

IOV-2001 showed strong anti-tumor activity against autologous leukemia cells compared to pre-ibrutinib PBL, suggestive of beneficial effect of ibrutinib.

HLA blockade reduced the cytotoxicity of IOV-2001 confirming antigen specificity and CD4, CD8 mediated killing of leukemia cells

IOV-2001 generated by short (9 days) expansion process has gene profile indicative of higher potency

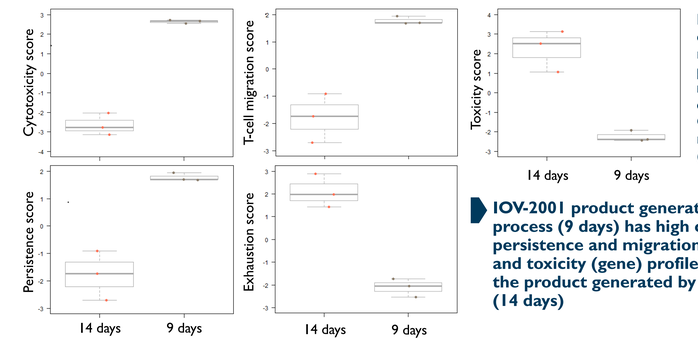


Figure 6. Box plots show expression levels of genes related to different T cell pathways measured by nCounter CAR-T characterization panel. Gene expression is represented by score (y-axis).

IOV-2001 product generated by shorter process (9 days) has high cytotoxicity, persistence and migration, low exhaustion and toxicity (gene) profiles compared to the product generated by longer process (14 days)

CONCLUSIONS

- IOV-2001 is a non-genetically modified, polyclonal T cell product called PBL.
- IOV-2001 can be reproducibly generated from 50 mL of blood over a 9-day manufacturing duration to yield billions of PBLs.
- Compared to pre-ibrutinib and treatment-naïve PBL, IOV-2001 has high fold expansion from initial limited clinical starting material (simple blood draw, no pheresis required) and secretes high levels of IFNγ in response to non-specific TCR stimulation.
- IOV-2001 demonstrated superior cytotoxicity against autologous tumor (leukemia) cells.
- First-in-patient testing of IOV-2001 is planned for the treatment of CLL/SLL patients.
- Future testing of this approach in broader array of hematologic malignancies is being explored.

DISCLOSURE
• This study and poster are sponsored by Iovance Biotherapeutics, Inc.

ACKNOWLEDGMENT
• All the listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors
• The authors would like to acknowledge Kranthi Kunkalla for his contribution to gene expression analysis studies
• The authors would like to thank the participating patients and their families for donation of material used in this study