Expansion of human γδ T cells for adoptive immunotherapy using a bisphosphonate prodrug

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Supplemental Information

Table S1.

Characteristics of the prostate cancer patients studied in this report.

Patient	**PC01**	**PC02**	**PC03**	**PC04**	**PC05**
Age at diagnosis	75	75	87	76	73
Initial PSA	79.79	35.27	37.86	8.52	263
Gleason's score	4 + 4 = 8	5 + 4 = 9	3 + 3 = 6	5 + 4 = 9	3 + 4 = 7
Clinical stage	cT2bN0M0	cT3aN0M0	cT2aN0M0	cT3aN1M0	cT3bN0M0
D'Amico risk	High risk	High risk	Low risk	High risk	High risk
Initial therapy	Radical prostatectomy	Radiation therapy	Hormonal therapy	Radiation therapy	Radiation therapy
Prognosis	Salvage radiation therapy, hormonal therapy	No recurrence	Stable PSA	Hormonal therapy, chemotherapy	Hormonal therapy, chemotherapy

Table S2.
Characteristics of the breast cancer patients studied in this report.

Patient	**BC29**	**BC33**	**BC35**	**BC36**	**BC37B**	**BC38**	**BC42**
Age at diagnosis	41	41	46	62	59	56	54
Characteristics	IDC[†]	MBC	IDC	DCIS	MBC	IDC	IDC
TNM classification	T2N0 M0	N/A	T1N0 M0	TisN0 Mx	TD1N0 M1	T2N0 M0	T1N0 M0
Clinical stage	2	4	1	0	4	2	1
Pathological subtype	pap-tub	N/A	pap-tub	DCIS	sci	pap-tub	sci
Clinical grade	G1	N/A	G1	N/A	G2	G1	G1
Estrogen receptor (ER) expression	100	0	100	N/A	100	100	100
Progesterone receptor (PgR) expression	100	0	100	N/A	100	100	100
HER2 expression	0	0	0	N/A	0	0	0
Ki-67 expression	5%	50%	<1%	N/A	15%	<5%	<5%
Molecular Subtype	Lum A	Triple negative	Lum A	N/A	Lum A	Lum A	Lum A

[†]Abbreviations used: DCIS, ductal carcinoma in situ; ER, estrogen receptor; HER2, human epidermal growth factor 2, IDC, invasive ductal carcinoma; Lum A, luminal A; MBC, metastatic breast cancer; N/A, not available; pap-tub, papillotubular carcinoma; PgR, progesterone receptor; sci, scirrhous carcinoma.

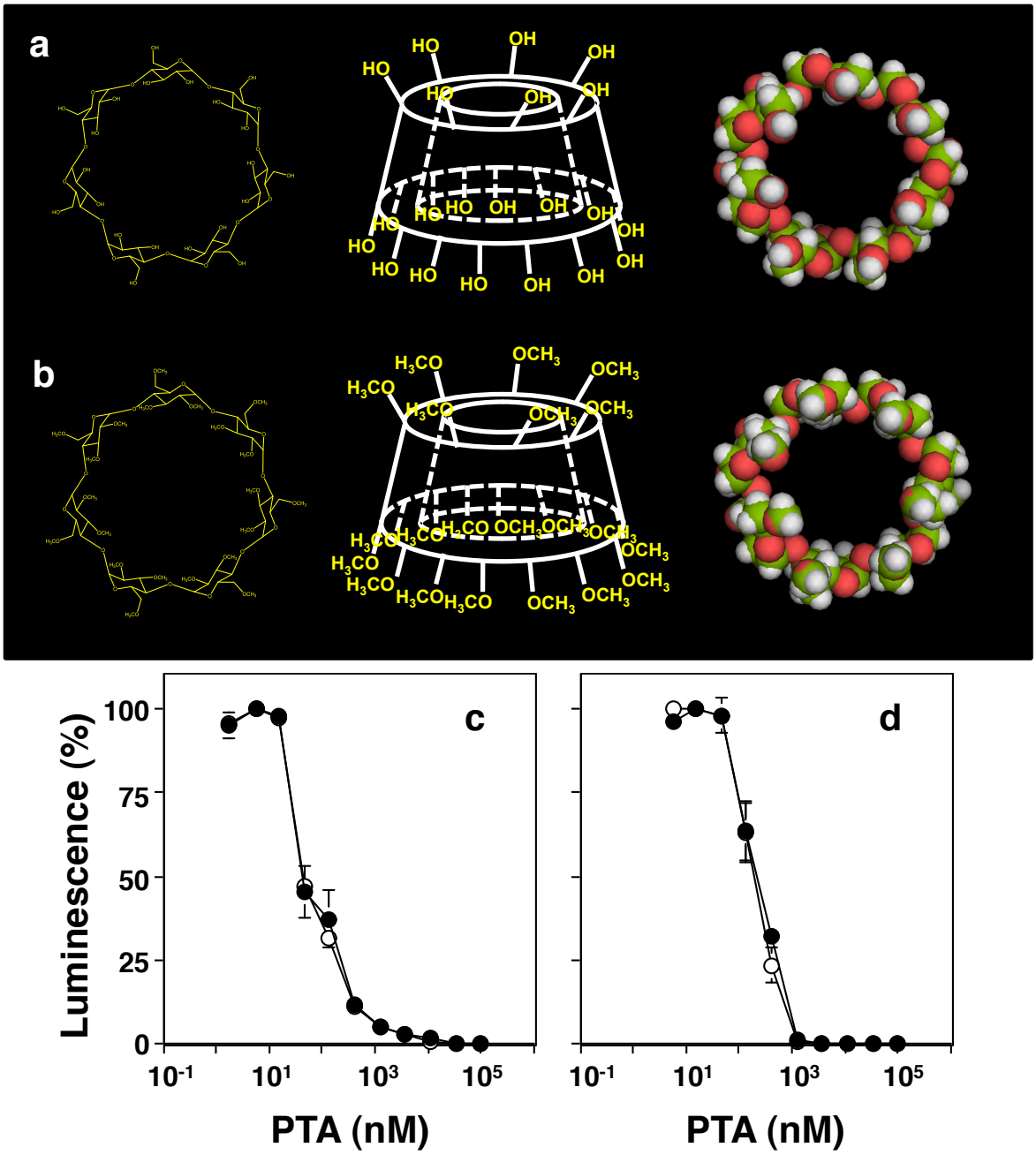

Figure S1. Solubilization of PTA with trimethyl β-cyclodextrin in ethanol. (a) Structure of β-cyclodextrin (βCD). Structural formula of βCD (left), schematic diagram of βCD (middle), and space-filling model of βCD (right) are shown. (b) Structure of trimethyl β-cyclodextrin (TMβCD). Structural formula of TMβCD (left), schematic diagram of TMβCD (middle), and space-filling model of βCD (right) are shown. (c, d) Effect of TMβCD solubilization on PTA inhibition of the proliferation of EJ-1 bladder carcinoma and U937 histiocytic lymphoma cells. EJ-1 (c) or U937 (d) cells were incubated with PTA that had been solubilized either in TMβCD with ethanol (open circles) or in dimethylsulfoxide (solid circles). After 4 days of culture, cell growth was determined by measuring cellular ATP using a luciferase assay. Note that PTA exhibited identical activity with either method of solubilization.

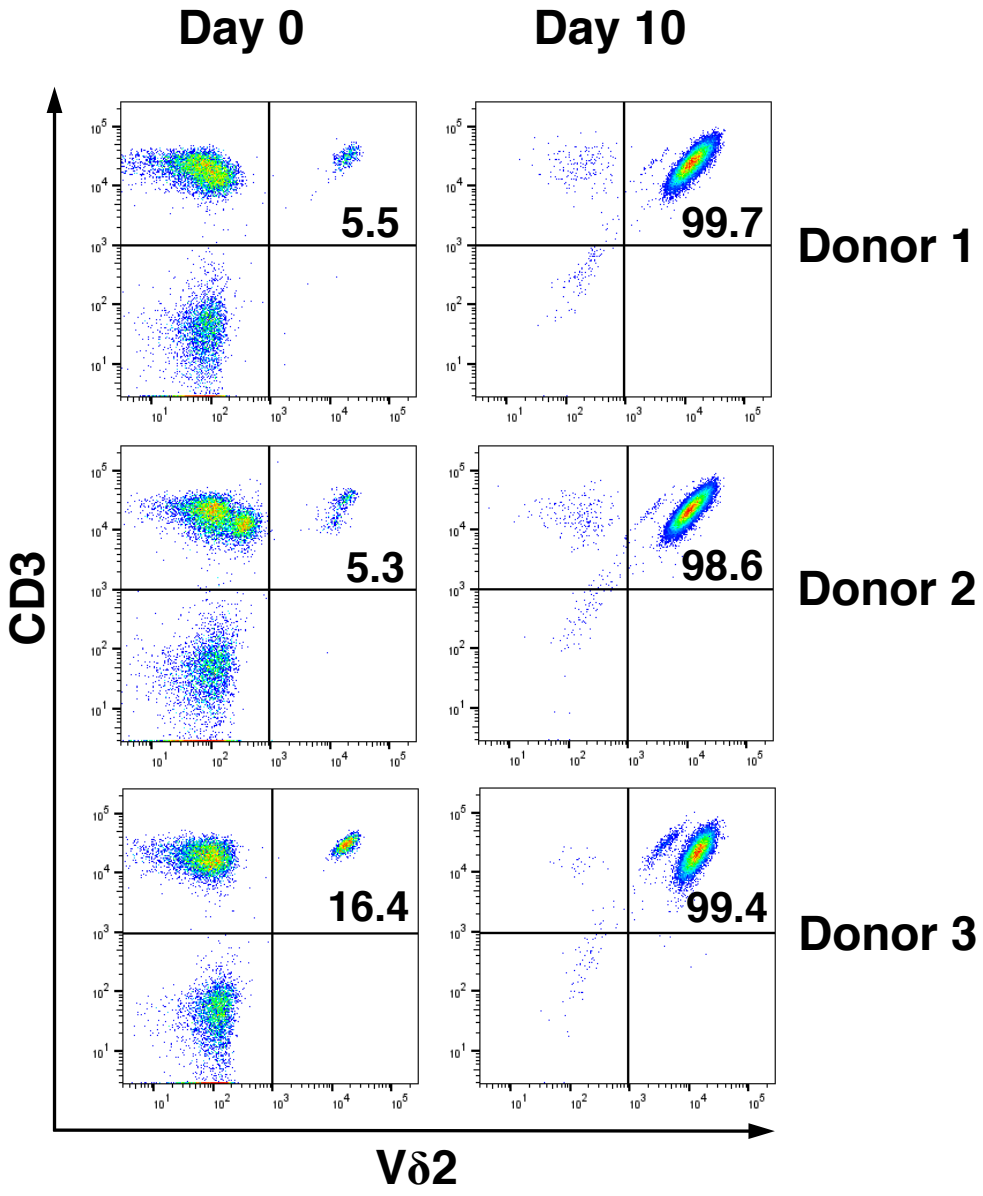

Figure S2. Expansion of Vγ2Vδ2 T cells from healthy donors using PTA with IL-2. PBMC derived from healthy adult donors were stimulated with PTA and IL-2 for 10 days in vitro. The cells were then harvested, stained with mAbs to Vδ2 and CD3, and analyzed by flow cytometry. Dot plots for the starting populations (left panels) and the ending populations (right panels) are shown. Numbers represent Vδ2 T cells as a percent of CD3 T cells. The absolute number of Vδ2 T cells increased from 4.1×10^5 to 4.7×10^8 for Donor 1, 1.7×10^5 to 9.2×10^7 for Donor 2, and 2.1×10^5 to 7.0×10^7 for Donor 3, with the expansion rates being 1,134-fold, 550-fold, and 329-fold, respectively.

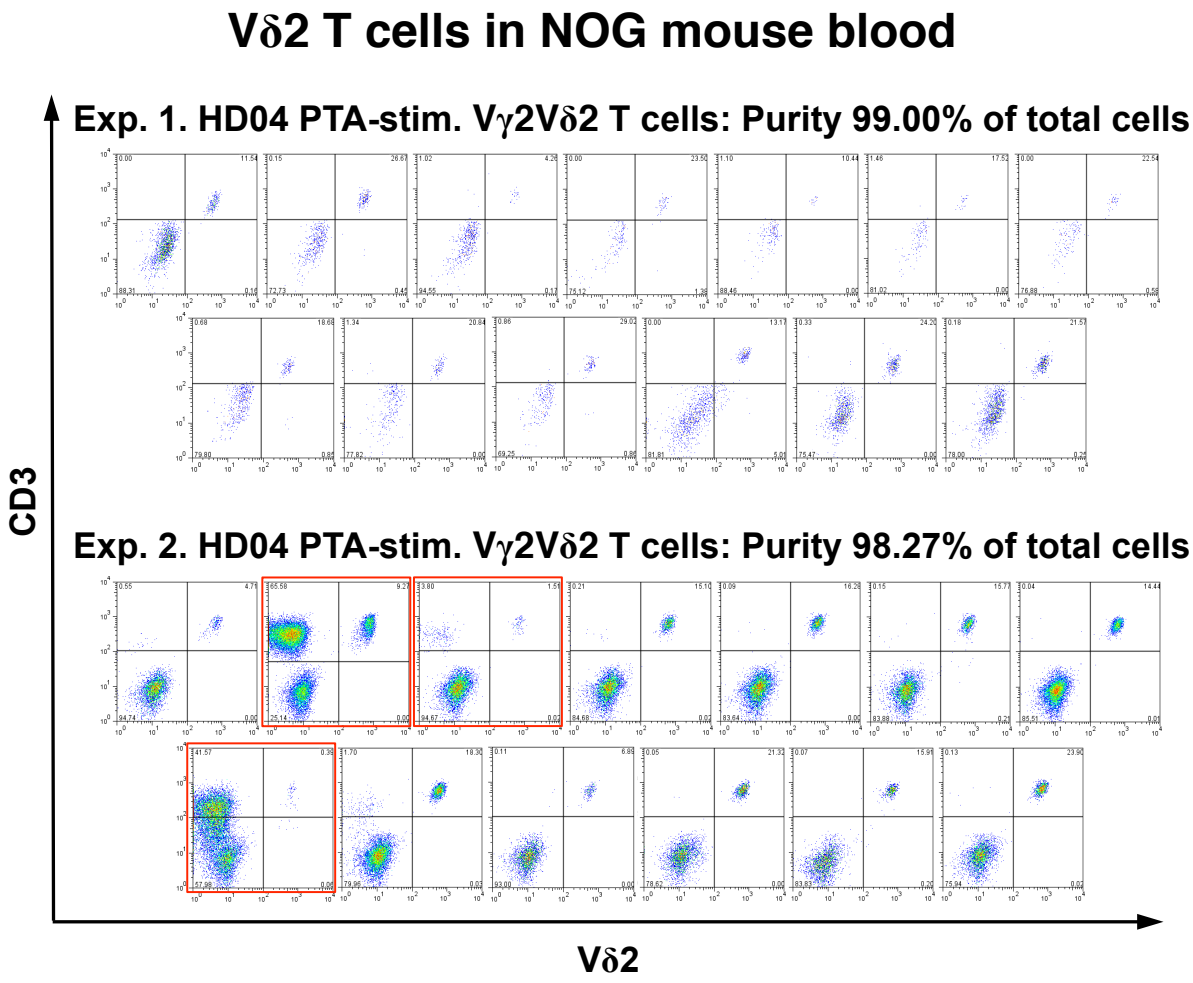

Figure S3. Purity of Vγ2Vδ2 T cells from a healthy donor helps to determine the success of engraftment in the blood of NOG mice after adoptive transfer. PBMC derived from a single healthy donor were stimulated with PTA and IL-2. After 10 days, Vδ2 T cells constituted 99.64% in Exp. 1 and 99.58% in Exp. 2 of total cells in the lymphocyte gate. 5×10^7 expanded Vγ2Vδ2 T cells (never frozen) were i.p. injected into each of 13 NOG mice. Fourteen days later, peripheral blood was obtained, treated with ACK lysis buffer, and washed with PBS/2% FCS. The resulting cells were then stained with mAbs to the Vδ2 TCR and human CD3 and analyzed by flow cytometry. Two experiments are shown (top and bottom panels). Mean ± SD of Vγ2Vδ2 T cells as a percent of total cells in the lymphocyte gate were for Exp. 1. 18.8% ± 7.1%, n = 13 Exp. 2. 12.6% ± 7.4%, n = 13. Each dot plot represents one NOG mouse. Mice with less than 80% Vγ2Vδ2 T cells of total T cells are outlined in red.

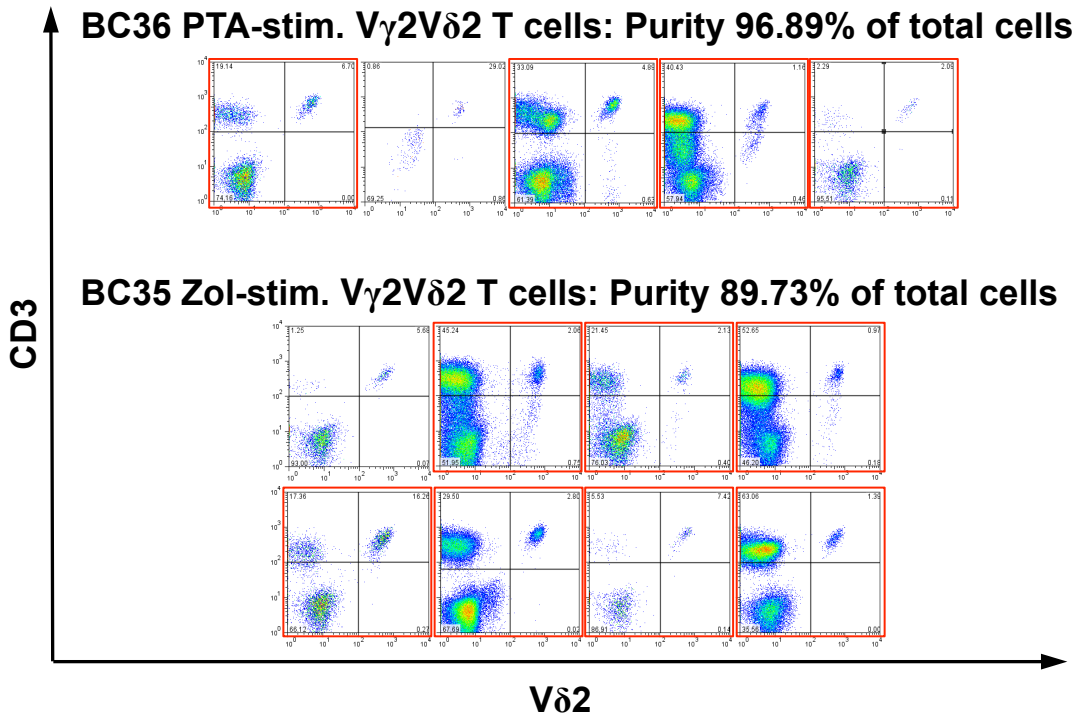

Figure S4. Purity of Vγ2Vδ2 T cells from breast cancer patients helps to determine the success of engraftment in NOG mice after adoptive transfer (continued). PBMC derived from two breast cancer patients were stimulated with either PTA and IL-2 (BC36) or Zol and IL-2 (BC35). After 10 days, Vδ2 T cells constituted 96.89% and 89.73% of total cells in the lymphocyte gate, respectively and were frozen for later transfer. For transfer, cells were thawed and 5×10^7 Vγ2Vδ2 T cells were i.p. injected into each of 5 NOG mice for BC36 and 8 NOG mice for BC35. Fourteen days later, peripheral blood was obtained, treated with ACK lysis buffer, and washed with PBS/2% FCS. The resulting cells were then stained with mAbs to the Vδ2 TCR and human CD3 and analyzed by flow cytometry. Each dot plot represents one NOG mouse. Mice with less than 80% Vγ2Vδ2 T cells of total T cells are outlined in red.