Supplementary Data for:

Selective Photodepletion of Malignant T Cells in Extracorporeal Photophereses with Selenorhodamine Photosensitizers

Zachariah A. McIver, Mark W. Kryman, Young Choi, Benjamin N. Coe, Gregory A. Schamerhorn, Michelle K. Linder, Kellie S. Davies, Jacqueline E. Hill, Geri A. Sawada, Jason M. Grayson, and *Michael R. Detty*

Table of Contents **Page**

Table S1. Mean fluorescence intensity (MFI) in arbitrary units (a.u.). from a 5:1 mixture of non-stimulated PMBCs and selenorhodamine photosensitizers **5-10** following uptake and extrusion.

T-cell subset	Dye	MFI, a.u. uptake	MFI, a.u. extrusion	Dye	MFI, a.u. uptake	MFI, a.u. extrusion
HUT-78	**5**	174000 ± 9000	126000 ± 1100	**6**	22800 ± 1200	10000 ± 300
CD3+	**5**	63900 ± 19400	42600 ± 2200	**6**	8150 ± 200	3690 ± 60
CD4+	**5**	62600 ± 2100	39900 ± 3600	**6**	7800 ± 300	3170 ± 120
CD8+	**5**	42000 ± 2800	18000 ± 3900	**6**	1980 ± 300	600 ± 100
HUT-78	**7**	90000 ± 7200	64000 ± 4700	**8**	90000 ± 3600	48000 ± 4000
CD3+	**7**	34800 ± 2100	21800 ± 1100	**8**	30000 ± 700	3110 ± 80
CD4+	**7**	33900 ± 2100	21000 ± 1600	**8**	29300 ± 1300	13600 ± 1400
CD8+	**7**	22600 ± 1400	11200 ± 1800	**8**	16100 ± 2100	3470 ± 670
HUT-78	**9**	30400 ± 2400	19800 ± 1500	**10**	43900 ± 1800	24900 ± 700
CD3+	**9**	13200 ± 600	7900 ± 400	**10**	16400 ± 300	8000 ± 50
CD4+	**9**	12800 ± 700	7300 ± 500	**10**	15700 ± 600	7300 ± 400
CD8+	**9**	8400 ± 800	3600 ± 500	**10**	7600 ± 800	1900 ± 300

[a] A 5:1 mixture of non-stimulated PMBCs and HUT-78 cells was treated with 7.5×10^{-8} selenorhodamine for 20 min (uptake). A second 5:1 mixture of non-stimulated PMBCs and HUT-78 cells was treated with 7.5×10^{-8} selenorhodamine for 20 min followed by 30 min in photosensitizer-free media (extrusion). Values of MFI were determined by flow cytometry following both uptake and extrusion and are the mean of three independent experiments. Error limits are ± SEM.

Table S2. Extrusion kinetics for thioamide/amide pairs **5/6**, **7**/**8**, and **9/10** as measured by the change in mean fluorescence intensity (ΔMFI) for a mixture of non-stimulated PMBCs and HUT-78 cells.

T-cell subset	Thioamide	ΔMFI ± SEM, %	Amide	ΔMFI ± SEM, %	Paired t-test *p*-value
HUT-78	**5**	28.1 ± 5.4	**6**	55.7 ± 1.9	0.009
CD3+	**5**	33.1 ± 5.5	**6**	54.6 ± 1.6	0.02
CD4+	**5**	36.1 ± 5.2	**6**	59.1 ± 2.0	0.015
CD8+	**5**	59.2 ± 7.3	**6**	69.0 ± 2.0	0.27
HUT-78	**7**	28.2 ± 3.2	**8**	47.1 ± 3.9	0.02
CD3+	**7**	36.8 ± 2.4	**8**	52.7 ± 2.9	0.013
CD4+	**7**	37.7 ± 3.3	**8**	54.1 ± 2.8	0.02
CD8+	**7**	51.0 ± 6.3	**8**	79.6 ± 1.6	0.012
HUT-78	**9**	34.6 ± 2.4	**10**	43.1 ± 0.9	0.03
CD3+	**9**	40.1 ± 1.9	**10**	51.3 ± 0.6	0.005
CD4+	**9**	42.8 ± 1.2	**10**	53.3 ± 1.7	0.007
CD8+	**9**	57.5 ± 1.5	**10**	76.2 ± 3.3	0.007

[a] A 5:1 mixture of non-stimulated PMBCs and HUT-78 cells was treated with 7.5×10^{-8} selenorhodamines **5-10** for 20 min (uptake). A second 5:1 mixture of non-stimulated PMBCs was treated with 7.5×10^{-8} selenorhodamines **5-10** for 20 min and cells were then washed and placed in photosensitizer-free media for 30 min (extrusion). Values of MFI were determined by flow cytometry following both uptake and extrusion. The change in MFI following uptake and extrusion was calculated as the mean of three independent experiments. Error limits are ± SEM. The significance of the difference in ΔMFI for thioamide/amide pairs is reported as the paired Student t-test *p* value.

Table S3. Mean fluorescence intensity (MFI) in arbitrary units (a.u.). from a 5:1 mixture of SEB-stimulated PMBCs and selenorhodamine photosensitizers **3-10** following uptake and extrusion.

T-cell subset	Dye	MFI, a.u. uptake	MFI, a.u. extrusion	Dye	MFI, a.u. uptake	MFI, a.u. extrusion
HUT-78	**3**	39300 ± 1500	37600 ± 1100	**4**	15300 ± 80	5270 ± 80
CD25+	**3**	45800 ± 2400	32200 ± 500	**4**	5390 ± 150	3110 ± 80
CD25-	**3**	10500 ± 360	4800 ± 210	**4**	900 ± 20	270 ± 10
HUT-78	**5**	81000 ± 2700	66000 ± 1700	**6**	6800 ± 170	4500 ± 210
CD25+	**5**	83300 ± 1800	69000 ± 1000	**6**	7510 ± 130	4500 ± 70
CD25-	**5**	25500 ± 600	16600 ± 530	**6**	1450 ± 70	580 ± 20
HUT-78	**7**	42000 ± 600	36000 ± 1700	**8**	33200 ± 900	20000 ± 900
CD25+	**7**	43300 ± 600	33800 ± 600	**8**	33800 ± 600	25000 ± 600
CD25-	**7**	14100 ± 120	9500 ± 130	**8**	9300 ± 400	3900 ± 240
HUT-78	**9**	13800 ± 300	11700 ± 300	**10**	9130 ± 360	6240 ± 320
CD25+	**9**	14500 ± 20	12100 ± 260	**10**	10900 ± 130	7300 ± 210
CD25-	**9**	5400 ± 20	3700 ± 40	**10**	2500 ± 60	1100 ± 40

[a] A 5:1 mixture of SEB-stimulated PMBCs and HUT-78 cells was treated with 7.5×10^{-8} selenorhodamine for 20 min (uptake). A second 5:1 mixture of non-stimulated PMBCs and HUT-78 cells was treated with 7.5×10^{-8} selenorhodamine for 20 min followed by 30 min in photosensitizer-free media (extrusion). Values of MFI were determined by flow cytometry following both uptake and extrusion and are the mean of three independent experiments. Error limits are ± SEM.

Table S4. Extrusion kinetics for thioamide/amide pairs **5/6**, **7/8**, and **9/10** from HUT-78 cells and SEB-stimulated PBMCs as measured by the change in mean fluorescence intensity (ΔMFI).[a]

T-cell subset	Thioamide	ΔMFI ± SEM, %	Amide	ΔMFI ± SEM, %	Paired t-test *p*-value
HUT-78	**5**	18.2 ± 3.2	**6**	34.0 ± 2.2	0.015
CD25+	**5**	16.8 ± 3.1	**6**	39.9 ± 0.5	0.02
CD25-	**5**	35.2 ± 0.9	**6**	59.8 ± 0.6	<0.0001
HUT-78	**7**	21.1 ± 1.2	**8**	32.9 ± 1.5	0.004
CD25+	**7**	22.0 ± 0.6	**8**	37.0 ± 2.5	0.004
CD25-	**7**	32.9 ± 1.5	**8**	58.6 ± 1.0	0.0001
HUT-78	**9**	15.3 ± 2.0	**10**	31.6 ± 2.5	0.007
CD25+	**9**	16.4 ± 1.8	**10**	33.5 ± 1.5	0.002
CD25-	**9**	30.7 ± 0.7	**10**	58.0 ± 0.8	<0.0001

[a] A 5:1 mixture of SEB-stimulated PMBCs and HUT-78 cells was treated with 7.5×10^{-8} selenorhodamines **5**-**10** for 20 min (uptake). A second 5:1 mixture of SEB-stimulated PMBCs was treated with $.5 \times 10^{-8}$ selenorhodamines **5**-**10** for 20 min and cells were then washed and placed in photosensitizer-free media for 30 min (extrusion). Values of MFI were determined by flow cytometry following both uptake and extrusion. The change in MFI following uptake and extrusion was calculated as the mean of three independent experiments. Error limits are ± SEM. The significance of the difference in ΔMFI for thioamide/amide pairs is reported as the paired Student t-test *p* value.

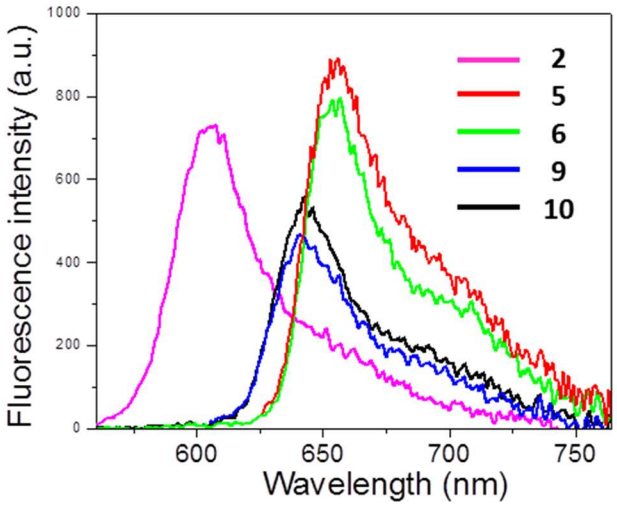


Figure S1. Fluorescence spectra of selenorhodamines **5**, **6**, **9**, and **10** compared to tetramethyl selenorosamine (**2**) used as a standard (Φ_{FL} = 0.09).

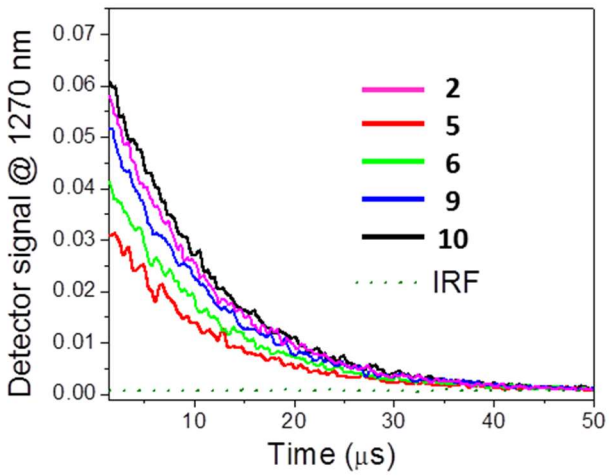
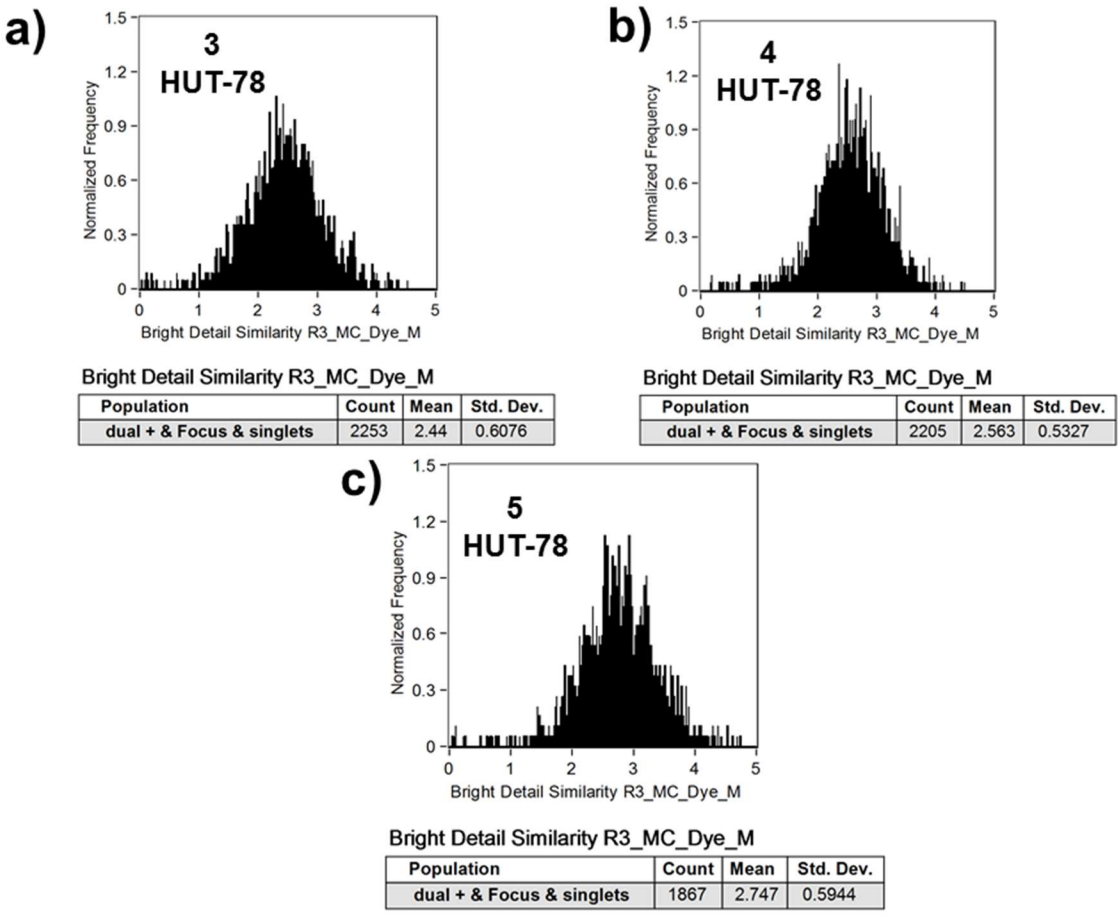


Figure S2. Decays of phosphorescence from $^1O_2$ sensitized by selenorhodamines **5**, **6**, **9**, and **10** used for determination of $\Phi(^1O_2)$. Signal obtained from air-saturated MeOH in the cuvette was used as the instrument response function (IRF). Tetramethyl selenorosamine (**2**) was used as a standard [$\Phi(^1O_2) = 0.87$].



Bright Detail Similarity R3_MC_Dye_M

Population	Count	Mean	Std. Dev.
dual + & Focus & singlets	2253	2.44	0.6076

Bright Detail Similarity R3_MC_Dye_M

Population	Count	Mean	Std. Dev.
dual + & Focus & singlets	2205	2.563	0.5327

Bright Detail Similarity R3_MC_Dye_M

Population	Count	Mean	Std. Dev.
dual + & Focus & singlets	1867	2.747	0.5944

Figure S3. A histogram of the pixel-by-pixel statistical analysis of each HUT-78 cell analyzed, in which the y-axis is number of cells and the x-axis is the similarity coefficient between MTG and selenorhodamines **3**, **4**, or **5**.

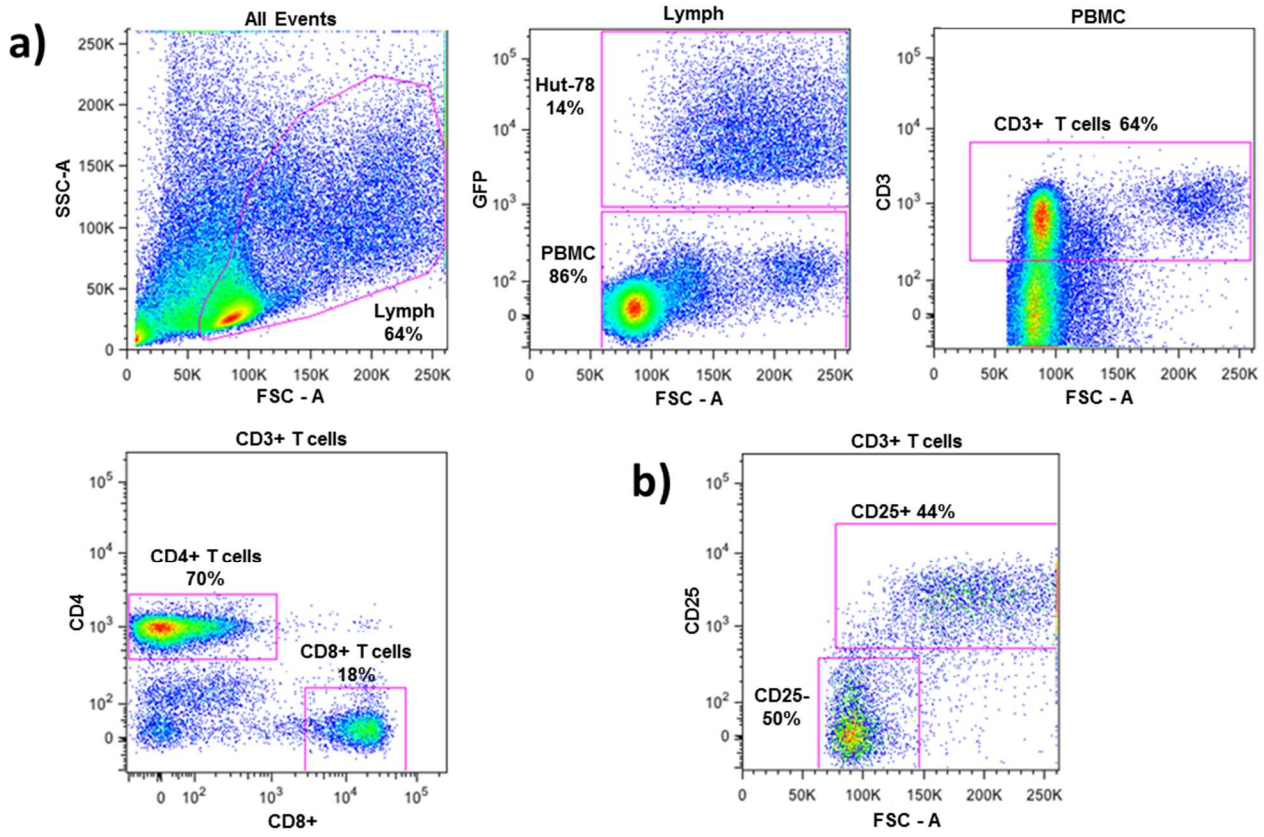


Figure S4. Flow cytometric analysis of resting, stimulated, and malignant T cells. Donor peripheral blood mononuclear cells (PBMC) were mixed with green fluorescent protein (GFP)-transfected HUT-78 malignant T cells prior to photodepletion. FACS analysis was then performed 18 hours after photodepletion to determine cell survival. All cells were then stained, acquired, and analyzed as outlined in the Experimental Section. A) Lymphocytes (Lymph) were identified in a side scatter-area (SSC-A) versus forward scatter-area (FSC-A) plot. Next, GFP+ HUT-78 malignant T cells and PBMC were identified in a GFP versus FSC-A plot. Within the PBMC population, T cells were identified by gating on CD3+ cells in a CD3 versus FSC-A plot. Finally, in resting PBMC containing samples, T cell subsets were identified by gating separately on the CD4+ and CD8+ populations in a CD4 versus CD8 plot. B) In SEB-stimulated PBMC containing

samples, T cell subsets were identified by gating separately on stimulated (CD25+) and resting (CD25-) populations in a CD25 versus FSC-A plot.