Supplemental Data

First-in-human Phase 1 Clinical Study of the IL-15 Superagonist Complex ALT-803 to Treat Relapse after Transplantation

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Target	Conjugate	Clone	Vendor
CD45	Horizon V500	HI30	BD Horizon
CD3delta	PE-Texas Red	7D6	Invitrogen
CD56	Brilliant Violet 605	HCD56	Biolegend
CD19	APC-ef780	HIB19	Invitrogen
CD4	PerCP-Cy5.5	RPA-T4	Biolegend
CD8	Pacific Blue	3B5	Invitrogen
CD25	APC	M-A251	BD Pharmingen
Foxp3	Alexa Fluor 488	259D	Biolegend
Ki67	PE	B56	BD Pharmingen

Table S1. Monoclonal antibodies used in flow cytometry assays.

Marker	Clone	Company	viSNE clustering
CCR7	G043H7	Biolegend	
CD117	104D2	Biolegend	Fig. 5A
CD14	M5E2	BD	Fig. 5A
CD3	UCHT1	Biolegend	Fig. 5A
CD33	WM53	Biolegend	Fig. 5A
CD34	581	BD	Fig. 5A
CD4	OKT4	BD	Fig. 5A
CD8	SK1	BD	Fig. 5A
CD94	DX22	Biolegend	Fig. 5B
CRTH2	BM16	Biolegend	Fig. 5A-B
DNAM1	DX11	BD	Fig. 5B
KIR2DL1/DS1 (CD158a,h)	EB6B	Beckman	Fig. 5B
KIR2DL2/2DL3 (CD158b)	CH-L	BD	Fig. 5B
KIR3DL1	DX9	Biolegend	Fig. 5B
Lag-3	11C3C65	Biolegend	Fig. 5B
NKG2A	Z199	Beckman	Fig. 5B
NKG2C	134591	R&D	Fig. 5B
NKG2D	1D11	Novus	Fig. 5B
NKp30	P30-15	Biolegend	Fig. 5B
NKp44	P44-8	Biolegend	Fig. 5B
NKp46	9E2	BD	Fig. 5A-B
PD-1 FitC	Keytruda ScFv	Miller Lab	Fig. 5B
Tim-3	F38-2E2	Biolegend	Fig. 5B
TRAIL	RIK-2	Biolegend	Fig. 5B
anti-FitC	FIT-22	Fluidigm	
CD11b	ICR44	Fluidigm	Fig. 5A-B
CD127	A019D5	Fluidigm	Fig. 5A
CD137	4B4-1	Fluidigm	Fig. 5B
CD16	3G8	Fluidigm	Fig. 5A-B
CD19	HIB19	Fluidigm	Fig. 5A
CD25	2A3	Fluidigm	Fig. 5A-B
CD45	HI30	Fluidigm	Fig. 5A-B
CD45RO	UCHL1	Fluidigm	
CD56	NCAM16.2	Fluidigm	Fig. 5A-B
CD57	HCD57	Fluidigm	Fig. 5B
CD62L	DREG-56	Fluidigm	Fig. 5B
GzmB	GB11	Fluidigm	Fig. 5B
Ki67	B56	Fluidigm	Fig. 5B
TIGIT	MBSA43	Fluidigm	Fig. 5B
Cell-ID 20-Plex Pd Barcoding Kit		Fluidigm	
DNA-IR		Fluidigm	
X8 Metal Conjugation Kits		Fluidigm	
Cisplatin		Enzo Life Science	
Goat Serum		Sigma	

Table S2. Monoclonal antibodies used in mass cytometry assays. When possible, directly conjugated antibodies were purchased (Fluidigm). Otherwise, purified antibodies were conjugated using Fluidigm X8 metal conjugation kits, according to manufacturer's instruction.

Table S3A. Mean (±SD) PK Parameters in Patients After a Single IV Dose of ALT-803

Dose (µg/kg)	Stat. Param.	$T_{1/2}$	T_{max}[1]	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$	CL	V_{ss}
		(hr)	(hr)	(ng/mL)	(hr*ng/mL)	(hr*ng/mL)	(mL/hr/kg)	(mL/kg)
1	**N**	3	6	6	6	3	3	3
	Mean	0.749	0.658	4.17	8.10	9.97	129	85.4
	SD	0.170	0.583, 0.800	3.02	5.42	6.30	71.6	24.6
	CV%	22.8	NA	72.5	66.9	63.2	55.5	28.8
3	**N**	3	3	3	3	3	3	3
	Mean	2.72	0.767	44.9	178	178	19.3	51.7
	SD	1.81	0.517, 0.817	10.9	73.2	73.0	9.37	3.58
	CV%	66.5	NA	24.4	41.1	40.9	48.5	6.9
6	**N**	4	4	4	4	4	4	4
	Mean	3.15	0.742	88.1	384	384	16.7	53.8
	SD	1.14	0.633, 2.17	37.7	128	128	4.25	21.2
	CV%	36.2	NA	42.8	33.3	33.3	25.5	39.4
10	**N**	3	3	3	3	3	3	3
	Mean	4.87	2.18	190	1040	1040	10.0	47.5
	SD	3.46	0.667, 4.22	75.5	222	221	2.41	12.2
	CV%	71.1	NA	39.8	21.4	21.4	24.1	25.6

[1] Median and range (Min, Max) presented; NA: not applicable.

Table S3B. Mean (±SD) PK Parameters Patients After a Single SQ Dose of ALT-803

Dose (µg/kg)	Stat. Param.	$T_{1/2}$	T_{max}[1]	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$	V_z/F	CL/F	F
		(hr)	(hr)	(ng/mL)	(hr*ng/mL)	(hr*ng/mL)	(mL/kg)	(mL/hr/kg)	(%)
6	**N**	1	8	8	8	1	1	1	8
	Mean	22.2	35.1	0.389	13.7	29.7	6470	202	3.56
	SD	NR	0.500, 55.7	0.317	8.10	NR	NR	NR	2.11
	CV%	NR	NA	81.6	59.2	NR	NR	NR	59.2
10	**N**	4	9	9	9	4	4	4	9
	Mean	29.3	8.25	0.921	30.9	44.5	10800	247	2.99
	SD	10.8	4.02, 46.1	0.375	16.6	15.3	6090	87.6	1.60
	CV%	36.9	NA	40.7	53.5	34.4	56.3	35.5	53.5

[1] Median and range (Min, Max) presented; NR: not reported (N<3); NA: not applicable.

Table S3. PK analyses following ALT-803 administration.

Patient	Dose	Route	Pre-dose	D7 Post Dose2	D7 Post Dose4
UMN-001	1	IV	0	0	0
UMN-002	1	IV	0	0	NC
UMN-003	1	IV	0	0	NC
UMN-004	1	IV	MS	0	NC
UMN-005	1	IV	MS	0	NC
UMN-006	1	IV	MS	0	NC
WSU-001	3	IV	0	0	0
UMN-007	3	IV	0	0	0
UMN-008	3	IV	0	0	0
UMN-009	6	IV	0	0	0
UMN-010	6	IV	0	0	NC
UMN-011	6	IV	0	0	0
WSU-002	6	IV	0	0	0
UMN-012	10	IV	0	0	0
UMN-013	10	IV	0	0	0
UMN-014	10	IV	0	0	0
UMN-015	6	SQ	0	0	NC
WSU-003	6	SQ	0	0	0
WSU-004	6	SQ	0	0	0
UMN-016	6	SQ	0	0	50
WSU-005	6	SQ	0	0	0
WSU-006	6	SQ			
WSU-007	6	SQ			
UMN-017	6	SQ	0	0	0
WSU-008	10	SQ			
WSU-009	10	SQ			
WSU-010	10	SQ			
UMN-018	10	SQ	0	0	0
UMN-019	10	SQ	0	0	0
UMN-020	10	SQ			
UMN-021	10	SQ			

Table S4. Immunogenicity analysis results from all ALT-803 patients and time points tested. Immunogenicity results are shown as average titer. NC, not collected. MS, missing sample.

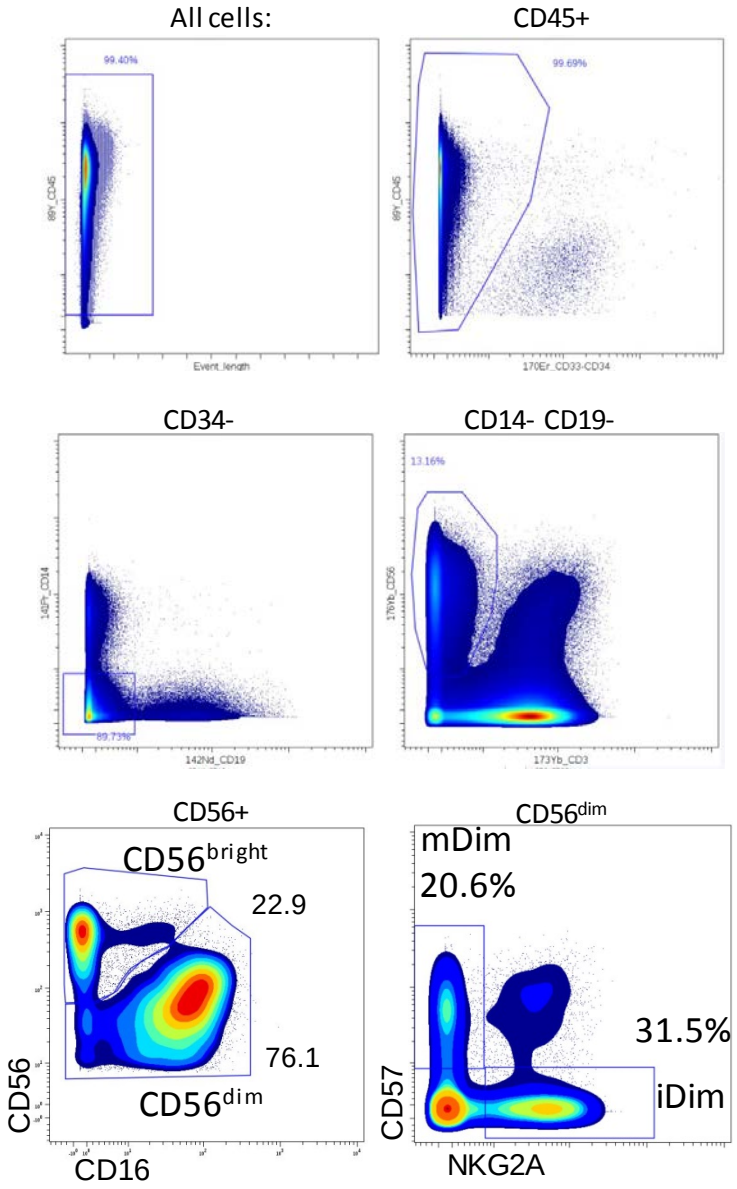

Figure S1. Gating strategy for NK cell subset analyses by mass cytometry.

$CD56^{bright}$ ($CD16^{-}$ $CD56^{bright}$) and $CD56^{dim}$ ($CD16^{+}$ CD56+) NK cells. $CD56^{dim}$ NK Cells were then assessed for NKG2A and CD57 expression. Mature Dims (mDim, CD57+ NKG2A-) and immature Dim (iDim; CD57- NKG2A+) were further examined. $CD56^{bright}$ NK cell immunophenotype was confirmed using addition markers (NKG2A, KIR, CD57) to ensure these cells represented $CD56^{bright}$, and not $CD56^{dim}$ NK cells with increased density of CD56 after ALT-803 administration.

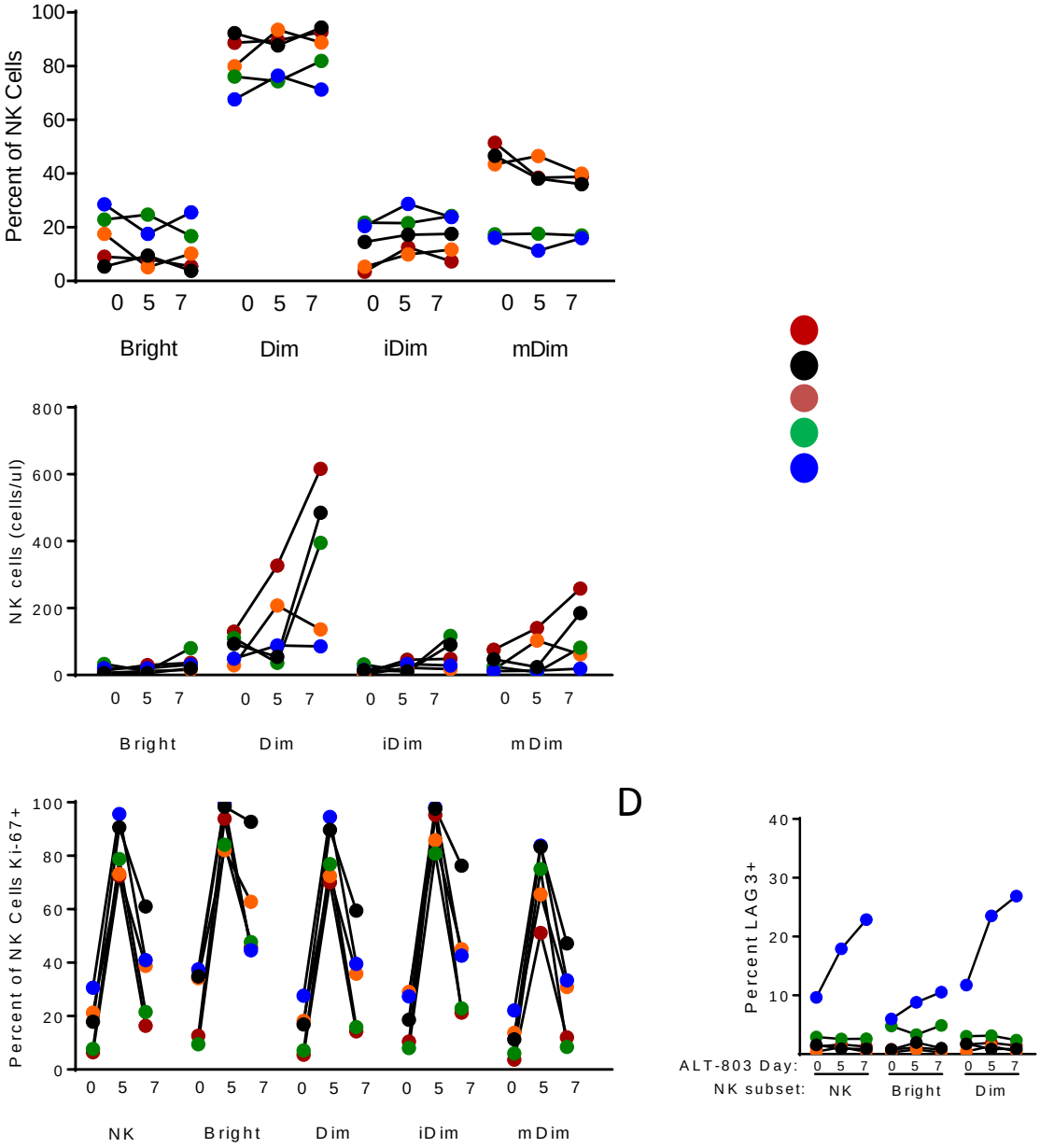

Figure S2. ALT-803 induced all stages of NK cell maturation, detected by mass cytometry. (A) Percentage of identified subset within NK cells. Bright, CD56^{bright}; Dim, CD56^{dim}; iDim, immature CD56^{dim}; mDim, mature CD56^{dim}. Gating on subsets was performed as shown figure S1. (B) Number of NK cells within the peripheral blood of each NK cell subset. (C) Percentage Ki67 positive within the peripheral blood of each NK cell subset. (D) Modulation of LAG-3 following ALT-803 administration is patient specific, with 1 of 5 patients tested with induced expression.

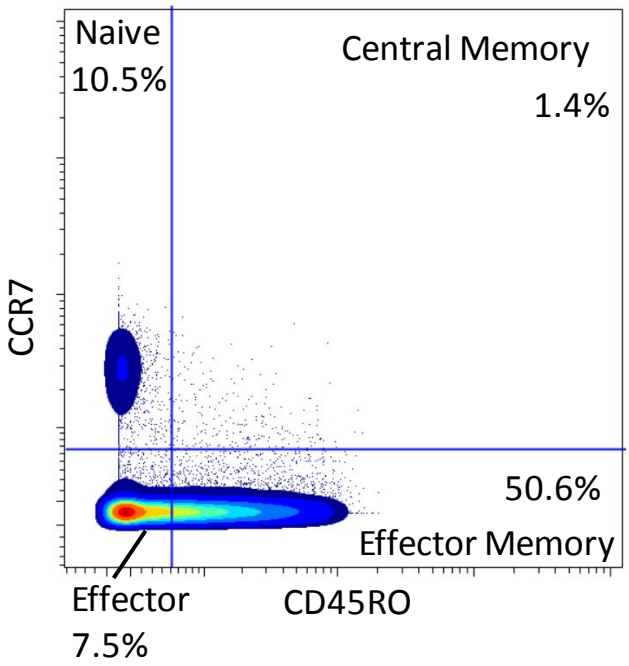

Figure S3. Gating strategy for CD8 T cell subset analyses by mass cytometry. CD8+ T cell gating strategy. CD8+ T cells were gated into Naïve (CCR7+ CD45RO-), Effector (CCR7- CD45RO-), Central memory (CCR7+ CD45RO+), and Effector memory (CCR7- CD45RO+).

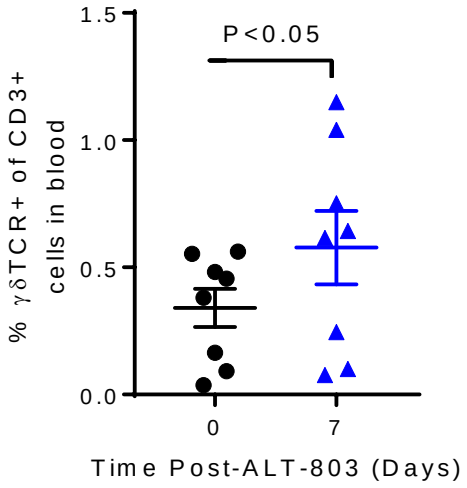

Figure S4. Analysis of γδT cells in blood of SQ ALT-803 patients. (A) γδT cells were identified in PBMC by gating on live CD3+ lymphocytes that were CD4-CD8-, as previously published (Wistuba-Hamprecht et. al, Cytometry Part A, 85A:522-524, 2014). The frequency of γδTCR+ T cells within CD3+ lymphocytes was a small in the blood and increased modestly yet significantly following ALT-803 administration (N=8, including patients with skin biopsies included in Figure 1). The average fold change from pre-therapy (0) to day 7 was 1.68-fold increase in frequency.