Supporting information

SN	File Designation	File Description
1	Figure-S1	Effect of delayed treatment with CTLA4-Ig plus Bortezomib on plasma cell subsets in mice immunized with donor spleen cells
2	Figure-S2	Kinetics of donor specific antibody responses post primary sensitization with (A) B/c spleen cells or (B) skin transplant
3	Figure-S3	Effect of delayed treatment with CTLA4-Ig plus Bortezomib on plasma cell subsets in mice immunized with 1° skin transplant
4	Figure-S4	Effect of delayed treatment with CTLA4-Ig plus bortezomib on plasma cell subsets in the spleen after 2° BALB/c skin transplantation
5	Figure-S5A	Clinical sequelae for Case 2
6	Figure-S5B	Clinical sequelae for Case 3
7	Figure-S5C	Clinical sequelae for Case 4
8	Figure-S5D	Clinical sequelae for Case 5
9	Figure-S5E	Clinical sequelae for Case 6
10	Figure-S6	MFI of DSA is not predictive of time to undetectable
11	Table-S1	Characteristics for all biopsies of patients treated with Belatacept plus bortezomib (B/B)
12	Table-S2	cPRA and DSA for individual patients
13	Table-S3	Changes in eGFR in AMR patients treated with Belatacept plus bortezomib
14	Method	A description of materials, methods and statistical analysis.

Fig S1: Effect of delayed treatment with CTLA4-Ig plus Bortezomib on plasma cell subsets in mice immunized with donor spleen cells

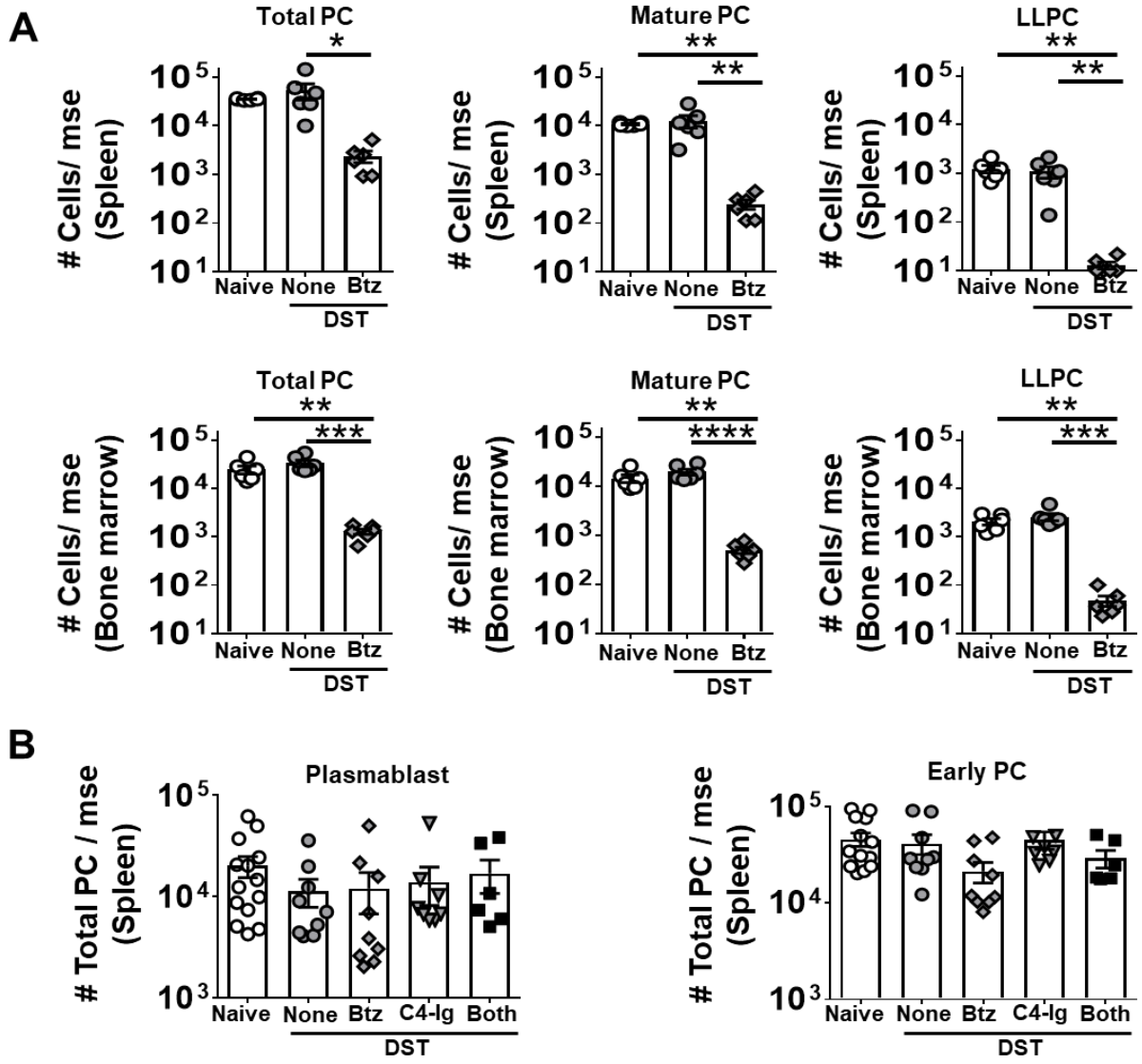


Figure S1: Effect of delayed treatment with CTLA4-Ig plus Bortezomib on plasma cell subsets in mice immunized with donor spleen cells. (A) Quantification of total PC, mature PC and long lived plasma cells (LLPCs: CD20-, CD19-, CD27-, CD38+, and CD138+) in spleen and bone marrow harvested from C57BL/6 mice immunized with BALB/c donor splenocytes (DST) and received no treatment (None) or Bortezomib (Btz; D5, D6). **(B)** Quantification of plasma cell subsets (plasmablast and early plasma cells (PC)) in spleens harvested from C57BL/6 mice immunized with BALB/c donor splenocytes (DST) and received no treatment (None), CTLA4-Ig (C4-Ig; from D5), Bortezomib (Btz; D5, D6) or CTLA4-Ig and Bortezomib (Both; from D5). (N=5-8/group). Y-axis represents total cells retrieved per mouse from naïve or mice immunized with DST, and each dot represents an individual mouse. Data are pooled from ≥2 independent experiments and presented as Mean ± SEM, and statistically significant differences were assessed by one way ANOVA. (**P* <0.05) (***P* <0.01) (****P* <0.001) (*****P* <0.0001).

Fig S2. Kinetics of donor specific antibody responses post primary sensitization with (A) B/c spleen cells or (B) skin transplant

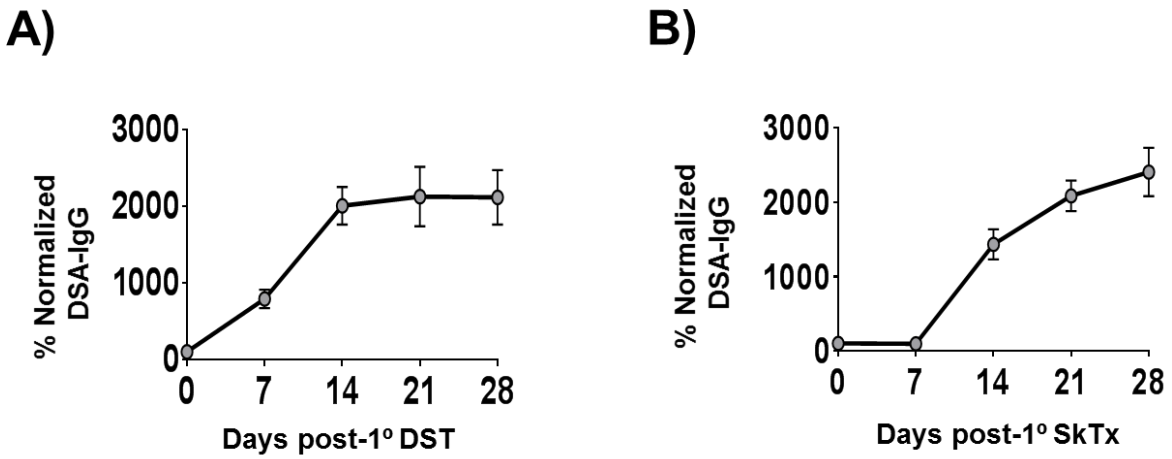


Figure S2. Kinetics of donor specific antibody responses post primary sensitization with (A) B/c spleen cells or (B) skin transplant: Anti-BALB/c IgG were assessed from D0-D28 post immunization with BALB/c **(A**) donor spleen cells (DST) **(B)** and skin allografts. (N=4-6/group). The Y-axis represents normalized MFI, calculated by considering the baseline MFI at day 0 to be 100% for each individual mouse. Data are presented as Mean ± SEM.

Fig S3: Effect of delayed treatment with CTLA4-Ig plus Bortezomib on plasma cell subsets in mice immunized with 1° skin transplant

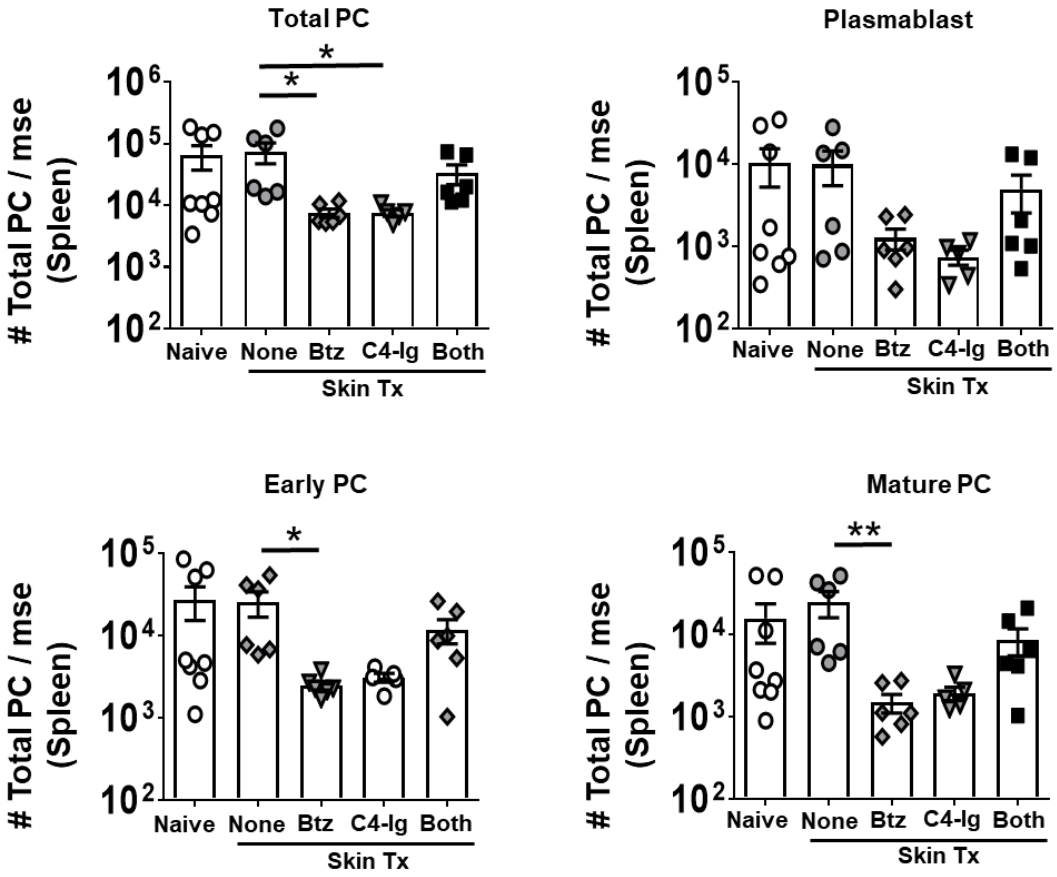


Figure S3: Effect of delayed treatment with CTLA4-Ig plus Bortezomib on plasma cell subsets in mice immunized with 1° skin transplant Quantification of plasma cell subsets in spleen harvested from C57BL/6 mice immunized with BALB/c skin allografts and received no treatment (None), CTLA4-Ig (C4-Ig; from D14), Bortezomib (Btz; D14, D15) or CTLA4-Ig and Bortezomib both (Both; from D14 or D35) (N=5-8/group). Y-axis represents total cells retrieved per mouse from naïve or mice immunized with DST or skin allograft, and each dot represents an individual mouse. Data are pooled from >2 independent experiments and presented as Mean ± SEM, and statistically significant differences were assessed by one way ANOVA. (**P* <0.05) (***P* <0.01) (****P* <0.001) (*****P* <0.0001).

Fig S4. Effect of delayed treatment with CTLA4-Ig plus Bortezomib on plasma cell subsets in the spleen after 2° BALB/c skin transplantation

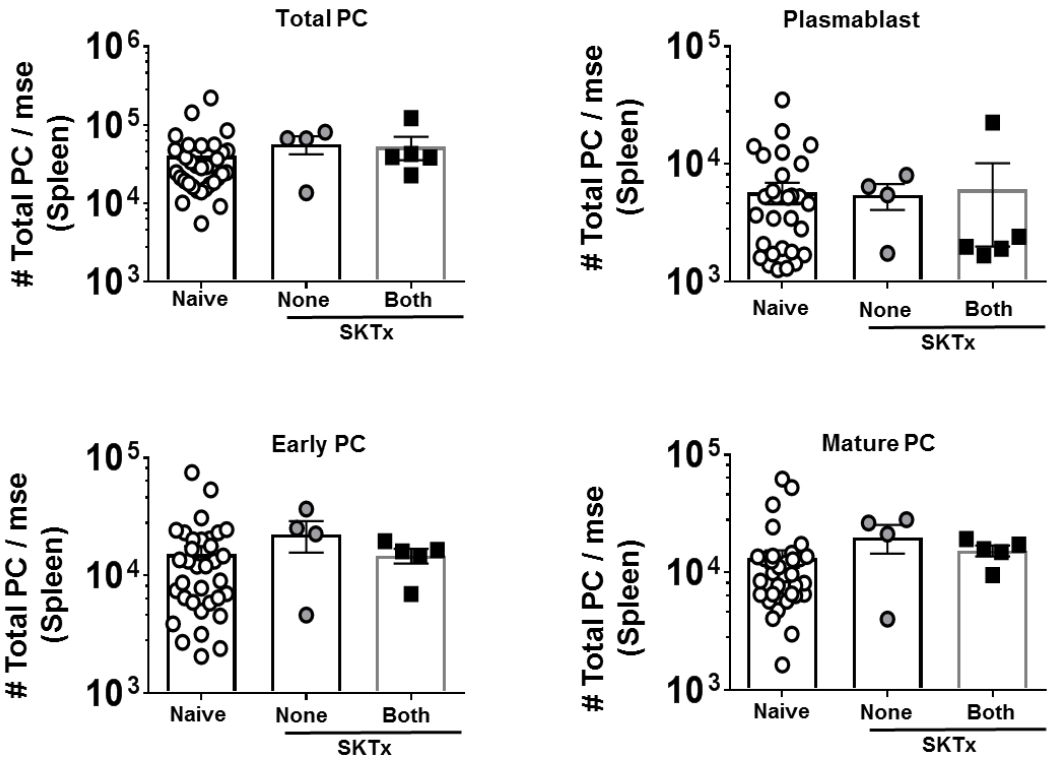
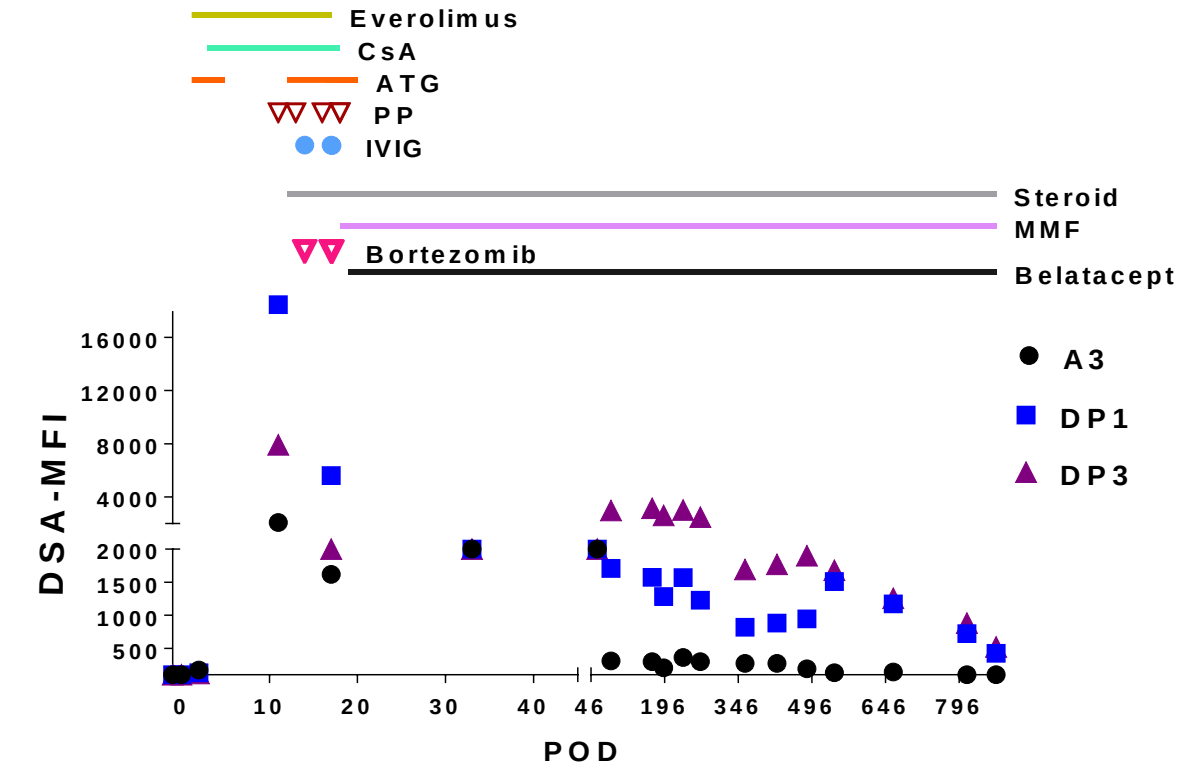
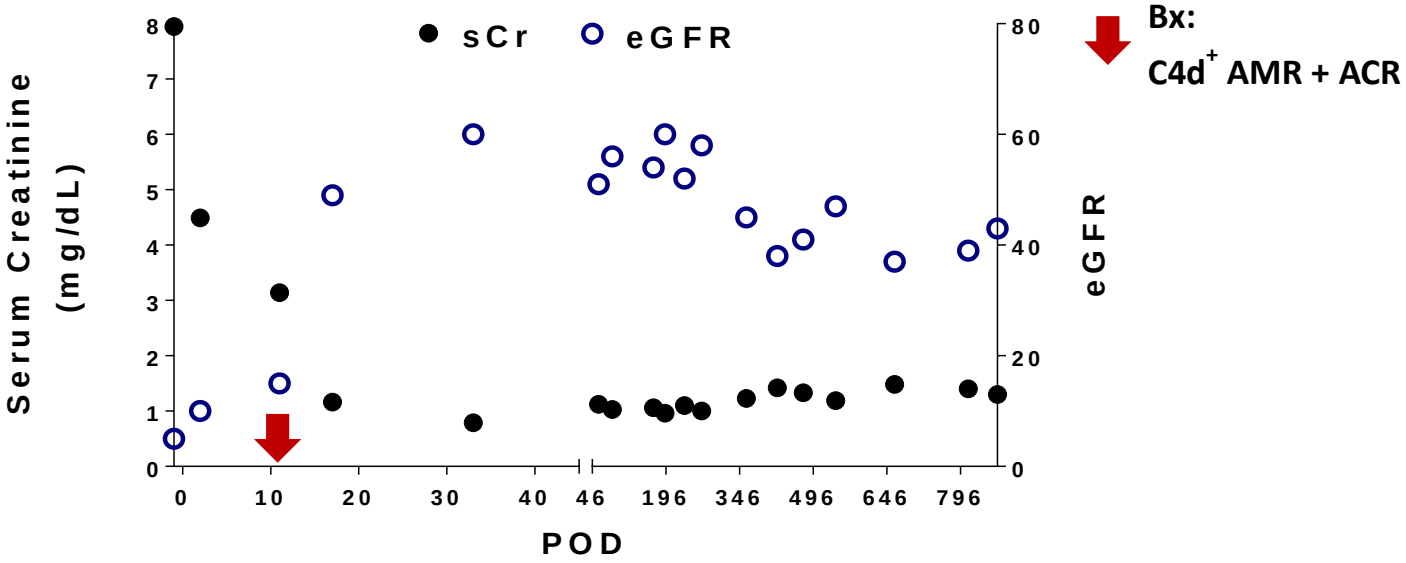


Figure S4: Effect of delayed treatment with CTLA4-Ig plus Bortezomib on plasma cell subsets in the spleen after 2° BALB/c skin transplantation. Quantification of plasma cell subsets in spleen harvested from naïve C57BL/6 or mice received BALB/c skin allograft on POD-70 and POD0. The Y-axis represents total cells retrieved per mouse on POD28. Cells retrieved from naïve or mice received 2°skin allograft, not receiving (None) or receiving CTLA4-Ig and Bortezomib from D14 post-2° skin transplant (Both; N=5-8/group). Each dot represents an individual mouse, pooled from >2 independent experiments. Data are presented as Mean ± SEM and statistically significant differences were assessed by one way ANOVA. (*P <0.05) (**P <0.01) (***P <0.001) (****P <0.0001).

Fig S5-A: Clinical sequelae for Case 2

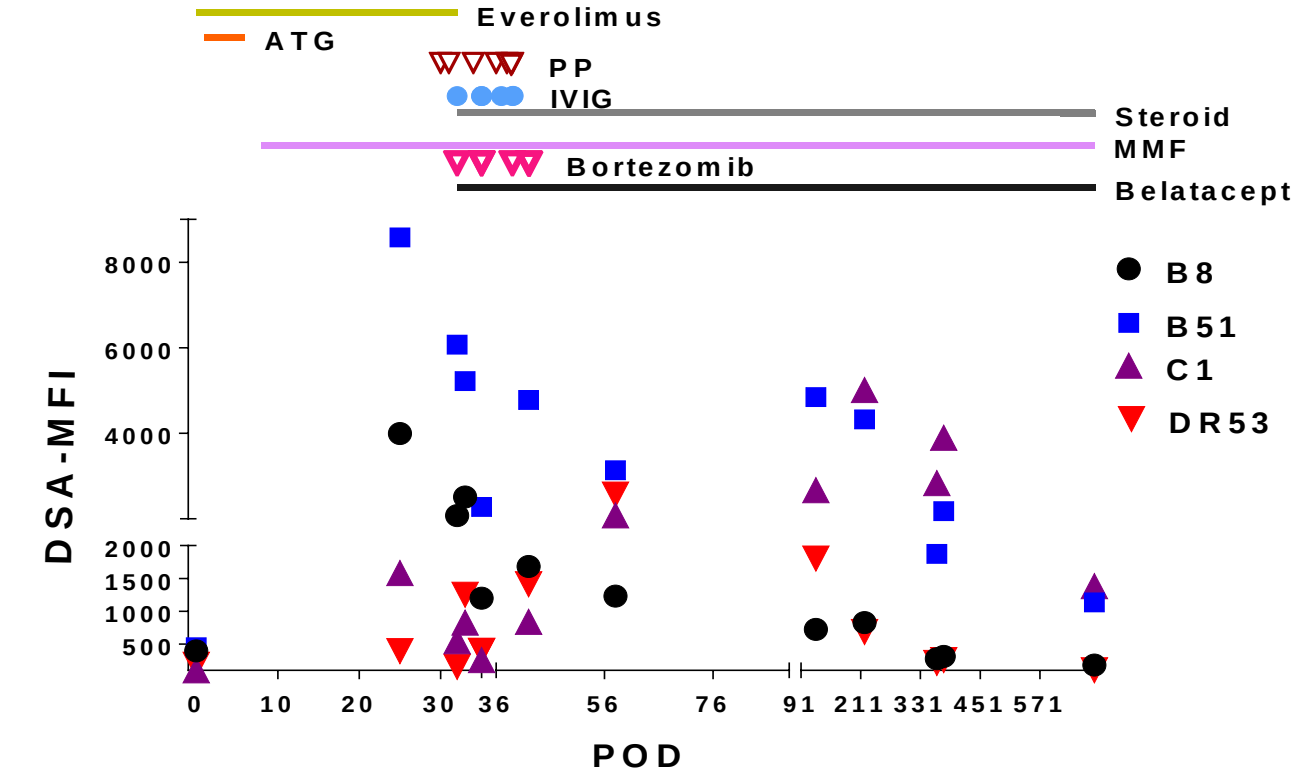


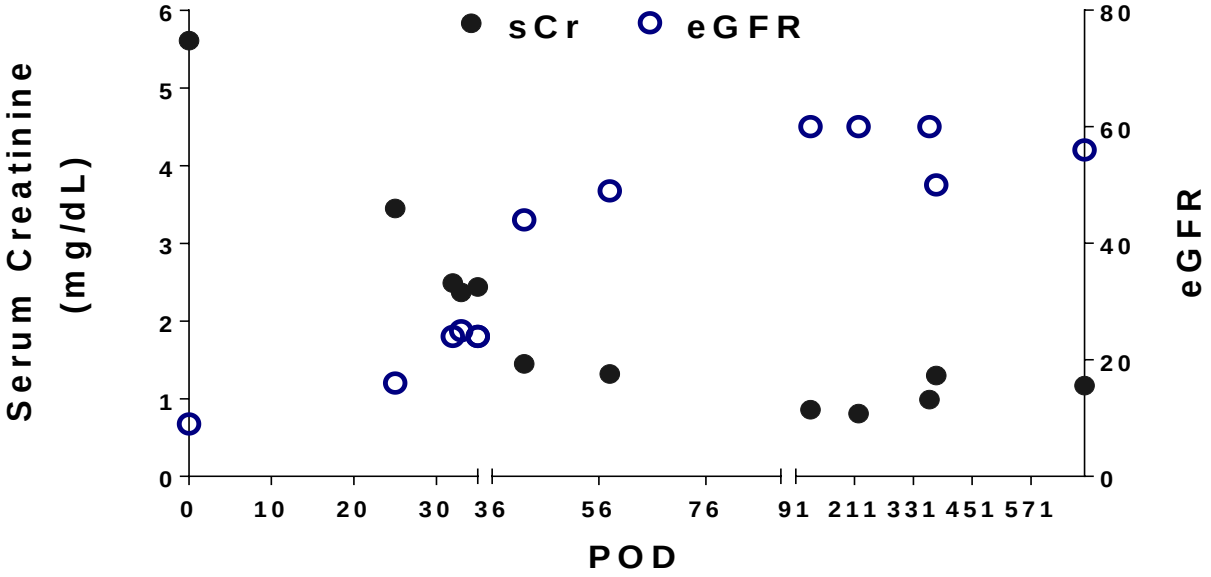


*Values higher than 60 for eGFR were plotted as 60.

**The MFI till POD59 were measured with SAB kit from Immucor Inc.

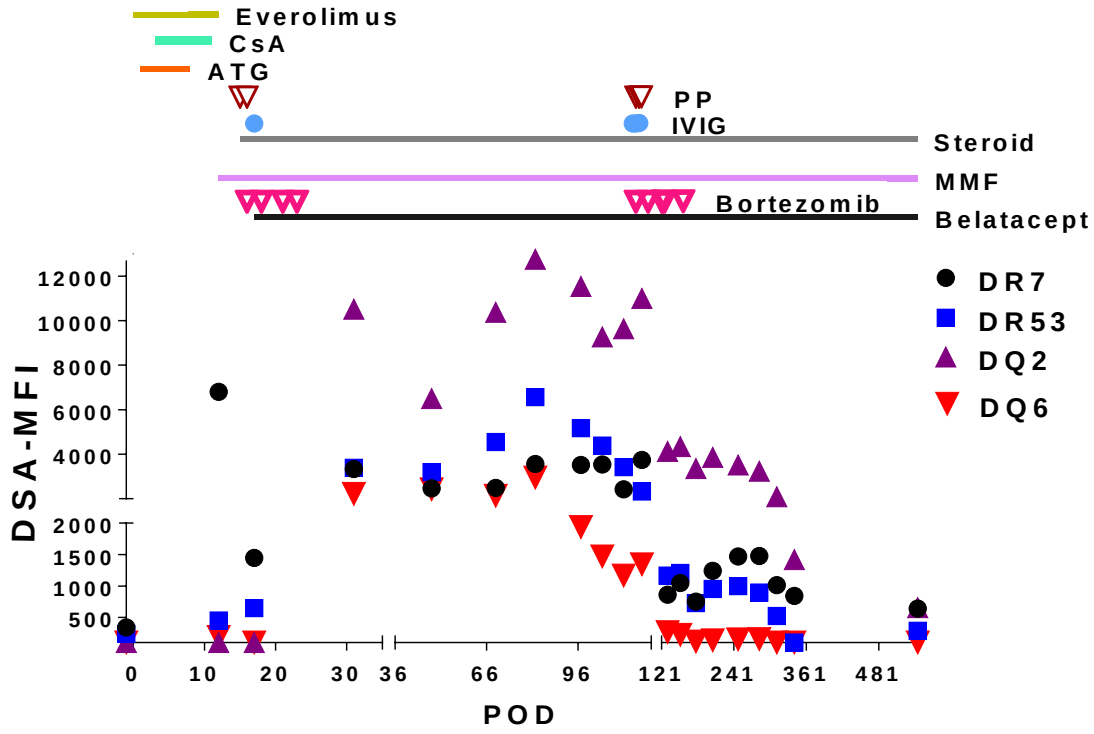
Fig S5-B: Clinical sequelae for Case 3

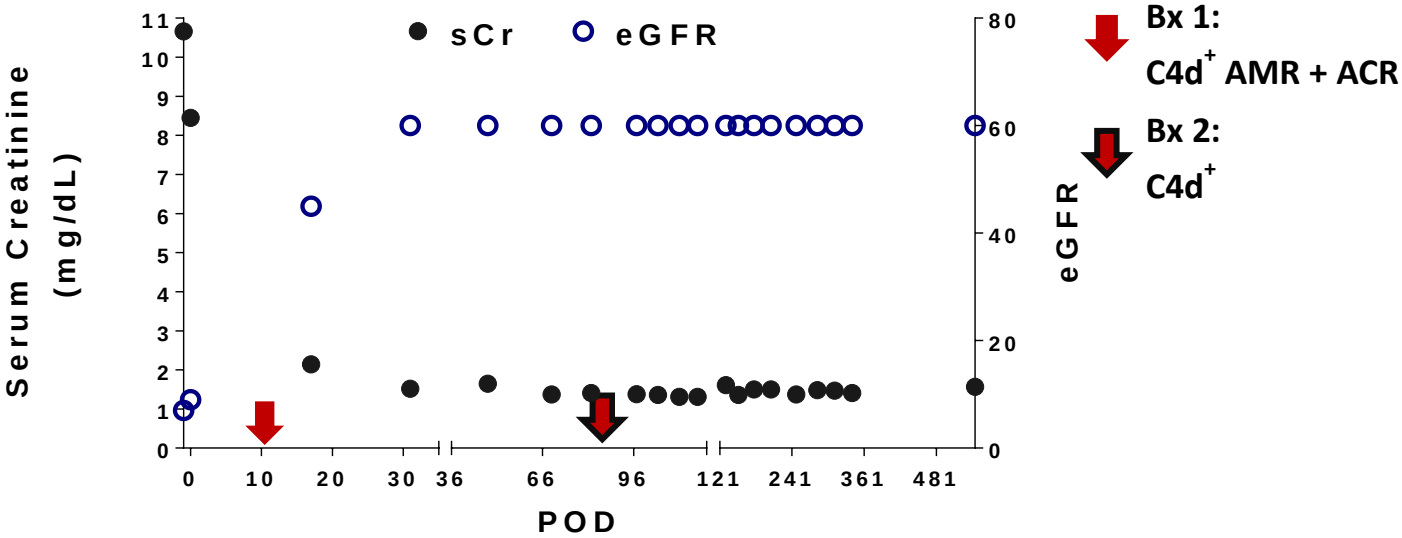




*Values higher than 60 for eGFR were plotted as 60.

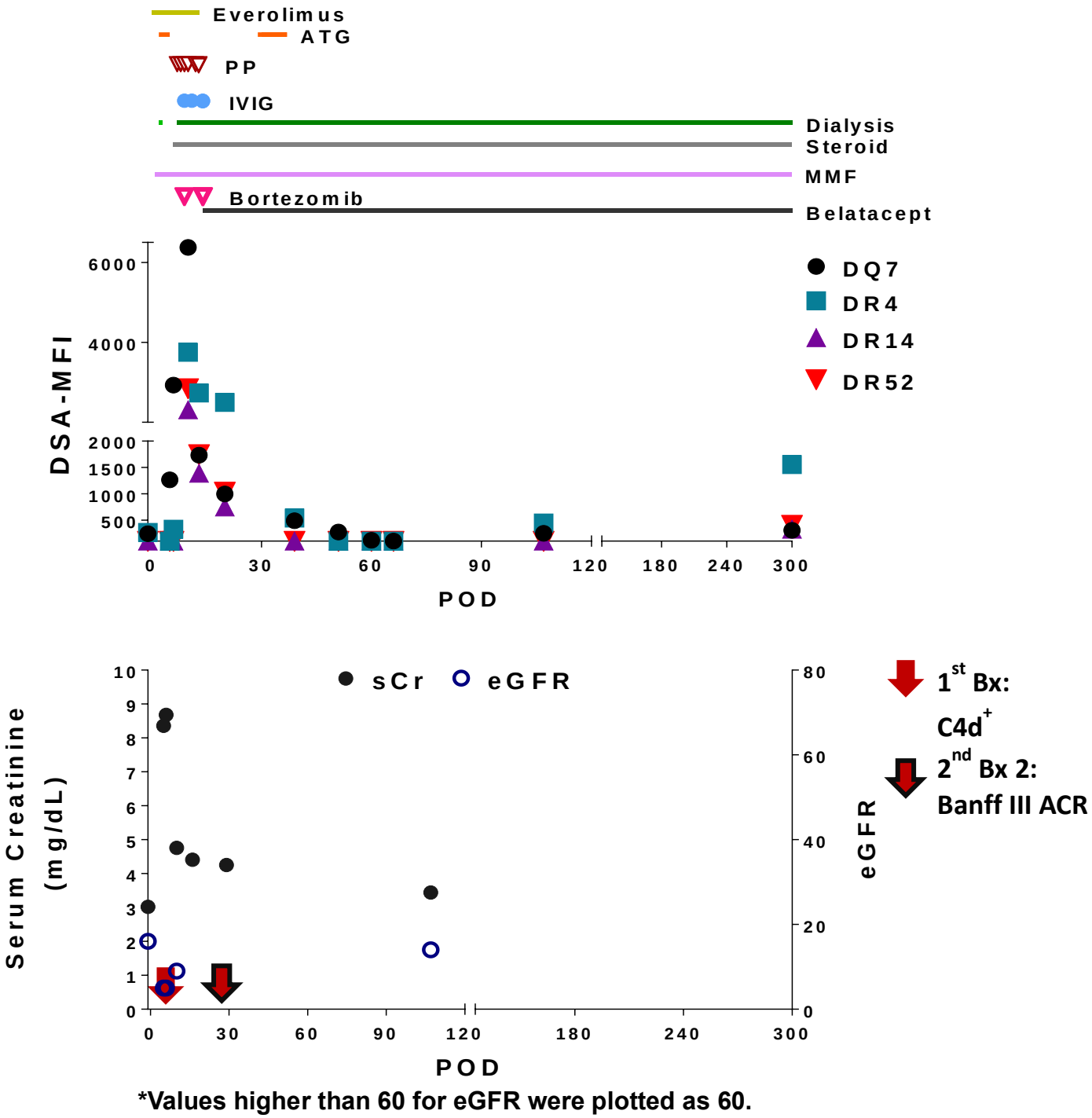
Fig S5-C: Clinical sequelae for Case 4





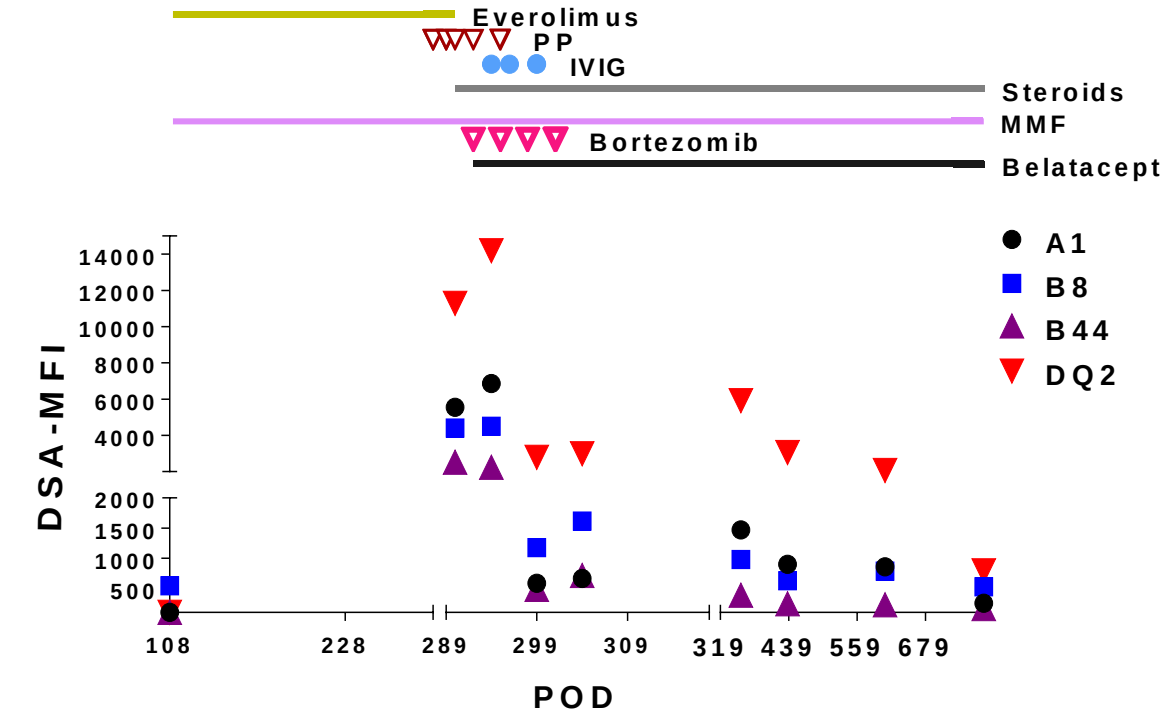
*Values higher than 60 for eGFR were plotted as 60.

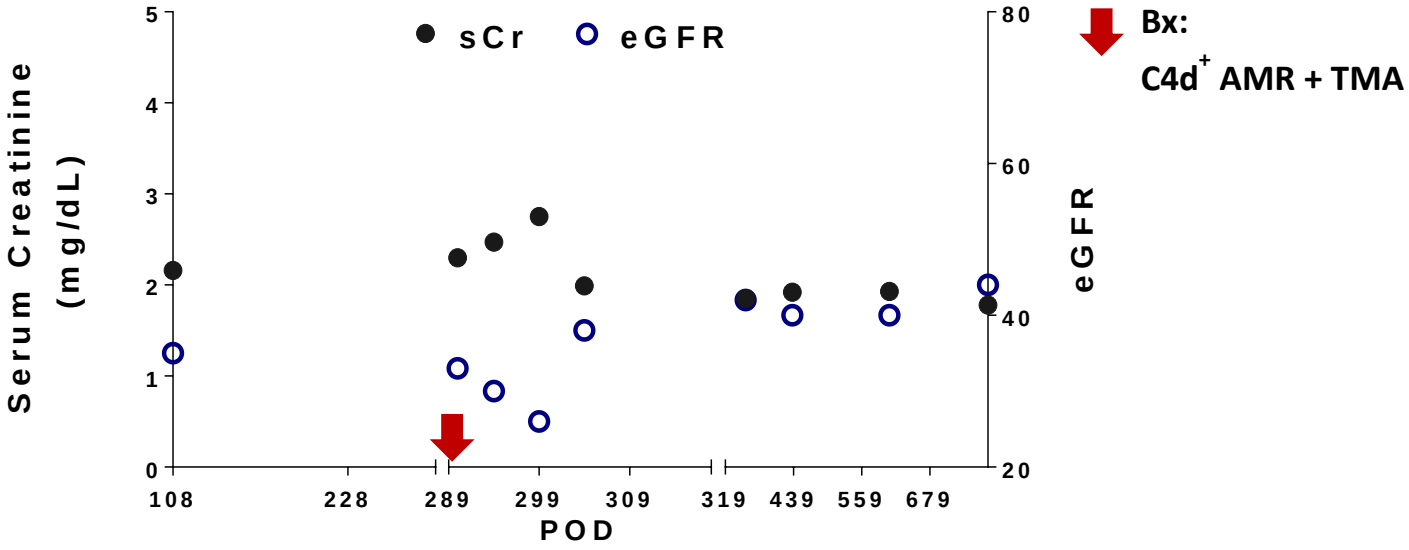
Fig S5-D: Clinical sequelae for Case 5



*Values higher than 60 for eGFR were plotted as 60.

Fig S5-E: Clinical sequelae for Case 6





*Values higher than 60 for eGFR were plotted as 60.

Fig S6: MFI of DSA is not predictive of time to reach undetectable levels

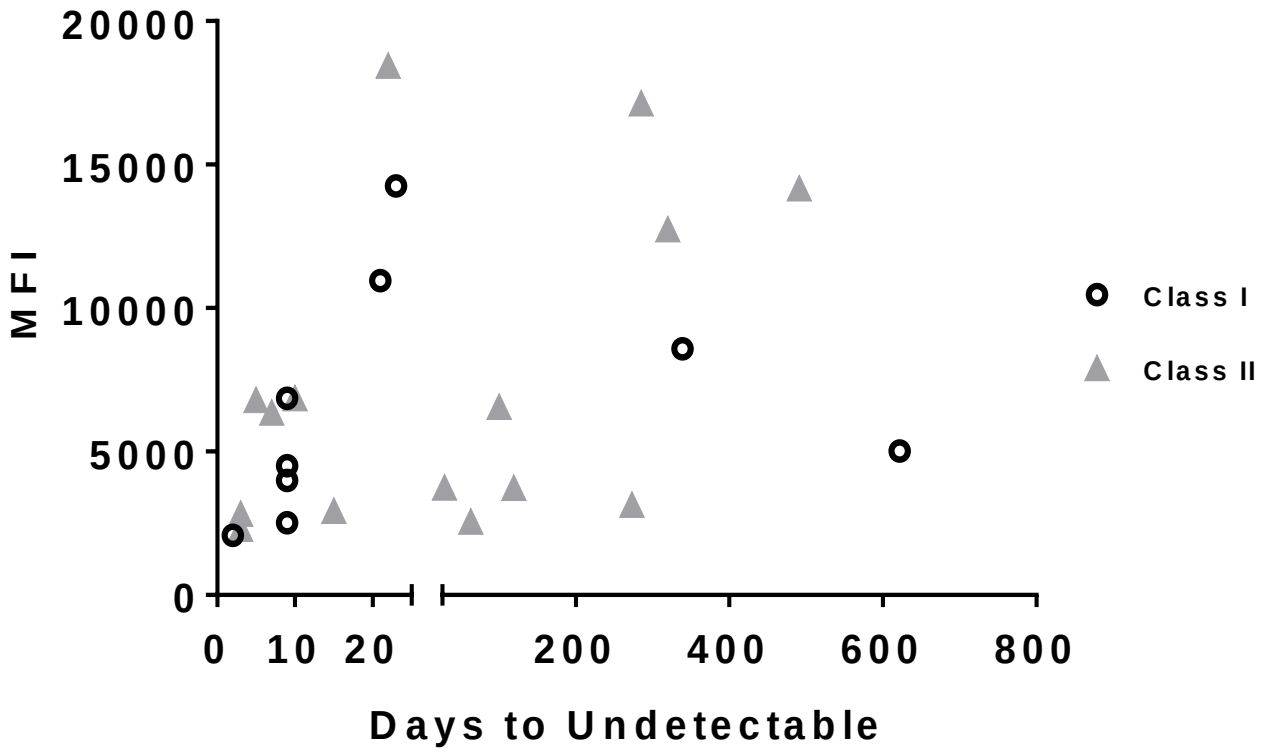


Figure S6 MFI of DSA is not predictive of time to undetectable.

MFI of HLA-Class I and Class II donor reactive antibodies for each single HLA antigen specificity were compared to days taken for each antibody to become undetectable from its first appearance. Each single dot represent the serum reactivity towards single HLA antigen and data were pooled from six transplant recipients with AMR.

Table S1. Characteristics for all biopsies of patients treated with belatacept plus bortezomib (B/B)

		Biopsy Findings												
Patient	Days post-transplant	t	v	i	g	ci	ct	cg	mm	cv	ah	ptc	C4d	other
1	0	0	0	0	0	0	0	0	0	0	0	0	ND	Baseline
1	2	0	0	0	1	0	0	0	0	0	0	2	0	ATN,TMA
1	12	0	-	0	1	0	0	0	0	0	0	2	0	ATN,TMA
1	20	0	1	1	1	0	0	0	0	1	0	2	3	ATN, TMA
1	35	0	1	0.5	1	0	0	0	0	2	0	3	3	
2	0	0	0	0	0	0	0	0	0	0.5	0	0	ND	Baseline
2	11	3	-	2	2	0	0	0	0	-	0	3	3	ATN, IH
2	662	0	0	0.5	1	0	0	1	0	1	0	1	0	
2	871	0	0	0	1	0	0	1	0	0	1	0	0	
3	ND	-	-	-	-	-	-	-	-	-	-	-	-	-
4 1st episode	0	0	0	0	0	0	0	0	0	0	1	0	ND	Baseline
4 1st episode	11	3	-	2	0	0	0	0	0	-	0	2	3	ATN
4 2nd episode	82	1	-	2	0	1	1	0	0	-	0	0	1	-
5	0	0	0	0	0	0	0	0	0	0	0	0	ND	Baseline
5	6	0	0	0	0	0	0	0	0	0	0	0	1	ATN,IH,Necrosis
5	28	1	0	0.5	1	1	1	0	0	2	0	3	0	ATN,IH,Necrosis
6	0	0	0	0.5	0	0.5	0.5	0	0	0	0	0	ND	
6	26	0	0	0.5	0	0.5	0.5	0	0	0	0	0	0	ATN
6	291	1	0	1	1	1	1	0	0	2	0	2	3	ATN, TMA

Table S2. cPRA and DSA for individual patients

Patient	Days Post Tx	cPRA or Class I, Class II PRA	Recipient DSA MFI for Donor HLA								Donor Typing Method/SAB Vendor
			A	B	C	DRB1	DRB3/4/5	DQB1	DPB1	DQA	
			3, 68	37, 62	7, 10	4, 10	53	5, 8	3, 20	NA	SSO/SSP
1	-1	79,37	<100,<100	<100,<100	<100,<100	190, <100	<100	<100,<100	1172,		One Lambda
	12	UNK	148, 1832	6907, 154	417, 306	214, 208	168	921, 971	12102,		**Immucor**
	14	UNK	294, 7206	12044, 407	825, 574	316, 651	234	2512, 1902	10987,		**Immucor**
	17	UNK	255, 10959	14251, 368	609, 513	447, 1063	208	6870, 237	11775,		**Immucor**
	19	UNK	244, 6231	9928, 332	562, 480	440, 346	218	2702, 333	12992,		**Immucor**
	24	UNK	156, 2712	6893, 224	409, 699	187, 160	112	1141, 177	17144,		**Immucor**
	28	UNK	291, 2567	6166, 288	517, 548	201, 181	192	557, 184	11794,		**Immucor**
	35	UNK	160, 491	1550, 187	251, 211	<100,<100	<100	193,<100	6352,		**Immucor**
	45	UNK	<100, 369	1149, 110	223, 180	<100,<100	<100	143,<100	3784,		**Immucor**
	49	UNK	<100, 495	1088,	202, 211	<100,<100	<100	164,<100	4825,		**Immucor**
	66	UNK	108, 468	1187,	227, 226	141, <100	<100	323,<100	4262,		**Immucor**
	108	UNK	112,415	1,099,220	221,227	185,<100	<100	213,<100	2733,		**Immucor**
	297	90	<100,<100	257, <100	<100,<100	<100,<100	<100	217,<100	1052,		One Lambda
	402	90	<100,<100	317,<100	<100,<100	<100,<100	<100	<100,<100	501,		One Lambda
	626	90	<100,<100	264,<100	<100,<100	<100,<100	<100	216,<100	820,		One Lambda
	902	88	<100,<100	<100,<100	<100,<100	<100,<100	<100	397, <100	235,		One Lambda
2			A	B	C	DRB1	DRB3/4/5	DQB1	DPB1	DQA	
			3, 11	7, 44	4, 7	7, 17	52, 53	2, -	1, 3	02, 05	SBT/SSO (for DQA)
	0	98	174, 187	<100,<100	<100,<100	101,<100	403, 201	<100,<100	132, 121		One Lambda
	11	98	2086, <2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	18452, 7920		**Immucor**
	17	98	1619, <2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	3153,<2000		**Immucor**
	33	99	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000		**Immucor**
	59	99	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000	<2000,<2000		**Immucor**
	87	98	311, 397	116,<100	778, 1432	<100,<100	<100,<100	<100,<100	1708, 2993		One Lambda
	171	98	299, 144	<100,<100	949, 1743	<100,<100	707, 122	<100,<100	1576, 3153		One Lambda
	194	98	206, <100	<100,<100	905, 1445	<100,<100	515,<100	<100,<100	1286, 2622		One Lambda
	234	98	361, 109	<100,<100	852, 1541	<100,<100	509, 128	<100,<100	1570, 3024		One Lambda
	269	99	298, 115	<100,<100	1133, 1997	<100,<100	335, 140	<100,<100	1229, 2484		One Lambda
	360	99	274, <100	<100,<100	<100,<100	<100,<100	110,<100	<100,<100	819, 1693		One Lambda
	425	98	273,<100	<100,<100	<100,<100	<100,<100	<100,<100	<100,<100	884, 1771		One Lambda
	486	98	190, <100	<100,<100	<100, 127	<100,<100	<100,<100	<100,<100	946, 1900		One Lambda
	542	99	133,<100	<100,<100	<100, 101	<100,<100	185, <100	<100,<100	1512, 1683		One Lambda
	662	98	144,<100	<100,<100	152, 154	<100,<100	155, 117	<100,<100	1174, 1259		One Lambda
	812	97	<100,<100	<100,<100	<100,<100	<100,<100	<100,<100	<100,<100	724, 883		One Lambda
	871	97	<100,<100	<100,<100	<100,<100	<100,<100	<100,<100	<100,<100	424, 523		One Lambda

			A	B	C	DRB1	DRB3/4/5	DQB1	DPB1	DQA	
			1, 11	8, 51	1, 7	7, 14	52, 53	2, 5	1, 10	01, 02	SSP
3	-1	35, 0	685, <100	398, 452	<100, 109	<100,<100	203, 186	137,<100	<100,<100		One Lambda
	25	96, 0	538, <100	3994, 8583	1582, 164	<100,<100	<100, 395	170,<100	<100,<100		One Lambda
	32	85, 0	241,<100	2079, 6072	545,<100	<100,<100	<100, 153	<100,<100	205,<100		One Lambda
	33	85, 0	115, <100	2513, 5223	829, 151	626, 1177	1550, 1252	345, 287	1147, 1408		One Lambda
	35	79, 0	137,<100	1201, 2280	258, 108	139, 336	323, 397	<100,<100	245, 536		One Lambda
	42	86, 49	172,<100	1682, 4780	839,<100	636, 958	1310, 1410	388, 210	769, 1001		One Lambda
	58	87, 49	423,<100	1230, 3138	2080, 319	300, 212	464, 2564	457,<100	366, 319		One Lambda
	121	93	516, <100	723, 4849	2672, 245	<100,<100	<100, 1804	376,<100	<100,<100		One Lambda
	219	92	293,<100	828, 4325	5015, <100	<100,<100	<100, 685	600,<100	<100,<100		One Lambda
	364	78	296,<100	277, 1873	2837,<100	<100,<100	<100, 214	155,<100	<100,<100		One Lambda
	378	81	389,<100	312, 2179	3895, <100	<100,<100	<100, 257	408,<100	<100,<100		One Lambda
	680	68	179,<100	182, 1136	1382,<100	<100,<100	<100,<100	307,<100	<100,<100		One Lambda

			A	B	C	DRB1	DRB3/4/5	DQB1	DPB1	DQA	
			3, 31	7, 44	7, 16	7, 15	51, 53	2, 6	02, -	01, 02	SSOP/DP by SBT
4 1st Episode	-1	0	<100,<100	<100, 165	<100,<100	343, 116	260, 240	<100,<100	<100		One Lambda
	12	22	147,<100	<100, 184	<100,<100	6803, 258	211, 450	<100, 188	<100,<100		One Lambda
	17	0	277,<100	<100, 224	<100,<100	1446,<100	164, 649	<100,<100	<100,<100		One Lambda
	31	68	1178, 250	414, 1367	<100,<100	3334, 584	810, 3392	10519, 2223	<100,<100		One Lambda
	48	50	767, 141	267, 940	<100,<100	2467, 419	775, 3199	6506, 2424	<100,<100		One Lambda
	69	73	1239, 618	1142, 1970	<100,<100	2491, 562	673, 4553	10380, 2137	<100,<100		One Lambda
	82	67	1082, 296	780, 1554	<100,<100	3557, 810	991, 6568	12756, 2949	<100,<100		One Lambda
	97	66	875, 239	661, 1453	<100,<100	3517, 519	718, 5169	11534, 1937	<100,<100		One Lambda
	104	66	728, 102	371, 1148	<100,<100	3542, 303	408, 4389	9269, 1468	<100,<100		One Lambda
	111	50	607, <100	208, 909	<100,<100	2420, 223	300, 3423	9635, 1165	<100,<100		One Lambda
4 2nd Episode	117	99	562, 908	644, 828	<100,<100	3744, 1317	1234, 2327	10995, 1344	<100,<100		One Lambda
	131	10	141, <100	<100, 287	<100,<100	861, 158	334, 1166	4127, 267	<100,<100		One Lambda
	152	10	159,<100	120, 349	<100,<100	1048, 139	275, 1206	4348, 223	<100,<100		One Lambda
	178	10	<100,<100	<100, 365	<100,<100	752,<100	<100, 727	3356, 118	<100,<100		One Lambda
	206	10	<100,<100	<100, 219	<100,<100	1241,<100	192, 953	3866, 135	<100,<100		One Lambda
	248	10	<100,<100	<100, 209	<100,<100	1471, 160	221, 1000	3522, 153	<100,<100		One Lambda
	283	10	<100,<100	<100, 226	<100,<100	1476, 205	215, 896	3240, 155	<100,<100		One Lambda
	312	10	<100,<100	146, 294	<100,<100	1012,<100	113, 523	2094, <100	<100,<100		One Lambda
	341	0	<100,<100	<100, 161	<100,<100	841, <100	<100,<100	1419,<100	<100,<100		One Lambda
	545	0	<100,<100	<100, 159	<100,<100	640, <100	<100, 287	656,<100	<100,<100		One Lambda

			A	B	C	DRB1	DRB3/4/5	DQB1	DPB1	DQA	
			31, -	**38, 44**	**5, 12**	**4, 14**	**52, 53**	**5, 7**	**04, -**	**01, 03**	**SSO/SSP**
5	-1	0	<100	<100,<100	<100,<100	268,	<100,<100	, 240	108		One Lambda
	5	0	<100	<100,<100	<100,<100	<100,<100	<100,<100	975, 1264	<100		One Lambda
	6	56	<100	<100,<100	<100,<100	326, <100	<100,<100	1993, 2929	<100		One Lambda
	10	56	148	<100, 565	851, 722	3751, 2314	2847, 1588	1910, 6373	1361		One Lambda
	13	0	118	123, 647	475, 330	2733, 1391	1749, 948	428, 1735	777		One Lambda
	20	0	<100	<100, 133	130, 139	2495, 749	1037, 713	262, 996	661		One Lambda
	39	0	<100	123, 140	<100, 129	540, <100	<100,<100	186, 489	110		One Lambda
	51	0	<100	<100,<100	<100,<100	<100,<100	<100,<100	<100, 275	<100		One Lambda
	60	0	<100	<100,<100	<100,<100	<100,<100	<100,<100	<100, 117	<100		One Lambda
	66	0	<100	<100,<100	<100,<100	<100,<100	<100,<100	<100,<100	<100		One Lambda
	107	0	<100	<100,<100	<100,<100	445,<100	<100,<100	<100, 250	<100		One Lambda
	300	56	<100	<100,<100	<100,<100	1556, 327	405, <100	583, 3005	<100		One Lambda

			A	B	C	DRB1	DRB3/4/5	DQB1	DPB1	DQA	
			1, -	**8, 44**	**5,7**	**13,17**	**52**	**2,6**	**04, 19**	**01, 05:01**	**SSP/SSO (for DQA)**
6	-1	0	<100	544,	<100,<100	<100,<100	<100	<100,0	<100,<100		One Lambda
	290	81	5548	4411, 2519	<100,<100	<100,<100	<100	11281, <100	<100,<100		One Lambda
	294	81	6861	4505, 2250	<100,<100	<100,<100	<100	14178, <100	<100,<100		One Lambda
	299	10	583	1177, 485	<100,<100	<100,<100	<100	2792, <100	<100,<100		One Lambda
	304	10	665	1,617,716	<100,<100	<100,<100	<100	2990, <100	<100,<100		One Lambda
	355	10	1471	978, 386	<100,<100	<100,<100	<100	5914, <100	<100,<100		One Lambda
	437	10	901	625, 244	<100,<100	<100,<100	<100	3053, <100	<100,<100		One Lambda
	608	10	856	787, 230	<100,<100	<100,<100	<100	2060, <100	<100,<100		One Lambda
	781	0	252	526, 164	<100,<100	<100,<100	<100	790, <100	<100,<100		One Lambda

Table S3. Changes in sCr and eGFR in patients treated with belatacept plus bortezomib (B/B)

Patient	Start	Last F/u	[a] ΔsCr ([b] eGFR)		
			Start->Best	Start->Last F/u	Best>Last F/u
1	21 d	30 m	-4.9(10.1)	-4.9(10.1)	0(1)
2	14 d	29 m	-2.35(4.1)	-1.84(2.9)	0.51(0.7)
3	32 d	22 m	-1.7(2.7)	-1.3(2.3)	0.4(0.9)
4	17 d	115 d	-0.79(2.2)	NA	NA
	115 d	18 m	-0.1(1.2)	0(1.0)	0(0.8)
5	9 d	10 m	-5.26(2.8)	[c]HDD	HDD
6	292 d	26 m	-0.5(2.0)	0.5(1.3)	0(0.7)

[a]ΔsCr is the difference in sCr and [b]ΔeGFR is the fold change in eGFR from B/B start to best or last follow-up (F/u). [C]HHD: Hemodialysis.

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Materials and Methods

Mice, immunization and immunosuppression: Female C57BL/6 (B6, H-2b), BALB/c (B/c, H-2d) mice, were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN), and received immunization with BALB/c splenocytes (DST; 25×10^6/mouse s.c. and i.p.). Full-thickness BALB/c skin were grafted onto the flank of recipient C57BL/6 mice. Mice were treated with Bortezomib (0.75-1mg/Kg, i.p.) (VELCADE, Millennium Pharmaceuticals, Inc) and/or CTLA4-Ig (25mg/kg; i.p.) (ORENCIA, abatacept, Bristol-Myers Squibb) followed by a 2x/week dosing of CTLA4-Ig (12.5mg/kg).

Flow cytometric analysis of PC subsets in mice: Spleen, femurs and tibiae were harvested, and erythrocytes were lysed (ACK lysing buffer, Quality Biological Inc.). Cells (5×10^6) were suspended in 2%FBS and stained for PC subset analysis, as described previously[18]. A futher gating was done on mature PC to analyze the long lived plasma cells (LLPCs: CD20-, CD19-, CD27-, CD38+, and CD138+). Data were acquired using a LSRFortessa™ flow cytometer (BD) and analyzed using FlowJo (FlowJo, LLC, Ashland OR).

Mouse DSA quantification: DSA were quantified using 1×10^6 BALB/c splenocytes, stained with 2µL of test serum and anti-IgG secondary antibodies. Mean fluorescence intensity (MFI) of anti-IgG were from non-$CD19^+$ B cells. The normalized MFI was calculated considering the baseline MFI at day zero to be one hundred percent for each individual mouse.

Antibodies for mouse PC subset and DSA analysis: The following antibodies were used: anti-CD138-PE (281-2, Cat#142503, Biolegend), anti-TACI-APC (ebio8F10-3, Cat#17594281, eBioscience), anti-CD45R (B220)-PerCP-Cy5.5 (RA3-6B2, Cat#45045280, eBioscience), anti-CD19 Alexa Fluor 488 (6D5, Cat#115524, Biolegend), anti-CD38 APC-Cy7 (90, Cat#102727, Biolegend), anti-CD20 PE-Cy7 (SA275A11 , Cat#150419, Biolegend), anti-CD27 BV510 (LG.3A10, Cat#563605, BD biosciences), anti CD19-APC (1D3, Cat#550992, BD biosciences), anti-IgG (H+L)-FITC (Cat#1031-02, Southern Biotech). Anti-CD3-eFluor450 (17A2,

Cat#48003282, eBioscience). LIVE/DEAD™ fixable violet dead cell stain kit (Invitrogen, Carlsbad, CA) and Fc block 2.4G2 (unconjugated CD16/CD32) were used.

Mouse IgG ELISpot: Total IgG and Anti K^d-IgG secreting cell were analyzed using a Mouse IgG ELISpot^{BASIC} HRP, Mabtech AB, Sweden). Briefly, cells were added to ELISpot plates (MultiScreen^{TM}, EMD Millipore, Burlington, MA) pre-coated with anti-IgG. Biotinylated K^d monomer (NIH, Tetramer core facility, Atlanta, GA) or anti-IgG were then added, followed by Streptavidin-HRP and TMB. Plates were analysed using CTL ImmunoSpot analyser (Cellular Technology Limited, Cleveland, OH).

Statistical analysis: The statistical significance of differences in mean values and cell numbers were analyzed by Student's *t* test, one way ANOVA using Graphpad Prism 7 (Graphpad, San Diego, CA). P values ≤0.05 were considered statistically significant.

Patients: A sequential series of 6 ABO-compatible renal kidney recipients transplanted between 9/2015 and 9/2016 that developed nascent AMR following renal transplantation were treated with bortezomib plus belatacept. Patient demographics and transplant characteristics are summarized in Table 1. All patients had a negative T and B cell flow cytometric cross-match with no discernable DSA in immediately pre-transplant sera by Luminex single antigen bead analyses (LABScreen Single Antigen, One Lambda, Canoga Park, CA and Immucor Inc. Peachtree Corners, GA). Five recipients received thymoglobulin induction, with one patient receiving basiliximab due to a history of ATG allergy. All 6 acute AMR episodes were treated with steroids and PP, and IVIG prior to initiating bortezomib and belatacept. Maintenance immunosuppression prior to AMR consisted of everolimus and microemulsion cyclosporine with steroid discontinuation after a rapid 5 day taper.

AMR treatment: Bortezomib was dosed at 1.3 mg/m^2/dose. Belatacept was dosed at 10 mg/kg on day 1, 5, weeks 2, 4, and 12 then 5 mg/kg monthly. The IVIG, was dosed at 1 gm/kg/dose (Privagen, CSL Behring,

King of Prussia, PA). All 6 acute AMR episodes were treated with steroids and PP prior to initiating bortezomib and belatacept, plus IVIG after PP in 3/6 cases. One patient was treated with ATG due to the presence of mixed acute cellular and antibody mediated rejection prior to bortezomib and belatacept therapy.

Qualitative Anti-HLA Antibody Screening and DSA identification: Solid-phase multiplex testing (FLOWPRA) was performed according to the manufacturer's recommendations (One Lambda, Canoga Park, CA). Bound antibody was detected with a PE-conjugated anti-human IgG using a Coulter Epics2 flow cytometer. A negative serum control was used to establish background cut-offs and a serum was considered positive if the MFI was 6% above the cut-off and exhibited peak architecture consistent with alloantibody binding. Sera which screened positive were then tested for Class I or Class II DSA using single antigen beads. The identification of DSA specificities were determined on a Luminex 100 reader, and MFI values above 2,000 were considered indicative of DSA presence (Table S2).

Biopsies: C4d staining was performed using indirect immunofluorescence (on frozen sections) using Quidel anti-C4d monoclonal antibody (Quidel Corporation, cat no. A213) (1:40 dilution) and FITC- goat anti-mouse IgG (Beckman Coulter, Marseille, France, cat no. PN IM0819) (1:50 dilution). Biopsies were scored using the Banff 2015 criteria by a single pathologist (TN at Ohio State University Hospitals). The Banff scoring of the indication biopsies used to confirm AMR are included in Table 2 and S3.

Estimated glomerular filtration rate (eGFR): The eGFR was calculated using the MDRD formula adjusted for patient height and weight.