Supplementary Materials for

Increased GVHD-related mortality with broad-spectrum antibiotics in humans and mice

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This file includes:

Supplementary Figure 1

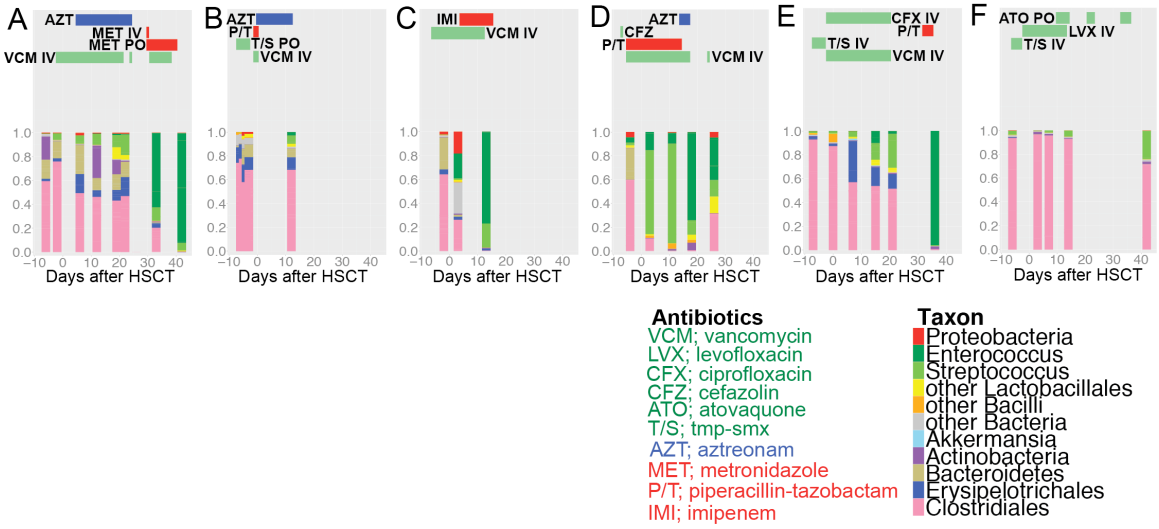

Figure S1. Six representative clinical cases with time courses depicted of fecal microbiota composition and administration of antibiotic treatments during the course of allo-HSCT. The dynamics of flora composition that occur in the setting of treatment are shown for aztreonam (**A and B**), imipenem-cilastatin (**C**), piperacillin-tazobactam (**D and E**), metronidazole (**A**), and minimal flora-perturbing antibiotics (**F**).

Supplementary Figure 2

Large intestine (day 21)

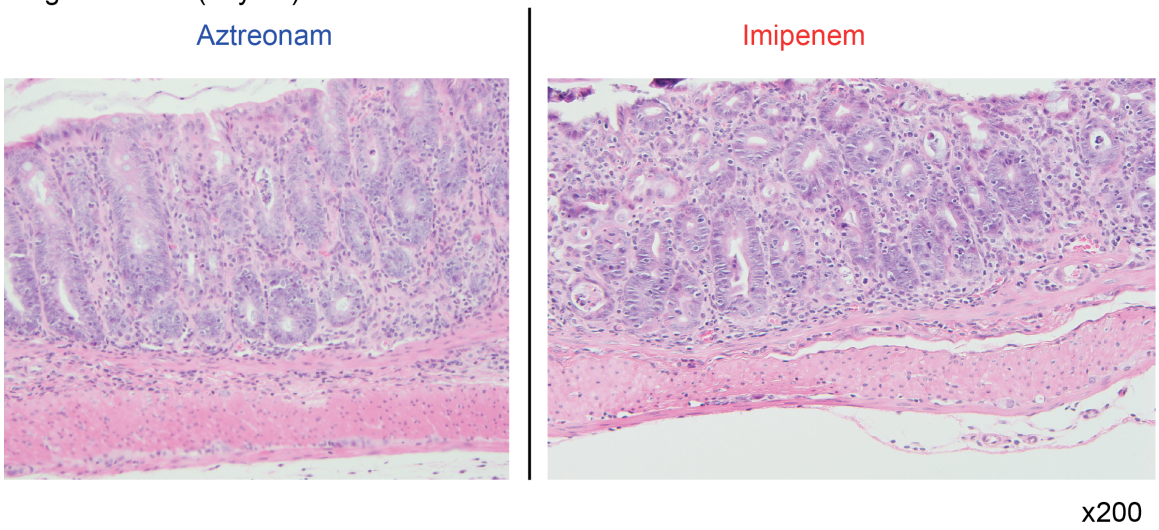

Figure S2. Recipients treated with imipenem-cilastatin exhibit enhanced histological GVHD on day 21. Lethally irradiated 129S1 recipients were transplanted with C57BL/6 TCD-BM cells with 1 × 10^6 C57BL/6 T cells. Recipients were treated with imipenem-cilastatin or aztreonam (100 mg/kg, SC, 3 times a week from day 10 to day 21). Colon tissues were harvested on day 21. Hematoxylin and eosin stained samples are shown (magnification, ×200). (Left) aztreonam treated recipients; (Right) imipenem-cilastatin treated recipients. Both show evidence of GVHD. The group treated with imipenem-cilastatin exhibits more inflammatory cell infiltration including neutrophils and lymphocytes, robust apoptosis and crypt destruction. Representative histology images from three independent experiments are shown.

Supplementary Figure 3

Large intestine (day 21)

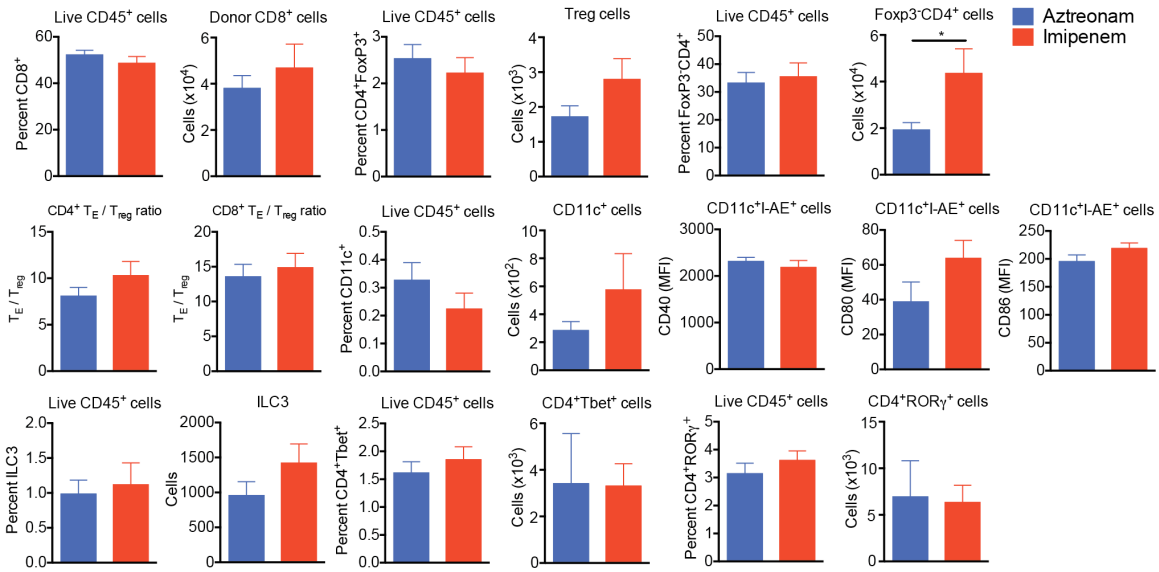

Figure S3. Flow cytometric analysis of colon tissues in the recipients on day 21. Lethally irradiated 129S1 recipients were transplanted with C57BL/6 TCD-BM cells with 1×10^6 C57BL/6 T cells. Recipients were treated with imipenem-cilastatin or aztreonam as described in **fig. S2**. Colon tissues from recipients were analyzed on day 21 by flow cytometry. Data are combined from two independent experiments. Values represent mean ± SEM (n = 10–14). *, P < 0.05.

Supplementary Figure 4

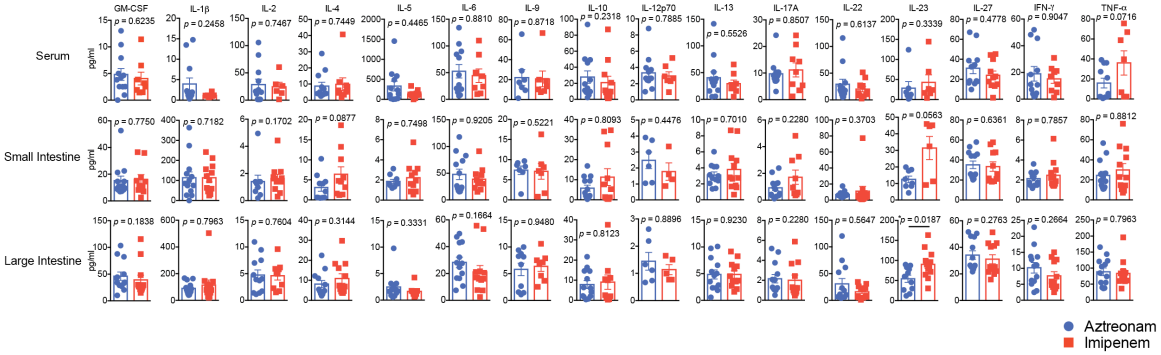

Figure S4. Recipients treated with imipenem-cilastatin show a higher level of IL-23 in the colon on day 21. Lethally irradiated 129S1 recipients were transplanted with C57BL/6 TCD-BM cells with 1 × 10^6 C57BL/6 T cells. Recipients were treated with imipenem-cilastatin or aztreonam as described in **fig. S2**. Serum, whole small intestine homogenate, and whole colon homogenate levels of 16 different cytokines are shown (Multiplex ELISA). Data are combined from two independent experiments. Values represent mean ± SEM (n = 10–12). *, P < 0.05.

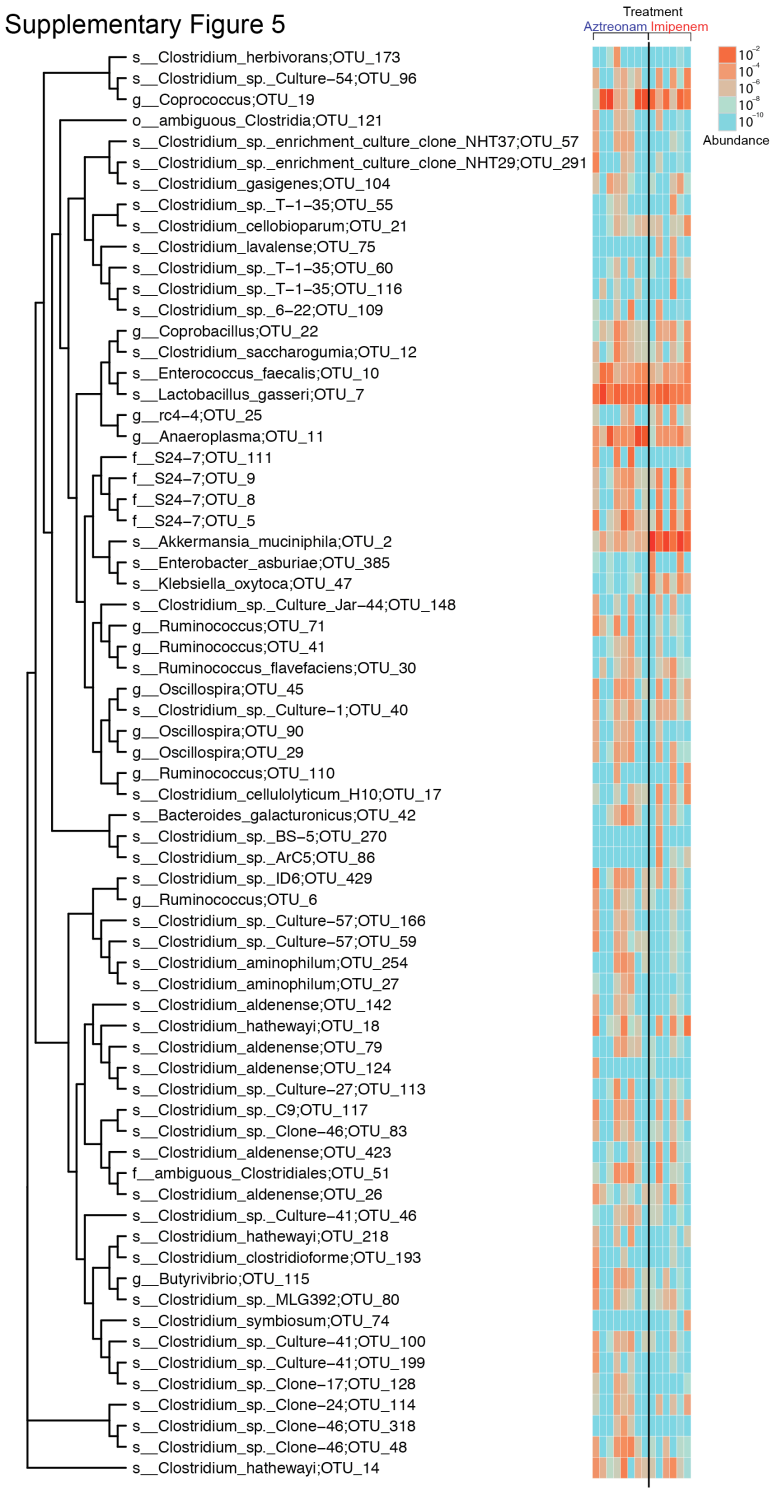

Figure S5. Abundance of bacterial OTUs, clustered by 16S rRNA sequence similarity, in recipients treated with imipenem-cilastatin or aztreonam. Recipients were treated with imipenem-cilastatin or aztreonam as described in **Fig. 4, D and E**. Data are representative from more than five independent experiments (n = 8

in aztreonam and $n = 6$ in imipenem-cilastatin treated recipients). This provides an alternative depiction of the data from **Fig. 4D and E**.

Supplementary Figure 6

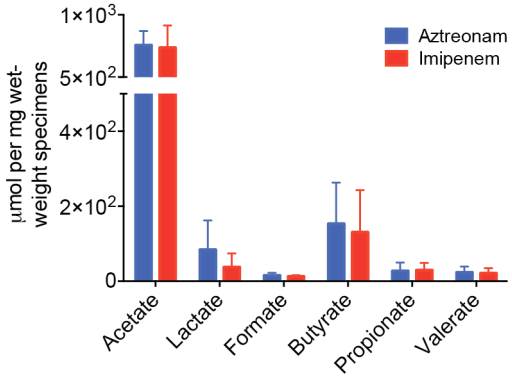

Figure S6. Analysis of SCFA levels in cecum stool specimens. Stool specimens from cecum were collected from recipients on day 21 after allo-HSCT and SCFA levels were determined by ^{1}H-NMR spectroscopy.

Supplementary Figure 7

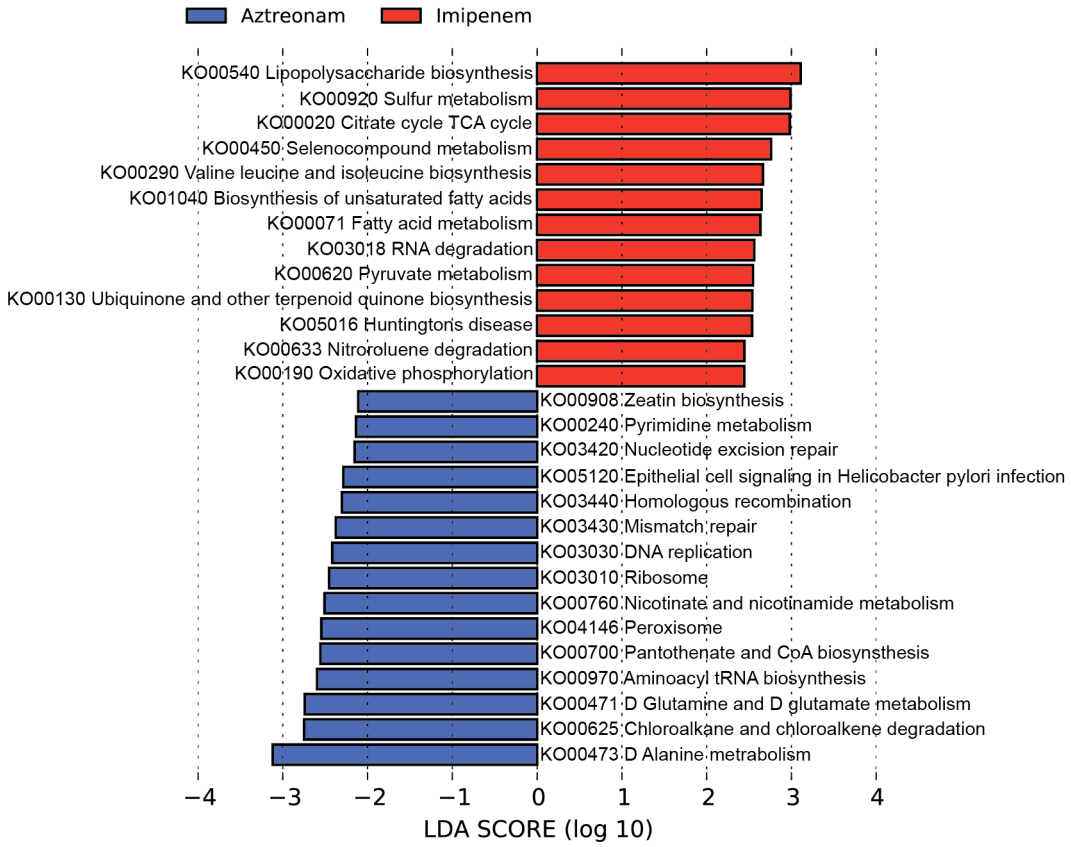

Figure S7. Linear discriminate analysis coupled with effect size measurements (LEfSe) analysis of relative numbers of sequences assigned to KEGG ortholog gene pathways following metagenomic shotgun sequencing. Recipients were treated with imipenem-cilastatin or aztreonam as described in **Fig. 4F** and stool specimens were collected on day 21.

Table S1. Summary of day 21 analyses* after allo-HSCT (aztreonam-treated vs. imipenem-treated recipients)

Cell Populations	Small Intestine LPL (n = 12-16)	Large Intestine LPL (n = 10-14)	Spleen (n = 12-16)
$CD4^+$ T cells (% in live $CD45^+$ cells)	p = 0.8282	p = 0.0162	p = 0.1050
$CD4^+$ T cells number	p = 0.6887	p = 0.0019	p = 0.0973
$CD8^+$ T cells (% in live $CD45^+$ cells)	p = 0.2754	p = 0.4604	p = 0.1593
$CD8^+$ T cells number	p = 0.5570	p = 0.4677	p = 0.1908
$CD4^+FoxP3^+$ (% in live $CD45^+$ cells)	p = 0.2189	p = 0.5327	p = 0.9254
$CD4^+FoxP3^+$ cells number	p = 0.3180	p = 0.2829	p = 0.2231
$CD4^+CD25^+$ $FoxP3^-$ (% in live $CD45^+$ cells)	p = 0.2744	p = 0.1878	p = 0.6377
$CD4^+CD25^+$ $FoxP3^-$ cells number	p = 0.3983	p = 0.0129	p = 0.5481
$CD11c^+$ cells (% in live $CD45^+$ cells)	p = 0.1967	p = 0.2498	p = 0.5615
$CD11c^+$ cells number	p = 0.5481	p = 0.2947	p = 0.0932
CD40 (MFI) in $CD11c^+I\text{-}AE^+$ cells	N/A	p = 0.8832	N/A
CD80 (MFI) in $CD11c^+I\text{-}AE^+$ cells	N/A	p = 0.0721	N/A
CD86 (MFI) in $CD11c^+I\text{-}AE^+$ cells	N/A	p = 0.2409	N/A
$CD4^+ROR\gamma^+$ (% in live $CD45^+$ cells)	p = 0.9291	p = 0.343	p = 0.3337
$CD4^+ROR\gamma^+$ cells number	p = 0.5481	p = 0.405	p = 0.2231
$CD4^+Tbet^+$ (% in live $CD45^+$ cells)	p = 0.4103	p = 0.6863	p = 0.2930
$CD4^+Tbet^+$ cells number	p = 0.8112	p = 0.4103	p = 0.5052
ILC3 ($RORg^+Thy1.2^+CD3^-$) (% in live $CD45^+$ cells)	p = 0.2231	p = 0.6520	N/A
ILC3 ($RORg^+Thy1.2^+CD3^-$) cells number	p = 0.3337	p = 0.1079	N/A

* Results shown are from comparison of the values between aztreonam-treated and imipenem-treated recipients (not adjusted for multiple comparisons).

LPL; lamina propria lymphocytes