SUPPLEMENTARY MATERIALS

Fig. S1: IL-6 measurements in SBI-101 treated participants

Fig. S2: Time to treatment inversely correlates with TNF-a reduction.

Table S1: Demographic Characteristics (Treated Set)

Table S2: Effect sizes of molecular and cellular PD Biomarkers

Table S3: Multiplex Panels

Table S4: Flow Cytometry Panels

Materials and Methods: Exploratory endpoint analysis

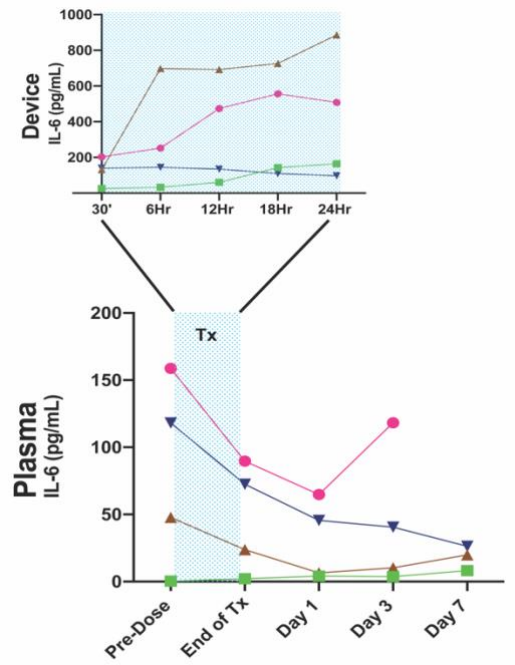

Fig. S1: IL-6 measurements in SBI-101 treated participants subjects.
IL-6 was quantified in the plasma of each participant (n=4, green, brown, blue and pink) pre and post treatment as well as in samples taken directly from the SBI-101 device during the 24 hours of treatment. Despite an increase in detectable IL-6 in the MSC microenvironment during treatment, plasma levels of IL-6 seem to decrease post treatment.

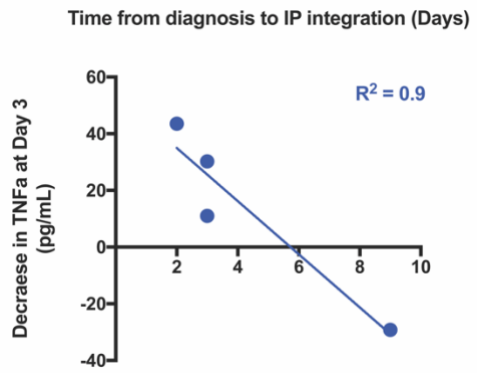

Fig. S2: Time to treatment inversely correlates with TNF-α reduction.
TNF-α was measure in plasma of subjects receiving SBI-101 therapy at baseline and at day 3 post treatment. The delta between the two time points was calculated and plotted against time from diagnosis of AKI to integration of SBI1-10 (IP- Investigational product). Strong correlation (r^2=0.9) indicates that patients that receive SBI-101 soon after their diagnosis may be more likely to have larger reductions of pro-inflammatory cytokines such as TNF-α.

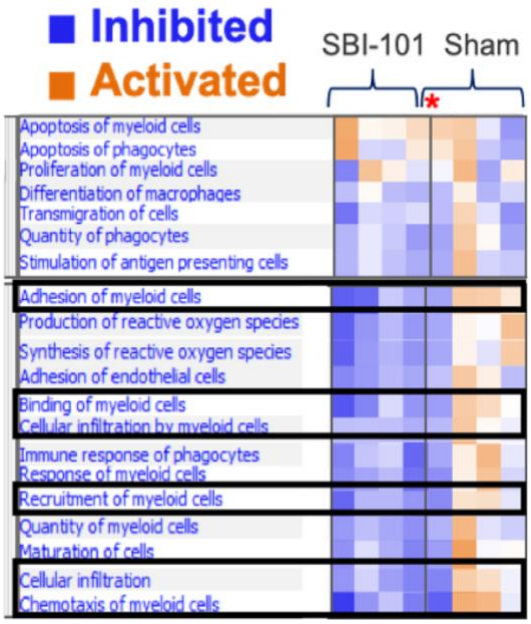

Fig. S3. Comparison Analysis of Monocyte-related Diseases and Functions of 250M SBI-101 Treated and Sham.

Disease and Function Analysis Comparison Heatmap was sorted by Hierarchical Clustering based on sample Activation Score. The activation score (Z-score) predicts direction of gene regulation of potential regulators assessing the match of observed and predicted upstream or downstream processes. Z-score serves as both a significance measure and a predictor for the activation state of the regulator (orange, +/activation, blue, -/inhibition). Monocyte-related Diseases and Functions are highlighted.

Table S1: Demographic Characteristics (Treated Set)

	Low Dose (N=12)	Sham (N=4)	Overall (N=16)
Age (years)			
N	12	4	16
Mean (SD)	55.9 (9.82)	61.1 (19.39)	57.2 (12.30)
Median	56.3	66.2	56.7
Q1, Q3	50.1, 62.2	47.5, 74.6	50.1, 64.2
Min, Max	38.1, 74.2	33.9, 77.9	33.9, 77.9
Sex, n(%)			
Male	7 (58.3)	1 (25.0)	8 (50.0)
Female	5 (41.7)	3 (75.0)	8 (50.0)
Race, n(%)			
American Indian/Alaska Native	0 (0.0)	0 (0.0)	0 (0.0)
Asian	0 (0.0)	0 (0.0)	0 (0.0)
Black/African American	2 (16.7)	0 (0.0)	2 (12.5)
Caucasian/White	10 (83.3)	4 (100.0)	14 (87.5)
Native Hawaiian/Pacific Islander	0 (0.0)	0 (0.0)	0 (0.0)
Multi-race	0 (0.0)	0 (0.0)	0 (0.0)
Other	0 (0.0)	0 (0.0)	0 (0.0)
Ethnicity, n(%)			
Hispanic or Latino	1 (8.3)	0 (0.0)	1 (6.3)
Not Hispanic or Latino	11 (91.7)	4 (100.0)	15 (93.8)
Weight (kg)			
N	12	4	16
Mean (SD)	112.9 (30.83)	96.1 (23.71)	108.7 (29.42)
Median	112.4	91.2	107.4
Q1, Q3	90.2, 132.4	78.4, 113.8	83.8, 129.0
Min, Max	65.8, 171.2	74.2, 127.9	65.8, 171.2
Height (cm)			
N	12	4	16
Mean (SD)	171.2 (8.49)	169.6 (4.87)	170.8 (7.63)
Median	174.0	171.5	172.7
Q1, Q3	161.3, 177.8	166.4, 172.9	162.6, 176.6
Min, Max	160.0, 182.9	162.6, 173.0	160.0, 182.9
BMI (kg/m^2)			
N	12	4	16
Mean (SD)	38.4 (9.73)	33.5 (8.22)	37.2 (9.38)
Median	37.2	32.7	36.3
Q1, Q3	32.6, 45.7	26.6, 40.3	30.2, 42.6
Min, Max	21.4, 54.2	25.6, 42.9	21.4, 54.2
Child Bearing Status, n(%)			
Yes	1 (8.3)	1 (25.0)	2 (12.5)
No	4 (33.3)	2 (50.0)	6 (37.5)
AKI etiology, n(%)			

Pre-renal azotemia due to hypovolemia, impaired cardiac output/shock	8 (66.7)	3 (75.0)	11 (68.8)
Decreased vascular resistance due to sepsis / anaphylaxis	3 (25.0)	1 (25.0)	4 (25.0)
Renovascular stenosis or occlusion	0 (0.0)	0 (0.0)	0 (0.0)
Other	4 (33.3)	0 (0.0)	4 (25.0)
Time from start of CRRT to treatment with IP n(%)			
< 3 Days	7 (58.3)	2 (50.0)	9 (56.3)
> 3 Days	5 (41.7)	2 (50.0)	7 (43.8)
> 5 Days	1 (8.3)	1 (25.0)	2 (12.5)
> 7 Days	0 (0.0)	0 (0.0)	0 (0.0)
Discontinuation from IP due to Clotting	5 (41.7)	1 (25.0)	6 (37.5)
Time to IP Discontinuation due to Clotting			
N	5	1	6
Mean (SD)	2.6 (1.77)	10.2 (--)	3.8 (3.49)
Geometric Mean	1.9	10.2	2.5
Median	3.0	10.2	3.6
Q1, Q3	0.8, 4.2	10.2, 10.2	0.8, 4.3
Min, Max	0.6, 4.3	10.2, 10.2	0.6, 10.2

Table S2: Effect sizes of molecular and cellular PD Biomarkers.
The most robust PD biomarkers were defined as those with effect sizes greater than one (absolute value). PD biomarkers with effect sizes greater than one on study days 0, 1, 3 or 7.

Molecular biomarkers

PD Endpoint	Direction of Response	Day	TWA Effect Size
6CKine	Decrease	0	-1.30
B2-Microglobulin	Increase	0	1.00
		1	1.01
Cathepsin D	Decrease	3	-1.00
		7	-1.07
Collagen IV	Decrease	0	-1.49
		1	-1.44
		3	-1.20
CTACK	Decrease	0	-1.80
		1	-1.20
Cystatin	Increase	3	1.08
		7	1.29
Eotaxin	Increase	3	1.23
		7	1.09
Eotaxin-1	Decrease	1	-1.25
		3	-1.09
Flt-3L	Decrease	7	-1.06
G-CSF	Increase	0	2.30
		1	1.90
		3	1.46
GRO alpha	Increase	0	1.14
		1	1.02
I-309	Decrease	0	-1.82
		1	-1.85
		3	-1.37
IFNa2	Decrease	0	-1.15
		1	-1.07
		3	-1.35
		7	-1.10
IFNy	Decrease	7	-1.42
IL-10/TNFa	Increase	0	1.03
		1	1.23
		3	1.45
		7	1.47
IL-13	Increase	0	1.27
		1	1.26
		3	1.24
		7	1.17
IL-15	Decrease	0	-1.28
		1	-1.21
IL-3	Increase	7	1.07
IL-5	Decrease	3	-1.02
IL-6/IL-10	Decrease	0	-1.14
		1	-1.43
KIM-1	Decrease	0	-1.12
		1	-1.14
		3	-1.10
MCP-2	Decrease	0	-1.31
		1	-1.79
		3	-2.23
		7	-1.74
MCP-3	Decrease	0	-1.64
		1	-1.06
		3	-1.02
Osteoactivin	Decrease	0	-1.85
		1	-2.15
		3	-4.34
		7	-2.34
PF4	Increase	0	1.73
		1	1.00
		7	1.00
SDF-1a+B	Decrease	0	-1.21
		1	-1.06
		3	-1.03
sIL-2Ra	Decrease	3	-1.09
sTNFRI	Decrease	0	-1.10
		1	-1.03
sTNFRII	Decrease	0	-1.34
		1	-1.18
sVEGFR1	Increase	0	1.10
		1	1.15
		3	1.04
		7	1.03
TGF-a	Decrease	0	-1.04
TGF-B1	Increase	7	1.04
TGF-B2	Increase	0	1.41
		1	1.42
		3	1.54
		7	2.39
TNFa	Decrease	0	-1.32
		1	-1.16
		3	-1.28
		7	-1.39
TRAIL	Decrease	3	-1.51
		7	-1.29

Cellular biomarkers

PD Endpoint	Direction of Response	Day	TWA Effect Size
B Cells (Marginal Zone B)	Increase	3	1.03
		7	1.26
B Cells (Unswitched Memory)	Increase	3	1.16
		7	1.55
Dendritic Cells (mDC2)	Increase	1	1.48
		3	2.41
		7	1.07
Dendritic Cells (pDCs)	Decrease	7	-1.27
Dendritic Cells (Total mDCs)	Increase	7	1.00
Monocytes Classical	Decrease	3	-1.15
Monocytes Intermediate	Decrease	0	-1.19
Monocytes Non-classical	Increase	0	1.46
		1	1.07
Monocytes Total	Decrease	3	-1.14
NK cells (CD56bright CD16low)	Increase	3	1.13
NK cells (CD56bright CD8+)	Increase	1	1.13
		3	1.05
NK cells (CD56dim CD16bright)	Increase	0	1.49
		1	1.18
NK cells (CD56dim CD8+)	Increase	0	1.36
CD4+ T Cells (Central Memory)	Increase	0	1.19
		1	1.02
CD4+ T Cells (Lag3+)	Decrease	0	-1.32
		1	-1.38
		3	-1.26
CD4+ T Cells (Naive CD38+)	Increase	0	1.16
CD8+ T Cells (CD69+)	Increase	1	1.02
		3	1.17
CD8+ T Cells (Central Memory)	Increase	3	1.18
		7	1.18
Treg (CD45+ CD4+ CD25-FoxP3+)	Decrease	1	-1.80

Table S3: Multiplex Panels

Panel	Eve Technologies Cat #	Analytes
Human Cytokine Array/Chemokine Array 65-Plex	HD65	EGF, Eotaxin, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO, IFN-α2, IFN-γ, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IL-1ra, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-3, MDC (CCL22), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGFα, TNF-α, TNF-β, VEGF, sCD40L, Eotaxin-2, MCP-2, BCA-1, MCP-4, I-309, IL-16, TARC, 6CKine, Eotaxin-3, LIF, TPO, SCF, TSLP, IL-33, IL-20, IL-21, IL-23, TRAIL, CTACK, SDF-1a+B, ENA-78, MIP-1d, IL-28A
Human TGFb 3-plex	TGFB1-3	TGF-beta 1, 2, and 3
Human Soluble Cytokine Receptor Array 14-plex	HDSCR14	sCD30, sEGFR, sgp130, sIL-1RI, sIL-1RII, sIL-2Ra, sIL-4R, sIL-6R, sRAGE, sTNF RI, sTNF RII, sVEGF R1, sVEGF R2, sVEGF R3
Human Supplemental Biomarker Array 10-plex	HDHSB10	BDNF, Cathepsin D, MPO, NCAM, PAI-1 (total), PDGF-AA, PDGF-AB/BB, RANTES, sICAM-1, sVCAM-1, Adipsin,
Human Cardiovascular Disease Panel 3 5-Plex	Costum	CRP, Fibrinogen, Haptoglobin, PF4, VWF
Human Kidney Injury Panel 4	Custom	Kim-1
Human Kidney Injury Panel 5	Custom	α-1-Microglobulin, Collagen IV, Lipocalin-2/NGAL, Osteoactivin, TIMP-1, Uromodulin
Human Kidney Injury Panel 6	Custom	β-2-Microglobulin, Cystatin C

Table S4: Flow Cytometry Panels

Panel	Subset	Markers
B Cells	Total	CD45+ CD19+
	Regulatory	CD45+ CD19+ CD38+ CD24+
	Unswitched Memory	CD45+ CD19+ IgD+ IgM+ CD27+ CD38-
	Transitional B Cells	CD45+ CD19+ IgD+ IgM+ CD27- CD38+ CD24+
	Plasmablasts	CD45+ CD19+ IgD- IgM- CD27+ CD38+
	Switched Memory	CD45+ CD19+ IgD- IgM- CD27+ CD38-
	Marginal Zone B	CD45+ CD19+ IgD+ CD27+
	Naïve B Cells	CD45+ CD19+ IgD+ CD27-
Dendritic Cells	Total mDCs	CD45+ Lin- CD11c+
	mDC1	CD45+ Lin- CD11c+ CD1c+ CD16-
	mDC2	CD45+ Lin- CD11c+ CLEC9A+ CD16-
	CD16+ mDCs	CD45+ Lin- CD11c+ CLEC9A- CD16+
	pDCs	CD45+ Lin- CD11c-CD123+
Monocytes	Total	3 monocyte subsets summed
	Classical	CD45+ HLA-DR+ CD14+ CD16-
	Intermediate	CD45+ HLA-DR+ CD14+ CD16+
	Non-classical	CD45+ HLA-DR+ CD14- CD16+
Natural Killer Cells	NK T Cells	CD45+ CD56+ CD3low
	NK T Cells CD3 Bright	CD45+ CD56+ CD3bright
	NK Cells	CD45+ CD56+ CD3-
	NK CD56bright CD16low	CD45+ CD56bright CD16low
	NK CD56bright CD16int	CD45+ CD56bright CD16int
	NK CD56dim CD16bright	CD45+ CD56dim CD16bright
	NK CD56dim CD8-	CD45+ CD56dim CD8-
	NK CD56dim CD8int	CD45+ CD56dim CD8int
	NK CD56dim CD8+	CD45+ CD56dim CD8+
	NK CD56bright CD8-	CD45+ CD56bright CD8-
	NK CD56bright CD8+	CD45+ CD56bright CD8+
Treg / Th17 Cells	Treg CD45+ CD4+ CD25- FoxP3+	CD45+ CD4+ CD25- FoxP3+
	Treg CD45+ CD4+ CD25+ FoxP3low	CD45+ CD4+ CD25+ FoxP3low
	Treg CD45+ CD4+ CD25+ FoxP3high	CD45+ CD4+ CD25+ FoxP3high
	CD45+ CD4+ CD25+	CD45+ CD4+ CD25+
	CD45+ CD4+ CD39+	CD45+ CD4+ CD39+
	CD45+ CD4+ CD127low	CD45+ CD4+ CD127low
	Treg CD45+ CD4+ CD127low FoxP3+	CD45+ CD4+ CD127low FoxP3+ CD25+
	Th17	CD45+ CD4+ ROTgt+
T Cells	CD4+ T Cells	CD45+ CD3+ CD4+ CD8-
	CD8+ T Cells	CD45+ CD3+ CD4- CD8+
	CD4+ Lag3+	CD45+ CD3+ CD4+ CD8- Lag3+
	CD4+ Lag3-	CD45+ CD3+ CD4+ CD8- Lag3-
	CD8+ Lag3+	CD45+ CD3+ CD4- CD8+ Lag3+
	CD8+ Lag3-	CD45+ CD3+ CD4- CD8+ Lag3-
	CD4+ Central Memory	CD45+ CD3+ CD4+ CD8- CD45RA+ CCR7-
	CD4+ Naïve	CD45+ CD3+ CD4+ CD8- CD45RA+ CCR7+
	CD4+ Effector Memory	CD45+ CD3+ CD4+ CD8- CD45RA- CCR7-
	CD4+ Effector	CD45+ CD3+ CD4+ CD8- CD45RA+ CCR7-
	CD4+ CD69+	CD45+ CD3+ CD4+ CD8- CD69+
	CD4+ Naïve CD69+	CD45+ CD3+ CD4+ CD8- CD45RA+ CD69+
	CD4+ CD38+	CD45+ CD3+ CD4+ CD8- CD38+
	CD4+ Naïve CD38+	CD45+ CD3+ CD4+ CD8- CD45RA+ CD38+
	CD4+ HLA-DR+	CD45+ CD3+ CD4+ CD8- HLA-DR+
	CD4+ Naïve HLA-DR+	CD45+ CD3+ CD4+ CD8- CD45RA+ HLA-DR+
	CD8+ Central Memory	CD45+ CD3+ CD4- CD8+ CD45RA+ CCR7-
	CD8+ Naïve	CD45+ CD3+ CD4- CD8+ CD45RA+ CCR7+
	CD8+ Effector Memory	CD45+ CD3+ CD4- CD8+ CD45RA- CCR7-
	CD8+ Effector	CD45+ CD3+ CD4- CD8+ CD45RA+ CCR7-
	CD8+ CD69+	CD45+ CD3+ CD4- CD8+ CD69+
	CD8+ Naïve CD69+	CD45+ CD3+ CD4- CD8+ CD45RA+ CD69+
	CD8+ CD38+	CD45+ CD3+ CD4- CD8+ CD38+
	CD8+ Naïve CD38+	CD45+ CD3+ CD4- CD8+ CD45RA+ CD38+
	CD8+ HLA-DR+	CD45+ CD3+ CD4- CD8+ HLA-DR+
	CD8+ Naïve HLA-DR+	CD45+ CD3+ CD4- CD8+ CD45RA+ HLA-DR+
	CD4+ Ratio of Lag3+/Lag3-	Manually calculate value
	CD8+ Ratio of Lag3+/Lag3-	Manually calculate value
	CD4+ Ratio of Naïve/Effector	Manually calculate value
	CD4+ Ratio of Central Memory / Effector Memory	Manually calculate value
	CD4+ Ratio of Naïve / Central Memory	Manually calculate value
	CD4+ Ratio of Effector / Effector Memory	Manually calculate value
	CD8+ Ratio of Naïve/Effector	Manually calculate value
	CD8+ Ratio of Central Memory / Effector Memory	Manually calculate value
	CD8+ Ratio of Naïve / Central Memory	Manually calculate value
	CD8+ Ratio of Effector / Effector Memory	Manually calculate value

Materials and Methods:

Analysis of the exploratory endpoints was undertaken at Cytel Inc (Waltham, MA). Biomarker venous sampling (e.g. from the patient) was performed during screen A, screen B, pre-dose baseline and days 1, 3, 7, 14, 21, and 28 visits. A ratio of concentrations from post treatment start at each timepoint to baseline, (value/baseline) was generated. Ratios were then log-transformed to approach a more normal distribution. Cmax was defined as the maximum ratio of value/baseline observed over the sampling duration.

The area under the curve (AUC) was generated using the linear trapezoid rule between baseline (Day -1) and each day after the end of treatment, starting with Day 1 (one day after the end of treatment). AUC designations were marked with the number of days after the end of treatment to which the AUC corresponds (i.e., AUC3 was the designation of the AUC from baseline to three days after the end of treatment). Time-weighted averages were then generated by dividing each AUC by the interval of time represented (2 days for AUC1, 4 days for AUC3, etc). For TWAs, the standard effect sizes were the mean difference between IP-treated and Sham treated TWAs divided by the pooled standard deviation.

Standardization of Biomarker Effects:

Because of differences in scale, Z scores from discrete biomarkers were used to standardize data from the desired biomarkers prior to pooling the data into single aggregate endpoints. Z-scores were obtained for each discrete parameter and timepoint endpoint as follows:

Z score = x – mean (across all subjects) / sd across all subjects), eq.1

where x was the observed value, mean was the mean of observed values across all subjects, and sd was the standard deviation of observed values across all subjects.

Next, effect sizes were computed as:

Effect Size = meanTRT -meanCNT / pooled sd, eq. 2

where meanTRT and meanCNT are the mean values for the treated group and the control groups, respectively; and where sd was the pooled standard deviation. The treatment effect directions for each biomarker were assigned such that a positive effect size indicated a positive change was observed and a negative effect size indicated a negative change.

Subsequently, the Z scores are multiplied by -1 or +1 according to the biological effect direction. Thus, if a negative effect is believed to indicate efficacy, negative effects will be converted into positive Z-scores and positive effects into negative Z-scores. Because of this standardization, biomarkers with anticipated changes in opposing directions may have been pooled appropriately, where increasing positive Z-scores indicate increases in desired biological activity and negative Z-scores indicate less desired biological activity.

Identification of Contending Biomarkers:

Absolute values of effect sizes were generated for each biomarker. A biomarker was considered a contender if the absolute value for the effect size was greater than or equal to one for both ratios of log-transformed values over baseline and TWA.

Composite Generation:

For each subject, a Composite Z score was computed. Composite Z scores were the average Z score across markers within a composite group (defined in section 2.3 above), for each subject and timepoint. Groupings were created based on both the magnitude of effect size (as shown in the first method) as well as the scientific plausibility of each combination. Although subjective, this approach was hoped to circumvent the likelihood of erroneous groupings due to small sample sizes, as well as to exclude the possibility of focusing on biomarkers unlikely to translate into clinical relevance.

Once composite scores were generated for each subject, composite endpoints were generated for each treatment group by averaging the individual composite Z-scores in each group. Finally, composite endpoints were used to generate composite effect sizes, calculated as the difference between treated and control composite endpoints divided by the pooled standard deviation.