Peripheral blood CD3+HLADR+ cells and associated gut microbiome species predict response and overall survival to immune checkpoint blockade

Joao Gorgulho[1,2], Christoph Roderburg[3,4], Fabian Beier[4,5], Carsten Bokemeyer[1], Tim H. Brümmendorf[4,5], Tom Luedde[3,4,#], Sven H. Loosen[3,4,#]

[1] Department of Oncology, Hematology and Bone Marrow Transplantation with Section of Pneumology, University Medical Centre Hamburg-Eppendorf, Martinistraße 52, 20251 Hamburg, Germany.

[2] Mildred Scheel Cancer Career Center, University Cancer Center Hamburg, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

[3] Department of Gastroenterology, Hepatology and Infectious Diseases, University Hospital Düsseldorf, Medical Faculty of Heinrich Heine University Düsseldorf, 40225 Düsseldorf, Germany

[4] Center for Integrated Oncology Aachen-Bonn-Cologne-Düsseldorf (CIO^{ABCD}), Germany

[5] Department of Medicine IV, University Hospital RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany

[#] These authors share last authorship

Table of Contents

Supplementary Table 1: Laboratory markers, peripheral T cell subsets and gut microbiota

Parameter	**ICI patients** median [range]
Frequency CD3+ cells [%]	70.60 [12.50-93.30]
Absolute count CD3+ cells [cells/µl]	877.0 [185.0-2745.0]
Frequency CD3+HLADR+ cells [%]	9.10 [1.40-46.90]
Absolute count CD3+HLADR+ cells [cells/µl]	67.5 [15.0-618.0]
Frequency CD3+HLADR+ cells early time-point [%]	11.40 [2.20-40.40]
Absolute count CD3+HLADR+ cells early time-point [cells/µl]	84.0 [12.0-494.0]
Frequency CD3+HLADR+ cells late time-point [%]	10.65 [3.1-50.3]
Absolute count CD3+HLADR+ cells late time-point [cells/µl]	73.5 [12.0-519.0]
Frequency CD3+CD4+ cells [%]	40.15 [9.30-70.30]
Absolute count CD3+CD4+ cells [cells/µl]	345.0 [53.0-1090.0]
Frequency CD3+CD8+ cells [%]	22.45 [3.60-53.70]
Absolute count CD3+CD8+ cells [cells/µl]	199.5 [19.0-1375.0]
Haemoglobin [g/l]	12.20 [5.30-17.60]
Platelets [cells/nl]	245.0 [114.0-693.0]
Leucocyte count [cells/nl]	6.9 [3.1-29.1]
Neutrophil count [cells/µl]	4567.5 [1693.0-26015.0]
Lymphocyte count [cells/µl]	1216.8 [311.0-6409.0]
NLR	4.37 [0.40-40.64]
NLR early time-point	3.98 [0.31-51.17]
NLR late time-point	3.60 [1.12-39.29]
Sodium [mmol/l]	138.0 [124.0-143.0]
Potassium [mmol/l]	4.50 [3.30-6.40]
Bilirubin [mg/dl]	0.37 [0.14-3.7]
AST [U/l]	27.0 [5.0-187.0]
ALT [U/l]	19.0 [7.0-179.0]
ALP [U/l]	94.0 [35.0-1439.0]
GGT [U/l]	49.0 [9.0-1591.0]
LDH [U/l]	228.0 [8.2-1273.0]
Creatinine [mg/dl]	0.85 [0.37-3.09]
Relative abundance Order Burkholderiales [%]	0.561 [0.054-3.231]
Relative abundance Order Burkholderiales late time-point [%]	0.759 [0.001-3.728]
Relative abundance Family Sutterellaceae [%]	0.351 [0.008-3.231]
Relative abundance Genus Sutterella [%]	0.254 [0.008-3.231]
Relative abundance Genus Bacteroides [%]	13.350 [0.305-47.747]
Relative abundance Genus Bacteroides late time-point [%]	13.477 [0.009-40.696]
Relative abundance species Bacteroides vulgatus (OTU3) [%]	5.499 [0.013-24.428]
Relative abundance species Bacteroides vulgatus (OTU3) late time-point [%]	4.630 [0.001-23.166]

NLR:neutrophil-lymphocyte ratio, AST: aspartate transaminase, ALT: alanine transaminase, GGT: γ-Glutamyl transpeptidase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase

Supplementary Figure 1.

Pretreatment freq. of CD3+HLA-DR+ cells do not significantly differ between different tumor entities (A), sex (B), UICC stage (C), smoking status (D), whether patients and been exposed to previous systemic cancer therapy (E). Pretreatment freq. of activated T-cells are significantly different according to ECOG PS status (F). Concerning chosen ICI drug, CD3+HLADR+ frequencies are not significantly different between patients at a pretreatment time-point when comparing dual to single immune checkpoint blockade (G) but when comparing early time-point frequencies, a significant difference can be observed with patients under Nivolumab/Ipilimumab therapy having higher levels than patients submitted to monotherapy (H).

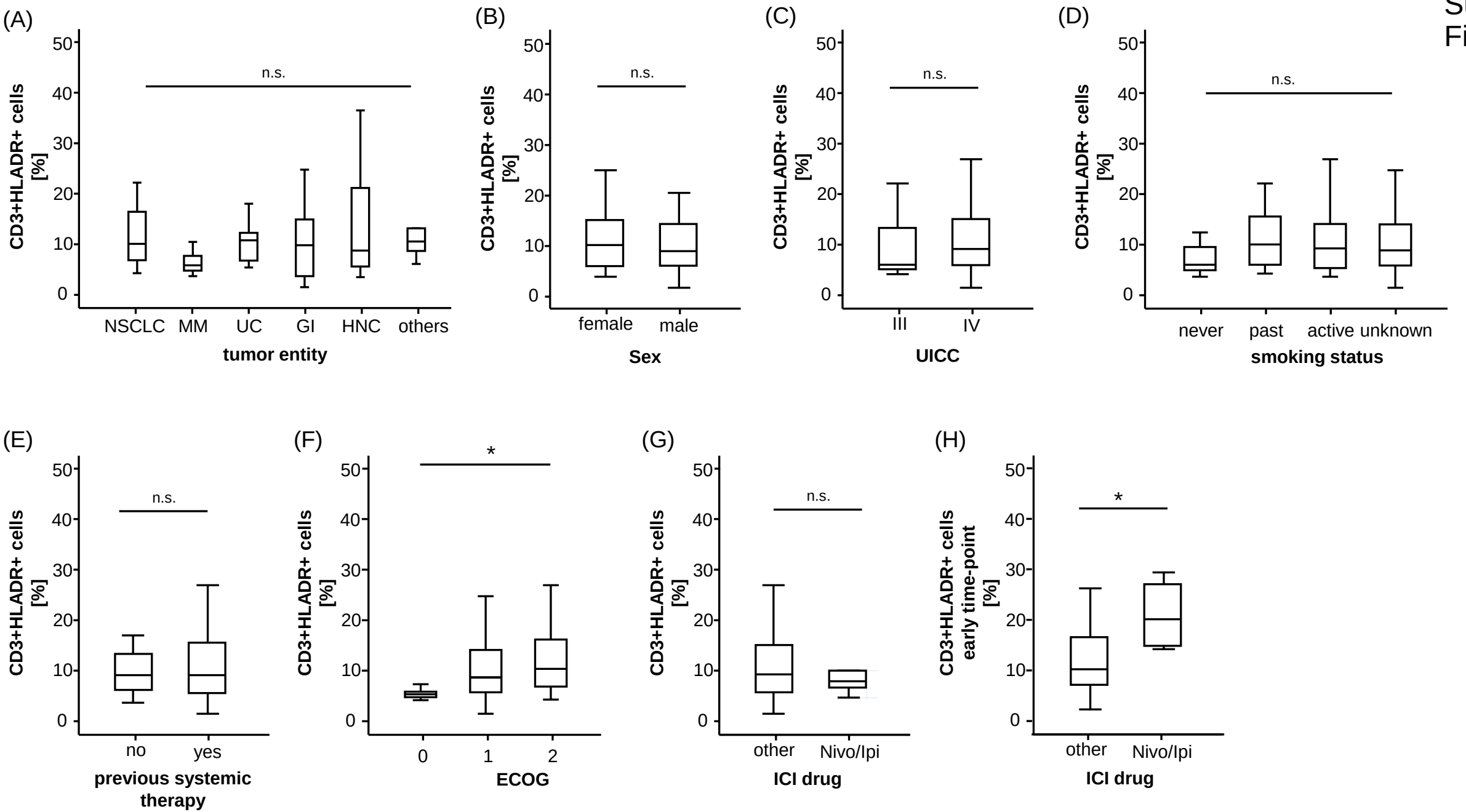
Supplementary Figure 2.

Freq. of CD3+HLA-DR+ cells at baseline positively correlate with frequency of CD3+CD8+ (CTLs) cells (A) and ECOG status (C) and negatively correlate with the frequency of CD3+CD4+ cells (B), as well as different gut microbiome taxa (D-G).

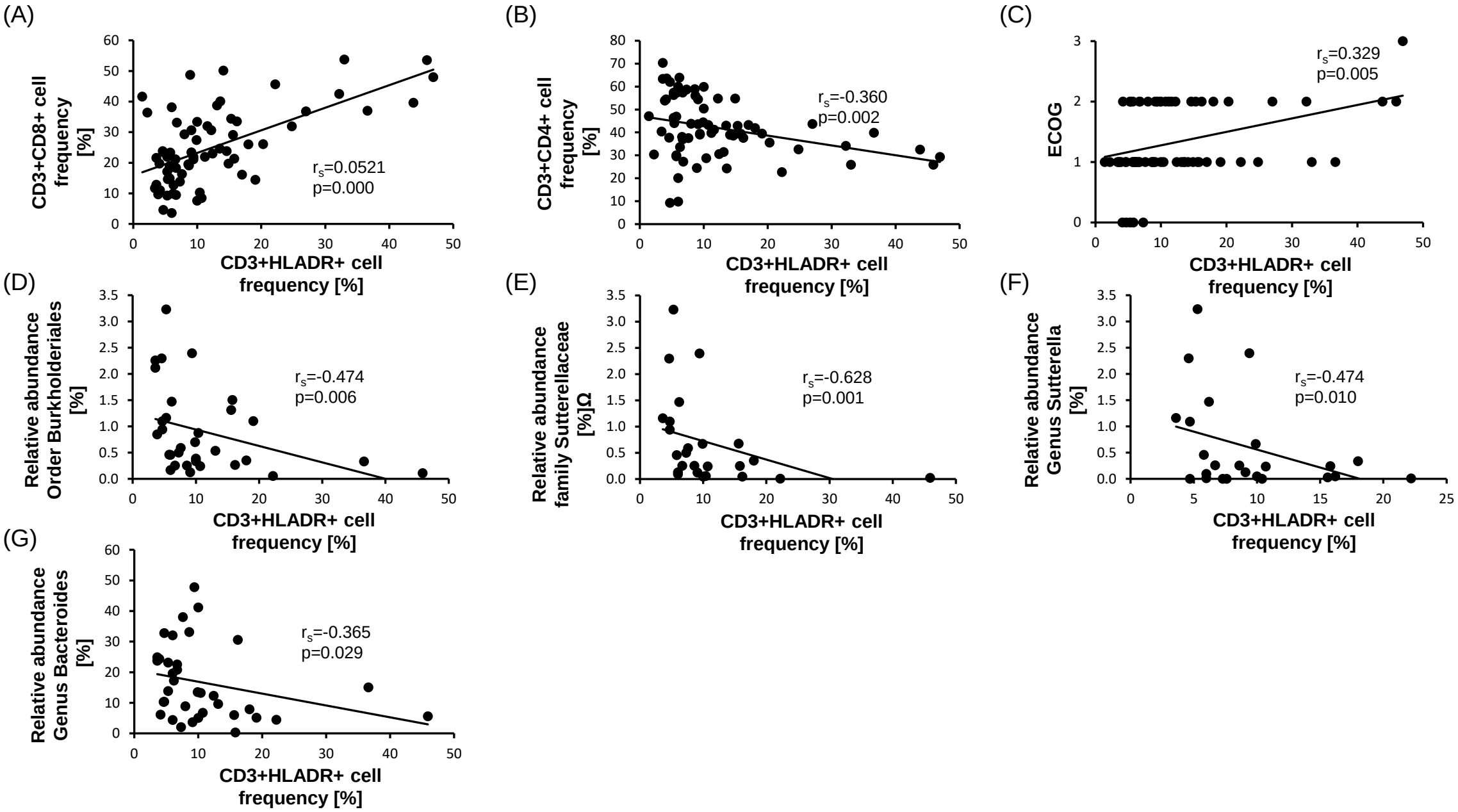
Supplementary Figure 3.

(A-B) Patients with a relative abundance of bacteria from the family Sutterellaceae and Genus Sutterella above an ideal cut-off have significantly improved OS compared to patients below this value, whereas concerning the Genus Bacteroides only a non-significant trend in the same direction can be depicted (C). Contrastingly, patients with a late-time point relative abundance of Bacteroides vulgatus above an ideal cut-off show a tendency towards impaired OS. (D) Patients with decreasing relative abundance of B. vulgatus during therapy show a tendency towards better OS.

Supp. Figure 1



Supp. Figure 2



Supp. Figure 3