

Supplemental Tables for:

A Randomized Phase II Trial of mFOLFOX6 + Bevacizumab Alone or with AdCEA Vaccine + Avelumab Immunotherapy for Untreated Metastatic Colorectal Cancer

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Supplemental Table 1. 138 peripheral immune cell subsets analyzed by flow cytometry. Ten parental phenotypes are identified as well as refined subsets of each relating to maturation and function. Expected function based on expression of specific markers within each subset is indicated in *italics*.

1. Total CD4⁺ T cells

- PD-L1⁺ CD4 - *activation/inhibition*
- PD-1⁺ CD4 - *activation/inhibition*
- CTLA-4⁺ CD4 - *inhibition*
- Tim-3⁺ CD4 - *inhibition*
- 41bb⁺ CD4 - *co-stimulation*
- Ki67⁺ CD4 - *proliferation*
- ICOS⁺ CD4 - *activation*
 - ICOS⁺ PD-L1⁺ CD4 - *activation/inhibition*
 - ICOS⁺ PD-1⁺ CD4 - *activation/inhibition*
- Total naïve (CCR7⁺CD45RA⁻) CD4
 - PD-L1⁺ naïve CD4 - *activation/inhibition*
 - PD-1⁺ naïve CD4 - *activation/inhibition*
 - CTLA-4⁺ naïve CD4 - *inhibition*
 - Tim-3⁺ naïve CD4 - *inhibition*
- Total central memory (CCR7⁺CD45RA⁻) CD4
 - PD-L1⁺ CM CD4 - *activation/inhibition*
 - PD-1⁺ CM CD4 - *activation/inhibition*
 - CTLA-4⁺ CM CD4 - *inhibition*
 - Tim-3⁺ CM CD4 - *inhibition*
 - Ki67⁺ CM CD4 - *proliferation*
- Total effector memory (CCR7⁻CD45RA⁺) CD4
 - PD-L1⁺ EM CD4 - *activation/inhibition*
 - PD-1⁺ EM CD4 - *activation/inhibition*
 - CTLA-4⁺ EM CD4 - *inhibition*
 - Tim-3⁺ EM CD4 - *inhibition*
 - Ki67⁺ EM CD4 - *proliferation*
- Total EMRA (CCR7⁻CD45RA⁺) CD4
 - PD-L1⁺ EMRA CD4 - *activation/inhibition*
 - PD-1⁺ EMRA CD4 - *activation/inhibition*
 - CTLA-4⁺ EMRA CD4 - *inhibition*
 - Tim-3⁺ EMRA CD4 - *inhibition*
 - Ki67⁺ EMRA CD4 - *proliferation*

2. Total CD8⁺ T cells

- PD-L1⁺ CD8 - *activation/inhibition*
- PD-1⁺ CD8 - *activation/inhibition*
- CTLA-4⁺ CD8 - *inhibition*
- Tim-3⁺ CD8 - *inhibition*
- 41bb⁺ CD8 - *co-stimulation*
- Ki67⁺ CD8 - *proliferation*
- Total naïve (CCR7⁺CD45RA⁻) CD8
 - PD-L1⁺ naïve CD8 - *activation/inhibition*
 - PD-1⁺ naïve CD8 - *activation/inhibition*
 - CTLA-4⁺ naïve CD8 - *inhibition*
 - Tim-3⁺ naïve CD8 - *inhibition*
- Total central memory (CCR7⁺CD45RA⁻) CD8
 - PD-L1⁺ CM CD8 - *activation/inhibition*
 - PD-1⁺ CM CD8 - *activation/inhibition*
 - CTLA-4⁺ CM CD8 - *inhibition*

- Tim-3⁺ CM CD8 - *inhibition*
- Ki67⁺ CM CD8 - *proliferation*
- Total effector memory (CCR7⁻CD45RA⁺) CD8
 - PD-L1⁺ EM CD8 - *activation/inhibition*
 - PD-1⁺ EM CD8 - *activation/inhibition*
 - CTLA-4⁺ EM CD8 - *inhibition*
 - Tim-3⁺ EM CD8 - *inhibition*
 - Ki67⁺ EM CD8 - *proliferation*
- Total EMRA (CCR7⁻CD45RA⁺) CD8
 - PD-L1⁺ EMRA CD8 - *activation/inhibition*
 - PD-1⁺ EMRA CD8 - *activation/inhibition*
 - CTLA-4⁺ EMRA CD8 - *inhibition*
 - Tim-3⁺ EMRA CD8 - *inhibition*
 - Ki67⁺ EMRA CD8 - *proliferation*

3. Total Tregs

- PD-L1⁺ Tregs - *activation/inhibition*
- PD-1⁺ Tregs - *suppression*
- CTLA-4⁺ Tregs - *suppression*
- ICOS⁺ Tregs - *suppression*
- CD45RA⁺ Tregs - *highly expandable in vitro*
- CD49d⁺ Tregs - *suppression*
- Ki67⁺ Tregs - *proliferation*
- CD38⁺ Tregs - *suppression*
- HLA-DR⁺ Tregs - *suppression*

4. Total B cells

- PD-L1⁺ B cells - *activation/inhibition*
- PD-1⁺ B cells - *activation/inhibition*

5. Total NK cells

- PD-L1⁺ NK - *inhibition*
- PD-1⁺ NK - *activation/inhibition*
- Tim-3⁺ NK - *activation/inhibition*
- Ki67⁺ NK - *proliferation*
- NKp30⁺ NK - *activation*
- NKp46⁺ NK - *activation*
- NKG2D⁺ NK - *activation*
- CD226⁺ NK - *adhesion/activation*
- Total mature (CD16⁺ CD56^{dim}) NK - *lytic*
 - PD-L1⁺ mature NK - *inhibition*
 - PD-1⁺ mature NK - *activation/inhibition*
 - Tim-3⁺ mature NK - *activation/inhibition*
- Total functional intermediate (CD16⁺ CD56^{br}) NK - *lytic, cytokine production*
 - PD-L1⁺ functional intermediate NK - *inhibition*
 - PD-1⁺ functional intermediate NK - *activation/inhibition*
 - Tim-3⁺ functional intermediate NK - *activation/inhibition*
- Total immature (CD16⁻ CD56^{br}) NK - *cytokine production*
 - PD-L1⁺ immature NK - *inhibition*

- PD-1⁺ immature NK - *activation/inhibition*
- Tim-3⁺ immature NK - *activation/inhibition*
- Total unconventional (CD16⁻ CD56^{dim}) NK - *non-lytic, non-cytokine production*
 - PD-L1⁺ unconventional NK - *inhibition*
 - PD-1⁺ unconventional NK - *activation/inhibition*
 - Tim-3⁺ unconventional NK - *activation/inhibition*

6. Total NK-T cells

- PD-L1⁺ NK-T - *inhibition*
- PD-1⁺ NK-T - *activation/inhibition*
- Tim-3⁺ NK-T - *inhibition*
- Ki67⁺ NK-T - *proliferation*

7. Total cDC

- PD-L1⁺ cDC - *inhibition*
- PD-1⁺ cDC - *activation/inhibition*
- Tim-3⁺ cDC - *inhibition*
- Ki67⁺ cDC - *proliferation*

8. Total pDC

- PD-L1⁺ pDC - *inhibition*
- PD-1⁺ pDC - *activation/inhibition*
- Tim-3⁺ pDC - *inhibition*
- Ki67⁺ pDC - *proliferation*

9. Total MDSC

- PD-L1⁺ MDSC - *inhibition*
- PD-1⁺ MDSC - *activation/inhibition*
- CD16⁺ MDSC - *immature/suppression*
- Total monocytic (CD14⁺ CD15⁻) MDSC
 - PD-L1⁺ mMDSC - *inhibition*
 - PD-1⁺ mMDSC - *activation/inhibition*
 - CD16⁺ mMDSC - *immature/suppression*
- Total granulocytic (CD14⁺ CD15⁺) MDSC
 - PD-L1⁺ gMDSC - *inhibition*
 - PD-1⁺ gMDSC - *activation/inhibition*
 - CD16⁺ gMDSC - *immature/suppression*
- Total lineage negative (CD14⁻ CD15⁻) MDSC
 - PD-L1⁺ lin neg MDSC - *inhibition*
 - PD-1⁺ lin neg MDSC - *activation/inhibition*
 - CD16⁺ lin neg MDSC - *immature/suppression*

10. Total Monocytes

- Classical Monocytes - *phagocytic*
- Intermediate Monocytes - *phagocytic/proinflammatory*
- Non-Classical Monocytes - *proinflammatory*
- PD-L1⁺ Monocytes - *inhibition*
- PD-1⁺ Monocytes - *activation/inhibition*

cDC, conventional dendritic cells; CM, central memory; CTLA-4, cytotoxic T lymphocyte-associated protein-4; EM, effector memory; EMRA, terminally differentiated effector memory; FoxP3, forkhead box P3; gMDSCs, granulocytic myeloid derived suppressor cells; ICOS, inducible T cell co-stimulator; lin neg MDSCs, lineage negative MDSCs; mMDSCs, monocytic MDSCs; NK, natural killer; pDC, plasmacytoid DC; PD-1, programmed cell death-1; PD-L1, programmed cell death ligand-1; Tim-3, T cell immunoglobulin and mucin domain-3; Tregs, regulatory T cells.

Supplemental Table 2. Effect of treatment on classic and refined PBMC subsets

A. SOC

Classic Subset	Direction of Change	Pre (median)	D29 (median)	P value	# Increasing >25%	# Decreasing >25%
CD4	=	34.24	36.99	0.95	3/8	1/8
CD8	=	11.34	12.74	0.95	1/8	1/8
Treg	=	0.98	0.65	0.55	3/8	3/8
NK	=	6.18	8.11	0.08	4/8	1/8
NK-T	=	0.92	1.29	0.08	2/8	1/8
B cells	=	10.92	9.48	1.00	3/8	2/8
cDC	=	0.29	0.32	0.38	4/8	2/8
pDC	=	0.16	0.22	0.31	4/8	2/8
MDSC	=	7.76	8.05	0.64	2/8	4/8
Monocytes	=	14.43	16.4	0.15	1/8	4/8
Refined Subsets	Direction of Change	Pre (median)	D29 (median)	P value	# Increasing >25%	# Decreasing >25%
CD4 ki67	↑	0.59	0.97	0.02	6/8	0/8
CD4 CM ki67	↑	0.17	0.31	0.04	5/8	0/8
NK Ki67	↑	0.30	0.61	0.04	7/8	1/8
NK NKp30	↑	0.70	1.32	0.01	8/8	0/8
NK Mature NKp30	↑	0.60	1.11	0.01	8/8	0/8

B. SOC + IO

Classic Subset	Direction of Change	Pre (median)	D29 (median)	P value	# Increasing >25%	# Decreasing >25%
CD4	=	32.57	27.71	0.03*	0/11	1/11
CD8	=	13.31	14.17	0.04*	4/11	0/11
Treg	=	0.62	0.48	0.05	2/11	4/11
NK	=	9.87	9.65	0.64	3/11	1/11
NK-T	=	2.94	4.67	0.05	6/11	1/11
B cells	=	7.37	6.31	0.03*	1/11	5/11
cDC	=	0.48	0.46	0.70	5/11	3/11
pDC	=	0.1	0.21	0.07	8/11	1/11
MDSC	=	7.21	8.64	0.47	5/11	3/11
Monocytes	=	16.49	15.09	0.58	4/11	3/11
Refined Subsets	Direction of Change	Pre (median)	D29 (median)	P value	# Increasing >25%	# Decreasing >25%
CD8 ki67	↑	0.28	0.76	0.003	9/11	0/11
CD8 EM ki67	↑	0.15	0.50	0.003	9/11	0/11
NK ki67	↑	0.46	0.81	0.02	8/11	1/11
Int Monocyte	↑	0.48	0.84	0.01	9/11	0/11

Median frequency of PBMC subsets before and after 1 month of treatment with SOC (A) or SOC + IO (B). Subsets with a potentially biologically relevant change (bold type) were defined as those with $p < 0.05$, most patients having a $> 25\%$ change, and difference in medians $> 0.05\%$ of PBMCs.

*Although $p < 0.05$, most patients did not change by $> 25\%$.

Abbreviations: Int, intermediate; IO, immuno-oncology; SOC, standard of care.

Supplemental Table 3. Effect of treatment on serum cytokines/soluble factors and complete blood count parameters

A. SOC

Serum Factor	Direction of Change	Pre (median)	D29 (median)	P value	# Increasing >25%	# Decreasing >25%
sCD27	=	80.00	93.00	0.23	1/7	0/7
sCD40L	↓	18.00	10.00	0.03	0/7	6/7
Ratio sCD27:sCD40L	↑	5.000	8.000	0.03	6/7	0/7
sPD-L1	=	78.80	79.00	0.94	0/7	0/7
IL-8	=	26.30	20.90	0.69	3/7	3/7
TGFβ	↓	27779	17646	0.02	0/7	6/7
IFNγ	=	0.000	0.000	1.00	0/7	1/7
CBC Measure	Direction of Change	Pre (median)	D29 (median)	P value	# Increasing >25%	# Decreasing >25%
ALC	=	1.440	1.170	0.03*	0/7	2/7
NLC	↓	4.850	2.130	0.02	0/7	7/7
NLR	↓	3.292	1.821	0.02	0/7	6/7
Platelet	↓	327.0	185.0	0.02	0/7	7/7

B. SOC + IO

Serum Factor	Direction of Change	Pre (median)	D29 (median)	P value	# Increasing >25%	# Decreasing >25%
sCD27	=	104.5	129.0	0.25	4/10	1/10
sCD40L	↓	23.50	16.50	0.02	0/10	6/10
Ratio sCD27:sCD40L	↑	5.000	8.000	0.03	7/10	0/10
IL-8	=	29.65	18.10	0.32	3/10	5/10
TGFβ	↓	32893	21308	0.002	0/10	6/10
IFNγ	=	0.000	0.000	0.50	0/10	2/10
CBC Measure	Direction of Change	Pre (median)	D29 (median)	P value	# Increasing >25%	# Decreasing >25%
ALC	=	1.535	1.635	0.81	1/8	2/8
NLC	=	4.960	2.745	0.06	1/8	5/8
NLR	=	2.746	2.015	0.46	2/8	4/8
Platelet	=	323.0	223.5	0.004*	0/10	4/10

Median frequency of measure before and after 1 month of treatment with SOC (A) or SOC + IO (B). Factors with a potentially biologically relevant change (bold type) were defined as those with $p < 0.05$, and most patients having a $> 25\%$ change.

*Although $p < 0.05$, most patients did not change by $> 25\%$. sPD-L1, TGF-β, IFN-γ, and IL-8 were measured in pg/mL, sCD27 in U/mL, and sCD40L in ng/mL.

Abbreviations: IO, immuno-oncology; SOC, standard of care; SOC + IO, AdCEA vaccine plus avelumab.

Supplemental Table 4. Frequency of patients with anti-Ad5 neutralizing antibodies pre- and post-SOC + IO treatment

Anti-Ad-5 Neutralizing Antibody Titer	Pre	~D29	~D56	~D111	~D167
Positive at $\geq 1:100$ serum dilution	0/10 (0%)	4/9 (44%)	6/10 (60%)	5/6 (83%)	5/5 (100%)
Positive at $\geq 1:500$ serum dilution	0/10 (0%)	4/9 (44%)	4/10 (40%)	2/6 (33%)	1/5 (20%)
Positive at $\geq 1:1,000$ serum dilution	0/10 (0%)	2/9 (22%)	2/10 (20%)	1/6 (17%)	1/5 (20%)

Abbreviations: IO, immuno-oncology; SOC, standard of care; SOC + IO, AdCEA vaccine plus avelumab.