

**Fluticasone impact on airway dendritic cells in smokers:  
a randomized controlled trial**

Marek Lommatzsch, Ulrike Kraeft, Laura Troebs, Katharina Garbe, Andrea Bier,  
Paul Stoll, Sebastian Klammt, Michael Kuepper, Kai Bratke, J. Christian Virchow

**SUPPLEMENT**

Antibody	Label	Company	Clone
Lineage Cocktail	FITC	BD Biosciences	SK7, M $\phi$ P9, 3G8, SJ25C1, L27, NCAM16.2
Anti-HLA-DR	PerCP	BD Biosciences	L243
Mouse IgG1	PE	BD Biosciences	X40
Mouse IgG1	APC	BD Biosciences	X40
Mouse IgG2a	PE	BD Biosciences	X39
Mouse IgG2a	APC	BD Biosciences	X39
Anti-CD1a	PE	Dako	NA1/34
Anti-CD11c	PE	BD Biosciences	S-HCL-3
Anti-CD11c	APC	BD Biosciences	S-HCL-3
Anti-CD40	APC	Invitrogen	HB14
Anti-CD80	APC	ImmunoTools	MEM-233
Anti-CD83	APC	BD Biosciences	HB15e
Anti-CD86	APC	BD Biosciences	BU63
Anti-CD123	PE	BD Biosciences	9F5
Anti-CD207/Langerin	PE	Beckman Coulter	DCGM4
Anti-BDCA-1	APC	Miltenyi Biotec	AD5-8E7
Anti-BDCA-3	APC	Miltenyi Biotec	AD5-14H12
Anti-BDCA-4	APC	Miltenyi Biotec	AD5-17F6
Anti-CCR5/CD195	APC	BD Biosciences	2D7

**Table S1. Antibodies used for four-colour flow cytometry**

*Abbreviations denote:* Blood Dendritic Cell Antigen (BDCA), Fluorescein isothiocyanate (FITC), Phycoerythrin (PE), Allophycocyanin (APC), Peridinin chlorophyll protein (PerCP). The Lineage cocktail contains antibodies against the following antigens: CD3, CD14, CD16, CD19, CD20, CD56.

Time Point	Placebo		Fluticasone		Fluticasone / Salmeterol	
	A	B	A	B	A	B
n	14	14	14	14	14	14
IVC	96.8 (42.8 - 124)	98.3 (89.1 - 121.6)	99.7 (79.2 - 121.8)	95.7 (72.9 - 118.5)	91.2 (77.4 - 119.5)	94 (81.3 - 123.4)
FVC	103.3 (88.6 - 121.1)	100.3 (83.3 - 122.3)	97.7 (80.3 - 119.6)	99.3 (79.5 - 114.5)	97.1 (80.9 - 125.9)	96.7 (83.5 - 128.7)
FEV <sub>1</sub>	93.9 (82.3 - 110.3)	92.8 (71.7 - 107.1)	91 (80.2 - 123.6)	87.6 (80.7 - 116.6)	90 (80 - 120.5)	90.9 (81.6 - 123.5)
FEV <sub>1</sub> %VC	74.1 (65 - 81.4)	74.2 (66.2 - 85)	79.9 (66.2 - 89.8)	77.7 (65.9 - 86.8)	76.1 (70.1 - 84.9)	76.5 (71.2 - 86.6)
MEF <sub>25</sub>	47.5 (26.3 - 97.3)	41.9 (15.9 - 92.8)	57.4 (16.1 - 137.9)	46.6 (11.9 - 122.9)	44.2 (29.3 - 102.3)	43.5 (33.1 - 128.1)
RV	113.2 (65.6 - 147.4)	107.5 (83.3 - 163.7)	87.8 (13.5 - 106.9)	95.4 (11.3 - 151.5)	92.5 (62.3 - 136.7)	93.8 (63.1 - 123.8)
TLC	103.9 (84.5 - 130.8)	98.1 (91.7 - 134)	95.4 (55.7 - 114)	99.8 (75.2 - 112.3)	95 (87.8 - 114.6)	97.5 (84.2 - 123.1)
RV%TLC	106.4 (74.9 - 119.9)	100.7 (78.9 - 121.6)	89.9 (58.3 - 116.9)	87.6 (68.7 - 128.3)	90.6 (59.3 - 125)	96 (55.8 - 114.4)

### Table S2 Lung function of the Participants

Shown are lung function parameters (median values, minimum – maximum) of the participants in the 3 study arms (Placebo, Fluticasone, Fluticasone/Salmeterol), at the time points A (prior to treatment) and B (directly after treatment). *Abbreviations denote:* Inspiratory Vital Capacity (IVC), Forced Vital Capacity (FVC), Forced Expiratory Volume in the first second (FEV<sub>1</sub>), Maximum Expiratory Flow when 75 % of the FVC is exhaled (MEF<sub>25</sub>), Residual Volume (RV), Total Lung Capacity (TLC). There were no significant differences between the time points in any parameter in any group.

	Placebo		Fluticasone		Fluticasone / Salmeterol	
Time Point	A	B	A	B	A	B
n	14	14	14	14	14	14
Hemoglobin (mmol / l)	9.1 (8 - 9.8)	9.1 (8.6 - 9.9)	9.5 (8.1 - 10.5)	9.3 (5.4 - 10.5)	9.4 (8.5 - 10.8)	9.3 (8.6 - 10.2)
Platelets (10 <sup>6</sup> / ml)	234 (146 - 338)	238 (157 - 341)	211 (176 - 391)	218 (178 - 312)	204 (142 - 320)	216 (147 - 341)
Leukocytes (10 <sup>6</sup> / ml)	6.7 (5.4 - 9.8)	6.3 (4.7 - 13.9)	7 (3.5 - 9)	6.7 (4.6 - 16.1)	7.2 (4.61 - 13.1)	7.2 (3.74 - 11.5)
Monocytes (%)	8.9 (6 - 10.6)	8.9 (6 - 14)	9.2 (6.4 - 13)	8.3 (6.2 - 13.8)	7.2 (4 - 12.4)	7.7 (5 - 11)
Lymphocytes (%)	32.7 (16.6 - 47.5)	29.1 (22 - 44.1)	32.9 (23.3 - 40.4)	33.3 (25 - 39.7)	24.9 (10.3 - 37.2)	27.6 (15 - 38.6)
Neutrophils (%)	56.6 (39.1 - 72.6)	54.8 (41.9 - 69)	54.7 (47.1 - 65.2)	54.5 (46.3 - 62)	64.0 (6 - 81.1)	61.8 (47.8 - 71)
Eosinophils (%)	2.2 (0.4 - 11)	2.9 (1.1 - 9.9)	3.2 (1.3 - 4.9)	2.8 (1.1 - 6.3)	2.4 (0.4 - 7.4)	2.0 (0.9 - 6.2)
CRP (mg / l)	1.3 (1 - 12.3)	1.6 (1 - 5.3)	2.0 (1 - 9.4)	2.0 (1 - 9.5)	2.0 (1 - 7.58)	1 (1 - 11.9)

**Table S3 Blood parameters of the Participants**

Shown are parameters in peripheral blood (median values, minimum – maximum) of the participants in the 3 study arms (Placebo, Fluticasone, Fluticasone/Salmeterol), at the time points A (prior to treatment) and B (directly after treatment). Monocytes, Lymphocytes, Neutrophils and Eosinophils are given in % of all leukocytes in peripheral blood. *Abbreviation denotes:* C-reactive Protein (CRP). There were no significant differences between the time points in any parameter in any group.

	Placebo		Fluticasone		Fluticasone/Salmeterol	
Time Point	A	B	A	B	A	B
n	14	14	14	14	14	14
<b>mDC</b>						
BDCA-1 [%]	89 (58 - 94)	89 (60 - 94)	88 (59 - 91)	85 (71 - 94)	89 (67 - 94)	88 (46 - 94)
BDCA-3 [%]	73 (38 - 91)	74 (42 - 90)	71 (44 - 92)	68 (43 - 91)	63 (24 - 90)	56 (29 - 78)
BDCA-4 [%]	51 (21 - 89)	46 (29 - 80)	43 (11 - 69)	51 (10 - 79)	47 (16 - 79)	41 (19 - 81)
CD40 [MFI]	1620 (1189 - 3091)	1733 (1238 - 3005)	1676 (865 - 2482)	1887 (1149 - 2430)	1603 (1173 - 2832)	1382 (919 - 3289)
CD80 [%]	73 (44 - 85)	65 (36 - 80)	63 (35 - 76)	63 (42 - 79)	60 (43 - 81)	58 (39 - 84)
CD83 [%]	17 (6 - 29)	14 (6 - 24)	13 (9 - 23)	14 (5 - 32)	13 (8 - 31)	12 (8 - 22)
CD86 [%]	75 (54 - 96)	68 (53 - 92)	61 (39 - 94)	65 (40 - 91)	68 (30 - 88)	59 (37 - 93)
CCR5 [%]	26 (6 - 56)	23 (3 - 48)	13 (0 - 61)	15 (2 - 45)	17 (1 - 44)	17 (0 - 44)
Langerin [%]	67 (33 - 76)	64 (33 - 82)	61 (44 - 75)	62 (41 - 73)	61 (41 - 76)	60 (33 - 75)
CD1a [%]	84 (44 - 87)	80 (42 - 90)	73 (58 - 85)	76 (65 - 83)	74 (47 - 91)	77 (48 - 91)

**Table S4 Surface molecule expression on BAL fluid myeloid DCs (mDCs)**

Shown is the expression of various surface molecules on BAL fluid mDCs (median values, minimum – maximum) of the participants in the 3 study arms (Placebo, Fluticasone, Fluticasone/Salmeterol), at the time points A (prior to treatment) and B (directly after treatment). The expression was calculated as the percentage of marker-positive mDCs in most cases. In case of CD40, the expression was calculated using the Mean Fluorescence Index (MFI). *Abbreviation denotes:* Blood Dendritic Cell Antigen (BDCA). There were no significant differences between the time points in any parameter in any group.