

Altered innate immune cell trafficking by dietary black raspberries in esophageal cancer

Dan Peiffer

Loyola-Stritch School of Medicine

dpeiffer1@luc.edu



Esophageal cancer

- 7th leading cause of death
 - Mostly SCC (90%) in China (50%)
 - Rates: China decreasing, US stable
 - ~1 in 125 men, 1 in 435 women in US
 - Low survival rate
 - ~15% 5 year
- Risk Factors
 - Alcohol and/or tobacco use
 - Eating salted or moldy foods
 - N-nitroso compounds
 - Currently use N-nitrosomethylbenzylamine (NMBA)-induced model

BRB, cancer, inflammation

- BRBs
 - Preventative agents (Kresty LA *et al.* 2001)
 - Vitamins and minerals (A, C, E, Ca, Se)
 - Phenolic compounds (ellagic acid, quercetin, **anthocyanins**)
 - Effects on inflammation
 - Reduce COX2 expression and angiogenesis (Chen T *et al.* 2006)
 - Additionally inhibits iNOS, sEH, HIF-1 α , and others

BRB components and inflammation

- Anthocyanins (AC)
 - Major phenolic compound of BRB
 - Part of plant innate immune system
 - Effective at reducing inflammation
 - Wang *et al.* 2009
- Major metabolite PCA
 - Similar anti-inflammatory activity as BRB and AC
 - Peiffer *et al.* 2014

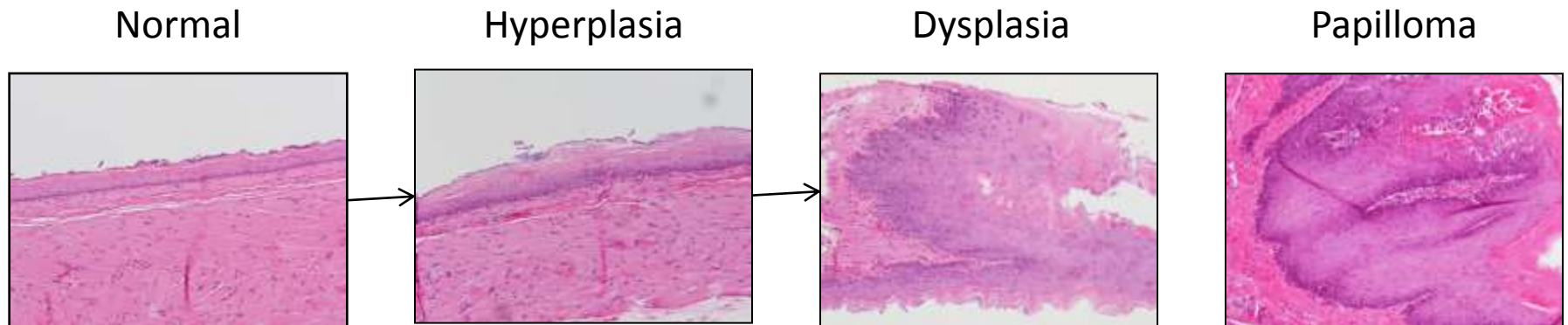


Inflammatory markers and esophageal cancer

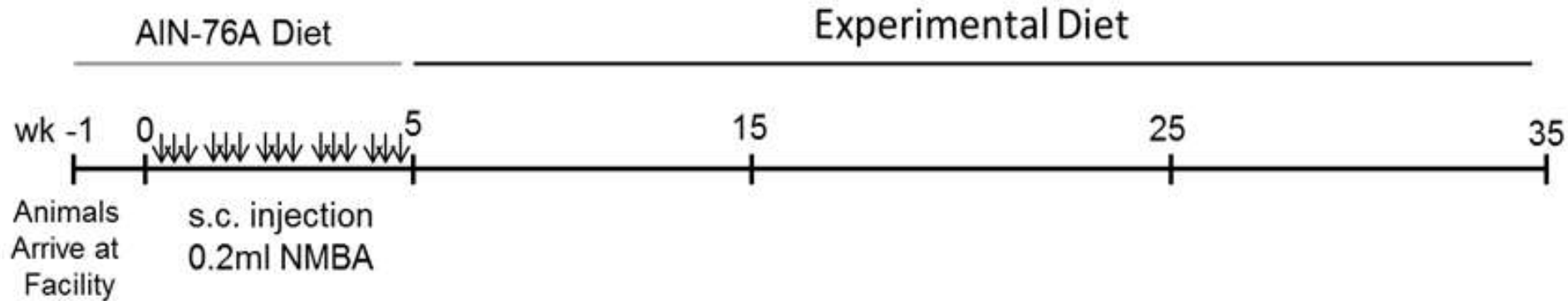
- IL-1 β and IL-6
 - Overexpressed in ESSC
 - Associated with M1 macrophage
 - Poor prognosis
- PTX3
 - Chemokine downregulated in ESCC
 - Inhibits neutrophil migration
- IL-12
 - Low levels considered risk factor for disease development
 - Associated with Th1 (Tumoricidal immune phenotype)

The model

- NMBA (0.35 mg/kg bw) in 20% DMSO solution
 - Metabolized by cytochrome p450
 - Causes DNA adduct formation
 - 100% tumor incidence
 - ~15-25 weeks
- Lesions closely mimic human SCC progression



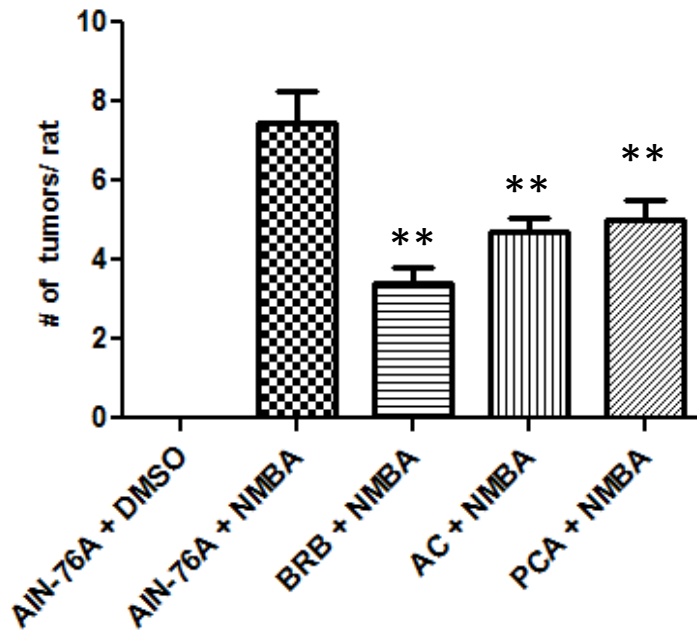
Study setup



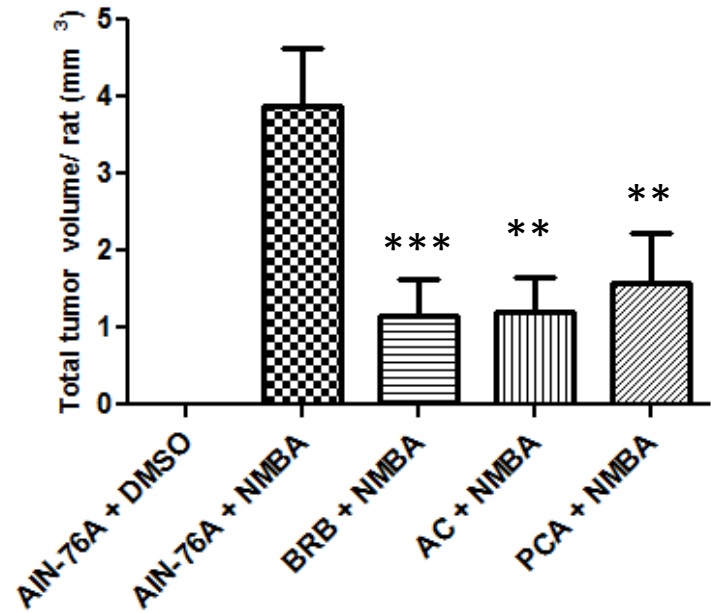
Group	Injection	Treatment	Concentration (mmol/g diet)	No. of rats (wks 15, 25, 35)
1	**Vehicle	-	-	(9, 9, 30)
2	NMBA	-	-	(9, 9, 30)
3	NMBA	6.1% BRBs	3.80	(9, 9, 30)
4	NMBA	Anthocyanin extract	3.80	(9, 9, 30)
5	NMBA	PCA (500 ppm)	3.24	(9, 9, 30)

Initial results...

Wk 25



Wk 35



Bioplex



**23+ cytokines
simultaneously!!!**

Effects on plasma cytokine expression...

Cytokine levels in pg/ml (\pm SE)

Cytokine	Cytokine levels in pg/ml (\pm SE)									
	Wk 15 DMSO	Wk 15 NMBA	Wk 15 BRB+ NMBA	Wk 15 AC + NMBA	Wk 15 PCA + NMBA	Wk 25 DMSO	Wk 25 NMBA	Wk 25 BRB + NMBA	Wk 25 AC + NMBA	Wk 25 PCA + NMBA
IL-1 β	^a 85.6 (3.3)	191.7 (1.8)	^a 112.8 (0.9)	133.1 (0.3)	^a 121.9 (5.3)	^a 90.6 (5.1)	216.5 (3.6)	^a 144.5 (4.5)	^a 129.4 (1.2)	^a 132.4 (3.3)
IL-2	^a 430.4 (9.2)	779.8 (12.4)	^a 314.2 (4.8)	^a 283.1 (6.2)	^a 239.5 (2.0)	485.9 (27.7)	901.5 (9.2)	^a 394.9 (7.6)	^a 382.3 (4.1)	^a 278.9 (4.9)
IL-4	^a 37.5 (2.8)	75.1 (0.4)	^a 46.9 (0.8)	^a 50.1 (0.2)	^a 35.2 (1.1)	^a 42.1 (1.5)	91.2 (1.0)	^a 46.9 (1.8)	^b 60.5 (2.5)	^a 31.6 (0.6)
IL-5	183.1 (2.2)	180.7 (5.0)	^b 682.7 (4.8)	^b 698.2 (3.1)	^b 754.6 (10.5)	181.5 (2.7)	171.5 (1.7)	^b 630.5 (17.1)	^b 605.1 (5.6)	^b 766.2 (6.8)
IL-6	^a 145.3 (3.8)	198.2 (6.6)	212.1 (5.2)	188.1 (8.5)	190.9 (5.0)	^a 179.7 (9.4)	307.2 (4.0)	^a 254.6 (8.6)	^a 232.1 (4.1)	^a 191.0 (2.5)
IL-10	269.0 (1.9)	166.2 (7.7)	182.5 (8.0)	170.4 (5.2)	174.5 (3.1)	147.5 (16.9)	124.3 (4.8)	^b 290.1 (9.0)	^b 298.1 (5.9)	^b 231.1 (3.4)
IL-12(p70)	47.6 (2.7)	49.7 (7.5)	^b 108.5 (0.7)	^b 99.1 (4.9)	^b 133.2 (4.2)	49.6 (6.5)	38.8 (4.1)	^b 98.8 (5.2)	^b 115.3 (6.1)	^b 93.4 (4.9)
IL-17A	25.4 (3.0)	19.9 (2.6)	^b 87.3 (1.8)	^b 90.9 (8.0)	^b 84.3 (0.6)	33.1 (4.6)	28.9 (0.2)	^b 88.5 (3.4)	^b 79.1 (1.8)	^b 79.4 (1.9)
IL-18	228.2 (26.5)	202.5 (22.5)	^b 358.9 (13.8)	^b 356.0 (1.9)	^b 397.8 (15.7)	327.0 (4.7)	309.9 (10.7)	^b 421.3 (3.3)	^b 417.1 (11.0)	^b 367.9 (11.8)
GM-CSF	43.1 (2.7)	36.6 (5.4)	^b 93.1 (0.7)	^b 96.8 (2.9)	^b 124.5 (5.1)	47.9 (1.2)	40.5 (0.9)	^b 130.7 (4.9)	^b 112.0 (3.1)	^b 145.8 (2.5)
IFN- γ	47.3 (2.8)	39.2 (0.6)	^b 113.9 (0.9)	^b 98.1 (0.5)	^b 114.7 (2.5)	59.7 (4.4)	49.8 (8.7)	^b 96.2 (2.6)	^b 94.2 (1.9)	^b 104.8 (1.3)
MIP-1 α	174.8 (4.0)	165.44 (9.7)	^b 414.1 (26.8)	^b 399.1 (5.1)	^b 384.5 (15.7)	279.7 (11.7)	265.5 (3.2)	^b 498.3 (13.5)	^b 423.9 (1.8)	^b 480.9 (17.6)
RANTES	^a 268.9 (6.2)	557.7 (4.5)	^a 212.3 (1.8)	^a 251.4 (8.1)	^a 313.1 (3.6)	^a 326.4 (4.6)	536.25 (5.7)	^a 319.5 (5.0)	301.8 (6.2)	^a 295.3 (1.6)
TNF- α	^a 25.5 (2.5)	72.9 (0.7)	^a 32.1 (0.8)	^a 44.0 (2.1)	^a 37.0 (0.7)	^a 30.8 (0.9)	106.3 (0.4)	^a 59.6 (1.0)	^a 50.1 (0.5)	^a 45.7 (1.0)
VEGF	^a 19.6 (1.0)	58.3 (0.2)	^a 34.10 (2.0)	^a 39.1 (2.1)	^a 41.3 (1.1)	^a 24.3 (0.1)	64.5 (0.2)	^a 47.4 (0.5)	^a 41.9 (3.2)	^a 37.5 (0.3)

^aSignificantly lower relative to NMBA-control (Group 2) (P< 0.05)

^bSignificantly higher relative to NMBA-control (Group 2) (P< 0.05)

All diets mixed with AIN-76A

Selected results

Significantly lower in
BRB/AC/PCA

Significantly higher in
BRB/AC/PCA

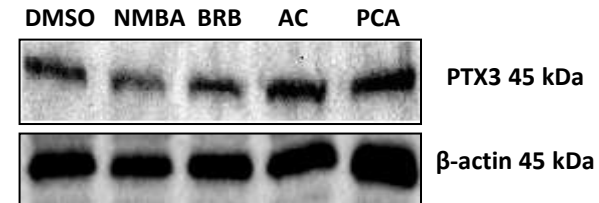
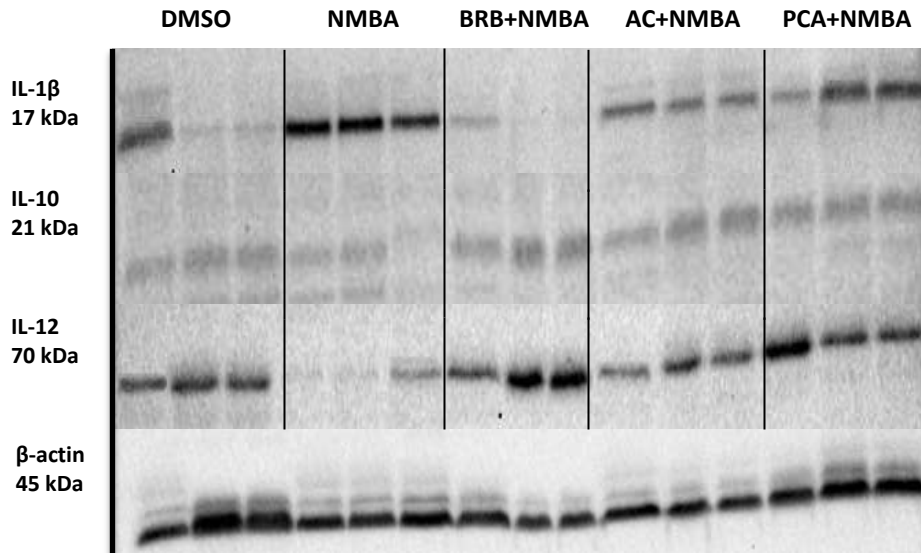
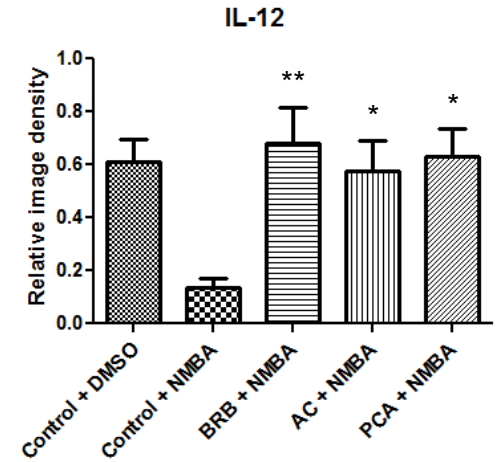
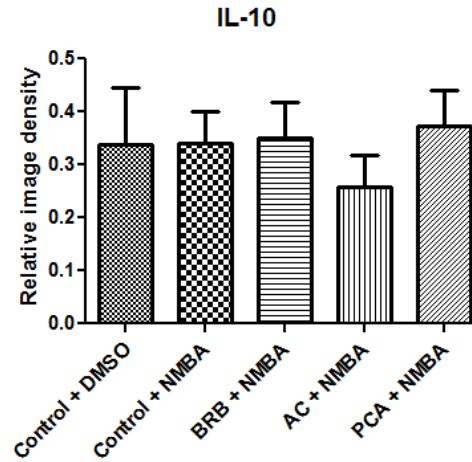
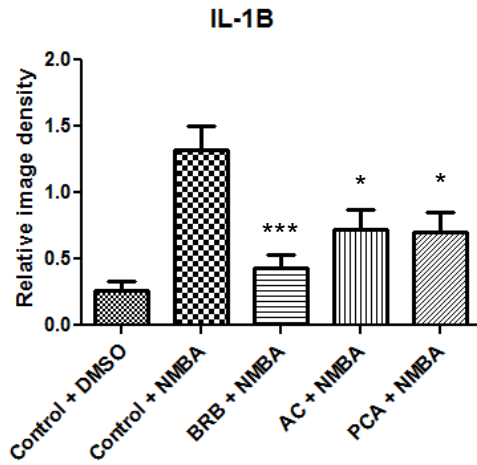
Significantly lower in BRB/AC/PCA		Significantly higher in BRB/AC/PCA	
Week 15	Week 25	Week 15	Week 25
IL-2	IL-2	IL-5	IL-5
RANTES (CCL5)	RANTES (CCL5)	IL-12	IL-12
TNF- α	TNF- α	IL-13	IL-13
IL-1 β	IL-1 β	IL-17A	IL-17A
IL-4	IL-4	IL-18	IL-18
	IL-6	GM-CSF	GM-CSF
		IFN- γ	IFN- γ
		PTX3	PTX3
			IL-10

Significance compared to NMBA-control (p < 0.05)

Week 35 Plasma

Cytokine	DMSO	NMBA	BRB	AC	PCA
IL-1 β	104.3 \pm 1.1	118.3 \pm 2.6	107.9 \pm 4.5	100.5 \pm 2.1	107.7 \pm 3.3
IL-10	110.1 \pm 2.9	96.7 \pm 3.8	115.5 \pm 4.0	111.1 \pm 2.1	108.7 \pm 3.4
IL-12	36.8 \pm 1.5	25.5 \pm 1.1	40.9 \pm 5.2	42.0 \pm 3.2	37.5 \pm 1.9
PTX3	0.34 \pm .01	0.14 \pm .02	0.32 \pm .02	0.33 \pm .03	0.35 \pm .01
Sign. higher than NMBA control (p<0.05)			Sign. lower than NMBA control (p<0.05)		

Week 35 esophageal lysate

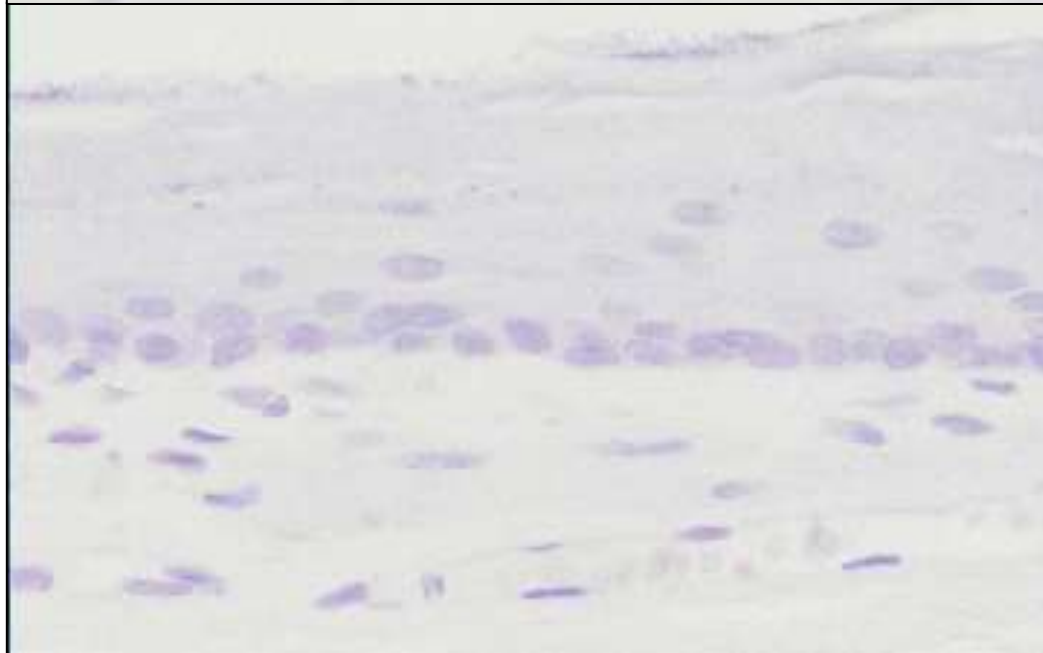


What effect does this have on innate immune cells?

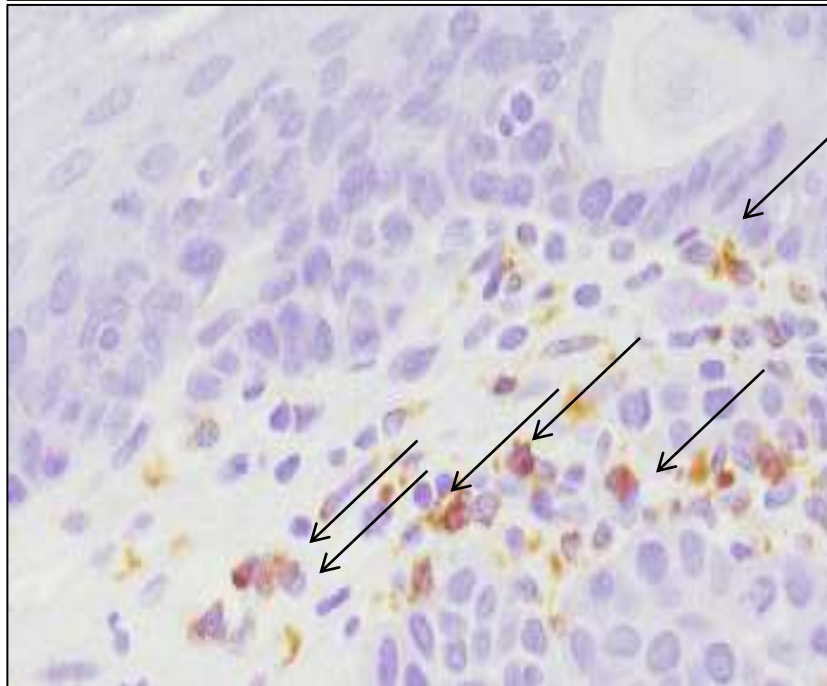
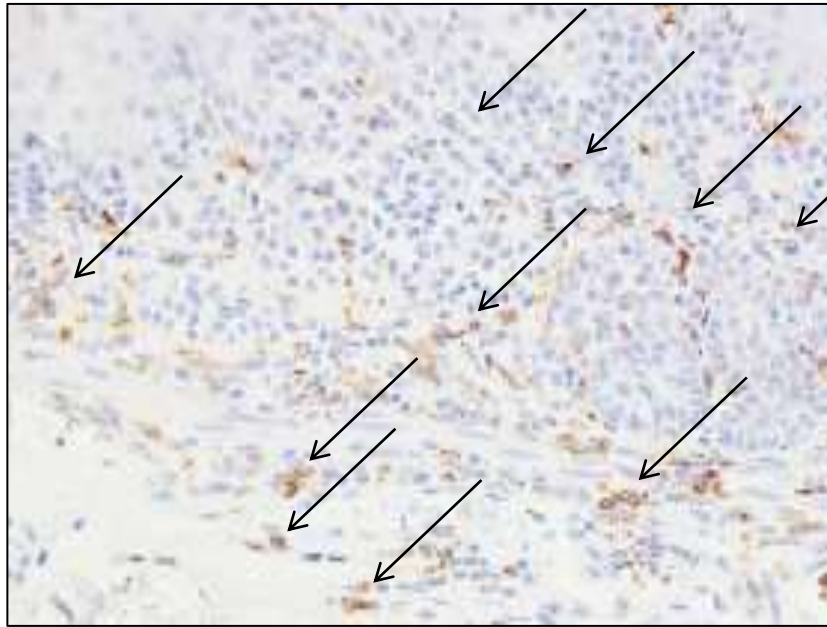
CD68

- General macrophage marker
- Higher expression in ESCC
 - Negative correlation w/ prognosis

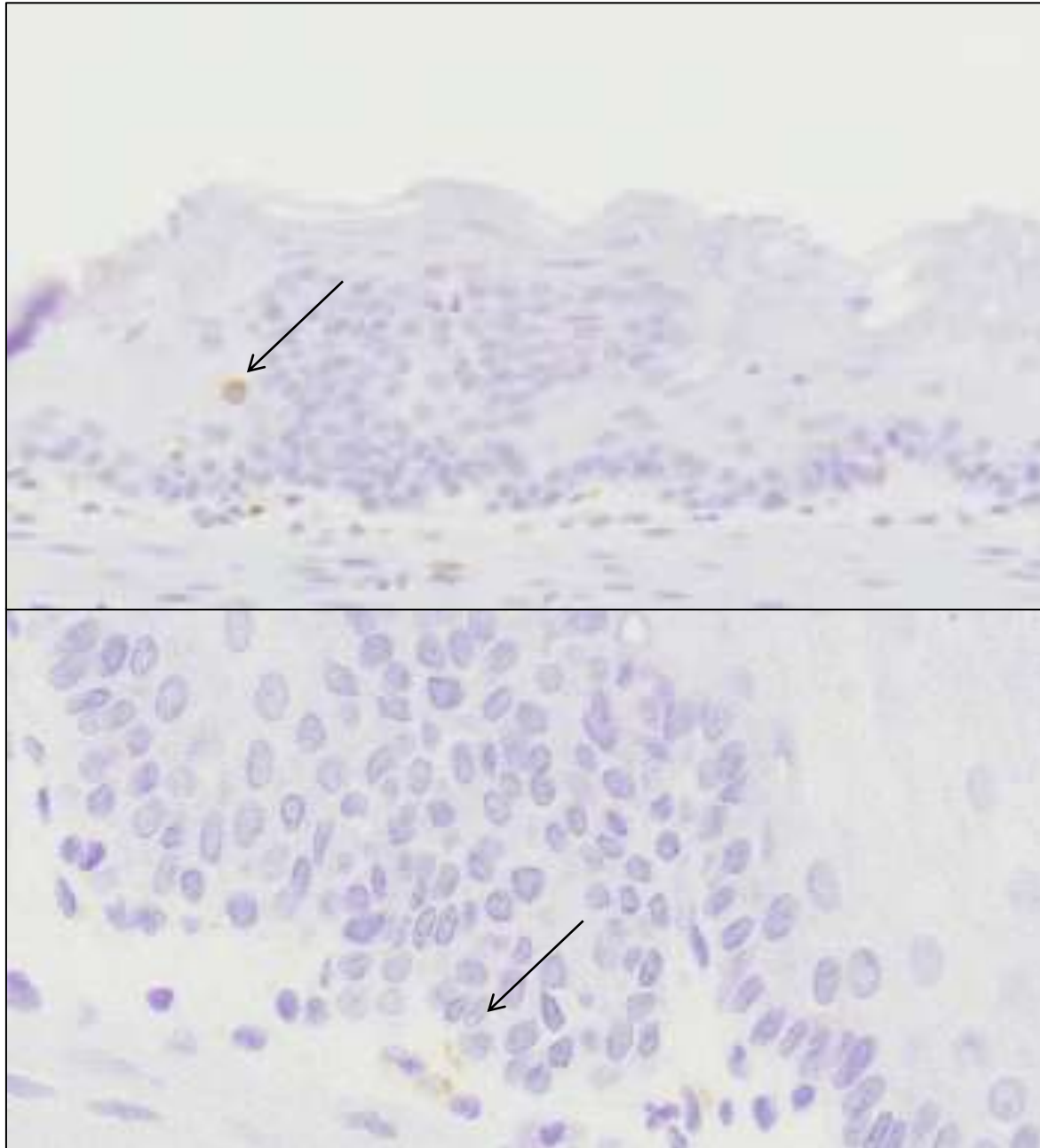
Vehicle



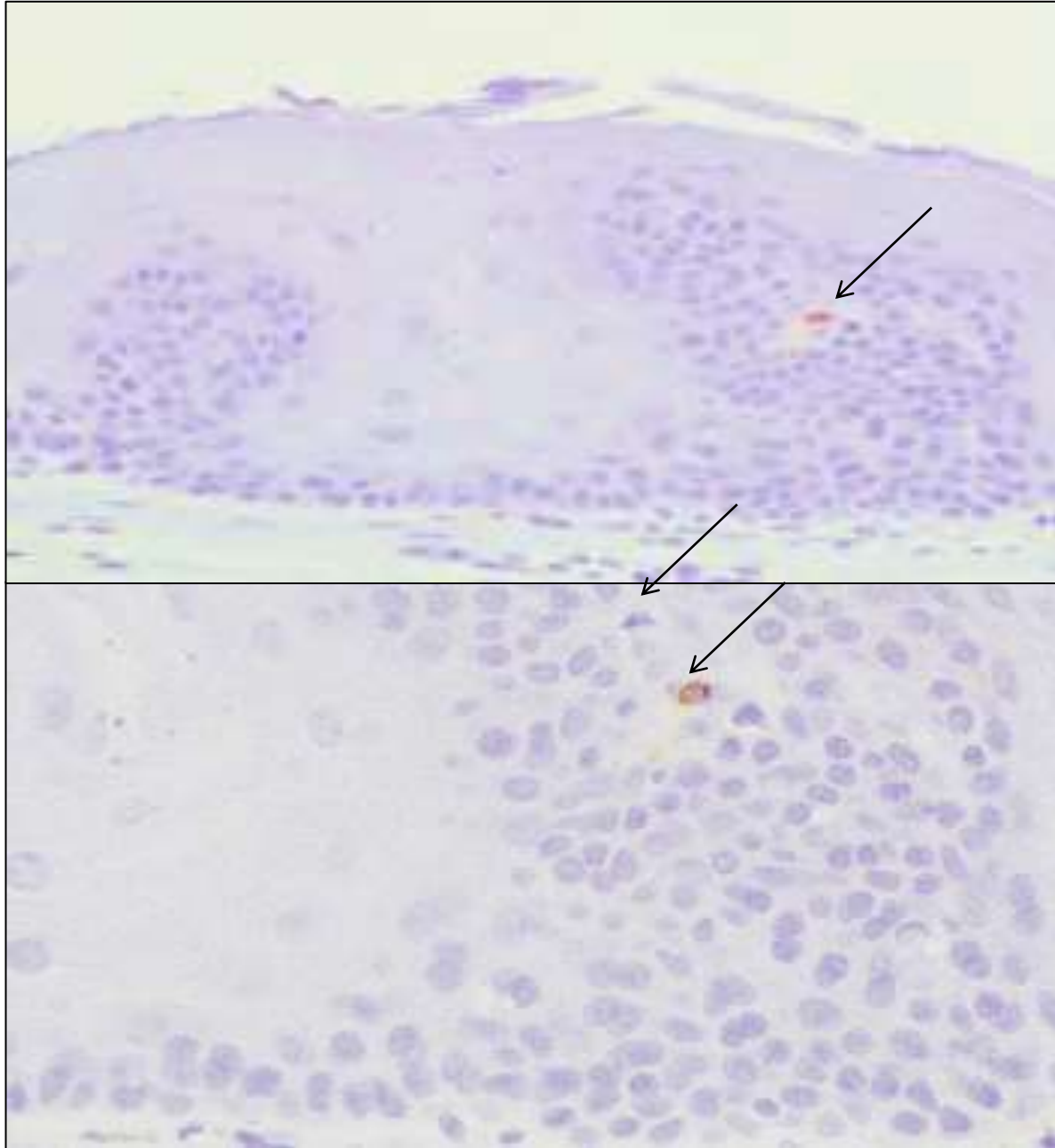
NMBA control



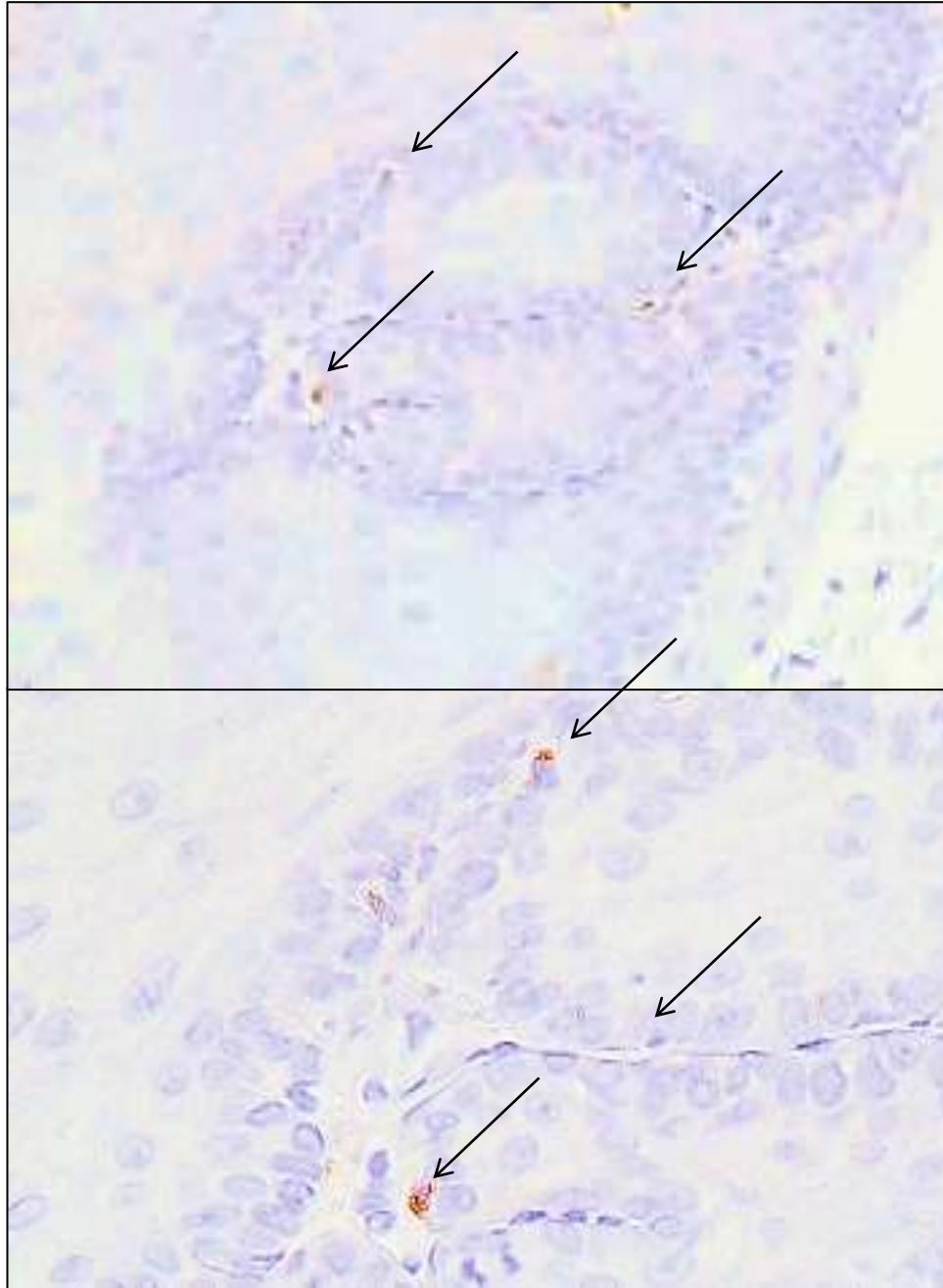
BRB



AC



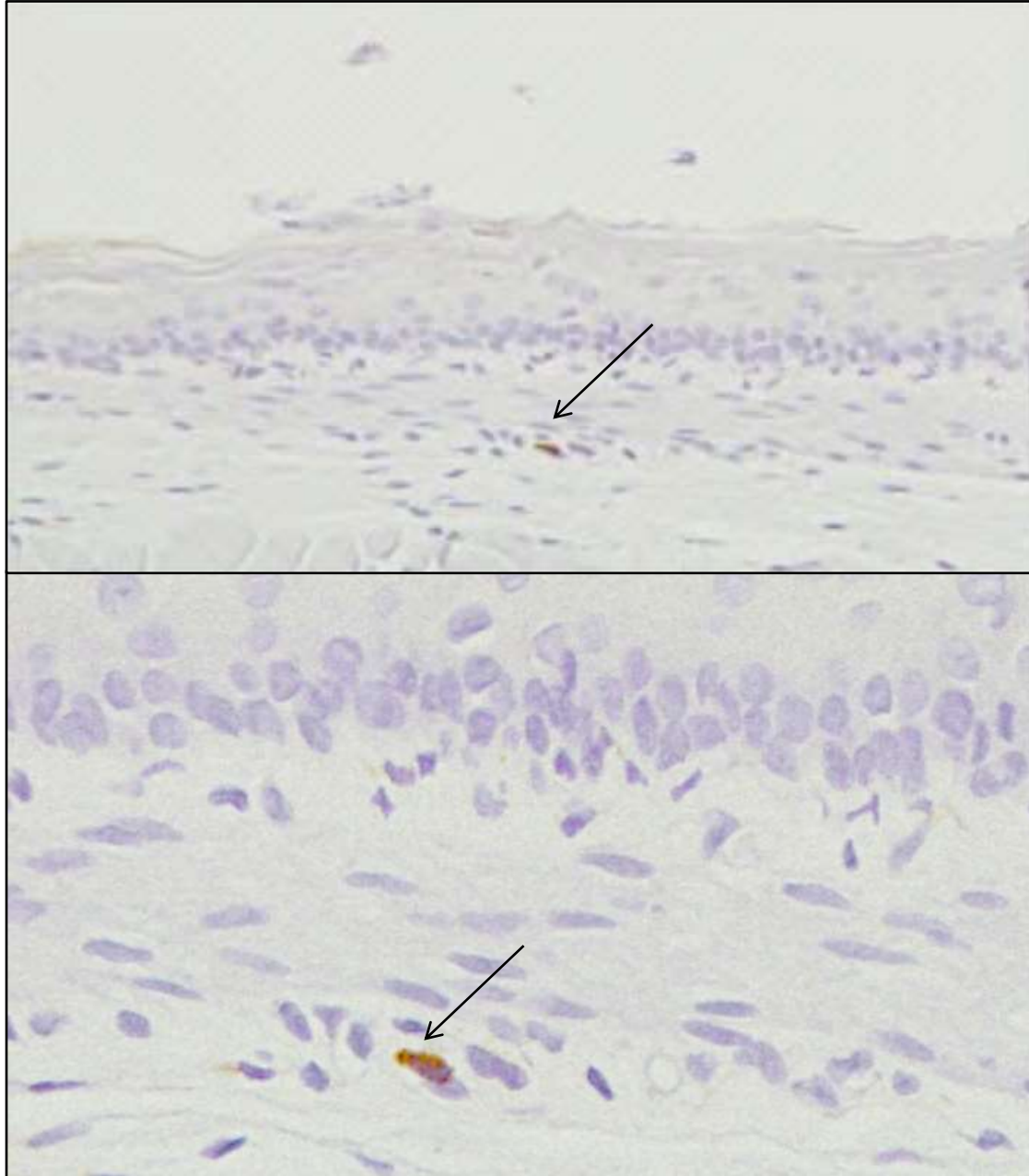
PCA



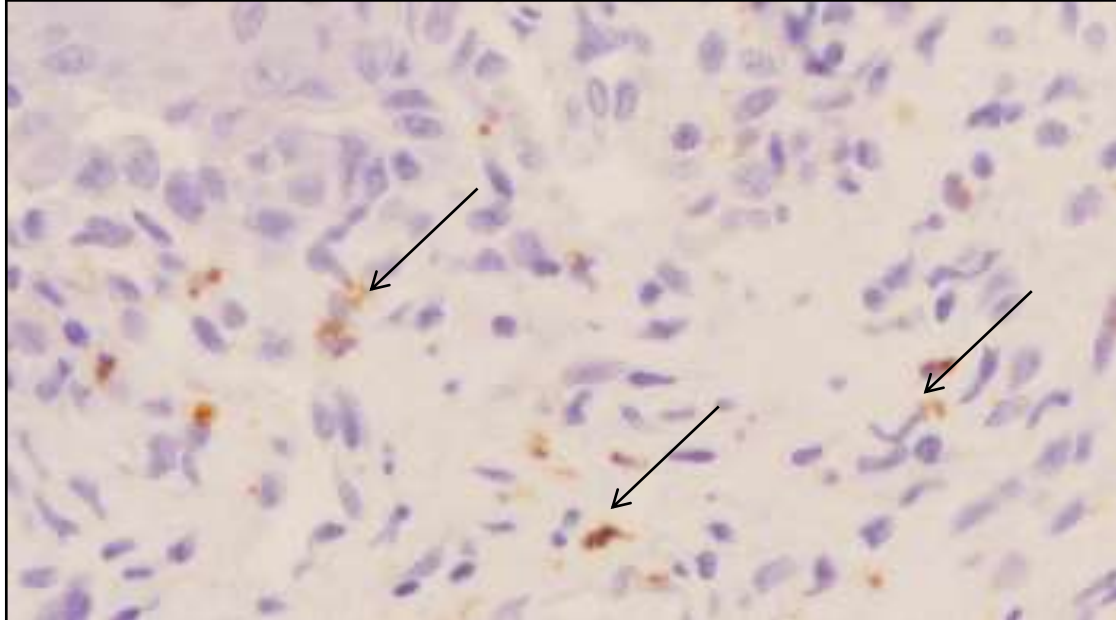
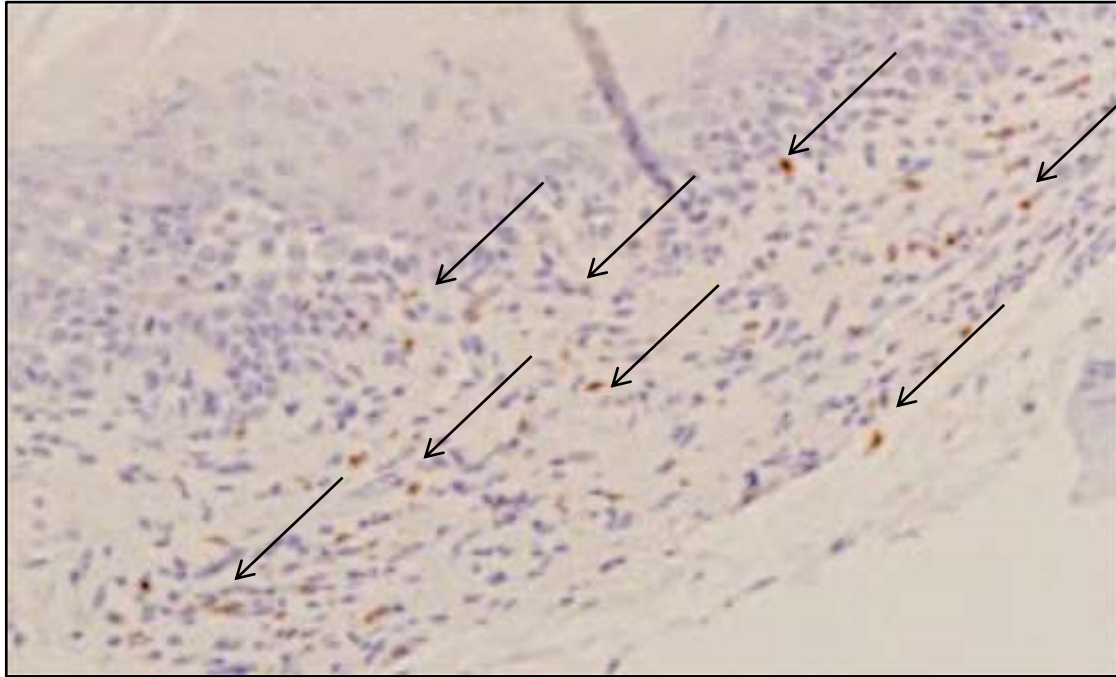
MPO

- Neutrophils
- More aggressive phenotype and poor prognosis in ESCC
- Migration inhibited by PTX3

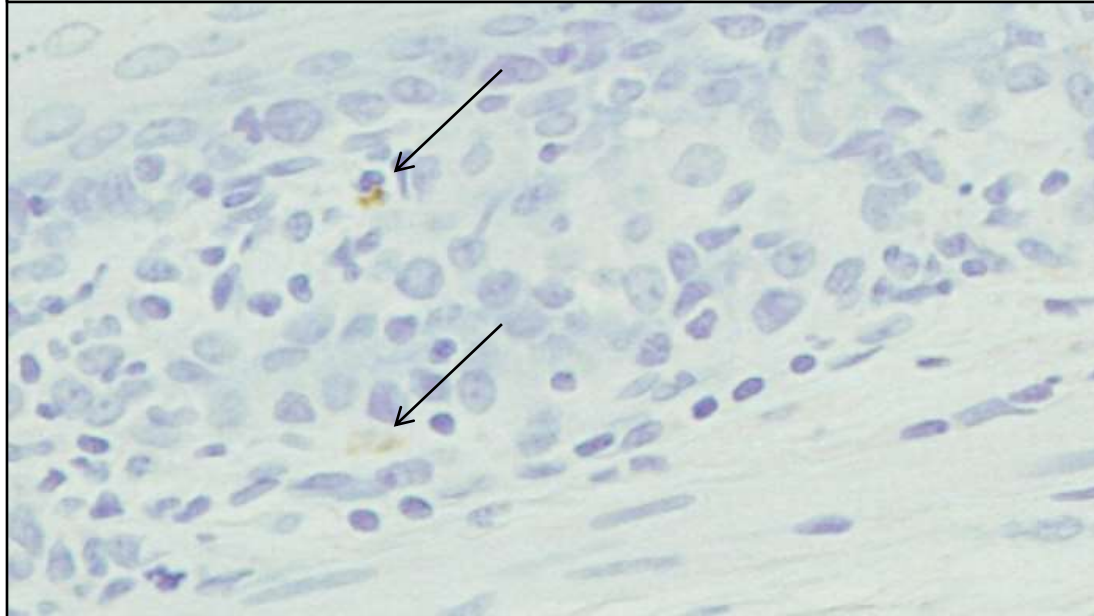
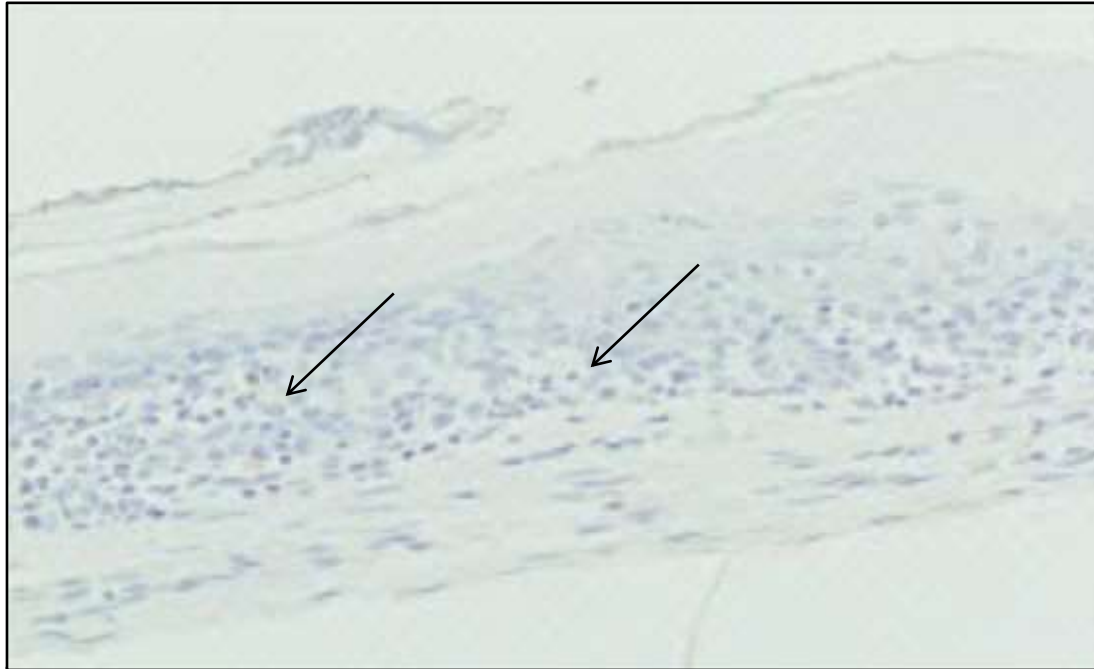
DMSO



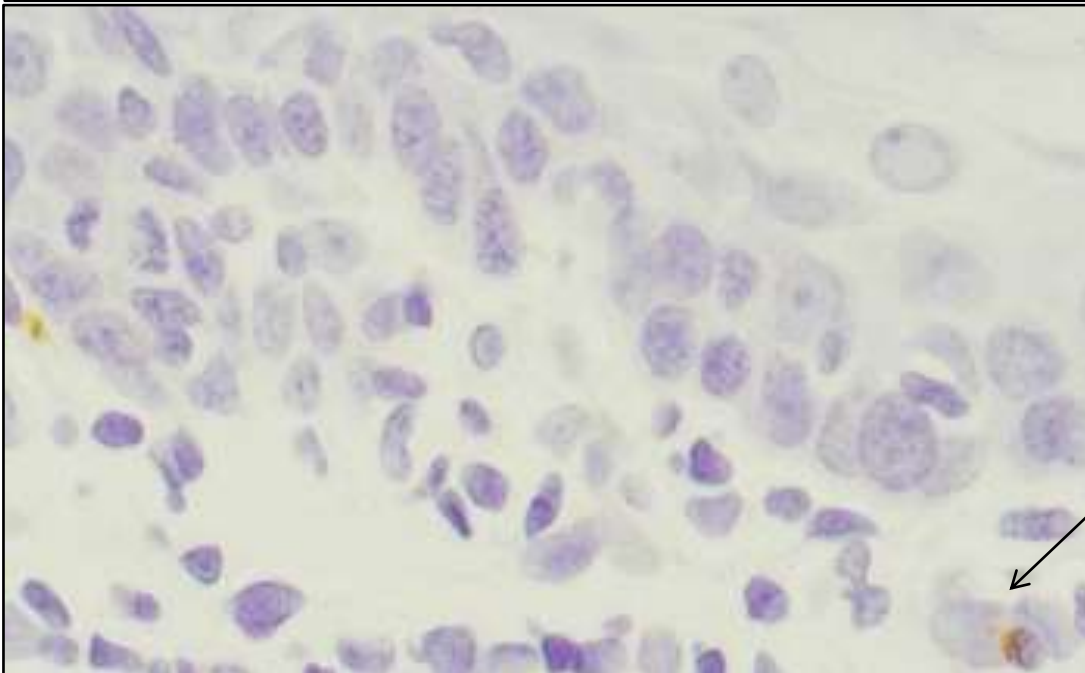
NMBA



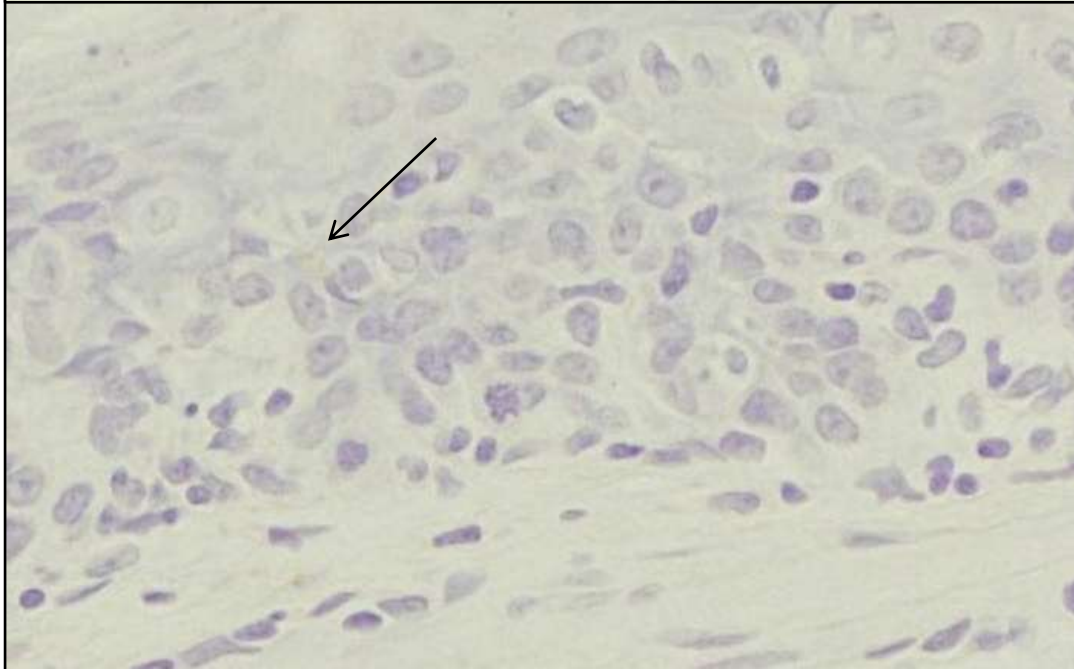
BRB



AC



PCA



Conclusions

- BRB, AC, PCA alter tumor microenvironment in a positive way
 - Decrease IL-1 β
 - Increase IL-12
 - Increase PTX3
 - Decrease macrophage infiltration
 - Associated w/ better prognosis
 - Inhibit neutrophil migration

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Thank you!!

Questions?

