Chemokines and chemokine receptor antagonists for airway disease

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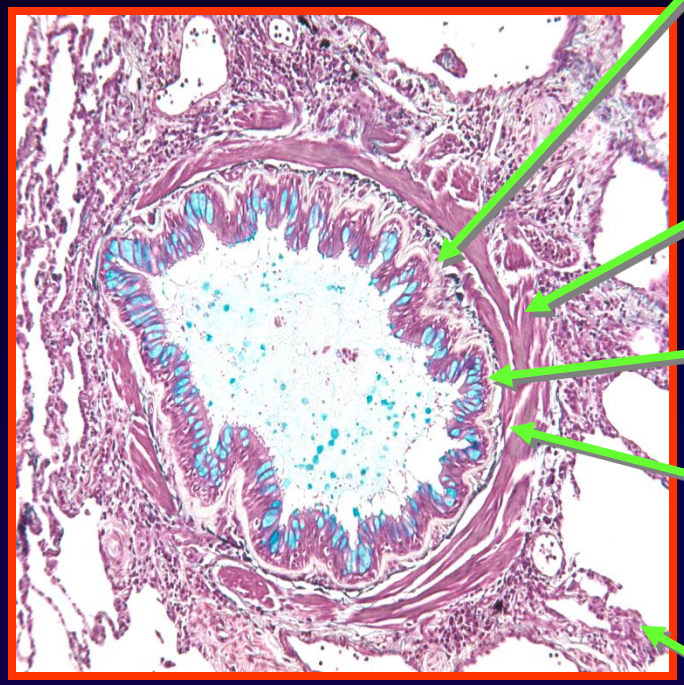
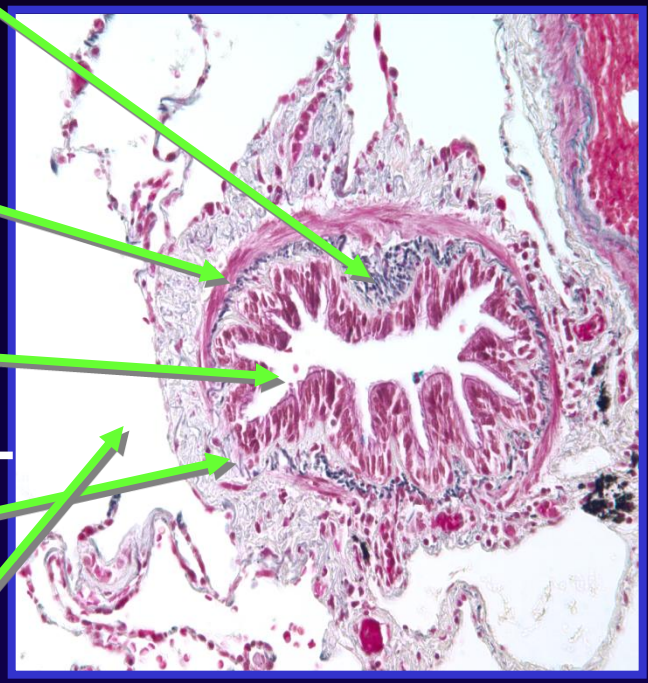
Asthma vs COPD: Histopathology



Asthma death

+++	Inflammation	+++
+++	ASM	+
+++	BM	+
+	Fibrosis	+++
-	Alveolar disruption	+++



Severe COPD

(Courtesy Professor Jim Hogg)

Changes in Lung Inflammatory Cells

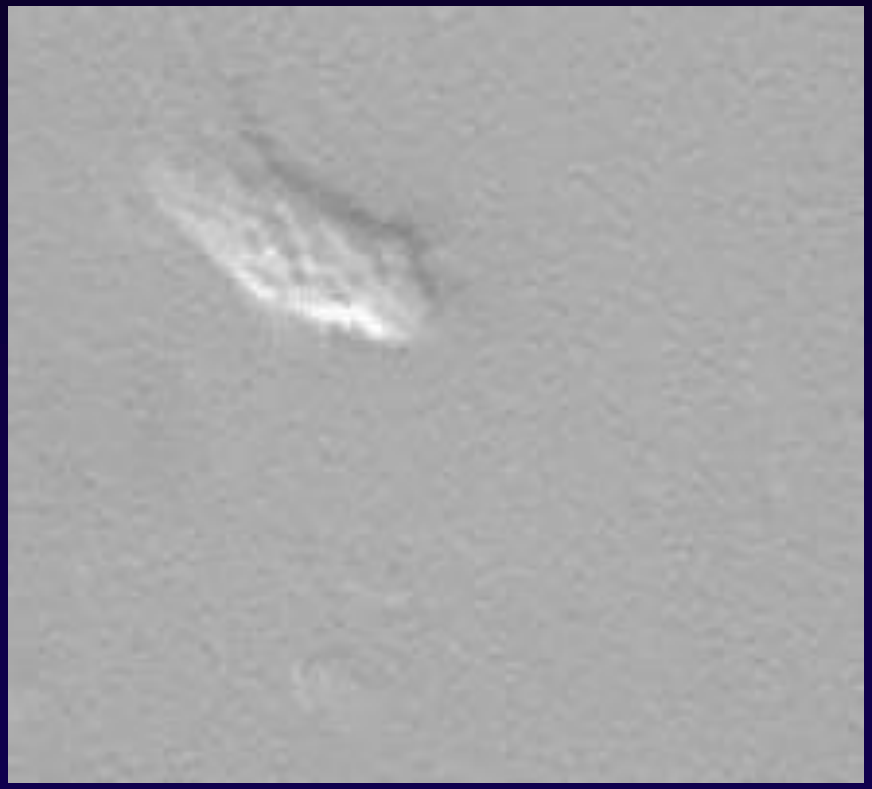
Inflammatory cells	Asthma	COPD
$CD45^+$	2	2
$CD3^+$	2	4
$CD4^+$	3	3
$CD8^+$	2	8
Macrophages	0	9
Neutrophils	-2	2
Eosinophils	93	4

Fold change in number of cells *vs* healthy controls

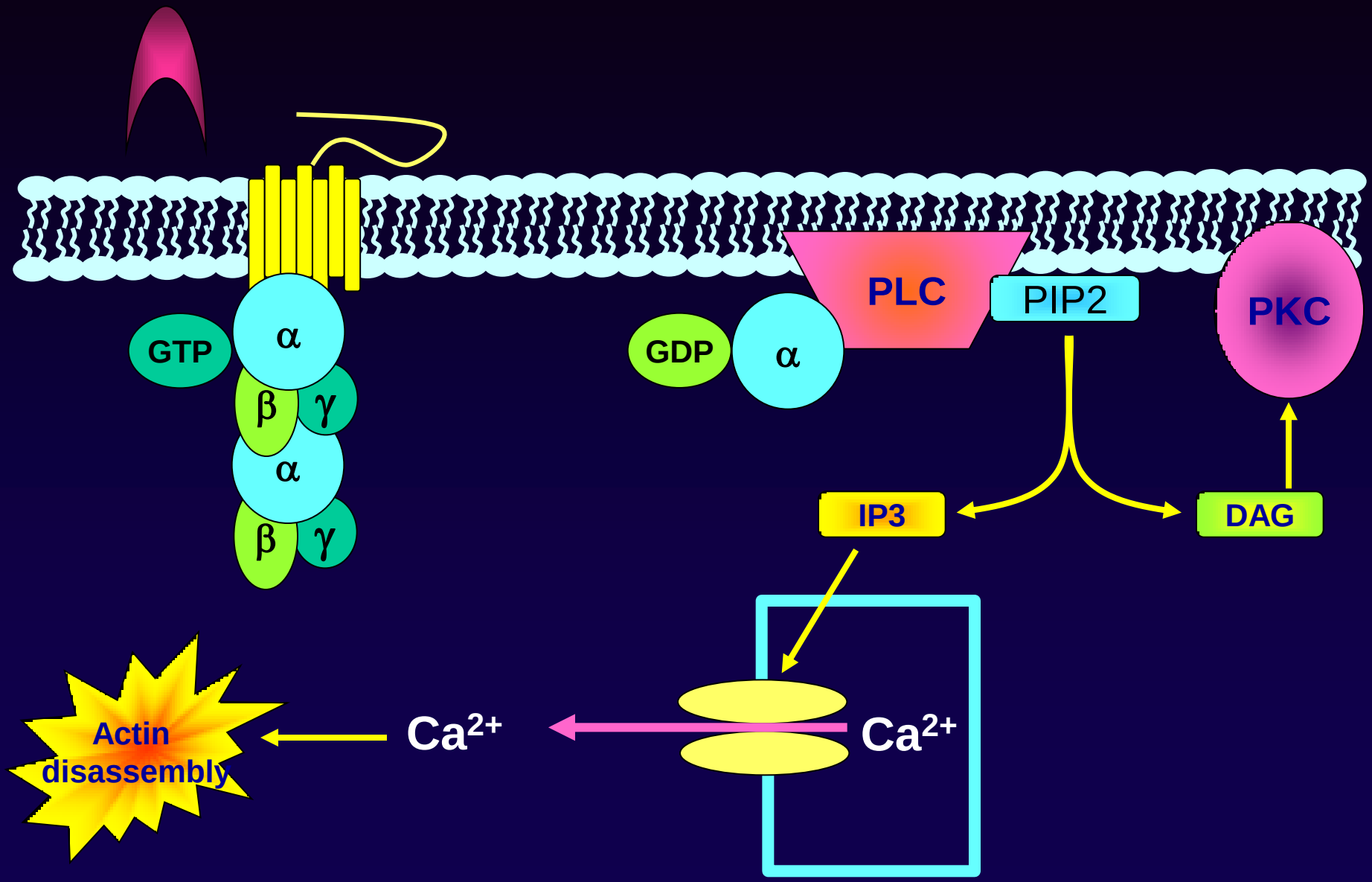
Jeffery, P.K (2000) Chest 117: 251S-260S

Chemokines

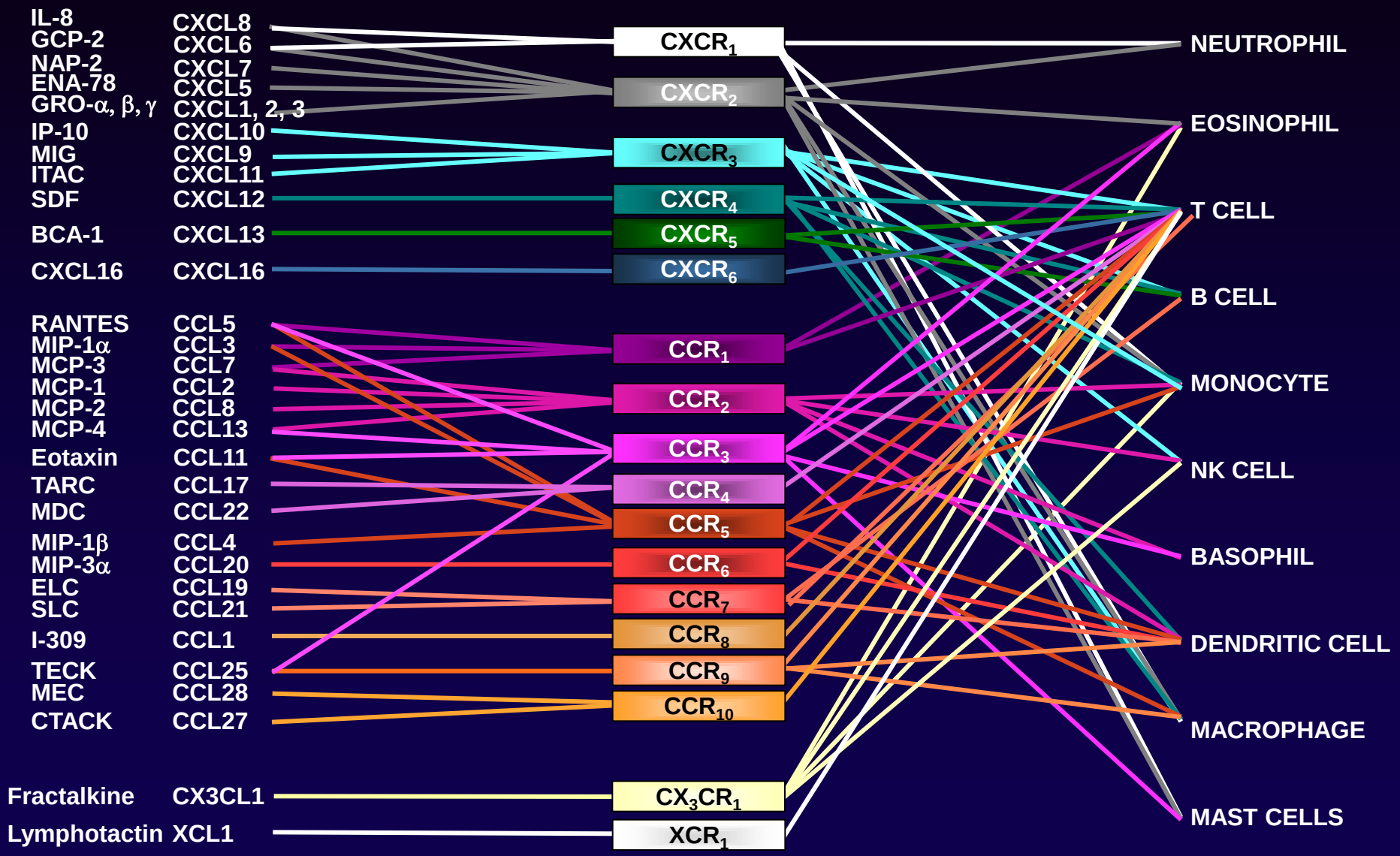
Cytokines that cause cell migration



Chemokine Receptor Signaling



Chemokine Receptor Redundancy

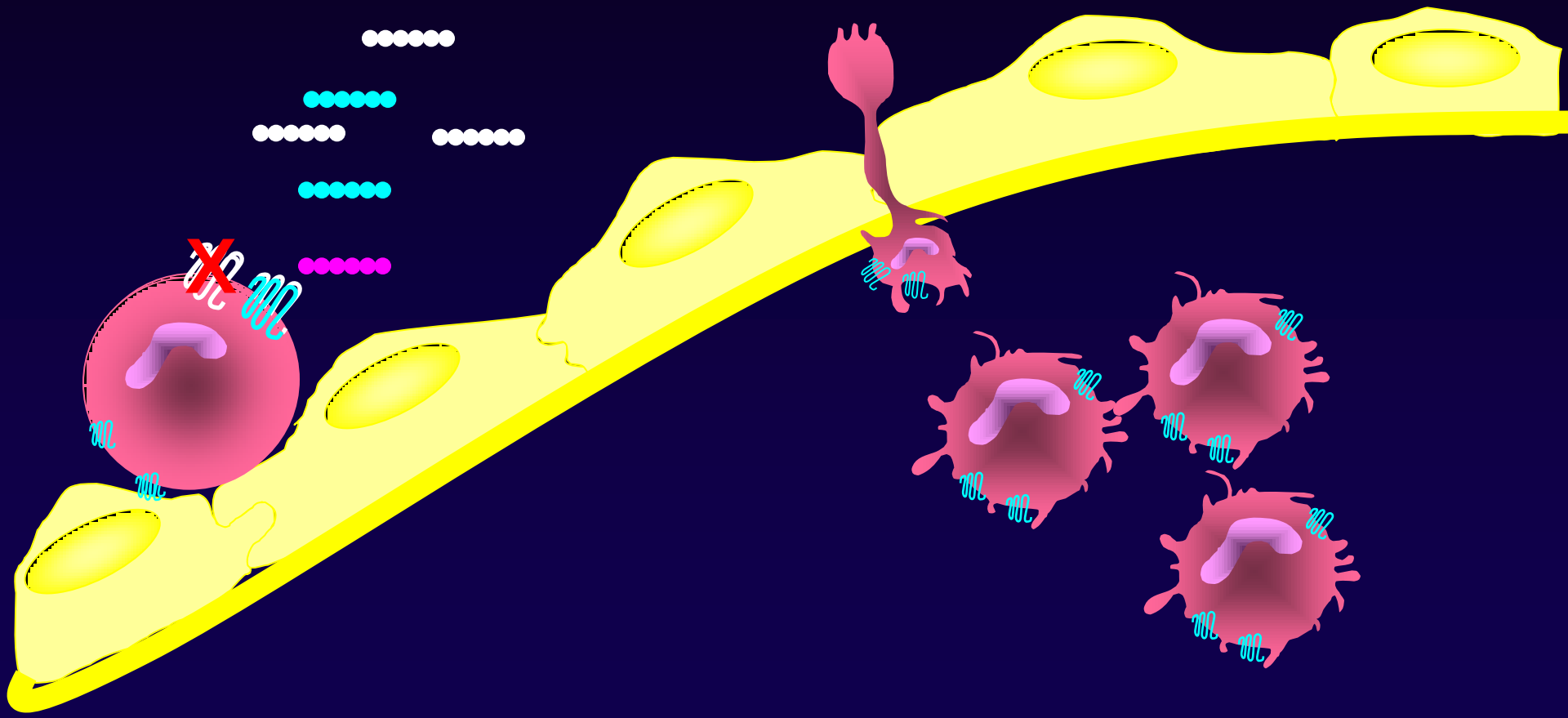


Redundancy

1. More than one chemokine/receptor

2. More than one receptor type/cell

Chemokine Receptor Redundancy



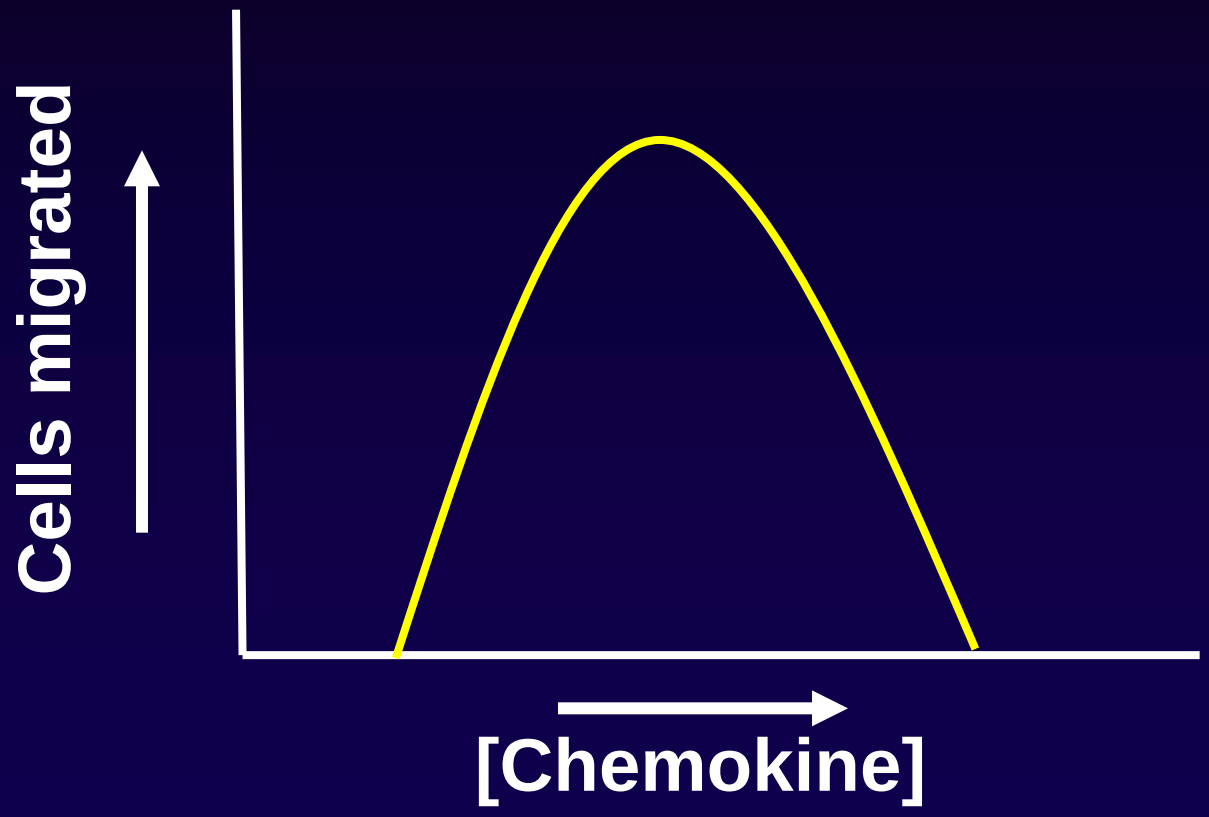
Redundancy - Assumptions

1. Different chemokines are equipotent at a single receptor

2. Different chemokines have the same function at a single receptor

3. Different chemokine receptors on a single cell have the same function

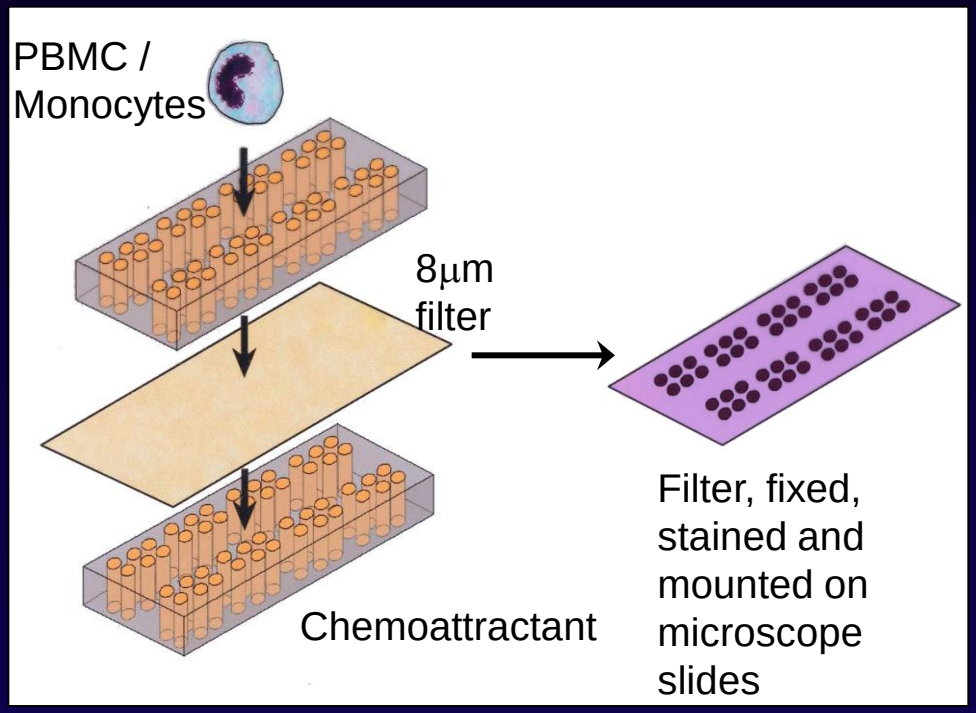
Bell-shaped Chemotactic Response



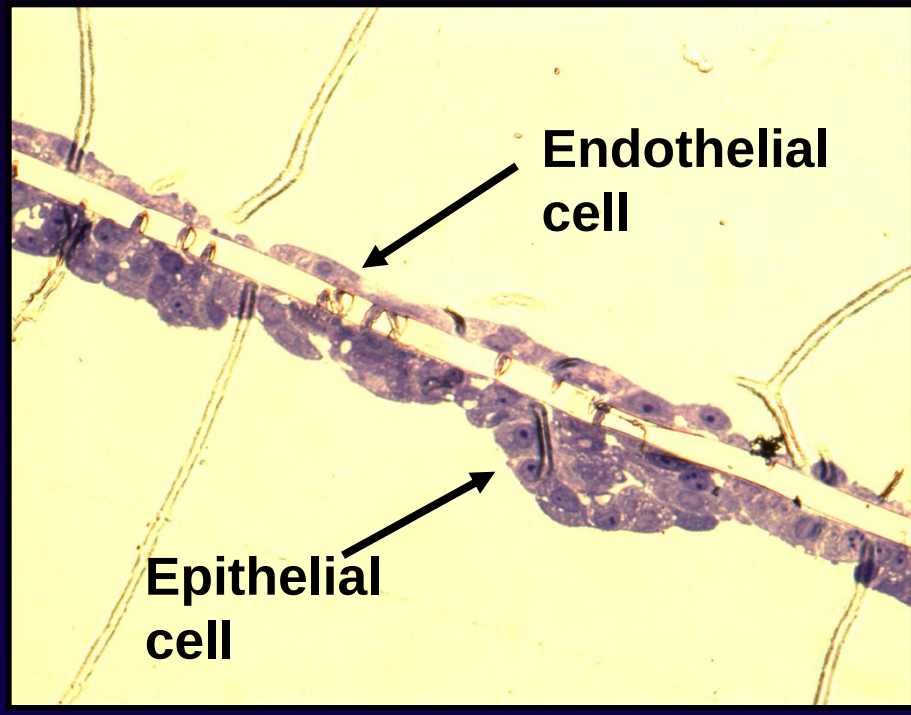
Measuring Migration

Chemotaxis assays

Boyden Chamber



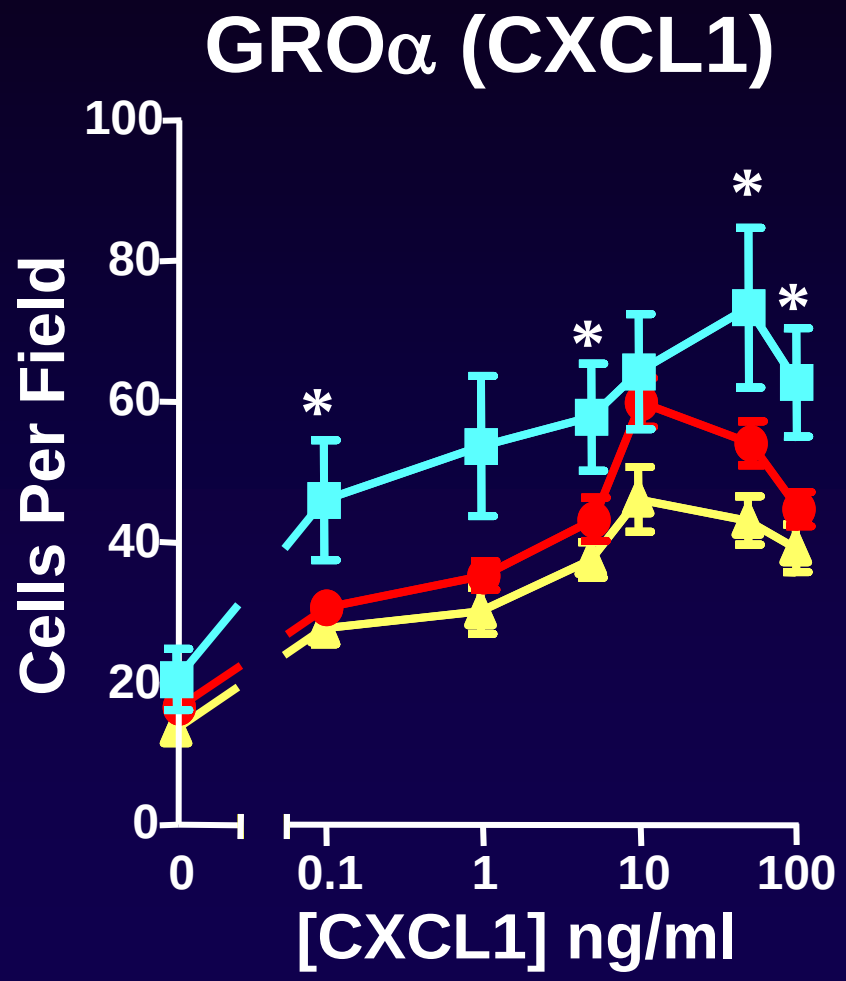
Transwell systems

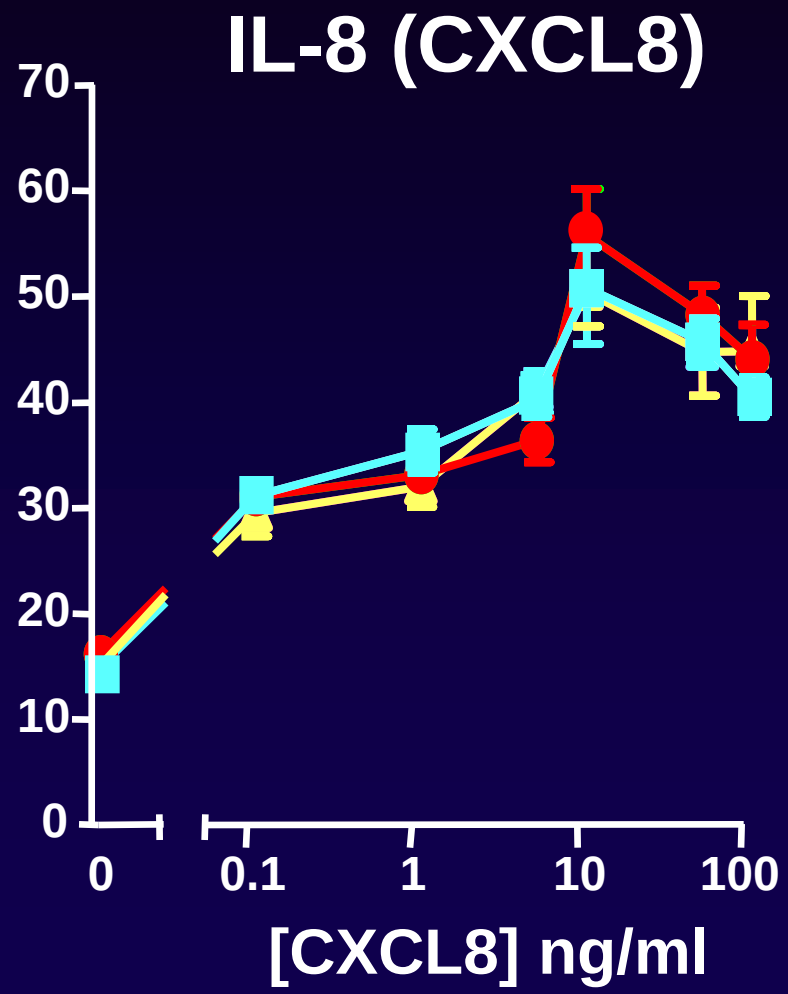


Surrogates – Shape change assays, Ca^{2+} mobilization

PBMC Migration

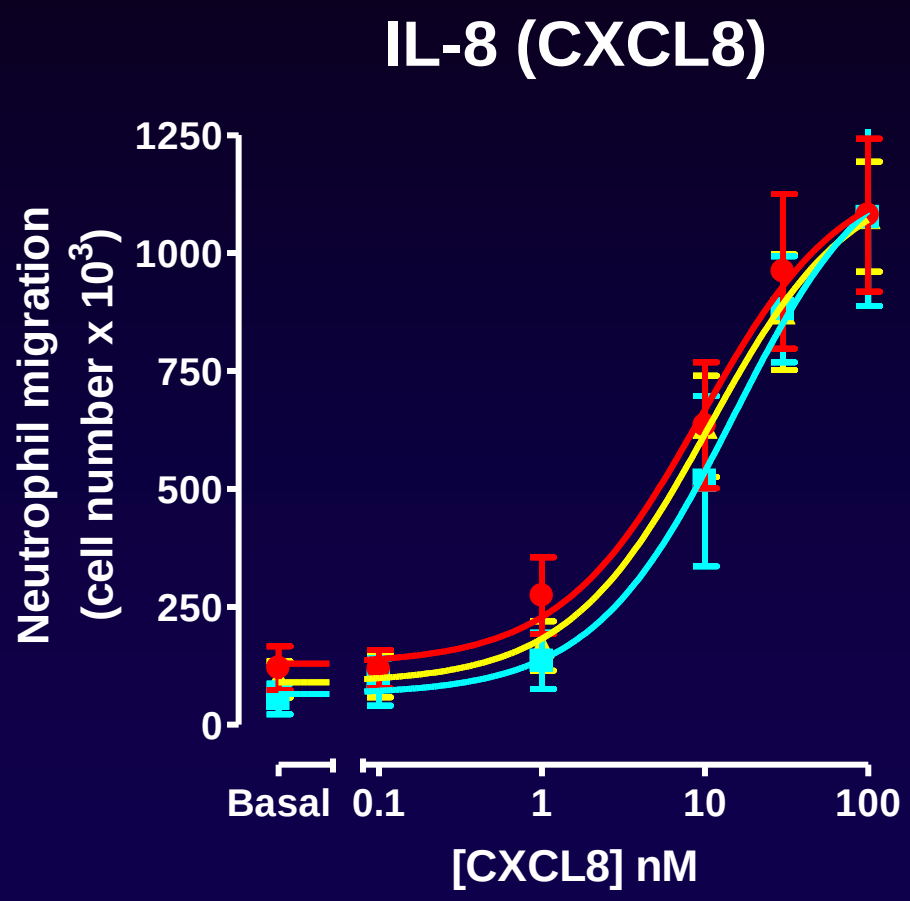
(Non-smokers (n=8), Smokers (n=8), COPD (n=10))

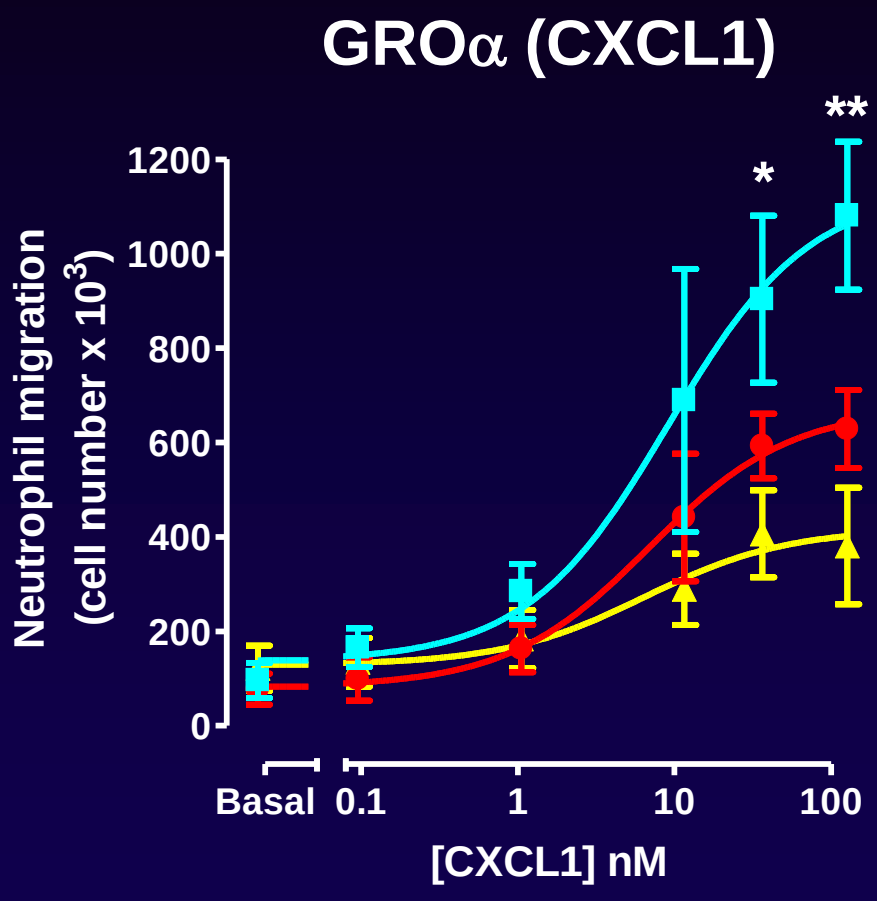




Traves *et al*., 2004 J. Leukoc. Biol

Neutrophil Migration to CXCL8 (IL-8) and CXCL1 (GROα)





▲ Non-smokers n=6 ● Smokers n=6 ■ COPD n=6

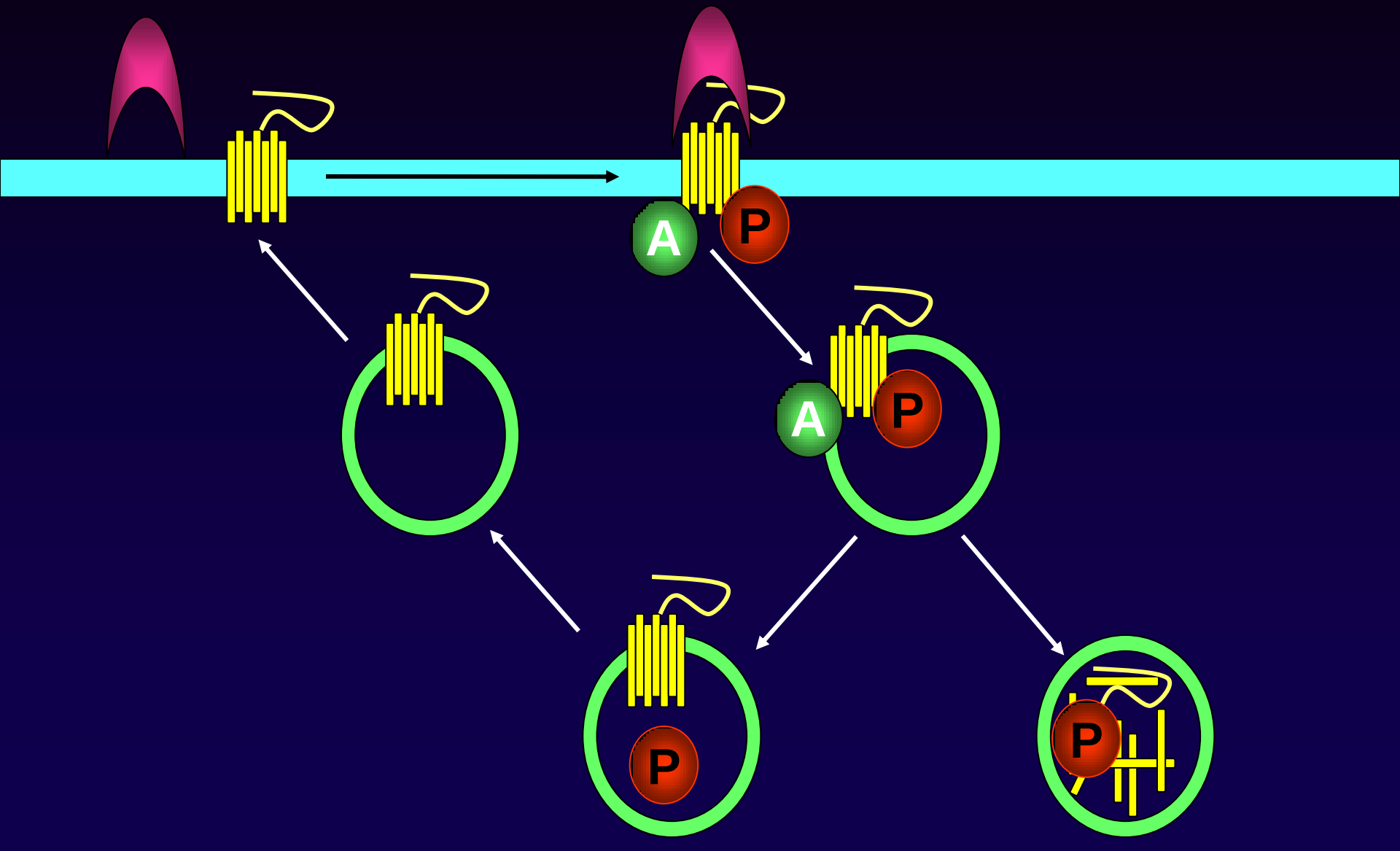
Receptor Expression by FACS

- $CXCR_1$ and $CXCR_2$
  - No difference in expression between the groups
  - Approx 30-40%

Are differences in migration due to differences in regulation of receptor expression?

Receptor recycling

Receptor Recycling



Summary

- **Differential effects of CXCL1 and CXCL8 suggest non-redundancy of chemokine function**

- **This may be due to differences in receptor recycling**

Supporting evidence:

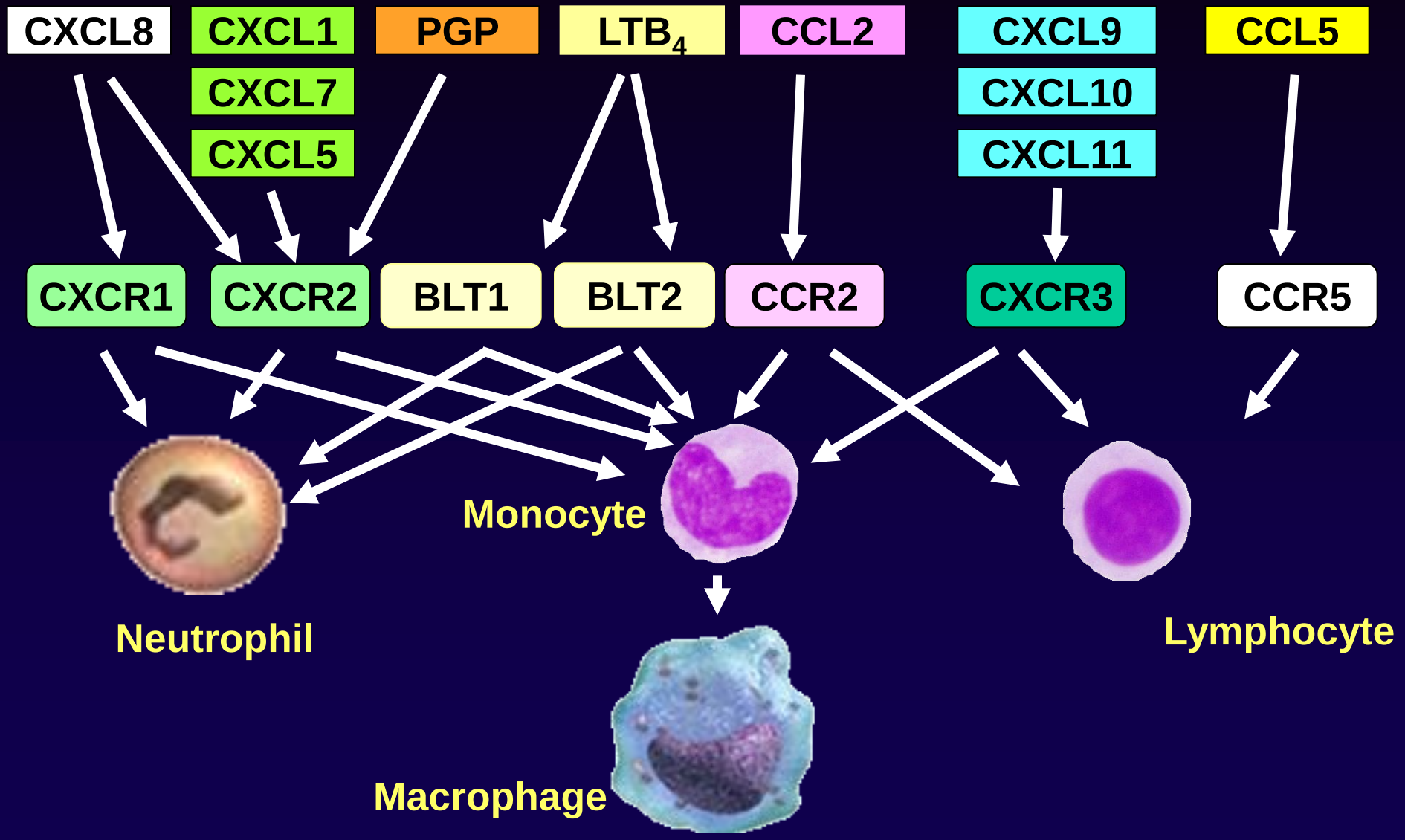
CXCL8, CXCL1 and CXCL7 bind to different amino acids in the CXCR2 receptor

Katancik *et al* 2000 Cytokine

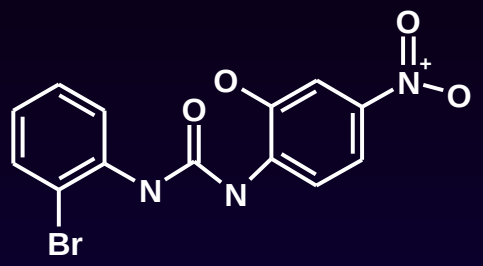
CXCL8 and CXCL7 lead to differential phosphorylation of the CXCR2 receptor

Ben-Baruch *et al* 1997 J. Immunol

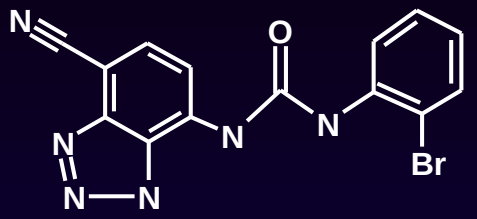
Which chemokine receptors to target? COPD



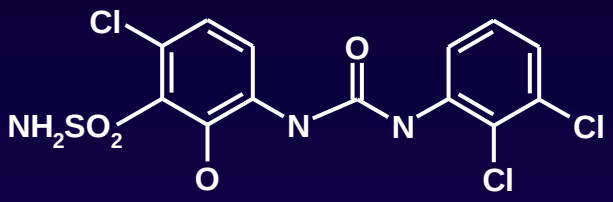
CXCR2 Antagonists



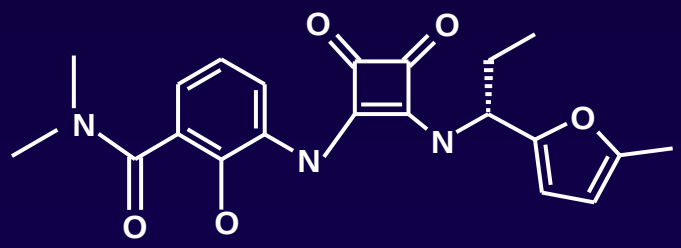
SB 225002



SB 265610



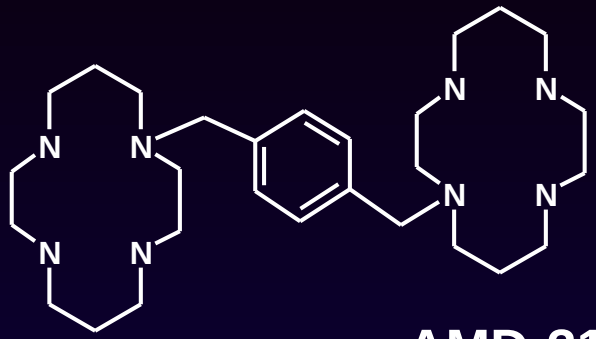
SB332235



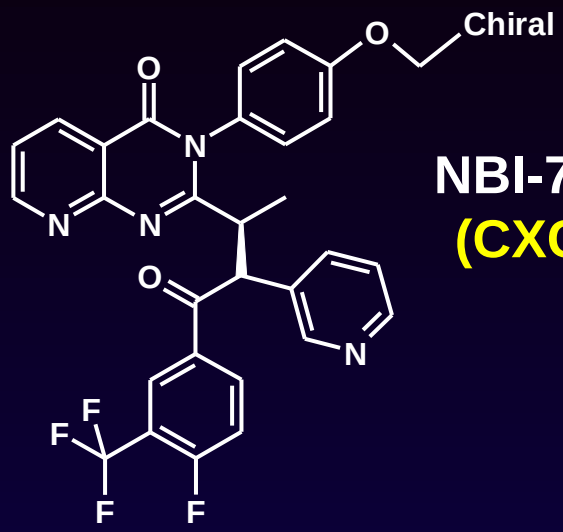
SCH527123
(CXCR1/CXCR2)

SB656933

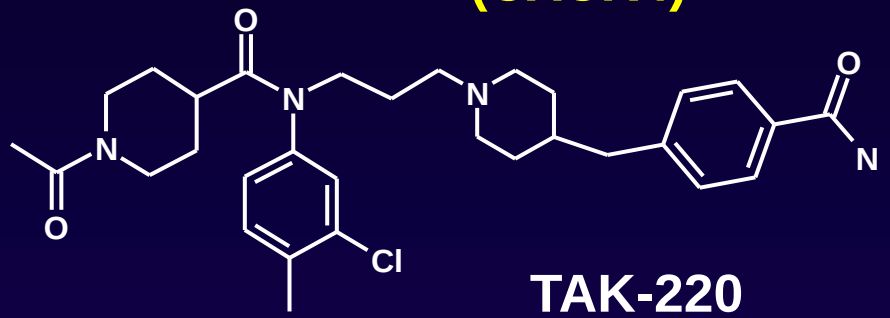
Antagonists



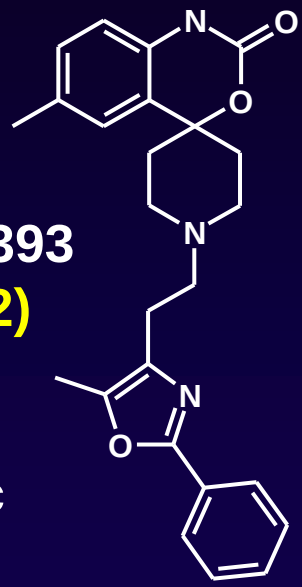
AMD-3100
(CXCR4)



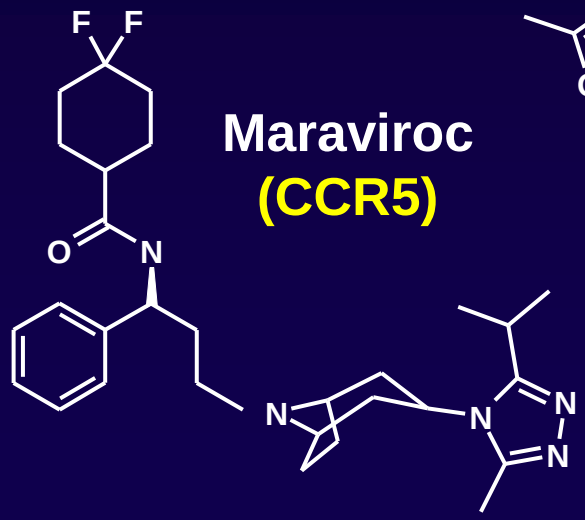
NBI-74330
(CXCR3)



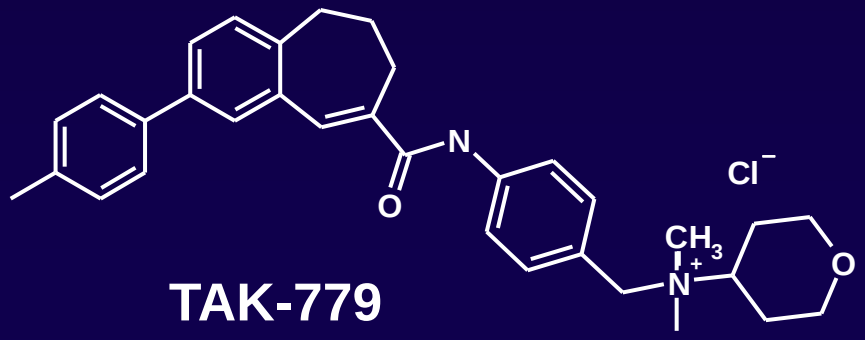
TAK-220
(CCR5)



RS 504393
(CCR2)

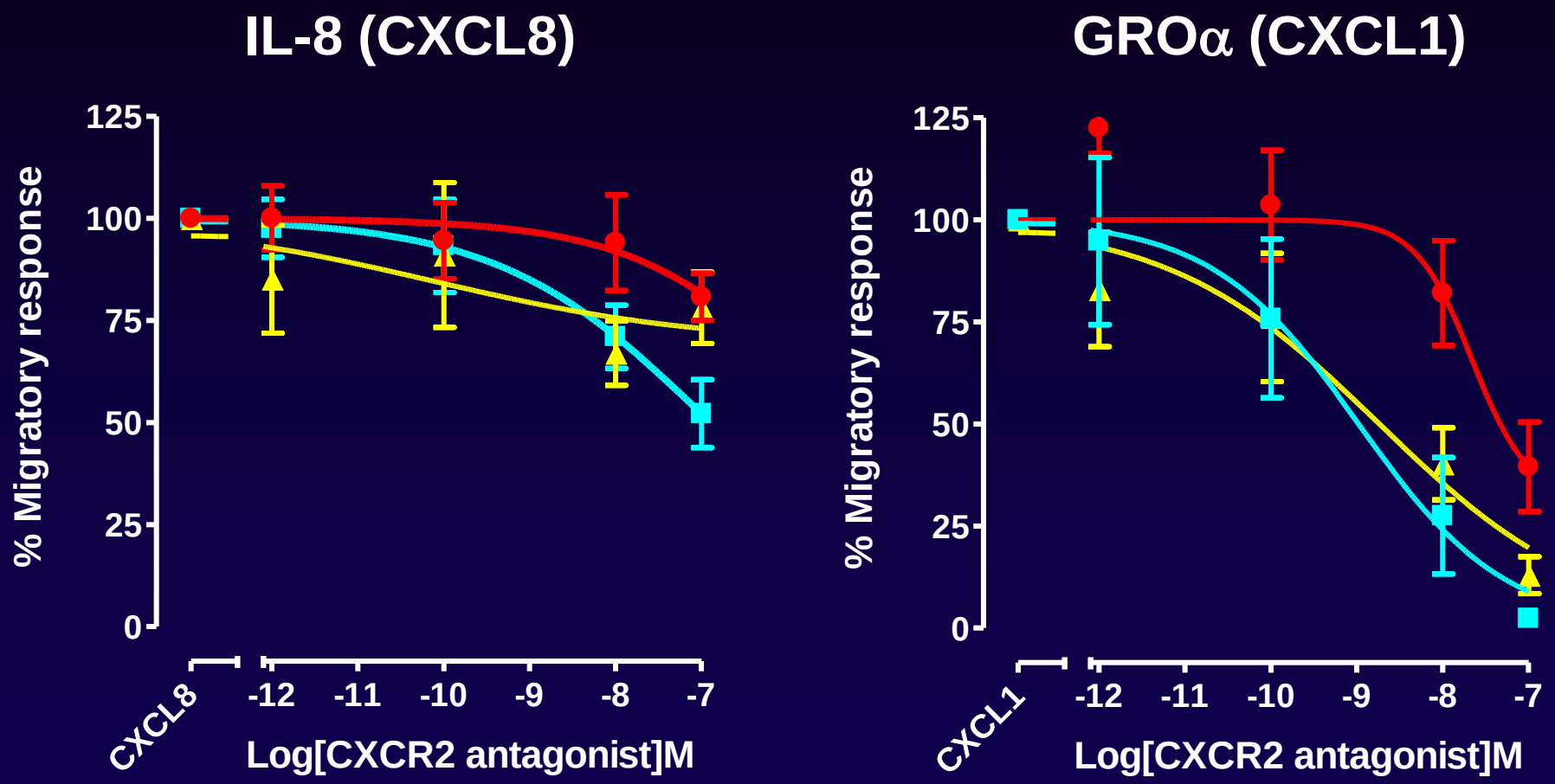


Maraviroc
(CCR5)



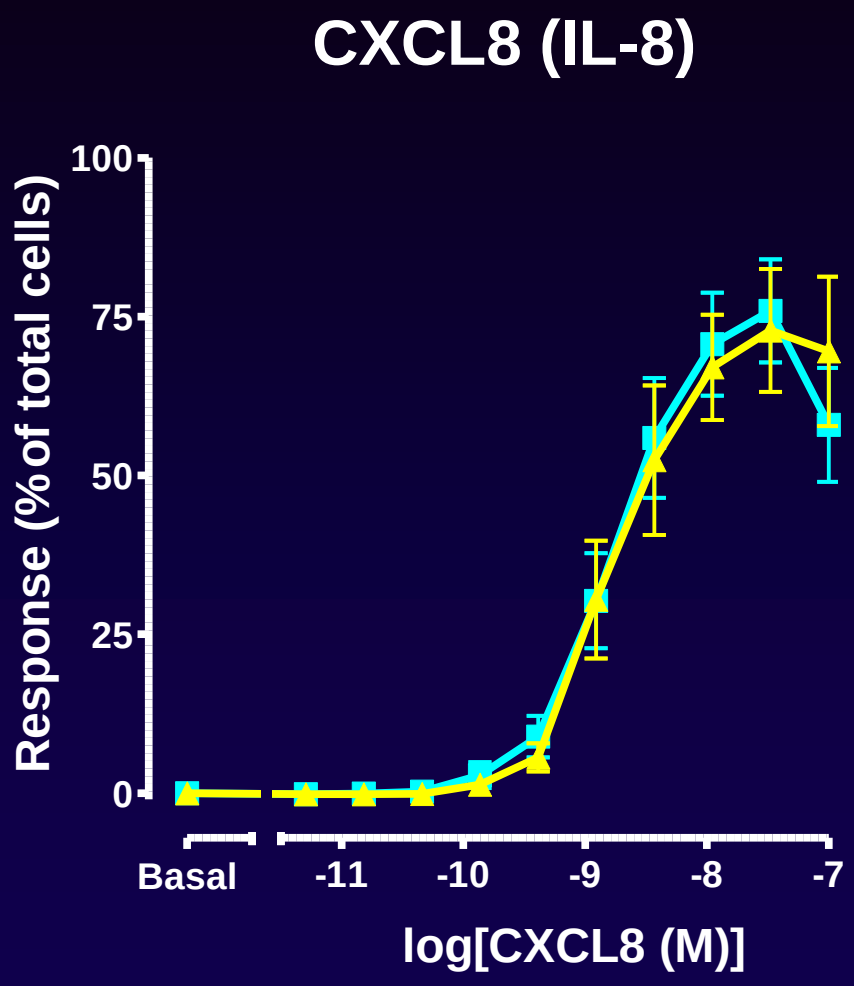
TAK-779
(CXCR3-CCR5)

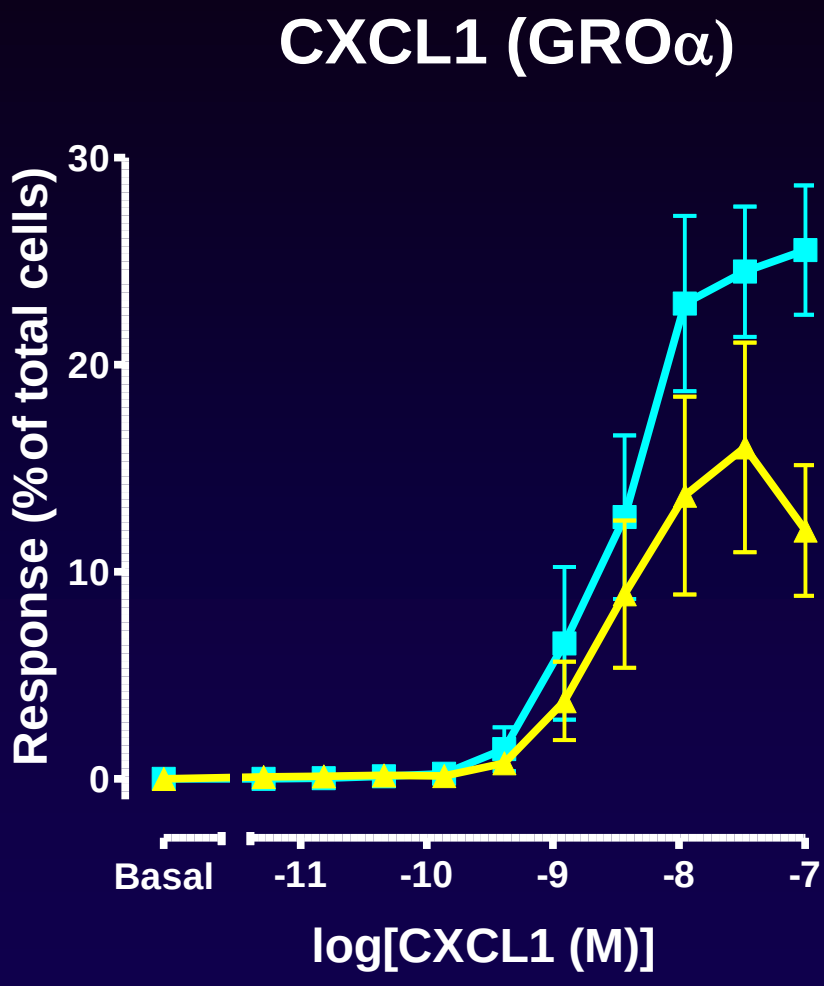
Effect of SB332235 on Neutrophil Migration to CXCL8 (IL-8) and CXCL1 (GROα)



▲ Non-smokers n=6 ● Smokers n=6 ■ COPD n=6

Neutrophil Migration to CXCL8 and CXCL1



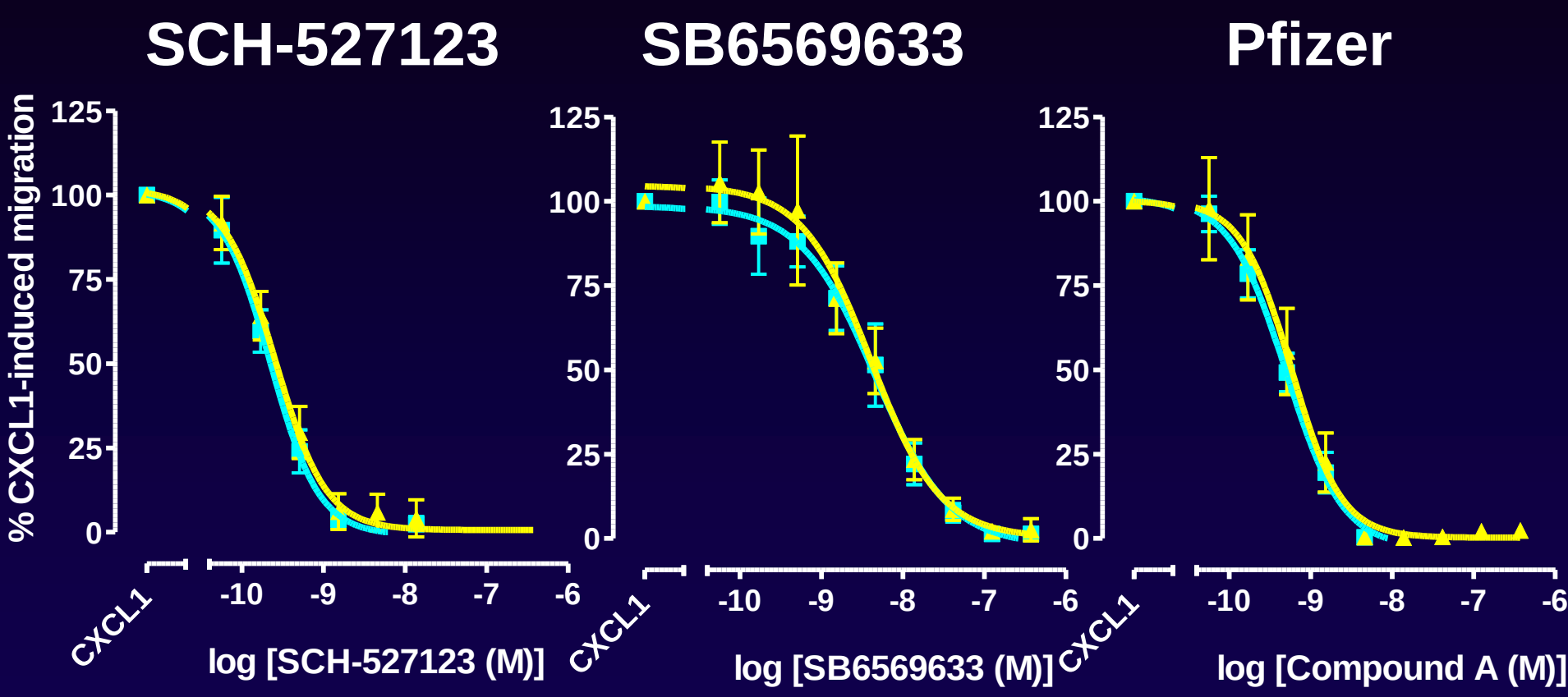


▲ Non-smokers n=7

■ COPD n=7

Stevens *et al*., 2010 ERS

Effect of CXCR1/2 antagonists on CXCL1 (GROα) – induced neutrophil migration

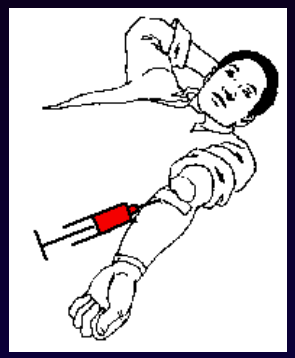


▲ Non-smokers n=6 ■ COPD n=7

Stevens *et al*., 2010 ERS

Assessing Antagonists – Whole Blood

Pre-clinical : *in vitro*

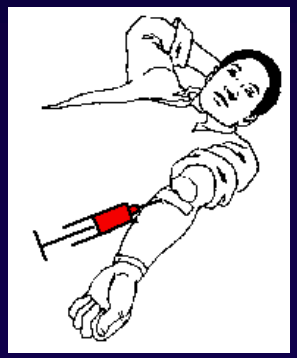


Venepuncture

DRUG

Stimulus

Clinical : *ex vivo*



DRUG

Venepuncture

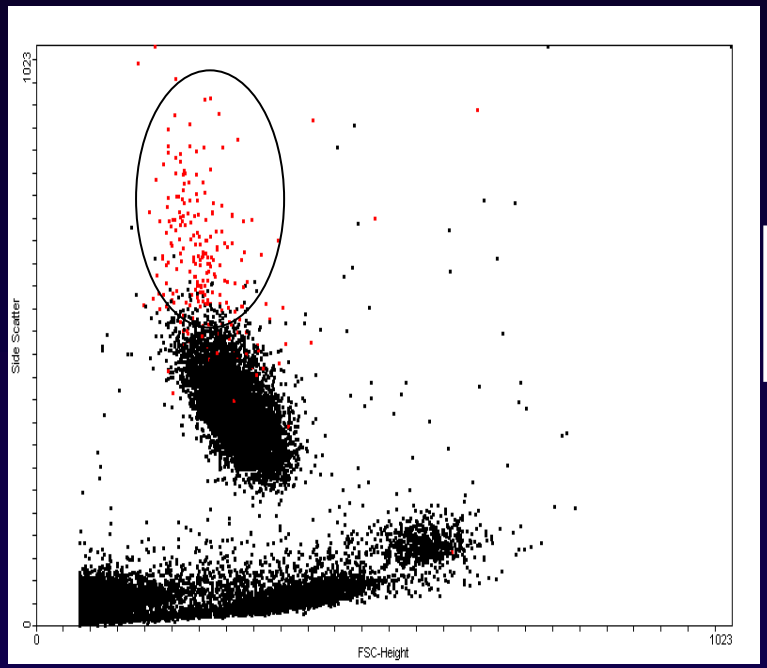
Stimulus

Flow cytometry:
- **Shape change**
- **CD11b**

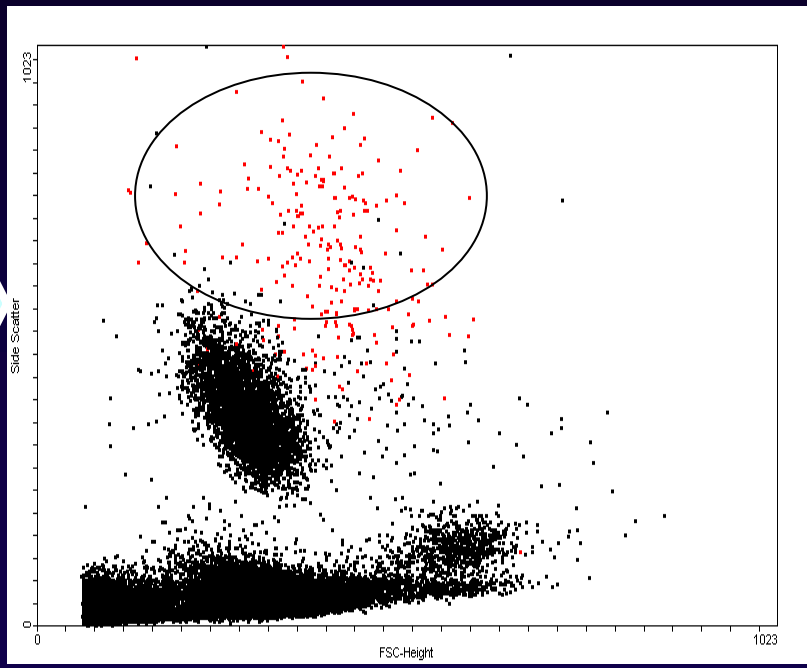
From: TT Hansel, NHLI

Gated Auto-Fluorescence Forward Scatter (GAFS)

SS : granularity



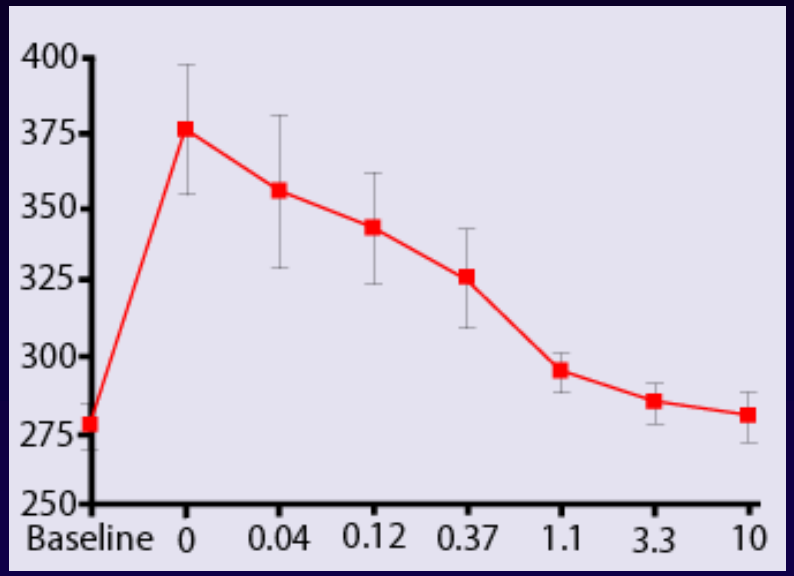
Eotaxin



FS : shape

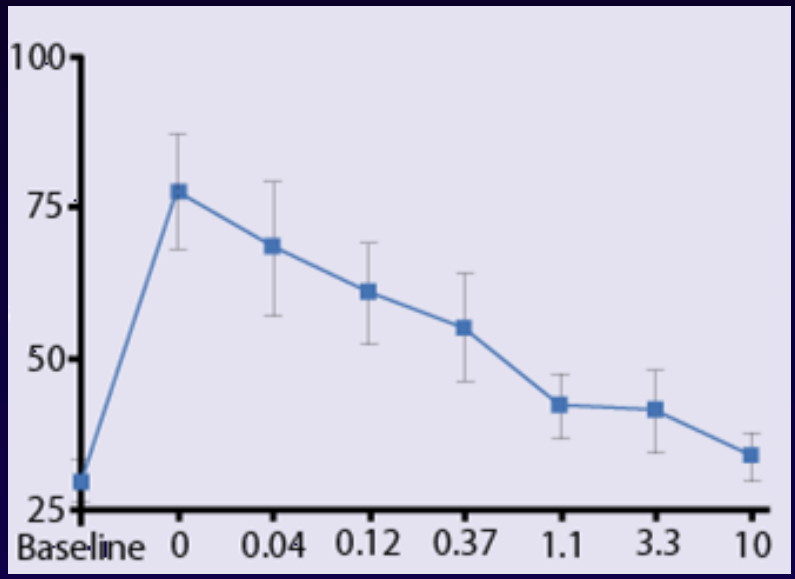
Effect of CXCR2 antagonist on CXCL1 (10 nM) stimulated COPD neutrophils

Shape Change



IC_{50} = 400 nM

CD11b



IC_{50} = 450 nM

Nicholson *et al*., 2007 PPT

Clinical Trials – CXCR1/2 Antagonist

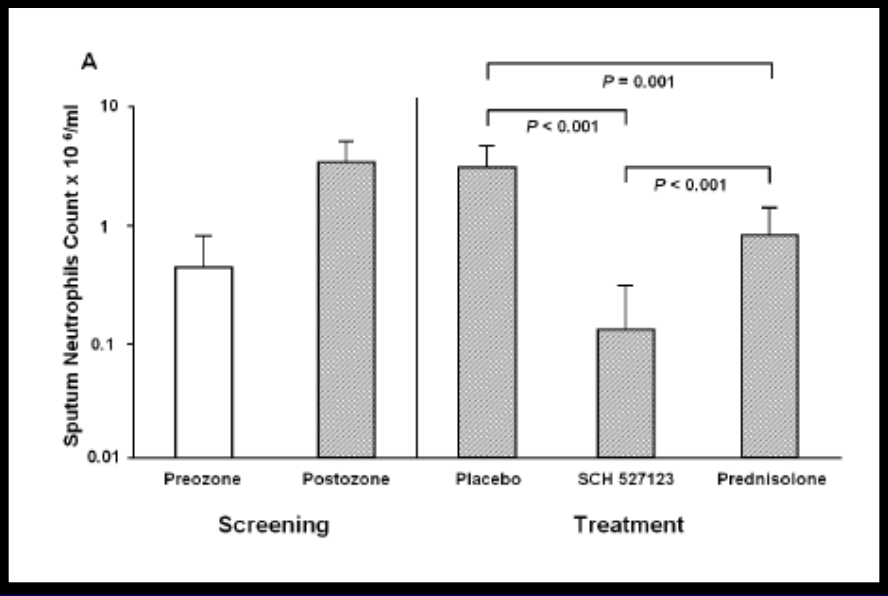
SCH 527123

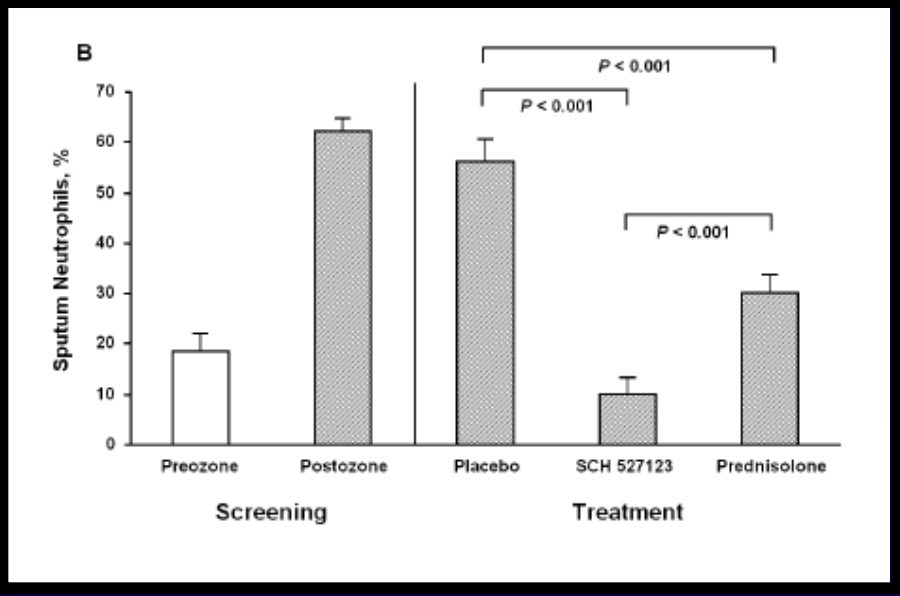
Double blind, placebo controlled trial

SCH 527123 50mg for 4 days – healthy volunteers

Ozone challenge – induce sputum neutrophilia

Results





Holz *et al.*, ERJ, 2010

Clinical Trials – CXCR1/2 Antagonist

SCH 527123

Double blind, placebo controlled, escalating dose trial

SCH 527123 – 12 weeks 3,10, 30mg – COPD patients

Results

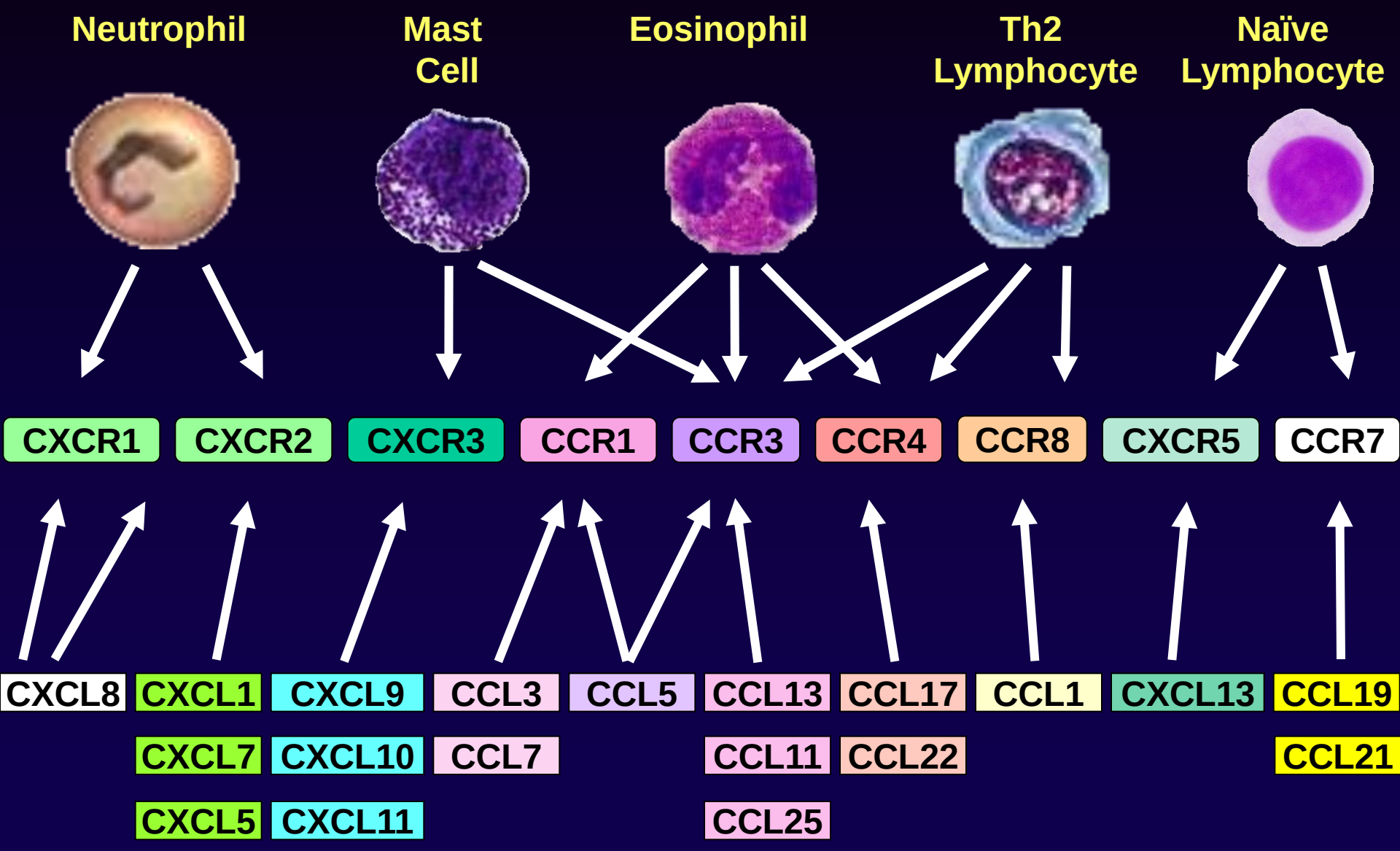
↓ Sputum neutrophils (47%)

↓ Sputum MMP-9 (59%)

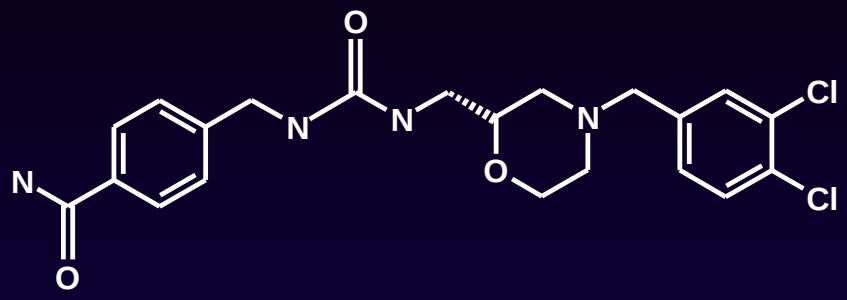
↑FEV_1 (91ml)

Magnussen *et al*., ERS, 2010

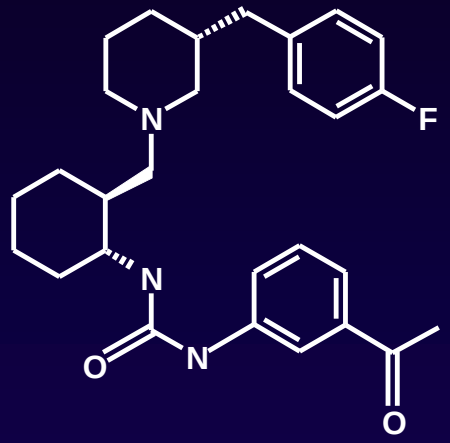
Which chemokine receptors to target? Asthma



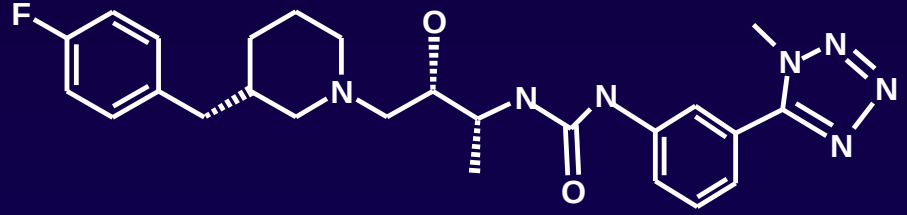
CCR3 Antagonists



GW-766994

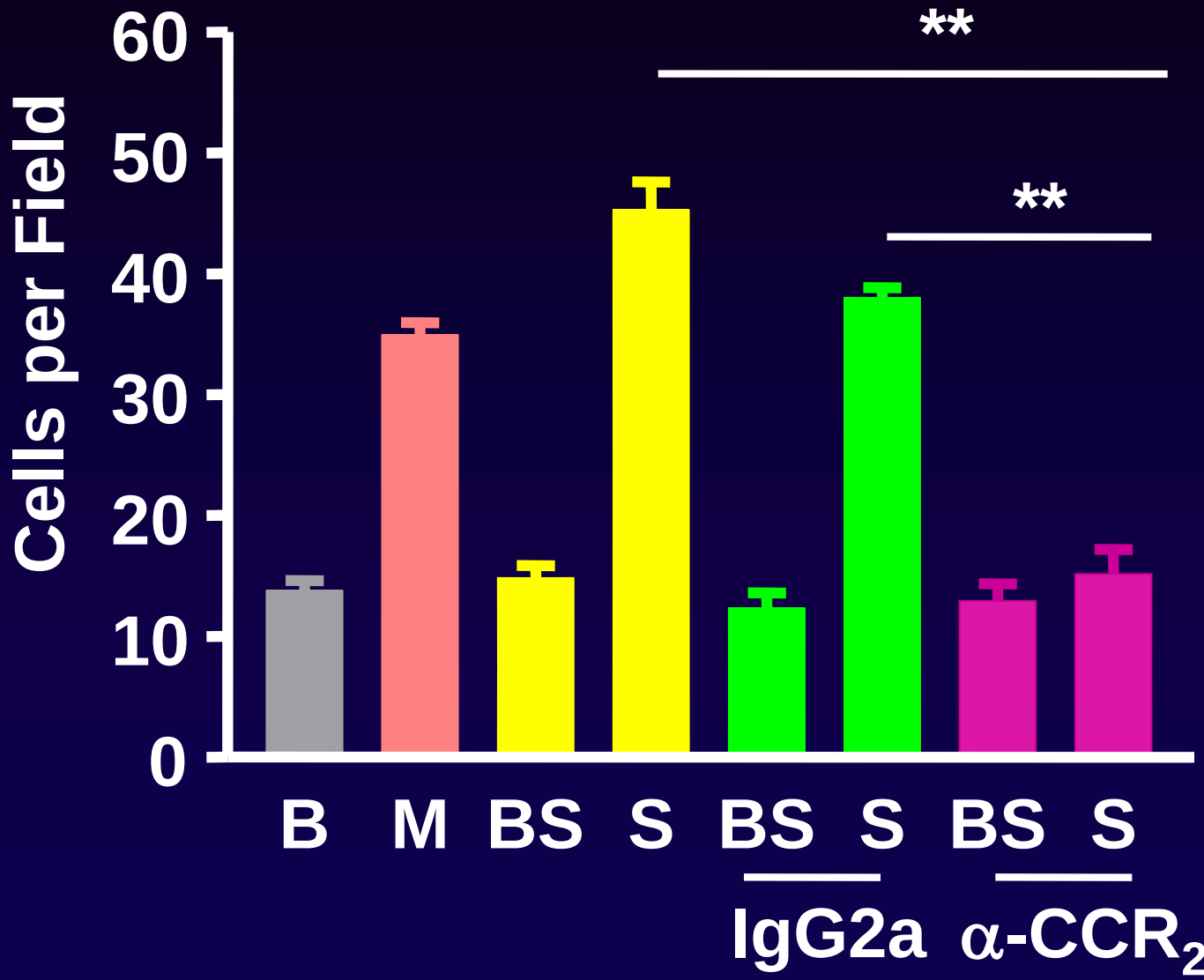


DPC-168

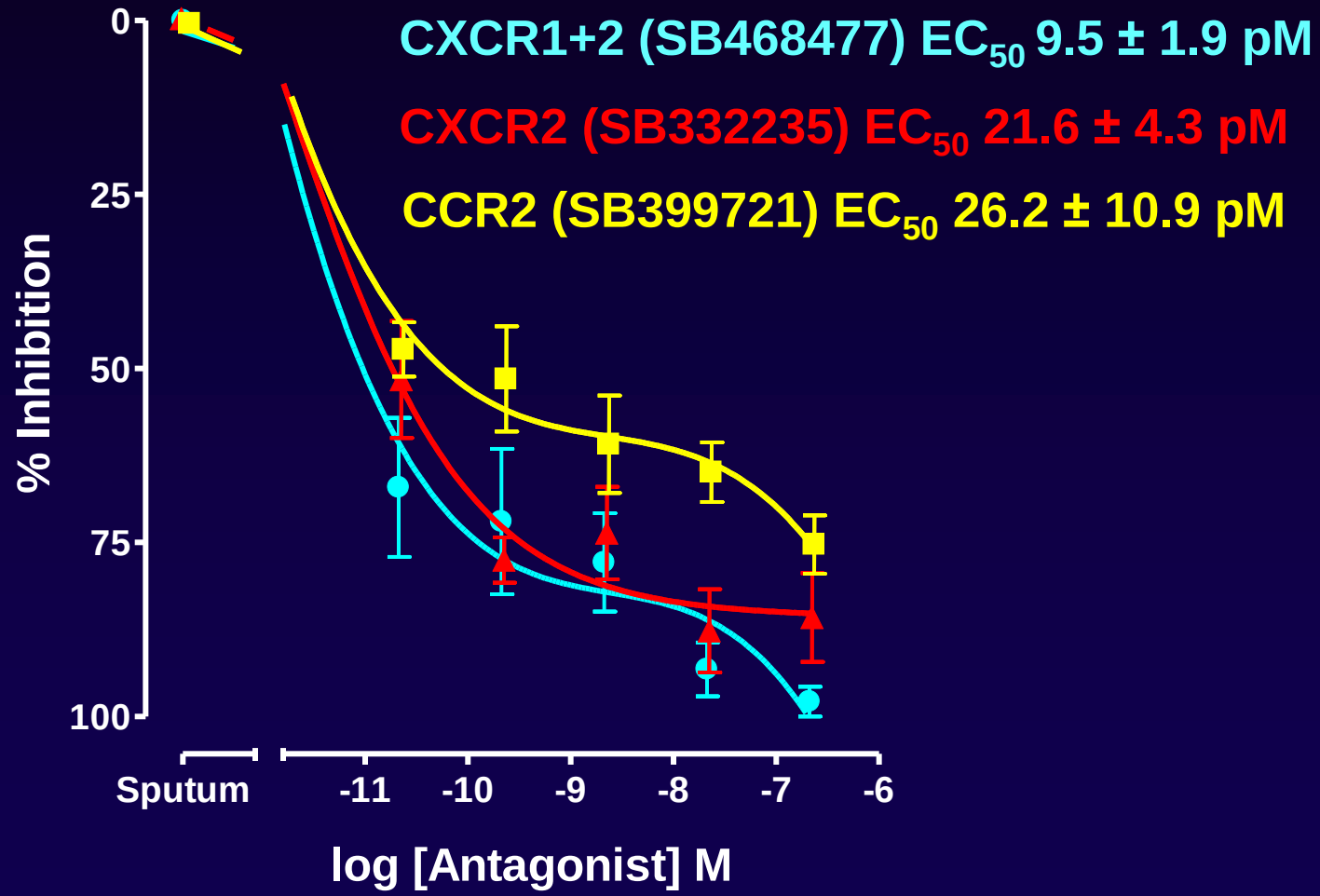


BMS-639623

Effect of 10μg/ml Antibody on Monocyte Chemotaxis to Sputum



Effect of Receptor Antagonists on Monocyte Chemotaxis to Sputum



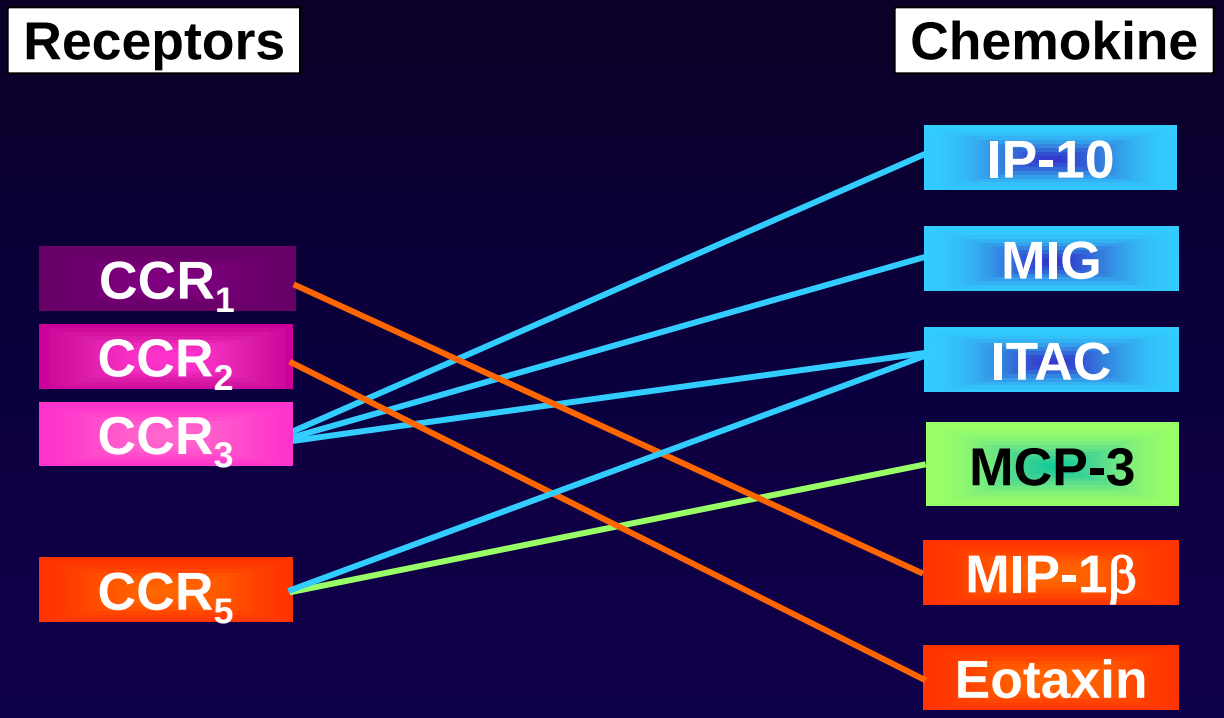
Chemokine Receptor Antagonism

Antagonists are more effective than IC_{50} values would suggest

SB468477:	**CXCR1 67 nM** **CXCR2 12 nM**
SB332235:	**CXCR2 19 nM**
SB399721:	**CCR2 219 nM**

More efficacious than would be predicted

Natural Chemokine Receptor Antagonists



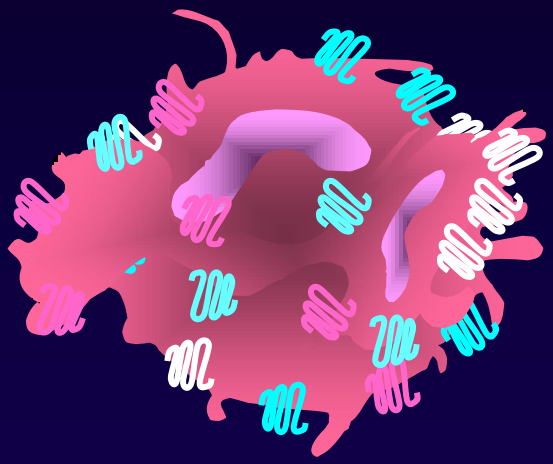
Summary

- Measuring effect of a chemokine receptor antagonist *vs* a single agonist does not predict efficacy *vs* a complex inflammatory infiltrate
- Chemokine receptor antagonists have increased efficacy *vs* complex inflammatory fluids (chemotaxis assay)

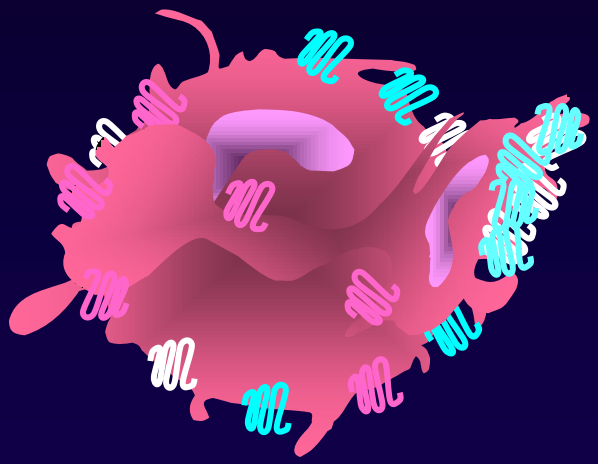
Why are antagonists more effective against mixtures?

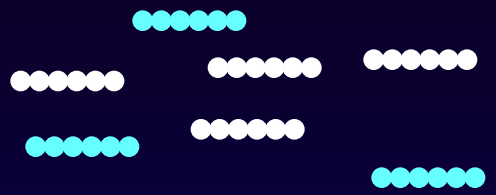
Wang *et al* 2002 Nature Cell Biol

Chemotaxis to a single agonist



Chemotaxis to a mixed agonists





Chemotaxis to mixed agonists in the presence of an antagonist

Steric hindrance?

Other reasons

Receptor-Receptor interactions

- homodimerization
- heterodimerization
- oligomerization

Chemokine-Chemokine interactions

- heterodimerization

Receptor-Receptor Interactions

Homodimers

- CCR2, CCR5, CXCR2, CXCR4

Heterodimers

- CCR2-CCR5 →
- CCR2-CXCR4
- CCR5-CXCR4 →

CCR5 ligands inhibit CCR2 ligand binding

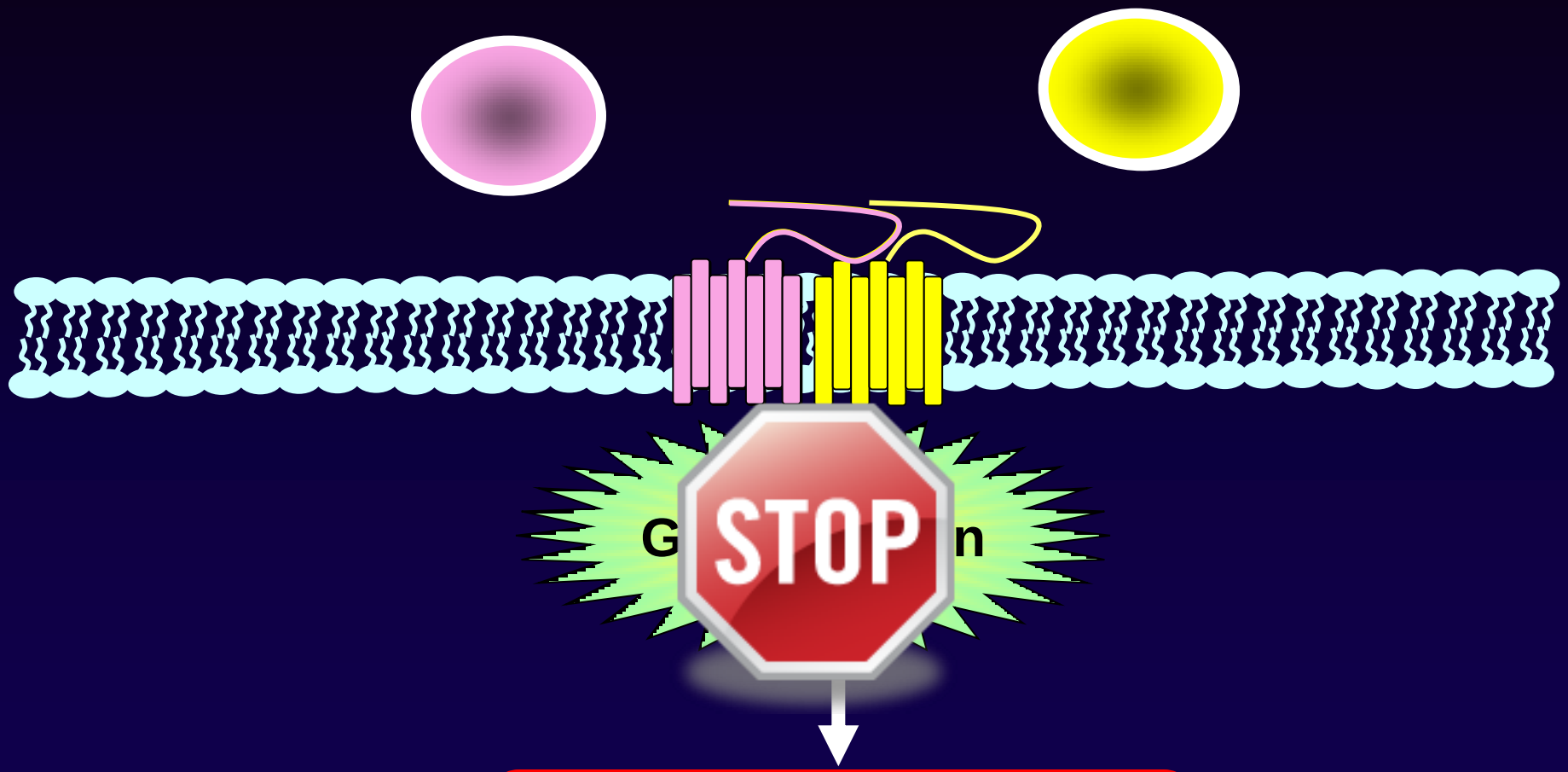
Modulate T cell function

Contento *et al 2008* PNAS

Oligomers

- CCR2/CXCR4/CCR5/CXCR2

CXCR4 Antagonist



Pello *et al 2008* Eur J Immunol

Chemokine Receptors – Dimers with other GPCR

CXCR2 - δ opioid receptor

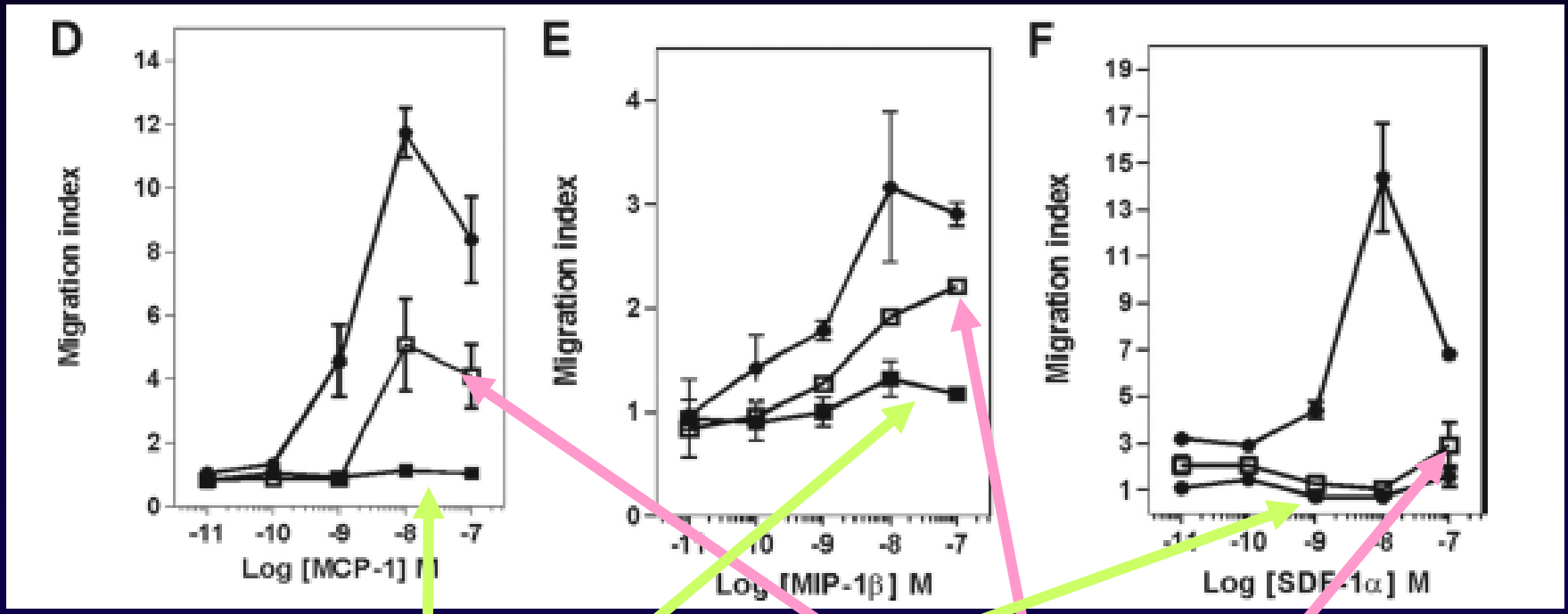
+ CXCR2 Antagonist

Enhance δ opioid receptor signalling

Parenty *et al 2008* Biochem J

Human CD4+ Lymphocytes

CCR2 CCR5 CXCR4



CCR2/5 antagonist

CXCR4 antagonist

Sohy *et al 2009* J Biol Chem

Chemokine-Chemokine Interactions

Homodimers

- IL-8, IP-10, MCP-1, RANTES, TARC, NAP-2 etc

→ **IL-8 dimers reduced CXCR1 binding**
Fernando *et al 2004* JBC

Heterodimers

- IL-8-PF-4
- MIP-1α-MIP-1β

→ **↑IL-8/CXCR2-dependent migration**
↑PF-4-dependent-anti-proliferative effect on EC
Nesmelova *et al 2005* JBC

Oligomers

TARC, PF-4, NAP-2 etc

Summary

Redundancy

- Little redundancy in the system
- Need to understand biology further

Target identification

- Need to understand receptor dimerization/oligomerization
- Cellular expression patterns

  eg. 40% monocytes express CXCR2
- Single antagonists *vs* dual antagonists(?)