# Discovery of Dual Antagonists of CRTH2 and DP Receptors: Novel Potential Treatment for Asthma

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## Asthma Overview

- **Asthma affects over 100 million people world-wide**
  - –Approximately 5%-10% of adults and 10%-15% of children suffer from asthma globally
  - –Prevalence continues to increase
  - –Annual expenditure for respiratory drugs in US alone totals $14 billion
- **Asthma is a chronic inflammatory disease of the airways with both:**
  - –Acute responses
    - •Contraction of airway smooth muscle
    - •Mucus hypersecretion
    - •Vasodilation
    - •Microvascular leakage
    - •Airway hyperresponsiveness
  - –Chronic
    - •Injury/repair of bronchial epithelium
    - •Recurrent exacerbations
    - •Airway remodeling
    - •Permanent airflow obstruction



## Prostaglandin $D_2$ ($PGD_2$)

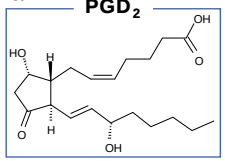
Synthesized by mast cells, Th2 cells and dendritic cells

Released by mast cells in large amounts during asthmatic responses

- Major prostanoid released by mast cells following immune challenge[1]
- Bronchoalveolar lavage fluid levels of PGD2 increase dramatically, reaching as much as ~30 ng/mL (~85 nM; mean ~9 ng/ml or ~26 nM)[2]

PGD2 mediates pathophysiological effects relevant to asthma:

- Bronchoconstriction
- Microvascular leakage
- Eosinophil, basophil and Th2 chemotaxis
- Mucus secretion




Transgenic mice overexpressing PGD synthase display elevated pulmonary infiltration of inflammatory cells and Th2 cytokine production after antigen challenge[3]

[1] *J Immunol* 1982; 129:1627.
[2] *Am J Respir Crti Care Med* 2000; 162:637.
[3] *J Immunol* 2002; 168:443.



## DP (DP1) Overview

**DP (D Prostanoid receptor)**

Gαs-type G protein-coupled receptor (cAMP)
Cloned in 1995[1]

**Expression**

Airway epithelium, smooth muscle, platelets and at low levels on basophils, eosinophils, mast cells and dendritic cells, NK T cells

**Functions**

Vasodilation and mucus secretion

DP ko mice display reduced hyperresponsiveness to acetylcholine, reduced pulmonary eosinophilia and reduced Th2 cytokine levels after antigen challenge[2]

DP antagonist decreases eosinophil infiltration in guinea pig asthma model and decreases nasal resistance in guinea pig rhinitis model[3]

**Genetic Validation**

SNPs in DP promoter suggest that increased expression of DP is linked to asthma while decreased expression of DP may be protective

[1] *J Biol Chem* 1995; 270:18910.
[2] *Science* 2000; 287:2013.
[3] *J Pharmacol Exp Ther* 2001; 298:411.

# CRTH2 (DP2) Overview

**CRTH2 (Chemoattractant receptor-homologous molecule expressed on TH2 cells)**

- Gαi-type G protein-coupled receptor (calcium)
- First reported in 1999[1]
- Prostaglandin D2 (PGD2) was identified as the natural ligand in 2001[2]

**Expression**

- TH2 cells, eosinophils, basophils, mast cells, neutrophils

**Functions**

- Chemotaxis of eosinophils, basophils, TH2 cells
- Eosinophil activation and degranulation
- Eosinophil mobilization from bone marrow
- Cytokine production from Th2 T cells
- Leukotriene production by mast cells

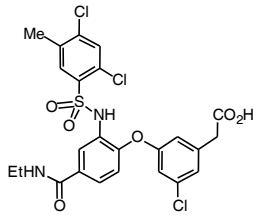
**Genetic Validation**

- SNPs in 3' UTR of CRTH2 gene result in higher stability mRNA, and are positively linked to asthma in African Americans and Chinese populations

[1] *J Immunol* 1999; 162:1278.
[2] *J Exp Med* 2001; 193:255.

# HTS Lead

**Phenylacetic Acid Derivative**

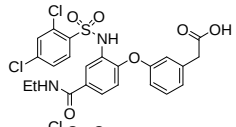
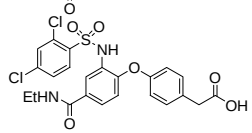
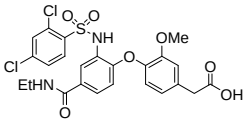
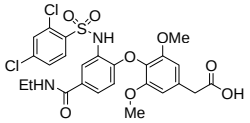
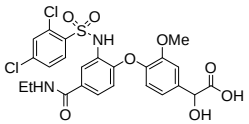


$^3$H-PGD2 binding assays
hCRTH2 $IC_{50}$ = 10 nM (buffer)
hCRTH2 $IC_{50}$ = 2,700 nM (100% h-plasma)
hDP $IC_{50}$ = 8,000 nM (buffer)

* $^3$H-$PGD_2$ binding assay with 293 transfected human cell line



# CRTH2 / DP Cross-Activity

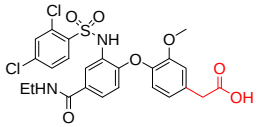
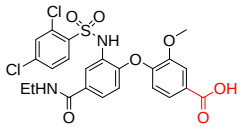
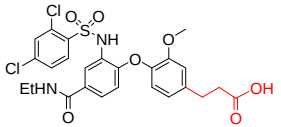
| Compound | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|
|  | 0.006 | >10.0 |
|  | 0.004 | 0.120 |
|  | 0.003 | 0.055 |
|  | 0.002 | 7.04 |
|  | 1.0 | 0.050 |

- *para*-Substituted phenylacetic acids offer good cross-activity between CRTH2 and DP
- The α-substitution of the phenylacetic acid gives a DP-selective compound

$^3$H-$PGD_2$ binding assay with 293 transfected human cell line



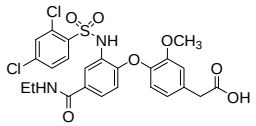
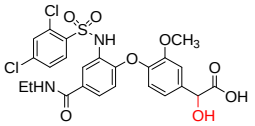
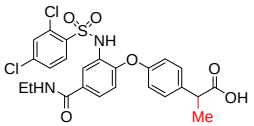
# Acid Linker

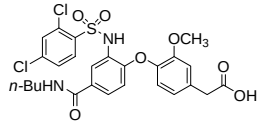
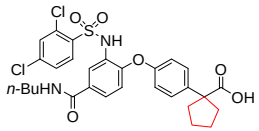
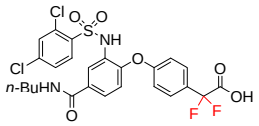
| Compound | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|
|  | 0.003 | 0.055 |
|  | >50 | >12.5 |
|  | 0.74 | 2.07 |

- The optimal distance between the acid and the phenyl ring is one carbon

* $^3$H-$PGD_2$ binding assay with 293 transfected human cell line

# α-Substitution of Phenylacetic Acid

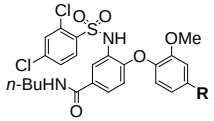
| Compound | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|
|  | 0.003 | 0.055 |
|  | 0.917 | 0.050 |
|  | 0.108 | 0.043 |

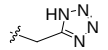
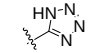
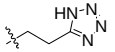
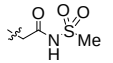
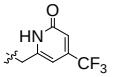
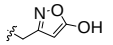
| Compound | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|
|  | 0.003 | 0.012 |
|  | >12.5 | 0.013 |
|  | 0.423 | 0.003 |

- α-Substitution tends to maintain DP activity but decrease CRTH2 activity



# Carboxylic Acid Replacements



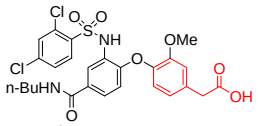
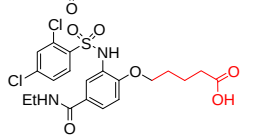
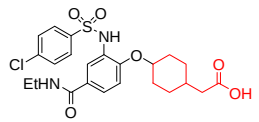
| R | CRTH2 binding $IC_{50}$ (μM)* | DP binding $IC_{50}$ (μM)* |
|---|---|---|
|  | 0.003 | 0.012 |
|  | 0.351 | 0.007 |
|  | 6.41 | 0.29 |
|  | 2.09 | 0.19 |
|  | 0.33 | 0.006 |
|  | 4.58 | 0.011 |
|  | 0.43 | 0.053 |

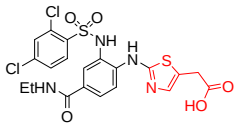
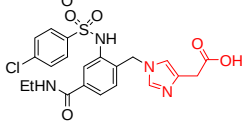
- Several carboxylic acid replacements maintain DP activity, decrease CRTH2 activity

* $^3$H-$PGD_2$ binding assay with 293 transfected human cell line



# Replacements of the Phenyl Acetic Acid Ring

| Compound | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|
|  | 0.003 | 0.012 |
|  | 0.345 | 1.683 |
|  | 5 | |

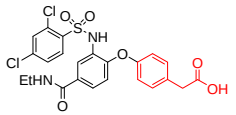
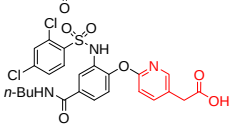
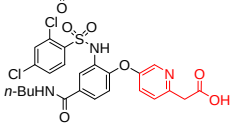
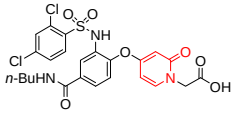
| Compound | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|
|  | 0.448 | >12.5 |
|  | 0.251 | >12.5 |

- Saturated alkyl groups impart decreased activity
- Some heterocycles show selective activity for CRTH2 over DP

* $^3$H-$PGD_2$ binding assay with 293 transfected human cell line

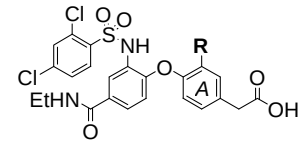
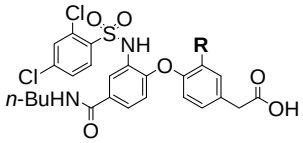


# Replacements of the Phenyl Acetic Acid Ring

| Structure | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|
|  | 0.006 | 0.120 |
|  | 0.315 | 0.479 |
|  | 0.010 | 0.231 |
|  | 8.25 | 11.37 |

- 2-Pyridine is a good replacement for the *A* ring

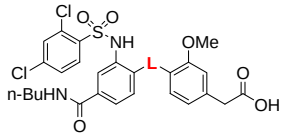
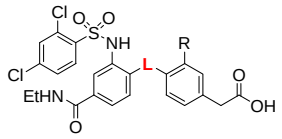
## 3-Substitutions on the *A* Ring

| Structure | R | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|---|
|  | H | 0.004 | 0.120 |
| | OMe | 0.003 | 0.055 |
| | OEt | 0.002 | 1.91 |
|  | OMe | 0.003 | 0.012 |
| | Cl | 0.006 | 0.008 |
| | F | 0.007 | 0.030 |
| | Me | 0.003 | 0.006 |

• 3-OMe, Cl, F, or Me enhance or maintain both activities



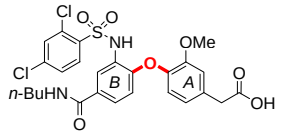
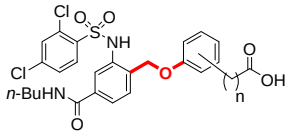
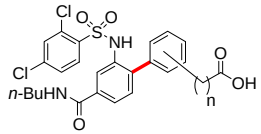
## Modifications of the Oxygen Linker

| Structure | L | R | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|---|---|
|  | O | | 0.003 | 0.012 |
| | $CH_2$ | | 0.002 | 0.005 |
|  | O | H | 0.004 | 0.045 |
| | $CH_2$ | H | 0.015 | 0.569 |
| | $SO_2$ | H | 1.73 | >12.5 |
| | NH | Br | 0.055 | 0.234 |

• Methylene linker is a good replacement for the oxygen linker



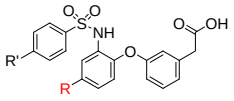
## Modifications of the Oxygen Linker

| Structure | Regio-isomer | n | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|---|---|
|  | | | 0.003 | 0.012 |
|  | *p* | 1 | >12.5 | >12.5 |
| | *m* | 1 | >12.5 | >12.5 |
| | *p* | 2 | >12.5 | >12.5 |
| | *o* | 2 | >12.5 | >12.5 |
|  | *p* | 1 | >12.5 | **2.81** |
| | *m* | 1 | **0.869** | >12.5 |
| | *p* | 2 | >12.5 | **1.23** |
| | *m* | 2 | >12.5 | **0.120** |

• The optimal distance between *A* ring and *B* ring is one atom



## Amide Moiety Replacements

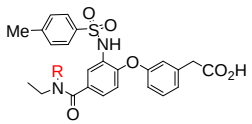
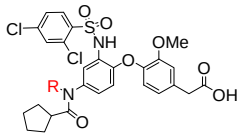
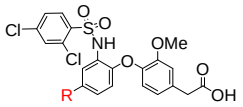
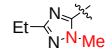


| R | R' | CRTH2 binding IC50 (μM) * |
|---|---|---|
| Cl | H | 0.675 |
| MeO | H | 1.40 |
|  | H | 0.380 |
|  | H | 2.00 |
|  | H | >10 |
|  | H | 0.047 |
|  | H | 0.013 |
|  | Me | 1.10 |
|  | Cl | 0.007 |
|  | Cl | 0.016 |

• The primary or secondary amide at the center phenyl ring can be replaced by reversed amide and urea while maintaining CRTH2 activity

* $^3$H-$PGD_2$ binding assay with 293 transfected human cell line.

# Amide Moiety: The Importance of an H-Donor

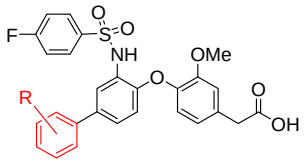
| Structure | R | CRTH2 binding $IC_{50}$ (μM)* | DP binding $IC_{50}$ (μM)* |
|---|---|---|---|
|  | H | 0.004 | |
| | Et | 1.10 | |
|  | H | 0.006 | 0.019 |
| | Me | >12.5 | 3.47 |
|  |  | 0.006 | 0.120 |
| |  | 0.006 | 0.015 |
| |  | 0.864 | 0.016 |
| |  | 0.159 | 0.010 |

• H-donor seems to be important to CRTH2 activity but does not necessarily affect DP activity

* $^3$H-$PGD_2$ binding assay with 293 transfected human cell line



# Amide Moiety Replacements: Aromatic Ring Without NH

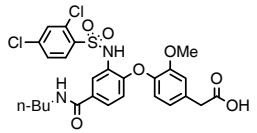
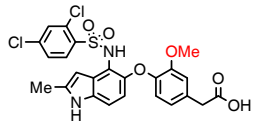
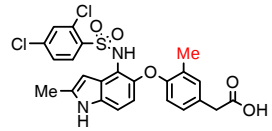
| Structure | R | CRTH2 binding $IC_{50}$ (μM)* | DP binding $IC_{50}$ (μM)* |
|---|---|---|---|
|  | 4-Et | 0.118 | 0.008 |
| | 4-n-Bu | 0.200 | 0.015 |
| | 3-i-Pr | 0.241 | 0.043 |
| | 4-Cl | 0.137 | 0.088 |
| | 3-Cl | 0.150 | 0.041 |

• Aryl replacements can improve DP activity, but generally decrease CRTH2 activity without NH

* $^3$H-$PGD_2$ binding assay with 293 transfected human cell line; N/A: data not available



# Indole as Amide Replacement

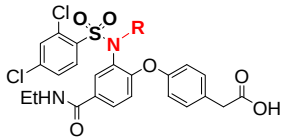
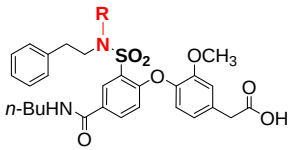
| Structure | CRTH2 binding $IC_{50}$ (μM)* | DP binding $IC_{50}$ (μM)* |
|---|---|---|
|  | 0.003 | 0.012 |
|  | 0.018 | 0.009 |
|  | 0.021 | 0.018 |

• Indole core compounds have comparable potency to that of amide analogs

* $^3$H-$PGD_2$ binding assay with 293 transfected human cell line.

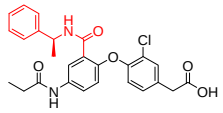
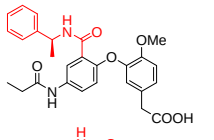
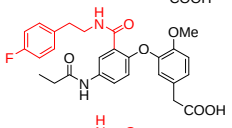
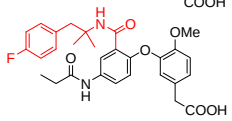


# Sulfonamide Methylation

| Compound | R | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|---|
|  | H | 0.004 | 0.045 |
| | Me | 0.354 | >12.5 |
|  | H | 0.136 | >12.5 |
| | Me | 0.029 | >12.5 |

• Sulfonamide NH may not be a requirement for CRTH2 activity, but it is important for DP activity

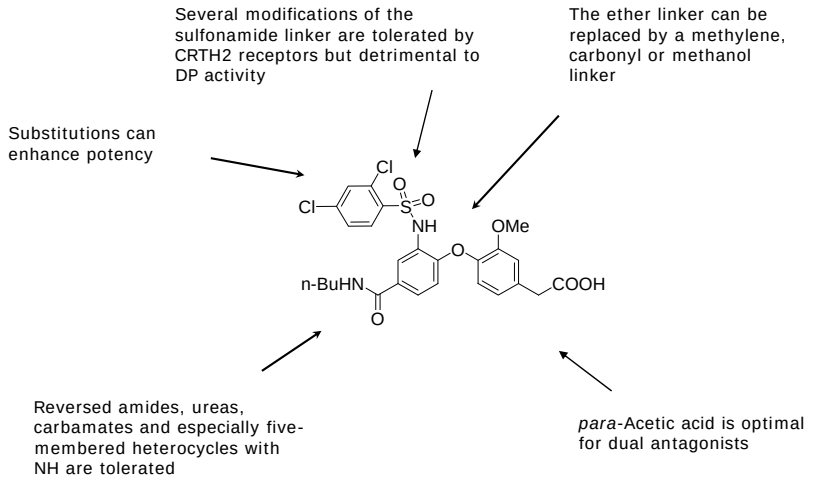
# Reversed Amide Replacement

| Structure | CRTH2 binding $IC_{50}$ (μM) | DP binding $IC_{50}$ (μM) |
|---|---|---|
|  | 0.003 | >10.0 |
|  | 0.001 | >10.0 |
|  | 0.002 | >12.5 |
|  | 0.002 | >12.5 |

- Reversed amides are excellent replacements with respect to CRTH2 activity but not with respect to DP activity

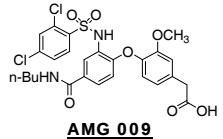


# SAR Summary






# CRTH2/DP Dual Antagonist: AMG 009



AMG 009

| CRTH2 Activities | $IC_{50}$(nM) |
|---|---|
| hCRTH2(293), Buffer Binding: | 3 |
| hCRTH2(293), 100% Plasma Binding: | 26 |
| hCRTH2(CEM), migration: | 5 |
| Sheep CRTH2, Buffer Binding: | 2 |
| Guinea Pig CRTH2, Buffer Binding: | 3 |

| DP Activities | (nM) |
|---|---|
| hDP(293), Buffer Binding ($IC_{50}$): | 12 |
| hDP(293), 50% Plasma Binding ($IC_{50}$): | 347 |
| hDP(293), cAMP (Ki, buffer): | 0.7 |
| hDP(293), cAMP (Ki, 33% plasma): | 143 |
| Sheep DP, cAMP (Ki, platelet rich plasma): | 1800 |

**Selectivity / Toxicity**

No activity against
- Receptor panel
- CYP450 (1A2, 2D6, 3A4)

HERG (patch clamp): 9% inhibition at 5μM

CYP450 2C9, $IC_{50}$: 44μM

| | |
|---|---|
| Ames test | negative |
| chromosal aber. (*in vivo/in vitro*) | negative |

| PK | Cls | MRT | Vdss | $F_{po}$ |
|---|---|---|---|---|
| Rat: | 1.2L/h/kg | 0.9h | 1.0L/kg | 28% |
| Dog: | 0.77L/h/kg | 1.4h | 1.0L/kg | 60% |
| Cyno: | 0.27L/h/kg | 1.3h | 0.37L/kg | 39% |

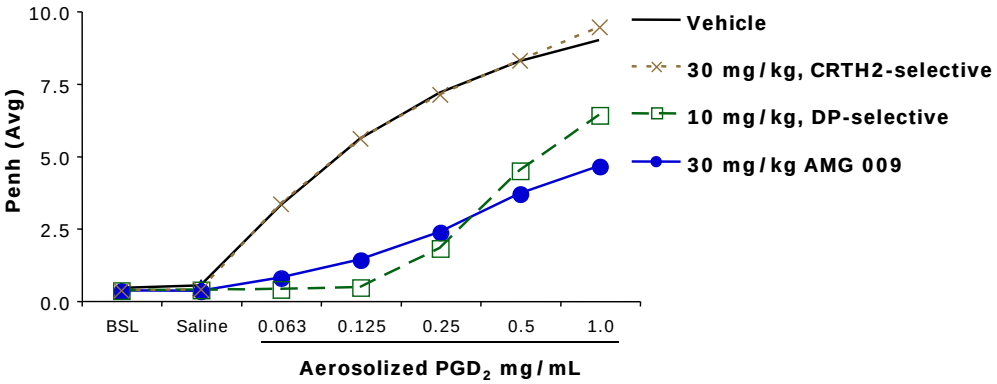
***Man: low predicted clearance (hepatocytes)***

**Plasma Protein Binding**

| | | | |
|---|---|---|---|
| Rat: | 99.8% | Sheep: | 99.4% |
| Monkey: | 99.7% | Human: | 99.2% |
| Dog: | 98.9% | | |



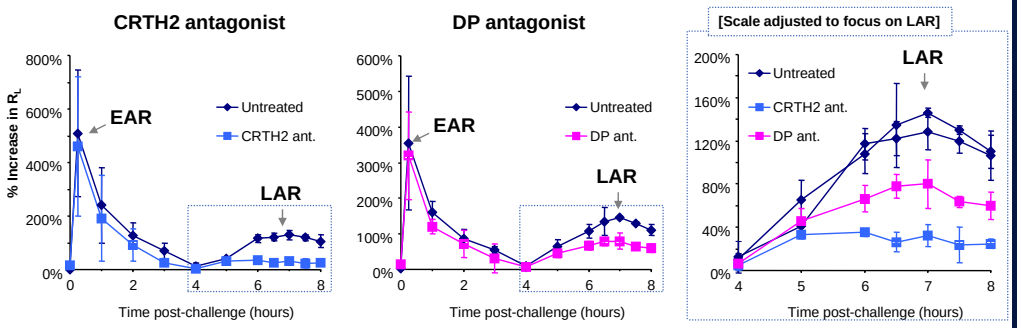
# Treatment with AMG 009 Blocks $PGD_2$-Induced Acute Airway-Constriction in Guinea Pigs




Compounds were dosed SC 15 minutes before challenge with aerosolized $PGD_2$
n=8 guinea pigs / group

## CRTH2 *and* DP are Involved in Late Airway Response to Allergen in Sheep (W.M. Abraham)

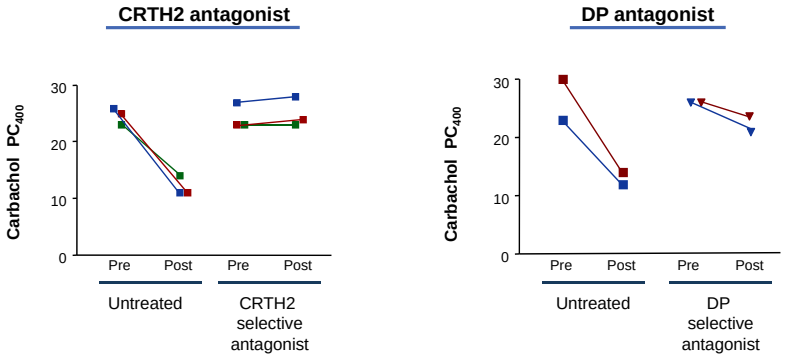
*Antagonism of either CRTH2 or DP reduces antigen-induced late airway response*




- $R_L$=Pulmonary resistance (L x cm $H_2O$/L/s)
- Mean values ± SEM
- Untreated group represents historical data for these individual sheep
- CRTH2 selective antagonist (15 mg/kg) was given SC BID for two days and 30 minutes prior to challenge with aerosolized *Ascaris suum* (n=3)
- DP selective antagonist (7.5 mg/kg) was give SC BID 60 minutes prior to antigen challenge (n=2)



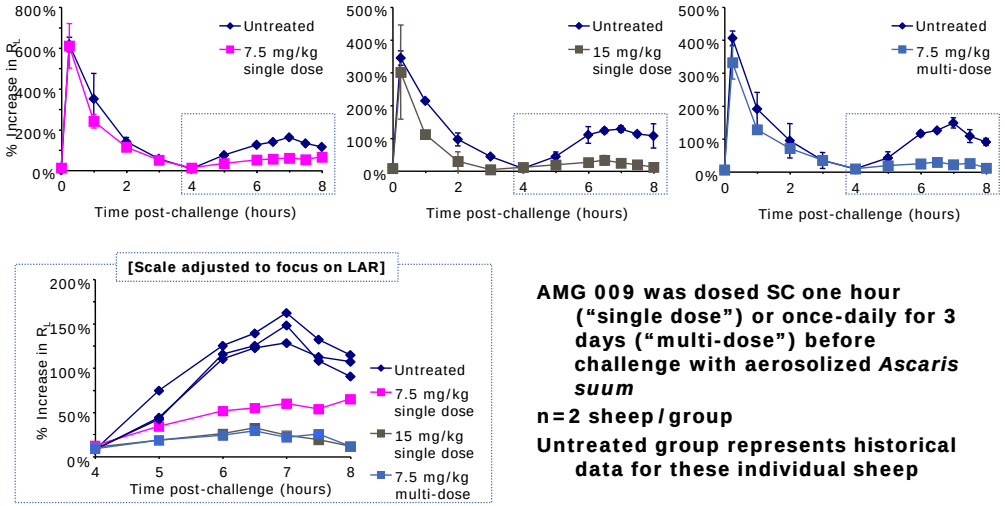
## CRTH2 *and* DP are Involved in Mediating Airway Hyper-reactivity to Carbachol (W.M. Abraham)




- CRTH2 selective antagonist (15 mg/kg) was given SC BID for two days and 30 minutes prior to challenge with aerosolized *Ascaris suum* (n=3 sheep/group)
- DP selective antagonist was given SC 1 h prior to challenge with aerosolized *Ascaris suum* (n=2 sheep/group)
- Untreated group represents historical data for these individual sheep



## Treatment with AMG 009 Inhibits Antigen-induced Late Airway Response in Sheep Allergic Airway Model



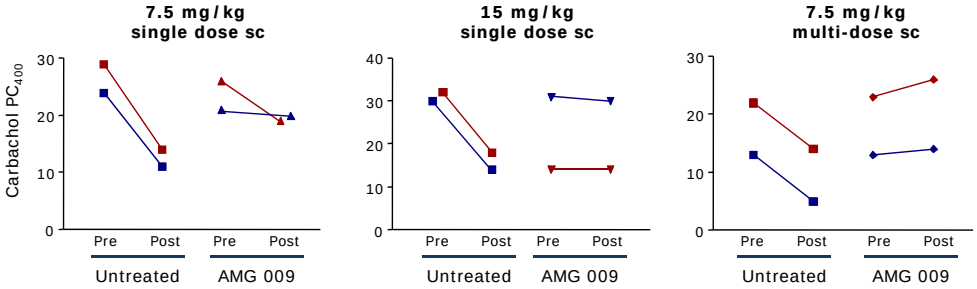

**AMG 009 was dosed SC one hour ("single dose") or once-daily for 3 days ("multi-dose") before challenge with aerosolized *Ascaris suum***

**n=2 sheep/group**

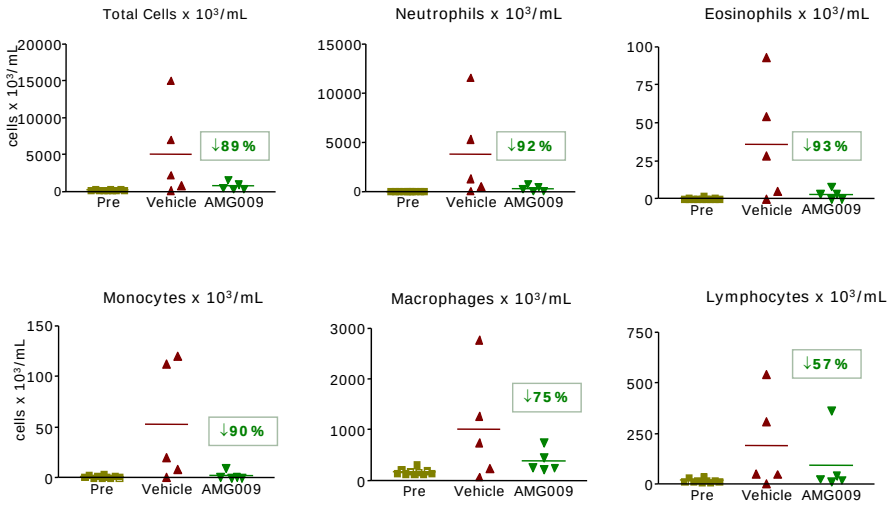
**Untreated group represents historical data for these individual sheep**



## Treatment with AMG 009 Blocks Antigen-Induced Development of Airway Hyperreactivity to Carbachol (W.M. Abraham)



## Treatment with AMG 009 Blocks Allergen-Induced Recruitment of Inflammatory Cells to the Lung in Sheep




AMG 009 was given SC once-daily for 3 days before segmental challenge with *Ascaris suum*

n=5 sheep/group in a crossover design



## Conclusions

- Identified AMG 009 as a potent dual antagonist of CRTH2 and DP receptors
- Demonstrated efficacy with AMG 009 in a guinea pig model of $PGD_2$-induced acute airway-constriction
- Demonstrated efficacy with CRTH2- and DP- selective antagonists and AMG 009 in sheep models of asthma
- AMG 009 was well tolerated in preclinical-safety models



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