Glenmark
A new way for a new world

Seventh Conference and Continued Education Program
of Society of Toxicologic Pathology India (STPI)
On Dermal, Juvenile and Immune Systems

Translational loss of PDE4 inhibitors induced cardiac changes in Mouse

Venkatesha Udupa
Glenmark Pharmaceuticals Limited
Mumbai, India

Acknowledgement

Glenmark R&D colleagues:

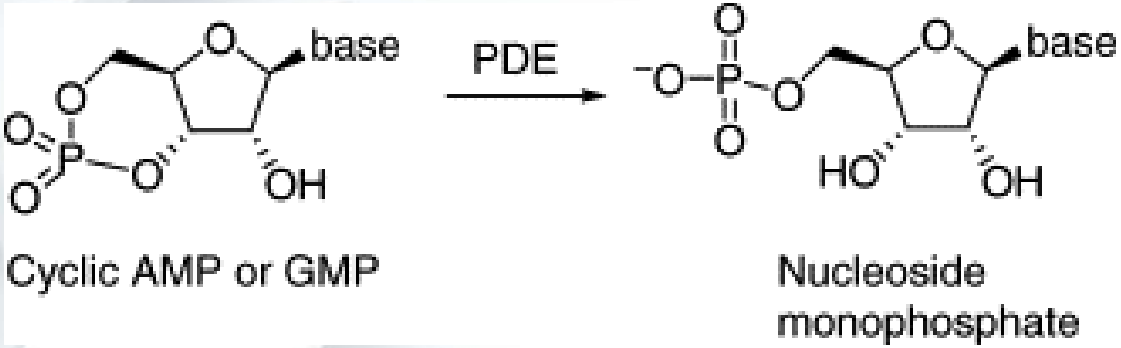
- Pramod Pawar
- Sanjay Gaikwad
- Vinod KR
- Pavankumar Sancheti
- Dattaprasad Bakre
- Zona Godsafe

Outline

- Introduction
  - Phosphodiestereases
  - PDE4 inhibitors in COPD and Psoriasis
- Heart lesions in various species with PDE4 inhibitors and their clinical outcome
- Materials and method for studying Compound A
- Heart lesions in mouse with Compound A
- Mechanism of injury
- Relevance/Translation of mouse heart lesions to man

PDE Inhibitors

- Phosphodiesterases (PDE) super family is large, complex and represents 11 gene families (PDE1 through PDE11).
- PDEs are enzymes responsible for the hydrolysis of cAMP and cGMP.

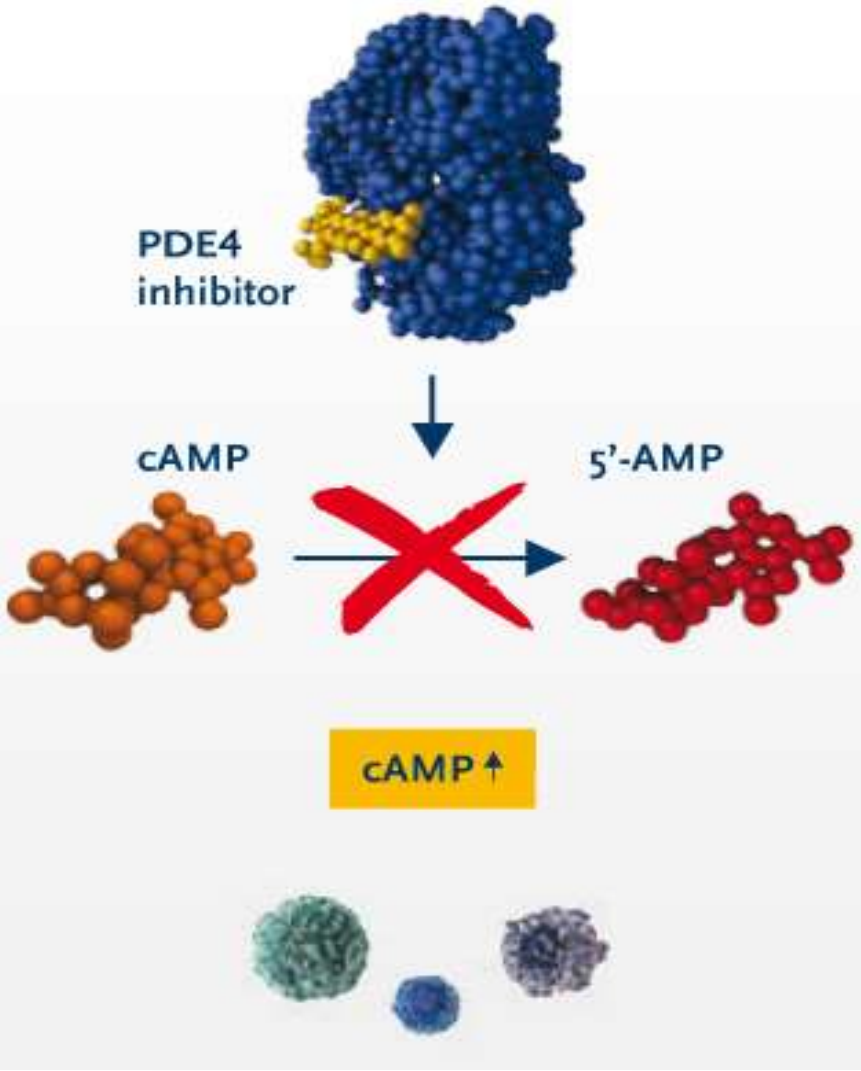


- cAMP and cGMP are intracellular secondary messengers involved in the regulation of multiple physiological processes, including **vascular resistance, cardiac output, visceral motility, immune response, inflammation, vision and reproduction**.
- PDE4 are **expressed in inflammatory cells, immune cells, airway smooth muscles, brain and cardiovascular tissue**.

PDE4 Inhibitors

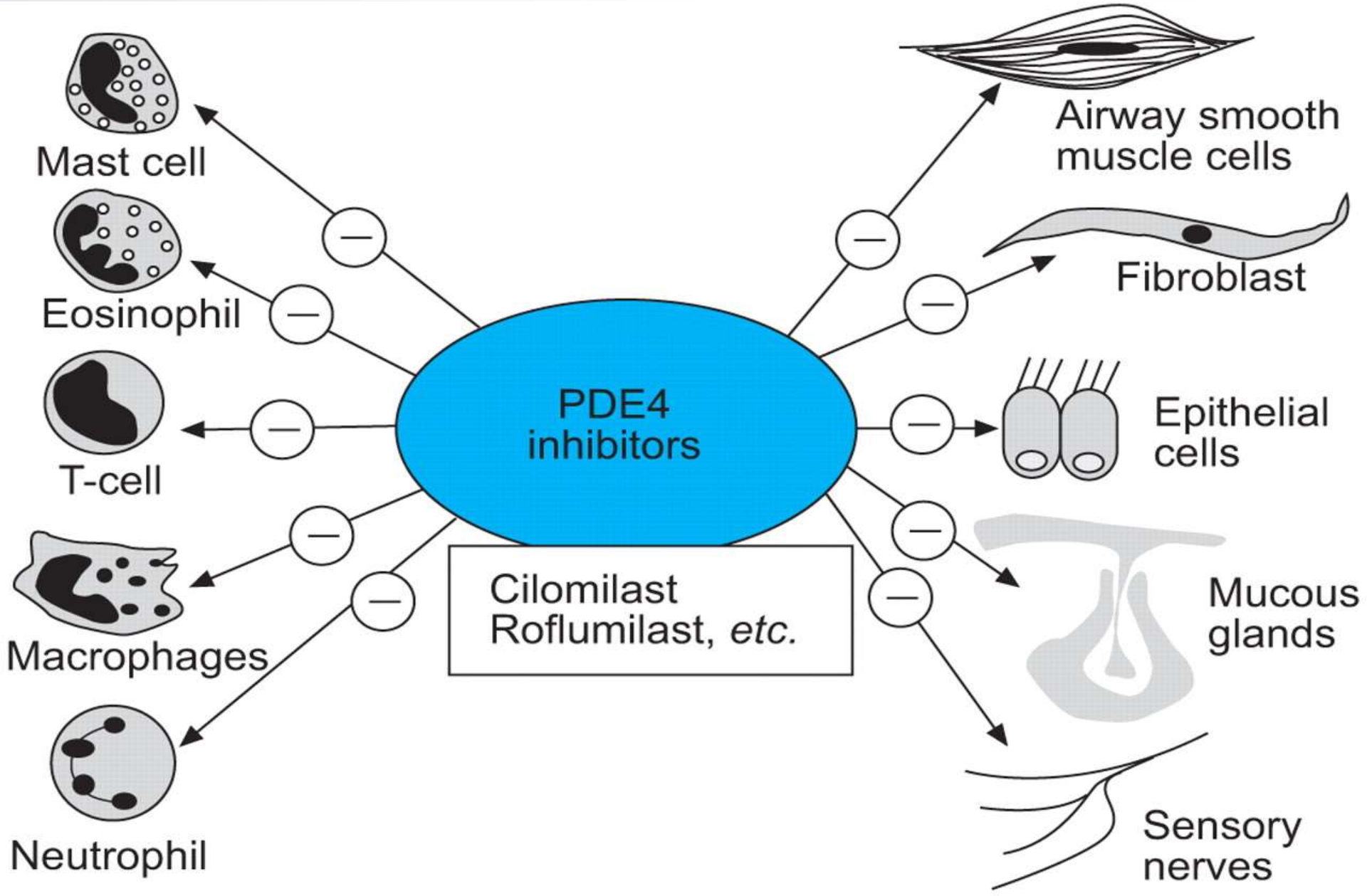
- Selective inhibition of PDE4 increases the cellular concentration of cAMP, which in turn
  - inhibits the chemotaxis,
  - inhibits cytotoxicity
  - activation of inflammatory cells and **causes indirectly relaxation of airway smooth muscle**.

- PDE4 activity has been recognized to contribute to a wide variety of diseases, like
  - asthma,
  - chronic obstructive pulmonary disease (COPD),
  - Psoriatic arthritis and
  - Psoriasis.



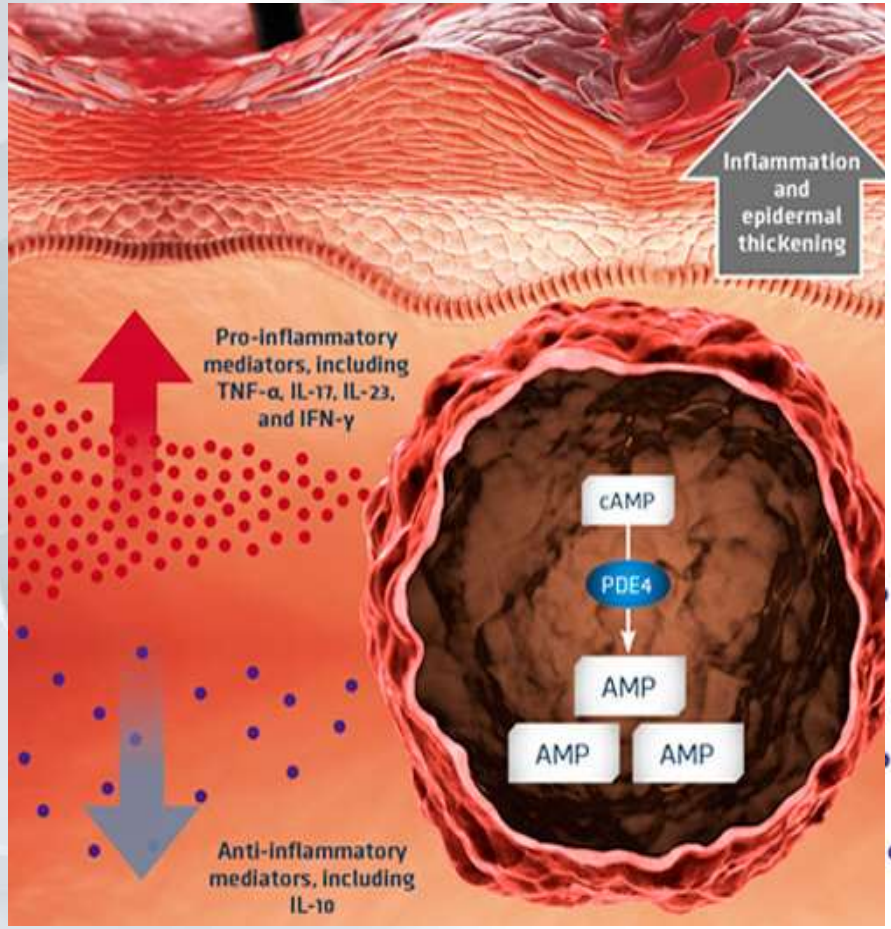
PDE4 Inhibitors and COPD

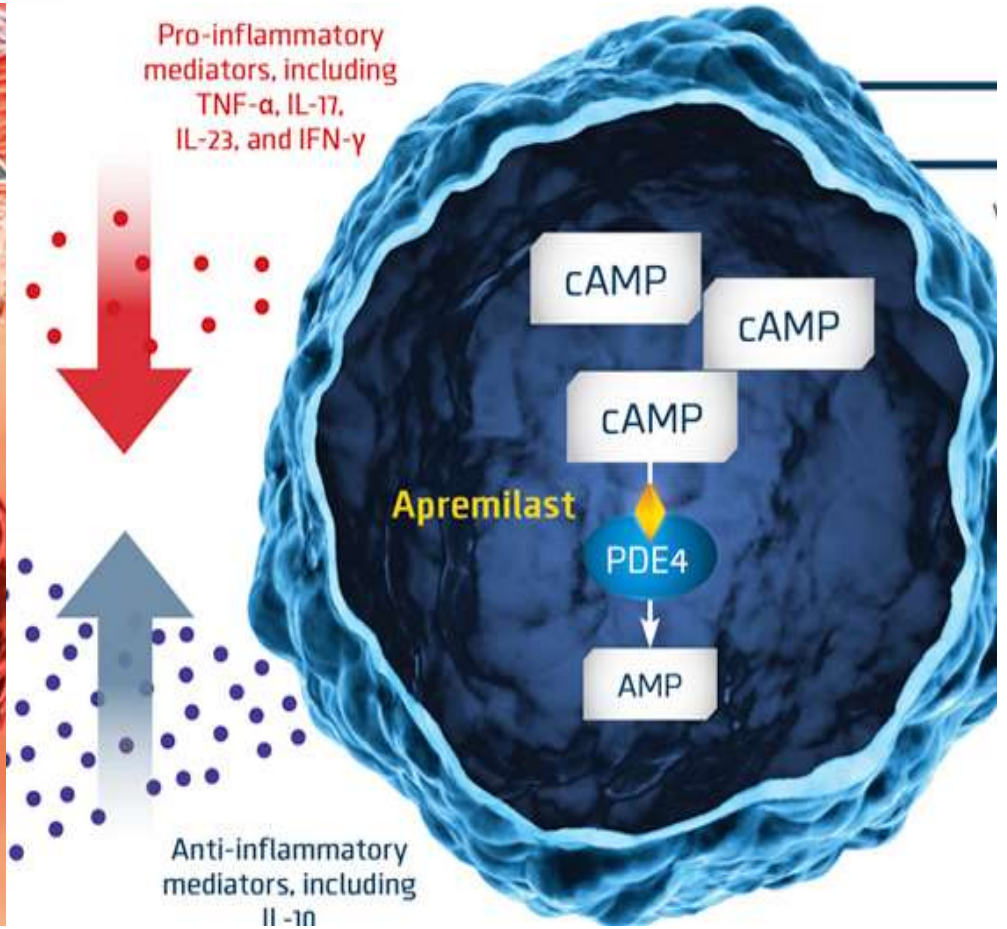
Glenmark
A new way for a new world



PDE4 Inhibitors and Psoriasis

Glenmark
A new way for a new world





https://www.otezla.com/

Currently available PDE4 Inhibitors

Glenmark
A new way for a new world

- Roflumilast (Daliresp, Daxas) - Initial US Approval 2011.
  - Indicated in **severe COPD** to decrease the number of flare-ups or the worsening of **COPD symptoms (exacerbations)**
  - Oral, may titrate dose from 250 mcg/day to 500 mcg/day (therapeutic dose) over 4 weeks to improve tolerability
- Apremilast (Otezla) - Initial US Approval 2014.
  - Approved for the treatment of patients with **moderate to severe plaque psoriasis** for whom phototherapy or systemic therapy is appropriate.
  - Approved for the treatment of adult patients with **active psoriatic arthritis at 10 to 30 mg/day.**
- Crisaborole (Eucrisa) - Initial US Approval 2016.
  - Topical (2% Ointment) treatment of mild to moderate atopic dermatitis in patients 2 years of age and older.

Heart Lesions with Roflumilast

Species	Study & doses (mg/kg/day)	Type of lesions
Mouse (B6C3F1)	6 month / 4, 12 & 36	Periarteritis at 12 & 36 mg/kg/day **with margin of >10x at NOAEL.**
Dog	1 month / 2, 6 & 18	Grossly bleeding in the right atrium at 6 & 18 mg/kg/day; nodule, scar and bleeding in left auricle at 18 mg/kg/day. Myocarditis, inflammation of nutritive vessels in right atrium and right auricle at 6 & 18 mg/kg/day; neutrophilic infiltration at 18 mg/kg/day.
	6 month / 0.2, 1 & 4	Chronic inflammation of right atrium & auricle at 1 & 4 mg/kg/day; pericardial haemorrhage, edema, degeneration and hemosiderin deposition at 4 mg/kg/day.
	12 month / 0.2, 0.6 & 2	Grossly cyst at 2 mg/kg/day. Epicardial hemosiderin and haemorrhages at 2 mg/kg/day.
Monkey	1 month / 0.1, 0.25 & 0.5	Myocarditis at 0.5 mg/kg/day. No heart lesions in long term study.

Heart Lesions with Apremilast

Species	Study & doses (mg/kg/day)	Type of lesions
Mouse	28 day /5, 25, 75 & 150	Arteritis at the root of aorta or around the cardiac arteries and in the myocardium at all dose levels.
	3 month /2, 4, 8 & 16	Arteritis at 16 mg/kg/day.
	6 month /10, 100 & 1000	Arteritis at 100 & 1000 mg/kg/day. Mortalities at 100 & 1000 mg/kg/day.
Monkey	14 day /200, 500 & 1000; 28 day /50, 180 & 650 3 month /25, 85 & 300 12 month /60, 180 & 600	Myocardial degeneration, inflammation, necrosis, fibrin deposition, fibroplasia, haemorrhage & arteritis (myocardium) at all doses. **No heart lesions in a 13-week study with doses up to 300 mg/kg or the chronic, 12-month study with doses up to 600 mg/kg**

- No neoplastic changes in heart both in rat and mouse.
- **The AUC exposure margin for the maximum recommended human dose of 30 mg b.i.d. at the NOAEL was 0.8 with 6 month mouse study**.

Heart Lesions with Crisaborole

- Crisaborole ointment is developed for the topical treatment of atopic dermatitis.
- Cardiovascular functions in minipigs treated with crisaborole, 5% ointment for three months remained normal.
- During 6-month of oral treatment **in rat**, crisaborole at the highest dose level of 450 mg/kg/day (NOAEL) did not cause any local or systemic toxicity.
- In 9-month minipig dermal study, two daily applications of 7% crisaborole ointment did not cause any changes in ECGs during the entire treatment and 1-month recovery period. No cardiac lesions seen.
- In 2-year carcinogenicity studies, no evidence of crisaborole-induced tumors was observed in mice at the maximal feasible dermal dose of 7% ointment or in rats at oral dose of 300 mg/kg/day in males and 100 mg/kg/day in females, respectively.

No cardiac lesions in animals. Route of administration, exposure, metabolism etc could be the reason for lack of cardiac lesion seen in Crisaborole as compared to other PDE4 inhibitors.

Heart Lesions with Cilomilast

Species	Study & doses (mg/kg/day)	Type of lesions
Mouse	1 month /400	Myocardial necrosis
Rat	10 day & 1 month /80	

- No cardiac lesions seen in 6 month rat study at highest dose of 20 mg/kg/day. Similarly, no cardiac lesions seen in 3 month mouse study.
- Safety margin for cardiac toxicity: less than 1x in rat, 7x in mouse.
- No cardiac changes in monkeys Or in Phase III studies
- Not approved in US (efficacy and GI safety issues)

Heart Lesions with SCH 351591

Species	Study & doses (mg/kg/day)	Type of lesions
Mouse	3 month /5, 15, 50, 100, 200, 400 & 800*	Myocardial inflammation (neutrophilic) – base of heart involving root of the aorta & coronary arteries, periarterial inflammation at >100 mg/kg/day. Inflammation affecting the atria and aortic outflow tract, oedema, thickening and malformation of the atrioventricular valve leaflets, hemorrhage and necrosis with mixed cellular infiltrates, left intramural coronary artery with endothelial hypertrophy and proliferation of fibroblastas and macrophages of all arterial layers at 800 mg/kg/day.
Monkeys	3 month /12, 24 & 48	Inflammatory and degenerative changes in heart. Arteropathy - edema, periarterial inflammation, hemorrhage and fibrinoid necrosis of vessel walls to chronic findings of sclerosis and medial and/or intimal hyperplasia at >24 mg/kg/day.

Materials and Method
Compound A, PDE4 inhibitor

Glenmark
A new way for a new world

8 and 13 week exploratory study in Swiss albino mouse:

Doses

- 8 week study: control, 3, 10 and 30 mg/kg/day as suspension formulation (n=6/gender/dose group)
- 13 week study: Control, 4.5/9, 6/12 and 9/15 mg/kg/day as suspension formulation. Four week recovery at control and 15 mg/kg/day (n=10/gender/dose group

Investigations Routine general toxicology parameters, Toxicokinetics

Result
Compound A, PDE4 inhibitor

Glenmark
A new way for a new world

8 and 13 week exploratory study in Swiss albino mouse:

Result

Well tolerated.

No treatment related changes in body weight, food consumption, Hematology, clinical chemistry, organ weights including heart and gross pathology.

Treatment related microscopic changes seen in heart >10 mg/kg/day.

Approximately 1.5x safety margin at NOAEL dose.

Heart Lesions with Compound A, PDE4 inhibitor

Glenmark
A new way for a new world

At 10 and 30 mg/kg/day (8 week study)
Minimal focal mycocardial fibrosis, minimal focal periaortic inflammation and fibrosis located at the base of the heart.

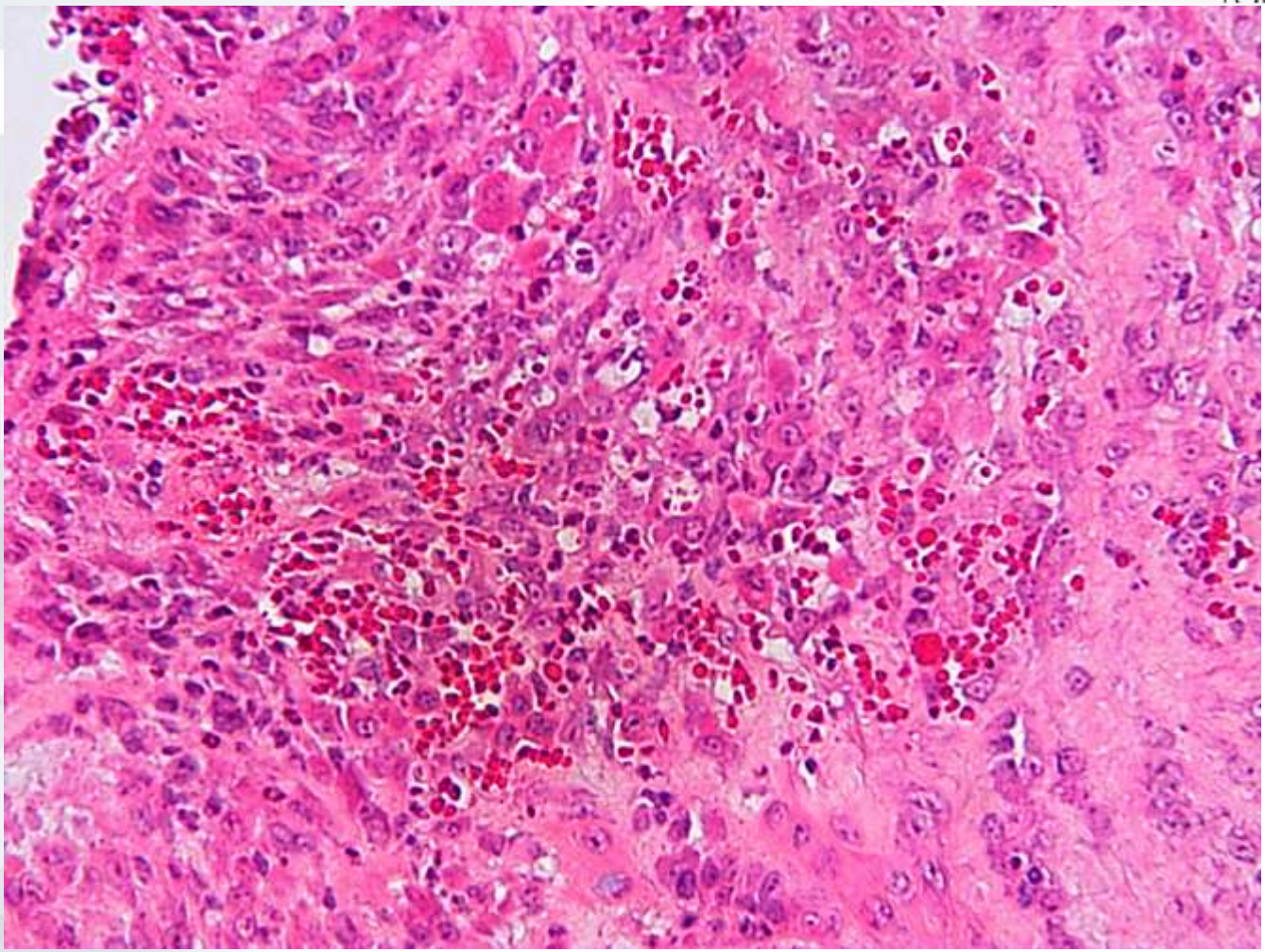
Additional findings at 30 mg/kg/day - myocardial haemorrhage, inflammation/ thickening of aortic valve and periarteritis and fibrosis of the artery.

At 12 and 15 mg/kg/day (13 week study)
Myocarditis, myocardial fibrosis, periaortic inflammation and fibrosis, inflammation/ thickening of aortic valve and inflammation and fibrosis around the artery. Changes were partially reversible.

These findings are **exposure dependent**.

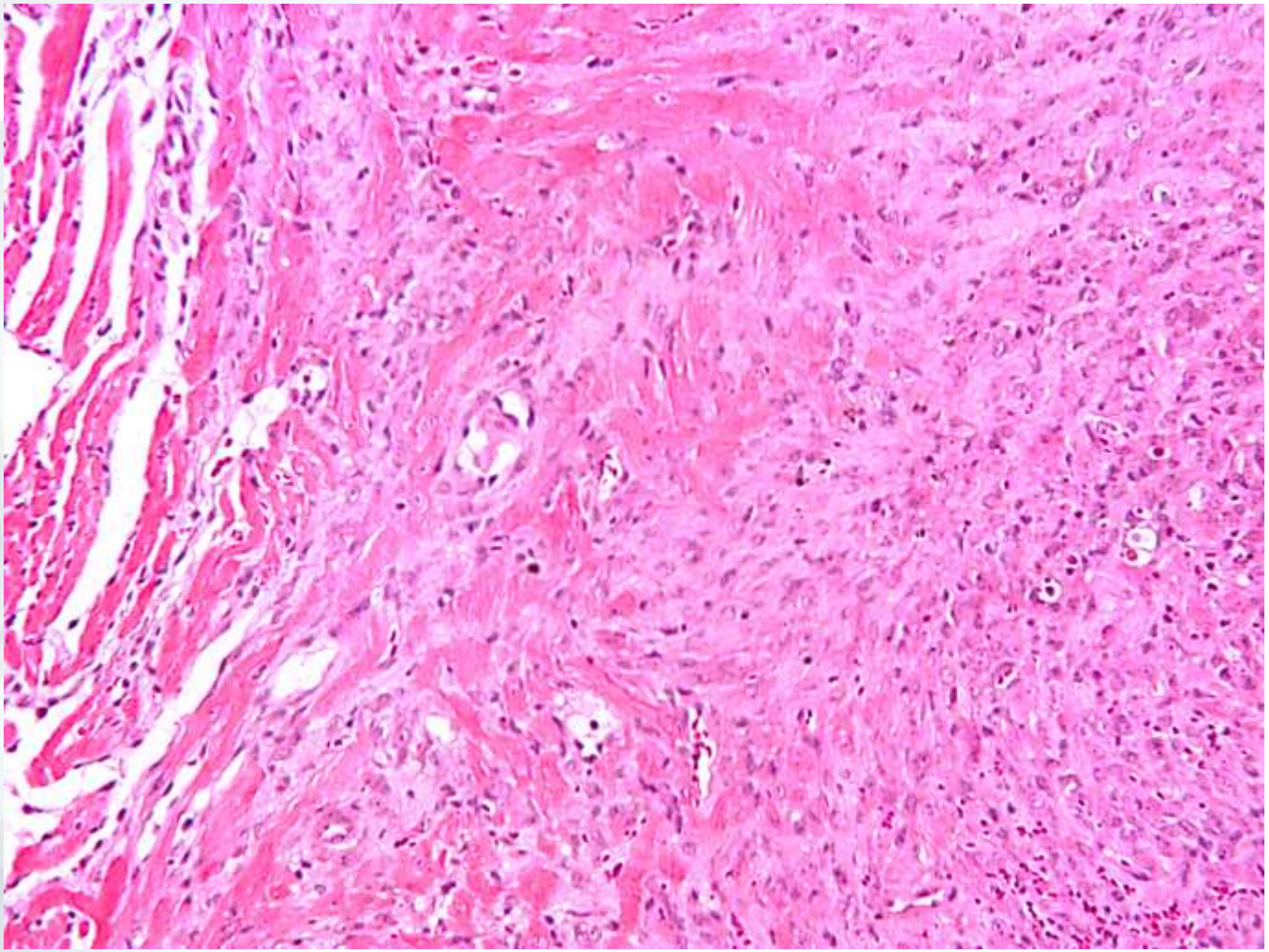
Haemorrhages and vacuolation

Glenmark
A new way for a new world



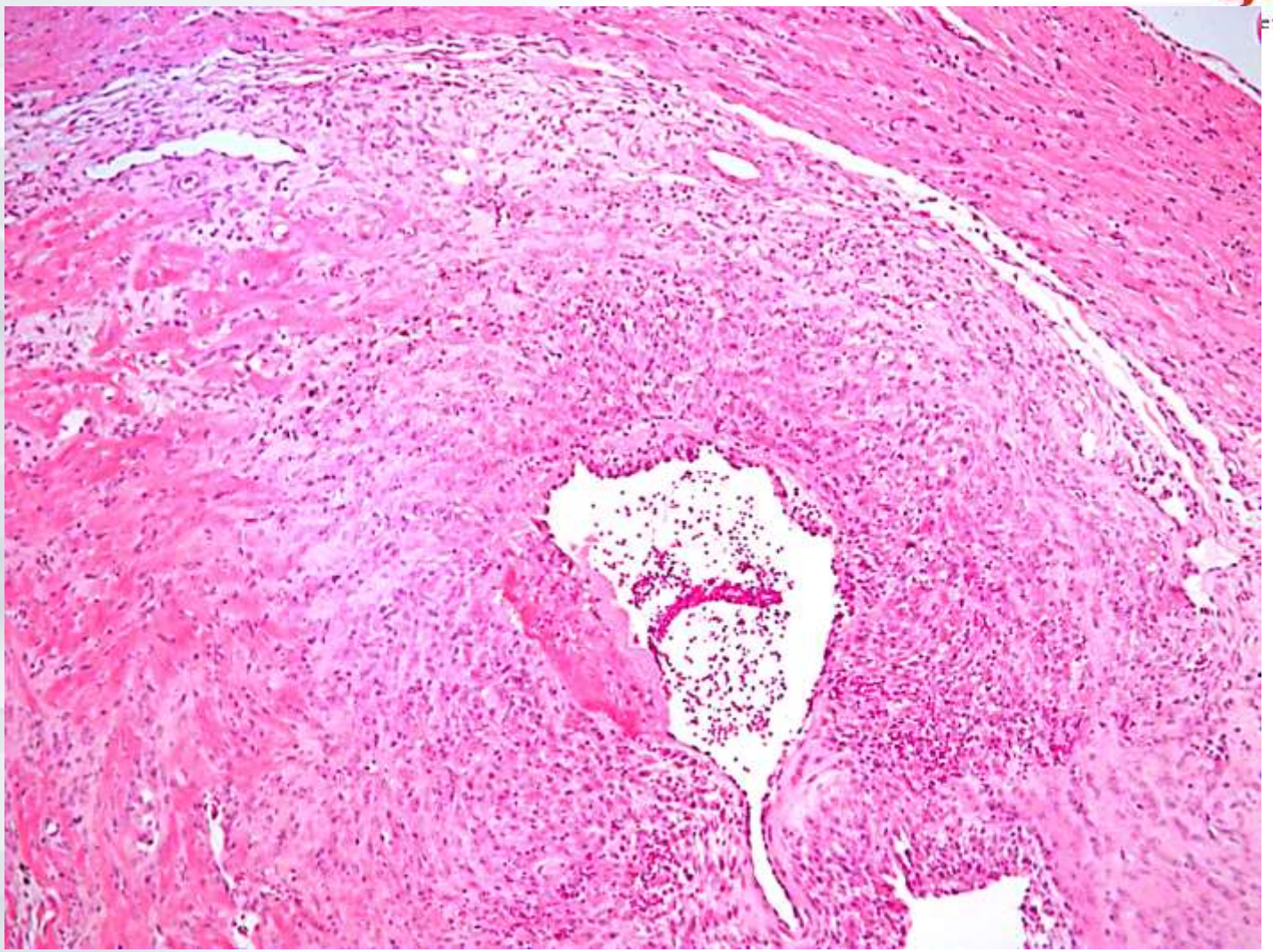
Heart, mouse, 8-week study, 10 mg/kg compound A, periaortic and aorta inflammation associated with haemorrhages and vacuolation (H&E, lens x20)

Myocardial fibrosis



Heart, mouse, 8-week study, 10 mg/kg compound A, myocardial fibrosis (H&E, lens x40)

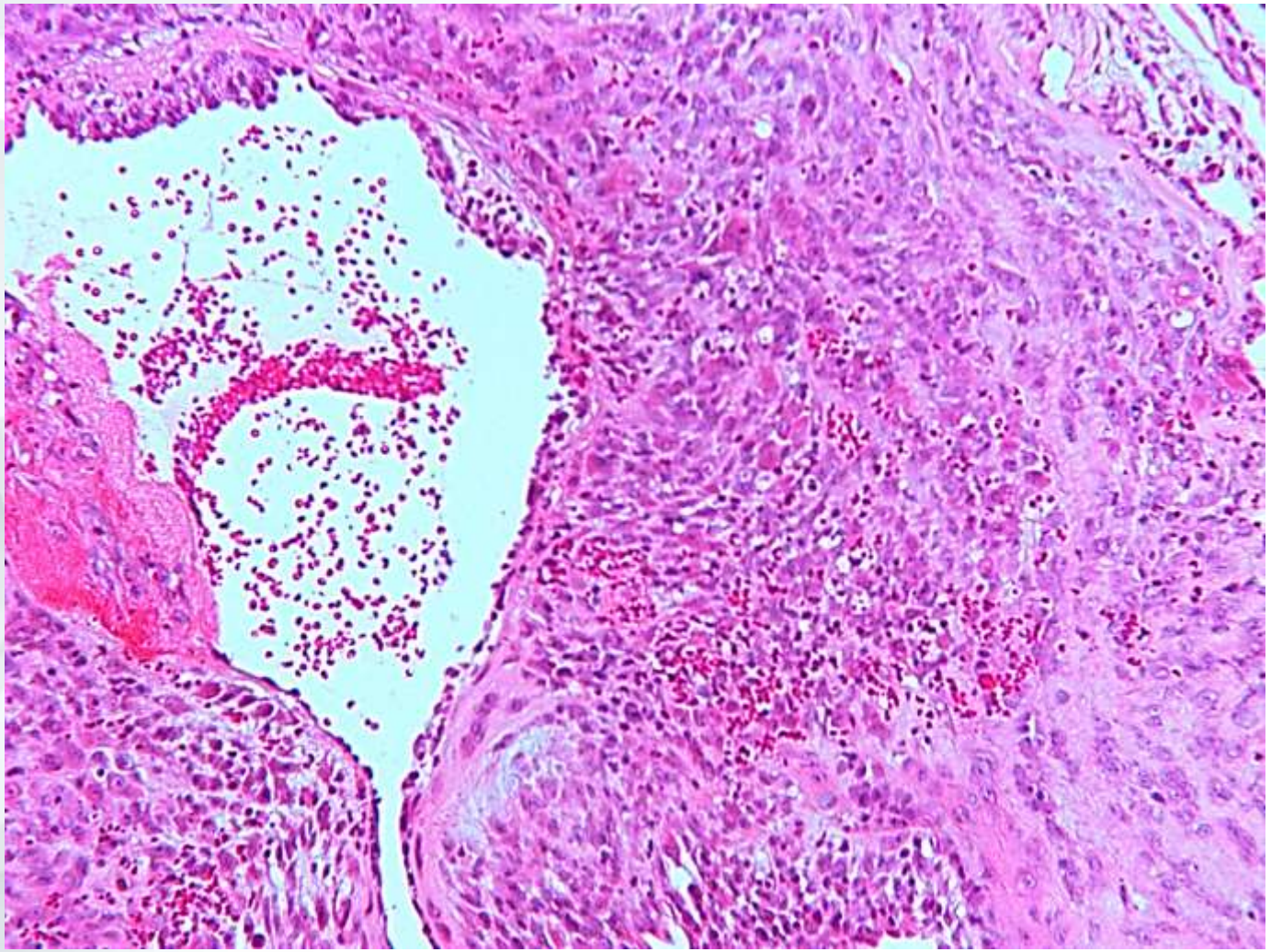
Periaortic and aorta inflammation



Heart, mouse, 8-week study, 10 mg/kg compound A, periaortic and aorta inflammation associated with haemorrhages and vacuolation (H&E, lens x10)

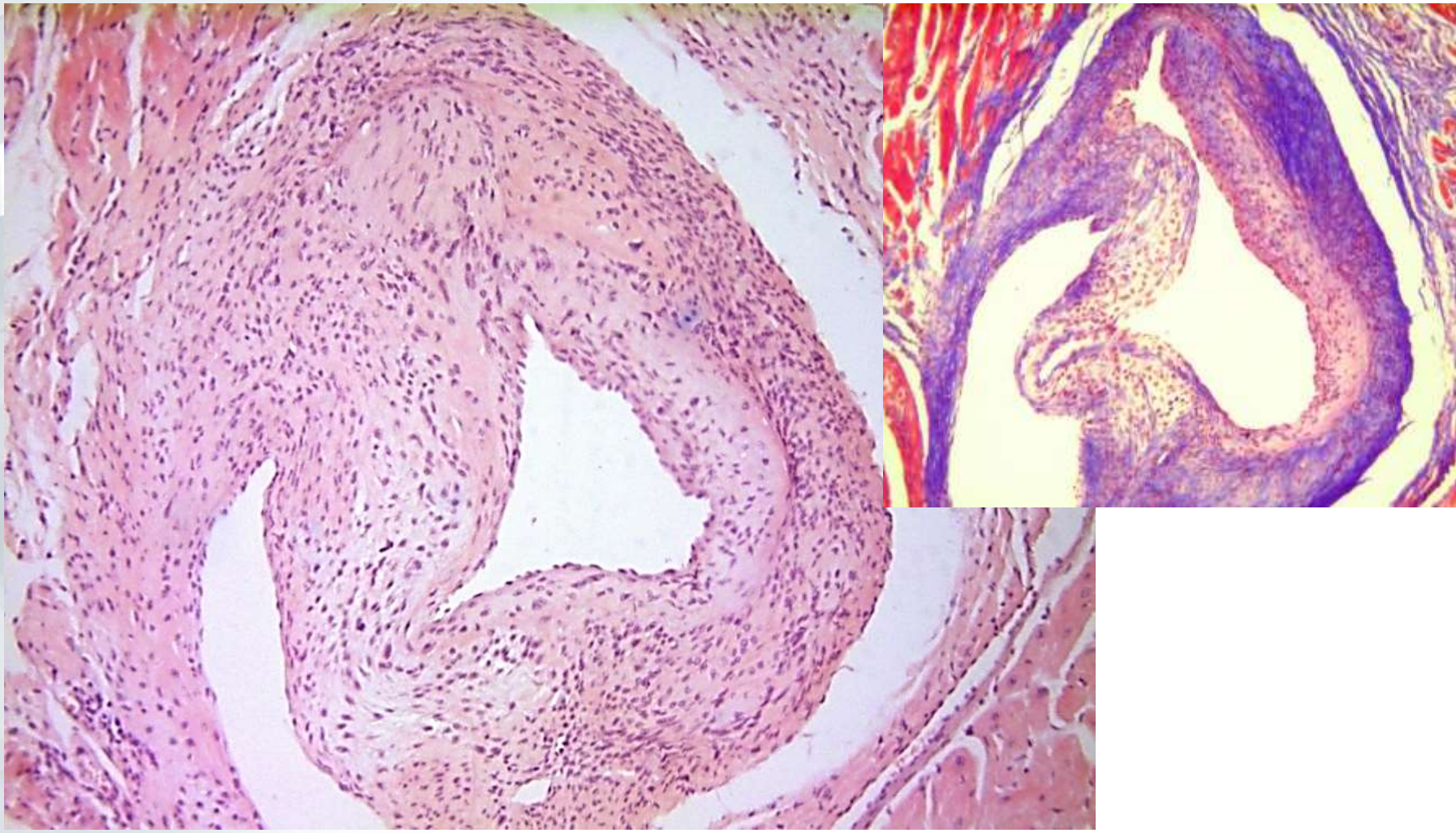
Periaortic and aorta inflammation

Glenmark
A new way for a new world



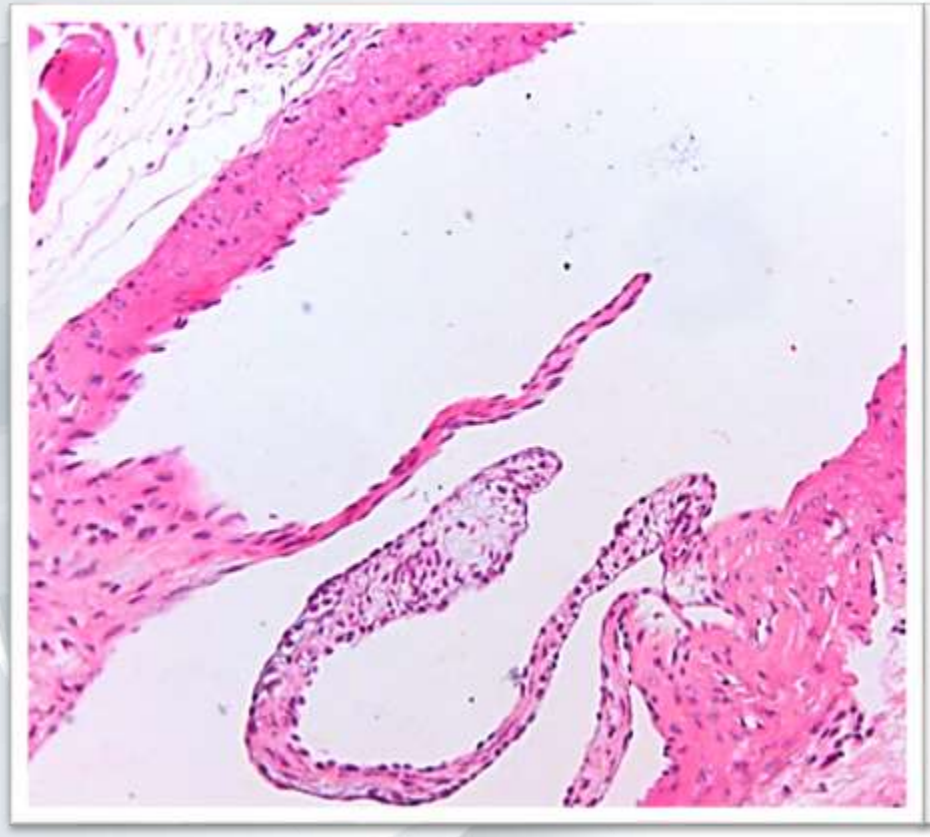
Heart, mouse, 8-week study, 10 mg/kg compound A, periaortic and aorta inflammation associated with haemorrhages and vacuolation (H&E, lens x20)

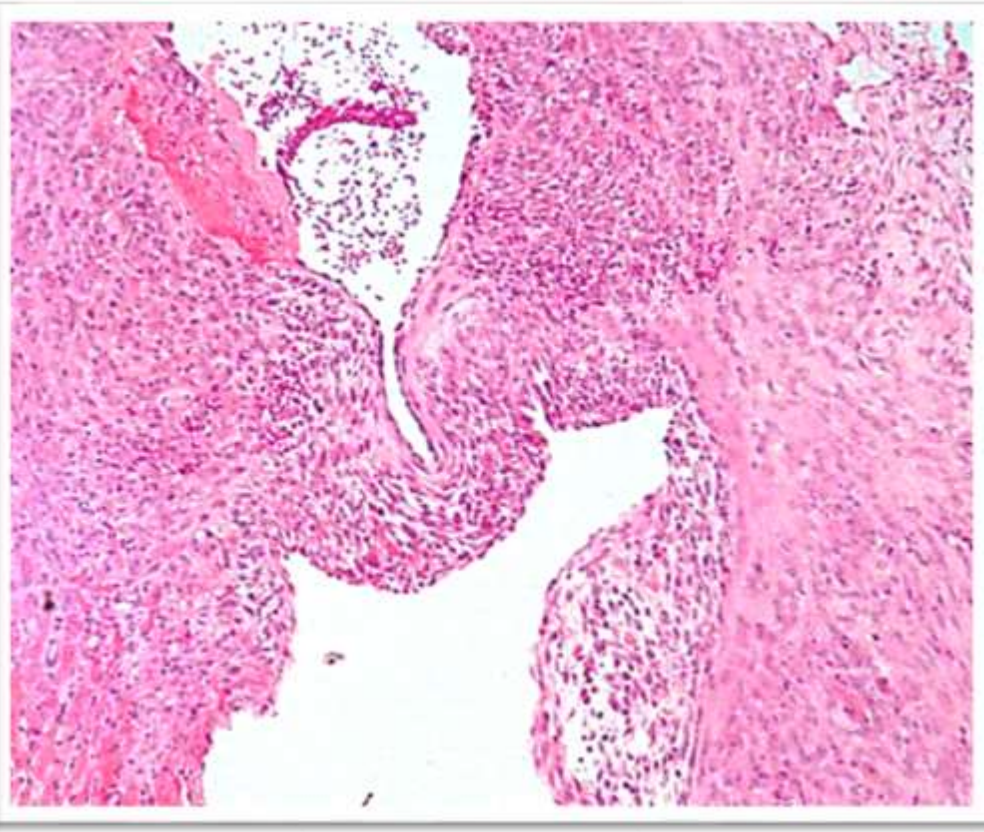
Periaortic fibrosis



Heart, mouse, 8-week study, 30 mg/kg compound A, periaortic fibrosis (H&E and Masson's Trichrome, lens x20)

Aortic valvular thickening and inflammation

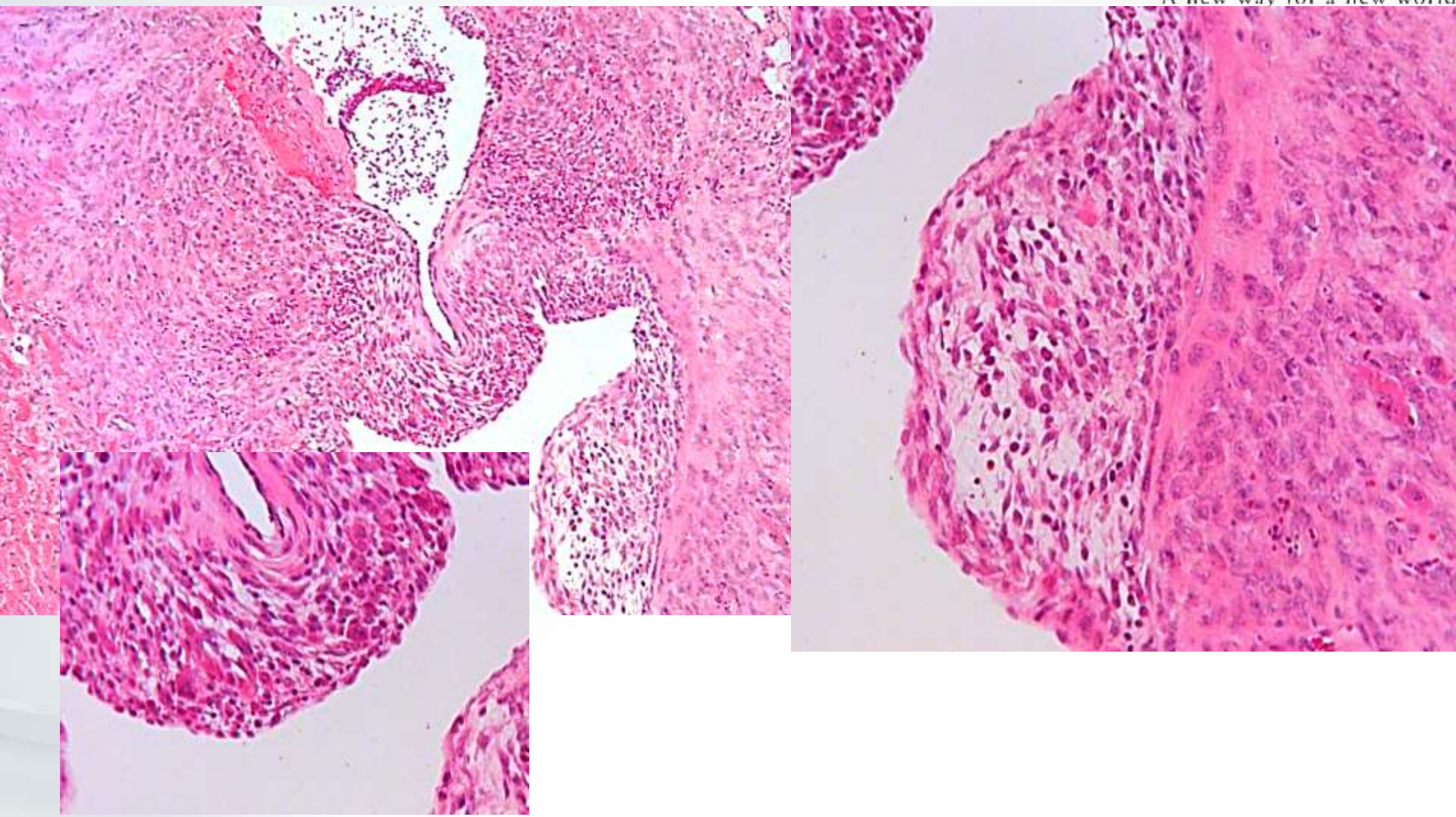




Heart, mouse, 8-week study, Control group, normal aortic valve (H&E, lens x20)

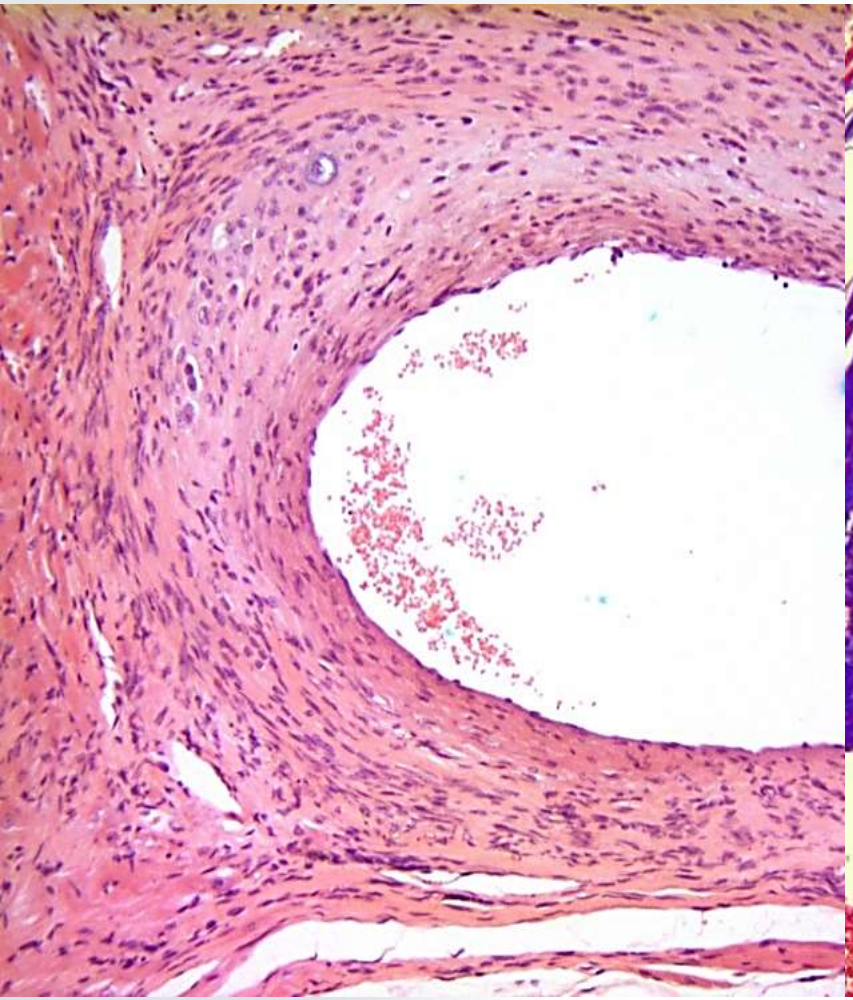
Heart, mouse, 8-week study, 30 mg/kg compound A, aortic valvular thickening and inflammation (H&E, lens x20)

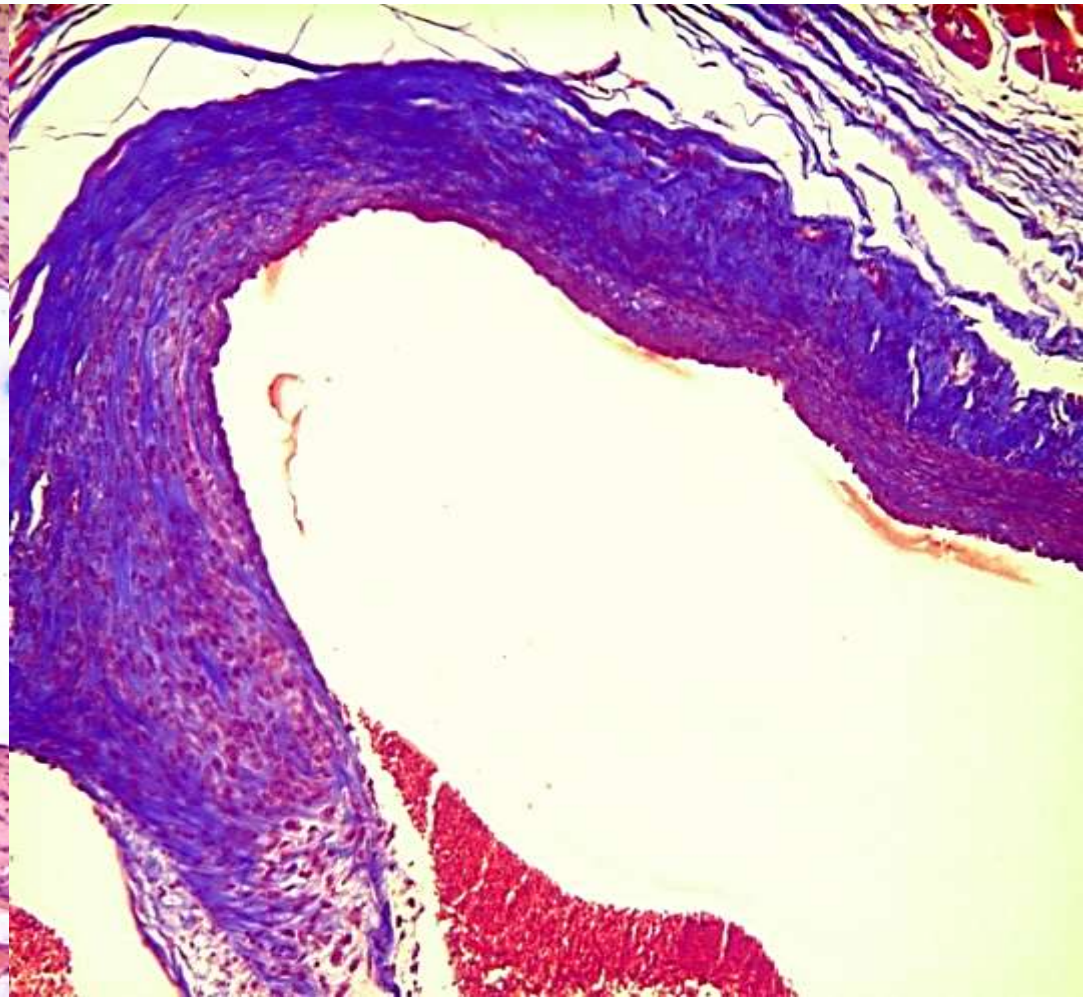
Aortic valvular thickening and inflammation



Heart, mouse, 8-week study, 30 mg/kg compound A, aortic valvular thickening and inflammation (H&E, lens x20, x40)

Periarterial fibrosis





Heart, mouse, 8-week study, 30 mg/kg compound A, fibrosis around artery (H&E and Masson's Trichrome, lens x20)

Mechanism of Injury

Following hypothesis proposed;

- Localization of the lesions and PDE4 isoform at the heart base and root of the major vessels.
- Local accumulation of cAMP and activation of molecular signaling.
  - Extreme vasodilatation of arteries, apoptosis and nitrative stress as a primary mechanism **Or**
  - Secondary to the release of inflammatory chemokines from activated neutrophils, mast cells, and/or endothelial cells and thus the vasculopathy.

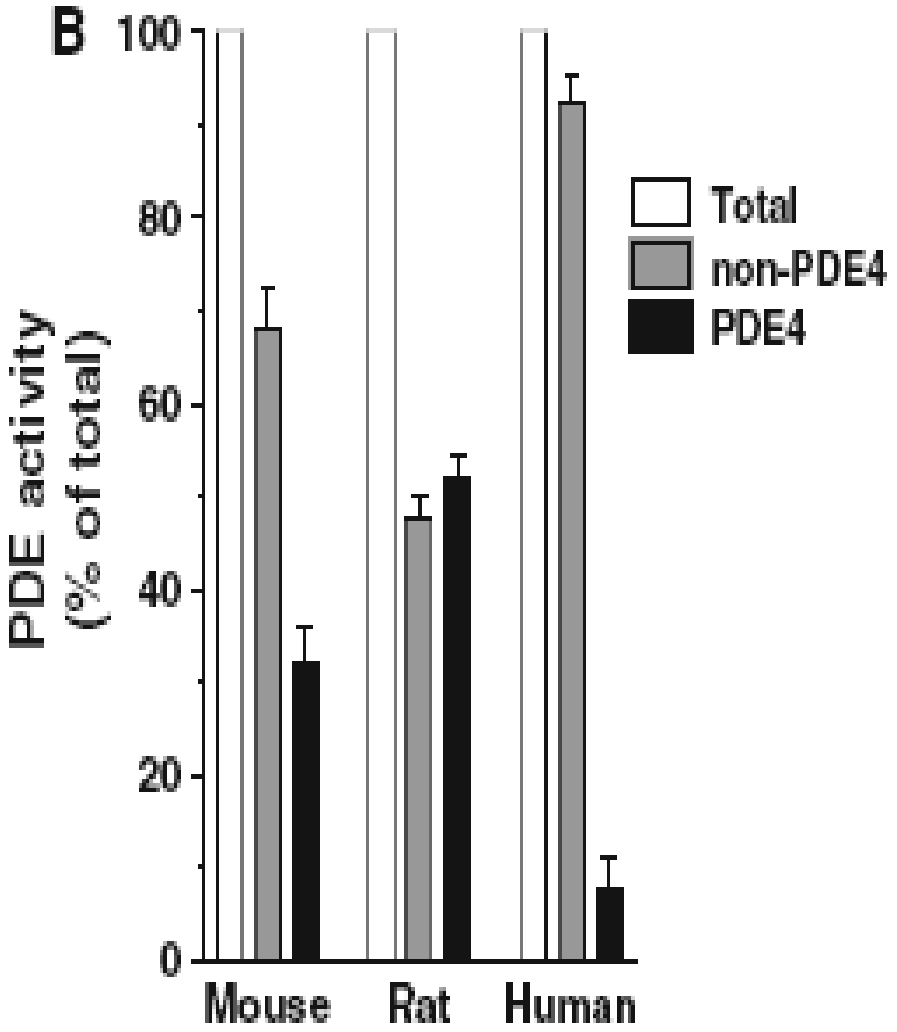
Joseph et al. 1996; Lugnier and Komas 1993; Slim et al. 2003; Zhang et al. 2002; Zhang et al. 2008

Relevance of mouse heart lesions to human

Glenmark
A new way for a new world

- PDE4 inhibition has 'global' effects on cAMP signaling **only in rodent heart, as PDE4 comprises a large fraction of the total cardiac PDE activity in rodents but not in humans.**

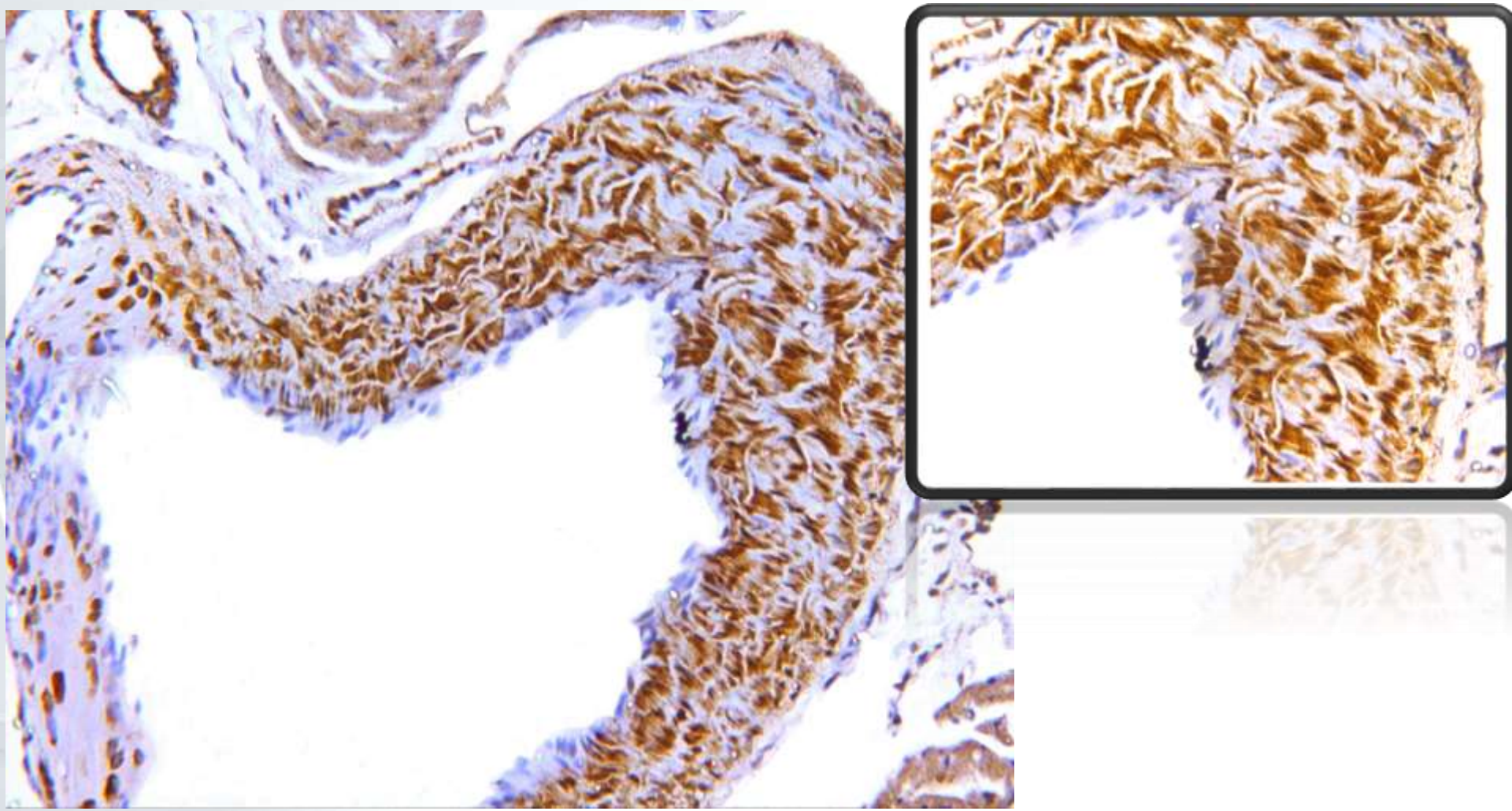
- PDE4 contributes a much larger portion of the total PDE activity in mouse and rat heart (32 and 52%,respectively) than in human heart (8%).



Wito Richter et al, 2011

Mouse aorta labelled with anti-PDE4 antibody

Glenmark
A new way for a new world



Heart, mouse, 8-week study, control group, Mouse, aorta labelled with anti-PDE4 antibody (lens x20 and 40)

Summary

- Cardiac lesions seen in animals with most of the Oral PDE4 inhibitors.
- **Cardiac lesions are commonly seen in mouse even at less than 1 fold safety margin**
- No cardiac lesions in long term studies with monkeys
- No cardiac neoplasms with any of the PDE4 inhibitors both in rats and mice
- **No CVS safety issues seen in Phase 2 (13 week) with Compound A.**
- There seems to be translational loss of Cardiac lesions in man due to difference in PDE4 expression in various species.

Glenmark
A new way for a new world

Thank You