Imperial College
Renal and Transplant Centre

Spleen Tyrosine Kinase (SYK) in IgA nephropathy

Dr Frederick W K Tam

譚偉强

MBBChir PhD FRCP FHEA
Hammersmith Hospital
London

Disclosures

- Research Grants
  - AstraZeneca
  - Baxter Bioscience
  - Human Genome Sciences
- Consultancy and Advisory Boards
  - Baxter
  - Medimmune
  - Rigel Pharmaceuticals

Outline

- Study of renal biopsies
- IgA1 stimulated primary human mesangial cells
- Experimental GN
  - Nephrotoxic nephritis
  - Autoimmune model
- clinical trial

- Jennifer Smith
- John McDaid
- Steve McAdoo
- Gurjeet Bhangal
- Ratana Chawanasuntorapoj
- Min Jeong Kim
- Karen Yu
- Theresa Page
- Prof. Terry Cook
- Prof. Charles Pusey

Rigel Pharmaceuticals, South San Francisco

Esteban Masuda

Dan Magilavy

Leicester

Jonathan Barratt

Karen Molyneux

Tricia Higgins

Prof. John Feehally

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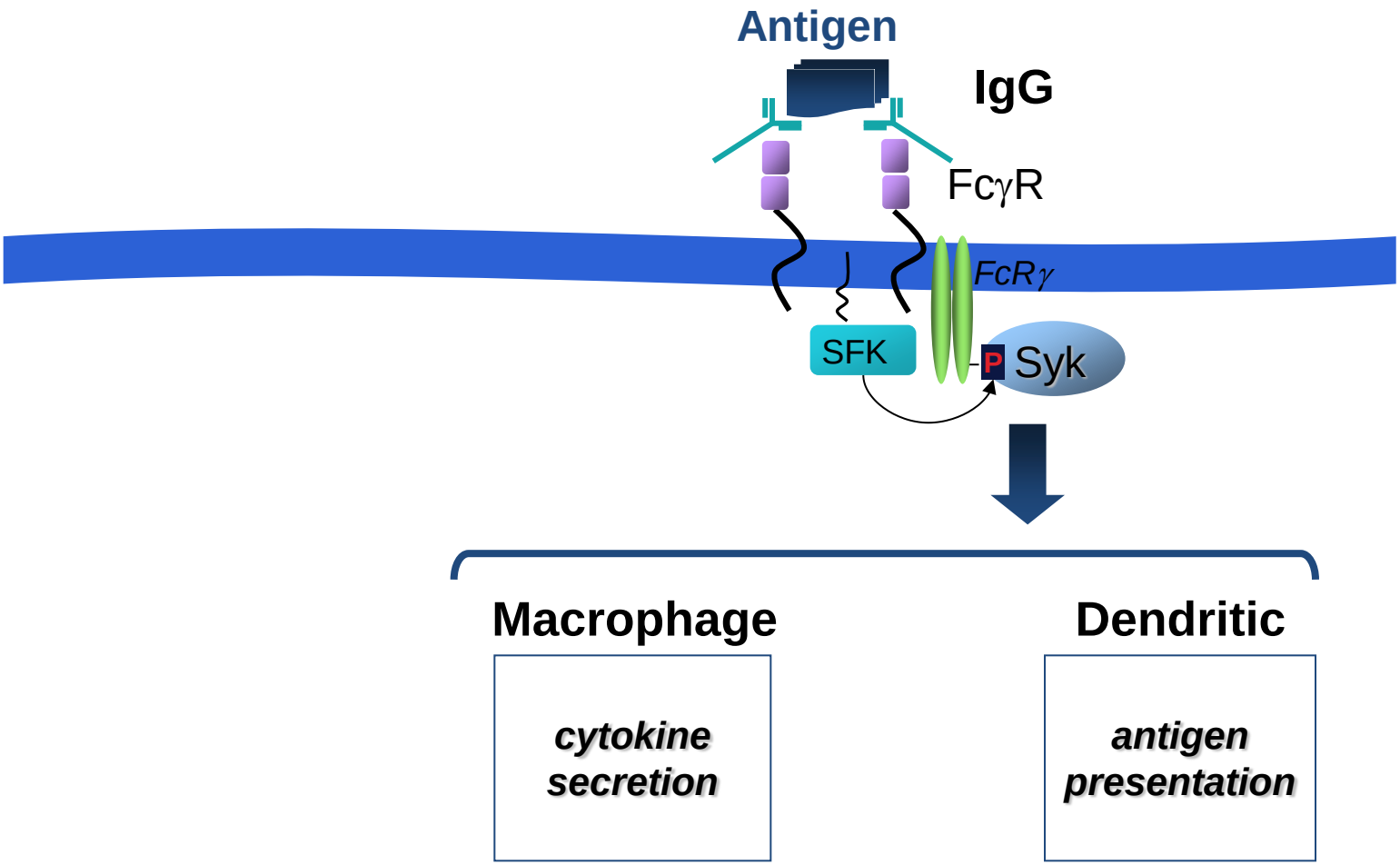
National Insitute for Health Research (NIHR) UK

Kidney Research UK

Syk in Fc Receptor Signalling



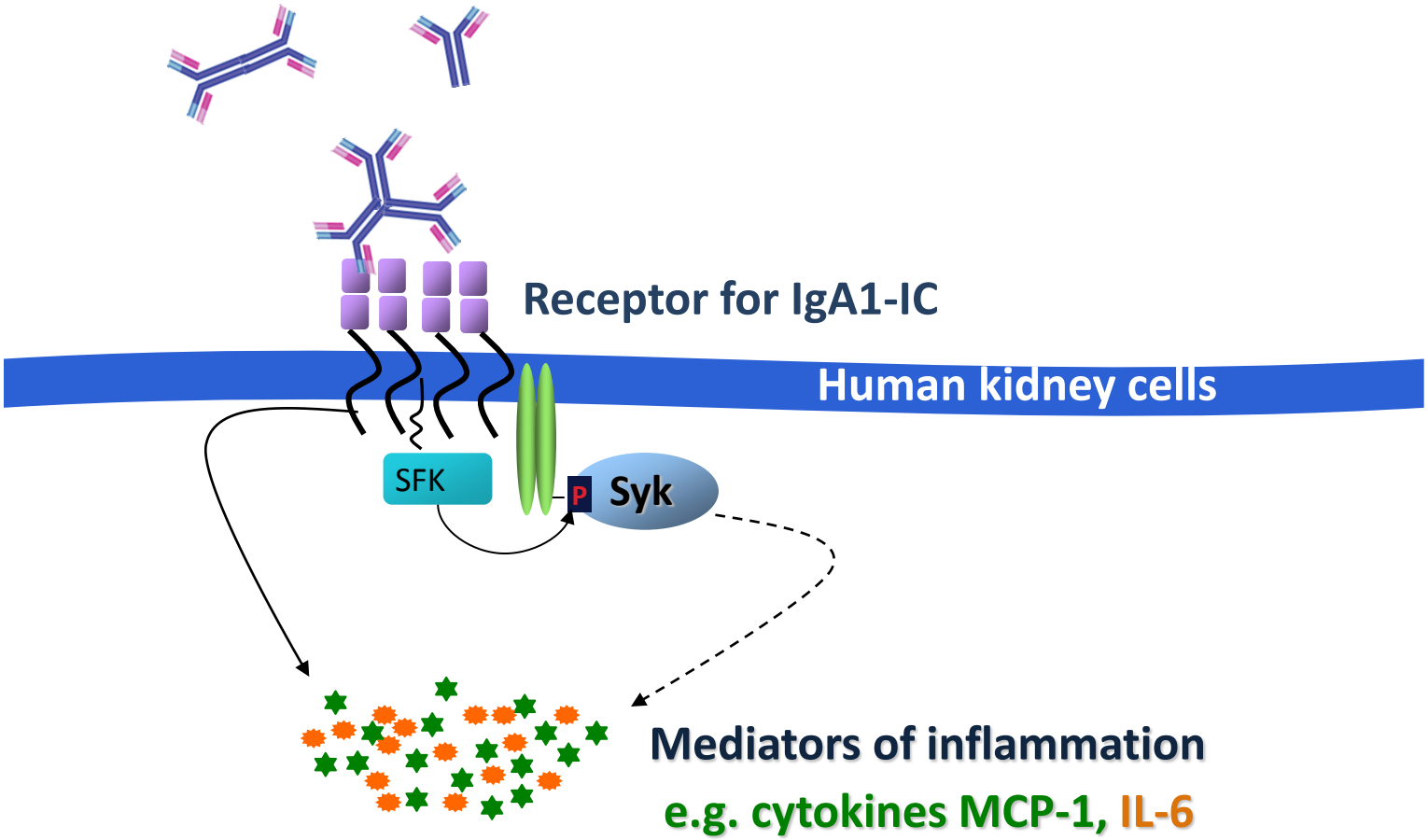
SFK Src family kinases

Modified from Rigel Pharmaceuticals.

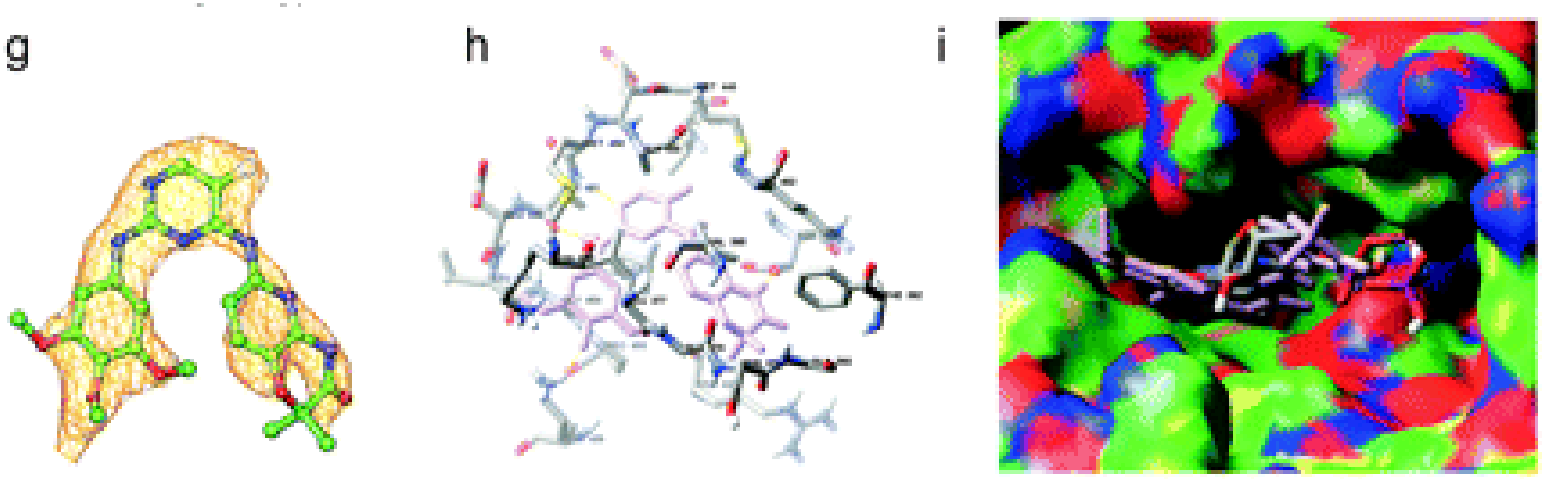
Spleen tyrosine kinase (Syk)

- Intracellular tyrosine kinase
- Present in white blood cells and kidney cells
- Can be activated during immune response or inflammation
- For example, binding of an antibody to specific receptors on cell surface

Hypothesis: IgA complex activates Syk and results in kidney inflammation



Fostamatinib: inhibit **SYK**



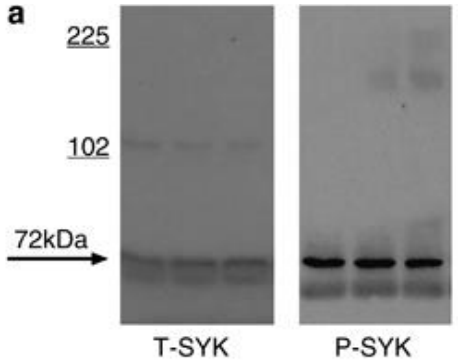
- Fostamatinib (R788) is an oral prodrug
(Provided by Rigel Pharmaceuticals & AstraZeneca)
- R406 is the active metabolite
- R406 – occupying ATP binding pocket of Syk
- Selective for Syk
- Off target effect: also inhibit Flt-3 with 5 fold less potency in cell based assays

Ref: Braselmann 2006 JPET 319:998-1008

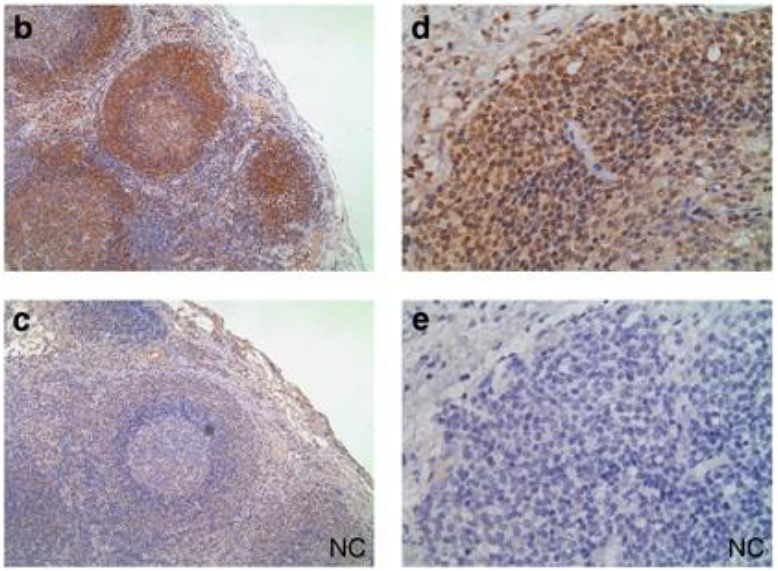
Clinical Translation

- **Does SYK increase in clinical IgA nephropathy?**
- **What is the evidence that patients‘ IgA will activate SYK?**
- **What is the consequence of blocking SYK in kidney cells?**

Method development and control immunohistochemistry for Syk (brown staining)



Western blot: human PBMC

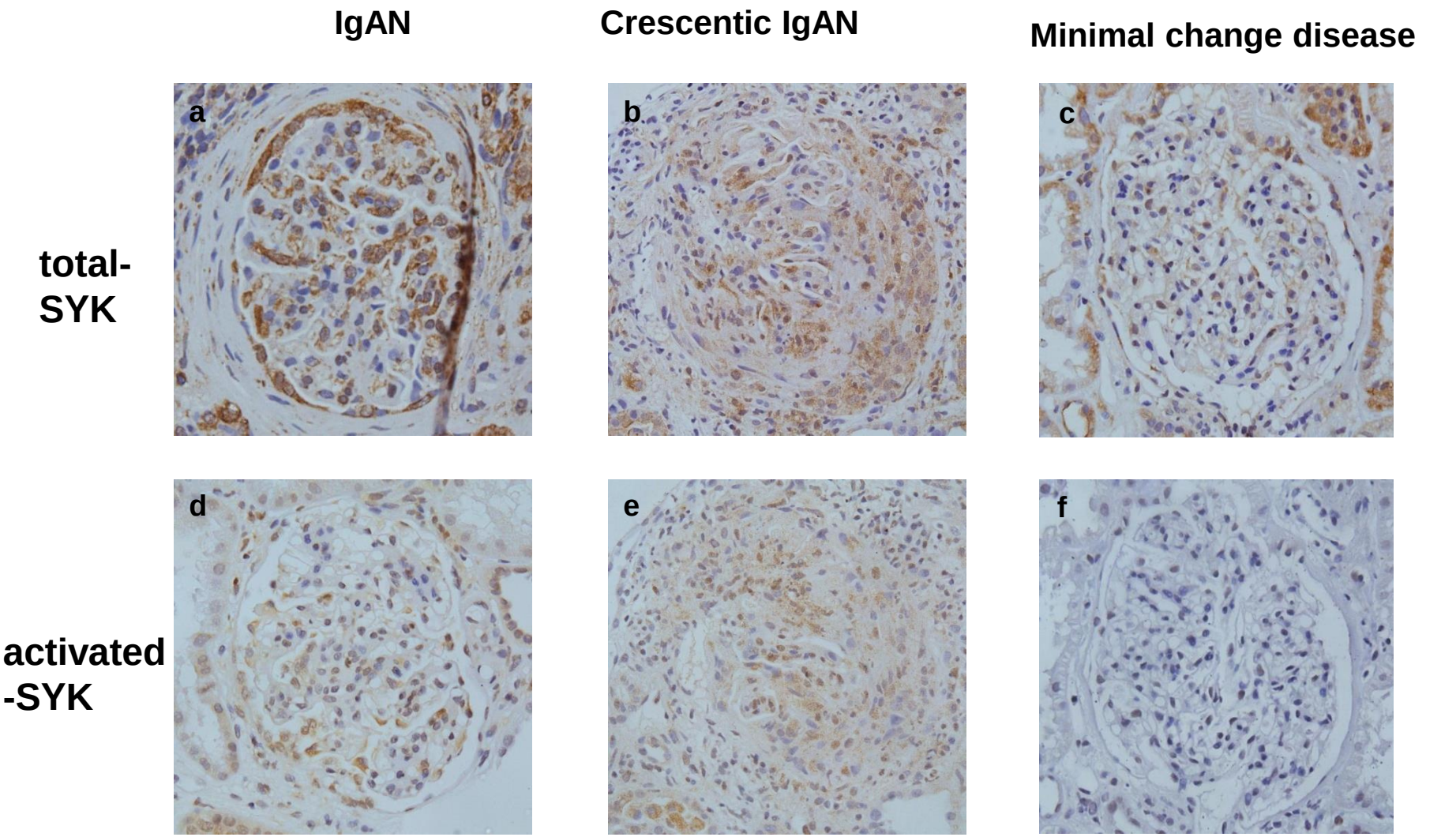


Lymph node

NC: negative control

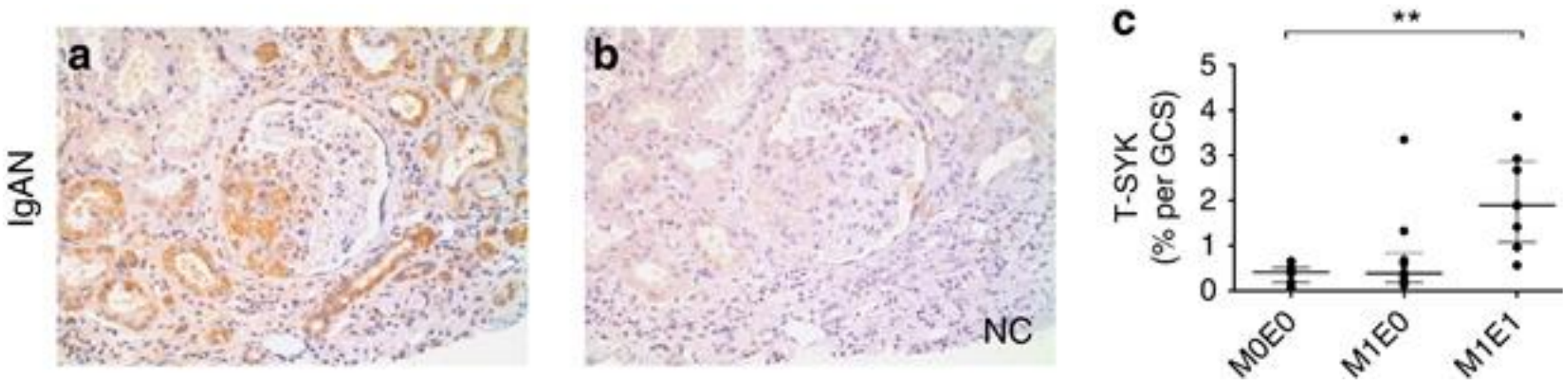
McAdoo et al 2015 ***Kidney Int*** 88: 52-60

Study of Kidney Biopsies from patients
Increase in SYK (brown staining) in IgA nephropathy



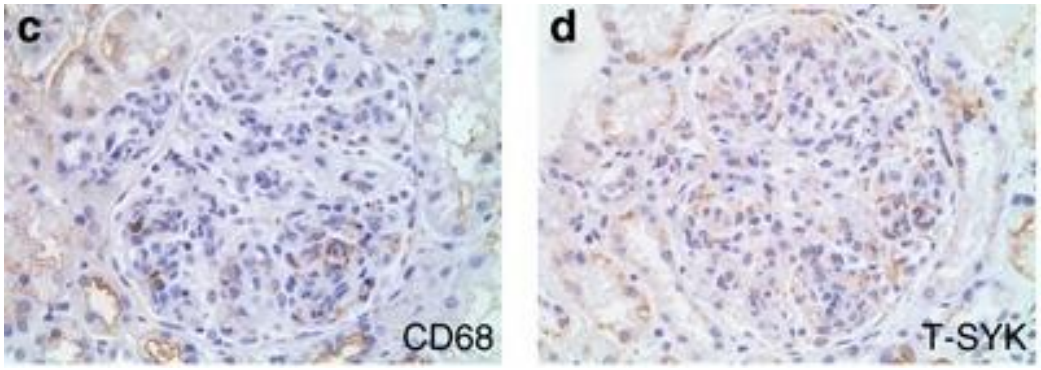
Kim MJ, McAdoo S et al J Immunol 2012;189:3751-8

Expression of SYK and severity of IgA nephropathy



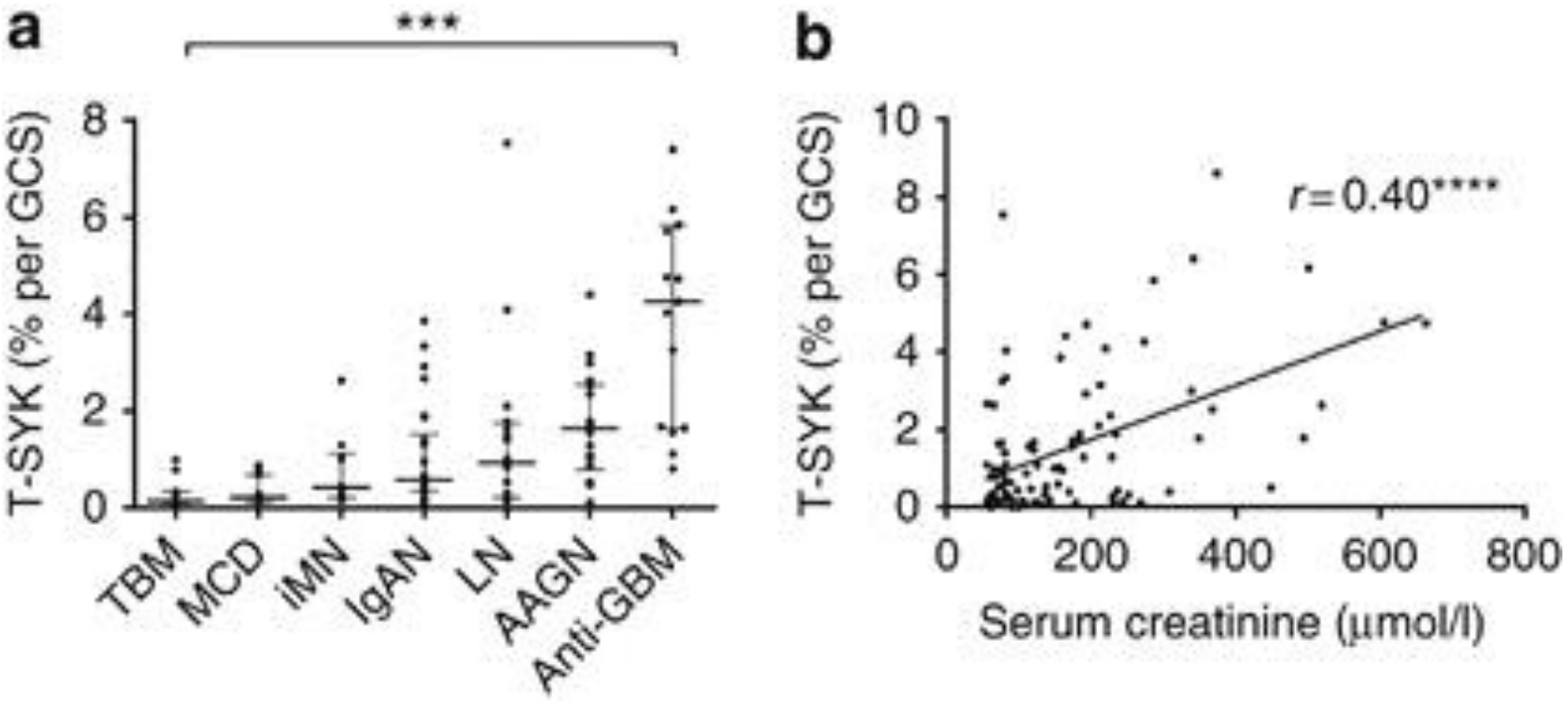
Oxford Classification

Cellular localisation of SYK: (CD68+) macrophages & other cells



McAdoo et al 2015 ***Kidney Int*** 88: 52-60

Expression of SYK in glomerular diseases - correlation with serum creatinine



McAdoo et al 2015 ***Kidney Int*** 88: 52-60

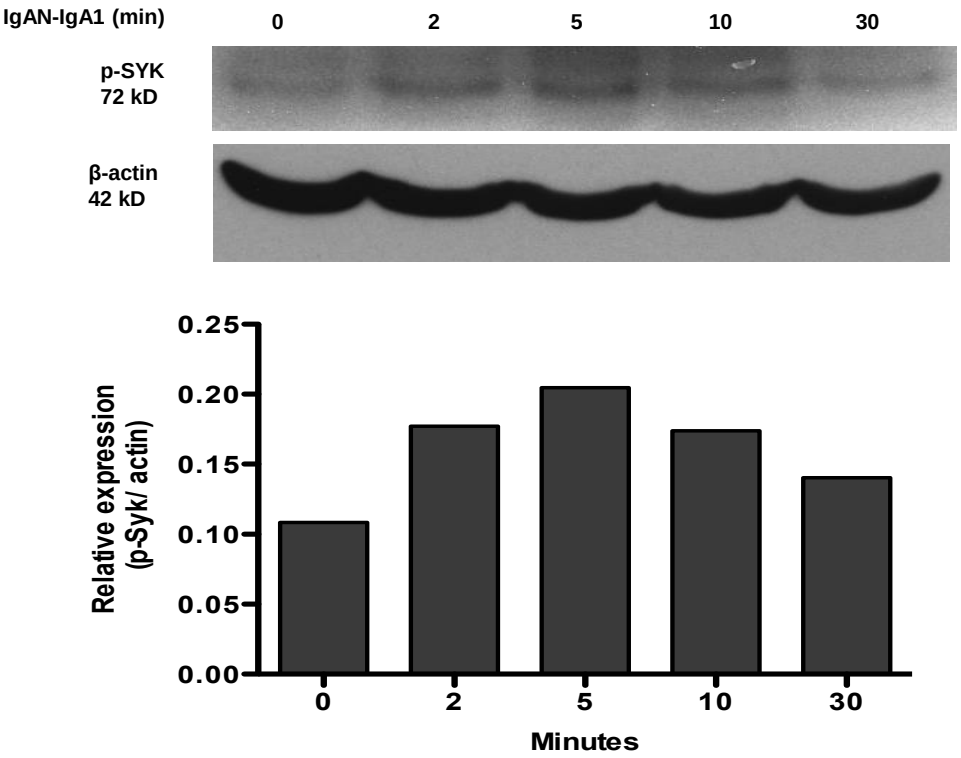
Clinical Translation

- **Does SYK increase in clinical IgA nephropathy?**
- **What is the evidence that IgA from patients will activate SYK?**
- **What is the consequence of blocking SYK in kidney cells?**

Collaboration with Renal Unit, Leicester

- To study the potential role of SYK in IgA1 stimulated mesangial cells

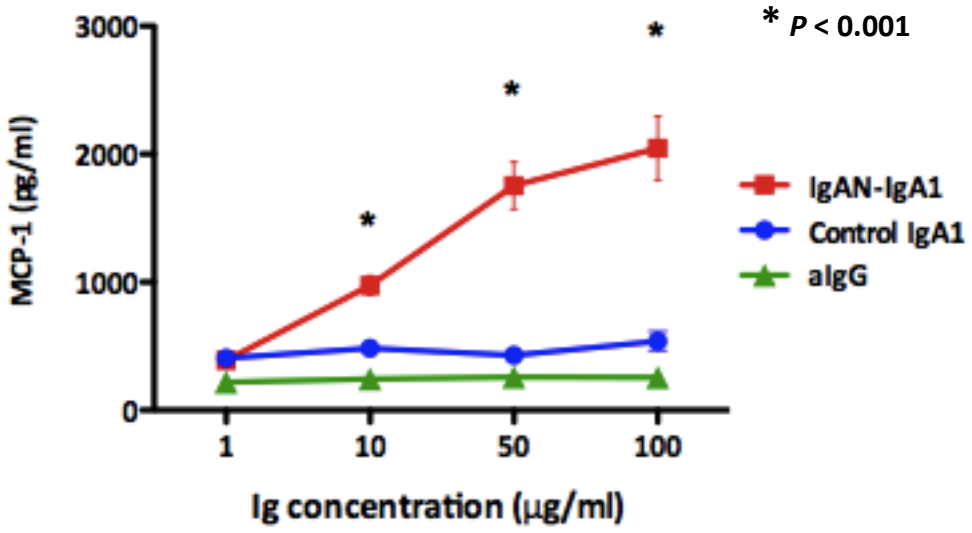
Induction of activated (phosphorylated) SYK in human kidney cells by patients' IgA



- IgA1 from patients with IgA nephropathy
- Induce expression of phospho-SYK in human mesangial cells

Kim MJ et al J Immunol 2012;189:3751-8

Patients‘ IgA stimulate production of inflammatory mediators In human kidney (mesangial) cells in culture



Kim MJ et al J Immunol 2012;189:3751-8

Clinical Translation

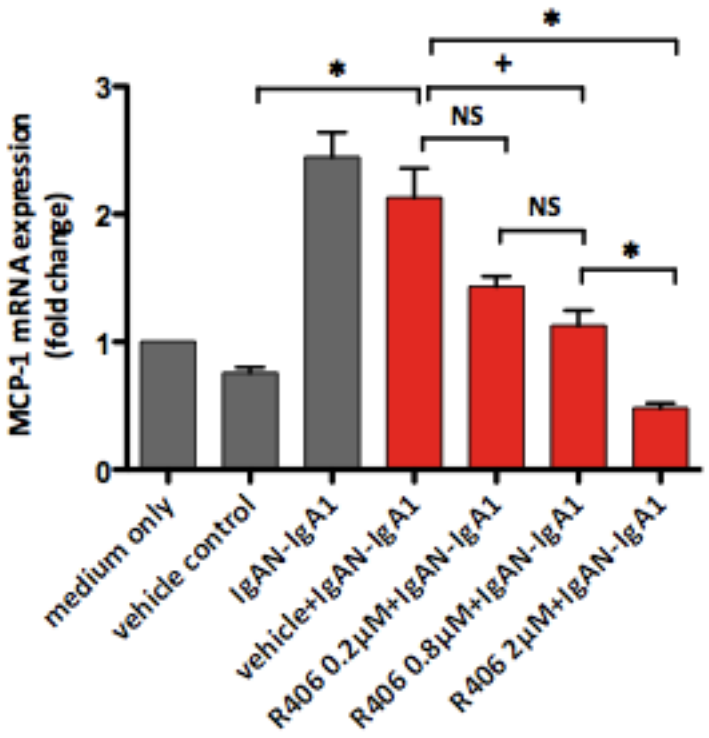
- Does SYK increase in the pathogenesis of clinical IgA nephropathy?
- What is the evidence that patients‘ IgA will activate SYK?
- What is the consequence of blocking SYK in kidney cells?

Does inhibiting SYK reduce inflammation?

- Study of human mesangial cells in culture
- Stimulations with IgA purified from patients' serum
- Inhibition with a the active metabolite of SYK inhibitor (R406)
- Check the specific role of SYK further using molecular biology method (small interfering RNA, siRNA)

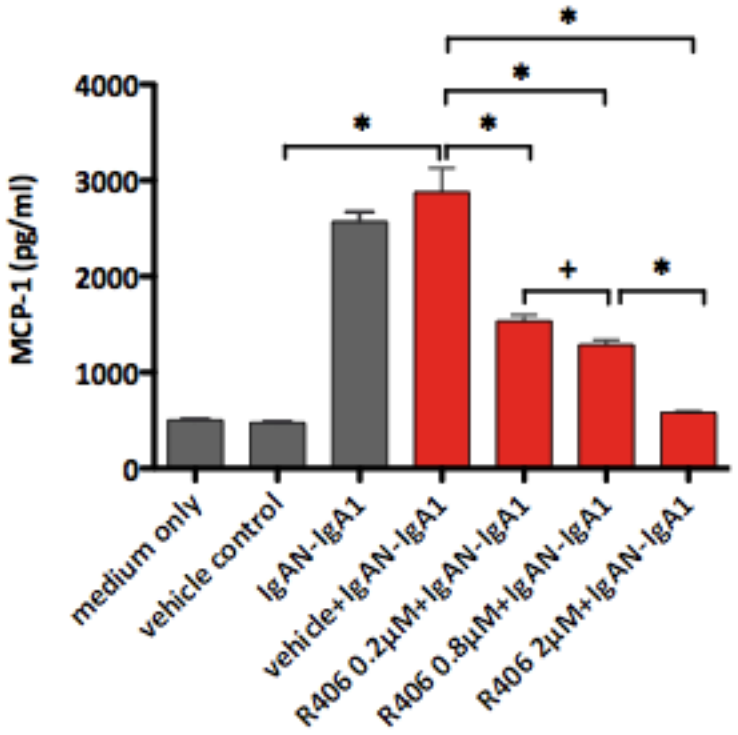
Syk inhibitor reduced MCP-1 synthesis in IgA stimulated human mesangial cells

mRNA



* $P < 0.01$, $^{+}$ $P < 0.05$

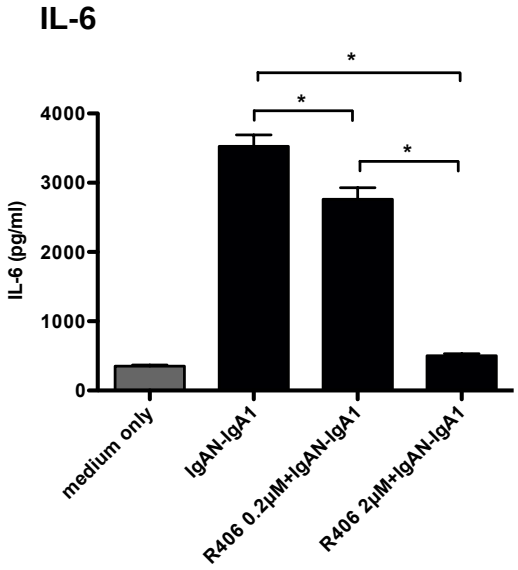
protein

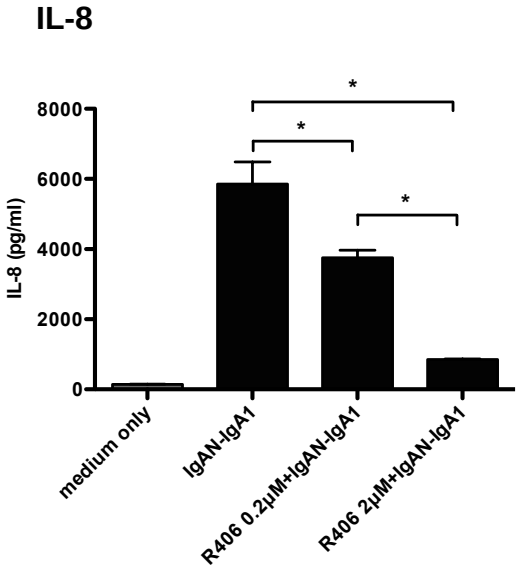


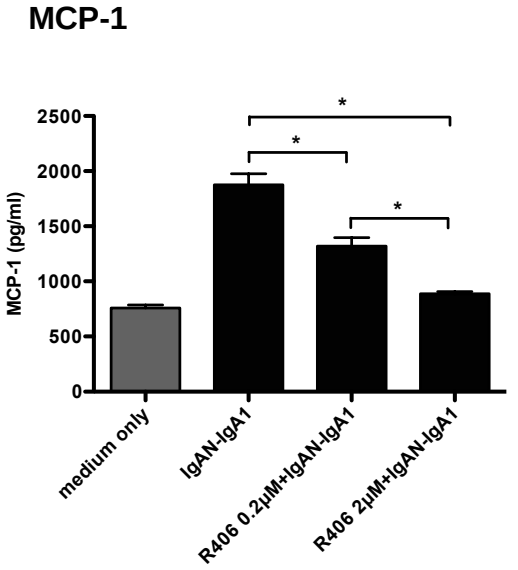
* $P < 0.01$, $^{+}$ $P < 0.05$

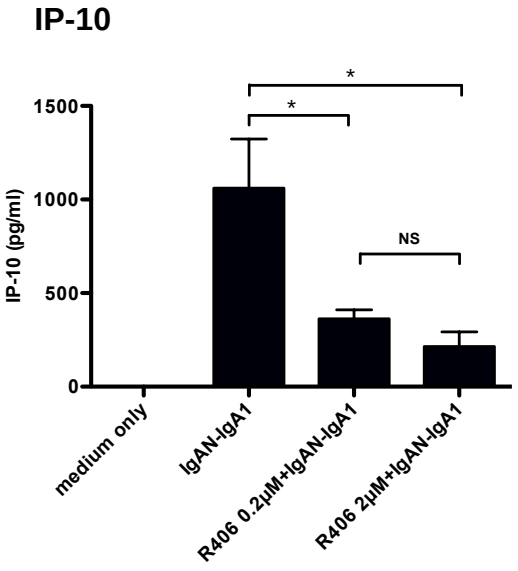
Kim MJ et al J Immunol 2012;189:3751-8

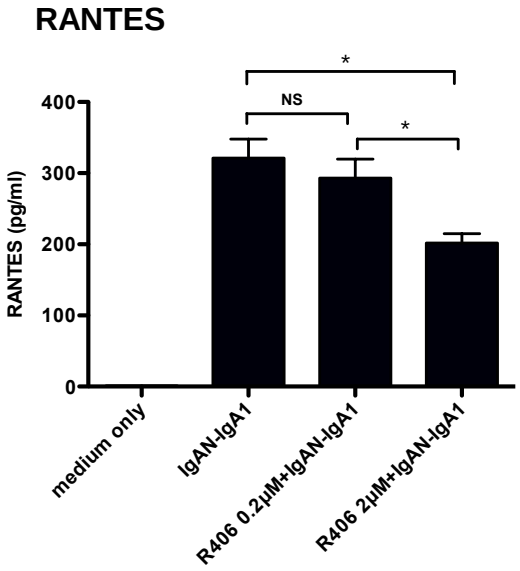
SYK inhibitor inhibit production of multiple cytokines from human mesangial cells

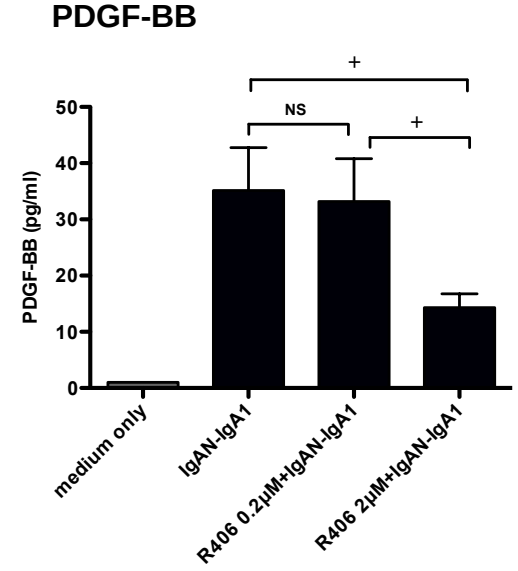








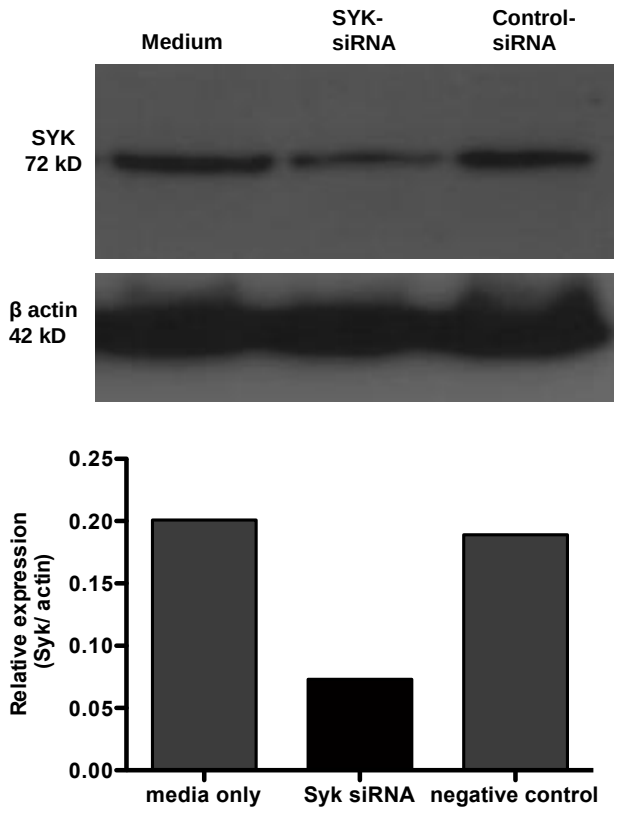




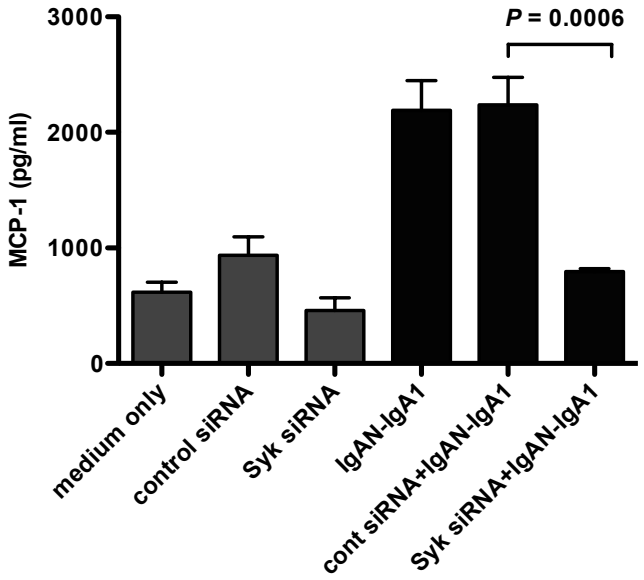
Kim MJ et al J Immunol 2012;189:3751-8

siRNA to SYK in mesangial cells

A

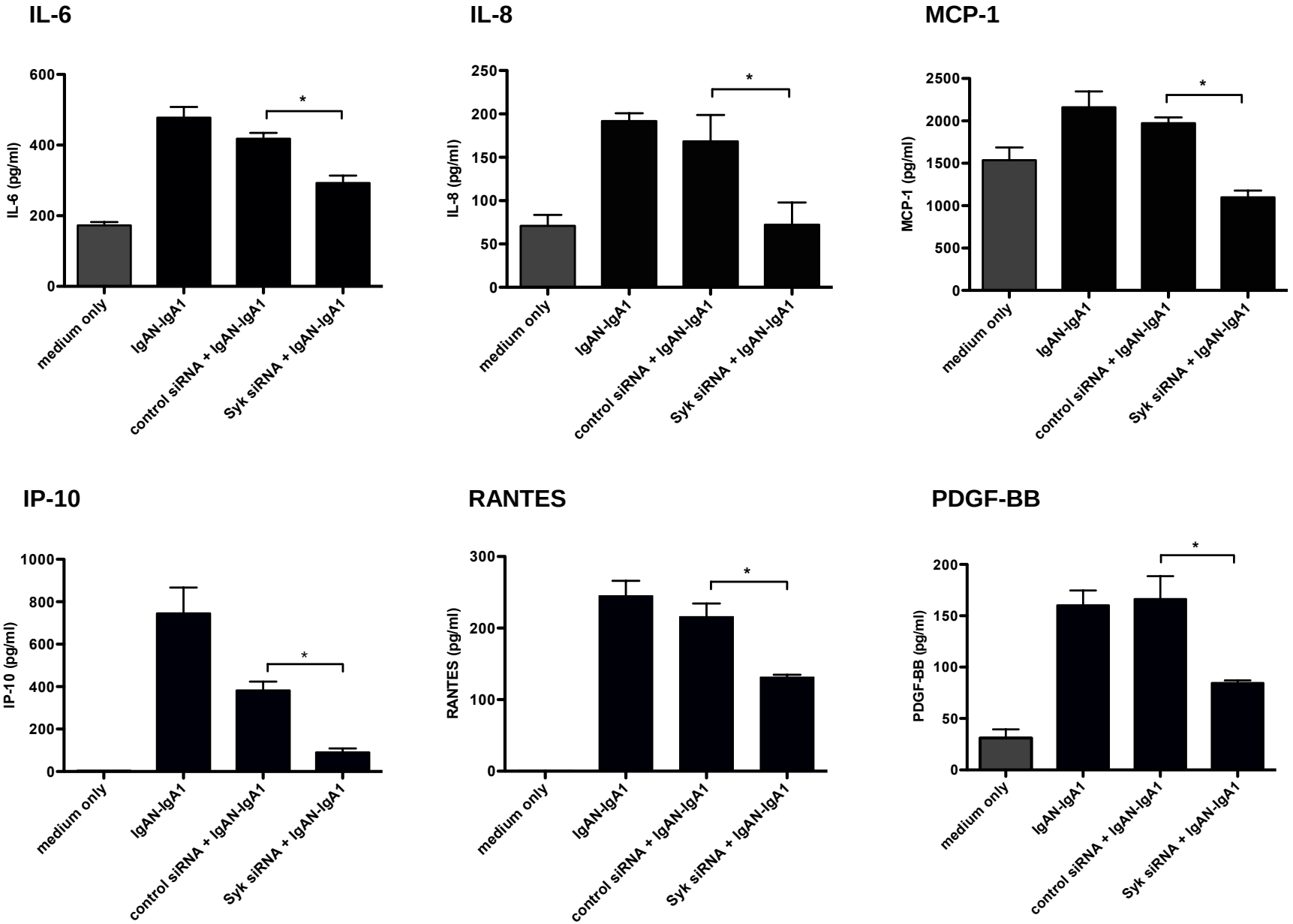


B



Kim MJ et al J Immunol 2012;189:3751-8

Effect of siRNA to SYK in human mesangial cells



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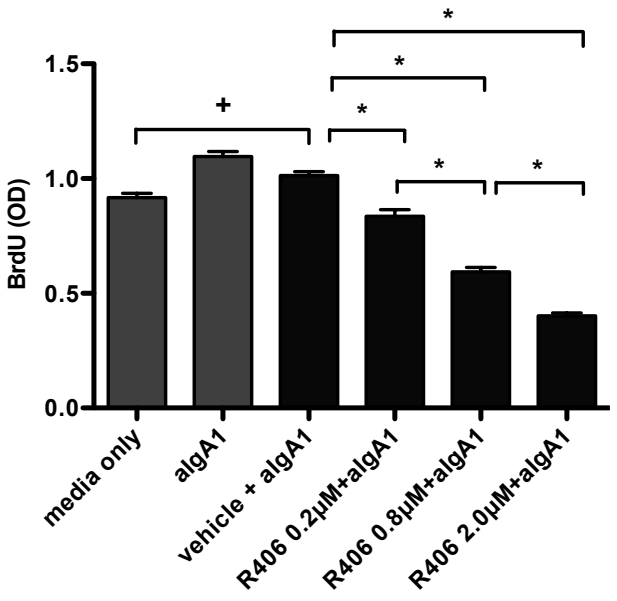
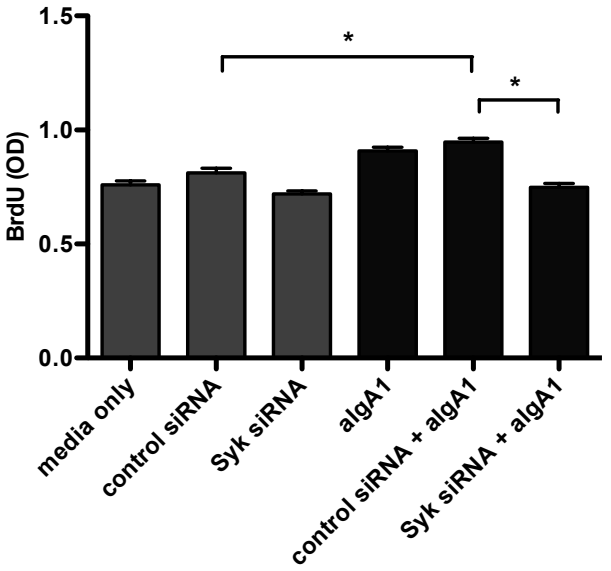
Effect of SYK inhibition on mesangial cell proliferation

inhibitor

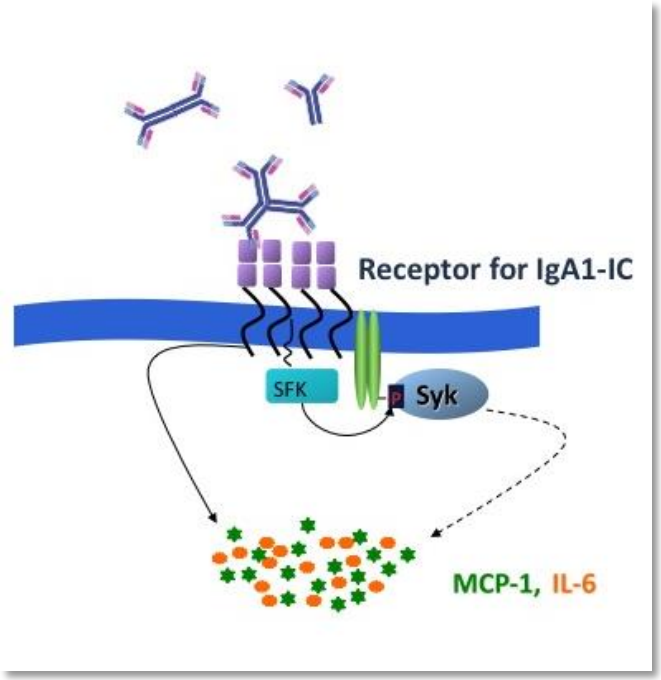


siRNA



Kim MJ et al J Immunol 2012;189:3751-8

Summary 1



- Increase p-SYK in the renal biopsies of patients with IgA nephropathy
- IgA1 from patients stimulates human mesangial cells to express p-SYK and produce pro-inflammatory mediators/growth factors.
- Both pharmacological inhibition using R406 and knockdown of Syk using siRNA decrease the cytokine and growth factor production and cell proliferation from IgA stimulated mesangial cells.
- SYK may play a key role in the pathogenesis of IgAN and be considered as a potential therapeutic target.

Is SYK inhibitor likely to be useful in treating glomerulonephritis (GN) in vivo?

- Research project: preclinical models of antibody mediated GN
  - It is challenging to have reproducible preclinical models of IgA nephropathy
  - We have studied two other models of experimental GN

Pre-clinical development

Antibody mediated glomerulonephritis

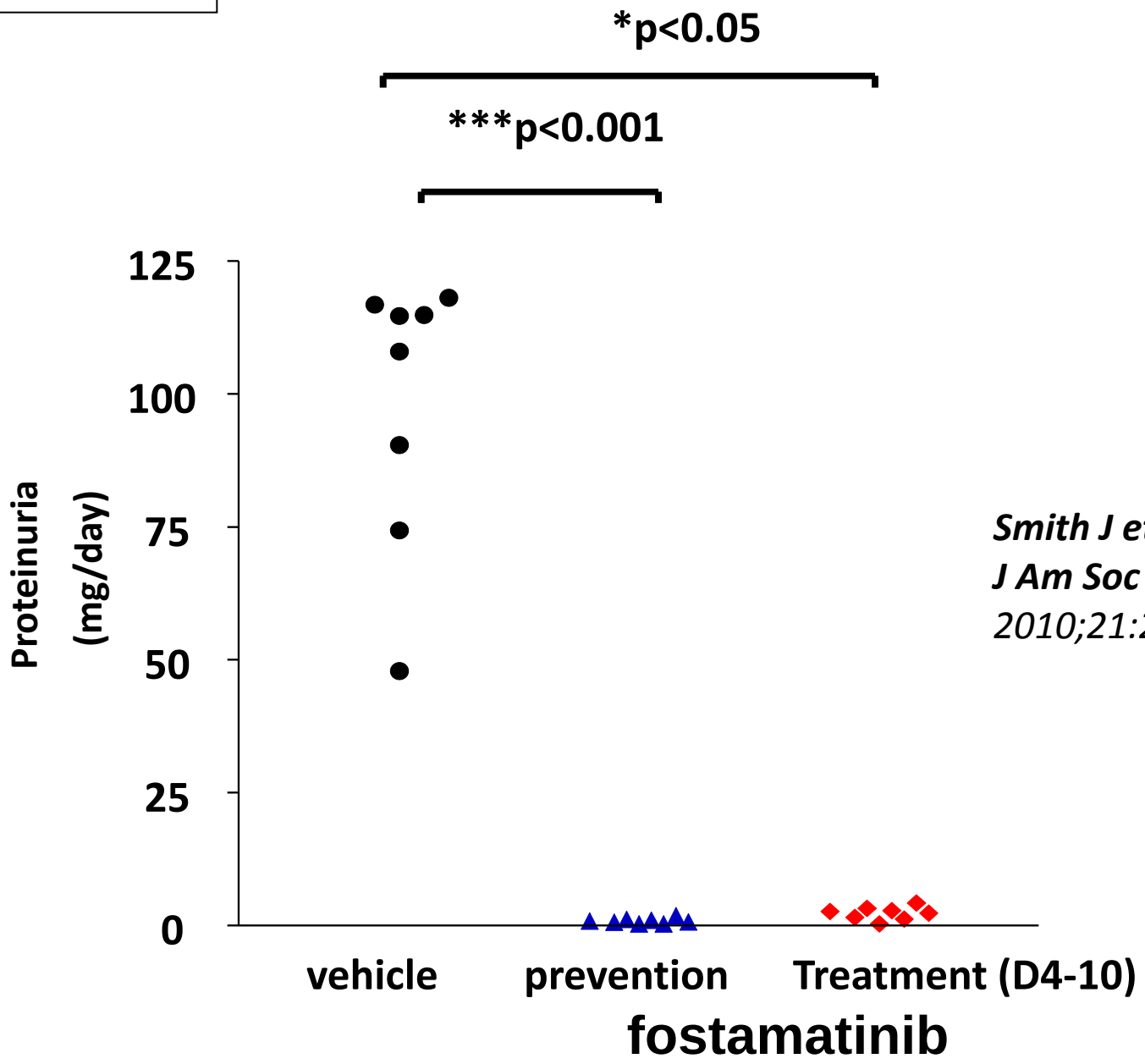
- Nephrotoxic nephritis (NTN) in WKY rats
- R788, fostamatinib (a Syk inhibitor, Rigel Pharmaceuticals, South San Francisco)

- *Smith J, McDaid JP, Bhangal G, Chawanasuntorapoj R, Masuda ES, Cook HT, Pusey CD, Tam FWK. A Spleen Tyrosine Kinase Inhibitor Reduces the Severity of Established Glomerulonephritis.* ***J Am Soc Nephrol****. 2010 (Feb);21(2):231-6*

- Is SYK inhibitor likely to be useful in treating glomerulonephritis (GN) after onset of disease?

Treatment of established GN

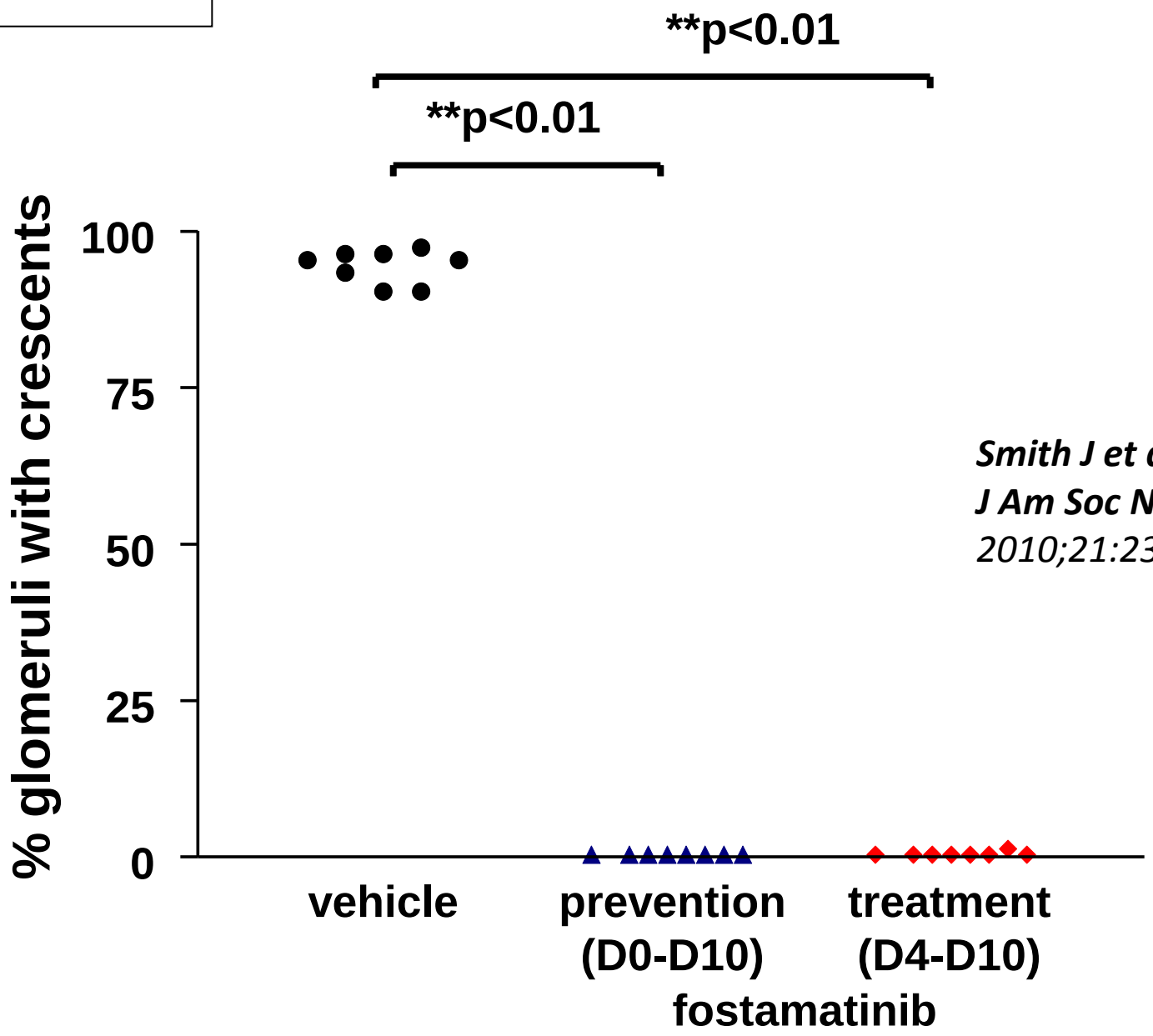
Reduction of proteinuria



Smith J et al.
J Am Soc Nephrol.
2010;21:231-6

Treatment of established GN

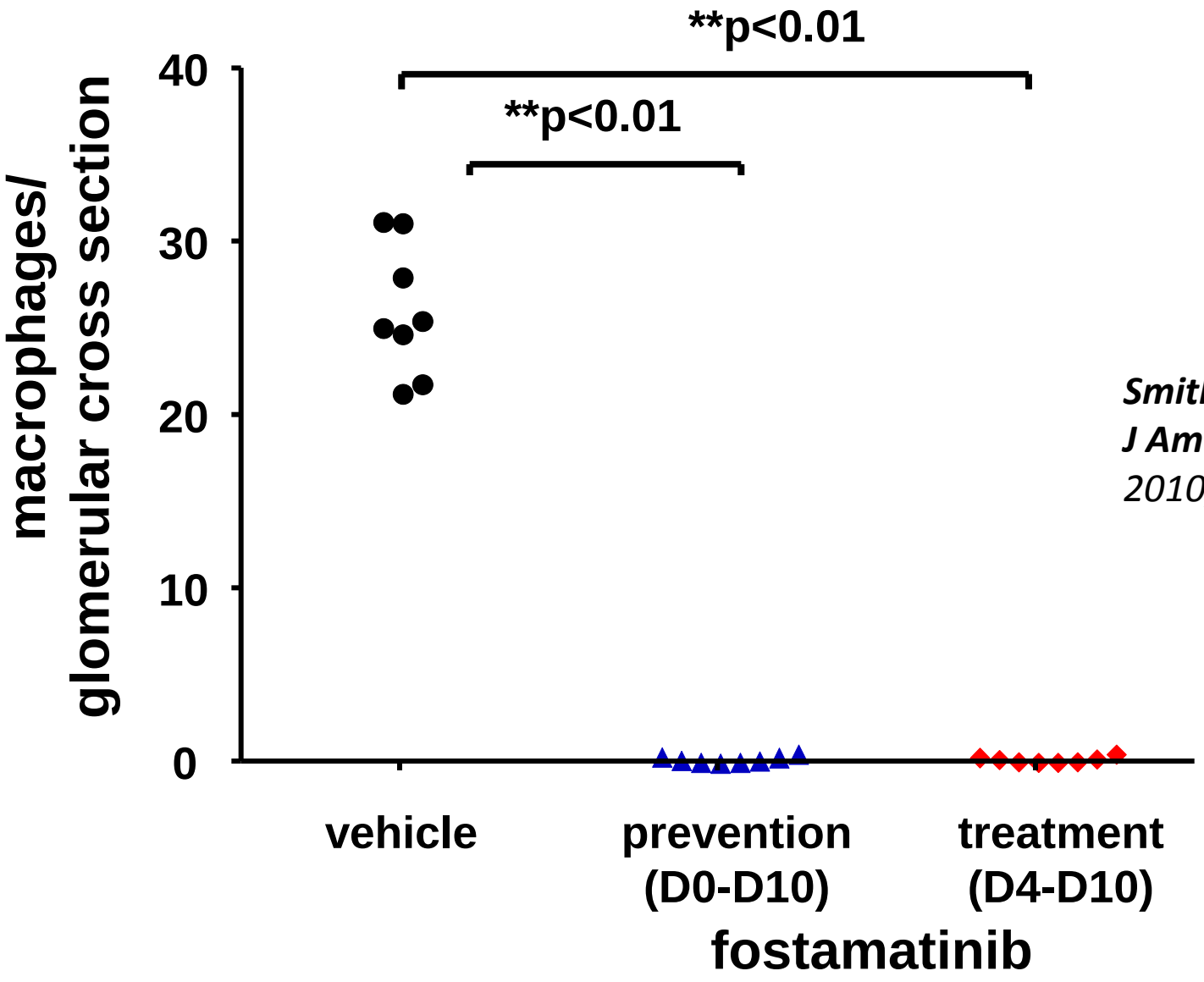
Glomerular crescents



Smith J et al.
J Am Soc Nephrol.
2010;21:231-6

Treatment of established GN

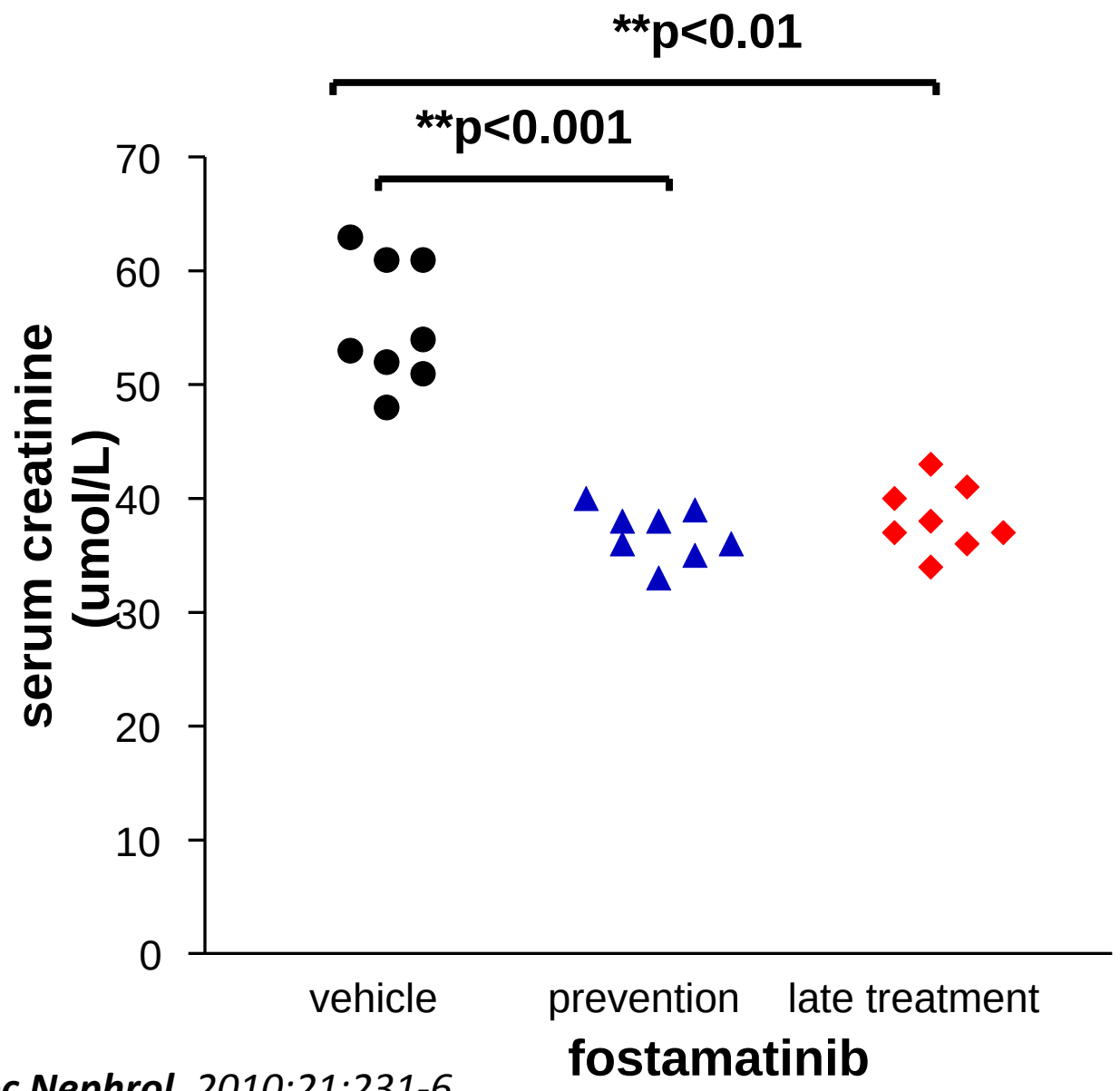
Glomerular macrophages



Smith J et al.
J Am Soc Nephrol.
2010;21:231-6

Treatment of established GN

Renal function



Smith J et al. J Am Soc Nephrol. 2010;21:231-6

Pre-clinical development

What is the effect of SYK inhibition on autoimmunity?

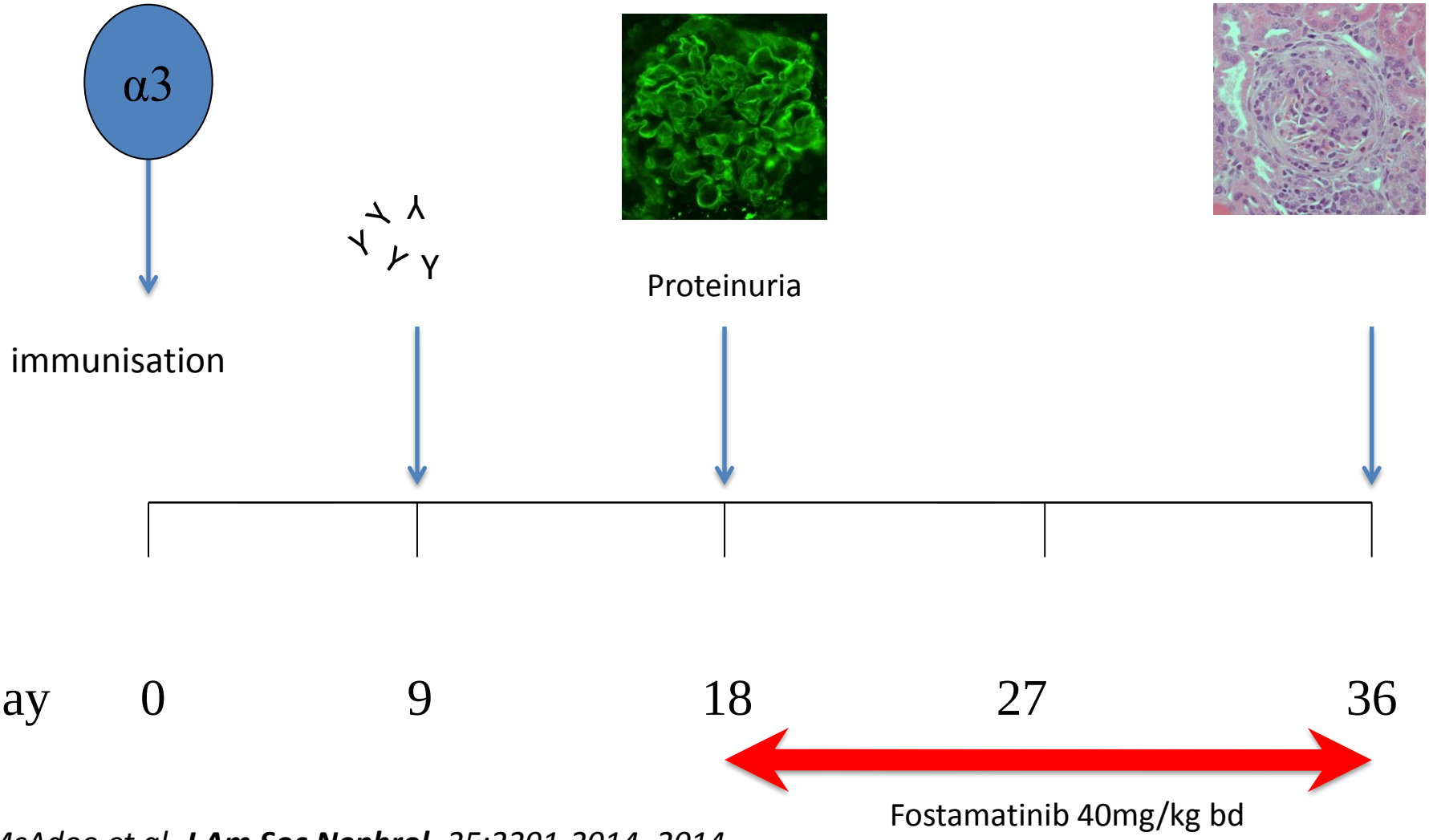
Experimental Autoimmune Glomerulonephritis (EAG)

- Genuine autoimmune model
- Characterised by ongoing autoantibody production
  - Recapitulates clinical diseases
  - Allows study of antibody production

McAdoo SP, Reynolds J, Bhangal G, Smith J, McDaid JP, Tanna A, Jackson WD, Masuda ES, Cook HT, Pusey CD, Tam FWK. Spleen Tyrosine Kinase Inhibition Attenuates Autoantibody Production and Reverses Experimental Autoimmune GN. ***J Am Soc Nephrol.*** *25:2291-2014, 2014*

Pre-clinical development

autommune: Treatment Study

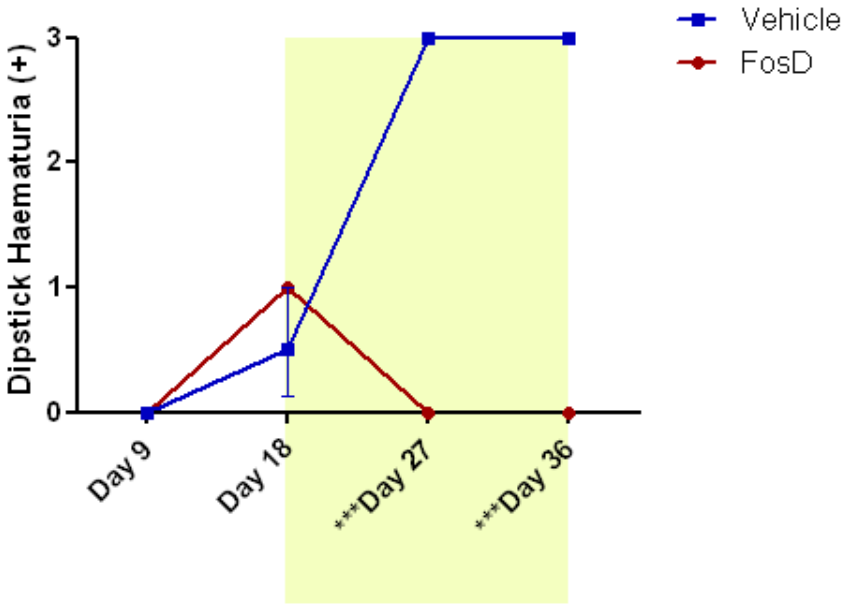


McAdoo et al, ***J Am Soc Nephrol.*** *25:2291-2014, 2014*

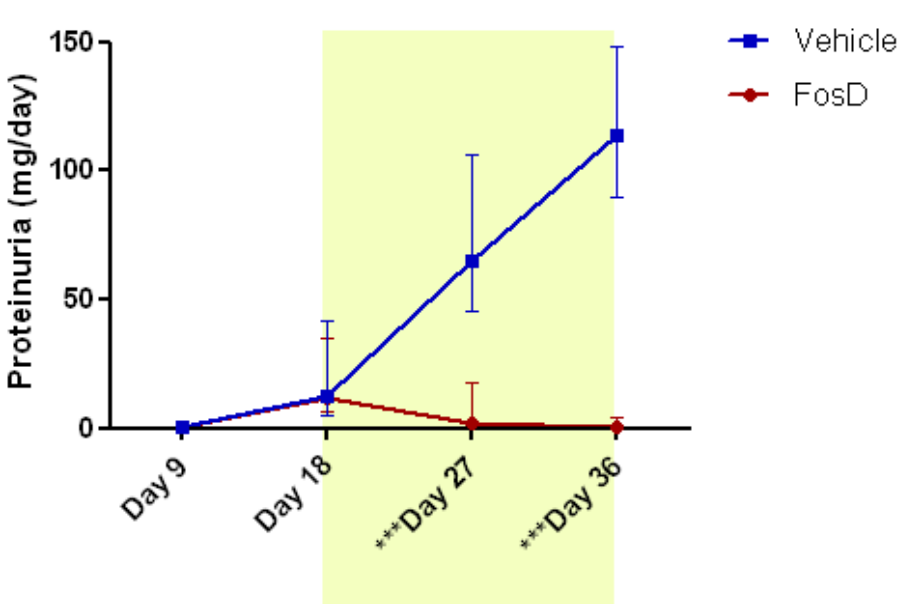
Pre-clinical development: autoimmune model

late treatment with fostamatinib reduced haematuria and proteinuria

haematuria



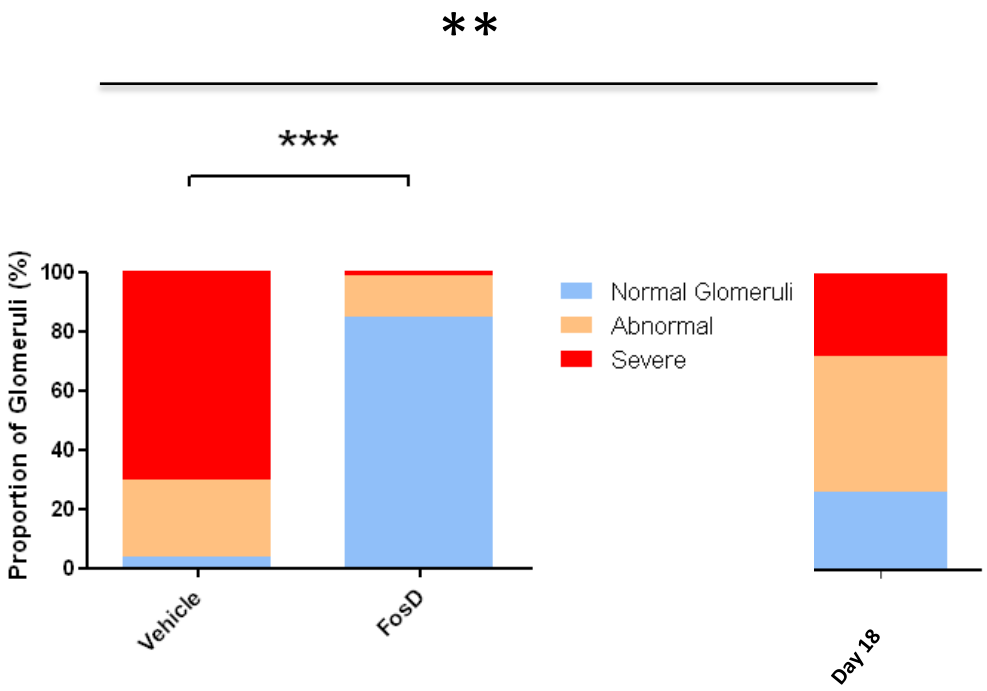
proteinuria

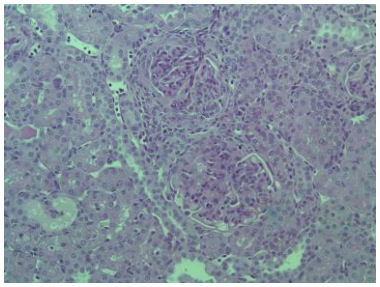


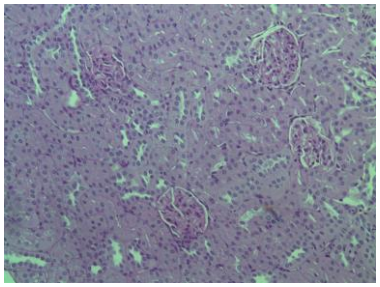
McAdoo et al, ***J Am Soc Nephrol.*** *25:2291-2014, 2014*

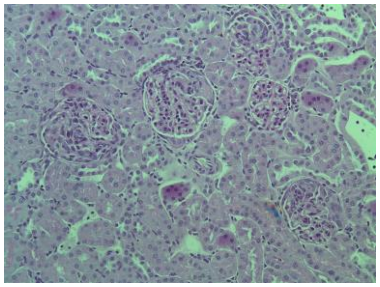
Pre-clinical development: autoimmune model

late treatment with fostamatinib : reverse histology damage of the kidney







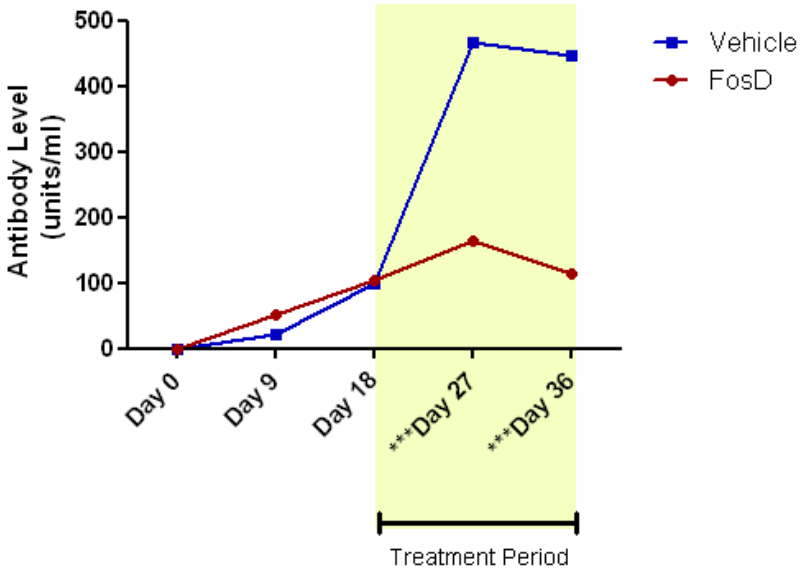


McAdoo et al, ***J Am Soc Nephrol.*** *25:2291-2014, 2014*

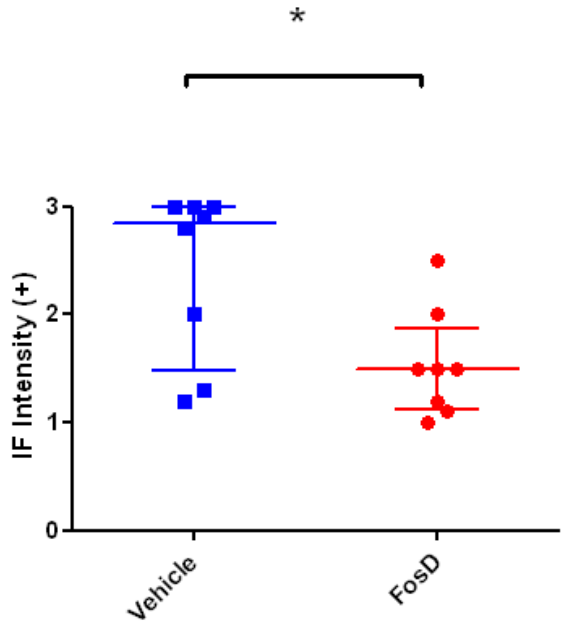
Pre-clinical development: autoimmune model

late treatment with fostamatinib : reduced production of autoantibody

Serum antibody



Antibody deposited in kidney



McAdoo et al, ***J Am Soc Nephrol.*** *25:2291-2014, 2014*

Summary 2 (preclinical models)

- Fostamatinib is an effective treatment in of established glomerulonephritis
  - Prevents and reverses histology of kidney damage
  - Inhibition of autoantibody production
  - Inhibition of inflammation
  - Reduction in proteinuria
  - Protected kidney function

Smith et al 2010, McAdoo et al, 2014

Clinical trial

Syk **I**nhibition for **G**lomerulo**N**ephritis

(NCT02112838)

- A Phase 2, Multi-Center, Randomised, Double-Blind, Ascending-Dose, Placebo-Controlled Clinical Study to Assess the Safety and Efficacy of Fostamatinib in the Treatment of IgA Nephropathy
  - industrial funding from the drug inventor (Rigel Pharmaceuticals)
  - in collaboration with Kidney Research UK
  - A patient support group
- More than 20 collaborating centres: Austria, Germany, Switzerland, UK, Singapore, Hong Kong, Taiwan, USA
- Recruitment started in December 2014

Objectives:

- To assess the efficacy of fostamatinib administered orally for 24 weeks to subjects with IgA nephropathy, as measured by change in renal function and histology.
- To investigate the safety and tolerability of fostamatinib administered orally for 24 weeks to subjects with IgA nephropathy.

Double-Blind Treatment

Treatment Group	Number of Subjects	Dose
A	25	Fostamatinib 100 mg bid
B	25	Fostamatinib 150 mg bid
C	25	Placebo bid

Proof of Principle (Phase 2) Clinical Trial

- Recent diagnosis of IgA nephropathy by kidney biopsy and has significant proteinuria
- Initial period (3-6 months) optimise treatment of blood pressure and proteinuria with angiotensin converting enzyme inhibitor or receptor blocker
- If still has significant proteinuria, then enter randomised controlled trial with the SYK inhibitor, Fostamatinib, or placebo for 24 weeks

Inclusion criteria

- Renal biopsy findings consistent with IgA nephropathy (within 6 months of randomisation)
- Treatment with an Angiotensin Converting Enzyme inhibitor (ACEi) and/or an Angiotensin II Receptor Blocker (ARB) for at least 90 days at the maximum approved (or tolerated) dose
- Proteinuria > 1 gm/day or sPCR > 100 mg/mmol at diagnosis of IgA nephropathy and Proteinuria > 0.50 gm/day (sPCR > 50 mg/mmol) at the second Screening Visit
- Blood pressure controlled to ≤ 130/80 with angiotensin blockade with or without other anti-hypertensive agents

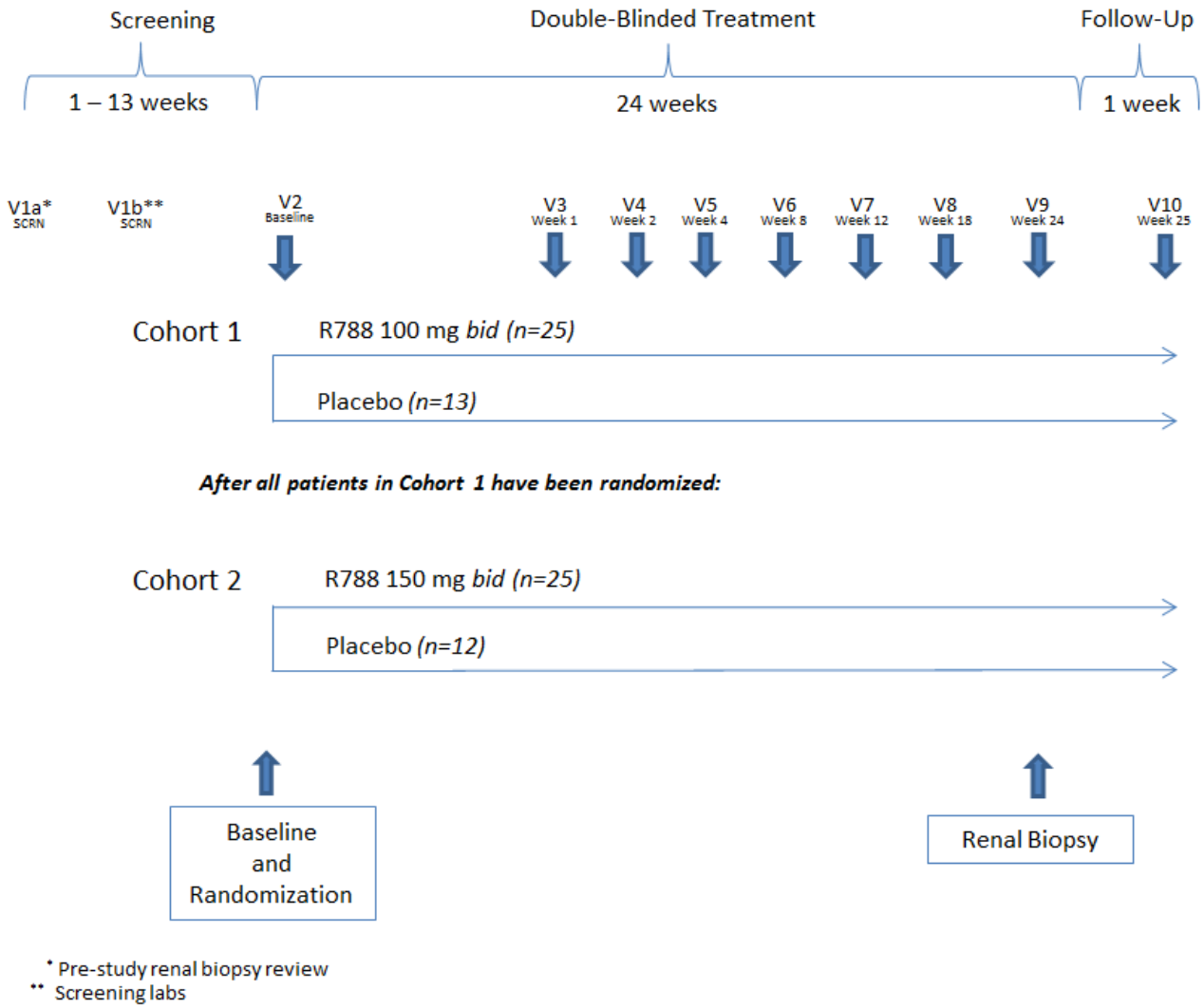
Exclusion criteria (1)

- History of or active, clinically significant, respiratory, gastrointestinal (including pancreatitis), hepatic, neurological, psychiatric, musculoskeletal, genitourinary, dermatological, or other disorder
- Have had any major cardiovascular event within the 180 days prior to randomisation, including but not limited to: myocardial infarction, unstable angina, cerebrovascular accident, pulmonary embolism, or New York Heart Association Class III or IV heart failure.
- Active bacterial or parasitic infections, including tuberculosis
- Virology: Positive serologic test for hepatitis B or hepatitis C, or subjects with suspected human immunodeficiency virus (HIV)
- Have a significant infection, an acute infection such as influenza, or who are known to have an active inflammatory process at the time of Screening
- .

Exclusion criteria (2)

- Henoch-Schonlein purpura
- use of > 15 mg/day prednisone
- Recent use (within 6 months prior to pre-study renal biopsy) of immunosuppressive agents including cyclosporine, cyclophosphamide, azathioprine, mycophenolate mofetil, Rituximab (or other anti-B cell therapies)
- eGFR < 30 ml/min/1.73 sqm

Study Design



* Pre-study renal biopsy review
** Screening labs

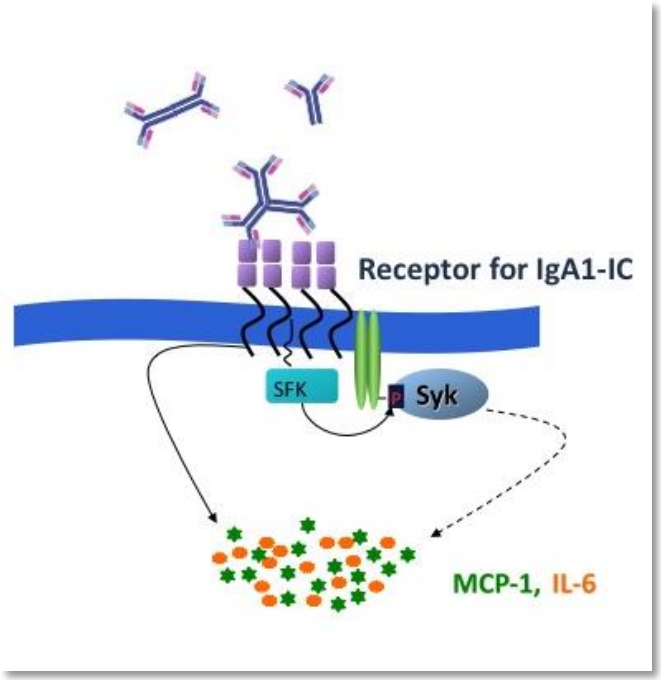
End points for clinical trial

- Improvement in proteinuria?
- Improvement in histology of kidney damage (kidney biopsy after 24 weeks of treatment)?
- Safety and tolerability assessment
- To ascertain what are the renal histology features predicting response to SYK inhibitor

Primary endpoint

- Mean change from Baseline of proteinuria as measured by spot protein-creatinine ratio (sPCR) at 24 weeks

Conclusions (selective SYK inhibition)



- Increase p-SYK in the renal biopsies of patients with IgA nephropathy
- Both pharmacological inhibition of SYK and molecular knockout of SYK reduced production of inflammatory mediators from kidney cells in culture
- SYK inhibitor was shown to be effective in reducing autoantibody production and kidney damage in preclinical models of glomerulonephritis
- Ongoing: a proof of principle clinical trials of fostamatinib for treatment patients with IgA nephropathy