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Spleen Tyrosine Kinase: A Novel Target in Autoimmunity

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1. Introduction

Spleen tyrosine kinase (Syk) is a non-receptor tyrosine kinase that is highly expressed in cells of haematopoietic lineage, where it has an important role in the intra-cellular signalling cascades for various immunoreceptors, such as the B cell receptor and the Fc receptor. As such, it is a potential target in allergic and autoimmune diseases. Emerging evidence also suggests that Syk may have additional roles beyond its well defined functions in classical immunoreceptor signalling, which may have implications, potentially useful or harmful, for therapies directed at this protein. Given these diverse functions in numerous cell types, it is unsurprising that Syk has been the subject of hundreds of original research articles, as well as several excellent reviews in recent years (Sada, Takano et al. 2001; Ruzza, Biondi et al. 2009; Mocsai, Ruland et al. 2010; Riccaboni, Bianchi et al. 2010). In this chapter we aim to summarise current understanding of the basic structure and function for Syk, before developing a rationale for targeting Syk in autoimmune disease. We will then review progress towards Syk-directed therapies in clinical practice, and finally we shall consider the emerging functions of Syk beyond the adaptive immune response, and what implications these may have for therapy.

2. Discovery

Syk was identified in the early 1990s in the cytosolic fractions of lysates from porcine spleen and bovine thymus as a 40kDa protein with intrinsic kinase activity. This 40kDa protein was subsequently shown to be a fragment, containing only the catalytic domain, of a larger 72kDa protein that was identified from a porcine spleen cDNA library (from whence it gained its name) using oligonucleotides designed according to the partial sequence of the purified 40kDa fragment (Taniguchi, Kobayashi et al. 1991). The *Syk* gene was subsequently mapped to chromosome 9q22 in humans (Ku, Malissen et al. 1994).

3. Basic structure and function

The Syk molecule has a multi-domain structure (Figure 1a) containing two N-terminal tandem Src Homology 2 (SH2) domains and a C-terminal kinase domain (Sada, Takano et al. 2001). Interdomains A and B connect the SH2-SH2 and SH2-kinase domains, respectively. At least 10 major phosphorylation sites have been identified within the molecule (Furlong,

Mahrenholz et al. 1997) – one located in interdomain A, five within interdomain B, two within the kinase domain, and two near the extreme C-terminus. An alternatively spliced form of Syk - SykB – lacks a 23 amino acid sequence in interdomain B, and in this respect is similar to ZAP-70, the only other member of the Syk family of kinases. ZAP-70 has approximately 60% overall homology to Syk and its expression appears to be more restricted, in particular to T lymphocytes and natural killer (NK) cells (Au-Yeung, Deindl et al. 2009).

In the resting state, it is thought that Syk assumes a closed, auto-inhibited structure (Figure 1b), wherein interdomain A and interdomain B bind to the C-terminal kinase domain, preventing its interaction with potential substrates, in what is termed a 'linker-kinase sandwich' (Deindl, Kadlecik et al. 2007; Kulathu, Grothe et al. 2009). Upon activation, structural changes within the molecule result in an open conformation that allows the exposed catalytic kinase domain to interact with downstream targets.

The canonical mechanism of Syk activation is via its interaction with immunoreceptor tyrosine-based activation motifs (ITAMs) (Turner, Schweighoffer et al. 2000). ITAMs are short peptide sequences characterised by a consensus sequence that contains two tyrosine residues 6-12 amino acids apart. As their name suggests, they are found in association with the cytoplasmic components of classical immunoreceptors, including the T-cell receptor (TCR), B-cell receptor (BCR) and Fc-receptor (FcR) for immunoglobulins, either as an associated adapter protein, or within the cytoplasmic region of the receptor itself.

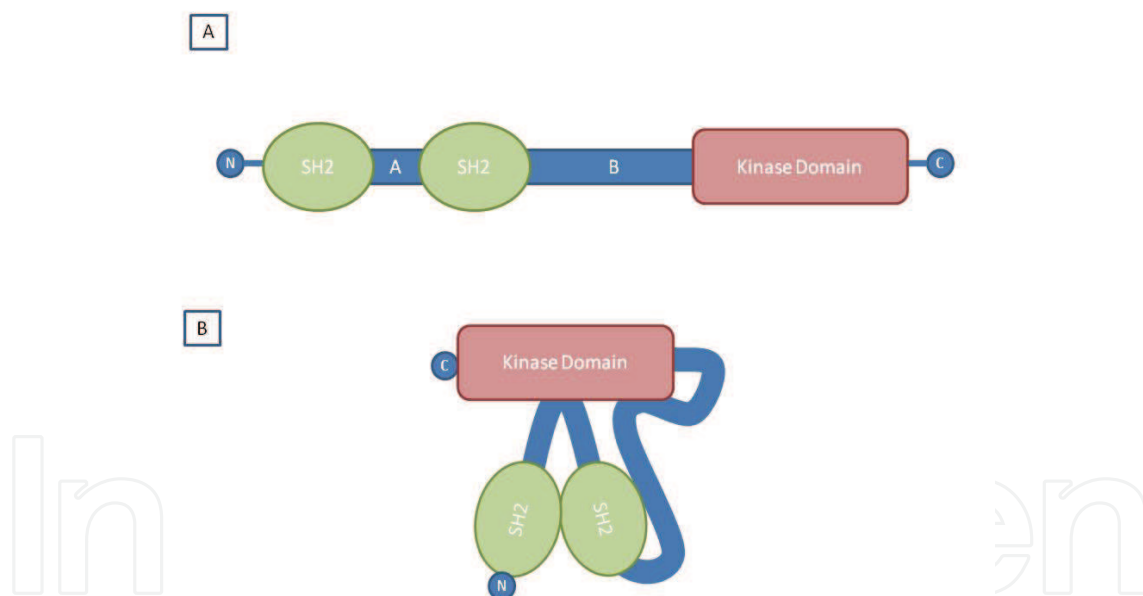


Fig. 1. a. Schematic diagram showing the multi-domain structure of Syk, including two N-terminal SH2-domains, C-terminal kinase domain, and interdomains A and B. Figure 1b: Schematic diagram of the 'linker-kinase sandwich' conformation that has been suggested for resting Syk (see text for details)

Upon receptor engagement, the tyrosine residues on ITAMs are rapidly phosphorylated, primarily by Lyn and other members of the Src family of kinases (Figure 2). The phosphorylated ITAM can now act as a docking site for the SH2 domains of Syk, resulting in conformational changes, exposure of the kinase domain, autophosphorylation and propagation of downstream signalling. In addition, disruption of the 'linker-kinase

sandwich' may occur upon phosphorylation of tyrosine residues alone, particularly those within interdomain B. This may occur by autophosphorylation following ITAM-mediated activation, or by transphosphorylation by other kinases, such as Lyn or other Src family kinases that are often co-localised with ITAMs at the cell membrane. As a consequence, positive feedback and sustained Syk activity is possible in the absence of phosphorylated ITAMs. This 'dual' mechanism of activation has recently led to the proposal of Syk as an 'OR' gate in signalling pathways (Tsang, Giannetti et al. 2008; Bradshaw 2010).

In addition to releasing the enzymatic domain of the protein from the 'linker-kinase sandwich', these changes in structure and phosphorylation, particularly within the tyrosine-rich interdomain B, create docking sites for downstream targets of Syk, for which it can perform both enzymatic and adapter functions (Kulathu, Grothe et al. 2009). These downstream targets include a host of adapter proteins and other enzyme targets (including LAT, SLP76, Vav1, PLC- γ , PI3K and MAP kinases) that are then able to effect complex cellular responses including proliferation, differentiation, phagocytosis and cytokine production (Mocsai, Ruland et al. 2010).

Two groups simultaneously reported the effects of targeted disruption of the *Syk* gene in mice in the mid-90s (Cheng, Rowley et al. 1995; Turner, Mee et al. 1995). *Syk* knockout resulted in perinatal death with a severe haemorrhagic phenotype. This was subsequently shown to be due to a failure of communication between developing vasculature and lymphatics during embryogenesis (Abtahian, Guerriero et al. 2003). Analysis of *Syk*-deficient lymphoid cells derived from these knock-out animals was critical in developing our early understanding of the functional role of Syk in immunoreceptor signalling in various cell types.

Bone marrow chimera animals, reconstituted with haematopoietic stem cells from *Syk*-deficient mice, showed no reduction in the numbers of circulating erythrocytes, platelets and total leucocytes. These animals had relatively normal reconstitution of T cells, however detailed analysis revealed impaired differentiation of the B cell lineage, with development arrested at the pro-B to pre-B cell stage, consistent with a role for Syk in pre-BCR signalling (Cheng, Rowley et al. 1995; Turner, Mee et al. 1995). Subsequent *in vitro* work, using a variety of cell lines and cell-based reconstitution systems, has defined a clear role for Syk in initiating downstream signalling following engagement of the BCR (Geahlen 2009), and indeed it was study in this area that was responsible for much of our understanding of the basic structure and function of Syk. The functional role of Syk in mature B cells (such as on antibody production by plasma cells) *in vivo*, however, is less well defined and future studies using small molecule inhibitors, or potentially conditional knockout in this cellular compartment, will be informative.

Analysis of myeloid cells, such as macrophages and neutrophils, from *Syk* knockout bone marrow chimeras showed ablation of FcR-mediated responses including phagocytosis and the generation of reactive oxygen intermediates (Crowley, Costello et al. 1997; Kiefer, Brumell et al. 1998). The role of Syk in signal transduction for activatory FcR in these and a variety of other cell types is now well established, including mast cells bearing Fc ϵ R (de Castro 2011). A critical role for Syk in Fc γ R-mediated antigen internalisation and maturation by dendritic cells has also been described (Sedlik, Orbach et al. 2003), and is notable given the important role of dendritic cells in initiating adaptive immune responses.

In addition to FcR-mediated responses, Syk has been implicated in integrin signalling in myeloid cells (Mocsai, Zhou et al. 2002; Mocsai, Abram et al. 2006). Integrins are

transmembrane receptors that have a critical role in cell adhesion and migration, via their interaction with adhesion molecules expressed on other cells, particularly the vascular endothelium. Syk deficient myeloid cells show impaired integrin-mediated responses, thought to be dependent on the association of integrins with ITAM-containing adapter proteins such as FcR γ -chain and DAP12, as myeloid cells deficient in these adapter proteins show similar defects.

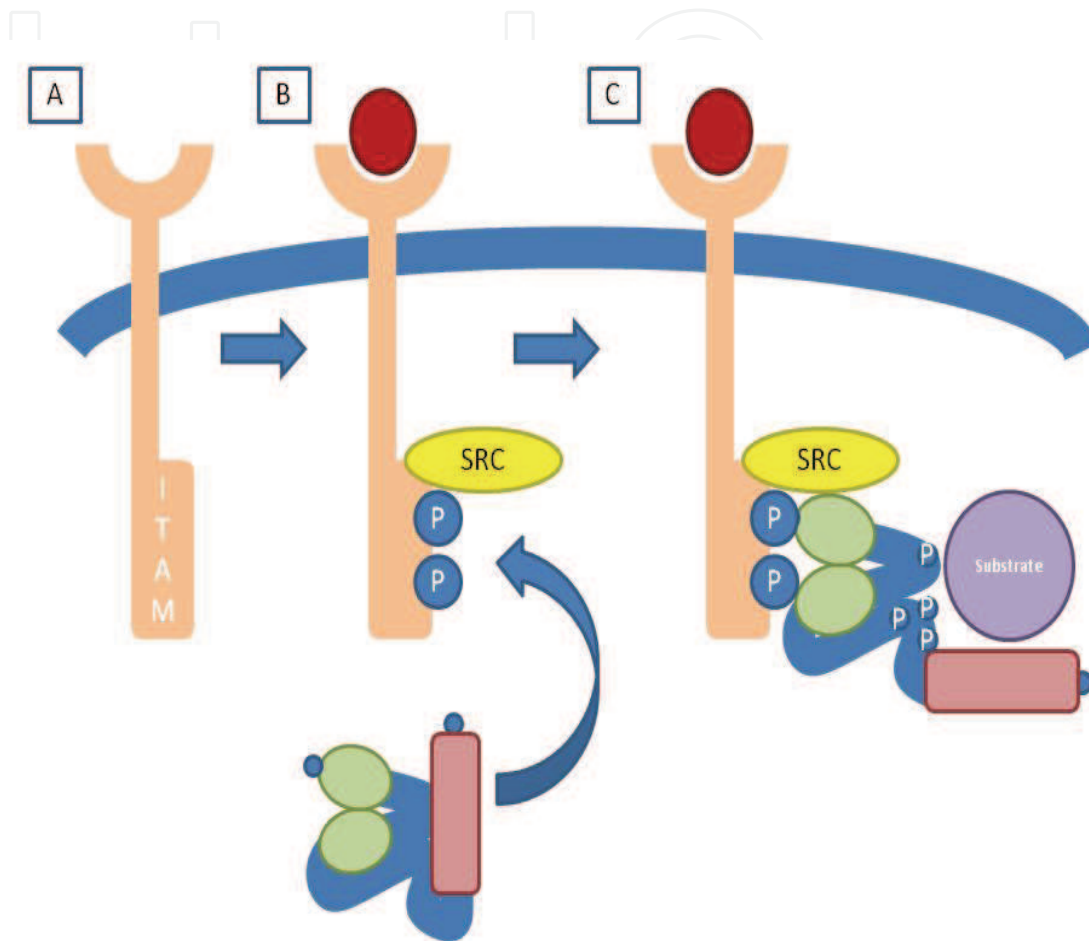


Fig. 2. Simplified schematic showing activation of Syk by interaction with ITAM.
 2a: Unengaged receptor bearing non-phosphorylated ITAM motif within its cytoplasmic tail.
 2b: Upon receptor engagement Src family kinases (SRC) phosphorylate (P) tyrosine residues within the ITAM motif.
 2c: Phosphorylated ITAM motif acts as a docking site for the SH2 domains of Syk, resulting in conformational changes, auto- and transphosphorylation of tyrosines within Syk, thus resulting in activation and phosphorylation of downstream targets

4. Syk in autoimmunity

Given our understanding of its role in BCR- and FcR-mediated signalling, the rationale for targeting Syk in autoimmunity is clear. The presence of autoantibodies is the hallmark of many autoimmune diseases. Whilst not universally pathogenic, these antibodies often contribute to disease via their interaction with FcR expressed on many immune effector cells (and other mechanisms including complement activation). Notably, FcR-deficient mice are

resistant to a variety of animal models of autoimmunity, and FcR polymorphisms have been shown to be important determinants of human autoimmune disease (Takai 2002; Nimmerjahn 2006). Syk inhibition may, therefore, have the desirable double effect of preventing the production of pathogenic autoantibodies, via inhibiting B cell activation via the BCR, and simultaneously inhibiting their downstream effects via disrupting signalling from their receptors.

It is encouraging that other therapeutic approaches that target B-cells have recently shown efficacy in clinical practice (Townsend, Monroe et al. 2010). Interestingly, however, clinical benefit cannot always be attributed to eradication of circulating autoantibody, and it is probable that effects on other B cell functions, such as antigen presentation, cytokine production, and provision of co-stimulation to other immune cells, contribute to the benefit seen. Disruption of Syk-dependent BCR signalling may provide similar benefits.

In addition, Syk inhibition may have the additional effect of inhibiting migration and recruitment of immune effector cells to sites of tissue inflammation, via inhibited integrin signalling.

Therapeutic strategies to target Syk, therefore, are desirable and can be broadly classified into two main approaches: pharmacological inhibition of Syk activity, and gene-based therapies aiming to silence Syk expression (Ruzza, Biondi et al. 2009). Whilst the application of the latter has yet to advance to clinical studies, we shall briefly review their future potential, before focusing on the progress of Syk inhibition therapies.

5. Gene-based therapies targeting Syk

5.1 Antisense oligonucleotides (ASO)

ASO are short, single stranded nucleic acid sequences that bind sense mRNA via complementary base-pairing, and thus inhibit the translation of the relevant protein. ASO directed against Syk have shown activity in a variety of cell types *in vitro*, including inhibitory effects of FcγR-mediated signalling in monocytes (Matsuda, Park et al. 1996). Published *in vivo* studies using Syk ASO are limited to animal models of allergic inflammation (Stenton, Kim et al. 2000; Stenton, Ulanova et al. 2002). These have shown that aerosolized Syk ASO, delivered in a liposomal complex, suppress Syk expression in, and inflammatory mediator release from, alveolar macrophages. In addition, markers of pulmonary inflammation were reduced in two distinct animal models. Whilst there have been no *in vivo* studies in autoimmune models, the proposed mechanism of action in these allergic models is via inhibition of activatory FcR-mediated responses, suggesting similar approaches may be effective in autoimmune disease. However, progress in the clinical use of ASO based therapy has been frustratingly slow since the introduction of Fomivirsen, the first antisense therapy to be licensed by the Food and Drug Administration (FDA), being approved for use in AIDS-related cytomegalovirus (CMV) retinitis over 10 years ago. This is due, in part, to the difficulty of producing reliable delivery systems to target the cells, tissues or organs of choice (White, Anastasopoulos et al. 2009) – a not insignificant problem given the multi-system nature of many autoimmune diseases.

5.2 RNA interference (RNAi)

RNAi is an innate cellular process that is thought to regulate endogenous gene expression and protect against viral infection. A variety of small RNA molecules, such as endogenous,

genetically encoded microRNA (miRNA) or exogenous small interfering RNA (siRNA), may bind target mRNA via Watson-Crick complementary base pairing, and then direct this target mRNA into an RNA-induced silencing complex (RISC), a natural degradation pathway, thus effecting gene silencing prior to translation. Since this first description of RNAi in 1998, advance in the field has been rapid, and there have been promising early phase clinical studies in a number of conditions, including retinal diseases, malignancies and viral infections. The most commonly used technique to harness RNAi for therapy has been to transfect siRNA into target cells. siRNA specific to Syk, for example, have been shown to inhibit antibody-mediated phagocytosis by human macrophages (Lu, Wang et al. 2011). Again, *in vivo* studies in this field are limited to models of allergic inflammation, and to date are only reported in international patent applications. Aerosolised delivery of Syk siRNA, using similar methods as used for ASO delivery, resulted in decreased pulmonary inflammation, as determined by recruitment of cells to bronchoalveolar lavage fluid, in a rat model of ovalbumin-induced asthma. Again, these findings augur well for the translation and use of RNAi in autoimmune disease. As with antisense therapy, outstanding challenges for harnessing RNAi include the development of effective delivery systems, escape of the innate 'interferon' response directed against foreign nucleic acids, and avoidance of 'off-target' gene silencing (Davidson and McCray 2011).

6. Pharmacological inhibition

A number of biotechnology and pharmaceutical companies are working to develop compounds to inhibit Syk (Ruzza, Biondi et al. 2009; Riccaboni, Bianchi et al. 2010). To date, two such inhibitors, both identified by Rigel Pharmaceuticals, and now in development by AstraZeneca, have progressed to clinical trials - initially R112, and more recently the related compound, R406 (and its respective prodrug, R788).

6.1 Preclinical studies with small molecule inhibitors

Cell based high-throughput screening of small molecules identified R112 as a potent inhibitor of Syk activity, as assayed by production and release of inflammatory mediators following FcR ϵ crosslinking by anti-IgE (Rossi, Herlaar et al. 2006). Subsequent characterization showed that R112 is an ATP-competitive inhibitor of Syk activity, as demonstrated by *in vitro* kinase assays (IC_{50} = 226 nmol/l). These assays also showed activity against other kinases such as Lyn (IC_{50} = 300nmol/l) and Lck (IC_{50} =645 nmol/l). However, when tested in cell-based assays, R112 was shown to be relatively selective for Syk as determined by phosphorylation of target proteins, despite the similar IC_{50} values in the *in vitro* assays.

Rigel subsequently developed the related compound R406, another potent competitive ATP inhibitor for Syk (*in vitro* IC_{50} = 41 nmol/l) (Brasemann, Taylor et al. 2006). Again, selectivity assessments using over 90 *in vitro* kinase assays showed an inhibitory effect on other kinases, although cell based assays confirmed selectivity for Syk - FLT3 was the next most potently inhibited kinase, though at 5-fold less activity. R406 has been shown to inhibit FcR-mediated responses in a variety of cell types *in vitro*, including mast cells, macrophages, neutrophils and dendritic cells (Brasemann, Taylor et al. 2006; Matsubara, Koya et al. 2006). In addition, activity against BCR-mediated responses has been shown in primary human B cells *in vitro*. Notably, R406 did not show significant activity in Syk-independent pathways (such as following lipopolysaccharide stimulation) at the concentrations necessary to inhibit

FcR- and BCR-mediated pathways, in keeping with the selectivity for Syk seen in the cell based phosphorylation assays. R788, or fostamatinib disodium (see McAdoo and Tam 2011 for review) was then developed as the methylene phosphate prodrug of R406, to improve its solubility and potential for oral dosing.

The efficacy of R406/R788 has been studied in numerous animal models of autoimmunity. In a murine model of immune cytopenias, for example, treatment with R788 prevented haemolysis and the development of thrombocytopenia following the administration of anti-red blood cell and anti-platelet antibodies respectively (Podolanczuk, Lazarus et al. 2009). Treatment with R406/R788 reduced clinical, histological and radiographic evidence of joint inflammation following the induction of collagen-induced arthritis, a rodent model of rheumatoid arthritis (Pine, Chang et al. 2007). R788 has shown efficacy in three animal models of SLE (Bahjat, Pine et al. 2008; Deng, Liu et al. 2010). Treatment of the lupus-prone NZB/NZW mouse strain reduced kidney disease, as determined by proteinuria and renal histology, and improved platelet counts and survival. In the BAK/BAX knockout mouse, treatment with R788 reduced lupus-like skin disease and lymphadenopathy. Similarly, in the MRL/lpr strain, treatment improved renal and skin disease, in addition to reducing lymphadenopathy. In a rodent model of antibody-mediated glomerulonephritis, treatment with R788 prevented the induction of disease (Smith, McDaid et al. 2010). In addition, treatment had a profound effect on established disease, reversing the histological features of crescentic glomerulonephritis even when initiated after the induction of disease. Finally, in an autoimmune diabetes model, R788 delayed the onset of insulinitis and spontaneous diabetes in NOD mice (Colonna, Catalano et al. 2010). Again, significant effects were seen even when treatment was initiated after the onset of glucose intolerance. Though not strictly a model of autoimmunity, treatment with R788 reduced local and remote inflammation in mesenteric ischaemia-reperfusion in mice (Pamuk, Lapchak et al. 2010), suggesting effects independent of the FcR and BCR, such as inhibition of leucocyte migration.

The safety of R788/R406 has also been assessed in detailed rodent toxicity and immunotoxicology studies (Zhu, Herlaar et al. 2007). At high doses, R406 was associated with a reduction in circulating lymphocyte count, bone marrow cellularity, and thymic and spleen weight. These changes generally resolved during a drug-withdrawal recovery period. In murine host resistance models, treatment with R788 did not significantly impair clearance of influenza, *Listeria* or streptococcal infection.

Other pharmaceutical companies have developed a number of other small molecule inhibitors. The majority of these, like R112 and R406, are competitive inhibitors for the ATP binding site of the catalytic domain. Bayer, for example, has developed the imidazopyrimidine analogue BAY 61-3606, which inhibits Syk-mediated responses *in vitro* and has demonstrated efficacy in animal models of allergy *in vivo* (Yamamoto, Takeshita et al. 2003). However, the published selectivity profile is limited to only 6 other kinases, and comparison with genetic knockdown suggests that BAY 61-3606 may have significant off-target effects (Lin, Huang et al. 2010).

Other groups have chosen to target the non-kinase domains of the Syk molecule. By inhibiting the interaction of the SH2-domains with their docking proteins, it has been proposed that Syk inhibition may be achieved whilst avoiding off-target effects on other kinases, and one such approach has been shown to inhibit IgE-mediated responses *in vitro* and *in vivo* (Mazuc, Villoutreix et al. 2008). Whilst the precise molecular mechanism of the

inhibitory effects of this molecule are yet to be definitively described, it should be noted that the SH2-domain is a highly conserved motif found in a large number of proteins involved in signal transduction, and targeting this domain may in turn have diverse off target effects.

6.2 Clinical studies

R112 was the first Syk inhibitor treatment to be assessed in clinical studies, where it showed benefit in relieving symptoms of allergic rhinitis when delivered as an intranasal preparation (Meltzer, Berkowitz et al. 2005). Subsequent work focused on R406/R788, with early phase clinical studies confirming that R406/788 is highly bioavailable following oral administration, rapidly absorbed systemically, and well tolerated at doses needed to achieve biological effects such as inhibition of basophil activation in response to anti-IgE *ex vivo* (IC₅₀ 1.06 microM) (Brasemann, Taylor et al. 2006; Sweeny, Li et al. 2010).

To date, the results of five phase II studies with R788 (fostamatinib) have been published. Three of these investigated the effects of fostamatinib in rheumatoid arthritis (RA) (Table 1). The first recruited almost 200 patients with active disease despite standard therapy with methotrexate (Weinblatt, Kavanaugh et al. 2008). Clinical responses were seen as early as one week following the initiation of treatment, and sustained at three months, with significant improvements in disease activity scores in the treatment groups versus placebo. The second study enrolled over 450 patients with active disease despite long-term methotrexate therapy, and this confirmed benefit at six months, with improved American College of Rheumatology 20% improvement criteria (ACR20), ACR50, ACR70 and DAS28 scores (Weinblatt, Kavanaugh et al. 2010). Notably, both these studies included a significant proportion of patients who had failed biological therapy with anti-TNF or anti-CD20 agents, and benefit with fostamatinib was seen in these subgroups (although overall response rates were lower than for the whole study population). A subsequent trial designed specifically to examine the efficacy of fostamatinib in this group of patients failing biologic therapy, however, did not achieve its primary endpoint of improved ACR20 in the treatment group (Genovese, Kavanaugh et al. 2011). There were, however, significant improvements in radiographic and biochemical markers of inflammation.

A small open label study in immune thrombocytopenic purpura (ITP) has also shown promising results (Podolanczuk, Lazarus et al. 2009). Although the numbers are small (n=16), the majority of patients had refractory disease with multiple previous ITP treatments (commonly steroids, intravenous immunoglobulin, rituximab and splenectomy). As such, the sustained (50%) and transient (25%) response rates seen during the average follow up time of 36 weeks represent encouraging results and further studies are planned.

In addition to these studies in autoimmune disease, a fifth phase II study examined the effects of fostamatinib in haematological malignancy (Friedberg, Sharman et al. 2010), the rationale for which is discussed below. Again, in a study population that included a significant proportion of patients with heavily pre-treated, relapsed or refractory disease, fostamatinib showed modest but significant clinical activity and a manageable side-effect profile.

The most frequent adverse event seen in these clinical studies was gastrointestinal toxicity, a common side effect of many kinase inhibitors. Symptoms were dose-related, and seen at rates of up to 45% in groups receiving the highest doses of fostamatinib, and this was the most common reason for patient withdrawal from the RA trials.

REF	N	ENTRY CRITERIA	FOLLOW UP	ENDPOINT	OUTCOMES
Weinblatt, Kavanaugh et al. 2008)	189	Active RA (≥ 12 months from diagnosis) despite ≥ 6 months methotrexate therapy	12 weeks	ACR20 response rate (ACR50, ACR70, DAS28 response rates)	Significant benefits in disease activity scores in patients treated with fostamatinib 100-150 mg bid. Clinical response noted as early as one week after initiation of treatment.
Weinblatt, Kavanaugh et al. 2010)	457	Active RA (≥ 6 months from diagnosis) despite ≥ 3 months methotrexate therapy	6 months	ACR20 response rate (ACR50, ACR70, DAS28 response rates)	Significant benefits in disease activity scores in patients treated with fostamatinib 100-150 mg bid. Again, response noted as early as one week.
Genovese, Kavanaugh et al. 2011)	229	Active RA (≥ 12 months from diagnosis) with disease unresponsive to current or previous biologic therapy	3 months	ACR20 response rate (ACR50, ACR70, DAS28 response rates; synovitis scores on MRI)	No difference in disease activity scores across treatment and placebo groups. Significant improvements in circulating inflammatory markers and synovitis scores on MRI noted in treatment groups.

Table 1. Summary of Phase II trials with fostamatinib in patients with rheumatoid arthritis (RA). ACR20/50/70, American College of Rheumatology 20/50/70% improvement criteria; DAS28, disease activity score using 28 joint counts; MRI, magnetic resonance imaging; DMARD, disease modifying anti-rheumatic drug

Neutropenia occurred in up to 30% of patients receiving fostamatinib in the RA trials. Again, this finding appeared to be dose-related and responded to dose reduction or temporary withdrawal of the drug. Although no direct pharmacokinetic interaction has been detected between fostamatinib and methotrexate (Baluum, Samara et al. 2011), synergistic effects on haematopoiesis beyond individual pharmacokinetic parameters (along with underlying bone marrow disease or other concomitant immunosuppressant therapy) may have contributed to this phenomenon, as neutropenia was not reported in the ITP trial. An increased rate of uncomplicated upper respiratory tract infections was seen in the treatment group of the largest RA trial. However, this was not associated with neutropenia. In addition, no opportunistic or atypical infections were reported in any of the clinical trials.

7. Beyond adaptive immunity: problems and potential for Syk-directed therapy

Recently, Syk has been implicated in a number of signalling pathways beyond the adaptive immune response that may have implications for Syk-directed therapy in clinical practice. These include the aforementioned role in cell adhesion, as well as possible roles in innate immunity, platelet function, bone metabolism and tumorigenesis (Mocsai, Ruland et al. 2010). Disruption of these functions may lead to significant toxicity, or alternatively open novel therapeutic avenues, in targeting Syk. The basic mechanism of these functions has been reviewed in detail elsewhere (Mocsai, Ruland et al. 2010); we shall here consider the potential clinical implications.

7.1 Innate Immunity and Infection

Given its diverse effects on adaptive immune responses, coupled to a role in inflammatory cell adhesion and migration, the preeminent concern with Syk directed therapies must be of over-immunosuppression and the associated risk of infection. It is also notable that Syk has recently been associated with a variety of pathogen recognition receptors (PRRs), important components of the innate immune response that recognise pathogen-associated molecular patterns (PAMPs). C-type Lectins, one such class of PRR, play an important role in antifungal immunity in particular, and Syk has been implicated in the intracellular signalling cascades for these receptors (Drummond, Saijo et al. 2011). Some, such as Dectin-1, contain an ITAM motif on their cytoplasmic domain, and others may associate with ITAM containing adaptor proteins such as FcR γ chain or DAP12. As such, Syk inhibition may potentially lead to excessive downregulation of multiple inflammatory, innate and adaptive immune responses, resulting in risk of overwhelming infection. The results of the preclinical toxicity assessments and host resistance models are reassuring in this respect (although a host resistance model of fungal infection has not been studied), as is the absence of severe, atypical or opportunistic infection in the clinical studies thus far. Larger and longer clinical studies are, however, needed to establish the long term and cumulative effects of Syk inhibition on innate immune responses and infection risk, particularly in patient groups who have had extensive treatment histories with other immunosuppressant agents.

7.2 Platelet function

Syk has been shown to be involved in a number of platelet activation pathways, including via the glycoprotein GPVI receptor (an FcR γ chain-associated receptor that bears an ITAM motif), integrin α IIb β 3, and C-type Lectin 2 (CLEC-2; a type II membrane protein containing

a single tyrosine-based motif in its cytoplasmic tail that has been termed a hemITAM) (Watson, Auger et al. 2005; Watson, Herbert et al. 2010). In addition, R406 has demonstrated inhibitory activity in these pathways (Spalton, Mori et al. 2009). Notably, however, very high dose exposure to R406 did not prolong bleeding time in mice, and in phase I human studies, R406 did not inhibit collagen or ADP-induced platelet aggregation *ex vivo* (Braselmann, Taylor et al. 2006), perhaps suggesting redundancy of Syk dependent pathways *in vivo*. Based on these observations, it would appear that targeting Syk in isolation would not be an effective anti-thrombotic therapy. However, synergistic effects, potentially both therapeutic and harmful, with other anti-platelet agents have not been explored in clinical studies.

7.3 Bone metabolism

Syk has been shown to regulate osteoclastic bone resorption, via its association with integrin $\alpha\beta3$ and ITAM bearing proteins, such as DAP12 and FcR γ chain, expressed at the osteoclast cell surface (Zou, Kitaura et al. 2007). In addition, Syk has recently been implicated in the suppression of osteoblast differentiation (Yoshida, Higuchi et al. 2011). Thus, Syk may represent a therapeutic target in disorders of bone metabolism such as osteoporosis, although potential effects of Syk inhibition on normal bone, such as osteosclerosis and increased fragility and fracture risk, have yet to be investigated *in vivo* or in clinical studies.

7.4 Tumorigenesis

It has been suggested that Syk is a negative regulator of progression in various types of malignancy (including breast, gastric and melanoma) based on the observation of decreased Syk expression in these tumour types and experimental studies where Syk transfection and re-expression in tumour cell lines suggested a tumour- and metastasis- suppressive effect (Coopman and Mueller 2006). The molecular mechanism of this suppressive role has yet to be established. Conversely, fostamatinib has shown activity in NCI-60 (a panel of 60 diverse human cancer cell lines), although this may be due to off-target effects, rather than Syk inhibition specifically. On this basis, however, a broad multi-histology Phase II study is currently recruiting (NCT00923481).

Syk signalling through the BCR has also been implicated as an important survival signal in various lymphoid malignancies, and R406 has shown antiproliferative and proapoptotic activity in B cell lymphoma and CLL lines *in vitro* (Chen, Monti et al. 2008; Buchner, Fuchs et al. 2009; Quiroga, Balakrishnan et al. 2009). Furthermore, R788 is highly active in animal models of non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukaemia (CLL) (Young, Hardy et al. 2009; Suljagic, Longo et al. 2010), and in a Phase II clinical trial, fostamatinib showed significant clinical activity in a heterogeneous group of NHL and CLL (Friedberg, Sharman et al. 2010). Based on these findings, larger Phase II trials in haematological malignancy are ongoing (NCT00446095, NCT00798096). Interestingly, some oncogenic viruses have been shown to encode ITAM-containing proteins - for example, Epstein Barr virus (EBV) latent membrane protein 2A (LMP2A) contains an ITAM motif, and has been shown to promote B cell development and survival (Caldwell, Wilson et al. 1998).

7.5 Cardiovascular risk

Hypertension was a commonly reported adverse event in clinical studies with fostamatinib. It is unclear whether this was due to Syk inhibition *per se*, or potentially due to off-target

effects on other kinases – the vascular endothelial growth factor receptor 2 being a putative candidate. Whilst usually mild, and responsive to dose reduction of fostamatinib or augmented antihypertensive therapy, the long-term effects of even small increases in blood pressure in patients with autoimmune rheumatic diseases such as RA and lupus, who already have dramatically increased cardiovascular risk, need to be considered. Interestingly, administration of fostamatinib was recently shown to attenuate plaque development in a rodent model of atherosclerosis, an effect thought to be mediated by impaired recruitment of inflammatory cells, suggesting that Syk inhibition is a potential target in atherosclerotic cardiovascular disease (Hilgendorf, Eisele et al. 2011). Syk has also been implicated in the mechanism of high-glucose induced NF- κ B activation in glomerular endothelial cells, suggesting a potential role of Syk inhibition in preventing end-organ complications of diabetes mellitus (Yang, Seo et al. 2008).

8. Conclusions

The rationale, the experimental data, and the clinical experience to date augur well for the efficacy of Syk inhibition in autoimmune disease. Several large phase II-III trials in RA are currently in progress (NCT01242514, NCT01264770, NCT01197521, NCT01197534, NCT01197755) and it is hoped that if successful, efficacy could translate to a wide range of organ-specific and systemic autoimmune diseases. Indeed, it is tempting to propose that Syk-directed therapy, with its proven clinical efficacy in early trials, along with potential benefits in cardiovascular disease, hyperglycaemia, bone metabolism and malignancy (particularly those related to oncogenic viruses) is the proverbial 'Holy Grail' of immunosuppressant therapy following 40 years of steroid- and cytotoxic-based regimes complicated by hypertension, diabetes, osteoporosis and lymphoproliferative disease. However, the widespread role of Syk in numerous immune functions raises serious concerns regarding the risk of opportunistic infection, and some questions about the oncogenic potential of Syk disruption in certain cell types remain unanswered. In addition, it is disappointing that Syk inhibition with fostamatinib, the drug furthest through clinical development, showed only improvement by objective assessment using MRI or biochemical markers, rather than clinical benefit in patients who had not responded to biologic therapy. Future clinical studies will need to establish both the long-term safety and superiority of Syk inhibition in practice.

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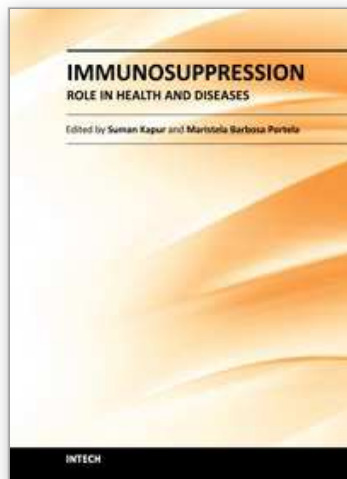
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A need for a book on immunology which primarily focuses on the needs of medical and clinical research students was recognized. This book, "Immunosuppression - Role in Health and Diseases" is relatively short and contains topics relevant to the understanding of human immune system and its role in health and diseases. Immunosuppression involves an act that reduces the activation or efficacy of the immune system. Therapeutic immunosuppression has applications in clinical medicine, ranging from prevention and treatment of organ/bone marrow transplant rejection, management of autoimmune and inflammatory disorders. It brings important developments both in the field of molecular mechanisms involved and active therapeutic approaches employed for immunosuppression in various human disease conditions. There was a need to bring this information together in a single volume, as much of the recent developments are dispersed throughout biomedical literature, largely in specialized journals. This book will serve well the practicing physicians, surgeons and biomedical scientists as it provides an insight into various approaches to immunosuppression and reviews current developments in each area.

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