Targeting Janus Kinases: That’s a Fact Jak

John O’Shea of NIAID/NIH gave a status report on Janus kinase inhibitors (Jakinibs) as modulators of host defense and immune-mediated diseases acting through various cytokines. Janus kinases are tyrosine kinases that contain a kinase and kinase-like domain. The kinase-like domain exhibits enzymatic activity. There are 4 members including Jak1, Jak2, Jak3, and Tyk2 that generally appear to work in pairs in their association on cytokine receptors. In vivo functions of Jaks include the following:

- Jak1: used by IFNs, γc, and gp130 cytokines
- Jak2: used by EPO, TPO, Prl, GH. βc cytokines, IFNγ, IL-12, IL-23, and IL-6
- Jak3: used by γc cytokines
- Tyk2: used by IL-6, IL-12, IL-23, and IFNα/β

Other effects of first generation Jakinibs include actions on T and B cells (including T cell metabolism), blockade of innate and adaptive immune responses as well as RankL production, and reductions in pro-inflammatory cytokine production.

Numerous Jakinibs are under preclinical and clinical development including tofacitinib (acting through modulation of Type I/II cytokines by Jak3, Jak1, and Jak2), which exhibited efficacy comparable to the biologics in RA and is being tested in psoriasis, IBD, dry eye, and renal transplantation. Some clinical issues to consider for the Jakinibs include: 1) mechanism of action (selectivity/multikinase inhibition), 2) mechanisms of toxicity (e.g., infection, cytopenias, BK nephropathy, increased lipids, etc.), and 3) whether effects are actually class or drug-specific.

Tofacitinib (CP-690,550) Mechanism of Action

James Clark from Pfizer reviewed the preclinical and clinical work done with the pan-JAK inhibitor, Tofacitinib (CP-690,550), which is summarized below.

Preclinical

- Tofacitinib exhibited selectivity against 64 kinases.
- Structural characterization of Tofacitinib bound to JAK3 has been elucidated.
As a pan-JAK inhibitor, Tofacitinib modulated cytokine signalling in multiple JAKs (IC₅₀s of 3.2, 4.1, and 1.6 nM for JAK1, JAK2, and JAK3 enzymes, respectively).

In a whole blood assay in humans and mice, Tofacitinib inhibited IL-15 signalling (JAK1/3 driven) with IC₅₀s of 56 and 42, respectively. In general, the IC₅₀ was dependent on the JAK/Stats mediating signalling.

Tofacitinib modulates innate and adaptive immunity (i.e., cytokine signalling including IL-7, IL-15, IL-21, IL-6 and IFNα and β) in various cell types.

In rodent arthritis models (CIA in the male DBA/1J mouse and AIA in rat) using a prophylactic or therapeutic dosing design, Tofacitinib reduced various markers of systemic inflammation (e.g., cytokines and chemokines) in the mouse model and bone resorption (osteoclast-mediated structural damage) in the rat model.

In a xenograft model of psoriasis in SCID mice, Tofacitinib reduced disease measured as clinical scores, histopathology (decreased epidermal thickening, parakeratosis, and cellular infiltrate), and JAK STAT3 phosphorylation as well as chemokine and other inflammatory biomarkers.

Clinical

- Tofacitinib is under development for RA (phase III; under regulatory review for moderate to severe RA in the US, EU, and Japan), psoriasis (phase III), transplant (phase II), Crohn’s disease (phase II), and ulcerative colitis (phase III).
- The most common serious side effect associated with Tofacitinib is infection.
- In patients with RA, Tofacitinib administered for 52 weeks reduced INFγ and IL-17 generated in CD4+ T cells.
- Tofacitinib was efficacious in patients with moderate to serve chronic plaque psoriasis.

Dual Inhibition of p38 Kinase Activation and Activity Provides Efficacy in Treatment of Rheumatoid Arthritis

The final speaker, Gary Schievin of BMS, described work in the area of p38 kinase inhibitors and specifically BMS-582949. P38 kinase is activated by various stimuli (antigen-antibody complexes, TNFα, IL-1β, IL-17, IL-23, T cell receptor, and RANKL) and mediates inflammation through production of various cytokines, chemokines, and MMPs. P38 inhibitors have generally been ineffective in diseases such as RA due to toxicity (liver) and tachyphylaxis (transient clinical activity possibly due to cellular resistance) issues.

BMS-582949 is a potent (IC₅₀ of 13 nM) and selective (5-fold versus p38β and 100-fold versus 382 other kinases) p38α inhibitor that binds both activated and unactivated p38 with similar affinity. BMS-582949 is thought of as a dual action compound that inhibits p38 activation in cells and inhibits kinase activity of any p38
that is activated. Other p38 compounds exhibiting tachyphylaxis (pamipimod and VX-702) don’t inhibit p38 activation. The tachyphylaxis/resistance noted with some p38 compounds may be related to a cellular compensatory mechanism for p38 inhibition by modulation of regulatory pathways to increase p38 activation. The BMS-582949 compound is also capable of rapid reversal of p38 activation.

In humans, BMS-582949 mediated inhibition of p38 was maintained over a 28 day dosing schedule (300 mg PO QD). In a phase II clinical study in RA, BMS-582949 (300 mg QD administered orally for 12 weeks in RA patients on MTX), was well tolerated and safe when administered in combination with MTX. It also met the primary efficacy endpoint (ACR20 at 12 weeks) with no evidence of tachyphylaxis.

Further development of this compound was halted due to evidence of QT prolongation at higher exposure levels.
Symposium VI: Novel Therapeutics

Mavrilimumab: A Fully Human IgG4 Monoclonal Antibody to GM-CSFR Alpha Chain for the Treatment of Rheumatoid Arthritis.

Mathew Sleeman of MedImmune spoke about mavrilimumab (CAM3001) and the rationale for targeting the GM-CSFRα chain (which is specific for GM-CSF) to treat patients with RA. He showed both preclinical and clinical data that supported a central role for GM-CSF in RA:

- Depletion of macrophages using clodronate-laden liposomes in a RA disease model in mice (mBSA/IL-1-induced) was associated with disease improvement.
- Anti-GM-CSFRα (CAM3003) treatment at 1 and 10 mg/kg reduces clinical scores and cytokine level (TNFα and IL-1β) in a rodent RA model.
- GM-CSF regulates differentiation, survival, and activation of macrophages.
- CD14+ve monocytes were found in arthritic joints in humans.
- GM-CSF and GM-CSF receptor are elevated in the synovial fluid and synovial tissue in RA patients.
- Clinical improvement in RA was associated with reductions in macrophages infiltration.

In vitro properties of Mavrilimumab (expressed as Kd/IC50) were summarized and included GM-CSF Rα binding (290 pM), inhibition of colony formation, TF1 cell proliferation (15 pM), granulocyte shape change (41 pM), and no binding to IL-3 Rα or IL-5 Rα. Mavrilimumab was demonstrated to be a competitive antagonist (affinity of 27 pM) when assessed against GM-CSF-induced human granulocyte shape change.

There was limited in vivo cross reactivity between different species for mavrilimumab. Mavrilimumab administered IV (1, 10, and 30 mg/kg) was shown to inhibit hGM-CSF mediated leukocyte margination and increases in circulating leukocytes in a non-human primate model.

A mavrilimumab phase I–FTIH single ascending dose (0.01 to 10 mg/kg in half-log increments) study in humans with adult onset RA ≥ 6 months was described. Primary, secondary, and exploratory objectives were safety, PK and PD, and disease activity, respectively. There were no drug related AEs noted and PK appeared to be non-linear following IV administration.

A phase II Earth Study to assess safety, tolerability, and efficacy of multiple doses (10, 30, 50, and 100 mg) of mavrilimumab SC in moderately to severe RA patients was conducted. The primary endpoint was DAS28-CRP (decrease >1.2 from BL to week 12). Secondary endpoints were DAS28-CRP remission, ACR20/50/70, HAQ-
D1, and safety profile. Significant clinical effects of mavrilimumab compared to placebo were noted with in 2 weeks in the absence of adverse safety effects.

**Personalized Medicine in Asthma: Co-Development of an IL-13 Inhibitor and Companion Diagnostic**

Joseph Arron of Genentech described 4 reasons why drug candidates fail (i.e., due to the wrong target, molecule, outcomes, and patients. He described asthma as a syndrome of loosely related pathophysiologic processes that elicit reversible airway obstruction and hyperreactivity. Asthma is currently treated empirically based on clinical severity/response to inhaled steroids as opposed to being based on underlying biology. Severe asthma is characterized as a heterogeneous, multiple mediator disease combining bronchoconstriction and mixed inflammation (Th2 eosinophilic and neutrophilic). The question raised was how to link targets, pathophysiology, and clinical outcomes in asthma using biomarkers.

One potential key mediator, IL-13 (derived from Th2, Tc2, NKT, macrophages, eosinophils, basophils, mast cells, etc.,) targets the epithelium, fibroblasts, airway smooth muscle, B cells, and VECs to produce mucus hypersecretion, eosinophilia, fibrosis, smooth muscle hyperplasia, hyperreactivity, and angiogenesis.

The following points relative to biomarker approaches were discussed:

- **Using biomarker discovery tools (microarray and qPCR),** major genes upregulated in asthma including periostin, serpinB2, and CLCA1 were demonstrated to be IL-13 inducible and steroid responsive under in vitro conditions in bronchial epithelial cells.
- **Based on an IL-13 responsive bronchial epithelial gene signature,** Type 2-high and low asthmatic responders were identified according to the degree of airway eosinophilia, presence of subepithelial fibrosis, mucus composition, and responsiveness to inhaled steroid exhibited.
- **IL-13 was elevated in the sputum of mild and severe asthmatics.** The question was whether these asthmatics were potential anti-IL-13 responders.
- **Measurement of systemic periostin concentrations** can be utilized as a biomarker to identify mild to moderate asthmatics according to the airway Th2 epithelial signature and eosinophilia.
- **In severe asthmatics on inhaled steroids with residual airway eosinophilia,** serum periostin was elevated (BOBCAT study).

In a small phase IIa bronchial allergen challenge study using an anti-IL-13 antibody (lebrikizumab) in mild allergic asthmatics, levels of IgE, blood eosinophils, IgE/eosinophil composite, and blood periostin correlated with the degree of clinical efficacy noted in the study. In a phase IIb (MILLY), IL-13 blockade reduced blood periostin and FEV1 only in patients with elevated
periostin prior to treatment. Based upon these data, blood periostin levels appear to be a non-invasive biomarker of airway eosinophilia.

**GLPG0634 Shows Efficacy and Safety in a Rheumatoid Arthritis Phase II Study**

Frederic Vanhoutte of Galapagos summarized disease facts surrounding RA and current (MTX, NSAIDS, steroids, biologics) or future (JAK- and SYK-inhibitors) therapeutics available for treatment. His focus was on JAK inhibitors, which act by modulating cytokine signalling. Clinically efficacious JAK inhibitors under development in phase II or III include tofacitinib (JAK3>JAK1>JAK2), baricitinib (JAK1=JAK2), and VX-509 (JAK3).

GLPG0634 is a highly selective JAK1 inhibitor. Phase 1 results in healthy volunteers administered 25-100 mg BID or 100-450 mg QD GLPG0634 demonstrated acceptable safety, acceptable oral pharmacokinetics (dose proportionality), and in vitro JAK1 efficacy (measured as IL-6/pSTAT1 in whole blood from phase I patients).

In a phase IIA study, RA patients with inadequate responses to MTX and naïve to biologics were administered 100 mg BID or 200 mg QD GLPG0634 for 4 weeks. The primary endpoint ACR20, changes in DAS28 (CRP) score, and reduction in serum CRP were achieved. Pharmacokinetics were similar to those noted in healthy volunteers. GLPG0634 was generally safe (minor changes in hemoglobin, platelets, and neutrophils were noted) and well-tolerated. Under these experimental conditions, GLPG0634 appeared to be efficacious with rapid onset of action.