

Abstract # 437 ( For contact for partnering: hsong@genosco.com )

## ABSTRACT

### Background

Spleen tyrosine kinase (SYK) is known to be a key mediator of immunoreceptor signaling in various cell types. Those immunoreceptors are to play important roles in the pathological changes of immune disease, such as rheumatoid arthritis (RA), allergy and asthma. Currently SYK is considered as one of the promising targets for RA treatment. A novel oral SYK-selective inhibitor, SKI-O-282, is under development as an inflammation modulator for the treatment of rheumatoid arthritis.

### Method

*In vitro* efficacy and selectivity of SKI-O-282 were evaluated in kinase assays. Effects on the immune cells were assessed in cell-based assays and western blot analyses for FcγR-, FcεR- and BCR-mediated signaling. *In vivo* efficacy of SKI-O-282 was carried out in the mouse CIA model. Pharmacokinetic studies, CYP450 inhibition assay, hERG inhibition assay and mouse toxicity studies were also performed. FosD (R788, a pro-drug of R406)<sup>3,4</sup> was used as a reference to compare pharmacological profiles with SKI-O-282.

### Result

SKI-O-282 showed potent inhibitory effect on SYK (IC<sub>50</sub> of 1.03 nM) in a cell-free assay and was highly selective to SYK in a panel of 298 kinases at 10 nM. Its potency and specificity were also confirmed from isolated cell-free assays. SKI-O-282 effectively inhibited FcγR-, FcεR- and BCR-mediated signaling pathways with IC<sub>50</sub> ranging from 38 to 237 nM. Interestingly *in vitro* SYK inhibition activities were maintained relatively constant in the presence of 10% serum and in the presence of physiologically relevant concentration of ATP (1.5 mM). Orally administered SKI-O-282 showed significant and dose-dependent inhibition of paw edema, which was better than R788 in the mouse CIA models. SKI-O-282 showed good *in vivo* pharmacokinetic profiles after single oral administration (at 10mg/kg, C<sub>max</sub>: 491 ng/ml, AUC: 1,837 ng•h/mL, T<sub>1/2</sub>: 3.1 hr and BA: 28.4% in rat). No significant inhibitions of CYP450 isozymes and hERG were observed at pharmacological concentration. Oral maximum tolerated doses were higher than 2,000 mg/kg in the single dose toxicity study and higher than 1,000 mg/kg in the 5-day repeated dose toxicity study in mice.

### Conclusion

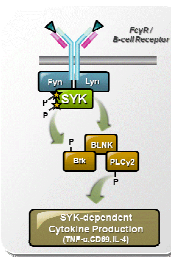
Our results suggest that SKI-O-282 is a potent and selective SYK inhibitor. Oral administration of SKI-O-282 displays potent anti-arthritis effect along with good pharmacokinetic and safety profiles. Therefore, SKI-O-282 is a novel and promising candidate for orally available RA drug.

## INTRODUCTION

### Biological features of SYK<sup>1</sup>

- SYK (spleen tyrosine kinase) is an intracellular protein tyrosine kinase.
- SYK is related to ZAP-70, and also demonstrates similarity with Jak, Src, and Tec families.
- SYK is expressed in most of hematopoietic cells.
- SYK serves as a key mediator of Fc receptor and B cell receptor signaling in inflammatory cells including macrophages, mast cells, dendritic cells, NK cells, and neutrophils.

- In vivo* genetic evidence of the role of SYK in the development of autoimmune arthritis<sup>2</sup>
- SYK KO mouse study reveals the complete inhibition of all macroscopic and microscopic signs of RA.



## RESULTS

### *In vitro* selectivity and potency

- SKI-O-282 showed 56-fold higher potency against SYK than R406 and superior selectivity to that of R406 (active form of R788)

| Kinase (isolated cell-free assay) | R406                  |              | SKI-O-282             |              |
|-----------------------------------|-----------------------|--------------|-----------------------|--------------|
|                                   | IC <sub>50</sub> (nM) | Ratio to SYK | IC <sub>50</sub> (nM) | Ratio to SYK |
| SYK                               | 56.5                  | 1.0          | 1.03                  | 1.0          |
| JAK1                              | 74.6                  | 1.3          | 1772.0                | 1720.4       |
| JAK3                              | 16.3                  | 0.3          | 78.1                  | 75.8         |
| RET                               | 10.7                  | 0.2          | 67.8                  | 65.8         |
| KDR                               | 16.8                  | 0.3          | 289.9                 | 281.5        |
| ZAP-70                            | 89.2                  | 1.4          | 53.9                  | 52.3         |
| FGFR1                             | 85.9                  | 1.6          | 2386.0                | 2316.5       |
| FGFR2                             | 22.4                  | 0.4          | 9082.0                | 8917.5       |
| FGFR3                             | 32.2                  | 0.6          | 1480.0                | 1392.2       |
| PKC2                              | 24.3                  | 0.4          | 536.1                 | 514.7        |
| AuroraB                           | 164.7                 | 2.9          | 1711.0                | 1661.2       |

- SKI-O-282 downregulated SYK-dependent pathways selectively in various immune cell lines.

| Signal pathways                               | Cell line | R406                  | SKI-O-282             |
|---|-----------|-----------------------|-----------------------|
|   |           | IC <sub>50</sub> (nM) | IC <sub>50</sub> (nM) |
| <b>SYK cell-free assay</b>                    |           |                       |                       |
|   |           | 56                    | 1.03                  |
| <b>SYK-dependent pathway</b>                  |           |                       |                       |
| IgE-induced TNF-α production                  | THP-1     | 231                   | 38                    |
| IgE-induced β-hexosaminidase release          | RBL-2H3   | 678                   | 237                   |
| IgM-induced CD89 up-regulation                | Ramos     | 858                   | 87                    |
| LPS-induced TNF-α production                  | THP-1     | 562                   | >2520                 |
| Thapsigargin-induced β-hexosaminidase release | RBL-2H3   | 47                    | 1227                  |
| <b>SYK-independent pathway</b>                |           |                       |                       |
| PMA-induced CD89 up-regulation                | Jurkat    | 14320                 | 3737                  |
| EGF-induced EGFR phosphorylation              | Hela      | >30000                | 22000                 |
| Insulin-induced AKT phosphorylation           | Hela      | 2740                  | >30000                |

- SKI-O-282 also downregulated SYK-dependent signal pathways in primary human cells.

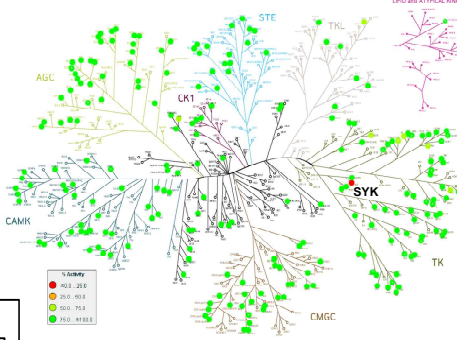
| Signal pathways                        | Cell type | R406                  | SKI-O-282             |
|--|-----------|-----------------------|-----------------------|
|  |           | IC <sub>50</sub> (nM) | IC <sub>50</sub> (nM) |
| <b>Human CD14<sup>+</sup> monocyte</b> |           |                       |                       |
| IgE-induced TNF-α production           | THP-1     | 231.2                 | 38.4                  |
| <b>Human CD19<sup>+</sup> B cell</b>   |           |                       |                       |
| IgM-induced TNF-α production           | Ramos     | 858.0                 | 86.6                  |

### Physicochemical and DMPK properties.

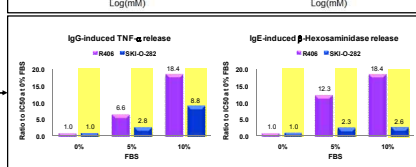
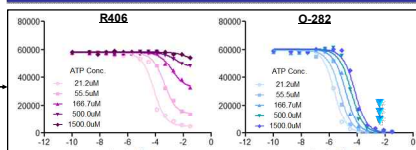
- SKI-O-282 has a good toxicity profile with low cardiovascular and genotoxic risks.

| Physicochemical property                             |                                  |                     |
|--|----------------------------------|---------------------|
| No chiral center                                     |                                  |                     |
| Starting materials commercially available            |                                  |                     |
| No violating the Rule of Five                        | Molecular weight                 | < 500               |
|  | CL <sub>int</sub> /PSA           | 4.38/79.06          |
| <b>ADME/Safety property</b>                          |                                  |                     |
| Human microsomal CYP inhibition (% at 10 μM)         | 3A4, 2C9, 2C19, 2D6, 2C8, 1A2    | < 50%               |
| Permeability (Caco-2, Papp: 10 <sup>-6</sup> cm/sec) | A - B, B - A, Efflux ratio       | 2.7, 3.9, 1.4       |
|  | P-gp interaction                 | Negative            |
| Plasma protein binding (%)                           | human, rat, mouse                | 99.9, 99.8, 99.8    |
| hERG (μM)  | <i>in vitro</i> IC <sub>50</sub> | 24.2                |
| Ames test  | TA97, TA98, TA100, TA102, TA1538 | No reverse mutation |
| 5-Day repeat dose toxicity, Mouse                    | MTD                              | > 1000mg/kg         |

- O-282 showed remarkable SYK selectivity in a full kinase panel assay : 10nM treatment of O-282 against 298 kinases (KinaseProfiler™, Millipore)



### Change of SYK inhibition at physiologically relevant ATP or serum concentration



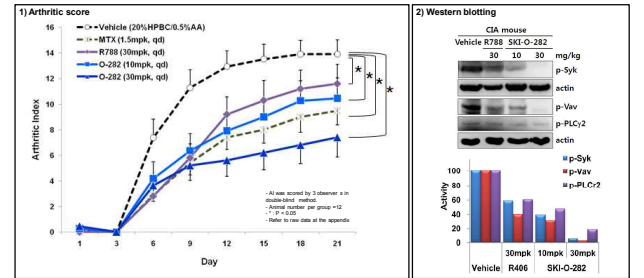
- SKI-O-282 shows good PK profiles in rodent and non-rodent models.

| Rat PK                          |                      |                                 |                     |
|---------------------------------|----------------------|---------------------------------|---------------------|
| Parameters                      | SKI-O-282 PO 10mg/kg | Parameters                      | SKI-O-282 IV 5mg/kg |
| C <sub>max</sub> (ng/ml)        | 491.0                | T <sub>1/2</sub> (hr)           | 3.6                 |
| T <sub>1/2</sub> (hr)           | 3.1                  | AUC <sub>0-24h</sub> (ng•hr/ml) | 3233.0              |
| AUC <sub>0-24h</sub> (ng•hr/ml) | 1837.1               | Cl (ml/min/kg)                  | 25.7                |
| F(%)                            | 28.4                 |                                 |                     |
| Dog PK                          |                      |                                 |                     |
| Parameters                      | SKI-O-282 PO 10mg/kg | Parameters                      | SKI-O-282 IV 5mg/kg |
| C <sub>max</sub> (ng/ml)        | 465.3                | T <sub>1/2</sub> (hr)           | 4.6                 |
| T <sub>1/2</sub> (hr)           | 7.9                  | AUC <sub>0-24h</sub> (ng•hr/ml) | 2052.7              |
| AUC <sub>0-24h</sub> (ng•hr/ml) | 1941.1               | Cl (ml/min/kg)                  | 40.4                |
| F(%)                            | 31.6                 |                                 |                     |
| Comparison of Oral PK profiles  |                      |                                 |                     |
| Parameters (PO 10mg/kg)         | Mouse                | Rat                             | Dog                 |
| C <sub>max</sub> (ng/ml)        | 808.3                | 491                             | 465.30              |
| T <sub>1/2</sub> (hr)           | 2.1                  | 3.1                             | 7.90                |
| AUC <sub>0-24h</sub> (ng•hr/ml) | 3372.7               | 1837.1                          | 1941                |
| F(%)                            |                      | 28.4                            | 32                  |

## RESULTS

### *In vivo* Efficacy in the mouse CIA model

- SKI-O-282 showed potent efficacy in an arthritis model, collagen-induced arthritis mouse (CIA mouse)
- In vivo* efficacy in order: O-282(30mpk) ≈ MTX (1.5mpk) > O-282 (10mpk) ≈ R788(30mpk)
- In the western blot analysis of hind limbs from the same CIA mouse, SYK signaling pathway is also potently inhibited in the same order as above.



## CONCLUSIONS

- SKI-O-282 is a highly potent and selective small molecule inhibitor of SYK that is known as a promising target for RA treatment. (Patent applied: USP, 2009 & PCT, 2010)
- SKI-O-282 inhibits SYK-dependent FcγR, FcεR and BCR pathways potently and selectively in various immune cell lines. Moreover, SYK-dependent FcγR and BCR pathways in human primary immune cells are also significantly inhibited by SKI-O-282.
- In addition, its SYK inhibition activity is maintained at physiological ATP concentration or in the presence of serum.
- SKI-O-282 shows good PK profiles in rodent (mouse, rat) as well as non-rodent animal (dog) and also shows a good toxicity profile with low cardiovascular and genotoxic risks.
- In the mouse CIA model, SKI-O-282 shows potent and dose-dependent inhibition of arthritis index, which was better than R788, a first-in-class SYK inhibitor.
- Based on current results, SKI-O-282 will be expected to yield promising outcome in clinical study.
- Preclinical study will be started at the end of 2011.

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Disclosure: All Oscotec and Genosco affiliated authors are full-time employees of Oscotec and Genosco For contact for partnering, Ho-Juhn Song, Ph.D., hsong@genosco.com, 1-617-494-1460