# A computational high throughput screening approach of iNKT agonists: a novel tool to find optimized iNKT cell ligands.

*Anton De Spiegeleer[1], Evelien Wynendaele[2], Sofie Stalmans[2], Nele Van Den Noortgate[3], Matthias Vandekerckhove[1], Serge Van Calenbergh[2], Sandrine Aspeslagh[1], Koen Venken[1] and Dirk Elewaut[1]*

[1]Laboratory for Molecular Immunology and Inflammation, Department of Internal medicine, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, 9000 Ghent, Belgium.
[2]Drug Quality and Registration (DruQuaR) group and Laboratory for Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium.
[3]Geriatrics unit, Department of Internal medicine, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, 9000 Ghent, Belgium.

## Introduction

Depending on the environment and the activating glycolipids, **iNKT cells** are known to induce **T-helper 1 and/or T-helper 2** cytokines. This highly versatile nature makes these innate-like cells very interesting targets for **immunomodulation**. The design of iNKT cell ligands with selective Th1 and Th2 properties requires refined structural insights. Therefore, the **chemical space** of 333 currently known iNKT-activators, including several newly tested analogs, was visualized by chemical descriptors which were calculated for each individual analog. The **immunological space** consisted of cytokines in different test-systems. With these two information-sets, **structure-activity models** were developed using a system biology computational approach. We present highly sensitive and specific predictive models that can be further exploited for *in silico* **screening of potential glycolipids**, thereby reducing the attrition rate.

## Dataset

✓ **Web of Science**, keywords 'α-GalCer-analogs' and 'iNKT cell activators'

✓ Period: **till the end of 2012**

✓ Articles containing defined chemical structures accompanied with quantitative cytokine-values + new analogs from our research group => **333 glycolipids**

✓ Minimal energy **3D optimalization** of chemical structures using Hyperchem software

✓All of the immunological responses were **normalized to the response of α-GalCer**

✓The immunological space consists of the **cytokines IL-2, IFN- γ, IL-4 and IL-13** in **five test-systems**: *mice/in vivo, mice/in vitro/cell-cell, mice/in vitro/cell-plate, human/in vitro/cell-cell and human/in vitro/cell-plate*

## Chemical space

Hyperchem 8.0.8 + Dragon 5.5 software programs

↓

3228 chemical descriptors

↓ Removal of constant descriptors

1656 chemical descriptors

↓

Multivariate data-analyses: Principal Component Analysis (PCA) + Hierarchical Cluster Analysis (HCA)

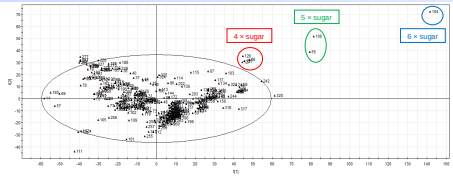


**Figure 1.** PCA score plot with the two first principal component vectors t(1) and t(2). Each PCA vector represents a specific combination of the 1656 chemical descriptors.

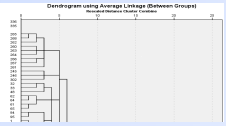

**Figure 2.** An illustration of the physico-chemical hierarchical cluster analysis (HCA).

## Immunological space

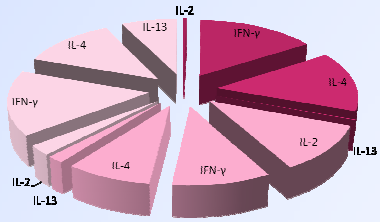


**Figure 3.** The immunological space in literature for the 3 most important test-systems:
*mice/in vivo* (■), *mice/in vitro* (■) and *human/in vitro* (■).

Before modelling, the immunological responses were transformed in dimensionless **desirability (D) values**, normalized combinations of desired cytokine-values (d). For example, an analog with high IFN-γ and low IL-4 values has a high Th1 D-value.

$$d(Y) = \frac{0.9-0.1}{Y_{max}-Y_{min}} \times (Y_i - Y_{min}) + 0.1$$

for parameters to be maximized

$$d(Y) = \frac{0.1-0.9}{Y_{max}-Y_{min}} \times (Y_i - Y_{min}) + 0.9$$

for parameters to be minimized

$$D = \sqrt[n]{\prod_{i=1}^{n} d_i^{z_i}}$$

## Structure-immune modelling

✓ Chemical space + immunological space => **structure-immune models** were computed

✓ Goodness-of-fit $R^2$ values + predictive $Q^2$ values (table 1) => **our models well explain the variability observed**

✓ In our laboratory, we **analyzed 16 new analogs** using *mice/in vivo* experiments and found only compounds 2 and 3 to have a higher Th1 desirability than α-GalCer 1

✓ Our computed model also indicated only these two analogs as more desirable than α-GalCer 1, which examplifies the good $R^2$ and $Q^2$

**Table 1.** The explained variability of the models.

| Functionality | $R^2$ | $Q^2$ |
|---|---|---|
| Th1 *mice/in vivo* | 0.941 | 0.742 |
| Th2 *mice/in vivo* | 0.962 | 0.783 |
| Th1 *human/in vitro* *mice/in vitro* | 0.722 | 0.512 |
| Th2 *human/in vitro* *mice/in vitro* | 0.640 | 0.512 |

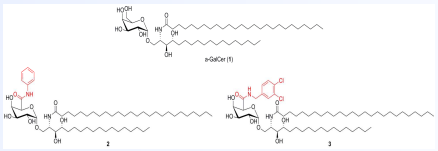

**Figure 4.** α-GalCer (1) with two novel strong Th1 analogs.

## Conclusion

**The *in silico* analysis combining the chemical and immunological space of iNKT activators as described here, provides a novel tool in predicting the functional impact of altered iNKT agonists, decreasing analysis time and costs for functionality analysis.**

## References

*An in silico approach for modelling Th polarizing iNKT cell agonists. Anton De Spiegeleer, Evelien Wynendaele, Matthias Vandekerckhove, Sofie Stalmans, Maxime Boucart, Nele Van Den Noortgate, Serge Van Calenbergh, Sandrine Aspeslagh, Koen Venken and Dirk Elewaut (2013) Manuscript in preparation.*