

# Licensing Opportunity



## INHIBODY<sup>©</sup>: an ultra-specific labeling tool for use in Gaucher patients and an alternative for GFP-labeling

- Measuring active glucocerebrosidase (GBA)
- Determining the biodistribution of therapeutic enzyme
- Amphiphilic inhibobodies easily penetrate cells by diffusion, enabling labelling in vivo

Gaucher | Diagnostics, Therapy monitoring | Research tool, protein labeling in vivo

2011

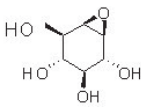
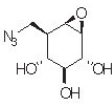
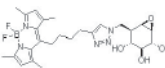
### Background

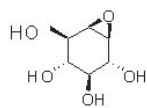
1. Gaucher's disease, a lysosomal storage disease, is caused by the lack of functional glucocerebrosidase (GBA). Enzyme replacement therapy is available, but is very costly (€ 300-600K/year). Treating physicians have a need for a test that would allow monitoring of level of active enzyme in patients, enabling them to properly adjust the amount of enzyme needed and thus prevent overdosing (and thus limit treatment costs).

2. Fluorescent visualization of proteins in living cells currently requires the generation of a fusion protein between the protein of interest and GFP.

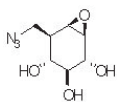
### The Technology

Prof. Hans Aerts of the AMC and Prof. Hermen Overkleeft from Leiden University have generated a GBA inhibitor that binds to active glucocerebrosidase only and thus provides for the first time a means to accurately determine the level of active enzyme. This allows more exact dosing of the very costly therapeutic enzyme, which could result in major savings in the treatment of Gaucher patients. Labeling of trace amounts of IR-labeled therapeutic enzyme can be used to determine the biodistribution of GBA and allow rational ERT.

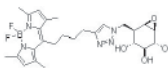
Structure	Compound	Apparent IC <sub>50</sub> (nM) rec. GBA1: 30 min, 37C pH 5.2 +Tch, Tr
	Conduritol B-epoxide	10,000 ±250
	Azido-cyclophellitol	110 ± 30
	BODIPY-cyclophellitol INHIBODY <sup>©</sup>	2 ± 0.5



Conduritol B-epoxide 10,000 ±250



Azido-cyclophellitol 110 ± 30



BODIPY-cyclophellitol  
INHIBODY<sup>©</sup> 2 ± 0.5

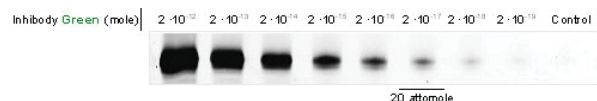


Figure: Rec.GBA labeled with just 2-20 attomoles INHIBODY<sup>©</sup>Green/Red can still be detected (average cell contains 1 femtomole (= 1000 attomole) GBA)

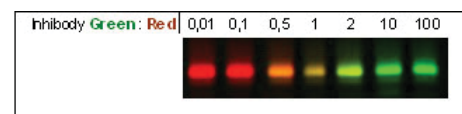


Figure: INHIBODY<sup>©</sup>Green and Red compete equally for binding to GBA. Pulse-chase in vivo labelling exps. allow determination of half-life of GBA in different tissues

Fusion proteins between a protein of interest and glucocerebrosidase can be detected by addition of the INHIBODY<sup>©</sup> that due to its amphiphilic characteristics is able to easily penetrate cells and bind the GBA part of the fusion protein, allowing detection of minute quantities of the protein of interest.

## Applications

1. Diagnosis of Gaucher patients, monitoring of therapy efficacy;
2. Labeling of minute amounts of proteins in cells, even in vivo!

## R&D Status

Overwhelming amount of in vitro data that this concept should work in the applications indicated.

## Intellectual Property

Priority filed in February 2010 (now PCT).

## Inventors

Prof. Hans Aerts is Professor of Medical Biochemistry, Head Department of Biochemistry at the Academic Medical Center, University of Amsterdam, International expert on biochemistry of inherited lysosomal storage disorders, including Gaucher Disease, and Former Chairman and Honorary Board Member European Working Group on Gaucher Disease.

Prof. Hermen Overkleeft is Professor of Bioorganic Synthesis, Head Department of Bioorganic Synthesis at the Leiden Institute of Chemistry, University of Leiden. His research interests include organic synthesis, bioorganic chemistry and chemical biology, with a focus on glycobiology and immunology. Recipient Gouden KNCV Medaille 2008.



### Ultrasensitive *in situ* visualization of active glucocerebrosidase molecules

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## Key publications

1. Witte MD, Kallemeijn WW, Aten J, Li KY, Strijland A, Donker-Koopman WE, van den Nieuwendijk AM, Bleijlevens B, Kramer G, Florea BI, Hooibrink B, Hollak CE, Ottenhoff R, Boot RG, van der Marel GA, Overkleeft HS, Aerts JM. Ultrasensitive *in situ* visualization of active glucocerebrosidase molecules. *Nat Chem Biol.* 2010 Dec;6(12):907-13.
2. Witte MD, Walvoort MT, Li KY, Kallemeijn WW, Donker-Koopman WE, Boot RG, Aerts JM, Codée JD, van der Marel GA, Overkleeft HS. Activity-Based Profiling of Retaining  $\beta$ -Glucosidases: A Comparative Study. *Chembiochem.* 2011 May 16;12(8):1263-9.
3. Aerts JM, Kallemeijn WW, Wegdam W, Joao Ferraz M, van Breemen MJ, Dekker N, Kramer G, Poorthuis BJ, Groener JE, Cox-Brinkman J, Rombach SM, Hollak CE, Linthorst GE, Witte MD, Gold H, van der Marel GA, Overkleeft HS, Boot RG. Biomarkers in the diagnosis of lysosomal storage disorders: proteins, lipids, and inhibodies. *J Inherit Metab Dis.* 2011 Jun;34(3):605-19.
4. Aerts JM, Yasothan U, Kirkpatrick P. Velaglucerase alfa. *Nat Rev Drug Discov.* 2010 Nov;9(11):837-8.
5. Wennekes T, van den Berg RJ, Boot RG, van der Marel GA, Overkleeft HS, Aerts JM. Glycosphingolipids--nature, function, and pharmacological modulation. *Angew Chem Int Ed Engl.* 2009;48(47):8848-69.