# Preface

Symposium 'Galectin Function and Therapeutics', which took place from September 17-19, 2012 in Boston, has had an important mission: to show the participants and the world where we stand with understanding galectins and their functions and the direct applications of galectin management in practical medicine.

This is a very complex and challenging issue. As is shown in Chapter 1, there are more than 3900 articles on galectins; on their structure and functions, published in academic literature since the middle of the 1990's. Most of these papers came from academia. As a result, we have a very advanced science on galectins, 15 of which were identified; on their chemical structure, interactions of galectins with their ligands of both carbohydrate and peptide nature, (alleged) mechanisms of their intracellular and intercellular operations, interception of various biochemical processes, their expression and regulatory effects of the expression, etc. Significant amounts of work have been done with experimental animals, including knockout gal(-/-) mice, as well on effects of galectins and their inhibitors on experimental pathologies in mice, rats, dogs, monkeys. In a few instances galectin inhibitors (galectin blockers) are being tested in human clinical trials, or will be tested soon.

It all sounds very encouraging. However, a bird's eye view at these vast research areas reveals a somewhat troubling, though not an unusual pattern. An observant eye will notice that the galectin-related field of science, which is essentially biomedical science, consists of four largely disconnected areas. They are connected by only one feature – galectins themselves, which is the main object of attention for the researchers. However, methodologically, by experimental design, by aims set, and by essence of conclusions advanced, these four areas are practically disconnected, and the translation of data and conclusions from one area to another is still in its infancy.

One area of research aims at studying isolated and purified galectins and their (desirably) specific inhibitors, that is galectin blockers. It has resulted in wonderful science. Great synthetic chemistry has been accomplished. Some very potent galectin blockers were designed, tested, and their (apparent) binding constants (actually, dissociation constants) have been measured; the best of which occur in the low nanomolar range (10-40 nM). However, those great numbers stay, as a rule, within that *in vitro* area. Commonly, they are not translated to the next (disconnected) area, which is essentially the cell biology of galectins.

The second area is comprised of studies into model cell systems, such as those focused on (alleged) galectin ligands and galectin interactions inside the cells, protein-carbohydrate and protein-protein interactions and recognitions. This is a highly sophisticated and advanced scientific endeavor, which brings


In Galectins and Disease Implications for Targeted Therapeutics; Klyosov, Anatole A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2012.

about remarkable fundamental knowledge, particularly when produced by skillful hands and minds. However, the same galectin blockers, when tested in cell cultures, show much less impressive (apparent) binding constants, often some 10,000-100,000 times less efficient, that is in the high micromolar range (100-600 μM). In other words, something is not right with the translation from the purified galectins to the intra- and/or extracellular galectins. Could it be that in the "chemical" system we study the effects of direct galectin blockers, and in the cellular system we study the effect of the same galectin blockers but at the output of the "rate-limiting" signaling system? Or maybe *in vitro* we study the "wrong binding", with a "wrong binding site", albeit very tight, while *in vivo* we see only those types of binding which produce specific biological effects which we actually observe and measure? This effect is well known in enzyme kinetics, for example, where it is called "non-productive binding". Probably it could be, however, it is seemingly beyond the current research interest. Maybe because of that the areas are sufficiently disconnected.

The thoughts described above do not appear to be some groundless suggestions. In 2009 the Glycobiology journal published a paper "The α-galactomannan Davanat binds galectin-1 at a site different from the conventional galectin carbohydrate binding domain". Then, another paper was published in the same 2009, now in Biochemical Journal, entitled "The carbohydrate-binding domain on galectin-1 is more extensive for a complex glycan than for simple saccharides…". It is hard to tell how significant those findings might be for other galectins and for their *in vivo* effects. However, it should be noticed here, that for the polymer α-galactomannan the translation between *in vitro* and *in vivo* effects, including those in human clinical trials, is appear to be more direct. For said polymer-based galactomannan its apparent $K_d$ *in vitro* (with purified galectin-1 and -3) is 0.9 and 0.2 mg/mL, respectively, while in humans the galactomannan dose in the clinical trials was 0.5 mg/mL (280 mg/m$^2$, that is 7 mg/kg, or 0.5 mg/mL of blood, taking 7% for the amount of blood in humans, which is about the same 7% in the rats). With mice, the employed dose of the galactomannan was 120 mg/kg (Preclinica, 2003), that is 2.5 mg/mouse, or about the same 0.5 mg/mL of blood.

It seems that a careful consideration of data obtained with low-molecular and high-molecular galectin blockers as drug candidates might shed more light on translational regularities for *in vitro* - *in vivo* systems.

The third area is associated with animal experiments and attempts to handle the respective galectin management *in vivo*. Terms such as "intracellular signaling", "cross-linking by galectins of the cell surface receptors", "segregated membrane microdomains", "galectins in pre-mRNA splicing" are "left behind", in the preceding area of cell studies. They are not used in work with experimental animals and in the respective data interpretations. A galectin blocker is administered by one or other means, and a readout such is commonly tumor shrinkage, reduction of a pathological manifestation, such as liver fibrosis stage and grade, portal blood pressure, biomarker levels, etc., is recorded. Translation of the cellular data from the "preceding" area to experimental animals is commonly absent. A mode of administration of a galectin blocker, such as IV, IP, sub-Q, oral, often (or always) rules a significant part of the outcome of the animal experiments.




In Galectins and Disease Implications for Targeted Therapeutics; Klyosov, Anatole A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2012.

The "highest" in that hierarchy, the fourth area, concerns human clinical trials. Some translation from the preceding level is obvious, such as with respect to toxicity of the galectin blocker. If it is toxic in experimental animals, it is automatically assumed to be toxic in humans, and it is commonly so. Efficacy of the galectin blocker is hard to translate from animal to man, and these data are practically absent. Hence, the disconnection again. Even the dosages are hard to translate from the animals to humans, and mg/kg in animals is now often mg/m2 (typically in cancer patients) in humans, being recalculated using some standard coefficients. Again, the mode of administration (IV, IP, sub-Q, oral) and bioavailability of the drug (if not administered IV) can completely change the pattern of the drug behavior in the human body.

Without telling examples, what is said above can appear to be just some theorization (except the example with the galactomannan polysaccharide *in vitro* and *in vivo*, on mice and humans, presented above). However, it is not a unique situation related only to galectins and galectin blockers. It is a rather common situation in biomedicine and drug design, when translation is often difficult to achieve.

At this point I would recall when – a long time ago - I was working with alcoholdehydrogenase (ADH) in a laboratory at Harvard Medical School, using ethanol as the principal substrate for the enzyme. It seemed strange that the highest amount of ADH was found in the horse liver, since horses were not known as heavy drinkers of alcohol. Soon, however, I learned that ethanol was not the "right" substrate for ADH in most of mammals; though a very standard one in biochemical studies. Some steroids were right substrates for ADH. Therefore, ethanol would not have been a good choice for translational studies, unless we specifically consider ethanol for the translation. By the way, octanol (with eight carbon atoms in the molecule), which is thousands of times better substrate of ADH compared to ethanol, would not have been a right substrate either for translational studies. So, being good is not necessarily being right in that area.

Following are some specific examples with respect to galectins:

Thiodigalactoside diesters TD131 and TD139 are very potent inhibitors of galectin-3 with an estimated $K_d$ of 30-50 nM (Nilsson, Leffler et al, see Chapter 2 in this book), that is approximately 0.00002-0.00003 mg/mL. This value has been determined employing competitive fluorescence polarization assays, apparently via competition with lactose and/or other standard low-molecular weight ligands, that is the binding was to the "conventional binding site" in the galectin. An attempt to translate this binding constant to mice via IP (intraperitoneal) injection led to injection of 0.3 mg of the inhibitor to mouse (Hepatology, 2012), that was approximately 10,000-15,000 times higher concentration compared to the above $K_d$ value. In cell culture experiments the biological effect was reached at the amount of the inhibitor 100-600 μM, that is 0.06-0.40 mg/mL, or 3,000-15,000 times higher concentration compared to the above $K_d$ value. The apparent $K_d$ in the cell culture, which was measured as $IC_{50}$, that is a concentration of the compound which results in the 50% inhibitory (or other biological) effect, was 300 μM, or 5,000-10,000 higher compared with the $K_d$ in vitro. Taking into account what was said above about two binding sites at some (or all?) galectins, it might be suggested that small molecular weight galectin inhibitors first saturate the tightly binding


In Galectins and Disease Implications for Targeted Therapeutics; Klyosov, Anatole A., et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2012.

"non-productive" site (which binds with the $K_d$ in a sub-μM and even low nM range), and only then, at concentrations of the inhibitor of ~ 10,000 times higher, they begin to occupy the second, "right" binding site of the galectin. Galectin-based polysaccharides, however, bind to the "right" substrate in the first place, and, being not so tight inhibitors, match the low-molecular weight inhibitors with their efficacy *in vivo*. This apparent match in efficacy might turn out for the low-molecular weight galectin blockers to be an impressive mismatch in the price of the drug candidate and its toxicity. This, of course, has to be studied in detail.

These considerations are given here, in the preface of the book, to tune the reader to the complexity of the therapy-related issues described in various chapters of this book. The most important mission of the Symposium was in realizing this complexity not only from the fundamental standpoint, but also – and foremost – in terms of the potential therapeutic applications of galectin-related therapy.

The above were just quick examples showing that the translation from *in vitro* to *in vivo* data needs to be addressed more attentively. Right now a choice of galectin blockers for studies in galectin therapy is highly empirical, on strictly a "trial and error" basis. The overall aim of the Symposium was to consider the situation and offer some directions to make the search for the right galectin blockers more justified and stemming from *in vitro* experimental data, if possible. To show that it is not possible, if it turned out that way, would also be a step in the right direction, meaning that we cannot rely on data obtained with purified galectins. This will save the biomedicinal industry time and money. To forego many *in vitro* data on galectins would not be the best outcome, at least for the time being. However, it would spare us from hopes based on wrong experimental systems. We are not there yet, and that is why we need translational data as described above.

Leo Tolstoy wrote in the first line of his "Anna Karenina" – "Happy families are all alike in their happiness; every unhappy family is unhappy in its own way". I would dare to say that happy drugs are all alike in their happiness, while every unhappy drug is unhappy in its own way.

**Anatole A. Klyosov**
Galectin Therapeutics, Inc.
4960 Peachtree Industrial Blvd., Suite 240
Norcross, Georgia 30071