

In vivo Neutralization of Naturally Existing Antibodies against Linear $\alpha(1,3)$ -Galactosidic Carbohydrate Epitopes by Multivalent Antigen Presentation: A Solution for the First Hurdle of Pig-to-Human Xenotransplantation

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Dedicated to Professor Daniel Belluš on the occasion of his 70th birthday

Abstract: Pig-to-human xenotransplantation of islet cells or of vascularized organs would offer a welcome treatment alternative for the ever-increasing number of patients with end-stage organ failure who are waiting for a suitable allograft. The main hurdle are preexisting antibodies, most of which are specific for ‘Linear-B’, carbohydrate epitopes terminated by the unbranched Gal- $\alpha(1,3)$ Gal disaccharide. These antibodies are responsible for the ‘hyper-acute rejection’ of the xenograft by complement mediated hemorrhage. For depletion of such antibodies we have developed an artificial injectable antigen, a glycopolymer (**GAS914**) with a charge neutral poly-lysine backbone (degree of polymerization $n = 1000$) and 25% of its side chains coupled to Linear-B-trisaccharide. With an average molecular weight of 400 to 500 kD, presenting 250 trisaccharide epitopes per molecule, this multivalent array binds anti- α Gal antibodies with at least three orders of magnitude higher avidity on a per-saccharide basis than the monomeric epitope. *In vivo* experiments with non-human primates documented that rather low doses – 1 to 5 mg/kg of GAS914 injected *i.v.* – efficiently reduce the load of anti-Linear-B antibodies quickly by at least 80%. This treatment can be repeated without any sensitization to GAS914. Interestingly, although the antibody levels start raising 12 h after injection, they do not reach pretreatment levels. The polymer is degraded and excreted within hours, with a minute fraction remaining in lymphoid tissue of anti- α Gal producing animals only, probably binding to and inhibiting antibody-producing B-cells. The results of pig-to-non-human primate xenotransplantations established GAS914 as a relevant therapeutic option for pig-to-human transplantations as well. The synthesis of GAS914 was successfully scaled up to kg amounts needed for first clinical studies. Key was the use of galactosyl transferases and UDP-galactose for the synthesis of the trisaccharide.

Keywords: Carbohydrate antigens · Enzymatic glycosylation · GAS914 · Glycopolymer · Xenotransplantation

The availability of human organs for transplantation to treat end-stage organ failure is limited to about 25% of patients on waiting lists. This has led to a great interest in the possibilities of xenotransplantation with the main focus on the pig as donor. Although organs from the larger non-human primates would pose the least immunogenic barrier, ethical considerations as well as the high risk of cross-species virus transmission preclude such an option.^[1] On the other hand, the immune reaction towards a pig-to-human xenograft includes all branches of the immune system, resulting in hyper-acute rejection, acute vascular rejection, and – after a period of accommodation – cellular rejection as well as chronic rejection. The first hurdle – the hyper-acute rejection – is initiated within minutes upon contact with monoreactive naturally existing antibodies directed to

carbohydrate epitopes, leading to complement activation and destruction of the organ by hemorrhage and thrombosis within hours. These preexisting antibodies originate from cross-immunization by bacterial flora upon colonization of the intestine after birth. The predominant antigen is related to a carbohydrate epitope found on proteins and lipids of all mammals except primates.^[2] This epitope – also called ‘Linear B’ (**1**) – is terminated with an $\alpha(1,3)$ -galactosidic linkage on an unbranched galactose, *i.e.* the $\alpha(1,2)$ -linked fucose of human blood group epitopes (**2, 3**) is missing (Fig. 1). Such variations in glycosylation patterns are the result of species-specific differences in glycosyl transferase genes. The fraction of preformed antibodies directed to these antigens widely differs between individuals, and can reach 3% of all preexisting antibodies.

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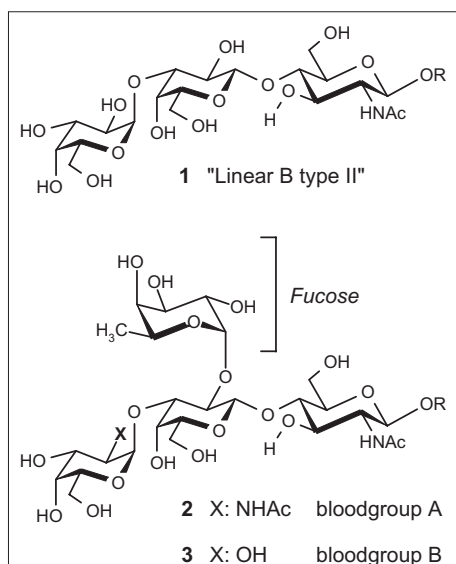


Fig. 1. Structures of Linear-B and Human Bloodgroup Carbohydrate Epitopes.

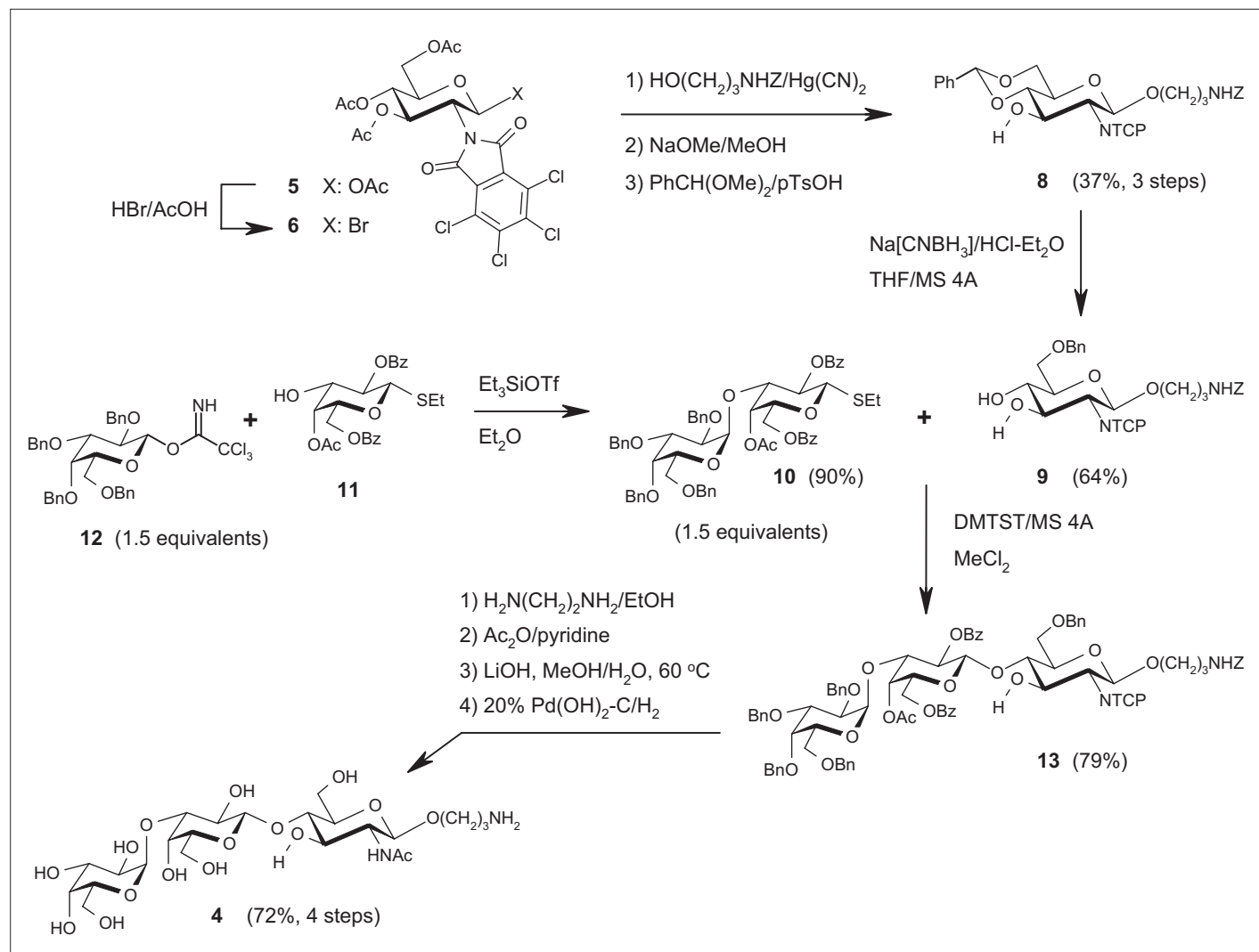
The hyper-acute rejection and to a large part also the acute vascular rejection are caused by anti-Linear-B antibodies and the connected complement activa-

tion. One way to overcome this hurdle is to breed transgenic animals expressing factors regulating complement activation such as 'hDAF', human decay accelerating factor,^[1a,3] or by depleting the xeno-reactive antibodies using extracorporeal plasmapheresis with affinity columns.^[4] Another strategy is to 'inhibit' these naturally existing antibodies by injection of carbohydrate antigen.^[5] Such monovalent epitopes have, however, to compete with a multivalent presentation on cell surfaces, e.g. of endothelial cells on blood vessels. High concentrations of at least 1 mM have to be reached to compete for the xenoantibodies, which are divalent receptors in the case of the IgG subclass, and decavalent for the pentameric IgM subclass, which plays a major role in hyper-acute rejection. The well-established principle of multivalent potentiation of weak carbohydrate-protein interactions^[6] should also be operative for anti-carbohydrate antibodies. For this purpose attempts with high molecular weight oligosaccharides from pig-stomach mucins,^[7] with serum albumin conjugates,^[8] with multivalent arrays on oligo-ethylene

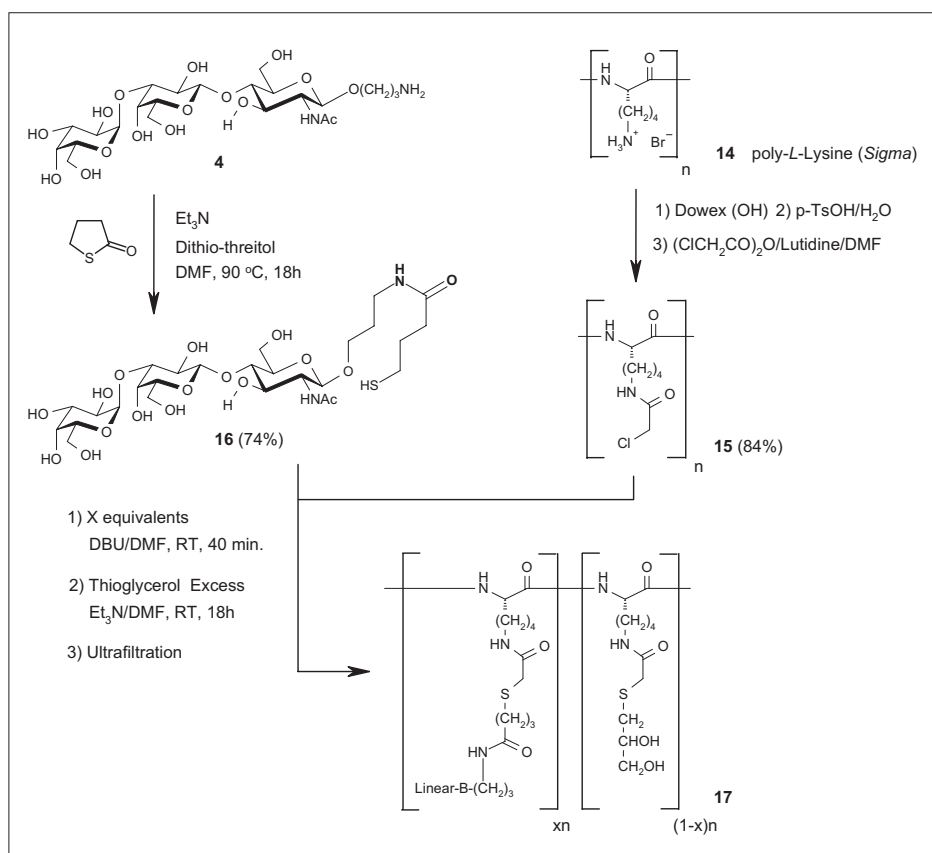
glycol scaffolds,^[9] and with poly-acrylamide conjugates^[10] were made.

Our poly-lysine based system, which had already been successfully applied for potent E-selectin ligands,^[11] would be well-suited for a multivalent presentation of Linear-B. This scaffold has several advantages over many of the other approaches for multivalency. Poly-lysine is commercially available with different degrees of polymerization. After derivatization a charge-neutral and hydrophilic amide linked polymer results, which has a low propensity for immunogenicity and is degradable, as opposed to poly-acrylates or poly-ethylene glycols. On the other hand, albumin conjugates^[8] and other proteins may be immunogenic, the ethylene glycol oligomer carries only eight carbohydrate epitopes,^[9] and poly-acrylamide with a lipophilic backbone is not degradable, and the molecular weights have upper limits.

To prepare poly-lysine conjugates a Linear-B oligosaccharide with a 3-aminopropyl aglycon had to be synthesized. A purely chemical synthesis using for the most part conventional transformations is



Scheme 1. Chemical synthesis of Linear-B type II trisaccharide 4 with an amino-propyl aglycon.

Scheme 2. Preparation of Linear-B poly-(L)-lysine conjugates **17**.Table 1. *In vitro* Data of poly-(L)-lysine Linear-B type II trisaccharide **4** conjugates **17**

Entry	Compound	MW (average)	# Lin-B per Molec.	Equiv. Weight	IC ₅₀ IgG (μM)	IC ₅₀ IgM (μM)	IC ₅₀ Hemolysis (μM)
1	Trisaccharide 4	602	1	602	0.7	390.0	397.0
2	Polymer 17 n: 36 x: 0.26	15.5 kD	~ 9	1657	0.02	1.2	1.0
3	Polymer 17 n: 250 x: 0.10	83.9 kD	~ 25	3356	0.005	6.0	0.11
4	Polymer 17 n: 250 x: 0.25	106.3 kD	~ 62	1700	0.003	0.45	0.02
5	Polymer 17 n: 250 x: 0.60	158.4 kD	~ 150	1056	0.2	0.005	0.08
6	Polymer 17 n: 1050 x: 0.25	446.3 kD	~ 263	1700	0.007	0.002	0.004
7	HSA-Conjugate (Dextra)	~ 70 kD	~ 11	~ 6000			0.7

depicted in Scheme 1. The per-acetylated tetrachloro-phthalimide-protected glucosamine derivative **5**^[12] is converted to the anomeric bromide **6**. Helferich-glycosylation of N-benzyloxycarbonyl-protected propanolamine **7**, deacetylation and trans-acetalization affords the 4,6-benzal derivative **8**, which is reduced to the 6-O-benzyl protected glucosamine **9**. By virtue of the bulky tetrachloro-phthalimido residue the 3-hydroxy group need not be protected for the regioselective glycosylation of

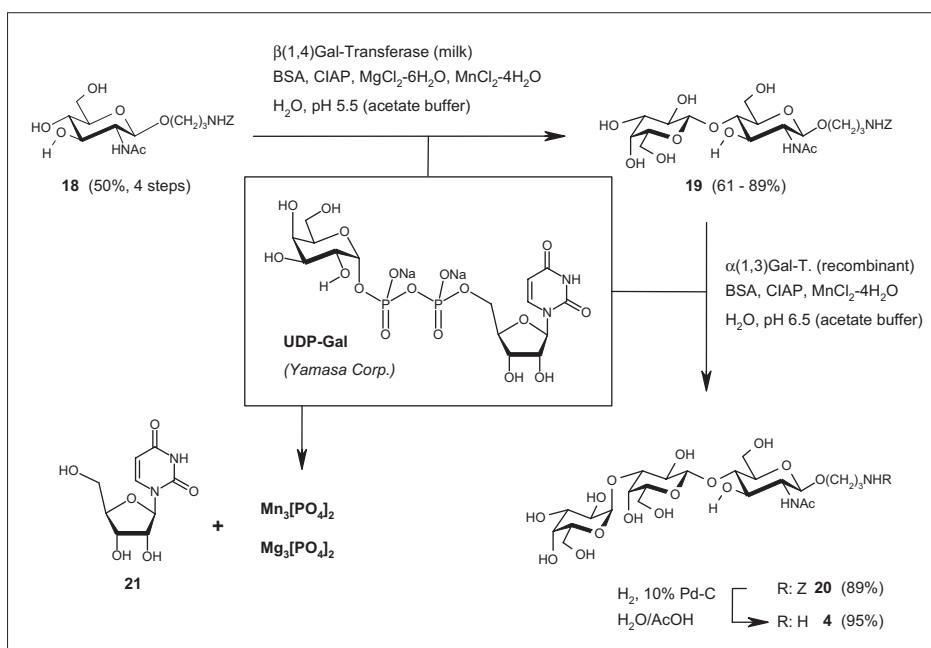
the 4-hydroxy group with the Gal-α(1,3) Gal disaccharide **10**. This intermediate is obtained by glycosylation of the selectively protected S-ethyl-1-thiogalactose derivative **11**^[13] with trichloro-acetimidate **12**.^[14] The crucial glycosylation of **9** with glycosyl donor **10** is mediated by activation with dimethyl-thiomethyl-sulfonium trifluoromethyl-sulfonate (DMTST) affording the protected trisaccharide **13** in 79% yield. Cleavage of the tetrachloro-phthalimide with ethylene diamine in eth-

anol, acetylation, and deprotection finally gives the trisaccharide **4**. The conjugation of Linear-B-propanolamine **4** to poly-lysine is described in Scheme 2. Reaction of **4** with thio-butylactone gives thiol **16**. Commercially available poly-(L)-lysine hydrobromide batches **14** with average degree of polymerization *n* around 40, 250, or 1000 are then converted to the DMF-soluble per-chloroacetamides **15**, which in turn are coupled with different equivalent amounts *x* of thiolated trisaccharide **16** as described previously.^[11] The final Linear-B conjugates **17** are obtained by capping the remaining chloroacetamide groups with racemic thioglycerol.^[15] The composition of these polymeric materials is given by the specification of the starting batch of **14** (vendor), and by ¹H-NMR measurements at elevated temperatures. Assessment by integration of selected signals, assigned to either backbone, thioglycerol, or trisaccharide, closely matched the stoichiometry of reactants (*cf.* ref. [11]).

These polymers were then tested *in vitro* for binding avidity with anti-αGal antibodies, and in a functional assay for inhibiting complement dependent hemolysis of pig erythrocytes by human serum.^[16] In binding assays competition for anti-Linear-B antibodies (pooled human AB-serum) between antigenic carbohydrate coated on well plates and the soluble antigen test compounds is measured with an ELISA (enzyme-linked immunosorbent assay) format, selective either for the divalent IgG or the decavalent IgM subtypes. The results shown in Table 1 list inhibitory IC₅₀ concentrations based on equivalent weight, *i.e.* trisaccharide concentration, rather than on molecular weight of the polymer. Thus, these equivalent weights are only dependent on the fraction *x* of lysine residues coupled to saccharide, determined by NMR, and are independent of the degree of polymerization *n*, determined by the vendor (SIGMA) by physical methods (size exclusion chromatography coupled to low angle laser light scattering). Polydispersities also specified by the vendor vary between 1.1 and 1.2 (*cf.* also refs [11b,c]). It is evident that multivalent arrays **17** inhibit anti-Linear-B antibodies better than monovalent trisaccharide **4** (entry 1), and for most cases also better than the human serum albumin (HSA) conjugate tested (entry 7). For IgM-binding and inhibition of cytotoxicity the potency increases with the degree of polymerization *n*: 36 (entry 2), 250 (entry 4), and 1050 (entry 6). Also 25% loading *x* is better than either 10% or 60% (entries 3, 4, 5). For the IgG subtype a less distinct but similar trend can be seen. For the best composition of **17** (entry 6) a potency gain of several orders of magnitude (2 to 6), compared to monovalent **4**, is observed. Further experiments led to the con-

clusion that the configuration of lysine and thioglycerol, as well as the spacer length between carbohydrate and backbone play a less significant role (data not shown).

First *in vivo* experiments confirmed the superiority of high molecular weight poly-lysine conjugates **17**. Therefore the polymer with 25% carbohydrate loading ($x: 0.25$) and a degree of polymerization n between 900 to 1200 entered development with the code **GAS914**. A major technical hurdle turned out to be the chemical synthesis of trisaccharide **4** (Scheme 1). In a Prep Lab-setting the 24-step sequence with six chromatographic separations could be scaled up to 20 g of **4**. Still, some toxic, smelly, or touchy reagents, such as DMTST (see above), and the amount of waste estimated to 10 tonnes per kg of trisaccharide would make this route a daunting task. After some attempts at optimization^[17] it was decided to resort to enzymatic glycosylations with glycosyl transferases and sugar nucleotides as glycosyl donors. The advantages of total stereo- and regioselectivity without the need of protecting groups has to be balanced against cost and availability of enzymes and activated sugars, difficulties of reaction control, reproducibility, and process scale up. A research procedure starting with Z-protected 1(3-aminopropyl)-*N*-acetyl-glucosamine **18**^[18] had been elaborated,^[19] but needed to be optimized for robustness, and toxic buffer (Na-cacodylate) should be replaced. Also a risk assessment concerning proteins of ruminant origin – bovine serum albumin, $\beta(1,4)$ Gal-transferase from cow milk, calf intestine alkaline phosphatase – was needed. As shown in Scheme 3, a first galactosylation of **18** with commercially available $\beta(1,4)$ Gal-transferase from cow milk gave *N*-acetyl-lactosamine **19** in 61–89% yield. The pH 7.5 cacodylate buffer was successfully replaced by acetate buffering at pH 5.5, a measure which at the same time alleviated substrate inhibition. Addition of Mg^{2+} solved the issue of excess of cofactor Mn^{2+} , which otherwise was depleted by precipitation of its phosphate. Equally successful was the optimization and scale up of the second galactosylation with recombinant $\alpha(1,3)$ Gal transferase giving the Z-protected trisaccharide **20** in 89% yield. Crucial here was the quality of the commercial enzyme in respect of minimized β -galactosidase activity, which leads back to monosaccharide **18**. Again the arsenic buffer could be replaced by a pH 6.5 acetate buffer. In this case addition of Mg^{2+} was avoided, as it appeared to be connected with the unwanted glycosidase activity. Besides inorganic phosphates uridine **21** is the only byproduct of these enzymatic glycosylations. It is formed by phosphate cleavage with CIAP (calf intestine alkaline phosphatase) from uridine diphosphate, an



Scheme 3. Chemo-enzymatic synthesis of Linear-B type II trisaccharide **4** with an amino-propyl aglycon.

inhibitor of galactosyl transferases. The intermediates **19** and **20** were purified by reversed phase chromatography, and a crystallization from ethanol/water yielding products of >98% purity. A membrane filtration before hydrogenolytic cleavage to the target Linear-B propanolamine **4** ensured removal of any larger entities such as endotoxins, prions, and viral particles. The deprotected 3-aminopropyl-trisaccharide **4** was also amenable to purification by recrystallization from ethanol/water. The remaining steps to the glycopolymer **17** (**GAS914**) followed the research procedure (Scheme 2) with some adjustments. The thiolated oligosaccharide **16** could be isolated by crystallization from the concentrated reaction mixture, thereby alleviating the separation from high boiling thio-butylolactone. This thiol was protected from oxidation to disulfide by the addition of antioxidant. Isolation of chloro-acetylated poly-(L)-lysine **15** and the final glycopolymer **17** by precipitation was a major hurdle. Precipitates were finally obtained in reproducible quality by direct precipitation in a stirrable pressure filter under high dilution, and redissolving of the product-cake directly on the filter. For the final purification by tangential flow ultra filtration compatible filter material had to be evaluated. Characterization of the product after lyophilization was done with NMR, gel permeation chromatography, and *in vitro* biochemical assays for biological properties. The optimized procedure thus allowed the preparation of kg-quantities of drug substance of sufficient quality to prepare injectable solutions for first clinical studies.

In vivo experiments were first done with non-human primates – *Cynomol-*

gus monkeys and baboons – both species with preexisting natural antibodies against the Linear-B epitope. As shown in Fig. 2 (results of one animal) 1 mg/kg *i.v.* injections into *Cynomolgus* monkeys on days 1, 4, and 7 resulted in immediate disappearance of circulating antigen specific antibodies, and also quenched the complement dependent hemolytic activity of treated *Cynomolgus* monkey serum on pig erythrocytes. Antibody levels and serum cytotoxicity slowly recovered after 12 h, but never reached pretreatment levels, even after 28 days. When the same animals were treated again at days 105, 108, and 111, the same depletion/inhibition was observed, notably without any immune response, *i.e.* without sensitization to **GAS914**.^[16] For pharmacokinetic studies **GAS914** was radiolabelled with ^{14}C , using ^{14}C -chloroacetic acid for the preparation (*cf.* Scheme 2). *In vivo* experiments with β -Gal-T knockout and wild type mice, rats, as well as with *Rhesus* monkeys showed a biphasic elimination of drug with 95% of radioactivity excreted within minutes in the first phase. The second slower phase was species dependent with a half life of 30 min for mice and 70 h for *Rhesus* monkeys. In the case of animals without anti-Linear-B specific antibodies – wild type mice and rats – the radioactivity was completely cleared by degradation in the liver and further degradation and excretion by the kidneys. For animals with preformed anti-Gal specific antibodies – *Rhesus* monkeys and Gal-T knockout mice – clearance was essentially identical, but a minute residual level of radiolabel was retained in lymphoid tissue, spleen and lymph nodes.

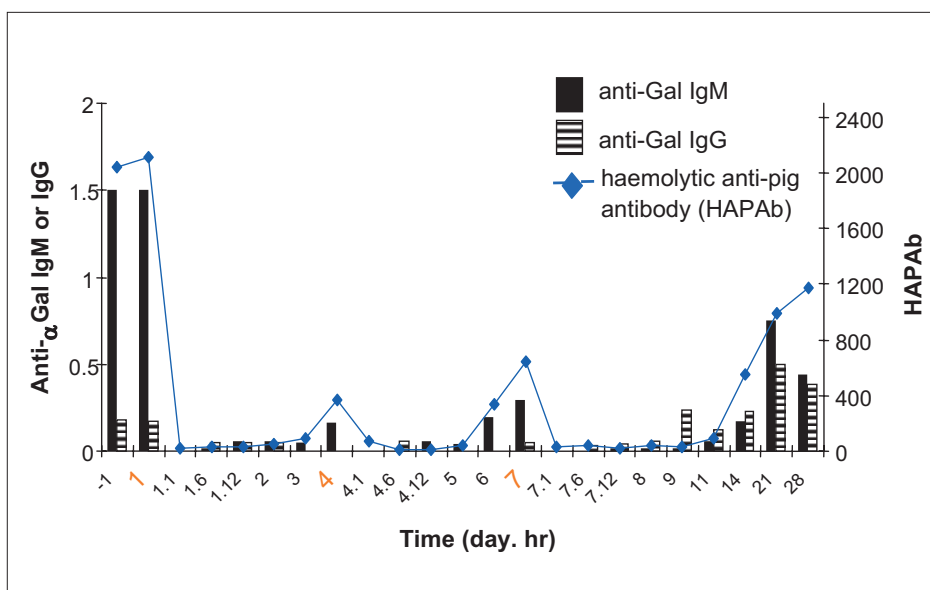


Fig. 2. Anti-Gal antibody titers and cytotoxicity levels of a single *Cynomolgus* monkey (Y119) receiving 1 mg/kg *i.v.*-doses of *GAS914* at days 1, 4, and 7. Plotted are antibody titers (IgG and IgM) relative to standard human serum (1.0), and hemolytic activity (pig erythrocytes) relative to standard human serum (1000).

With the aid of monoclonal antibodies raised against the poly-(L)-lysine backbone of *GAS914* it could be shown that the radioactivity remaining in lymphoid tissue was associated with intact *GAS914* and also co-staining with B-cell regions.^[16] It might therefore be speculated that such collocation indicates binding of *GAS914* to B-cells, and suppression of anti-Gal antibody production, resulting in the observed long term lowering of antibody levels.^[16] During these treatments no adverse side effects such as immune complex mediated glomerulonephritis were observed, and complement activation remained very low. To our knowledge, *GAS914* is the most potent inhibitor of anti- α Galactosidic carbohydrate antibodies reported. In the case of oligo-ethylene glycol conjugates 50 mg/kg doses have to be injected for similar effects,^[9] and poly-acryl amide conjugates appear to be even less efficient, especially for the IgG-type antibodies.^[10] Furthermore the lipophilic backbone of poly-acryl amide poses a high risk for immunogenicity, and biodegradation is difficult at best.

Polymers analogous to *GAS914*, also with other Gal-(1,3)Gal carbohydrate epitopes – disaccharide and pentasaccharide – have been prepared, with an additional low percentage of lysine side chains coupled to a reactive linker (amino group) for immobilization on solid supports such as *Sepharose*[®]. Similar to other immuno adsorbents,^[4] columns filled with such material were less efficient for depleting serum from anti-Linear-B antibodies than *GAS914* injections.^[20] Subsequent pig-to-primate xenotransplantations dem-

onstrated that *GAS914* injections consistently removed at least 80% of preexisting natural anti-Linear-B antibodies, thus offering a relevant therapeutic option for xenotransplantation in general.^[21] Even better results can be expected, when, in addition to *GAS914* with Linear-B type II carbohydrate epitopes, analogous glycopolymers with the related Linear-B type VI epitopes would be applied as well. Yet another principle to present carbohydrate ligands in multivalent arrays is self-assembly of small entities, *e.g.* lipids or other bipolar compounds aggregating to form vesicles or liposomes, mimics of cell surfaces. We recently discovered that small glyco-dendrimers, based on an aromatic scaffold, also form aggregates, which bind anti-Linear-B antibodies with high avidity, comparable with *GAS914*.^[22]

In the past years interest in pig-to-human xenotransplantation has to some extent diminished mainly because of the risk of transmitting infections by the *Porcine* endogenous retrovirus (PERV).^[23] Then the successful production of $\alpha(1,3)$ -galactosyl transferase doubly knockout pigs by nuclear transfer cloning^[24] was another leap forward towards clinical xenotransplantation. Currently the interest is focused on transplantation of pig islet cells to treat diabetes, or to bridge the waiting time for an allograft by 2–6 months with a pig heart transplant, thereby replacing mechanical devices currently in use. From recent pig-to-primate transplantations with $\alpha(1,3)$ -Gal-T knockout animals it became evident that further genetic modifications would be necessary to prolong survival of xenografts beyond the six months currently achieved. Along these lines anti-coagulant genes

should prevent thrombotic microangiopathy, causing the majority of organ losses.^[25] Yet another complication might be inflammation and loss of pig-xenografts by infection with *Human* cytomegalovirus.^[26] Other options are tolerance induction through chimeric bone marrow cells, so far restricted to transplantations in early infancy, before establishment of full immune competency, or organogenesis from developing animal organ primordials rather than from *Human* embryonic stem cells.^[27] Under these circumstances the clinical development of *GAS914* for eliminating xeno-antibodies was discontinued. It should, however, be noted, that the poly-lysine backbone of *GAS914*^[11,15] offers an excellent base for other antigen-specific therapies in antibody mediated diseases, be it as injectables or as ligands for immunapheresis.

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- [1] a) 'Xenotransplantation', Eds. D. K. C. Cooper, E. Kemp, K. Reemtsma, D. J. G. White, Springer Verlag, Heidelberg, **1991**; b) M. Cascalho, J. L. Platt, *Immunity* **2001**, *14*, 437; c) H.-J. Schuurman, R. N. Pierson III, *Frontiers in Bioscience* **2008**, *13*, 204.
- [2] a) U. Galili, B. A. Macher, J. Buhler, S. B. Shohet, *J. Exp. Med.* **1985**, *162*, 573; b) U. Galili, S. B. Shohet, E. Kobrin, C. L. M. Stults, B. A. Macher, *J. Biol. Chem.* **1988**, *263*, 17755; c) E. Yuriew, M. Agostino, W. Farrugia, D. Christiansen, M. S. Sandrin, P. A. Ramsland, *Expert Opin. Biol. Ther.* **2009**, *9*, 1017.
- [3] E. Cozzi, F. Bhatti, M. Schmoekel, G. Chavez, K. G. Smith, A. Zaidi, J. R. Bradley, S. Thiru, M. Goddard, C. Vial, D. Ostlie, J. Wallwork, D. J. White, P. J. Friend, *Transplantation* **2000**, *70*, 15.
- [4] a) D. Lambrights, P. Van Calster, Y. Xu, M. Awwad, F. A. Neethling, T. Kozlowsky, A. Foley, A. Watts, S. J. Chae, J. Fishman, A. D. Thall, M. E. White-Scharf, D. H. Sachs, D. K. C. Cooper, *Xenotransplantation* **1998**, *5*, 274; b) A. Watts, A. Foley, M. Awwad, S. Treter, G. Oravec, L. Buhler, I. P. Alwayn, T. Kozlowsky, D. Lambrights, S. Gojo, M. Basker, M. E. White-Scharf, D. Andrews, D. H. Sachs, D. K. C. Cooper, *Xenotransplantation* **2000**, *7*, 181.
- [5] a) U. Galili, K. L. Matta, *Transplantation* **1996**, *62*, 256; b) P. M. Simon, F. A. Neethling, S. Taniguchi, P. L. Goode, D. Zopf, W. W. Hancock, D. K. C. Cooper, *Transplantation* **1998**, *65*, 346.
- [6] N. Jayaraman, *Chemical Society Reviews* **2009**, *38*, 3463; and literature cited therein.
- [7] S. F. Li, F. A. Neethling, S. Taniguchi, J.-C. Yeh, T. Kobayashi, Y. Ye, E. Koren, R. D. Cummings, D. K. C. Cooper, *Transplantation* **1996**, *62*, 1324.
- [8] a) R. G. Warner, WO Patent Appl. No. WO 97/07823, **1997**; b) K. Teranishi, B. Gallackner, L. Buhler, C. Knosalla, L. Correa, J. D. Down, M. E. White-Scharf, D. H. Sachs, M. Awwad, D. K. C. Cooper, *Transplantation* **2002**, *73*, 129.
- [9] a) A. Schwarz, T. A. Davis, L. E. Diamond, J. S. Logan, G. W. Byrne, WO Patent Appl. No. WO 99/52561, **1999**; b) L. E. Diamond, G. W. Byrne, A. Schwarz, T. A. Davis, D. H. Adams, J. S. Logan, *Transplantation* **2002**, *73*, 1780.
- [10] a) J.-Q. Wang, X. Chen, W. Zhang, S. Zacharek, Y. Chen, P. G. Wang, *J. Am. Chem. Soc.* **1999**, *121*, 8174; b) R. Rieben, N. V. Bovin, E. Y.

- Korchagina, R. Oriol, N. E. Nifant'ev, D. E. Tsvetkov, M. R. Daha, P. J. Mohacsi, D. H. Joziassse, *Glycobiology* **2000**, *10*, 141.
- [11] a) G. Thoma, R. Duthaler, B. Ernst, J. L. Magnani, J. T. Patton, WO Patent Appl. No. WO 97/19105, **1997**; b) G. Thoma, J. L. Magnani, R. Oehrlein, B. Ernst, F. Schwarzenbach, R. O. Duthaler, *J. Am. Chem. Soc.* **1997**, *119*, 7414; c) G. Thoma, J. T. Patton, J. L. Magnani, B. Ernst, R. Oehrlein, R. O. Duthaler, *J. Am. Chem. Soc.* **1999**, *121*, 5919; d) G. Thoma, R. O. Duthaler, J. L. Magnani, J. T. Patton, *J. Am. Chem. Soc.* **2001**, *123*, 10113.
- [12] J. C. Castro-Palomino, R. R. Schmidt, *Tetrahedron Lett.* **1995**, *36*, 5343.
- [13] P. J. Garegg, S. Oscarson, *Carbohydrate Res.* **1985**, *136*, 207.
- [14] B. Wegmann, R. R. Schmidt, *J. Carbohydrate Chem.* **1987**, *6*, 357.
- [15] R. Duthaler, A. Katopodis, W. Kinzy, R. Oehrlein, G. Thoma, WO Patent Appl. No. WO 98/47915, **1998**.
- [16] A. Katopodis, R. G. Warner, R. O. Duthaler, M. B. Streiff, A. Bruelisauer, O. Kretz, B. Dorobeck, E. Persohn, H. Andres, A. Schweitzer, G. Thoma, W. Kinzy, V. F. J. Quesniaux, E. Cozzi, H. F. S. Davis, R. Mañez, D. White, *J. Clin. Invest.* **2002**, *110*, 1869.
- [17] S. Hanessian, O. M. Saavedra, V. Mascitti, W. Marterer, R. Oehrlein, C.-P. Mak, *Tetrahedron* **2001**, *57*, 3267.
- [18] E. Kamst, K. Zegelaar-Jaarsveld, G. A. van der Marel, J. H. van Boom, B. J. J. Lugtenberg, H. P. Spaink, *Carboh. Res.* **1999**, *321*, 176.
- [19] a) J. Fang, J. Li, X. Chen, Y. Zhang, J. Wang, Z. Guo, W. Zhang, L. Yu, K. Brew, P. G. Wang, *J. Am. Chem. Soc.* **1998**, *120*, 6635; b) N. Brinkmann, M. Malissard, M. Ramuz, U. Römer, T. Schumacher, E. G. Berger, L. Elling, C. Wandrey, A. Liese, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2503; c) R. Oehrlein, G. Baisch, unpublished results, cf. also G. Baisch, R. Oehrlein, *Angew. Chem.* **1996**, *108*, 1949, *Angew. Chem. Int. Ed.* **1996**, *35*, 1812.
- [20] a) R. Mañez, F. Crespo, E. Gonzales, A. Centeno, A. Juffe, F. Arnal, E. Cozzi, D. J. G. White, R. Duthaler, W. Kinzy, R. Oehrlein, A. Katopodis, *Transplantation Proceedings* **2000**, *32*, 888; b) R. Mañez, N. Domenech, A. Centeno, E. Lopez-Pelaez, F. Crespo, A. Juffe, R. O. Duthaler, A. G. Katopodis, *Xenotransplantation* **2004**, *11*, 408.
- [21] a) K. Teranishi, I. P. J. Alwayn, L. Buhler, B. Gollackner, C. Knosalla, L. Correa, J. D. Down, M. E. White-Scharf, D. H. Sachs, R. Duthaler, A. Katopodis, M. Awwad, D. K. C. Cooper, *Transplantation Proceedings* **2002**, *34*, 2757; b) R. Zhong, Y. Luo, H. Yang, B. Garcia, A. Ghanekar, P. Luke, S. Chakrabarti, G. Lajoie, M. J. Phillips, A. G. Katopodis, R. O. Duthaler, M. Cattral, W. Wall, A. Jevnikar, M. Bailey, G. A. Levy, D. R. Grant, *Transplantation* **2003**, *75*, 10; c) T. T. Lam, B. Hausen, K. Boeke-Purkis, R. Paniagua, M. Lau, L. Hook, G. Berry, J. Higgins, R. O. Duthaler, A. G. Katopodis, R. Robbins, B. Reitz, D. Borie, H.-J. Schuurman, R. E. Morris, *Xenotransplantation* **2004**, *11*, 517; d) K. Kuwaki, C. Knosalla, K. Moran, A. Alt, A. G. Katopodis, R. O. Duthaler, H.-J. Schuurman, M. Awwad, D. K. C. Cooper, *Xenotransplantation* **2004**, *11*, 210.
- [22] a) G. Thoma, A. G. Katopodis, N. H. Voelker, R. O. Duthaler, M. B. Streiff, *Angew. Chem.* **2002**, *114*, 3327; *Angew. Chem. Int. Ed.* **2002**, *41*, 3195; b) G. Thoma, M. B. Streiff, A. G. Katopodis, R. O. Duthaler, N. H. Voelcker, C. Ehrhardt, C. Masson, *Chemistry, A European Journal*, **2006**, *12*, 99.
- [23] a) D. Butler, M. Wadman, S. Lehrman, Q. Schiermeier, *Nature* **1998**, *391*, 322; b) F. H. Bach, H. V. Fineberg, *Nature* **1998**, *391*, 326; c) R. A. Weiss, *Nature* **1998**, *391*, 327; d) K. Paradis, G. Langford, Z. Long, W. Heneine, P. Sandstrom, W. M. Switzer, L. E. Chapman, C. Lockey, D. Onions, The XEN 111 Study Group, E. Otto, *Science* **1999**, *285*, 1236; e) L. J. W. van der Laan, C. Lockey, B. C. Griffith, F. S. Frasier, C. A. Wilson, D. E. Onions, B. J. Hering, Z. Long, E. Otto, B. E. Torbett, D. R. Salomon, *Nature* **2000**, *407*, 90.
- [24] a) L. Lai, D. Kolber-Simonds, K.-W. Park, H.-T. Cheong, J. L. Greenstein, G. S. Im, M. Samuel, A. Bonk, A. Rieke, B. N. Day, C. N. Murphy, D. B. Carter, R. J. Hawley, R. S. Prather, *Science* **2002**, *295*, 1089; b) Y. Dai, T. D. Vaught, J. Boone, S.-H. Chen, C. J. Phelps, S. Ball, J. A. Monahan, P. M. Jobst, K. J. McCreath, A. E. Lamborn, J. L. Cowell-Lucero, K. D. Wells, A. Colman, I. A. Polejaeva, D. L. Ayares, *Nature Biotechnology* **2002**, *20*, 251.
- [25] a) Z. Ibrahim, M. Ezzelarab, R. Kormus, D. K. C. Cooper, *Xenotransplantation* **2005**, *12*, 168; b) D. K. C. Cooper, A. Dorling, R. N. Pierson III, M. Rees, J. Seebach, M. Yazair, H. Ohdan, M. Awwad, D. Ayares, *Transplantation* **2007**, *84*, 1; c) R. N. Pierson III, *J. Amer. Med. Assoc. (JAMA)* **2009**, *301*, 967.
- [26] M. Ghielmetti, A.-L. Millard, L. Haeberli, W. Bosshard, J. D. Seebach, M. K. J. Schneider, N. J. Mueller, *Transplantation* **2009**, *87*, 1792.
- [27] M. R. Hammermann, *Transplant Immunology* **2005**, *15*, 1.