α-Galactosylceramide in chronic hepatitis B infection: results from a randomized placebo controlled phase I/II trial.

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Abstract

**Background:** The glycosphingolipid alpha-galactosylceramide (α-GalCer) is known to stimulate invariant natural killer T (NKT) cells and is able to induce powerful antiviral immune responses. The present dose-escalating randomized placebo-controlled phase I/II trial aimed to investigate antiviral activity and safety of α-GalCer as a novel class of treatment for chronic hepatitis B patients.

**Methods:** Patients were randomly assigned to 0.1 µg/kg (n=8), 1 µg/kg (n=6) or 10 µg/kg (n=6) α-GalCer or placebo (n=7).

**Results:** Almost all α-GalCer-treated patients showed a rapid and strong decline in NKT cell numbers. Especially patients with high baseline NKT cell numbers showed immune activation, including NK cell activation, elevated serum TNF-α and IL-6 levels and development of fever. Three patients demonstrated a transient decline in HBV DNA. Only one α-GalCer-treated patient had a sustained drop in HBV DNA at the end of follow-up. Four patients discontinued therapy because of fever shortly after drug administration. Otherwise no significant side effects were observed.

**Conclusions:** α-GalCer (0.1-10 µg/kg) used as monotherapy for chronic hepatitis B infection resulted in a strong decline of NKT cells but did not clearly affect HBV DNA and ALT levels. α-GalCer was poorly tolerated and is unlikely to provide an alternative as monotherapy to the current treatment regimen.

**Key words:** KRN7000, HBV, NKT cells, alpha-GalCer, viral hepatitis, human
Introduction

Chronic hepatitis B remains a major health problem. Worldwide 2 billion people show evidence of infection with hepatitis B virus (HBV) and it chronically affects around 400 million people.\textsuperscript{1} Approximately 15-40\% of these will develop serious complications such as liver cirrhosis and hepatocellular carcinoma.\textsuperscript{2} Pegylated interferons have shown to induce a sustained response in about 35-45\% of patients.\textsuperscript{3-5} Because of the importance of immune control over the hepatitis B virus, immunomodulatory drugs are of special interest in the treatment of chronic hepatitis B infection.

The glycosphingolipid \(\alpha\)-galactosylceramide (\(\alpha\)-GalCer) has been shown to induce potent antiviral as well as antitumor immune responses.\textsuperscript{6-8} \(\alpha\)-GalCer, originally derived from marine sponge, activates invariant natural killer T (iNKT) cells, which recognize \(\alpha\)-GalCer in the context of the MHC-like molecule CD1d.\textsuperscript{9} iNKT cells constitute a distinct lymphocyte subpopulation characterized by expression of both NK receptors and a restricted T cell receptor repertoire, which in humans consists of a \(V\alpha24\) chain preferentially paired to \(V\beta11\).\textsuperscript{10} Upon activation these cells rapidly secrete large amounts of both Th-1 and Th-2 type cytokines, which subsequently enhance innate as well as adaptive immune cells.\textsuperscript{11-13} Besides their pivotal role in anti-tumor and anti-viral immune responses, NKT cells have also been implicated in several other antimicrobial immune responses, as well as in autoimmunity and allergy.

Approximately 20-30\% of intrahepatic lymphocytes consist of NKT cells.\textsuperscript{14} Interestingly, NKT cells activated by \(\alpha\)-GalCer have been shown to inhibit hepatitis B virus replication in HBV transgenic mice.\textsuperscript{15} This antiviral effect of \(\alpha\)-GalCer is associated with a rapid induction of IFN-\(\gamma\) and IFN-\(\alpha/\beta\) in the liver even before a significant number of inflammatory cells are recruited to the liver, suggesting that intrahepatic NK and NKT cells are involved in this
In addition, it has been suggested that α-GalCer also exerts direct anti-viral activity against HBV.\textsuperscript{17}

KRN7000 is a synthetic α-GalCer that has been most frequently used in experimental mouse studies, but also in some human oncology trials and in hepatitis C.\textsuperscript{18-22} Also in the human setting, α-GalCer administration has been shown to induce immune activation in individuals, which depended on pretreatment circulating iNKT cell numbers.\textsuperscript{19} The aim of the present study was to investigate the safety, tolerability and the antiviral effect of α-GalCer for the treatment of patients with chronic hepatitis B infection.
Methods

Patients
Male and female patients aged 18 to 70 years with either HBeAg-positive or -negative chronic hepatitis B infection were enrolled. All patients had an HBV DNA level above $10^5$ copies/ml at screening and two alanine transaminase (ALT) values of $>1.2$ times the upper limit of normal (xULN) within 8 weeks before initiation of treatment. The ULN for ALT was 40 U/L for males and 30 U/L for females. A liver biopsy obtained within 3 years prior to screening, consistent with chronic hepatitis B infection and without cirrhosis, was required.

Exclusion criteria were the evidence of decompensated liver disease, as indicated by bilirubin $>20$ $\mu$mol/L, serum albumin $<35$ g/L, prothrombin time prolonged by $>3$ seconds, history of bleeding esophageal varices, ascites or hepatic encephalopathy; ALT level $>10$ xULN; pregnancy or the inability to practice adequate contraception; clinically significant or major illnesses; history of autoimmune disease; systemic interferon-\(\alpha\) treatment, systemic antiviral agents or another investigational drug within 3 months prior to enrollment in the study; immune suppressive treatment; pre-existing severe cytopenia (i.e. hemoglobin $<7$ mmol/L, white blood cell count $<3.0*10^9$/L, lymphocytes $<0.5*10^9$/L or platelets $<100*10^9$/L); evidence of hepatocellular carcinoma as indicated by alpha fetoprotein $>50$ ng/ml and/or ultrasound demonstrating a mass suggestive of liver cancer; other acquired or inherited causes of liver disease.

The study was approved by the ethics committees at our hospital according to the Declaration of Helsinki, and all patients gave written informed consent before enrollment.
Study design

This phase I/II dose-escalation trial was performed in a randomized, double-blind, placebo-controlled manner. Patients with chronic hepatitis B who met the inclusion criteria were assigned to receive three dosages of α-GalCer (KRN7000 ((2S,3S,4R)-1-O-(α-D-galactopyranosyl)-N-hexacosanoyl-2-amino-1,3,4-octadecanetriol), Kirin Pharma Co., Ltd., Gunma, Japan) or placebo intravenously, with intervals of 4 weeks. Patients were enrolled into 3 dose escalating groups with 11 patients in the first group (8 verum; 3 placebo) and 8 patients in the second and third group (both 6 verum; 2 placebo) (Figure 1). After enrollment, patients were randomized to receive either α-GalCer or placebo. The dosage of α-GalCer was 0.1 µg/kg body weight in the first, 1 µg/kg in the second and 10 µg/kg in the third group.

After completion of 8 weeks of treatment, with injections at 0, 4 and 8 weeks, patients were monitored without further therapy for an additional 16 weeks. Dose escalation to the next cohort was decided after evaluation by a safety review board of all the safety data collected on all the patients who had completed 3 weeks after the first injection in the preceding dose cohort. The safety review board consisted of three experienced hepatologists who were not involved in the study.

Study objectives

The primary objective of the study was to evaluate the safety and tolerability of the 3 ascending doses of α-GalCer. The secondary objective was to evaluate the effectiveness of α-GalCer, immunological responses, reduction of HBV DNA and ALT normalization.

Biochemical and virological assessments

The extent of liver inflammation was determined by measuring serum alanine aminotransferase (ALT) levels. HBV DNA levels were measured using an in-house developed
TaqMan real-time PCR assay (dynamic range 400-10^{10} copies/ml). The Eurohep HBV DNA standard was used for validation of HBV DNA levels. Hepatitis B ‘e’ antigen (HBeAg), hepatitis B ‘s’ antigen (HBsAg), antibodies to ‘e’ antigen (anti-HBe) and antibodies to ‘s’ antigen (anti-HBs) were measured using a commercially available immunoassay (Abbott Laboratories, Abbott Park, IL).

**Immunology testing**

Lymphocyte numbers were determined by adding fixed volumes of FlowCount™ fluorospheres (Beckman-Coulter, Miami, USA) to the leukocytes after erythrocyte lysis (BD Biosciences, San Jose, USA) just before flow cytometric evaluation. FACS analysis was performed using monoclonal antibodies against CD3 (SK7), CD4 (SK3), CD8α (SK1), CD45 (2D1), CD69 (L78), and the isotype controls mouse IgG1 (X40) and IgG2a (X39)(all purchased from BD Biosciences), Vβ11 (C21) and Vα24 (C15), CD8β (2ST8.5H7)(all from Immunotech, Marseille, France), and CD56 (MOC-1; IQ products, Groningen, The Netherlands) before and 2 and 7 days after each injection as well as at the end of treatment (EOT; day 84) and at the end of follow up (EFU; day 168). For staining with α-GalCer or vehicle loaded CD1d-tetramers (Gemini Science Inc., San Diego, CA), 150 µl of whole blood was incubated with the tetramers for 10 min at 37°C, followed by staining for CD3 and Vα24 and erythrocyte lysis. Whole blood analysis of myeloid and plasmacytoid dendritic cell (DC) numbers was performed, as described previously, before and 7 days after each injection as well as at EOT and EFU. Flow cytometric analysis was performed on a FACS Calibur using CELL Quest software (BD Biosciences).

Serum levels of IFNγ, TNFα, IL-6, IL-1β, IL-10, IL-5 and GM-CSF were measured by flow cytometry using the CBA Human Soluble Protein Flex Set system (BD Biosciences) and ELISA (IFNγ and TNFα, R&D Systems, Abingdon, UK) before and 4 hours and 2 days after
injection in all patient groups. Depending on the number of cells, IL-12 production by polyI:C (20 µg/ml; Sigma-Aldrich, St. Louis, MO) and IFNγ (1000 U/ml; Strathmann Biotech) stimulated BDCA1+ myeloid DC, which were isolated from PBMC by negative depletion with anti-CD19-conjugated microbeads, followed by positive selection using anti-BDCA1-PE and PE-conjugated microbeads using the mini-MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany) and stimulated in the presence of GM-CSF during 24 hr, was examined as described before.25 mDC function was analysed in all patient groups (placebo: n=5, level 1: n=5, level 2: n=3, level 3: n=2) before and 7 days after start of treatment and at EOT.

Statistical analyses
Because of the explorative character of the study, power analysis was not considered. Patients were randomized by the Clinical Research Bureau of the Erasmus MC University Medical Center Rotterdam using a computer generated randomization list. All analyses were performed on an intention-to-treat basis. For analysis purposes, the patients treated with placebo therapy from the different dose levels will be considered as one treatment group. Paired and unpaired Student T tests, Wilcoxon matched pairs test, repeated measures ANOVA and Pearson’s correlation coefficient were used where appropriate. P-values of <0.05 were considered statistically significant.
Results

Patients

A total of 30 patients were screened between August 2003 and January 2006 at the Erasmus MC University Medical Center Rotterdam. Twenty-seven patients met the criteria for enrollment into the study. In total, 8 patients were allocated to a dose of 0.1 \( \mu \)g/kg body weight (dose level 1), 6 were allocated to 1 \( \mu \)g/kg body weight (dose level 2), 6 were allocated to 10 \( \mu \)g/kg body weight (dose level 3) and 7 to placebo (Fig. 1). One patient in the highest dosage group withdrew his informed consent before study medication was administered.

The median age at inclusion was 35 years (range, 21-58). At baseline the median ALT level was 103.5 IU/L (range 35-356) and the median HBV DNA level 8.1 \( \log_{10} \) copies/mL (\( = 7.4 \log_{10} \) IU/mL) (range 5.0-9.5 \( \log_{10} \) copies/mL). Further demographics and baseline characteristics for the different dose levels are given in table 1. There were no significant differences between the patient characteristics of different treatment groups prior to therapy.

NKT cell numbers decline after \( \alpha \)-GalCer administration

At baseline, the number of circulating NKT cells, defined as CD3\(^+\)V\(\alpha\)24\(^+\)V\(\beta\)11\(^+\) cells, did not significantly differ between the different groups (Fig. 2). The first administration of \( \alpha \)-GalCer induced a rapid decrease in circulating NKT cells in all dose levels (day 0 vs day 2: dose level 1, \( p=0.0156 \)); level 2, \( p=0.0313 \); level 3: \( p=0.1250 \)), which was followed by a recovery of NKT cell numbers (Fig. 2BC). Although less pronounced, this decline in NKT cell numbers was also observed after the second and third administration of \( \alpha \)-GalCer (Fig. 2B). Furthermore, albeit not significantly different, the number of NKT cells was still decreased at the end of treatment (day 84) and approached baseline levels at the end of follow up (day 168). The NKT cell numbers in patients receiving placebo did not significantly differ during
the study period (Fig. 2AC). Similar findings were observed with α-GalCer CD1d-tetramer staining, which was evaluated in 201 blood samples (data not shown).

Of note, all patients exhibiting high baseline NKT cell levels that received ≥ 1 µg/kg α-GalCer developed fever and severe rigors 1 hour to 2 days after drug administration (see safety, Fig. 2D). NKT cell subset analysis in these patients revealed that after the first administration of α-GalCer the proportion of CD4⁺ NKT cells decreased and the proportion of CD8⁺ NKT cells increased, whereas in placebo treated patients with high baseline NKT cell levels the proportion of NKT cell subsets did not differ.

Analysis of circulating NK cells, T cells and DC
To evaluate α-GalCer induced indirect immune activation, peripheral blood NK cells, T cells and DC were analyzed. The analysis of circulating NK cells revealed that α-GalCer treatment significantly changed the number of NK cells 2 days post-injection (Fig. 3). Surprisingly, in patients receiving 0.1 or 1.0 µg/kg α-GalCer, NK cell numbers significantly decreased (p=0.02 and p=0.03), whereas the highest dosage induced an increase in NK cells (p=0.03). Activated NK cells, as defined by CD69⁺ cells, were observed in all treated patient groups, but the most pronounced increase in CD69 expressing NK cells was observed in patients with high NKT cell numbers at baseline (%CD69⁺ NK cells: t=0, median 0.6, range 0.1-4.1, versus t=2, median 2.9, range 0.0-11.5; p=0.05). Significant differences in circulating T cells and DC were not observed. Of note, the only patient with a sustained drop in HBV DNA levels during treatment demonstrated an increased number of circulating myeloid DC at the end of treatment (t=0: 0.32%, t=84: 0.74%). Moreover, these myeloid DC displayed an increased capacity to produce IL-12 (t=0: 60 pg/ml, t=84: 1478 pg/ml), which is in line with our previous findings demonstrating that viral load reduction increases the number of circulating myeloid DC as well as their function.²⁵
Serum cytokine levels

Serum cytokine levels were determined at baseline, 4 hr and 2 days after each \(\alpha\)-GalCer administration. Cytokine levels remained undetectable in the patient group with low NKT cell numbers. However, in 5 of 9 patients with high NKT cell levels, a transient increase in TNF\(\alpha\) was observed. The patient exhibiting the highest TNF\(\alpha\) level (35 pg/ml) experienced severe fever shortly after \(\alpha\)-GalCer administration (see Safety). In addition, the patients exhibiting a period of fever shortly after \(\alpha\)-GalCer administration demonstrated an increase in IL-6 from 2 ± 3 pg/mL at baseline to 719 ± 906 pg/mL 4 hours after drug administration that returned to baseline levels at day 2. No detectable levels of IFN\(\gamma\), IL-1\(\beta\), IL-10, IL-5 and GM-CSF were observed in serum of those patients.

Virological and biochemical response

No significant decreases in HBV DNA following the first administration of \(\alpha\)-GalCer were observed in any of the three dosages groups (Fig. 4). Four patients did show a more than 0.5 log\(_{10}\) copies/mL drop in HBV DNA levels following the first administration (1 \(\mu\)g/kg n=2, 10 \(\mu\)g/kg n=2) with a median decline in the first week of 1.09 log\(_{10}\) copies/mL (range 0.54 – 2.96; n=4) from baseline.

For the whole group during follow-up of 168 days, also no statistically significant changes in HBV DNA level were observed among the different dose levels (Fig. 4). One patient in dose level 2, who displayed a low circulating NKT cell number and experienced a viral load drop of 0.54 log\(_{10}\) copies/mL one week after the first injection, had a sustained drop in HBV DNA level of 4.02 log\(_{10}\) copies/mL at the end of follow-up. The other three patients with a drop in HBV DNA level after the first administration, of whom 1 patient displayed low and 2 patients displayed high baseline NKT cell numbers, had no decline after the second and third injection.
of α-GalCer and HBV DNA levels returned to baseline. HBeAg-seroconversion was not observed in any of the α-GalCer treated patients. One HBeAg-positive patient in the placebo group had an HBeAg-seroconversion and a drop in HBV DNA of 4.48 log_{10} copies/mL at the end of follow-up. There were also no clear and significant differences in the ALT values over time in the 3 different dose levels of α-GalCer treated patients compared to placebo (Fig. 5).

Safety
A total of 122 adverse events were reported in this study. The most frequent reported adverse events were flu like symptoms: fever (78%) and headache (63%). Other frequently reported side effects were abdominal pain (37%), and nausea and vomiting (30%) (Table 2). Two patients had an ALT flare above 10 times the ULN, both not resulting in hepatic decompensation. Four patients discontinued therapy prematurely due to an episode of fever and severe rigors 1 hour to 2 days after drug administration; one patient in the 1 µg/kg dose level, the other three in the 10 µg/kg dose level group. All these episodes of fever resolved within 1 week after onset.
Discussion

This phase I/II randomized, double-blind, placebo-controlled trial provides unique data on the antiviral activity and safety of the immune-modulating glycosphingolipid α-GalCer for the treatment of chronic hepatitis B infection. In this trial, three administrations of α-GalCer with a 28-day interval were given in chronic hepatitis B patients in three different dose levels. Although almost all patients responded to α-GalCer as shown by a rapid and strong decline in NKT cell numbers, proper immune activation with a clear and sustained antiviral activity was however not observed.

As expected from literature, iNKT cells rapidly disappeared from the circulation upon α-GalCer administration. This decline in circulating iNKT cell numbers was observed after each α-GalCer injection but also rapidly returned close to pretreatment values. Whether circulating NKT cells die or migrate to the liver, as described previously, remains to be determined.

In contrast to the study of Veldt et al. in which chronic hepatitis C patients showed only a moderate decrease in circulating iNKT cell numbers upon α-GalCer treatment, the current study on chronic hepatitis B patients showed a profound decrease in circulating iNKT cells upon similar doses of α-GalCer. Therefore, the effect of α-GalCer treatment on the immune system in the current study surpasses the effects observed in α-GalCer-treated hepatitis C patients.

The initial number of the circulating iNKT cells in chronic hepatitis B patients appears to be comparable to the number present in healthy controls and chronic hepatitis C patients. The reduction of circulating iNKT cells in chronic hepatitis B patients was most pronounced after the first injection of α-GalCer and seemed to decline over time. This may suggest that the potential antiviral effect of α-GalCer in chronic hepatitis B patients diminishes over time, which may be related to the decreasing iNKT cell pool during treatment. In line with previous
studies, we observed the strongest immune activating effects on patients with high NKT cell levels.\textsuperscript{19,22} We defined the iNKT-high group by using the median number of circulating iNKT cells in patients (1100 CD3\(^+\)V\(\alpha\)24\(^+\)V\(\beta\)11\(^+/\)10\(^6\) CD3\(^+\) cells). This NKT-high group showed stronger immune activation upon \(\alpha\)-GalCer administration than the other patients as demonstrated by stronger iNKT cell decline, transient rises in serum TNF-\(\alpha\) and IL-6 levels and increased circulating activated NK cells. None of the immunological parameters tested showed clear \(\alpha\)-GalCer dose-dependent effects. However, some dose-response effects were observed in relation to circulating NK cell numbers and the development of fever. Patients with high level of circulating iNKT cells developed fever, as reported before,\textsuperscript{19} but only when treated with at least 1 \(\mu\)g/kg \(\alpha\)-GalCer. The specific, but transient, increase in the proportion of CD8\(^+\) NKT cells in these patients may reflect a shift towards a more pronounced Th-1 phenotype,\textsuperscript{29} but also in these patients IFN\(\gamma\) serum levels remained undetectable.

Although the biological activity of \(\alpha\)-GalCer in chronic hepatitis B patients seemed to be superior compared to chronic hepatitis C patients, \(\alpha\)-GalCer did not significantly affect HBV DNA or ALT levels. Four patients treated with \(\alpha\)-GalCer had a pronounced decline in HBV DNA in the first week of treatment, but this decline was not sustained and also not observed after the second and third administration of \(\alpha\)-GalCer. One patient in dose level two had a sustained drop in HBV DNA at the end of follow-up (4 log\(_{10}\) copies/mL reduction). Since \(\alpha\)GalCer is thought to act through an immune mediated mechanism, the study population may have been to heterogeneous to observe clear \(\alpha\)GalCer-induced immune regulation as demonstrated by differences in HBeAg status, ALT levels, HBV genotypes and presumed route of infection which may reflect different immunological stages in HBV infection as well as different immunological activities. In HBV transgenic mice \(\alpha\)-GalCer was able to inhibit HBV replication by directly activating NKT cells and by consequent activation of NK cells to
secrete antiviral cytokines, such as IFN-γ and IFN-α/β in the liver. However compared to chronic HBV infection in patients, these mice, which express products of the HBV genome and also show some signs of HBV replication, do not have an active HBV infection and contain disproportional high numbers of intrahepatic NKT cells making these mouse studies difficult to translate to the human situation. Although we were not able to study intrahepatic NK cell activation, patients with high circulating NKT cell levels did show enhanced levels of activated circulating NK cells upon α-GalCer injection, which is in line with previous reports. This enhanced activation status of NK cells did not result in significant increases in serum IFNγ levels and/or HBV-DNA decline. We did notice signs of immune activation as determined by increased serum IL-6 level in the patients exhibiting an episode of fever and severe rigors. This suggests that α-GalCer is able to induce immune activation in the liver since IL-6 is one of the primary inducers of the acute-phase response in liver. Four α-GalCer treated patients discontinued therapy early due to an episode of fever short after drug administration. All these episodes resolved spontaneously. These side effects limit further development of treatment with α-GalCer in chronic hepatitis B patients. Furthermore, there is no clear and consistent effect of α-GalCer on the HBV DNA and ALT levels. This can be due to the relatively low NKT cell levels in humans compared to mice, where α-GalCer was able to inhibit HBV replication. Serious side effects were not observed in a trial with α-GalCer treatment in patients chronically infected with hepatitis C. This suggests that there is a different NKT-cell response in chronic hepatitis B patients. A higher dosage of α-GalCer might be more effective, but the α-GalCer dosage is probably limited by its side effects. Alternatively, administration of α-GalCer bound to dendritic cells has been shown to be much more potent than α-GalCer itself and seems to be well tolerated.
Nevertheless, previous studies on α-GalCer in humans did show immune responses with the relatively low levels of α-GalCer used in this trial.¹⁹

In conclusion, α-GalCer resulted in a strong decline of circulating NKT cells but had no effect on HBV DNA and ALT levels and was poorly tolerated in chronic hepatitis B infected patients when used as monotherapy at the doses 0.1-10 μg/kg. It is unlikely that α-GalCer will provide an alternative, at least as monotherapy, to the current treatment options.
Acknowledgements

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References


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Legends to the figures

Figure 1: Overview of the trial profile

Figure 2: NKT cell numbers decline upon α-GalCer treatment.

AB. Peripheral blood CD3⁺Vα24⁺Vβ11⁺ NKT cell numbers within CD3⁺ T cell population in placebo (A) and α-GalCer-treated (B) patients. α-GalCer (0.1 µg/kg) was administered at day 0, 28 and 56 (arrows).

C. Median NKT cell numbers within CD3⁺ T cell population in placebo and α-GalCer-treated patients at day 0, 2, 7 and at the end of treatment (EOT). *p<0.05, Wilcoxon matched pairs test.

D. Pre-treatment NKT cell numbers within CD3⁺ T cell population in the different treatment groups. Dashed line represents the median of pre-treatment NKT cell number calculated from the patients included.

E. NKT cell subset analysis was performed at day 0, 2 and 7. Three representative patients (placebo, 1.0 µg/kg α-GalCer and 10 µg/kg α-GalCer) with high NKT cell levels at baseline are shown.

Figure 3. NK cell numbers change upon α-GalCer treatment

Peripheral blood CD3⁻CD56⁺ NK cell numbers present in the lymphocyte population in placebo and α-GalCer-treated patients at day 0, 2 and 7. *p<0.05, Wilcoxon matched pairs test.

Figure 4: Median HBV DNA (log₁₀ copies/ml) in the three different treatment arms and the placebo group.
Figure 5: Median ALT (IU/L) in the three different treatment arms and the placebo group.
Table 1: Baseline characteristics.

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<th>0.1 μg/kg (n=8)</th>
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<th>10 μg/kg (n=6)</th>
<th>Placebo (n=7)</th>
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<td>Male gender (%)</td>
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<td>Age, years*</td>
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<td>Baseline ALT (IU/L)*</td>
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* median (range)
Table 2: Most common adverse events, defined as those occurring in at least 2 patients in any dosing group.

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<td>ALT &gt;10 xULN</td>
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<tr>
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<td>2</td>
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<tr>
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<tr>
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<td>Hypertension</td>
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<td>1</td>
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</table>

Total number of adverse events | 122 | 36 | 31 | 31 | 24 |

CNS = central nervous system
27 patients randomized

11 patients assigned in dose level one

- 8 patients 0.1 μg/kg α-GalCer
- 3 patients placebo
- 8 patients completed Rx and FU

8 patients assigned in dose level two

- 6 patients 1.0 μg/kg α-GalCer
- 2 patients placebo
- 5 patients completed Rx and FU

8 patients assigned in dose level three

- 6 patients 10 μg/kg α-GalCer
- 2 patients placebo
- 2 patients completed Rx and FU

1 discontinued therapy due to side-effects

3 discontinued therapy due to side-effects 1 withdrew consent

1 withdrew consent

8 patients completed Rx and FU

3 patients placebo

6 patients placebo

2 patients placebo

2 patients placebo

Rx = treatment; FU = follow-up
Figure 3

![Graphs showing NK cell counts (%) over time (0, 2, 7, EOT) for different dosages: placebo, 0.1 µg/kg, 1 µg/kg, and 10 µg/kg.](image-url)
Figure 4

- **α-GalCer 0.1 μg/kg**
- **α-GalCer 1.0 μg/kg**
- **α-GalCer 10 μg/kg**
- **Placebo**

**Drug administration**

**HBV DNA (log₁₀ copies/ml)**

**Time (days)**: 0, 28, 56, 84, 112, 140, 168

<table>
<thead>
<tr>
<th>Drug Administration</th>
<th>HBV DNA (log₁₀ copies/ml)</th>
<th>Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-GalCer 0.1 μg/kg</td>
<td>6, 7, 8, 9, 10</td>
<td>0, 28, 56, 84, 112, 140, 168</td>
</tr>
<tr>
<td>α-GalCer 1.0 μg/kg</td>
<td>6, 7, 8, 9, 10</td>
<td>0, 28, 56, 84, 112, 140, 168</td>
</tr>
<tr>
<td>α-GalCer 10 μg/kg</td>
<td>6, 7, 8, 9, 10</td>
<td>0, 28, 56, 84, 112, 140, 168</td>
</tr>
<tr>
<td>Placebo</td>
<td>6, 7, 8, 9, 10</td>
<td>0, 28, 56, 84, 112, 140, 168</td>
</tr>
</tbody>
</table>
Figure 5

**α-GalCer 0.1 μg/kg**

Drug administration

**α-GalCer 1.0 μg/kg**

Drug administration

**α-GalCer 10 μg/kg**

Drug administration

**Placebo**

Drug administration