A new therapeutic approach for type 1 diabetes: rationale for GNbAC1 an anti-HERV-W-Env monoclonal antibody

Francois Curtin MD[1,2+], , Corinne Bernard PhD[1], Sandrine Levet PhD[3], Hervé Perron PhD[1,3,5+], Hervé Porchet MD[1,4], Julie Médina PhD[3], Sam Malpass MSc[6], David Lloyd PhD[6], Richard Simpson DM[7] on behalf of the RAINBOW-T1D investigators

[1] GeNeuro SA, Chemin Pré-Fleuri 3, CH 1228 Plan-les-Ouates/Geneva, Switzerland

[2] Division of Clinical Pharmacology and Toxicology, Rue Perret-Gentil, University of Geneva, Geneva, Switzerland

[3] Geneuro-Innovation, Avenue Rockefeller 60, 69008 Lyon, France.

[4] Department of Pharmacology; University of Pretoria; Pretoria, South Africa

[5] Faculty of medicine Laënnec, University of Lyon, Lyon, France.

[6] Southern Star Research Pty Ltd, Gordon NSW 2072 Australia

[7] Eastern Clinical Research Unit Level 1, 5 Arnold St, Box Hill VIC 3128, Australia

Running Title: Anti-HERV-W-Env antibody in Type 1 Diabetes

Revision 8 May 2018

[+] Corresponding authors: François Curtin, MD, Hervé Perron PhD

Corresponding author's address: GeNeuro SA, 3 Chemin Pré-Fleuri, CH-1228 Plan-les-Ouates, Geneva, Switzerland; email address: fc@geneuro.com

Word count: abstract: 252, core text: 4272, no of references: 59, no of figures: 5

ABSTRACT

We describe a newly identified therapeutic target for type 1 diabetes: an envelope protein of endogenous retroviral origin called Human Endogenous Retrovirus W Envelope (HERV-W-Env). HERV-W-Env was found to be detected in the blood of around 60% of type 1 diabetes (T1D) patients and is expressed in acinar pancreatic cells of 75% of T1D patients at *post-mortem* examination. Preclinical experiments showed that this protein displays direct cytotoxicity on human β-islet cells. *In vivo* HERV-W-Env impairs the insulin and glucose metabolism in transgenic mice expressing HERV-W-Env.

GNbAC1, an IgG4 monoclonal antibody has been developed to specifically target HERV-W-Env and to neutralize the effect of HERV-W-Env *in vitro* and *in vivo*. GNbAC1 is currently in clinical development for multiple sclerosis and more than 300 subjects have been administered with GNbAC1 so far. GNbAC1 is now tested in T1D in the RAINBOW-T1D study: a randomized placebo controlled study with the objective of showing the safety and pharmacodynamics response of GNbAC1 in patients suffering from T1D with a maximum duration of 4 years. GNbAC1 is tested versus placebo at the dose of 6 mg/kg in 60 patients during 6 repeated administrations during 6 months, a 6-month open-label extension will follow. The primary endpoint will assess safety and secondary endpoints the pharmacodynamic responses to GNbAC1.

GNbAC1 targeting HERV-W-Env is currently in clinical development in T1D with the first safety and pharmacodynamic study. If the study results are positive, this may open the door to the development of an innovative non-immunomodulatory disease modifying treatment for T1D.

Registration: clinicalTrials.gov Identifier: NCT03179423

Key words: monoclonal antibody, endogenous retrovirus, HERV, Phase II study, type 1 diabetes, disease modifying drug

1. INTRODUCTION

Despite three decades of intense clinical research and development, there is currently no registered disease-modifying treatment for type 1 diabetes (T1D).[1] In line with the therapeutic approach used in several auto-immune disorders, most development efforts have focused on immunosuppressive/immunomodulatory drugs, with the objective of preserving the β-islet cells from immune-mediated cytotoxicity. The following immunomodulatory approaches have been tested in clinical development: blocking tumor necrosis factor alpha (TNF-α) by etanercept or blocking Interleukin-1 (IL-1) by canakinumab, inactivating IL-1β with gevokizumab, depleting -lymphocytes with rituximab, blocking T-cells with abatacept or alefacept, depleting T-cell with anti-CD3 antibodies such as teplizumab or otelixizumab or enhancing Treg response with low dose of Interleukin-2 (IL-2). However, encouraging results with these treatments in early clinical development were either not confirmed in late phase trials or significant safety issues related to the targeted immune functions and cells were reported and therefore none of these treatments has yet reached the registration stage for T1D.[2] Other therapeutic strategies use agents or drugs potentially favoring β -islet cell regeneration, for example imatinib or the islet neogenesis associated protein peptide (INGAPP),[3] peptide immunotherapies derived from proteins such as pro-insulin or T1D autoantigens attempting to induce tolerance by different mechanisms: these strategies are currently being tested in early clinical development.[4] It is likely that the heterogeneity of the immunopathogenic mechanisms involved in T1D, reflected by varying levels of functional β-cell reserve, types of auto-antibodies or of immune pancreatic infiltrations for example, may explain the failure or the slow progresses of drug development in this indication.[5,6] Possibly to obtain a real therapeutic efficacy, this would necessitate stratification of patients and the availability of therapeutic approaches, alone or in combination, on different targets, immunologic or not, such as antigen presenting cells, effector T-cells, cytokine production and/or B lymphocytes.[4,5,7]

We are presenting here the rationale having led to the development of an innovative therapeutic approach for T1D. This rationale is based on the identification of a new pathogenic target, a protein called human endogenous retrovirus-W envelope (HERV-W-Env), which has been found involved in other auto-immune disorders such as multiple sclerosis (MS)[8] and chronic inflammatory demyelinating polyneuropathy (CIDP).[9] This protein, devoid of any

physiological role, is encoded by a member of retroviral families that have colonized genomic DNA through insertions in primate germ line cells over many millions of years during evolution. HERV-W Env is an envelope protein from the HERV type W family and was found to be significantly detected in subgroups of patients with T1D. Its expression also appears to have pathophysiological effects in T1D models. A humanized IgG4 monoclonal antibody, GNbAC1, has been developed to neutralize this non-physiological target. If the data supporting the involvement of HERV-W-Env in the pathophysiology of T1D are confirmed in patients, GNbAC1 could be the first disease modifying therapy for T1D without impacting on the immune system, unlike therapeutic strategies usually proposed for a range of autoimmune diseases.

2. Targeting HERV-W-Env, a potent TLR4 agonist

The gene encoding the HERV-W-Env protein belongs to the HERV-W family of retroviral elements, which are widely and heterogeneously dispersed in the human genome following the integration events of a corresponding exogenous retrovirus that first infected the germ line of primates (Catharrhines) about 25 million years ago.[10,11] HERV elements represent approximately 8% of the human genome and they are closely related to other remnants of "mobile genetic elements" that have contributed to genomes recombination and evolution which altogether represent at least one-half of the human genome.[12,13] HERV elements may have evolved towards physiological contributions[14] or have retained pathogenic potential that is normally epigenetically silenced.[15] This duality is also observed with other mobile genetic elements such as retrotransposons.[12,16] HERV elements nonetheless have phylogenic homologies with exogenous retroviruses as shown in Figure 1 (for a review see reference 17) and the role of the different HERV families in the causation of human disorders and in particular of autoimmune diseases is an active field of investigation.[11,18,19] Several families of HERVs have been identified, among which HERV-W is potentially implicated in human diseases, such as T1D and Multiple Sclerosis (MS).[20] In particular, the protein HERV-W-Env originates from a retroviral envelope protein and appears to play a pathogenic role in MS.[8, 18, 21-23]

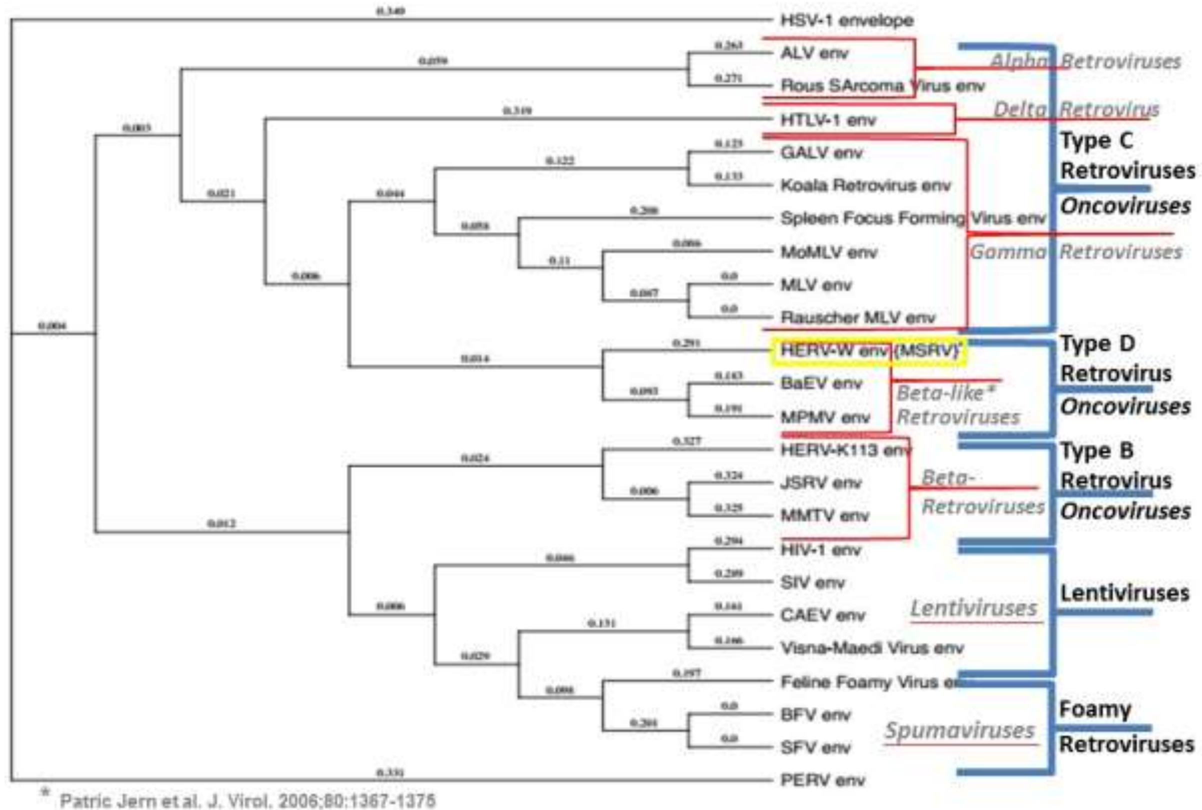

Figure 1. Alignments of HERV-W env gene sequences with other endogenous and exogenous retroviruses illustrating phylogeny among Retroviridae.

Abbrevations:

HSV-1: Herpes simplex virus type 1; ALV: avian leucosis virus; RSV: Rous sarcoma virus; HTLV: human T-lymphotropic virus; GALV: gibbon ape leukemia virus (gibbon monkey leukemia); Koala retrovirus: koala retrovirus; SFFV: spleen focus-forming virus (virus forming foci in the spleen); MoMLV: Moloney virus of murine leukemia (MLV variant); MLV: murine leukemia virus (murine leukemia); Rauscher MLV: Rauscher virus of murine leukemia (variant from MLV); HERV-W: human endogenous type W retrovirus (W type endogenous human retrovirus); BaEV: baboon endogenous virus (endogenous baboon retrovirus); MPMV: Mason-Pfizer monkey virus (monkey Mason-Pfizer virus); HERV-K113: human endogenous retrovirus type K, K113 copy (human endogenous retrovirus type K, copy K113); JSRV: Jaagsiekte retrovirus (adenomatosis virus) pulmonary mutton); MMTV: mouse mammary tumor virus; HIV: human immunodeficiency virus (human immunodeficiency virus); SIV: simian immunodeficiency virus (simian immunodeficiency virus); CAEV: caprine arthritis-encephalitis virus); MVV: Maedi-Visna virus causing pulmonary, neurological, joint or mammary diseases in sheep; FFV: feline foamy virus; BFV: bovine foamy virus; SFV: simian foamy virus (simian "foamy" virus); PERV: porcine endogenous retrovirus (endogenous porcine retrovirus).

*beta-like retroviruses, as defined according to: Jern P et al. [17]

The target protein HERV-W-Env, also named MSRV-Env from original Multiple Sclerosis associated RetroVirus isolates,[24] has three domains: the signal peptide, the surface domain or ectodomain (SU) and the transmembrane domain (TM). HERV-W-Env is expressed on

monocytes, NK cells and B lymphocytes of patients with MS.[25] Its SU domain comprises sites responsible for the potent agonist properties on the Toll-like receptor 4 (TLR4), an upstream receptor in the innate immune pro-inflammatory system. Following the initial observation that the *in vitro* abnormal immune response characterized by T-lymphocyte polyclonal activation was associated with HERV-W-Env,[21] the cytokines induced by the interaction between the SU domain of HERV-W-Env protein and TLR4 were studied in peripheral blood mononuclear cells (PBMC): these cytokines include, IL-1 β, IL-6 and IL-12p40 initially secreted, ahead of the IFN-γ release, reflecting a delayed but potent T-cell immune reaction. Therefore an innate immune activation by HERV-W Env started before T-cell engagement. It was shown that the mechanism of release of the pro-inflammatory cytokines such as IL-1ß, IL-6 or TNF-α from PBMC was mediated through the engagement of TLR4, and CD14 which are respectively pattern recognition receptor and co-receptor of primary importance for the innate immunity. HERV-W-Env also triggers a maturation process in human dendritic cells endowing these cells with the capacity to promote a Th1-like type of Th cell differentiation.[26- 27] *In vivo*, HERV-W-Env TLR4 mediated immune activation pathways can lead to acute and chronic inflammation as well as to autoimmunity in MS-like animal models induced with HERV-W Env.[28]

It has further been shown that HERV-W-Env induces a concentration-dependent release of IL-6 and TNF-α by human PBMC in culture, or when injected i.v. in mice, with affinities in the picomolar or low nanomolar range.[29]

HERV-W-Env appears to exert superantigen-like effects downstream the innate immune activation as shown *in vitro* in human PBMC cultures and *in vivo* in humanized SCID mice grafted with a functional human lymphoid system.[21, 30] The ability of superantigens to cause antigen-independent polyclonal activation of T lymphocytes can result in abnormal autoreactive T-cell activation and an over-secretion of pro-inflammatory cytokines.[31] Superantigen properties of bacterial or viral antigens have been suggested as a causative factor of autoimmune diseases.[32,33] Nonetheless, the HERV-W-Env superantigen-like effect appears to be dependent upon the upstream TLR4 activation, as it is no longer observed after blocking TLR4 receptors with an appropriate antibody, which results in the inhibition of both TNF-α and IFN-γ secretion.[27]

3. Mechanisms of HERV activation by exogenous infections

Epidemiologically, an association between the occurrence of T1D and infections is suggested.[34] Viruses such as influenza, rhinovirus, Epstein-Barr and in particular Coxsackie B virus are epidemiologically linked to T1D[35,36]. Antigenic mimicry or immune tolerance breakdown could be the mechanisms leading to T1D induction by these viruses, although paradoxically a protective effect from autoimmunity was paradoxically suggested.[37] It has been observed that HERV expression can be induced by viruses, notably by Herpesviridae in multiple sclerosis.[38] Recently, the CVB-4E2 strain of a Coxsackie Virus isolated from T1D pancreas when compared to a control CVB-4 strain isolated from a non-T1D patient induced a higher magnitude of expression of the HERV-W genes *in vitro* compared to the control strain. These results suggest that only certain enteroviral strain have the potency to transactivate HERV-W and may "turn-on" a self-sustaining and expanding HERV-W expression in cells they have infected, i.e. in the pancreas.[39] This observation is compatible with mild or subacute infections of target tissues by environmental viruses as a cause of endogenous retroviral-mediated pathogenesis in type 1 diabetes.

4. HERV-W-Env as a potential target and biomarker for type 1 diabetes

A series of epidemiological and pathological observations and experiments support the hypothesis of the role of the protein HERV-W-Env in the pathological processes leading to T1D. From an epidemiological perspective, HERV-W-Env protein has been detected in human T1D patients by two different methods. An antigenemia study was conducted with an ELISA method on sera from 30 T1D patients and 93 non-T1D blood donors: it was shown that 21 over 30 T1D (70%) patients had detectable HERV-W-Env protein in comparison to 11 of 93 (12%) non-T1D controls. In parallel a real time quantitative PCR study was conducted on peripheral blood mononuclear cells (PBMCs) from 23 T1D patients and from 26 non-T1D blood donors. HERV-W-Env RNA was detected in 57% of T1D patients versus 12% of controls.[40]

Immunohistochemical analyses were subsequently performed on pancreas biopsies obtained from 39 donors with cerebral death: 20 suffered from T1D, and 19 controls presented various disorders. The HERV-W-Env protein was expressed in the pancreas of 75% of T1D patients but only 16% of control patient. Typical HERV-W-Env staining on pancreatic tissue slides are

presented in Figure 2, revealing that HERV-W-Env expression is mostly restricted to pancreatic acinar cells. HERV-W-Env is likely to be secreted in the extracellular space by producing cells[4, 21] and exerts its pathogenic effects through the interaction with the innate immune receptor TLR4[29] which is expressed by pancreatic β-cells.[41,42] This suggests that acinar cells may be the permissive cells for HERV-W-Env expression in human pancreas and that pancreatic beta cells may be target cells of HERV-W-Env.

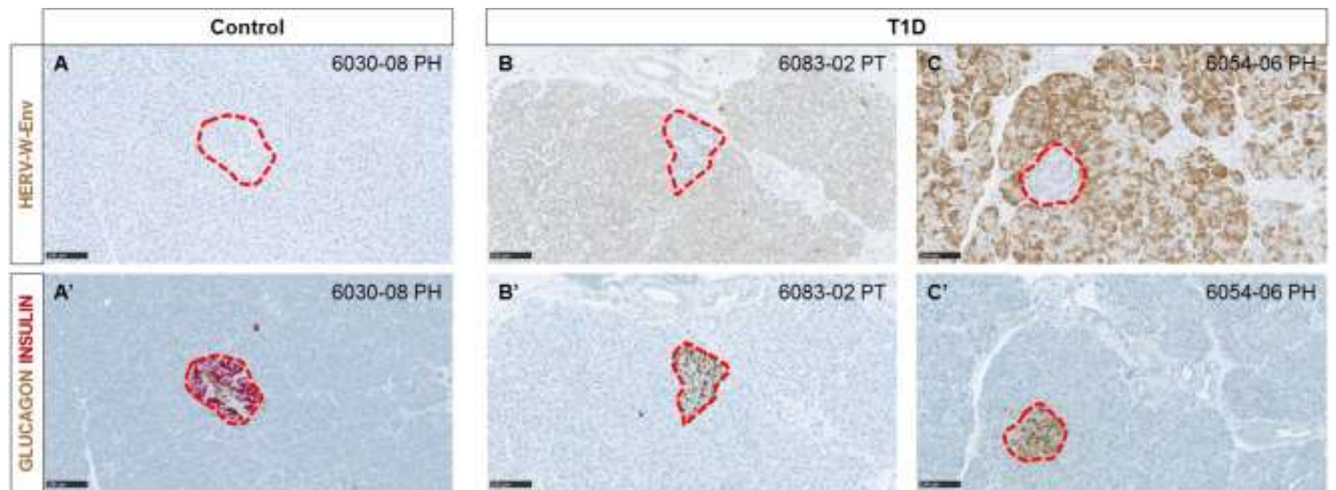

Figure 2: Expression of HERV-W-Env in pancreas from T1D patients, in the vicinity of Langerhans islets. Pancreas slices of three nPOD (network for Pancreatic Organ Donors) donors stained with GN_mAb_Env03 are presented at 20X magnification (A, B, C). Adjacent slices were coimmunostained with insulin (red) and glucagon (brown) (A', B', C'). Pancreatic Langerhans islets are highlighted with red dotted line. One non-T1D control individual is presented in A and A', and two T1D patients are presented in B, B', C and C'. nPOD case ID is indicated in the upper left corner, with block number and pancreas zone (PH: pancreas head, PT pancreas tail). Scale bars: 100µm.

In vitro data showed that HERV-W-Env is involved in two probable pathological components of T1D: 1) HERV-W-Env displays autoimmune and pro-inflammatory effects and 2) a direct cytotoxic effect on β-cells affecting insulin production. HERV-W-Env induces immunopathogenic effects engaging both innate and adaptive immune activation with the release of pro-inflammatory cytokines via an upstream TLR4 activation. TLR4 is also expressed by certain non-immune cells, and particularly by pancreatic β-cells.[43] For this reason, the pathogenic effect of HERV-W-Env has been assessed *in vitro* on primary human Langerhans islets and on rat insulinoma cells INS1E. It has been shown that HERV-W-Env impairs insulin secretion by pancreatic β-cells in a dose-related fashion[40] in pancreatic beta cells of both species.

These *in vitro* data were completed by *in vivo* observation in a transgenic mice model expressing HERV-W-Env originally cloned from MS isolate. These transgenic mice, named CAG-Env mice, displayed both hyperglycemia and hypoinsulinemia, as seen in T1D pathology. On average, the 7 weeks old CAG-Env transgenic mice displayed insulin levels 28% below than that of control mice and a glycemia 29% above the controls.[40] The hyperglycaemia concomitant with hypoinsulinaemia in the transgenic mice constitutes an *in vivo* model with hallmarks of T1D clinical features.

5. Selection and humanization of a monoclonal antibody, GNbAC1, neutralizing HERV-W Env pathogenic effects

The above described pathophysiological findings led to the search for a specific HERV-W-Env antagonist drug, which could specifically bind to this endogenous retroviral protein and neutralize its pathogenic activity. A monoclonal antibody (mAb) was isolated which selectively binds to a linear and non-glycosylated epitope of the SU domain of the HERV-W-Env protein and neutralizes its TLR4 binding potential without interacting with this receptor. Following murine and chimeric antibody versions, a humanized recombinant antibody of the IgG4/kappa subclass, called GNbAC1, was produced. Site-directed mutagenesis within the corresponding encoding nucleotide sequence was performed to stabilize the interchain disulfide bridges of the core region of IgG4. GNbAC1 has a molecular weight of approximately 147 KDa. The expected mode of action of GNbAC1 is to bind the HERV-W Env protein and thereby to act upstream of the immune-activated pro-inflammatory cascade or of the direct cytotoxic effects on non-immune cells expressing TLR4. This approach represents a potential new therapeutic solution to the pathogenic process avoiding to directly target molecular or cellular components of the immune system. The details of the selection and preclinical development of GNbAC1 are described in reference 44. GNbAC1 is developed in parallel for an indication in multiple sclerosis.

5.1. *In vitro* testing of GNbAC1 in diabetes-related models

GNbAC1 was tested *in vitro* for its ability to block the release of pro-inflammatory cytokines induced by HERV-W-Env. It was shown that the release of TNF-α by PBMC stimulated by HERV-W-Env is inhibited by GNbAC1, however GNbAC1 does not suppress PBMC activation by lipopolysaccharide (LPS), a TLR4 agonist: this shows that the GNbAC1 mode of action is specific of the target and does not involve any direct TLR4 inhibition.[26] Therefore, the neutralizing activity of GNbAC1 does not interfere with this receptor, neither by itself nor when bound to the target epitope on its ligand.

Another study used the HEK-Blue^{TM} human TLR4 cells designed for studying the stimulation of recombinant human TLR4 by monitoring the activation of NF-κB and AP-1. In this model, it was confirmed that HERV-W-Env induces a strong and highly potent TLR4 activation with a 50% maximal effect concentration (EC_{50}) in the picomolar range. It was further shown that HERV-W-Env effect is significantly and specifically inhibited by GNbAC1 in a dose-response manner.[29]

In the *in vitro* models of T1D, GNbAC1 has been shown to inhibit the more direct toxic effect of HERV-W-Env: the dose-related toxic effect on primary human pancreatic beta cells *in vitro* is blocked by GNbAC1 (Figure 3) up to high concentrations (100 ng/mL) of HERV-W-Env.[40]

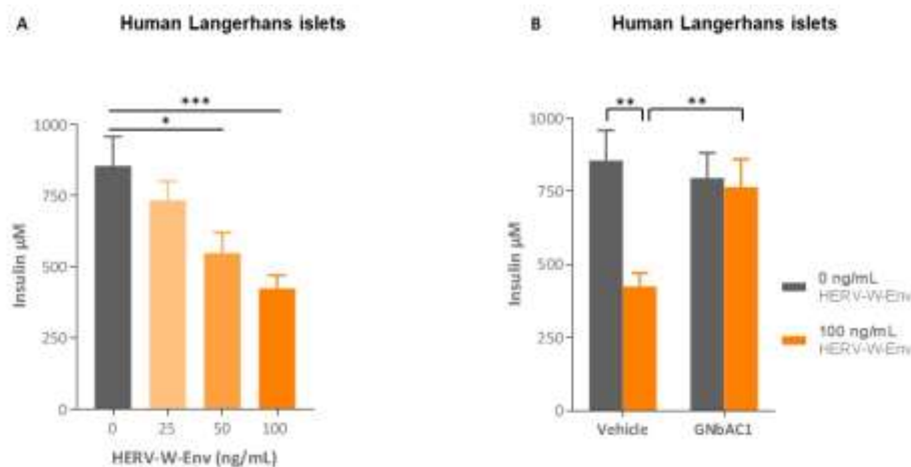

Figure 3: Direct pathogenic effects of HERV-W-Env toward human pancreatic β cells.

(A) Human Langerhans islets have been exposed for 48h to HERV-W-Env (0, 25, 50, 100ng/mL) and insulin secretion has been measured in response to 20mM glucose stimulation. (B) Human Langerhans islets have been exposed for 48h to HERV-W-Env (0 and 100ng/mL) and to GNbAC1 (3µg/mL) or Vehicle. Insulin secretion has been measured in response to 20mM glucose stimulation. Results are presented as individual values and as mean ±SEM. Each condition has been performed in triplicate and in three independent experiments. Significance determined by Bonferroni's test (A) or by unpaired t-test (B).

5.2. Effect of GNbAC1 *in vivo*

It has been shown that HERV-W-Env induces a strong release of pro-inflammatory cytokines such as IL-6 and TNF-α in mice in the blood, two cytokines found elevated in T1D[45]. HERV-W-Env injected intravenously induces a strong release of IL-6 and TNF-α in mice blood 2 h after its administration. This general effect is inhibited by the administration of GNbAC1[29] (Figure 4).

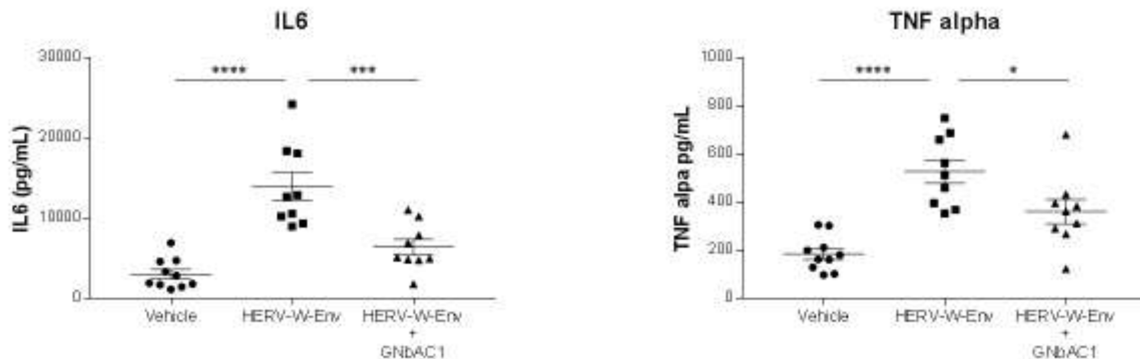

Figure 4. Figure 6: Release of pro-inflammatory cytokines induced by HERV-W-Env is reversed by GNbAC1 treatment in mice.

C57Bl6 mice have been injected intravenously with vehicle or with HERV-W-Env (3.3 mg/kg) alone or together with GNbAC1 (1.6mg/kg), 2 hours prior blood collection, serum preparation, and cytokines IL6 and TNF alpha dosage. n=9 or 10 mice per group. Data are presented as individual values and as mean ± SEM. Significance determined by Tukey's test. * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$.

5.3. Clinical data on GNbAC1

So far three clinical trials have been performed and completed with GNbAC1: two were pharmacology studies in healthy volunteers[46,47] and one was a clinical study in multiple sclerosis patients.[48, 4]. Currently a Phase IIb clinical trial is underway in approximately 260 patients with MS.[50]

5.4. Safety data

In clinical development, GNbAC1 has been tested so far in more than 300 healthy subjects or patients suffering from MS with a favorable safety profile.[51] There is no evidence for specific patterns of adverse events or for any dose relationship with the severity or the frequency of adverse events. Serious adverse events have remained rare. Interim safety data from the

Phase IIb clinical study in multiple sclerosis after 6 months of follow-up confirm these observations with no difference in the frequency of adverse events and serious adverse events between groups receiving GNbAC1 or placebo. No infusion reactions have been observed and to date there is no evidence of induction of anti-drug antibodies during treatment.[49, 52] In particular, GNbAC1 does not impair the immune system and the preservation of an immune response has been shown to a series of antigen during treatment with GNbAC1.[53]

5.5. Pharmacokinetics of GNbAC1

GNbAC1 was assessed for the first time in Humans in a Phase I trial in 33 healthy subjects up to a dose of 6mg/kg.[46] Another Phase I study where single doses of GNbAC1 of 6 mg/kg, 18 mg/kg and 36 mg/kg were tested in 21 healthy male subjects with a confirmation of the pharmacokinetic data observed at lower dose.[47] The pharmacokinetics parameters are in line with the parameters usually observed with similar monoclonal antibodies. The geometric mean elimination half-life was estimated between 21 to 26 days across all dose levels up to 36 mg/kg. Geometric mean residence time values by dose were calculated to a range from 29 to 36 days.[47] The median time to maximal concentration (t_{max}) is observed at times ranging from 2 to 4 hours. GNbAC1 exposure increases with increasing doses in a dose proportional manner for the ratios of the means, for the peak concentration and the areas under the curve.[46, 47] When repeated doses of GNbAC1 are administered, the accumulation factor is estimated to be around two fold.[49]

5.6. Pharmacodynamic response to GNbAC1

Messenger RNA transcribed from the genes HERV-W-Env and HERV-W-Pol, two pharmacodynamic markers related to HERV-W, significantly decreased during long-term treatment with GNbAC1 in a one-year study in MS.[48] In addition, a TLR4 hyper-activity in peripheral monocytes was observed among patients with multiple sclerosis and this TLR4 activity was normalized during the course of GNbAC1 treatment.[53] These pharmacodynamic effects suggest that GNbAC1 acts on the expression of the HERV-W genes and tend to decrease the inflammatory syndrome as illustrated at the cytokine and immune activation levels, at least in MS patients.

6. GNbAC1 in T1D

We postulate that GNbAC1 by its neutralising action on HERV-W-Env may reduce its proinflammatory action and the cytotoxic effects of secreted HERV-W-Env onto pancreatic beta cell in perhaps a subgroup of T1D patients. Therefore, GNbAC1 may 1) slow down or stop the auto-immune process in early diabetic patients and 2) preserve the insulin function. Blocking HERV-W-ENV expressed in diabetic pancreas, GNbAC1 should preserve the insulin secreting potential of beta islet cells and possibly restore some of the islet capacity in T1D patients. While we can currently only speculate on the full potential of GNbAC1, perhaps if given early enough in the diabetic process, this mAb may limit the extent of the disease and the need for insulin supplementation in responsive patients and thus influence the later development of complications or co-morbidities.

6.1. First study testing GNbAC1 in type 1 diabetes patients

The study RAINBOW-T1D (clinicaltrials.gov no NCT03179423) standing for, **RA**ndom**I**sed, Double-**B**lind, Placeb**O**-Controlled Study to Investigate GNbAC1 in Patients **W**ith Onset of Type 1 Diabetes Within 4 Years, is a Phase IIa study to assess the safety of repeated doses of GNbAC1 and its effect on the insulin secretion and the autoimmune T1D process. The primary objective of the study is to assess the safety and tolerability of GNbAC1 in patients with recent onset of T1D: GNbAC1 will be tested versus placebo in a randomised multicentre double-blind phase at the dose of 6 mg/kg in 60 patients for 6 repeated 4-weekly doses. Patients will be randomised at a ratio 2:1 (40 receiving GNbAC1 and 20 placebo). Patients will continue to receive their usual insulin therapy. This will be followed by an optional open-label extension phase where all patients will receive GNbAC1 at the dose of 6 mg/kg for additional 6 repeated administrations. This will give up a total of 48 weeks of treatment. The secondary objective is to determine the pharmacodynamic response to GNbAC1 on biomarkers of T1D. These are biomarkers assessing the insulin secretion and the auto-immune processes. The overall design of the study is shown in Figure 5.

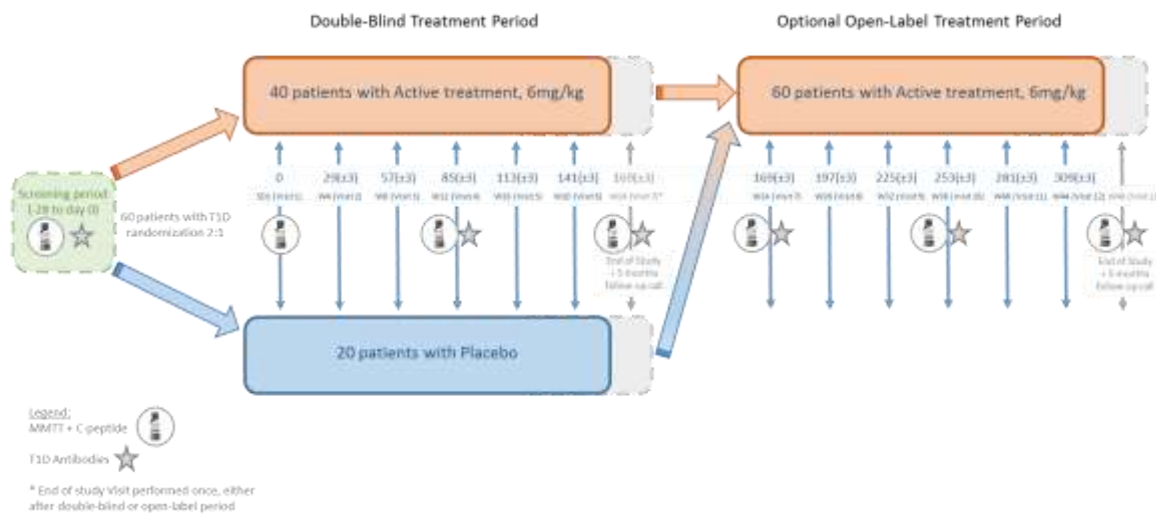

Figure 5 Design of the RAINBOW-T1D study with the timing of the main assessments

The patient population will satisfy the following criteria: Male or female (if female willing to use highly effective contraceptive methods) and with a definite diagnosis of T1D up to a maximum of 4 years prior to the signed informed consent, with a peak stimulated C-peptide of ≥0.2 nmol/L during a mixed meal tolerance test (MMTT) performed during the screening period. They will be between 18 to 55 years of age and test positive for at least one diabetes-associated auto-antibody (anti-glutamic acid decarboxylase-65 (anti-GAD-65) antibody, anti-islet cell antibody, anti-insulin antibody, anti-zinc transporter 8 (ZnT8) antibody or islet-cell antibody).

The primary endpoints are related to safety: Serious Adverse Events (SAE), Adverse Events (AE), physical examination, vital signs and clinical laboratory values. The secondary endpoints are 1) pharmacodynamics and related to diabetes control and insulin secretion: glycated haemoglobin (HbA1c) blood levels; C-peptide levels as assessed by Area Under the concentration Curve after the MMTT; change from baseline in percentage of subjects not requiring insulin; daily use of insulin (total units); 2) changes in auto-immunity will be assessed; levels of anti-glutamic acid decarboxylase-65 (anti-GAD-65) antibody, anti-islet cell antibody, anti-insulin antibody, anti-zinc transporter 8 (ZnT8) antibody. The following exploratory endpoints will be assessed: levels of HERV-W-Env biomarkers (protein and mRNA); anti-GNbAC1 antibodies, triglycerides and adiponectin. Anti-thyroglobulin and anti-thyroperoxidase (TPO) antibodies, anti-transglutaminase antibodies, total Immunoglobulin A (IgA) and anti-21-hydroxylase antibodies will be assessed to examine any possible impact on

auto-immune thyroid and coeliac disease which are known to be associated with type 1 diabetes.[54,55]

The tolerability/safety and pharmacodynamics data will be evaluated descriptively. Exploratory analyses will be performed on pharmacodynamic endpoints to assess differences between treatment groups supporting a potential therapeutic effect. In particular, stratification taking into account the duration of the disease before entering study will be performed to analyse possible differential responses. Subgroups defined by baseline HERV-W-Env blood levels will be used to perform exploratory stratified analyses on the pharmacodynamics and safety endpoints to identify whether blood HERV-W-Env biomarkers may be predictive of responses with the objective of developing a potential companion diagnostic.[51]

The study is currently recruiting in 13 centres in Australia and the the 6-month interim results are expected for the second half of 2018.

7. DISCUSSION

GNbAC1 is the first monoclonal antibody developed as an antagonist of a protein of endogenous retroviral origin from the HERV-W family. It aims at neutralizing a target which may play a role in the pathophysiological process of T1D. The HERV-W-Env target is particularly interesting as it is expressed by monocytes, B lymphocytes and pancreatic acinar cells in post-mortem T1D pancreas and it has a pathophysiological action promoting inflammation and dysregulation of glucose/insulin metabolism by interaction on the TLR4 receptor of β-cells. Based on histopathological observations,[40] it is proposed that HERV-W-Env has a dual pathophysiological effect in T1D pancreas. First, by a direct functional effect on β-cells, HERV-W-Env expressed in the vicinity on acinar cells, and also by a possible paracrine effect, may inhibit insulin secretion and possibly may reduce cell viability by activation of their TLR4 receptors.[41] Second, HERV-W-Env induces an indirect immune-mediated effect favouring $CD3^+$ and $CD68^+$ cell infiltrates in the pancreas in particular its exocrine part as well as a release of pro-inflammatory cytokines.[40] Further exploration of the pathologic role of pancreatic HERV-W-Env expression is warranted. By neutralizing HERV-W-Env protein, it is expected that GNbAC1 could inhibit the potential inflammatory and cytotoxic actions of HERV-W-Env which

may play a role in T1D pathophysiology, and thereby open the door to an efficacious and truly disease-modifying treatment of T1D without impact on the immune system. As T1D appears as an heterogenous disorder in its clinical presentation as well as in its immunopathogenesis,[5,6,56] it is not clear at present whether the HERV-W-Env is expressed systematically in all forms of the disease or if it concerns only a subgroup of patients. The data collected so far suggest an expression limited to a significant subgroup of T1D patients, however only the collection of more epidemiological data and clinical evidence will confirm this.

The high target specificity expected with a mAb such as GNbAC1 is an advantage in terms of safety, as GNbAC1 will only neutralize the effect of the protein HERV-W-Env, which is not known to play any role in the normal Human physiology.[18] Initial clinical studies performed in healthy subjects and in multiple sclerosis patients showed a favorable safety profile for GNbAC1, notably supporting the absence of modulation of the immune system. Naturally the safety profile of GNbAC1 has to be confirmed during the ongoing clinical development.

The current Phase IIa study is performed in T1D patients who have some preserved beta cell function as assessed by C-peptide levels after MMTT. The pharmacodynamic responses assessed in these patients with the measurement of the C-peptide, HbA1c and T1D related auto-antibodies will be key to assess the accuracy of the HERV-W-Env postulate in this disease condition. It is expected that any inhibitory effect of GNbAC1 on the pro-inflammatory and β-cell cytotoxic effect induced by HERV-W-Env should start within few weeks, though with a progressive effect on HERV-W Env depletion and on the downregulation of its positive feedback loop on mRNA expression, as observed to be significant at 6 months in MS patients. [48] It is therefore expected that signs of a pharmacodynamic response could already be observed after a period of 6 months. The study design has some limitations: first of all, allowing for T1D to be diagnosed in patients up to four years before the start of the trial is a potential weakness of the design. However, at this stage, the aim is to assess whether the mAb would have an action on the remaining β-cell function; subgroup analyses based on disease duration since diagnosis will help assess this question. Also the sample size of the study is limited but should be sufficient to assess a pharmacodynamics effect. Based on the previous experience of GNbAC1 in MS, this study should allow to collect the first pieces of evidence in this new indication. It is anticipated that even the preservation of some remaining

β-cell function may help in the glycemic control and possibly ultimately support more effective β-cell regeneration and replacement therapies.[4] Future T1D trials with GNbAC1 will aim at treating patients much earlier in the course of the disease.

An interesting element of the HERV-antagonist therapeutic strategy is the ability to measure the target HERV-W-Env in the blood either expressed as mRNA extracted from PBMCs or as a protein in the serum.[40] This is of interest as these HERV-related biomarkers have been shown to have some prognostic value on the evolution of MS[4751, 57] and may also have some predictive power in the response to immunomodulators in MS.[58, 59] There is currently no longitudinal data to assess if the level of expression in T1D varies over time and whether there is some correlation with the clinical evolution; this question will be investigated. The HERV-W-Env test is not specific for T1D : in the two control cohorts discussed here,[40] about 12% of healthy subjects express some level of HERV-W-Env and HERV-W-Env over-expression is also found in patients with MS[8] and CIDP.[9] The clinical significance of the level of peripheral expression of the target is still unclear. The level of expression may identify a subgroup of T1D patients or it may be, more generally, the expression of a currently ongoing auto-immune or inflammatory process. Clearly, a primary objective of this programme is to assess whether it may be possible to identify sub-groups of responders to GNbAC1 based on HERV-W-Env biomarkers, as the target may not be expressed in all T1D patients. If the use of biomarkers turns out to be meaningful, a companion diagnostic test to the GNbAC1 treatment could be developed and implemented in future trials in a precision medicine approach.

There are now more and more observations supporting an association between endogenous retroviruses of different families with the etiopathogenesis of several disorders with autoimmune or inflammatory components. Autoimmune disorders such as systemic lupus erythematous have been found to be associated with HRES-1,[60] rheumatoid arthritis and amyotrophic lateral sclerosis with HERV-K[61-63] and Sjögren syndrome.[64] For the specific case of HERV-W, HERV-W-Env has been shown to be overexpressed in patients with multiple sclerosis, schizophrenia presenting an inflammatory syndrome[65] as well as in CIDP.[9] There exists some level of epidemiological association between T1 and MS or CIDP[66-67] but this not the case for schizophrenia,[68] and data collected so far suggest that the expression of HERV-W-Env remains organ specific for a given condition without increasing the risk of expression in

other organs. However the epidemiological association between HERW-W markers and a higher risk of diseases, especially their combination, deserves further work.

The GNbAC1 therapeutic development program is the first and so far the only therapeutic development program specifically targeting a possible HERV related pathogenic protein. If any evidence for improving the insulin secretion and/or reducing the auto-immune processes is obtained during the current trial, this will pave the way to the development of the first non-immunomodulatory disease modifying treatment for T1D. If the assumptions presented above on the role of HERV-W-Env in T1D are confirmed, neutralizing such a protein with a monoclonal antibody and monitoring the response with related biomarkers could be a complete change of the paradigm for the treatment of T1D. Considering the heterogeneity of T1D presentations and possible therapeutic targets as well as the trend towards personalized treatments, the place in the T1D therapeutic armamentarium of a HERV-W-Env neutralizing approach, alone or in combination, will need to be determined.

Funding Sources

The RAINBOW-T1D study is financed by GeNeuro Australia Pty Ltd, a subsidiary of GeNeuro SA, Switzerland.

Conflict of Interest

F Curtin, C Bernard, H Porchet, S Levet, J Medina and H Perron are employees and/or shareholders of GeNeuro SA.

S Oates, D Lloyd are employees of Southern Star Research Pty Ltd.

R Simpson has received research grants from GeNeuro.

References

1 Lord S Greenbaum CJ Disease modifying therapies in type 1 diabetes: Where have we been, and where are we going? Pharmacol Res. 2015 ;98:3-8.

2 Davis IC, Randell J, Davis SN. Immunotherapies currently in development for the treatment of type 1 diabetes. Expert Opin Investig Drugs. 2015;24:1331-41.

3 Mittermayer F, Caveney E, De Oliveira C, Fleming GA, Gourgiotis L, Puri M, Tai LJ, Turner JR Addressing Unmet Medical Needs in Type 1 Diabetes: A Review of Drugs Under Development. Curr Diabetes Rev. 2017;13(3):300-314.

4 Smith EL, Peakman M. Peptide Immunotherapy for Type 1 Diabetes-Clinical Advances. Front Immunol. 2018;9:392. doi: 10.3389/fimmu.2018.00392.

5 Arif S, Leete P, Nguyen V, Marks K, Nor NM, Estorninho M, Kronenberg-Versteeg D, Bingley PJ, Todd JA, Guy C, Dunger DB, Powrie J, Willcox A, Foulis AK, Richardson SJ, de Rinaldis E, Morgan NG, Lorenc A, Peakman M. Blood and islet phenotypes indicate immunological heterogeneity in type 1 diabetes. Diabetes. 2014;63(11):3835-45.

6 Gomez-Tourino I, Arif S, Eichmann M, Peakman M. T cells in type 1 diabetes: Instructors, regulators and effectors: A comprehensive review. J Autoimmun. 2016 Jan;66:7-16

7 Peakman M. Immunological pathways to β-cell damage in Type 1 diabetes. Diabet Med. 2013 Feb;30(2):147-54.

8 Perron, H., R. Germi, C. Bernard, M. Garcia-Montojo, C. Deluen, L. Farinelli, R. Faucard, F. Veas, I. Stefas, B. O. Fabriek, J. Van-Horssen, P. Van-der-Valk, C. Gerdil, R. Mancuso, M. Saresella, M. Clerici, S. Marcel, A. Creange, R. Cavaretta, D. Caputo, G. Arru, P. Morand, A. B. Lang, S. Sotgiu, K. Ruprecht, P. Rieckmann, P. Villoslada, M. Chofflon, J. Boucraut, J. Pelletier and H. P. Hartung Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease. Mult Scler 2012;18(12): 1721-1736.

9 Faucard R, Madeira A, Gehin N, Authier FJ, Panaite PA, Lesage C, Burgelin I, Bertel M, Bernard C, Curtin F, Lang AB, Steck AJ, Perron H, Kuntzer T, Créange A. Human Endogenous Retrovirus and Neuroinflammation in Chronic Inflammatory Demyelinating Polyradiculoneuropathy. EBioMedicine. 2016 Apr;6:190-198.

10 Voisset C, Blancher A, Perron H, Mandrand B, Mallet F, Paranhos-Baccala G Phylogeny of a novel family of human endogenous retrovirus sequences, HERV-W, in humans and other primates. AIDS Res Hum Retroviruses 1999; 15 : 1529-33.

11 Feschotte C, Gilbert C. Endogenous viruses: insights into viral evolution and impact on host biology. Nat Rev Genet. 2012 Mar 16;13(4):283-96.

12 Kazazian, H. H., Jr. and J. V. Moran (2017). Mobile DNA in Health and Disease. N Engl J Med 377(4): 361-370.

13 Medina, J. and H. Perron (2017). [DNA sequences from mobile genetic elements, a hidden half of the human genome]. Med Sci (Paris) 33(2): 151-158.

14 Chuong, E. B., N. C. Elde and C. Feschotte (2016). Regulatory evolution of innate immunity through co-option of endogenous retroviruses. Science 351(6277): 1083-1087.

15 Engel, M. E. and S. W. Hiebert (2010). The enemy within: dormant retroviruses awaken. Nat Med 16(5): 517-518.

16 Richardson, S. R., S. Morell and G. J. Faulkner (2014). L1 retrotransposons and somatic mosaicism in the brain. Annu Rev Genet 48: 1-27.

17 Weiss, R. A. (2006). The discovery of endogenous retroviruses. Retrovirology 2006; 3: 67.

18 Perron H, Lang A. The human endogenous retrovirus link between genes and environment in multiple sclerosis and in multifactorial diseases associating neuroinflammation. Clin Rev Allergy Immunol. 2010;39:51-61

19 Volkman HE, Stetson DB. The enemy within: endogenous retroelements and autoimmune disease. Nat Immunol. 2014 May;15(5):415-22. doi: 10.1038/ni.2872

20 Dolei A. Endogenous retroviruses and human disease. Expert Rev Clin Immunol. 2006 Jan;2(1):149-67.

21 Perron H, Jouvin-Marche E, Michel M, Ounanian-Paraz A, Camelo S, Dumon A, Jolivet-Reynaud C, Marcel F, Souillet Y, Borel E, Gebuhrer L, Santoro L, Marcel S, Seigneurin JM, Marche PN, Lafon M. Multiple sclerosis retrovirus particles and recombinant envelope trigger an abnormal immune response in vitro, by inducing polyclonal Vbeta16 T-lymphocyte activation. Virology. 2001 Sep 1;287(2):321-32.

22 Kremer, D., T. Schichel, M. Forster, N. Tzekova, C. Bernard, P. van der Valk, J. van Horssen, H. P. Hartung, H. Perron and P. Kury (2013). "Human endogenous retrovirus type W envelope protein inhibits oligodendroglial precursor cell differentiation." Ann Neurol 74(5): 721-732.

23 van Horssen, J., S. van der Pol, P. Nijland, S. Amor and H. Perron (2016). "Human endogenous retrovirus W in brain lesions: Rationale for targeted therapy in multiple sclerosis." Mult Scler Relat Disord 8: 11-18.

24 Perron, H., J. A. Garson, F. Bedin, F. Beseme, G. Paranhos-Baccala, F. Komurian-Pradel, F. Mallet, P. W. Tuke, C. Voisset, J. L. Blond, B. Lalande, J. M. Seigneurin and B. Mandrand (1997). "Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. The Collaborative Research Group on Multiple Sclerosis." Proc Natl Acad Sci U S A 94(14): 7583-7588.

25 Mameli G, Poddighe L, Mei A, Uleri E, Sotgiu S, Serra C, Manetti R, Dolei A. Expression and activation by Epstein Barr virus of human endogenous retroviruses-W in blood cells and astrocytes: inference for multiple sclerosis. PLoS One. 2012;7(9):e44991.

26 Rolland, A., Jouvin-Marche E, Saresella M, Ferrante P, Cavaretta R, Créange A, Marche P, Perron H. Correlation between disease severity and in vitro cytokine production mediated by MSRV (multiple sclerosis associated retroviral element) envelope protein in patients with multiple sclerosis. J Neuroimmunol 2005 ; 160: 195-203.

27 Rolland A, Jouvin-Marche E, Viret C, Faure M, Perron H, Marche PN. The envelope protein of a human endogenous retrovirus-W family activates innate immunity through CD14/TLR4 and promotes Th1-like responses. J Immunol 2006; 176: 7636-44.

28 Perron H, Dougier-Reynaud HL, Lomparski C, Popa I, Firouzi R, Bertrand JB, Marusic S, Portoukalian J, Jouvin-Marche E, Villiers CL, Touraine JL, Marche PN. Human endogenous retrovirus protein activates innate immunity and promotes experimental allergic encephalomyelitis in mice. PLoS One. 2013 Dec 6;8(12):e80128.

29 Madeira A, Burgelin I, Perron H, Curtin F, Lang AB, Faucard R. MSRV envelope protein is a potent, endogenous and pathogenic agonist of human toll-like receptor 4: Relevance of GNbAC1 in multiple sclerosis treatment. J Neuroimmunol. 2016 Feb 15;291:29-38.

30 Firouzi R, Rolland A, Michel M, Jouvin-Marche E, Hauw JJ, Malcus-Vocanson C, Lazarini F, Gebuhrer L, Seigneurin JM, Touraine JL, Sanhadji K, Marche PN, Perron H. Multiple sclerosis-associated retrovirus particles cause T lymphocyte-dependent death with brain hemorrhage in humanized SCID mice model. J Neurovirol. 2003 Feb;9(1):79-93.

31 Arad G, Levy R, Hillman D, Kaempfer R. Superantigen antagonist protects against lethal shock and defines a new domain for T-cell activation. Nat Med. 2000 Apr;6(4):414-21.

32 Wucherpfennig KW, Sethi D. T cell receptor recognition of self and foreign antigens in the induction of autoimmunity. Semin Immunol. 2011 Apr;23(2):84-91

33 Delogu LG, Deidda S, Delitala G, Manetti R. Infectious diseases and autoimmunity. J Infect Dev Ctries. 2011 Oct 13;5(10):679-87.

34 Christen U1, Bender C, von Herrath MG Infection as a cause of type 1 diabetes? Curr Opin Rheumatol. 2012 Jul;24(4):417-23.

35 Tauriainen, S., S. Oikarinen, M. Oikarinen and H. Hyoty (2011). Enteroviruses in the pathogenesis of type 1 diabetes. Semin Immunopathol 2011; 33(1): 45-55.

36 Yeung, W. C., W. D. Rawlinson and M. E. Craig (2011). "Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies." BMJ 342: d35.

37 Laitinen, O. H., H. Honkanen, O. Pakkanen, S. Oikarinen, M. M. Hankaniemi, H. Huhtala, T. Ruokoranta, V. Lecouturier, P. Andre, R. Harju, S. M. Virtanen, J. Lehtonen, J. W. Almond, T. Simell, O. Simell, J. Ilonen, R. Veijola, M. Knip and H. Hyoty (2014). "Coxsackievirus B1 is associated with induction of beta-cell autoimmunity that portends type 1 diabetes." Diabetes 63(2): 446-455.

38 Perron H et al. Endogenous retroviral genes, Herpesviruses and gender in Multiple Sclerosis. J Neurol Sci. 2009; 286:65-72.

39 Dimier J, Bertin A, Medina J, Charvet B, Portoukalian J, Perron H, Hober D, Germi R, Morand P. Coxsackievirus B4-E2 strain from Type 1 Diabetes transactivates Human Endogenous Retrovirus type W: organ and virus-specific activation? American Diabetes Association Congress2017 , San-Diego USA.

40 Levet S, Medina J, Joanou J, Demolder A, Queruel N, Réant K, Normand M, Seffals M, Dimier J, Germi R, Piofczyk T, Portoukalian J, Touraine JL, Perron H. An ancestral retroviral protein identified as a therapeutic target in type-1 diabetes. JCI Insight. 2017 Sep 7;2(17). pii: 94387. doi: 10.1172/jci.insight.94387.

41 Garay-Malpartida HM, Mourão RF, Mantovani M, Santos IA, Sogayar MC, Goldberg AC. Toll-like receptor 4 (TLR4) expression in human and murine pancreatic beta-cells affects cell viability and insulin homeostasis. BMC Immunol. 2011 Feb 28;12:18.

42 Li M, Song L, Gao X, Chang W, Qin X. Toll-like receptor 4 on islet β cells senses expression changes in high-mobility group box 1 and contributes to the initiation of type 1 diabetes. Exp Mol Med. 2012 Apr 30;44(4):260-7

43 Vives-Pi M1, Somoza N, Fernández-Alvarez J, Vargas F, Caro P, Alba A, Gomis R, Labeta MO, Pujol-Borrell R. Evidence of expression of endotoxin receptors CD14, toll-like receptors TLR4 and TLR2 and associated molecule MD-2 and of sensitivity to endotoxin (LPS) in islet beta cells. Clin Exp Immunol. 2003;133(2):208-18.

44 Curtin F, Perron H, Kromminga A, Porchet H, Lang AB. Preclinical and early clinical development of GNbAC1, a humanized IgG4 monoclonal antibody targeting endogenous retroviral MSRV-Env protein. MAbs. 2015;7(1):265-75.

45 Qiao YC, Chen YL, Pan YH, Tian F, Xu Y, Zhang XX, Zhao HL. The change of serum tumor necrosis factor alpha in patients with type 1 diabetes mellitus: A systematic review and meta-analysis. PLoS One. 2017 ;12(4):e0176157.

46 Curtin F, Lang AB, Perron H, Laumonier M, Vidal V, Porchet HC, Hartung HP. GNbAC1, a humanized monoclonal antibody against the envelope protein of multiple sclerosis-associated endogenous retrovirus: a first-in-humans randomized clinical study. Clin Ther. 2012 Dec;34(12):2268-78

47 Curtin F, Vidal V, Bernard C, Kromminga A, Lang AB, Porchet H. Serum pharmacokinetics and cerebrospinal fluid concentration analysis of the new IgG4 monoclonal antibody GNbAC1 to treat multiple sclerosis: A Phase 1 study. MAbs. 2016 Jul;8(5):854-60.

48 Derfuss T, Curtin F, Guebelin C, Bridel C, Rasenack M, Matthey A, Du Pasquier R, Schluep M, Desmeules J, Lang AB, Perron H, Faucard R, Porchet H, Hartung HP, Kappos L, Lalive PH. A phase IIa randomized clinical study testing GNbAC1, a humanized monoclonal antibody

against the envelope protein of multiple sclerosis associated endogenous retrovirus in multiple sclerosis patients - a twelve month follow-up. J Neuroimmunol. 2015 ;285:68-70

49 Derfuss T, Curtin F, Guebelin C, Bridel C, Rasenack M, Matthey A, Du Pasquier R, Schluep M, Desmeules J, Lang AB, Perron H, Faucard R, Porchet H, Hartung HP, Kappos L, Lalive PH. A phase IIa randomised clinical study of GNbAC1, a humanised monoclonal antibody against the envelope protein of multiple sclerosis-associated endogenous retrovirus in multiple sclerosis patients. Mult Scler. 2015 ;21(7):885-93.

50 Curtin F, Porchet H, Glanzman R, Schneble HM, Vidal V, Audoli-Inthavong ML, Lambert E, Hartung HP. A placebo randomized controlled study to test the efficacy and safety of GNbAC1, a monoclonal antibody for the treatment of multiple sclerosis - Rationale and design. Mult Scler Relat Disord. 2016 Sep;9:95-100.

51 Curtin F, Perron H, Faucard R, Porchet H, Lang AB. Treatment against human endogenous retrovirus: a possible personalized medicine approach for multiple sclerosis. Mol Diagn Ther. 2015 Oct;19(5):255-65.

52 Hartung HP, Curtin F, Schneble HM, Porchet H, Glanzman R, Lambert E, Selmaj K, on behalf of the GNC-003 investigators, Barkhof F. GNC-003: an international, double-blind, randomized, placebo-controlled phase IIb trial to assess the efficacy, safety and pharmacokinetics of GNbAC1 in patients with relapsing remitting multiple sclerosis. ECTRIMS Congress Paris 2017

53 Zimmermann M, Sanderson NS, Rasenack M, Lalive PH, Lang AB, Curtin F, Lindberg RL, Kappos L, Derfuss T. Immunologic monitoring during a phase 2a trial of the GNbAC1 antibody in patients with MS. Neurol Neuroimmunol Neuroinflamm. 2015 Aug 20;2(5):e144

54 Kurien M, Mollazadegan K, Sanders DS, Ludvigsson JF. Celiac Disease Increases Risk of Thyroid Disease in Patients With Type 1 Diabetes: A Nationwide Cohort Study. Diabetes Care. 2016 Mar;39(3):371-5

55 Shun CB, Donaghue KC, Phelan H, Twigg SM, Craig ME Thyroid autoimmunity in Type 1 diabetes: systematic review and meta-analysis. Diabet Med. 2014 Feb;31(2):126-35.

56 Von Herrath M, Peakman M, Roep B. Progress in immune-based therapies for type 1 diabetes. Clin Exp Immunol 2013;172,186-202

57 Sotgiu S1, Mameli G, Serra C, Zarbo IR, Arru G, Dolei A. Multiple sclerosis-associated retrovirus and progressive disability of multiple sclerosis. Mult Scler. 2010 ;16:1248-51

58 Arru G, Leoni S, Pugliatti M, Mei A, Serra C, Delogu LG, Manetti R, Dolei A, Sotgiu S, Mameli G Natalizumab inhibits the expression of human endogenous retroviruses of the W family in multiple sclerosis patients: a longitudinal cohort study. Mult Scler. 2014;20:174-82.

59 Mameli G, Serra C, Astone V, Castellazzi M, Poddighe L, Fainardi E, Neri W, Granieri E, Dolei A. Inhibition of multiple-sclerosis-associated retrovirus as biomarker of interferon therapy. J Neurovirol. 2008;14:73-7.

60 Perl A, Nagy G, Koncz A, Gergely P, Fernandez D, Doherty E, Telarico T, Bonilla E, Phillips PE.Molecular mimicry and immunomodulation by the HRES-1 endogenous retrovirus in SLE. Autoimmunity. 2008;41:287-97.

61 Nelson PN1, Roden D2, Nevill A2, Freimanis GL2, Trela M2, Ejtehadi HD2, Bowman S2, Axford J2, Veitch AM2, Tugnet N2, Rylance PB2. Rheumatoid arthritis is associated with IgG antibodies to human endogenous retrovirus gag matrix: a potential pathogenic mechanism of disease? J Rheumatol. 2014;41:1952-60.

62 Tugnet N Rylance P Roden D Trela M Nelson P Human Endogenous Retroviruses (HERVs) and Autoimmune Rheumatic Disease: Is There a Link? Open Rheumatol J. 2013;7:13–21.

63 Douville R, Liu J, Rothstein J, Nath A. Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. Ann Neurol. 2011;69:141-51.

64 Sander DM, Szabo S, Gallaher WR, Deas JE, Thompson JJ, Cao Y, Luo-Zhang H, Liu LG, Colmegna I, Koehler J, Espinoza LR, Alexander SS, Hart DJ, Tom DM, Fermin CD, Jaspan JJ, Kulakosky PC, Tenenbaum SA, Wilson RB, Garry RF. Involvement of human intracisternal A-type retroviral particles in autoimmunity. Microsc Res Tech. 2005;68:222-34

65 Perron H, Mekaoui L, Bernard C, Veas F, Stefas I, Leboyer M. Endogenous retrovirus type W GAG and envelope protein antigenemia in serum of schizophrenic patients. Biol Psychiatry. 2008 Dec 15;64(12):1019-23

66 Laughlin RS1, Dyck PJ, Melton LJ 3rd, Leibson C, Ransom J, Dyck PJ. Incidence and prevalence of CIDP and the association of diabetes mellitus. Neurology. 2009 Jul 7;73(1):39-45.

67 Tettey P, Simpson S Jr, Taylor BV, van der Mei IA. The co-occurrence of multiple sclerosis and type 1 diabetes: shared aetiologic features and clinical implication for MS aetiology. J Neurol Sci. 2015 Jan 15;348(1-2):126-31. doi: 10.1016/j.jns.2014.11.019. Epub 2014 Nov 20.

68 Juvonen H, Reunanen A, Haukka J, Muhonen M, Suvisaari J, Arajärvi R, Partonen T, Lönnqvist J. Incidence of schizophrenia in a nationwide cohort of patients with type 1 diabetes mellitus. Arch Gen Psychiatry. 2007 Aug;64(8):894-9.