TG1050, an HBV-targeted immunotherapeutics, efficiently decreases HBV viremia and antigenemia in a preclinical model; a meta-analysis and the determination of the involvement of CD4 and CD8 T cells.

Poster ID: 1868

Corresponding author: R.Kratzer : kratzer@transgene.fr

Roland Kratzer[1], Alexei Evlachev[1]*, Karine Lélu-Santolaria[1]*, Benoît Sansas[2]*, Doris Schmitt[3], Clarisse Dubois[1], Yasmin Schlesinger[3], Jean-Baptiste Marchand[3], Michel Geist[3], Renée Brandely[3], Annie Findeli[3], Thierry Menguy[3], Nathalie Silvestre[3], Marie-Louise Michel[4,5], Geneviève Inchauspé[1], Perrine Martin[1]

**All these authors equally contributed to this work.*

*[1]**Transgene** SA, Dept. of Infectious Diseases, Lyon, France - [2]**Transgene** SA, Smart Data Lab, Strasbourg, France - [3]**Transgene** SA, Smart Virus Lab, Strasbourg, France - [4]**Institut Pasteur**, Laboratoire de pathogénèse des virus de l'hépatite B, Paris, France - [5]**INSERM** U994, Paris, France*

INTRODUCTION

Purpose: Current therapies (nucleos(t)ide analogs or peg-IFNa) for chronic hepatitis B virus (HBV) infection rarely achieve virus clearance. Cohort studies have shown the critical role of cellular immune responses to control HBV infection. We developed an HBV-targeted immunotherapeutic called TG1050 and have shown the induction of multispecific T cells in an HBV-persistent mouse model (AAV-HBV) together with antiviral properties (Martin, Gut 2015 Dec;64(12):1961). We report here a meta-analysis of 5 experiments performed with the AAV-HBV model. Furthermore, we have started to dissect in this HBV model TG1050-associated mechanism of action in particular the role of CD4 and CD8 T-cells.

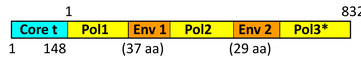
TG1050 : OBJECTIVE AND DESCRIPTION

OBJECTIVE

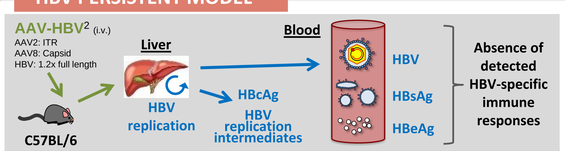
Develop a viral vector-based HBV-specific immunotherapeutic inducing potent, multispecific, sustained and cross reactive T cell responses displaying properties of immune responses detected in HBV resolving patients

TG1050

Based on non-replicative E1 and E3 deleted human adenovirus serotype 5 encoding a fusion protein comprising truncated HBV Core fused to a deleted and mutated HBV polymerase and 2 selected HBsAg domains (genotype D sequence)



HBV PERSISTENT MODEL



Absence of detected HBV-specific immune responses

Following TG1050 injection in AAV-HBV C57BL/6 mice:

- Induction of HBV-specific functional T cells in spleens and livers and sustained antiviral effects including anti-HBsAg seroconversion[1]

MATERIAL & METHODS

TG1050 treatment in AAV-HBV mice : C57BL/6 mice were injected intravenously with recombinant AAV-HBV, encoding 1.2 copies of the HBV genome. In this model HBV antigens and DNA replication intermediates (HBV mRNA incl. pgRNA) are detected within the liver as well as HBsAg, HBeAg and HBV infectious particles in the blood of injected mice, with a persistence of around 1 year[2]. In parallel no HBV-specific T cells or antibodies were detected in AAV-HBV injected mice whereas an increased number of regulatory T cells and IL-10 producing T cells are present in the liver. This model mimics to some extent the HBV chronic infection. Four to 5 weeks after AAV-HBV injection, TG1050 was injected multiple times by sub-cutaneous route at a dose of $2x10^9$ vp/injection/mice and viral parameters were monitored at various time points. HBsAg in mouse sera was quantified using a BioRad kit (Monolisa HBsAg plus using a recombinant HBsAg protein for quantification). HBV DNA in mouse sera was quantified using a qPCR assay (limit of quantification : 100 copies/reaction). Anti-HBsAg/HBcAg antibodies were detected by an « in house » ELISA assay.

Meta-analysis: For the meta-analysis 5 preclinical experiments, lasting in total between 11 weeks to 20 weeks, were considered for statistical analyses: Female mice, C57BL/6 strain, AAV only or AAV+TG1050 treatment (at $2x10^9$ vp/injection/mice), at least 3 time points after TG1050 administration.

Global Mixed model: A global mixed model was done with all experiments considering the following covariates: Time, Treatment, the interaction between Time and Treatment and the HBsAg value at baseline as fixed effects and the Experiment as random effect.

Meta-Analysis for interaction term: The meta-analysis was done by estimating for all experiments the interaction term Day*Treatment and the associated standard error. Then a meta-analysis using a fixed effect model was done by weighting estimation with the inverse-variance.

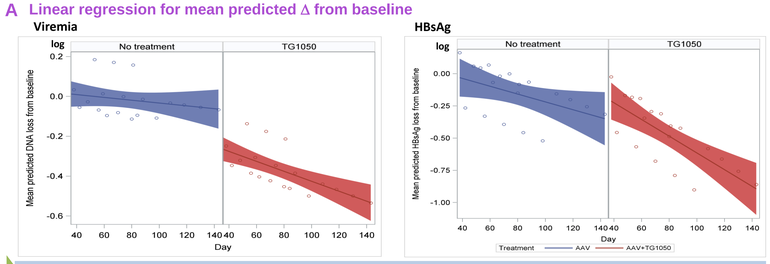
Percentage of responder: A mouse was considered as "Responder" if it presented an HBsAg/DNA decrease higher than 0.5 log to the baseline value for two or more time points during the study (consecutive or not). The percentage of responders was calculated for each experiment and then the mean average percentage of responders was calculated for each treatment group and compared with a non-parametric Wilcoxon-Mann-Whitney test.

TTR: The Time-To-Response (TTR) was defined as the time between the first TG1050/AAV administration and the time of response (defined as the second time point presenting a decreased more than 0.5 log). If a mouse did not present a response, the TTR was censored at the last blood sample measurement. A Cox model was done to estimate the Hazard Ratio and the estimated confidence interval.

TG1050 mode of action experiments : In similar experimental settings, CD4 and/or CD8 T cells were depleted (twice a week, 150 µg, starting at D34 before 3 TG1050 immunizations (D40, D47, D54) until the end of the experiment D77) or adoptively transferred ($7.5x10^6$ CD8+ or CD4+ cells purified by magnetic beads from HBV-free mice immunized by 1 TG1050 immunization 2 weeks before into AAV-HBV mice) to determine their involvement in antiviral efficacy of TG1050. Transferred CD8+ T cells contained ~$1\text{-}2x10^5$ HBV-specific (polymerase and core) multimer positive T cells. Livers have been sampled 4 weeks post transfer to analyze intracellular IFNg secretion by intrahepatic CD8 T cells. (Serum HBsAg and viremia has been analyzed as described before.)

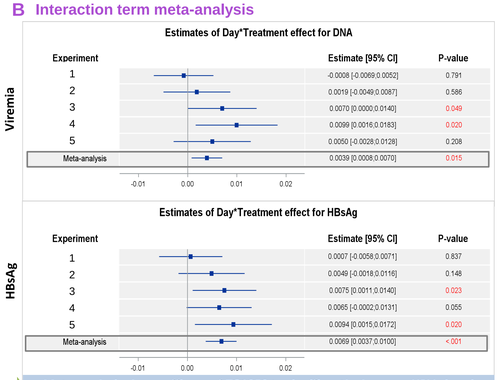
RESULTS (I): META-ANALYSIS OF TG1050 ANTIVIRAL EFFECTS IN AAV-HBV MODEL

A Linear regression for mean predicted Δ from baseline



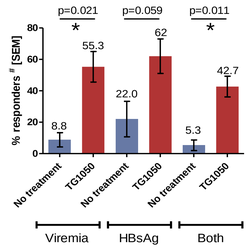
TG1050 administration leads to a persistent decrease in HBV viremia and HBsAg levels up to 110 days post immunization

B Interaction term meta-analysis



Meta-analysis shows efficacy of TG1050 to significantly decrease HBV viremia and HBsAg levels

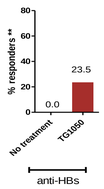
C Percentage of responders#



percentage of mice displaying at least a 0,5 log decrease or more on ≥ 2 time pts

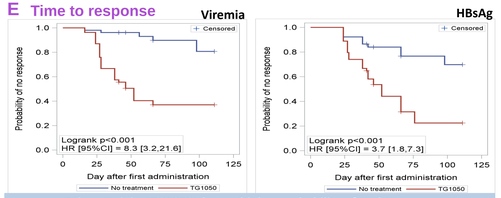
D Percentage of responders** (anti-HBsAg antibodies)

long experiments, 16-18 weeks post 1st immunization, N=2



** percentage of mice displaying detectable anti HBsAg Abs on ≥ 2 time pts

E Time to response

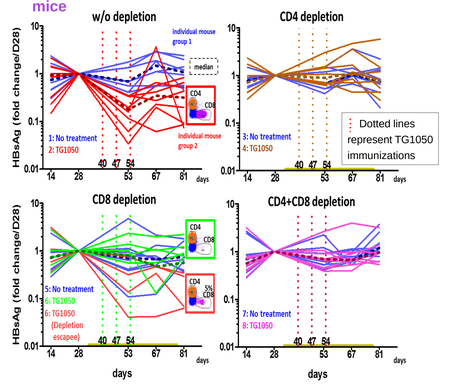


Mice receiving TG1050 show a higher probability of early response (HBV viremia and antigenemia decrease)

RESULTS (II): MODE OF ACTION OF TG1050 IN THE AAV-HBV MOUSE MODEL

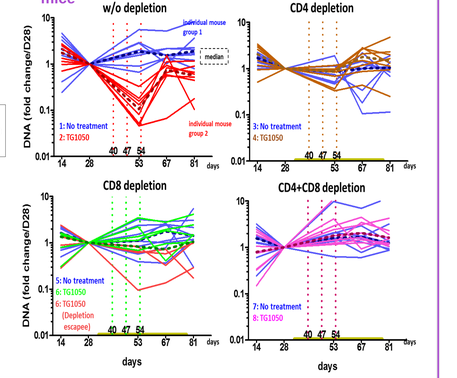
T cell depletion experiment

F HBsAg levels w/ and w/o TG1050 treatment in AAV-HBV mice



Dotted lines represent TG1050 immunizations

G DNA levels w/ and w/o TG1050 treatment in AAV-HBV mice

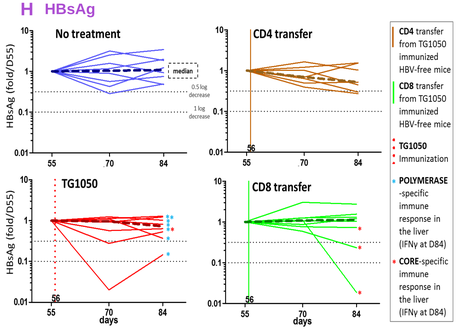


CD8 and/or CD4 depletion leads to an abolishment of antiviral activity of TG1050 confirming the expected mechanism of action. Residual antiviral activity is observed in mice escaping CD8 depletion (lower left graph F+G)

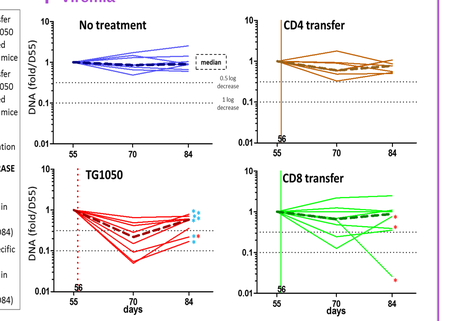
Adoptive transfer of TG1050-educated T cells in AAV-HBV mice

CD8 or CD4 transfer (7.5×10^6 splenocytes sampled at D14 post TG1050 immunization from HBV-free mice) into AAV-HBV mice 56 days post-AAV-HBV administration

H HBsAg



I Viremia



CD8 T cell transfer results in a decrease in viral titers in a subset of mice. This decrease is concomitant with the detection of IFNg+ CORE-specific CD8 T cells in the liver. Whereas antiviral activity due to direct injection of TG1050 is associated with the detection of IFNg+ POL-specific CD8 T cells.

CONCLUSION

- **META-ANALYSIS OF PRE-CLINICAL DATA FOR TG1050 IN AN HBV TOLERANT MOUSE MODEL SHOWS :**
  - ✓ ANTIVIRAL EFFECT: Significant treatment effect on viremia and HBsAg levels (**p=0.015** and **p<0.001** resp.)
    Decreasing (≥ 0.5 log) viremia and HBsAg levels in **55%** and **62%** of mice, resp. (75% and 64%, resp., in long experiments >16Wp1)
    Mice receiving TG1050 were found to have **19** or **10** times higher chance to present a DNA or HBsAg response, resp. using a logistic regression (not shown)
    Mice receiving TG1050 show a higher probability of early response (decline in viral parameters; time to response)
  - ✓ HUMORAL IMMUNE RESPONSE: Anti-HBs seroconversion (clinical goal of HBV therapies) in **24%** of mice (in long experiments, >16Wp1)
- **ANALYSIS OF TG1050 MODE OF ACTION IN AN HBV TOLERANT MOUSE MODEL SHOWS :**
  - ✓ CD8 AND CD4 T CELLS INDUCED BY TG1050 ARE INVOLVED IN ANTIVIRAL EFFICACY.
  - ✓ FURTHER STUDIES ARE ONGOING TO VALIDATE THESE PRELIMINARY DATA
- **A PHASE 1/1B STUDY (FIRST-IN-MAN AND DOSE FINDING) IS UNDERWAY[3]**

DISCLOSURE

All authors except MLM are or were employees of Transgene SA.

REFERENCES

[1] Martin et al., Gut, Dec 2015; 64(12):1961-71
[2] Dion et al., J Virol, May 2013; 87(10):5554-63
[3] ClinicalTrials.gov: NCT02428400

AASLD 2016 Liver Meeting – Boston, MA – November 2016