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.

### INTRODUCTION

Purpose: Current therapies (nucleos(t)ide analogs or peg-IFN) 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 immunotherapeutics (called TG1050) and have shown the induction of multispecific T-cells in an HBV-particle mouse model (HBV-Ab) with antiviral properties (Martin, Gut 2015 Dec;64(12):1961). We report here a meta-analysis of 5 experiments performed with the Ab/AIV model. Further studies are aimed to dissect in this HBV model TG1050-associated mechanism of action and in particular the role of CD4 and CD8 T-cells.

### MATERIAL & METHODS

**TG1050 treatment in AAV-HBV mouse:** C57BL/6 mice were injected intravenously with recombinant Ab/AIV-HBV encoding 1.5 copies of the HBV genome. In this model, HBV antigen and DNA replication intermediates (HBV DNA) are detected within the liver as well as HbsAg, HBsAg, and HBV infectious particles in the blood of infected mice, with a persistence of around 1 year. To parallel to HBV-specific T cells or antibodies were detected in AAV-HBV infected mice as an increased number of regulatory T cells and B-10 producing T cells are present in the liver. This model mimics some aspect the HBV chronic infection. Four to 5 weeks after AAV-HBV injection, TG1050 was injected multiple times by subcutaneous route (4x/injection/mouse and viral parameters were monitored at various time points. HbsAg in mouse sera was quantified using a sandwich ELISA (Diasorin; DHRD kit) using a recombinant HBsAg as standard. Quantification of HBV DNA was performed using a qPCR assay (limit of quantification: 100 copies/reaction). Anti-HBsAg/HBsAg antibodies were detected by an in-house ELISA assay.

**Meta-analysis:** For the meta-analysis, 5 preclinical experiments, lasting in between 12 weeks to 20 weeks, were considered for statistical analyses. Female mice, C57BL/6, AAV or not TG1050 administration (at 2xIDp of apheresis/mouse), at least 2 time points after TG1050 administration.

A global mixed model was done with all experiments considering the following covariates: Time, Treatment, and the interaction Between Time and Treatment, and the HBV load as baseline as fixed effects as the response 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 each experiment 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 times during the study (parametric or not). The percentage of responders was calculated for each experiment and then the mean average of percentages of responders was calculated for each treatment group and compared with a non-parametric Wilcoxon-Mann-Whitney test.

**Time to Response (TR):** was defined as the time between the first TG1050/AIV administration and the time of response (defined as the second time point presenting a decrease more than 0.5 log). If a mouse did not present a response, the TR 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, 100 μg, starting at 5 days before 1 TG1050 administrations (D16, D17, D18), ending until the end of the experiment (D77) or adoptively transferred (7x25μg CD4 or CD8 cells purified by magnetic beads from HBV mice inoculated by TG1050 immunization 2 weeks before into HBV-Ab mice) to determine their involvement in antiviral effects of TG1050. Transferred CD8 T-cells contained ~2×10^6 HBV-specific polytropic and only multipotent P1 cells. Lymphocytes were sampled 5 days post transfer to analyze intracellular IFNγ secretion by polytropic CD8 T-cells. (Serum HbsAg and viremia have been analysed as described below).

### RESULTS

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

**A. Linear regression for mean predicted a from baseline**

**B. Interaction term meta-analysis**

**C. Percentage of responders**

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

**F. HbsAg levels w/ and w/o TG1050 treatment in AAV-HBV mice**

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

**H. HbsAg transfer results in a decrease in viral load in a subset of mice.**

This decrease is concentration-dependent with the detection of HbsAg in CD4+CD8+ AAV-Cells in the liver. Whereas antiviral activity due to depletion of TG1050 is associated with the detection of HbsAg in CD4+CD8+ AAV-Cells in the liver.

**CONCLUSION**

- **META-ANALYSIS OF PRE-CLINICAL DATA FOR TG1050 IN AN HBV TOLERANT MOUSE MODEL SHOWN**
  - **ANTIVIRAL EFFECT:** Significant treatment effect on viremia and HbsAg levels (p=0.0015 and p=0.001 respectively). 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 20 times higher chance to present a DNA or HbsAg response, resp. using a logistic regression (not shown).

- **HUMORAL IMMUNE RESPONSE:** Anti-HbsA immunization (clinical goal of HBV therapy) in 24% of mice (in long experiments, 10±2%)

- **ANALYSIS OF TG1050 MODE OF ACTION IN AN HBV TOLERANT MOUSE MODEL SHOWN**
  - **CD4 AND CD8 T CELLS PRODUCED ARE IN VITRO INACTIVITY**
  - **FURTHER STUDIES ARE ONGOING TO VALIDATE THESE PRELIMINARY DATA**

**REFERENCES**

1. Martin et al., Gut, 2015 Dec;64(12):1961
2. Bran et al., Virol. May 2013; 87(10):5554
3. ClinicalTrials.gov: NCT02065830

**DISCLOSURE**

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