CD3ε Humanized mice:
A new tool to validate your bi-specific T cell engager molecule

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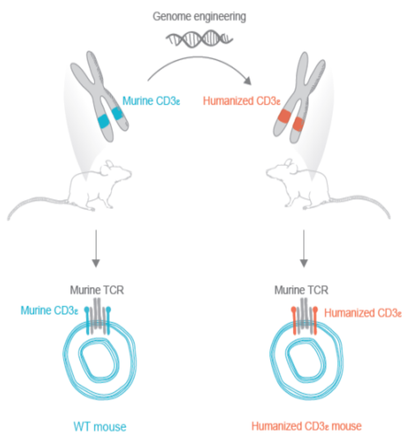
Abstract

Oncology is characterized by a desperate medical need for new drugs. Once identified, a cancer drug target is validated by demonstrating that a given therapeutic agent is clinically effective. In this way, **finding the good preclinical animal model is one of the prerequisites for achieving success**. These last decades saw the emergence of monoclonal antibodies as therapeutics, and recently the development of **bispecific T cell engagers** as promising drugs such as blinatumomab (anti-CD19/anti-CD3)[1] which bridges **cytotoxic T cells** (via CD3 T-cell co-receptor) to tumor cells (via specific target receptors) thereby leading to tumor cell death[2].
Among the several CD3 co-receptor chains, **CD3ε** is **the main target of bispecific T-cell engagers** on the effector side. **This poster shows a first validation at MI-mAbs of a CD3ε humanized KI mouse model obtained by replacing mouse CD3ε by human CD3ε, allowing *in vitro and in vivo* evaluation of bispecific T cell engager targeting human CD3ε.**

A CD3ε humanized KI mouse model



In vitro immunophenotyping

There is a good expression level of human CD3ε in Heterozygous and Homozygous mice (cf. Figure 1).
The immunophenotyping at steady state shows that heterozygous mice are quite similar to wild type (WT) in cell numbers in blood, lymph node (LN) and thymus. Homozygous are slighty different to Heterozygous and WT.
An impaired thymic maturation (reduced cell number) in Homozygous is observed, but almost similar cellularity in all other peripheral organs (LN, Blood) at all ages. A decreased number of T cells is observed but B cells are unaffected. Effector Memory subsets in proportion are increased and CD4/CD8 proportion are decreased.

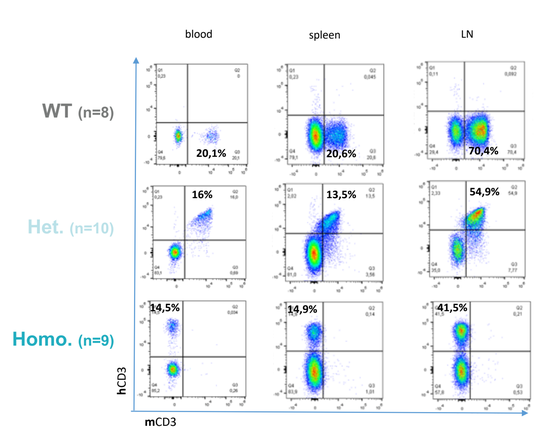


Figure 1: Heterozygous (HET.) mice T-cells express both human and mouse CD3e Homozygous (HOM.) mice T-cells only express human CD3e

In vitro validation

T cells of CD3ε humanized mice respond to *in vitro* human CD3 activation

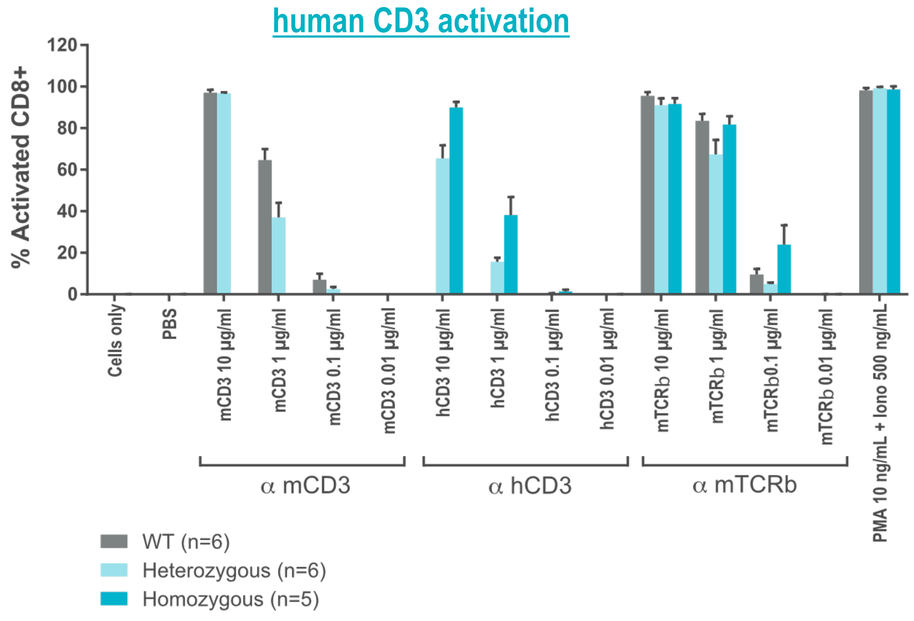


Figure 2: T cells enriched from hCD3ε Wild type (WT), Heterozygous (Het.) and Homozygous (Homo.) splenocytes were activated with coated mCD3, hCD3, anti-mTCRβ plus soluble anti-CD28, or PMA/Ionomycin (positive control) for 48h.
% of activated mCD8+ T cells (CD69+ CD25+) were studied by flow cytometry.

T cells of CD3ε humanized mouse display *in vitro* cytotoxic activity when triggered via human CD3

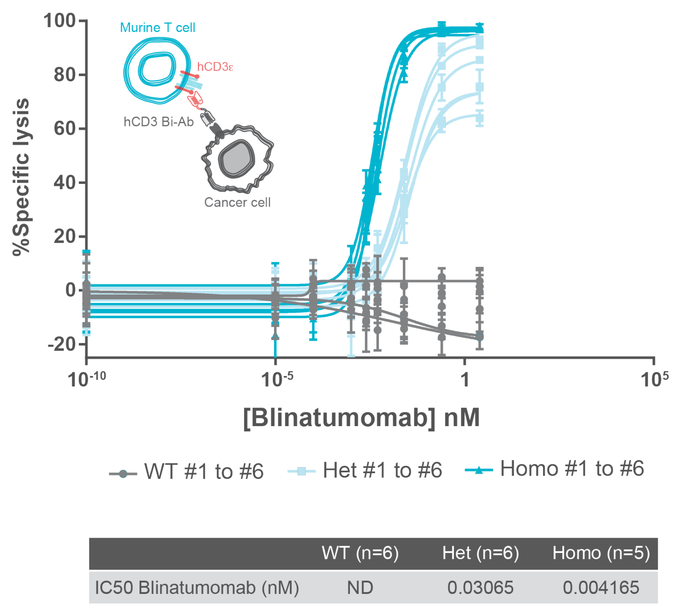


Figure 3: T cells enriched from hCD3ε WT, Het. and Homo. splenocytes were **pre-activated for 3 days** with anti-mTCRb and anti-mCD28.
Specific Lysis % of **Raji-Luc** (=Target cells expressing hCD19 & luciferase) by **pre-activated T cells** (=Effector cells) in presence of **Blinatumomab** (hCD19-hCD3 bispecific) after **overnight incubation at 37°C** was calculated as follow :

% Sp. lysis = 100 × (E − S) / (M − S)

E : experimental lysis
S: spontaneous lysis (Raji-Luc + T cells signal)
M: maximum lysis

	WT (n=6)	Het (n=6)	Homo (n=5)
IC50 Blinatumomab (nM)	ND	0.03065	0.004165

- Dose effect activation of hCD3ε Het. and Homo. T cells with anti-hCD3 + anti-CD28
- Cytotoxic activity of HCD3ε Homo. and Het. T cells when triggered via hCD3 receptor
- Better activation and cytotoxic activity of hCD3ε Homo. vs Het.

In vivo validation

Blinatumomab exhibits *in-vivo* anti-tumoral activity in CD3ε humanized mice injected with B16F10-hCD19^{Hi} cells

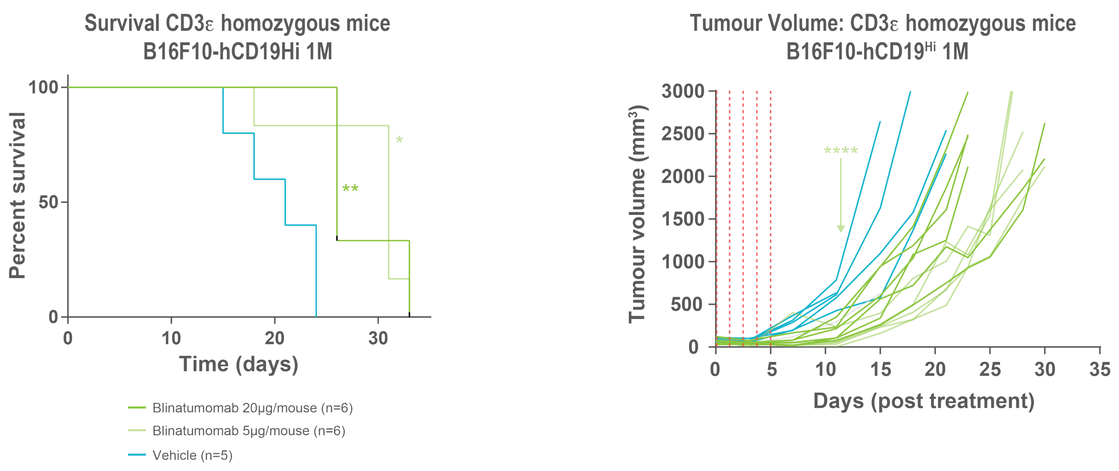


Figure 4: **CD3ε humanized mice (Homo.)** were injected sub-cutaneously with 1.10^6 B16F10 murine melanoma cells expressing hCD19. When tumors reached 50-100 mm^3, mice were treated intravenously daily with **Blinatumomab** (hCD19-hCD3 bispecific, 5 or 20 µg/mouse) or vehicle for 5 days (salmon dotted lines). Survival and tumor volume ((length x width^2)/2) along time are represented in the left & right panels respectively. P-values were calculated according to Mantel–Cox test (survival) or Tukey's test (tumor volume at D11 for Blina. 20 or 5µg/mouse vs. vehicle). * P < 0.05 | ** P<0.005 | **** P<0.00005

Response to immunization

Week	1							2							3							
Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
TNP-KLH immunization	■														■							
Serum collection	■							■														■

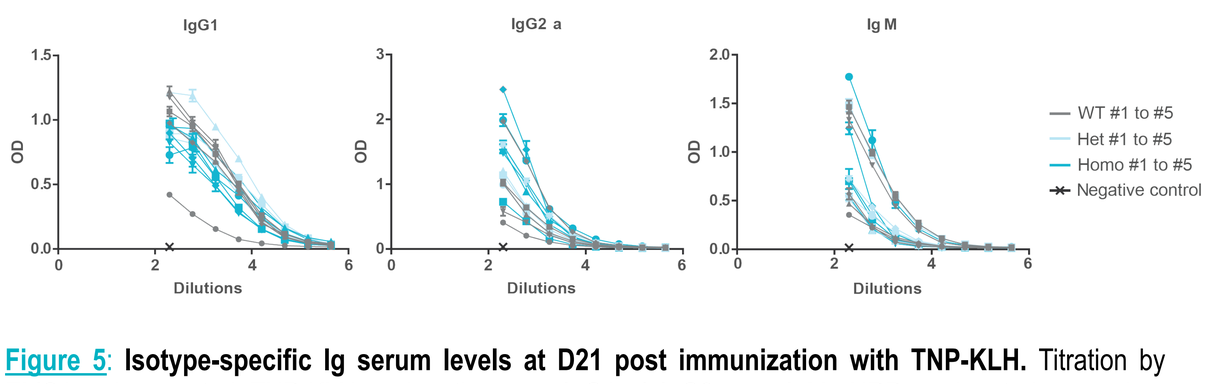


Figure 5: **Isotype-specific Ig serum levels at D21 post immunization with TNP-KLH.** Titration by ELISA using coated TNP-KLH and anti-mouse IgG1- / IgG2a- or IgM- HRP conjugated secondary antibodies.

- Anti-tumoral activity of blinatumomab in hCD3ε Homo mice bearing CD19-tumor
- **CD3ε humanized mouse available at MI-mAbs is a suitable model for *in vivo* testing of Bispecific T Cell Engager compounds.**
- **Similar response to immunization** in WT, heterozygous and homozygous hCD3ε mice

- 1st model available on the market, available at MI-mAbs to test all bi-specifics engaging T cells.
- Performed by an experienced team (in collaboration with CIPHE)

MI-mAbs validates your target using a bi-specific format on hCD3ε mice:
- Builds with you the most adequate scientific program
- Provides sufficient data to be able to select the right candidate, including on-demand preliminary PK/PD and efficacy package in this model

1- Fan G, Wang Z, Hao M, Li J. Bispecific antibodies and their applications. Journal of Hematology & Oncology. 2015;8:130. 2- Klinger M, Benjamin J, Kischel 1, Stienen S, Zugmaier G. Harnessing T cells to fight cancer with BiTE® antibody constructs--past developments and future directions. Immunol Rev. 2016; 270(1):193-208.

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