UNIVERSITÉ DE STRASBOURG

Inserm
Institut national
de la santé et de la recherche médicale

5° Forum du Cancéropôle Grand Est

03/11/2011

Adoptive allogeneic immunotherapy of hepatocellular carcinoma (HCC) by infusion of suicide gene-modified cells (GMC) : *in vitro* and *in vivo* proof of concept

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Current treatments of hepatocellular carcinoma (HCC)

- TransArterial ChemoEmbolization (TACE)
- Radiofrequency
- Surgical ablation

} Limited efficiency (recidive, no treatment of the sub-jacent cirhosis)

- Transplantation : reserved to patients with a low risk of recidive
  (1 tumour < 5cm or 2-3 tumours < 3cm = Milan criteria)

ADJUVANT TREATMENTS REQUIRED

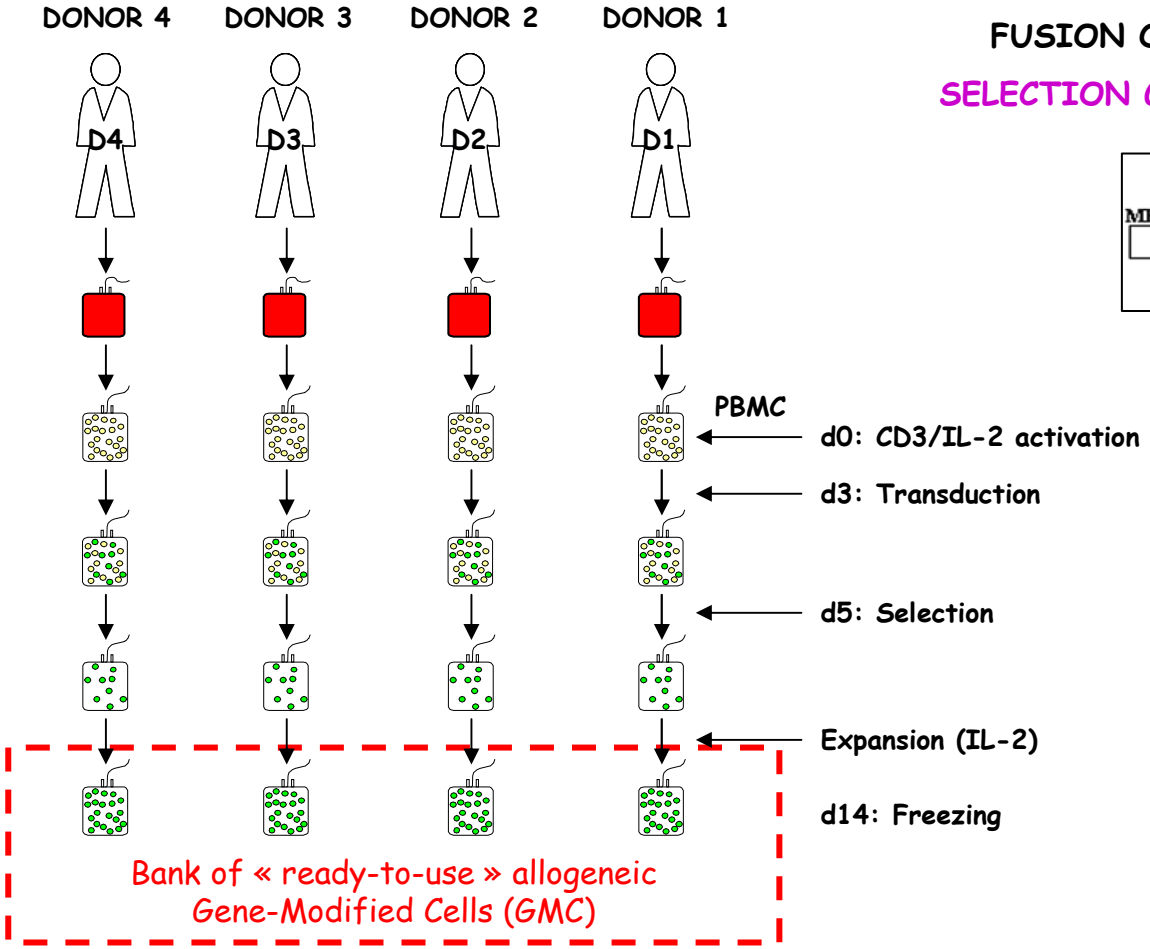
Our aim: Evaluate suicide gene-modified lymphocytes as a new adoptive allogeneic immunotherapy of HCC

How? Injection of alloreactive lymphocytes from normal blood donors

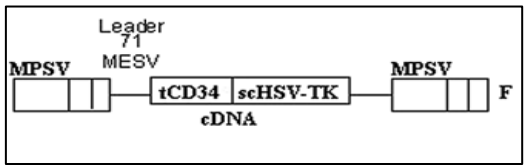
→ cytotoxic activity against tumor cells = **GvT (Graft-vs-Tumor) effect**

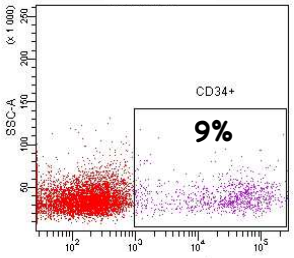
↻ Recognition of tumor cells based on genetic mismatches between the donor and the recipient

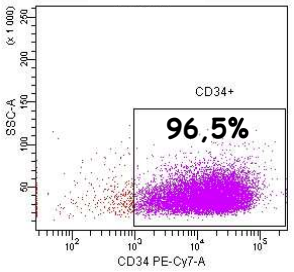
Bank of "ready-for-use" allogenic gene-modified cells (GMC)



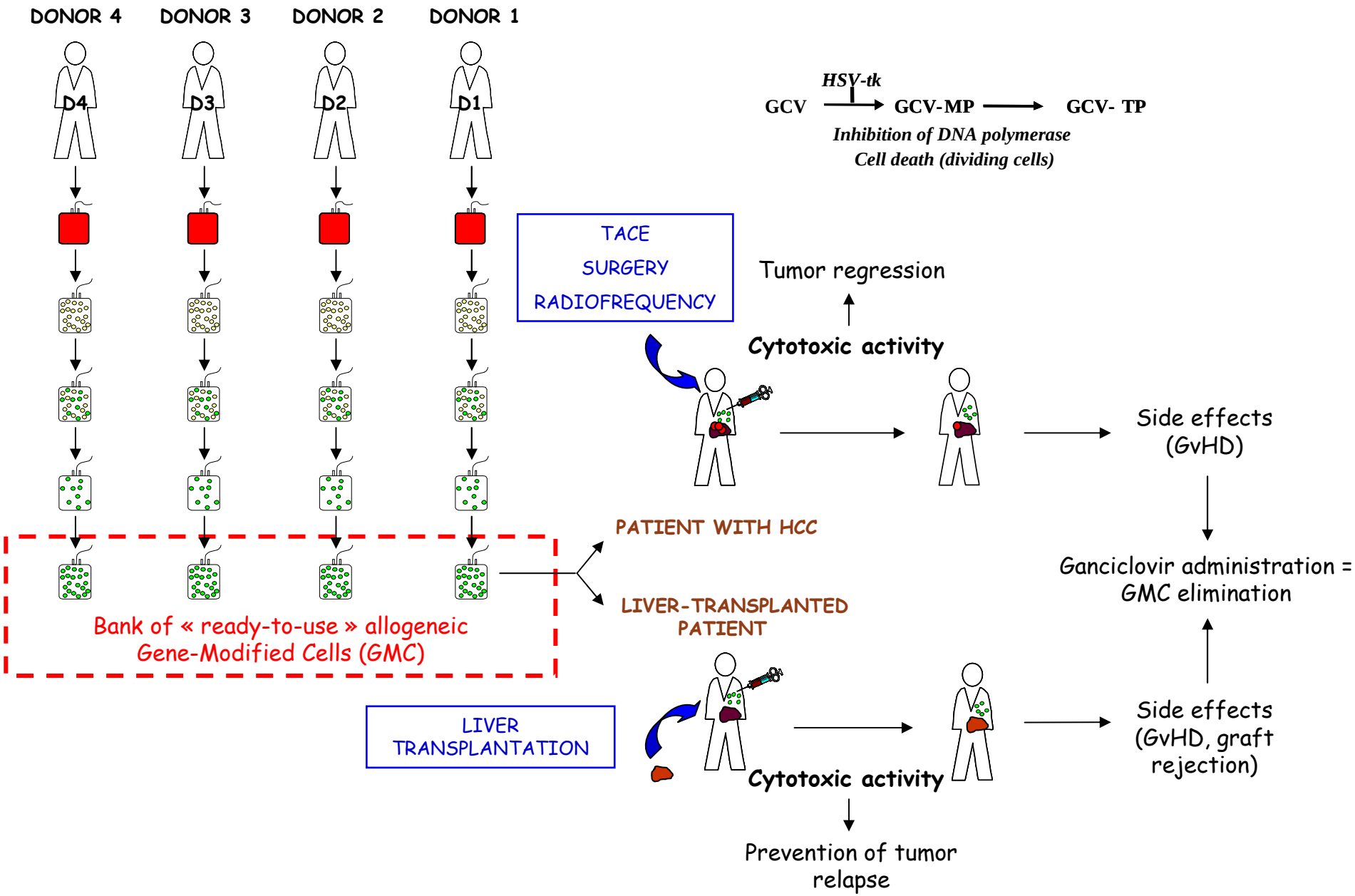
FUSION OF A SUICIDE GENE (HSV-tk) AND A SELECTION GENE (CD34) IN A RETROVIRAL VECTOR







Bank of "ready-for-use" allogenic gene-modified cells (GMC)



In vitro efficacy of GMC against HCC cell lines

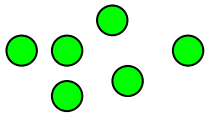
Cytotoxicity assay

Effector cells :

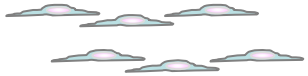
GMC

Co (non-transduced expanded cells)

PBMC

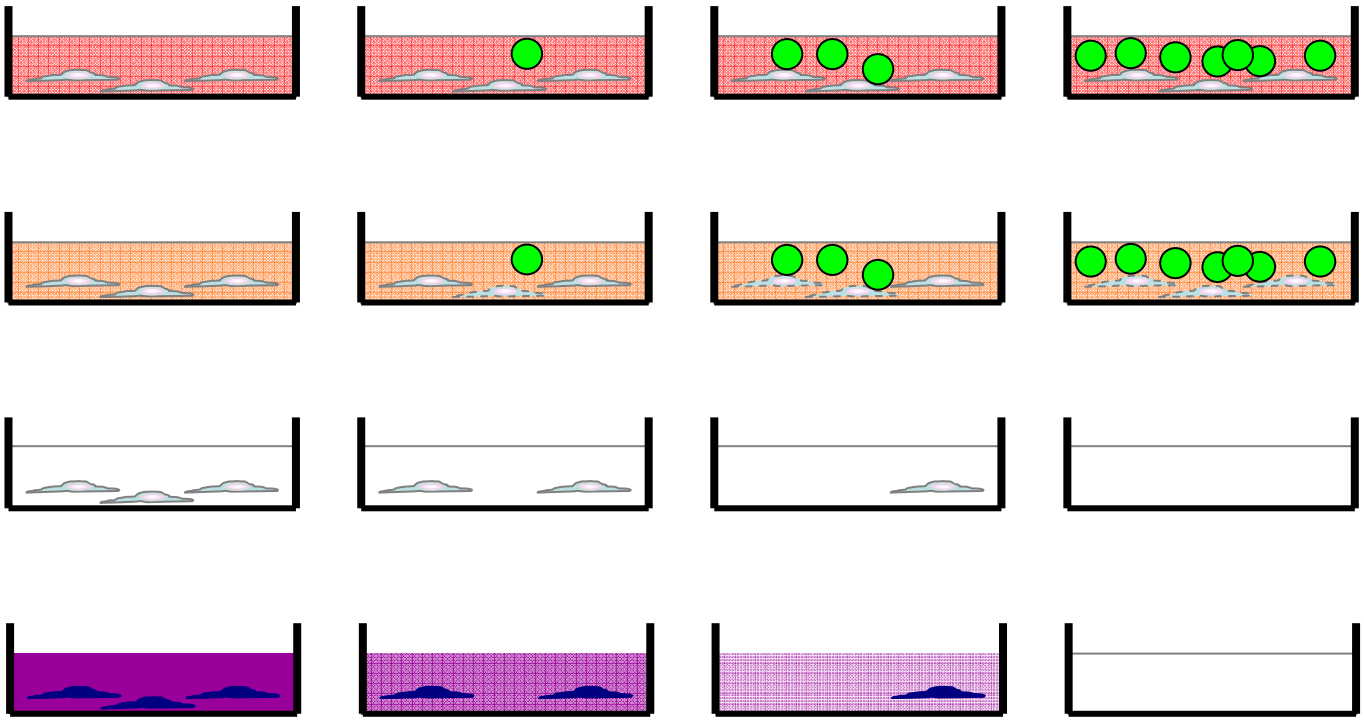


Target cells :

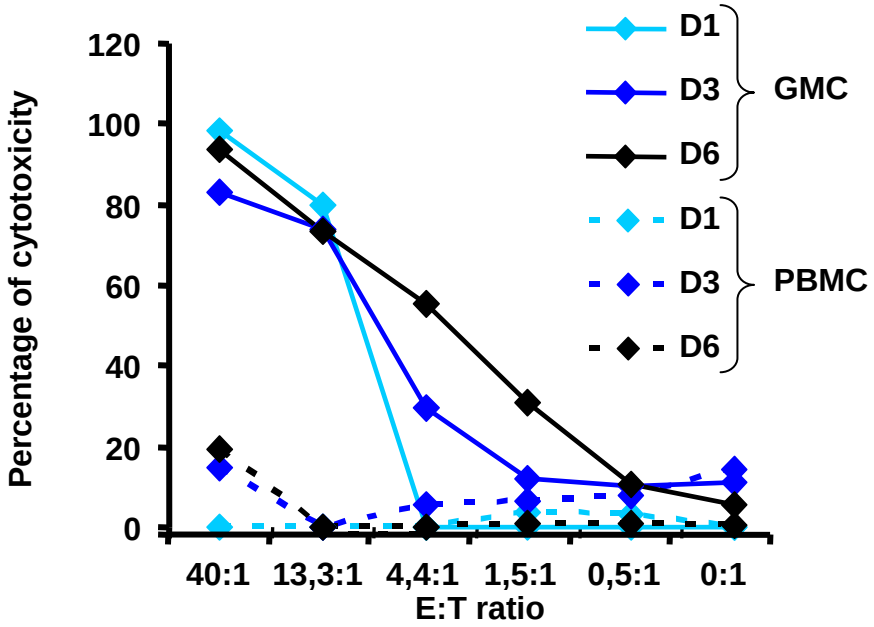


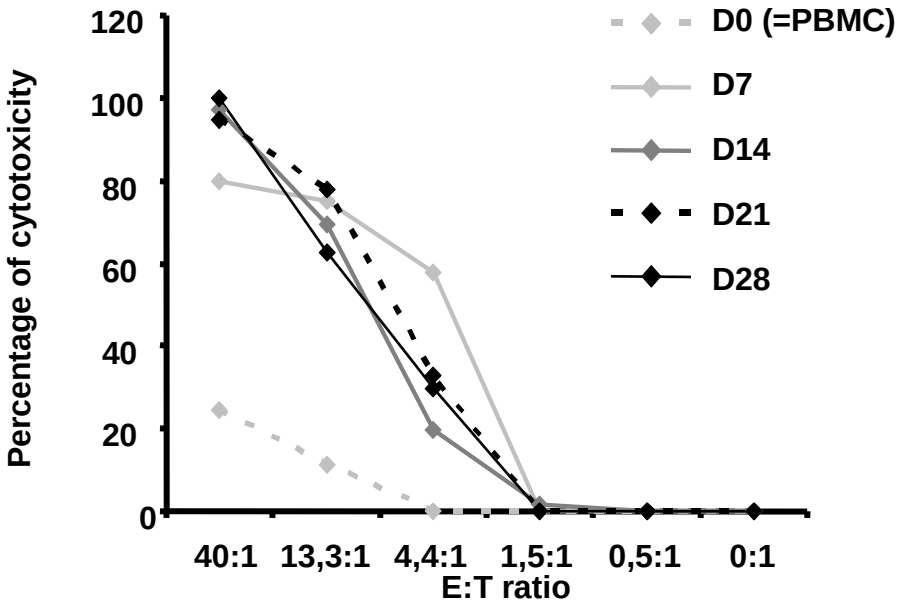
Huh7.5

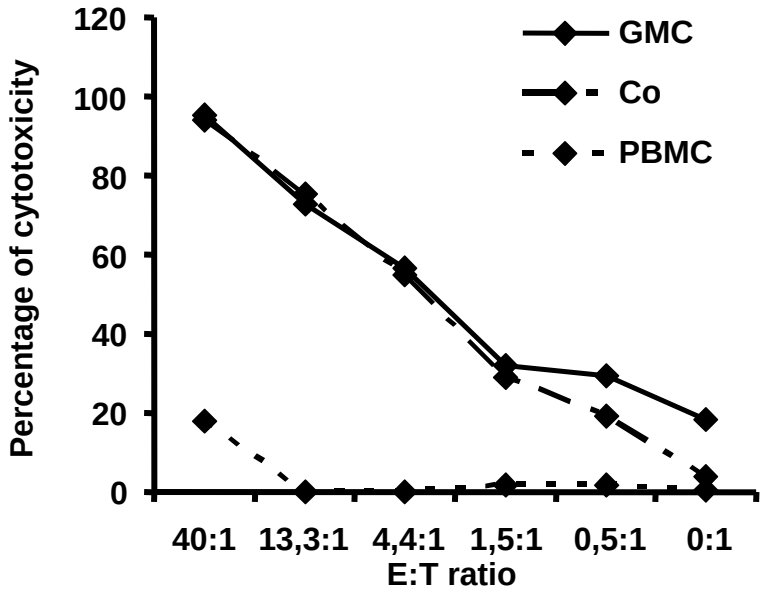
HeLa

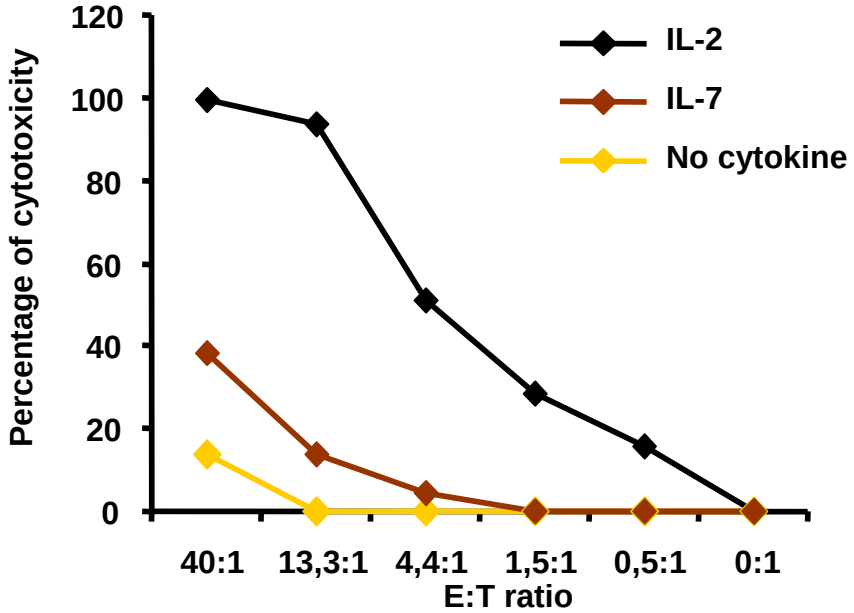


The GMC's cytotoxicity is rapidly acquired in culture and is IL-2-dependent

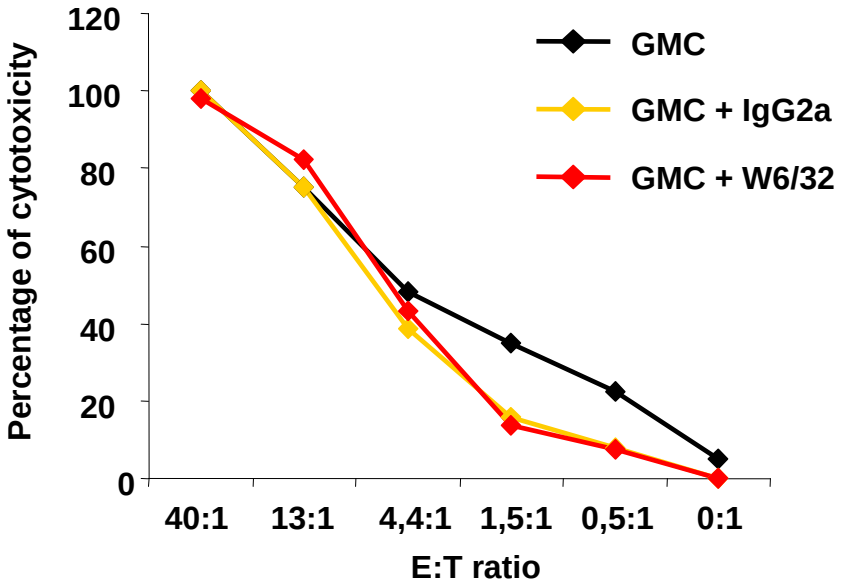


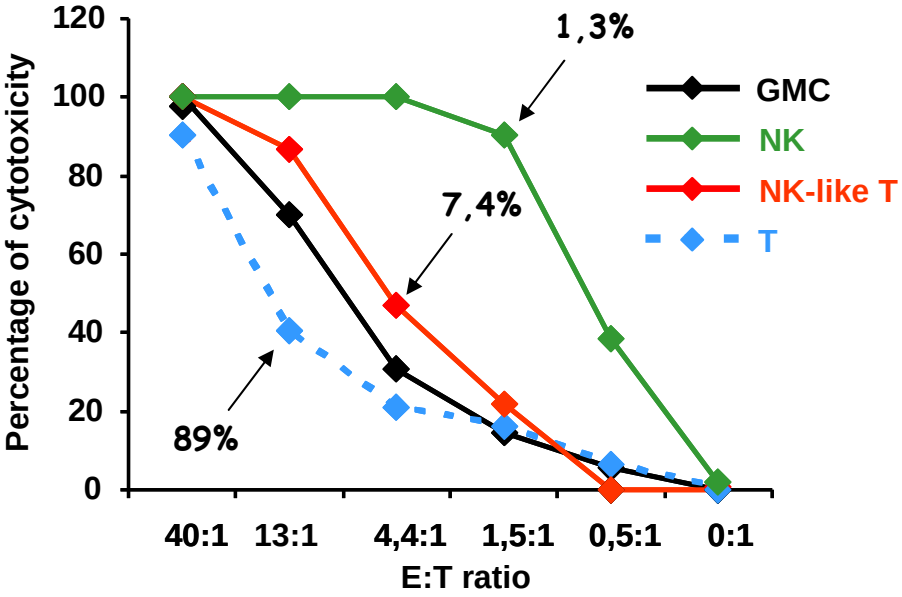


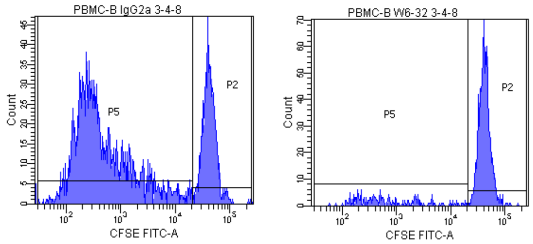




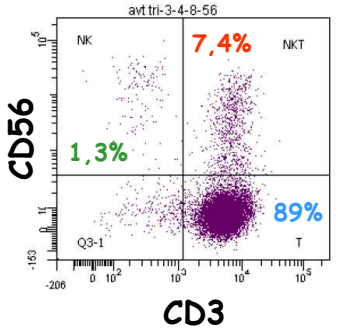
Characterization of effector cells







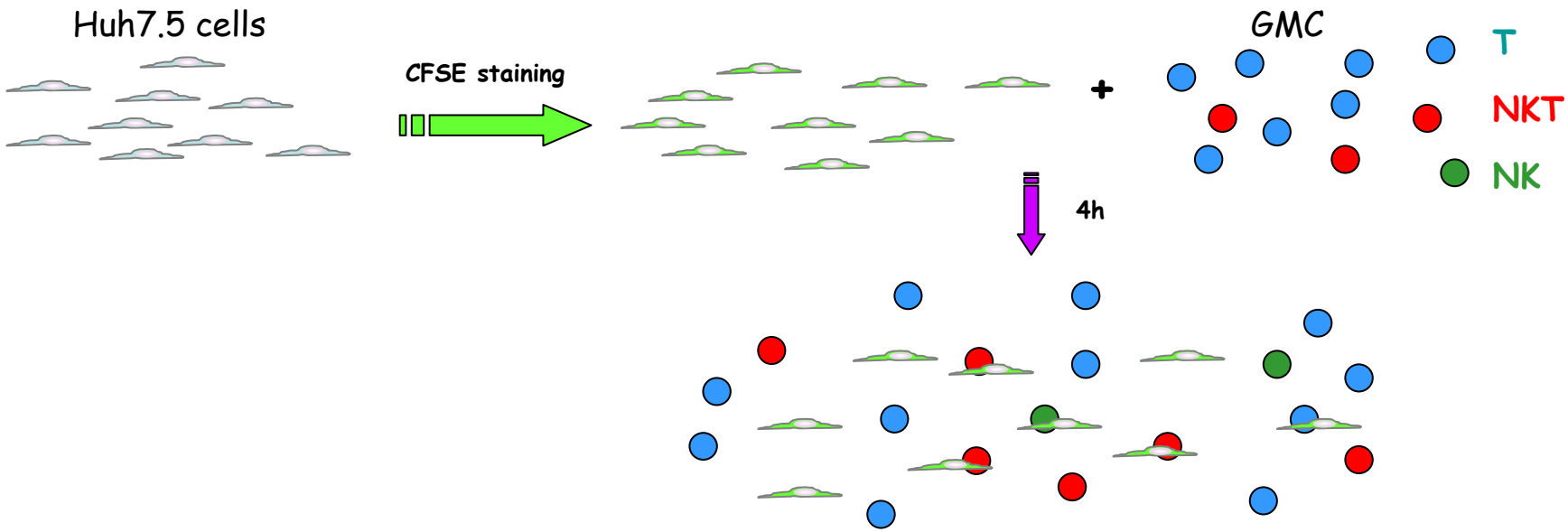
PBMC x B-EBV + IgG2a + W6/32

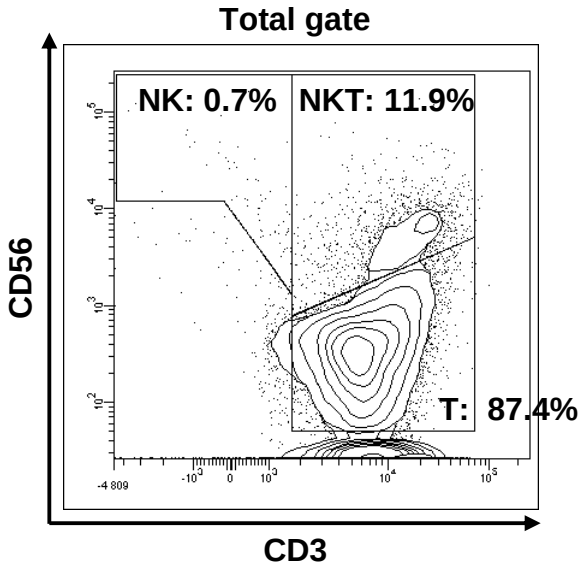


The cytotoxic activity is not MHC class I-restricted

NK cells are more cytotoxic than NK-like T cells or T cells

CD3+ CD56+ cells are the main effector cells





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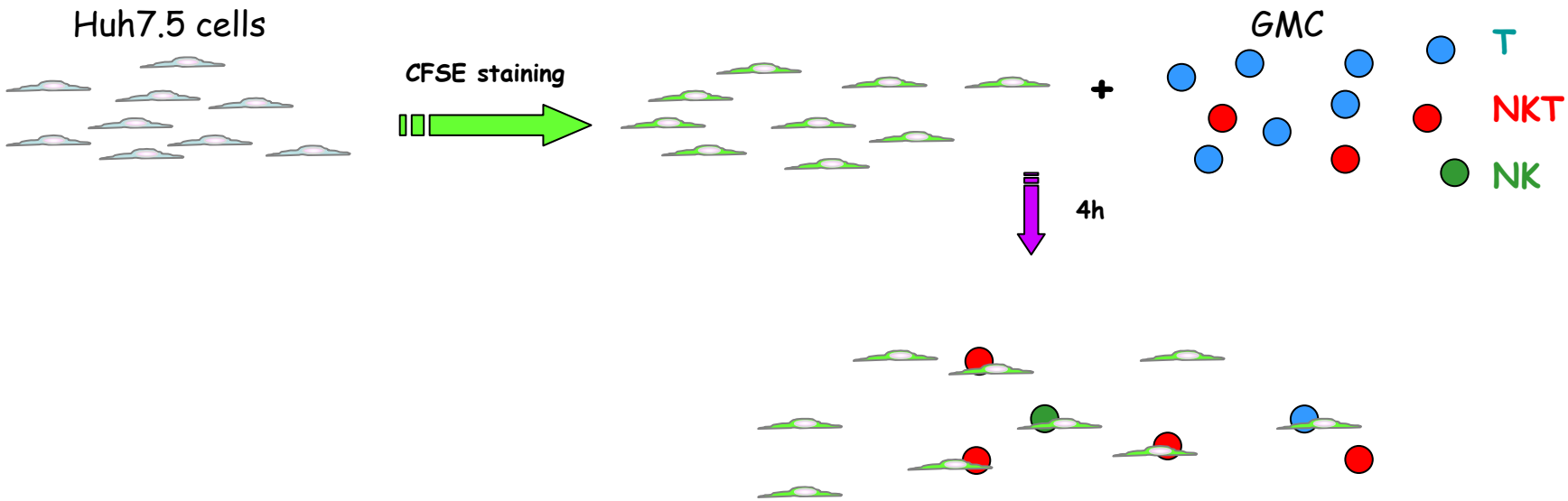
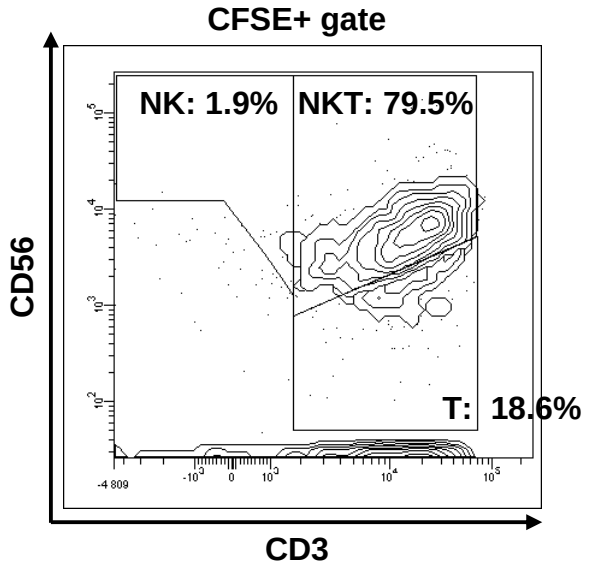




In vivo assessment of GMC-mediated anti-tumor activity

Orthotopic HCC humanized mouse model

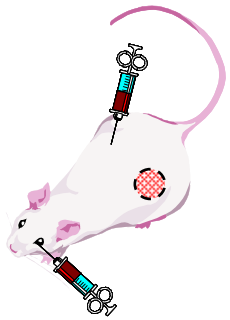
IL-2 (daily i.p. injection)

Huh7.5-luc

Intrasplenic injection

Huh7.5-luc homing to the liver

4 days



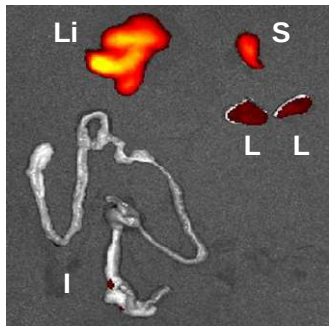
GMC

Immunodeficient mice

SCID/bg

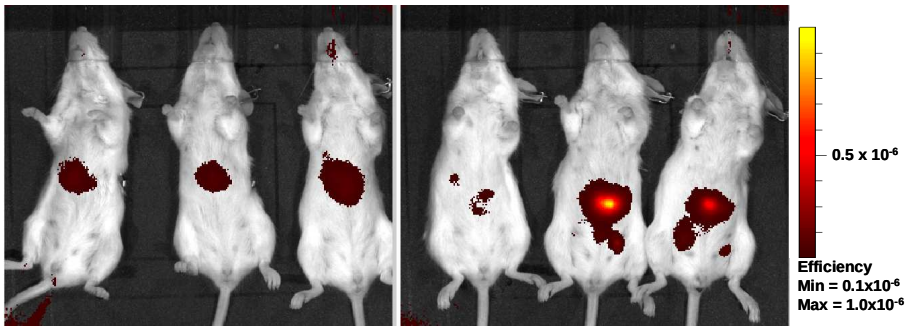
Monitoring of bioluminescence (IVIS 50, Xenogen Caliper)

D0, D3, D7

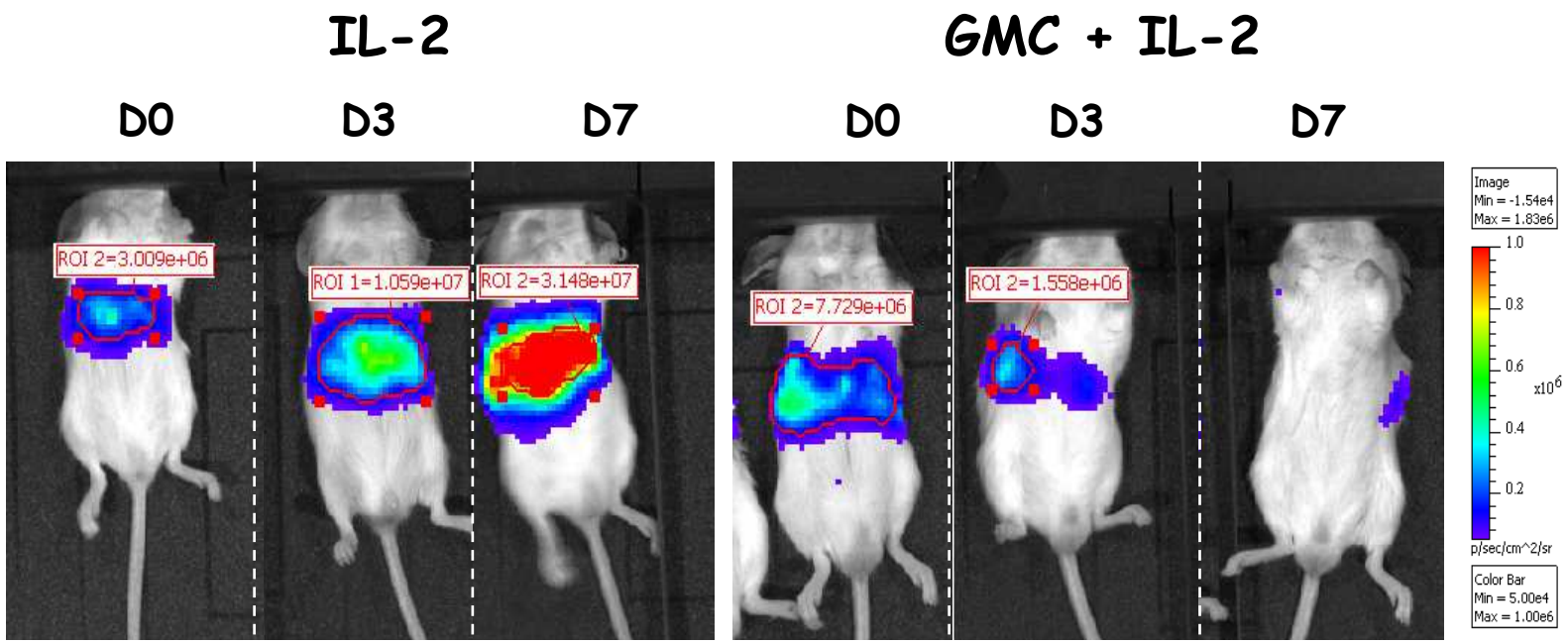


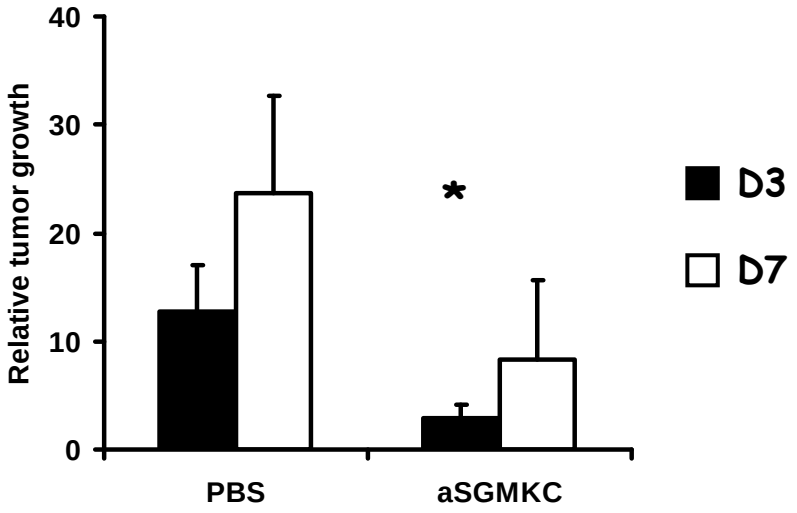
Li: Liver
S: Spleen
L: Lung
I: Intestine

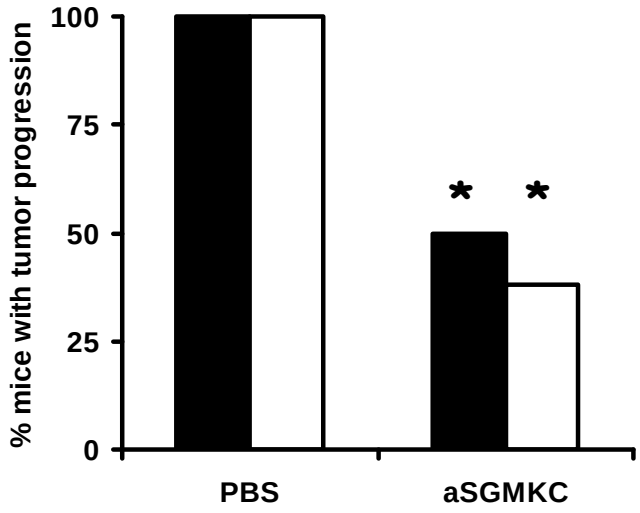
i.v. i.p.



GMC are highly cytotoxic against Huh7.5 cells *in vivo* (orthotopic model)





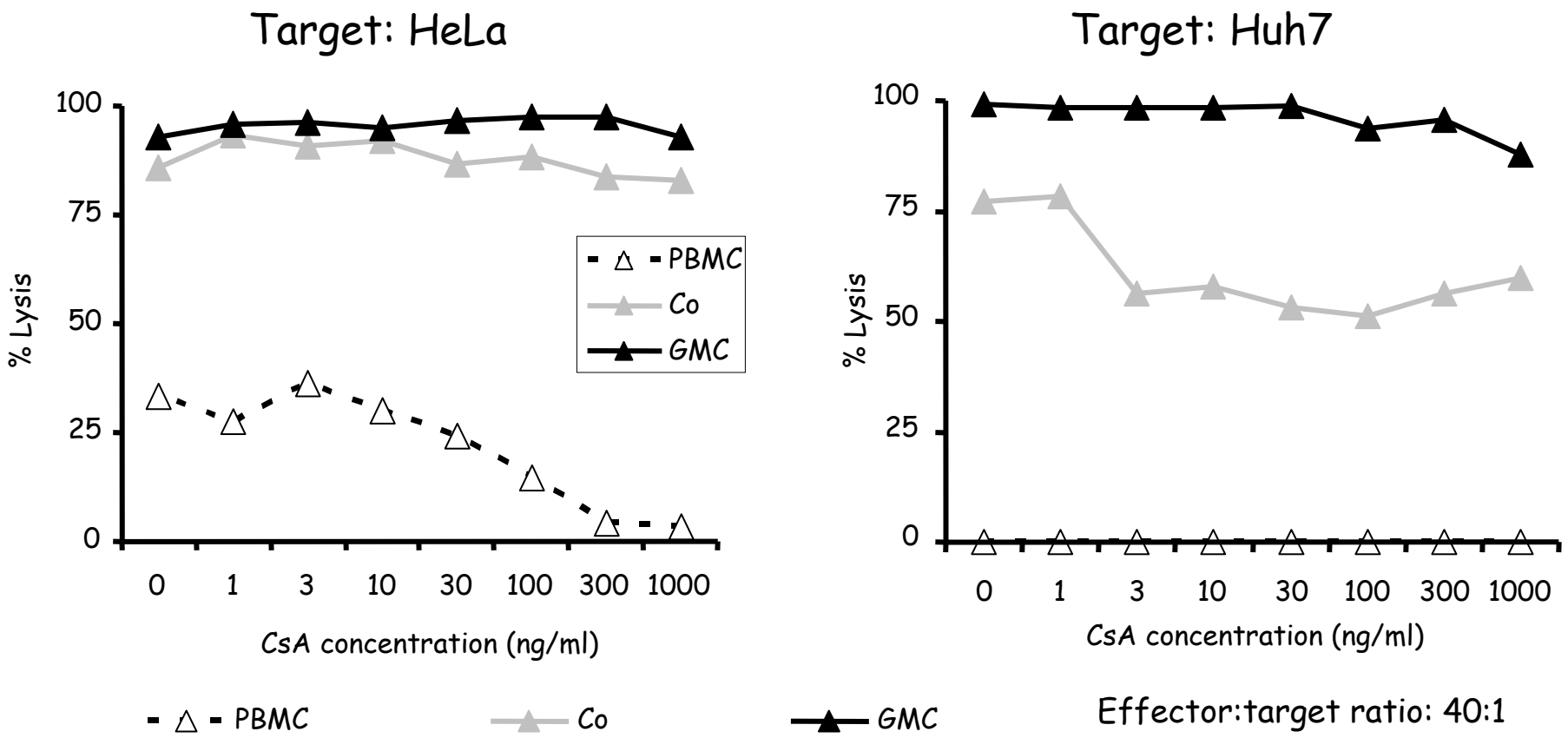


* p<0.05 vs PBS

Prevention of the allo-immunization against GMC

Cyclosporin A effect on cytotoxic activity of PBMC, Co and GMC

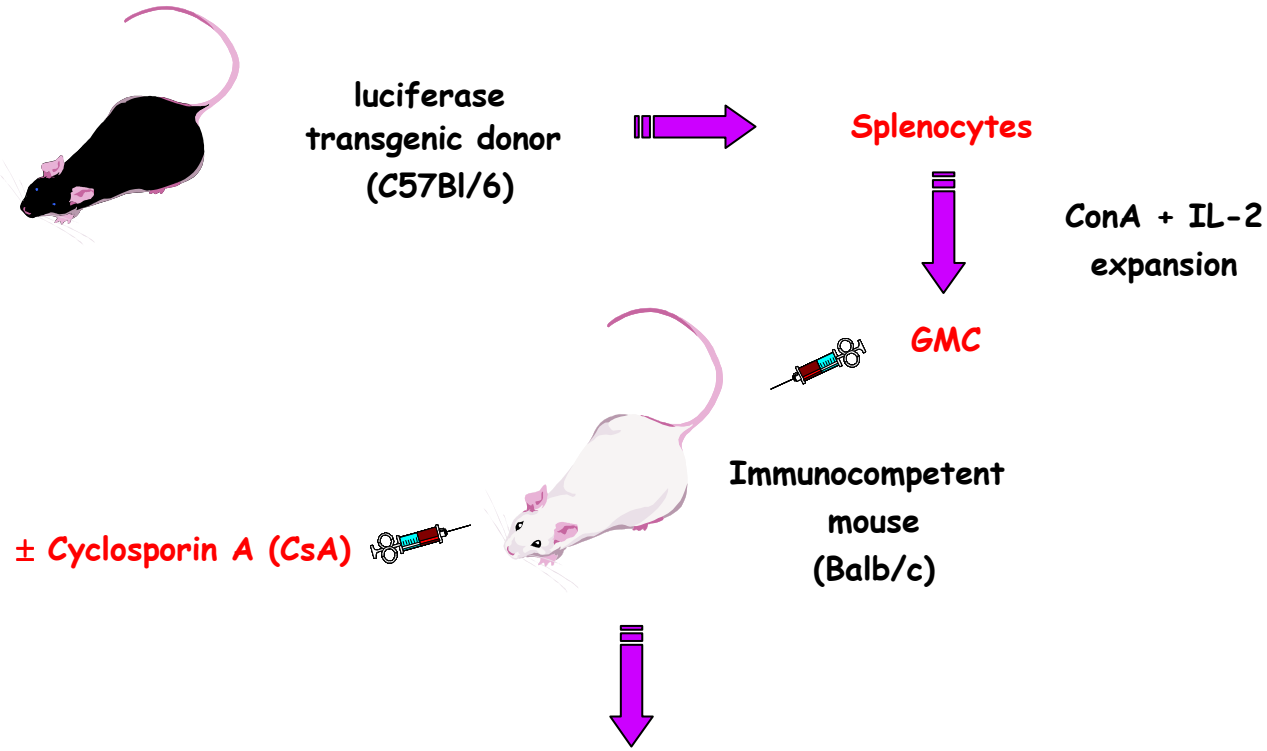
- Patients are immunocompetent : risk of GMC rejection by the immune system of the recipient ⟶ use of Cyclosporin A (CsA) to prevent this rejection



➡ GMC, as Co cells, are resistant to Cyclosporin A

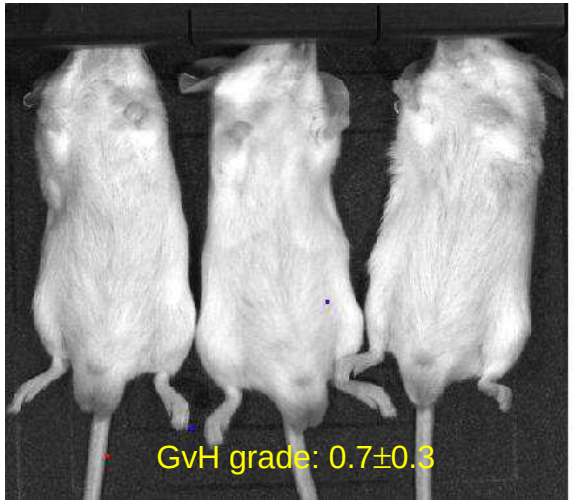
Immunocompetent mouse model

⇨ Immune cells of the recipient (resting T cells) could be inhibited by CsA, while GMC (cultured cells) are resistant to CsA



CsA prevents GMC rejection *in vivo*

GMC-luc + IL-2

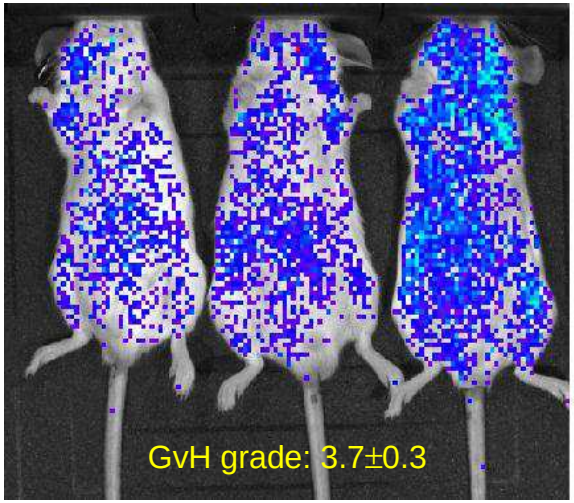


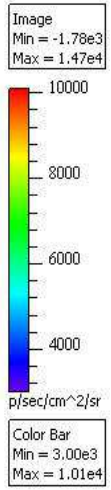
The immune system of the recipient is efficient

GMC are rejected

No GvH

GMC-luc + IL-2 + CsA





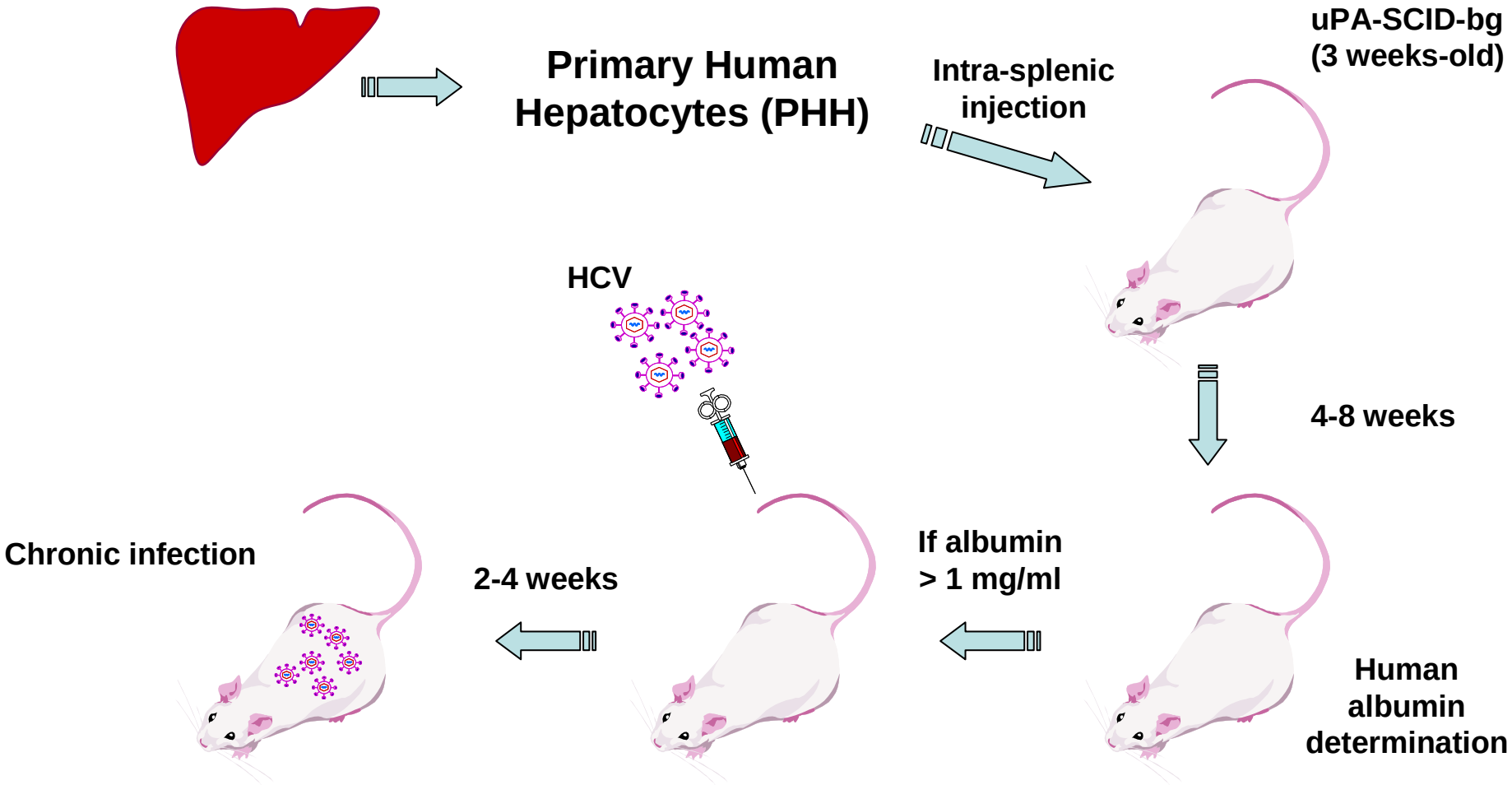
The immune system of the recipient is inhibited by CsA

GMC are not rejected and not inhibited by CsA

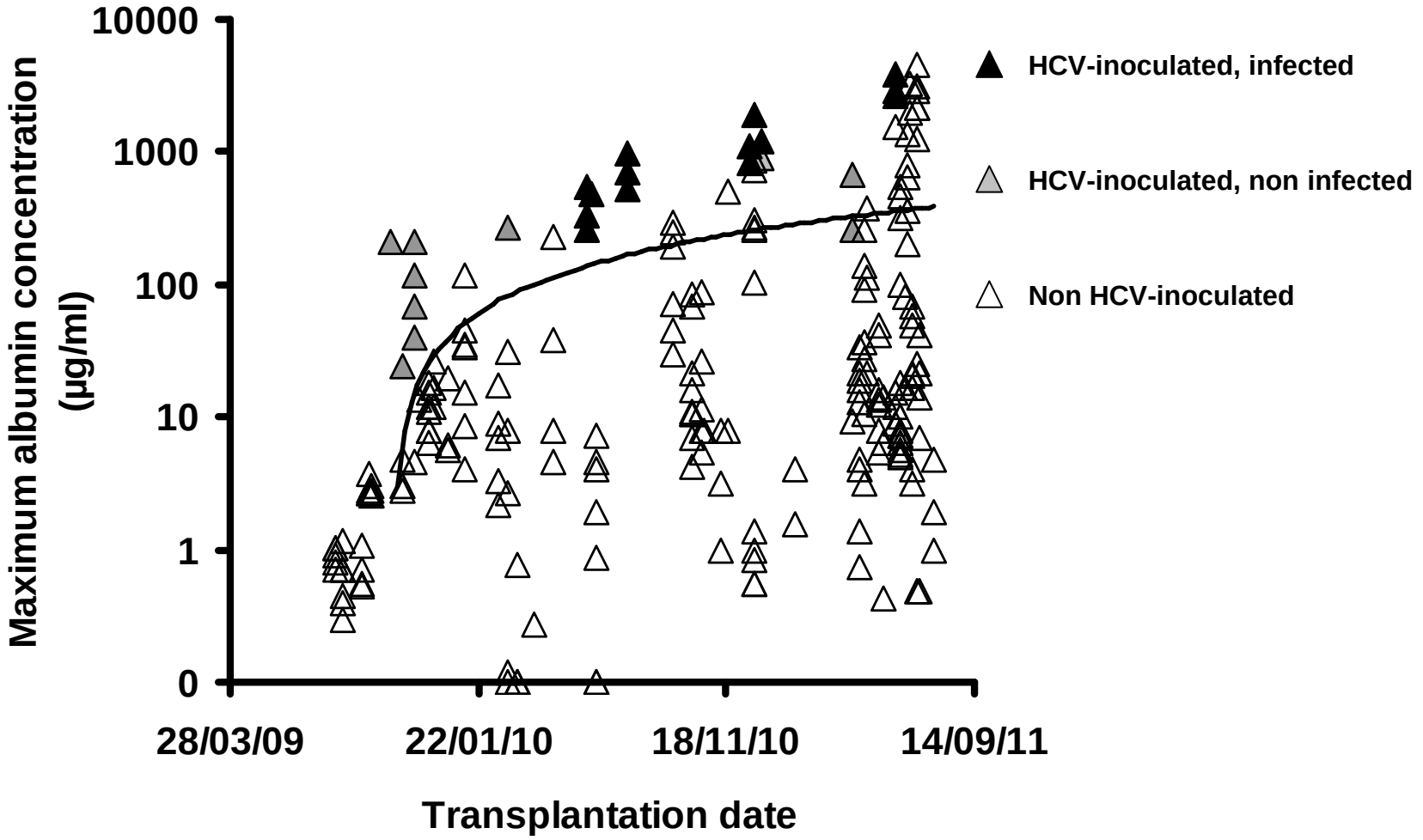
GvH

Xenogenic transplantation of primary human hepatocytes: a possible model for HCC ?

The Human Liver-Chimeric (HLC) uPA/SCID mouse: a model of *in vivo* HCV infection



PHH transplantation: the learning curve



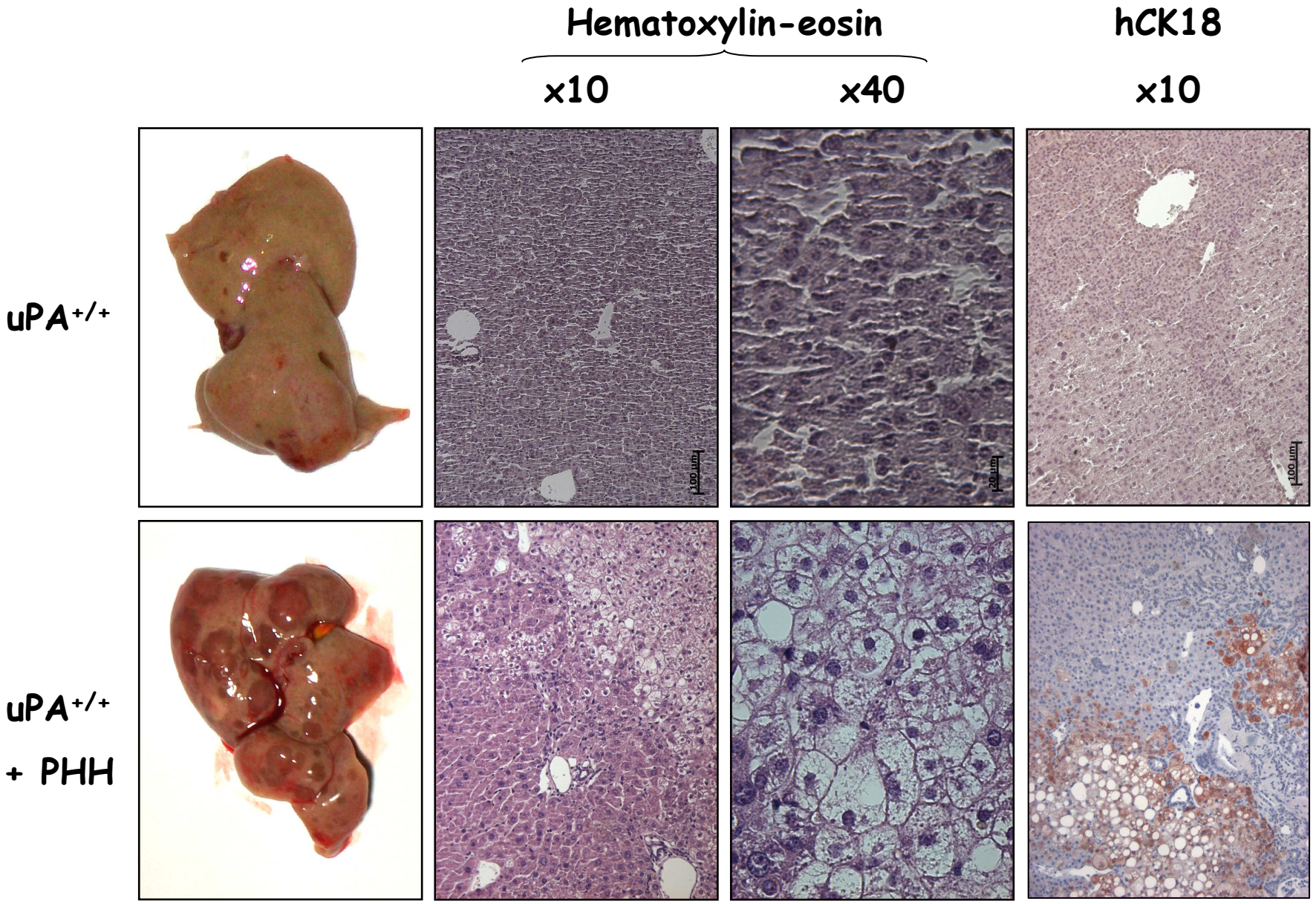
42 transplant experiments

17 donors

> 400 transplanted mice

249 mice analyzed

Histology of PHH-transplanted livers



Possible use of humanized liver-chimeric mice

Pharmacokinetic experiments

Hepatotropic viruses infection (HBV, HCV)

Human liver-specific graft-versus-host disease model ?

→ soluble human Cytokeratin-18 = marker of PHH apoptosis

→ histology

Hepatocellular carcinoma model ?

→ conditional induction of HCC by gene transfer into PHH ?

A Humanized Mouse Model to Study Hepatitis C Virus Infection, Immune Response, and Liver Disease

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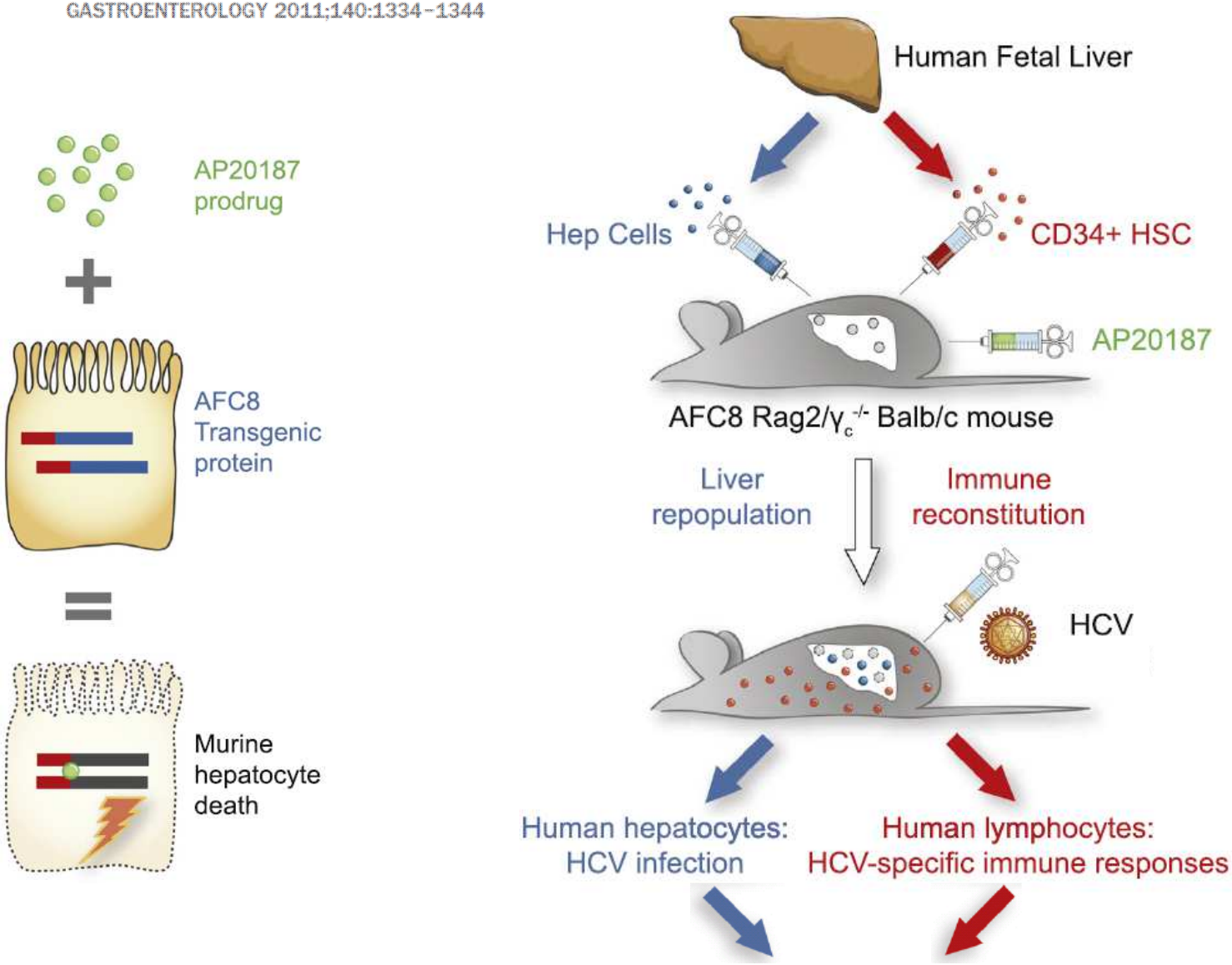
GASTROENTEROLOGY 2011;140:1334–1344



Conclusion / Perspectives

Conclusions

GMC cytotoxicity is:

- **rapid and highly efficient *in vitro* and *in vivo***
- **not altered by retroviral transduction**
- **non-MHC-restricted**
- **mainly mediated by NK-like T (CD3+ CD56+) cells**
- **resistant to calcineurin inhibitors**

  → may allow to prevent the allo-immunization against GMC

Bank of allogeneic GMC:

- **"ready for use" effector cells**
- **clinical batch available for several patients: no loss !**
- **conditional elimination of effector cells**

Perspectives

Prevention of allo-immunization against GMC

- **calcineurin inhibitors**
- **FTY720** *(Marcus et al., Blood 2011)*
- **fludarabin and/or cyclophosphamide-based lympho-ablative conditioning regimen** *(Ghiringhelli et al., Cancer Immunol. Immunother. 2007; Slavin et al., Cancer Immunol. Immunother. 2010)*

Human liver-chimeric mice:

- **GMC toxicity against normal PHH ?**
- **control of GMC's toxicity by the suicide gene ?**
- **model of primary HCC ?**
- **immuno-reconstituted, liver-chimeric mice: primary HCC + fibrosis ?**

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