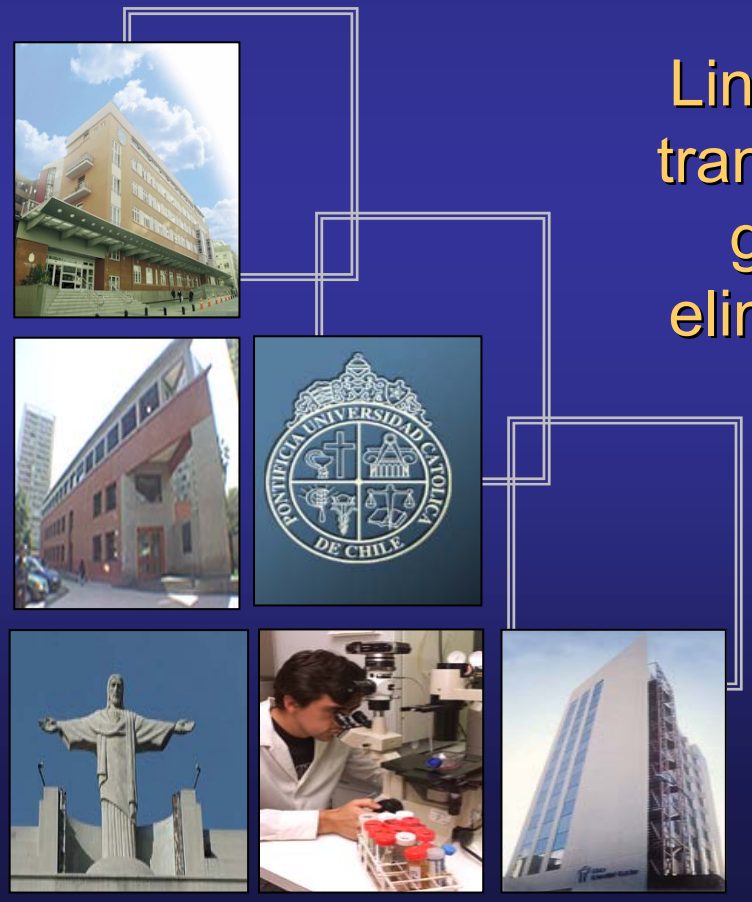
XV Congreso Sociedad Chilena de Hematologia

Mantagua, 26 de Octubre 2006



Linfocitos T humanos activados *ex vivo* y transducidos con el gen suicida ΔCD34-tk generan GVHD y son eficientemente eliminados *in vivo* en un modelo de raton

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PROGRAMA DE CANCER

PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

INTRODUCCION

- El GVHD es la mayor causa de morbi-mortalidad en trasplante alogeneico de precursores hematopoyeticos.
- Modelos animales para el GVHD humano ayudarian en el desarrollo de mejores tratamientos, estos no existen.
- La manipulacion *ex vivo* de linfocitos disminuye su funcionalidad.

OBJETIVOS

1. Desarrollar un modelo de GVHD humano en ratones inmunosuprimidos

2. Transducir linfocitos T humanos (huT) con el gen suicida CD34/tk preservando su funcion (huT $^{CD34/tk}$).

3. Demostrar que es posible eliminar eficientemente in vivo los huT $^{CD34/tk}$ para prevenir GVHD.

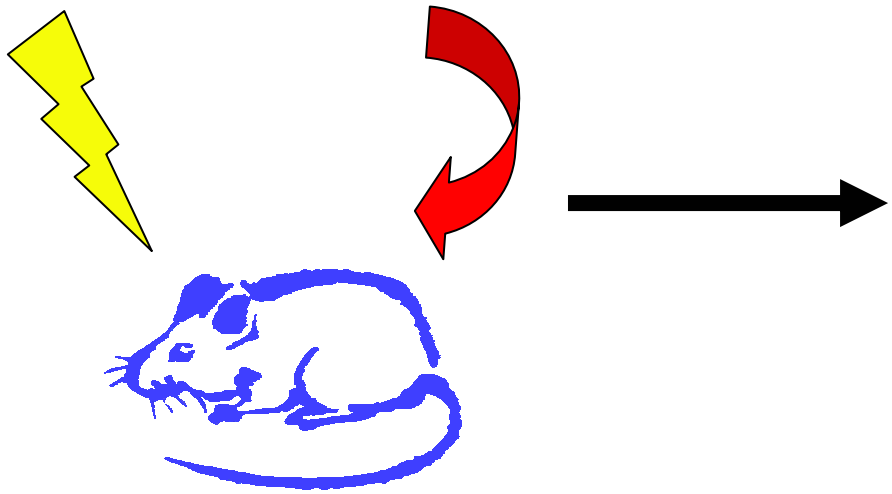
Ratones NOD SCID β2m $^{-/-}$

- Cruza entre ratones:
  NOD SCID x β2-microglobulina $^{-/-}$
- No expresan MHC clase I
- Inmunidad reducida por perdida de linfocitos B, T y celulas NK

DISENO EXPERIMIENTAL

250 cGy
(dia 0,-1)

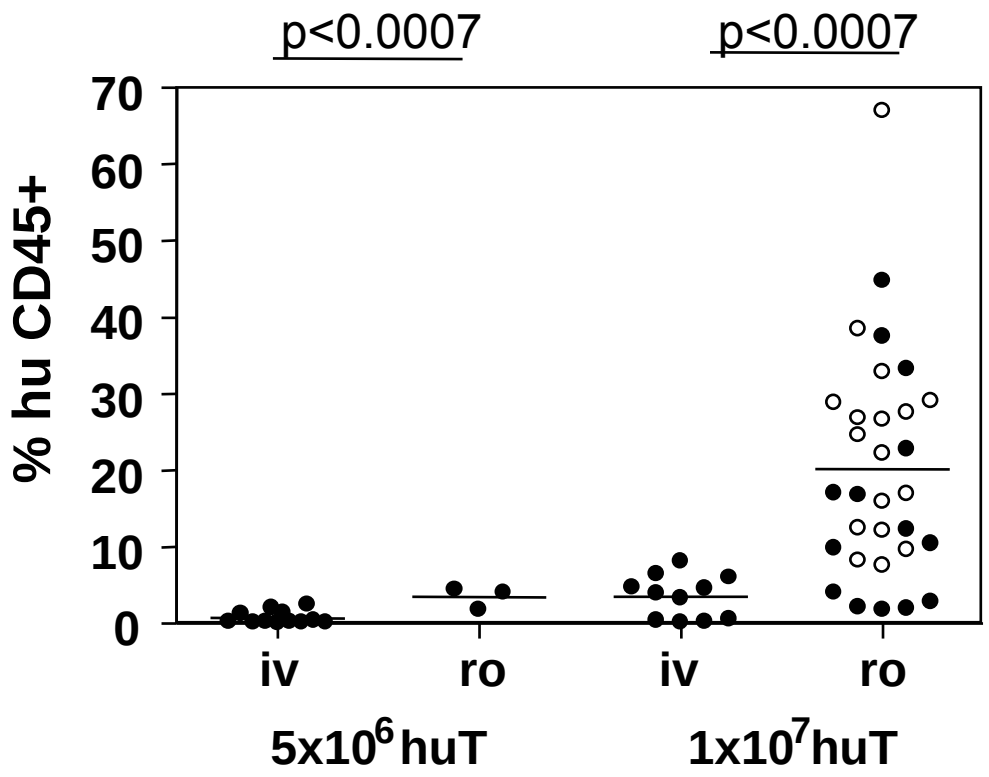
huT
iv o ro
(dia 0)



- Signos de GVHD
  - ↓ actividad
  - jorobados
  - Pelo desordenado
  - ↓ peso > 20%
- Celldyn semanal
- Prendimiento (FACS)
- Citokinas humanas en suero
- Patologia

NOD SCIDβ2m null

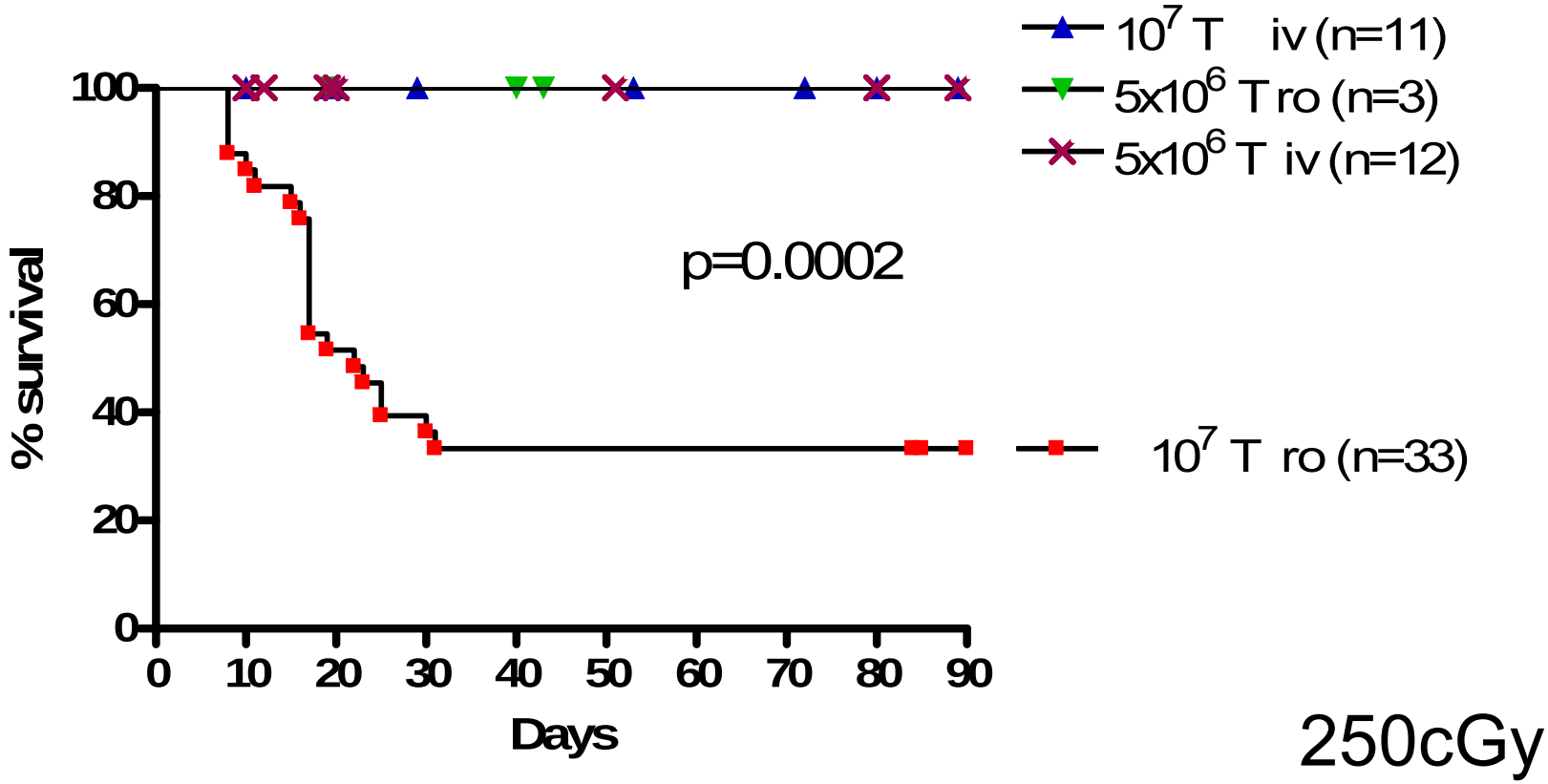
EXPANSION DE huT EN SANGRE DE RATONES



- ◦ GvHD
- • No GvHD

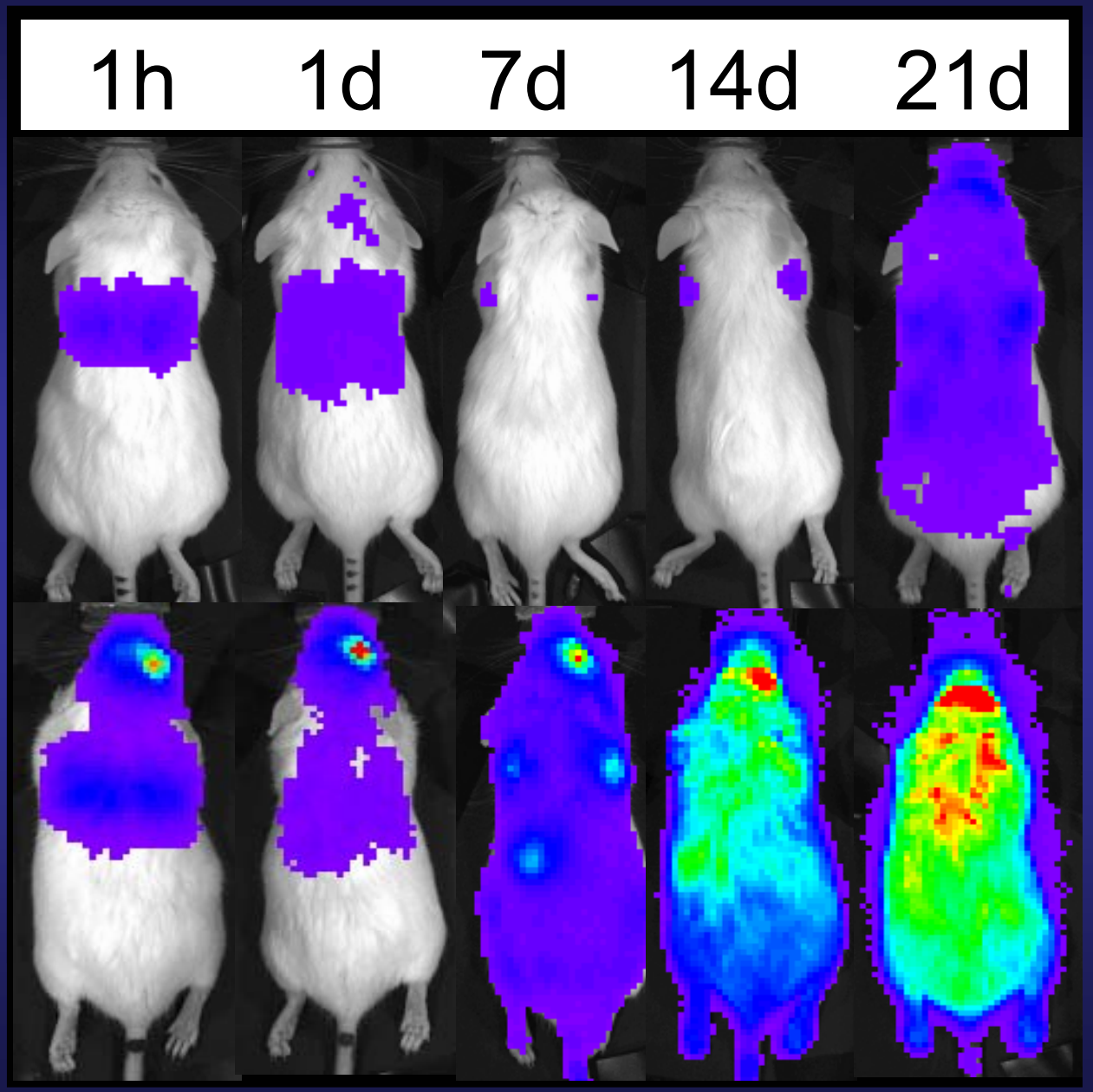
iv: inyeccion x vena de la cola
ro: inyeccion retro-orbital

Kaplan Meier
analisis de sobrevida



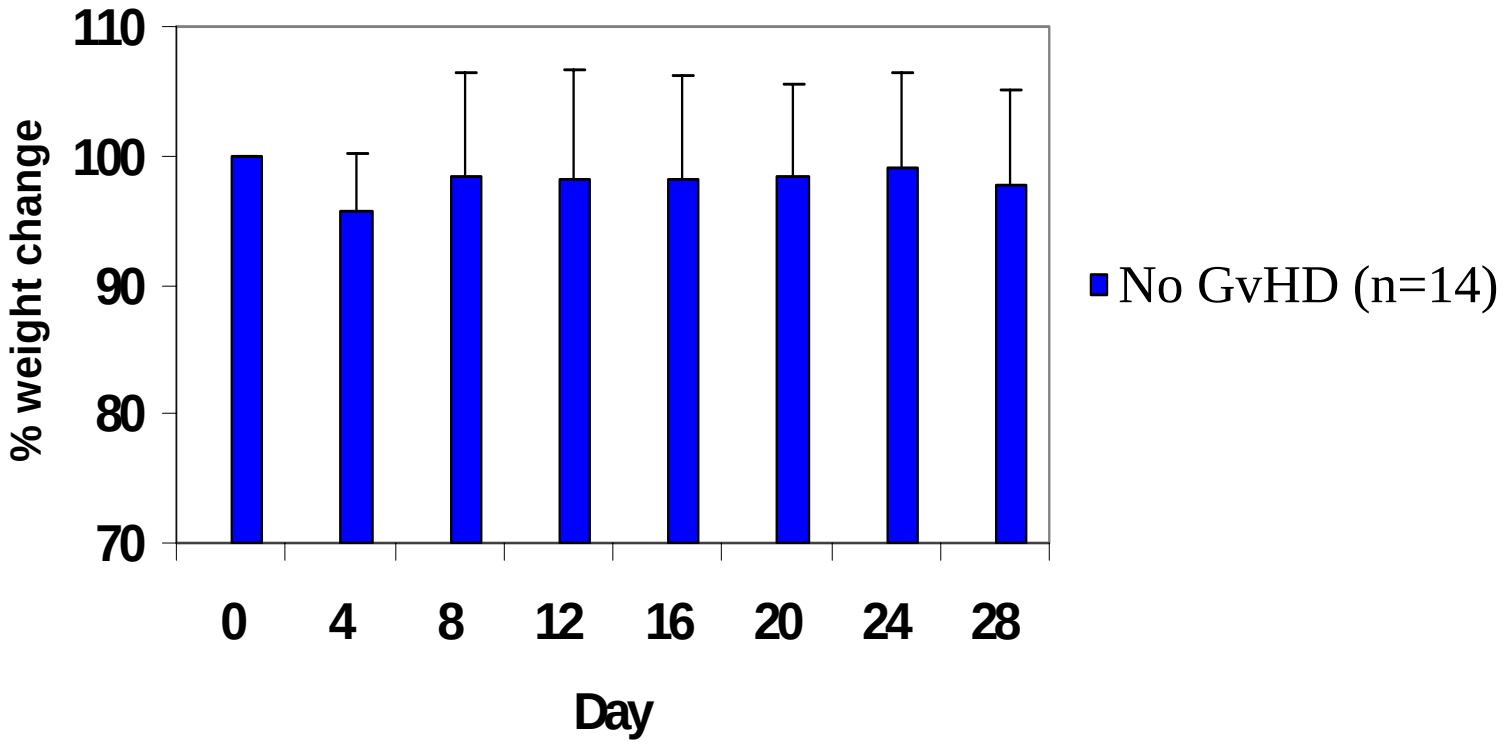
Inyeccion vena de la cola

Inyeccion retro-orbital



Cambios en el peso

(10^7 huT ro - 250cGy)



Cambios en el peso

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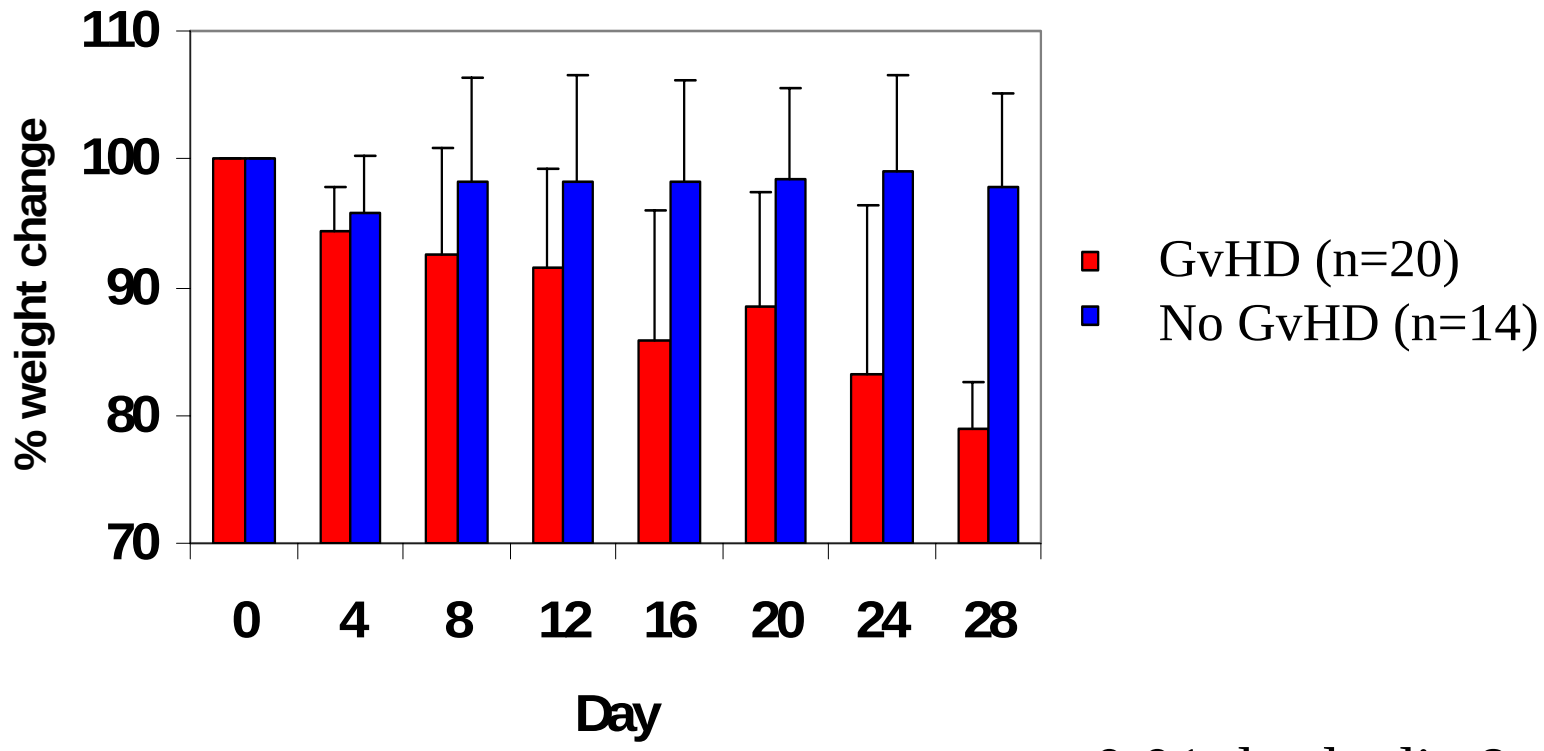
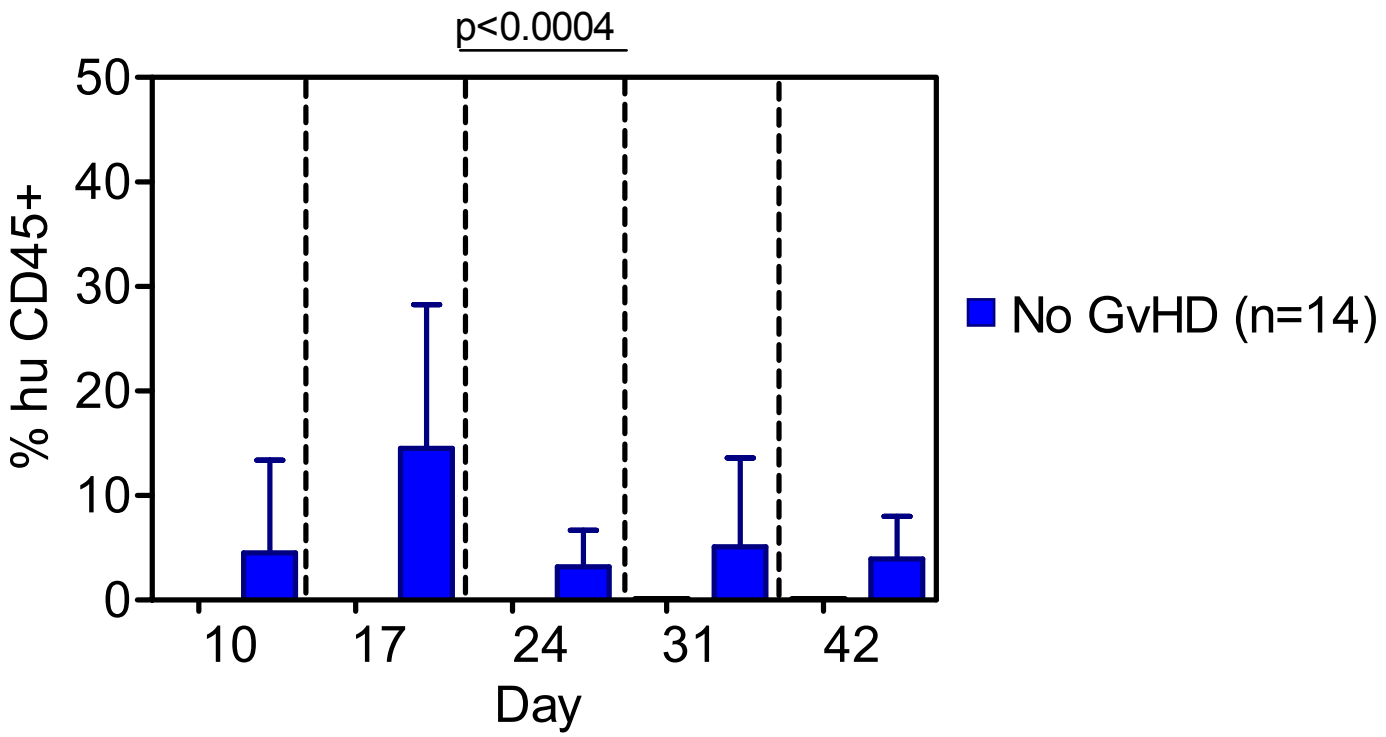


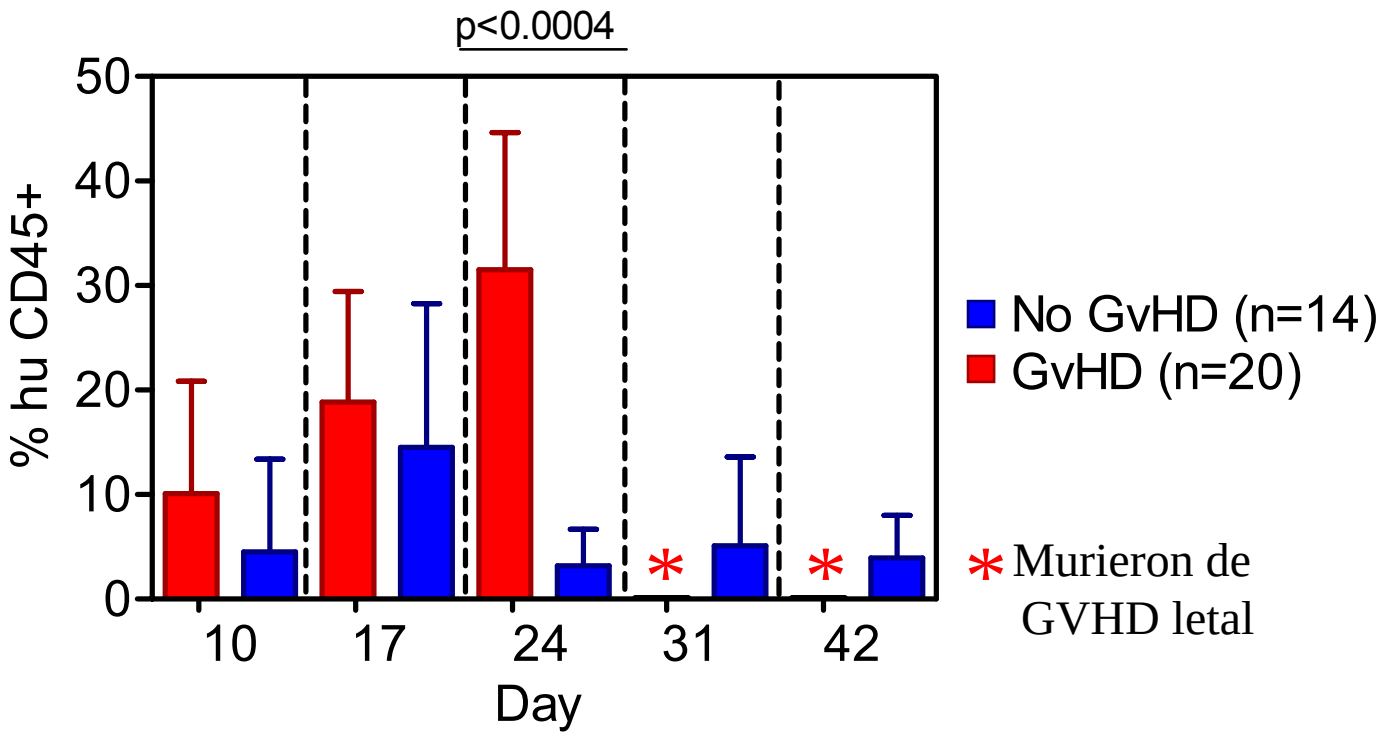
$p < 0.01$ desde dia 8

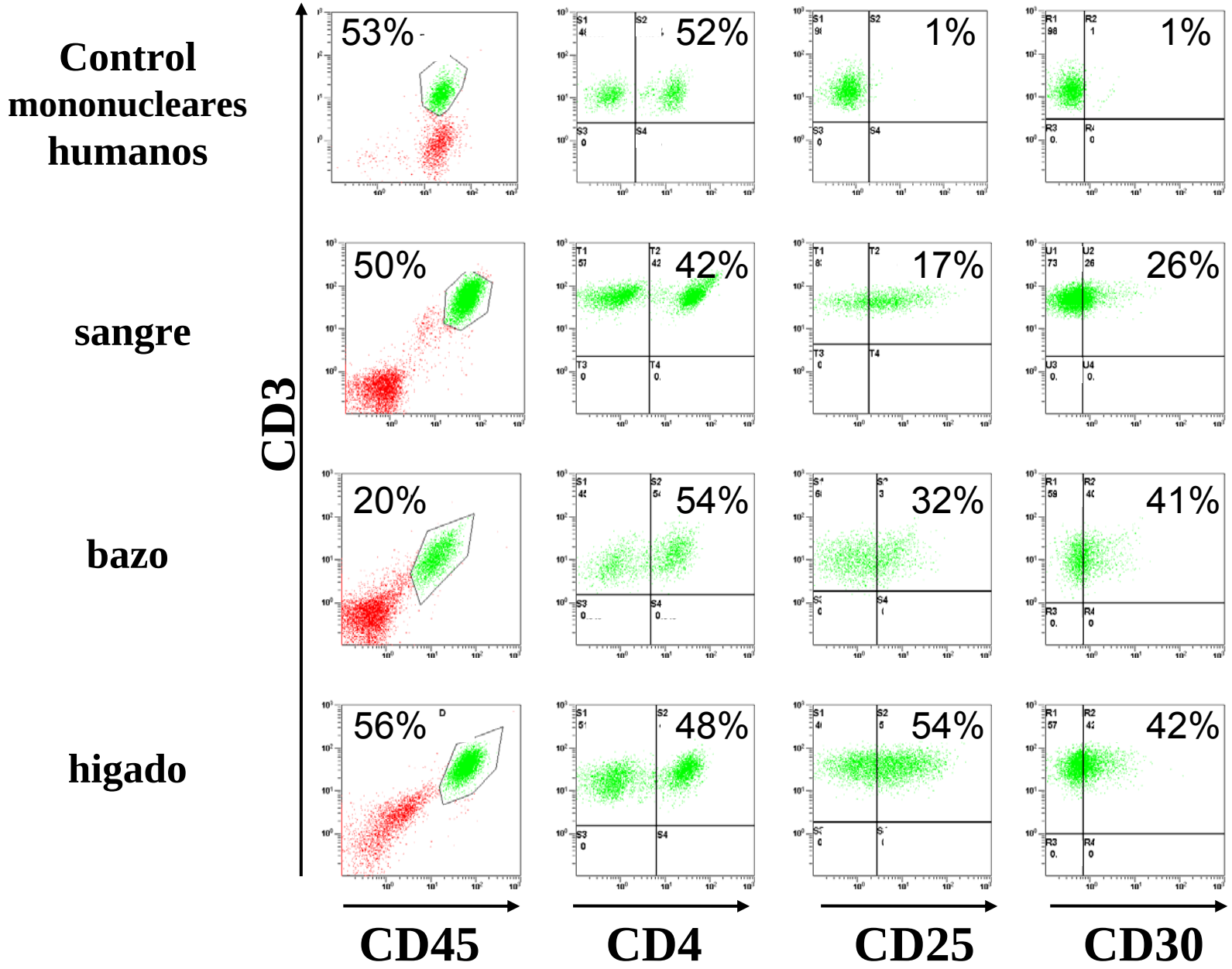
Expansion de huT en sangre
(10^7 huT ro - 250cGy)



Expansion de huT en sangre

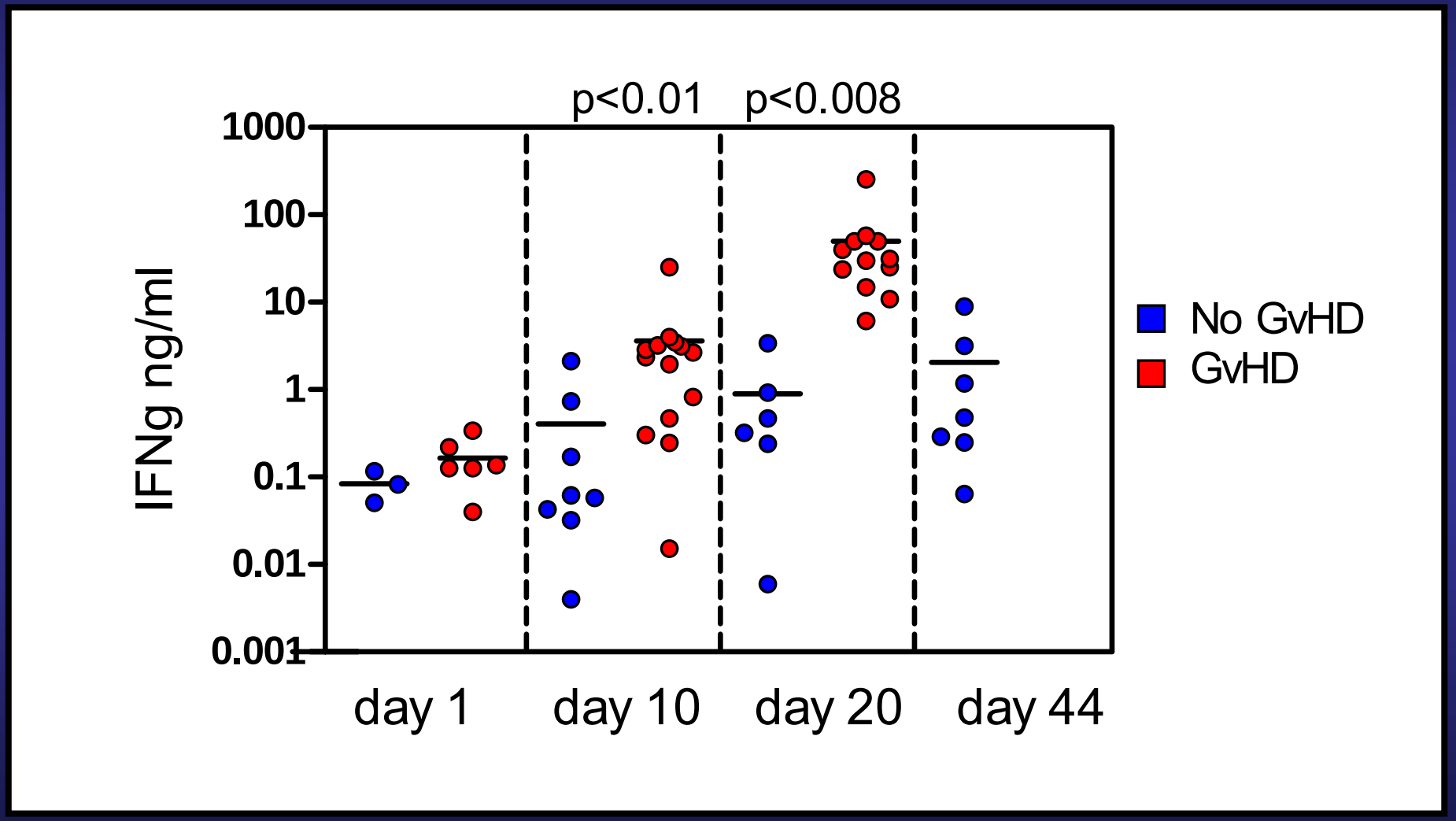
(10^7 huT ro - 250cGy)



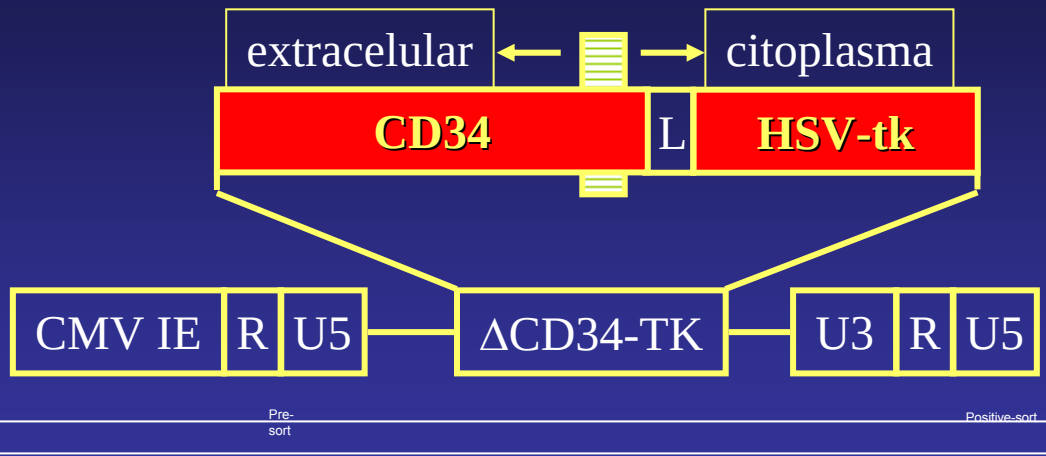

Control mononucleares humanos
sangre
bazo
higado
CD3
53%
52%
1%
1%
50%
42%
17%
26%
20%
54%
32%
41%
56%
48%
54%
42%
CD45
CD4
CD25
CD30

Niveles sericos de IFNγ

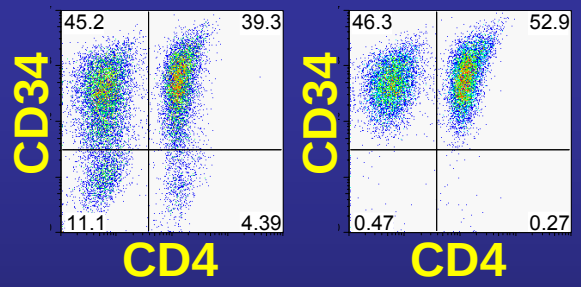
(10^7 huT ro - 250cGy)



Gen suicida



Protocolo de activacion y transduccion



huPBMC
anti-CD3/CD28 beads
IL-2

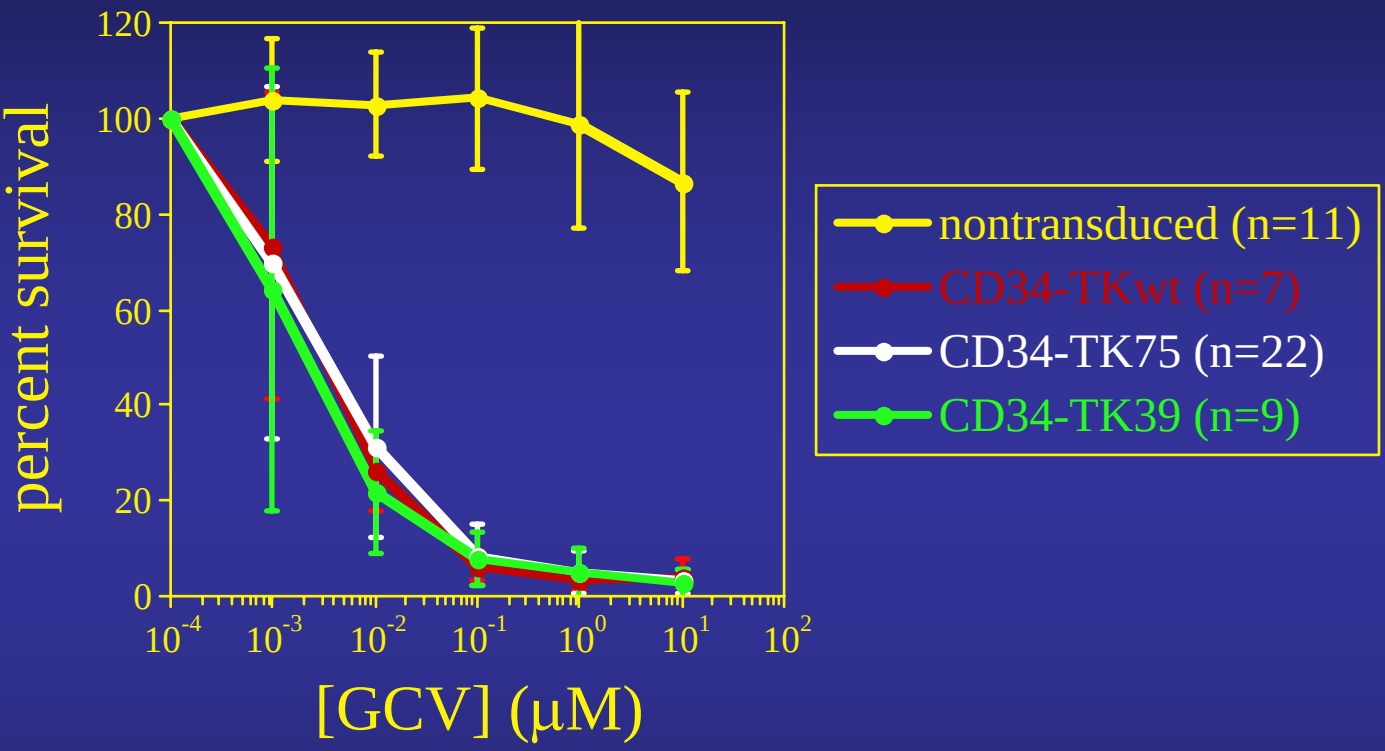
VSV-G pseudotyped
MLV-based retrovirus
CD34-tk

CD34 MACS
99% pureza

Activacion | Infeccion | Expansion | Aislamiento

0 48 56 96 hours

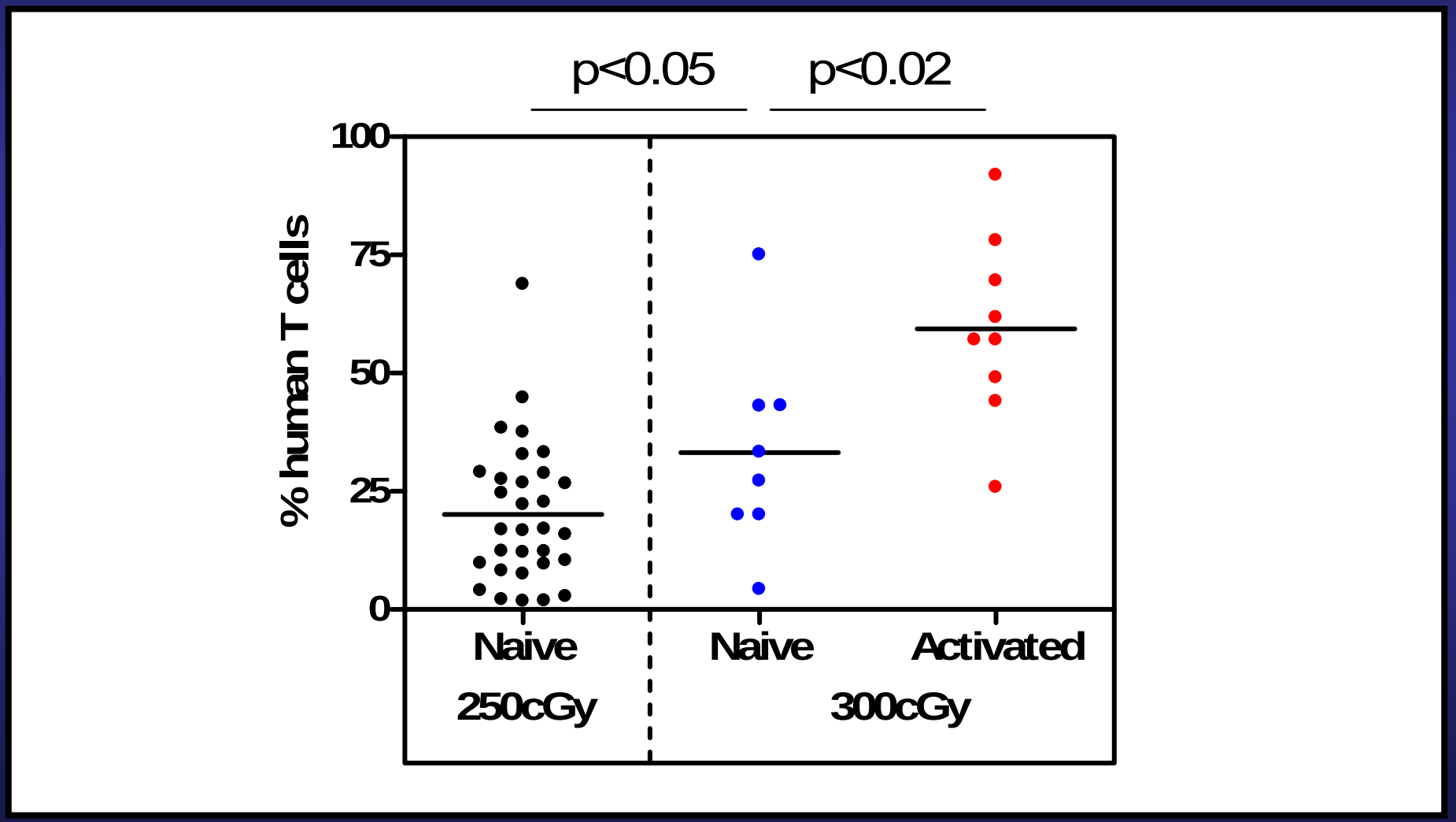
Sensibilidad de huT al Ganciclovir



	IC_{50} (μM)
CD34-TKwt	0.0031
CD34-TK75	0.0033
CD34-TK39	0.0022

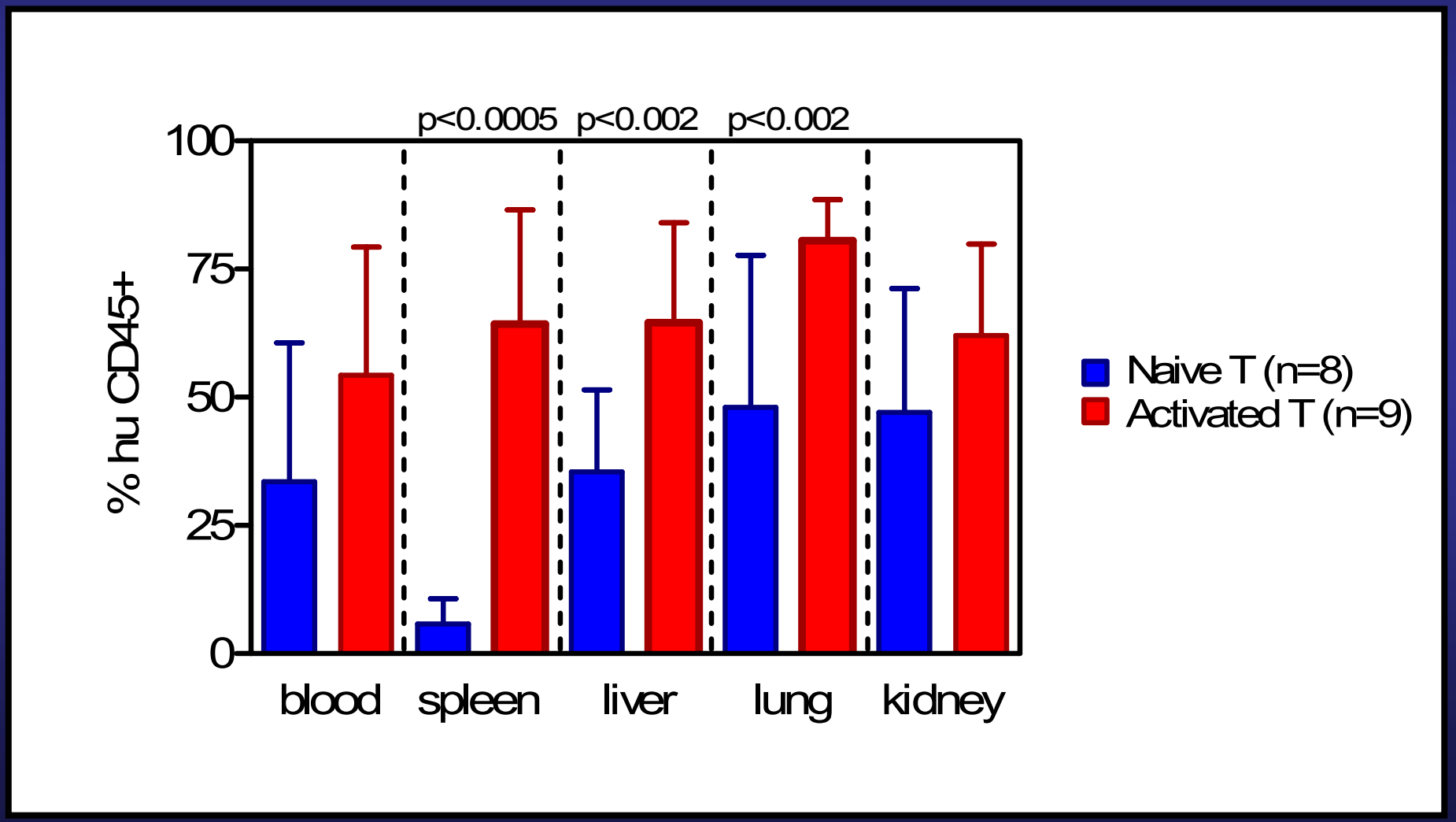
Maxima expansion de huT en sangre

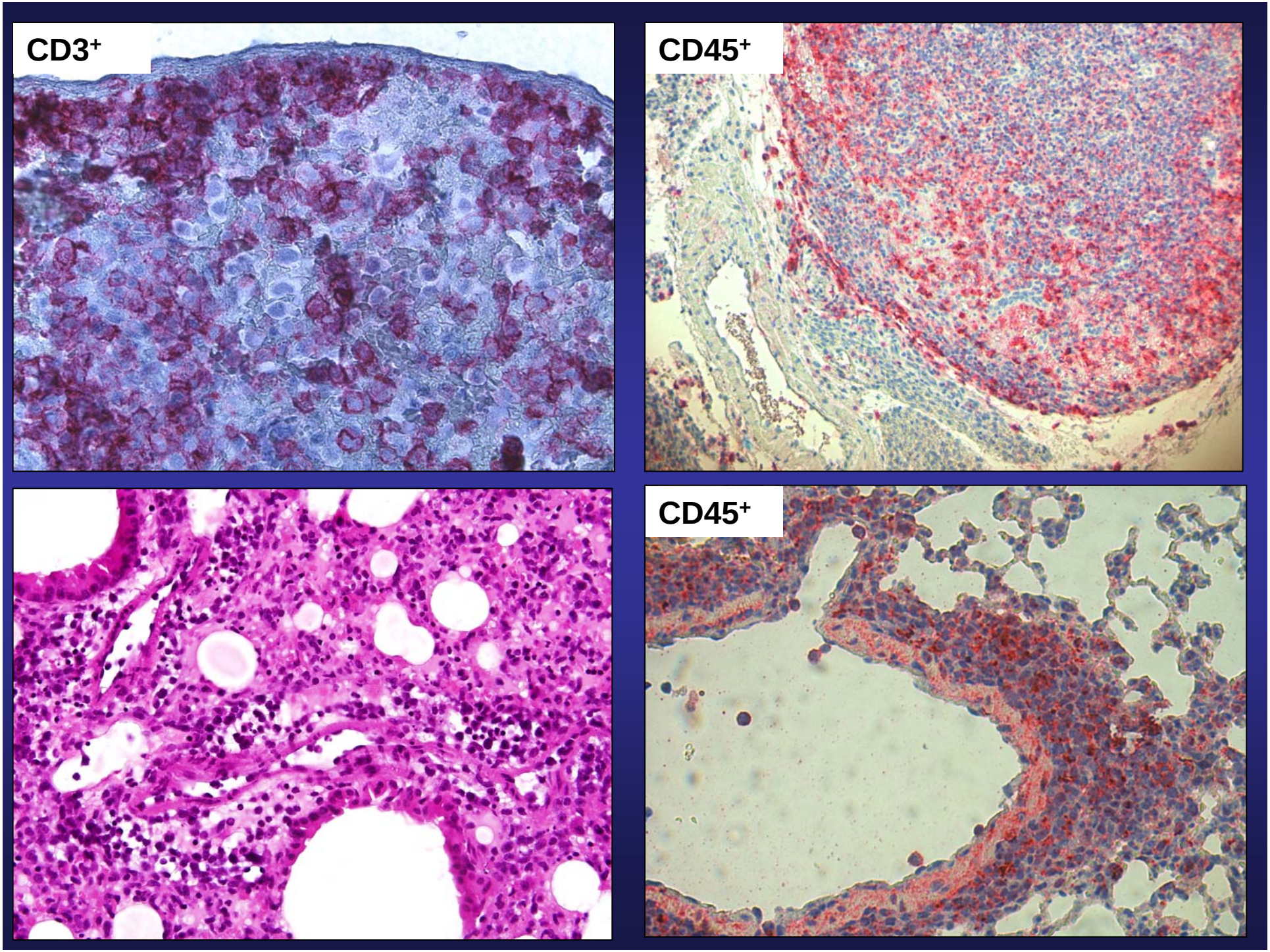
(10^7 huT ro – 250 or 300cGy)



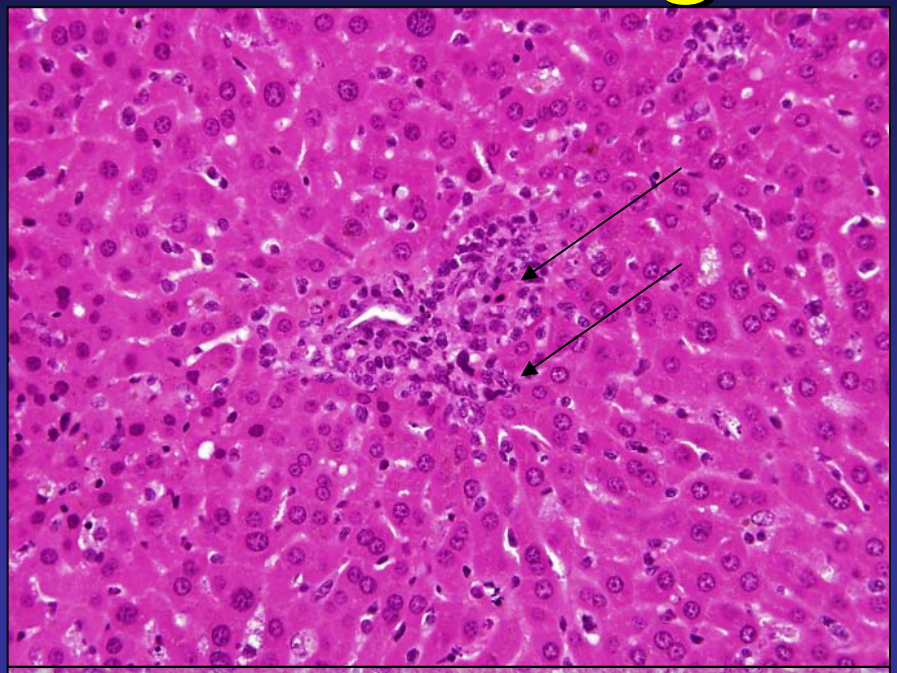
Infiltracion de huT en distintos tejidos

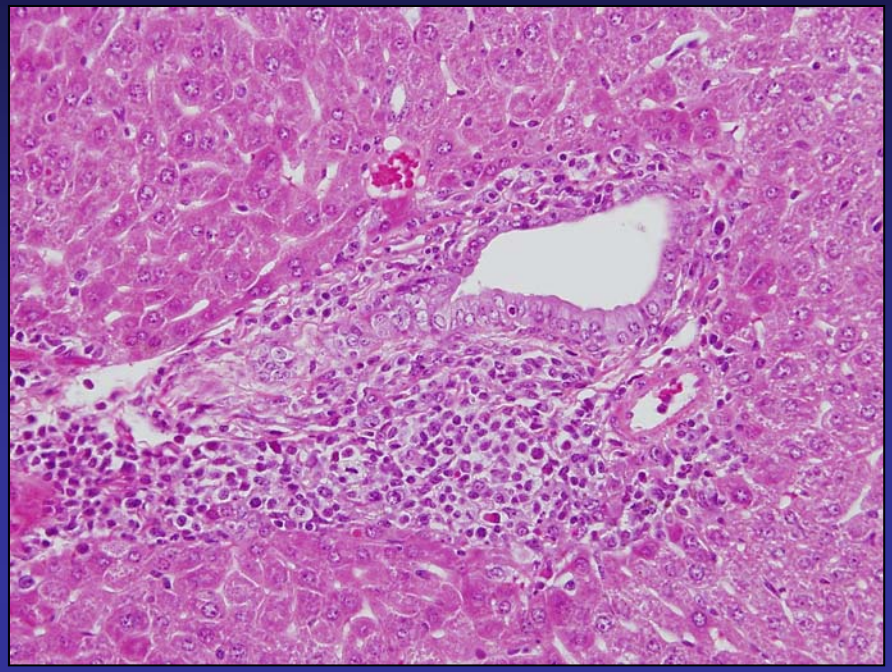
(10^7 huT ro - 300cGy)

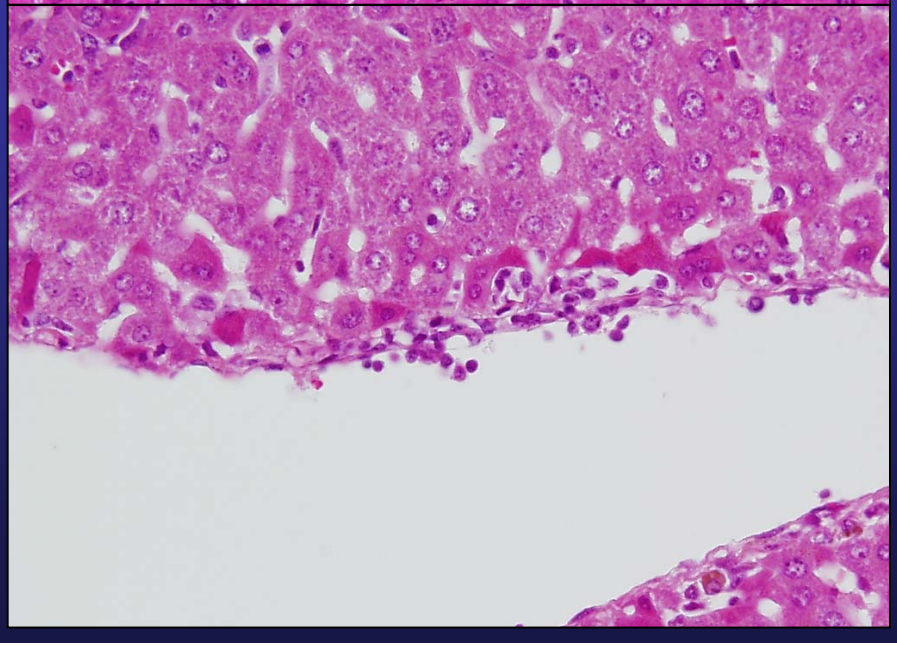



CD3+
CD45+
CD45+

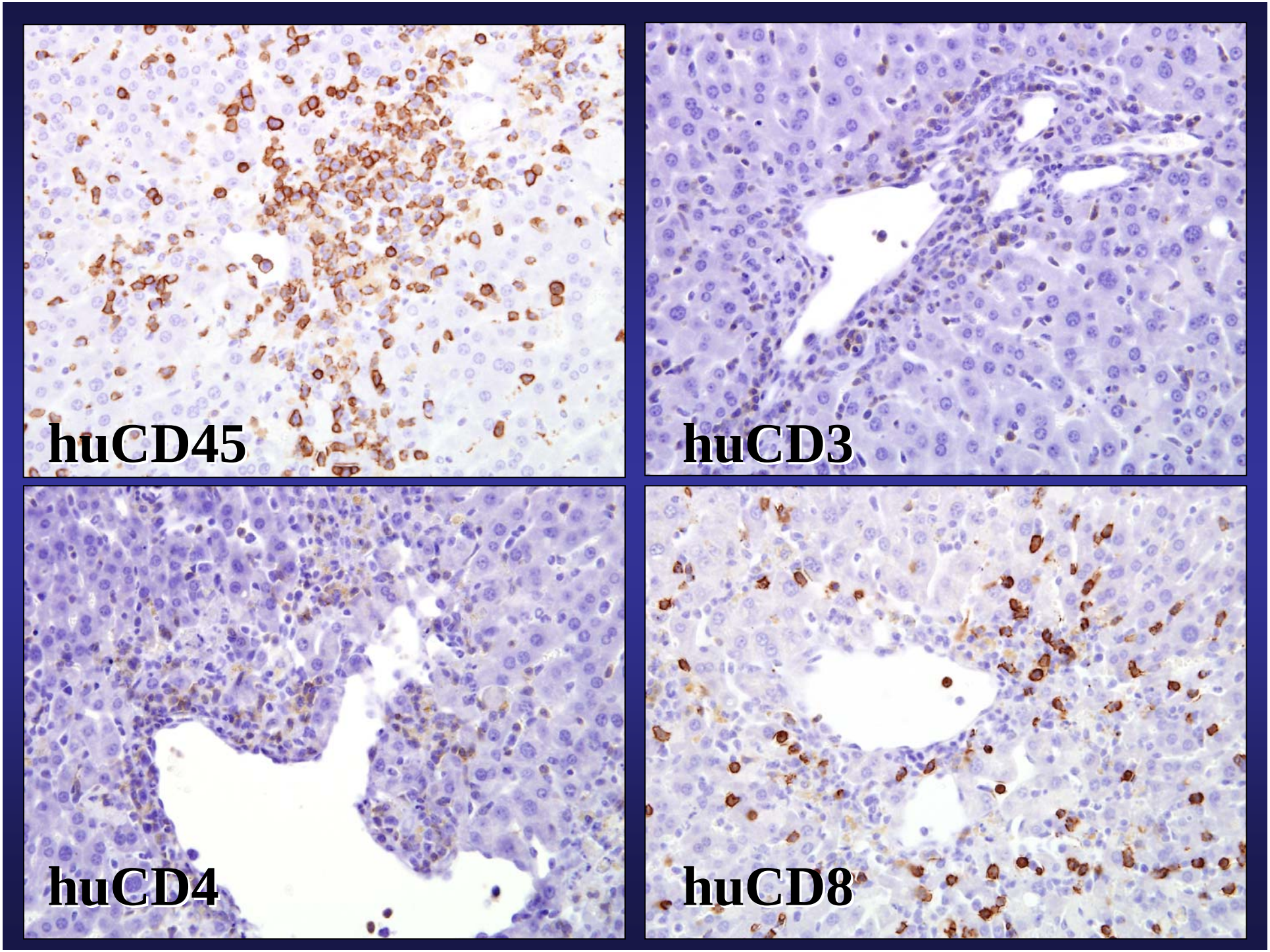
Higado H&E







Infiltrado linfocitario portal
Infiltrado linfocitario ducto biliar
Apoptosis epitelio ducto biliar
Alteracion arquitectura epitelio ductal
Endotelitis
Apoptosis hepatocitos
Microabcesos


huCD45
huCD3
huCD4
huCD8

Organ	Histologic findings
Skin	Basal vacuolar damage
	Epidermal lymphocytic infiltrate
	Apoptosis in epidermis/follicle
	Dermal lymphocytic infiltrate
	Cleft and microvesicle formati
	Separation epidermis from dermis
Liver	Portal lymphocytic infiltrate
	Bile duct lymphocytic infiltrate
	Bile duct epithelial apoptosis
	Bile duct epithelial sloughing
	Endotheliitis
	Hepatocyte apoptosis
	Microabscess
	Hepatocyte mitosis
	Cholestasis
	Steatosis
Salivary gland	Epithelial apoptosis
	Interstitial lymphocytic infiltrate
	Vasculitis
	Perivascular lymphocytic infiltrate
	Regeneration
	Luminal debris
	Loss of duct or acinus
	Necrosis

Organ	Histologic findings
Small bowel	Villous blunting
	Crypt regeneration
	Luminal sloughing of celldebris
	Crypt cell apoptosis
	Outright crypt destruction
	Lamina propria infiltrate
	Mucosal ulceration
Colon	Crypt regentation
	Surface colonocyte vacuolatio
	Surface colonocyte attenuati
	Crypt cell apoptosis
	Outright crypt destruction
	Lamina propria infiltrate
	Mucosal ulceration
Lung	Perivascular infitrate
	Endotheliitis
	Peribronchiolar infiltrate
	Interstitial infiltrate
	Bronchial epithelial apoptosis
	Bronchial epithelial detachment
	Alveolar edema
	Alveolar debris
	Alveolar damage

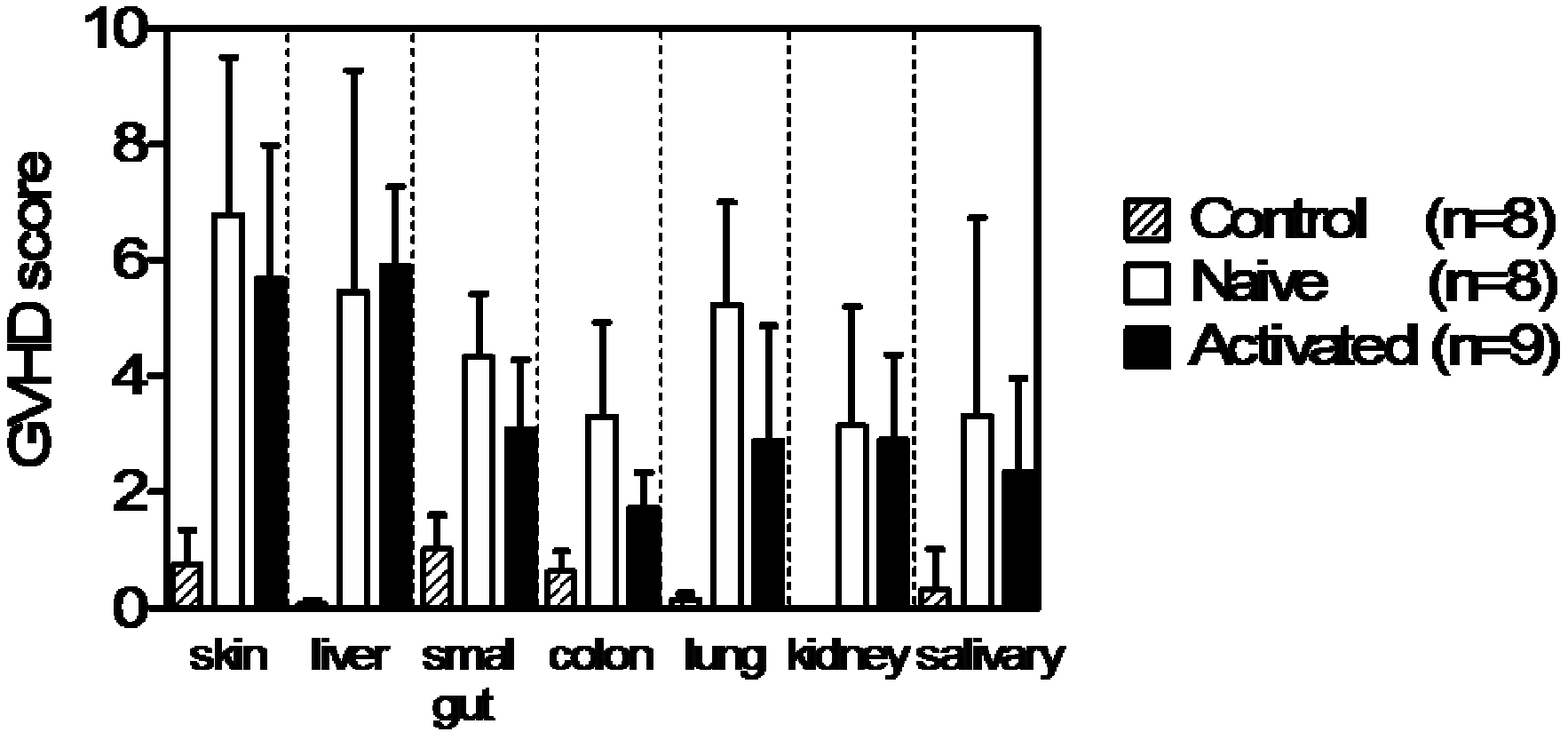
Organ	Histologic findings
Kidney	Interstitial lymphocytic infiltrate
	Tubulitis
	Vasculitis
	Perivascular infiltrate
	Glomerulitis
	Interstitial fibrosis
	Tubular atrophy
	Glomerular sclerosis
	Apoptosis
Spleen	Splenomegaly
	Structural damage
	Fibrosis
	Vasculitis

Score	GvHD
0	normal
0.5	focal and rare
1	focal and mild
2	fiffuse and mild
3	diffuse and moderate
4	diffuse and severe

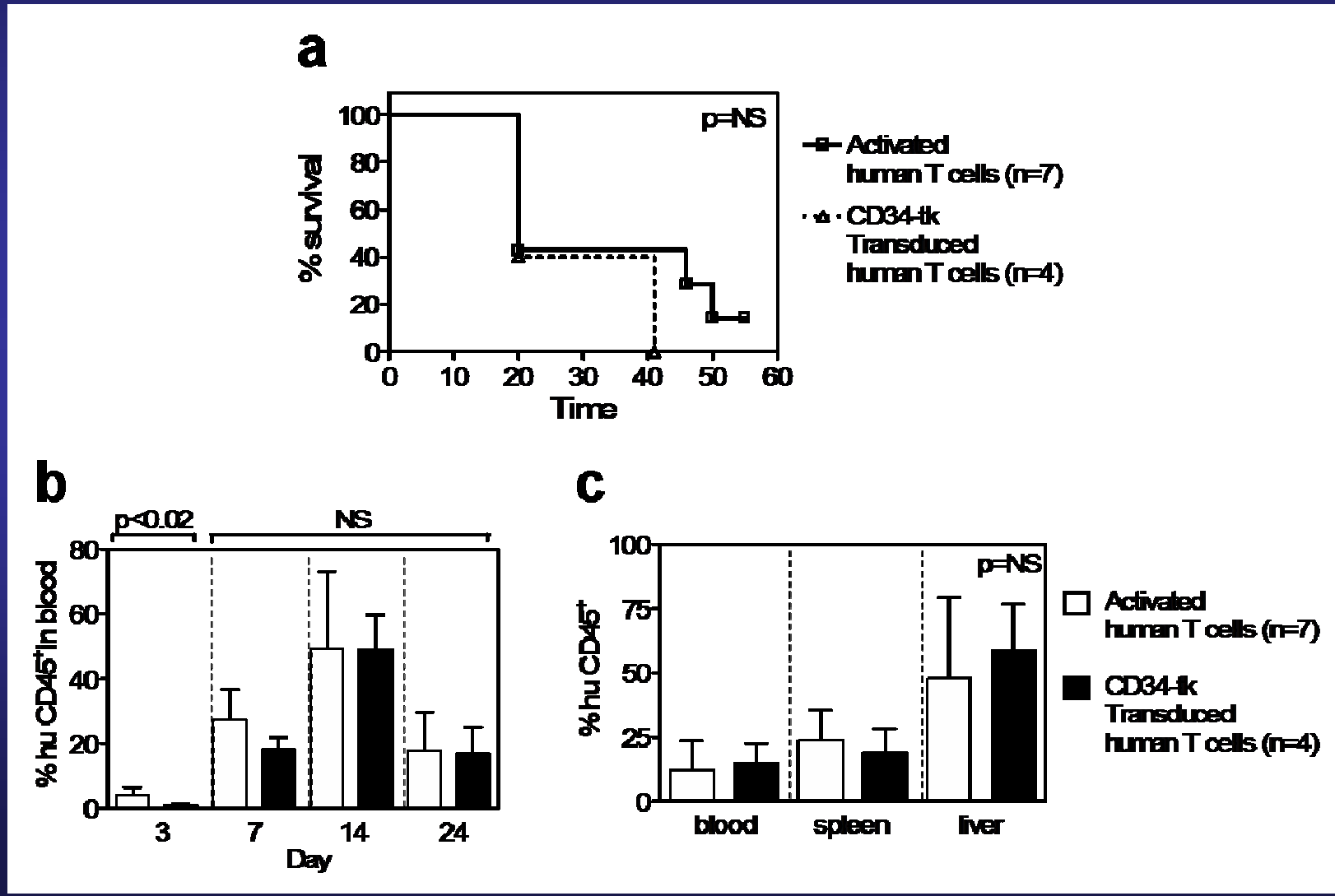
GVHD score

los ratones que bajan de peso y tienen expansion de huT en sangre desarrollan dano tisular

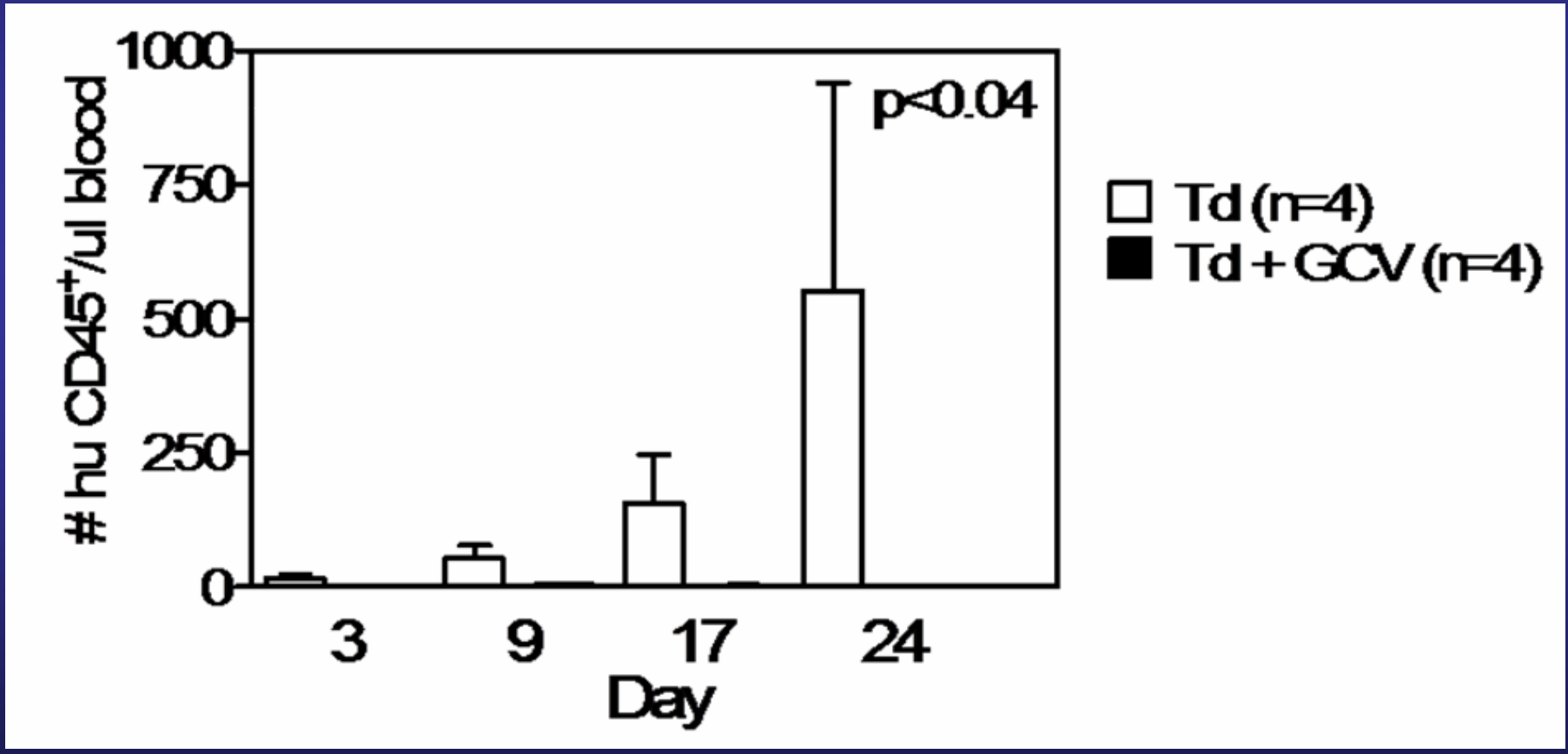
Figure 7



Los huT $^{CD34\text{-}tk}$ conservan el potencial de producir GVHD



Terapia genica suicida para controlar GVHD



Prevencion de GVHD usando terapia genica suicida (ΔCD34-TK/GCV)

No Ganciclovir

Ganciclovir dias 1-7

DISCUSION

1. Desarrollamos un modelo de GVHD con linfocitos humanos en ratones inmunodeprimidos.

2. Consistente con GVHD humano, huT se activan contra alo-antigenos murinos, expanden in vivo, producen IFNγ, infiltran y danan tejidos murinos.

3. Es posible eliminar eficientemente huT $^{CD34\text{-}tk}$ con terapia genica suicida.

4. Comenzaremos a reclutar pacientes para estudio Fase I.

AGRADECIMIENTOS

GMP facility

- *Gerhard Bauer*
- *Jon Walker*

Pathology

- *Hanlin Wang*

Xcyte Therapies

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Stem Cell Biology Division

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- *Julie K Ritchey*