Highly efficient hematopoietic reconstitution by *in vivo* chemoselection of HPRT-deficient bone marrow with 6-thioguanine

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Presenter Disclosure

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Scientific Advisor to CalImmune which has licensed the technology from UCLA

Hematopoietic Stem Cell Transplantation (HSCT)

- Performed in preconditioned recipients

- Conditioning therapy:

  - Total body irradiation
  - Chemotherapy (e.g. busulfan, cyclophosphamide)

  → suppresses immune system
  → myeloablation – create niche for graft cells

⇨ Successful HSCT

Complications to Successful Hematopoietic Stem Cell Transplantation (HSCT)

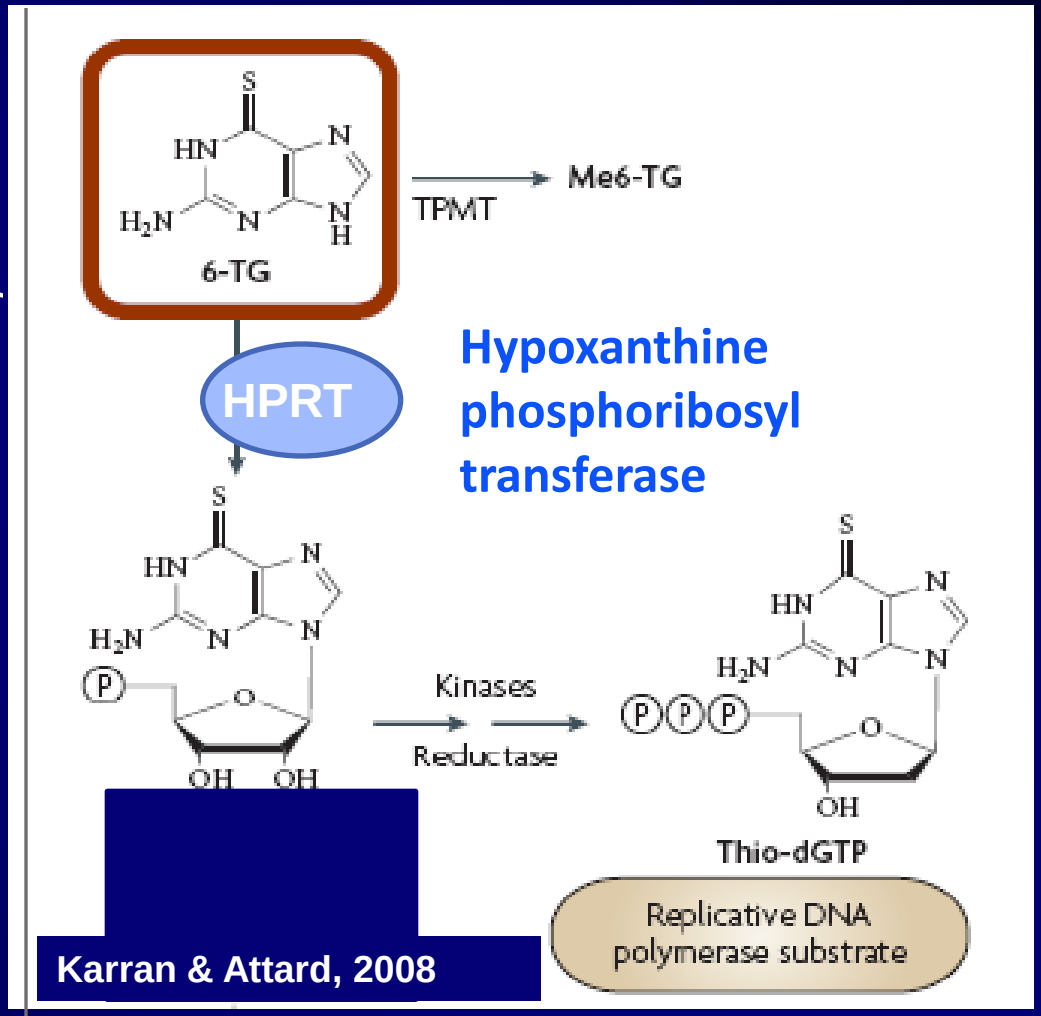
- High treatment related mortality
- Infection
- Graft versus host disease (GVHD)
- Lack of suitable donor

Thiopurines

- Anti-inflammatory, anticancer and immunosuppressive drugs
- Available in clinical practice for over half a century
- Treatment for: - leukemia
  - chronic inflammatory and auto-immune disorders
  - solid organ transplanted patients

Novel and highly efficient strategy for combined preconditioning and chemoselection using 6TG

- 6-Thioguanine
  - Thiopurine drug family
  - Available in clinical practice for over half a century
  - Anti-inflammatory, anticancer and immunosuppressive drugs
  - Cytotoxicity is dependent on its conversion to thioguanine nucleotides by HPRT



HPRT-deficient Mice

(Hooper *et al*.,1987)

- C57BL/6J genetic background
- Deletion of exons 1 and 2 of the *Hprt* gene
- Lethal dose of 6TG in HPRT-deficient mice:

23-fold > wildtype mice (Aubrecht *et al*. 1997)

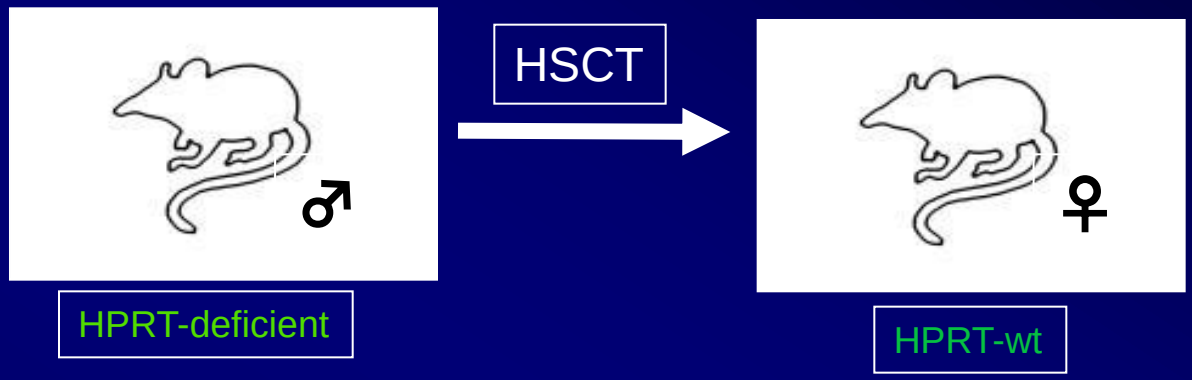
Hypothesis:

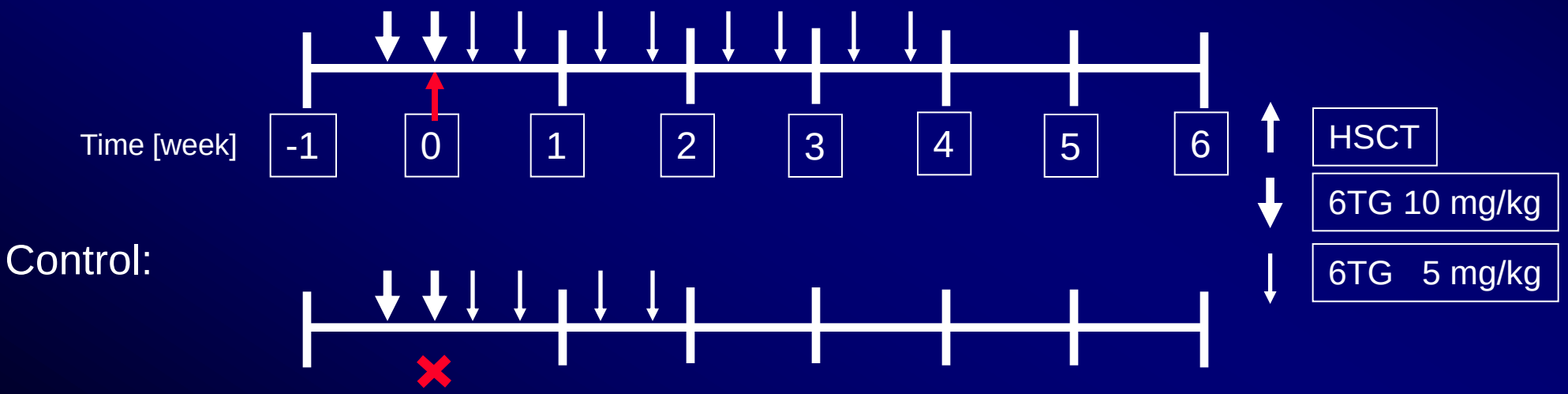
HPRT-deficient bone marrow

can be selected *in vivo* by

applications of sublethal doses of 6TG.

Scheme: 6TG *in vivo* selection





6TG *in vivo* Selection

- Control group:
  - Typical clinical symptoms of 6TG toxicity
    → loss of body weight
    → anemia
    → death after 10-12 days

  - Depletion and necrosis of hematopoietic tissues:
    → femoral bone marrow
    → splenic red pulp
    → mandibular/mesenteric lymph nodes

- HSC transplanted group:
  - Without immediate toxic symptoms or distress

Comparison of engraftment efficacy

(pre) treatment 6TG *in vivo* selection	Engraftment 95% in BM	Comment 4 weeks selection
No ablation	10-40%	(Zhong etal.’02)
TBI syngeneic	+90% at >6.5 Gy (at 3mo)	Almost linear corr. (Down etal.’91) 0% below 5Gy
TBI allogeneic	90% at >6Gy (at 3mo)	
Busulfan + cyclophosphamid	Max. 78% in BM on day 60	Reaches plateau (Sadeghi etal. ’08)
Reduced intensity cond. (RIC)	dependent on GvHD,	Non –myeloablative, high relapse rates (Kato etal; ’07)
MGMT *in vivo* selection (BG/BCNU)	30% after BMT, 60-100% after selection	Traditional BMT, dependent selection time (Davies etal. ’97)
other *in vivo* selection (e.g. MDR-1)	5-15%, prob. low transduction efficiencies	transient levels of $MDR1^{+}$ cells (Southgate & Fairbairn, ‘04

6TG toxicity: HPRT-Ko mice treated with 6TG

Day	0.25 mg/kg	0.5 mg/kg	1 mg/kg	2.5 mg/kg	5 mg/kg	Control
0	100%	100%	100%	100%	100%	100%
5	100%	100%	100%	100%	100%	100%
10	100%	100%	100%	100%	100%	100%
15	100%	100%	100%	100%	100%	100%
20	100%	100%	100%	100%	100%	100%
25	100%	100%	100%	100%	100%	100%
30	100%	100%	100%	100%	100%	100%
35	100%	100%	100%	100%	100%	100%
40	100%	100%	100%	100%	100%	100%
45	100%	100%	100%	100%	100%	100%
50	100%	100%	100%	100%	100%	100%
55	100%	100%	100%	100%	100%	100%

Treatment scheme: every 3rd day ip injection with 6TG concentration as indicated

6TG toxicity: C57/BL6 mice treated with 6TG

Day	0.25 mg/kg	0.5 mg/kg	1 mg/kg	2.5 mg/kg	5 mg/kg	Control
0	100%	100%	100%	100%	100%	100%
5	100%	100%	100%	100%	100%	100%
10	100%	100%	100%	100%	100%	100%
15	100%	100%	100%	100%	100%	100%
20	100%	100%	100%	100%	100%	100%
25	100%	100%	100%	100%	0%	100%
30	100%	100%	100%	0%		100%
35	100%	100%	100%			100%
40	100%	100%	66%			100%
45	100%	100%	33%			100%
50	100%	100%	0%			100%

Treatment scheme: every 3rd day ip injection with 6TG concentration as indicated

Optimal Performance of 6TG *in vivo* Selection

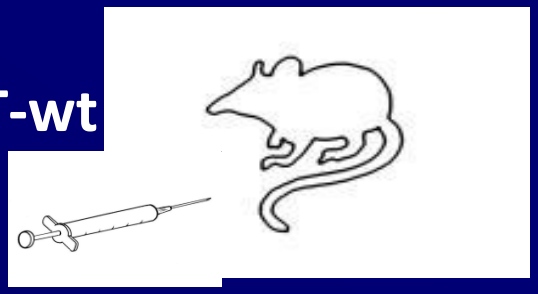
- HSC engraftment :
  - XY-chromosome FISH
  - q-RT-PCR for mouse TSPY
  - EGFP/HPRT-KO mice

- Distribution transplanted HSCs vs. residual host HSCs

- Longevity of transplanted HSCs in recipients

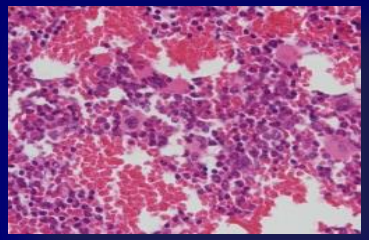
HPRT-wt BM is sensitive to 6TG

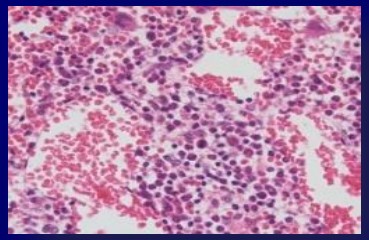
HPRT-wt

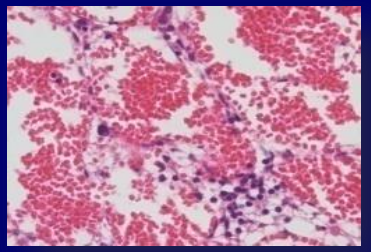


6TG - 10 mg/kg

analysis

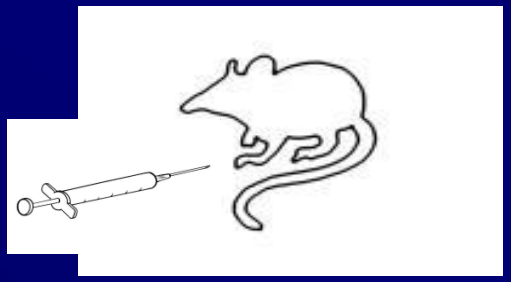






Time [Day] 1 3 4 6 7

HPRT-deficient marrow is resistant to 6TG



6TG
10 mg/kg

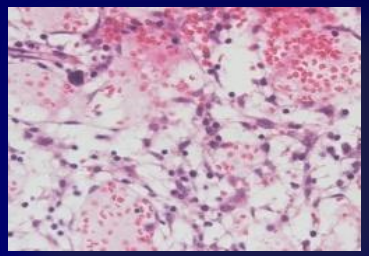
6TG
10 mg/kg

6TG
5 mg/kg

analysis

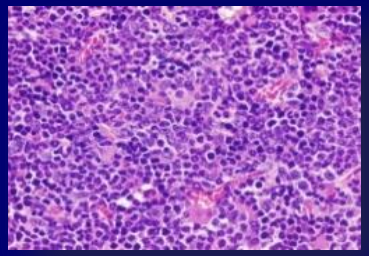
HPRT-wt

Time [Day] 1 3 4 6 7

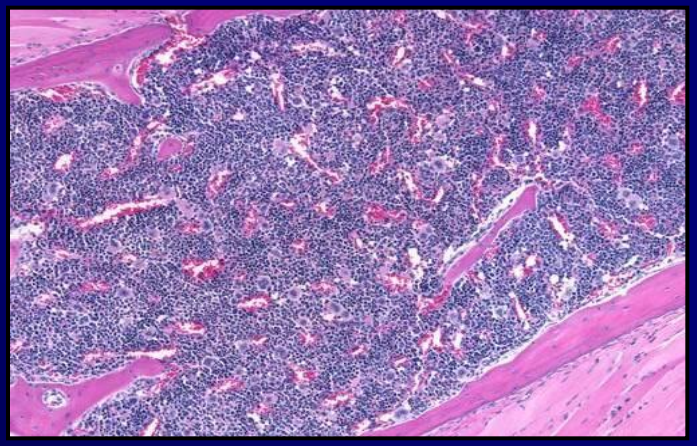


HPRT-KO

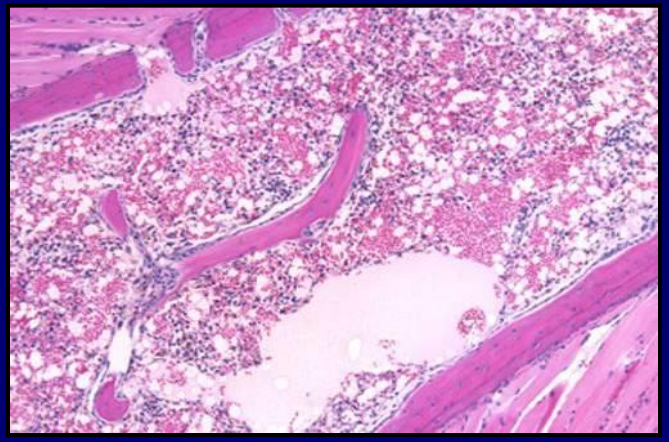
Time [Day] 1 3 4 6 7



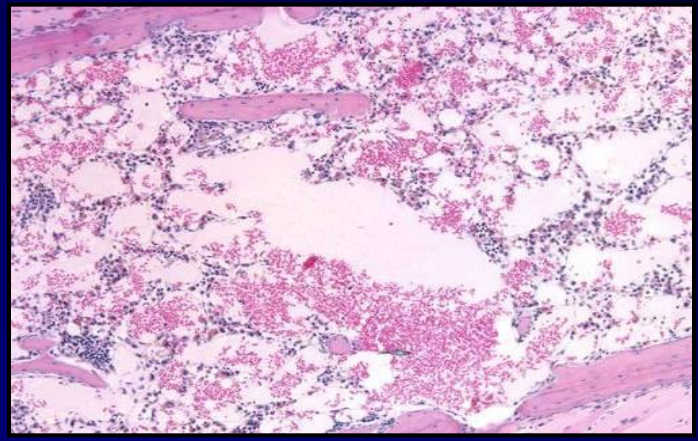
Histopathology mouse BM



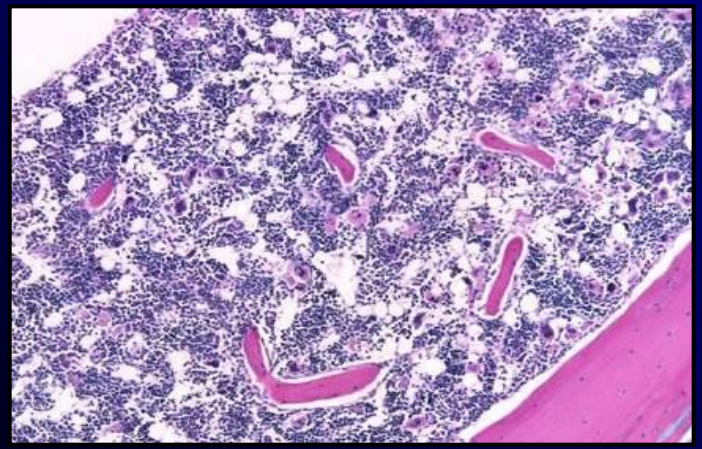
untreated



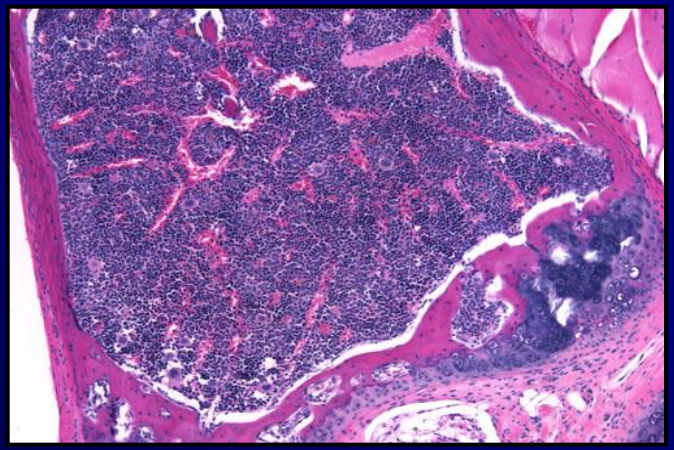
6TG (25 mg/kg total)
treatment after 7 days



6TG (25 mg/kg total)
treatment after 12 days



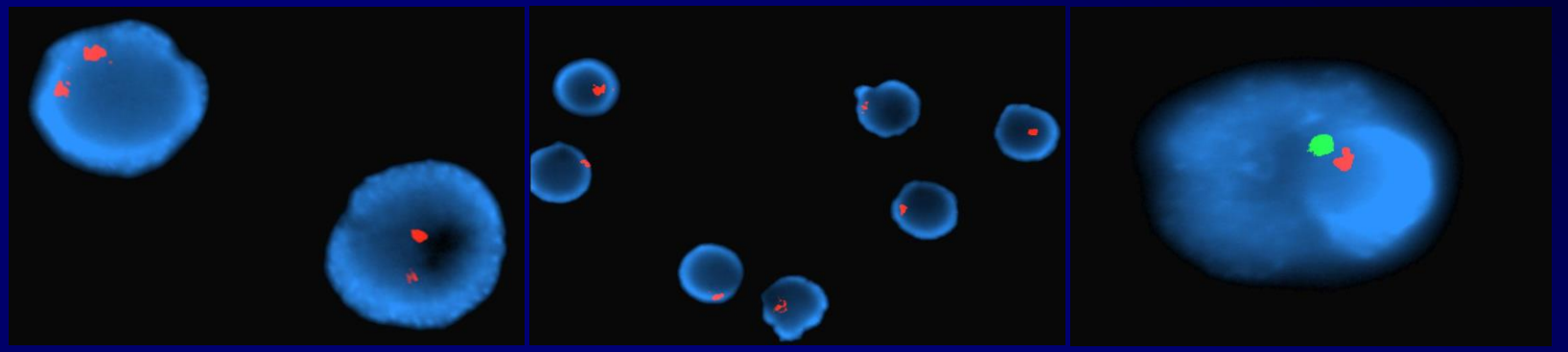
HSCT + 6TG *in vivo* selection



HSCT +6TG *in vivo* selection
4 weeks after last treatment

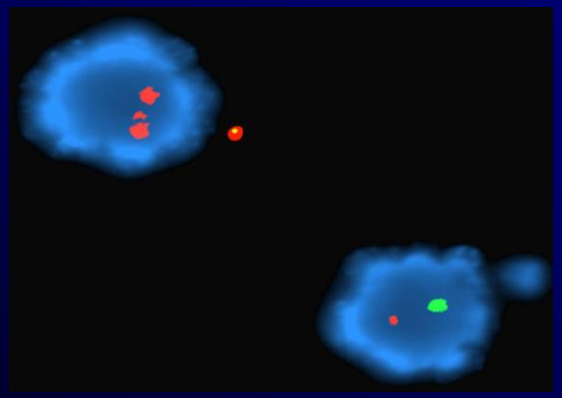
XXXY-chromosome FISH

Controls:

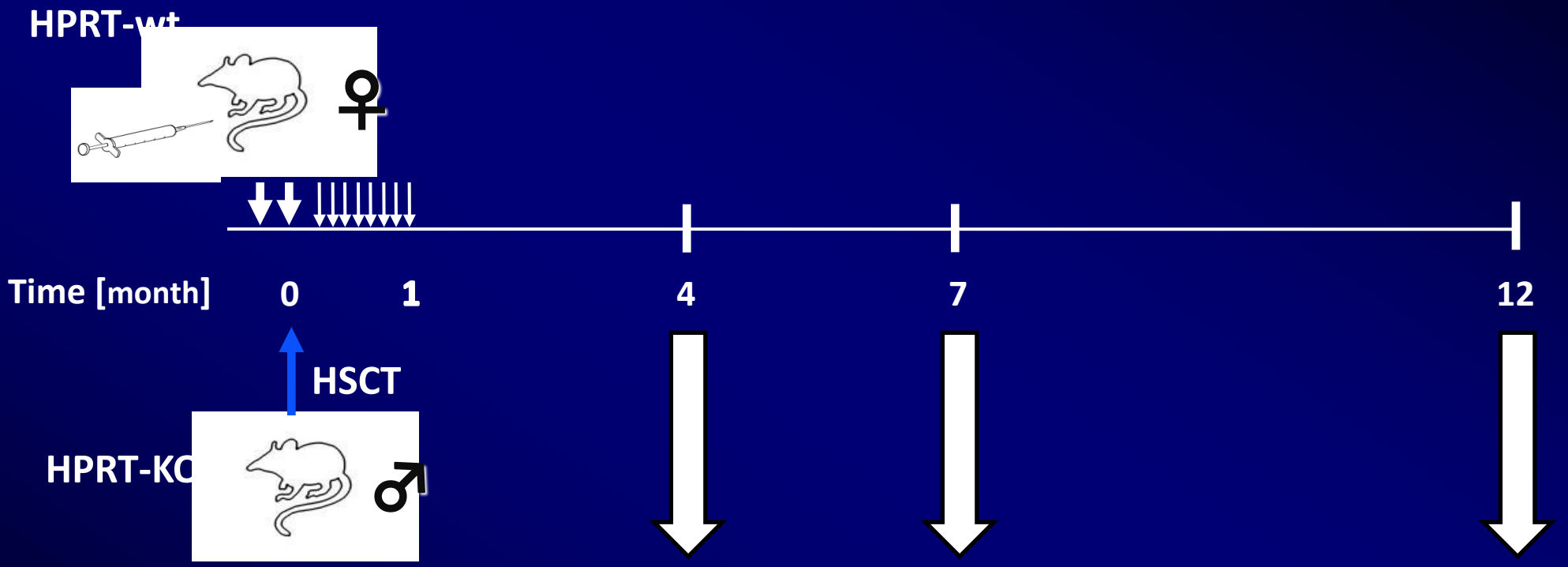


HSC transplanted:

♀



Combined 6TG conditioning and *in vivo* chemoselection results in long-term reconstitution of HPRT-deficient BM



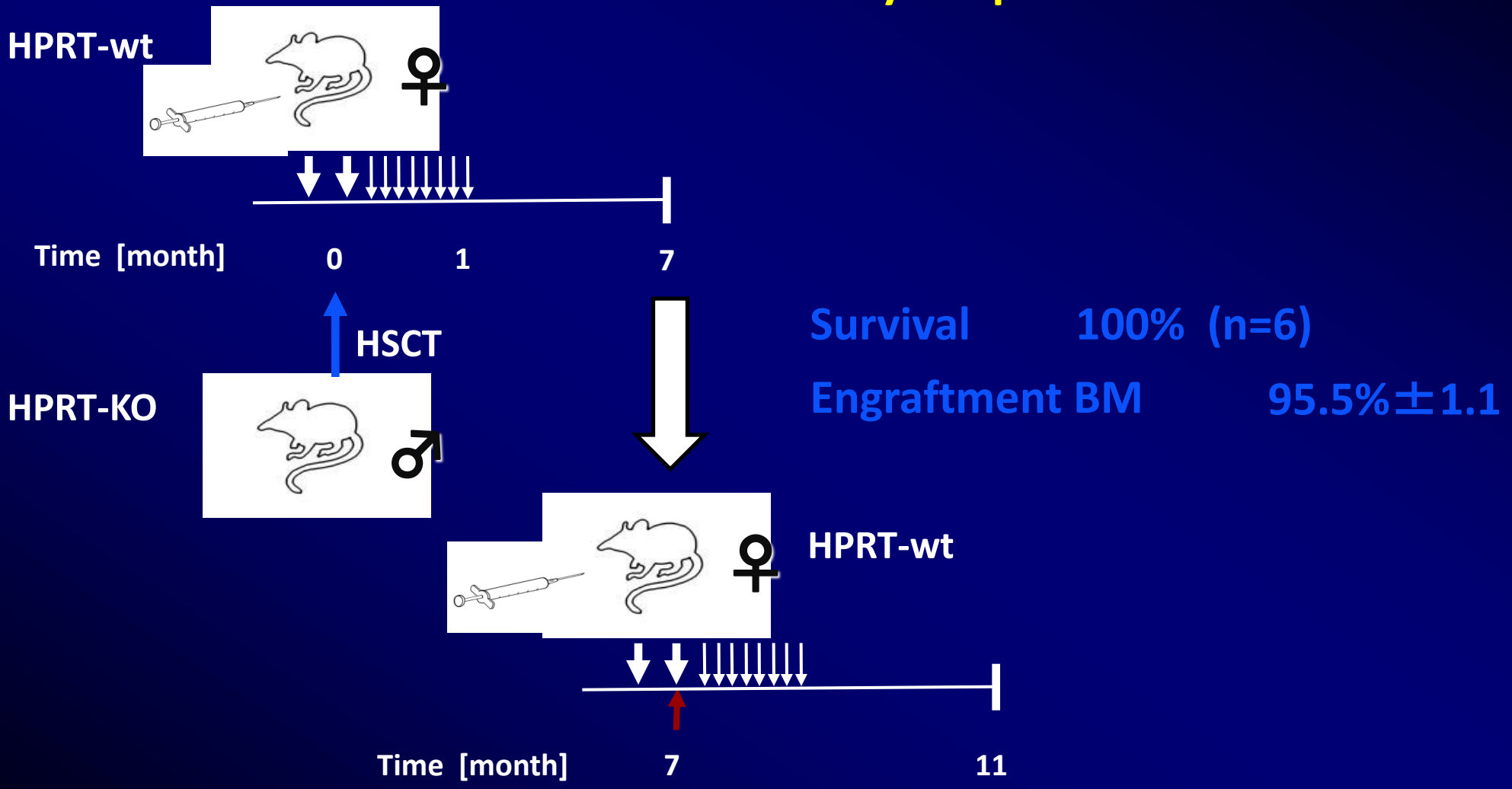
Survival	100% (n=8)	100% (n=6)	100% (n=5)
Engraftment BM	97.7% ±0.5	94.7% ±1.9	93.0% ±0.8
Engraftment PBL	67.4% ±10.6	73.3% ±8.1	73.0% ±10.7

Combined 6TG conditioning and *in vivo* chemoselection results in multi-lineage reconstitution of immunophenotypically normal lymphohematopoiesis

Cell population	BM (%)	PBL (%)	Thymus (%)	Spleen (%)
$CD45.2^+$	76.1±9.3	73.8±5.2	89.7±8.3	65.9±5.2
$CD4^+$	3.2±1.3	17.0±3.0	10.4±2.8	22.6±3.6
$CD8^+$	3.0±1.3	12.7±0.9	4.3±0.8	14.9±2.1
$CD4^+CD8^+$			79.3±4.2	
$B220^+$	29.3±6.3	45.4±3.8		58.2±8.6
$Mac1^+Gr1^+$	80.68±6.1	28.2±3.3		14.1±1.6

- High engraftment of donor-derived CD45.2 cells
- Relative percentages of hematopoietic cell populations comparable to those of controls

HPRT deficient BM after 6TG conditioning and *in vivo* chemoselection reconstitutes secondary recipients



Survival 100% (n=6)

Engraftment BM 95.5%±1.1

Applicability of 6TG *in vivo* selection as disease treatment for lymphoma in AT-patients

- **Atm-deficient mice as lymphoma model:**

  - Acquire lymphoma between 2 and 4 months of age

  → PET/CT scans (Dr. Johannes Czernin)

  → Mice with lymphoma: HSC transplantation + 6TG *in vivo* selection

  → Tracking the healing progress with PET/CT scans

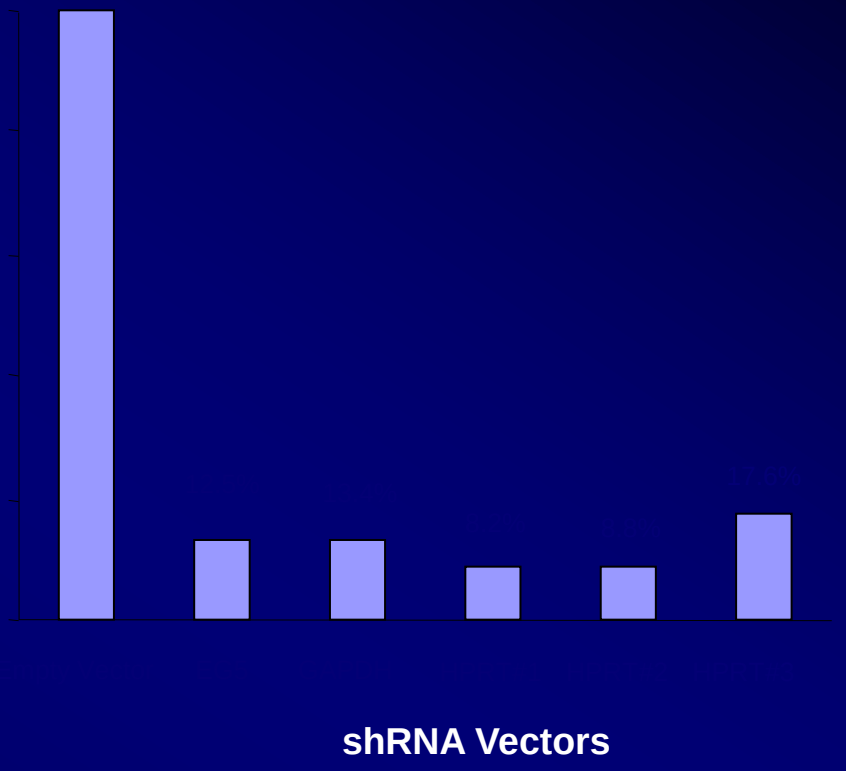
Summary of results

- Highly efficient engraftment of HSC of HPRT-deficient BM after preconditioning and *in vivo* chemoselection with 6TG alone
- Additional myeloablative conditioning by radiation or other chemotoxins is not required for stable engraftment
- Long-term multilineage reconstitution of lymphohematopoiesis has been achieved

Efficient HPRT Knock Down by shRNA Sequences

Experimental Procedure:

- HEK293T cells were purchased from Open Biosystems (Huntsville, AL.)
- Transfection of different shRNA vectors was performed with FuGene6 (Roche, Switzerland) reagent
- Cells were exposed to puromycin one day after transfection at 3 ug/ml for one week
- pooled surviving clones were analyzed for respective RNA level by real time RT-PCR analysis
- results are expressed relative to that from the empty pGIPZ vector

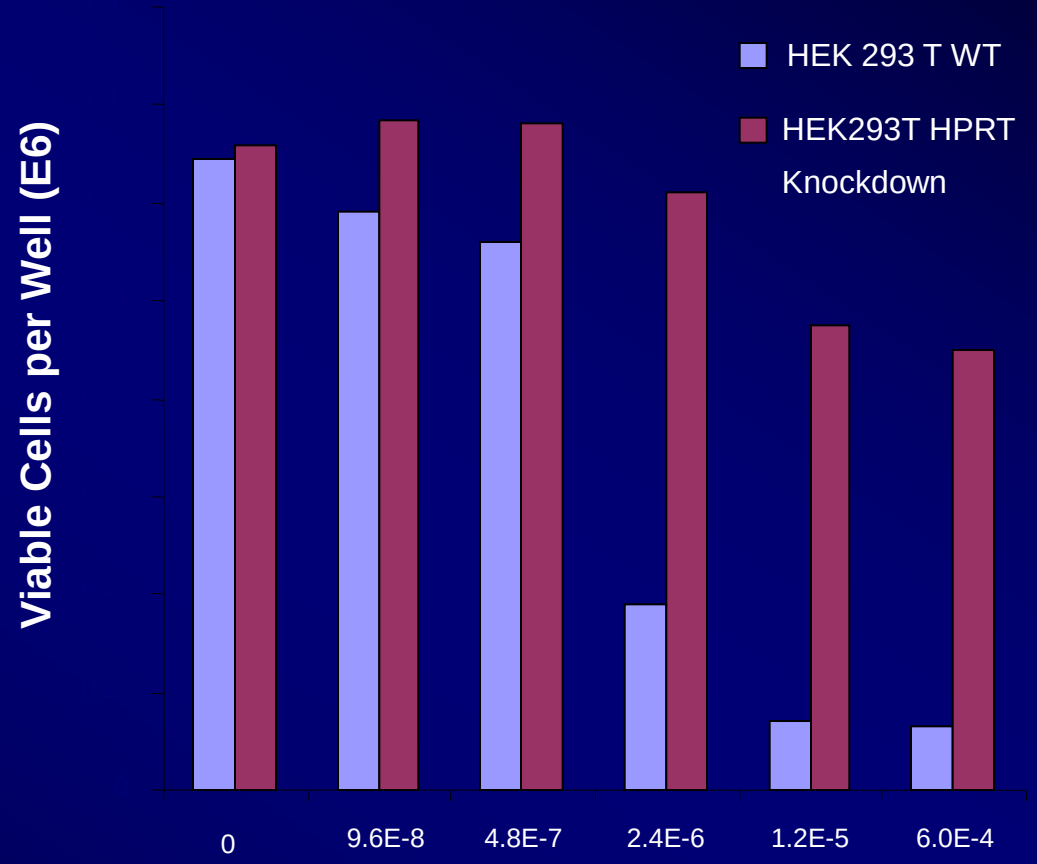


HPRT shRNA vector #1 (Clone ID V2LHS_82406, Open Biosystems, Huntsville, AL.) has the most efficient HPRT knockdown (8.2% residual RNA level) and is chosen for subsequent experiments.

HPRT Status Determines 6TG Sensitivity

Experimental Procedure:

- HEK293T cells were purchased from Open Biosystems (Huntsville, AL) and maintained in 90% Dulbecco's MEM and 10% FBS supplemented with gentamycin
- Cells with or without HPRT knock down were plated at 0.12E6 per well in a 12 well plate
- Cells were dosed with 6-TG the day after plating at concentrations indicated
- Cells were counted 48 hours later (Cedex Automated Cell Counter, Innovatis, Germany) and viable number of cells were expressed as indicated



Combined 6TG conditioning and *in vivo* chemoselection

- Provides a competitive advantage for the graft
- Removes requirement for high levels of gene transduction
- Selects at stem cell level enabling stable engraftment with discontinuation of 6TG
- Permits *in vivo* selection and virtually complete hematopoietic replacement without severe systemic toxicity

Acknowledgments

Nori Kasahara
Janet Treger
Brooke Bogan
Nathan Lemp

Akos Szakmary
Jiri Aubrecht

Gay Crooks
Andrew Cuddihy

Nagesh Rao
Nora Rozengurt
Gregory Lawson
Donald Kohn

MNIT Foundation

NCI-supported UCLA *In Vivo* Cellular and Molecular Imaging Center (ICMIC)

NIAID-funded UCLA Center for Biological Radioprotectors

California Institute of Regenerative Medicine (CIRM)