Hematopoietic Stem Cell Gene Therapy: Safety and Efficacy in Large Animal Studies

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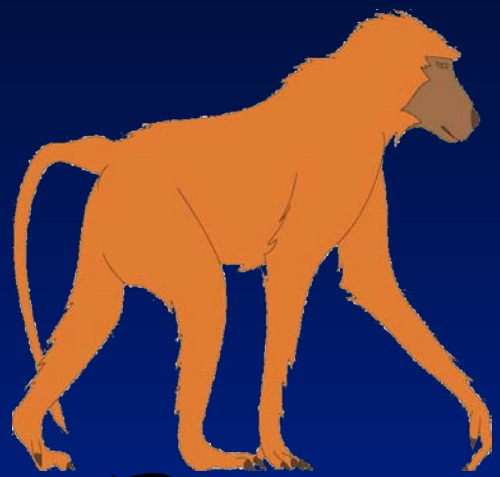
Overview - Purpose

- HSC gene transfer levels with gamma, lenti and foamy virus vectors
- Integration site profiles
- In vivo selection
- Clinical study with MGMTP140K

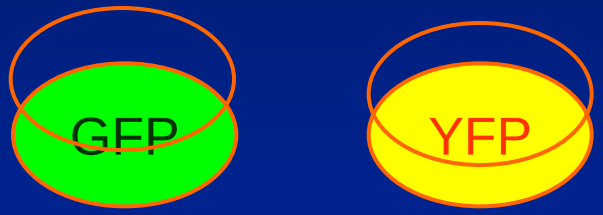
Testing of Multiple Variables - Competitive Repopulation Assay



Stem cell harvest
and
CD34 enrichment

split cells in
two aliquots

Transduction

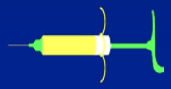


Vector A

Vector B

Fibronectin Fragment
Growth factors
Envelopes
Packaging cells
Vector systems
Stem cell sources

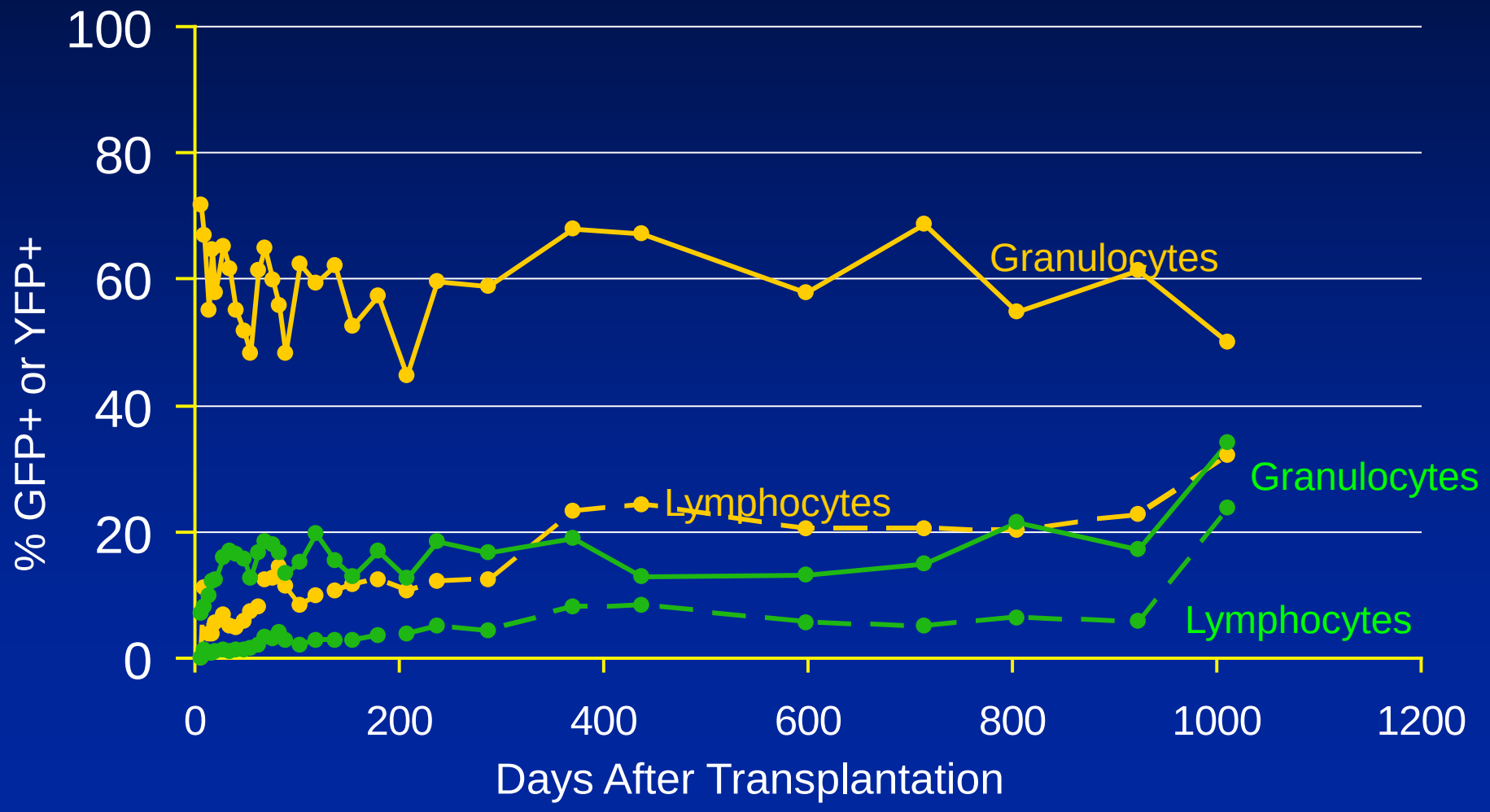
Conditions tested:



Infusion
of pooled cells

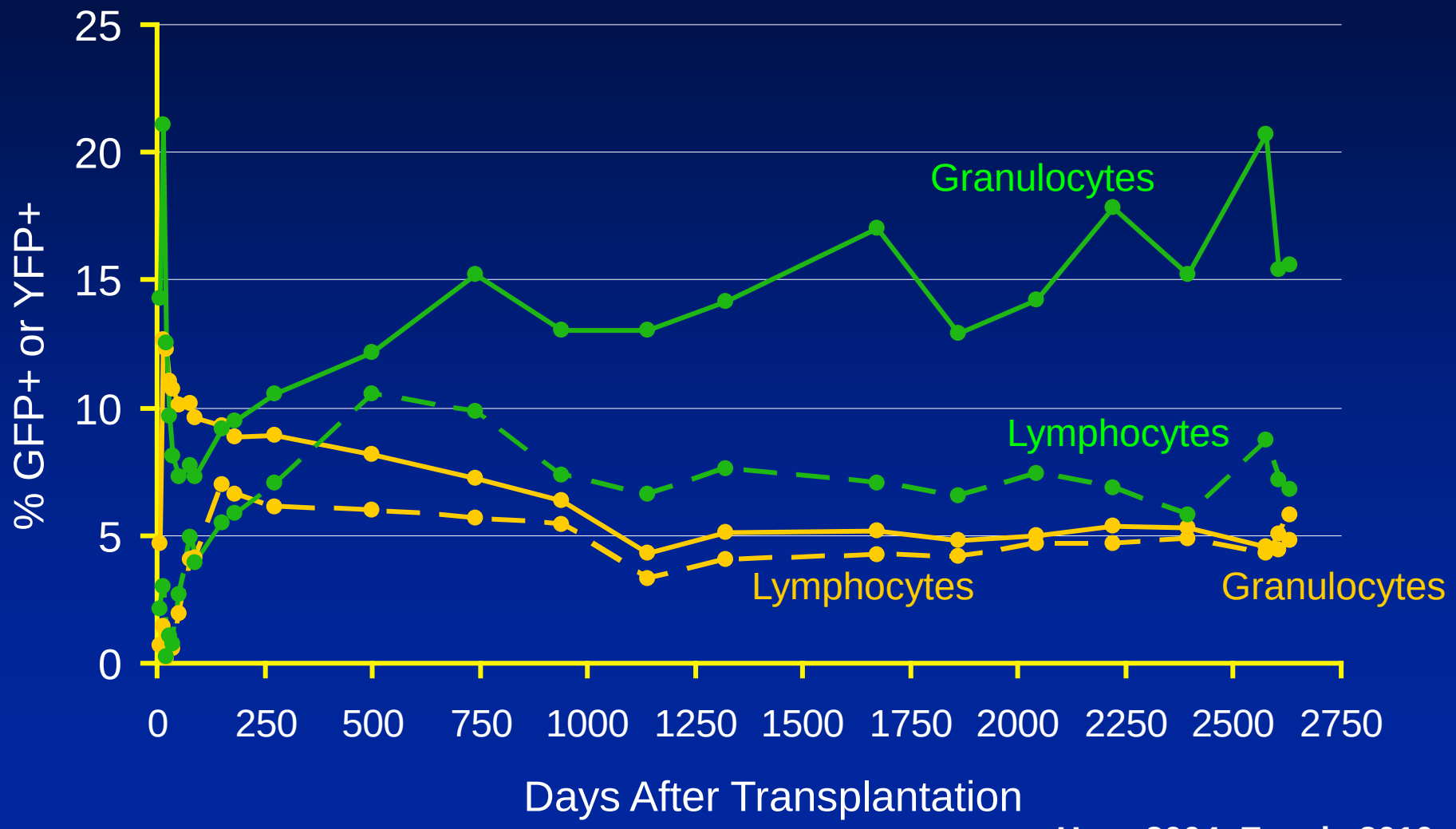
Myeloablative
Irradiation or other
Conditioning Regimens

Gammaretrovirus Gene Marking in the Monkey



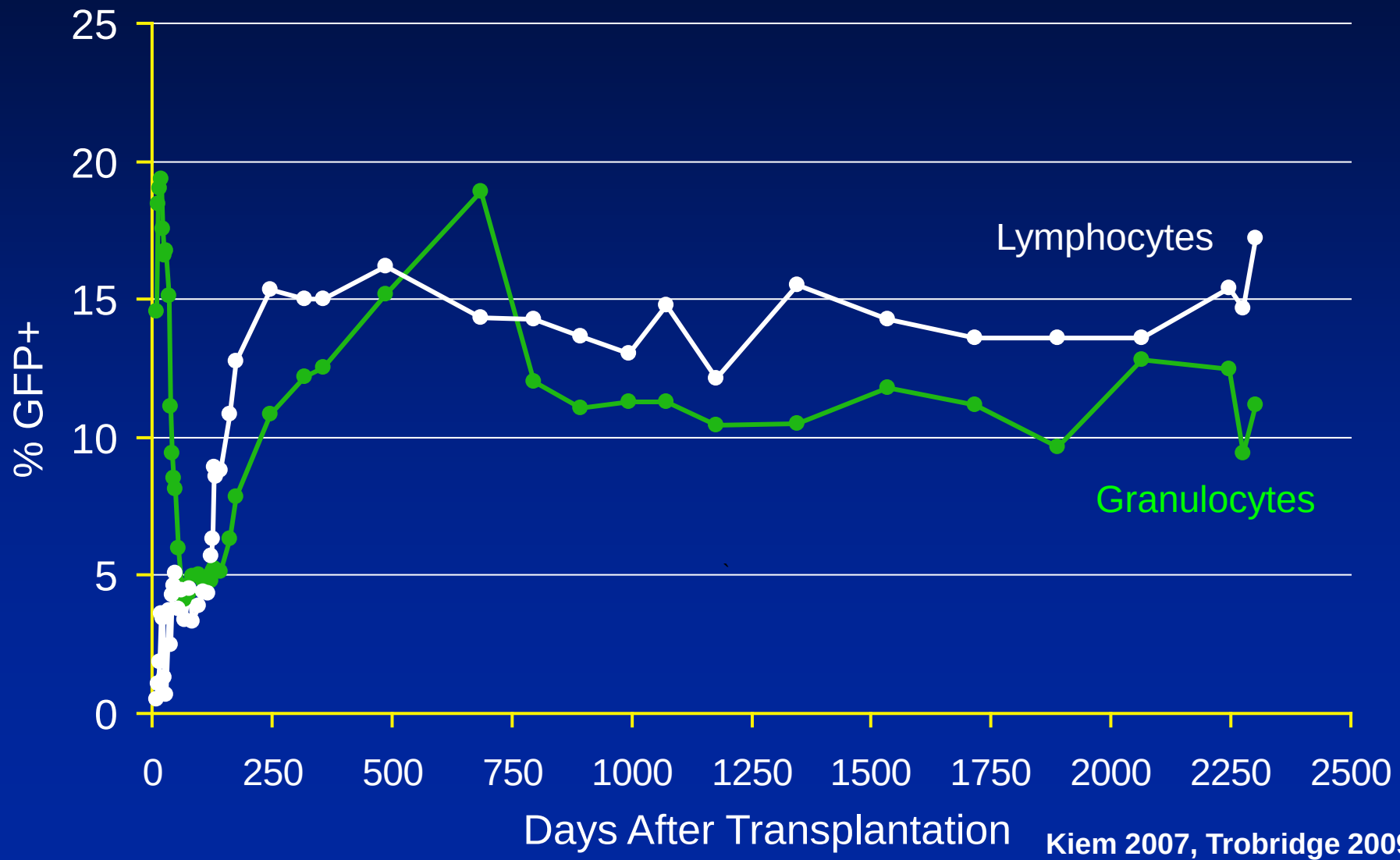
Beard et al. Mol Therapy 2006

Lentivirus Gene Marking in the Dog



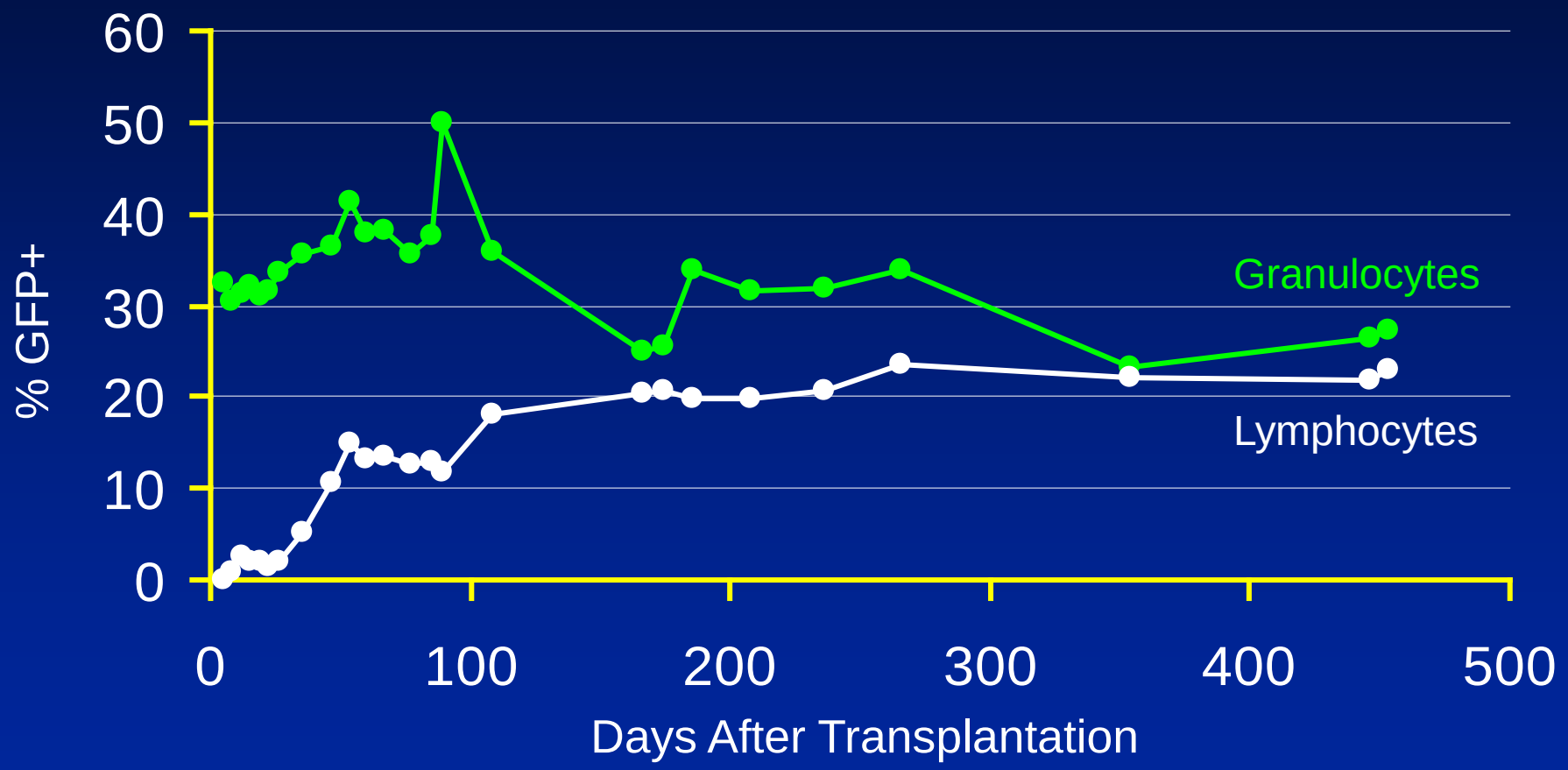
Horn 2004, Enssle 2010

Foamy Virus Gene Marking in the Dog



Kiem 2007, Trobridge 2009

High Level Lentiviral Marking in the Pigtailed Macaques



MOI 10

Trobridge et al. Blood 2008

Marking Summary

- High level marking with all 3 integrating viral vectors
- Multilineage marking
- Stable marking over many years
- Large animal models good for testing long-term stability of expression in different lineages

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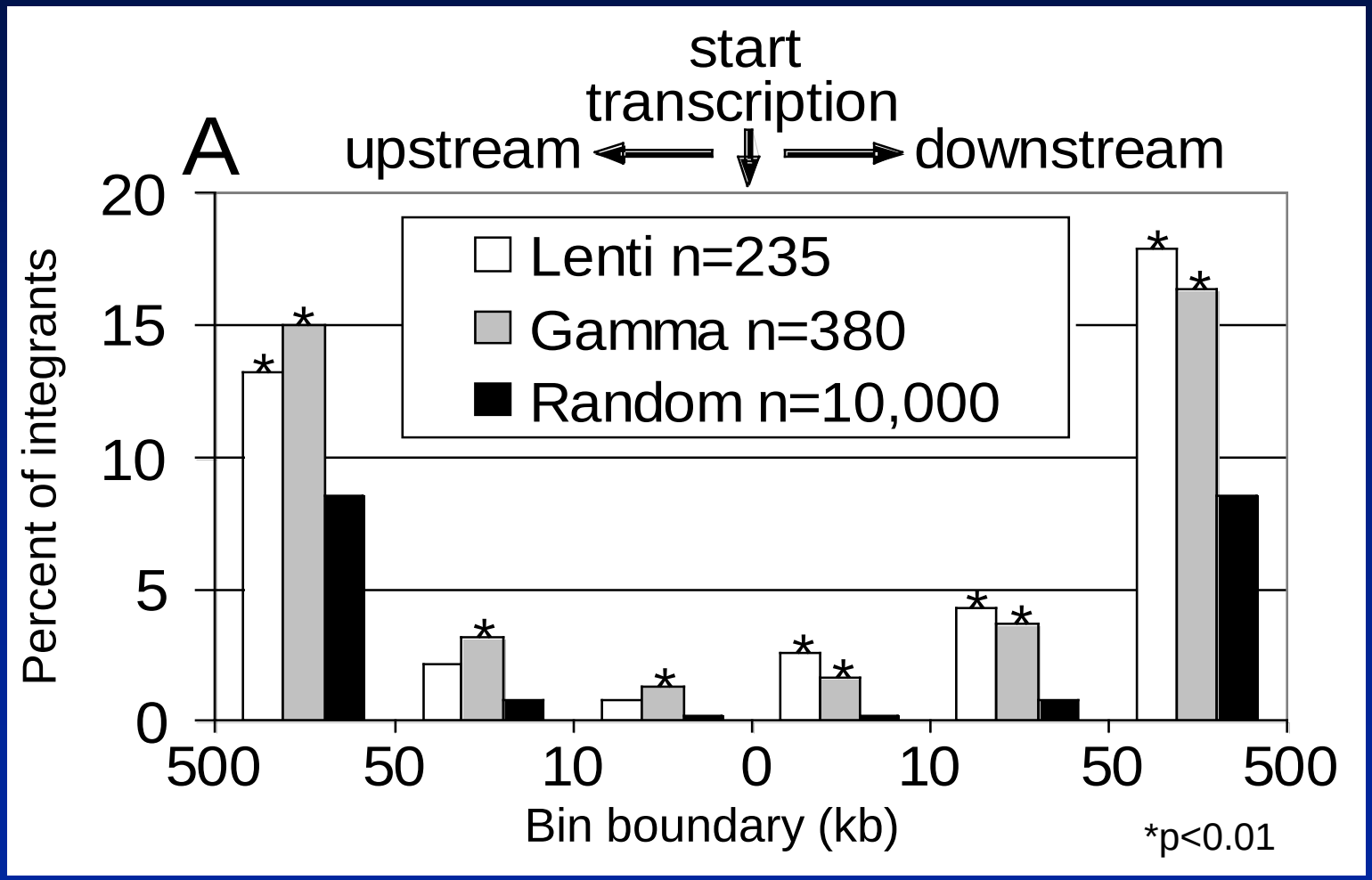
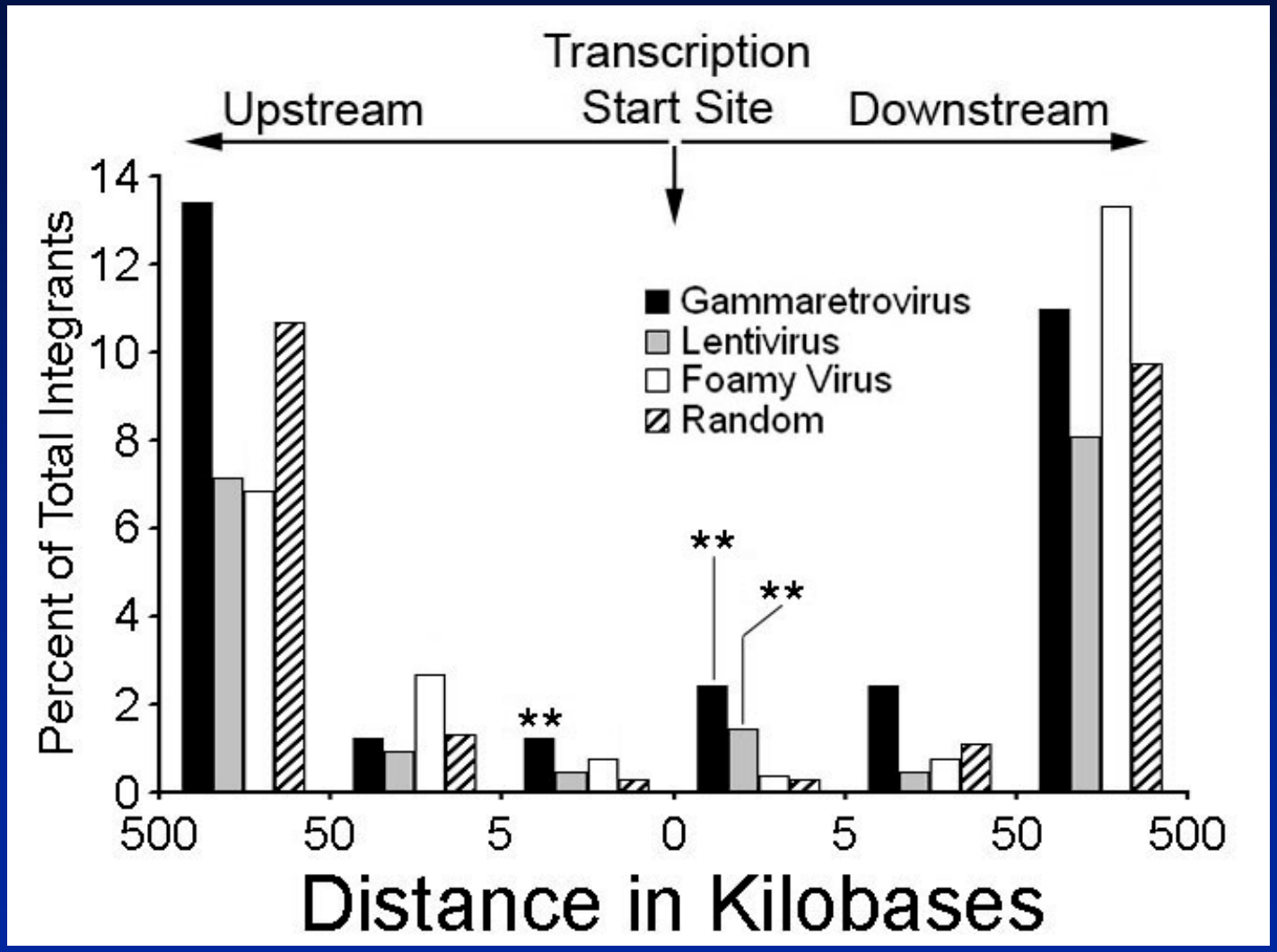
Distribution of Vector Integrants in Primate Long-Term Repopulating Cells Relative to Proto-oncogenes



Beard et al Mol Therapy

FV Do Not Integrate Preferentially Near Proto-Oncogenes in Dogs



Beard et al., Hum Gene Ther 18: 423-434, 2007

Long-term Follow-up in Large Animals: (No) Insertional Mutagenesis

- **We have followed 42 rhesus macaques, 23 baboons, and 17 dogs with marking levels > 1% for a median of 3.5 years (range 1 to 7)**
- **None developed abnormal hematopoiesis or leukemia**
- **Integration site analysis confirmed stable polyclonal retrovirally marked hematopoiesis**

Kiem et al. Mol. Ther. 2004

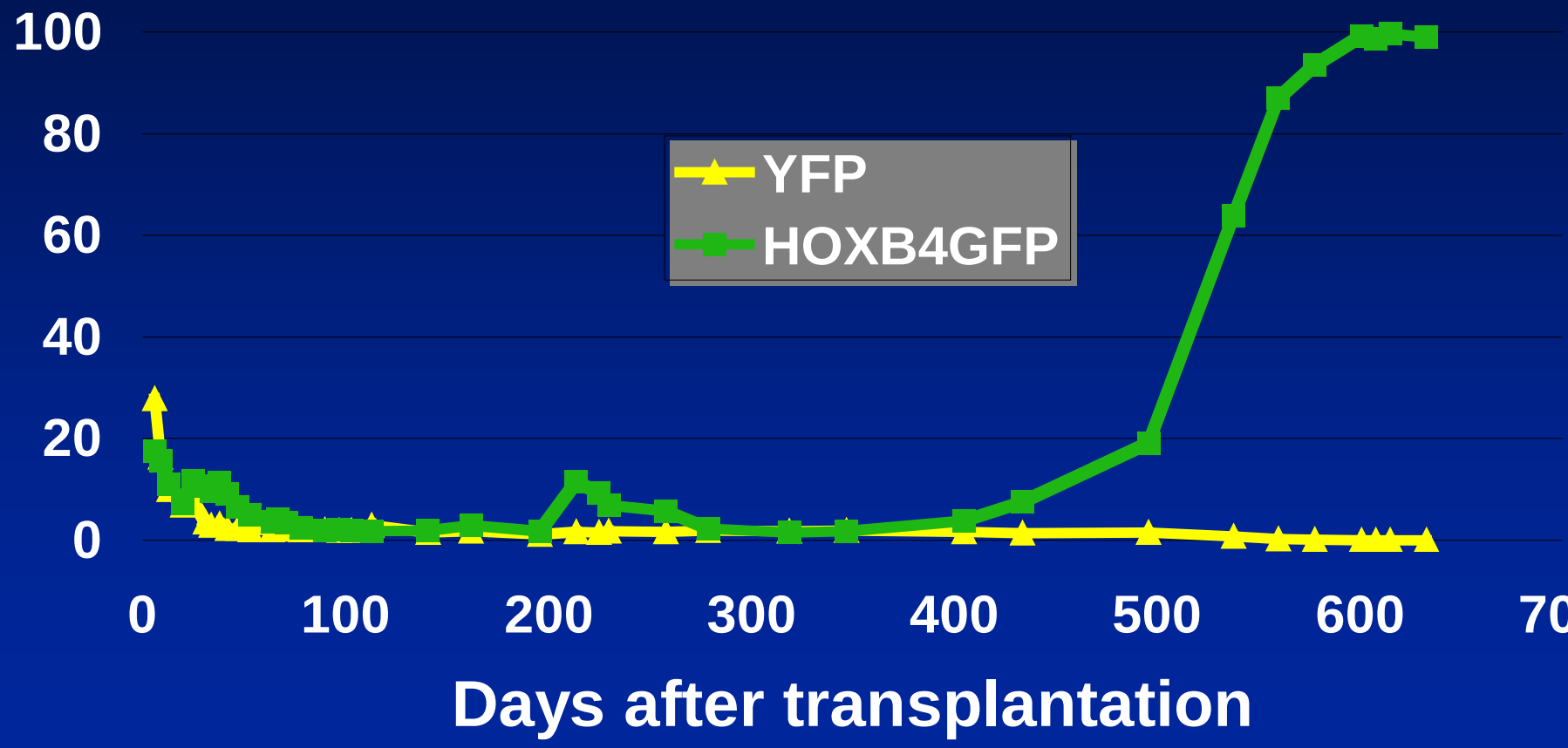
Long-term Follow-up in Large Animals: Insertional Mutagenesis

- Seattle
  - > 20 dogs marking levels > 1% with follow-up between 1 and 8 years
  - > 20 monkeys with levels >1% and follow up between 1 and 5 years
  - Only 1 monkey abnormal hematopoiesis after chemotherapy with DTIC
  - Otherwise integration site analysis confirmed stable polyclonal retrovirally marked hematopoiesis
- NIH
  - > 60 monkeys
  - 2 animals with myeloid malignancy, one with insertion in Bcl2-A1 and a second animal in HDAC7 and after repetitive busulfan treatments

Integration Sites Summary

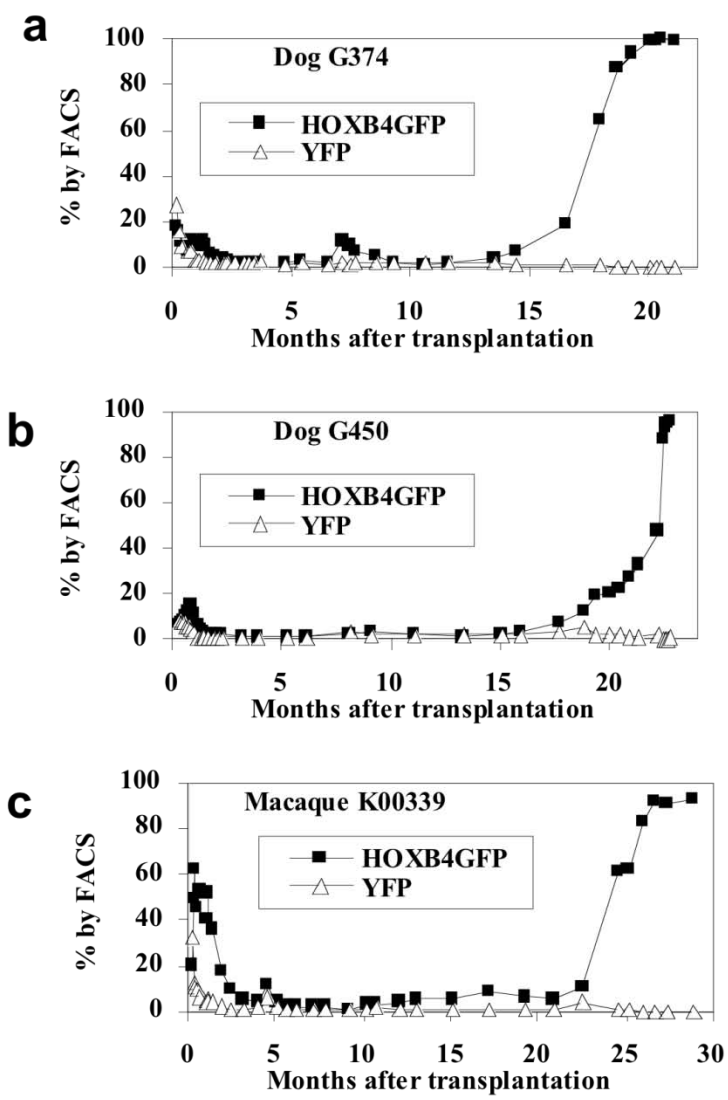
- Polyclonality in all but one animal (Seattle)
- Integration sites close to proto-oncogenes likely with all vectors
- Differences in vector backbones – foamy maybe least likely to integrate close to proto-oncogenes

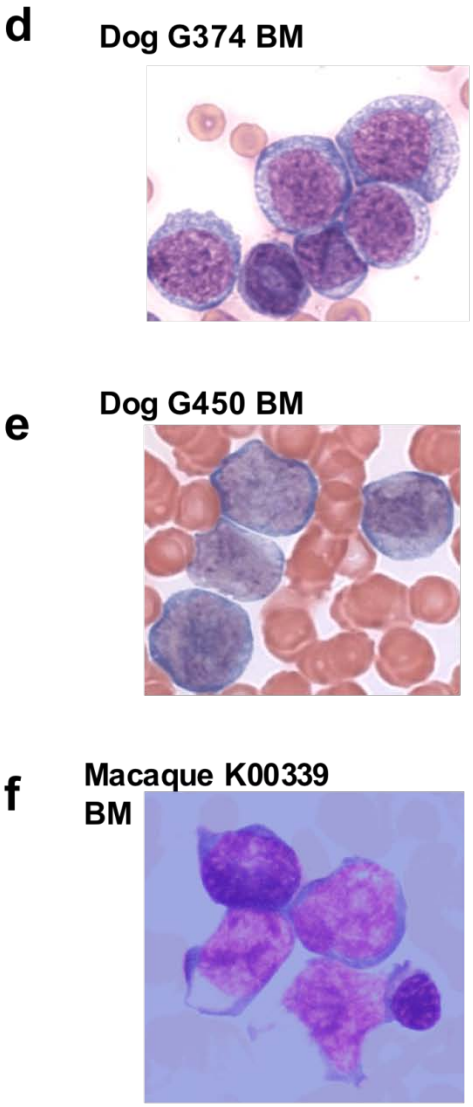
Leukemia in Dogs and Monkeys when growth promoting HOXB4 is used



Zhang et al. JCI 2008

HOXB4-mediated Leukemia





Zhang et al. JCI 2008

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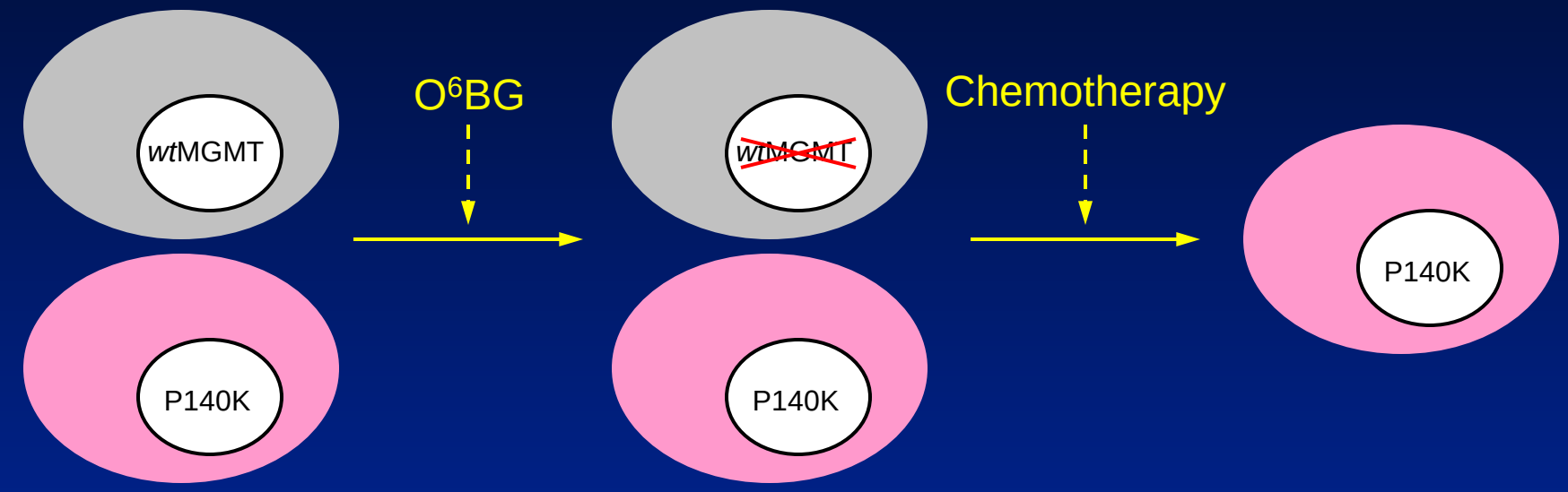
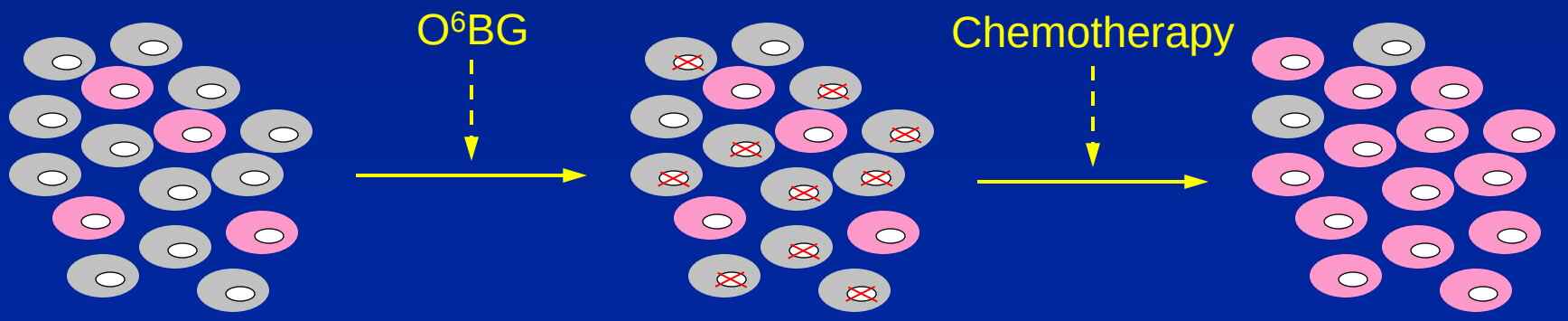
MGMT-Mediated Chemoprotection

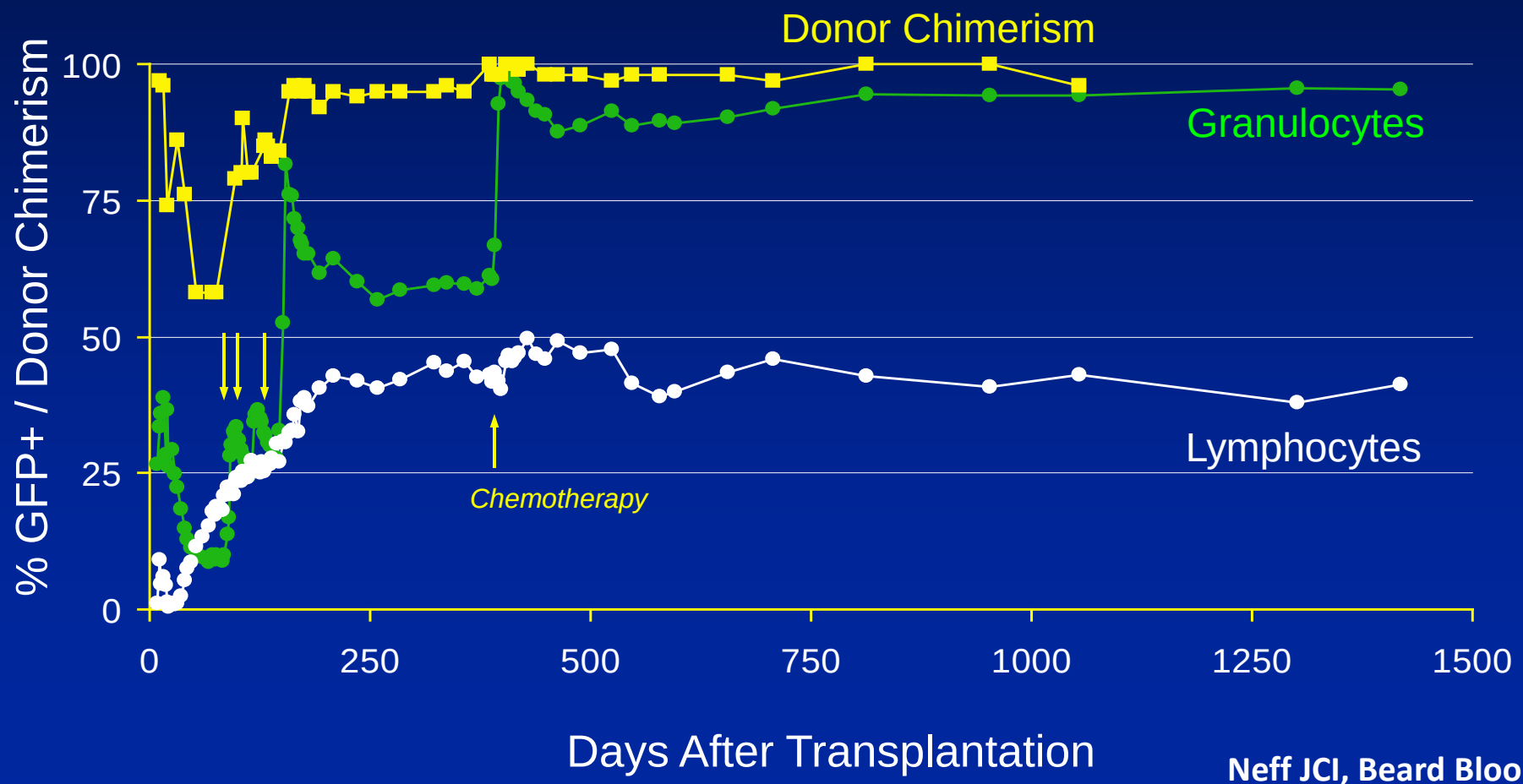




MGMT-mediated in Vivo Selection

- O^6-methylguanine-DNA methyltransferase (MGMT) repairs O^6 guanine alkylation damage formed by a variety of alkylating agents (BCNU, temozolomide)
- O6-benzylguanine inactivates MGMT, thus enhancing the effects of chemotherapy with BCNU or temozolomide
- Mutant MGMT P140K does not bind BG and thus allows for selection of gene-modified cells by BG/BCNU or BG/temozolomide

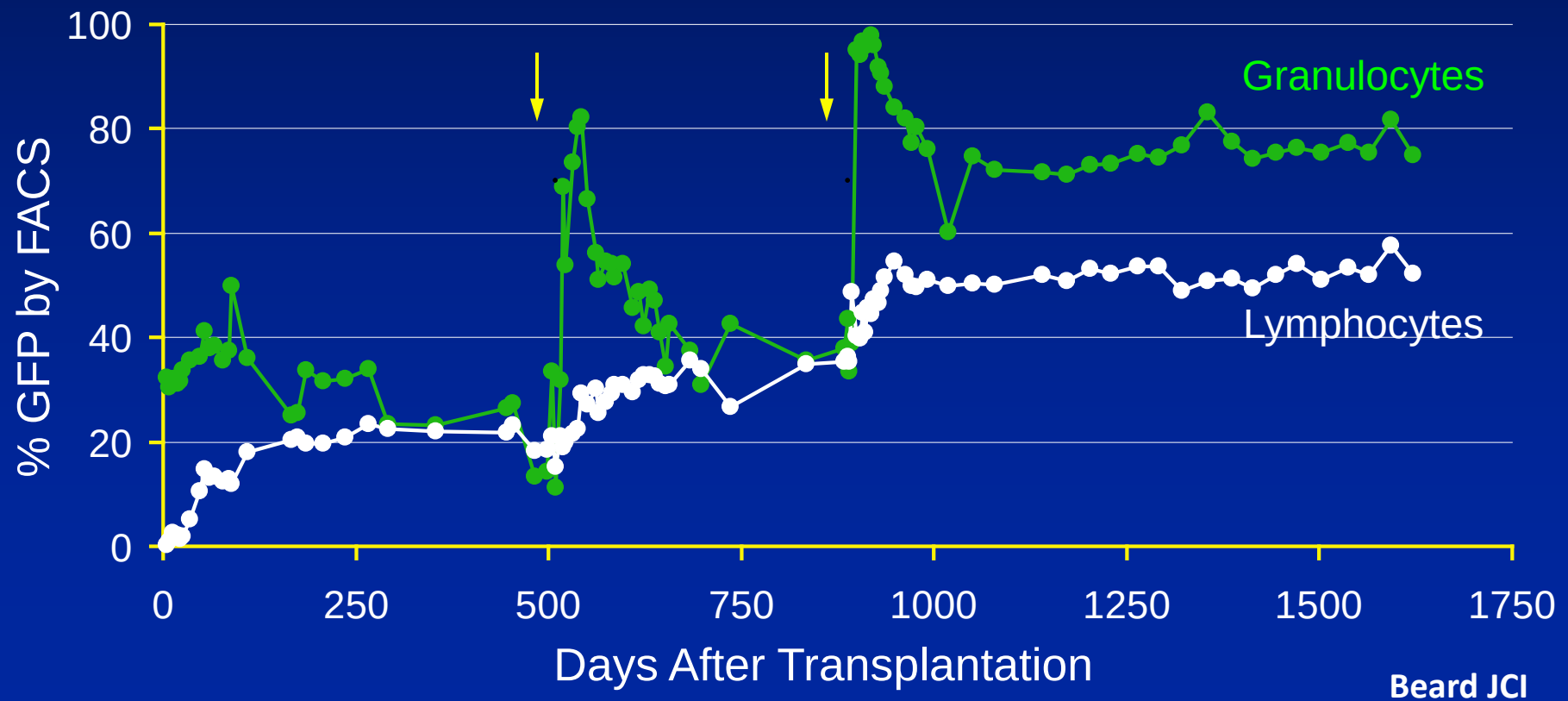
P140K-Mediated *In vivo* Selection



Neff JCI, Beard Blood

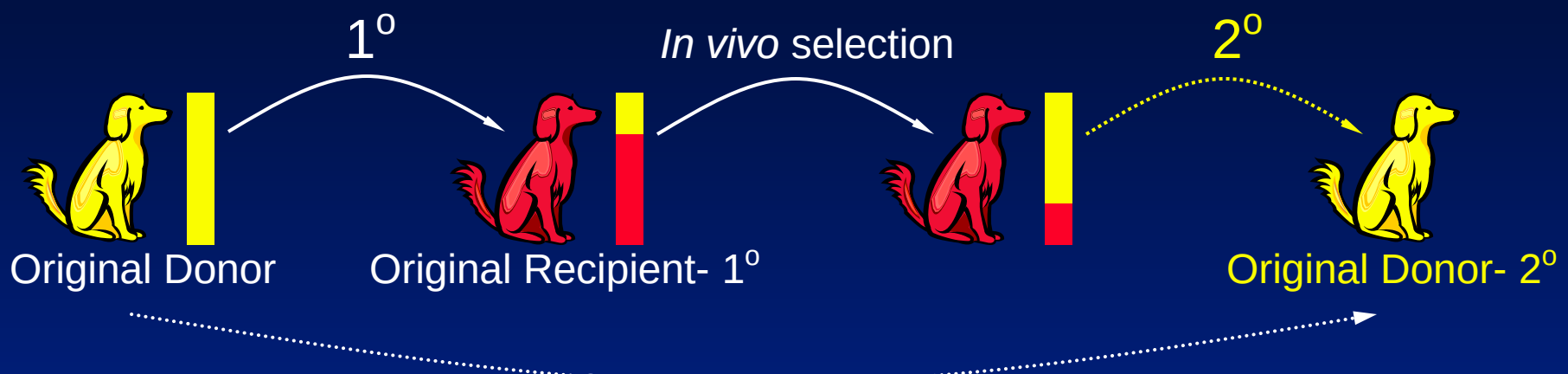
P140K-Mediated *In vivo* Selection in the Macaque

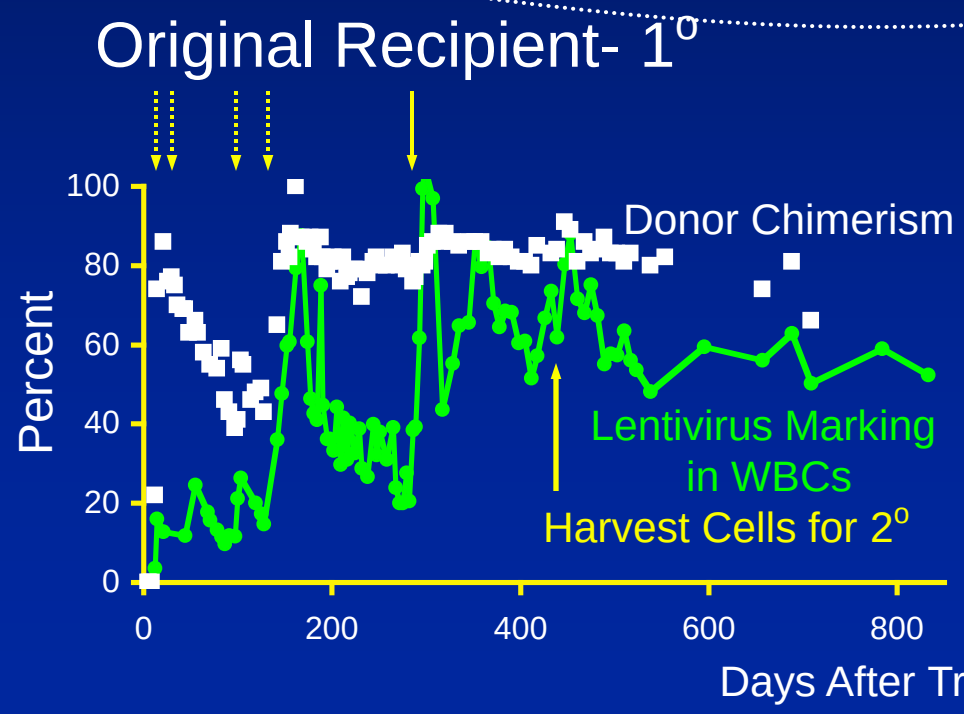
- Ablative monkey autologous gene-modified transplant
  - Lentivirus vector expressing P140K-GFP

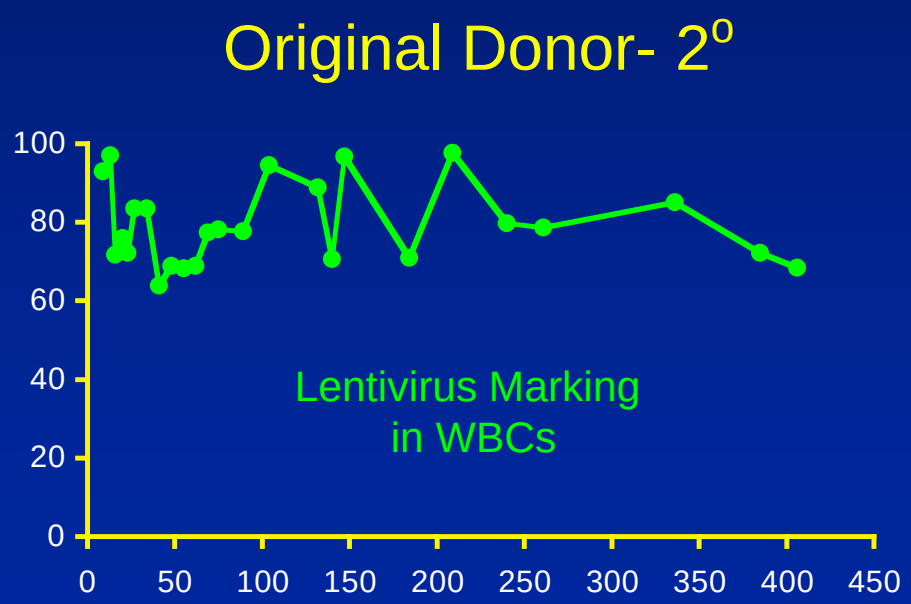


Beard JCI

Transfer of Selected Repopulating Cells to the Original Donor

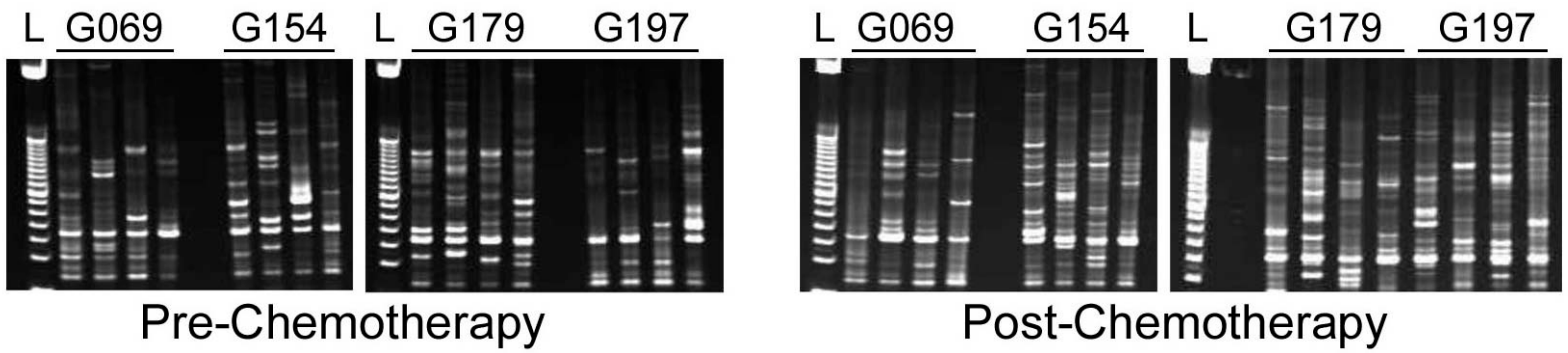


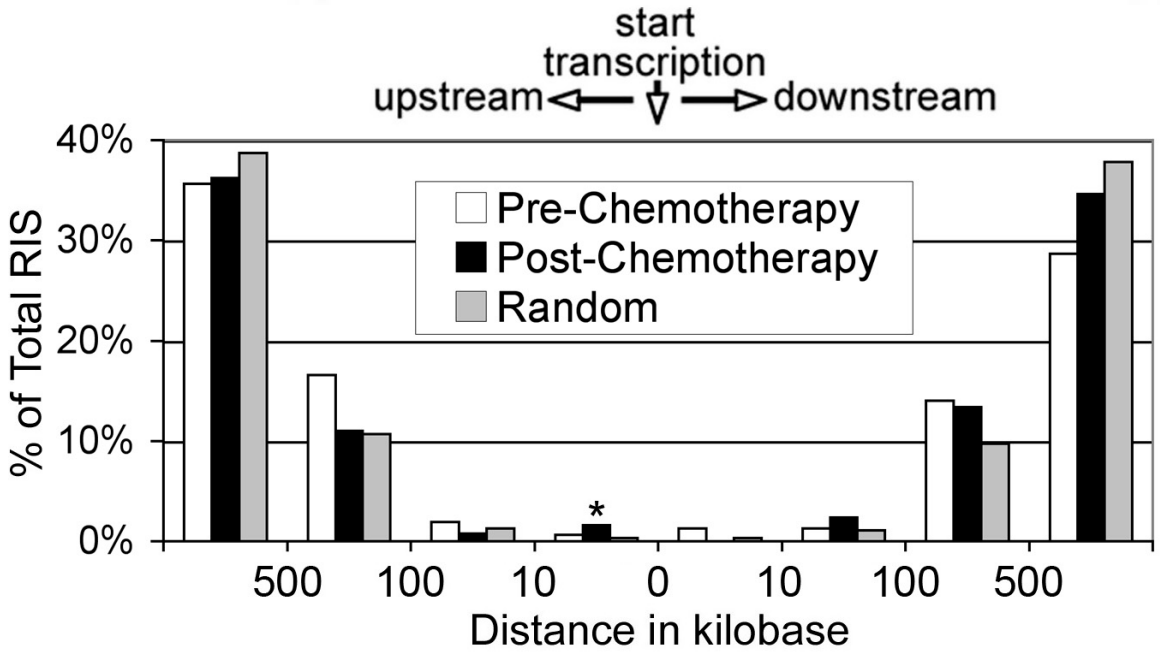




Beard et al. Blood 2009

Distribution of RIS Near Proto-Oncogenes Before and After Chemotherapy





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Use of Large Animals in Gene Therapy Studies

- Engraftment kinetics of gene-modified cells
  - Impact of stem cell dose
  - Impact of conditioning regimens
- Long-term follow up
  - Clinical
  - Vector function, expression in different lineages
  - Integration site profiles
  - Clonality
- Intact immune system allows testing of transgene or vector specific immune responses

Use of Large Animals in Gene Therapy Studies – Disease models

- Dogs–
  - Canine leukocyte adhesion deficiency
  - Canine pyruvate kinase deficiency
  - Canine SCID-X1
  - Canine hemophilia
- Nonhuman primate
  - HIV/AIDS studies

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Kiem lab

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