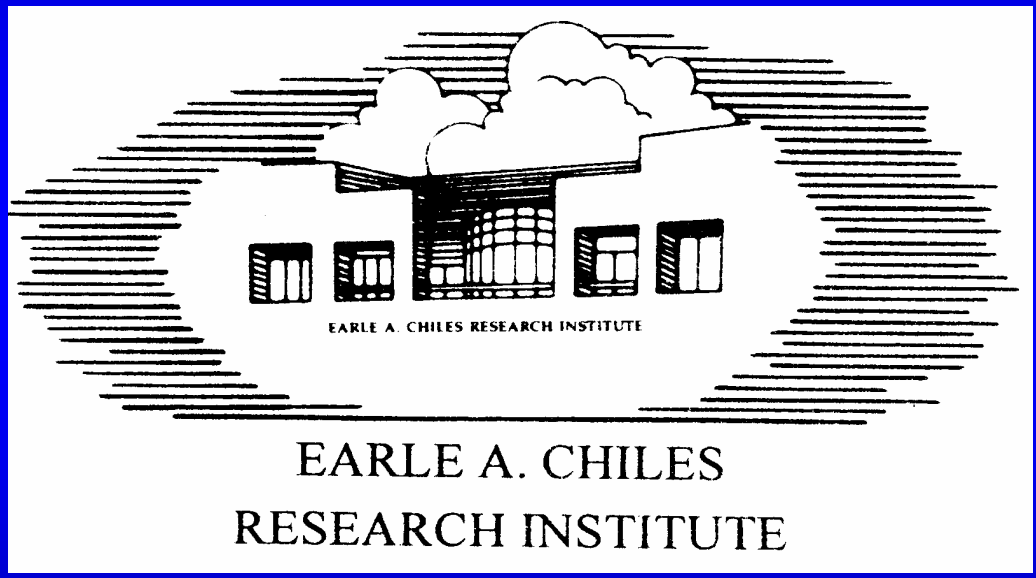
Vaccination of Reconstituted Lymphopenic Mice (RLM) Improves the Generation of Anti-tumor T cells for Active-Specific and Adoptive Immunotherapy



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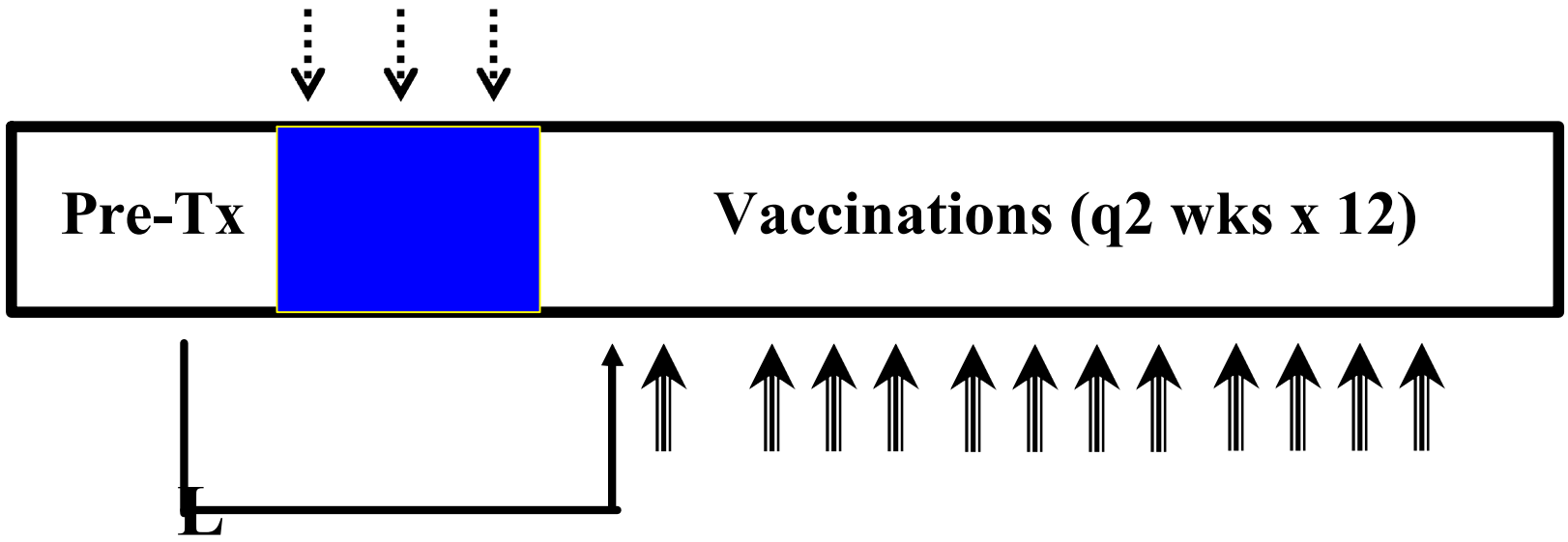
Phase I/II study of allogeneic prostate GVAX™ in advanced prostate cancer patients made lymphopenic by chemotherapy and infused with autologous PBMC - DOD PC020094 / PHS 02-200

1) None
2) Cytoxan 350 mg/m² d 1-3
3) Cytoxan + Fludarabine 20 mg/m² d 1-3



GVAX - GM-CSF secreting allo-Prostate vaccine

Homeostasis-driven Proliferation

Findings:

Naïve T-cell repertoire can be skewed toward a specific antigen, resulting in a dramatic expansion of antigen-specific T cells.

Mackall, CL et al. J Immunol 1996.
Borrello, IK, et al. Blood 2000.
Hu, H-M, et al. J. Immunother, 2000
Asavaroengchai and Mule. PNAS 99:93, 2002
Dummer et al., J. Clin Inv. 110:185, 2002
Hu, H-M, et al. Cancer Research, 2002

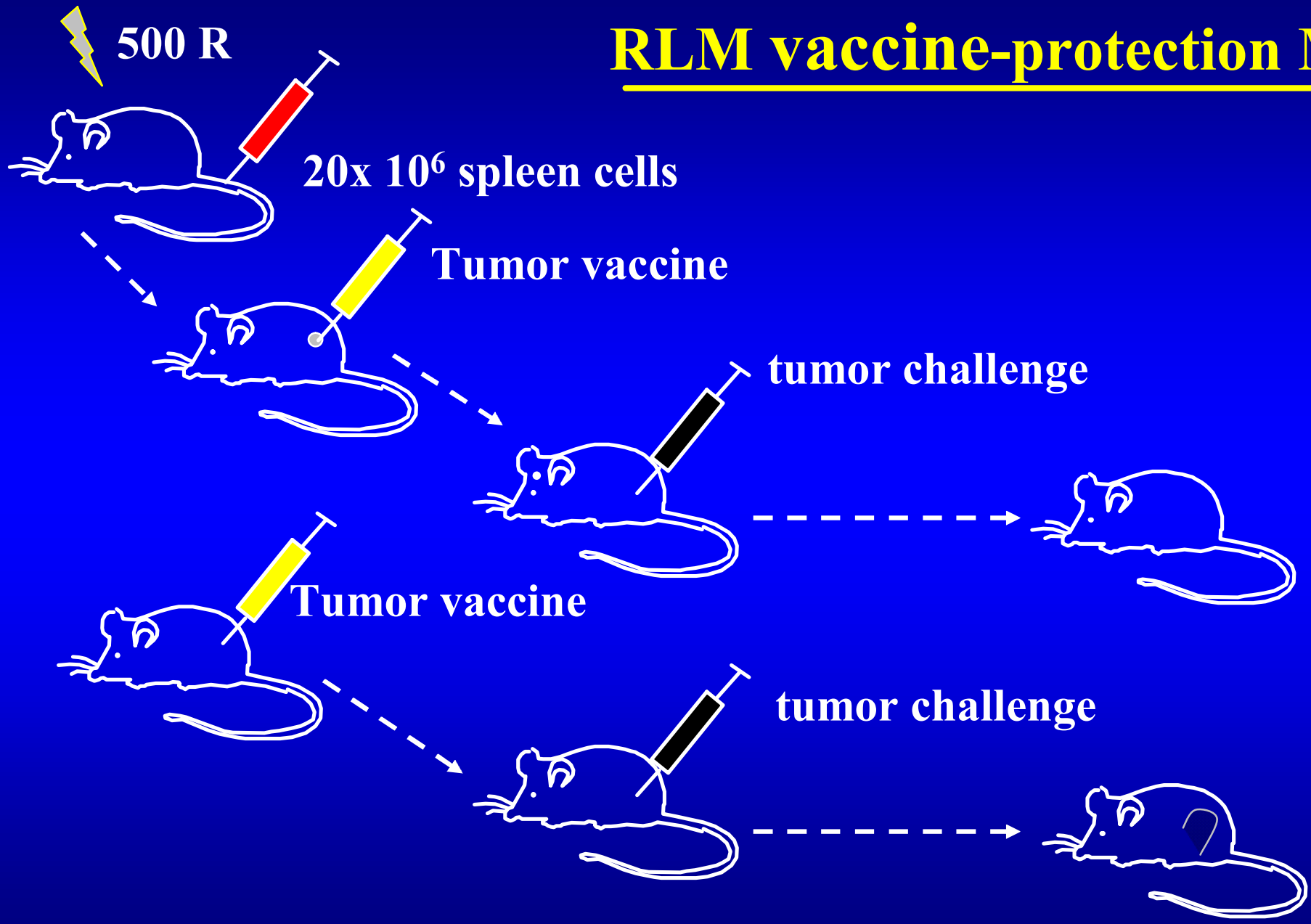
Preclinical Results:

1) Vaccination of reconstituted lymphopenic mice (RLM) is more effective at generating T cells for adoptive immunotherapy.

2) Active specific immunotherapy is also more effective in RLM mice.

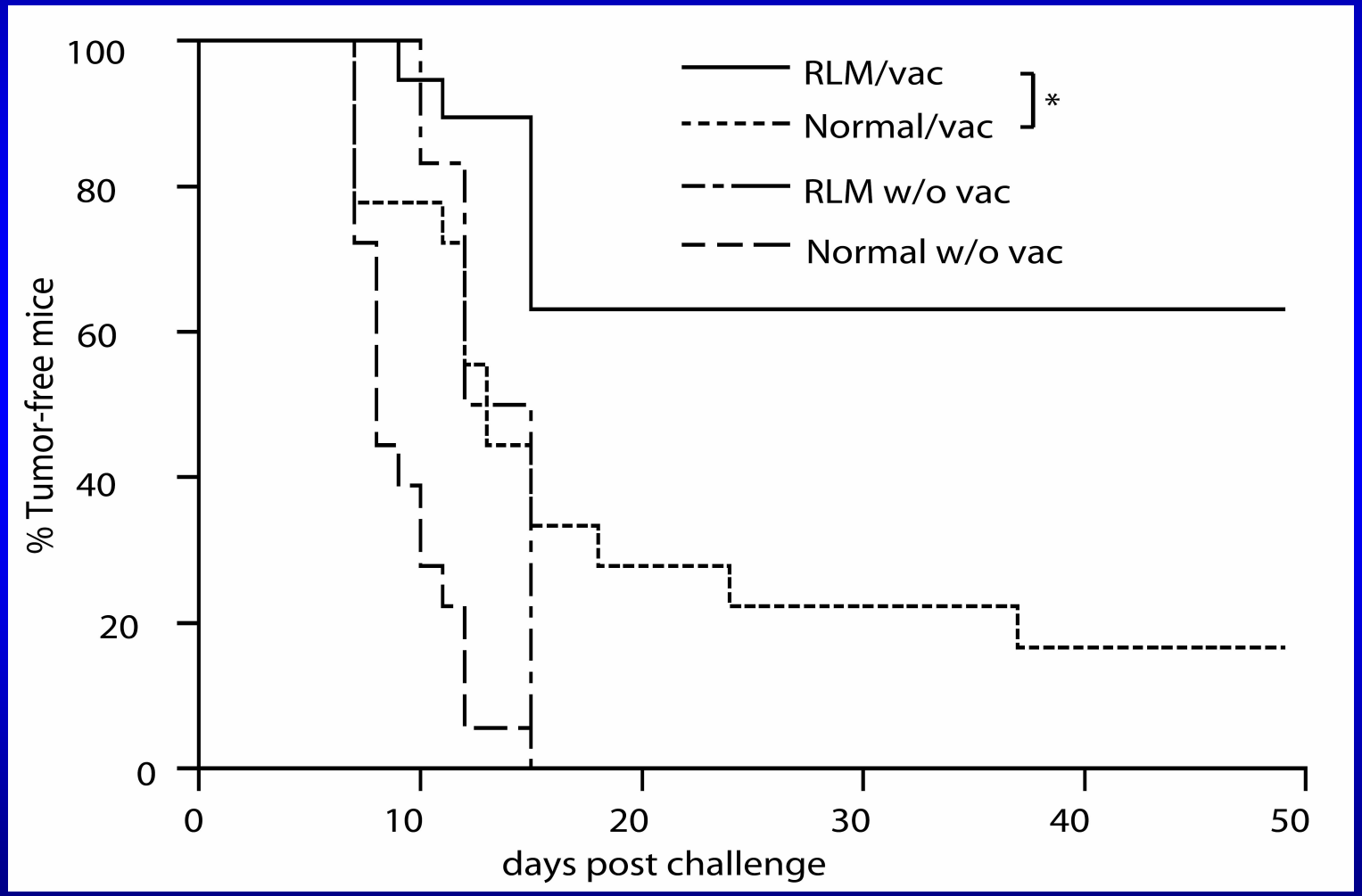
3) The method of induction of lymphopenia (e.g. genetic [RAG-/-], radiation or chemotherapy) does not affect the efficacy of this approach.

RLM vaccine-protection Model



Ma et al., Eur. J. Immunol. 33:2123, 2003

Vaccinated RLM exhibit enhanced protection from a subsequent tumor challenge



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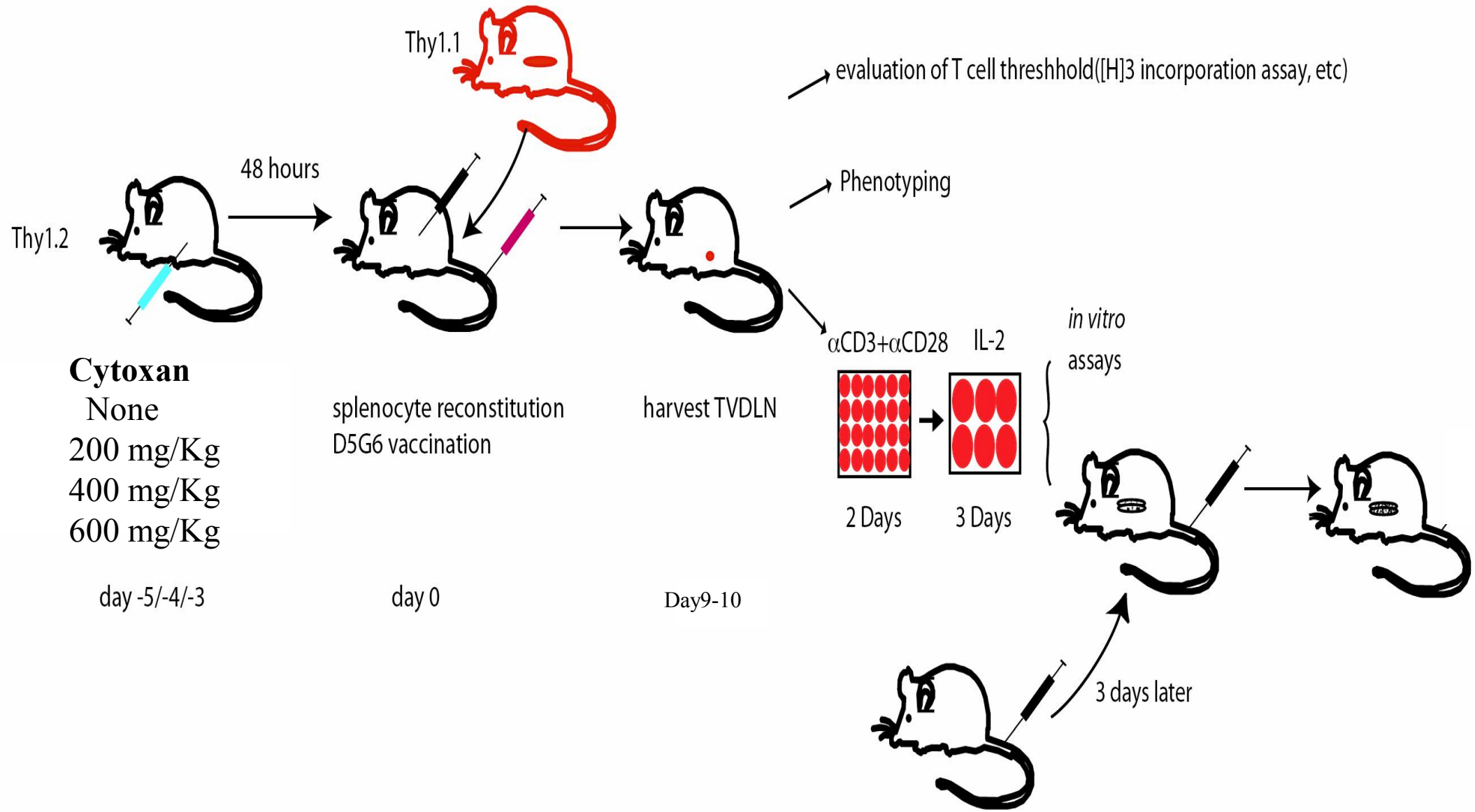
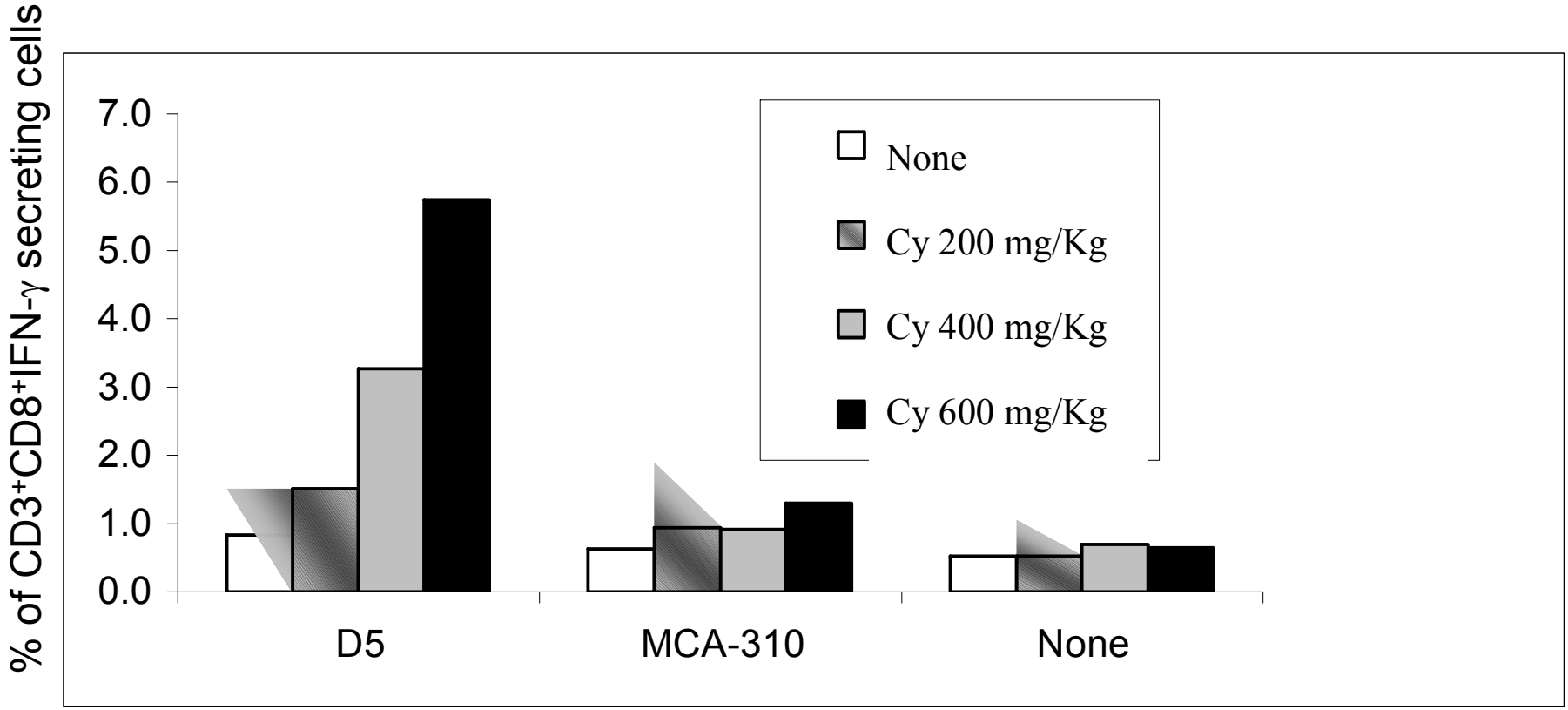
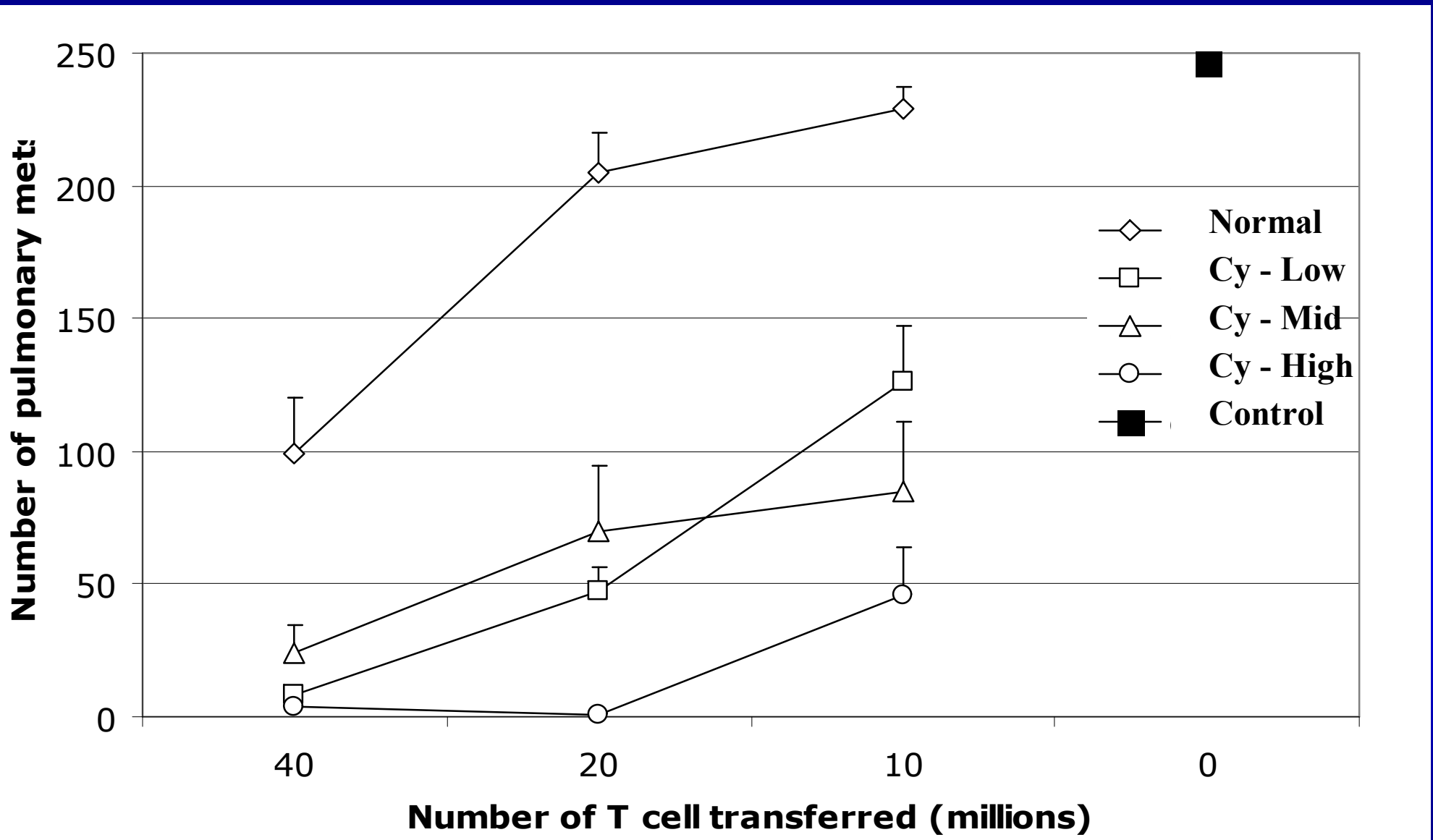
Experimental Design:



Preferential expansion of tumor-specific T cells increases with increased lymphopenia.

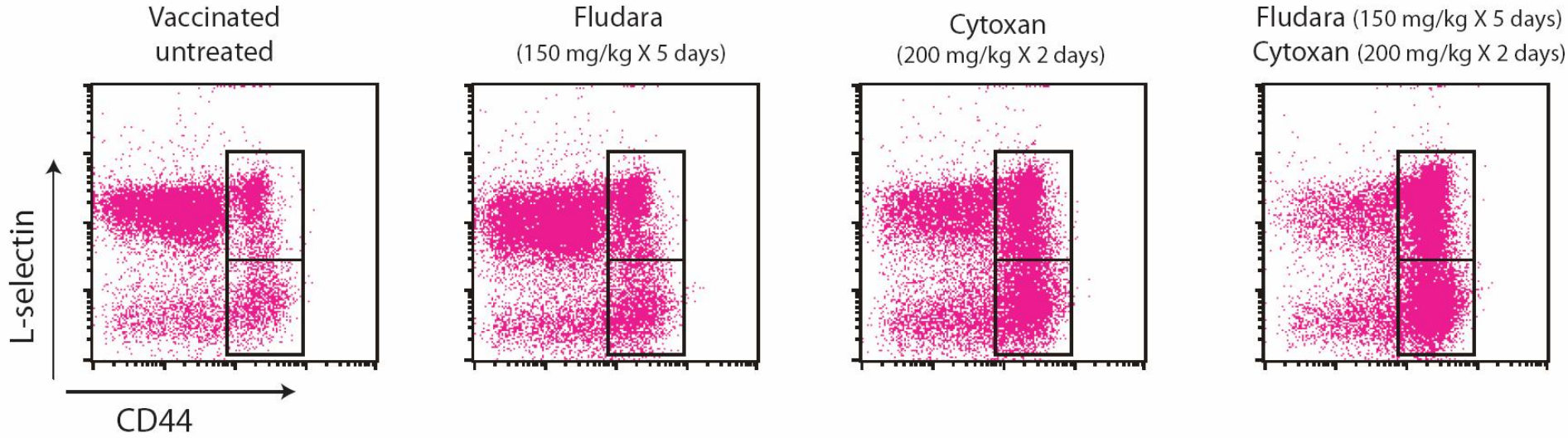



250
200
150
100
50
0
Number of pulmonary met
40
20
10
0
Number of T cell transferred (millions)
Normal
Cy - Low
Cy - Mid
Cy - High
Control

Cyclophosphamide and Fludarabine

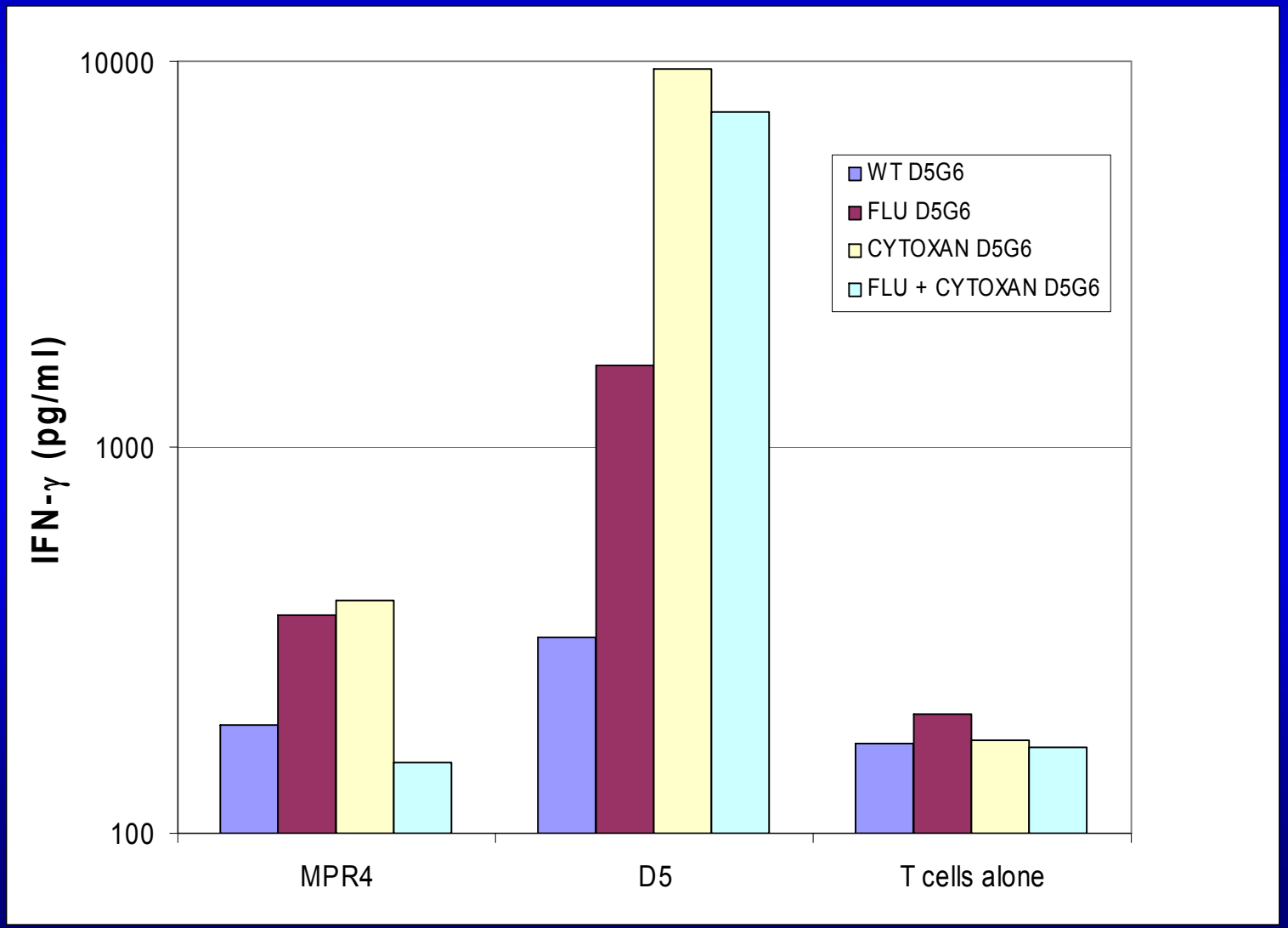
- Extent of lymphopenia appears to correlate with T-cell response, and efficacy, as long as PBMC reinfusion is employed.
- Fludarabine is known to be an excellent lymphodepleting agent in humans.
- Experience with CTX/FLU in patients suggested that it could be employed safely.

Frequency of ‘activated’ T cells Highest in CTX/FLU – treated Mice



$CD44^{hi}$	25.4	34.0	57.3	78.0
$CD44^{hi}$ / $CD62L^{lo}$	9.7	14.2	26.7	32.0

Functional Activity of 'Effector' T cells from Chemotherapy-induced Lymphopenic Mice



Summary of Rationale

- Vaccination of RLM significantly augments generation of therapeutic T cells
  - -Adoptive immunotherapy
  - -Active-specific immunotherapy

- Increased frequency of tumor-specific CD4+ and CD8+ effector T cells.

- Increased lymphopenia (by whatever means: RT; RAG-/-; or chemotherapy) leads to an increased frequency of therapeutic tumor-specific T cells.

Current Strategy

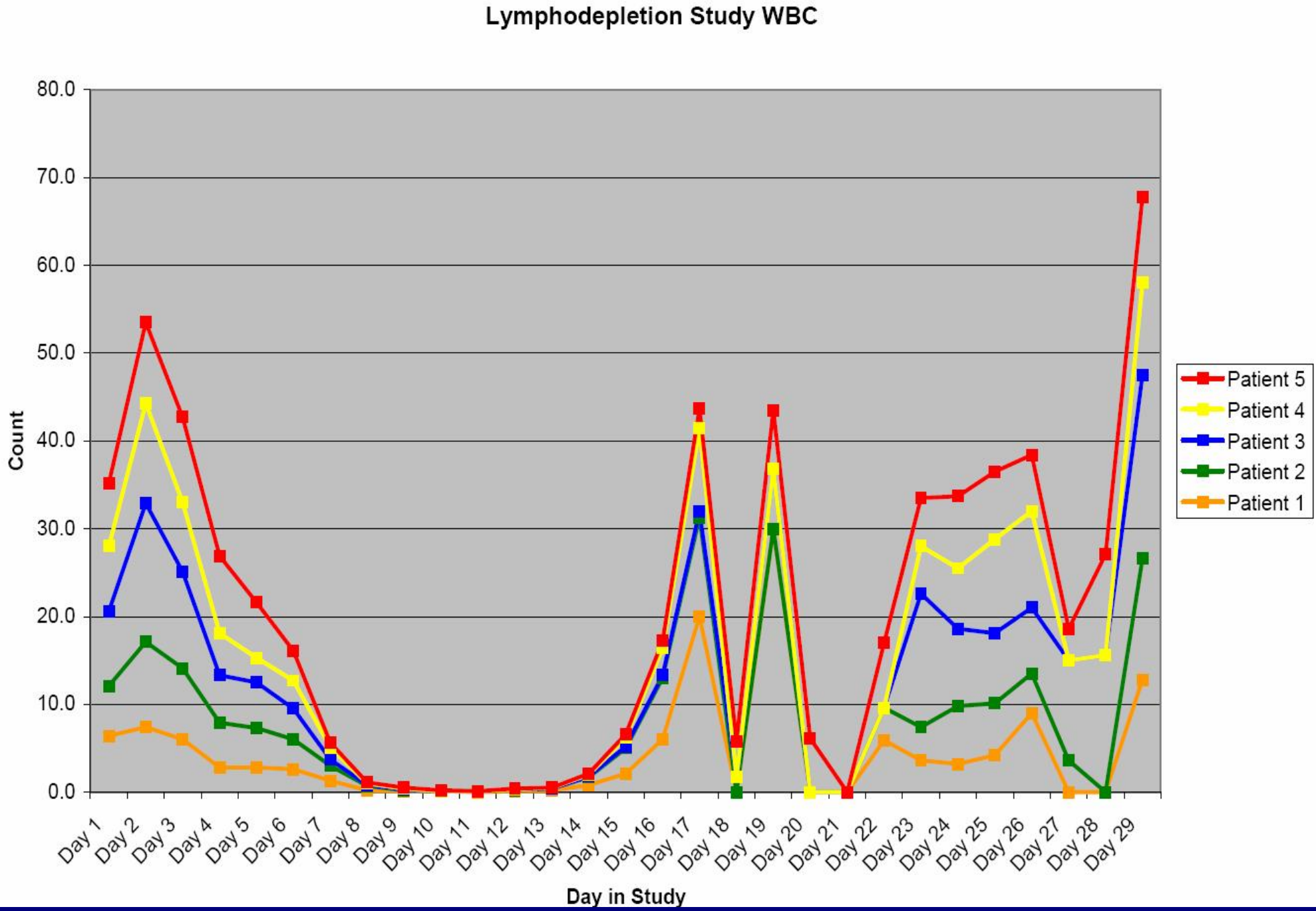
- Translate preclinical results to clinical trial.
- Chemotherapy is most readily translatable to patients with cancer.
- CTX/FLU has been used safely and successfully as non-myeloablative therapy in the transplant setting and as a preparative regimen for adoptive immunotherapy with TILs.
- CTX/Flu was chosen as the lymphodepleting regimen for our study in prostate cancer.

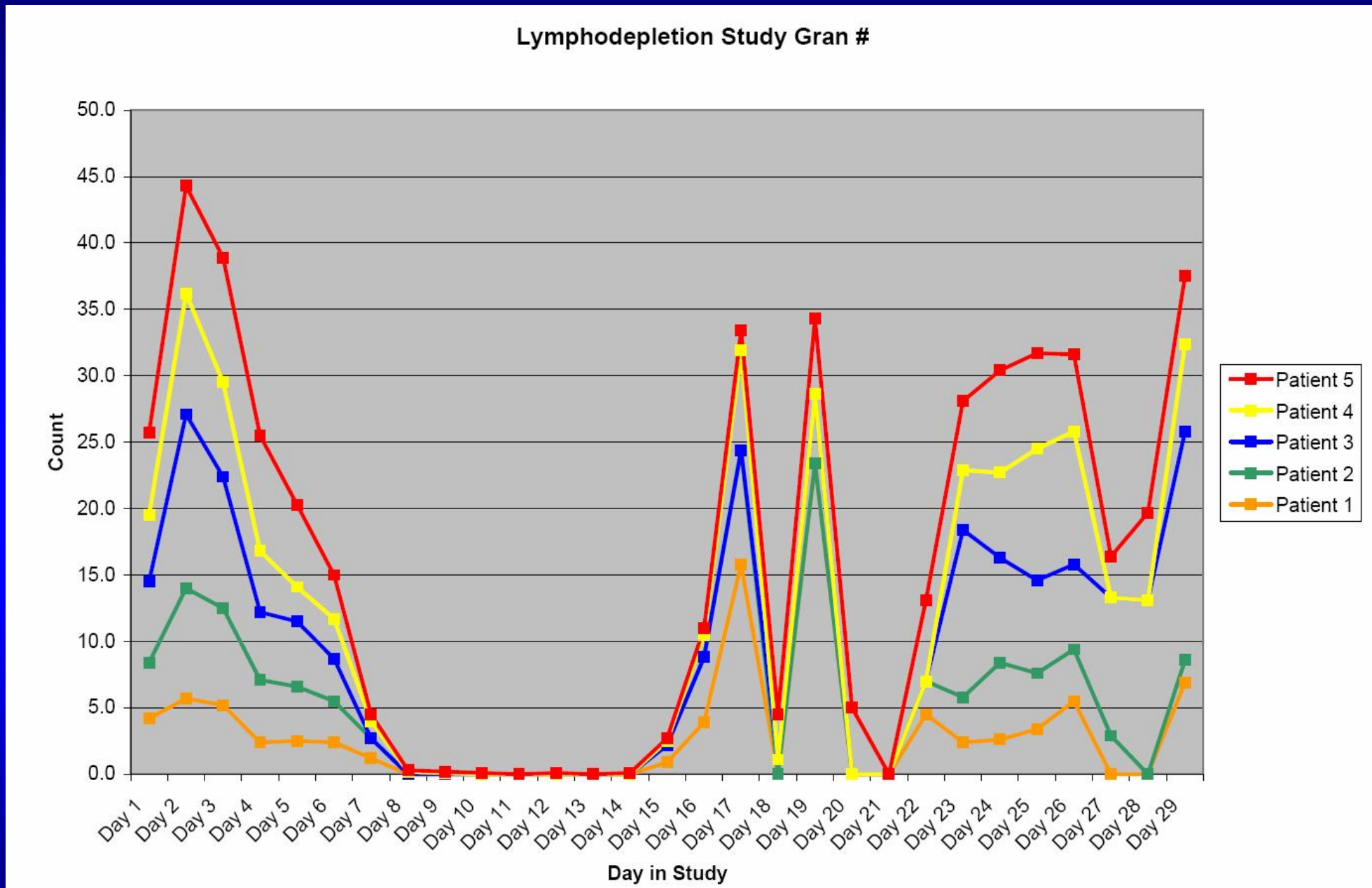
Surgery Branch Studies

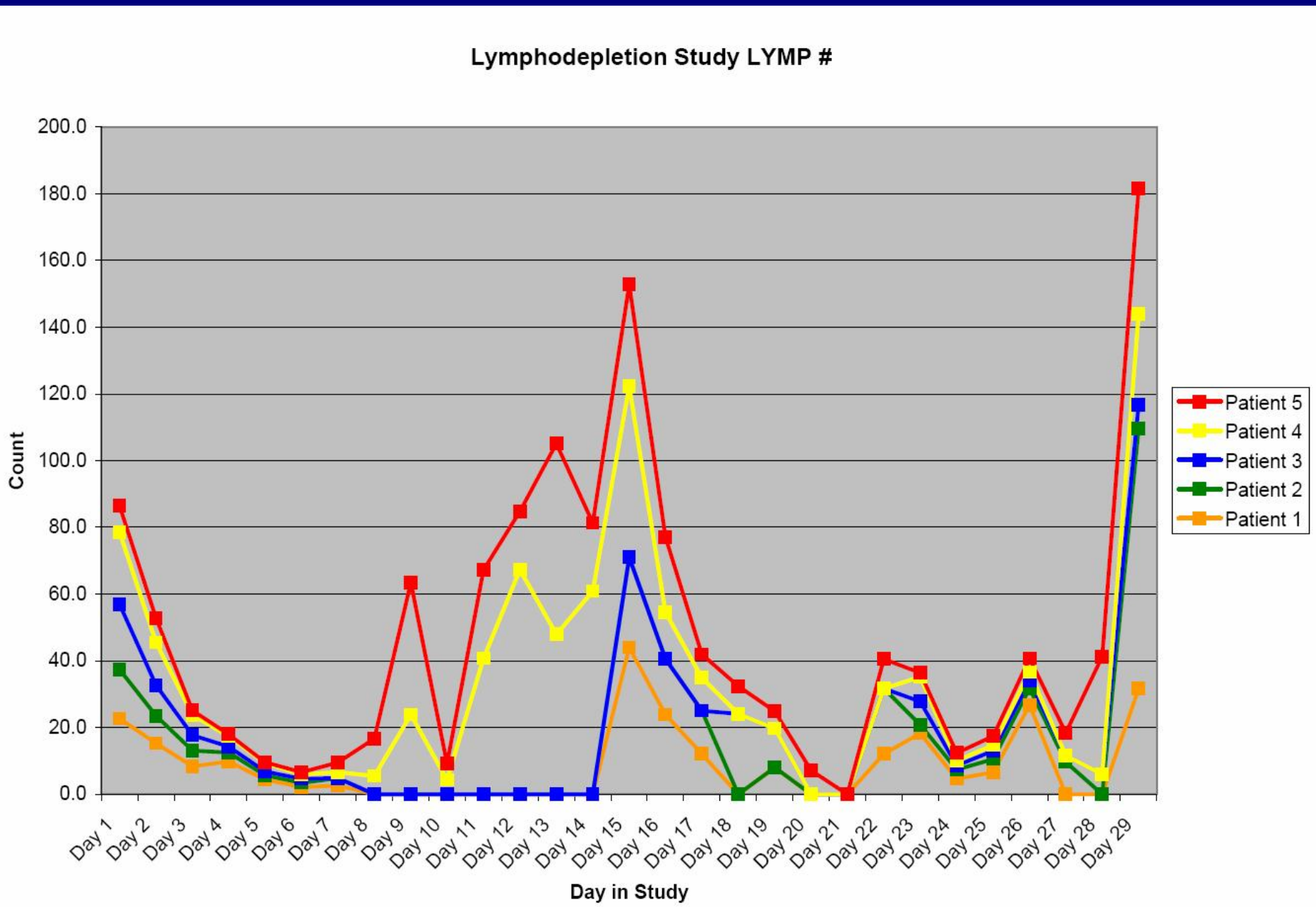
- CTX 60mg/kg for two days; Fludarabine 25mg/m2 for 5 days.
- Highly enriched tumor-specific TILs plus high-dose IL-2.
- 18 responders (including some CRs) among 35 patients.
- Neutropenia seen in all patients – recovered by day 11 after T-cell infusion.
- One EBV-related lymphoma; one RSV.
- Vitiligo and uveitis seen in a small numbers of patients.
- One patient had more than 70% of PBLs of one clonotype specific for melanoma antigen; survived for > 4 years without infection.
- Surgery Branch has concluded this is a safe regimen and are trying to increase lymphopenia by adding 200 cGy of TBI.

Ernstoff Study

- CTX 60mg/kg for two days; Fludarabine 25mg/m2 for five days.
- No cell infusion.
- High-dose IL-2 600,000 IU/kg every 8 hours X 14 .
- GM-CSF also administered.
- Neutropenia seen in all 5 treated patients.
- Myelosuppression recovered by day 16 or 17.
- No unusual infections or septicemia.


Lymphodepletion Study WBC
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Count
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Day 15
Day 16
Day 17
Day 18
Day 19
Day 20
Day 21
Day 22
Day 23
Day 24
Day 25
Day 26
Day 27
Day 28
Day 29
Day in Study
Patient 5
Patient 4
Patient 3
Patient 2
Patient 1


Lymphodepletion Study Gran #
Count
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Day 15
Day 16
Day 17
Day 18
Day 19
Day 20
Day 21
Day 22
Day 23
Day 24
Day 25
Day 26
Day 27
Day 28
Day 29
Day in Study
Patient 5
Patient 4
Patient 3
Patient 2
Patient 1


Lymphodepletion Study LYMP #
Count
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Day 8
Day 9
Day 10
Day 11
Day 12
Day 13
Day 14
Day 15
Day 16
Day 17
Day 18
Day 19
Day 20
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Patient 5
Patient 4
Patient 3
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Phase I/II study of allogeneic prostate GVAX™ in advanced prostate cancer patients made lymphopenic by chemotherapy and infused with autologous PBMC - DOD PC020094 / PHS 02-200

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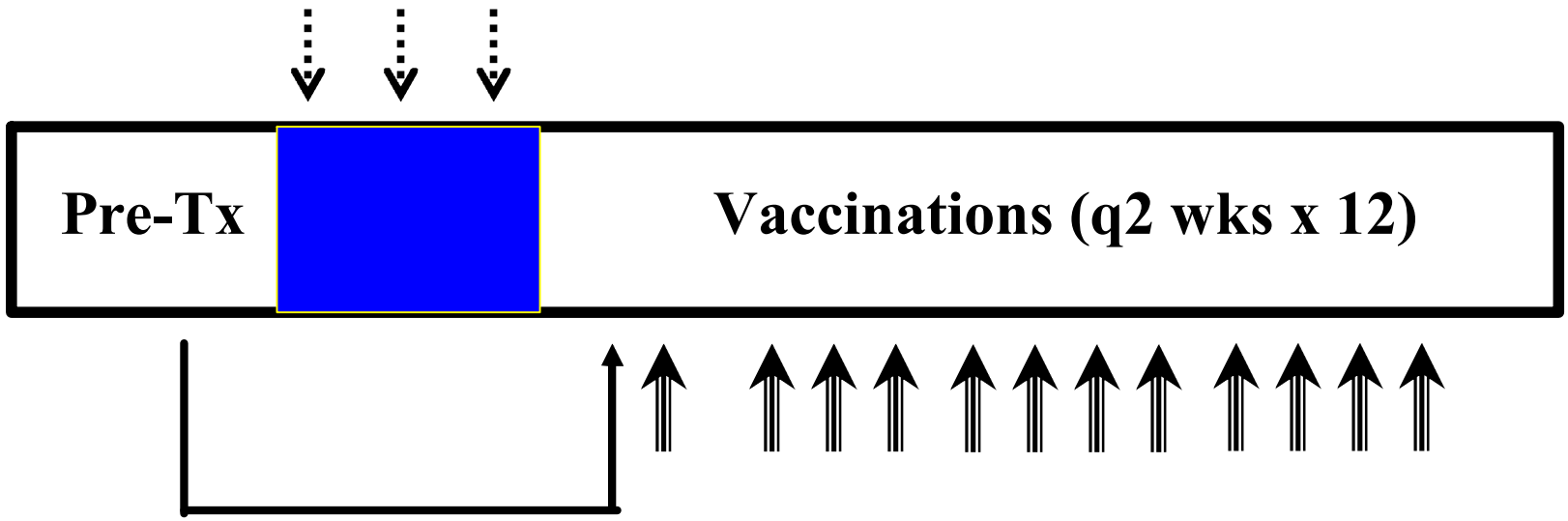

Challenges of the Trial Design

1) Control lymphopenia-driven proliferation

2) Maintain T-cell repertoire

3) Minimize risk of infections

4) Manage potential problems with autoimmunity

Challenges of the Trial Design:

1) Control lymphopenia-driven proliferation
2) Maintain T-cell repertoire

- Reconstitute patients with leukapheresis product obtained before chemotherapy at the time of vaccination.

- Infusion of “normal” lymphocytes may help control both the timing of lymphocyte recovery and maintain a significant component of the pretreatment repertoire.

Challenges of the Trial Design:

3) **Minimize risk of infection:**

- **Total dose of drugs much lower in our study: CTX about 20% and fludarabine about 50%.**
- **Higher doses have already been shown to be safe.**
- **Infusion of "normal" PBMC will help marrow recovery time and maintain a large component of pretreatment repertoire.**
- **Cell infusion could limit "dangerous" skewing of repertoire, which is unexpected.**
- **Monitor recovery of EBV and CMV responses.**

Challenges of the Trial Design:

4) Manage potential problems of autoimmunity:

- Autoimmunity is an expected outcome if immune responses are potent.
- Autoimmunity may cause disease, which if localized to the prostate is not expected to be a major problem.
- Systemic autoimmune disease is a possibility.
- Patients will be observed closely for disease and treated with appropriate measures – e.g. steroids.
- Precedence exists for association of autoimmune disease and clinical efficacy in patients treated with anti-CTLA4 (steroids are very effective in this setting).

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Xi'an
Jun Ma
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Cell Genesys
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