*Presenter Disclosure*

Richard G. Vile

Nothing To Disclose

# VSV as an Adjuvant for Tumor Viro/Immunotherapy

Society for Immunotherapy of Cancer,
SITC
Bethesda, MD
October, 2012

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# Oncolytic Virotherapy: The Paradigm

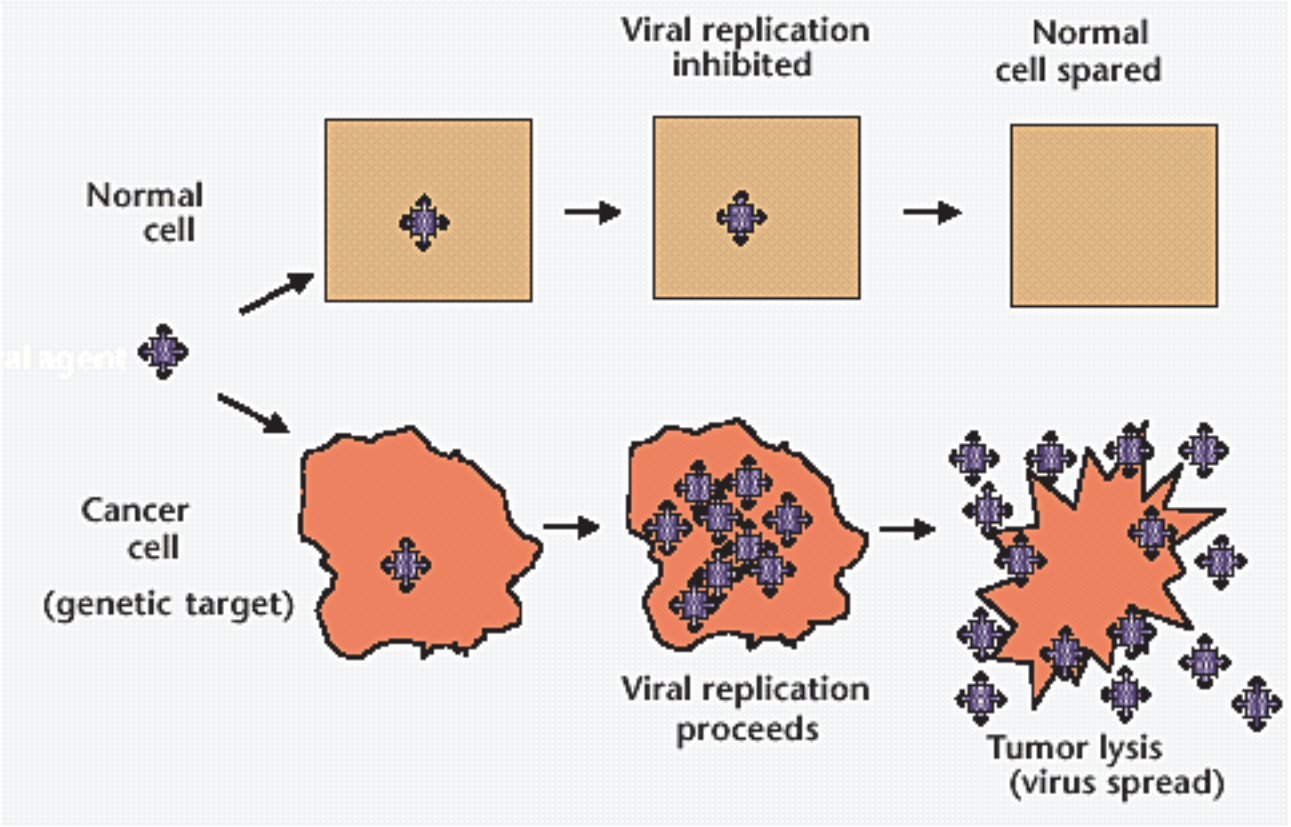


Figure 1. Schematic representation of tumor-selective viral replication and oncolysis

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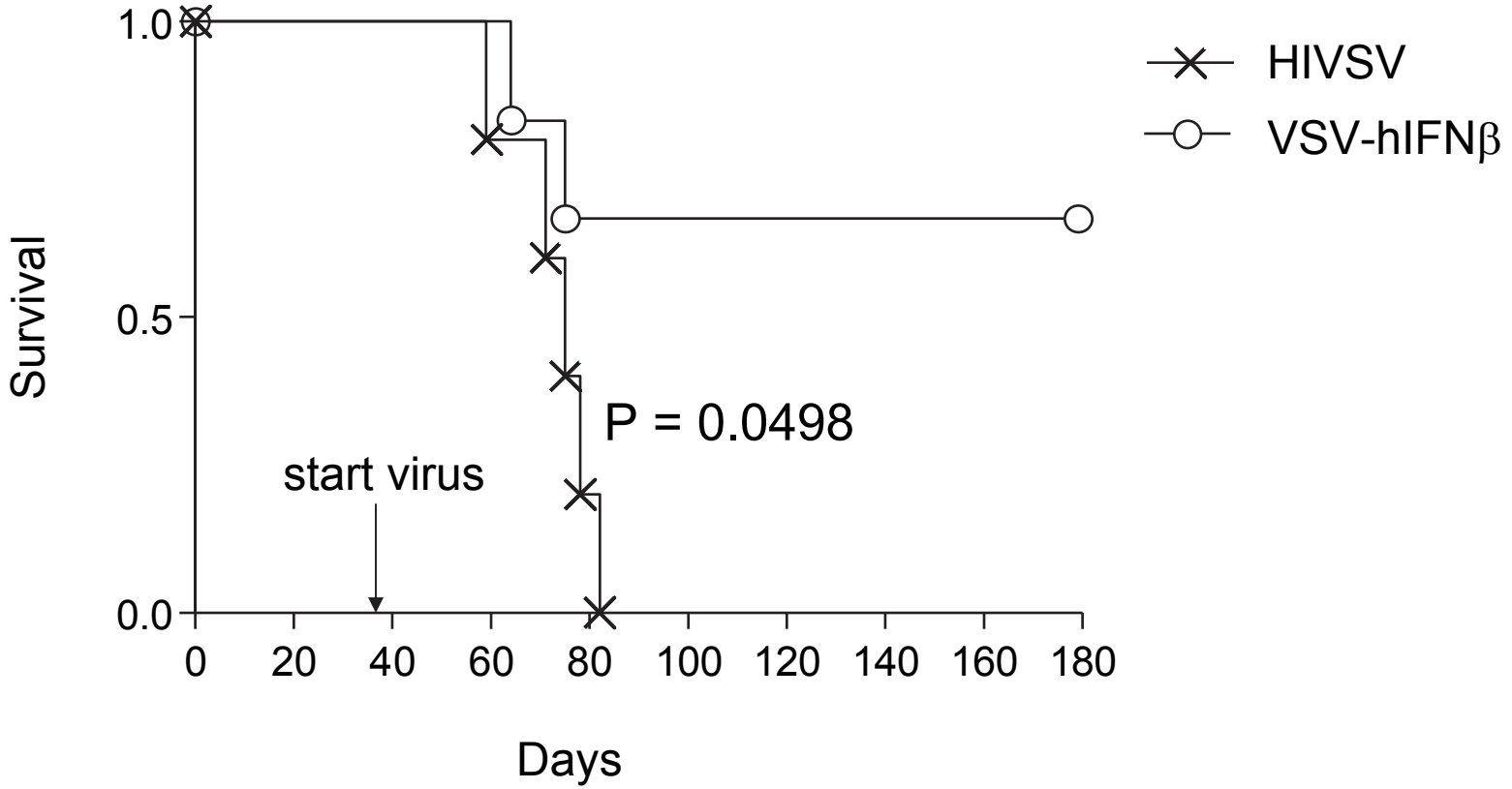
Nature Medicine 7, 781 - 787 (2001)
David Kirn, Robert L. Martuza & James Zwiebel

# VSV-hIFNβ has Anti-Tumor Activity Against Established Human Hep3B HCC Xenografts




Nude mice bearing large established (50-80mm$^3$) Hep3B subcutaneous tumors were injected intratumorally with 5x10$^8$ pfu of VSV-hIFNβ for a total of 6 injections every other day for 2 weeks (5-6 mice/group). Survival of mice with time is shown. HI = heat inactivated control.

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# MC1148 – FIRST IN HUMAN STUDY OF VSV-hIFNβ

## Phase I Trial of Intratumoral Injection of VSV-hIFNβ in Patients with Sorafenib Refractory/Intolerant Hepatocellular Carcinoma

Mitesh J. Borad, Mayo Clinic, Scottsdale, AZ

Stephen Russell, Mayo Clinic, Rochester, MN

Kah-Whye Peng, Mayo Clinic, Rochester, MN

Jorge Rakela, M.D., Mayo Clinic, Scottsdale, AZ

Mark Federspiel, Mayo Clinic, Rochester, MN

Richard G. Vile, Mayo Clinic, Rochester, MN

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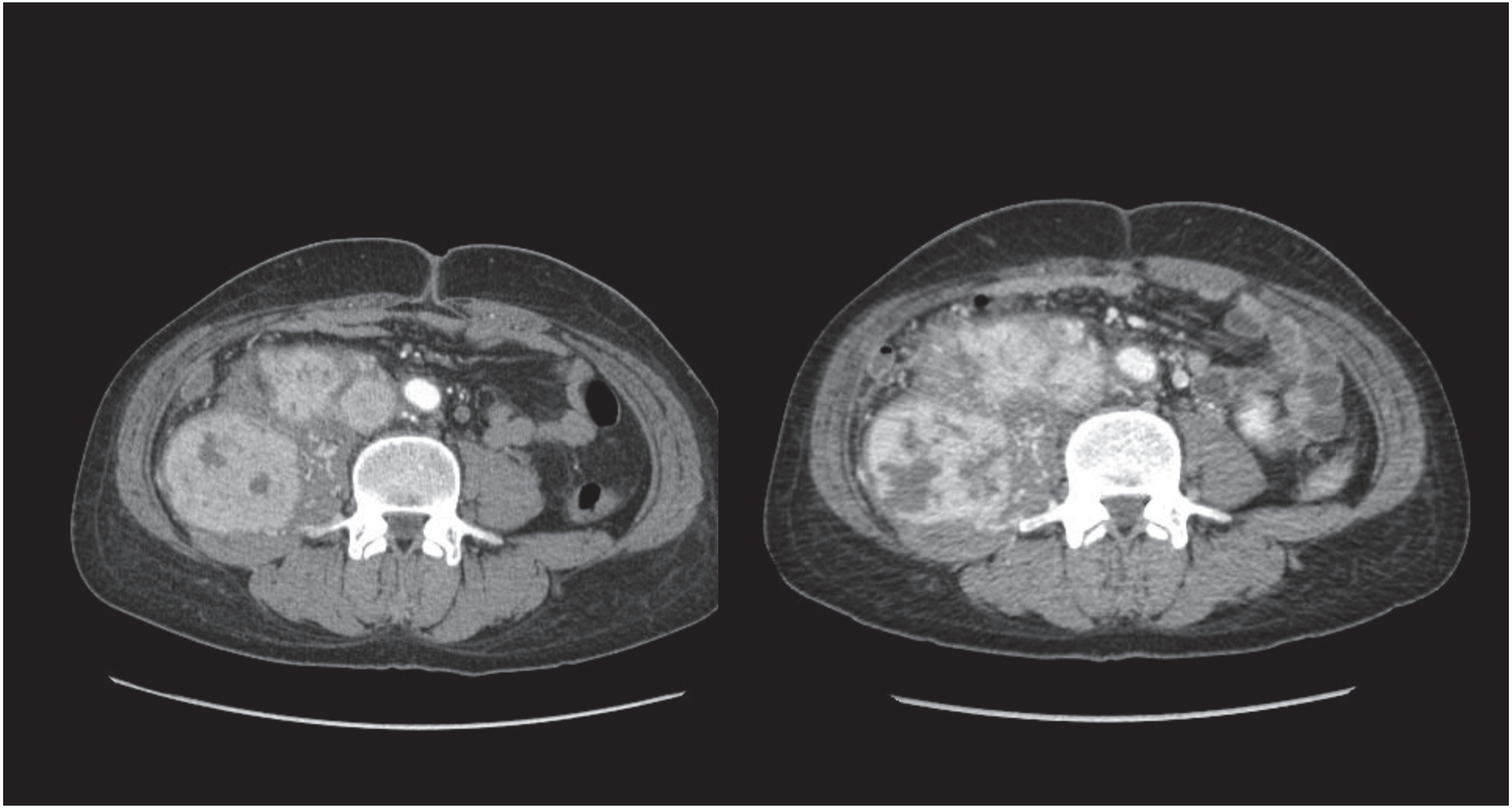
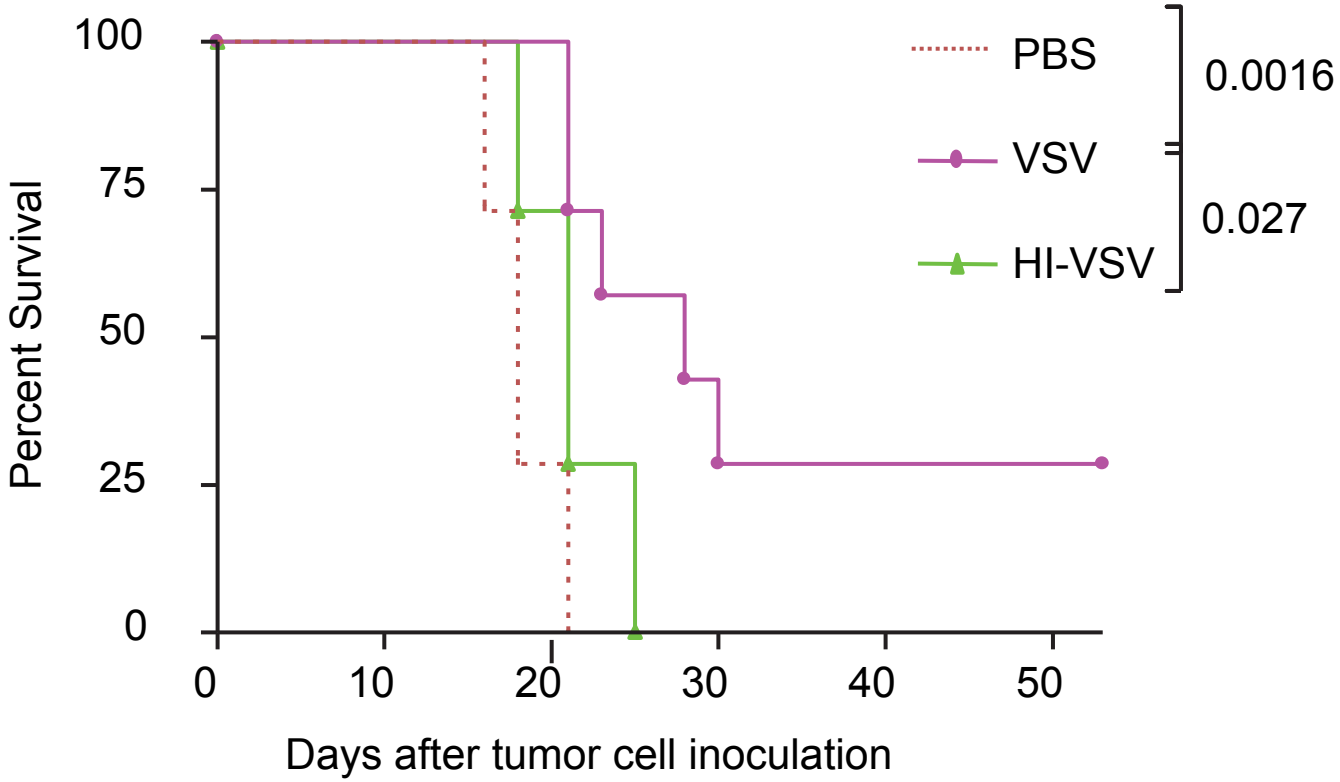
# Patient 1



PRE-TREATMENT

DAY 28

# VSV is Oncolytic Against B16ova Melanomas in C57BL/6 Mice

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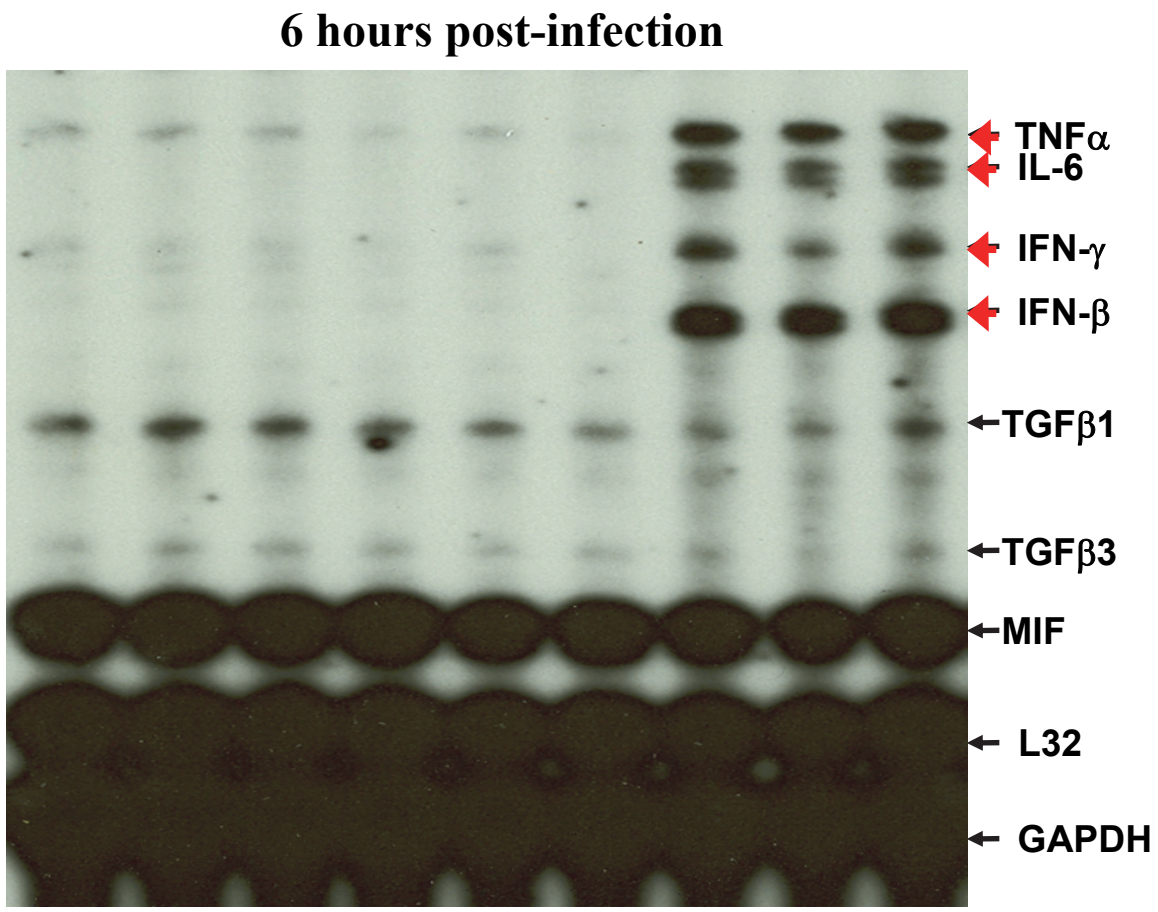
# VSV induces a strong innate immune response in the tumour microenvironment




Tumor RNA → Ribonuclease Protection Assay

*one lane corresponds to tumor RNA sample from 1 mouse (3 mice/group)

Galivo *et al.*, Gene Therapy,2009

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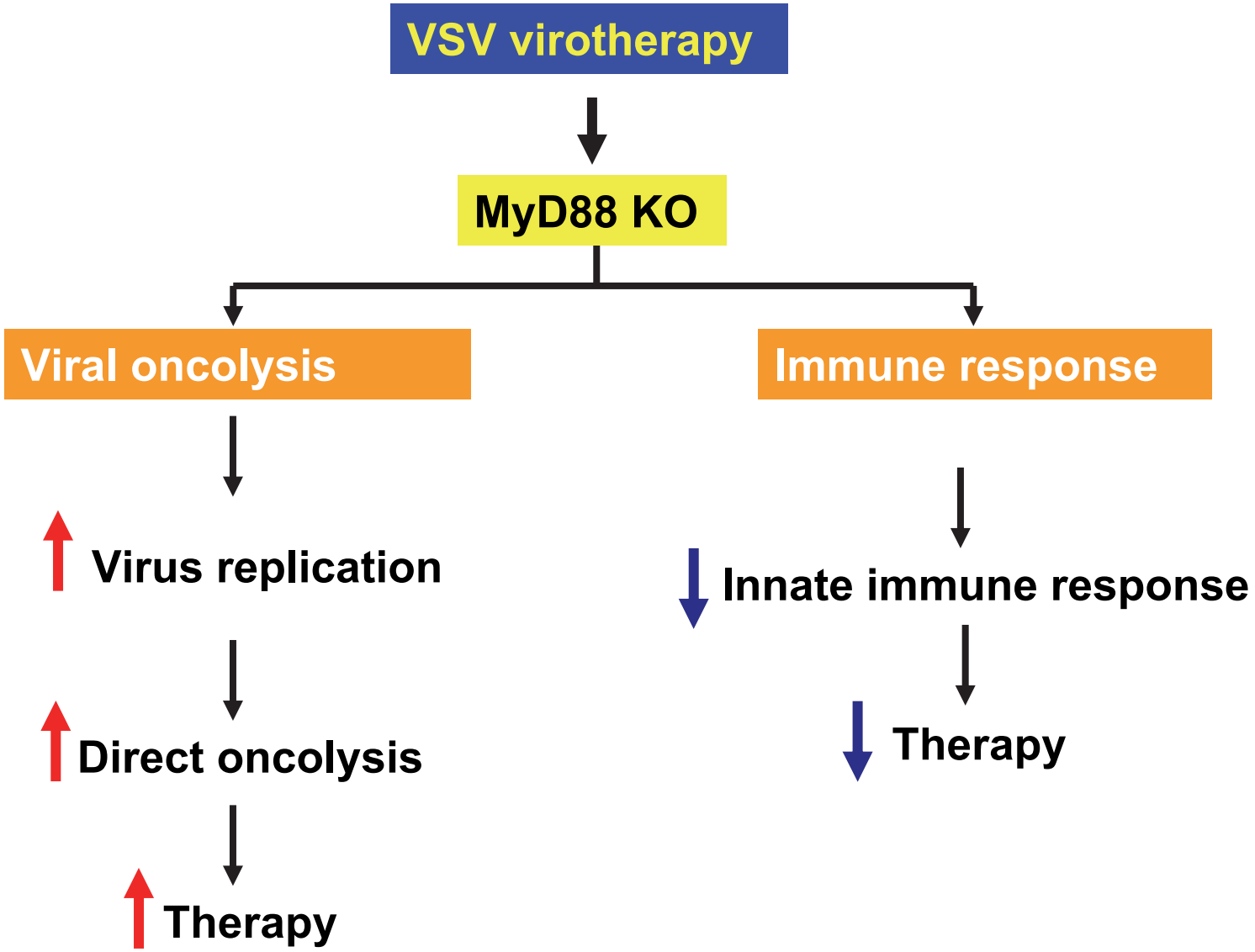
VSV virotherapy
MyD88 KO
Viral oncolysis
Immune response
Virus replication
Direct oncolysis
Therapy
Innate immune response
Therapy

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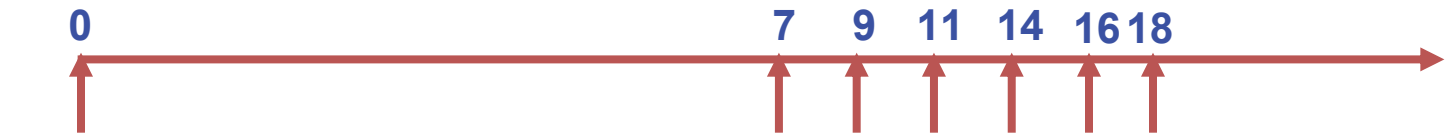
# VSV therapy is significantly decreased in MyD88/KO mice

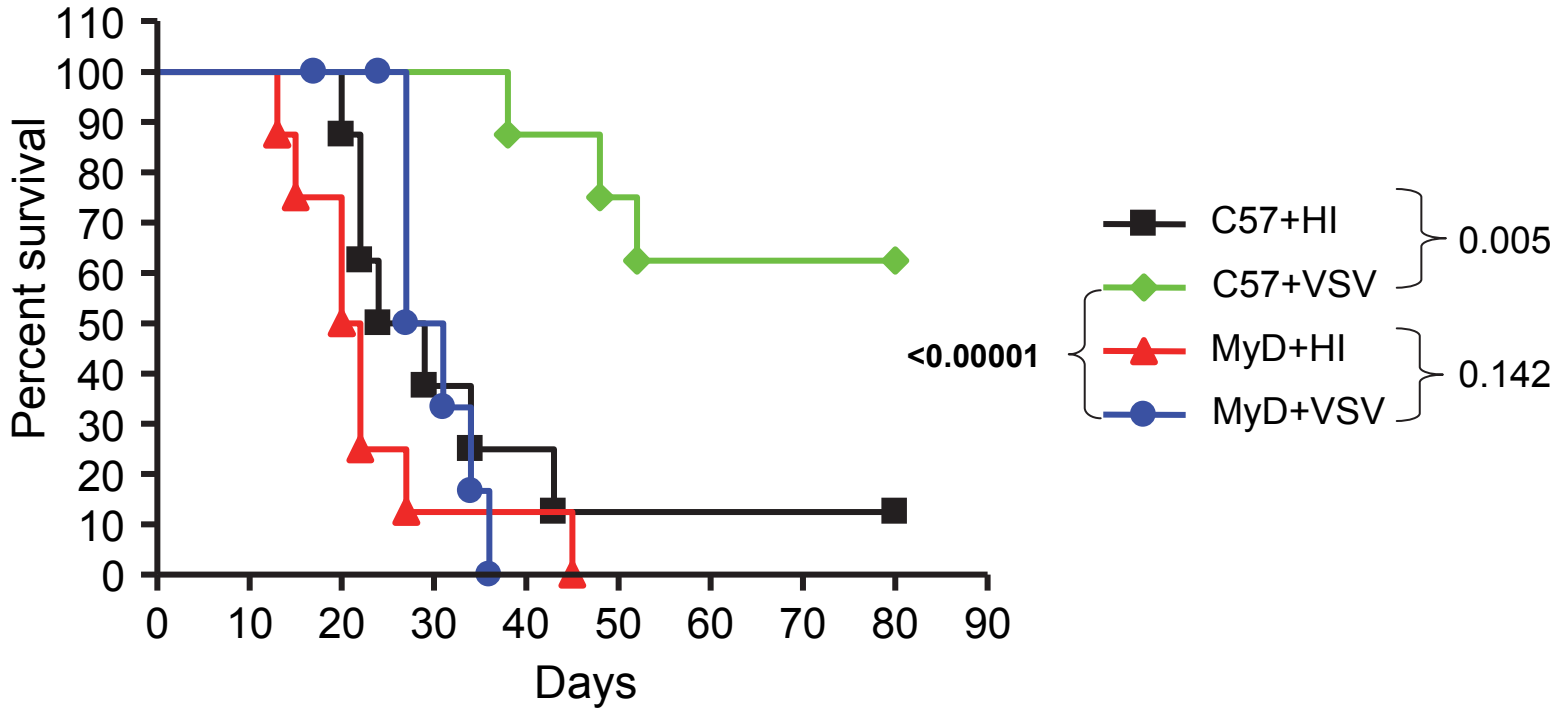



Sc Injection of B16ova cells

$5x10^5$ cells/100 µl

IT Injection of VSVs

$5x10^8$ pfu/50 µl




Therefore, MyD88 signaling provides a significant signal associated with the therapy of VSV

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Wongthida *et al, Cancer Research*

Anti tumor therapy in the B16ova model is mediated through viral-induced activation of innate immune signaling which is then responsible for killing of both infected and non infected bystander tumor cells.

Diaz *et al.*, Cancer Research, 2007
Willmon *et al.*, Cancer Research, 2009
Galivo *et al.*, Human Gene Therapy, 2010
Galivo *et al.*, Gene Therapy, 2010
Wongthida *et al.*, Cancer Research, 2010
Wongthida *et al.*, Molecular Therapy, 2011

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## VSV as a Pure Oncolytic:

-Highly sensitive to the host anti-viral IFN Response;

-***IF*** tumor cells are truly, and completely, defective in all aspects of the IFN response, VSV will be an excellent oncolytic.

-*In reality*, many tumour cells still have the ability to produce, and/or respond to (stroma-produced) host anti-viral IFNs upon infection with VSV;

-The host inflammatory response to viral infection/replication will act to inhibit viral spread

## VSV as an Oncolytic Adjuvant:

-The host inflammatory response to viral infection/replication will act as an excellent adjuvant to prime adaptive T cell responses against virally/tumor encoded antigens

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# Hypothesis

**By encoding multiple TAA in VSV, the strong immunostimulatory properties of VSV will allow for the priming of effective anti-tumor T cell responses against those TAA**

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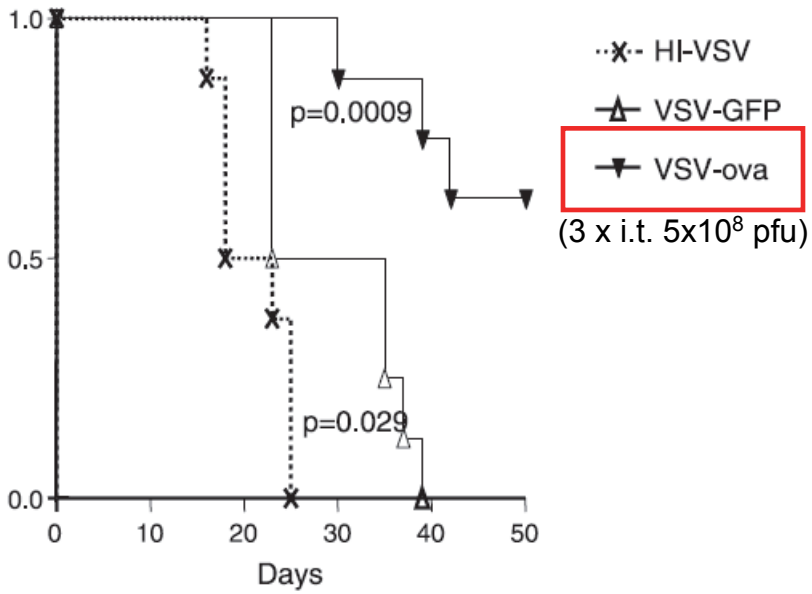
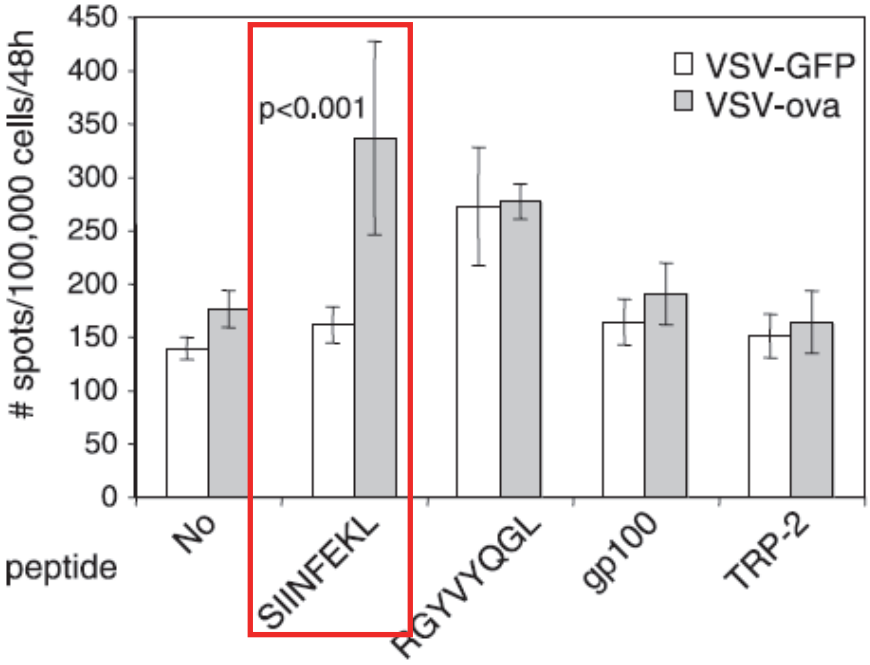
# Enhancing adaptive antitumor immunity with VSV-TAA

**B16ova melanoma**




Diaz R., *et al.* (2007) ***Cancer Res***. 67: 2840-2848

Viral-encoded TAA enhances the generation of activated antitumoral CD8 T cells

Anti-ova priming is associated with VSV-ova trafficking to the lymph nodes directly

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A close correlation between the development of anti tumor immune responses, and the development of autoimmune manifestations, has been anecdotally reported for many years in both pre-clinical and clinical settings…

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

# Prognostic Significance of Autoimmunity during Treatment of Melanoma with Interferon

Helen Gogas, M.D., John Ioannovich, M.D., Urania Dafni, Sc.D., Catherine Stavropoulou-Giokas, M.D., Konstantina Frangia, M.D., Dimosthenis Tsoutsos, M.D., Petros Panagiotou, M.D., Aristidis Polyzos, M.D., Othonas Papadopoulos, M.D., Alexandros Stratigos, M.D., Christos Markopoulos, M.D., Dimitrios Bafaloukos, M.D., Dimitrios Pectasides, M.D., George Fountzilas, M.D., and John M. Kirkwood, M.D.

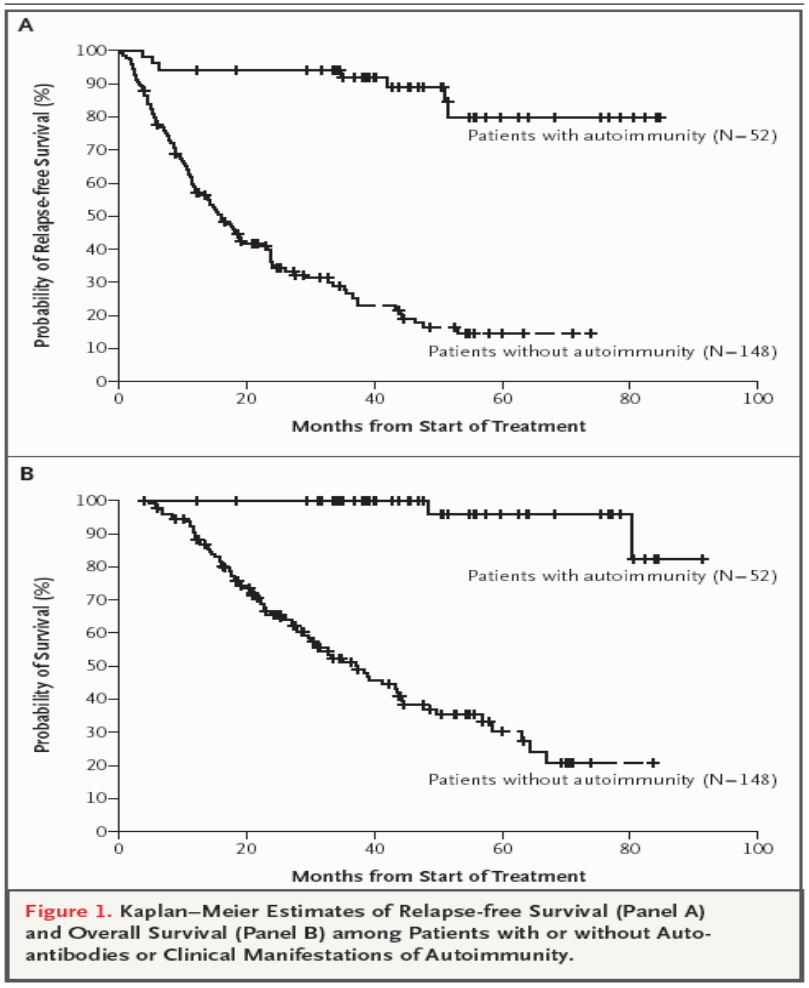


Figure 1. Kaplan–Meier Estimates of Relapse-free Survival (Panel A) and Overall Survival (Panel B) among Patients with or without Autoantibodies or Clinical Manifestations of Autoimmunity.

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# VSV: beyond direct oncolysis

| Requirements for successful Tumor Immunotherapy | Provisions of VSV Oncolytic Virotherapy |
|---|---|
| Identify relevant TAA | cDNA library |
| Release TAA for presentation to APC | Viral-mediated infection in lymph nodes |
| Recruit/Activate APC for presentation to TAA-specific T cells | Immunogenicity of VSV (adjuvant) |
| Increase the frequency of fully activated specific T cells | Viral-associated presentation of TAA |

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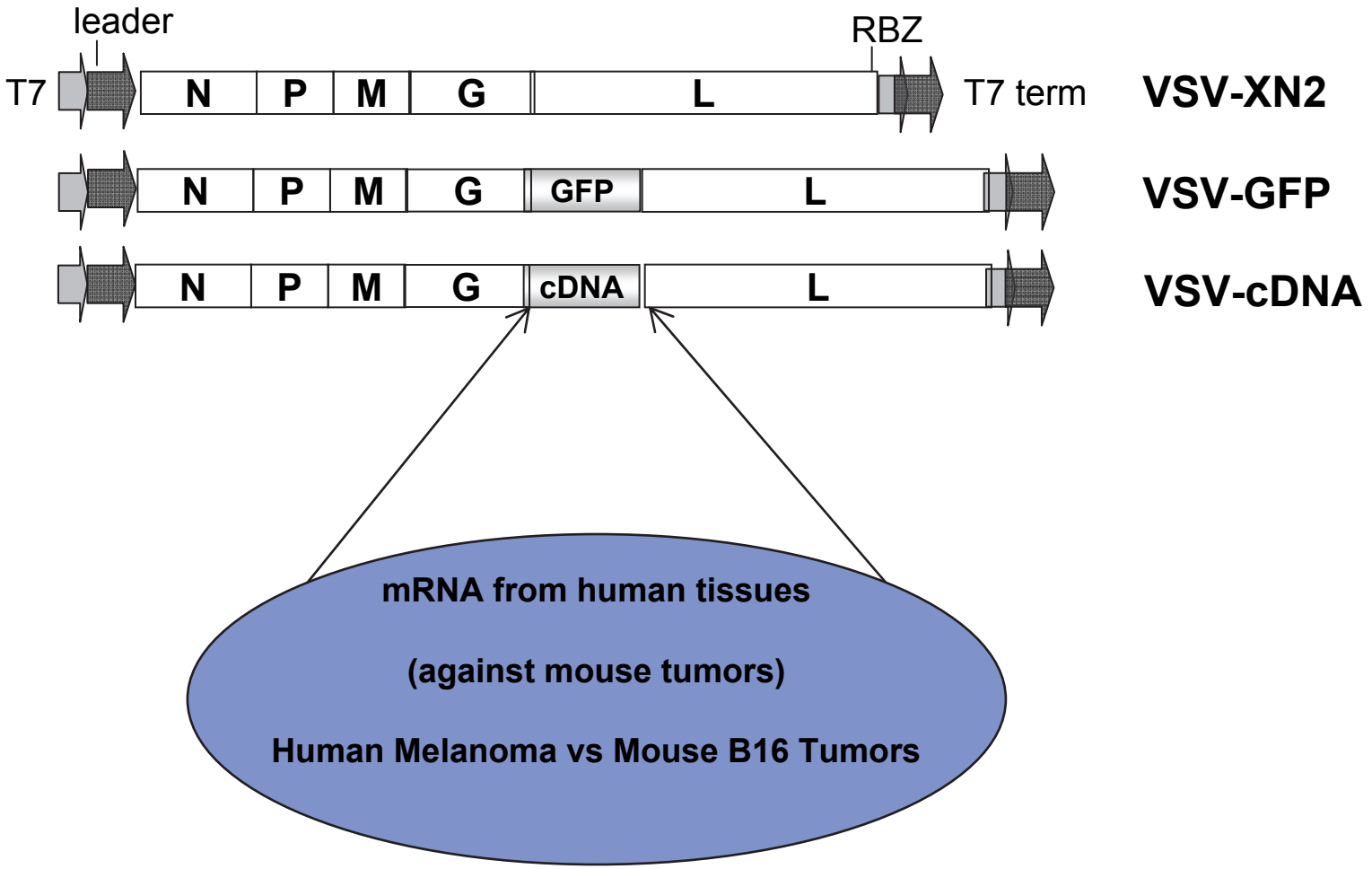
# Library Approach

**Altered Self-Epitope VSV-cDNA Library (ASEL)**

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# Construction of VSV-cDNA Libraries:

## 3rd Generation:

cDNA size fractionated to below 4kbp prior to cloning into pXN-2 plasmid

Complexity of plasmid cDNA library was ~4.75x$10^6$ cfu (@ dilutions of $10^{-6}$ and $10^{-5}$ gave 5 and 45 colonies respectively)

20 colonies picked at random:
3 no insert; 5 had inserts of <0.5kbp; 12 had inserts between 0.5kbp and 4kbp

All 5 tissue specific genes detected by rtPCR from infected BHK cells

PCR based estimates of 1-100 VSV-cDNA(PSA) pfu / 6x$10^5$ pfu of overall stock

DI particles still abundant (enhanced adjuvant/immunogenicity?)

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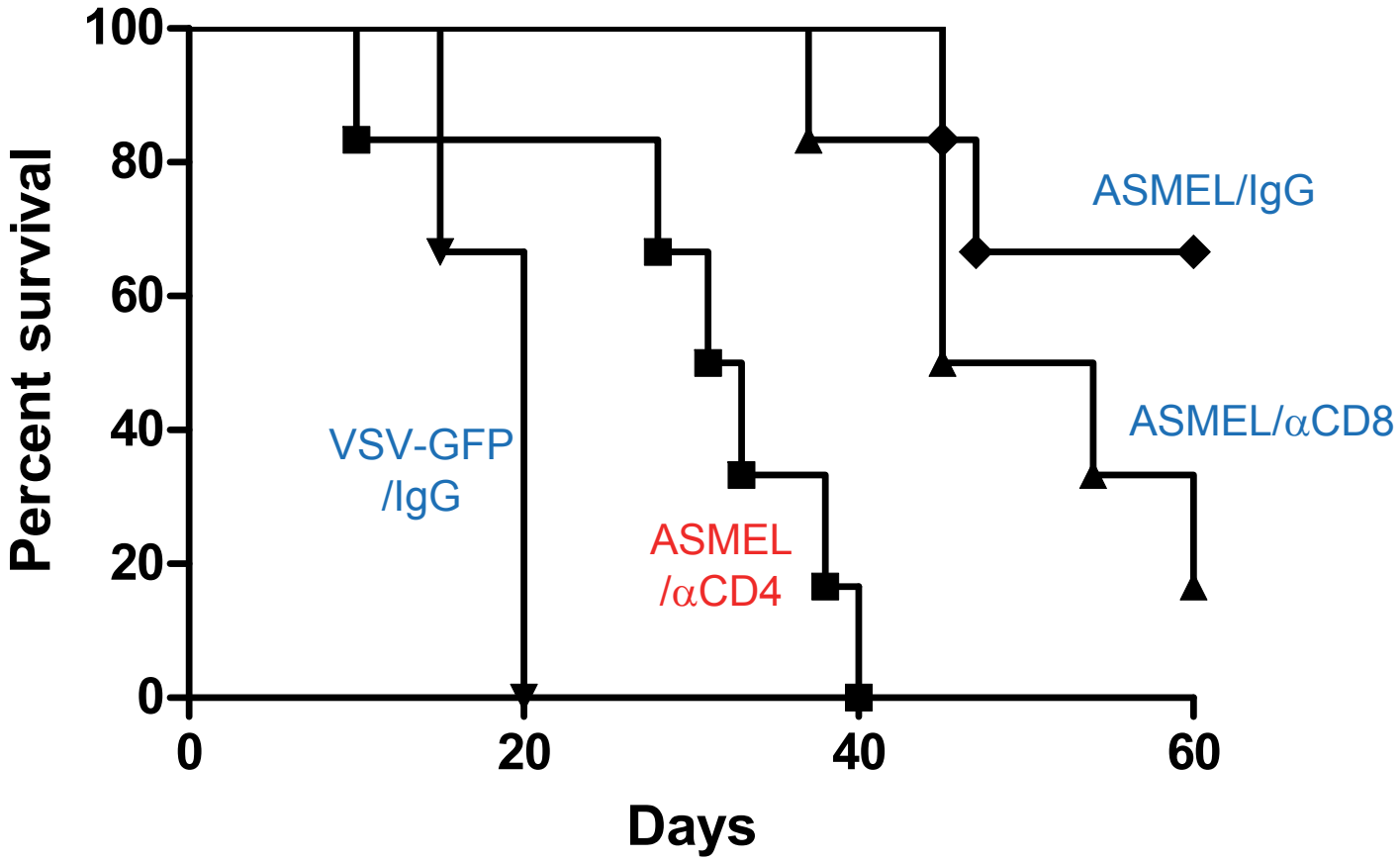
## VSV-cDNA library - Melanoma




Therapy dependent on CD4+ Th17 response

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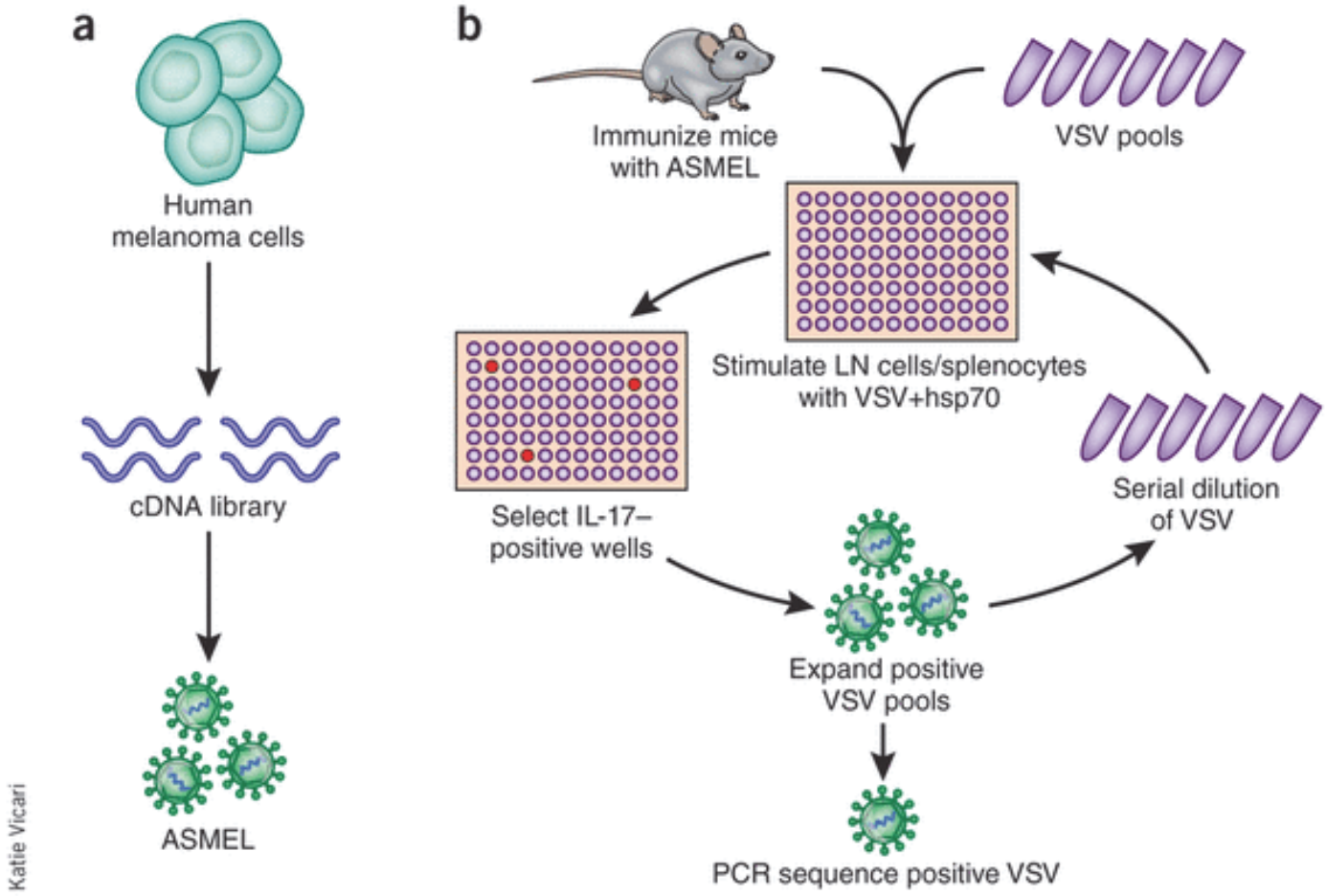
# TAA Identification




Three relevant TAA identified for melanoma model: **N-RAS, TYRP1, Cytochrome c-1**

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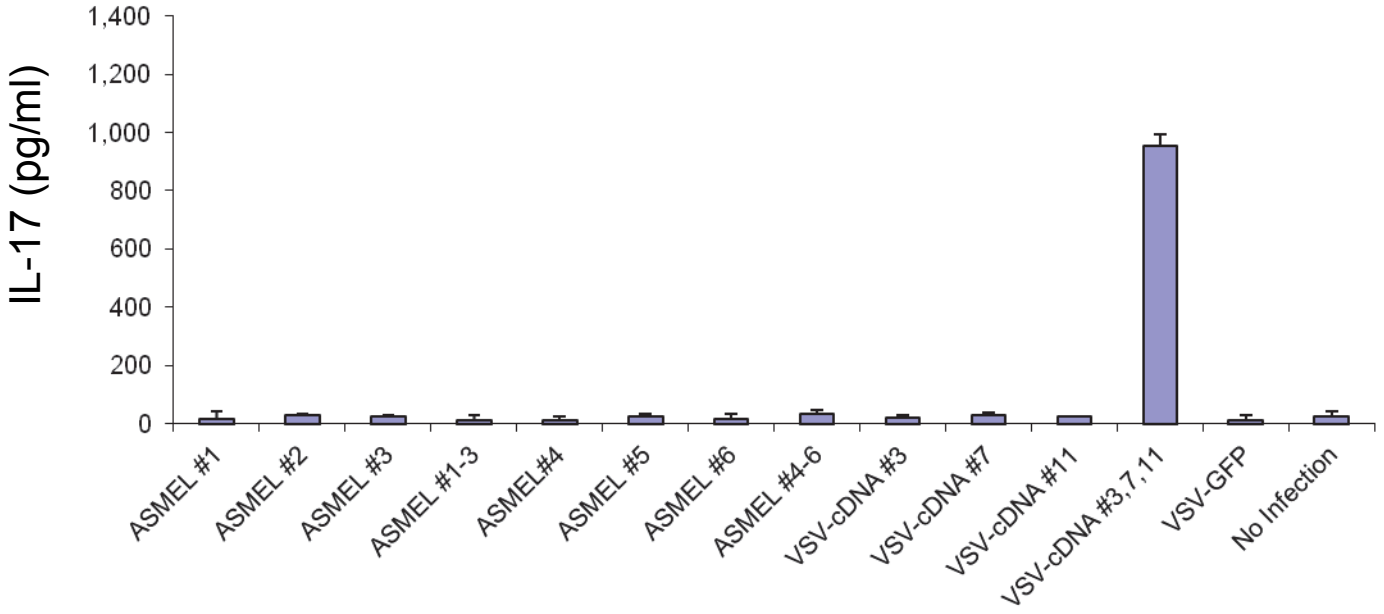
Avogadri and Wolchok (2012) **Nat Biotechnol**. 30:328-29




Only a Combination of all Three VSV-cDNA Viruses
Can Stimulate the IL-17 Recall Response

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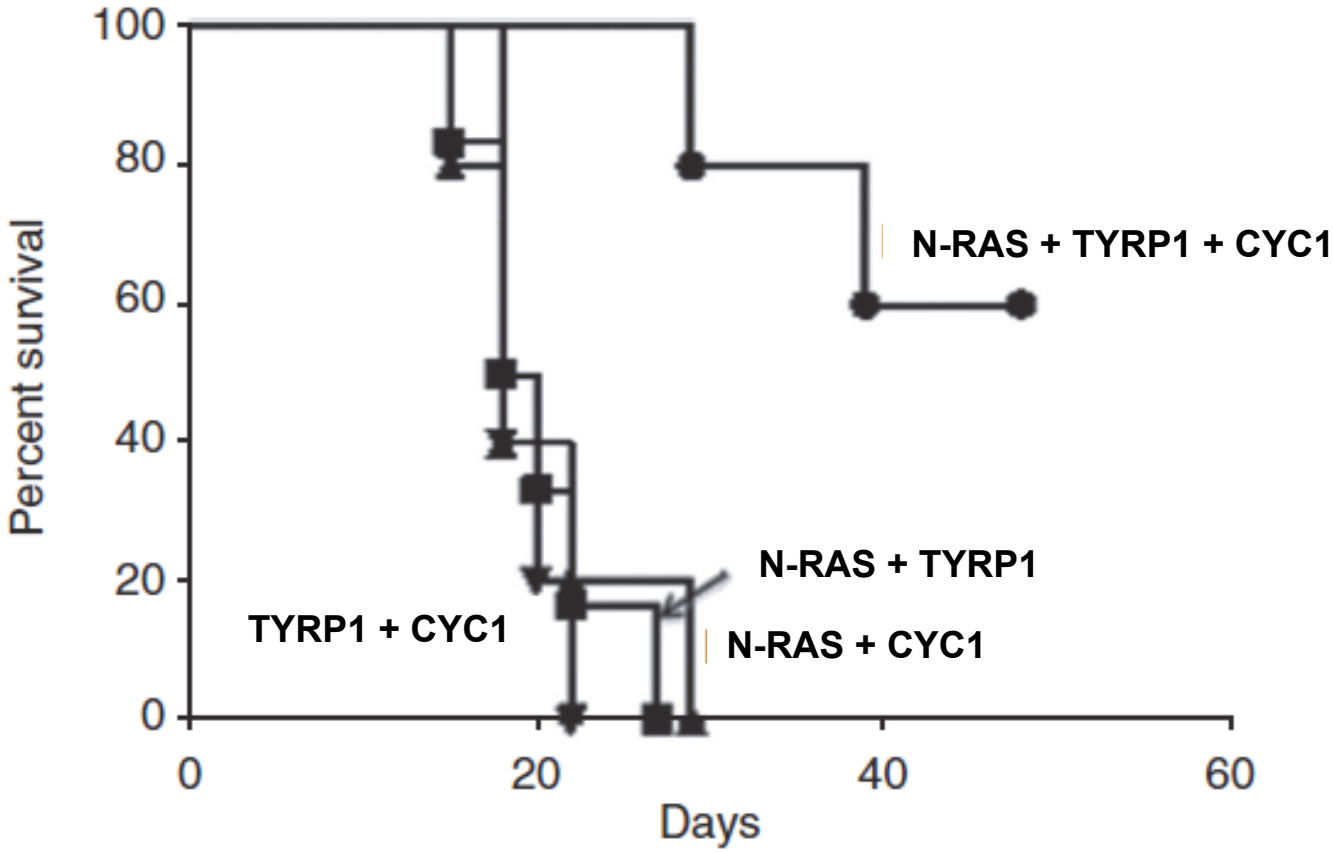
# VSV-TAA combination




Only a combination of all three VSV-cDNA viruses can stimulate the IL-17 recall response and treat established B16 melanomas

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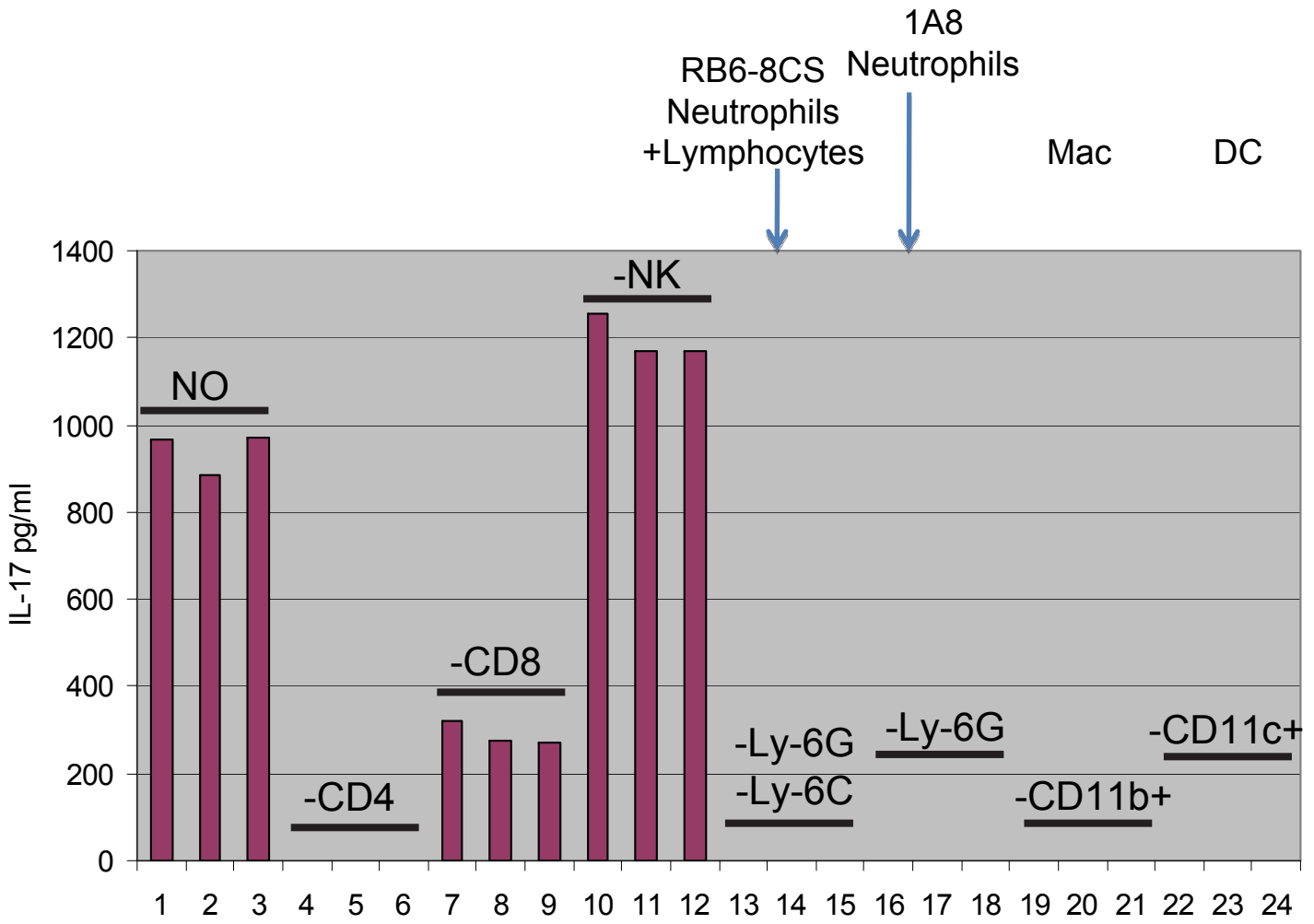
*Pulido, et al (2012)* ***Nat Biotechnol****. 30:337-43*

# IL-17 Recall Response Depends Upon CD4+ T Cells, Neutrophils, Macrophages and DC

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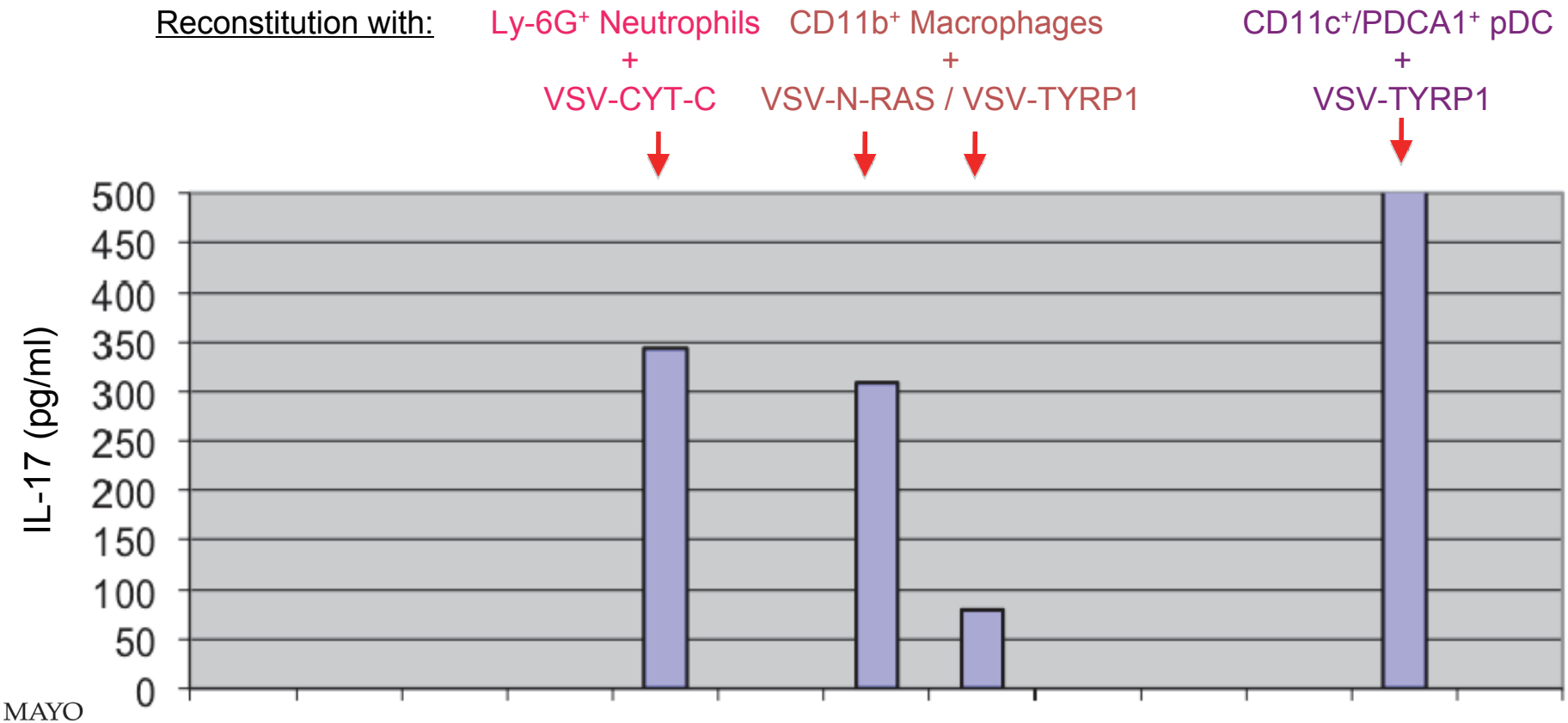
# Antigen Presenting Cells involved in activation of Th17 response

Splenocytes depleted of:

- Ly-6G$^+$ (neutrophils)
- CD11b$^+$ (macrophages)
- CD11c$^+$ (plasmacytoid DC)

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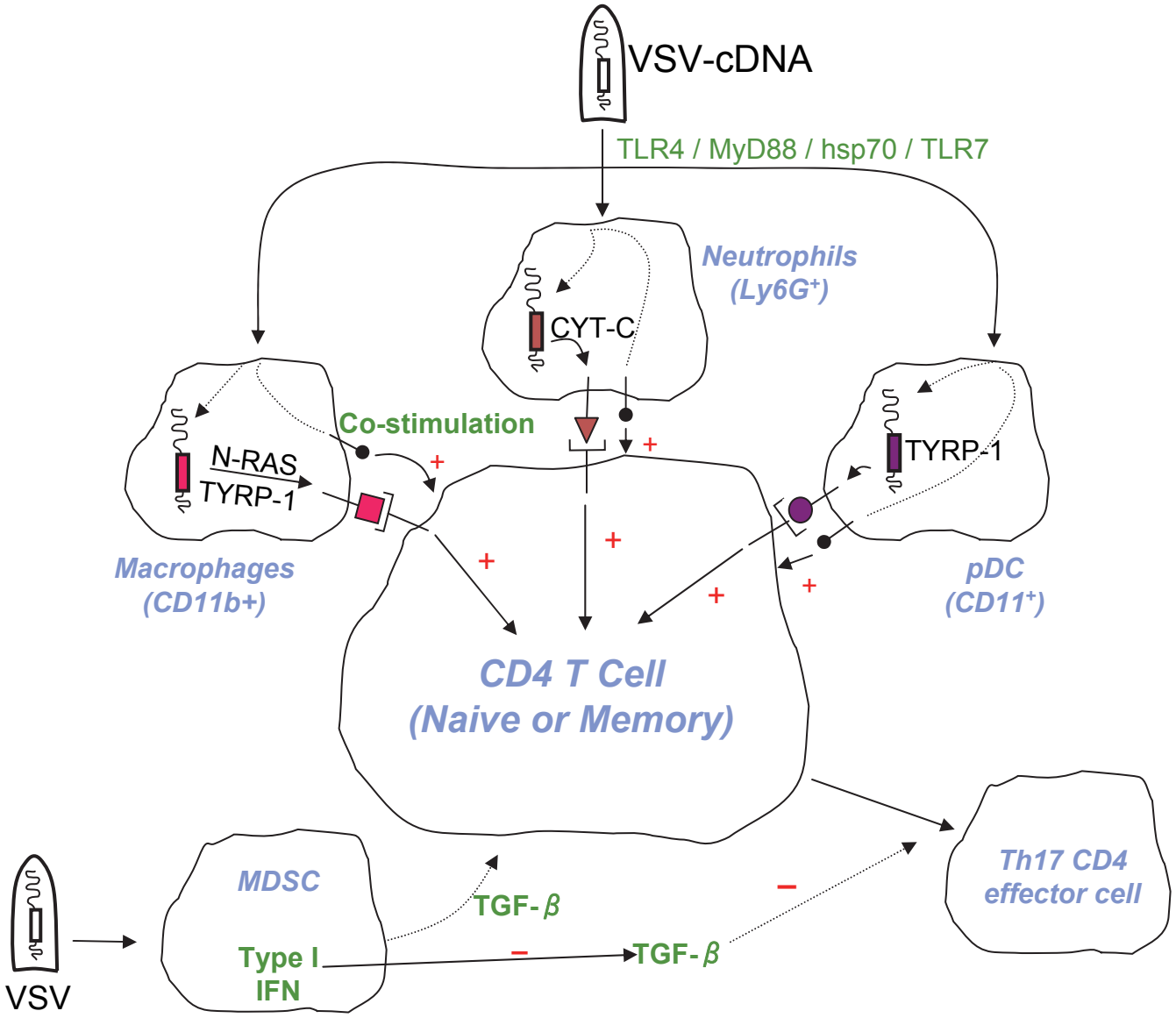
# Proposed Model

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**EFFICACY** of VSV as an Adjuvant for Enhancing T cell Activation Against Weak Tumor Associated Antigens:

-Potent Immunological *Danger Signal* (TLR 4,7 Activation, hsp induction)

-*Wide tropism* for different APC/immune cell types allows for Delivery of a wide range of potential TAA to the most appropriate APC for their presentation to T cells.

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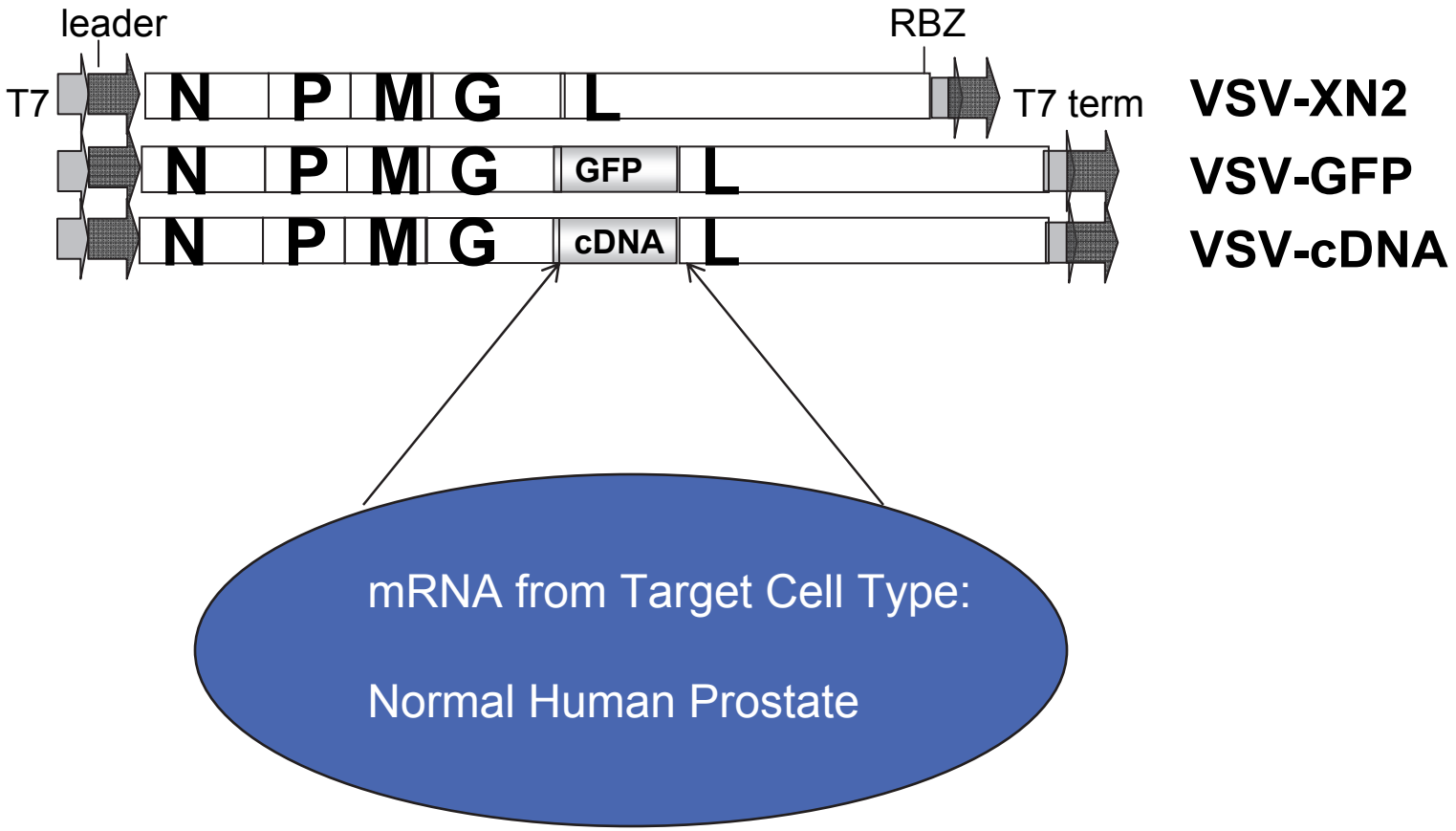
# Altered Self Epitope Library (ASEL) – cDNA of normal *Human* prostate in VSV to treat *Murine* TC2 Prostate Tumors

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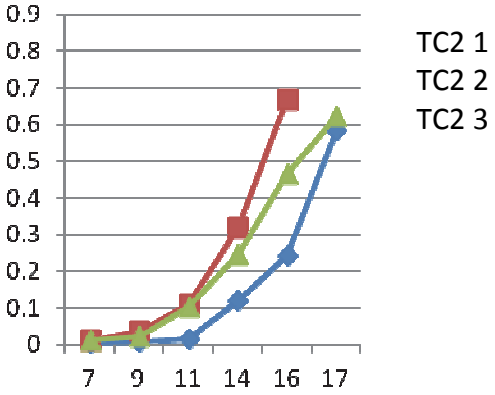
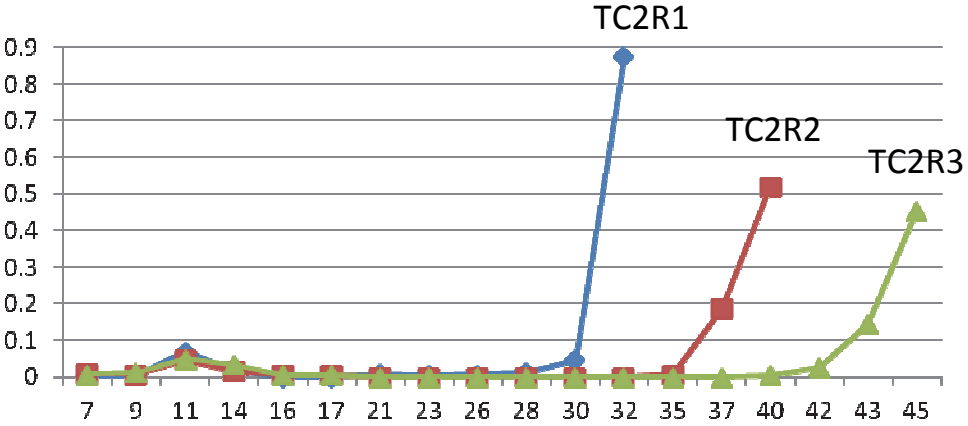
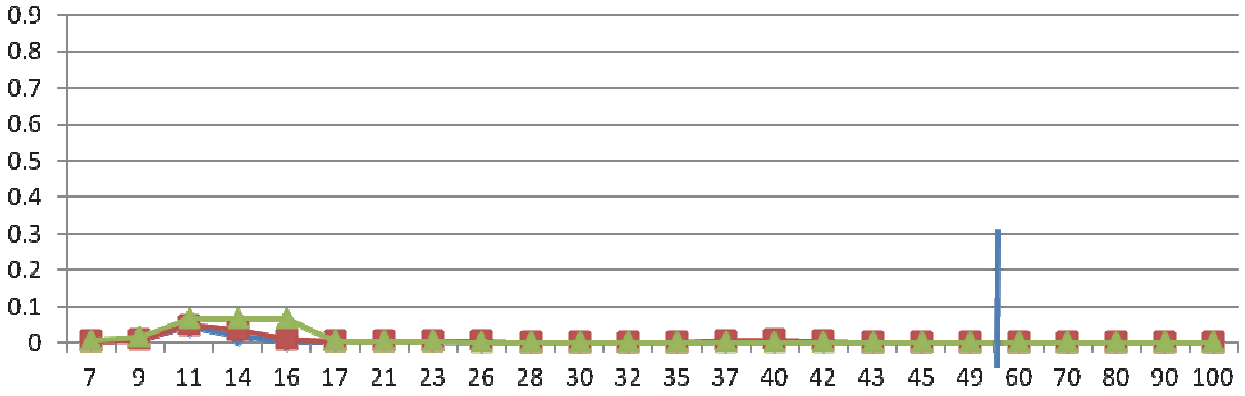



PBS Days 7,9,11, 14,16




6 Injections ASEL,
Days 7,9,11, 14,16,18



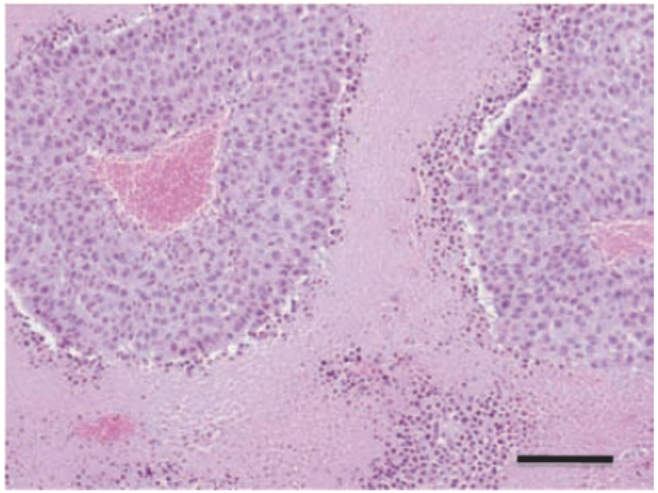

9 injections ASEL
Days 7,9,11,14,16,18,21,23,25

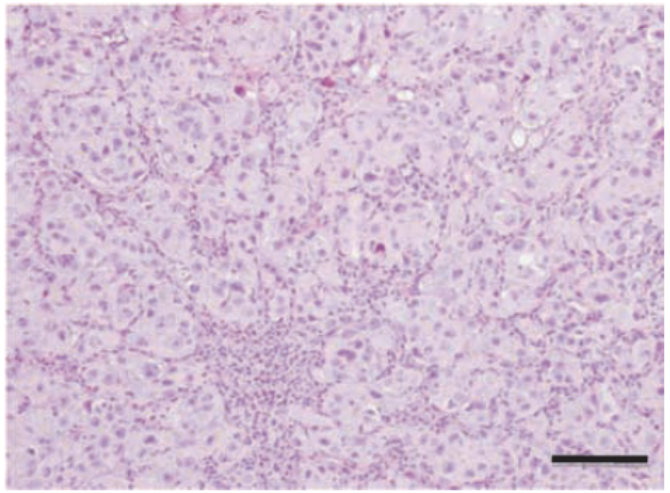
Sub optimal ASEL Therapy Causes Tumor Regressions Followed by Aggressive Recurrence

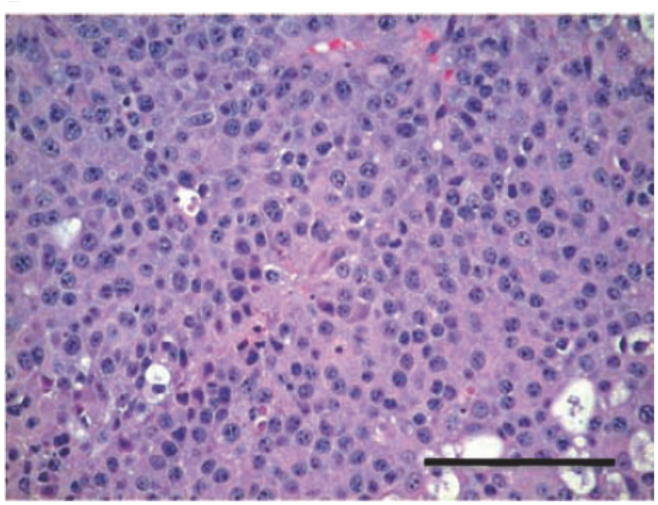
# Morphological changes

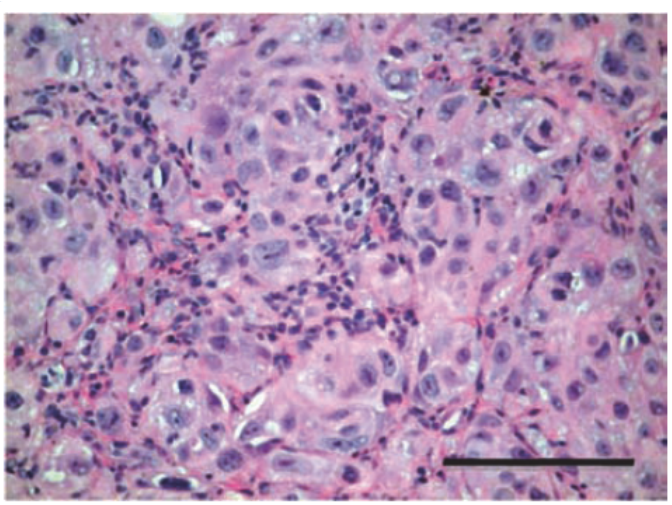
TC2 tumors

TC2R tumors









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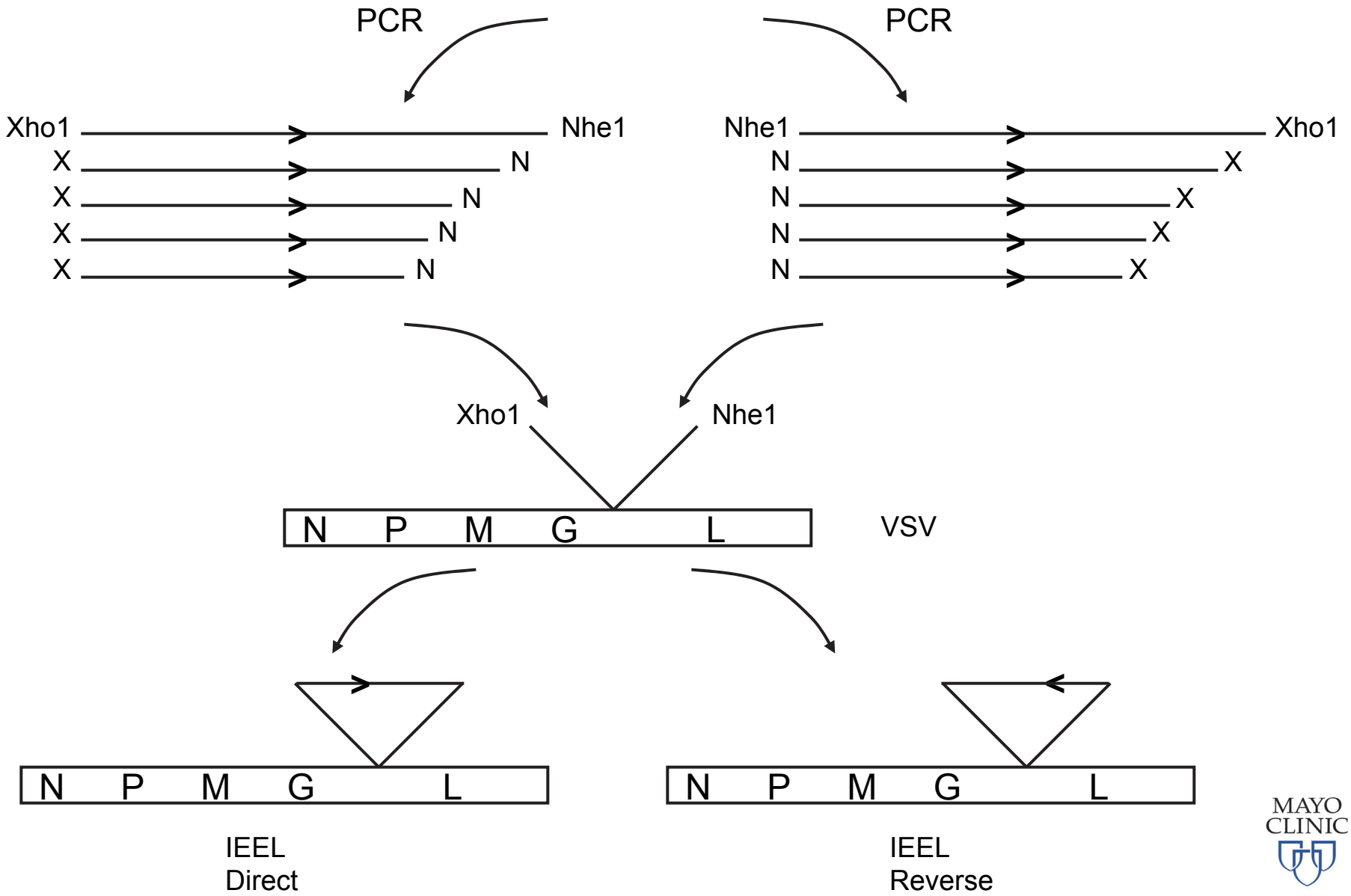
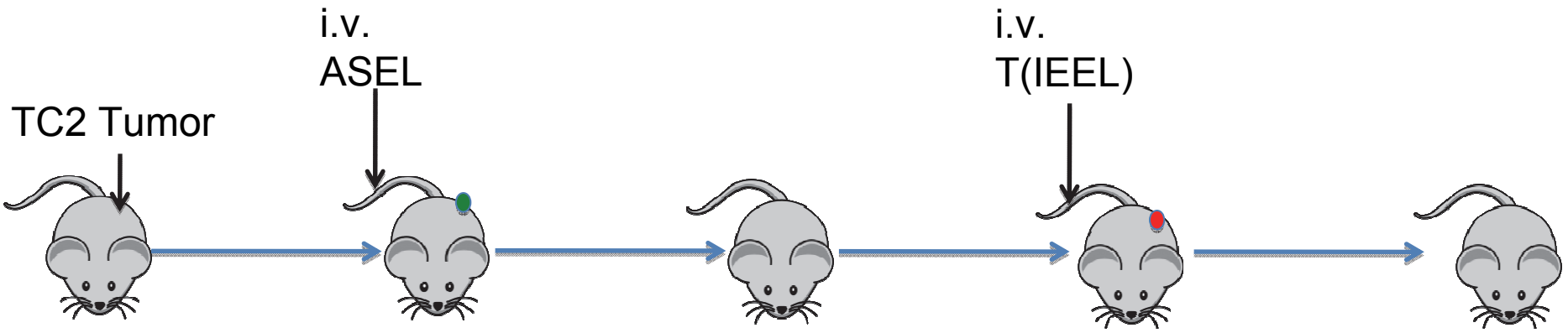
TC2 tumors which undergo regression and re-growth *in vivo*
have a very different morphology from VSV- or control-treated TC2 tumors

# cDNA Library from 3 pooled populations of TC2R Tumors – Immune Escape Epitope Library (IEEL)



# Primary TC2 Tumors Treated by Vaccination with the ASEL, and the Subsequent Recurrent TC2R Variants Treated with the IEEL, are Rejected Through Activation of Different Immunological Effector Mechanisms




Vaccination with altered self cDNA

Tumor clearance (CD4+T cells/ Th17 response vs TC2 and normal prostate) followed by tumor recurrence

Vaccination with recurrent antigen cDNA

Tumor cures

Th1 IFN-γ response vs TC2R BUT not vs TC2 or normal prostate

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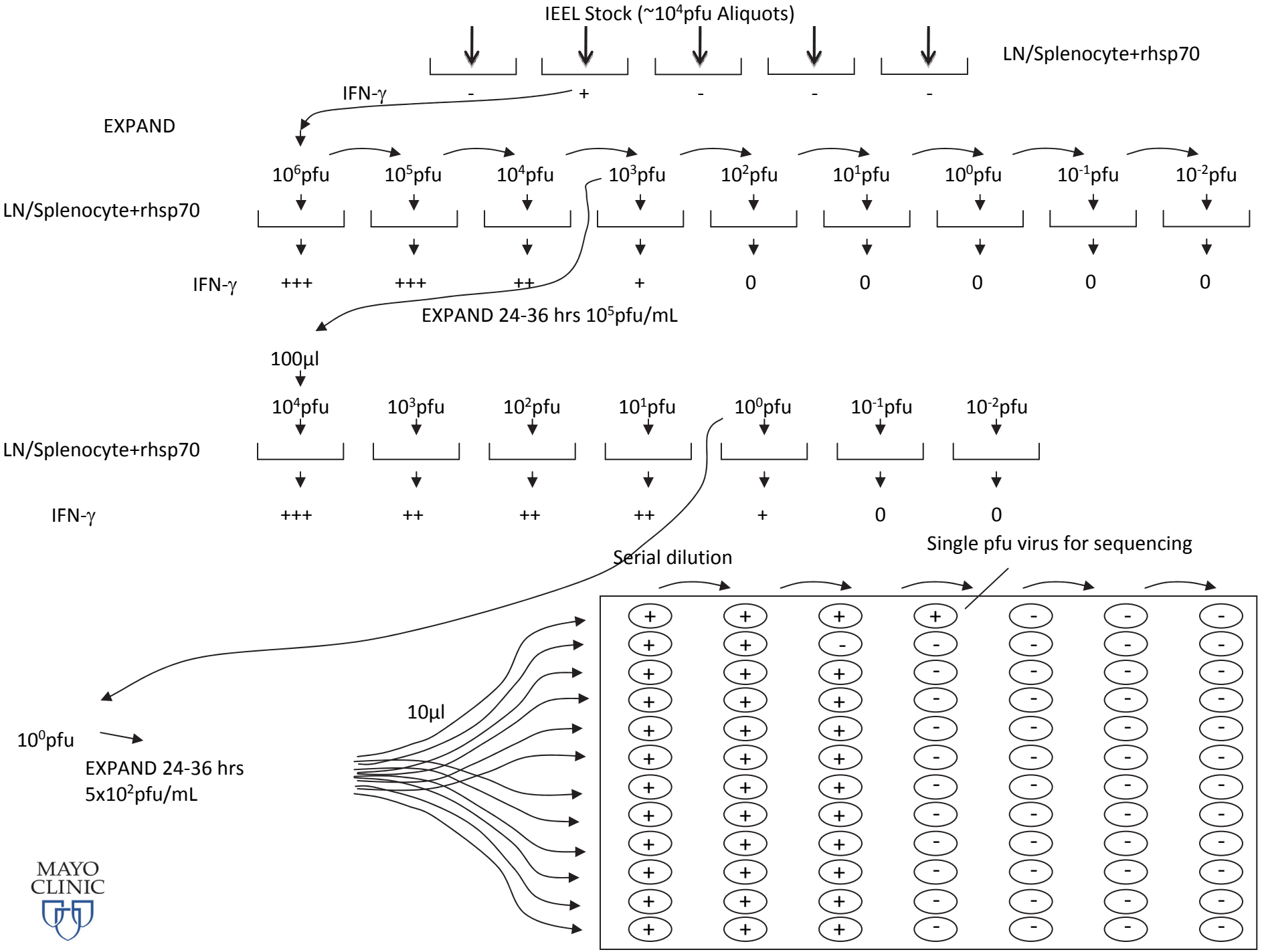
IEEL Stock (~10^4pfu Aliquots)
LN/Splenocyte+rhsp70
IFN-γ - + - - -
EXPAND
10^6pfu 10^5pfu 10^4pfu 10^3pfu 10^2pfu 10^1pfu 10^0pfu 10^-1pfu 10^-2pfu
LN/Splenocyte+rhsp70
IFN-γ +++ +++ ++ + 0 0 0 0 0
EXPAND 24-36 hrs 10^5pfu/mL
100µl
10^4pfu 10^3pfu 10^2pfu 10^1pfu 10^0pfu 10^-1pfu 10^-2pfu
LN/Splenocyte+rhsp70
IFN-γ +++ ++ ++ ++ + 0 0
Serial dilution
Single pfu virus for sequencing
10µl
10^0pfu
EXPAND 24-36 hrs
5x10^2pfu/mL
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-Screen of IEEL #1:

Of those virus clones, recovered from the IEEL, which stimulate the IFN-$\gamma$ recall response *in vitro* from splenocytes/LN of IEEL-cured mice:

**-5 contain cDNA sequences from CD44:**

**-9 contain cDNA sequences from DNA Topoisomerase II$\alpha$**

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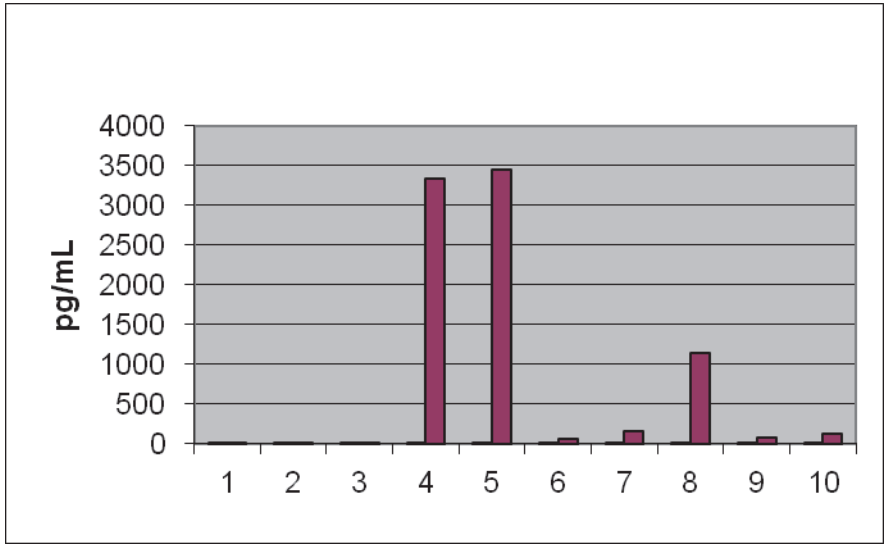
IFN-γ




Splenocytes/LN of IEEL Vaccinated Mice, Stimulated with:
Lane 1: Nothing
Lane 2: VSV-GFP
Lane 3: TC2 cells
Lane 4: TC2R1 cells
Lane 5 TC2R2 cells
Lane 6: VSV-CD44 #2
Lane 7: VSV-TOPO #17
Lane 8: #2+#17
Lane 9: #2 + VSV-GFP
Lane 10: #17 + VSV-GFP

*A Combination* of VSV-CD44 and VSV-Topo IIα Induce a Memory Recall Response From Splenocytes/LN of IEEL Vaccinated Mice
– CD44 and TOPO IIα Can Act as Tumor Antigens

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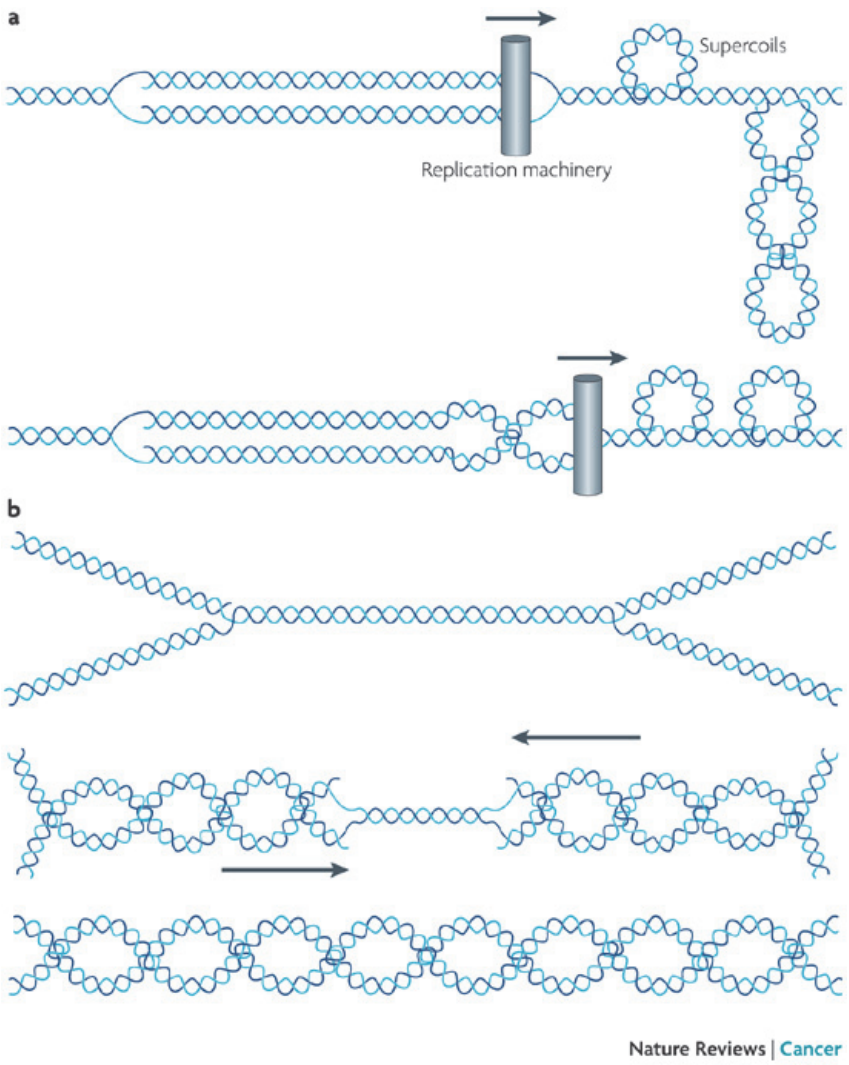


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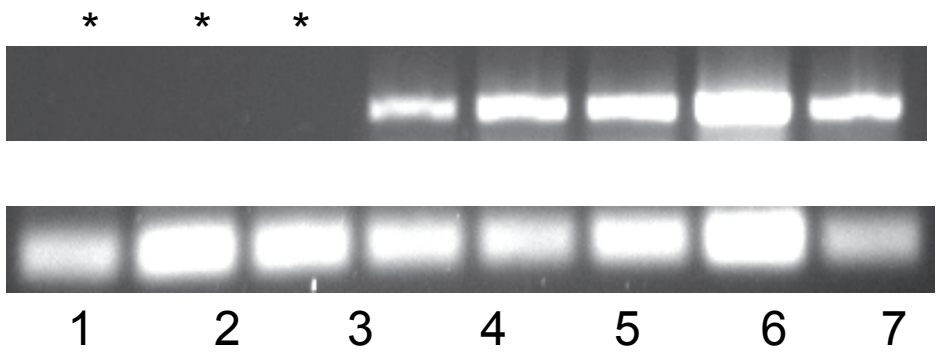
DNA Topoisomerase IIα

# *Freshly* Explanted TC2R Recurrent Tumours, Which Escape ASEL Therapy, Express High Levels of Topoisomerase IIα mRNA

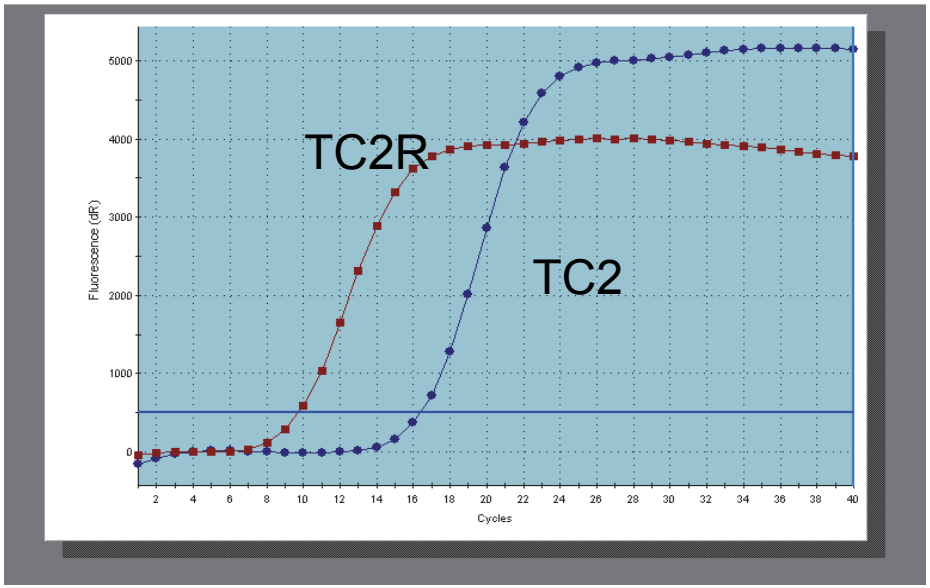



* +ve with 30 cycles

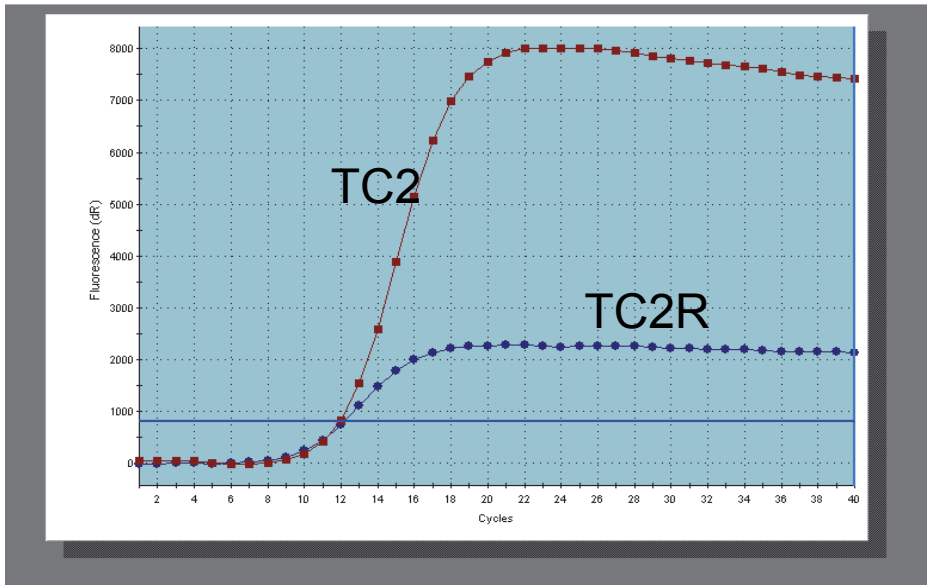
-48hrs; serum free culture; 15 cycles

qrt-PCR Amplification Curves:

TOPO IIα cDNA




GAPDH



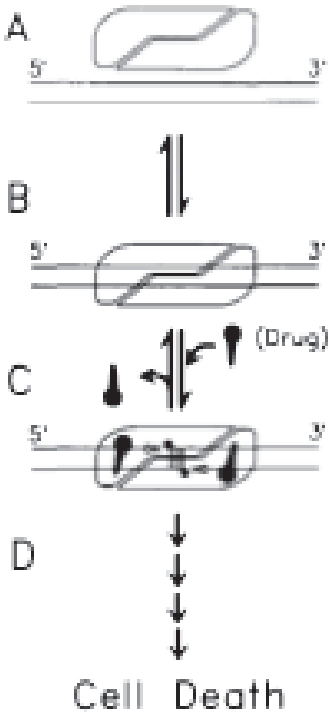



*Figure 3* Cellular processing of topoisomerase II–DNA cleavable complexes. Mammalian DNA topoisomerase II poisons stabilize the cleavable complex in the topoisomerase II reaction by forming a drug-enzyme-DNA ternary complex on chromosomal DNA in cultured cells. Each subunit of topoisomerase II is presumed to have one drug-binding site. Drug-DNA interaction is also shown. The drug-stabilized cleavable complex represents reversible DNA damage. Cellular processing of the drug-stabilized cleavable complex is required to trigger the lethal effect of topoisomerase II poisons.

*Doxorubicin interacts with DNA by intercalation and inhibition of macromolecular biosynthesis. This inhibits the progression of the enzyme topoisomerase II, which relaxes supercoils in DNA for transcription. Doxorubicin stabilizes the topoisomerase II complex after it has broken the DNA chain for replication, preventing the DNA double helix from being resealed and thereby stopping the process of replication.*

Inhibition of Topoisomerase II in Cancer Chemotherapy

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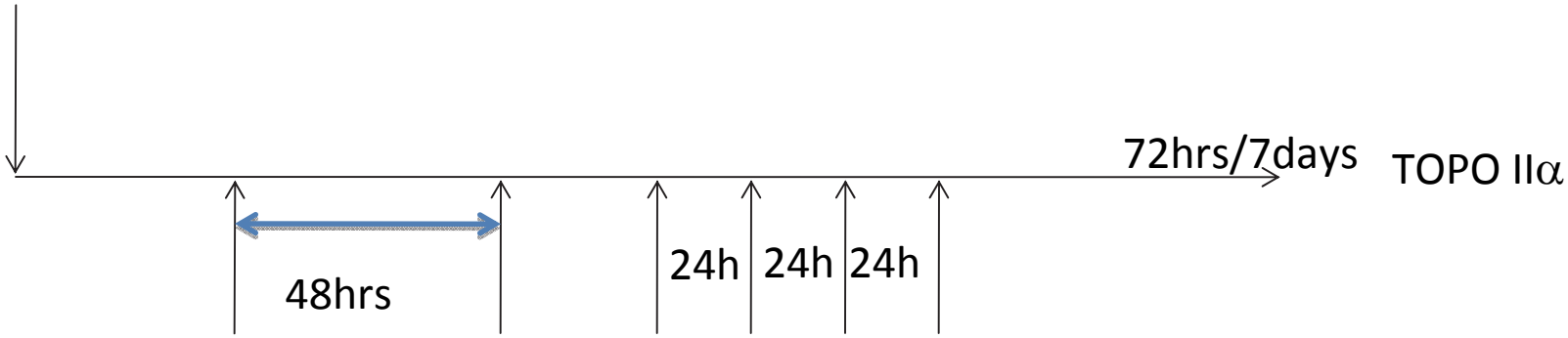
TC2 ($10^4$ cells)




| | |
|---|---|
| PAC | Th17 Splenocytes/LN (ASEL MOI 1) |
| DOX | Th17 Splenocytes/LN (ASMEL MOI 1) |
| None | C57BL/6 Splenocytes/LN |
| | None |

The TOPO IIα Hi Phenotype Can Be Induced by T Cell Co-culture *in vitro*
And Can be Inhibited by Doxorubicin

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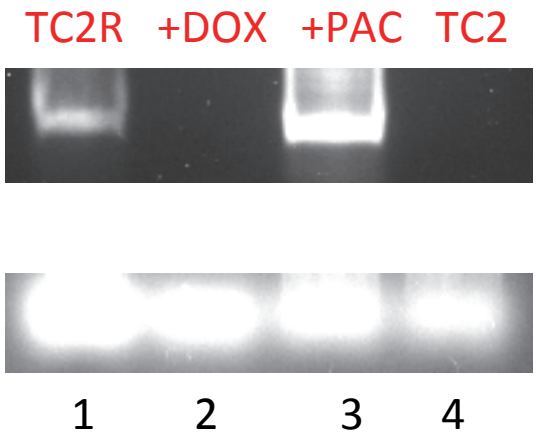
48hrs; serum free culture; 15 cycles




Transient Treatment of TC2R Cells *in vitro* with DOXORUBICIN Purges a TOPO IIα$^{Hi}$ Population of Cells

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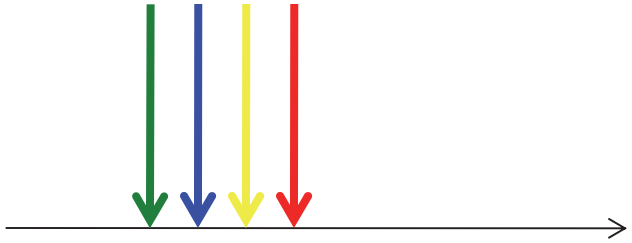
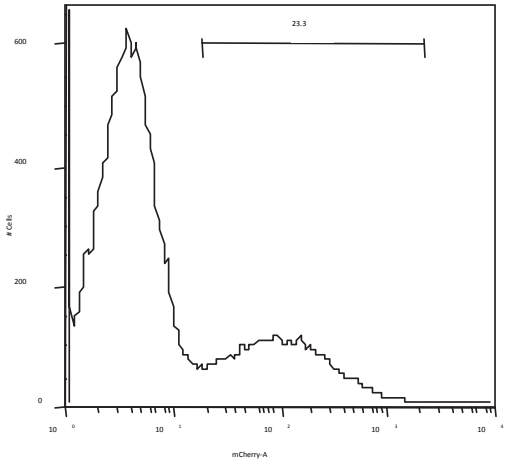
Lenti-GFP
Lenti-CFP
Lenti-YFP
Lenti-Cherry

TC2 cells



TC2 Rainbow population




25.5% CHERRY

TC2 (Rainbow)

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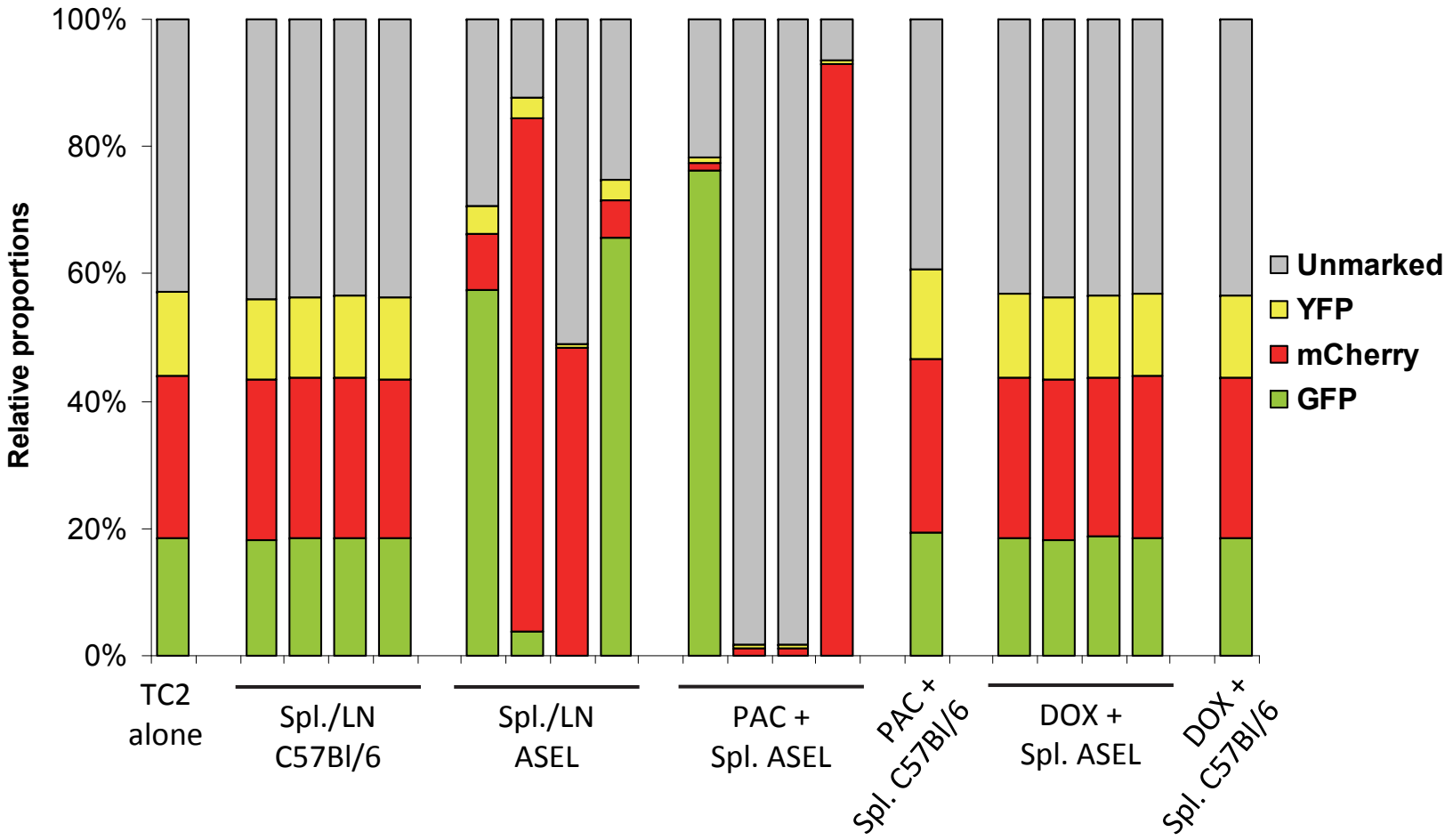
Relative proportions
100%
80%
60%
40%
20%
0%
Unmarked
YFP
mCherry
GFP
TC2 alone
Spl./LN C57Bl/6
Spl./LN ASEL
PAC + Spl. ASEL
PAC + Spl. C57Bl/6
DOX + Spl. ASEL
DOX + Spl. C57Bl/6

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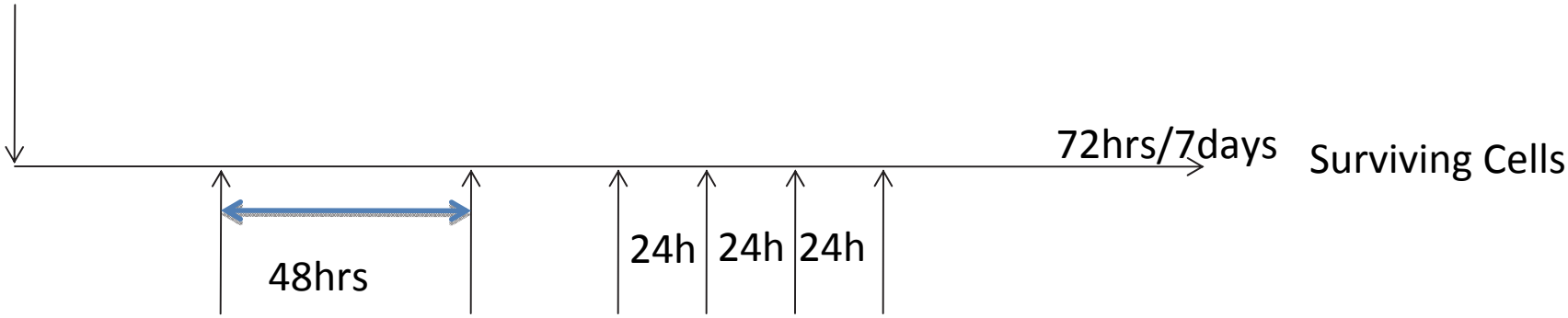
TC2 ($10^4$ cells)




| | |
|---|---|
| PAC | Th17 Splenocytes/LN (ASEL MOI 1) |
| DOX | Th17 Splenocytes/LN (ASMEL MOI 1) |
| None | C57BL/6 Splenocytes/LN |
| | None |

A Dox-sensitive Population of Cells within TC2 Cultures Allows Tumor Cell Expansion/Recovery Following Antigen Specific Splenocyte Killing of Tumor Cells

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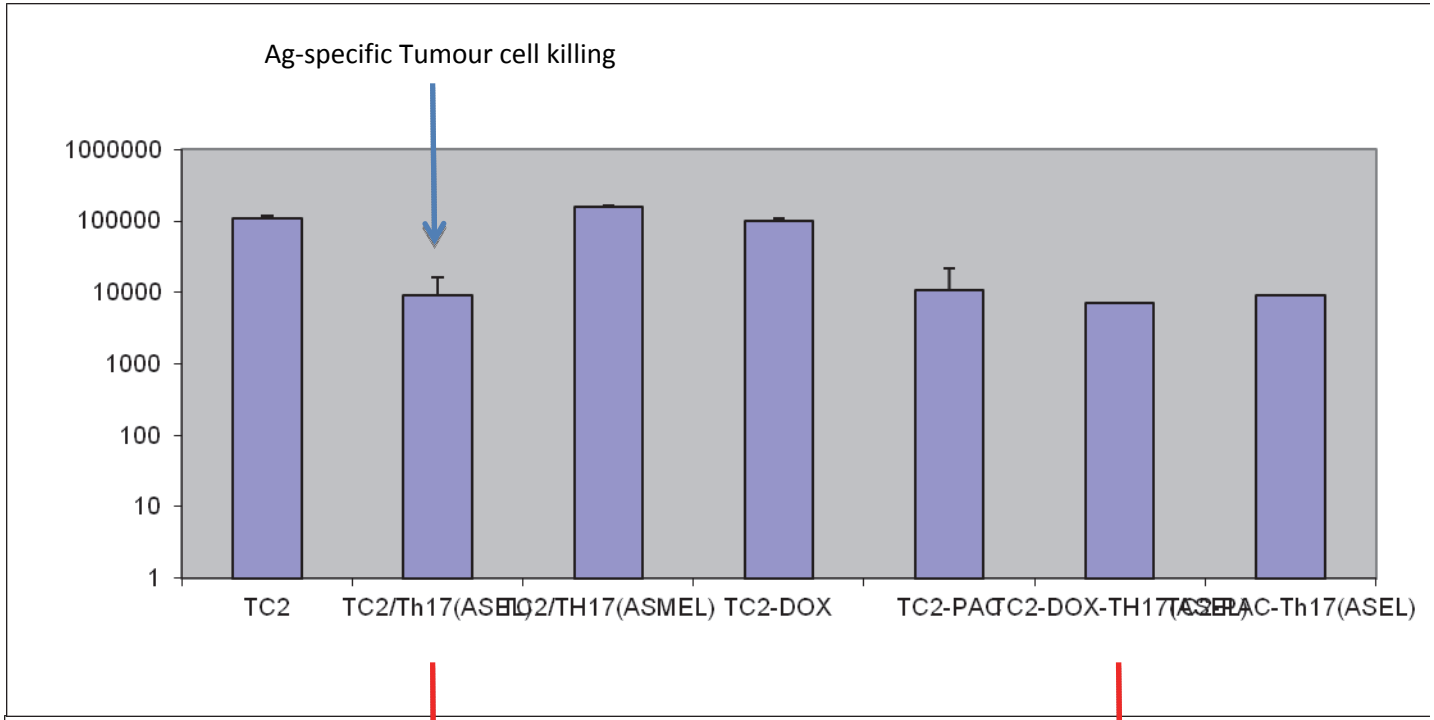
**I.**

72hrs after Co-culture

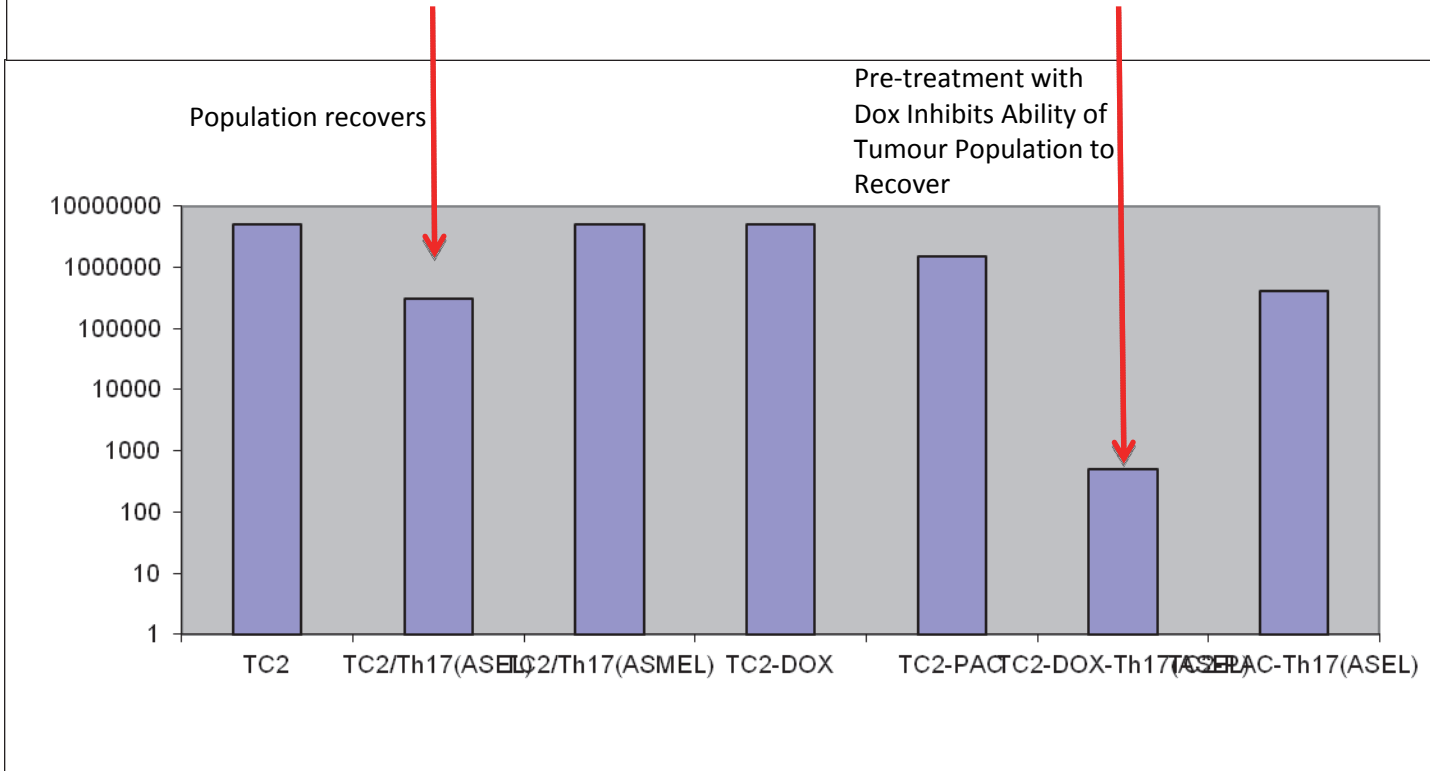
# of Surviving Cells




**J.**

7 Days after Co-culture

# of Surviving Cells

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**Hypothesis:**

Doxorubicin Purges TC2 and TC2R Cells of a Low Frequency Population of TOPO-II$\alpha^{Hi}$ Expressing, 'Plastic'/Stem Cell-Like Cells Which Respond to Applied Selective Pressure

-DOX may be an effective 'recurrence purging' chemotherapy, which may have apparently low activity against the tumor cell population as a whole, but high activity against a low frequency population which may be responsible for treatment failure, recurrence

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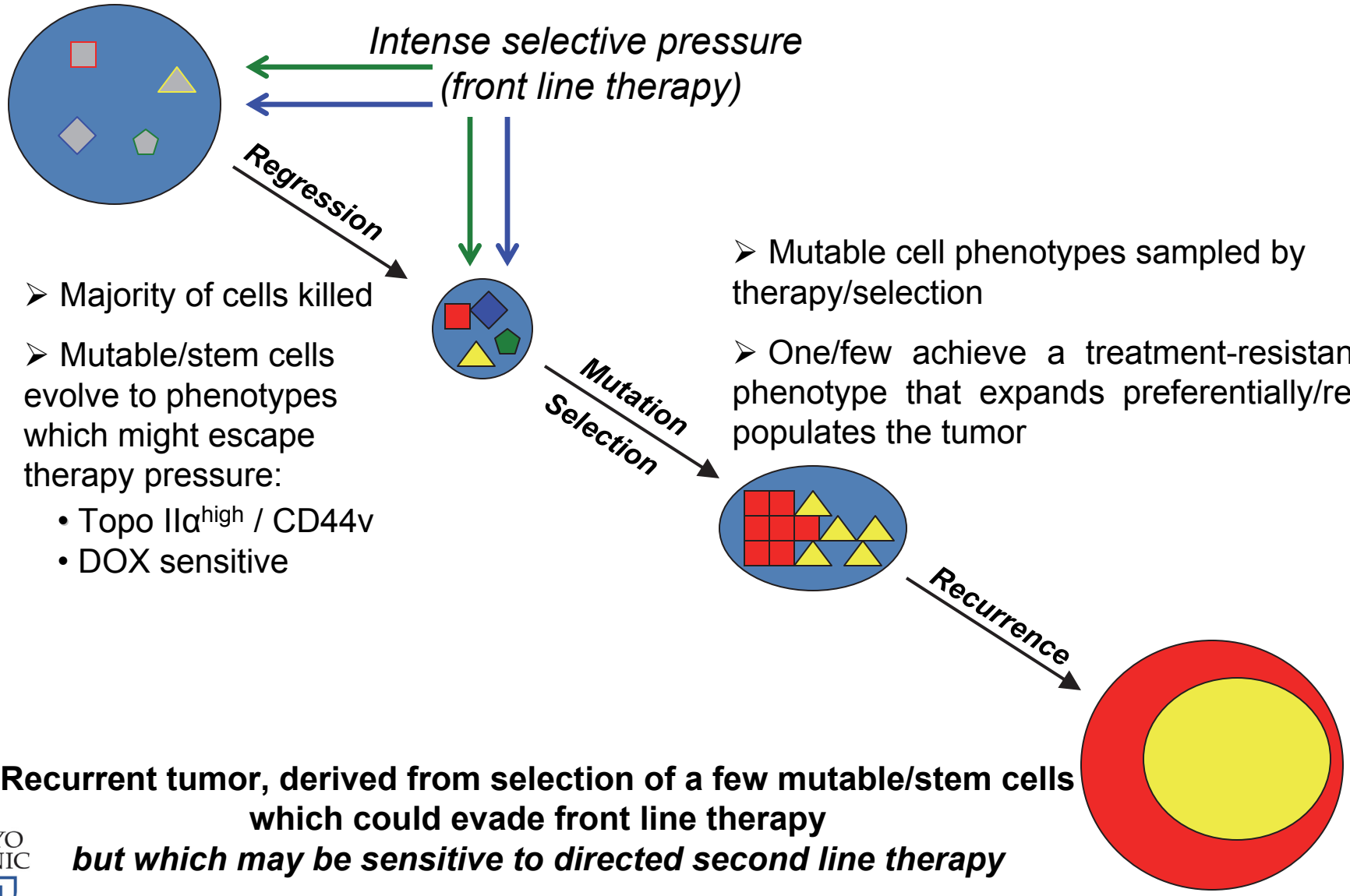
# Proposed model




- Majority of cells killed
- Mutable/stem cells evolve to phenotypes which might escape therapy pressure:
  - Topo IIα$^{high}$ / CD44v
  - DOX sensitive

- Mutable cell phenotypes sampled by therapy/selection
- One/few achieve a treatment-resistant phenotype that expands preferentially/re-populates the tumor

**Recurrent tumor, derived from selection of a few mutable/stem cells which could evade front line therapy**
***but which may be sensitive to directed second line therapy***

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# Targeting TOPO-IIa$^{Hi}$ Plastic/Stem Cells in Tumors For Therapy

-*Chemotherapy*: Doxorubicin, others

-*Vaccines* – VSV-TOPO II$\alpha$

-*Virotherapy* – Reovirus, others

**Hypothesis**:

Can DOX chemotherapy be combined with front line viro-immunotherapy to treat recurrences derived from these TopoII$\alpha^{Hi}$ mutable/stem cells?

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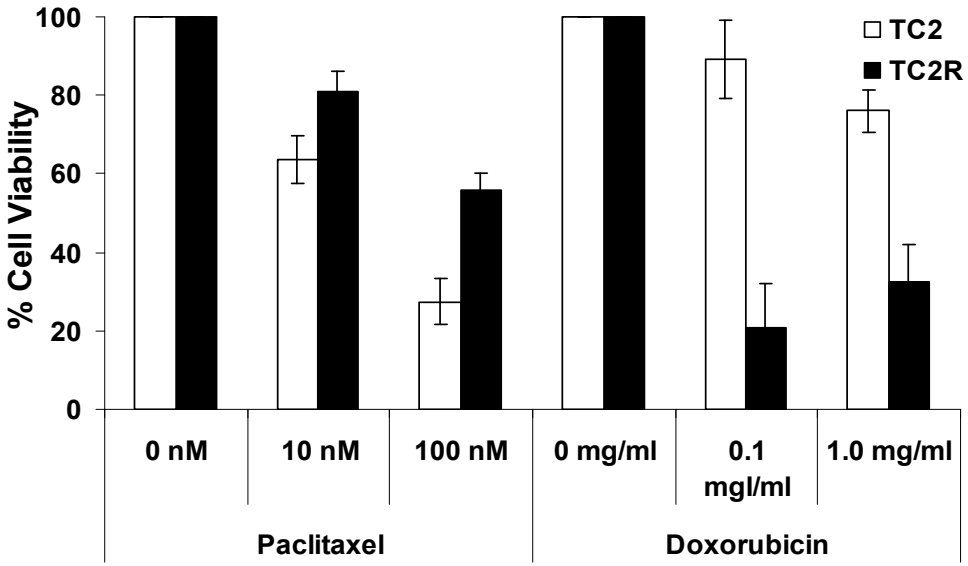
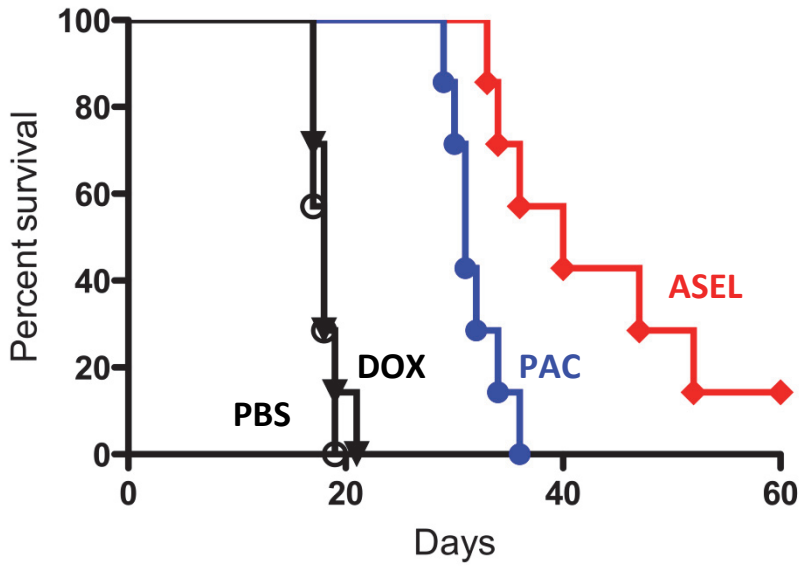
# Sensitivity to chemotherapy

***In vitro***




***In vivo***




TC2 and TC2R cells

have different sensitivities to chemotherapy *in vitro and in vivo*

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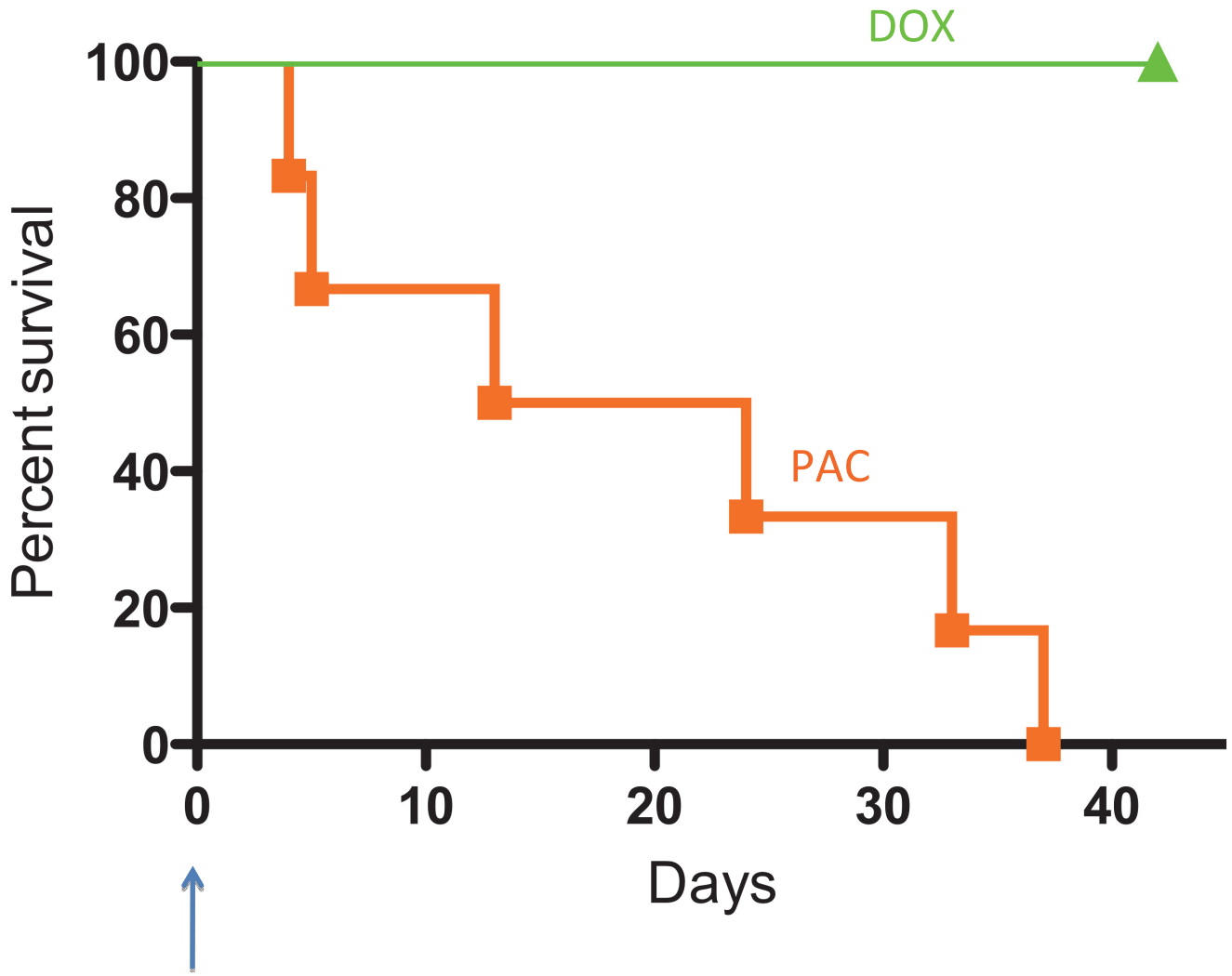
# Doxorubicin Chemotherapy Combines with (suboptimal) ASEL Viro-Immunotherapy to Prevent Tumor Recurrence




START of 2nd Line Therapy Following ASEL Vaccinations (All Mice With Tumors)

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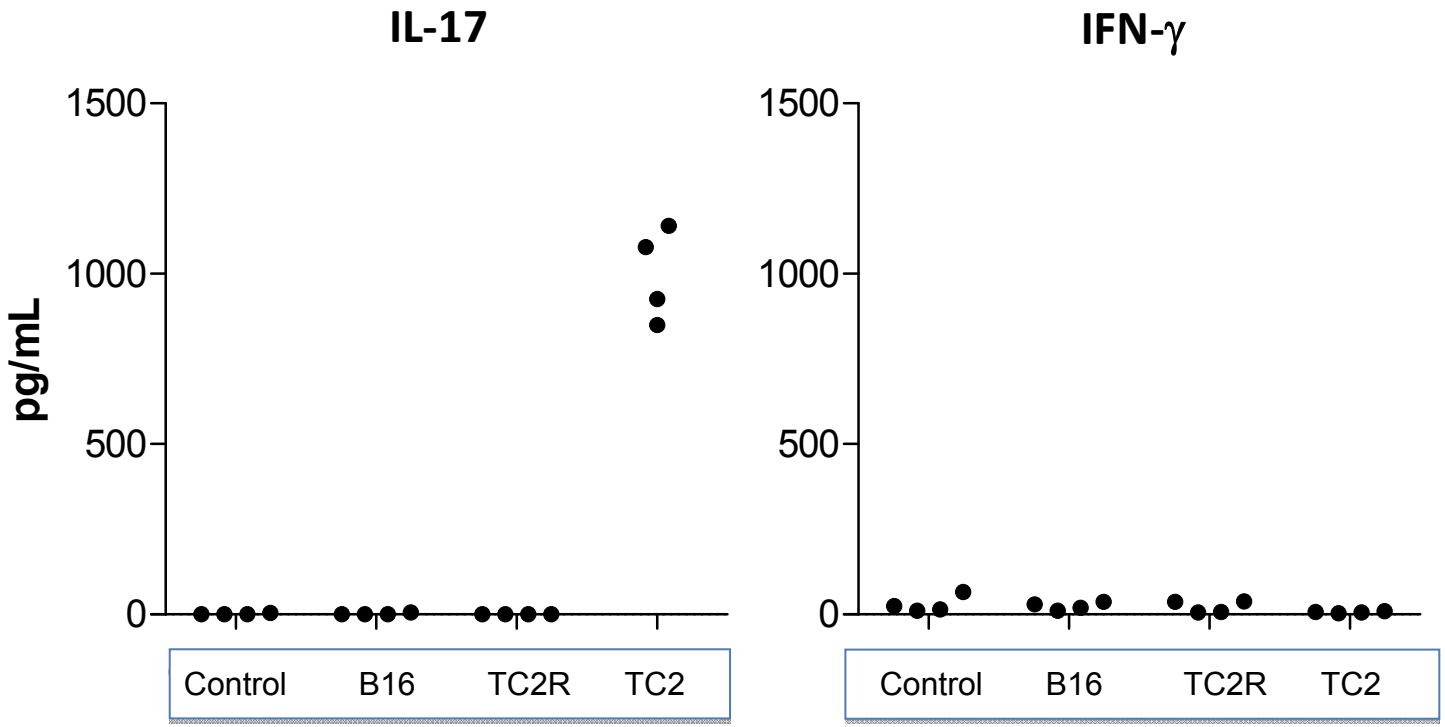


Survivors of ASEL+DOX Combination Therapy Have a Th17 Response to TC2 Cells But No IFN-γ Response to TC2R Cells

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# Hypothesis:

A TOPO-II$\alpha^{Hi}$ sub-population of Plastic/Stem Cell Like
Cells Exists in Tumors, Which Can Respond To Selective Pressures and Change Phenotypically To Escape These Pressures.

Is this Model Dependent (TC2, C57BL/6)?

Is this therapy dependent? (Strong T cell therapy)?

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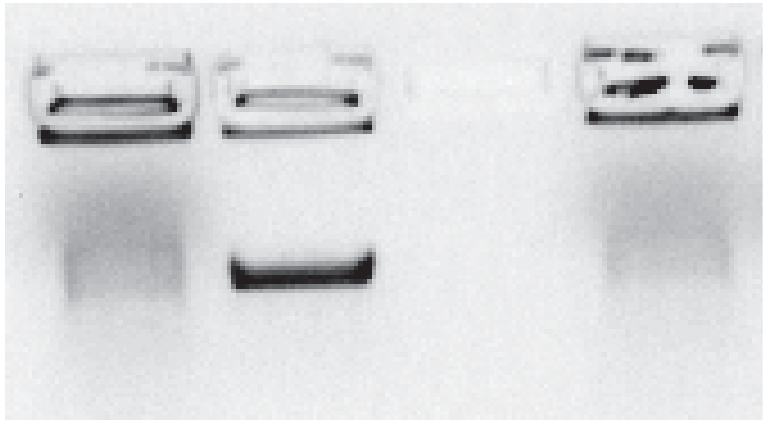

| 1 | 2 | 3 |
|---|---|---|
| +DOX | +PBS | +PBS |
| +GCV | +GCV | +PBS |

-B16tk Cells Which Survive GCV Chemotherapy are TOPO II$\alpha^{Hi}$
-DOX Purges the Ability of These Cells to Predominate in Escape Cultures

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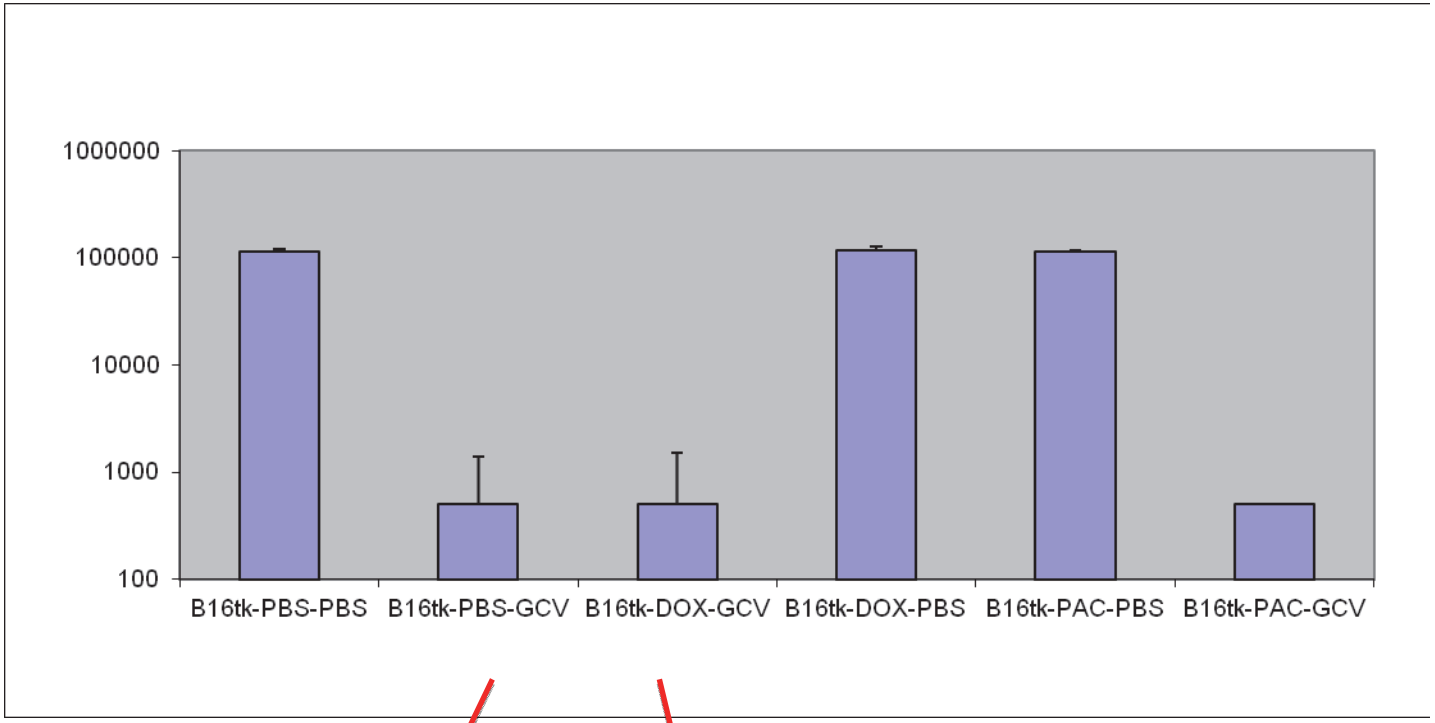
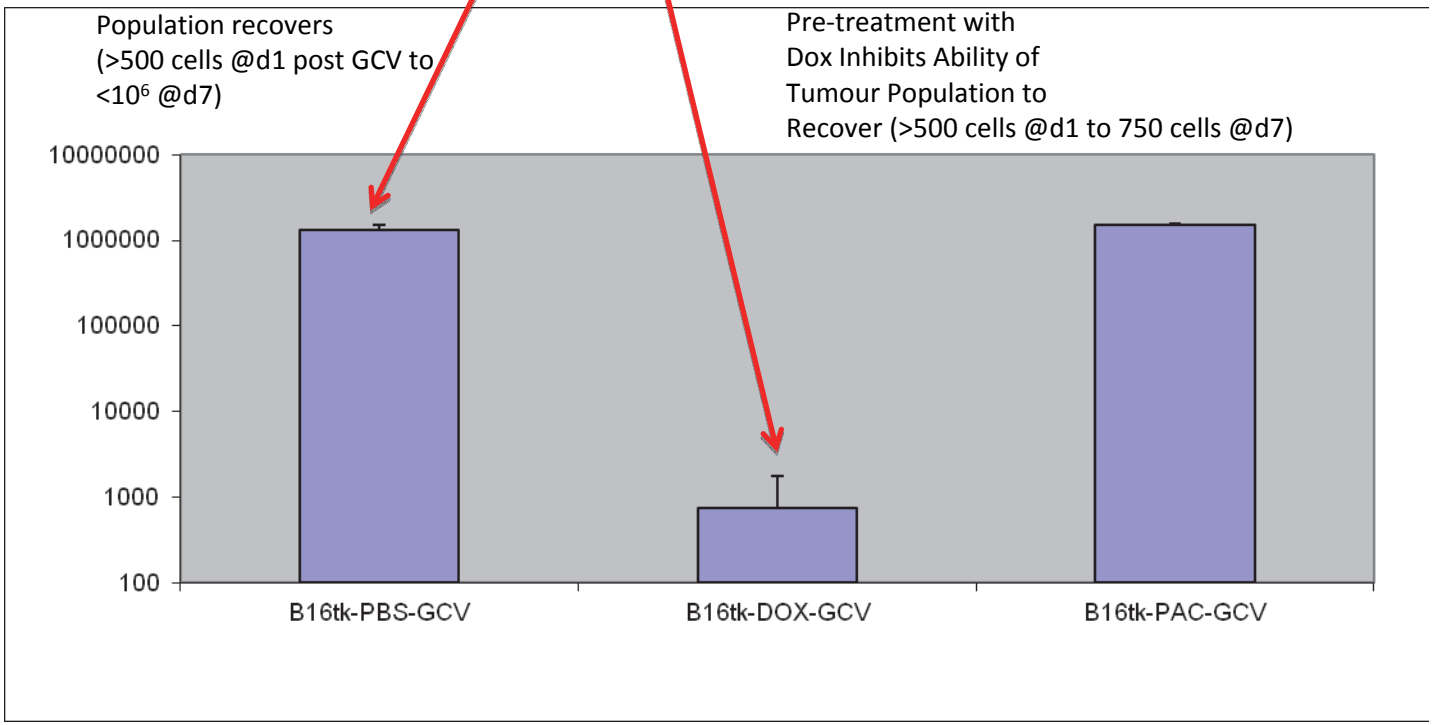
-CHEMOTHERAPY
-MELANOMA

A. 5 Days of GCV




# of Surviving Cells

B. 7 Days after Cessation of GCV

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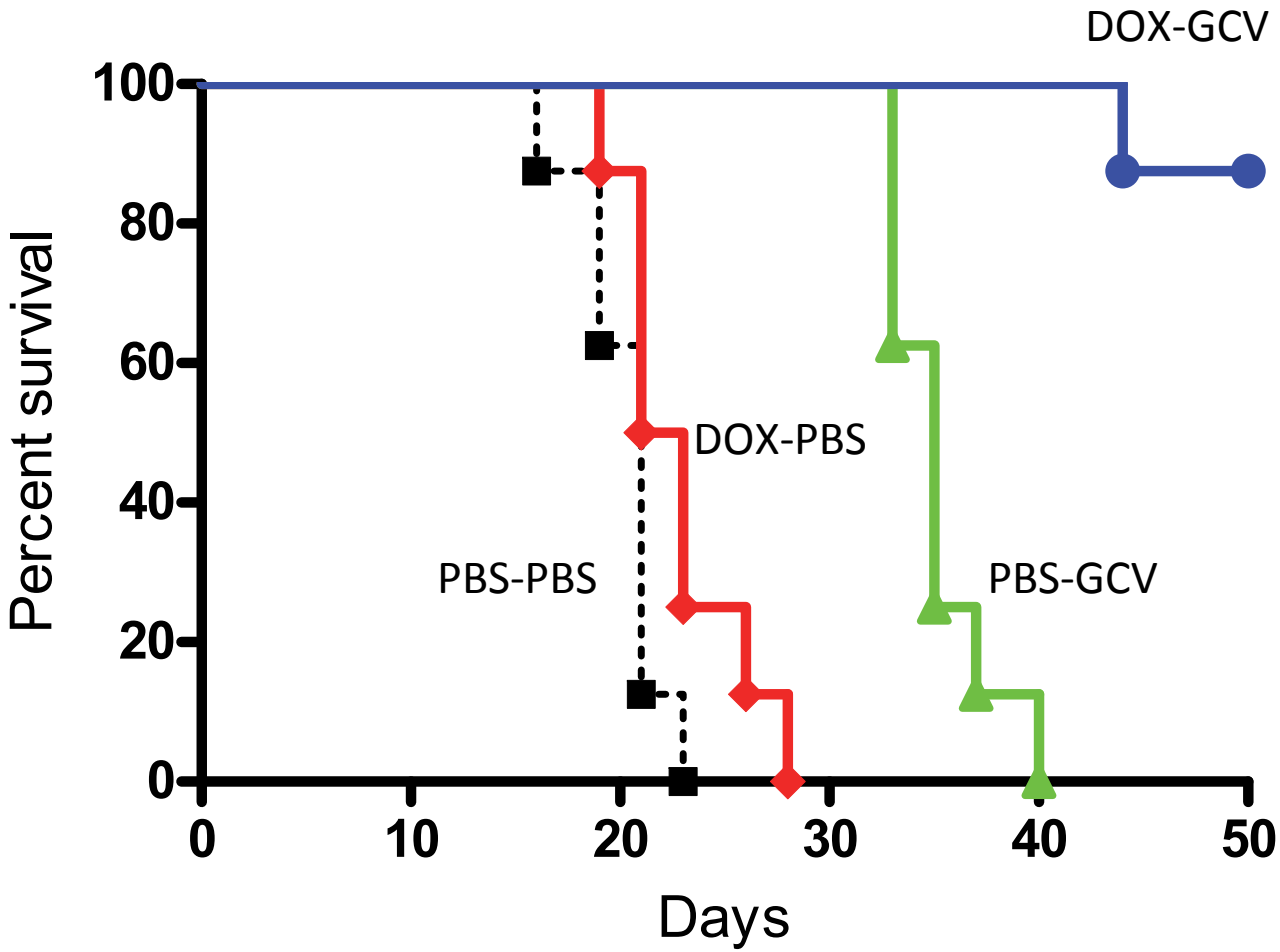
DOX-GCV
DOX-PBS
PBS-PBS
PBS-GCV
Percent survival
Days
100
80
60
40
20
0
0
10
20
30
40
50

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Antigens identified from the IEEL (which treats recurrent but not primary prostate tumors):

TOPOII$\alpha$; CDC-7 kinase; YB-1;

CD44v6B

-Up-regulation of *proteins of DNA replication* may be critical to drive tumor recurrence;

-*A subset of these proteins* may serve as tumor associated antigens specific to (early) recurrent tumors.

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## Conclusions: Combination Viro-/Immunotherapy with Chemotherapy

-Viral mediated expression of a broad antigenic repertoire (cDNA library) generates a wide-ranging T cell response against multiple antigens, leading to tumor rejection;

-Suboptimal vaccination is still sufficient to force the tumor cells to evolve an immune escape phenotype which is radically different from the parental tumor phenotype;

-This immune escape phenotype is *predictable and reproducible*, at least in certain phenotypic respects;

-By characterizing the molecular footprint of the escape phenotype, partly through antigen identification from the VSV-cDNA technology, it is possible to develop *rational, mechanism based* second line therapies to target tumor recurrences

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# Uses of VSV-cDNA Library Technologies:

-*Anti tumor Therapy*: multiple tumor types.

-*Tumor Antigen Discovery*: Arrays of antigens associated with T cell mediated tumor rejection.

-*Antigen Presentation*: Co-operative presentation by multiple types of APC to generate cumulative T cell activation.

-*Mechanisms of tumor recurrence*: antigenic targets that lead to rejection of recurrent tumors across tumor types and treatment barriers

-*Aetiology of autoimmune disease*

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Geoff Hall
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<u>University of Surrey, Guildford, UK</u>
Hardev Panda

Antigens identified from the IEEL (which treats recurrent but not primary prostate tumors):

-TOPOII$\alpha$; CDC-7 kinase; YB-1;

-CD44v6B

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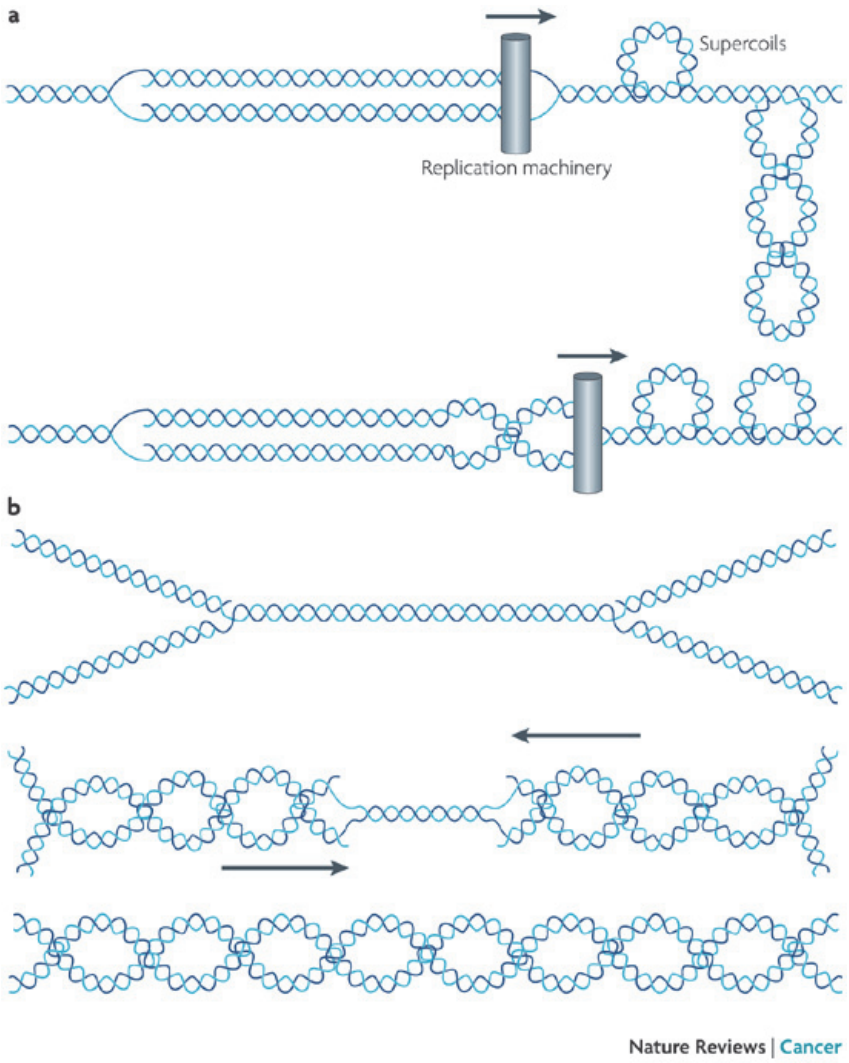


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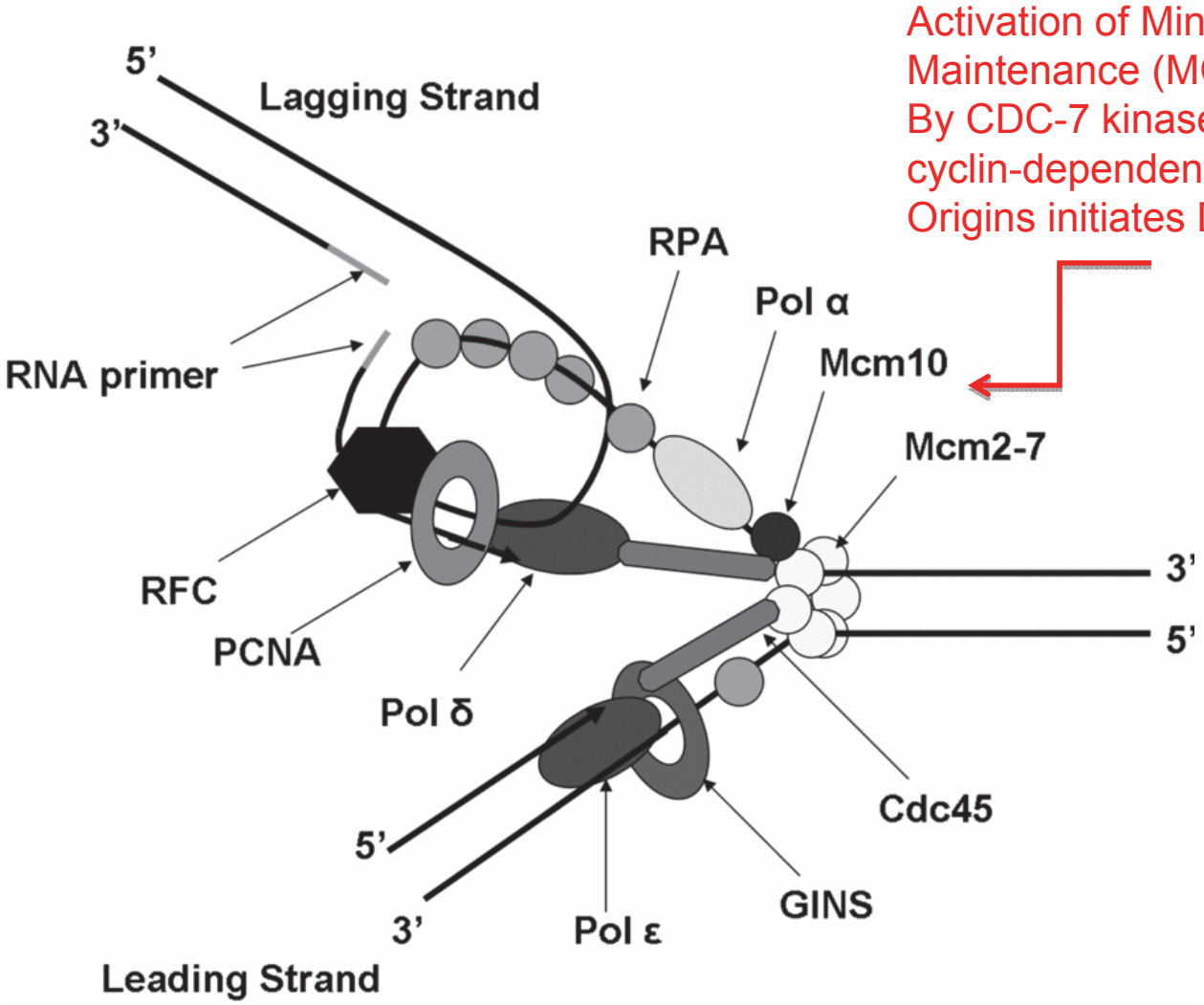
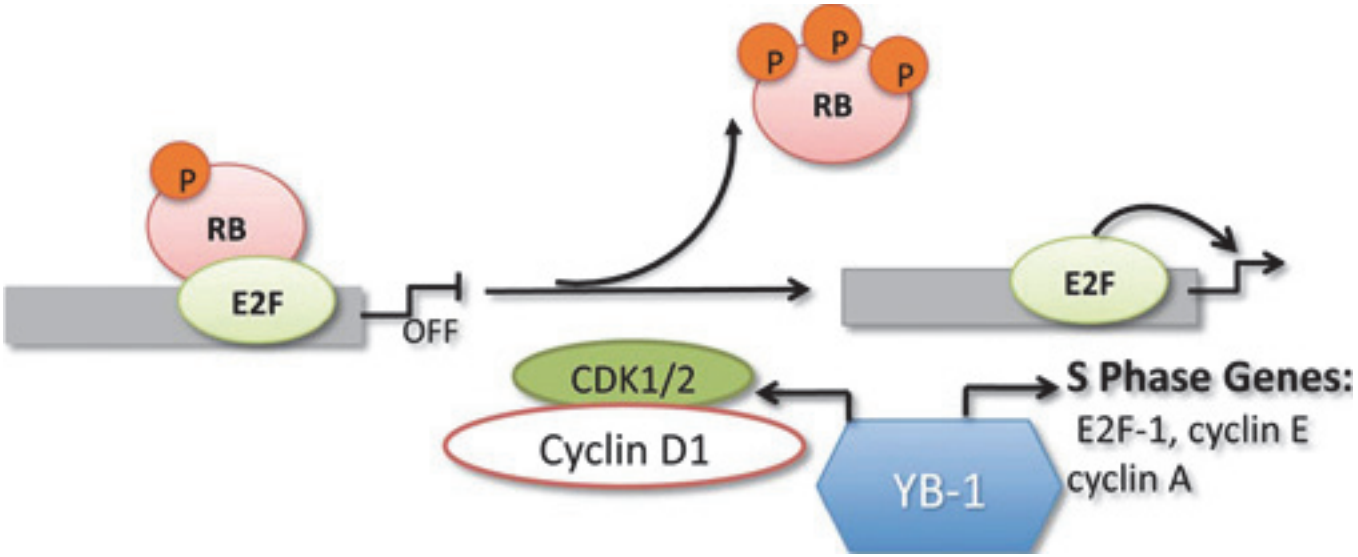
DNA Topoisomerase IIα

Figure 1 Cdc45 function at the replication fork during the elongation stage of eukaryotic DNA replication




Biochemical Society Transactions (2009) 37, 926-930
- Ronan Broderick and Heinz-Peter Nasheuer
www.biochemsoctrans.org




**YB-1 modulates RB tumor suppressor activity**

The diagram illustrates the regulation of RB function and how YB-1 affects this process. YB-1 transactivates the upstream regulators of RB, cyclin D1 and CDK1/2, which promote hyperphosphorylation of RB leading to release of E2F1 (and the transfection factors). YB-1 also directly activates expression of S-phase genes including those encoding E2F1, cyclin E and cyclin A. Both these processes promote cell-cycle progression. P, phosphorylation.

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