Presenter Disclosure Information

Susan M. Kaech

The following relationships exist related to this presentation:

Symphogen Inc., Collaborative Research Project

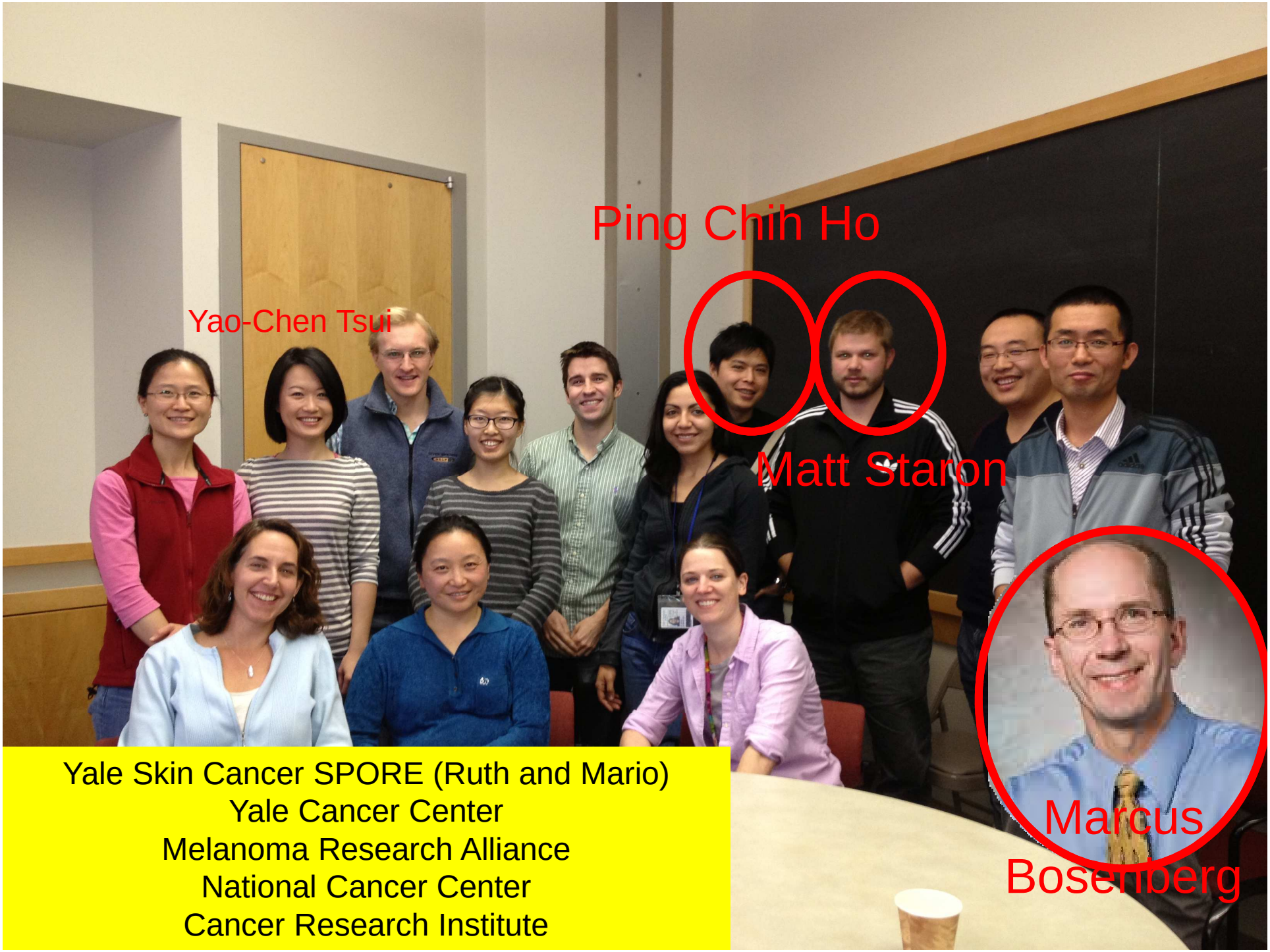
AbbVie, Collaborative Research Project

Immune-based antitumor effects of BRAF inhibitors rely on signaling by CD40L and IFNγ

Susan Kaech

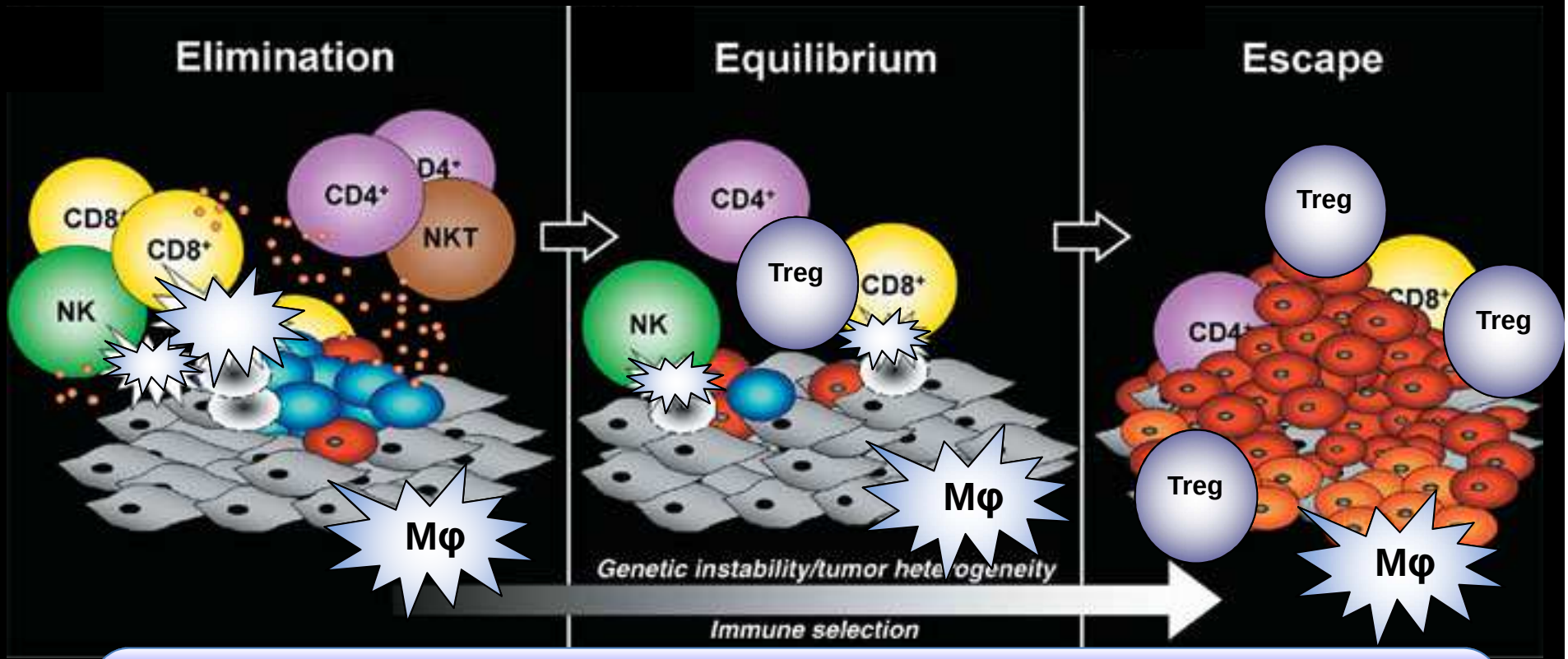
Dept. Immunobiology

Yale University



Yale Skin Cancer SPORE (Ruth and Mario)
Yale Cancer Center
Melanoma Research Alliance
National Cancer Center
Cancer Research Institute

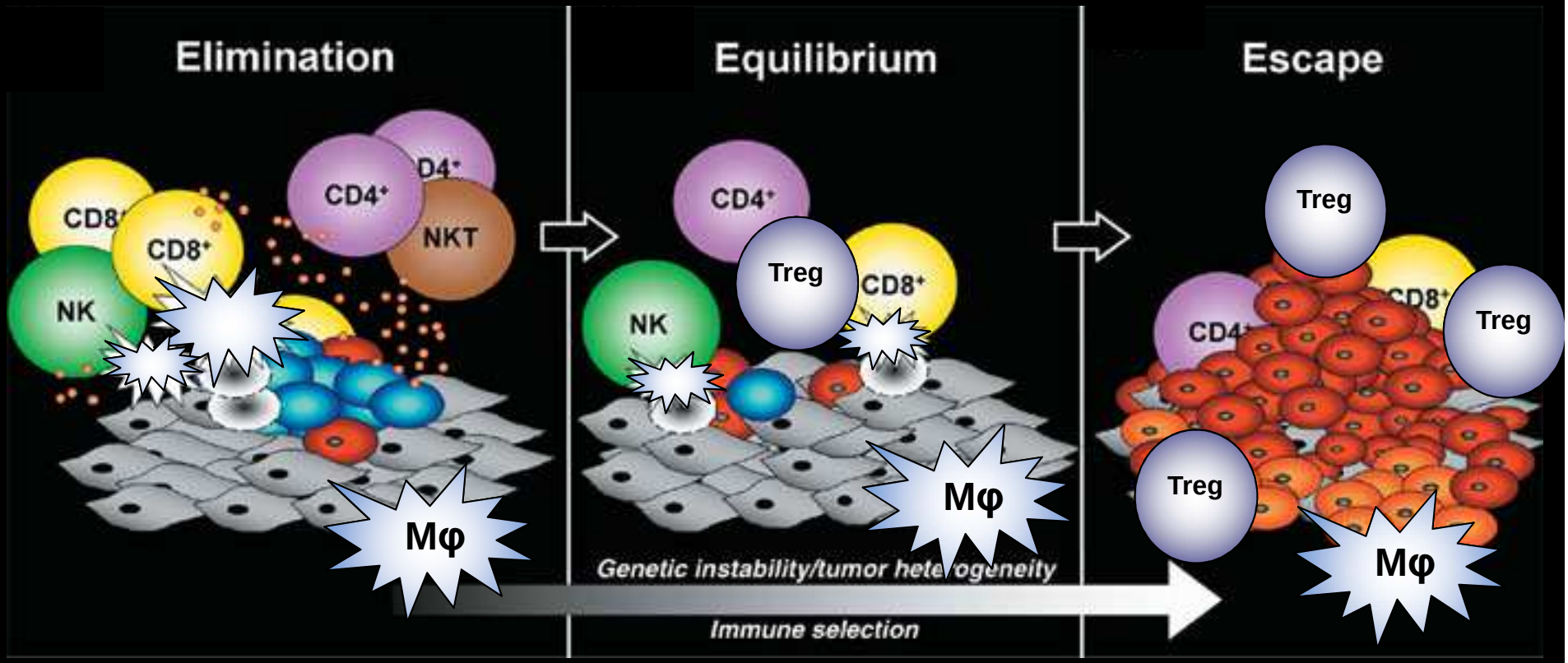
Immune surveillance and escape



How is an immunosuppressive tumor microenvironment formed and maintained?

Can this be reversed to promote anti-tumor immune responses?

Immune surveillance and escape



What alterations occur in the immune cells in the tumor microenvironment and how is this affected by anti-tumor therapies?

Inducible $Braf^{V600E}$/Pten murine melanoma model

ER-Cre

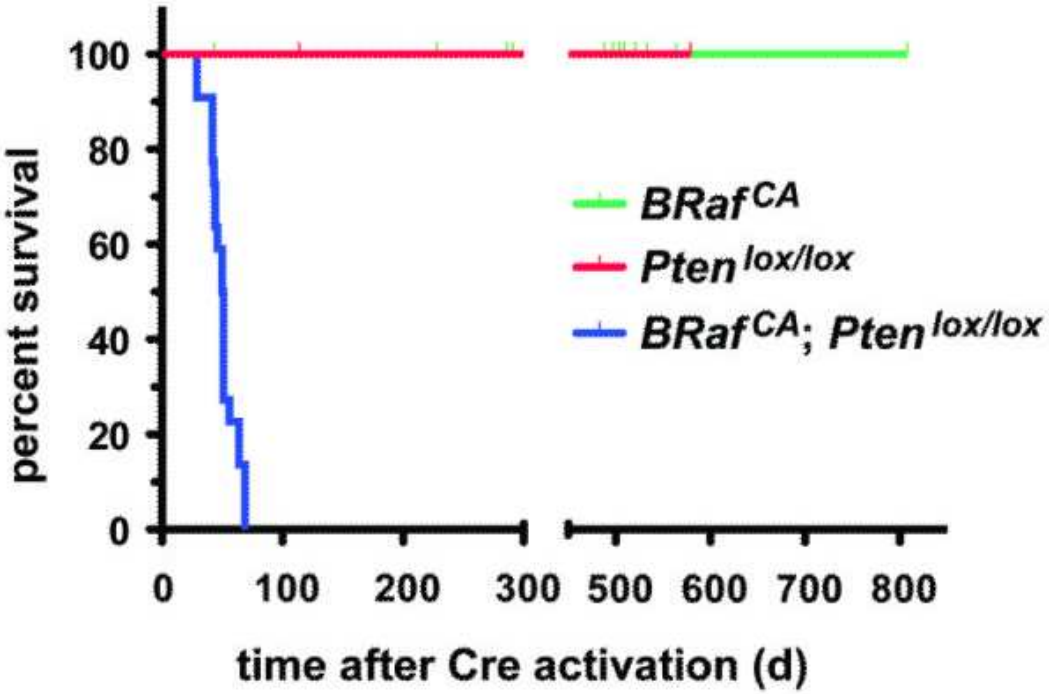
$BRAF^{WT}$

$PTEN^{fl/fl}$

Active ER-Cre

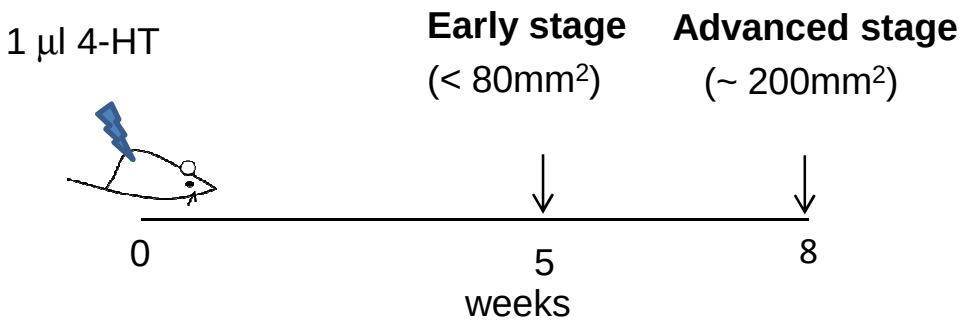
$BRAF^{V600E}$

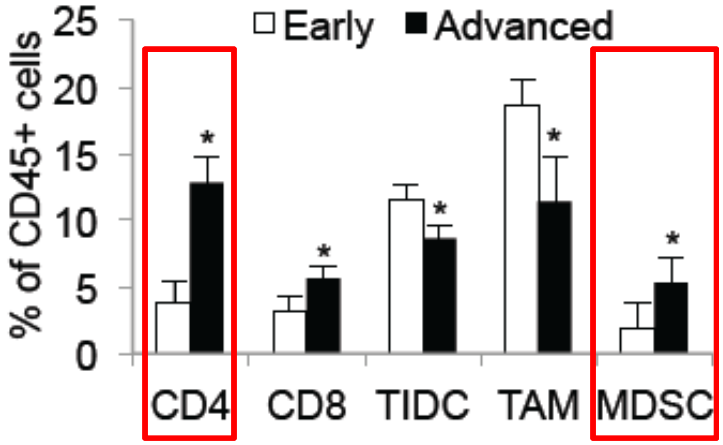
PTEN deletion

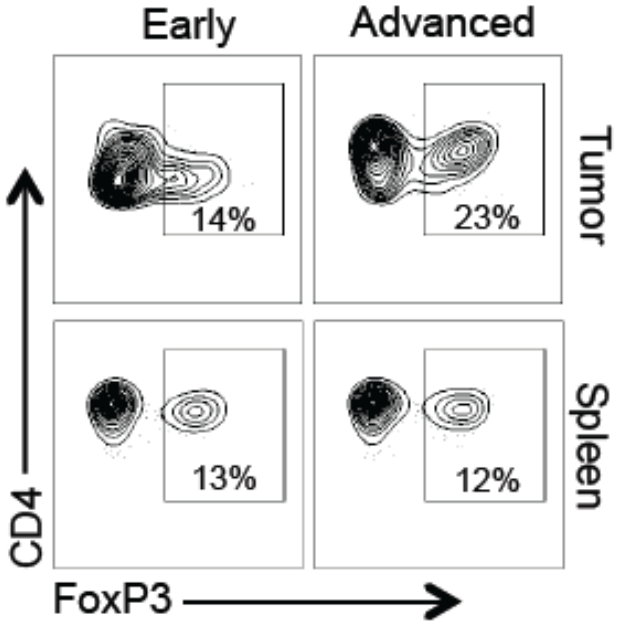


Dankort et al., Nat. Genetics 41; 544

Accumulation of immunomodulatory cells within melanomas during tumor growth





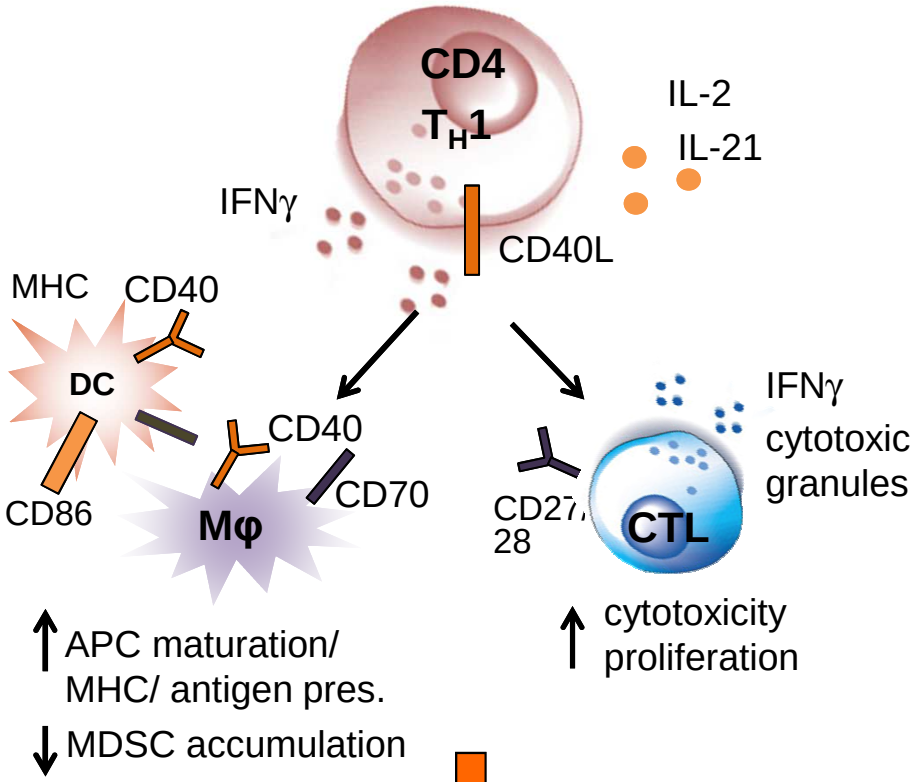


TIDC: tumor-infiltrating dendritic cell

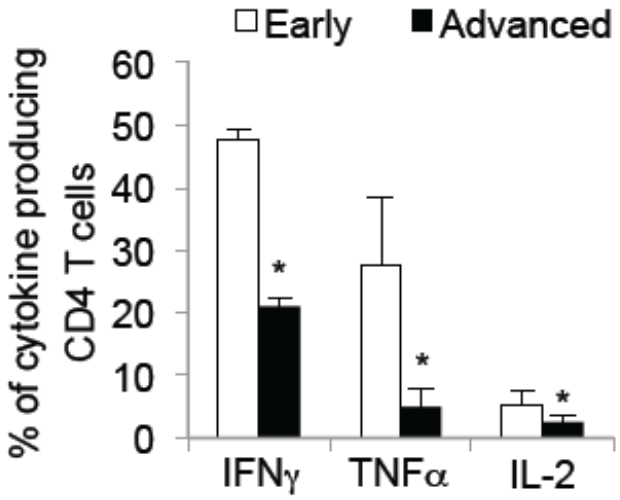
TAM: tumor-associated macrophage

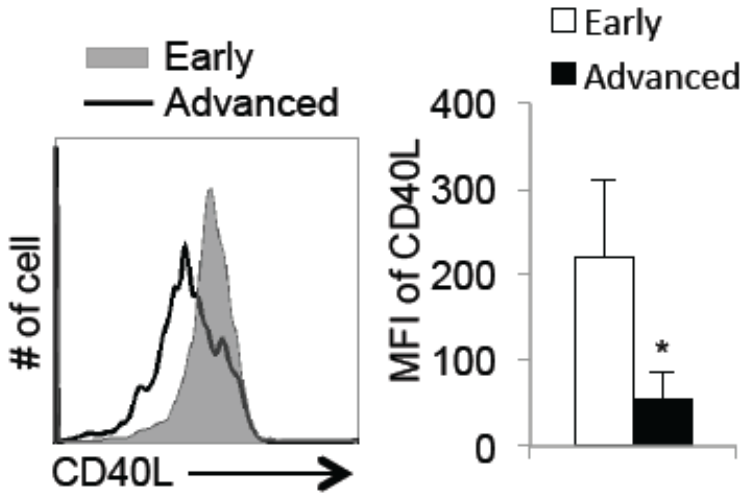
MDSC: myeloid-derived suppressor cell

Diminished effector and helper functions on intratumoral CD4 T cells during tumor progression

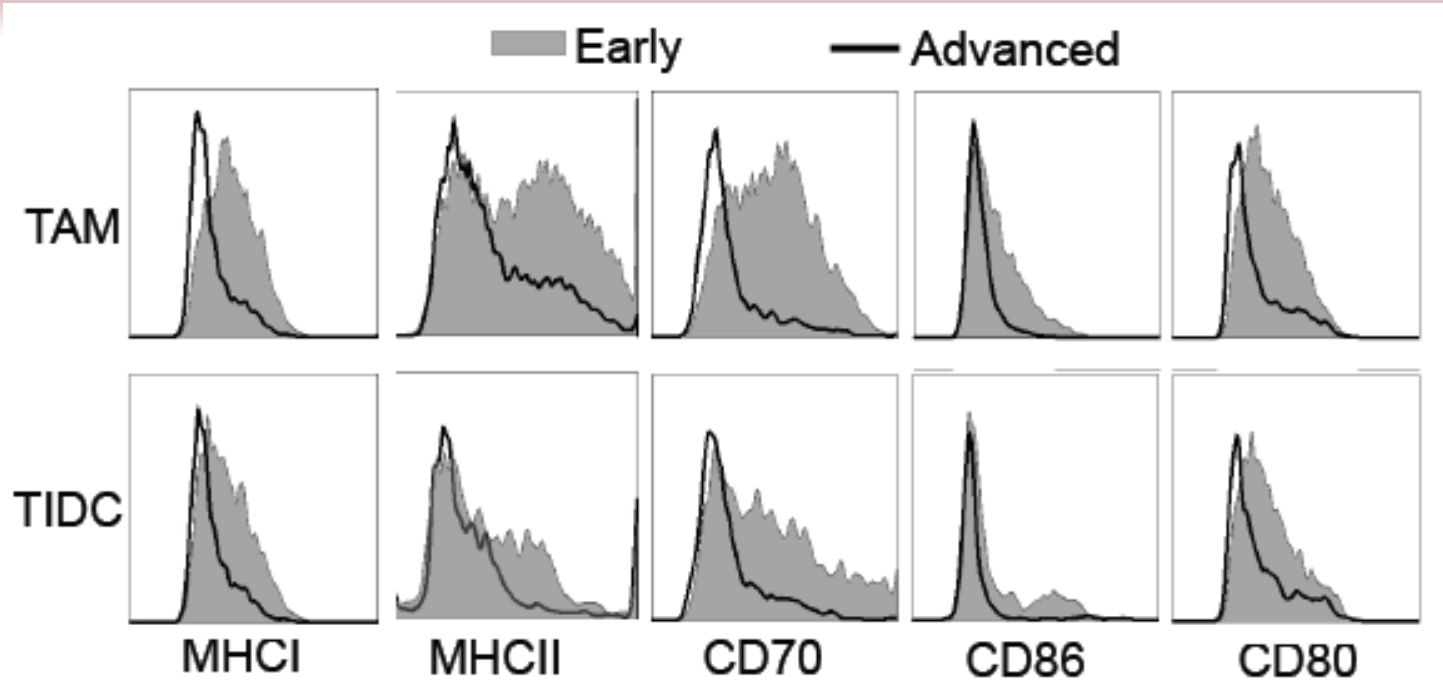


Tumor regression



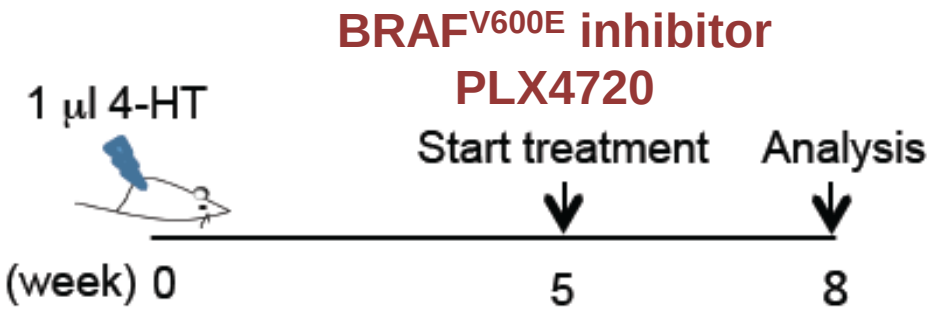


Tumor infiltrating APCs acquired tolerogenic phenotype during tumor progression



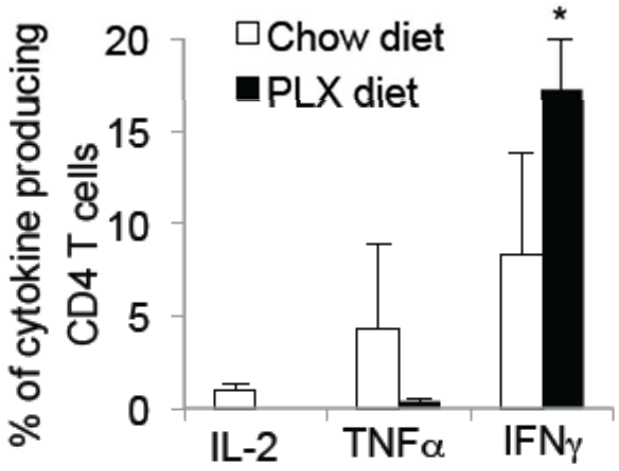
- TIDCs isolated from advanced staged melanomas lost antigen-presenting ability.
- TAMs acquired M2-skewed phenotype during tumor growth and inflammatory properties declined.
- Agonistic anti-CD40 mAb promoted M1-skewed phenotype of TAMs and elevated antigen-presenting ability of TIDCs in the *in vitro* cultures.

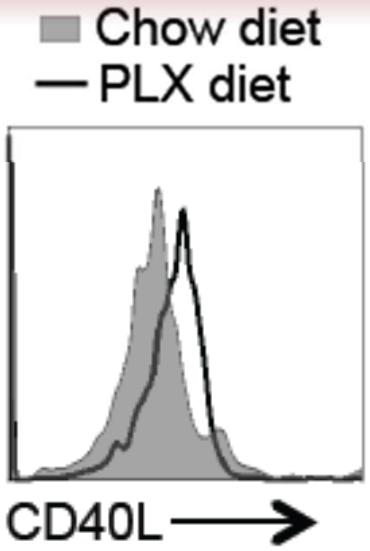
What happens to the immune cells during $BRAF^{V600E}$ inhibitor treatment?

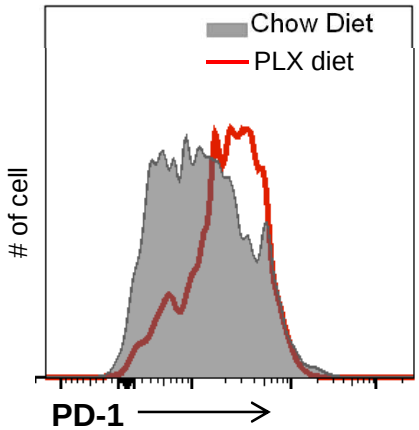


- **PLX4720 boosts tumor infiltration of CD8 T cells and enhances antitumor responses.**
  (Clin Cancer Res. 2013; 19: 393, Clin Cancer Res. 2012; 18: 1386 Cancer Immunol Res 2014 2:643& Cancer Res. 2013; 72: 3928)
- **PLX4720 promotes tumor regression via CCR2- and CD8 T cell-dependent mechanisms** (JCI 2013; 123:1371)**.**
- **PLX4720 had no effect or reduced infiltration of T cells.**
  (Oncoimmunology 2012 5:609).
- **Vemurafenib treatment can directly stimulate T cell MAPK signaling**
  (Clin Cancer Res. 2013; 19:598)**.**

Targeting $BRAF^{V600E}$ augmented IFNγ- and CD40L-expressing population of intratumoral CD4 T cells





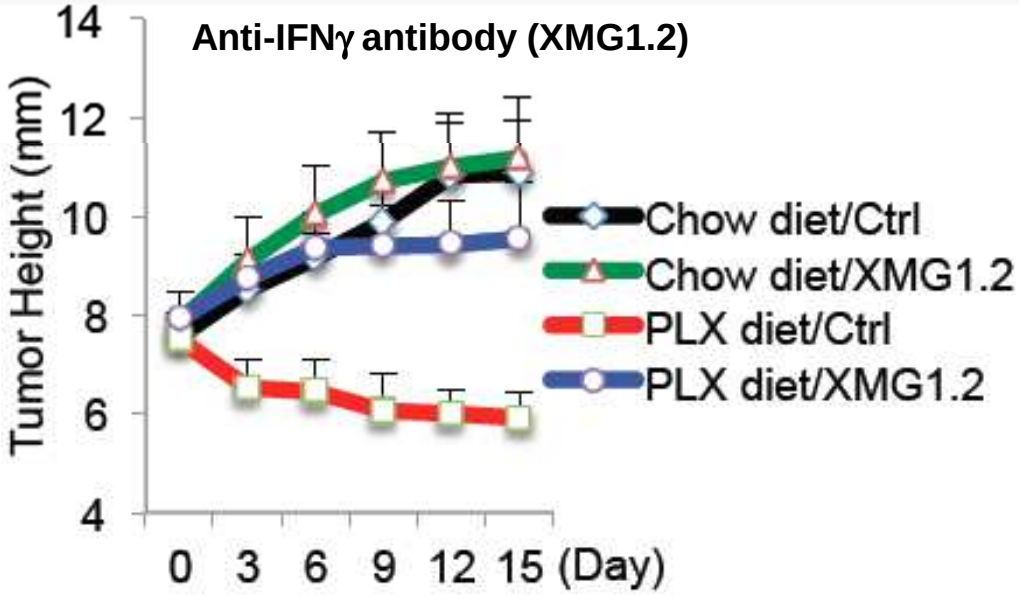


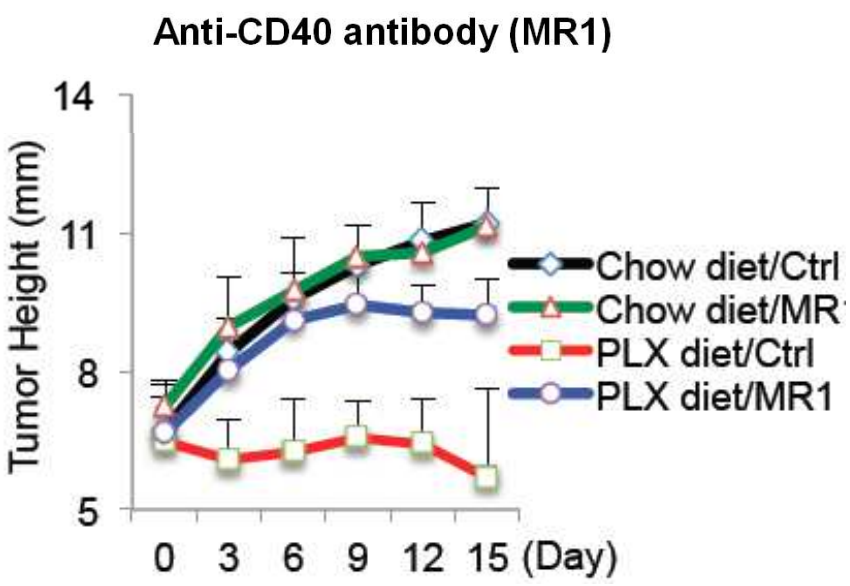
Braf-inhibitor Treatment

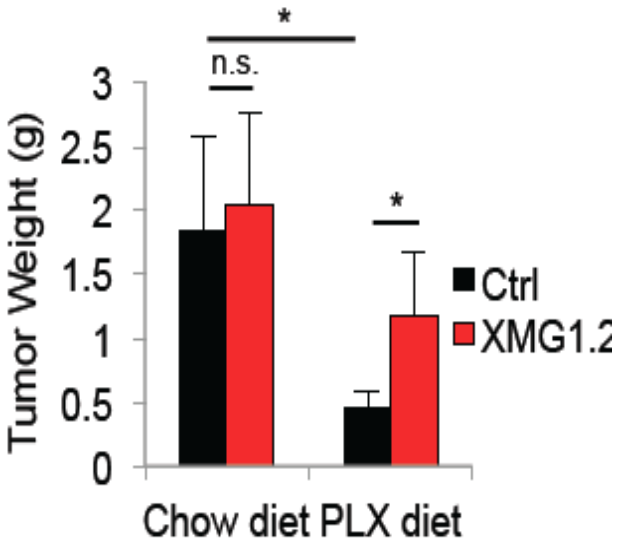
- ↑ IFNγ T cells
- ↑ CD40L+ T cells
- ↑ PD-1+ T cells

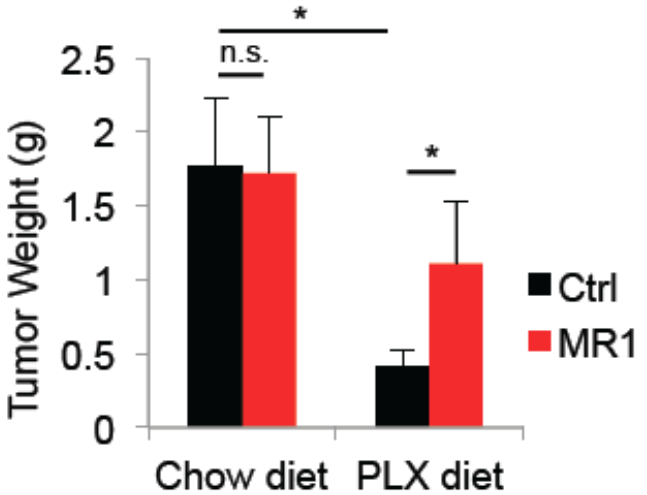
- ↑ Activated Macs
- ↓ MDSCs
- ↓ Tregs

IFNγ– and CD40L-signaling is required for PLX4720-mediated antitumor response





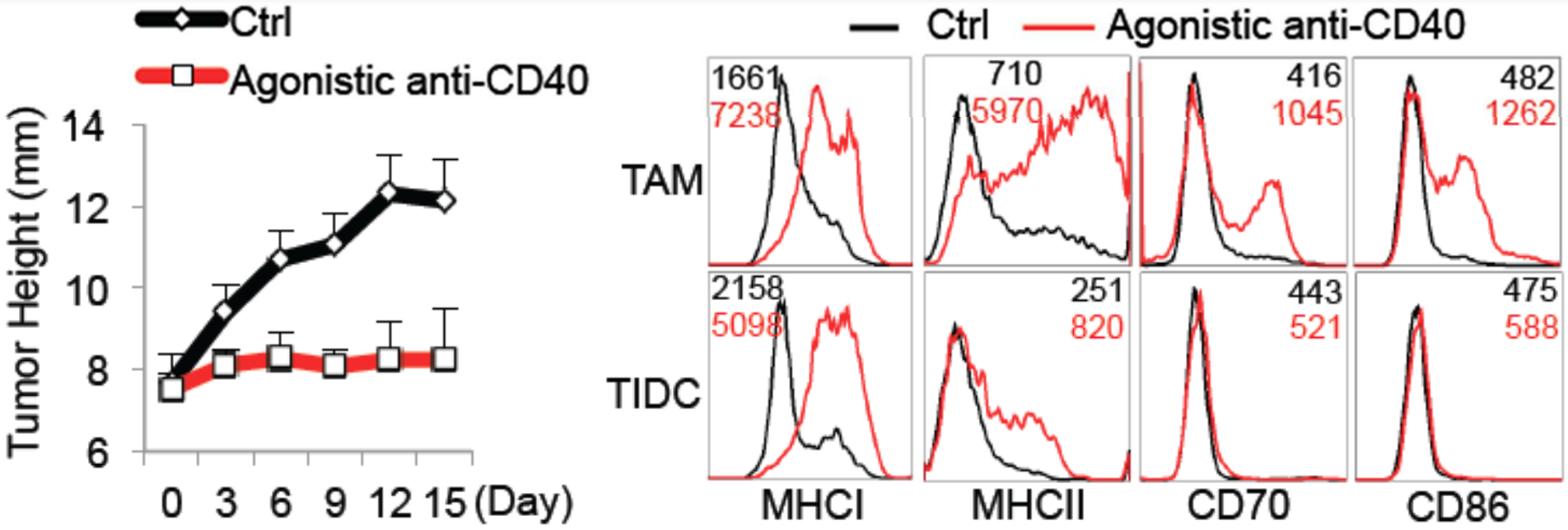




If CD40:CD40L is a critical pathway for promoting anti-melanoma immunity,

then is agonistic anti-CD40 mAb treatment therapeutic?

Agonistic anti-CD40 mAb alone inhibited melanoma progression

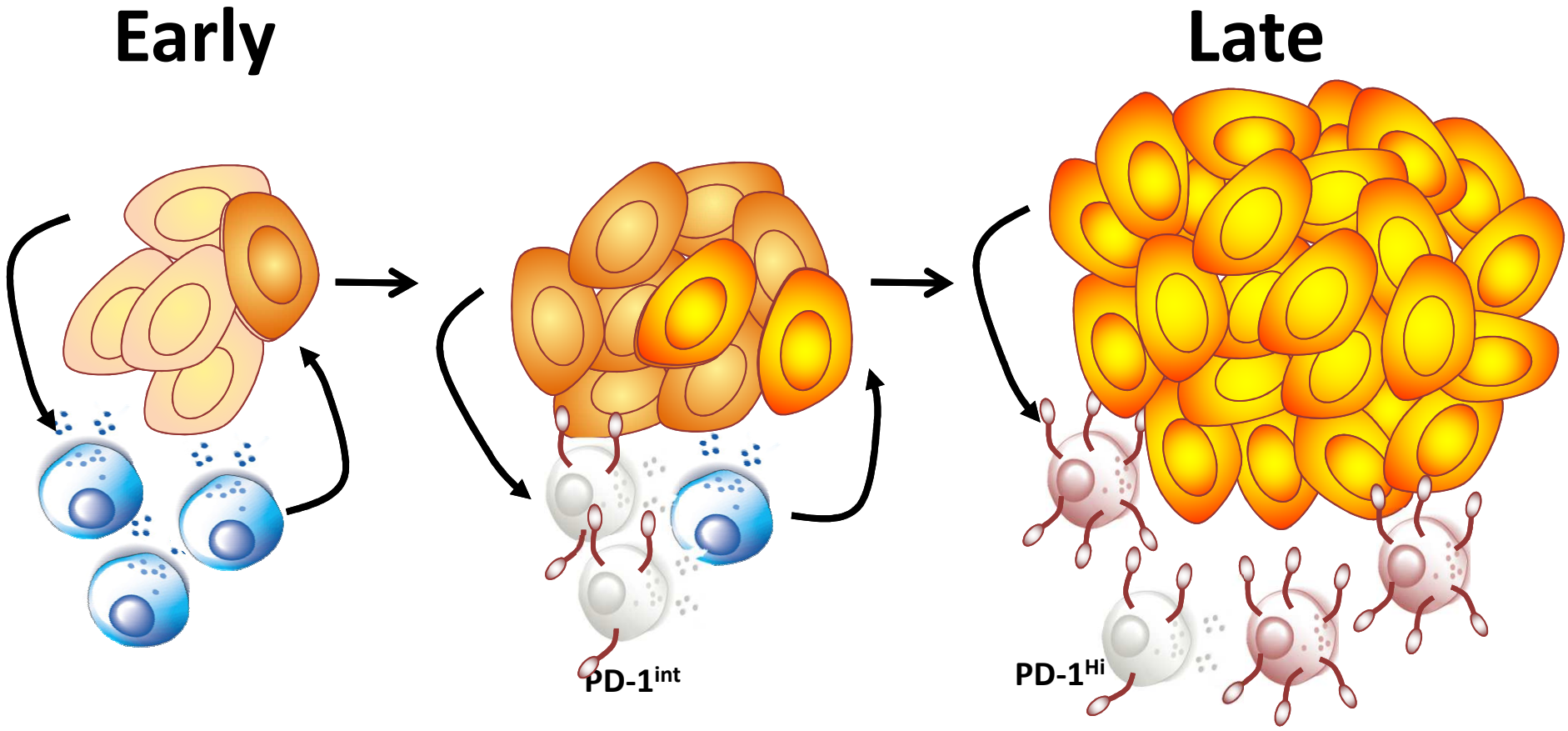


Effects of anti-CD40 agonistic antibody are T cell independent

(similar to Beatty et al. Science 2011 in pancreatic cancer models).

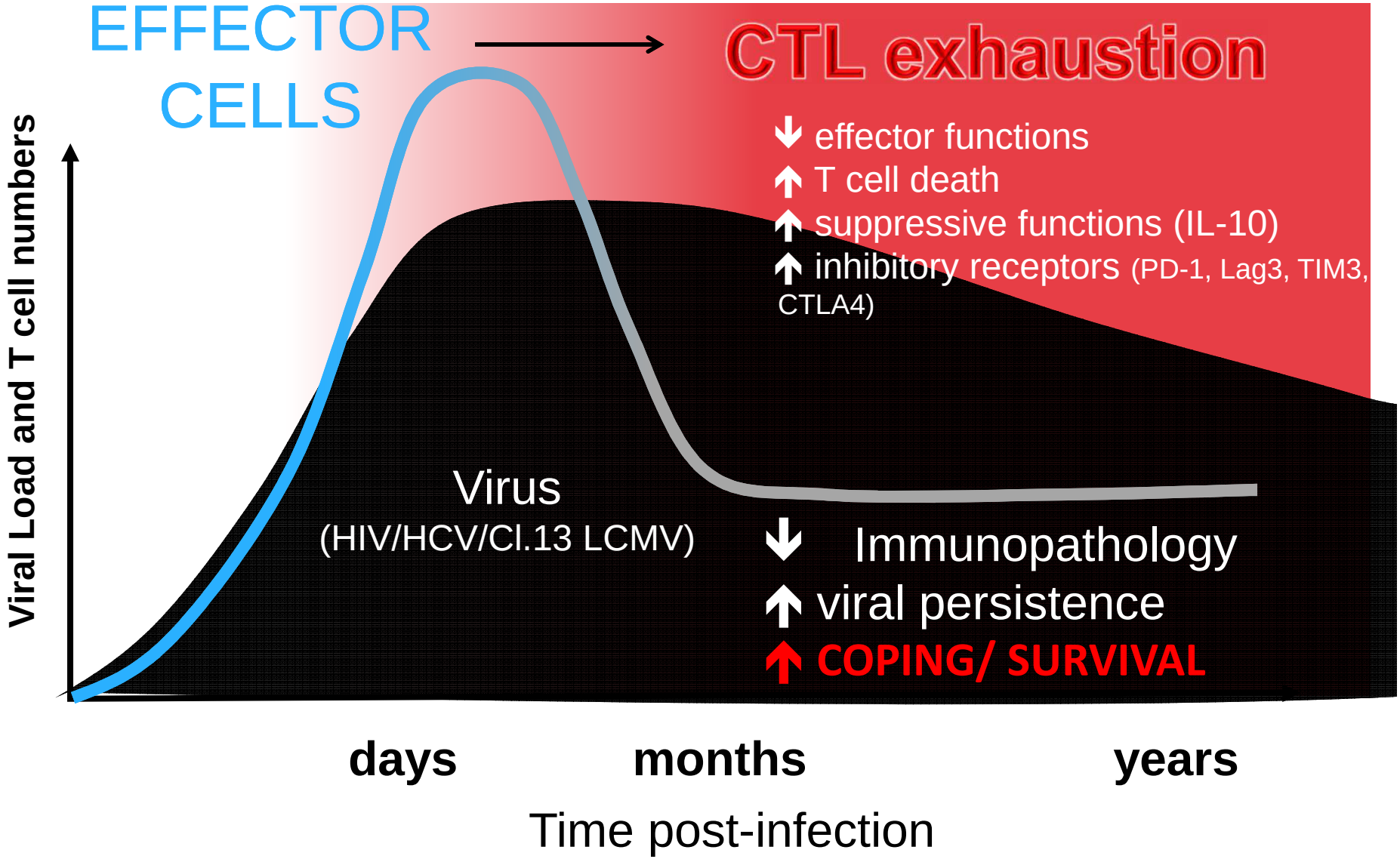
Durability?

Combo immunotherapies…innate + adaptive
+ targeted therapies

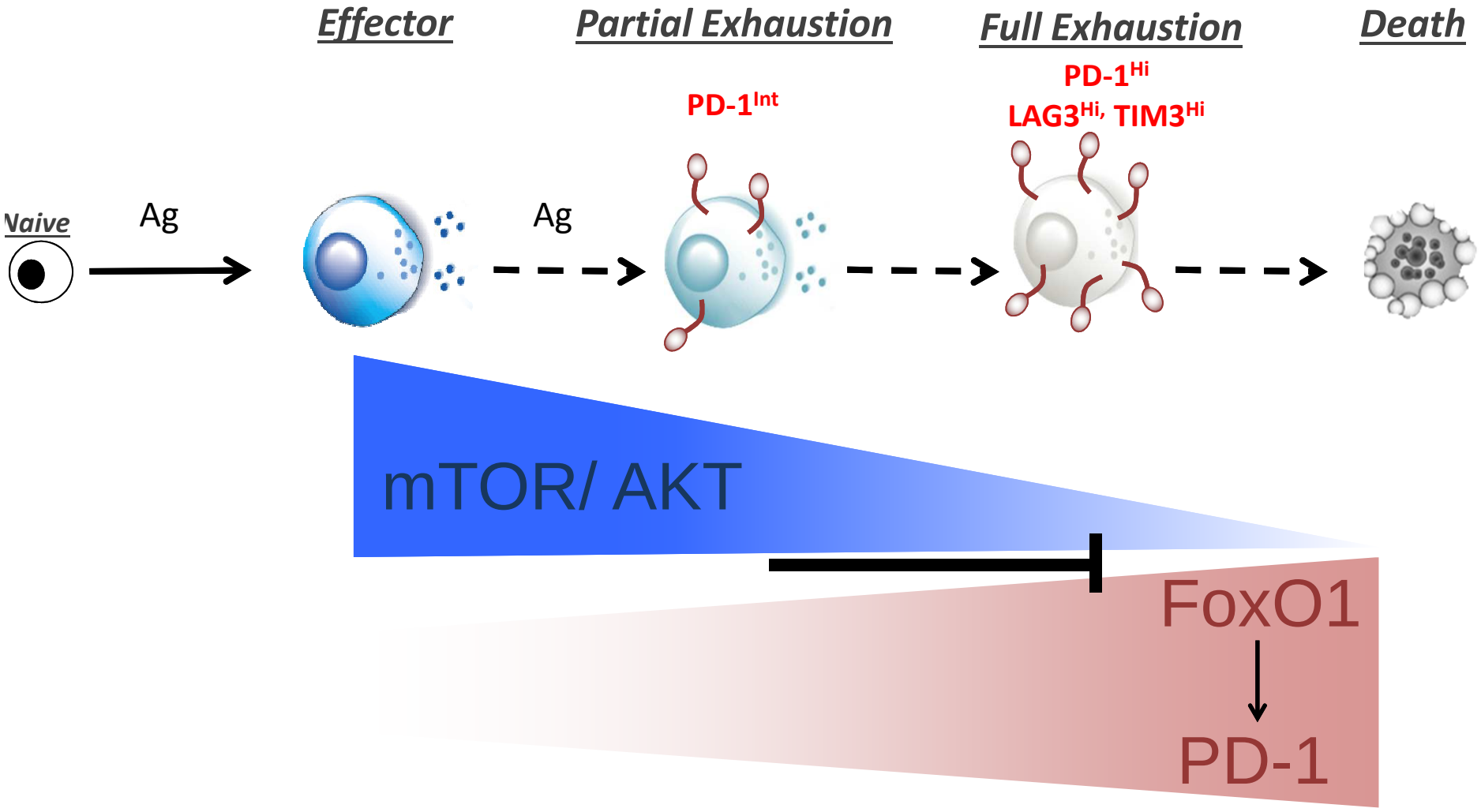


How is T cell exhaustion controlled in tumors?

CTL exhaustion during chronic infection

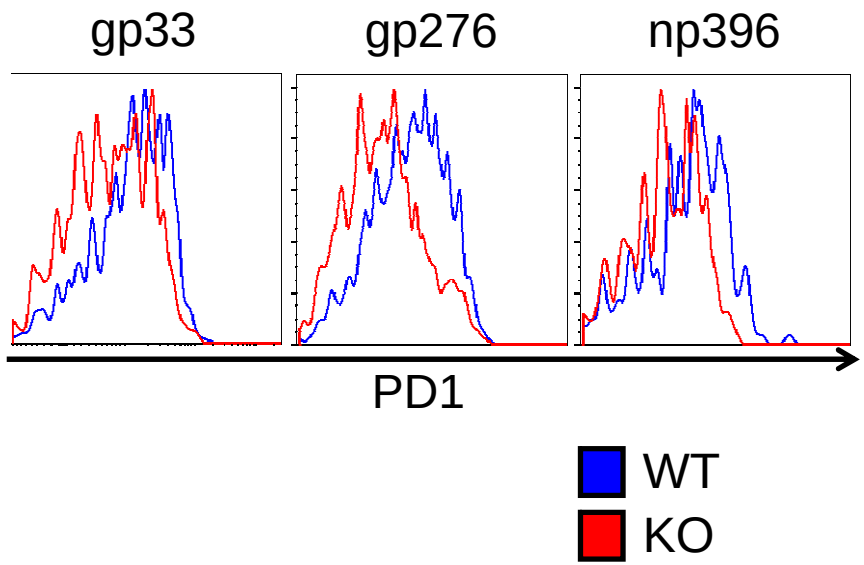


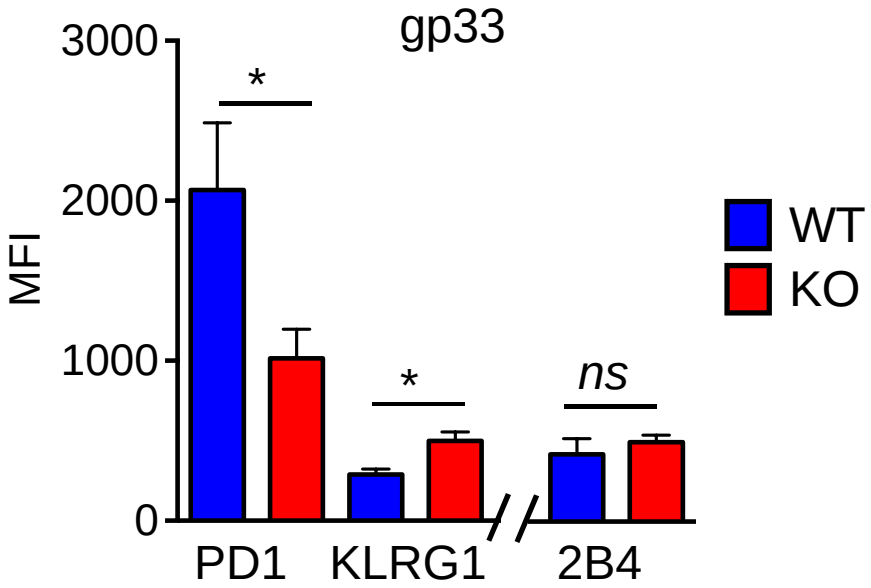
PI3K- AKT-mTOR signaling declines during T cell exhaustion



Staron et al., in press *Immunity*

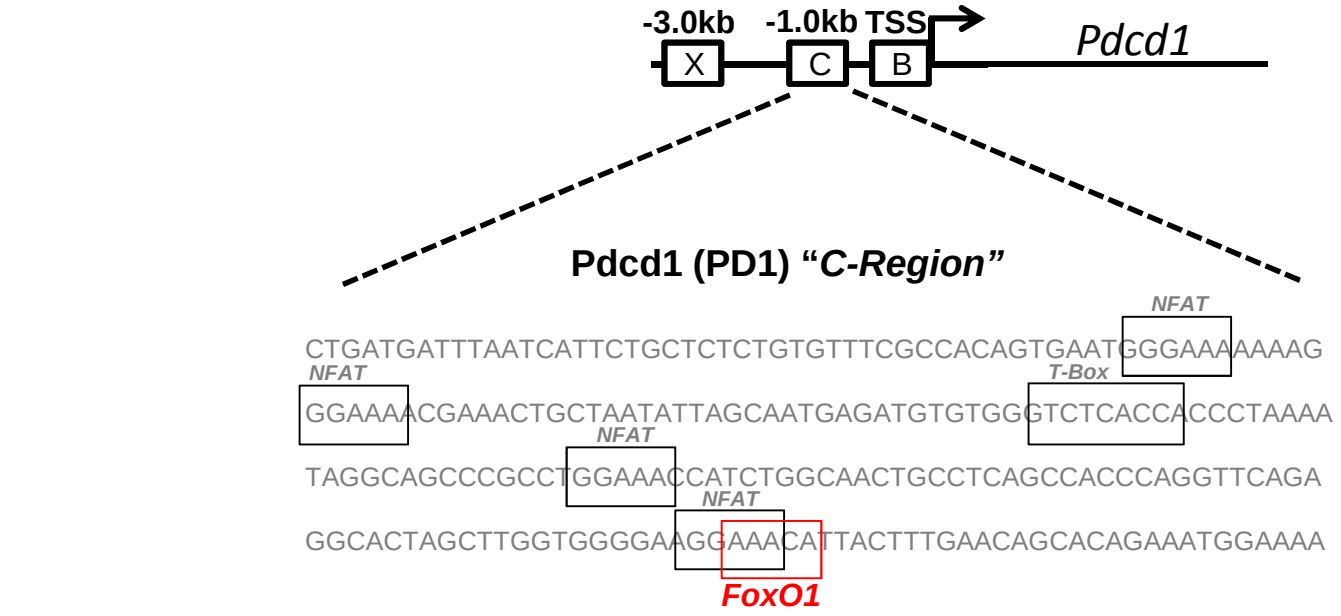
FoxO1 is required for increased PD1 expression in virus-specific CTLs during persistent infection.

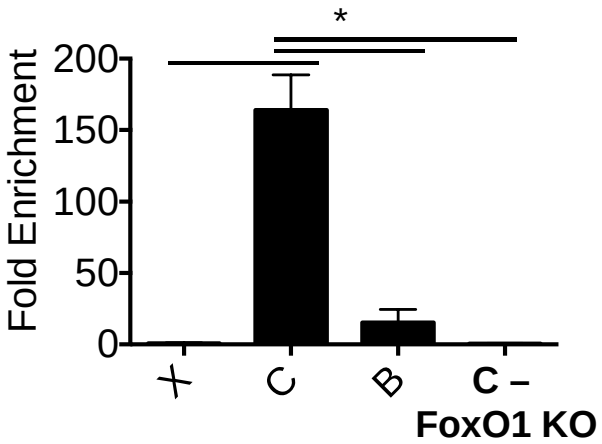


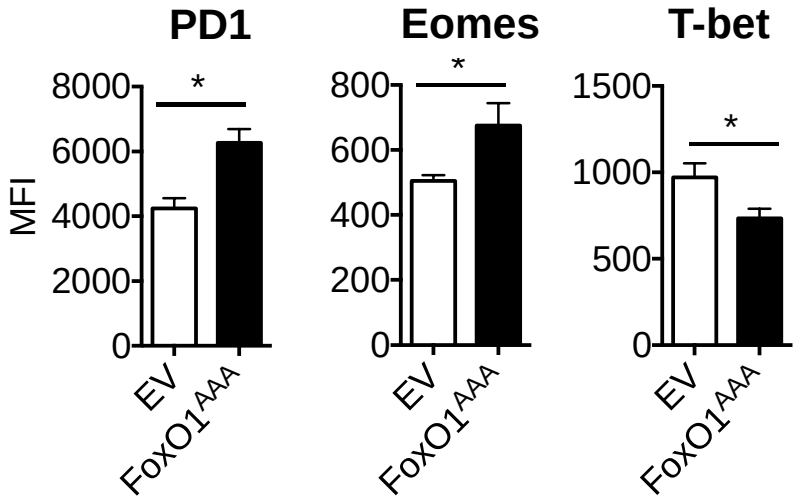


Does FoxO1 regulate PD-1 expression directly?

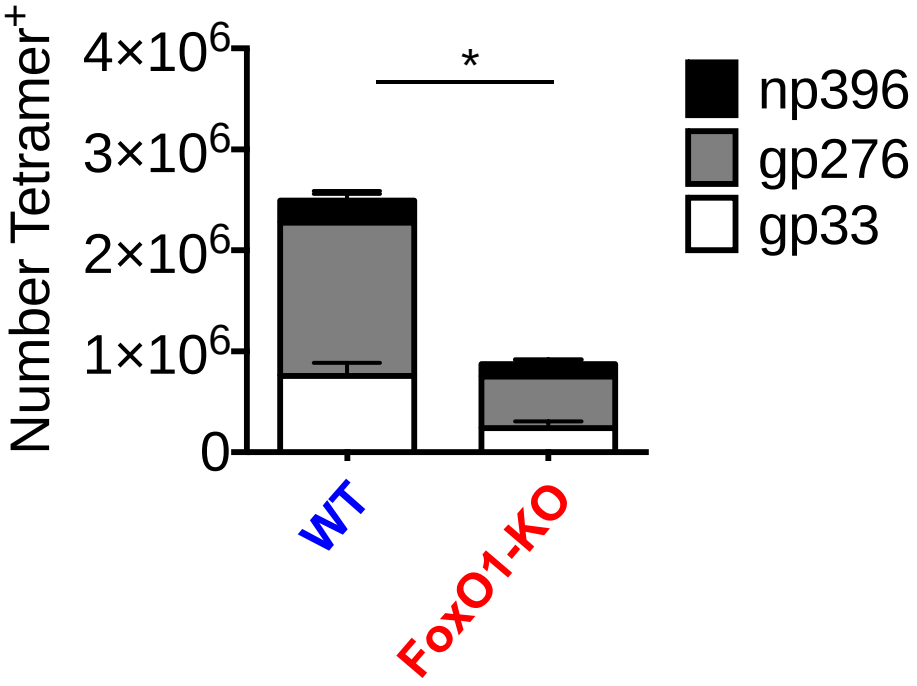
FoxO1 controls PD1 expression during CD8 T cells





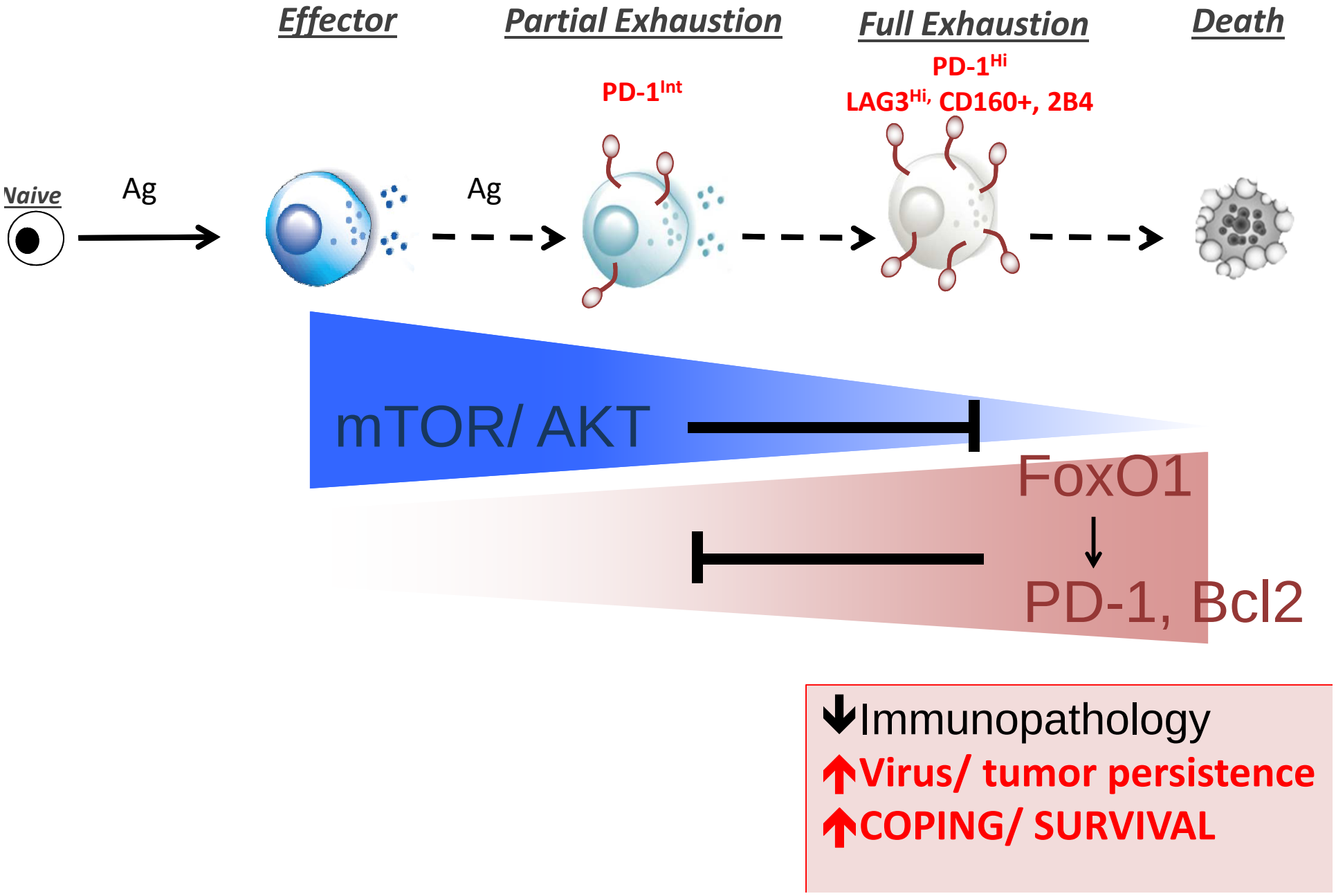


FoxO1 is required to sustain virus-specific CTLs during persistent infection.



↓PD-1
↓Bcl-2
↓Proliferation

PI3K- AKT-mTOR signaling declines during T cell exhaustion



Summary

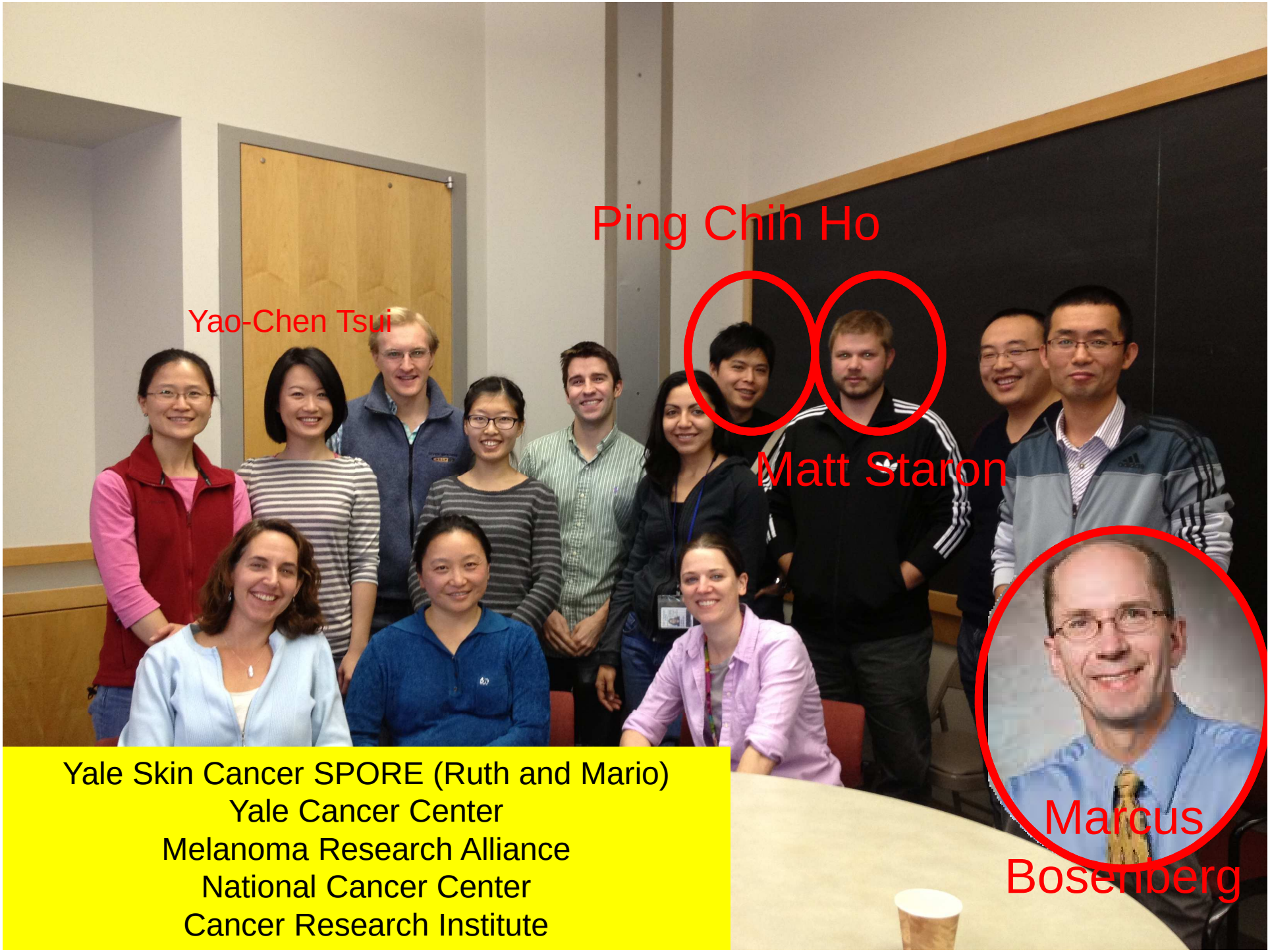
- B-raf inhibitors can have an immunostimulatory effect on the tumor micro-environment. Loss of Tregs and MDSCs, gain of T cell function and maturation of TAMs and TIDCs.

- IFNγ and CD40:CD40L signaling is critical to sustaining an immunosupportive tumor microenvironment, especially during B-raf inhibitor treatment.

- Treatment with CD40 agonistic mAbs is sufficient to suppress melanoma growth in a T cell independent manner.
    - *Will drugs that target both innate and adaptive responses be more effective?*

- PD-1 expression in "exhausted" CTLs is dependent on FoxO1, but this also needed to sustain the pool of CTLs.

- The development of an "exhausted" phenotype in the face of persistent antigen is important to maintain the pool of CTLs.
    - *Can we reinvigorate the CTL response via ↑ mTOR/AKT?*



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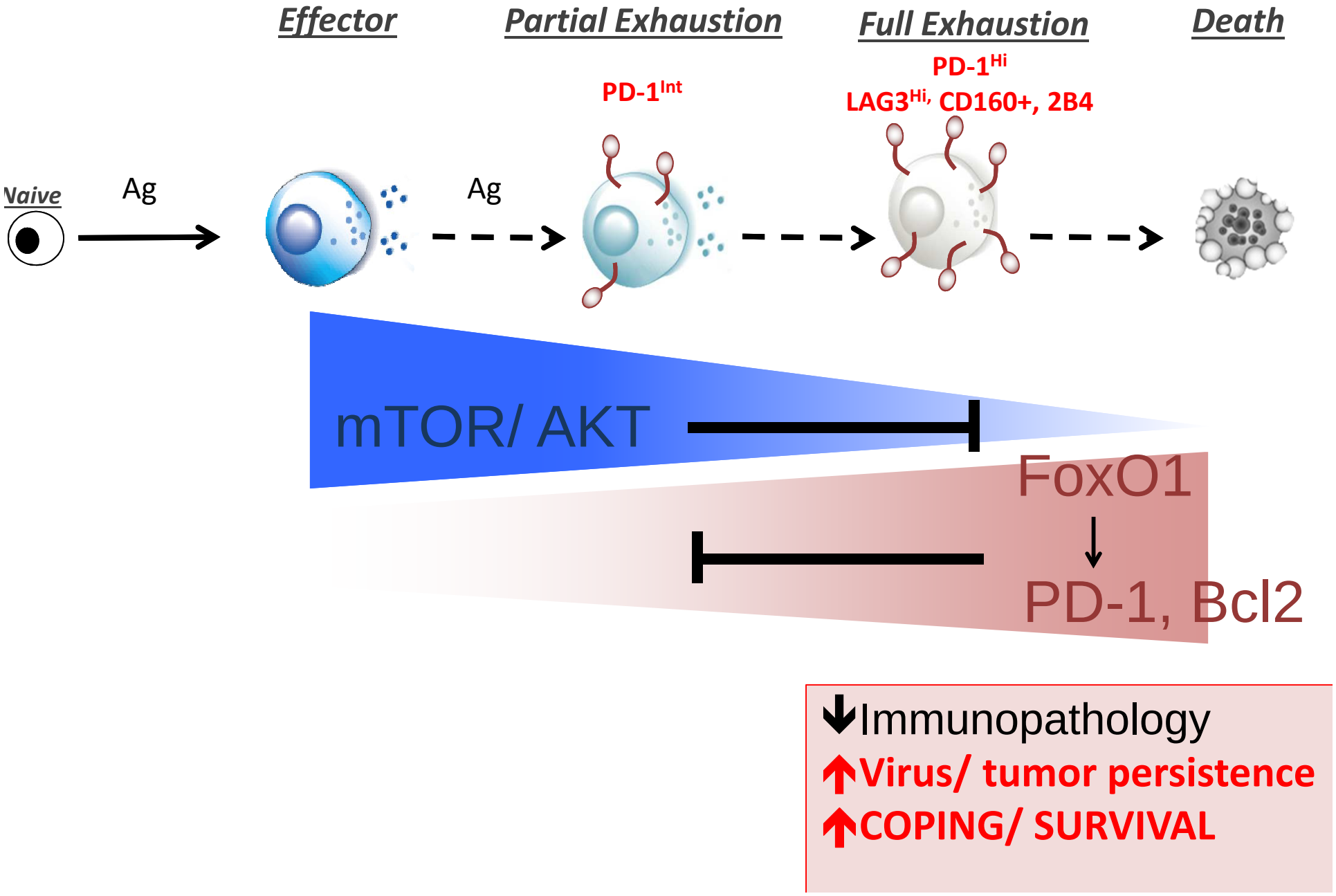
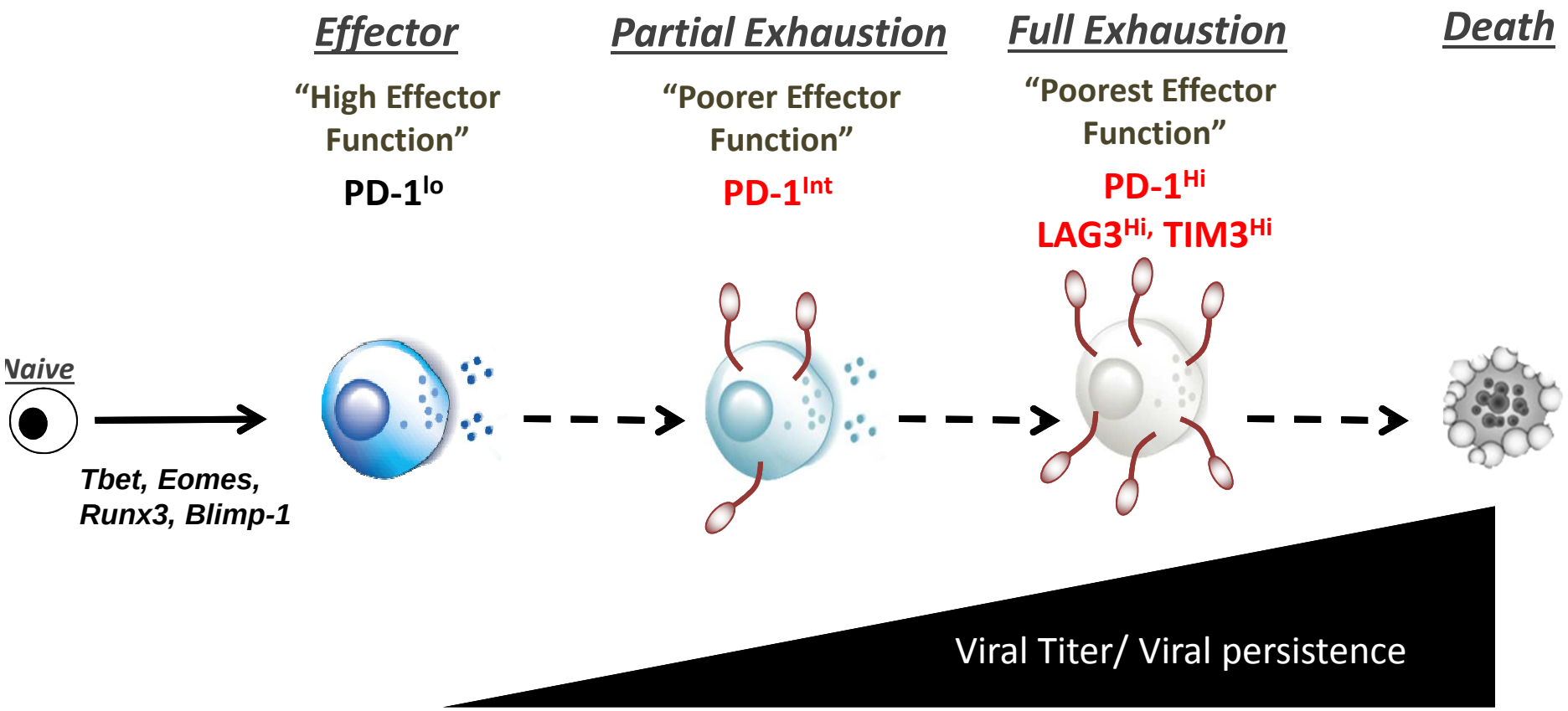
PI3K- AKT-mTOR signaling declines during T cell exhaustion

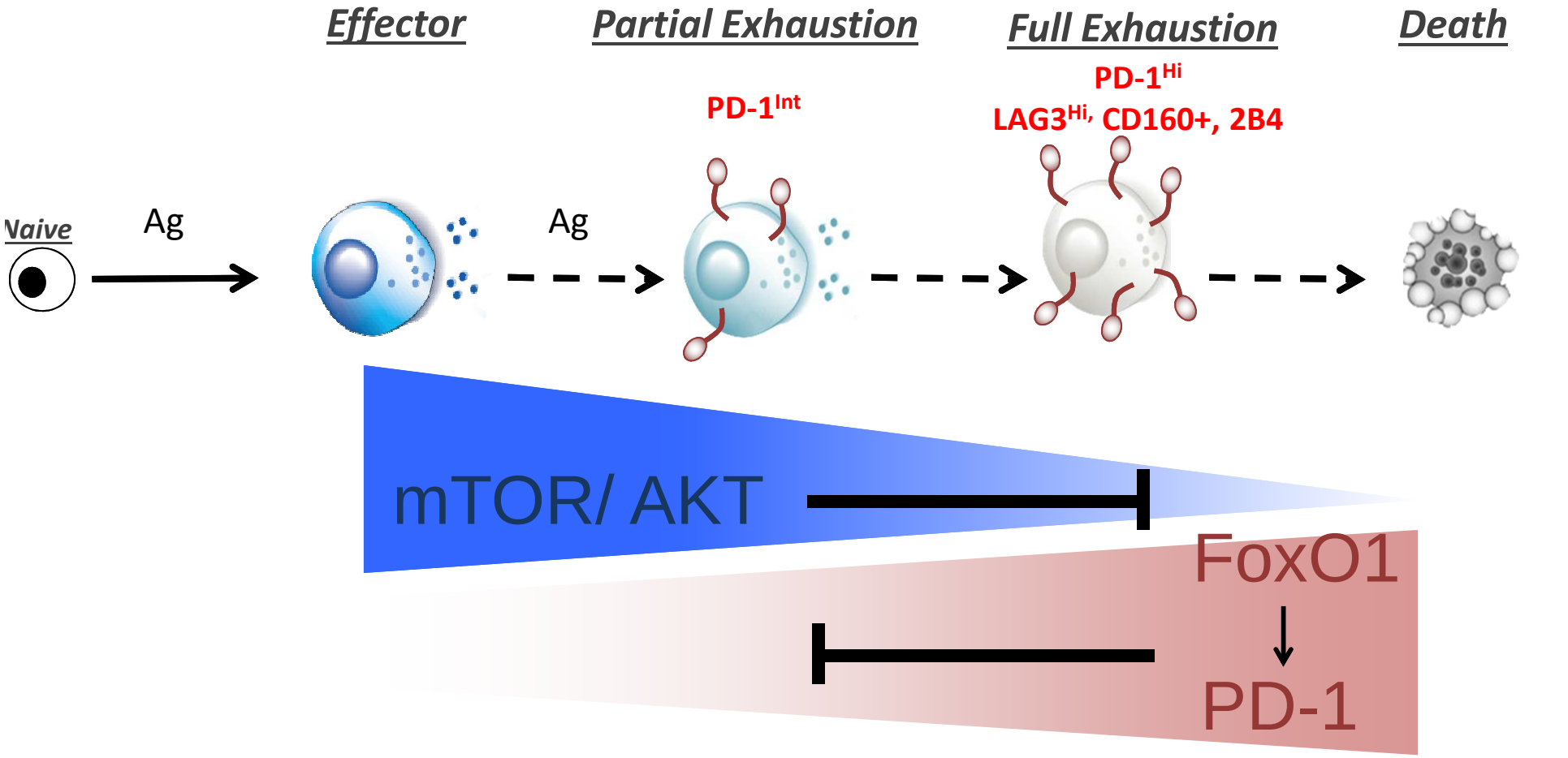


Progressive loss of T cell function during chronic infection



Can we intervene and prevent or reverse CTL exhaustion to improve viral control or develop anti-cancer therapies?

PI3K- AKT-mTOR signaling declines during T cell exhaustion

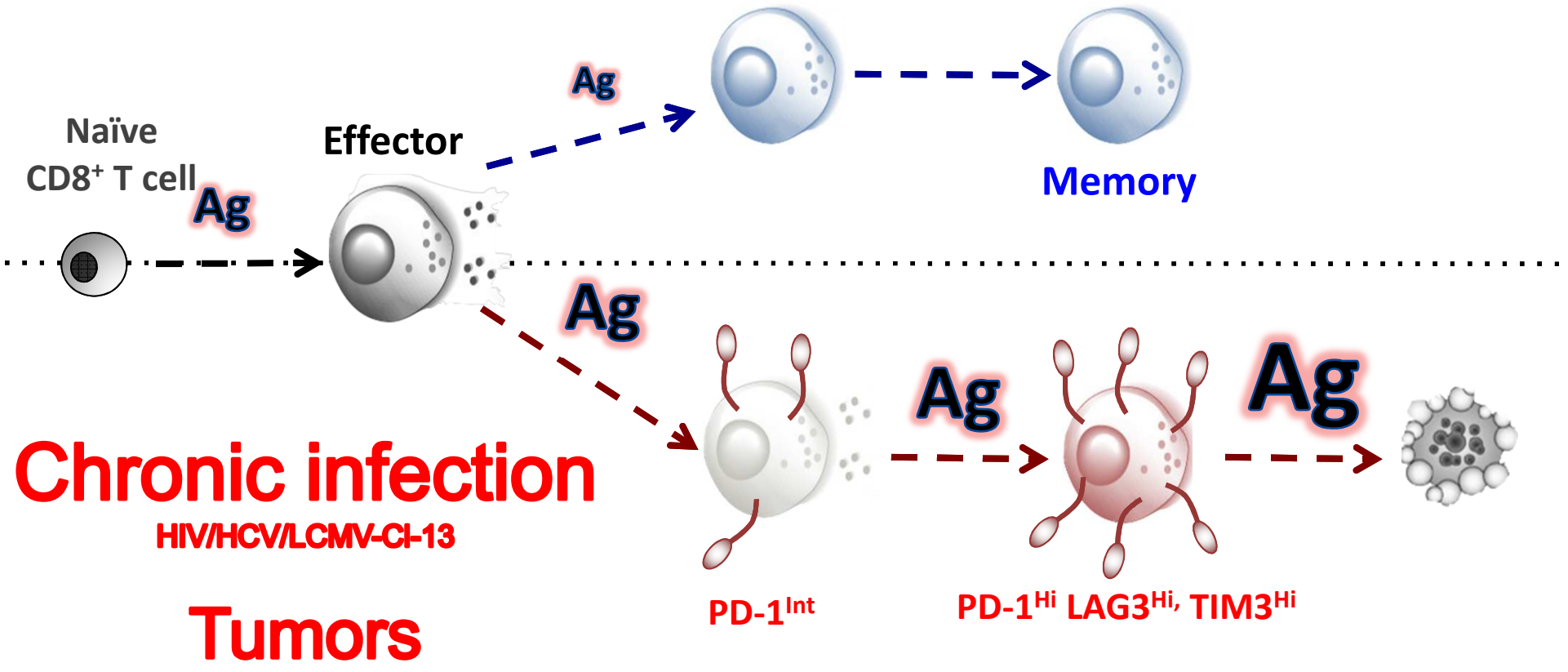


↓Immunopathology

↑Virus/ tumor persistence

↑COPING/ SURVIVAL

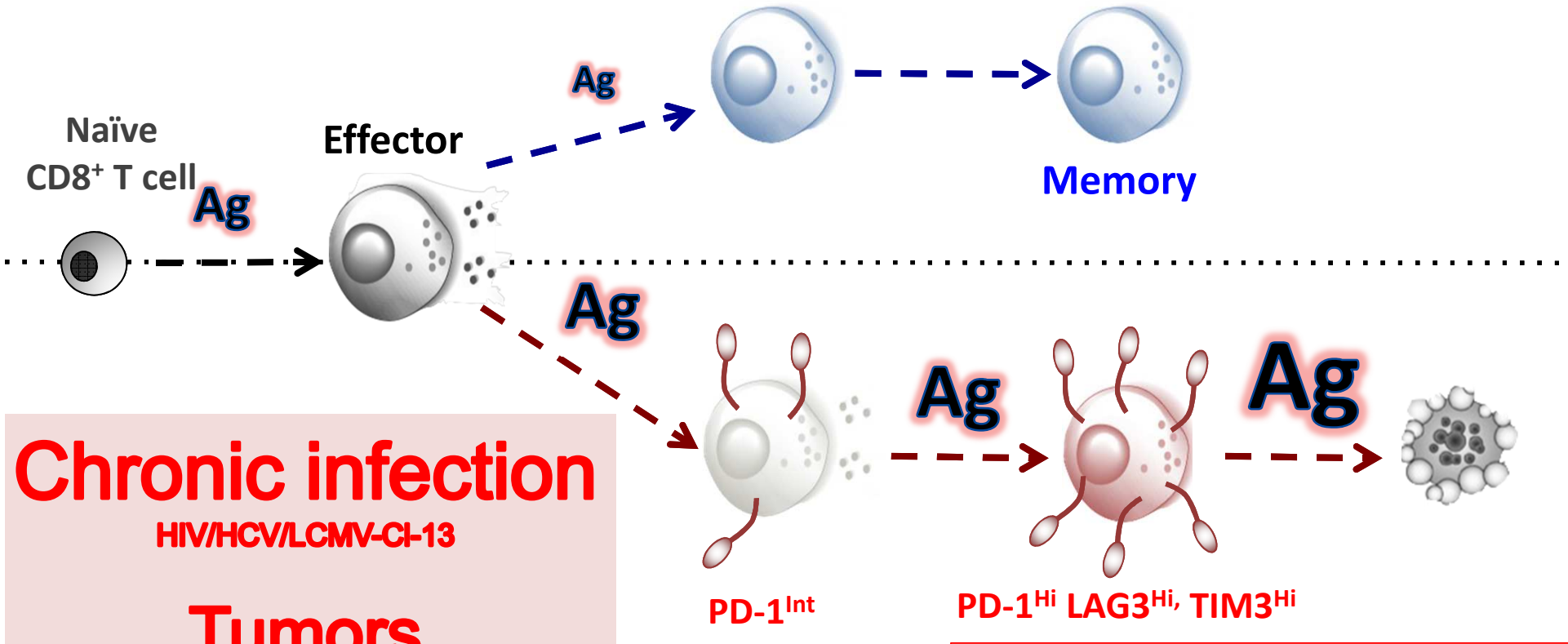
Acute



DYFUNCTIONAL

"EXHAUSTED"

Acute



Chronic infection

HIV/HCV/LCMV-Cl-13

Tumors

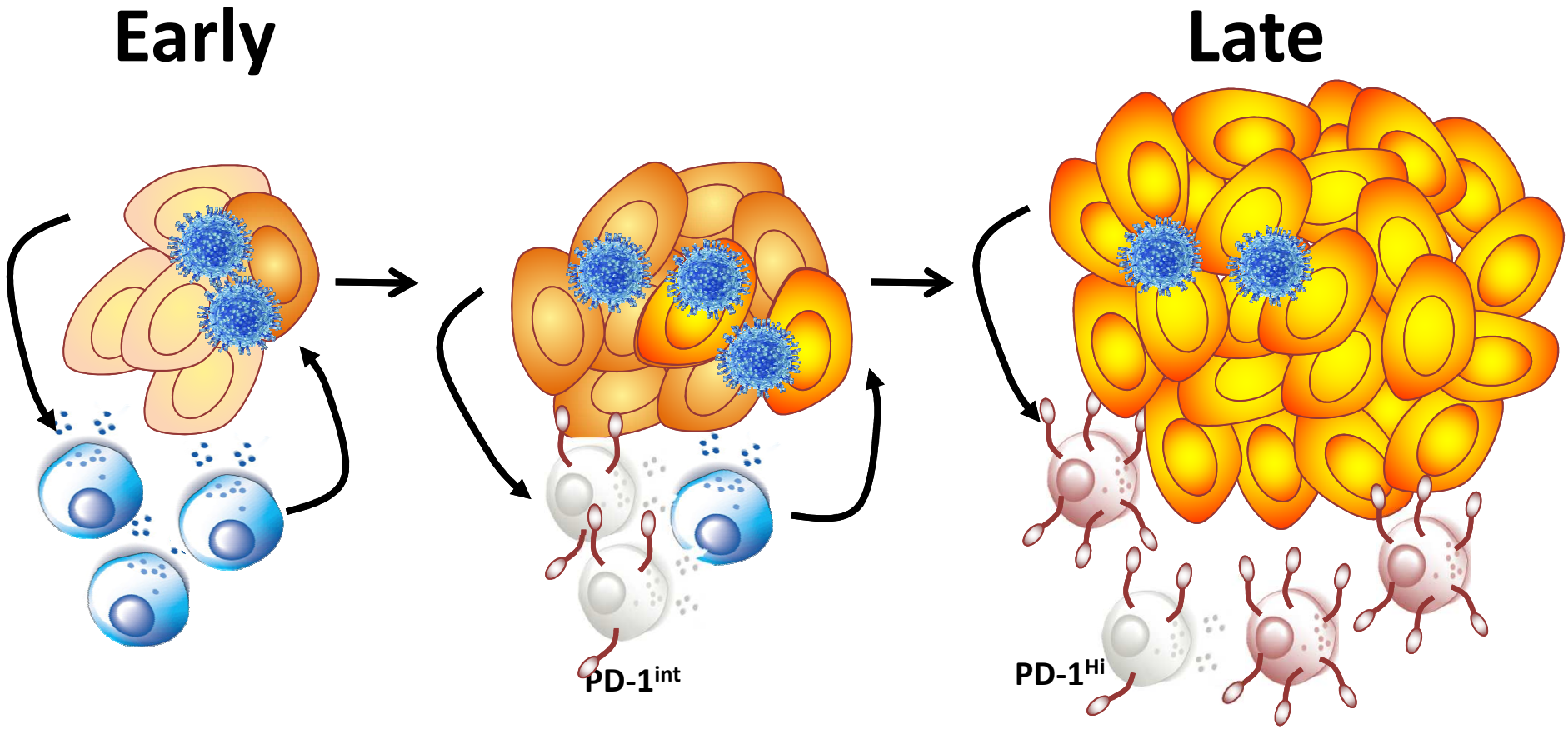
DYFUNCTIONAL

"EXHAUSTED"

↓Immunopathology

↑Virus/ tumor persistence

↑COPING/ SURVIVAL



How is T cell exhaustion controlled in chronic viral infection?

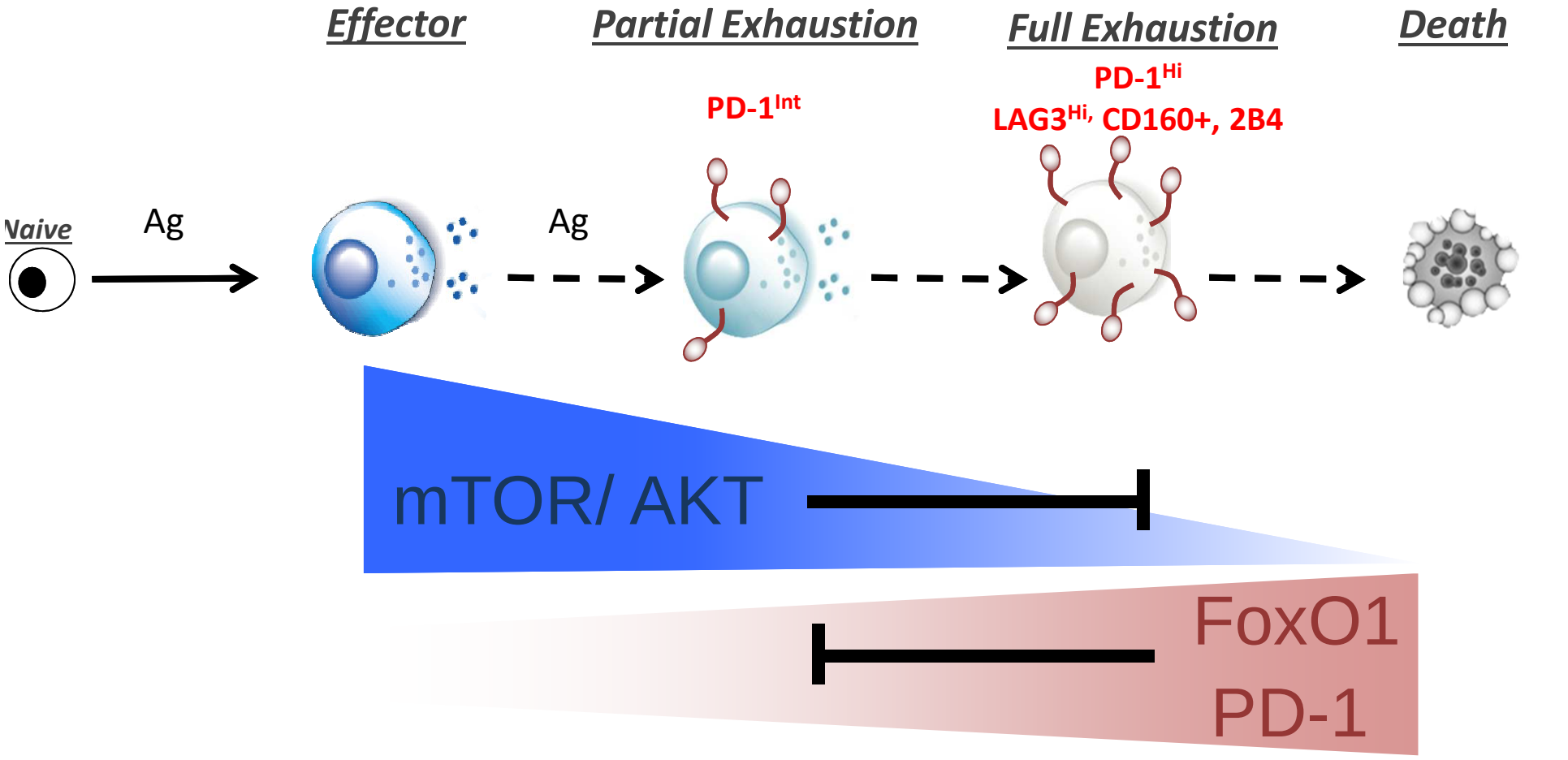
Major goals of study

- To characterize changes in the immune cell infiltrates in melanoma as tumors progress.

- To examine how anti-cancer drugs and immunotherapies affect the phenotype and function of the infiltrating immune cells.

- To identify immune-mediated pathways that suppress tumor growth.

PI3K- AKT-mTOR signaling declines during T cell exhaustion



↓Immunopathology

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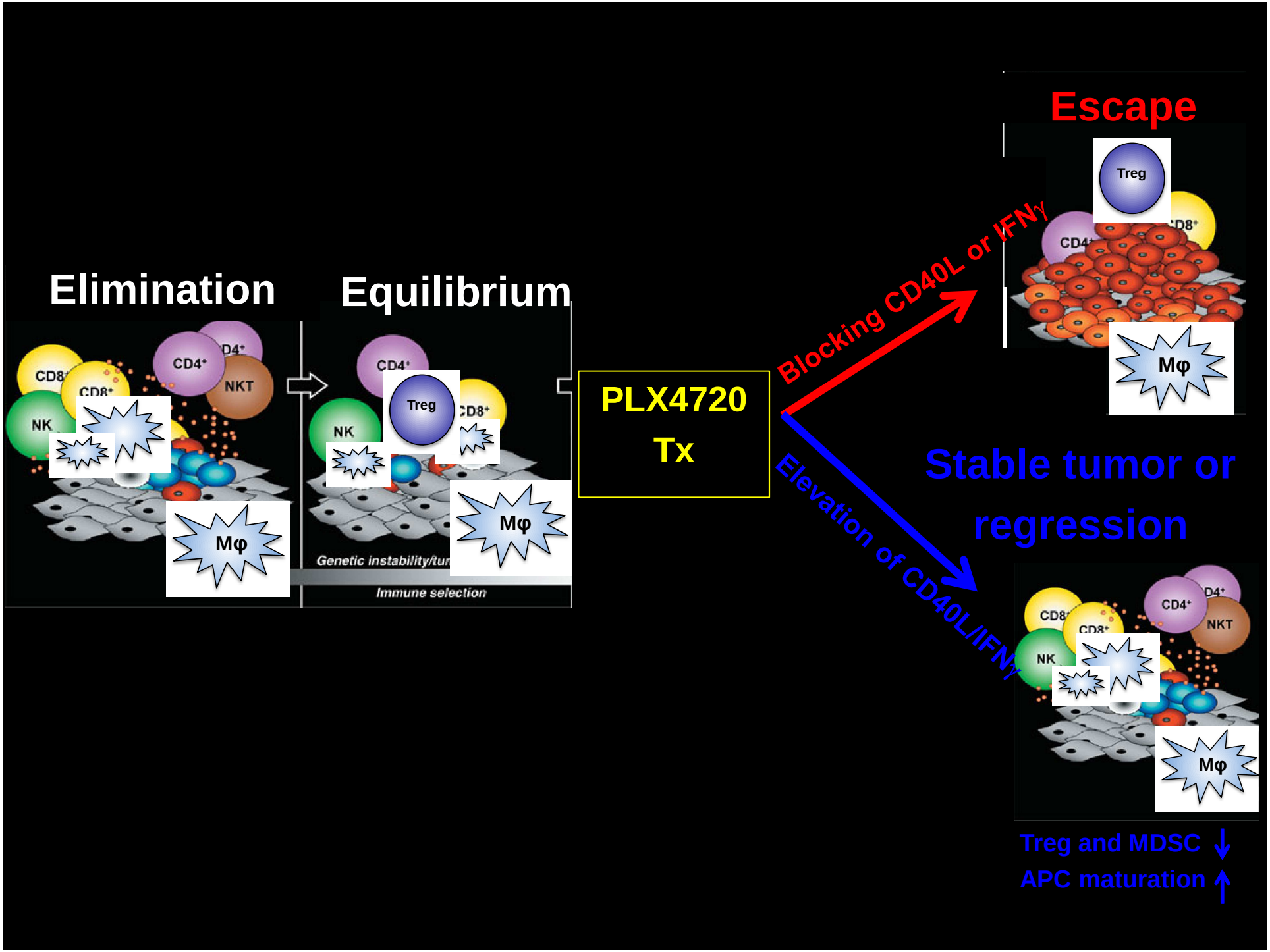
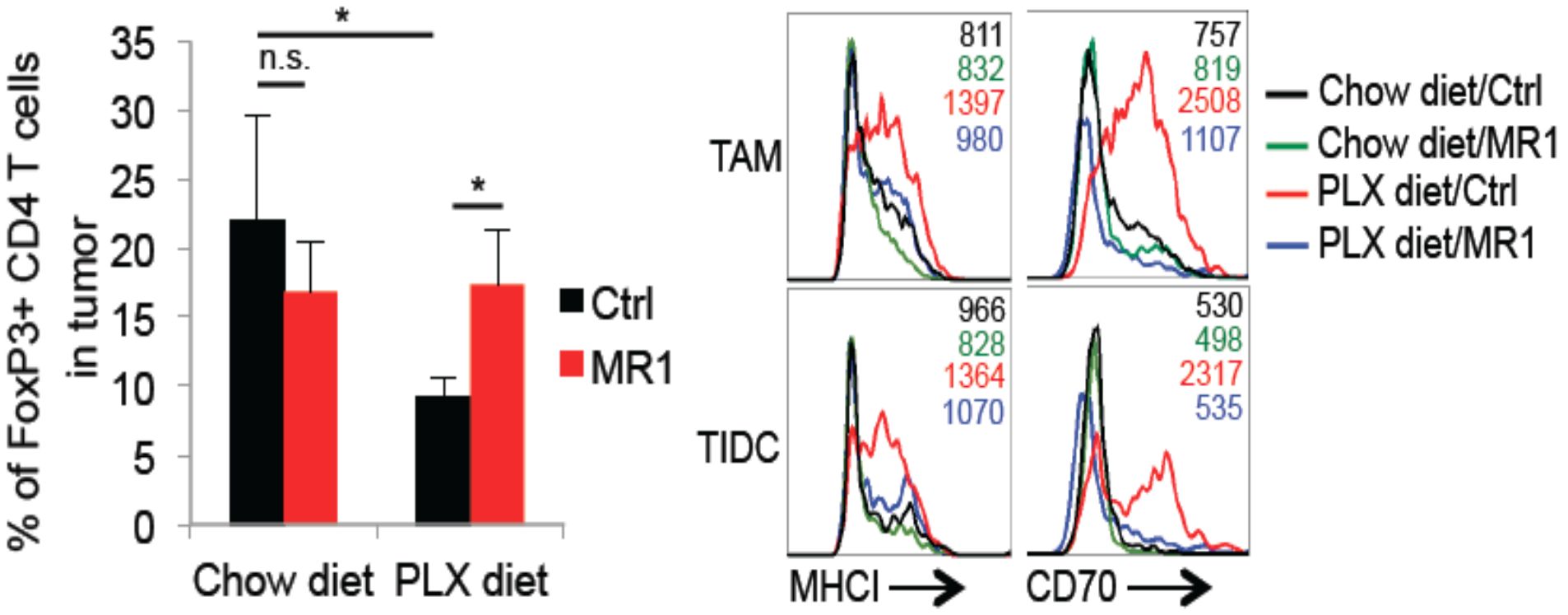
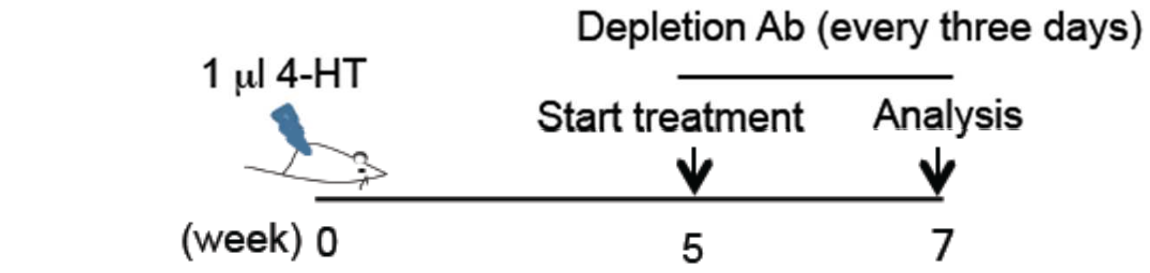
↑**COPING/ SURVIVAL**

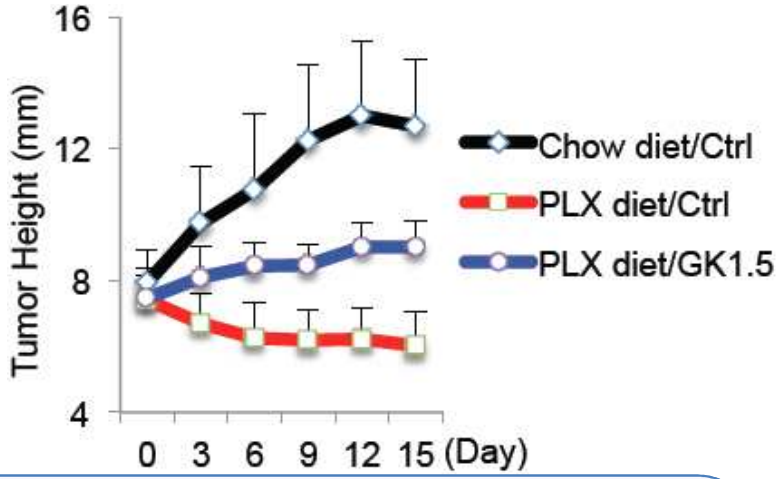

Elimination
Equilibrium
CD4+
CD8+
NKT
NK
Mφ
Treg
Genetic instability/tur
Immune selection
PLX4720
Tx
Blocking CD40L or IFNγ
Escape
Elevation of CD40L/IFNγ
Stable tumor or
regression
Treg and MDSC ↓
APC maturation ↑

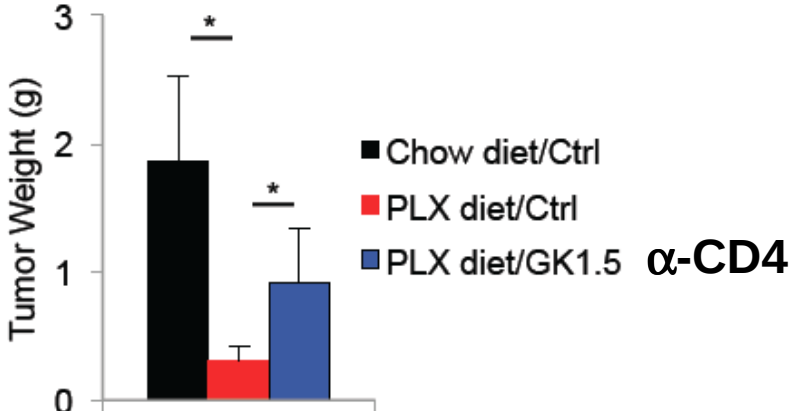
CD40L was critical for PLX4720-mediated reduction of intratumoral Tregs and APC maturation



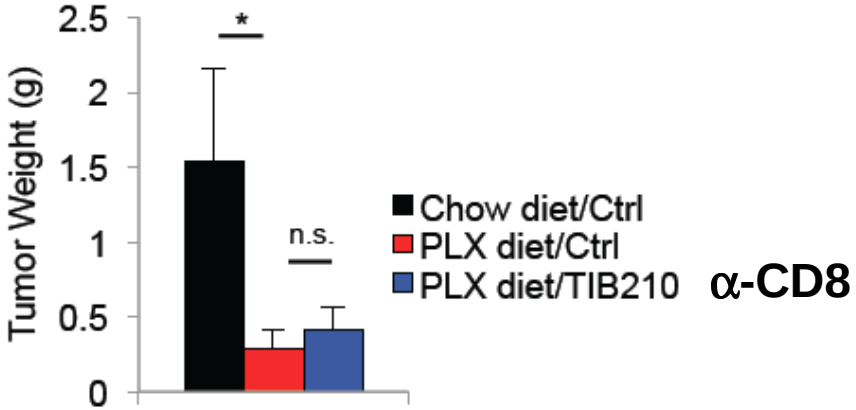
PLX4720-mediated anti-tumor response was partially CD4 T cell-dependent

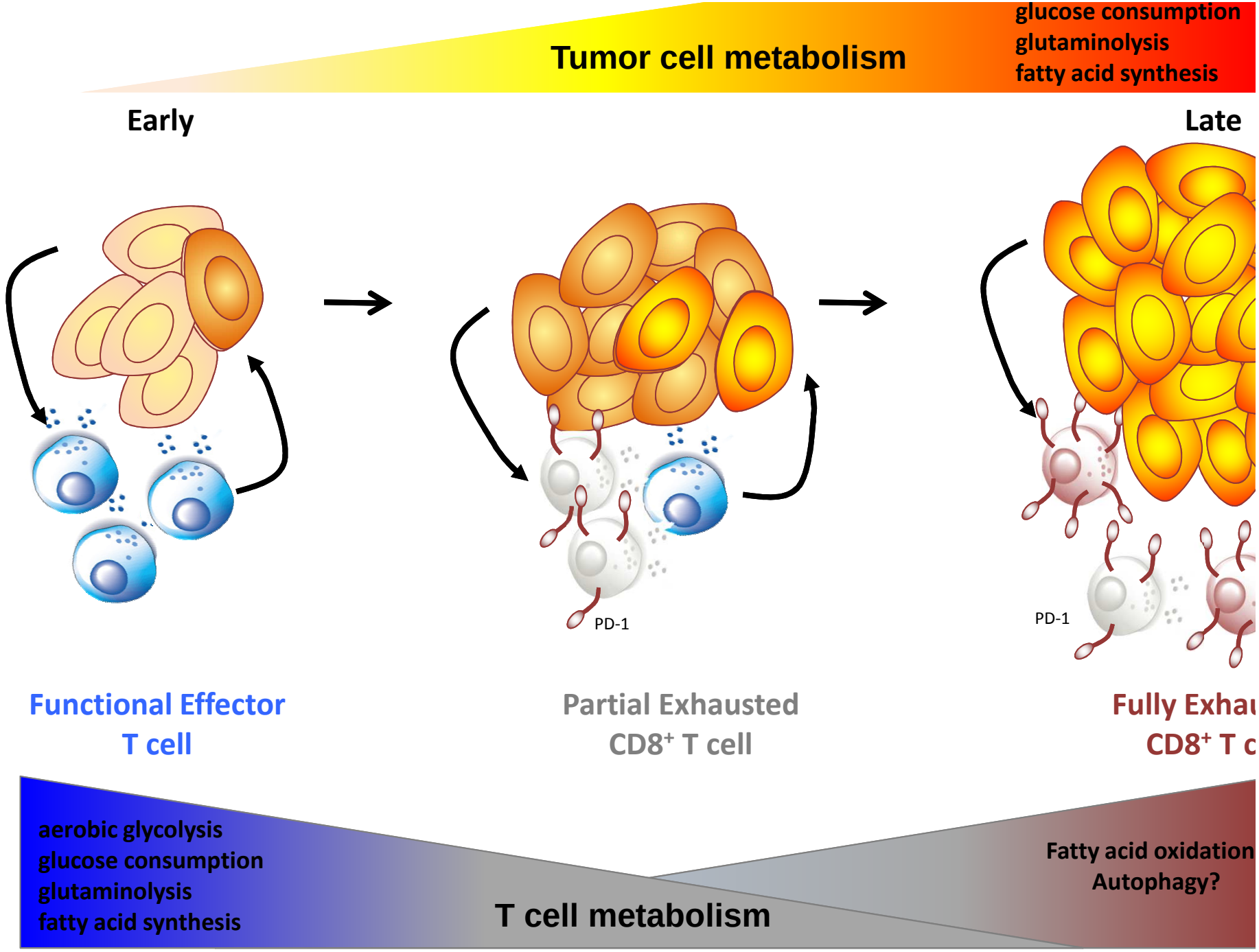




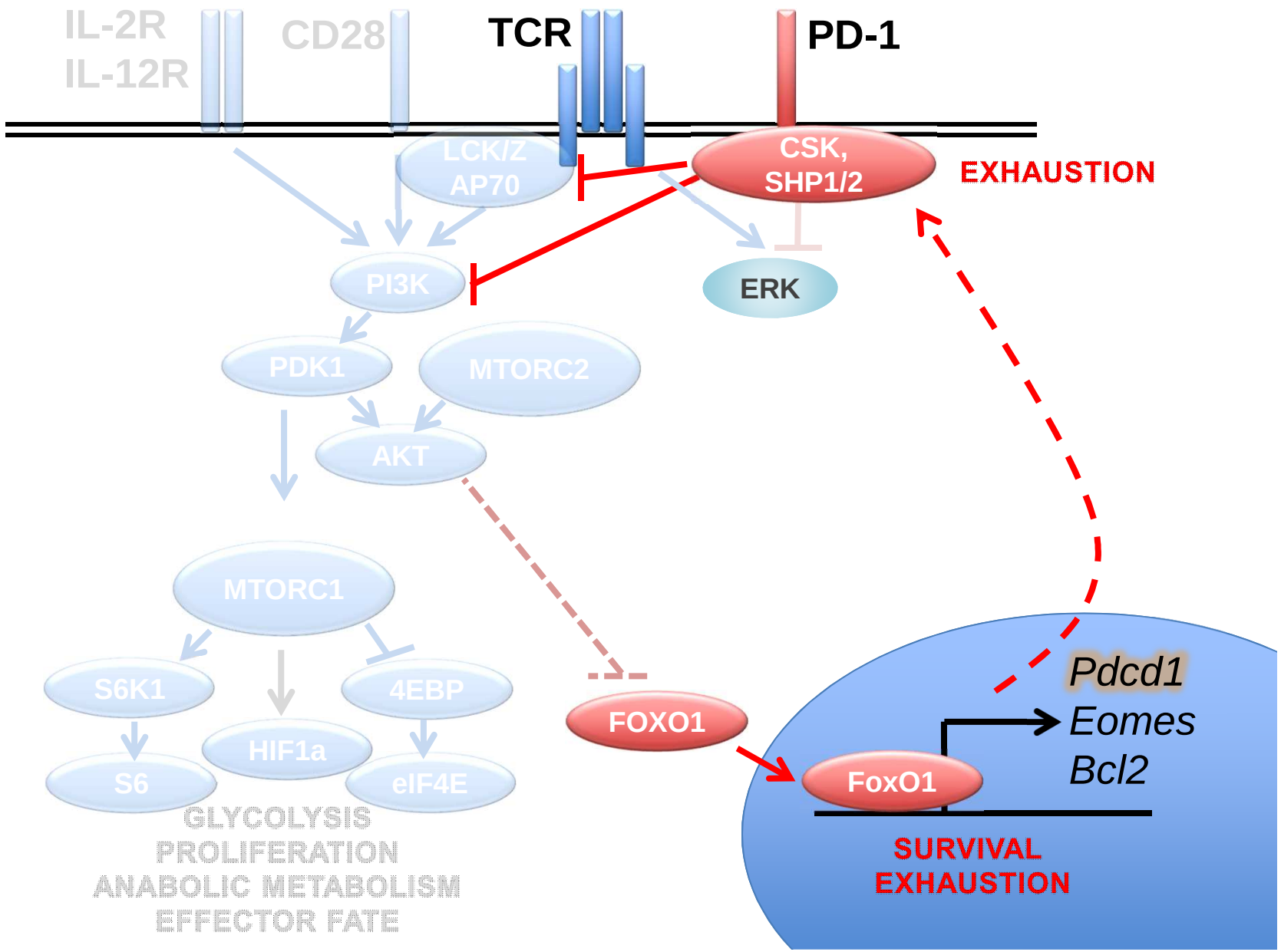


Do CD4 T cells contribute to PLX4720-induced anti-tumor immunity through their effector and helper functions?

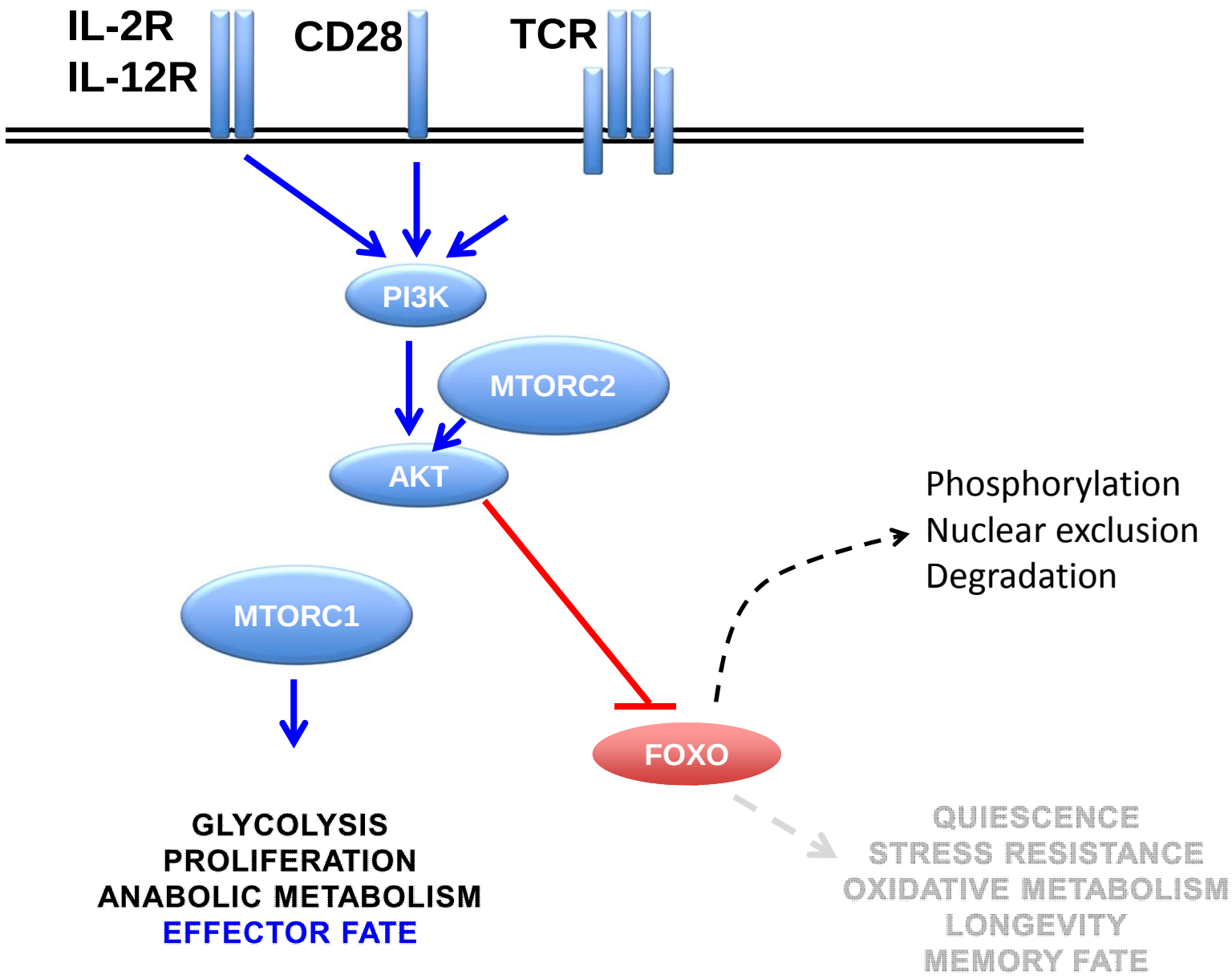


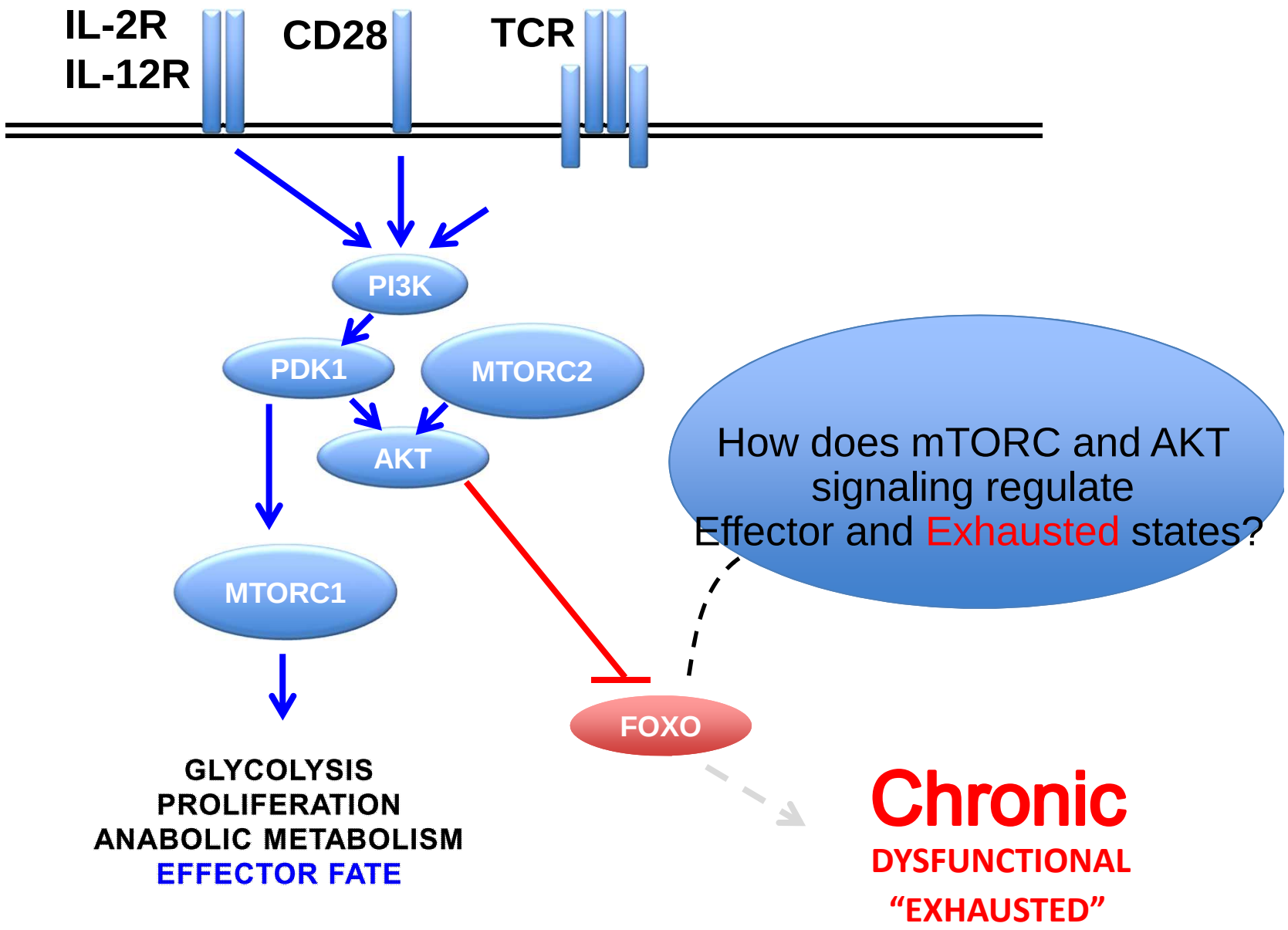

Tumor cell metabolism
glucose consumption
glutaminolysis
fatty acid synthesis
Early
Late
PD-1
PD-1
Functional Effector
T cell
Partial Exhausted
CD8+ T cell
Fully Exhau
CD8+ T c
aerobic glycolysis
glucose consumption
glutaminolysis
fatty acid synthesis
T cell metabolism
Fatty acid oxidation
Autophagy?

Suppression of PI3K/Akt/mTOR promotes a positive feedback pathway that promotes CTL exhaustion

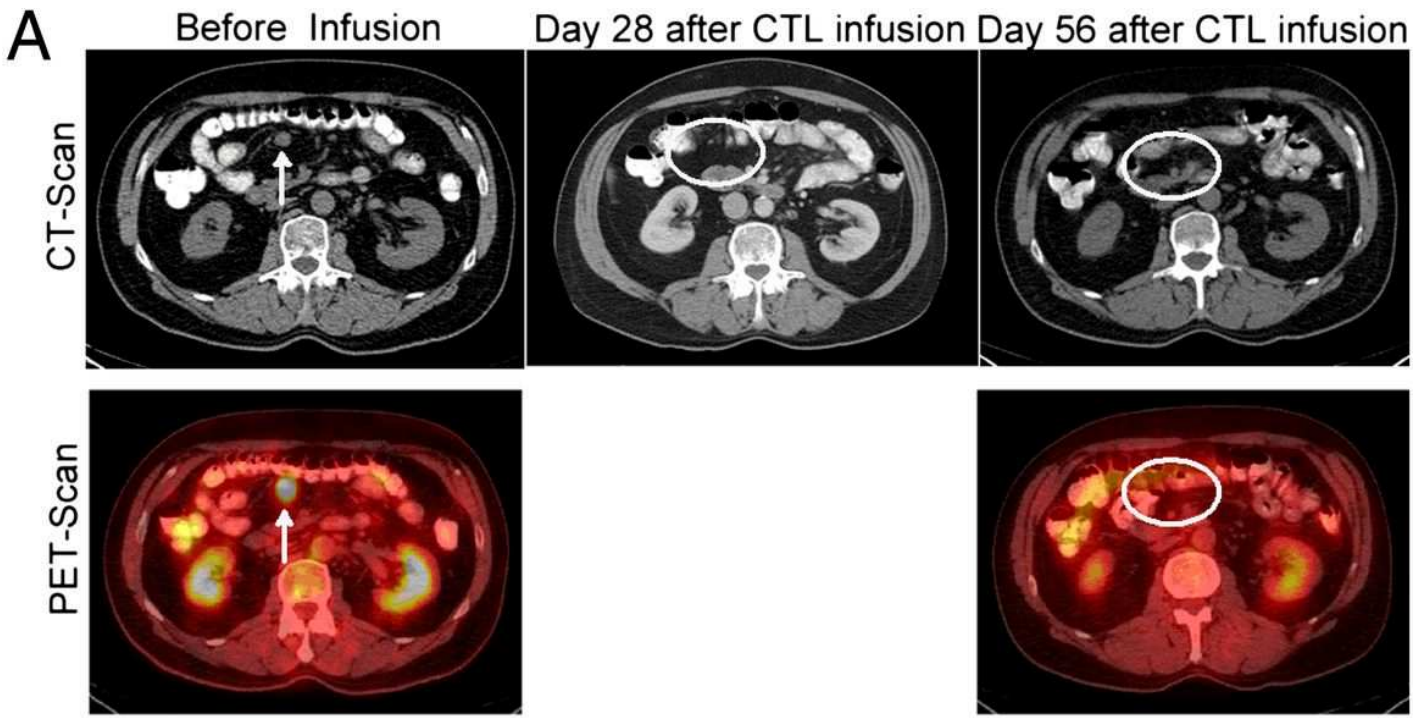


PI3K signaling regulates Effector and Memory states




IL-2R
IL-12R
CD28
TCR
PI3K
PDK1
MTORC2
AKT
MTORC1
FOXO
How does mTORC and AKT signaling regulate Effector and Exhausted states?
GLYCOLYSIS
PROLIFERATION
ANABOLIC METABOLISM
EFFECTOR FATE
Chronic
DYSFUNCTIONAL
"EXHAUSTED"

Melanoma is an immunogenic cancer



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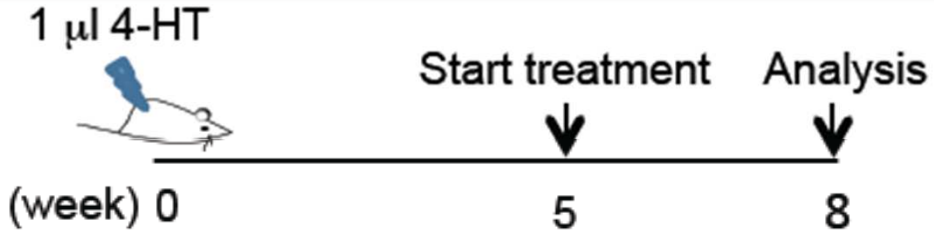
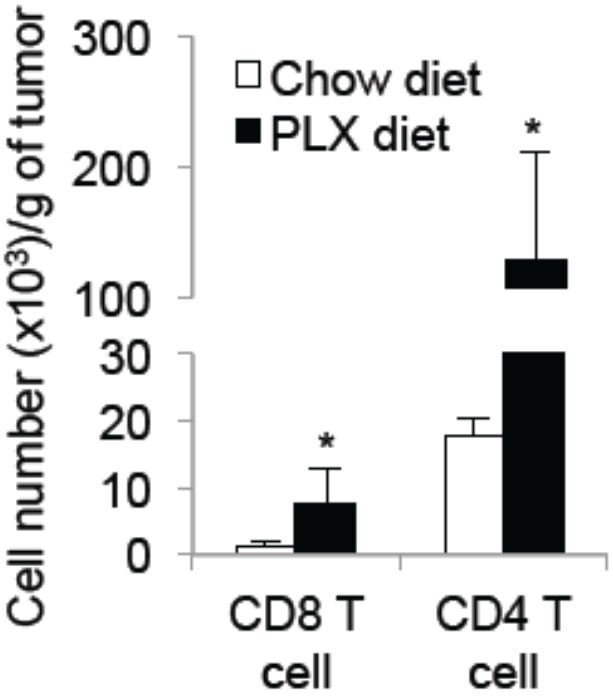
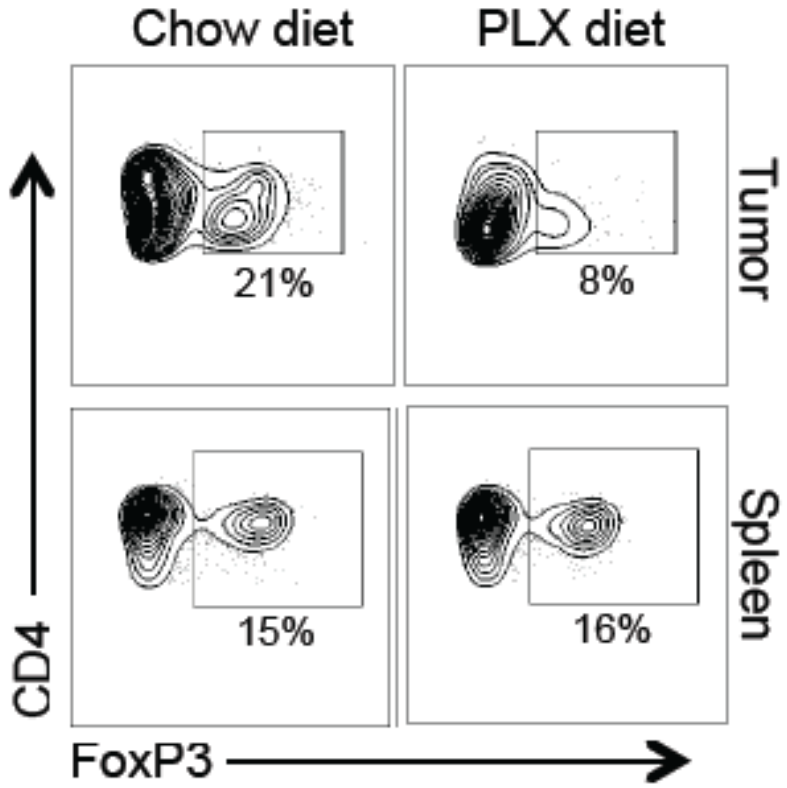
- ✓ Tumor bearing patients contain antigen-reactive T cells to melanoma antigens
- ✓ Relatively large number of mutations in tumors
- ✓ Minority of patients respond to high-dose IL-2
- ✓ Larger fraction of patients are responsive to ACT or anti-CTLA4 or anti-PD-1 immunotherapy

BRAF^{V600E} inhibitor, PLX4720, inhibited tumor progression and promoted tumor infiltration of T cells in Braf^{V600E}/Pten melanoma model

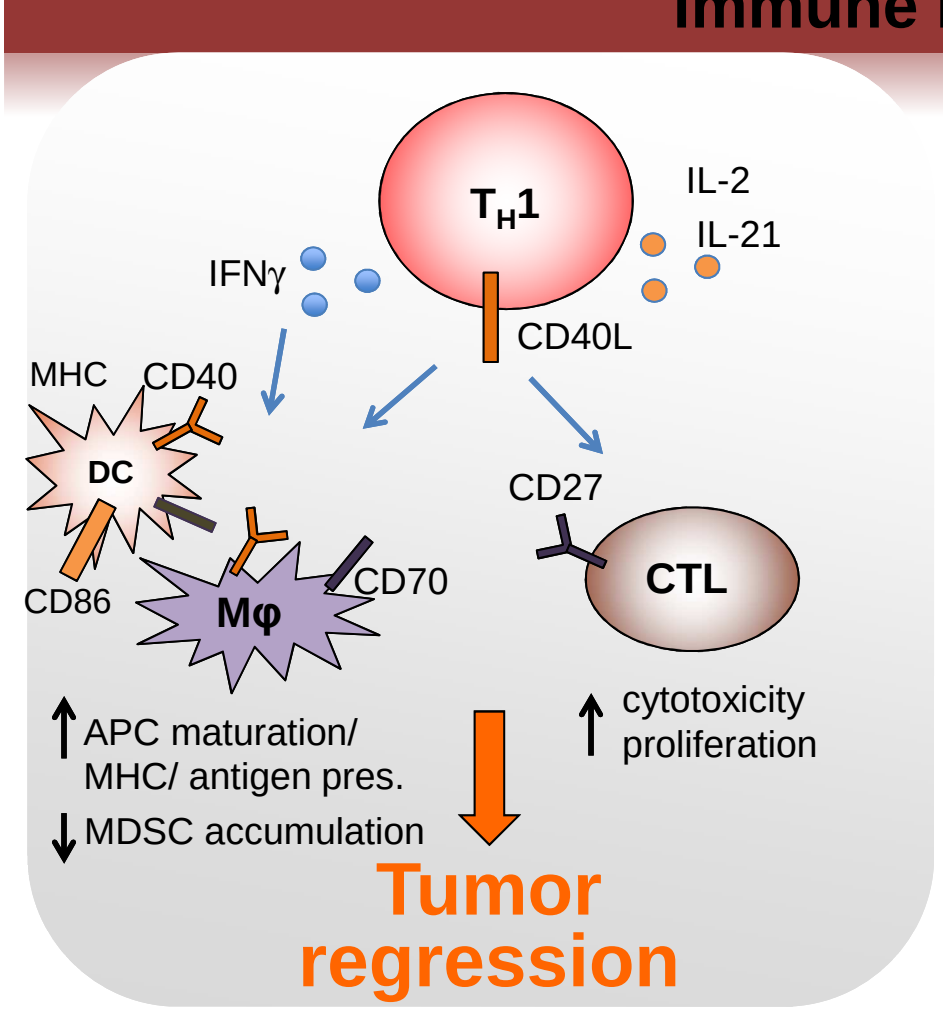


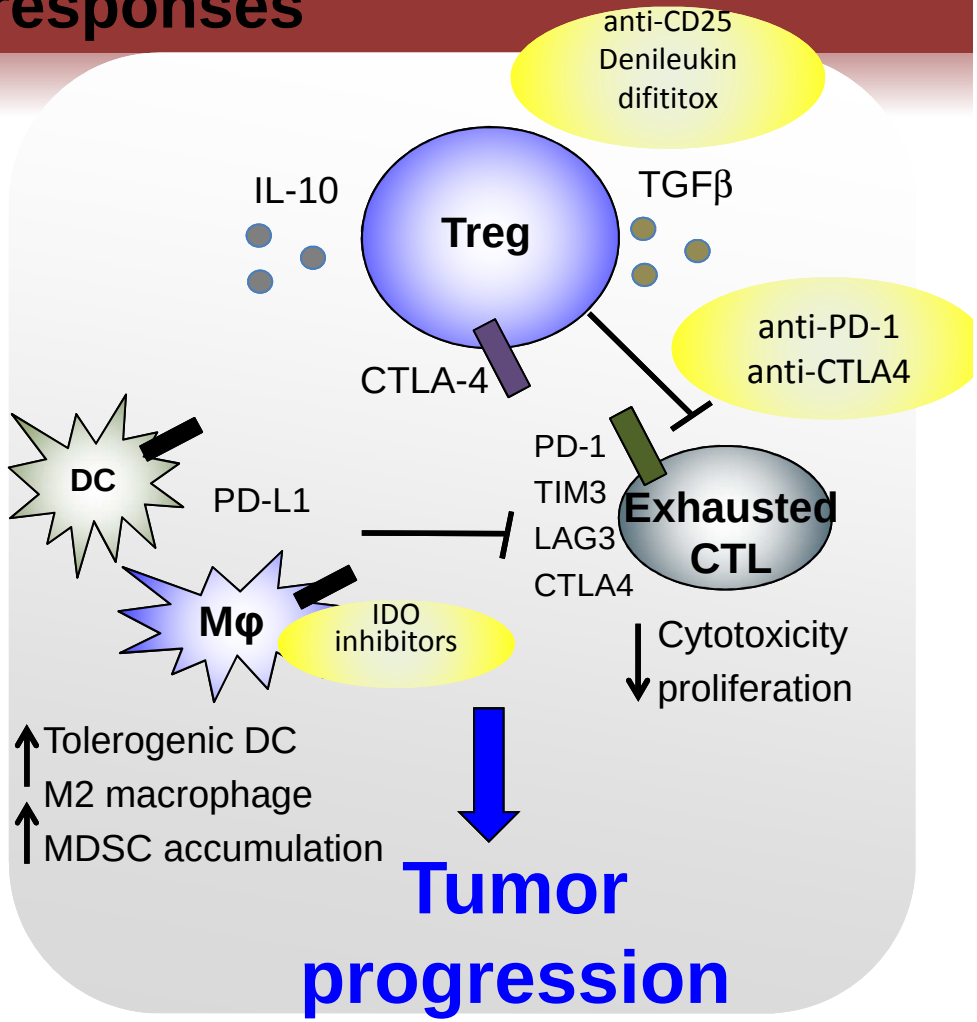




Also saw a drop in MDSCs.

Notable immune checkpoints that regulate anti-tumor immune responses





How is immunosuppressive microenvironment formed and maintained?

Can this be reversed to promote anti-tumor immune responses?

Targeted Immunotherapies