Tumor Microenvironment at a Genetic Level

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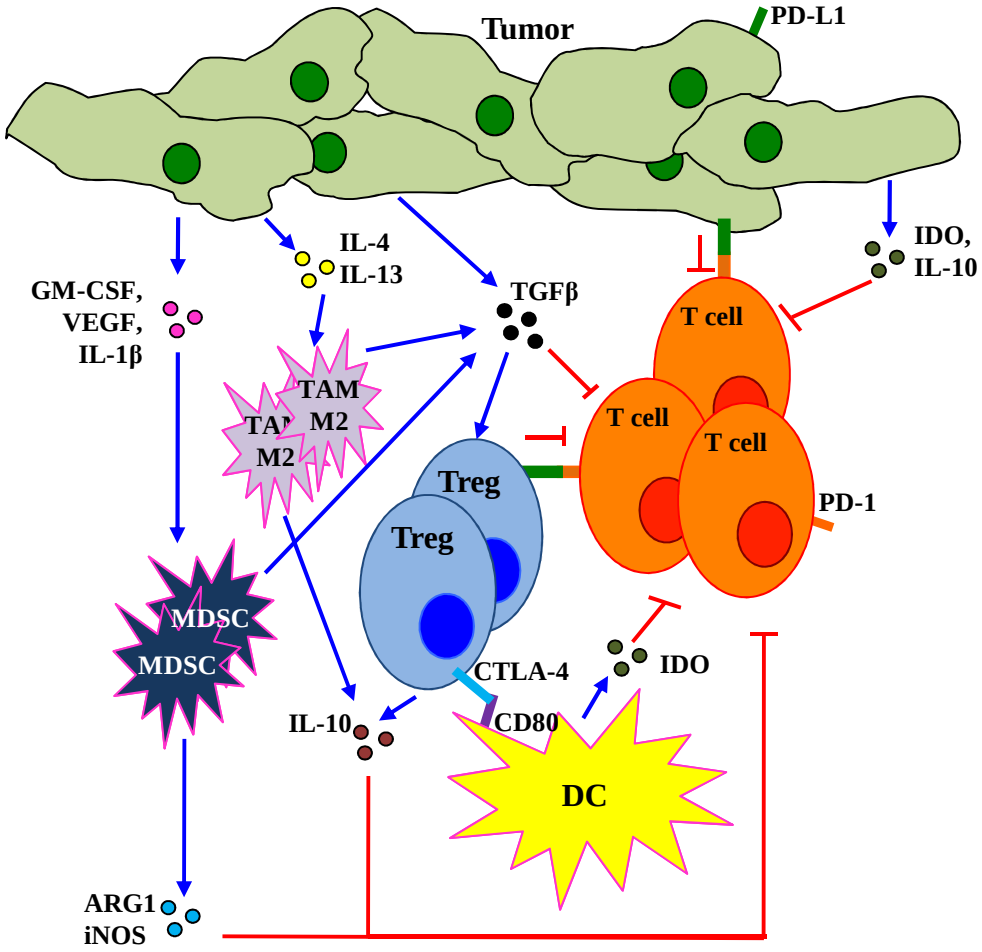
Tumor-Immune Interaction



Biomarkers

- Prognostic
- Predictive
- Metabolic
- Outcome

Immune Biomarkers

- Prognostic
- Immune Target BM– HLA/Ag-
- Predictive
- Metabolic

- Outcome

Immune Biomarkers

- Prognostic
- Immune Target BM– HLA/Ag- mutant antigens
- Predictive
- Metabolic

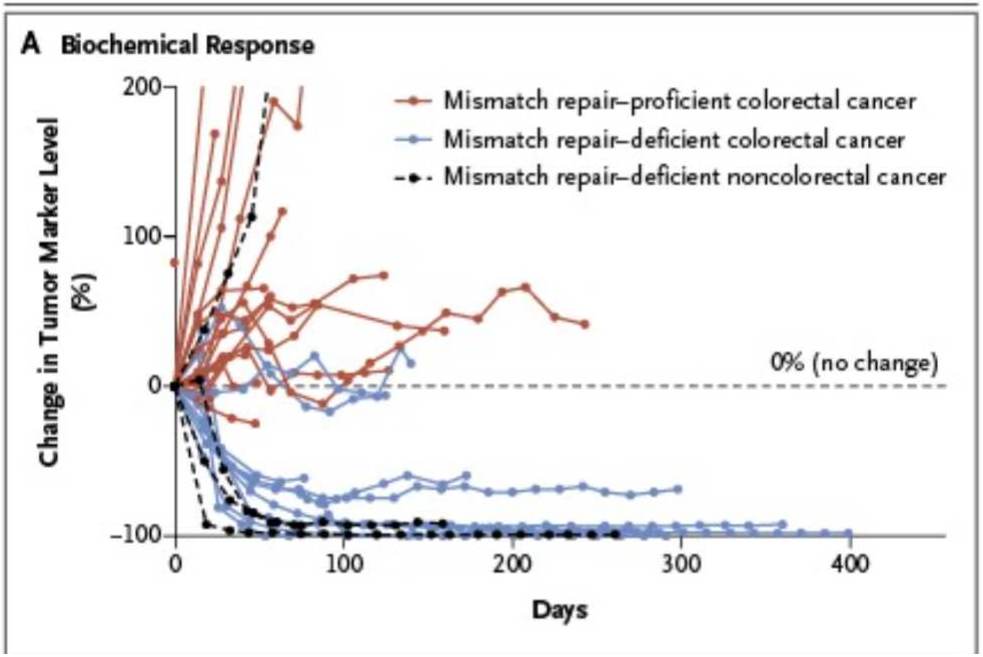
- Outcome

Immune Biomarkers

- Prognostic
- Immune Target BM– HLA/Ag--- PDL1
- Predictive
- Metabolic

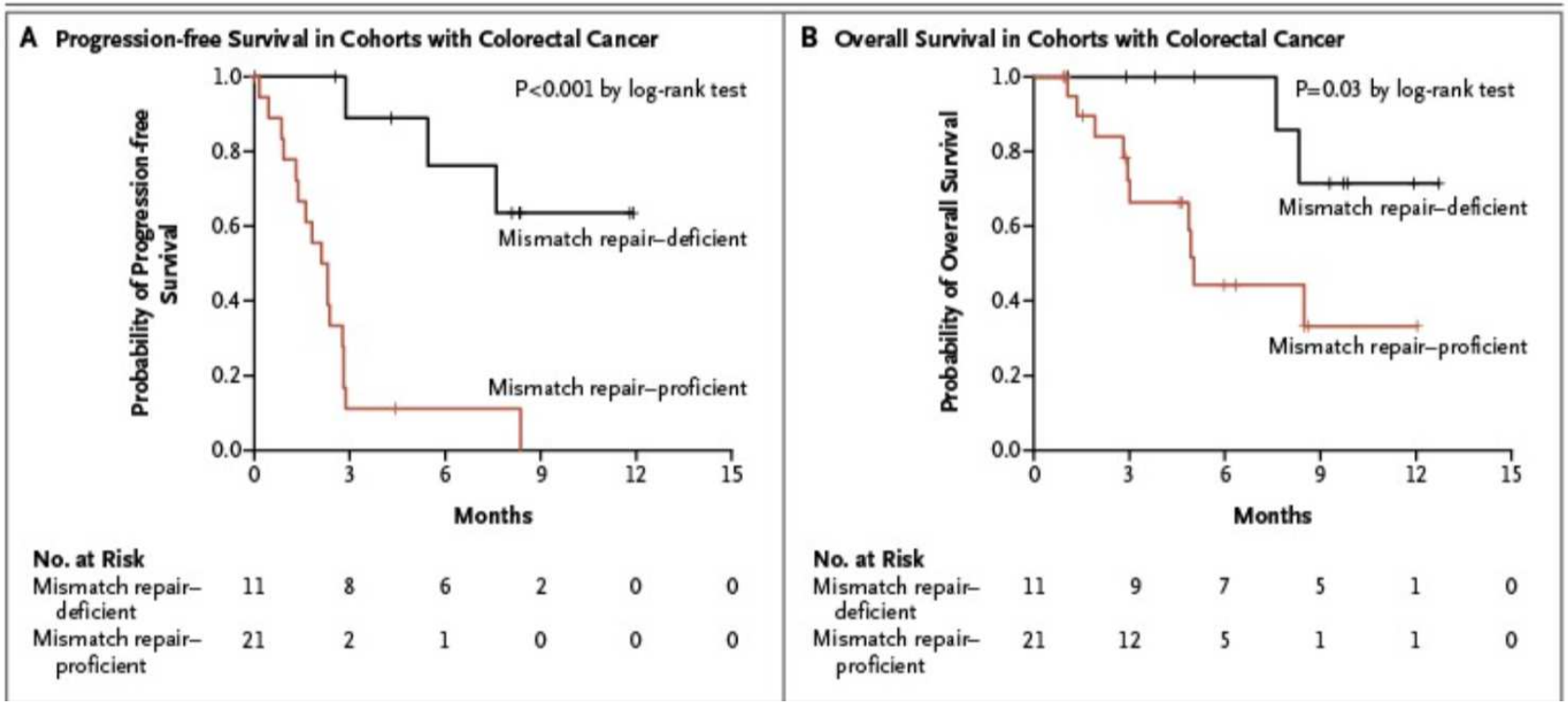
- Outcome

MSI high colorectal cancer - biochemical response with



Le et al. N Engl J Med 2015;372:2509-20

MSI high colorectal cancer PFS and OS with pembrolizumab



Le et al. N Engl J Med 2015;372:2509-20

Immune Biomarkers

- Prognostic
- Immune Target BM– HLA/Ag--- PDL1
- Predictive-- MSI
- Metabolic

- Outcome

Immune Biomarkers

- Prognostic
- Immune Target BM– HLA/Ag--- PDL1
- Predictive– MSI—mutational load
- Metabolic

- Outcome

Immune Biomarkers

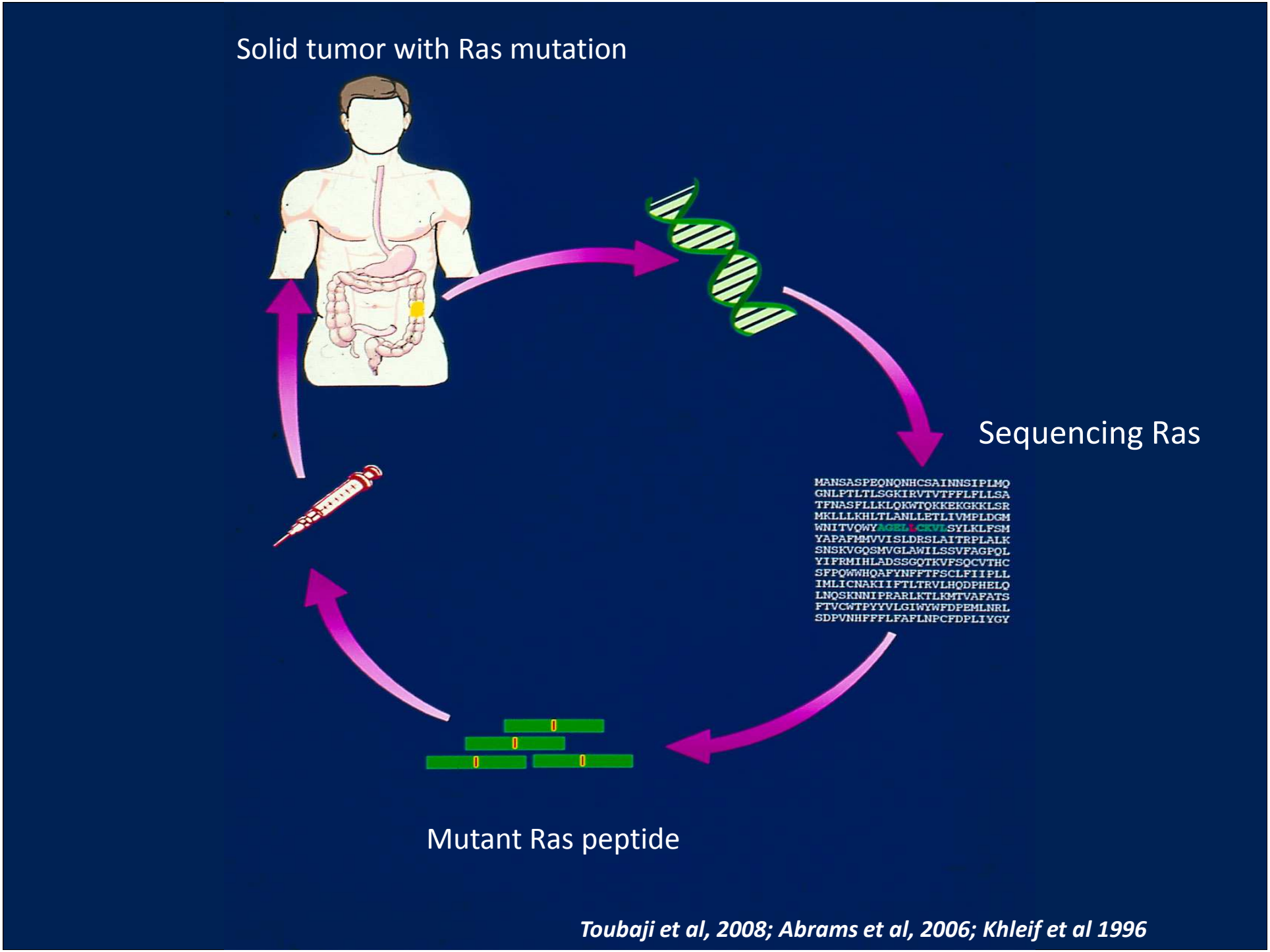
- Prognostic
- Immune Target BM– HLA/Ag--- PDL1
- Predictive– MSI-- – 9p24 amplification
- Metabolic

- Outcome

Biomarkers for Combination Immunotherapy

- Prognostic
- Immune Target – Mutant Ras
- Predictive
- Metabolic

- Outcome



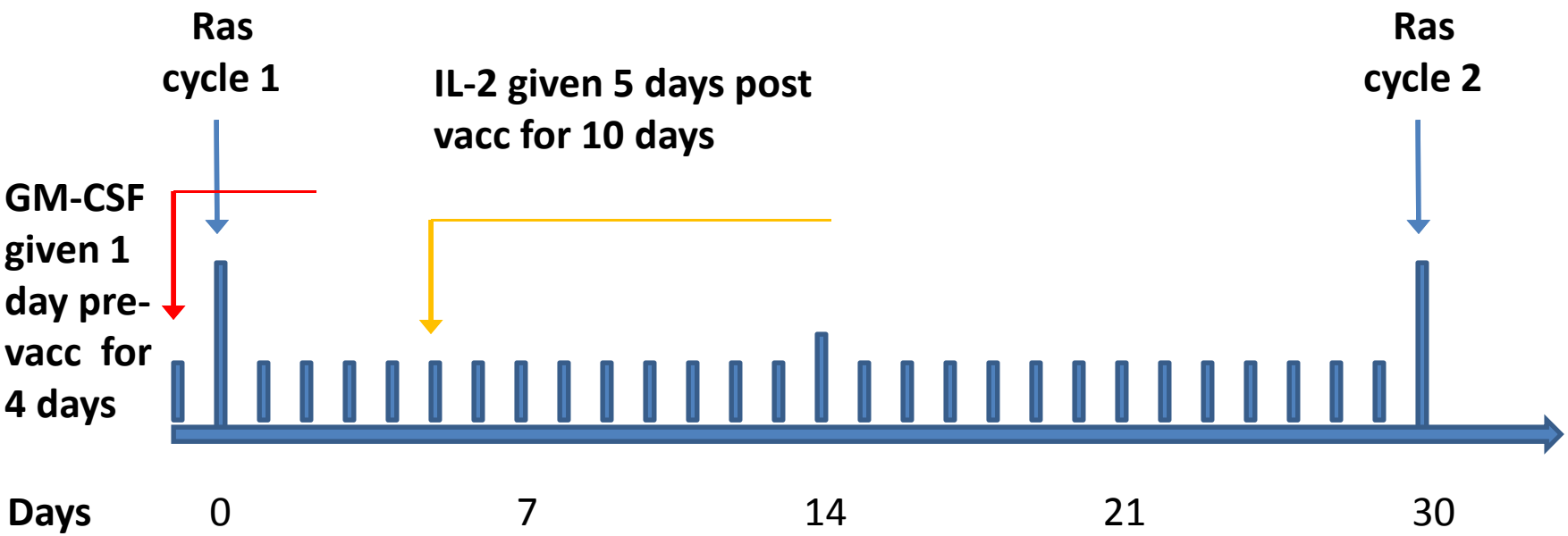
Toubaji et al, 2008; Abrams et al, 2006; Khleif et al 1996

Phase II Trial with Tumor Specific Mutated Ras Peptides and IL-2, GM-CSF, or both for Adult Patients with Solid Tumors

Eligibility

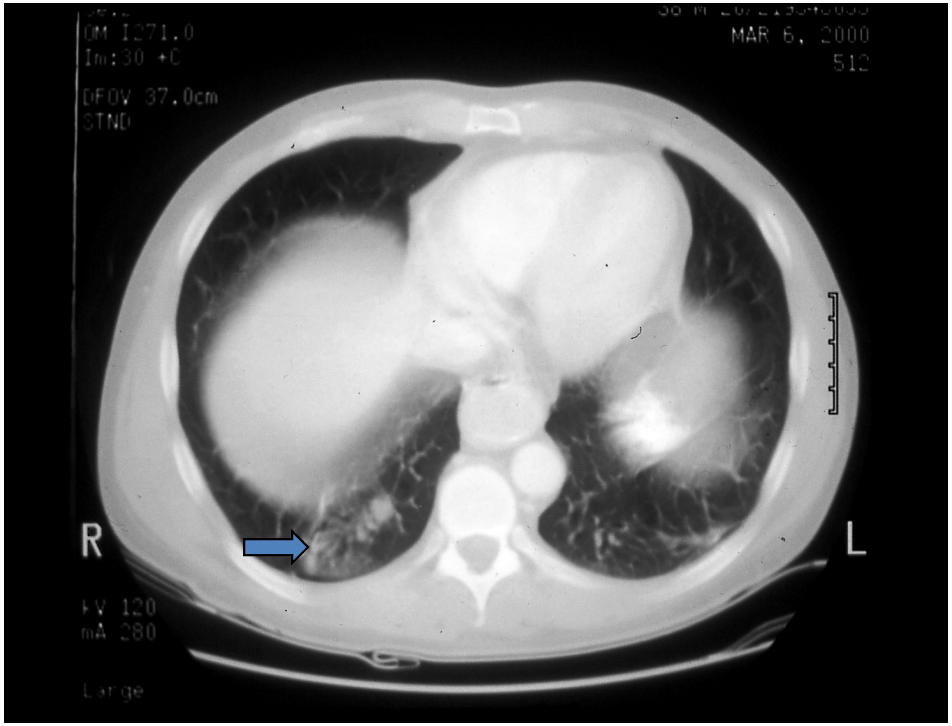
- **Ras Mutation: Gly to Cys, Asp, or Val**
- **Metastatic disease except CNS metastasis**
- **Failed prior treatment**

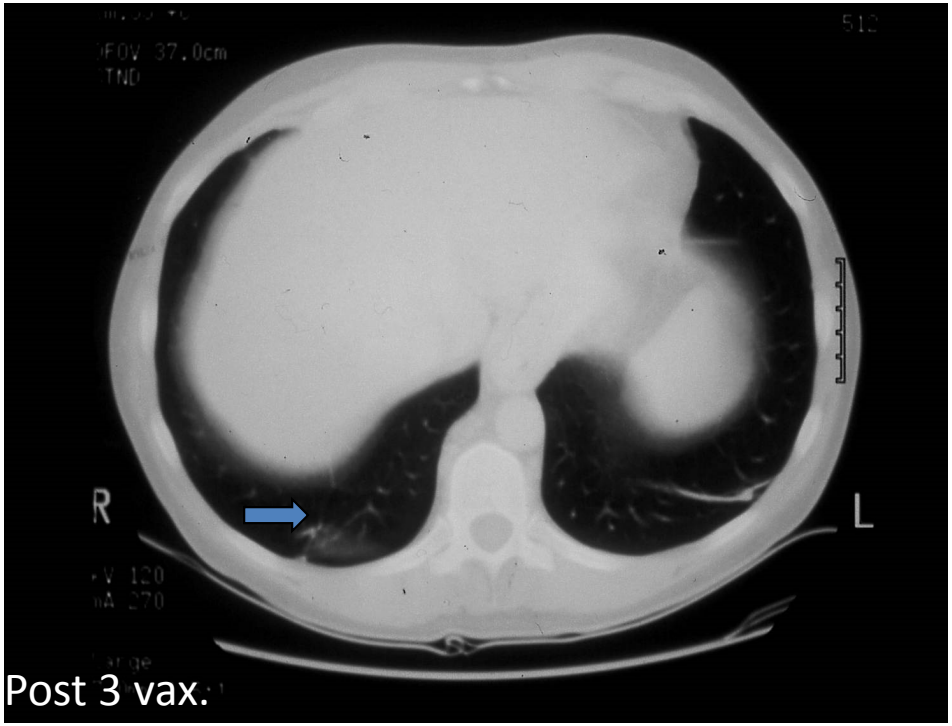
Vaccination Schedule



If SD continue vaccination up to 15 cycles

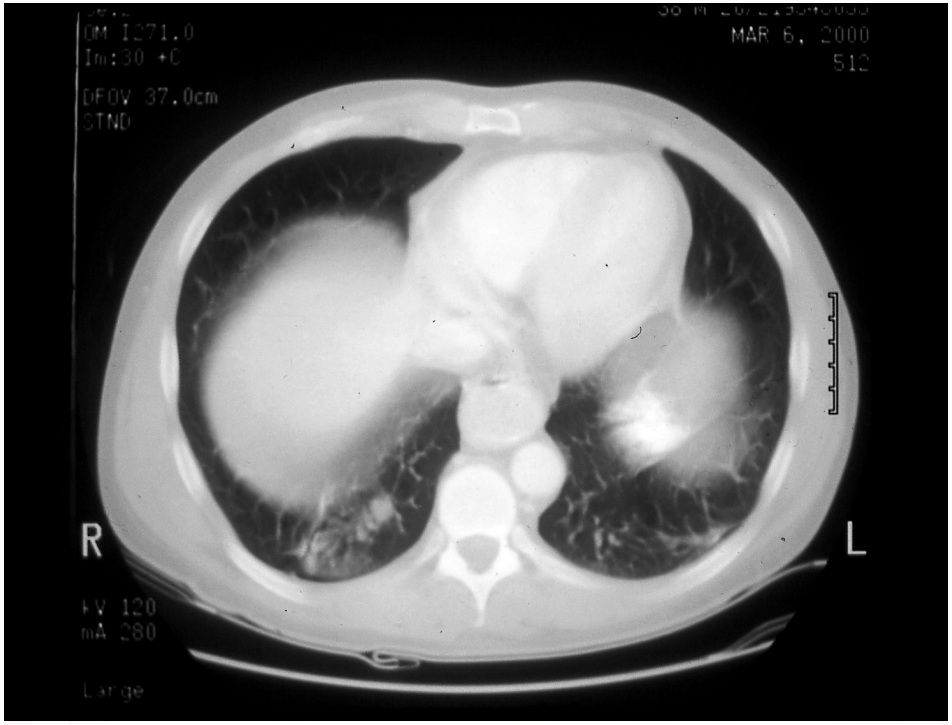
Rahma et at, JTM 2014

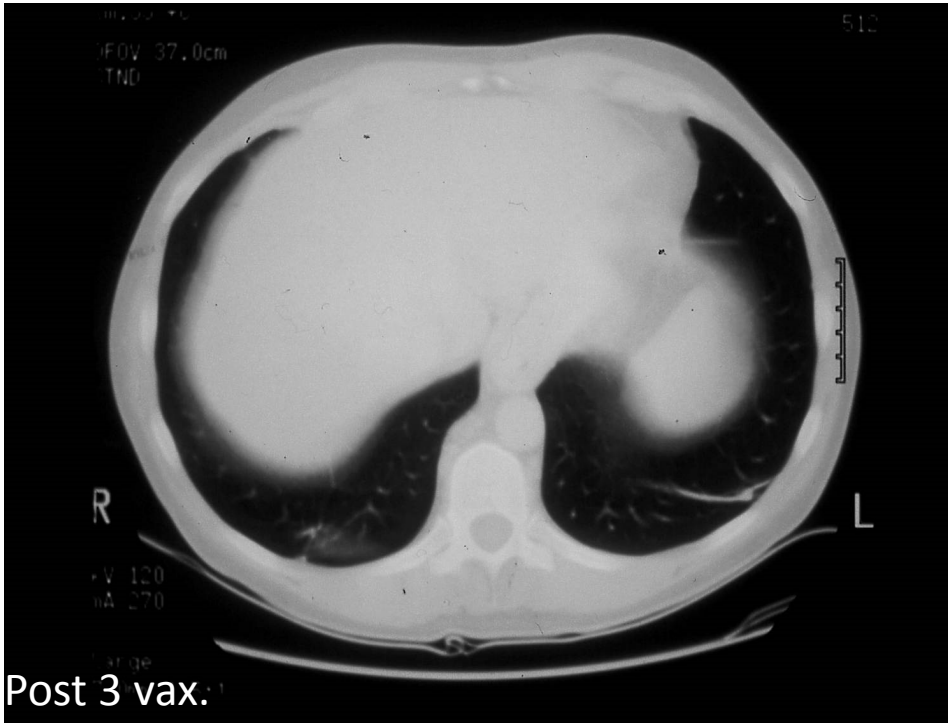




Post 3 vax.

Rahma et at, JTM 2014





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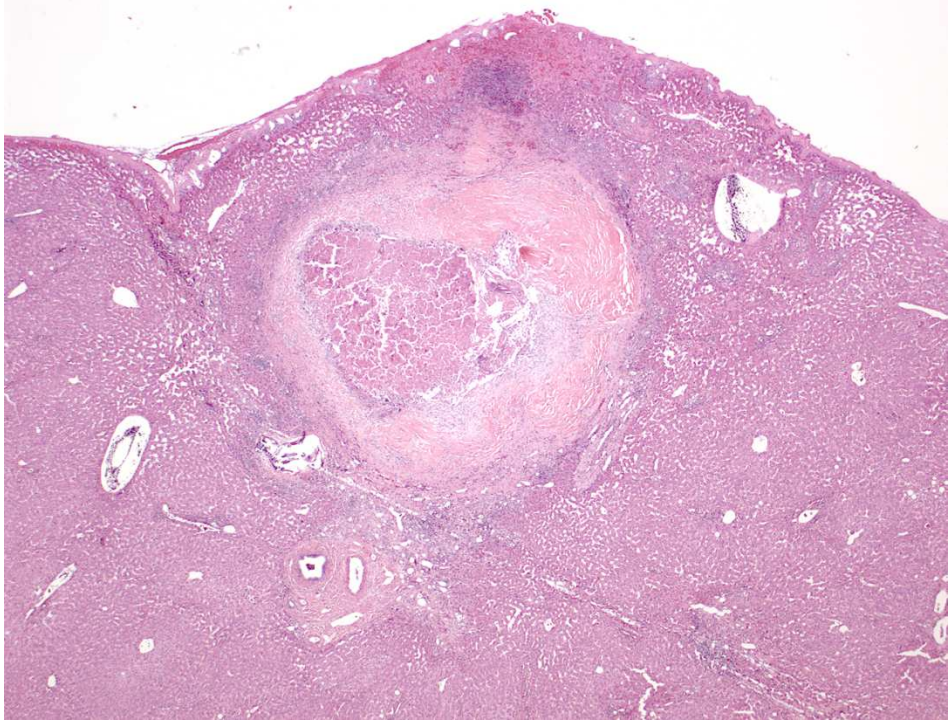
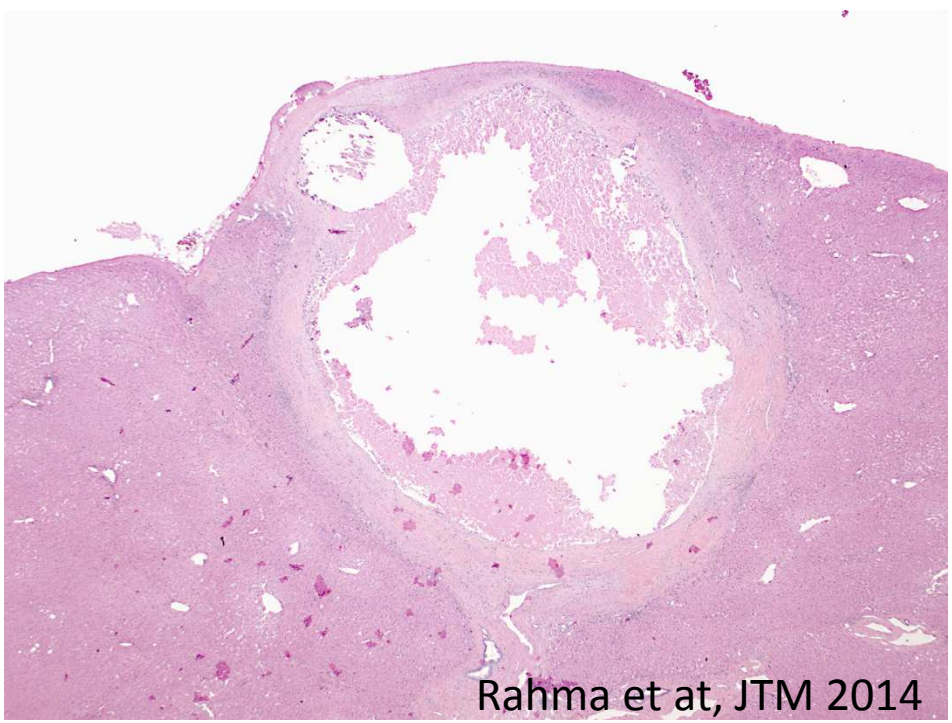
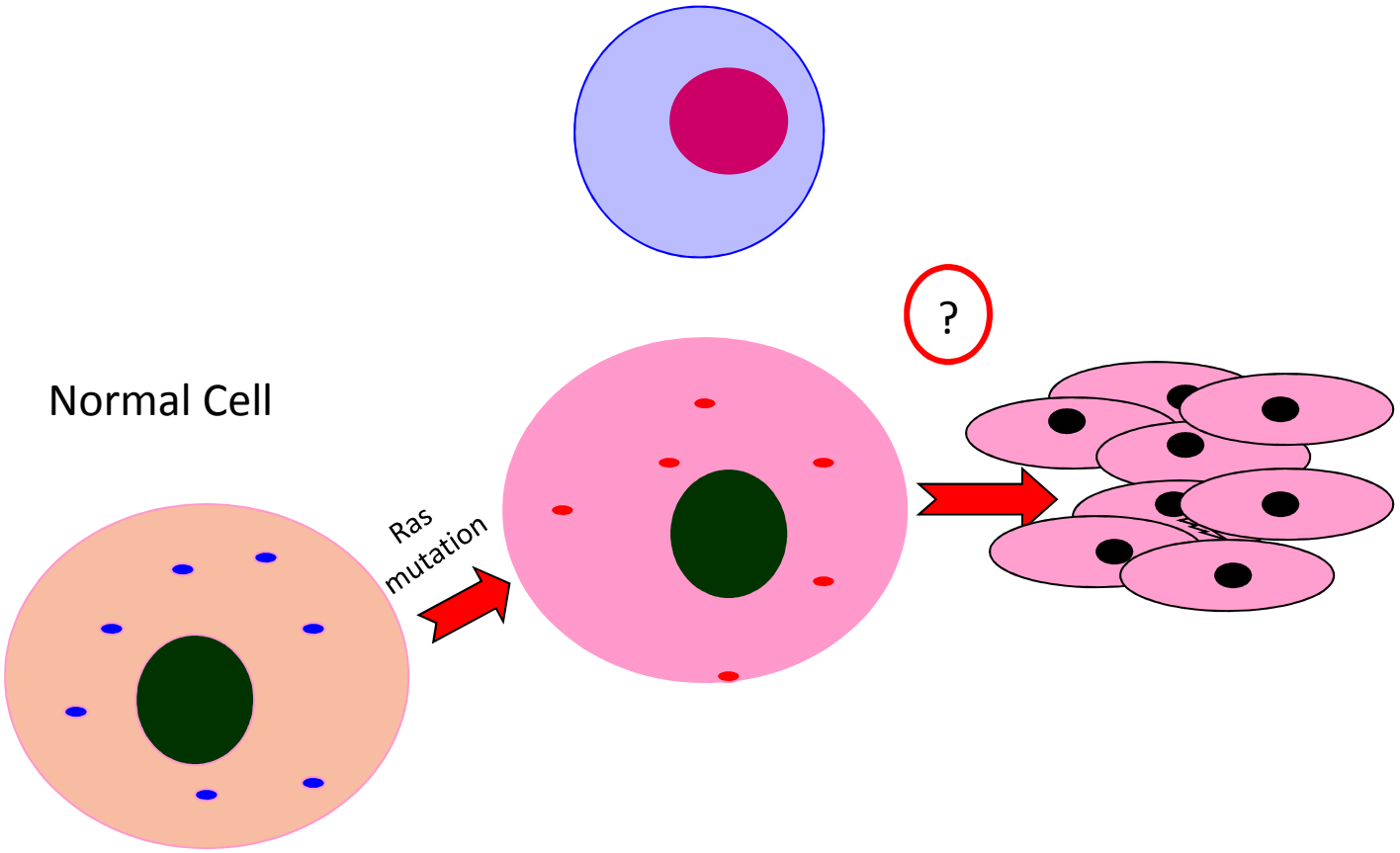
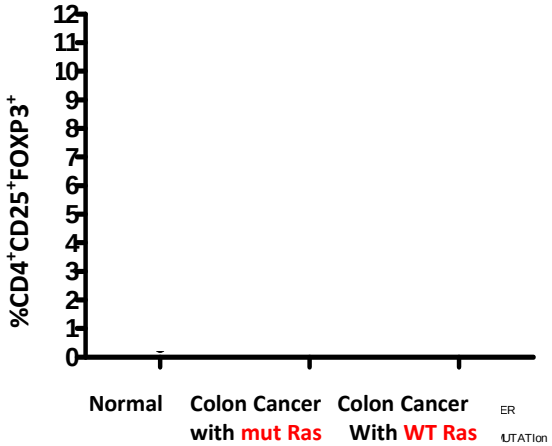




Rahma et at, JTM 2014

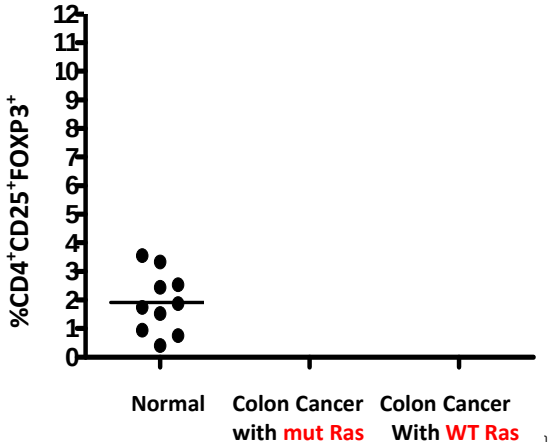

Normal Cell
Ras
mutation
?

T regs in Colon Cancer Patients



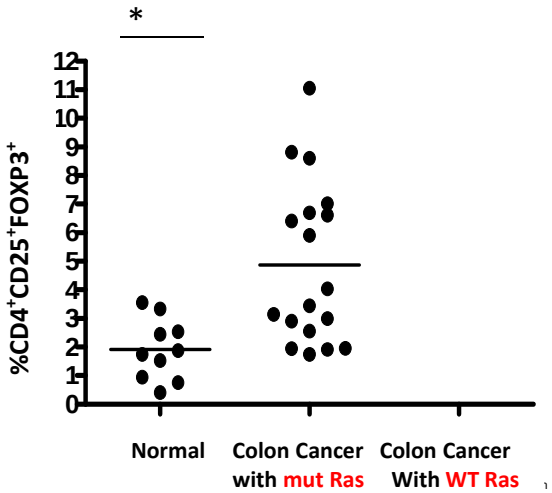
Zdanov et at, CIR 2016

T regs in Colon Cancer Patients



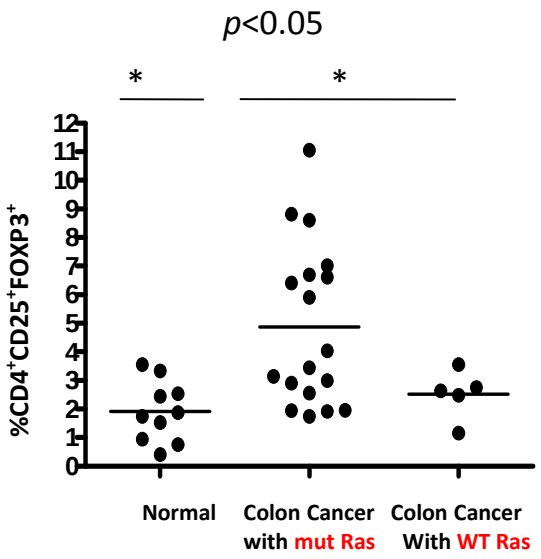
Zdanov et at, CIR 2016

T regs in Colon Cancer Patients



Zdanov et at, CIR 2016

T regs in Colon Cancer Patients



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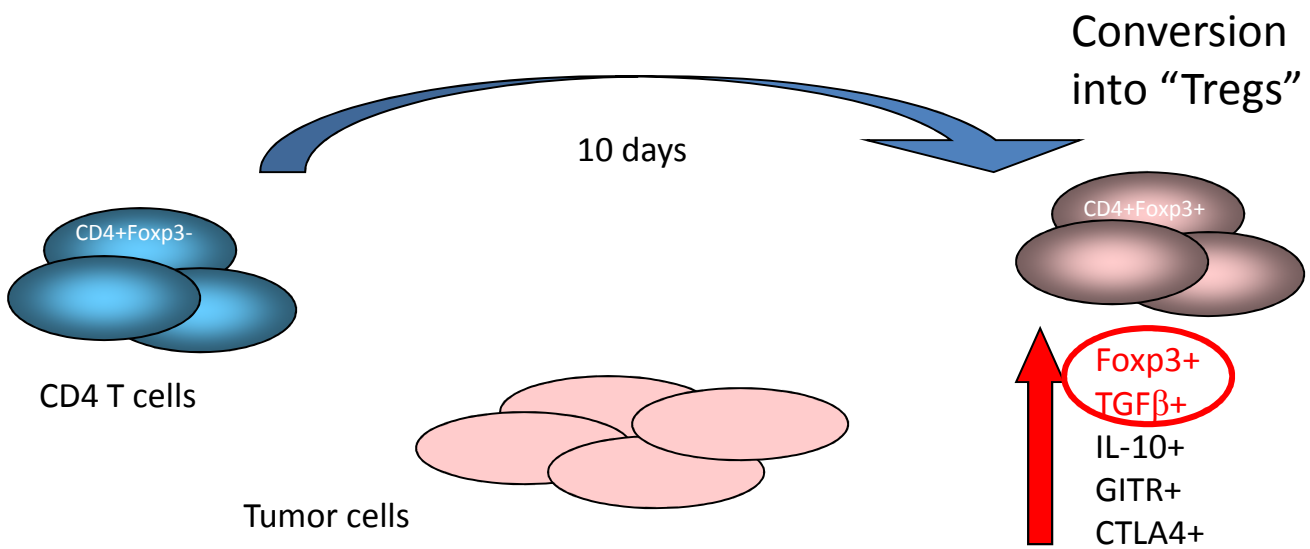
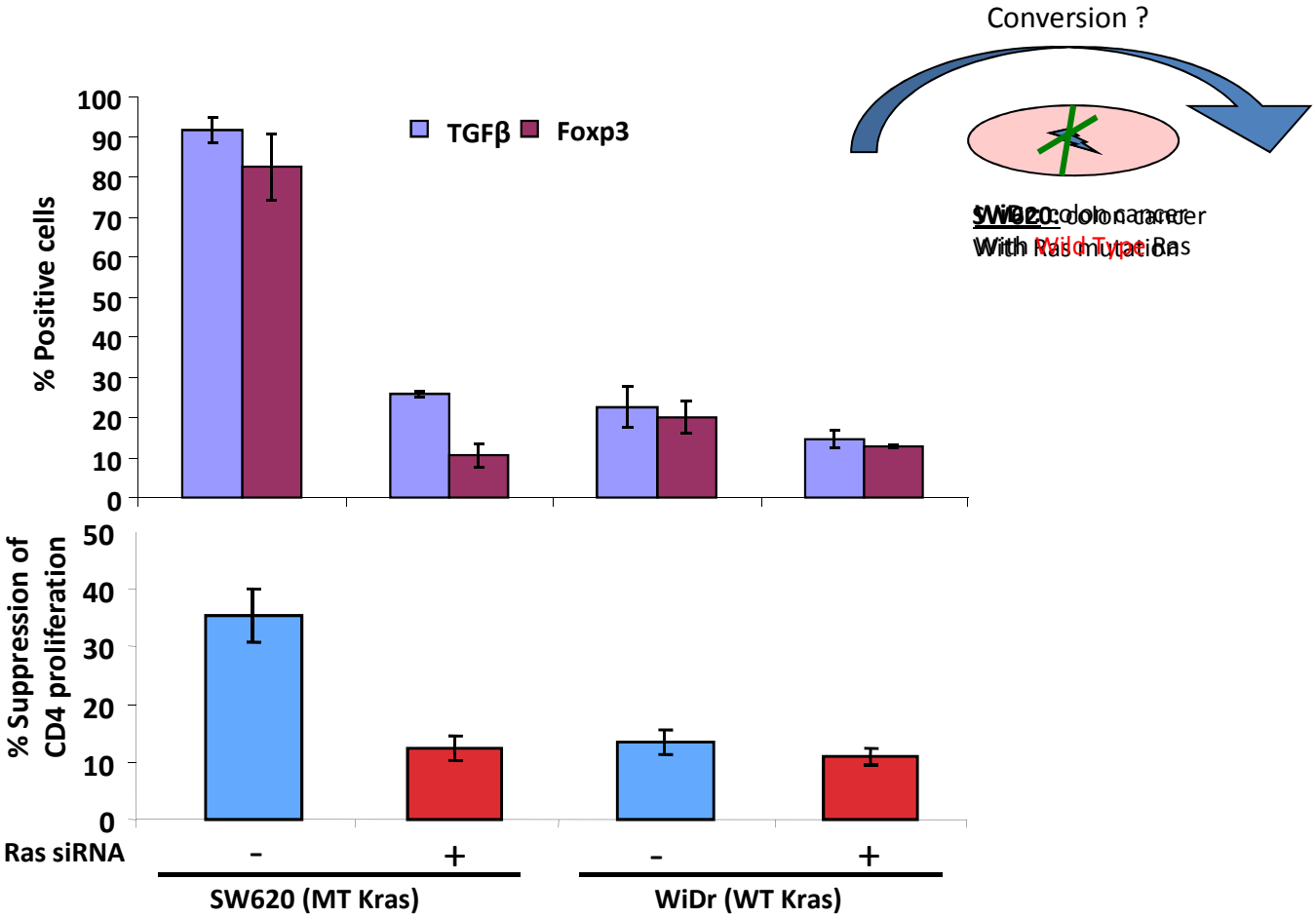
Treg induction





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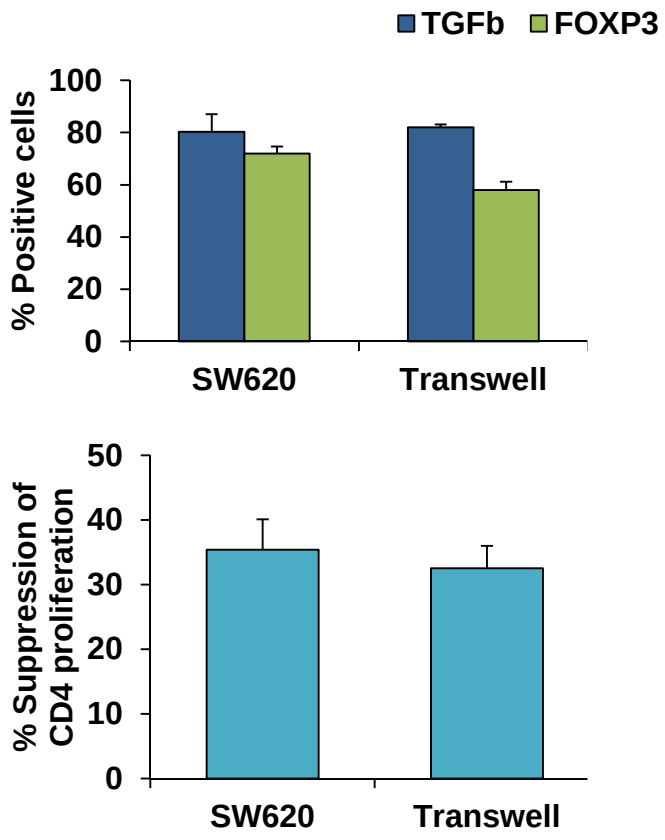
Conversion of Tregs by mutant Ras is IL-10 &TGF-b1 dependent



Zdanov et at, CIR 2016

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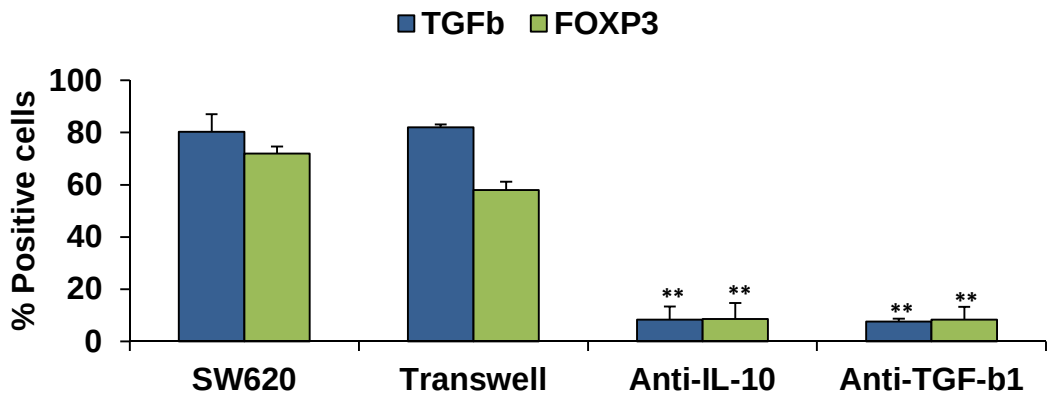

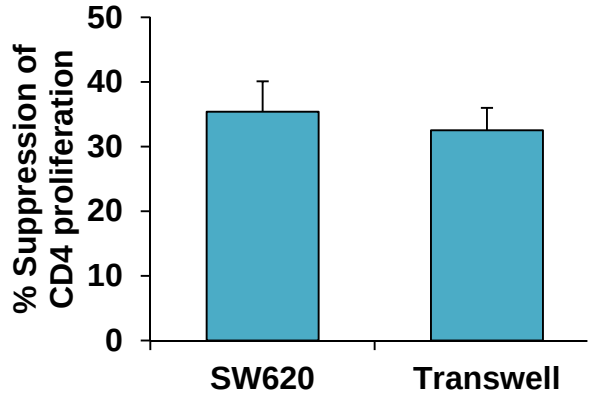

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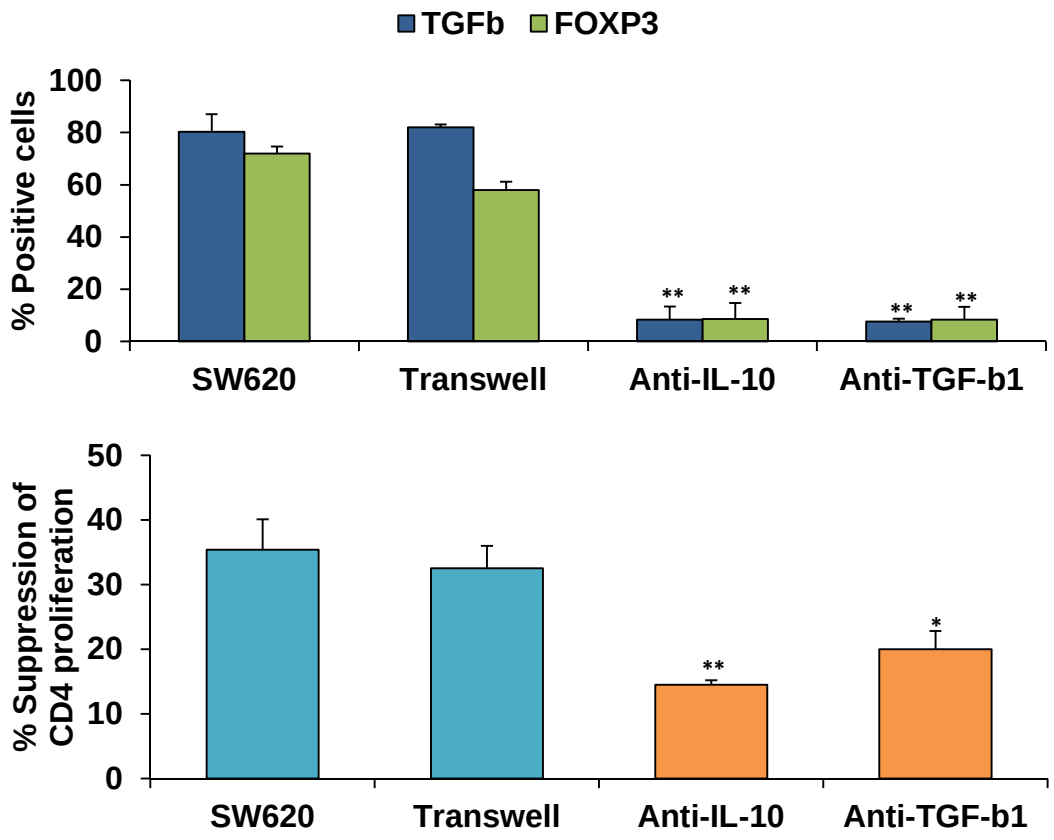

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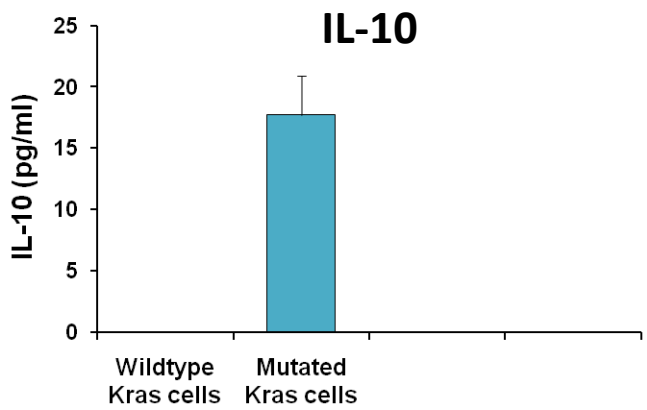
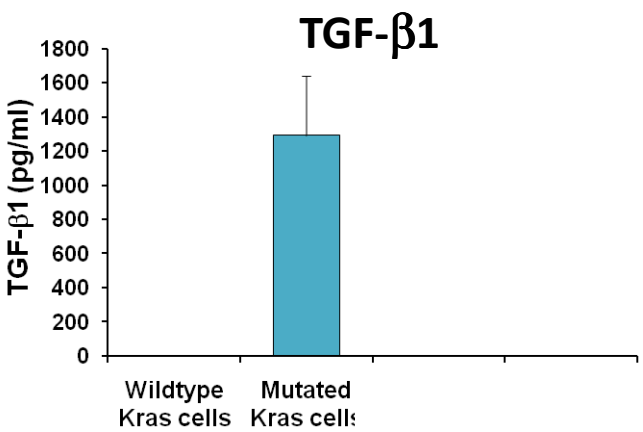
Tumor with mutant Kras release IL-10 and TGF-b1





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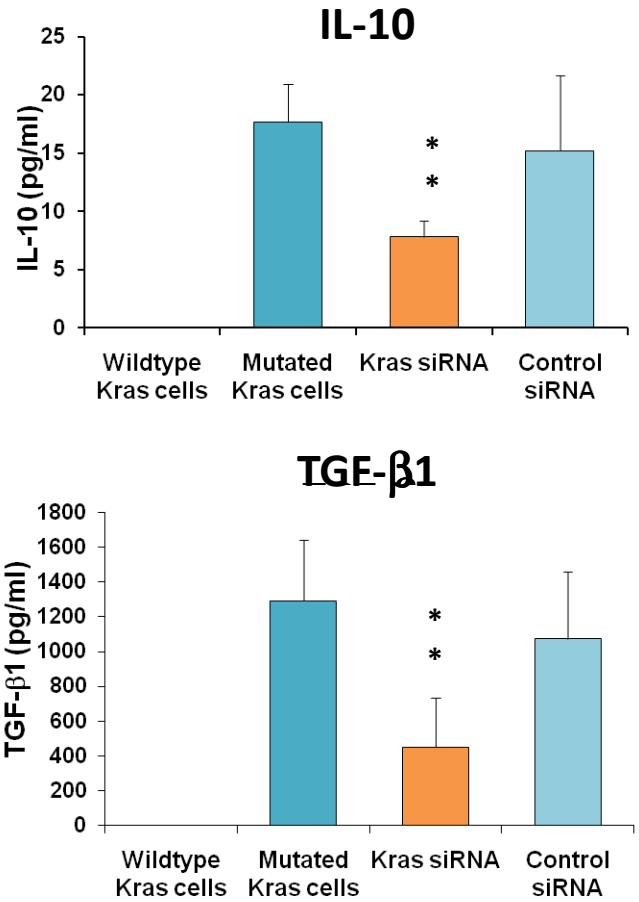

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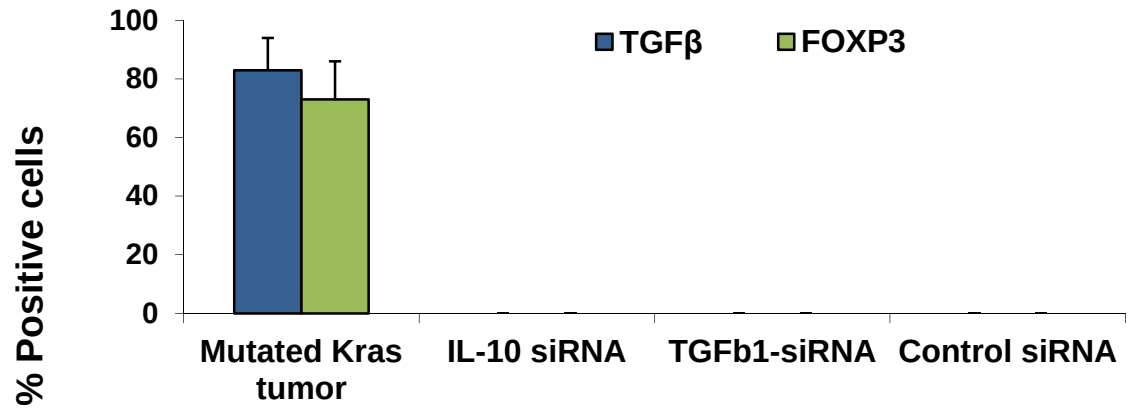

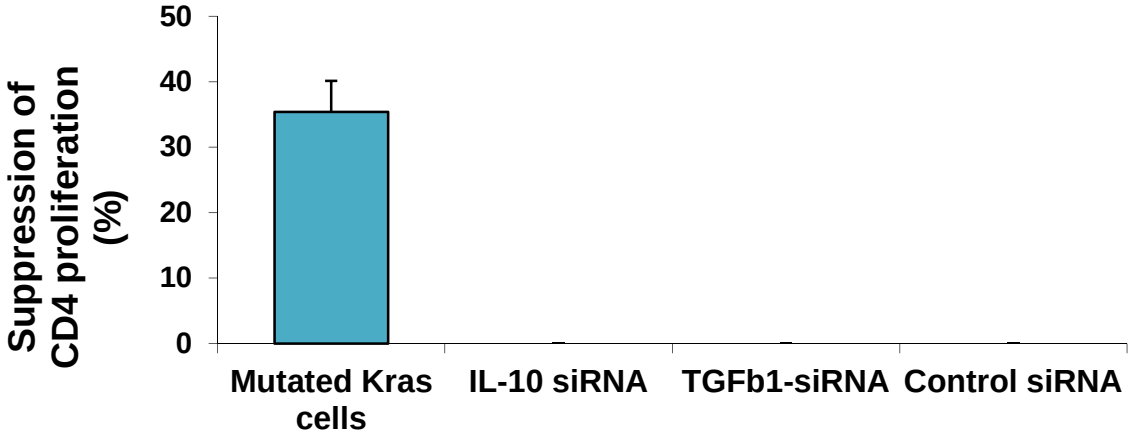

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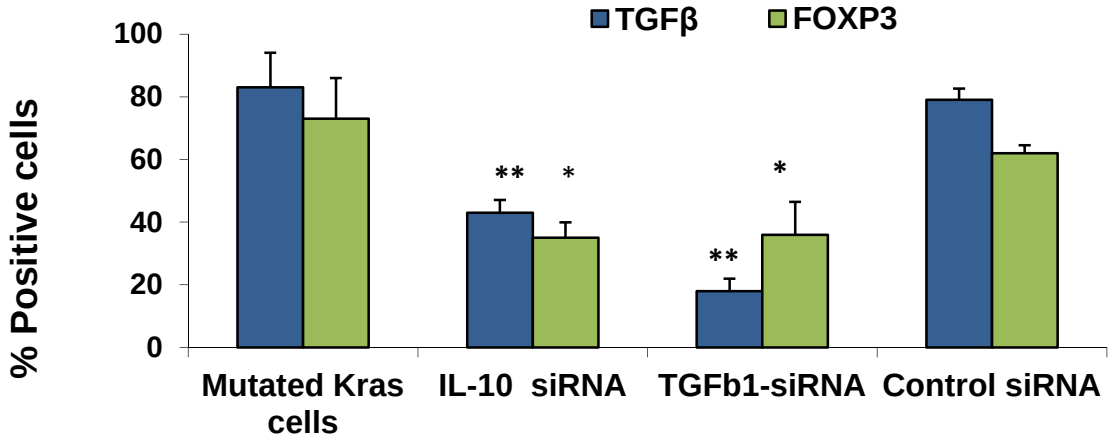

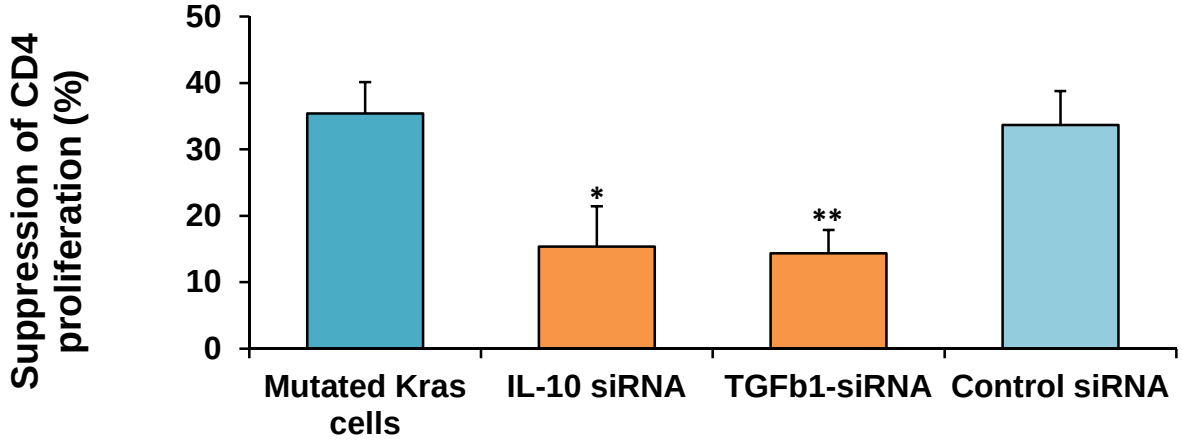

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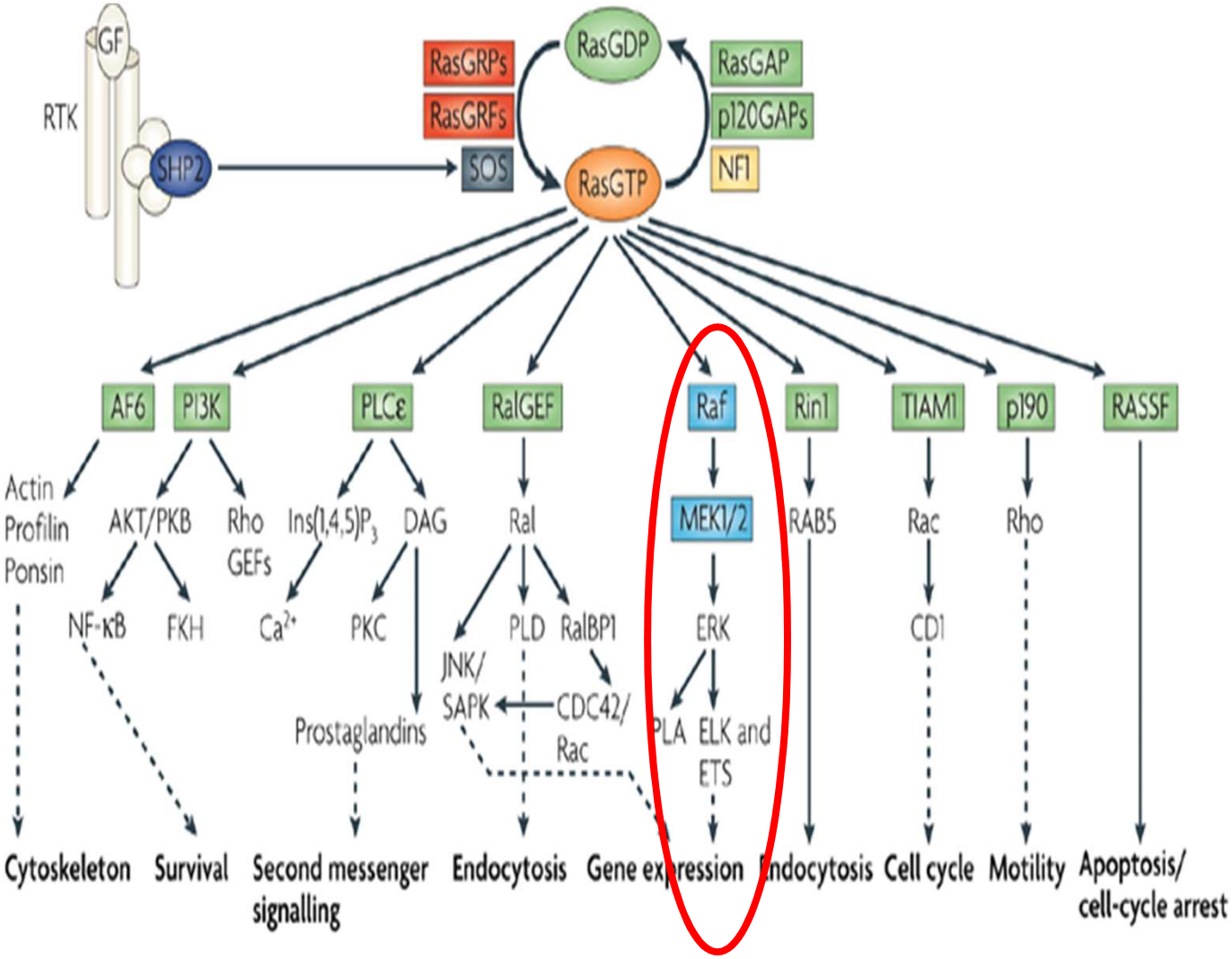
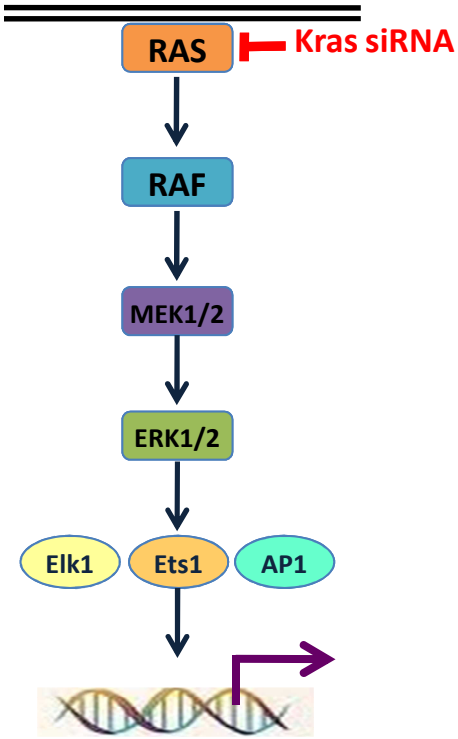
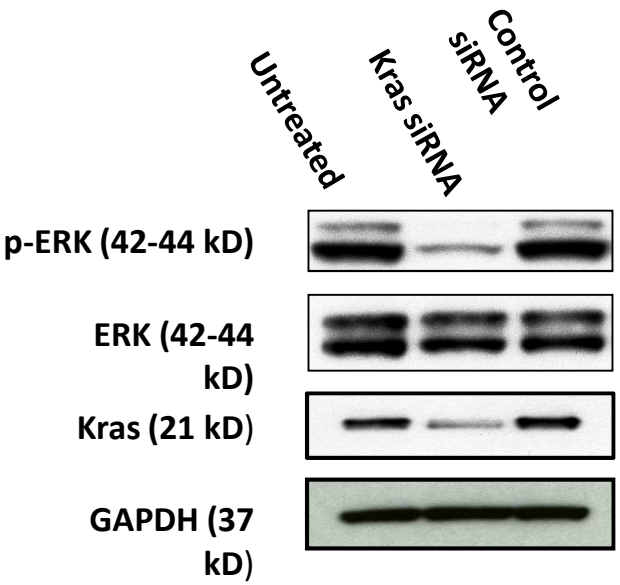

GF
RTK
SHP2
RasGRPs
RasGRFs
SOS
RasGDP
RasGTP
RasGAP
p120GAPs
NF1
AF6
PI3K
PLCε
RalGEF
Raf
Rin1
TIAM1
p190
RASSF
Actin
Profilin
Ponsin
AKT/PKB
Rho
GEFs
Ins(1,4,5)P3
DAG
Ral
MEK1/2
RAB5
Rac
Rho
NF-κB
FKH
Ca2+
PKC
PLD
RalBP1
ERK
CD1
JNK/
SAPK
CDC42/
Rac
Prostaglandins
PLA
ELK and
ETS
Cytoskeleton
Survival
Second messenger
signalling
Endocytosis
Gene expression
Endocytosis
Cell cycle
Motility
Apoptosis/
cell-cycle arrest

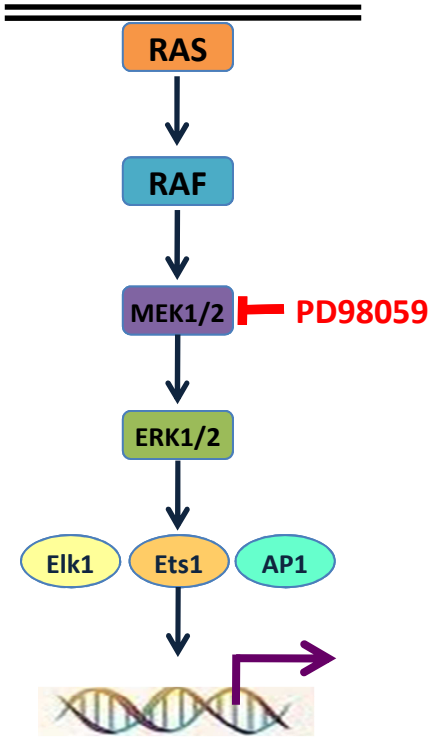
Silencing of Kras prevents ERK activation



Ras oncogene signaling pathway

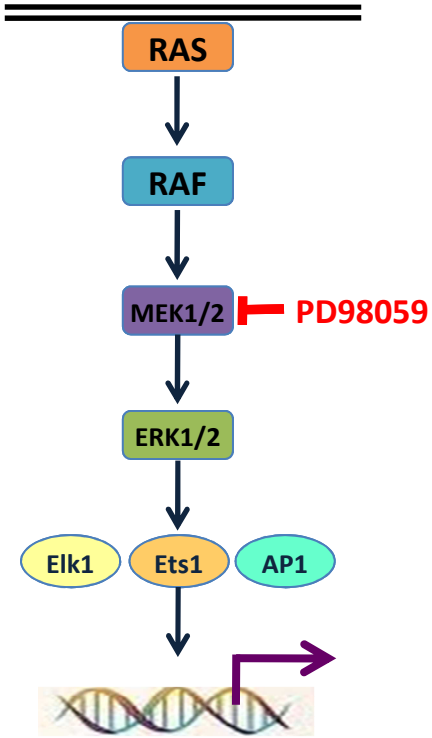


Zdanov et at, CIR 2016

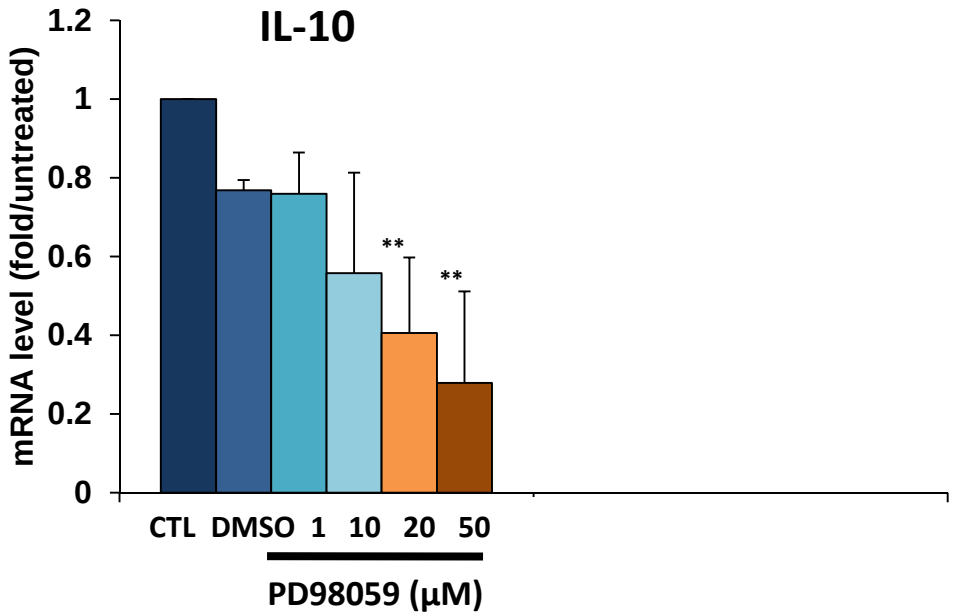


Ras oncogene signaling pathway

MEK1/2 controls the expression of IL-10 and TGF-β1

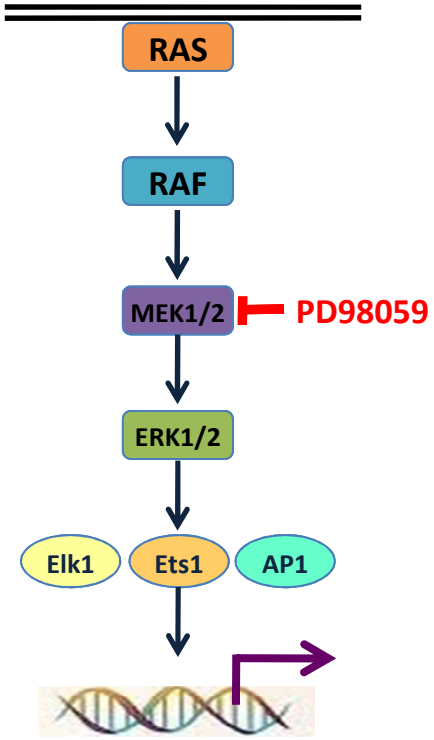


Ras oncogene signaling pathway

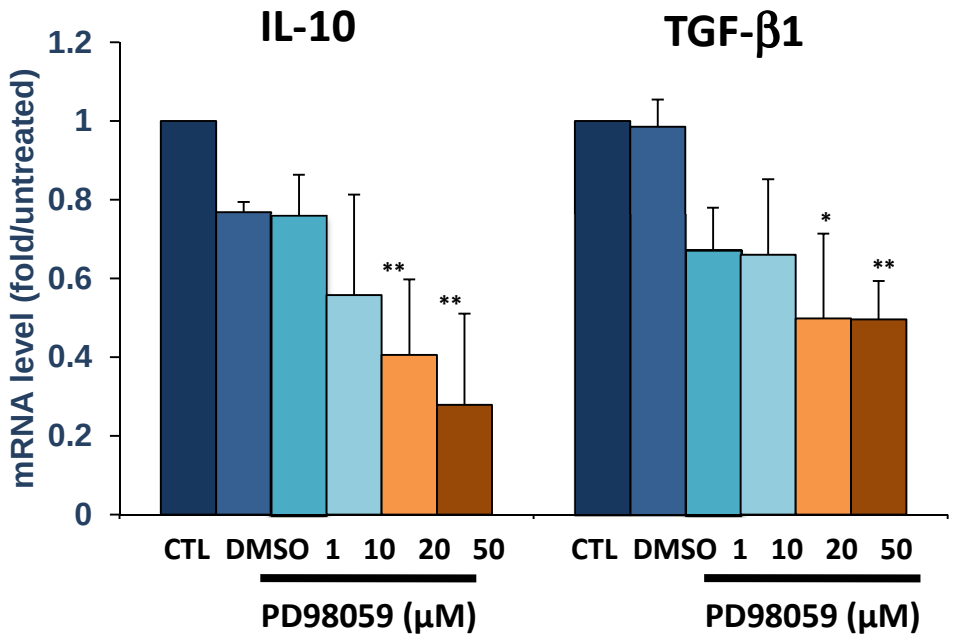


Zdanov et at, CIR 2016

MEK1/2 controls the expression of IL-10 and TGF-β1



Ras oncogene signaling pathway



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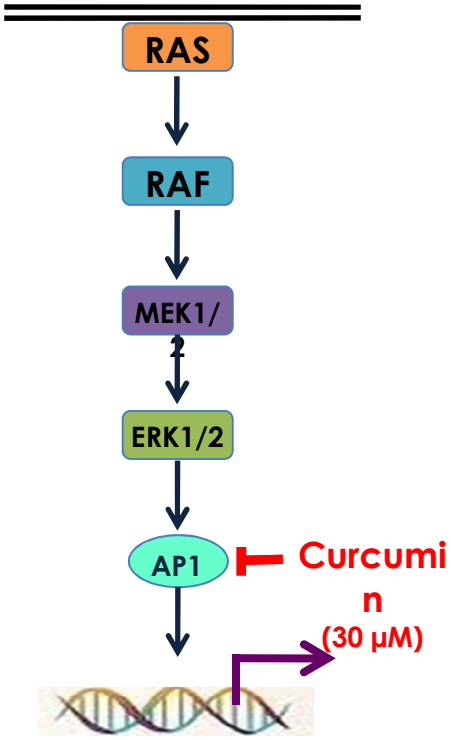
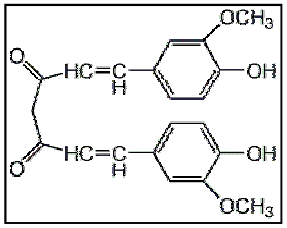
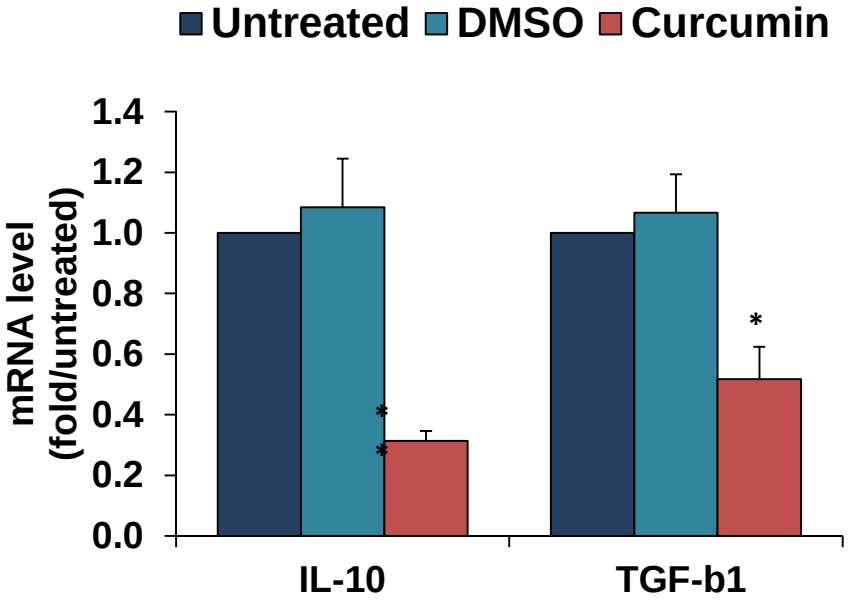
AP1 controls the expression of IL-10 and TGF-β1



Ras oncogene signaling pathway



Cell permeable and irreversible inhibitor
IC50 = 8μM Lipooxygenase
IC50 = 25μM AP-1
IC50 = 52μM COX



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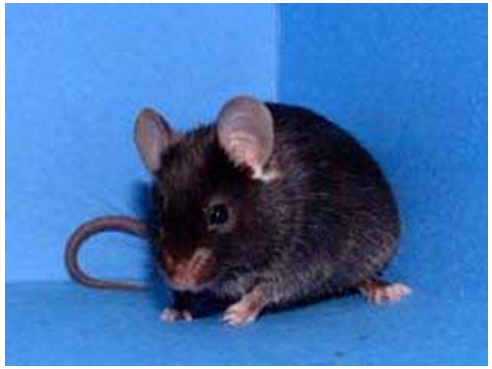
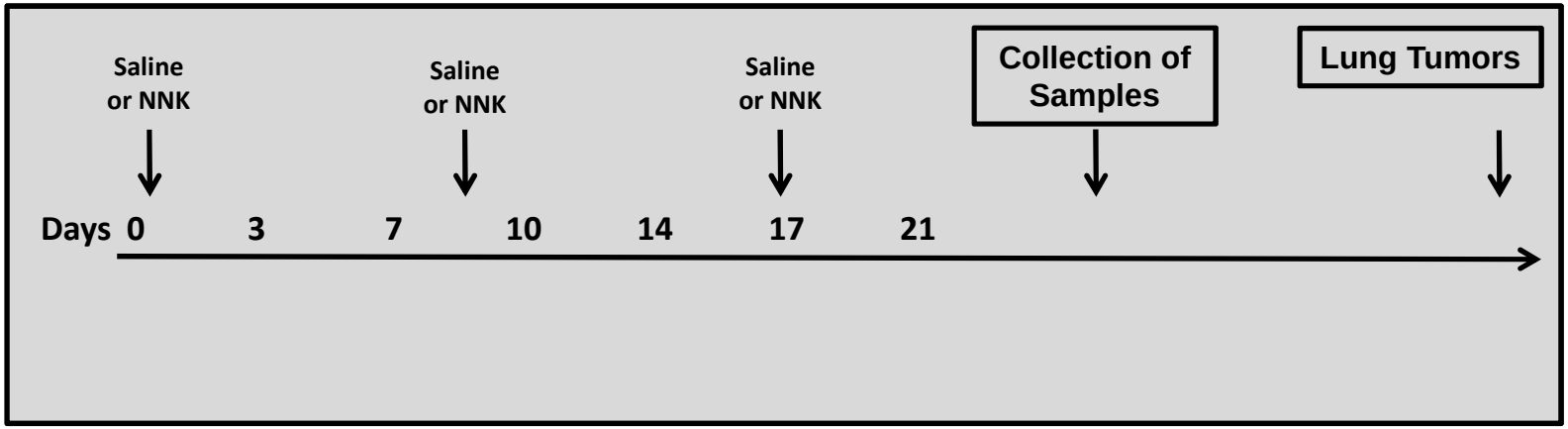


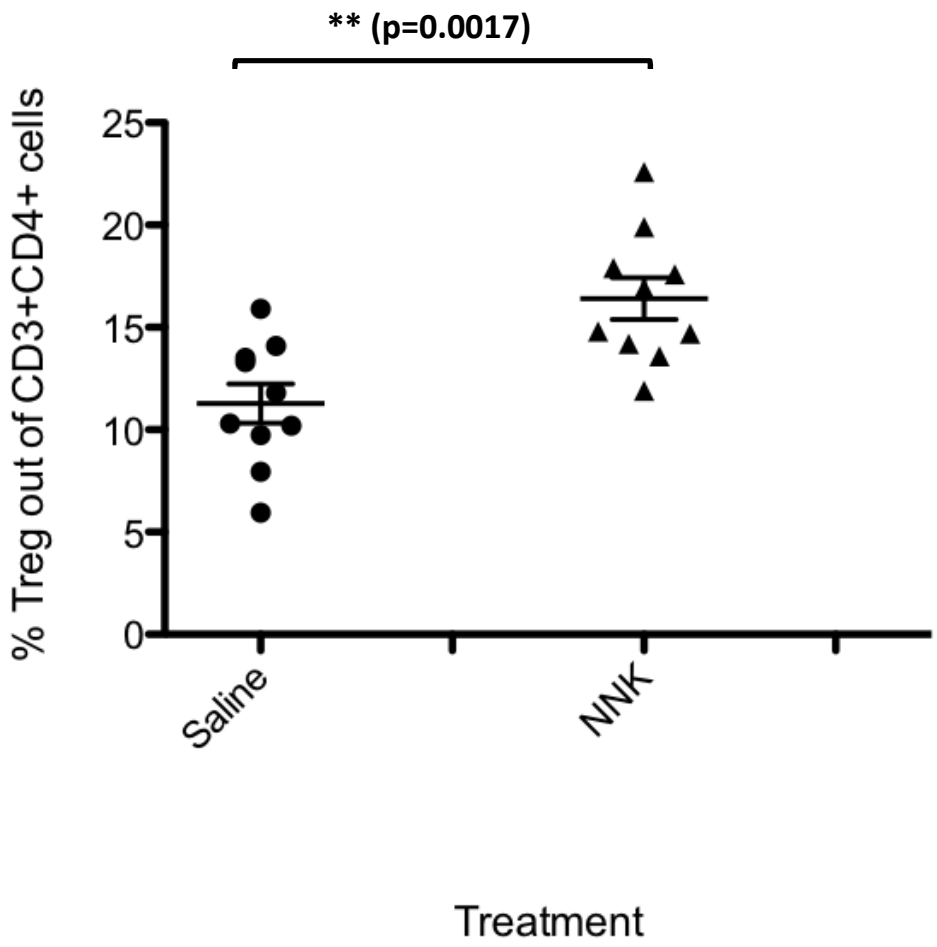
Groups of mice

1. Saline
2. Saline + kR-A4-4
3. NNK
4. NNK + kR-A4-4



(Collaboration with Dr. N. Tarasova (NCI Frederick)

Kras Mutation Increase Tregs in Lung Tissue



Zdanov et at, CIR 2016

➢ **To demonstrate the direct correlation between Kras mutation and Treg infiltration using KRas inhibitor in the NNK lung cancer model**

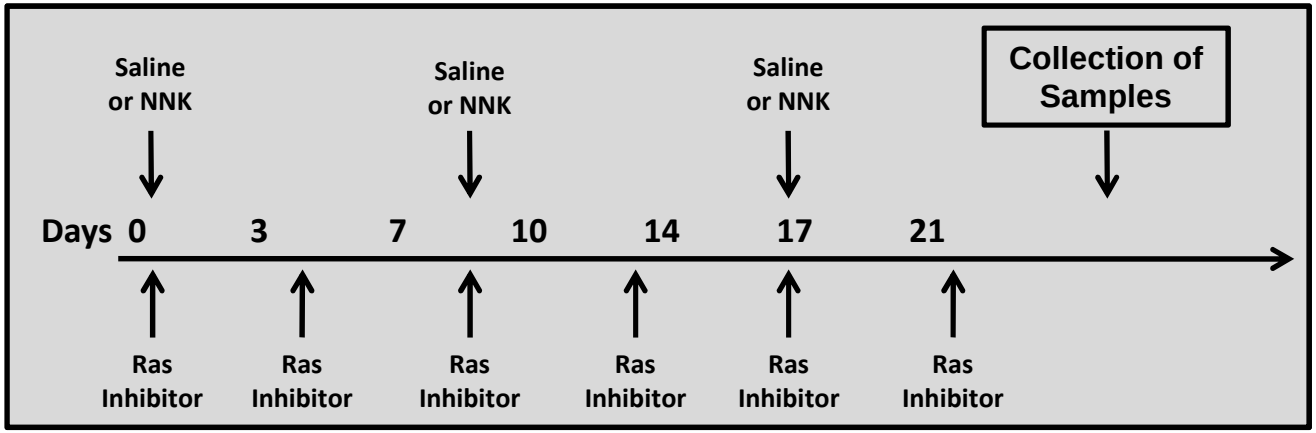
KRas Inhibitor [kR-A4-4]

=> Lipopeptide, analog of the C terminal tail of Kras

- **Administration:** IV Injection at 12.5mg/kg
- **Half life:** Unknown in animals but no degradation in cell medium
- **Mechanism of Action:** Analog of the C-terminal alpha helix, binds directly to Kras
- **GI50:** Low nanomolar range

Groups of mice

1. Saline
2. Saline + kR-A4-4
3. NNK
4. NNK + kR-A4-4



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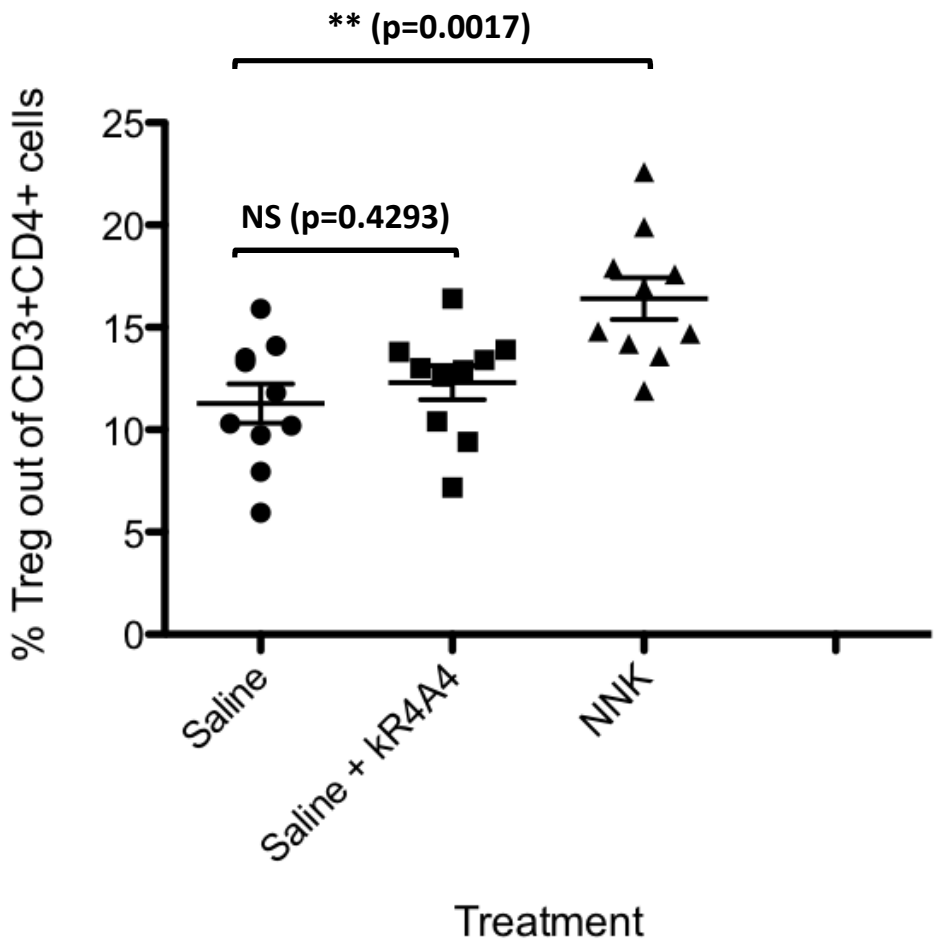
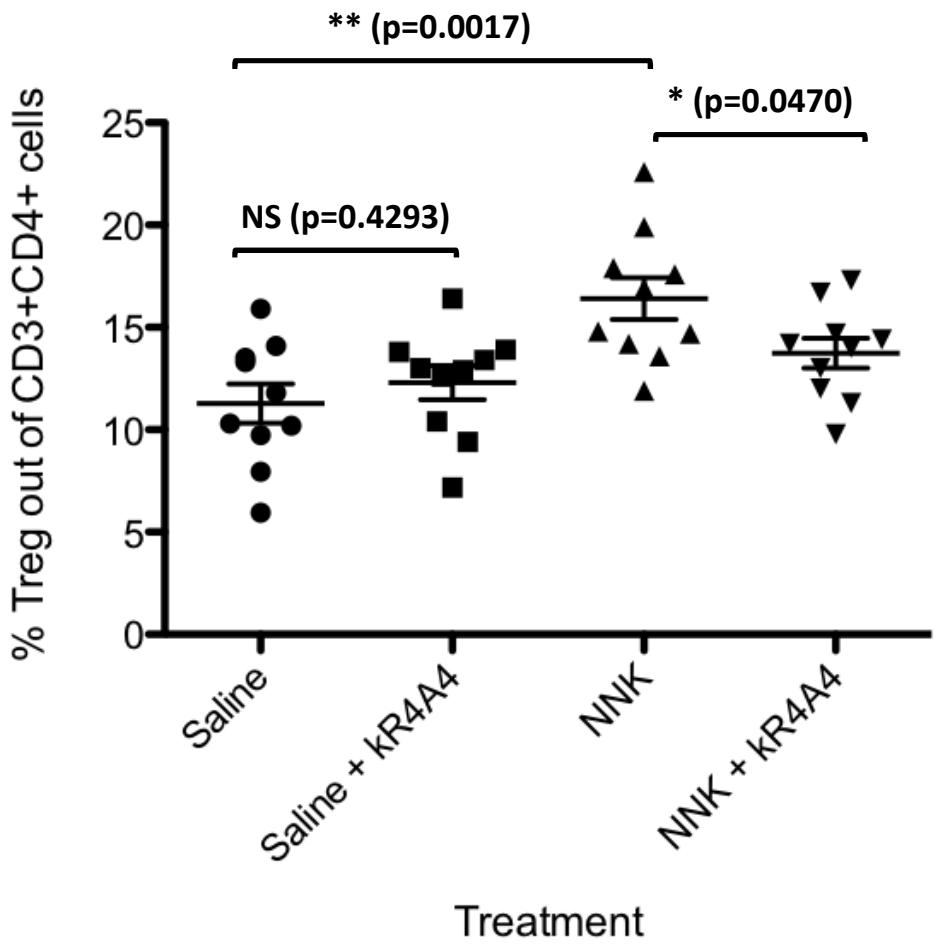
Kras Mutation Increase Tregs in Lung Tissue



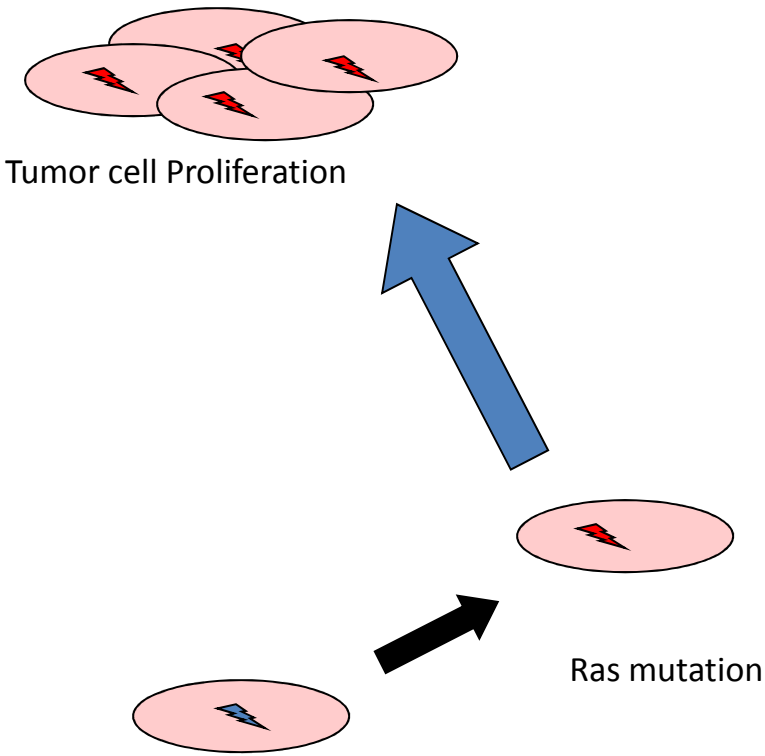
Zdanov et at, CIR 2016

Inhibition of Kras reverse Tregs in Lung Tissue



Zdanov et at, CIR 2016

Tregs induction is a potentially new mechanism of mutant Ras oncogenesis



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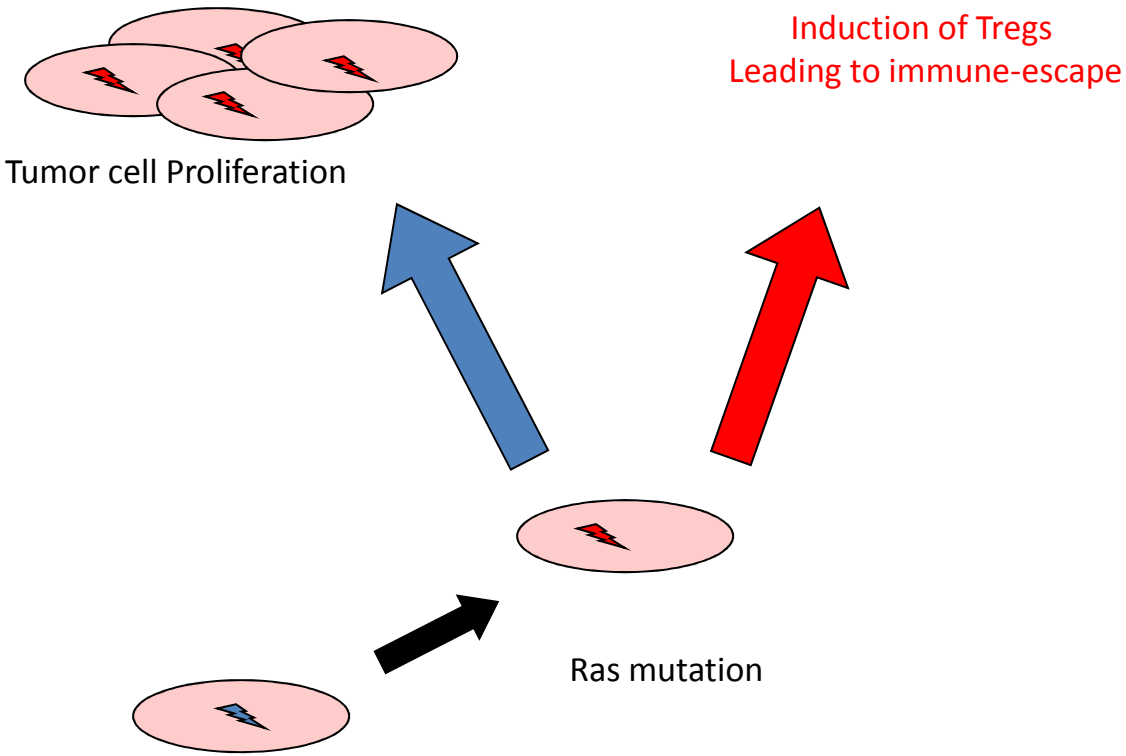

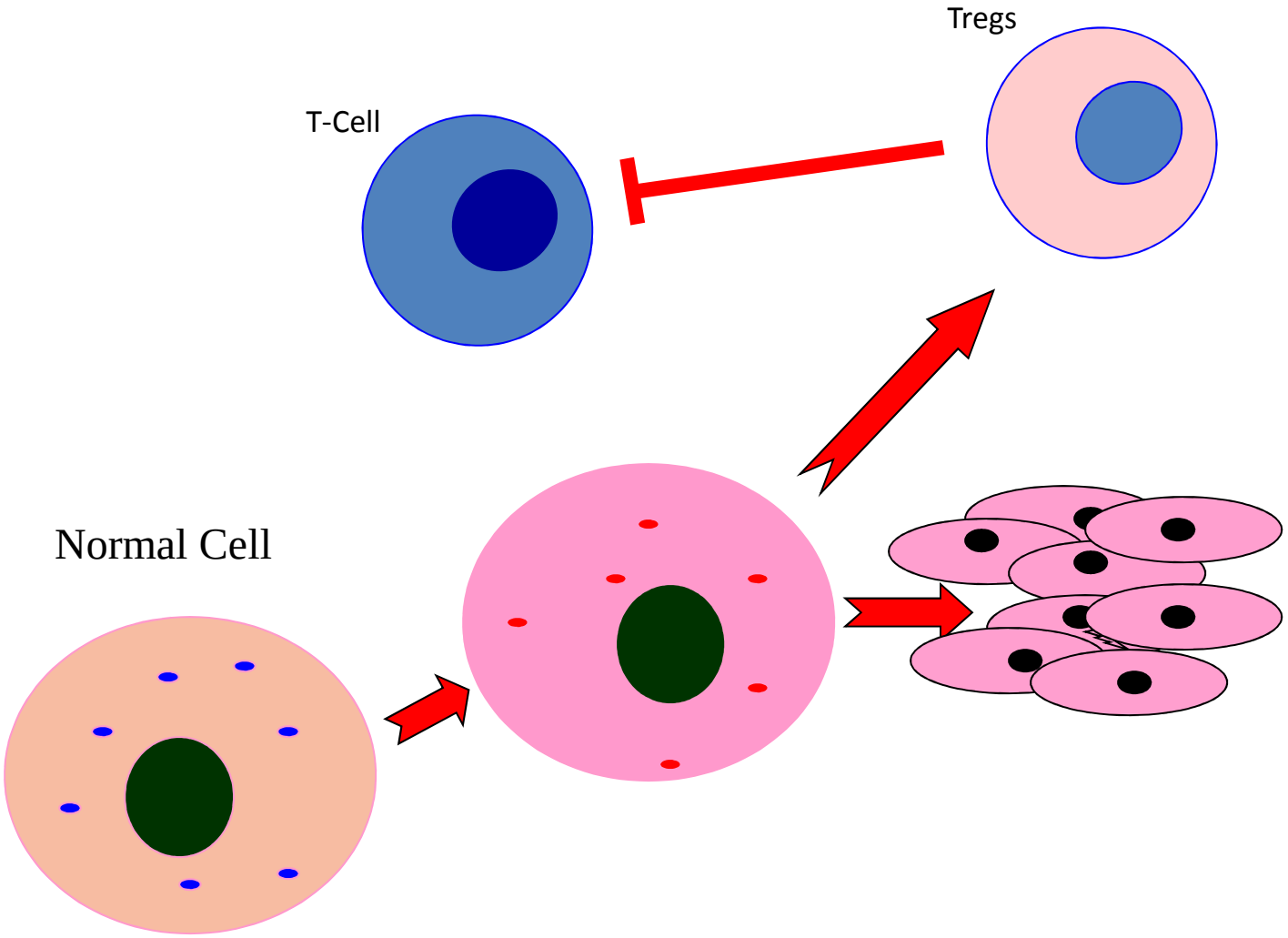
Tregs
T-Cell
Normal Cell

Biomarkers for Combination Immunotherapy

- Prognostic
- Immune Target – Mutant Ras
- Predictive-- MSI
- Metabolic
- Biologic outcome – generated an immune response– ME infiltration– change in the ME
- Outcome

Biomarkers for Combination Immunotherapy

- Prognostic
- Immune Target – Mutant Ras -- indication for the need to other type of agents e.g. CPM

Biomarkers for Combination Immunotherapy

- Prognostic
- Immune Target – Mutant Ras
- Combination IT Guiding Biomarkers
  - Immune bystander biologic effect of the ME
- Predictive-- MSI
- Metabolic
- Biologic outcome – generated an immune response– ME infiltration– change in the ME
- Outcome

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