# Inhibitory effects of targeted cancer therapeutics on T cell function

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# Rationale

- Pharmacologic agents that inhibit specific oncogenic signaling pathways are entering the clinical arena at a rapid pace
- Many of the same signaling molecules involved in cancer cell proliferation and survival are also involved in T cell activation, proliferation, and differentiation
- It may be desirable to combine such agents with immunotherapeutics in the future, or at least to avoid overt immunosuppression when anti-tumor immune responses are sought to be preserved
- It is therefore important to determine the functional and biochemical effects of these inhibitors on T cell function, to consider optimizing future approaches for combination therapy

# Example in melanoma: the farnesyltransferase inhibitor R115777

- Ras-pathway signaling is “on” in melanoma by several distinct mechanisms
- Even melanoma cell lines with mutant B-Raf often show constitutive Ras activation
- R115777 is a potent farnesyltransferase inhibitor (FTI) that should inhibit proper post-translational modification of Ras and other signaling proteins
- Single agent clinical activity of R115777 has been observed in hematologic malignancies
- R15777 inhibits melanoma cell line proliferation in vitro, even those with wildtype Ras
- These observations motivated exploration of this FTI in patients with advanced melanoma

# R115777 in melanoma: Brief eligibility

- Histologically confirmed metastatic melanoma
- PS=1 or 0
- No prior chemotherapy, and at most 1 prior immunotherapy
- No brain metastases
- Intact organ function
- At least 2 cutaneous tumors amenable to excisional biopsies for correlative assays

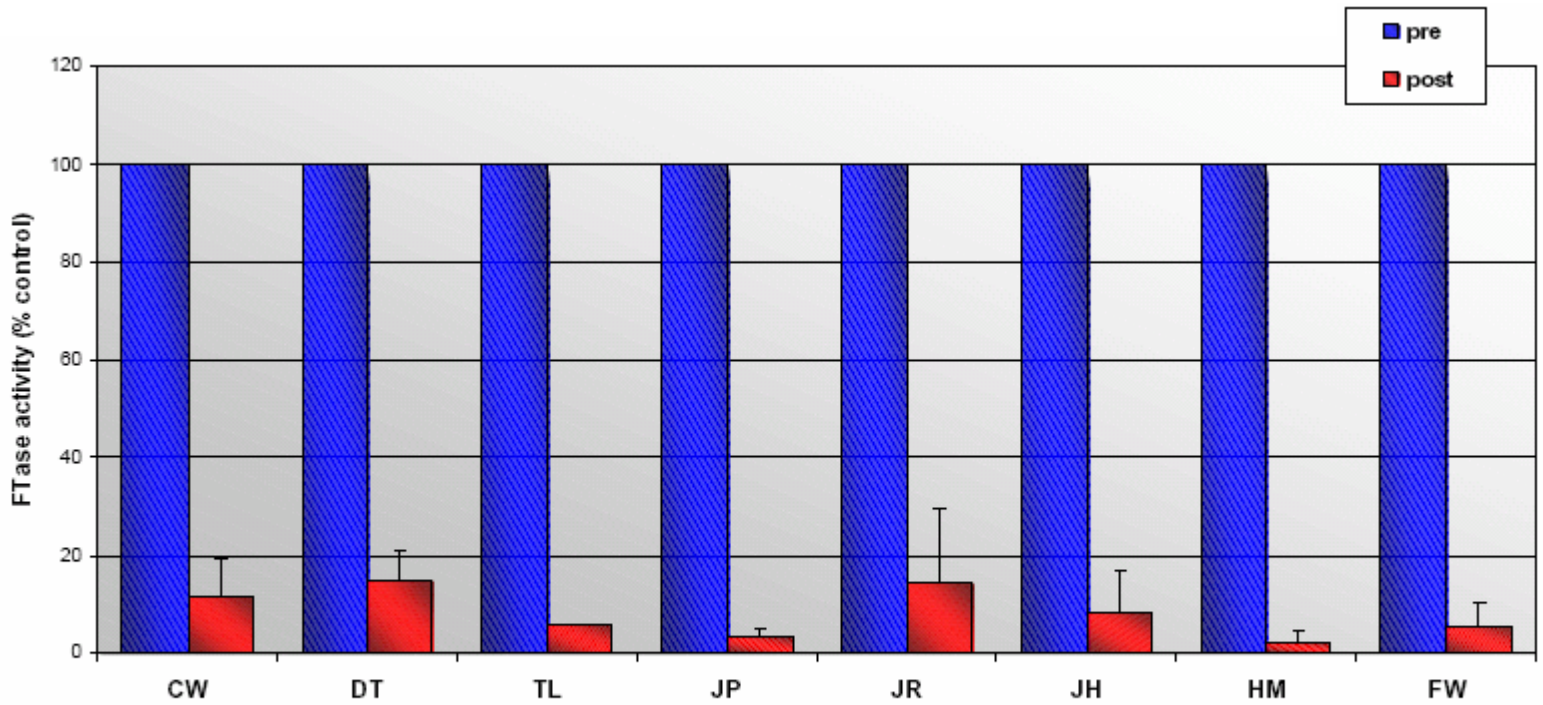
# R115777 in melanoma: Treatment plan

- R115777 given 300 mg po BID for 21 days of 28-day cycle
- Tumor response evaluation every 2 cycles
- Excisional biopsy required pre-treatment and post-2 cycles Rx
- Correlative assays:
  - HDJ-2 gel shift by Western blotting on PBMCs
  - Direct farnesyltransferase assay in tumor biopsies
  - Analysis of downstream signaling in tumor biopsies
  - Measurement of T cell function ex vivo

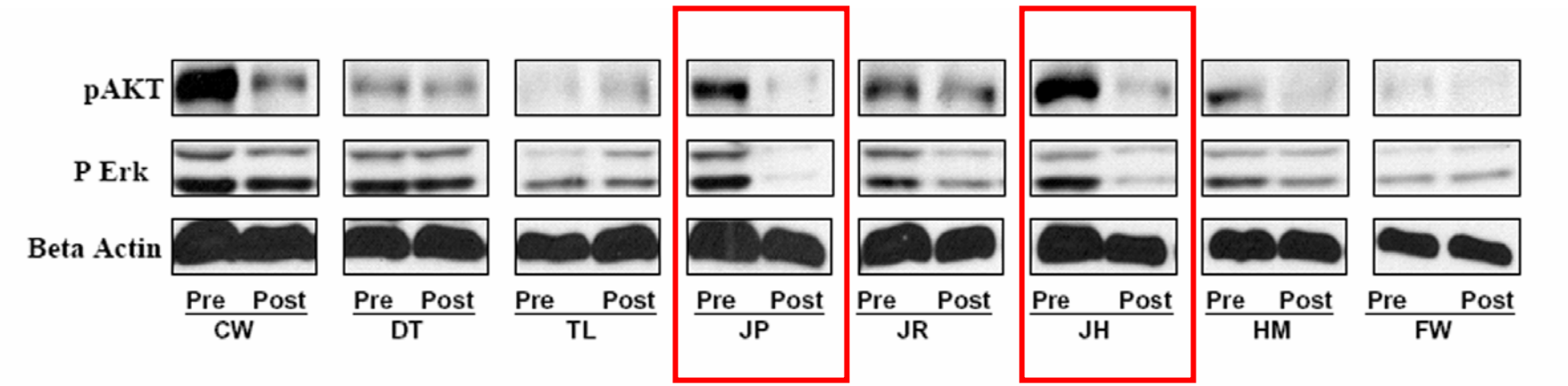
# R115777 in melanoma: Clinical results

- 14 patients enrolled and treated
- Toxicities
  - Generally well tolerated
  - 2 patients with grade 3 toxicities
    - Nausea/vomiting, elevated BUN
    - Myelosuppression, anorexia
- Response
  - No objective clinical responses out of 14 patients treated

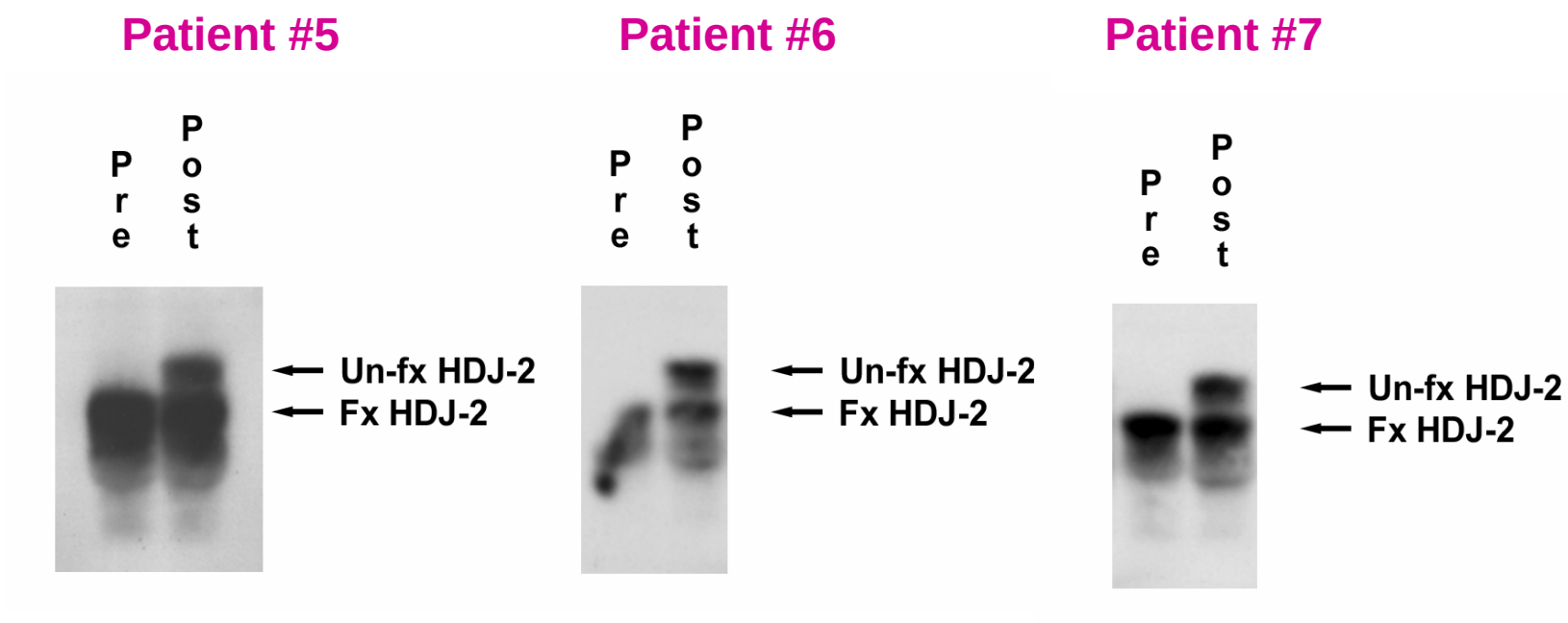
# R115777 blocked farnesyltransferase activity measured by direct assay in all melanoma biopsies tested



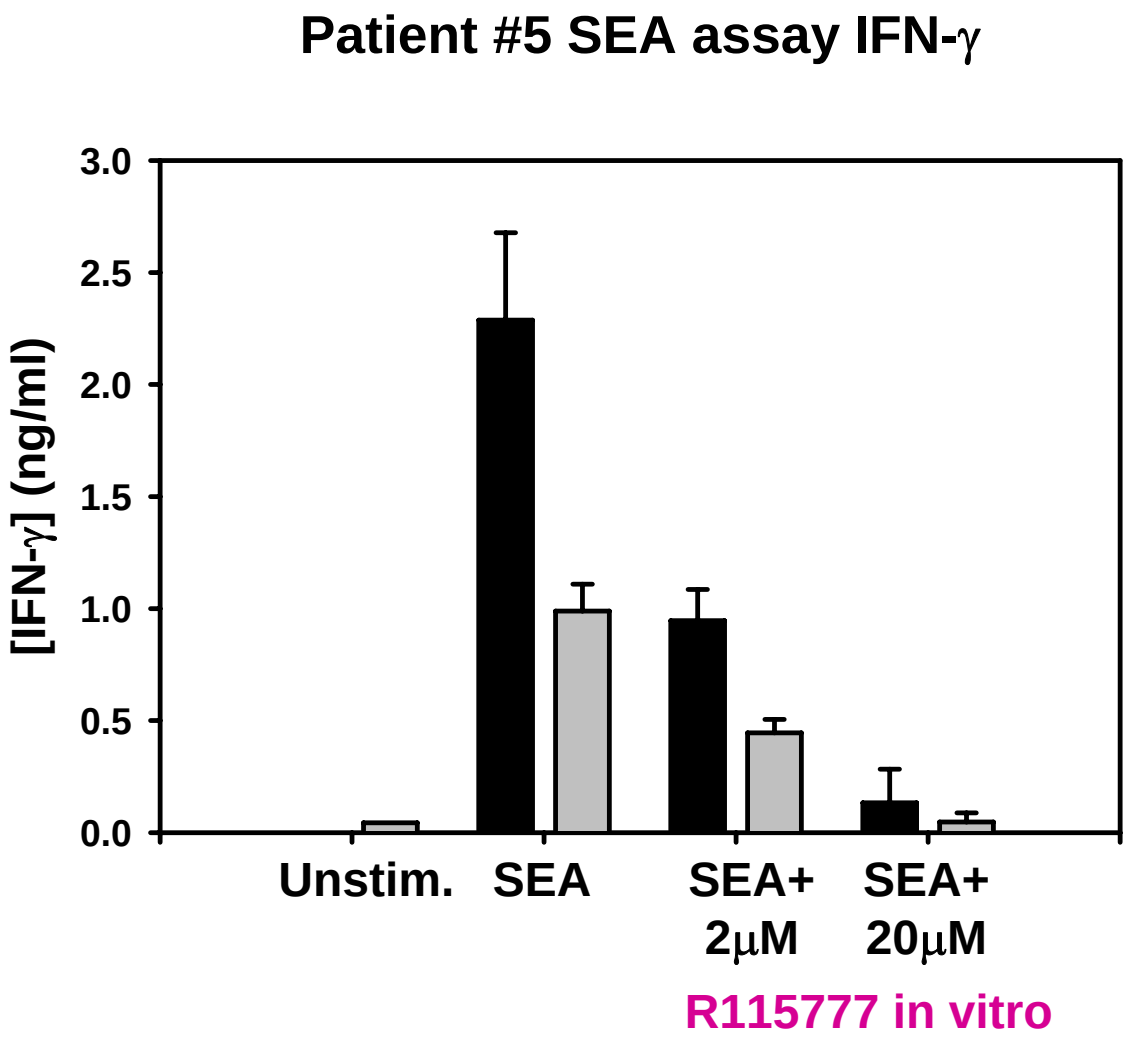
# R115777 potently blocked ERK and Akt phosphorylation in a subset of tumors



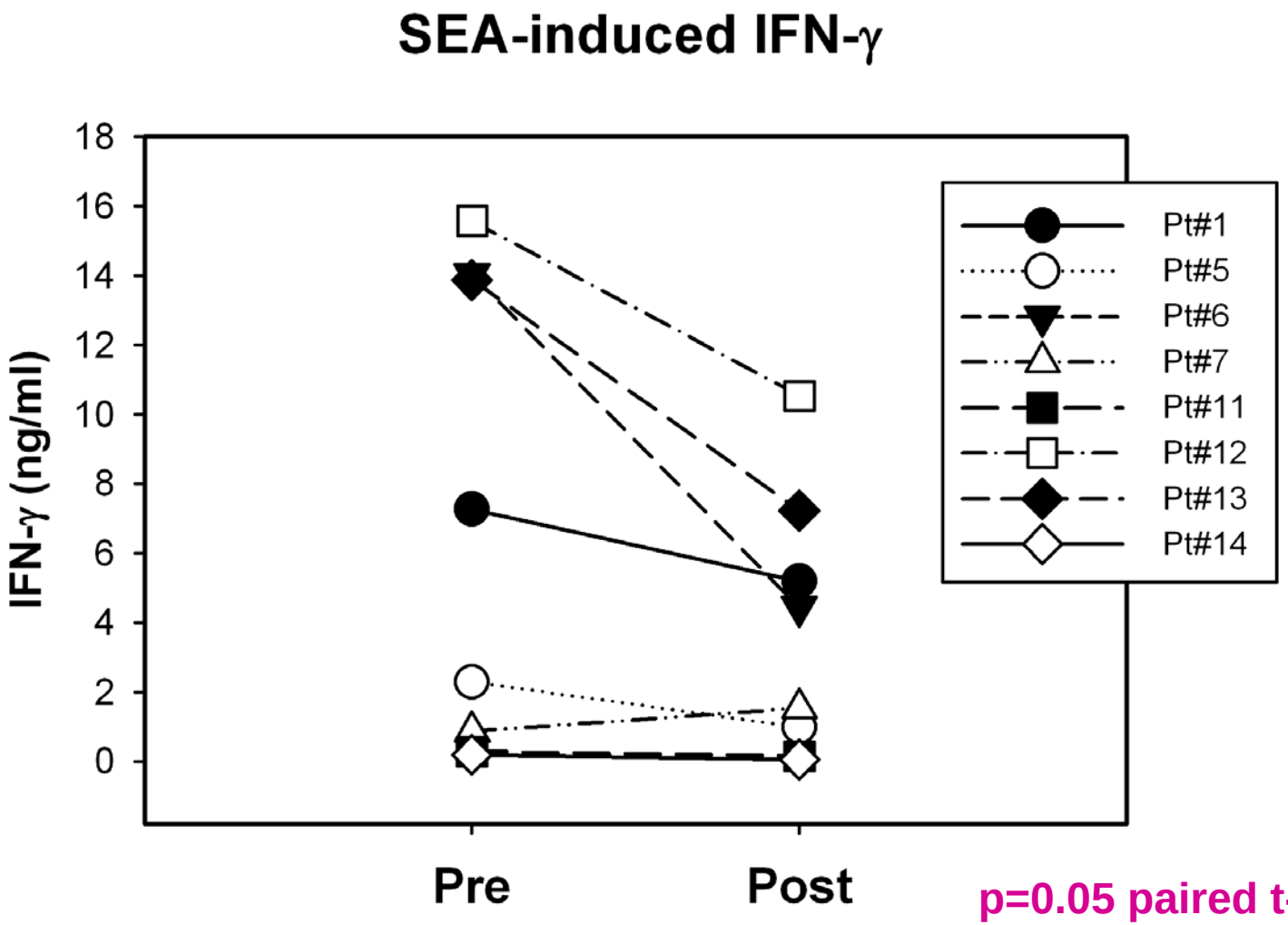
# R115777 effect on PBL: Accumulation of unfarnesylated HDJ-2



# Representative ex vivo IFN-γ production assay pre- and post-R115777

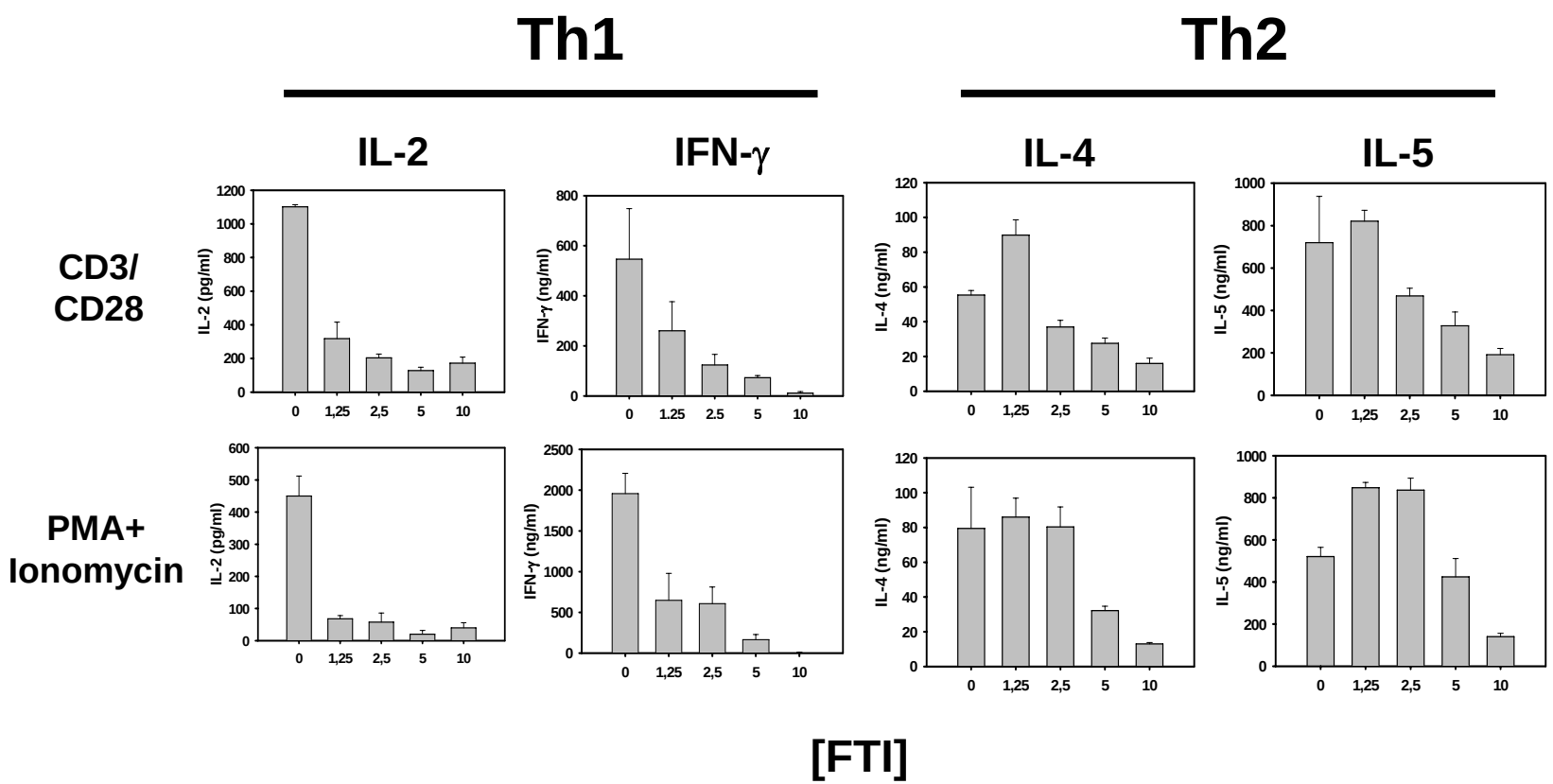


# Ex vivo IFN-γ production from PBMC in all assayable patients

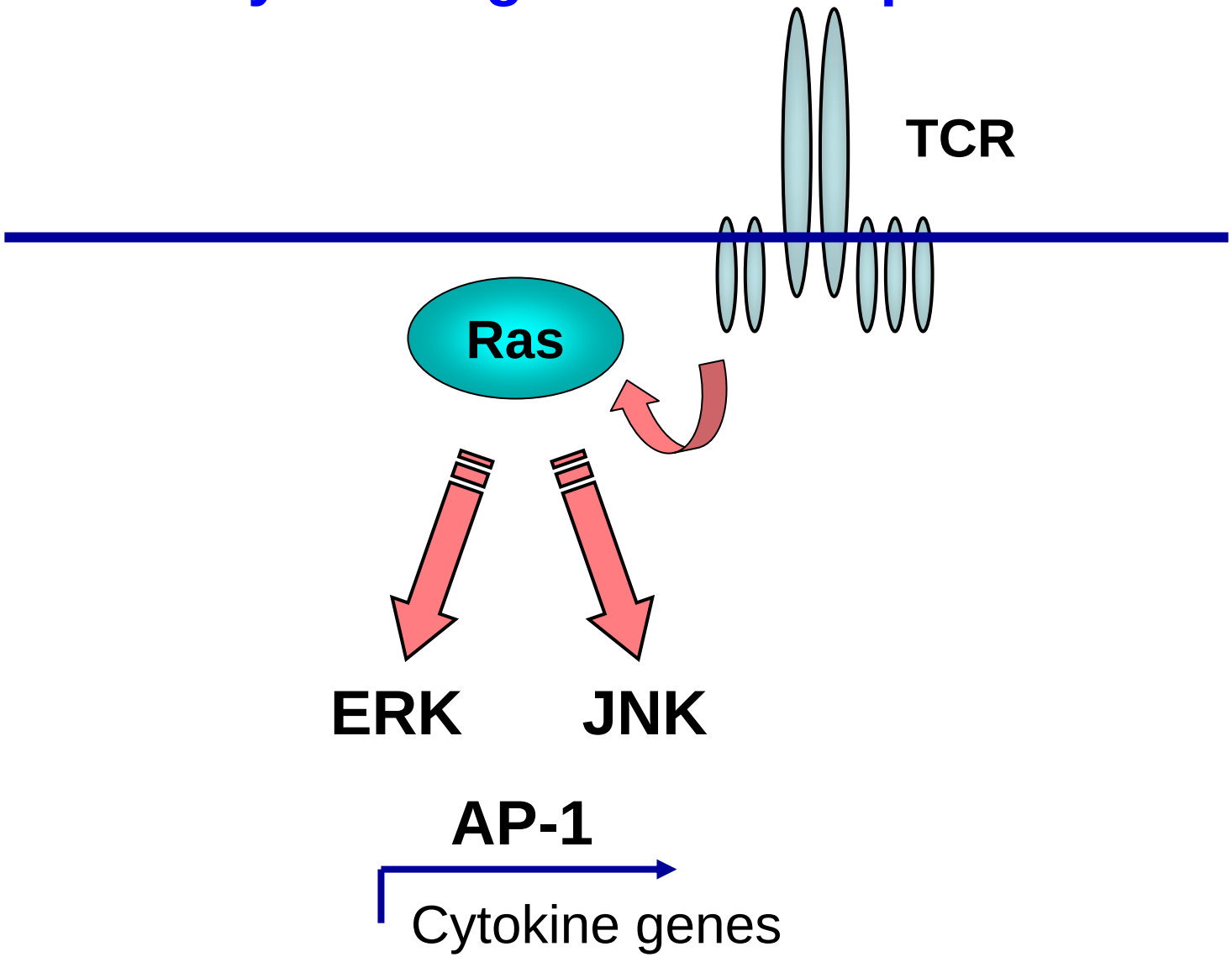



p=0.05 paired t-test

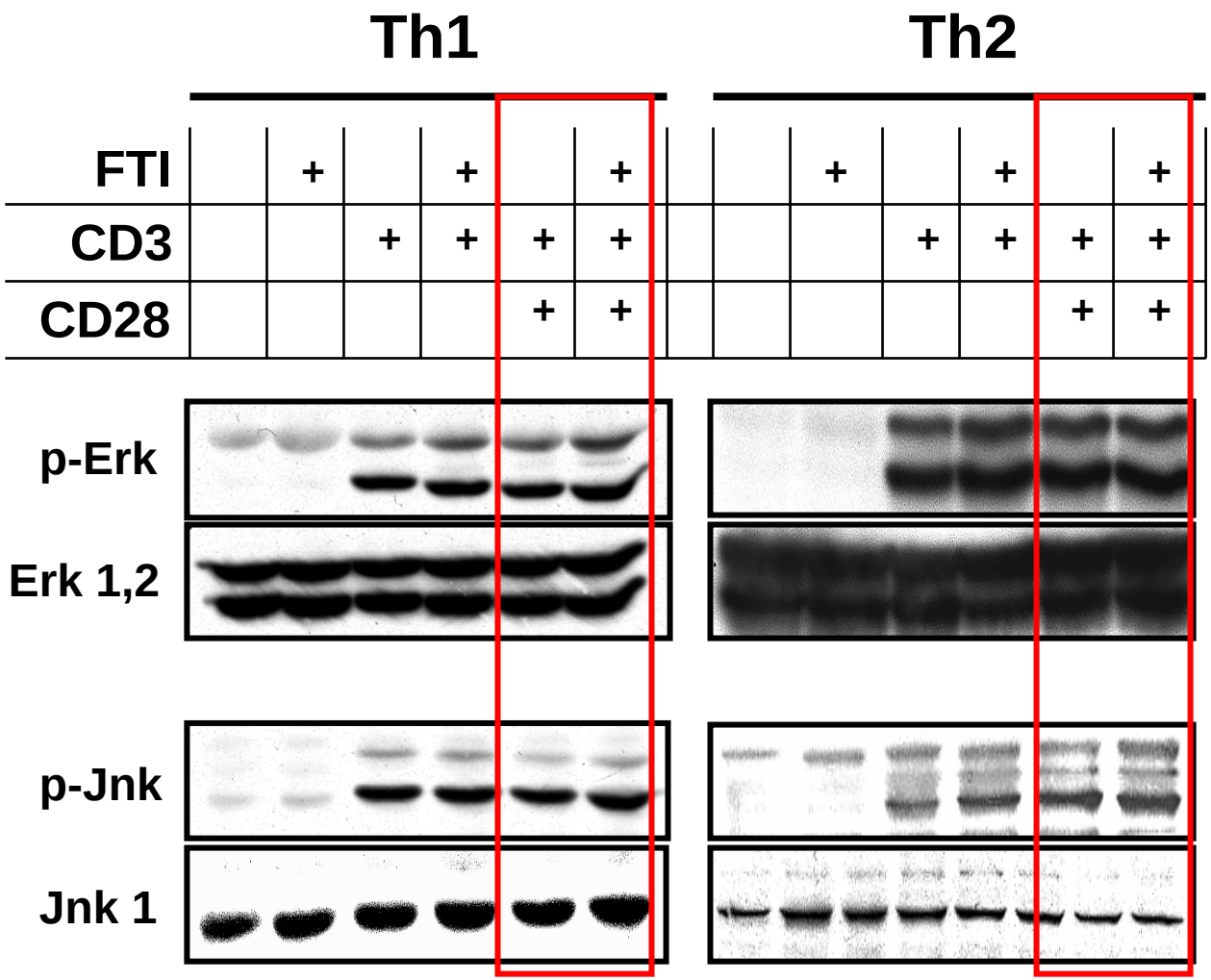
# Farnesyltransferase inhibition blocks Th1 and Th2 cytokine production in vitro



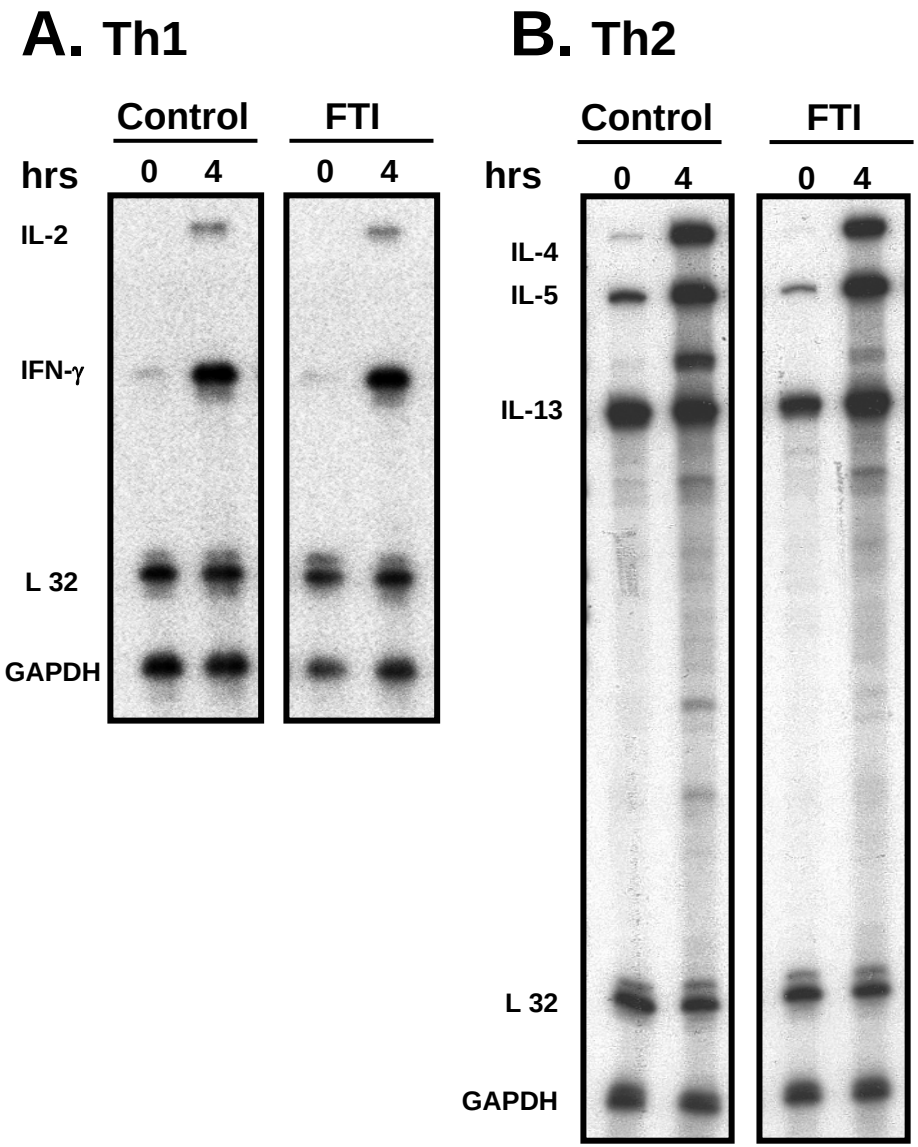
# Presumed mechanism of FTI action would be at the level of Ras, upstream from MAP kinases and cytokine gene transcription



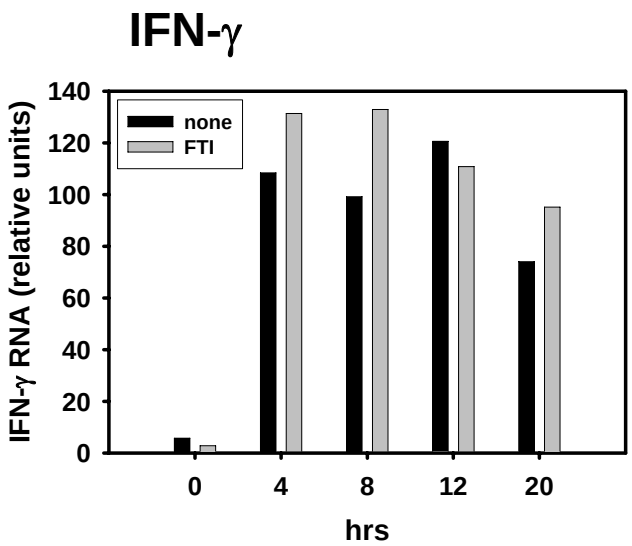
# Farnesyltransferase inhibition does not block ERK or JNK activation in response to CD3/CD28 ligation

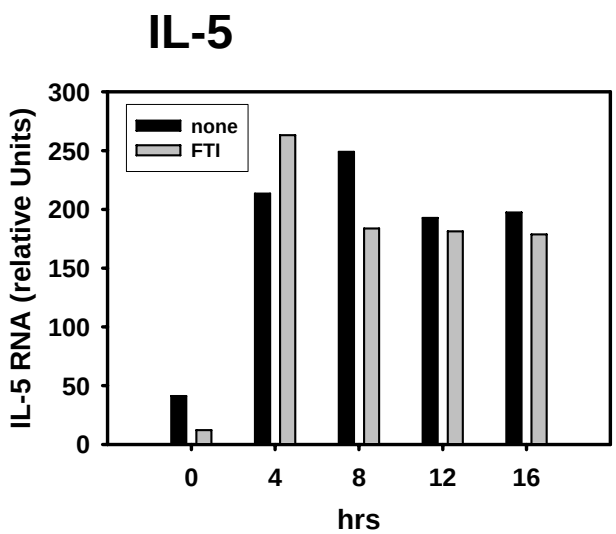


# Farnesyltransferase inhibition does not inhibit cytokine mRNA induction as assessed by RPA



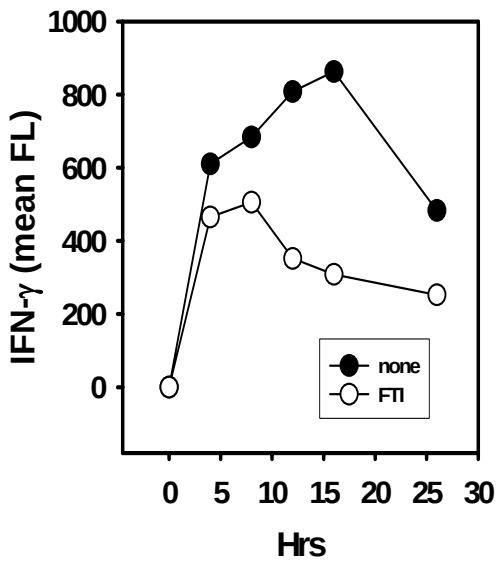
# Kinetic analysis of cytokine mRNA: lack of effect of FTI over time



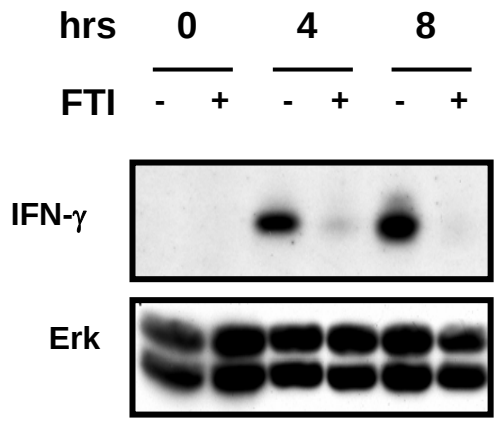



# Despite lack of inhibition of cytokine mRNA induction, cytokine protein synthesis is reduced

**A. Intracellular FACS**

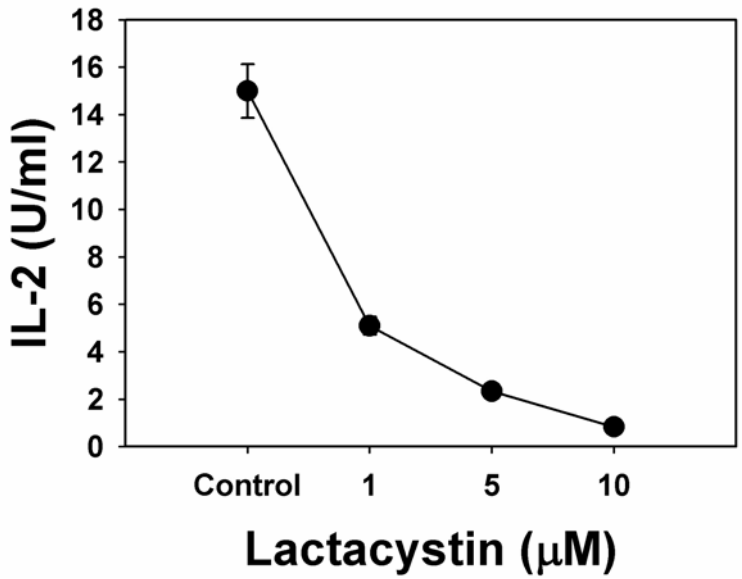



**B. Western Blot**

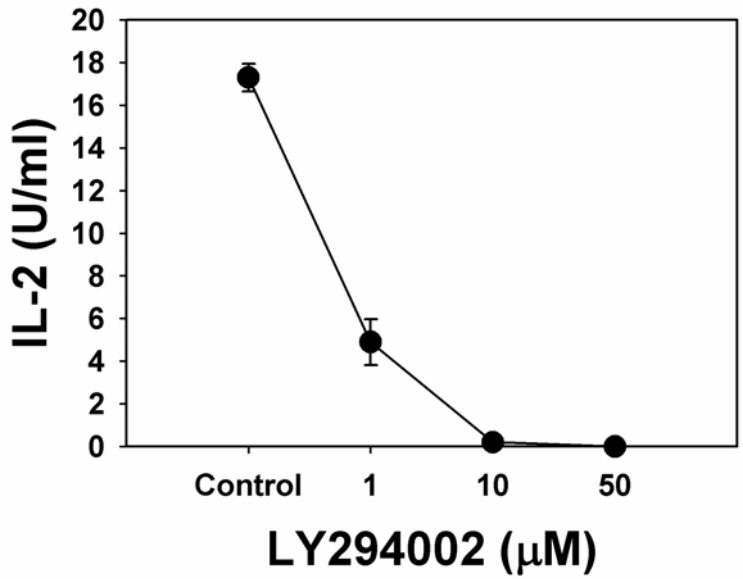


# Numerous targeted inhibitors inhibit T cell activation

Proteasome inhibitor




PI3K/Akt inhibitor




Inhibition of T cell activation also seen with inhibitors of MEK, p38MAPK, JNK, mTOR/p70S6K, Src kinases, pan-tyrosine kinases

# Conclusions

- R115777 in melanoma:
  - R115777 clearly inhibited farnesyltransferase activity in melanoma tumor tissue
  - Evidence suggests that downstream Ras effectors ERK and Akt were also inhibited substantially in several patients
  - However, suppression of these signaling events in patients' tumors was not necessarily sufficient to halt melanoma growth in vivo
  - R115777 modestly inhibits SEA-induced IFN-$\gamma$ production by T cells measured ex vivo
- FTIs and T cell activation
  - Farneslytransferase inhibitors block Th1 and Th2 cytokine production
  - However, mechanism is not at level of Ras pathway signaling or cytokine gene expression
  - Rather, inhibitory effect is at post-transcriptional level
  - Novel immunosuppressive drugs? Similar mechanism in cancer?
- Bottom line:
  - Care will need to be taken when integrating targeted inhibitors into immunotherapeutic regimens to choose dose and schedule that do not compromise immunity

# Acknowledgments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**Signaling inhibitors and T cells**
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