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

Patient #5  Patient #6  Patient #7

Pre Post Pre Post Pre Post

Un-fx HDJ-2 Un-fx HDJ-2 Un-fx HDJ-2
Fx HDJ-2   Fx HDJ-2   Fx HDJ-2
Representative ex vivo IFN-γ production assay pre- and post-R115777

Patient #5 SEA assay IFN-γ

R115777 in vitro
Ex vivo IFN-\(\gamma\) production from PBMC in all assayable patients

SEA-induced IFN-\(\gamma\)

\(p=0.05\) 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

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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

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-γ production by T cells measured ex vivo

• FTIs and T cell activation
  – Farnesyltransferase 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

R115777 trial
Helena Harlin
Todd Kuna
Donna Niedzwiecki
Jeffrey Johnson
Gerald Linette
Cynthia Bucher
Michelle Blaskovich
Said Sebti
Frank Haluska
Julianne Buenting
CTEP

FTI effect on T cells
Reinhard Marks
Allen Ho

Signaling inhibitors and T cells
Sujit Janardhan
Fabiola Rivas
Candace Cham
Yuan-yuan Zha
Kesavannair Praveen