Drug Discovery

Vemurafenib

Vemurafenib Review

And

British Journal of Cancer (2014) 111, 640-645

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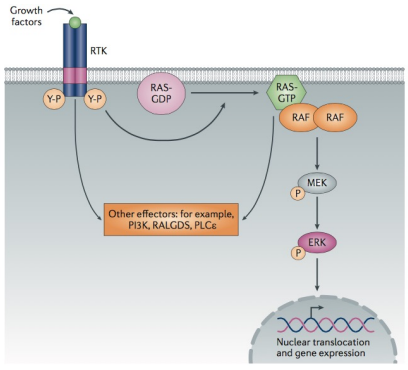
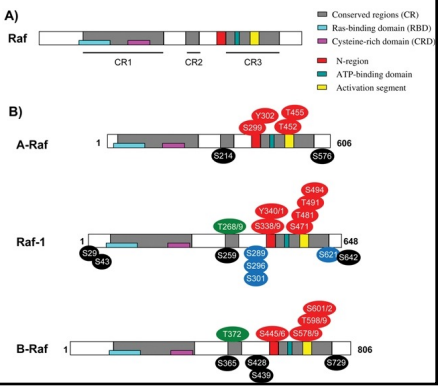
RAF

- Raf kinase family – (rapidly accelerated fibrosarcoma) activate canonical ERK/MAPK signaling from a tyrosine kinase receptor
- Three different Raf genes 1/c-Raf, B-Raf and A-Raf





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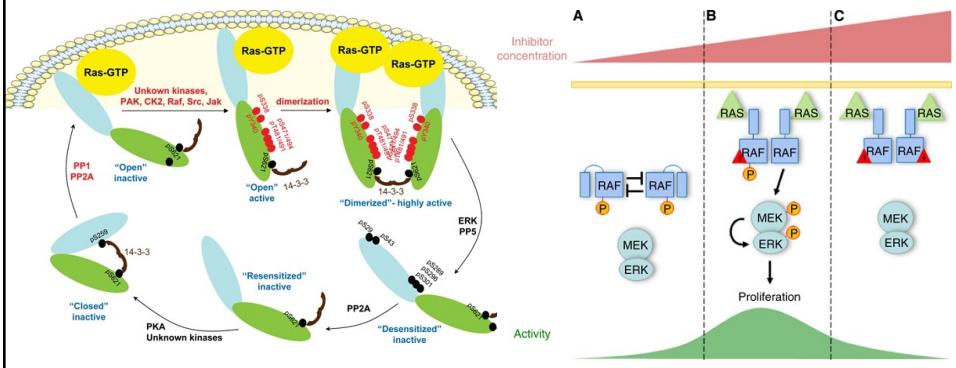
RAF

- Regulation of phosphorylation and **dimerizing (drug induced????)**.
- Different activation by each isoform
- Autophosphorlyation inhibits. Low drug conc may block autophosphorylation and high drug inhibitors binds and inhibits both Raf promoters and kinase activity



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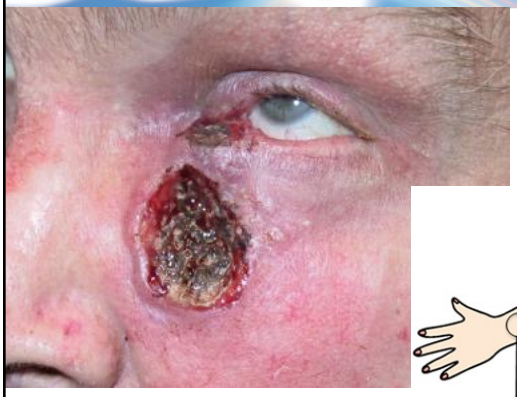
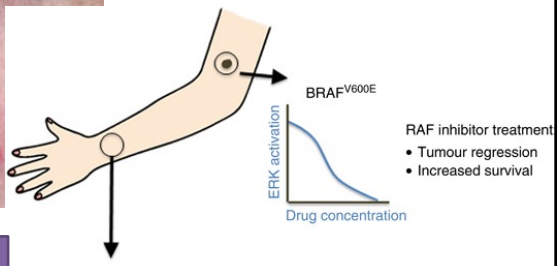
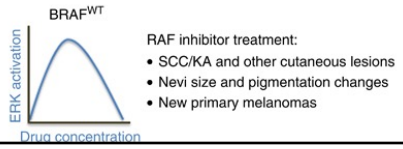
Cutaneous squamous cell carcinoma



Second most common skin cancer after basal cell carcinoma
- >50% of melanomas harbor BRAF mutation (90% are at Val 600 – Glu)



BRAF V600E constitutively activates BRAF independent of receptor activation or dimarization
- Inhibitors can both promote and inhibit wild-type RAF not mutant



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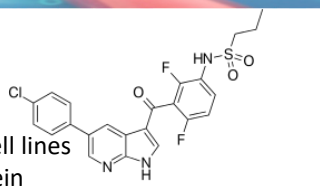
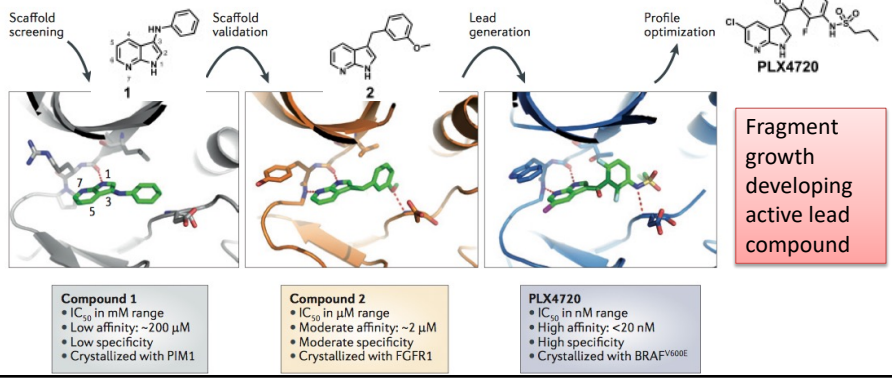
Vemurafenib

- Fragmentation screen generated
  - Bind to both V600E and V600K
  - Poor binding to wild type
  - Induces apoptosis in melanoma but not cancer cell lines
  - Modest selectivity (wt vs mutant) with pure protein
  - HUGE difference in cells harboring mutation





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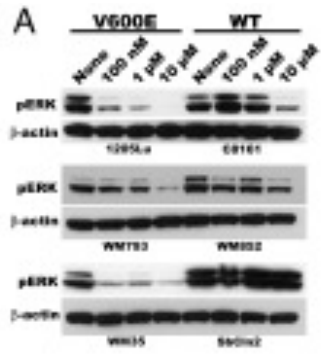
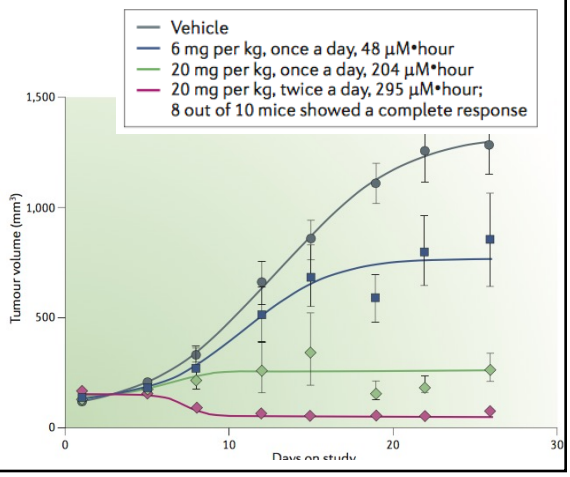
Vemurafenib

- Parent compound blocked ERK phosphorylation in mutation vs wild type and well in melanoma cell lines
- Nude mouse studies (oral route) show dose response loss of tumor mass!





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Vemurafenib

- Used “scaffold” aka fragment based drug development to generate a lead compound that co-crystalized with PIM1.
  - They were looking for a BRAF inhibitor but BRAF would not crystalize, so they used other recombinant kinase domains to build an inhibitor while figuring out how to crystalize BRAF
  - They mutated the kinase domain of BRAF to make the protein more soluble for co-crystlalization

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Vemurafenib

- “DFG-in” (three amino acids)
  - Bind to the and regulate active site.
  - Inactive BRAF has the motif occupying the ATP binding pocket in place of ATP causing inhibition
  - Active BRAF has a Mg that binds to D and ATP phosphate groups opening the motif from the active site.
  - DFG-in refers to the inactive conformation
  - Vemurafenib binds into the ATP binding site only inhibiting the active form.
  - Drug binding moes the helix blocking Arg 509 required for dimerization
  - BRAF (gene) wiki page and review gives good details

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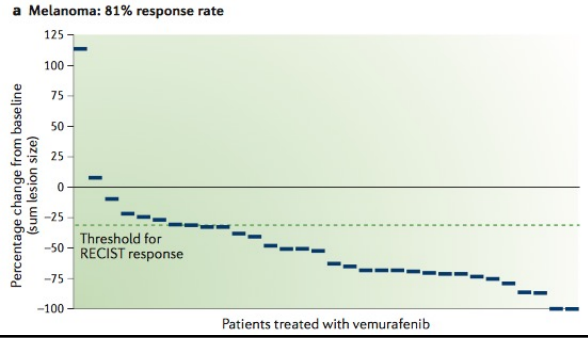
Vemurafenib

Clinical Trials

- Phase I Small Group, looking for safety and side effects at increasing doses
  - 81% response in metastatic melanoma
  - Colorectal cancer (low 5 yr survival) show less impact



RECIST – Response evaluation criteria in solid tumors.
- Looking at tumor changes, not patient improvement
- CT, NMR, Xray measure lesion changes

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Vemurafenib

Clinical Trials – moved to duel phase II & III

- Phase II 30-120 patients, 2 years different treatment options
  - 53% response with 6% complete in about 7 months!
- Phase III more than 300 – 1000 randomized patients take several year
  - 675 patients positive for BRAF V600 (K or E) mutations
  - Compared to older less effective drug
  - > 50% response compared to control old drug
  - Positive results shifted from dacarbazine to just vemurafenib.
  - 63% reduction in risk of death

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Vemurafenib

Near complete regression of tumors

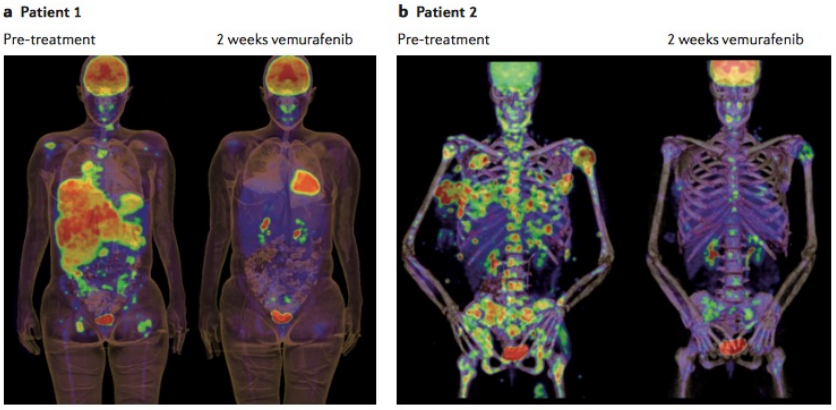

Figure 7 | **PET scans of patients treated with vemurafenib.** Positron emission tomography (PET) scanning was used to visualize uptake of ^{18}F-deoxyglucose, as shown in two patients (panel **a** and panel **b**) from the Phase I extension cohort[96]. In both panels **a** and **b**, the left image is taken before initiating therapy and the right image is taken after 2 weeks of vemurafenib treatment. All evaluable patients from this cohort experienced reduction of PET signal within 2 weeks.

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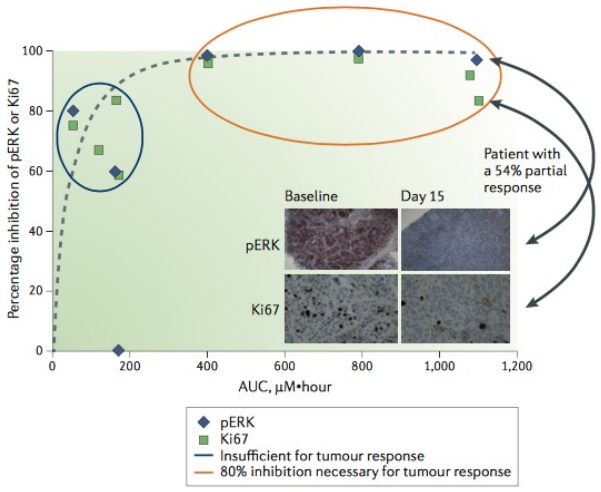
Vemurafenib

80% reduction in pERK and lower Ki67 (proliferation marker) staining in higher drug doses



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Vemurafenib

Most patients with melanoma tumors develop resistance sometimes within 6 months

- Some tumors become drug dependent for tumor survival! (drug increases BRAF expression)
  - Loss of drug leads to regression.
  - Options to pulse drug on and off to kill but not build resistance?
- There are other impacting factors that over-ride and produce resistance including growth factor activation and possible dimerization

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Learning Objectives

- Be able to outline and discuss the drug discovery process – big picture
- Know the key terms of the process
- Describe target identification and validation
- Know what a HTS and the workflow to develop lead compounds
- Describe fragment based drug discovery and how NMR is often used in its screen
- BE ABLE TO CREATE A FBDD USING AN UNKNOWN PROTEIN/DNA INTERACTION…
- Know how vemurafenib was designed and how it binds and regulates mutant BRAF vs wild type BRAF
- Explain the data presented in the ppt using information from the linked paper(s)

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