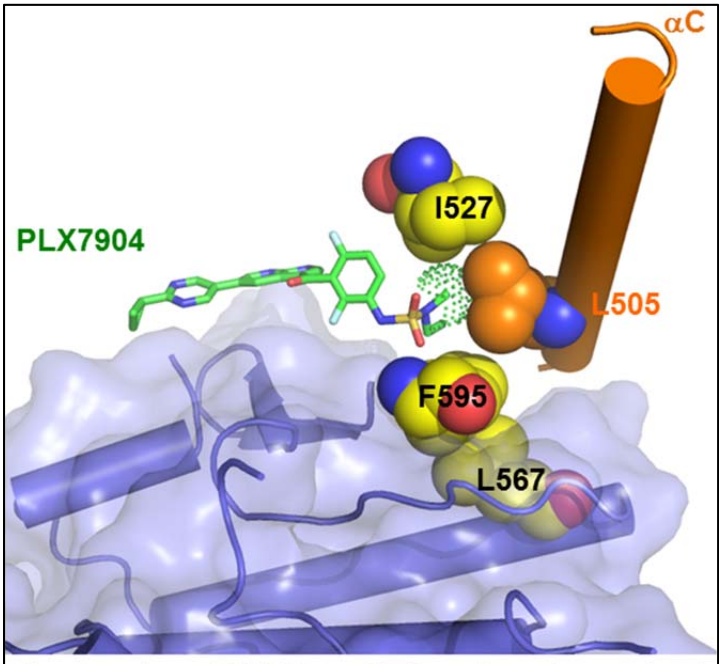
Discovery of Next Generation RAF Inhibitors that Dissociate Paradoxical Activation from Inhibition of the MAPK Pathway

Genes encoding members of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway are frequently mutated in human cancer. RAS (a small GTPase) and RAF (a serine/Threonine kinase) are two major nodes on this important signaling axis. Constitutive activation of BRAF, a member of the RAF family, has been shown to promote RAS independent MAPK pathway up-regulation[1]. First generation RAF selective inhibitors, including vemurafenib (PLX4032) and dabrafenib, have demonstrated clinical benefits in $BRAF^{V600}$ mutant driven melanoma and were approved by FDA for treating metastatic melanoma[2,3]. However these inhibitors paradoxically activate the MAPK pathway in cells bearing wild-type RAF and oncogenic RAS mutations. This paradoxical activation can promote cellular proliferation and occasionally appear to accelerate the progression of certain RAS driven cancers. Therefore Plexxikon has pursued the discovery of next generation RAF inhibitors (dubbed "paradox breakers") that avoid paradoxical activation on RAS driven tumors while maintaining or improving efficacy on BRAF mutant tumors. Since paradoxical activation by first generation inhibitors is a result of inhibitor binding to one protomer of a RAF dimer that allosterically activates the other protomer, Plexxikon's strategy to overcome paradoxical activation was to find small molecule inhibitors that are able to disrupt RAF dimer formation.

Using x-ray diffraction data collected from SSRL and ALS beam lines, Plexxikon has obtained co-structures of mutant BRAF bound to different classes of small molecule inhibitors. Based on these co-structures, it was hypothesized that interactions with Leu505 on the C-helix, one of the four residues comprising the so called regulatory spine of kinases, could alter the BRAF oligomerization state through long range structural effect. PLX7904 and its analogs were thus designed to form close contacts with Leu505, thereby forcing conformational changes in the associated C-helix (a major component of RAF dimer interface) and disrupting the RAF dimer. Co-structures of PLX7904 and its analogs with mutant BRAF determined using data collected at both SSRL and ALS showed that the unique N-methyl-ethyl tail of this group of compounds optimally displaced Leu505, farther than any other known RAF inhibitor. *In vitro* assays and *in vivo* studies validated that PLX7904 and its analogs such as PLX8394 are indeed paradox breakers and proved for the first time that that the two opposing modes of action of RAF inhibitors, either blocking or activating the MAPK pathway, can be uncoupled.



Close-up view of PLX7904 with its carbon atoms colored green bound to mutant BRAF. The N-lobe is removed to show the inhibitor and its interaction with the four-residue R-spine (Ile527, Leu505, Phe595 and Leu567. A dotted surface around the N-methyl group in PLX7904 illustrates its close contact with the R-spine residue Leu505 of the aC helix (orange).

Crystallographic studies enabled by SSRL and ALS facilities not only have yielded fundamental new insights into mechanistic understanding of the RAF-RAS signaling axis in the MAPK-ERK pathway, but also have helped in devising strategies to design next generation RAF inhibitors that could dissociate MAPK pathway inhibition from paradoxical activation. Clinical studies to test if this approach will translate to improved safety and efficacy in cancer patients are ongoing.

Primary Citation

C. Zhang, W. Spevak, Y. Zhang, E. A. Burton, Y. Ma, G. Habets, J. Zhang, J. Lin, T. Ewing, B. Matusow, G. Tsang, A. Marimuthu, H. Cho, G. Wu, W. Wang, D. Fong, H. Nguyen, S. Shi, P. Womack, M. Nespi, R. Shellooe, H. Carias, B. Powell, E. Light, L. Sanftner, J. Walters, J. Tsai, B. L. West, G. Visor, H. Rezaei, P. S. Lin, K. Nolop, P. N. Ibrahim, P. Hirth and G. Bollag, "RAF Inhibitors that Evade Paradoxical MAPK Pathway Activation", *Nature* **526**, 583 (2015) DOI: 10.1038/nature14982.

References

1. H. Davies *et al.*, "Mutations of the BRAF Gene in Human Cancer", *Nature* **417**, 949 (2002).
2. P. B. Chapman *et al.,* "Improved Survival with Vemurafenib in Melanoma with BRAF V600E Mutation", *N. Engl. J. Med*. **364**, 2507 (2011).
3. A. Hauschild *et al.*, "Dabrafenib in BRAF-mutated Metastatic Melanoma: A Multicentre, Open-label, Phase 3 Randomised Controlled Trial", *Lancet* **380**, 358 (2012).

Contact

Ying Zhang, Plexxikon Inc.

SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences.