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COMMERCIALIZATION OF RESEARCH
Institute for Research in Immunology and cancer

RAF INHIBITORS DEVOID OF PARADOXICAL ACTIVATION IN K-RAS MUTANT CANCERS

THERAPEUTIC AREA - Oncology

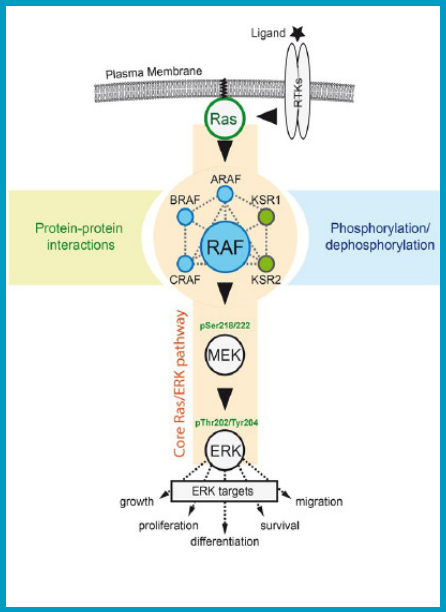
INDICATION - Ras driven cancers (lung, colon, pancreas, etc.)

OBJECTIVES - To identify within a proprietary series, high-affinity pan-RAF inhibitors that a- limit dimerization and/or transactivation of RAF; b- potently and consistently inhibit ERK signaling; and c- block the growth of Ras-mutant tumors.

STAGE OF DEVELOPMENT - Advanced Lead Optimization

PRINCIPAL INVESTIGATOR - ***Marc Therrien, Ph.D.***

RAS-RAF-MEK-ERK SIGNALING PATHWAY



Adapted from Lavoie and Therrien, Nat Rev Mol Cell Biol. 2015; PMID: 25907612

MARKET ENVIRONMENT AND UNMET NEEDS

The Ras-RAF-MEK-ERK signaling pathway is a major regulator of cell proliferation and is dysregulated in a large proportion of cancers by constitutively activating mutations in K-Ras (30%) or BRAF (8%). The prevalence of K-Ras mutations is high in pancreatic (>90%), colorectal (50%), and lung (30%) cancers while BRAF mutations, found in about 8% of human tumors are frequent in melanomas (50%).

To address these important medical needs, efforts have been mostly focused on the most common Ras-independent BRAF mutation ($BRAF^{V600E}$), leading to the approval of vemurafenib and dabrafenib for $BRAF^{V600E}$-driven metastatic melanoma. However, responses to these treatments are commonly followed by clinical resistance caused by re-activation of Ras-RAF-MEK-ERK and development of new tumors resulting from the paradoxical activation of downstream ERK signaling in patients' healthy tissues. Furthermore, $BRAF^{V600E}$ inhibitors are ineffective against K-Ras-driven cancers and to date, therapeutic options remain unfulfilled in this area. Second generation $BRAF^{V600E}$ inhibitors such as the so-called paradox-breakers (e.g. PLX-8394 from Plexxikon) or highly potent type-II pan-RAF inhibitors such as LXH-254 (Novartis) and BGB-283 (BeiGene) are currently in clinical trials. However, despite displaying improved profiles, these inhibitors are either weakly potent or still induce paradoxical ERK activation in the context of K-Ras mutant cell lines.

COMPLETED WORK

We have developed a series of pan-RAF inhibitors that potently inhibit ERK signaling (IC50 < ~100nM) and proliferation in a panel of K-Ras mutant cancer cell lines (CCLs) and unlike current $BRAF^{V600E}$ inhibitors, are completely devoid of paradoxical induction of the Ras-ERK signaling pathway. To our knowledge, these compounds possess a unique biological profile, distinct from competing approaches. These inhibitors bind with high affinity (IC50 < ~10nM) to WT B- and C-RAF as well as $BRAF^{V600E}$ and inhibit ERK phosphorylation in $K\text{-}Ras^{MUT}$ CCLs with undetectable induction of the pathway at all tested concentrations.

A co-crystal structure of a lead inhibitor in complex with the BRAF kinase domain was successfully solved leading to the formulation of a mechanistic hypothesis that is consistent with the observed biological profile and Structure-Activity-Relationships (SAR) generated in the series. The inhibitors appear to lock the RAF kinase conformation in an inactive state and block dimerization and/or transactivation of RAF, a phenomenon that has been linked to paradoxical activation of the pathway. Optimization is currently progressing toward the identification of molecules suitable for *in vivo* proof of concept studies in animal models.

COMPETITIVE ADVANTAGES

We provide a series of pan-RAF inhibitors that potently block ERK signaling in K-Ras-mutant cell lines with a novel binding mode that precludes paradoxical pathway induction as inferred from a crystal structure of a lead proprietary RAF inhibitor in complex with BRAF kinase domain.

NEXT STEPS

+ Continue to improve pERK and anti-proliferative potency to achieve a uniform potency profile across a panel of K-Ras mutant cancer cell lines.
+ Identify analogs with suitable ADME-PK properties for an *in vivo* proof of concept in a K-Ras tumor xenograft model.

PUBLICATIONS

+ Lavoie et al. Nat Chem Biol. 2013 PMID: 23685672.
+ Thevakumaran, Lavoie et al. Nat Struc Mol Biol. 2015 2015 PMID: 25437913.
+ Lavoie and Therrien, Nat Rev Mol Cell Biol. 2015 PMID: 25907612.

LEAD SCIENTIST

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Dr. Therrien is a principal investigator at the Institute for Research in Immunology and Cancer (IRIC). With his team, he devotes his research to the study of the signalling mechanisms involved in cell proliferation and differentiation. After completing his Ph.D. in Biochemistry at the Université de Montréal, Dr. Therrien wanted to develop a greater understanding of intracellular signalling processes using the fruit fly Drosophila; a highly effective model to explore fundamental questions in human biology. For this reason, he went on to do his postdoctoral training at the University of California at Berkeley under the guidance of Gerald M. Rubin (a world-renowned fly geneticist currently Vice President and Executive Director of HHMI Janelia Farm Research Campus). His work focused on how cells perceive extracellular signals and how these are then conveyed within the cells to stimulate specific responses. The research group Dr. Therrien identified several genes that function in concert with Ras; a notorious human oncogene. Upon establishing his own group in Montreal, Dr. Therrien pursued his work on the Ras/MAPK pathway and focused his attention on describing in molecular terms the inner workings of the pathway. This led him to discover that RAF activation critically depends on dimerization of its kinase domain. Because RAF is a major human oncogene, this finding represents a completely novel avenue to explore for potential anticancer therapeutic applications.

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