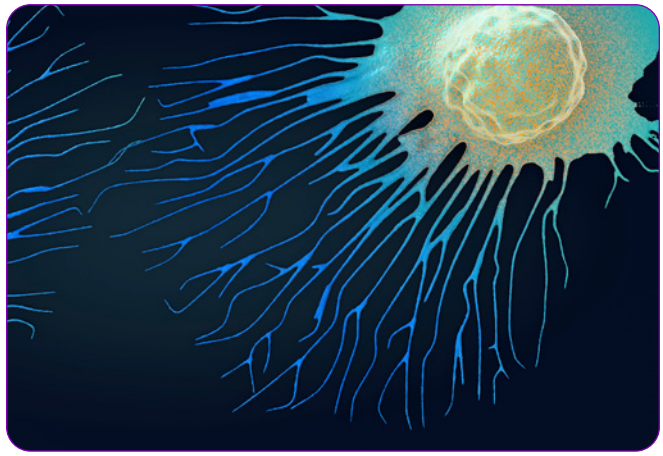
The impact of pan-KRAS inhibitors on cancer drug discovery

Introduction

The significance of KRAS mutations in certain types of cancers, and their influence on fundamental cellular processes such as cell growth, differentiation, and survival cannot be overstated. Treatment options are often limited, and the prognosis is typically poor for patients with colorectal, lung, pancreatic, and other cancers commonly associated with KRAS mutations. The inhibition of KRAS signaling specifically targets the disease's molecular driver, minimizing potential harm to normal cells. This selective inhibition strategy holds great potential for improving patient outcomes and reducing the toxicity often associated with non-specific therapies. While the targeting of KRAS mutations has long been considered a daunting task, recent advances have led to the emergence of inhibitors specifically designed to combat certain KRAS variants.

Challenges associated with KRAS inhibition are present due to its complex structure and affinity for guanosine triphosphate (GTP) which plays a critical role in controlling KRAS activation and downstream signaling. KRAS is a protein that acts as a GTPase, which means it helps hydrolyze GTP. When KRAS is in the state bound to GTP, it promotes cell growth and proliferation.[1] In contrast, when GTP is hydrolyzed into guanosine diphosphate (GDP), KRAS becomes inactive, leading to the termination of signaling.

Amid the cancer landscape, a recent study uncovered a breakthrough in KRAS inhibition.[1] By discovering, characterizing, and introducing a non-covalent small molecule inhibitor, the researchers achieved a notable reduction in cell growth across an array of diverse KRAS mutations. This breakthrough has far-reaching implications for the creation of targeted therapies in the realm of KRAS-related cancers. Here, we explore the approach the researchers took to develop and evaluate pan-KRAS inhibitors for their ability to combat carcinogenesis.



Development of Pan-KRAS inhibitors

The assessments performed by the researchers utilized methods to explore the potential of BI 2865, a KRAS inhibitor as a therapeutic agent, to gain insights into its effects on KRAS signaling. To evaluate the inhibitors' efficacy against diverse KRAS mutations, the researchers employed a cell panel screen. The panel consisted of a diverse array of isogenic cell lines housing specific KRAS mutations, enabling a comprehensive assessment of inhibitor selectivity, potency, and activity across various genetic backgrounds. The platform enabled the researchers to determine the effects of KRAS mutations in a clinical context, offering insights into the inhibitors' application, and illuminating the structural components essential for successful KRAS inhibition. This knowledge guided subsequent rounds of medicinal chemistry optimization, enabling refinement of the lead compound's structure for improved potency and selectivity, and led to the development of a highly potent and selective Pan-KRAS inhibitor.

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Mechanism of Action

The inhibitor's mode of action was intricately tied to the nucleotide cycling of KRAS mutants in cancer cells. Contrary to being locked in a constitutively active state, common KRAS mutants were found to undergo nucleotide cycling, a property essential for the inhibitor to access the GDP-bound conformation required for its activity. This cycling occurred with slower kinetics in comparison to WT KRAS, potentially influencing the inhibitor's rate of action.

Binding interactions between BI 2865 and its target protein were investigated using nucleotide exchange assays as a way of examining the underlying mechanisms of KRAS activation. Valuable insights into binding kinetics were obtained by conducting surface plasmon resonance experiments. Protein preparation and crystallization provided information that helped visualize the interaction between BI 2865 and different KRAS mutants. The inhibitors' extraordinary potency was revealed through an ability to suppress nucleotide exchange in KRAS variants, with a potency several orders of magnitude higher than the effect on HRAS or NRAS. A critical test of the inhibitors' selectivity in cells involved "RASless" murine embryonic fibroblasts expressing a single RAS variant, demonstrating that the inhibitor effectively suppressed the activation of KRAS splice variants 4A and 4B, whereas NRAS and HRAS activation remained largely unaffected.

Selectivity

Selectivity for KRAS over other RAS isoforms led to unforeseen results given the minimal evolutionary divergence in the GTPase domain of RAS isoforms. The researchers discovered that the potency of the inhibitor's effects was tied to specific amino acid differences between KRAS, HRAS, and NRAS. A pivotal amino acid substitution, H95 in the α3 helix of KRAS, appeared crucial for this selectivity, with mimetic substitution at the 95th residue in NRAS (L95H) sensitizing NRAS to the inhibitor's effects. Additionally, residues 121 and 122 (AA, PT, and PS) distinguished HRAS from NRAS and KRAS. By introducing KRAS-mimetic substitutions at these positions, the researchers unveiled an intricate allosteric network that contributes to selective inhibition.

The researchers evaluated KRAS inhibition selectivity through a combination of genetic, structural, and functional methodologies. This comprehensive approach enabled them to map an allosteric network comprising residues 95, 121, and 122, intertwined with the dynamics of KRAS's nucleotide cycle. The importance of this network was further affirmed by saturation mutagenesis experiments, where amino acid substitutions in the G4 and G5 motifs, as well as specific α5 contacts, were positively selected under the inhibitor's influence. The role of these residues in modulating the dynamics of KRAS's nucleotide cycle highlighted their central role in the inhibitor's selectivity.

Downstream effects

The inhibitor's impact on downstream signaling and proliferation exhibited correlations with its antiproliferative effects in a broad range of cancer cell lines. The correlation was especially pronounced in KRAS-amplified cell lines, revealing a potential therapeutic avenue for specific cancer subtypes. Not all KRAS mutants were equally susceptible to the inhibitor, with some, like KRAS G12R and KRAS Q61L/K/R, showing limited inhibition. The drug's potency was also influenced by cellular conditions, with serum deprivation enhancing its effect.[1] Remarkably, the pan-KRAS inhibitor demonstrated an increase in caspase activation in KRAS mutant models, further highlighting its potential for inducing apoptotic pathways.

The research team elucidated the biochemical and functional aspects of the pan-KRAS inhibitor but also paved the way for its translational potential. Structural analogs optimized for in vivo administration, such as BI-2493, exhibited similar binding and inhibitory properties, offering promise for potential clinical use. Encouragingly, BI-2493 exhibited favorable pharmacokinetic profiles and demonstrated significant antitumor effects in mouse models with various KRAS mutations.

Research summary

These interconnected and comprehensive methods allowed for an assessment of the potential of BI 2865 as a KRAS inhibitor, shedding light on its mechanisms of action and therapeutic possibilities. The findings underscore the potential of the pan-KRAS inhibitor to provide a therapeutic breakthrough for a wide range of KRAS-driven cancers, including lung, colorectal, and pancreatic cancers. The inhibitor's selectivity for KRAS over other RAS isoforms and its ability to disrupt downstream signaling pathways point toward a promising avenue for targeted cancer therapies.

Role of the Revvity Cell Panel Screen

As highlighted by Dr. Philipp Jaeger, Director of High-Throughput Biology at Boehringer Ingelheim, Vienna, Revvity's (formerly known as Horizon Discovery) cell panel screen played a pivotal role in the published study. He stated,

> "We included data generated by [Revvity] as part of this Nature publication because they have consistently provided high quality, actionable data that we trust, and we look forward to continuing our work with them."

The ability to evaluate drug candidates across a wide range of cell lines is particularly advantageous in the context of KRAS inhibitors. KRAS mutations exhibit functional diversity, with different mutations conferring distinct sensitivities to therapeutic interventions. The cell panel screen provided the researchers with a comprehensive platform to assess the activity profiles of Pan-KRAS inhibitors against different KRAS mutations, facilitating a deeper understanding of the structural requirements for effective KRAS inhibition.

Conclusion

The development of Pan-KRAS inhibitors was characterized by a multidisciplinary approach that unraveled the intricate molecular interactions underpinning remarkable selectivity for KRAS over other RAS isoforms. This holistic understanding offers new avenues for precision cancer therapies by providing insights into the complex interplay between KRAS mutants and potential inhibitors.

These discoveries and enlightening data shed light on the development of a new class of Pan-KRAS inhibitors and their potential impact on cancer drug discovery. The cell panel screen platform played a vital role in this research, providing invaluable insights into KRAS inhibition and facilitating the identification and optimization of a potent and selective Pan-KRAS inhibitor. This breakthrough represents a significant advancement in the field, bringing us closer to developing effective targeted therapies for patients with KRAS-mutant cancers.

Reference

1. Kim, D., Herdeis, L., Rudolph, D., Zhao, Y., Böttcher, J., Vides, A., Ayala-Santos, C. I., Pourfarjam, Y., Cuevas-Navarro, A., Xue, J. Y., Mantoulidis, A., Bröker, J., Wunberg, T., Schaaf, O., Popow, J., Wolkerstorfer, B., Kropatsch, K. G., Qu, R., de Stanchina, E., Sang, B., Chuanchuan, L., McConnell, D.B., Kraut, N., Lito, P. (2023). Pan-KRAS inhibitor disables oncogenic signalling and tumour growth. Nature. https://doi.org/10.1038/s41586-023-06123-3

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