8-10 october

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Book of abstracts

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SHORT TALKS

ABSTRACTS SELECTED FOR SHORT TALKS

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"How SHP2 Signals to RAS"

SESSION 1: RAS signaling

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Abstract

SHP2 (PTPN11) is required for activating the RAS/ERK pathway in receptor tyrosine kinase (RTK) and cytokine receptor signaling and mediates adaptive resistance. Consequently, SHP2 inhibitors are in trials for RTK/RAS/ERK pathway-driven tumors. Germline PTPN11 mutations cause the related developmental disorders Noonan syndrome (NS) and Noonan syndrome with multiple lentigines (NS-ML). Somatic mutations are found in juvenile myelomonocytic leukemia (JMML), neuroblastoma, and adult solid tumors. NS and cancer-associated mutants have increased phosphatase activity, while NS-ML mutations are catalytically impaired.

For more than 30 years, how SHP2 promotes RAS activation has remained unclear. The prevailing model holds that SHP2 dephosphorylates a key substrate to activate SOS1/2. Many substrates have been proposed, yet none clearly shows this function. By combining biochemistry, cell biology, NMR, super-resolution microscopy (SRM), and new Ptpn11 knock-in mice affecting its catalytic cysteine (CysCAT) and/or tyrosine phosphorylation sites, we obtained new insights into SHP2 action.

We found that the SHP2 C-terminal tyrosines are essential for normal mouse development and are differentially required for EGF-, PDGF-, and FGF-evoked RAS/ERK activation. These sites act solely to recruit GRB2. As expected, CysCAT mutants are phosphatase-dead, but surprisingly, they have distinct effects on RAS/ERK activation. Remarkably, the CysCAT□Asp mutant uniquely mediates enhanced RTK-evoked SOS1 and GRB2/SOS1 cluster formation (by SRM) and sustains RAS/ERK activation in multiple cell types. This mutant shows distinct NMR behavior, including numerous residues in two states in slow exchange. Intriguingly, many of these features are induced by mild oxidation of wild-type SHP2.

Homozygous CysCAT□Asp mice are obtained at a normal Mendelian ratio and appear healthy, while grafting CysCAT□Asp into a JMML-associated allele confers cytokine independence. In concert, our results show that SHP2 can—and likely does—signal to RAS as an adaptor via an oxidation-induced conformational shift, while its phosphatase activity helps terminate signaling. These findings suggest new ways to design SHP2 inhibitors for human disease.

"Oncogenic KRAS activation induces CRC epithelial plasticity leading to therapeutic resistance in vivo"

SESSION 10: Tumour heterogeneity: naïve & post-treatment

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Abstract

The colorectal epithelium is a rapidly renewing tissue, with a remarkable capacity to regenerate following injury. In colorectal cancer (CRC), this regenerative capacity can be co-opted to drive epithelial plasticity. Activation of oncogenic KRAS in CRC is common, with mutations found in 40-50% of patients. Nonetheless, inhibition of the RAS/MAPK pathway is often linked to rapid therapeutic resistance.

Given the recent development of effective targeted therapies, we have investigated mechanisms of resistance to these agents in complex mouse models of CRC. We find that MAPK signalling supports a regenerative/revival stem cell phenotype in vivo, with targeted inhibition of mutant KRAS (or mutant BRAF) leading to a rapid transcriptional adaptation and adoption of a WNT-high phenotype marked by expression of the canonical stem marker Lgr5. This process provides a mechanism for the acute therapeutic resistance of KRAS-driven cancers observed clinically.

Importantly, where epithelial plasticity is restrained, for example in early metastatic seeding, under chemotherapeutic pressure, or where WNT signalling has been suppressed, therapeutic efficacy is restored. Together, our data provide clear insight into the mechanisms underpinning resistance to targeting oncogenic KRAS in CRC. Moreover, we demonstrate that strategies which aim to break plasticity/adaptive stem cell responses may improve efficacy of MAPK targeting agents in CRC and beyond.

KRAS blockade elicits a DNA damage repair deficiency exploitable with PARP inhibitors.

SESSION 2: Unveiling RAS biology

Connor Welch[1], Gabriela Novoa[1]*, Carlos Vasquez[1], Sofia Llorente[2], Alessia Subrizio[3], Irati Macaya[1], Rosario Prados-Carvajal[4], Paolo Battuello[3], Giovanni Crisaful[3], Elizabeth Guruceaga[1], Pablo Huertas[4], Alberto Bardelli[3], Tian Tian[2], Mariangela Russo[3], Silve Vicent[1]

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Abstract

Background: The generation of direct KRAS inhibitors (KRASi) represented a milestone in oncology offering targeted treatment to vast patient cohorts with KRAS mutations. Nevertheless, escape mechanisms based on tumour compensatory activation, genetic evolution, or phenotypic switch limit their antitumor efficacy and restrict survival benefit. Thus, it is of paramount importance to uncover drug combinations which could maximize the antitumor effect of KRASi across the largest fraction of patients.

Methods: Bulk and single cell transcriptome data of human and mouse mutant KRAS (mtKRAS) lung and pancreatic cancer cell lines exposed to genetic and pharmacological KRAS inhibition was interrogated, and a reporter system employed to assess DNA repair functionality. Combination therapies consisting of KRASi and PARP inhibitors (PARPi) were tested in vitro, as well as in cell line-derived xenograft (CDX), allografts (CDA) or PDX in vivo. Integration of CRISPR screening data of genes sensitizing to PARPi with the Connectivity Map was employed to nominate KRASi-induced transcriptional nodes regulating genome instability coupled with functional validation. In vitro and in vivo models of lung and pancreatic cancer were deployed to test the efficacy of KRASi + PARPi.

Results: We report a commonly downregulated gene signature upon KRAS blockade related to DNA damage repair (DDR) integrity, and show a consistent deficit in homologous recombination (HR) functionality. Single cell analysis shows that early tumoral populations tolerant to pharmacological KRAS inhibition abrogate key elements of HR. This early response to KRASi is vulnerable to therapeutic attack with PARPi, eliciting a lethality in numerous lung and pancreas cancer models reflected as impaired cell proliferation and delay of the onset of resistance in vitro and potent tumor regressions in vivo. At the cellular level, HR depletion exacerbates DNA damage in the context of PARPi which may underscore the synthetic lethal phenotype. Mechanistically, we unveil that the transcription factor FOSL1, a known key effector of KRAS signalling, is involved in the BRCAness phenotype since: 1) FOSL1 genetic abrogation depletes BRCA2/RAD51 levels in addition to vast elements of genomic integrity, 2) FOSL1 levels correlate to PARPi efficacy, and 3) FOSL1 genetic inhibition sensitizes to PARPi. Lastly, mtKRAS cancer patients with high FOSL1 levels display poor prognosis when treated with DNA damaging agents.

Conclusions: Our data provide nominate a mechanistically driven combination for hard-to-treat mtKRAS cancers consisting of KRASi and PARPi which defines a path for clinical translatability. In addition, we provide molecular and cellular data positioning the transcription factor FOSL1 as a key player in tumoral genome integrity and response to DNA damaging therapies.

Isoform-specific regulation of KRAS signaling and lysosomal function by RAP1GDS1-607 in lung adenocarcinoma

SESSION 3: Decoding RAS-Driven tumours

Marta Roman, Alex G. Lee, Kari Herrington, Janos Demeter, Truc Dinh, Juan Antonio Camara Serrano, Rushika M. Perera, Kaja Kostyrko, Peter K Jackson, E. Alejandro Sweet-Cordero

Abstract

We use biochemical, proteomic, and genetic approaches to dissect the isoform-specific roles of RAP1GDS1, a critical regulator of protein prenylation, in KRAS-mutant lung adenocarcinoma. RAP1GDS1 exists in two isoforms, the long isoform (RAP1GDS1-607) and the short isoform (RAP1GDS1-558), each playing distinct roles in cell signaling.

Using an isoform-specific mouse knockout model, we demonstrate that RAP1GDS1-607 plays a specific and non-redundant role in KRAS signaling and lysosomal function. Mechanistically, loss of RAP1GDS1-607 leads to decreased membrane localization of KRAS and altered signaling downstream of KRAS.

In addition, we find that RAP1GDS1-607 knockout leads to altered endocytosis and dysregulation of lysosomes specifically in cells harboring KRAS mutations but not in cells expressing wild-type KRAS. This altered lysosomal function is linked to the role of RAP1GDS1 in regulating RAB7A.

Moreover, using in vitro and in vivo models, we demonstrate that RAP1GDS1-607 knockout significantly improves the response to pharmacological inhibition of KRAS-G12C. These findings support a combinatorial strategy for KRAS-driven tumors, highlighting the therapeutic potential of targeting RAP1GDS1-607 specifically in KRAS-driven LUAD.

Together, these results suggest that RAP1GDS1-607 plays a unique role in KRAS-driven oncogenesis through a dual mechanism that involves both changes in KRAS membrane localization and changes to lysosomal function and endocytosis.

VS-7375 (GFH375): An oral, selective KRAS G12D dual ON/OFF inhibitor with superior anti-tumor efficacy relative to ON-only KRAS inhibitors

SESSION 6: Emerging treatments (Preclinical perspective)

Silvia Coma[1], Cristina Caffarra Malvezzi[2], Feng Yan[3], Fusheng Zhou[3], Yu Wang[3], Nathan Sanburn[1], Chiara Ambrogio[2], Jonathan A. Pachter[1]

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Abstract

KRAS G12D is the most prevalent KRAS mutation in human cancers, present in 37%, 12.5%, and 4.9% of pancreatic, colorectal, and lung cancers, respectively. Currently, there are no FDA-approved RAS inhibitors effective for patients with KRAS G12D mutant cancers. VS-7375 is an orally available KRAS G12D inhibitor (G12Di) currently being evaluated in patients with KRAS G12D mutant solid tumors.

In 3D proliferation assays among a panel of human tumor cell lines, VS-7375 showed improved KRAS G12D potency and selectivity relative to the G12Di RMC-9805 and MRTX1133. In contrast to ON-only RAS inhibitors, VS-7375 binds KRAS G12D in both the active (ON; GTP-bound) and inactive (OFF; GDP-bound) states with low nM potency.

To assess potential benefits of dual ON/OFF inhibition, we compared efficacy relative to ON-only inhibitors RMC-9805 (G12Di) and RMC-6236 (pan-RAS inhibitor) in KRAS G12D in vivo models. In a KP4 G12D pancreatic cancer model, VS-7375 (50 mg/kg twice daily orally) showed similar initial tumor regression relative to the ON-only inhibitors RMC-9805 (100 mg/kg once daily orally) and RMC-6236 (25 mg/kg once daily orally). However, after approximately 2 weeks of dosing, tumors continually treated with RMC-9805 or RMC-6236 began to grow (mean tumor volume >850 mm^3 by day 30), in contrast to those treated with the ON/OFF inhibitor VS-7375 which showed sustained tumor regression (mean tumor volume ~80 mm^3 by day 30).

In an LS513 G12D colorectal cancer model, VS-7375 showed tumor regression, whereas the ON-only inhibitors RMC-9805 and RMC-6236 showed tumor growth inhibition, but not tumor regression. Clinically, in patients with KRAS G12D pancreatic and lung cancers, VS-7375 monotherapy exhibited compelling initial overall response rates (confirmed and unconfirmed) of 52% (12/23) and 42% (5/12), respectively, with a manageable safety profile (NCT06500676, China). VS-7375 is now being evaluated as monotherapy and in combinations in the US (NCT07020221).

Targeting hyaluronic acid in pancreatic ductal adenocarcinoma uncovers novel therapeutic opportunities

SESSION 9: Tumor microenvironment

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related deaths, with a five-year survival rate of ~12%. Cancer-associated fibroblasts (CAFs) contribute to PDAC's dense, immunosuppressive tumor microenvironment (TME). CAFs produce intratumoral hyaluronic acid (HA), which increases interstitial fluid pressure (IFP), impairing drug delivery. Systemic PEGPH20 hyaluronidase reduces HA and IFP, improving gemcitabine efficacy in mice.

Here, we targeted HA synthesis rather than degradation by genetically ablating the three HA synthases (Has1, Has2, Has3). Has1 and Has3 null alleles were generated by CRISPR in mouse embryos, as these genes are dispensable for embryonic development. Conditional Has2^lox alleles were combined with Rosa26-CreERT2 to allow systemic deletion in adult mice via tamoxifen (TAM) diet. These alleles were introduced into the standard KPF PDAC model (KRas^+/FSFG12V; P53^F/F; Pdx1-FlpO). TAM exposure in tumor-bearing mice induced strong HA depletion, leading to reprogramming of tumor, stromal, and immune compartments, and significantly slowing tumor progression.

Tumor cells became more differentiated, with increased cytokeratin-19 and epithelial adhesion molecules, and showed reduced proliferation, EMT, migration, and invasion—yet paradoxically upregulated KRas expression. In the stroma, CAF activation decreased, fibrosis and collagen deposition were reduced, and CAF composition shifted toward iCAFs (inflammatory) with fewer myCAFs (myofibroblastic). Notably, CD8+ T cell infiltration increased.

HA depletion enhanced responses to gemcitabine, anti-CTLA-4 therapy, and the pan-RAS inhibitor darasonrasib, with the strongest effects seen when darasonrasib was combined with anti-CTLA-4. In summary, genetic elimination of HA synthesis in PDAC remodels the TME, suppresses tumor aggressiveness, promotes anti-tumor immunity, and improves sensitivity to multiple therapies, highlighting new avenues for treatment.

POSTER SESSION

ABSTRACTS SELECTED FOR POSTER PRESENTATION

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C3G deregulation uncovers a dual role in B-cell lymphoma: tumor suppression and enhanced metastasis via Rap1 and Rac2 signaling

SESSION 1: RAS signaling

POSTER Nº: 1

Alba Morán-Vaquero[12], Óscar Herranz[12], Ana Dávila-Hidalgo[12], Antonio Rodríguez-Blázquez[12], Cristina Fernández-Infante[12], Ignacio García-Tuñón[123], Elena Vuelta[123], Femke van der Meer[4], Coert Margadant[4], Carmen Guerrero[125], José M. de Pereda[1]

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Abstract

C3G (RapGEF1) is a guanine nucleotide exchange factor that activates Rap1, a small GTPase implicated in hematologic malignancies. C3G regulates cell adhesion, migration, proliferation, apoptosis, and differentiation. We previously showed that the GEF activity of resting C3G is self-repressed via its AIR (autoinhibitory region). A missense mutation (Y554H) found in a non-Hodgkin's lymphoma, targeting the AIR, results in constitutive C3G activation (previously published in Carabias et al., 2020, Sci Signal 13, eabb7075). This study aimed to investigate the impact of C3G deregulation in B-cell lymphoma.

To this end, we introduced the equivalent murine mutation (Y564H) into the A20 B-cell lymphoma line using CRISPR/Cas9. A20-C3G-Y564H cells exhibited increased Rap1 activation compared to A20-C3G-WT cells, under both basal and stimulated conditions. Hyperactivation of the C3G-Rap1 pathway impaired proliferation, promoted apoptosis, and was associated with reduced ERK1/2 phosphorylation. Furthermore, Rac2 activity was diminished, correlating with altered adhesion properties.

Consistently, cell migration and invasion were enhanced, in correspondence with an increased number of metastatic foci in the liver following tail vein injection into syngeneic BALB/c mice. Notably, reduced C3G expression further augmented the metastatic potential of A20 cells.

In summary, C3G plays a dual role in B-cell lymphoma cells, acting as either a tumor suppressor by inhibiting cell growth and promoting apoptosis, or as a tumor promoter by facilitating metastasis via enhanced motility. This dual effect likely reflects a functional balance between Rap1 and Rac2 signaling. These findings underscore the complexity of C3G-regulated pathways in B cells and point to new therapeutic targets in hematologic malignancies.

Decoding Kinase Networks: Uncovering Hidden Heterogeneity in Glioblastoma

SESSION 1: RAS signaling

POSTER Nº: 2

Alejandra Macías[1], Maruan Hijazi[1]

[1] Department of Biochemistry and Molecular Biology, Institute for Neuroscience of Castilla y León (INCyL), Institute for Biomedical Research of Salamanca (IBSAL), University of Salamanca, Salamanca, Spain

Abstract

Glioblastoma is an aggressive brain tumor with limited treatment options and poor prognosis, despite the latest advances in basic and translational research in the past decades. Receptor tyrosine kinases and their downstream pathways are altered in almost 90% of glioblastomas. All these alterations are known to contribute to progression, dissemination, resistance to therapy, and glioma stem cell maintenance.

We obtained a cohort of 28 frozen tissue samples subjected to LC-MS/MS based phosphoproteomics, which is the high-throughput method implemented here. Then, the approach named KSEA was developed to infer kinase activity, calculating enrichment of substrates identified in phosphoproteomics experiments for all the kinases expressed across samples. PCA analysis showed two well-separated clusters referring to tumor versus peritumoral tissue, which matched perfectly with the pathologist's information regarding the percentage of tumor cells in each sample.

Additionally, we found in tumor samples a higher enrichment of GO terms related to the regulation of gene transcription, indicative of the recruitment and assembly of the entire transcription machinery, accessibility to chromatin, and other epigenetic mechanisms. We also found that the activity of some kinases such as mTOR, PKC, CK2A1, CDC7, and CDK2, among others, increased significantly in tumor samples. Kinase substrate enrichment analysis allows patient-specific kinase networks to be reconstructed through computational pipelines. Some identified kinases could play a key role in the regulation of the biology of primary glioblastoma cells.

This study will serve to make predictions for each of the patients analyzed. In summary, we aim to identify predictive biomarkers to accurately select patients for therapy.

Genetically encoded PDE6D inhibitors modulate their affinity at the entrance of the lipid binding pocket

SESSION 1: RAS signaling

POSTER Nº: 3

Atanasio Gómez-Mulas[1], Rohan Chippalkatti[1], Elisabeth Schaffner-Reckinger[1], Daniel Kwaku Abankwa[1]*

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Abstract

The trafficking chaperone PDE6D plays a crucial role in facilitating the solubilization of prenylated proteins and their trafficking toward the plasma membrane or primary cilium. One such cargo is K-Ras, which is associated with cancer and developmental diseases known as RASopathies. In an effort to indirectly target K-Ras, several potent small-molecule inhibitors have been developed against PDE6D. These inhibitors insert into the hydrophobic prenyl-binding pocket of PDE6D. However, it has been shown that molecules with a higher affinity for this hydrophobic pocket often have poor solubility and an increased risk of off-target effects.

To address the limitations of previous PDE6D inhibitors, we have designed inhibitory peptides inspired by the C-terminal sequences of K-Ras and INPP5E, a high-affinity cargo of PDE6D. These genetically encoded inhibitors exploit contacts at the entrance of the prenyl-binding pocket, increasing their affinity for PDE6D. They significantly reduce the interaction between K-Ras and PDE6D, as shown by a ~60% decrease in BRET experiments. The inhibitors also disrupt normal K-Ras plasma membrane organization and downstream signaling, as measured by BRET and Western blot analysis. Furthermore, they affect the differentiation of mouse muscle C2C12 cells, which requires downregulation of Ras-MAPK signaling.

In summary, we introduce a new approach for the development of PDE6D inhibitors where the affinity is modulated at the entrance of the hydrophobic pocket. These genetically encoded inhibitors will serve as a template for the development of cell-penetrating peptides and, in the next steps, peptidomimetics..

Cell signalling mediated by kinase inhibitors in a cellular model of glioblastoma

SESSION 1: RAS signaling

POSTER Nº: 4

Maruan Hijazi[1], Beatriz Fernández-Roldán[1], Elisa Arias[1], Lucía García[1], Alejandra Macías[1]

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Abstract

Glioblastoma (GBM) is the most common malignant brain tumour in adults, characterised by its high aggressiveness and a median survival of less than 15 months after diagnosis. Aberrant activation of signalling pathways involved in cell survival and proliferation, such as the MAPK pathway, promotes tumour recurrence and resistance to current treatments.

This study hypothesises that inhibition of MEK1/2 with trametinib may reduce the viability of tumour stem cells by interfering with MAPK signalling. To this end, the effect of trametinib on cell viability and intracellular signalling was evaluated in murine glioblastoma stem cells (NPE-IE line). Trametinib treatment significantly reduced cell viability and inhibited the phosphorylation of ERK, its direct target, while also modulating other associated signalling pathways.

The results indicate that trametinib alters global kinase activity in these cells, suggesting a broad disruption of tumour signalling. In conclusion, this study supports the potential of trametinib as a therapeutic agent, particularly in combination therapies against the compensatory mechanisms of GBM.

Mechanistic insights by phosphoproteomics to determine responses to PI3K in combination with MEK inhibitors in glioblastoma

SESSION 1: RAS signaling

POSTER Nº: 5

Elisa Arias[1], Maruan Hijazi[1]

[1] Department of Biochemistry and Molecular Biology, Institute for Neuroscience of Castilla y León (INCyL), Institute for Biomedical Research of Salamanca (IBSAL), University of Salamanca, Salamanca, Spain

Abstract

Signalling pathways driven by PI3K-mTOR and MAPK are often dysregulated in glioblastoma, the most common and malignant primary brain tumor. As a result, pharmaceutical companies are actively developing inhibitors targeting this network to treat these brain tumors. However, these pathways converge to regulate downstream functions and often compensate for each other, leading to drug resistance and transient therapeutic responses.

To overcome this resistance, co-treatment therapies targeting both the PI3K/mTOR and MAPK pathways are being investigated in clinical trials, although the mechanisms determining sensitivity to co-treatment are not fully understood. Here, we have explored the mode of action of these co-treatments by using mass spectrometry-based phosphoproteomics together with other methodologies, such as immunoblotting and viability assays. We have examined the relationship between the activity of kinases affected by the inhibitors and their anti-cancer efficacy.

In this sense, some kinase activity signatures were highly associated with sensitivity to co-treatment in some cellular models of glioblastoma. Additionally, our study uncovers phosphosites as key mediators of response to PI3Ki plus MEKi. These studies are likely to uncover drug targets active in cells resistant to kinase inhibitors, potentially offering new treatment options for patients resistant to current therapies. Overall, these findings will advance our knowledge and treatment of this aggressive brain cancer, which remains extremely difficult to treat.

Establishing a Quantitative Proteomics Pipeline for Systematic RAS Network Quantitation

SESSION 1: RAS signaling

POSTER Nº: 6

Fiona E. Hood[1], Theo Redfern-Nichols[2], Graham Ladds[2], Ian A. Prior[1]

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Abstract

RAS is a key driver of cancer, with activating mutations in RAS genes found in ~20% of human cancer cases. Further RAS network dysregulation via RAS or other network members is found in many other cancer cases. It is therefore critical to understand the topology of the RAS signalling network and how it behaves in different cells, tissues, and disease and treatment contexts. Current models do not fully account for this, lacking key data on relative concentrations and activity levels of nodes within the network and in different contexts.

We aim to use quantitative proteomics to measure levels and activity of core RAS network nodes to generate data our collaborators can use to parameterise systems biology models. We will use the recently developed Triggered by Offset, Multiplexed, Accurate mass, High resolution, and Absolute Quantitation (TOMAHAQ) method, with companion Tomahto workflow software to maximise multiplexed detection and quantification of peptides labelled with TMTpro reagents.

We will begin with a proof-of-concept experiment set profiling the MAPK and PI3K arms of the RAS signalling network, using 99 peptides to quantify expression level of 49 proteins, and 22 peptides to report on levels of key activation or feedback post-translational modifications.

Identifying RAF1 degraders as a therapeutic approach in KRASdriven lung tumors

SESSION 1: RAS signaling

POSTER Nº: 7

Gonzalo Aizpurua[1], Laura de-la-Puente-Ovejero[1], Lucia Lomba-Riego[1], Mariano Barbacid[1], Sara García-Alonso[1]

[1] Experimental Oncology Group, Tumor Biology Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernández Almagro 3, 28029 Madrid, Spain

Abstract

RAF kinases are critical effectors of the MAPK pathway, and ablation of RAF1 in mouse models of KRAS-driven lung adenocarcinoma promotes tumor regression. Cryo-EM analysis of RAF1 has revealed that it forms a complex with HSP90 and CDC37, which is crucial for RAF1 stability. Since RAF1 has emerged as a promising therapeutic target, both the purified protein and its atomic structure have proven to be invaluable tools for early drug discovery.

In order to identify molecules that can disrupt the RAF1-CDC37 interaction, in silico studies were conducted. In this study, we have identified a molecular pocket in the interaction site that could potentially be targeted by molecules to prevent the interaction. Three different in vitro and in cellulo screening platforms were designed and developed. Two of the platforms are able to study the disruption of the interaction between RAF1 and CDC37 within the purified complex and inside the cells. A third strategy has been developed that involves the fusion of a luminescence protein to RAF1, allowing the detection of RAF1 degradation through luminescence measurements.

One of the compounds revealed a good response in all the orthogonal assays, showing RAF1 protein degradation by Western blot. The following steps include performing medicinal chemistry to improve the efficiency of this compound to pass from hit to lead. This compound holds promise as a strategy for treating KRAS-driven cancers.

Characterization of cell models in response to kinase inhibitors relevant to glioblastoma

SESSION 1: RAS signaling

POSTER Nº: 8

Maruan Hijazi[1], Lucía García[1], Elisa Arias[1], Alejandra Macías[11]

[1] Department of Biochemistry and Molecular Biology, Institute for Neuroscience of Castilla y León (INCyL), Institute for Biomedical Research of Salamanca (IBSAL), University of Salamanca, Salamanca, Spain

Abstract

Survival for patients with glioblastoma multiforme (GBM) typically ranges from 12 to 18 months after diagnosis, largely due to high relapse rates and treatment resistance. GBM is known for its heterogeneous population of cancer cells, including glioma-initiating cells (GICs), which makes them a key therapeutic target for improving clinical outcomes.

In this study, we evaluated the expression and localization of stem cell marker proteins such as connexin 26, Id-1, and Sox2 in two GBM cell models: adherent GL261 cells and neurospheres. Our findings indicate that neurospheres acquired stem cell characteristics. Both models were then treated with kinase inhibitors targeting PI3K (Alpelisib and Copanlisib) and MAPK (Trametinib) signaling pathways, which are both deregulated in GBM.

We observed distinct activity patterns: adherent cells exhibited higher PI3K pathway activity, whereas neurospheres showed higher MAPK pathway activity. Furthermore, combined treatments notably reduced cell viability, with a stronger effect in neurospheres. Overall, these results highlight fundamental differences between neurospheres and adherent cells in their response to therapy and support the use of dual PI3K/MAPK inhibition to overcome compensatory mechanisms in GBM.

Nanobodies targeting the kras-raf1 interaction as a novel therapeutic strategy for lung cancer

SESSION 1: RAS signaling

POSTER Nº: 9

Lucia Lomba-Riego[1], L. de la Puente-Ovejero[1], G. Aizpurua[1], S. Garcia-Alonso[1], M. Barbacid[1]

[1] Experimental Oncology Group, Molecular Oncology Program, Spanish National Cancer Research Centre (CNIO), Madrid 28029, Spain

Abstract

RAF1 is a serine/threonine kinase acting downstream of RAS in the MAPK signaling pathway. Our group has demonstrated that genetic ablation of RAF1, but not ARAF or BRAF, leads to lung tumor regression in KRAS^G12V; Trp53^KO-driven lung cancer mouse models. The interaction between RAF1 and KRAS is mediated by the RAS Binding Domain (RBD) of RAF1, and disruption of this interaction through a point mutation (R89L) disrupts their binding, highlighting it as a promising therapeutic target.

Here, we aimed to develop nanobodies targeting the RAF1-RBD to block its interaction with KRAS. Using a phage display library, we obtained 13 VHHs specific for the RBD of RAF1. Surface plasmon resonance (SPR) confirmed nanomolar affinities in several candidates. Intracellular functionality was assessed via yeast two-hybrid assays, identifying seven functional intrabodies.

To evaluate the nanobody binding in mammalian cells, we optimized a NanoBRET assay based on FRET, which revealed a lead candidate displaying the strongest intracellular binding to RAF1. This correlated with the CDR3 sequence of the nanobody showing the highest in vitro affinity.

We are currently assessing the biological activity of this intrabody in lung cancer cell lines and patient-derived models, focusing on MAPK pathway inhibition. In the NanoBRET system, the lead intrabody reduced KRAS–RAF1 interaction by 51%. We are also generating doxycycline-inducible cell lines to express the intrabody in xenograft models for in vivo validation and evaluating the possibility to develop a bioPROTAC against RAF1.

Our findings will define the potential of targeting the KRAS–RAF1 interaction with nanobodies as a novel therapeutic strategy for KRAS-driven lung cancers.

Systema-c quan-ta-on of Ras ac-vity using NMR

SESSION 1: RAS signaling

POSTER Nº: 10

Madison T. Chappel, Fiona E. Hood, Marie M. Phelan, Ian A. Prior

Abstract

RAS proteins are a family of small GTPases that play a pivotal role in regulating cell growth, differentiation, and survival. They behave as binary molecular switches by interconverting between the active GTP-bound state and the inactive GDP-bound state. Despite high sequence homology, Ras mutants are not equally capable of driving Ras biology. It is commonly believed that these isoform-specific tendencies are influenced by "Ras dosage," which encompasses the total protein abundance and the amount of active Ras generated by mutations.

While previous studies have explored Ras protein abundance and recent efforts have quantified Ras activity, only a limited subset of mutants have been studied. This project aims to expand on the existing work by using NMR to quantitate Ras activity across a full panel of variants to understand their relative dosage of active, signaling-competent Ras.

NMR is a powerful tool that can be used to measure GTP hydrolysis and nucleotide exchange. This study is important as it will refine the Ras activity model, thus facilitating the design of novel targeted treatments for Ras-driven diseases. In a wider context, this study of Ras activity provides a principle that can be applied to study the activity and function of other GTPases, ultimately improving treatment strategies across various diseases.

Baseline expression of c-Myc shapes the tissue specificity of oncogenic K-Ras

SESSION 1: RAS signaling

POSTER Nº: 11

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Abstract

KRAS is among the most frequently mutated oncogenes in cancers. Yet, mutations in KRAS are common only in tumors originating in a subset of tissues. Dissecting the molecular mechanisms underlying this mutation tropism is fundamental for our understanding of tissue-specific Ras signaling in particular and the phenomenon of oncogene tropism in general. Nevertheless, comprehensive analyses that experimentally define tissue permissivity to oncogenic K-Ras are missing.

We utilized a series of genetically engineered mouse models to force the expression of active K-Ras in adult tissues and performed a systematic assessment of the tissue-specific responses. In addition, we mapped the pre-existing tissue-specific signaling network using untargeted and targeted MS-based proteomic approaches.

Our results show that K-Ras permissive tissues are characterized by an increased expression of proteins across the entire Ras signaling pathway compared to non-permissive tissues. Nevertheless, we found that the ability of oncogenic K-Ras to affect the fitness of cells in a given tissue is independent of canonical signaling through the MAPK pathway. Further, the proliferative index as well as the induction of senescence and apoptosis – or lack thereof – do not determine tissue permissivity to K-Ras. Instead, we discovered that low baseline expression of c-Myc renders tissues non-permissive to oncogenic K-Ras, a context that can be reversed in the liver by ectopically expressing c-Myc.

Collectively, our findings highlight the importance of the basal signaling network – and critical nodes within it – for determining the tissue specificity of oncogenes.

RasV12 signaling depends on EGFR activity in vivo

SESSION 1: RAS signaling

POSTER Nº: 12

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Abstract

Oncogenic Ras mutants have long been considered constitutively active proteins locked in a GTP-bound state. However, their relationship with upstream modulators remains controversial, with an emerging role of EGFR as a central player in Ras-driven tumorigenesis. Understanding the impact of Ras mutants on downstream pathway activation, as well as the specific cellular requirements they depend on, is crucial for the development of novel therapeutic strategies with clinical relevance.

In this work, we addressed how RasV12 mutant signals in vivo and its relationship with EGFR. We took advantage of the genetic tractability of Drosophila melanogaster, in which the members of the Ras signaling pathway are conserved with low genetic redundancy, enabling the dissection of genetic interactions in vivo. We generated transgenic lines that express the Drosophila Ras1V12 and the human KRASBV12 alleles from the Ras1 promoter.

Germline expression of these oncogenic Ras variants induces a range of gain-of-function phenotypes and lethality, consistent with hyperactivation of the Raf/ERK pathway. However, global phospho-ERK levels are not modified, and the normal spatial pattern of ERK phosphorylation in epithelial cells is maintained. RasV12 mutant cells exhibit enhanced responsiveness to upstream EGFR activation compared to wild-type cells, and in the absence of EGFR, Ras1V12 fails to promote full ERK phosphorylation.

Our findings challenge the prevailing view of RasV12 as a constitutively active mutant, demonstrating instead that its activity remains dependent on upstream EGFR input in vivo when expressed at physiological levels. These results suggest that targeting upstream activators like EGFR is a valid strategy in RasV12-mutant tumors.

Wild-type RAS signaling is an essential therapeutic target in RAS-mutated cancers

SESSION 1: RAS signaling

POSTER Nº: 13

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Abstract

Mutated RAS proteins activate downstream RAF/MEK/ERK and PI3K/AKT effectors to drive oncogenic transformation; combined inhibition of RAF/MEK/ERK and PI3K/AKT signaling is required for effective treatment of RAS-mutated cancers. RAS family members show differential activation of downstream effectors: HRAS effectively activates PI3K but poorly activates RAF, whereas KRAS potently activates RAF but poorly activates PI3K.

Differential activation of RAS effectors by RAS family members underlies a dependence on wild-type (WT) RAS to promote mutant RAS-driven transformation and defines synergy between inhibitors of mutant RAS and RAS effector pathways. WT RAS family members promoted mutant RAS-driven transformation by activating RAS effectors poorly engaged by mutated RAS. Specifically, KRAS/NRAS promoted RAF/MEK/ERK signaling in cells expressing mutated HRAS, whereas HRAS/NRAS promoted PI3K/AKT signaling in cells expressing mutated KRAS.

Inhibitors of the RAS effector pathways activated poorly by mutated RAS further synergized with mutant RAS inhibitors in a WT RAS-dependent manner. The farnesyltransferase inhibitor tipifarnib blocked mutant HRAS–PI3K signaling and synergized with MEK inhibitors in HRAS-mutated cells. KRAS^G12C inhibitors blocked mutant KRAS–MEK signaling and synergized with PI3K inhibitors in KRAS^G12C-mutated cells. Inhibition of NRAS^G12C using sotorasib synergized with inhibitors of proximal RTK signaling (SHP2, SOS1) or combined inhibition of WT HRAS and KRAS, but not with MEK or PI3K inhibitors, consistent with the ability of NRAS to activate both RAF/MEK/ERK and PI3K/AKT signaling.

Synergy was abolished in RASless MEFs and in cancer cell lines where mutant-independent WT RAS proteins were deleted. These data highlight the critical role of WT RAS family members in supporting mutant RAS signaling and the importance of inhibiting WT RAS signaling for effective therapeutic targeting of RAS-mutated cancers.

Dynamic network modelling uncovers TP53–AKT activation loop, CDK4/6, and ferroptosis as key vulnerabilities in pancreatic cancer

SESSION 1: RAS signaling

POSTER Nº: 14

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with poor prognosis and limited therapeutic options, despite the high prevalence of oncogenic KRAS mutations. To uncover targetable vulnerabilities beyond classical RAS pathway inhibition, we applied the cSTAR dynamic network modelling pipeline to integrate systematic perturbation data from RPPA phosphoproteomics and global proteomics across three KRAS-mutant PDAC cell lines (PSN1, PANC1, MiaPaCa2) and one non-transformed pancreatic epithelial cell line (H6C7).

The reconstructed models revealed a self-amplifying feedback loop between AKT and TP53 in all PDAC cell lines, reinforcing oncogenic signalling. In contrast, this regulatory motif formed a negative feedback loop in the normal cell line. This shift from homeostatic to oncogenic signalling highlights the AKT–TP53 axis as a key feature of malignant transformation. Additionally, CDK4/6 was identified as a central node driving oncogenicity and proliferation in all PDAC models. Situated at the intersection of the RAS-MAPK and TP53 pathways, CDK4/6 represents a strategic point of convergence for therapeutic intervention to suppress tumour growth.

In parallel, network modelling identified RAS-ERK and TP53-AKT as dominant oncogenic drivers in PDAC. As ferroptosis is regulated by both pathways, this pointed to ferroptosis induction as a promising approach to bypass apoptosis resistance, particularly in refractory cell lines such as PANC1. This hypothesis was experimentally validated: PDAC cell lines showed significantly greater sensitivity to ferroptosis inducers than the normal epithelial cell line, confirming ferroptosis as a selective vulnerability.

Altogether, our study highlights two independent but complementary therapeutic strategies: CDK4/6 inhibition to constrain oncogenic signalling and ferroptosis induction to trigger non-apoptotic cell death, both informed by the structure of the oncogenic signalling network in PDAC.

Morphological Profiling Characterises KRAS Inhibitor Sensitivity in Pancreatic Cancer

SESSION 1: RAS signaling

POSTER Nº: 15

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Abstract

Ras-targeting agents represent a significant advancement for pancreatic ductal adenocarcinoma (PDAC), a disease characterised by driver KRAS mutations, poor prognosis, and limited therapeutic options. Due to the complexity and adaptability of signalling pathways, both acute and intrinsic resistance to Ras inhibition in PDAC are likely. We examined how acute Ras inhibition produces different outcomes in individual cells, including apoptosis, cell cycle exit, or maintenance of a proliferative phenotype.

We tracked PDAC cells expressing markers of the cell cycle and apoptosis using long-term time-lapse microscopy. Automated brightfield segmentation and unbiased pattern recognition (CellPhePy toolkit) revealed distinct morphological phenotypes within quasi-mesenchymal PDAC lines. Cells exhibited morphological plasticity, switching between long, thin, highly adherent forms and rounded, blebbing, low-adherent cells with a highly contractile actin cortex enriched in phosphorylated ezrin-radixin-moesin and myosin light chain 2.

Under standard conditions, no differences in cell cycle or death were observed between morphological phenotypes. However, treatment with RAS inhibitors led to apoptosis that was selective for low-adherent cells, despite similar reductions in p-ERK across both phenotypes. This occurred with KRASG12C, KRASG12D, and pan-RAS inhibitors across multiple cell lines with different KRAS mutations. Sensitivity to Ras inhibition increased when cells were cultured on soft hydrogels, where they adopted a rounded, low-adherent morphology. Conversely, inhibition of actin contractility decreased cell death.

We identified non-cleaved procaspase-3 as a marker for low-adherent cells, which was highly enriched at membrane blebs. RNA sequencing and immunofluorescence showed that KRAS inhibition decreased apoptotic inhibitor expression and retained signalling receptors, thereby promoting caspase-3 activation and apoptosis. More adherent cells exited the cell cycle, enhancing survival and resistance. These findings demonstrate that the impact of Ras signalling inhibition varies between cell morphologies, providing insights into potential resistance mechanisms and highlighting morphological plasticity as a therapeutic target.

Exploring the role of secondary KRASG12C mutations identified in KRASi-resistant patients

SESSION 10: Tumour heterogeneity: naïve & post-treatment

POSTER Nº: 16

Debora Caprella[12], Rossella Scardaci[1], Sandra Vietti Michelina[1], Edoardo Garbo[34], Alessandro Di Federico[3], Matteo Cereda[25], Biagio Ricciuti[3], Mark M. Awad[6], Chiara Ambrogio[1]

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Abstract

RAS is a family of small GTPase proteins involved in cell proliferation, migration, apoptosis, and survival. KRAS is one of the RAS isoforms frequently mutated in cancer, particularly in non-small cell lung cancer (NSCLC), where the KRASG12C variant is the most prevalent.

KRAS has long been considered an undruggable target due to its high affinity for GTP and the absence of canonical binding pockets. However, the identification of a cryptic pocket in the GDP-bound form of KRASG12C has changed the field. Sotorasib (AMG 510) was the first KRASG12C inhibitor approved in 2021, followed by adagrasib (MRTX 849) in 2022. Initial responses to both drugs were promising, but resistance developed in patients within 6–8 months.

In this context, a non-interventional, single-coordinating center, decentralized bio-specimen collection study in USA-based adult subjects with acquired resistance to KRAS inhibitors is currently ongoing (SPARK trial, NCT05272423). Liquid biopsies from patients are performed at baseline and after the development of resistance to KRAS inhibitors. Among the identified co-mutations, we are focusing on secondary KRAS mutations in cis with G12C.

We firstly generated the models from RASless (Kras^lox/lox, Hras-/-, Nras-/-, RERT^ert/ert) mouse embryonic fibroblasts (MEFs), rescued with KRASG12C and mutations in cis, in which 4-OHT treatment allows the deletion of endogenous KRas and the evaluation of the exclusive expression of the variant of interest.

Then, we characterized the models by viability assays, live-cell imaging, Western blot, RAS-GTP pull-down assays, and RT-qPCR. Growth kinetics and IC50 results show that secondary KRAS mutations in cis with G12C displayed different levels of resistance to KRAS inhibitors. MAPK activation and RAS-GTP levels did not significantly change after treatment, suggesting that resistance mechanisms may not rely on reactivation of the canonical MAPK pathway. Notably, one mutant showed increased KRAS transcript levels, suggesting a potential role of transcriptional regulation in determining resistance.

Our data warrant further investigations into transcriptome alterations to better understand the gene expression and signaling pathways implicated in resistance to KRAS inhibitors.

Phosphoproteomic of cancer stem cells using in vivo models of lung cancer brain metastasis

SESSION 10: Tumour heterogeneity: naïve & post-treatment

POSTER Nº: 17

Jesús Gómez-Escudero[12], Elisa Arias[12], Pilar Cerveró[12], Arantxa Tabernero[12], Maruan Hijazi[12]

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Abstract

Metastasis in the central nervous system is the most common type of brain tumor and has a median survival of only 2 years. This is partly due to the presence of cancer stem cells (CSCs) that initiate and maintain the neoplasm of malignant tumors. Studying this cell subpopulation is essential, as they promote the progression of brain tumors, contribute to therapy resistance, and their interaction with the microenvironment makes metastases complex to study.

Additionally, the brain microenvironment is very different from other organs due to its specialized cells (neurons and glia), the blood-brain barrier, and its unique metabolism. In this study, we apply a phosphoproteomics approach to investigate the interaction of the brain microenvironment with lung CSCs using in vivo models of lung cancer brain metastasis.

Preliminary data indicate that the brain microenvironment modifies the phenotype of CSCs. We study the signaling pathways that mediate cell plasticity and participate in the interaction between the brain microenvironment and CSCs. The goal is to design new therapies that improve patient responses to current treatments and advance our understanding of the molecular basis of brain metastases, which remain extremely difficult to treat.

Decoding SOS: eIF2B as a Key Driver of Mutant KRAS Signaling and Tumorigenesis

SESSION 2: Unveiling RAS biology

POSTER Nº: 18

Hyungdong Kim[12], Shiqi Diao[12], Kwang-Jin Cho[3], Hyon-Ro Lee[4], Pascal Egea[5], Junchen Liu[67], Milla Kurki[8], Tatu Pantsar[8], Shuo Wang[1], Jia Yi Zou[12], Mehdi Amiri[910], Nour Ghaddar[1], Ritchel Gannaban[67], John Hancock[67], Kylie M. Rice[3], Qiyun Deng[910], Atsuo Sasaki[1112], John Asara[1314], Brajendra Tripathi[15], Douglas Lowy[15], Rosalie Lawrence[16], Maria Hatzoglou[17], Carlos R. Azpilcueta-Nicolas[18], Jean-Philip Lumb[18], John Columbus[19], Thomas J. Turbyville[19], Christopher B. Marshall[20], Mitsuhiko Ikura[20], Jay T. Groves[4], Nahum Sonenberg[910], Peter Walter[5], Antonis E. Koromilas[121],#

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Abstract

Beyond its canonical function as a guanine nucleotide exchange factor (GEF) in mRNA translation initiation, eIF2B forms a distinct complex preferentially with oncogenic mutant KRAS, rather than wild-type KRAS, and Son of Sevenless (SOS) in cells. Mechanistically, the catalytic ε subunit of eIF2B cooperates with mutant KRAS to engage the allosteric RAS-binding domain of SOS, enhancing KRAS GTP loading and activation.

In vitro assays using recombinant proteins support a model in which eIF2B binding to GTP-bound RAS relieves SOS autoinhibition via its allosteric site, promoting GDP–GTP exchange on RAS. eIF2B co-localizes with SOS and mutant KRAS at the plasma membrane (PM), amplifying mutant KRAS activity and driving MAPK pathway signaling.

Concurrently, eIF2B enhances translation of B4GALT5 mRNA, stimulating the glycosphingolipid (GSL) biosynthesis pathway, which facilitates mutant KRAS localization and clustering at the PM. Genetic inactivation of the eIF2Bε subunit significantly suppresses growth of mutant KRAS-driven tumors in xenograft and autochthonous lung adenocarcinoma models. Elevated eIF2Bε expression correlates with poor prognosis in human cancers harboring KRAS mutations.

These findings reveal an unexpected link between a core translation initiation factor and oncogenic KRAS function, offering new mechanistic insights and identifying potential therapeutic avenues for mutant KRAS-driven malignancies.

Exploring KRAS allelic imbalances in pancreatic cancer

SESSION 2: Unveiling RAS biology

POSTER Nº: 19

Blanca Rosas-Pérez[1], Juan Carlos López-Gil[1], Vasiliki Liaki[1], Carmen Guerra[1], Mariano Barbacid[1]

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most heterogeneous and highly aggressive malignancies. Its high lethality is attributed to late diagnosis and lack of effective treatment options. Therefore, new strategies and approaches are essential to enhance patient outcomes and overall survival.

Among the main driver genes of PDAC, KRAS is the most frequently mutated oncogene, usually altered in exon 2, codon 12. Complex genomic rearrangements, such as chromothripsis, have also been detected as common occurrences in human PDAC. These mitotic events result in an increase of KRAS gene dosage and may lead to loss of the wild-type allele (loss of heterozygosity, LOH).

The loss of the wild-type allele, as well as amplification of the mutant allele in KRAS-driven PDAC tumors, influences tumor progression, metastasis, and therapeutic response. However, the functional and therapeutic implications of such KRAS allelic imbalances remain poorly understood.

This study focuses on a comprehensive and integrative approach to uncover the molecular mechanisms by which KRAS allelic imbalances drive rapid metastasis and poor therapy response. We aim to investigate the impact of these imbalances on current treatment modalities and explore their potential as prognostic markers.

Proteomics- and BRET-screens identify SPRY2 as interactor of active Ras that impacts on its membrane organisation

SESSION 2: Unveiling RAS biology

POSTER Nº: 20

Blanca Rosas-Pérez[1], Juan Carlos López-Gil[1], Vasiliki Liaki[1], Carmen Guerra[1], Mariano Barbacid[1]

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Characterization of the subcellular localization determinants, biological role, and drug vulnerabilities of the r-ras2 gtpase

SESSION 2: Unveiling RAS biology

POSTER Nº: 21

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Abstract

R-RAS2 (also known as TC21) is a small GTPase located in focal adhesions that is closely related to classical RAS proteins (H-, K-, and N-RAS) at the structural and signalling level. However, emerging evidence indicates that it plays overlapping but not fully redundant functions with these GTPases. In this study, we set out to further characterize the subcellular localization, biological roles, and therapeutic vulnerabilities of R-RAS2.

Through the generation and transfection of RAS chimeric constructs, we identified that the C-terminal region of R-RAS2, which includes a conserved "R-RAS box", is responsible for its specific localization in focal adhesions. Using R-RAS2-deficient mouse embryonic fibroblasts (MEFs), we also demonstrated that R-RAS2 is required for efficient cell migration and for the proper deposition and structural organization of the extracellular matrix (ECM).

Genome-wide gene expression analysis further revealed that the lack of R-RAS2 leads to profound changes in the transcriptome of MEFs, both at the level of gene expression and differential splicing. Finally, we found that oncogenic R-RAS2 mutants (Q72L, G23V) confer sensitivity to the pan-RAS(ON) inhibitor RMC-6236 but not to K-RAS-specific compounds, highlighting a distinct drug response profile. Interestingly, RMC-6236 induced cytotoxic effects in R-RAS2-driven tumour cell lines while causing cytostatic responses in non-transformed cells.

These findings establish R-RAS2 as a structurally unique and pharmacologically targetable GTPase that regulates fibroblast motility and stromal architecture, with implications for tumour progression and therapy..

K-Ras controls ciliation during cell differentiation – A mechanistic commonality between RASopathies and ciliopathies

SESSION 2: Unveiling RAS biology

POSTER Nº: 22

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Abstract

The impact of Ras-MAPK signaling on cell proliferation is well studied, whereas its role in cell differentiation remains unclear. We have recently shown that the C2C12 mouse muscle cell line represents a hierarchical cell differentiation model, with a minor population of stem cells dividing asymmetrically to replenish the pool of committed myoblasts. Evidence suggests that asymmetric cell divisions of muscle stem cells in vivo are guided by the primary cilium, an antenna-like organelle that coordinates developmental signaling pathways in stem and progenitor cells. Protein trafficking into the cilium is mediated by the chaperone protein PDE6D that binds prenylated cargo, including K-Ras4B (hereafter K-Ras). Because we observed that K-Ras depletion decreases the stem cell population of C2C12 cells, we hypothesized that K-Ras impacts ciliation during muscle cell differentiation.

Here we show that active K-Ras localizes to the primary cilium in a PDE6D-dependent manner in C2C12 cells. Supported by mathematical modelling, our data demonstrate that K-Ras promotes re-ciliation, thus protecting the stem cell subpopulation during asymmetric cell divisions, while N-Ras is neutral and H-Ras is inhibitory. Restricting K-Ras exclusively to the cilium is sufficient to promote re-ciliation.

Single-cell RNA sequencing revealed that the C2C12 differentiation trajectory mirrors that observed in vivo and recapitulates key muscle cell differentiation stages. Gene expression data allowed us to identify putatively ciliated subpopulations at the top of the differentiation hierarchy. These data support that high K-Ras expression and activity are required for re-ciliation in highly proliferative, ciliated subpopulations. Both K-Ras depletion or expression of oncogenic K-RasG12C perturb normal differentiation. Transformation with oncogenic K-RasG12C inhibited terminal differentiation and trapped cells in a committed, low-proliferative state.

Our study describes a novel fundamental role of K-Ras-MAPK signaling in the primary cilium, which we postulate is broadly conserved in vertebrates and explains the phenotypic similarities between RASopathies and ciliopathies.

Oncogenic mutant KRAS: redox sensitive cysteines biology, from inhibition to drug resistance mechanisms

SESSION 2: Unveiling RAS biology

POSTER Nº: 23

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Abstract

KRAS (Kirsten rat sarcoma virus) is one of the most frequently mutated GTPase signaling proteins in human cancers, with oncogenic alterations—most commonly G12D, G12V, and G12C—found in more than 20% of adult tumors across various cancer types. Selective inhibitors targeting mutant KRAS, such as sotorasib (Lumakras) and adagrasib (Krazati), have recently transformed the clinical management of KRAS-mutant NSCLC patients by enabling personalized targeted therapies. However, both adaptive and acquired resistance mechanisms often arise following an initial period of therapeutic response, underscoring the urgent need for alternative strategies to target KRAS-mutant isoforms.

Recent evidence suggests that specific reactive oxygen species (ROS) can modulate KRAS function through redox-sensitive cysteine residues, particularly cysteine 118 (C118) within the NKCD motif. To investigate this, we engineered a redox-mimetic KRAS variant by substituting C118 with aspartic acid (C118D), simulating constitutive oxidation. Our findings demonstrate that oxidation at C118 selectively inhibits oncogenic KRAS in the context of non-small cell lung cancer (NSCLC), both in vitro and in vivo.

Furthermore, we showed that combined treatment with the pro-oxidant paraquat and the nitric oxide synthase inhibitor L-NAME (N^G-nitro-L-arginine methyl ester) induces selective redox-mediated inhibition of KRAS activity via C118 oxidation. Lastly, based on the hypothesis that oxidation of solvent-exposed cysteine residues may impair covalent inhibitor binding, we investigated the impact of G12C oxidation on resistance to sotorasib. Our results suggest that oxidative modification of the G12C residue could contribute to resistance mechanisms, with implications for therapeutic efficacy.

A targeted treatment approach of mutant KRAS in genetically engineered mouse models of colorectal cancer

SESSION 2: Unveiling RAS biology

POSTER Nº: 24

Laura Millett[12], Catriona Ford[2], Arafath K. Najumudeen[3], Kathryn Gilroy[1], Rosalin Simpson[1], Sarah Ross[4], Simon Barry[4], Andrew Campbell[2], Owen Sansom[12]

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Abstract

Intestinal tumorigenesis driven by loss of the WNT suppressor gene Apc, co-occurring with a point mutation in the KRAS oncogene, represents approximately 40% of colorectal cancer (CRC) cases. These mutations drive tumour growth via the MAPK and PI3K/AKT pathways, rendering this subset of CRC patients ineligible for current targeted therapies. KRAS inhibitors as monotherapies are suboptimal clinical tools against CRC. We posit that co-targeting both KRAS (G12C or G12D mutants) and the PI3K/AKT pathway at multiple nodes could be an effective treatment strategy to extend survival in genetically engineered mice and orthotopic models.

We generated a clinically relevant model of Apc-deficient, KRASG12C-mutant CRC in genetically engineered mice (VillinCreER Apc^fl/wt^ KRASG12C/wt^). Guided by the response of an initial compound screen in 3D intestinal organoids derived from tumors of these mice, we identified an additive effect of the clinically approved AKT inhibitor (Capivasertib, AZD5363) in combination with KRASG12C inhibition (AZD4625). We assessed the pro-survival impact of this treatment regimen in vivo and explored the underlying mechanisms using western blotting and bulk RNA-seq techniques both in vitro and in vivo.

Additionally, we evaluated the utility of KRAS inhibition in complex genetically engineered mouse models (GEMMs), modeling frequent genetic alterations (allelic imbalance) and cooperative co-mutations (PIK3ca^H1047R^) in KRAS-mutant CRC, to capture responses relevant to patient subsets. We also assessed tumor biomarkers in VillinCreER Apc^fl/fl^ KRASG1D/wt^ GEMMs following treatment with the KRASG12Di inhibitor (MRTX1133) in combination with AZD5363, demonstrating a shared mechanism of response across KRAS point mutations.

Finally, we recapitulated the synergistic effect of KRASG12Ci and AKTi in reducing metastasis following intrasplenic injection of tumoroids from VillinCreER KRASG12C/wt^ Trp53^fl/fl^ Rosa26^N1icd/wt^ mice. These results indicate that co-targeting the MAPK and PI3K/mTOR pathways offers a powerful approach to limit disease progression in CRC.

KRAS4A Directly Regulates Methionine Synthase

SESSION 2: Unveiling RAS biology

POSTER Nº: 25

Mercedes Fissore-O'Leary[1], Wenjuan Su[1], Juan Kochen-Rossi[1], Cristina Nuevo-Tapioles[1], Cristina Branco[1], Anthony Belanger[2], Ruma Banerjee[2], Mark Philips[3]

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Abstract

The excitement generated by the advent of direct KRAS inhibitors (KRASi) has been tempered by the observation that they do not produce durable responses in KRAS-driven cancers. This has stimulated renewed interest in understanding KRAS biology to uncover vulnerabilities that could synergize with KRASi.

The KRAS locus produces two proteins, KRAS4A and KRAS4B, both oncogenic when the locus carries an activating mutation. The isoforms differ exclusively in their hypervariable regions (HVR), which dictate differential trafficking within the cell. We performed a proximity labeling screen for each KRAS splice variant and defined their distinct interactomes. Among the 24 proteins preferentially labeled by KRAS4A was methionine synthase (MTR).

MTR is one of only two mammalian enzymes that utilize vitamin B12 as a cofactor and is essential for sustaining the folate cycle and one-carbon metabolism, which has been a chemotherapeutic target for over seven decades. Using co-immunoprecipitation and affinity capture, we validated a direct, isoform-specific interaction between KRAS4A and MTR that depends on GTP-binding and the HVR of KRAS4A. The interaction requires the B12-binding domain of MTR.

We developed an in vitro radiometric assay for the B12-dependent activity of MTR. Strikingly, KRAS4A markedly stimulated MTR activity in a GTP-dependent manner. Kinetic analysis revealed an increase in V_max, suggesting that KRAS4A acts as an allosteric activator of MTR. Lysates of RAS-less HEK cells rescued with KRAS4A showed higher MTR activity compared to cells rescued with other RAS isoforms.

Our data indicate that KRAS4A directly regulates one-carbon metabolism and highlight MTR as an attractive target for inhibitors that may synergize with KRASi.

KRAS4A Directly Regulates Methionine Synthase

SESSION 2: Unveiling RAS biology

POSTER Nº: 26

Mercedes Fissore-O'Leary[1], Wenjuan Su[1], Juan Kochen-Rossi[1], Cristina Nuevo-Tapioles[1], Cristina Branco[1], Anthony Belanger[2], Ruma Banerjee[2], Mark Philips[3]

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Our data indicate that KRAS4A directly regulates one-carbon metabolism and highlight MTR as an attractive target for inhibitors that may synergize with KRASi.

Investigating the role of CRL5 E3 Ubiquitin ligase complex in KRAS inhibitor resistance in non-small cell lung cancer

SESSION 2: Unveiling RAS biology

POSTER Nº: 27

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Abstract

Oncogenic KRAS is a prevalent driver of non-small cell lung cancer (NSCLC), with KRAS^G12C representing a significant therapeutic target due to FDA approval of two small molecule inhibitors, adagrasib and sotorasib. While these inhibitors have demonstrated clinical efficacy, resistance remains a critical challenge, necessitating deeper understanding of pathways that modulate KRAS signaling and therapeutic sensitivity.

Genetic screening efforts revealed Cullin RING Ubiquitin Ligase 5 (CRL5) as a regulator of RAS/MAPK inhibitor sensitivity, with loss of CRL5 members conferring resistance. This implicates CRL5 in modulating oncogenic signaling networks that support tumor cell survival. We hypothesized that stabilization of one or more CRL5 substrates promotes RAS inhibitor resistance.

To identify candidate substrates, proteomics analyses were performed on parental and CRL5 gene knockout (CUL5, SOCS3, or ARIH2) NSCLC cell lines. Among many upregulated proteins in CRL5 knockout cells, the focal adhesion protein talin-2 (TLN2) was the only protein commonly upregulated across all CRL5 knockouts. TLN2 mRNA and protein were also upregulated upon acute and long-term RAS/MAPK inhibition, suggesting a compensatory mechanism.

Genetic depletion of TLN2 in parental and CUL5 knockout cells increased RAS inhibitor sensitivity. TLN1/2 isoforms are critical for beta integrin activation and focal adhesion kinase (FAK) activity, indicating that TLN2-mediated RAS inhibitor resistance may act through the FAK–YAP/TEAD axis.

In conclusion, this work highlights the importance of auxiliary regulators of oncogenic pathways and provides insights into mechanisms contributing to RAS inhibitor resistance in NSCLC.

Assessing the differential impact of Ras mutations in tissue mechanics and tumour formation

SESSION 2: Unveiling RAS biology

POSTER Nº: 28

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Abstract

Different Ras mutant isoforms show variable mutational rates across cancers. KRAS^G12 mutations are commonly found in lung cancer, whereas NRAS^Q61 mutations are prevalent in melanoma. However, it remains unclear why specific Ras mutations confer a greater tumorigenic burden in one tissue type over another.

Hyperactivated Ras has been shown to increase cell contractility, which is linked to multiple tumorigenic processes, including dysregulated cell division, live-cell extrusion, and metastasis. The extent to which different Ras mutations alter the physical properties of cells during early tumorigenesis, however, remains to be elucidated.

Using targeted microinjection of Ras-mutant RNA in developing Xenopus laevis embryos, tumour-like structures (ITLS) can be induced in fate-restricted cells, enabling the study of early tumorigenesis in complex tissue environments. This study focuses on four Ras variants: KRAS^G12V, KRAS^G12D, NRAS^G12V, and NRAS^Q61K. Preliminary data show that different Ras variants generate ITLS with distinct sizes, compositions, and intracellular Ras localization patterns.

Laser ablation experiments indicate that KRAS^G12V, KRAS^G12D, and NRAS^G12V increase junctional tension in tissues, whereas NRAS^Q61K does not. Western blot analyses reveal that different Ras mutations differentially activate downstream signaling pathways. Specifically, KRAS^G12V, KRAS^G12D, and NRAS^Q61K are associated with MAPK hyperactivation, while KRAS^G12V and KRAS^G12D—mutations linked to the greatest junctional tension—also show elevated PI3K/AKT pathway activity.

These results suggest that distinct Ras mutations exert differential tumorigenic and mechanical effects during early tumorigenesis, potentially explained by their variant-specific signaling profiles.

Molecular mechanisms of high-fat diet as a risk factor for pancreatic ductal adenocarcinoma (PDAC) initiation and progression

SESSION 3: Decoding RAS Driven tumours

POSTER Nº: 29

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer-related deaths, primarily due to late diagnosis and limited treatment options. High-fat diet (HFD)-induced obesity is a significant risk factor for PDAC. However, the mechanisms linking diet-induced obesity to tumor initiation and progression remain poorly understood.

Previous studies have shown that KRAS mutant cells and precursor lesions are relatively common among healthy individuals. Most genetically-engineered mouse models (GEMMs) for PDAC carry these mutations from embryonic development. In contrast, our work demonstrated that expression of a Kras mutation in adult acinar cells alone does not induce acinar-to-ductal metaplasia (ADM) or PanIN/PDAC, except when chronic pancreatitis is present, which promotes transformation in a subset of cells. Our aim is to elucidate the molecular mechanisms by which HFD influences adult pancreas tumorigenesis.

Using Kras+/LSLG12Vgeo; Trp53lox/lox; Elas-tTA/tetO-Cre (KpeC) mice, we induced postnatal (P21) oncogenic Kras expression and fed them a HFD. Unlike embryonic Kras activation, which leads to tumors by 12–16 weeks, postnatal Kras expression reduced tumor incidence, supporting the notion of adult resistance to tumorigenesis. In postnatal controls, tumor detection was delayed, whereas HFD-fed mice did not show this delay.

Tumors from HFD-exposed mice and primary PDAC cells derived from these tumors exhibited mesenchymal morphology, in contrast to the epithelial characteristics observed in control tumors. RNA sequencing revealed enrichment of gene signatures associated with epithelial-mesenchymal transition (EMT), invasiveness, epithelium development, and cancer stemness. Functional assays confirmed increased migration and invasion capabilities in HFD tumor cells. HFD tumors also showed Gata6 downregulation, suggesting induction of a hybrid phenotype expressing both epithelial and mesenchymal markers.

These findings indicate that HFD promotes aggressive PDAC with hybrid epithelial-mesenchymal phenotypes and enhanced tumor plasticity. Understanding the obesity-mediated molecular mechanisms may inform preventive or therapeutic strategies for high-risk obese patients.

Personalized medicine in metastatic pancreatic cancer (PANMET)

SESSION 3: Decoding RAS Driven tumours

POSTER Nº: 30

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death worldwide, with a 5-year survival rate of only 5%. Early-stage disease is usually asymptomatic, and over 80% of patients present with local or distant metastases at diagnosis. Current treatments, such as gemcitabine and FOLFIRINOX, extend survival to only 6–11 months and are highly toxic. Surgery remains the only curative option, but recurrence rates reach 85%, and most patients are ineligible due to late diagnosis. No targeted therapies are currently approved for PDAC.

This CNIO project aims to evaluate a triple therapy targeting RAF1/EGFR/STAT3, previously shown to induce complete regression in KRAS/TP53-driven primary tumors. The strategy will be tested across multiple KRAS contexts (WT, G12D, G12V, G12C, G12R, and WT allele loss) and in tumors lacking key suppressors such as CDKN2A or SMAD4. Experimental models include human and murine cell lines, patient-derived organoids (PDOs), genetically engineered mouse models (GEMMs), and orthotopic models.

In cases of resistance, whole-exome sequencing (WES) and RNA sequencing (RNA-seq) will be performed to identify resistance mechanisms and new therapeutic targets, which will then be validated in vitro and in vivo. The approach will also be applied to metastatic PDAC using metastasis-prone GEMMs, patient-derived xenografts (PDXs), and PDOs. To promote spontaneous metastasis, a surgical resection model will be used to extend survival.

Additionally, genetic and molecular profiling of tumors and metastases from both mouse and human samples will be conducted to identify predictive biomarkers of treatment response. Liquid biopsies will be analyzed in collaboration with Hospital Gregorio Marañón.

Expected outcomes include advancing the molecular understanding of PDAC and KRAS biology, identifying new therapeutic targets and biomarkers, and paving the way for more effective, less toxic treatments ready for clinical translation.

Identification of new therapeutic vulnerabilities in aneuploid RAS-driven tumors

SESSION 3: Decoding RAS Driven tumours

POSTER Nº: 31

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Abstract

In Non-Small Cell Lung Cancer (NSCLC), about 30% of patients present KRAS mutations, correlating with a more aggressive phenotype and poor prognosis, and 77% are characterized by aneuploidy, which contributes to tumor heterogeneity and resistance to therapies. KRAS-driven cancer cells are characterized by a hyper-activated CRAF-MEK-ERK axis, which is even more enhanced in aneuploid cancer cells.

Aneuploidy, by activating the DNA damage response, limits the efficacy of DNA damage inducers such as Topotecan and Etoposide, impairing further DNA damage induction. Targeting the MAPK pathway with small molecules, in combination with Topotecan or Etoposide, could overcome this limitation and allow effective DNA damage induction by these Topoisomerase inhibitors.

Using different human KRAS-driven lung adenocarcinoma (LUAD) cell lines with varying aneuploidy scores, such as NCI-H23, H358, and H1373, we tested the hypothesis that these cellular vulnerabilities could be exploited to selectively eradicate aneuploid tumors. This study revealed a novel aspect of KRAS inhibition specifically in aneuploid LUAD.

To exploit their potential dependence on the MAPK pathway, we tested a combinatorial treatment using Adagrasib together with Topotecan or Etoposide. These combinations demonstrated a synergistic effect, enabling further DNA damage induction by Topoisomerase inhibitors and modulation of the MAPK pathway.

The aim of the study is to establish a foundation for a new combinatorial therapy based on the aneuploidy status of different KRAS-driven cancer cells.

Mapping Stage-Driven RAS Effector Dynamics in Colon Adenocarcinoma

SESSION 3: Decoding RAS Driven tumours

POSTER Nº: 32

Loretta László[1 2], Anna Lovrics[1], Álmos Tilajka[1 2], Tamás Takács[1 2], László Buday[1 3], Virag Vas[1]

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Abstract

Cancer emerges from disruptions in complex cellular networks rather than isolated gene defects. From a Network Medicine perspective, this study integrated transcriptome and patient tissue analyses to elucidate RAS-driven molecular transitions in colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD), and lung squamous cell carcinoma (LUSC). RNA-seq data of 43 bona fide RAS effectors were acquired from The Cancer Genome Atlas, and Pearson's correlation profiling was performed to assess co-expression changes. Tumor samples exhibited a pronounced loss of co-regulation across the RAS interactome, with COAD showing the greatest reduction even at Stage I, indicating early perturbation of RAS-mediated signaling.

Focusing on COAD, logistic regression with cross-validation identified a core five-RAS effector signature (RAF1, PLCE1, RGL1, RIN1, GRB7) achieving balanced accuracy > 0.90 for early tumor detection. Validation by RT-qPCR on patient-derived colon tissue cDNA samples confirmed significant downregulation of PLCE1 and RAF1, and upregulation of GRB7, recapitulating the co-expression loss observed in the TCGA cohort.

In contrast, comparisons between Stage I and Stage IV COAD failed to uncover any single effector or combination of genes with sufficient discriminatory power, yielding low balanced accuracy values (< 0.70) even with multigene models. These findings suggest that COAD progression beyond Stage I involves extensive genetic and phenotypic diversification, diminishing the utility of RAS-centric markers for late-stage stratification.

Overall, this work defines a robust RAS effector signature driving the earliest transition in colorectal tumorigenesis and underscores the critical importance of targeting early-stage molecular alterations—particularly RAF1, PLCE1, RGL1, RIN1, and GRB7—for effective therapeutic intervention before the tumor evolves into a highly heterogeneous disease state.

A graphical network model illustrates the dysregulation of 43 RAS-interacting effector molecules in early-stage COAD compared to normal tissue, with nodes grouped by functional category and downstream effects. Significant expression changes are color-coded (red for upregulation, green for downregulation), and core RAS effectors with high diagnostic accuracy are highlighted in bold.

This research was supported by grants from the National Research, Development, and Innovation Fund of Hungary (K124045, 2020-1.1.6-JÖVŐ-2021-00004), and Project no. RRF-2.3.1-21-2022-00015 (National Laboratory of Pharmaceutical Research and Development, PharmaLab), with support from the European Union. L.L. received additional support through the Joseph Cours Scholarship from Eötvös Loránd University and the Dr. Bodzsár Éva Foundation. V.V. acknowledges the HAS fellowship, which assists researchers with children in obtaining the title of Doctor of the Hungarian Academy of Sciences.

Targeting NRAS-Mutated Melanoma: Mechanisms of Sensitivity and Resistance to RAS(ON) Multi-Selective Inhibitors

SESSION 3: Decoding RAS Driven tumours

POSTER Nº: 33

Mona Foth[1], Wontak Kim[1,2], Kayla O'Toole[1,2], Brandon Murphy[1], Montserrat Justo-Garrido[1], Sanjana Boggaram[1,2], Phaedra Ghazi[1,2], Euan Brennan[1], M. Isaac Wright[1], Tate Shepherd[1], Emilio Cortes Sanchez[1], Yingyun Wang[5], Jennifer A. Roth[6], Matthew Rees[6], Melissa Ronan[6], Jingjing Jiang[5], Eric Smith[1], Robert Judson-Torres[1,4], Mark Silvis[1], David Lum[1], Kasey Couts[5], Siwen Hu-Lieskovan[1,3], Conan Kinsey[1,3], Jeffery Russell[1,3], Aparna Hegde[6], Ignacio Garrido-Laguna[1,3], Matthew Holderfield[6], Mallika Singh[6], Martin McMahon[1,2,4*]

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Abstract

Most patients with advanced BRAF- or NRAS-driven melanoma receive front-line immunotherapy. However, if immunotherapy fails, BRAF-driven melanoma patients have effective second-line pathway-targeted therapies available, whereas NRAS-mutated patients lack any pathway-targeted options.

RAS(ON) multi-selective inhibitors, such as RMC-7977 and the investigational agent daraxonrasib (RMC-6236), inhibit RAS[GTP] signaling by forming an inhibitory complex with cyclophilin A (CYPA) and GTP-bound RAS (RAS(ON)). Both compounds demonstrate potent anti-proliferative activity against cultured NRAS-mutated cutaneous or acral melanoma cell lines, and robust anti-tumor activity against preclinical melanoma models in vivo.

However, resistance to RMC-7977 monotherapy arose in some preclinical models of NRAS-driven cutaneous melanoma through mutations in the Ppia gene (encoding CYPA) or Map2k1 (encoding MEK1). Additionally, two clinical case studies in patients with NRAS-mutated melanoma treated with daraxonrasib demonstrated clear anti-tumor activity in one patient, and progressive disease in another patient for whom co-occurring NRAS and MAP2K1 mutations were detected in tumor at baseline.

These findings support the clinical evaluation of daraxonrasib in patients with NRAS-mutated melanoma and reveal candidate mechanisms of monotherapy resistance, underscoring the need for combination therapies to improve outcomes.

Unraveling transcriptomic mechanisms of resistance to KRAS-G12C inhibitors

SESSION 3: Decoding RAS Driven tumours

POSTER Nº: 34

Paraskevi Lagkada[1,2], Sandra Vietti Michelina[1], Debora Caprella[1,2], Mariachiara Grieco[2,3], Chiara Ambrogio[1,2], Matteo Cereda[2,3]

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Abstract

Oncogenic RAS mutations drive approximately 30% of all carcinomas, with KRAS being the most commonly altered isoform. Although KRAS has been considered undruggable, the development and FDA approval of KRAS-G12C (OFF) inhibitors, sotorasib and adagrasib, have marked a significant breakthrough in the treatment of KRASG12C-mutated Non-Small Cell Lung Cancer (NSCLC). Nevertheless, the durability of response is limited by the emergence of resistance mechanisms.

In this study, we identify transcriptomic adaptations—including changes in gene expression and alternative splicing—that underlie resistance to KRAS-G12C inhibition, uncovering mechanisms of resistance and suggesting targets for improved intervention.

We used human lung cancer cell lines (H23, CALU1) and generated RAS-less mouse embryonic fibroblasts (MEFs) engineered to express the human KRAS-G12C substitution. All cells were then exposed to escalating doses of sotorasib and adagrasib until resistance emerged. Total RNA sequencing was employed to map transcriptomic adaptations at the gene and exon expression level. Differential expression and alternative splicing analyses identified significant gene/exon expression dysregulation and pathway activation.

RNA-seq revealed transcriptomic reprogramming and key resistance-associated pathways after prolonged treatment. Notably, epigenetic regulation, translational control, and epithelial-to-mesenchymal transition were significantly altered upon resistance. Dysregulation of the MAPK signaling pathway was consistently observed across both mouse and human samples. Strikingly, a broad downregulation of transcription factors was detected in all resistant models, with AR and TP63 emerging as the most prominent candidates.

Cross-species pathway enrichment analysis of alternative splicing events further revealed strong enrichment of MYC target pathways in both mouse and human samples, suggesting potential regulation by RNA-binding proteins. These findings reveal that cells dynamically rewire their transcriptome through alternative splicing, highlighting conserved regulatory mechanisms driving resistance to sotorasib and adagrasib across human and mouse models.

Our results uncover new therapeutic opportunities to overcome KRAS-G12C inhibitor resistance by targeting RNA-based mechanisms.

Exploiting PP2A tumor suppressor inhibition in KRAS-driven NSCLC.

SESSION 3: Decoding RAS Driven tumours

POSTER Nº: 35

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Abstract

Among lung cancers, Non-Small Cell Lung Cancer (NSCLC) accounts for 85% of all cases, and KRAS mutations are found in 30% of patients, with G12C being the most frequent. KRAS-driven cancer cells are characterized by aberrant survival and proliferation due to the hyperactivation of the RAF-MEK-ERK axis of the MAPK pathway, leading to aggressive disease features and poor prognosis.

At today's date, resistance mechanisms against specific mutant KRAS inhibitors have been widely reported, highlighting the limited efficacy of NSCLC targeted therapies. In this context, we hypothesized that PP2A (Protein Phosphatase 2A) can be a targetable core hub regulating MAPK pathway activity. Indeed, PP2A plays a tumor suppressor role in NSCLC.

PP2A inhibition via small molecules such as LB100, coupled with drug withdrawal from KRAS inhibitors (KRASi), could further boost signaling hyperactivation and, as a consequence, lead to oncogene toxicity in resistant cells. Using different human KRAS-driven lung adenocarcinoma (LUAD) cell lines such as NCI-H23 and NCI-H358, we tested this hypothesis to determine whether LB100 treatment could impair cell growth and proliferation upon drug holiday in KRASG12C-mutated cells resistant to sotorasib or adagrasib, and potentially unveil a new strategy in KRAS-driven LUAD treatment.

We demonstrated that upon KRASi withdrawal, LB100 as a single agent impairs proliferation of resistant cells, confirming its potential role in overcoming KRASi resistance. The aim of this study is to pave the way for sequential therapies based on KRASi drug withdrawal upon development of resistance, followed by PP2A inhibition to induce and exploit oncogene toxicity.

Targeting tumor resistance to Raf1 deletion in NSCLC

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 36

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Abstract

Lung tumors are the leading cause of death among cancer patients. Oncogenic mutations in KRAS are present in one-third of all cases of lung adenocarcinoma. Although these mutations have been studied for four decades, effective therapies targeting the oncogenic downstream signaling of KRAS have not yet been approved.

In 2018, Sanclemente et al. found that targeting Raf1 induces significant regression of advanced KrasG12V/Trp53KO mutant lung tumors, leading to partial regression of most tumors and a high percentage of complete regressions. This effect occurs through a mechanism that induces massive apoptosis without affecting canonical MAPK signaling. However, despite the good responses, resistance to Raf1 ablation emerges.

Our recent data show that KrasG12V/Trp53KO/Raf1KO resistant tumors have amplification of the mutant Kras allele and subsequent KRAS protein upregulation, suggesting compensatory mechanisms restoring tumor growth in the absence of Raf1. Moreover, these Raf1KO resistant tumors are more sensitive to RAS inhibitors, indicating that KRAS signaling remains a critical vulnerability. The combination of Raf1 deletion and KRAS inhibition shows a higher percentage of complete regressions compared with monotherapies.

Given the lack of clinically available RAF1 inhibitors, we are shifting to a pharmacological approach using the RAF/MEK "clamp" inhibitor avutometinib (VS-6766) in combination with a FAK inhibitor. This strategy enables dual inhibition of both vertical (RAF/MEK) and parallel (FAK) pathways, enhancing anti-tumor efficacy.

Our current work aims to validate, in this pharmacological context, the KRAS dependency observed in the genetic model of Raf1 ablation resistance. Confirmation of this would support the development of clinically translatable sequential multimodal therapies capable of overcoming resistance that appears after targeting the MAPK pathway in KRAS-driven lung cancer.

SRC blockade to reverse resistance to RAS inhibitors in KRAS-mutant colorectal cancer (CRC): a combinatorial therapeutic approach

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 37

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Abstract

KRAS mutations, most frequently at codons G12D, G12V, G13D, followed by G12C, occur in 40–50% of colorectal cancer (CRC) patients and are linked to poor prognosis and resistance to EGFR-targeted therapies. KRAS inhibitors have shown limited efficacy in CRC patients, in part due to EGFR reactivation, but the comprehensive molecular mechanisms driving resistance remain poorly understood. SRC, a non-receptor tyrosine kinase associated with tumor progression, increased tumor aggressiveness, poor response to treatment, and adverse clinical outcomes in CRC, may play a role in this resistance. Here, we investigated the functional role of SRC in KRAS-mutant (KRASm) CRC preclinical models and its potential as a therapeutic target.

KRASm CRC cell lines (LS180KRASG12D and HCT116KRASG13D) and patient-derived xenograft organoids (PDXOs: KRASG12D, KRASG12V) were treated with RMC-6236 (pan-RAS inhibitor), MRTX1133 (KRASG12D specific inhibitor), dasatinib (SRC inhibitor), and/or cetuximab (EGFR inhibitor) as single agents, dual, or triple combinations. Protein levels were evaluated by Western Blot. Apoptosis was measured by FACS; colony formation, migration, and drug synergy were also evaluated in cell lines.

RASi and KRASG12Di induced SRC activation in KRASm CRC models (cell lines and PDXOs). Combining RAS/KRASG12Di with SRCi or EGFRi showed synergistic anti-tumor effects. Triple inhibition (RASi/KRASG12Di + SRCi + EGFRi) led to significantly reduced cell viability, colony formation, and migration, along with an increase in apoptosis. Consistently, PDXOs showed marked reduction in organoid size after triple therapy. Both preclinical models displayed a decrease in the activation of ERK and AKT after the triple strategy.

SRC activation may drive resistance to RAS-targeted therapies in KRASm CRC. Triple inhibition, including SRC blockade, enhances anti-tumor efficacy and attenuates pathway reactivation. These findings support SRC-targeted combination strategies as a promising therapeutic approach to improve outcomes for KRASm CRC patients.

MRTX1133 alone or in combination with Cetuximab effect in 2D and 3D KRAS G12D MSS colorectal cancer models

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 38

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Abstract

Recent advances in RAS inhibition primarily target KRAS G12C mutations, representing ~3% of metastatic colorectal cancer (mCRC). Conversely, KRAS G12D is the most prevalent mutation, yet selective inhibitors like MRTX1133 remain in early clinical development. Limited efficacy of KRAS G12C inhibitors is linked to EGFR–ERK pathway reactivation, supporting combination strategies with EGFR inhibitors such as cetuximab. Additionally, KRAS inhibitors show greater potency in 3D models. Here, we investigate the effects of MRTX1133 in 2D and 3D CRC models.

We tested MRTX1133 (provided by Mirati Therapeutics) ± cetuximab (5 µg/mL) in KRAS WT (SW48), KRAS G12D (SW48 G12D/+, LS513, Isreco1, LS174T), and KRAS G13D (HCT116) CRC cell lines. MAPK pathway activity was assessed through p-ERK detection (Western blot). Cell viability was measured using CellTiter-Glo assay at 72 h (2D) and 8 days (3D spheroids, ULA U-bottom 96-well plates). Dose-response curves were analysed by non-linear regression and two-way ANOVA. Transcriptomic differences between Isreco1 and LS513 cell lines were analysed via edgeR and KEGG pathway enrichment using public data (ENA: PRJEB57691).

MRTX1133 dose-dependently reduced p-ERK in KRAS G12D cells (10–100 nM at 3 h), showing pathway reactivation after 24 h. In 2D, G12D-mutant cells exhibited IC50 values of 1–15 nM (SW48 G12D: 1.25 nM; LS513: 14.43 nM; LS174T: 10.52 nM), except Isreco1 (100 nM). Cetuximab combination enhanced sensitivity in LS513 (IC50: 0.001 nM) and Isreco1 (IC50: 16.47 nM). RNA-seq revealed enrichment of resistance-related pathways (p53, Hippo-YAP, MAPK, focal adhesion) in Isreco1. Notably, Isreco1 3D models showed higher MRTX1133 sensitivity (IC50: 7.5 nM alone and 1.3 nM with cetuximab).

As previously described for KRAS G12C inhibitors, p-ERK levels rebound after MRTX1133 treatment for 24 h. KRAS G12D and MSS CRC cell lines show improved responses to MRTX1133 in combination with cetuximab. Moreover, for a cell line exhibiting moderate effects in 2D, likely driven by intrinsic resistance mechanisms, higher potency is seen in 3D assays, underscoring the importance of 3D cell–cell interactions in drug response.

VS-7375: A potent and selective inhibitor that targets both the active (GTP-bound, ON) and inactive (GDP-bound, OFF) states of KRASG12D

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 39

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Abstract

KRAS represents the most prevalent oncogenic mutation in solid tumors, including highly fatal cancers such as colorectal adenocarcinoma (50%), pancreatic adenocarcinoma (88%), and lung adenocarcinoma (32%). KRAS mutations are associated with poor prognosis and resistance to conventional therapies. KRASG12D is the most prevalent KRAS mutation, occurring in ~26% of human cancers, followed by KRASG12V (20.7%) and KRASG12C (13%). KRASG12D is particularly frequent in pancreatic cancer (37%), colorectal cancer (12.5%), endometrial cancer (8%), and non-small cell lung cancer (5%). Despite its high prevalence, no FDA-approved selective inhibitors for KRASG12D are currently available, creating a significant unmet clinical need.

VS-7375 is an orally administered, selective small molecule inhibitor of KRASG12D (G12Di) that targets both the active (GTP-bound, ON) and inactive (GDP-bound, OFF) states of KRASG12D. By inhibiting KRASG12D in both its ON and OFF conformations, VS-7375 may provide more comprehensive suppression of KRAS G12D-mediated signaling and tumor progression compared to inhibitors that target predominantly the OFF state (MRTX1133) or exclusively the ON state (RMC-9805).

We evaluated the antitumor activity of VS-7375 in a panel of RAS-less mouse embryonic fibroblasts (MEFs) engineered to express different KRAS mutation variants. In vitro, VS-7375 potently and selectively inhibited proliferation in MEFs expressing KRAS G12D by suppressing ERK and CRAF phosphorylation. Time-lapse proliferation assays confirmed VS-7375 specificity for KRASG12D, accompanied by morphological changes in the cells. Notably, VS-7375 was more potent than other KRAS G12D inhibitors (RMC-9805, MRTX1133) in reducing levels of active KRAS G12D-GTP (ON state) in MEFs expressing human KRAS G12D after 4 hours of treatment, with inhibition maintained up to 24 hours.

Our results support ongoing clinical trials assessing VS-7375 for the treatment of patients with KRAS G12D-mutant cancers (NCT07020221; NCT06500676).

Targeting oncogene cooperation: myc inhibition enhances response and overcomes resistance to kras inhibitors

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 40

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Abstract

Activating mutations in the KRAS oncogene, particularly at G12, are frequent in non-small cell lung cancer (NSCLC, ~30%), with G12C being the most prevalent. KRAS mutations are also highly represented in pancreatic ductal adenocarcinoma (PDAC, ~90%) and colorectal cancer (CRC, ~35%). Although direct KRAS-G12C inhibitors (KRASi), such as sotorasib and adagrasib, have been approved for NSCLC, their efficacy is often limited by both intrinsic and acquired resistance. MYC, a key transcription factor downstream of KRAS, is frequently deregulated in cancer and is known to cooperate with KRAS in tumorigenesis and therapy resistance. To interrogate this oncogenic interplay and evaluate novel combination strategies, we leveraged Omomyc, the only direct MYC inhibitor undergoing clinical evaluation, as a tool to explore the impact of MYC blockade in KRAS-driven cancers.

Using a panel of human NSCLC cell lines, we profiled KRAS-driven signaling and tested the effects of sotorasib, Omomyc, and their combination on proliferation, cell cycle, and apoptosis. Mechanistic insights were obtained through Western Blot and RNA-seq analyses. The combination was also tested in vivo in a NSCLC patient-derived xenograft (PDX) model.

Our results reveal that combined KRAS and MYC inhibition synergistically impairs proliferation and robustly induces apoptosis. Notably, Omomyc sensitized intrinsically resistant cells to KRASi and restored drug sensitivity in cells with acquired resistance to sotorasib. Transcriptomic and proteomic analyses showed a broad reprogramming of oncogenic pathways upon combination treatment. In vivo, the combination of Omomyc with adagrasib led to significantly enhanced tumor regression and delayed tumor growth in the NSCLC PDX model. These findings were corroborated in vitro in KRAS-mutant CRC and PDAC cell lines, highlighting the broad relevance of this therapeutic strategy.

In conclusion, this study underscores the critical cooperation between MYC and KRAS in cancer and demonstrates that direct MYC inhibition can enhance the efficacy of KRAS inhibitors and help overcome resistance.

Complete deletion of Kras in adult mice: a genetic model

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 41

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Abstract

KRAS is the initiating oncogenic event in 30% of lung adenocarcinomas and in 90% of human pancreatic ductal adenocarcinomas (PDACs). PDAC is the third leading cause of cancer-related death in the US, with a 5-year survival rate around 9%, mainly due to late diagnosis and lack of effective treatments. Currently, surgical intervention is the only effective means to improve prognosis. Lung adenocarcinoma, the most common primary lung cancer, remains the leading cause of cancer-related death in the US, with a 5-year survival rate around 12%. Surgical and chemotherapeutic approaches are available, but late diagnosis and tumor heterogeneity complicate treatment.

An important breakthrough has been the development of KRAS inhibitors targeting specific KRAS mutations (G12C and G12D). However, some patients do not respond to these treatments. Inhibitors against the G12V mutation and pan-KRAS inhibitors are also under development. Total ablation of all three RAS genes is lethal, as shown in previous studies, making pan-RAS drugs inevitably toxic. Since pan-KRAS inhibitors are in development, it is important to understand potential side effects of total KRAS inhibition.

To investigate this, genetically modified mice were developed allowing specific ablation of Kras without affecting other Ras isoforms (Hras and Nras). Systemic deletion of Kras in adult (two months old) and aged (one year old) mice did not result in major adverse effects, as mice remained healthy up to 12 months post-deletion. However, detailed histopathological analysis revealed the presence of myelomonocytic metaplasia in all mice. These studies aim to elucidate potential adverse effects of pan-KRAS inhibitors in humans.

Rational targeting of the RAS-MAPK pathway in high-risk recurrent neuroblastoma

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 42

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Abstract

Neuroblastoma is the most common pediatric extra-cranial solid tumor and recurs in over 50% of patients despite multimodal therapy. Sequencing studies have identified pathway alterations arising during treatment that may be associated with drug resistance. More than half of relapse-specific mutations are predicted to activate the RAS-MAPK pathway, suggesting RAS/MAPK inhibition as a therapeutic target for relapsed neuroblastoma.

Previous studies demonstrated that inhibition of SHP2, a bona fide RAS-MAPK pathway activator, is an effective strategy for neuroblastoma. SHP2 inhibitor (SHP2i) monotherapy is most effective in MAPK-altered neuroblastoma (e.g., ALK or NF1 aberrant), and these effects are enhanced with combination therapy with other MAPK inhibitors (e.g., MEKi, ERKi, or ALKi). However, innate SHP2i resistance was observed in tumors harboring an NRAS-Q61K mutation, the most prevalent RAS mutation in primary and relapsed neuroblastoma, and adaptive ALKi resistance emerged via secondary MAPK alterations.

To explore alternative MAPK inhibition strategies, the efficacy of the pan-RAF inhibitor tovorafenib, RAS-GTP inhibitor RMC-6236, RAF/MEK clamp inhibitor avutometinib, and RAS-cleaving engineered biologic RRSP-DTB was assessed in a panel of RAS/MAPK-aberrant neuroblastoma cell lines. Direct inhibition of RAS with RRSP-DTB reduced cell and xenograft tumor growth in MAPK-altered neuroblastoma, including NRAS-Q61K models. RMC-6236 and avutometinib selectively reduced cell growth in neuroblastoma cells harboring KRAS or NRAS mutations or NF1 alterations, and importantly, RAS-MAPK pathway activation was inhibited. Preclinical studies to determine the efficacy of these inhibitors, including tovorafenib, in neuroblastoma models with MAPK alterations and/or adaptive resistance are ongoing.

Overall, this work highlights the importance of genetics/genomics profiling to guide more precise and effective interventions for high-risk neuroblastoma patients.

Targeting oxidative metabolic vulnerabilities in sotorasib-tolerant lung cancer cells

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 43

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[4] Department of Medical Biophysics, University of Toronto, Toronto, Canada
[5] Department of Paediatrics, The Hospital for Sick Children, Toronto, Canada

Abstract

Neuroblastoma is the most common pediatric extra-cranial solid tumor and recurs in over 50% of patients despite multimodal therapy. Sequencing studies have identified pathway alterations arising during treatment that may be associated with drug resistance. More than half of relapse-specific mutations are predicted to activate the RAS-MAPK pathway, suggesting RAS/MAPK inhibition as a therapeutic target for relapsed neuroblastoma.

Previous studies demonstrated that inhibition of SHP2, a bona fide RAS-MAPK pathway activator, is an effective strategy for neuroblastoma. SHP2 inhibitor (SHP2i) monotherapy is most effective in MAPK-altered neuroblastoma (e.g., ALK or NF1 aberrant), and these effects are enhanced with combination therapy with other MAPK inhibitors (e.g., MEKi, ERKi, or ALKi). However, innate SHP2i resistance was observed in tumors harboring an NRAS-Q61K mutation, the most prevalent RAS mutation in primary and relapsed neuroblastoma, and adaptive ALKi resistance emerged via secondary MAPK alterations.

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Overall, this work highlights the importance of genetics/genomics profiling to guide more precise and effective interventions for high-risk neuroblastoma patients.

Preclinical Evaluation of RAS(ON) Multi-Selective Inhibitors as a Therapeutic Strategy for KRAS mutant Cholangiocarcinoma

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 44

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Abstract

Cholangiocarcinoma (CCA) comprises around 3% of all gastrointestinal cancers and features a highly dismal prognosis. Therapies predominantly rely on chemotherapy, and treatment advancements have been stagnant for decades, highlighting the need for innovative strategies. KRAS is among the most prevalent oncogenes in CCA, suggesting a potential role for novel KRAS inhibitors as treatment options. Here, we present preclinical data on RAS(ON) multi-selective inhibitors as a potential therapy for CCA with KRAS mutations.

Human and murine KRAS mutant cell lines, as well as immortalized cholangiocytes expressing KRAS wild-type and mutant alleles (4B, G12, G13, Q61), were used to study the effect of RAS(ON) multi-selective inhibitors: the investigational agent daraxonrasib (RMC-6236) and the preclinical tool compound RMC-7977, in vitro (2D and 3D) and in vivo (CDX, PDX, CDA). RNA-seq and Western blot analyses were conducted to investigate molecular changes and resistance mechanisms. Pharmacokinetic (PK) and pharmacodynamic (PD) analyses were performed in KRAS mutant xenografts treated with RMC-7977 to establish the PKPD relationship in vivo. The tumor immune microenvironment was assessed by multiplex flow cytometry, and compound combinations to overcome resistance were tested in vitro and in vivo. Human and murine cell lines resistant to RMC-7977 were generated and characterized by WES and Western blot.

KRAS mutant CCA cells were dependent on RAS signaling for growth, similar to other KRAS-driven cancers such as lung and pancreatic tumors. RMC-7977 decreased proliferation and viability of mouse and human CCA cells with KRAS mutations and immortalized cholangiocytes expressing various mutant alleles in vitro. Both RMC-7977 and RMC-6236 effectively inhibited tumor growth in human and mouse KRAS mutant CCA models in vivo, impairing proliferation and inducing apoptosis. Immune profiling of RMC-7977-treated CDA showed increased NK, CD8, and CD4 infiltration, and a reduction in PMN-MDSCs, indicative of a less immunosuppressive microenvironment. Combining RMC-7977 with the standard of care regimen (Gemcitabine+Cisplatin+antiPD1) exhibited synergistic antitumor effects, leading to deep regressions in vivo.

Early adaptive resistance to RMC-7977 was mainly driven by MAPK pathway reactivation, including KRAS upregulation, and RTK and SHP2 overactivation. Combination therapy with RMC-7977 and a SHP2 inhibitor showed benefits in both treatment-naïve and resistant CCA lines in vitro and in vivo. Genomic changes in resistant CCA cells involved KRAS, MYC, and EGFR amplification, guiding additional drug combinations with RMC-7977. In summary, these preclinical findings support clinical testing of RAS(ON) multi-selective inhibitors, alone or in combination with standard of care or KRAS-pathway inhibitors, as a promising therapeutic strategy for CCA.

Overcoming the challenges of targeting RAF1: functional and structural insights into PROTAC-mediated degradation

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 45

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Abstract

Despite advances in understanding KRAS-driven lung adenocarcinoma, effective therapies remain limited. RAF1 has emerged as a promising therapeutic target, as its genetic ablation induces tumor regression in vivo. Notably, these effects are related to its kinase-independent role, suggesting that conventional inhibitors may not fully suppress its oncogenic functions. However, development of RAF1 degraders has been limited by factors including insufficient structural knowledge, uncertainties regarding ubiquitination, and the need for in vivo models capable of mimicking RAF1 degradation to validate therapeutic potential.

In this work, several limitations were addressed. In silico predictions, structural mapping, and literature evidence supported the accessibility of potential ubiquitination lysines, reinforcing RAF1's suitability as a PROTAC-mediated target. While identification of selective RAF1 binders for PROTAC design is ongoing, initial efforts have been guided by structural and biochemical insights generated to date.

Due to the lack of selective degraders, the dTAG system was employed as a chemical-genetic tool to eliminate RAF1 in vivo, both to gain insight for degrader development and to validate its therapeutic relevance in a context closer to the clinic. Despite efficient target elimination, tumor regression was not observed, likely due to impairment of RAF1 scaffold functions caused by N-terminal tagging.

These findings underscore the critical role of RAF1's non-catalytic mechanisms and provide a structural and functional framework for rational design of selective degradation strategies. They also demonstrate that chemical-genetic TPD approaches like dTAG are powerful tools to validate targets in vivo; however, factors such as tag placement, protein context, or cellular function must be carefully considered, as overlooking them may prevent results from reflecting the true biological relevance of the target.

RAS/MAPK inhibition shows preclinical efficacy in both ALK and NRAS-mutated neuroblastoma cells

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 46

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Abstract

Neuroblastoma (NB) is a rare pediatric solid tumor of the peripheral sympathetic nervous system. High-risk cases frequently present metastasis and relapse, with poor response to current therapies, resulting in low overall survival. Activating mutations in the RAS/MAPK pathway are enriched in relapsed NB and are associated with poor prognosis, highlighting the potential of targeted inhibitors against key regulators of this pathway as novel therapeutic options.

This study assessed the potential therapeutic effects of RAS/MAPK inhibition in high-risk NB cell models. SOS1, SHP2, and pan-RAS(ON) inhibitors were tested in both ALK-mutated (RAS wild-type) and NRASQ61K NB cell lines. Single-agent SOS1 or SHP2 inhibition slightly reduced proliferation in ALK-mutated NB cells with variable impact on RAS/MAPK signaling. Combination treatment produced a significant antiproliferative effect via potent suppression of RAS/MAPK pathway activation, suggesting a synergistic mechanism. Preliminary migration assays indicated that dual SOS1/SHP2 inhibition also impaired cell migration in these cells.

NRASQ61K NB cells were resistant to SOS1/SHP2 inhibition individually or in combination. It was hypothesized that NRASQ61K impaired GTP hydrolysis underlies this resistance, but these cells were sensitive to pan-RAS(ON) inhibitors, such as RMC-6236, which potently reduced cell viability and disrupted RAS/MAPK signaling. This effect was milder in ALK-mutated NB cells.

Overall, these results emphasize the importance of characterizing mutational status to select appropriate therapeutic strategies in RAS-dependent high-risk NB and suggest potential targeted therapies for ALK- or NRASQ61K-driven NB with poor prognosis, supporting personalized medicine approaches in this patient population.

G-domain Divergence in KRAS, MRAS, RRAS, and RRAS2: New Insights into RAS Family Druggability Beyond KRAS

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 47

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Abstract

Extensive drug discovery efforts have focused on targeting KRAS, while its closely related small GTPases — MRAS, RRAS, and RRAS2 — remain relatively understudied. All three are implicated in RASopathies, and recent findings suggest an emerging role in cancer, particularly for RRAS2. Although these GTPases share high structural similarity, notable differences in effector protein signaling and nucleotide-binding properties have been reported. A detailed understanding of these differences is critical to uncover the unique characteristics of these small GTPases, which currently lack targeted therapies.

In this study, we investigate the G-domain behavior of KRAS and its close relatives — MRAS, RRAS, and RRAS2 — using long-timescale all-atom molecular dynamics (MD) simulations. These simulations capture the conformational dynamics of these highly flexible proteins at atomic resolution, revealing subtle shifts in their conformational ensembles. Over one millisecond of cumulative simulation time, complemented by selected biochemical assays, we observed substantial variation in G-domain dynamics among these related proteins.

We identified key differences in nucleotide binding and switch region behavior, which may influence effector interactions, downstream signaling, and the dynamics of the switch-II pocket, a known druggable site in KRAS. Our findings illuminate the structural and dynamic features that distinguish MRAS, RRAS, and RRAS2 from KRAS, providing insights for future drug discovery efforts targeting these understudied members of the RAS subfamily.

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Targeting sotorasib resistance in KRAS-mutant lung cancer

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 48

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Abstract

KRAS mutations are the most common oncogenic drivers in lung adenocarcinomas (LUADs), present in up to 32% of cases and associated with poor prognosis. The approval of sotorasib, a selective KRASG12C inhibitor, marks a therapeutic advance for this subset of patients. However, resistance develops rapidly, and the clinical benefit remains limited, highlighting the urgent need to define underlying mechanisms and design more effective treatments.

Previous work from our lab demonstrated that advanced LUADs display strong dependence on the KrasG12V allele, as its ablation induced extensive regression. To determine whether this dependency extends to other Kras variants, we developed a novel genetically engineered mouse model (GEMM) (Kras+/FSFloxG12Clox; Trp53FRT/FRT; Rosa26-CreERT2KI/KI; Tg.hUBC-CreERT2+/T), allowing for conditional deletion of the KrasG12C allele. Using this system, we demonstrated that both treatment-naïve and sotorasib-resistant tumors remain highly dependent on KrasG12C for initiation, survival, and maintenance. Importantly, genetic ablation of KrasG12C triggered nearly complete and durable tumor regression, highlighting persistent oncogenic addiction to this allele, even in the context of acquired resistance.

Since direct genetic deletion is not clinically feasible, and new generations of RAS inhibitors remain under study, we investigated combination therapies. Tipifarnib—a farnesyltransferase inhibitor—has shown promising results in patient-derived xenografts in combination with sotorasib. Using our GEMM, we tested sotorasib/tipifarnib in a physiologically relevant, immune-competent environment mimicking human disease. Prolonged treatment led to significant and sustained tumor regression for over six months, with only 8% tumor recurrence. This improvement over monotherapy highlights the value of targeting complementary pathways to extend KRAS-directed responses.

In conclusion, our results establish persistent KRAS oncogenic addiction in advanced LUADs and demonstrate that combination therapy can markedly improve the durability of KRAS-targeted treatment.

Characterising the therapeutic vulnerabilities of KRASG13X mutant lung adenocarcinoma

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 49

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Abstract

KRAS is the most commonly mutated driver oncogene in lung adenocarcinoma (LUAD; ~30% of patients). The biological diversity of KRAS signalling/function depends on the hotspot codons (12, 13, 61) and its specific point mutations. Codon 13 is the second most frequent mutational hotspot (~7% of KRAS-mutant LUAD). Co-occurring inactivating mutations in the NF1 GAP are frequent in KRASG13X mutant cancers (~20–50%) and may modulate tumorigenic potential and sensitivity to anticancer therapy.

There are currently no approved treatments for KRASG13X mutant LUAD. This project aims to investigate therapeutic vulnerabilities to single agents or drug combinations applicable to this patient population.

As no mutant-specific KRASG13D inhibitors are currently in development, the effects of treatment with a pan-KRAS degrader, ACBI3, were assessed regarding downstream signalling and cell viability changes. Treatment with ACBI3 caused a rapid drop in KRAS and MAPK cascade output in KRASG13D and KRASG12D human LUAD cell lines, whereas PI3K/Akt/mTOR signalling did not appear to change significantly. Both in KRASG13D and KRASG12D cell lines, treatment with ACBI3 led to ~30–40% reduction in cell viability.

Next, functional consequences of NF1 depletion were investigated by RNA interference in KRASG13D cell lines. Some trends toward higher MAPK and PI3K/Akt/mTOR cascade outputs were observed, although these were not conclusive. NF1 knockdown did not consistently increase viability; a follow-up experiment will compare therapeutic effects of KRAS inhibition in cells with normal and depleted NF1 expression.

Drug repurposing screens provide preclinical evidence of activity against new targets for drugs already tested for safety and dosage. An unbiased high-throughput drug screen on a KRASG13D cell line panel is planned to identify drugs with promising activity as single agents or in combination with a pan-(K)RAS targeting agent.

This initial work provides foundations for further investigation into KRASG13X-targeting therapies, with potential implications for addressing an unmet clinical need in precision oncology.

Direct comparison of inhibition versus degradation of oncogenic KRAS

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 50

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Abstract

KRASG12C inhibitors were recently approved for clinical use for treatment of non-small cell lung cancer (NSCLC), colorectal cancer (CRC), and pancreatic adenocarcinoma (PDAC). However, only 41% to 53% of NSCLC patients respond to KRAS inhibitors, and this percentage is lower in other cancer types. Furthermore, after 6 to 9 months, drug resistance to these therapies occurs. Proteolysis targeting chimeras (PROTACs) differentiate from classical inhibitors because they can degrade their target through recruitment of the ubiquitin-proteasome system, potentially making them more effective than inhibitors in KRAS-driven cancers.

To assess differences between inhibition and degradation of mutant KRAS, we applied a multi-omics approach on RAS-less cells expressing KRASG12C targetable with the PROTAC dTAG-13 (KRasloxKRASG12C-dTAG cells). Cells were treated up to 48 hours with KRASG12C inhibitors or the degrader, and RNA sequencing, quantitative expression proteomics, and phospho-proteomics were performed. Kinome analyses assessed differences in kinase activity, revealing distinct molecular rewiring between inhibition and degradation that could be exploited to improve treatment efficacy.

Using the same model, cells resistant to two approved KRASG12C inhibitors (sotorasib and adagrasib) were generated. These cells retained partial sensitivity to PROTAC treatment, with response variations depending on the inhibitor that caused resistance, indicating that sotorasib and adagrasib induce resistance through different mechanisms. In both cases, KRAS degradation remained a viable option for second-line treatment.

Further investigations will be conducted in vivo to determine whether PROTACs generate a more active immune environment than approved inhibitors, potentially enhancing the response to KRAS-targeted therapy.

Title: To be defined

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 51

C. Díaz-González, I. Fernández-Pisonero, C. Castilla-Rodríguez, M. Vicente-Manzanares, C. Cuesta, E. Castellano, and X. R. Bustelo

Abstract

RAS is the most frequently mutated oncogene across all human cancers, yet effective clinical targeting remains a challenge due to drug resistance and toxicity. The SHOC2-MRAS-PP1 (SMP) complex has emerged as a promising therapeutic vulnerability in RAS-driven cancers. SHOC2 knockout has been shown to impair proliferation in KRAS-mutant models and enhance sensitivity to MEK inhibitors, widening their therapeutic index.

Recent data from the Cancer Dependency Map (DepMap) and other studies suggest functional diversity among RAS mutations. Notably, KRAS Q61 and KRAS G13D mutations may confer increased dependency on the SMP complex and be more responsive to its inhibition.

We used a CRISPR knock-in approach to generate isogenic mouse models harboring the most prevalent RAS oncogenic mutations. Using CellTiter-Glo viability assays, we assessed the impact of SHOC2 genetic ablation, targeted protein degradation, and pharmacological inhibition alone and in combination with MAPK pathway inhibitors, including MEK and KRAS inhibitors.

Our results show that loss or inhibition of SHOC2 significantly sensitizes cells to both MEK and KRAS inhibitors. This sensitization was consistently observed across genetic, degradative, and pharmacologic strategies targeting the SMP complex. Importantly, the effect was particularly pronounced in KRAS Q61 and KRAS G13-mutant cells, aligning with predictions from the DepMap project.

These findings support the therapeutic potential of co-targeting the SMP complex with MEK or KRAS inhibitors to overcome resistance in RAS-driven cancers. This strategy may be especially beneficial for patients with KRAS G13D or KRAS Q61H mutations and merits further translational investigation.

Dual SOS1/BCR-ABL1 Inhibition: A Synergistic Strategy to Counter Imatinib Resistance in CML

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 52

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Abstract

Despite the success of BCR-ABL1 tyrosine kinase inhibitors (TKIs) like imatinib in treating chronic myeloid leukemia (CML), therapeutic resistance and disease persistence remain significant challenges. This study investigated the potential of BI-3406, a selective SOS1 inhibitor (a key activator of RAS signaling), either alone or in combination with imatinib.

Using preclinical CML models, including transgenic mice and patient samples, it was observed that BI-3406 and imatinib administration were well-tolerated. Although both drugs partially reduced splenomegaly and leukocytosis individually, combination therapy showed a significantly greater therapeutic effect. This combination extended survival, normalized spleen architecture, and sharply reduced leukemic stem and progenitor cells, suggesting a synergistic impact on leukemic burden and stem cell compartments.

In vitro assays confirmed that BI-3406 potentiated the cytotoxic effects of imatinib in CML cell lines, increasing apoptosis. Notably, in primary bone marrow cells from patients with the imatinib-resistant T315I mutation, BI-3406 not only synergized with ponatinib but also restored sensitivity to imatinib, offering a potential strategy to overcome drug resistance.

RNA sequencing revealed that BI-3406 alone triggered a compensatory anabolic transcriptional program, while imatinib suppressed key proliferative and metabolic pathways. However, combination therapy produced more profound reprogramming, simultaneously reinforcing anti-proliferative effects and activating immune and inflammatory pathways. These conserved effects across murine and human samples support the translational potential of this dual-targeting strategy.

In conclusion, SOS1 inhibition with BI-3406, in combination with TKIs, dismantles oncogenic signaling, suppresses leukemic stemness, and reprograms the immune microenvironment. This dual-targeting approach holds promise as a novel therapeutic strategy to enhance TKI efficacy and overcome resistance in CML.

Work supported by grants from Boehringer Ingelheim OpnMe, ISCIII-MCUI (FISPI22/01538); JCyL (SA264P18 & SA222P23 to UIC 076); ISCIII-CIBERONC (group CB16/12/00352). Research co-financed by FEDER funds and supported by the Programa de Apoyo a Planes Estratégicos de Investigación de Estructuras de Investigación de Excelencia of Castilla y León (CLC-2017–01) and AECC Excellence program Stop Ras Cancer.

Preclinical and clinical targeting of KRAS mutant cancers with the KRAS G12D (ON/OFF) inhibitor VS-7375 (GFH375) and the combination of the RAF/MEK clamp avutometinib with the FAKinhibitor defactinib

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 53

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Abstract

Multiple therapeutic strategies are being evaluated for KRAS mutant cancers. The combination of the RAF/MEK clamp avutometinib and FAK inhibitor defactinib is being assessed in several indications.

In a KRAS mutant low-grade serous ovarian cancer (LGSOC) PDX model, RNASeq analysis showed that addition of FAK inhibitor with avutometinib induced deeper inhibition of RAS/MAPK signaling and abrogated multiple adaptive resistance pathways including PI3K, MYC, and TEAD, which were each induced by avutometinib alone. Accordingly, in patients with recurrent KRAS mutant LGSOC, the combination of avutometinib and defactinib resulted in a greater response rate (44%, 25/57) than avutometinib alone (NCT04625270), leading to FDA accelerated approval of this combination for recurrent KRAS mutant LGSOC in May 2025.

The combination of avutometinib and defactinib is also being explored in pancreatic ductal adenocarcinoma (PDAC). In a KPC-based model of KRAS mutant PDAC, the combination of avutometinib, FAK inhibitor, and chemotherapy induced strong reductions in primary tumors and liver metastases, supporting an ongoing phase 2 study in patients with treatment-naïve metastatic PDAC (NCT05669482). The combination of avutometinib, defactinib, gemcitabine, and nab-paclitaxel showed an 83% (10/12) response rate, supporting an ongoing expansion phase. FAK inhibition also augmented the anti-tumor efficacy of KRAS inhibitors including sotorasib or RMC-6236 in preclinical models.Additionally, VS-7375, an oral selective dual inhibitor of the ON (GTP) and OFF (GDP) states of KRAS G12D, has shown encouraging preclinical and clinical efficacy. In KRAS G12D xenograft models, VS-7375 showed deeper regression and more sustained tumor growth inhibition compared with maximal doses of the ON-only inhibitors RMC-9805 or RMC-6236.

Clinically, in patients with KRAS G12D pancreatic and lung cancers, VS-7375 monotherapy exhibited compelling initial overall response rates (confirmed and unconfirmed) of 52% (12/23) and 42% (5/12), respectively, with a manageable safety profile (NCT06500676, China). Clinical evaluation of VS-7375 is now ongoing in the US (NCT07020221)..

Targeting SHP2 Mutants: Structural and Functional Challenges of Orthosteric Inhibition

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 54

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Abstract

Background: SHP2 is a non-receptor protein tyrosine phosphatase encoded by PTPN11, known for its critical role in RAS activation and MAPK/ERK pathway propagation. Gain-of-function mutations in PTPN11 lead to hyperactive signaling and are implicated in Noonan syndrome and various cancers. Although several SHP2 inhibitors have been developed, only allosteric inhibitors have reached clinical trials. However, these compounds are largely ineffective against SHP2 mutants with activating mutations, as they rely on stabilizing the protein's autoinhibited conformation. This underscores the need for effective and selective orthosteric inhibitors that can target mutant forms of SHP2.

Aims: Our study aimed to characterize the binding of orthosteric inhibitors to SHP2's catalytic domain and evaluate their effects on both wild-type and oncogenic mutant variants.

Methods: Using molecular docking, we modeled inhibitor binding, and employed a DIFMUP-based phosphatase assay to assess their functional impact on wild-type SHP2 and four activating mutants.

Results: Unexpectedly, some orthosteric compounds enhanced phosphatase activity in mutant SHP2 variants. Structural models suggest that certain inhibitors may disrupt the protein's autoinhibition by weakening the interface between the catalytic and SH2 domains. Moreover, inhibitors such as PHPS1 exhibited non-classical inhibition kinetics, with sigmoid-shaped dose–response curves, suggesting atypical binding stoichiometry and possible multivalent interactions.

Conclusion: These findings highlight that orthosteric inhibitors can unintentionally destabilize SHP2 autoinhibition and activate the protein, especially in its mutant forms. Therefore, future drug design should prioritize compounds that preserve or reinforce autoinhibition and consider interactions beyond the catalytic site, particularly with the nSH2 domain. Avoiding stacking behavior and achieving precise binding stoichiometry may also improve potency and selectivity against oncogenic SHP2 variants.

Funding: Project no. RRF-2.3.1-21–2022-00015 has been implemented with the support provided by the European Union. Supported by the KDP-2021 Program of the Ministry of Innovation and Technology from the source of the National Research, Development and Innovation Fund.

Observing the Switch-II Pocket via a Computational Microscope: Ligand-Specific Insights from Molecular Dynamics Simulations

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 55

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Abstract

Background: Targeting the small GTPase RAS has long posed a therapeutic challenge due to its cryptic and dynamic nature. The discovery of the switch-II pocket (SII-P) has enabled the development of small-molecule inhibitors, including two FDA-approved drugs and several candidates in clinical trials. Despite the availability of over 100 co-crystal structures of SII-P inhibitors in the RCSB Protein Data Bank, these static snapshots offer limited insight into the pocket's dynamic and enigmatic behavior.

Methods: Microsecond timescale all-atom molecular dynamics (MD) simulations provide a virtual alternative to capture the conformational flexibility of SII-P in atomistic detail. In this contribution, we highlight key findings from two studies leveraging this approach.

In the first study, we evaluated the similarities and differences in SII-P binding between sotorasib and adagrasib by applying biochemical, cellular, and computational methods. Approximately 180 µs of MD simulations across various RAS isoforms and mutations revealed mechanistic insight into isoform specificity and resistance mechanisms driven by secondary mutations.

In the second study, we predicted the binding modes of divarasib and olomorasib to KRAS(G12C) using 200 µs of MD simulations. These predictions were validated biochemically, confirming high-affinity binding and revealing distinct susceptibility profiles to resistance-associated co-mutations and isoform selectivity.

Conclusion: Our findings demonstrate that microsecond timescale MD simulations can accurately predict binding interactions and provide valuable mechanistic insight into resistance profiles and isoform selectivity within the dynamic SII-P context.

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Pharmacological targeting of kras signaling at three nodes leads to complete and durable pancreatic cancer regression

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 56

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Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) has one of the lowest cancer survival rates. Recent studies using RAS(ON) inhibitors as single agents have opened the door to better and more efficacious therapies.

Methods and Results: Previous work demonstrated that genetic ablation of three independent nodes involved in downstream (RAF1), upstream (EGFR), and orthogonal (STAT3) KRAS signaling pathways leads to complete and permanent disappearance of orthotopic PDACs induced by KRAS/TP53 mutations.

Similarly, a combination of selective inhibitors targeting RAS(ON) (daraxonrasib), EGFR (afatinib), and STAT3 (SD36) induced effective regression of orthotopic tumors, with no evidence of tumor relapse for over 200 days post-treatment. This combination therapy also led to significant regression of autochthonous tumors.

The efficacy of this therapeutic strategy was further validated using patient-derived organoids (PDOs) and tumor xenografts (PDX). Equally important, the combination therapy was well tolerated.

Conclusion: These results may support the development of clinical trials that could provide significant benefit to PDAC patients.

Defining a "one-two-punch" approach for RAS-targeting therapies

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 57

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Abstract

Background: Recent advances in targeting the previously "undruggable" RAS oncogenes have transformed lung cancer treatment; however, resistance almost invariably emerges, limiting durable responses.

Mechanism: Growing evidence highlights the role of non-genetic adaptations and cellular plasticity in enabling a rapid and reversible drug-tolerant state in response to RAS inhibition (RASi). This transient state allows cancer cells to evade initial therapy, facilitating the emergence of resistant clones and ultimately driving patient relapse. Targeting this reduced pool of RASi-tolerant cells could help achieve long-term responses in KRAS-mutant lung cancer patients.

Approach: A "one-two-punch" strategy has been proposed for the eradication of drug-resistant cells. This involves sequential administration of two drugs to maximize efficacy by targeting cells arrested by the initial treatment with a second "punch".

Methods and Preliminary Results: A high-throughput phenotypic screening workflow has been implemented to identify compounds that selectively kill lung cancer cells surviving treatment with RAS inhibitors. Preliminary results and ideas for further development will be presented.

Dysregulation of RAS degradation limits the efficacy of RAS inhibitors by reprogramming protein dosage reprograms amino acid sensing machinery

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 58

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Abstract

Activating KRAS mutations drive nearly 30% of lung adenocarcinomas. Despite promising advances in anti-RAS inhibitors, intrinsic resistance remains a critical barrier to achieving full therapeutic efficacy. Unraveling the mechanisms of this drug resistance is key to improving response rates and patient outcomes.

We show that increased dosage of wild-type KRAS (KRAS-WT) protein in KRAS-mutant lung adenocarcinomas leads to intrinsic resistance to RAS inhibitors. KRAS-WT dosage can increase through either loss of LZTR1, a regulator of RAS GTPase stability, or treatment with the pan-RAS inhibitor RMC7977, both of which impair RAS degradation.

Elevated KRAS-WT activates mTOR/HIF1α signaling, promoting vascular remodeling and contributing to therapeutic resistance. mTOR upregulation occurs via a SLC3A2/SLC7A5-dependent mechanism, highlighting the critical role of wild-type KRAS dosage in lysosomal amino acid transport. Shallow deletion of LZTR1 occurs in up to 50% of KRAS-mutant lung cancer cases and is associated with high mTOR activity, explaining the frequent intrinsic resistance to RAS inhibition.

Blocking the mTOR pathway with dactolisib or inhibiting the SLC3A2/SLC7A5 complex with JHP203 synergizes with RAS-targeted therapies, enhancing efficacy by overcoming resistance mechanisms. These combination approaches hold promise for maximizing therapeutic outcomes in KRAS-mutant lung adenocarcinomas..

b1 integrin inhibition inhibits vessel co-option in k-ras driven lung cancers

SESSION 9: Tumor microenvironment POSTER Nº: 59

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Abstract

During tumor growth, the demand for oxygen and nutrients increases, requiring a vascular network capable of supplying these resources to tumor cells. The main vascularization mechanism, tumor angiogenesis, involves the formation of new blood vessels from pre-existing ones. Antiangiogenic therapy, developed to counteract this process, has entered clinical practice but faces significant limitations. One such limitation is non-angiogenic tumor growth, observed in many tumors and often emerging as resistance to antiangiogenic therapy.

Vessel co-option (VCO) is a non-angiogenic vascularization mechanism occurring in highly vascularized tissues such as the lung, liver, and brain, where tumor cells hijack pre-existing blood vessels instead of generating new ones. A key factor regulating VCO is the adhesion of tumor cells to existing blood vessels, a process mediated by β1 integrin.

Our study aimed to identify VCO in non-small cell lung cancer (NSCLC) using commonly employed experimental models driven by K-ras mutations and to evaluate a VCO inhibition strategy based on β1 integrin blockade.

We found that the orthotopic injection of KPB6 cells (K-ras mutant, p53-deficient) in NSCLC models produces an alveolar VCO phenotype (cancer cells filling the lung alveoli), while the spontaneous K-ras^LA2 model displays an interstitial VCO phenotype (cancer cells growing in the alveolar walls). β1 integrin inhibition in the KPB6 model induces transdifferentiation toward neoangiogenesis, whereas in the K-ras^LA2 model it reduces VCO and thereby tumor growth.

In both models, the inhibition of β1 integrin promotes a favorable tumor microenvironment, allowing the combination of β1 integrin inhibitors with chemotherapy to enhance treatment efficacy.

Our findings highlight the utility of K-ras–driven lung tumor models to study human tumors that develop VCO and demonstrate the therapeutic potential of β1 integrin inhibition for their treatment.

Profiling Reveals Spatial KRAS Modulation of the Tumour Immune Microenvironment and Prognostic Networks in Lung Adenocarcinoma

SESSION 9: Tumor microenvironment POSTER Nº: 60

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Abstract

Oncogenic KRAS mutations modulate the tumour immune microenvironment (TIME) through multiple mechanisms, promoting both pro-inflammatory carcinogenesis and immunosuppression that facilitates immune escape. Investigating KRAS-induced immunomodulation is crucial for understanding cancer progression, identifying therapeutic targets, and overcoming resistance to immunotherapy.

Advancements in spatial imaging technologies now enable detailed investigation of TIME crosstalk. Here, we leveraged single-cell, multi-modal spatial data to profile the immune microenvironment of KRAS-mutant and wild-type lung adenocarcinoma (LUAD).

The TIME of 64 early-stage LUADs (35 KRAS-mutant, 29 KRAS wild-type) was analyzed using imaging mass cytometry (IMC). A 36-antibody panel, including phenotypic (tumour, lymphoid, myeloid, structural) and functional (cell signalling, cell state, immune checkpoint) markers, was used to characterize 180 tumour cores of 1 mm^2 each. Computational packages (Squidpy, QUICHE) were employed to identify cell populations, expression profiles, and spatial clusters. Differential spatial enrichment analysis was performed to identify cellular neighbourhoods associated with KRAS mutation status and patient survival outcomes.

Over 800,000 cells were annotated and classified into 17 distinct cell populations. While some populations—including pan-CK high, neutrophils, and monocytes—were enriched in KRAS-mutant tumours, overall population diversity did not significantly differ (Simpson's diversity index $p = 0.5833$). Higher expression of markers associated with proliferation (Ki67, pS6), adhesion/migration (CD44, ICAM1), and immunomodulation (CD38, CD74) was observed in KRAS-mutant cancers. Differences in cell-specific spatial organization were statistically associated with overall survival and KRAS mutation status (median FDR < 0.05). Patients with longer overall survival exhibited sparser cellular networks, whereas deceased patients displayed densely interconnected TIMEs enriched with myeloid cells.

In conclusion, KRAS mutations reshape the tumour immune microenvironment through distinct changes in cellular composition, marker expression, and spatial organization. The identified KRAS-driven immunosuppressive features and the prognostic value of spatial cellular networks offer key insights into disease progression and highlight novel targets for therapeutic intervention in lung adenocarcinoma..

Microbiota-driven impact on anticancer therapy: Lacticaseibacillus rhamnosus GG in KRAS G12C preclinical models

SESSION 9: Tumor microenvironment POSTER Nº: 61

Rossella Scardaci[1], Roberto Mignacco[1], Pietro Scaparone[1], Alessandro Scagliotti[1], Ilenia Savinelli[1], Enrico Patrucco[1], Paola Cappello[1], Chiara Ambrogio[1]

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Abstract

KRAS mutations are frequent in lung adenocarcinomas (LUADs) and correlate with poor prognosis. Although targeted therapies for G12C mutations have been approved, resistance remains a major clinical challenge. Emerging evidence suggests that tumor-induced changes in the microbiota may influence both disease progression and therapeutic response.

In KRAS-driven LUAD mouse models, tumor-associated microbiota shifts have been shown to exacerbate disease. Notably, pulmonary delivery of Lacticaseibacillus rhamnosus GG (GG) reduced metastasis and enhanced therapeutic efficacy. Here, we present novel findings on the effects of GG on KRASG12C-driven cancer cells, both in vitro and in vivo, aiming to elucidate tumor–microbiota interactions and their contribution to treatment resistance.

GG treatments, when combined with the targeted inhibitor sotorasib (AMG), partially restored drug sensitivity in KRASG12C-resistant LUAD cell models (AMGR cells). In a subcutaneous mouse model, the probiotic demonstrated efficacy both as a standalone therapy and in combination with AMG, whether administered topically or via oral gavage. Additionally, in a lung homing model, intranasal GG delivery modulated the tumor microenvironment toward a more pro-inflammatory state (M1 > M2 macrophages and TH1 > TH2 T helper cells), accompanied by activation of the humoral immune response.

In summary, GG treatment appears to overcome tumor resistance to AMG in vitro and in vivo, with its therapeutic effects potentially linked to immune system recruitment. While the key molecular effectors remain to be determined, these findings lay the groundwork for developing novel strategies to overcome therapeutic resistance and deepen our understanding of KRAS-driven tumorigenesis and microbiota–cancer interactions in LUAD.

Targeting the SHOC2–RAS interaction in RAS-mutant cancers

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 62

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Abstract

Activating mutations in the rat sarcoma (RAS) genes—HRAS, NRAS, and KRAS—collectively represent the most frequent oncogenic drivers in human cancer. While previously considered undruggable, recent advances have led to the clinical development of mutant-selective KRASG12C and KRASG12D targeting agents, which have demonstrated promising therapeutic responses at tolerated doses. However, clinical agents selectively targeting NRASQ61*—the second most frequent oncogenic driver in melanoma—are still lacking.

Here, we identify SHOC2, a component of the SHOC2–MRAS–PP1C (SMP) complex, as a dependency of RASQ61* tumors in a nucleotide-state-dependent and isoform-agnostic manner. Mechanistically, we found that oncogenic NRASQ61R forms a direct interaction with SHOC2, for which we provide the first X-ray co-crystal structure.

In vitro high-throughput screening enabled the discovery of small molecules that bind to SHOC2 and disrupt its interaction with NRASQ61*. Structure-based optimization led to a cellularly active tool compound showing inhibition of MAPK signaling and proliferation in RAS-mutant cancer models, most notably in NRASQ61* contexts.

These findings provide evidence for a neomorphic SHOC2–RAS protein interaction that is pharmacologically actionable and critical for cancer survival. Overall, this work establishes concept validation and a foundation for developing new therapies targeting the core of the RAS signaling pathway.

SOS1 & KRASG12D inhibition synergizes in vitro in KRASG12D-mutated PDAC cell lines

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 63

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Abstract

KRAS is the most frequently mutated RAS oncogene in human cancers, and numerous KRAS inhibitors have been developed in recent years. KRASG12C inhibitors are already in clinical use, and approval is also being sought for inhibitors targeting other prevalent KRAS mutations, including G12D and G12V. However, the therapeutic assessment of these inhibitors has shown limited efficacy and rapid resistance onset, suggesting that combination therapies targeting additional upstream or downstream components of the RAS pathway may be needed to improve therapeutic outcomes.

SOS1, the main RAS activator in mammals, is highly promising in this regard, since we previously demonstrated that SOS1 inhibition—either genetic or pharmacological—displays significant intrinsic and extrinsic antitumor activity and enhances the therapeutic efficacy of KRASmut inhibitors.

Here, we have assessed the potential therapeutic synergy of the specific SOS1 inhibitor BI3406 when combined with the KRASG12D inhibitor MRTX1133 in different pancreatic ductal adenocarcinoma (PDAC) cell lines harboring KRAS G12D and Q61H mutations. Using Incucyte assays, we determined the IC_{50} values for both BI3406 and MRTX1133 and performed synergy assays, observing a strong synergistic behavior of these drugs in 4 of 5 cell lines harboring the KRASG12D mutation. This was correlated with the specific cellular proliferation outcomes observed in each cell line.

To gain a deeper understanding of the mechanisms involved in SOS1 and/or KRASG12D inhibition, we are currently characterizing the effects on cellular signaling and the metabolic impact of this combinatorial drug strategy.

This work has received funding from the Asociación Española Contra el Cáncer Salamanca, ISCIII-MCUI (FISPI22/01538), JCyL (SA264P18 & SA222P23 to UIC 076), and ISCIII-CIBERONC (group CB16/12/00352). Research was co-financed by FEDER funds and supported by the Programa de Apoyo a Planes Estratégicos de Investigación de Estructuras de Investigación de Excelencia of Castilla y León (CLC-2017–01) and the AECC Excellence program Stop Ras Cancer.

3¨UTR Shortening in KRAS Oncogene in human cancer

POSTER Nº: 64

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Abstract

Many studies have shown that cancer cells favor shorter 3 UTRs in oncogenes and proliferation-related genes. Lengthening events also occur with variable frequency in human cancers. KRAS is a prognostic marker in many cancers, and its exceptionally long 3 UTR region possesses numerous cis- and trans-acting elements that serve as genetic biomarkers predictive of prognosis, diagnosis, and treatment outcomes.

In this study, we investigated the differential expression of KRAS 3 UTR isoforms in human cancers using an independent cohort of primary samples. We measured differential isoform abundance and its impact on encoded protein levels.

Methods:
PCR-based data on the relative expression of long and short 3 untranslated regions of KRAS were obtained by designing three specific primers (short 3 UTR, middle 3 UTR, and distal 3 UTR). RT-qPCR analysis was used to measure ΔΔCt KRAS in an independent cohort of human primary samples, including glioblastoma, lung adenocarcinoma and squamous carcinoma, infiltrating breast carcinoma, and colon carcinoma. Additionally, semiquantitative western blot analysis was performed to evaluate the impact on encoded protein levels.

Results:
We found that 3 UTR KRAS transcripts are expressed in a cell-type-specific manner. In particular, glioblastoma primary cultures showed extensive regulation of 3 UTR lengths. We quantified mRNA fold induction in primary and long-term cultures compared to normal human astrocytes (NHA) and across distinct glioblastoma subtypes (stem-like versus non-stem-like). The exceptionally long 3 UTR KRAS transcript was undetectable in almost all samples, with few exceptions such as the mammary epithelial cell line MCF-10. Human breast cancers showed a remarkable 3 UTR KRAS shortening in FFPE tissues compared to commercial cell lines.

Conclusion:
In contrast to its mutational status, the transcriptional landscape of the KRAS gene remains largely unexplored. Recently, multiple novel KRAS transcript isoforms (39 variants) have been identified, though their functional relevance is still unknown. Further analysis will help determine whether KRAS 3 UTR length changes can serve as a genetic biomarker or as a potential target in RNA-based anti-cancer therapeutics.

KRAS and EGFR Mutations Rewire Cancer Associated Fibroblast Programs to Shape the Tumor Microenvironment

SESSION 9: Tumor microenvironment POSTER Nº: 65

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Abstract

Lung adenocarcinoma (LUAD) remains the leading cause of cancer-related mortality worldwide, with KRAS and EGFR as the most frequent oncogenic drivers. Although targeted therapies have improved patient survival, relapse due to resistance mechanisms remains a major clinical challenge, underscoring the need for alternative strategies.

The tumor microenvironment (TME), particularly the extracellular matrix (ECM) and cancer-associated fibroblasts (CAFs), plays a fundamental role in shaping tumor progression, immune infiltration, and therapeutic response. Yet, how specific oncogenic mutations remodel the ECM and reprogram CAFs remains poorly understood.

We investigated this question by combining functional and transcriptomic analyses of primary CAFs co-cultured with lung cancer cells expressing defined oncogenic mutations (KRAS^G12C, KRAS^G12D, KRAS^G12V, and EGFR^L858R). RNA-seq profiling revealed that each oncogene imprints a distinct CAF program. KRAS mutations promoted transcriptional signatures associated with matrix remodeling, contractility, and inflammatory paracrine signaling, with individual variants biasing towards mechanical, immune, or secretory axes. In contrast, EGFR^L858R reprogrammed CAFs towards a secretory and electrogenic phenotype, with increased trafficking and ion channel activity coupled to reduced intercellular communication.

Together with differences observed in ECM organization, these results highlight that oncogenic drivers not only fuel tumor cell growth but also dictate stromal heterogeneity at both structural and transcriptional levels. Distinct mutations generate unique CAF phenotypes—ranging from pro-invasive and pro-inflammatory to secretory states—that shape the TME in functionally diverse ways.

By revealing how tumor genotype orchestrates stromal programs, this work provides insight into the reciprocal interactions between oncogenic signaling and the microenvironment, and points to new therapeutic opportunities aimed at disrupting tumor–stroma crosstalk in LUAD..

Disrupting RAS–PI3K signaling in CAFs reprograms the extracellular matrix and unveils microenvironmental vulnerabilities in KRAS-driven lung cance

SESSION 9: Tumor microenvironment POSTER Nº: 66

Cristina Cuesta[1], Marta Alcón[1], Nicole Prócel[1], Jie Zheng[2], Alejandro Rosell[1], Diana Loa-Mesón[3], Belén Martínez[1], Héctor Sanz-Fraile[4], Robert E. Hynds[5], Charles Swanton[5,6], Jordi Alcaraz[4], Pedro Cutillas[7], Constantino C. Reyes-Aldasoro[8], Haiyun Wang[2], Esther Castellano[1]

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Abstract

The tumor microenvironment (TME) is a critical driver of lung cancer progression and therapeutic resistance. Among its components, cancer-associated fibroblasts (CAFs) actively remodel the extracellular matrix (ECM) and regulate immune dynamics. Using functional 3D models, we demonstrate that disrupting the RAS–PI3K axis in CAFs reprograms their phenotype and the architecture of the ECM they produce, creating a microenvironment hostile to tumor progression.

In murine and human NSCLC-derived CAFs, genetic uncoupling of RAS from PI3K or pharmacological PI3K α inhibition induces a switch from contractile myCAFs to inflammatory CAFs (iCAFs), marked by elevated IL6, FAP, STAT3 and NF-κB signaling, and reduced α-SMA and ECM contractility. Resulting ECMs produced by these CAFs are thinner, disorganized, and deficient in collagen, fibronectin, and glycoproteins, with impaired stiffness and fiber alignment. These alterations disrupt key tumor cell functions—proliferation, EMT, adhesion and migration—when cells are cultured on mutant CAF-derived ECMs.

In vivo, fibroblast-specific RAS–PI3K disruption (Col1a2-CreER; Pik3caRBD/lox) delays KRAS-driven tumor growth, recapitulates CAF and ECM remodeling, hinders macrophage recruitment, and enhances CD8 T cell infiltration, reinforcing the causal role of stromal PI3K signaling.

Altogether, our work reveals a central role for RAS–PI3K signaling in dictating ECM composition and immunomodulatory capacity of the TME. By combining genetic models and 3D functional assays, we expose microenvironmental vulnerabilities that may be exploited to improve NSCLC therapy beyond tumor cell–intrinsic targets..

Functional screening of potential scaffold proteins controlling kras nanoclustering in lung adenocarcinoma

SESSION 2: Unveiling RAS biology

POSTER Nº: 67

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Abstract

Targeting KRAS has been a challenge for decades. After the development of various specific inhibitors against this protein, a new problem has emerged: resistance to these drugs. This has led us to focus on alternative molecular aspects of KRAS activation.

The ability of KRAS to organize into nanoclusters appears to be crucial for its biological activity. However, the mechanisms driving the formation of these complex structures remain unclear. Previous studies suggest that scaffold proteins may contribute to nanocluster assembly.

To address this, we used the A549 lung adenocarcinoma cell line to develop an inducible tripartite fluorescent readout (split-GFP model) triggered by KRAS nanoclustering. This system was coupled with CRISPR knock-out editing to test the role of 24 putative scaffold proteins. These results are being corroborated in KRAS-dependent and -independent lung cancer cell lines.

In parallel, confocal and total internal reflection fluorescence (TIRF) microscopy are being used to evaluate how interfering with the most promising scaffolds affects KRAS nanocluster dynamics. Additionally, the A549 split-GFP model has been employed for an unbiased chemical screen to identify compounds that reduce KRAS membrane clustering. The most promising candidates were characterized for their effects on the MAPK pathway and further evaluated in KRAS-dependent and -independent cell lines. Selected hits will undergo medicinal chemistry refinement to enhance their biological activity.

This research aims to increase mechanistic understanding of KRAS nanoclustering regulation and to develop alternative KRAS-targeted therapies for patients resistant to direct KRAS inhibitors..

Impact of KRASG12V signaling on the tumor microenvironment in autochthonous lung tumors and subcutaneous tumors

SESSION 9: Tumor microenvironment POSTER Nº: 68

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Abstract

Mutations in KRAS are well-established initiating events in lung adenocarcinoma, yet the precise mechanisms by which KRAS drives tumor development and therapeutic resistance remain poorly defined. Signaling through the MAPK/ERK pathway is critical for tumor induction, but additional downstream effectors have not been fully characterized. The transcriptional repressor CIC has recently been identified as an ERK substrate whose activity is suppressed by KRAS/MAPK signaling. One of its key functions is to block the expression of several tumor-promoting genes, including members of the PEA3 transcription factor family. Importantly, loss-of-function alterations in CIC have been identified in a subset of patients with KRAS-mutant lung adenocarcinoma, contributing to resistance against MAPK pathway inhibitors.

CIC inactivation in a Kras/p53 mutant mouse model (KPCic) reduced survival and promoted tumor initiation through lineage conversion of Club cells to an AT2-like phenotype. Tumors displayed reduced allelic imbalance, suggesting that Kras allelic amplification contributes to CIC loss. To test therapeutic strategies, we ectopically reactivated CIC using adenoviral vectors. CIC reactivation suppressed proliferation and re-sensitized cells to the MEK inhibitor trametinib, highlighting the potential of CIC restoration as a therapeutic approach.

RNA-seq analysis revealed that ETV4 and ETV5, two PEA3 family members, are key downstream targets derepressed upon CIC loss. Their combined silencing reduced proliferation in both CIC-deficient and CIC-wt KRAS-mutant cells, and importantly, restored trametinib sensitivity in resistant models. Furthermore, a pharmacological screen identified two inhibitors, PFK15 and Tx-1123, which preferentially impaired proliferation of CIC-deficient KRAS mutant lung cancer cells. When combined with trametinib these compounds restored sensitivity in cell lines and patient-derived xenograft (PDX) models.

Taken together, these findings establish that CIC loss drives resistance to MAPK pathway inhibition through constitutive activation of ETV4 and ETV5. Furthermore, this vulnerability can be therapeutically exploited, offering potential avenues for overcoming resistance in KRAS-driven lung adenocarcinoma.

Metabolic vulnerabilities as targets to enhance the antitumor effect of KRASG12V degradation in LUAD

SESSION 6: Emerging treatments (Preclinical perspective)

POSTER Nº: 69

Alberto Martín[1], Emilie Gaday[1], Sonia San José[1], Cristina Teodosio[3], Inés María García[2], Silvia Rodríguez-López[1], Cristóbal Castilla[1], Nerea Gestoso[1], Cristina Mayor-Ruiz[2], David Santamaría[1]

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Abstract

Unfortunately, the clinical efficacy of novel inhibitors targeting mutant KRAS (mKRAS) has been constrained by the rapid onset of resistance mechanisms. In this context, the advent of KRAS PROTACs (proteolysis-targeting chimeras), able to cause proximity-induced ubiquitination and degradation of KRAS oncoproteins, offers a compelling alternative.

To functionally mimic PROTAC-mediated degradation, we employed a chemogenetic approach utilizing the dTAG system to evaluate the short- and long-term in vivo effects of selective KRASG12V degradation in lung adenocarcinoma (LUAD). While treatment elicited a rapid and pronounced antitumor response, resistance invariably emerged, resulting in limited survival benefit.

Aiming to identify therapeutic vulnerabilities associated with KRAS loss, transcriptomic comparison between degrader-treated and untreated LUAD cells revealed cholesterol homeostasis among the most significantly downregulated categories following KRAS degradation. Cholesterol is one of the final products of the multistep mevalonate (MVA) pathway, which also provides non-sterol isoprenoids essential for the membrane localization of RAS proteins and other small GTPases. Interestingly, combined degradation of KRASG12V and MVA pathway inhibition using statins markedly enhanced cytotoxicity in vitro. Supplementation experiments with cholesterol, squalene, or ubiquinone failed to reverse this effect, whereas MVA, geranylgeranyl pyrophosphate (GGPP), and to a lesser extent farnesyl pyrophosphate (FPP), restored cell viability.

Notably, only inhibition of geranylgeranyl transferase (via GGTI-298), and not farnesyl transferase (via tipifarnib), partially recapitulated the statin-induced phenotype, implicating GGPP-mediated geranylgeranylation as a key mechanism. Among geranylgeranylated proteins, small GTPases such as Rho, Rac, and Rab—central to actin cytoskeleton dynamics—emerged as potential effectors. Preliminary data suggest that macropinocytosis, reliant on actin remodeling, might have a role under combined KRAS degradation and statin treatment since the induced cytotoxic phenotype is considerably alleviated by bovine serum albumin (BSA) supplementation.

Collectively, these findings indicate that disruption of cancer metabolic rewiring imposed by oncogenic KRAS can be therapeutically exploited in a context of mKRAS degradation..

Inactivation of Capicua confers resistance to MAPK pathway inhibition but exposes selective vulnerabilities in KRAS mutant lung cancer cells

SESSION 1: RAS signaling

POSTER Nº: 70

Alejandra A. Flores-Gómez[1], Morena Scotece[1], Irene Ballesteros[1], Matthias Drosten[1]

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Abstract

Activating mutations in KRAS are known to drive tumor cell proliferation. However, growing evidence indicates that KRAS-mutant tumor cells can directly modulate the tumor microenvironment to contribute to tumor progression, in part by promoting immune suppression. Yet, the specific effects of KRAS signaling on the tumor microenvironment in lung cancer and the mechanisms controlling its composition remain poorly understood.

To address this gap, we performed single-cell RNA sequencing of lung tumors grown in Kras+/FSFG12Vlox;Trp53lox/lox;Rosa26-CreERT2KI/KI;hUBC-CreERT2+/T (KG12VloxPC2) mice after intranasal infection with adeno-FLP, either left untreated or after a 2-week treatment with tamoxifen to eliminate the mutant Kras allele. Elimination of KrasG12V reversed the immune-suppressive environment, promoting T cell infiltration, specifically CD8+ cytotoxic T cells, as well as CD4+ T cells, along with an increase of TCR gamma/delta T cells and myeloid-derived cells such as neutrophils and eosinophils. Single-cell results were corroborated by immunohistochemistry assays.

Furthermore, we injected KG12VloxPC2 tumor cells subcutaneously into syngeneic wild-type mice. Tamoxifen treatment for two weeks also led to regression of these tumors, and RNA sequencing of untreated and tamoxifen-treated, regressing tumors confirmed the single-cell data from lung tumors. Moreover, using KG12VloxPC2 tumor cell lines, we determined changes in cytokine secretion in the presence and absence of KRASG12V expression.

In summary, our analysis reveals a direct impact of KrasG12V on the composition of the tumor microenvironment in both autochthonous lung tumors and subcutaneous tumors. Moreover, genetic elimination of KrasG12V affected secretion of a set of cytokines, which could partially explain the impact of KRASG12V signaling on immune suppression.

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