Therapeutic opportunities for Porcupine inhibition in Gastrointestinal cancer

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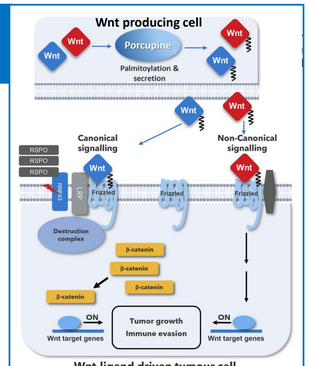
Background

- Gastrointestinal (GI) cancer (Upper/Lower GI and Hepatobiliary) causes more cancer deaths than any other body system, with 3.4 million related deaths in 2018[1].
- Wnt pathway activation promotes common GI cancers, but safely targeting this pathway is challenging.
- Inhibition of Porcupine, an enzyme essential for Wnt ligand activity, has demonstrated the clinical potential to suppress Wnt signalling in a safe and tolerated manner.
- Preclinically, GI tumours with upstream Wnt pathway variants (RNF43 loss-of-function (LoF), RSPO gain-of-function (GoF)) are Wnt ligand-dependent and exquisitely sensitive to RXC004[2], a small molecule Porcupine inhibitor.



Wnt-ligand driven tumour cell

Aims

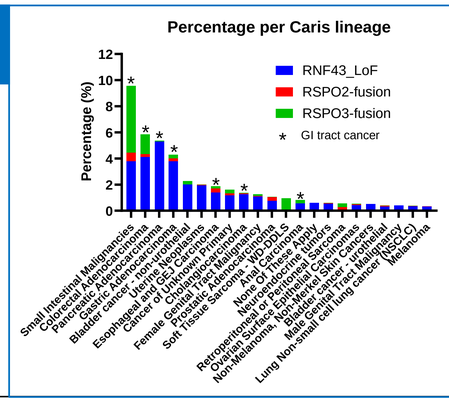
1. To determine the prevalence of upstream Wnt pathway variants across the 58 solid tumour lineages within the Caris Life Sciences database.
2. To determine if upstream Wnt pathway variants are associated with an altered co-mutational profile that could provide for rational combination therapies in the clinic.
3. To determine if upstream Wnt pathway variants are associated with a different real-world overall survival to aid interpretation of clinical data from RXC004 clinical trials.

Methods

- 278,649 human tumours were analysed by next-generation sequencing of DNA (592 genes, NextSeq; WES, NovaSeq) and RNA (WTS, NovaSeq) by Caris Life Sciences
- Stringent criteria were used to define LoF RNF43 and GoF RSPO variants in order to determine the prevalence of upstream Wnt pathway cases, with their molecular profiles compared with negative (WT) cases using Fisher-Exact analysis.
- Real world overall survival was obtained from insurance claims data and Kaplan-Meier estimates were calculated for defined cohorts from time of tissue collection to last contact.

Results

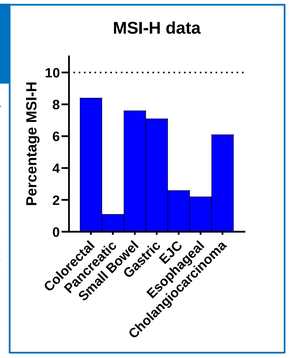
Upstream Wnt pathway drivers (RNF43_LoF variants or RSPO2/3_GoF fusions) were detected in ~1.7% of all solid tumours, increasing to 4.7% of GI cancers. The highest prevalence was seen in Small Bowel (SB) Malignancies (9.6%), followed by Colorectal (CRC; 5.9%), Pancreatic (5.4%), and Gastric (4.3%) Adenocarcinomas. Overall GI cancers accounted for 74% of all upstream Wnt pathway variant cases.

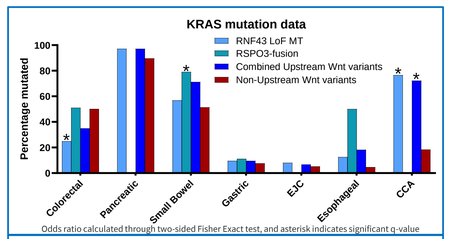


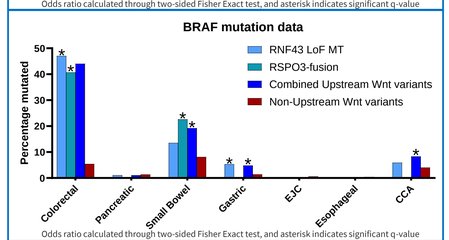
Results

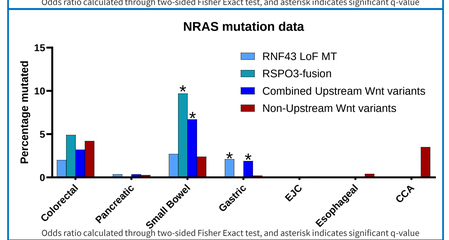
Prevalence of Microsatellite Instability detected across GI cancers

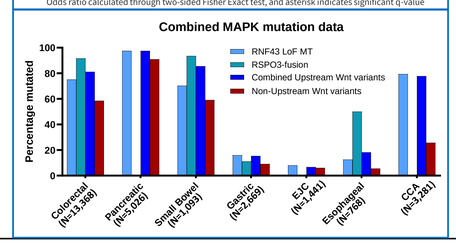
As GI cancers with high microsatellite instability (MSI-H) are immunogenic and receive clear benefit from anti-PD-1 treatment[3] (e.g. pembrolizumab and nivolumab), this reduces the need for novel therapeutics in MSI-H. We determined the prevalence of MSI-H across GI cancers with upstream Wnt pathway drive. MSI-H level were <10% throughout; with highest levels were seen in CRC (8.4%), SB cancer (7.6%), Gastric (7.1%) and Cholangiocarcinoma (CCA; 6.1%). Hence the vast majority of GI cancers are Microsatellite Stable (MSS).











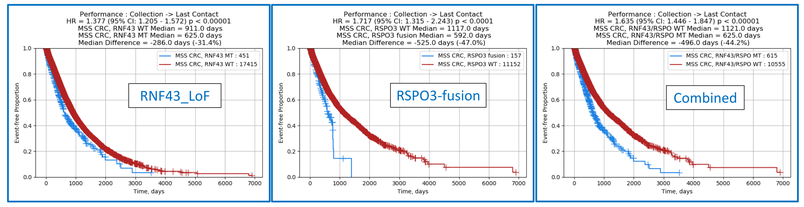
Detailed molecular profiling of Microsatellite Stable GI cancers

- Given the high unmet need in MSS GI cancer, and the limited efficacy of targeted therapies as single agents in GI cancer[4] compared with other cancer types, we assessed mutations that commonly co-occurred with upstream Wnt pathway variants in order to aid design of rational clinical combinations.
- We identified that mutations in three key members of the MAPK pathway (KRAS, BRAF and NRAS) co-occurred with upstream Wnt pathway variants (RNF43_LoF and/or RSPO_GoF) in two or more GI cancers:
  - KRAS mutations were significantly more likely to co-occur with RSPO3-fusions in SB cancer, and RNF43_LoF in CCA.
  - BRAF mutations were significantly more likely to co-occur with RNF43_LoF mutations and RSPO3-fusions in CRC, RSPO3-fusions in SB cancer, and RNF43_LoF in Gastric cancer.
  - NRAS mutations were significantly more likely to co-occur with RSPO3-fusions in SB cancer, and RNF43_LoF in Gastric cancer.
- Upstream Wnt pathway variants co-occurred with at least one MAPK mutation in 98% of pancreatic, 86% of SB, 81% of CRC and 78% of CCA Microsatellite Stable cancers.
- Across all assessed GI cancers with MSS (27,646 total cases), upstream Wnt pathway variants co-occurred with MAPK mutants in **77%** of cases.

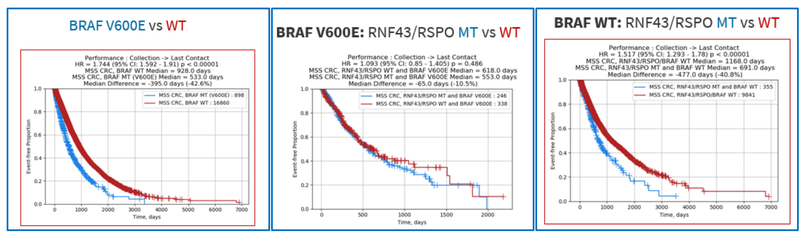
Results (continued)

Real-world OS data for upstream Wnt pathway variants in GI cancer

Detailed real-world data analysis for CRC cases demonstrated that RNF43_LoF mutations [HR 1.38] or RSPO3-fusions [HR 1.72] were associated with a significantly poorer real-world overall survival (OS). This poor real-world OS was also evident in a combined upstream Wnt pathway variant cohort (RNF43_LoF and RSPO_GoF; HR 1.64). In contrast, Pancreatic and Small Bowel cancer cases did not show any significant association of upstream Wnt pathway variants with OS (data not shown).



Given the significant co-occurrence of RNF43_LoF / RSPO3-fusions with BRAF mutations in CRC, and the known association of BRAF_V600E with poor survival in CRC [HR 1.74], we assessed if these effects were independent. Whilst RNF43/RSPO variant status did not affect the poor OS of BRAF-V600E cases, in BRAF wild-type cases these upstream Wnt pathway variants were associated with poor OS [HR 1.52].



Conclusion

1. The prevalence of upstream Wnt pathway variants (RNF43_LoF and RSPO2/3-fusion) was enriched in GI cancers (4.7%), in particular SB, CRC, Pancreatic and Gastric cancers.
2. Upstream Wnt pathway variants are significantly associated with co-mutation in the MAPK pathway (KRAS, BRAF, NRAS) across multiple GI cancers, guiding rational future combination opportunities for this patient subgroup.
3. In Microsatellite Stable CRC, upstream Wnt pathway variants are associated with a significantly poorer overall survival in this real-world data.

The Porcupine inhibitor RXC004 completed Phase 1 evaluation (NCT03447470), demonstrating a well-tolerated profile with differential clinical efficacy in Wnt-ligand dependent tumours[5] (including those with upstream Wnt pathway variants). Ongoing Phase 2 studies (NCT04907851 and NCT04907539) are assessing the efficacy of RXC004 +/- anti-PD-1 therapies in GI cancers.

Acknowledgements:

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References:

[1] Arnold et.al., Gastroenterology (2020); [2] Phillips et.al., Cancer Research Communications (2022); Shimozaki et.al., Cells (2023); [4] Yaeger et.al., NEJM (2023); [5] Cook et.al., Annals of Oncology (2021)

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