CGG/ERN GENTURIS/ICARE Monthly Journal Round-Up – June & July 2022

Translational science

Loss of PTEN-Induced Kinase 1 Regulates Oncogenic Ras-Driven Tumor Growth By Inhibiting Mitochondrial Fission. Zhu *et al.* (2022). *Frontiers in Oncology*; 12. https://doi.org/10.3389/fonc.2022.893396

- Abnormalities in mitochondrial metabolism and dynamics (fission and fusion) are implicated in both neurodegenerative disorders and cancer. Mitochondrial fission is necessary for the growth of mutant Ras-dependent tumors.
- The authors investigated whether loss of PTEN-induced kinase 1 (PINK1) - a mitochondrial kinase linked to recessive familial Parkinsonism - affects the growth of oncogenic Ras-induced tumor growth *in vitro* and *in vivo*.
- They show that Ras_{G12D}-transformed embryonic fibroblasts (MEFs) from PINK1-deficient mice display reduced growth, increased necrosis and decreased cell cycle progression, compared to Ras_{G12D}-transformed MEFs derived from wildtype mice. PINK1 re-expression (overexpression) at least partially rescues these phenotypes. Neither PINK1 deletion nor PINK1 overexpression altered Ras expression levels.
- Their findings suggest that PINK1 deficiency primarily inhibits Ras-driven tumor growth through disturbances in mitochondrial fission and associated cell necrosis and cell cycle defects.
- They also show that PINK1 is required for optimal growth of Ras-transformed cells
- Overall, the results support the importance of mitochondrial function and dynamics in regulating the growth of Ras-dependent tumor cells and provide insight into possible mechanisms underlying the lower incidence of cancers in Parkinson's disease and other neurodegenerative disorders.

In the clinic

The Multicenter Cancer of Pancreas Screening Study: Impact on Stage and Survival. Dbouk *et al.* 2022. *J Clin Oncol.* https://doi.org/10. 1200/JCO.22.00298

- Pancreatic cancer five-year survival is around 11%, and this poor survival is largely attributed to the late stage at diagnosis for most patients; very few patients are diagnosed with stage I PDAC.
- The Cancer of Pancreas Screening-5 (CAPS5) study is a multicentre, prospective cohort study involving eight academic medical centres in the United States. CAPS5 enrolled patients at elevated risk of developing pancreatic ductal adenocarcinoma (PDAC) as per the CAPS international consensus guidelines (surveillance if ≥5% lifetime risk).
    - A small % of patients were enrolled based on other risk criteria, such as a *BRCA2* or *ATM* PV without a FH, or those with other FH criteria (e.g. having 3 SDRs with pancreatic cancer, without an affected FDR). See table 1 in the paper for full characteristics of the cohort.

- Surveillance is typically annual and involves pancreas imaging surveillance with magnetic resonance imaging and endoscopic ultrasound.
- The primary endpoint was the stage distribution of PDAC detected, and the secondary outcome was overall survival after a diagnosis of PDAC or HGD for HRIs.
- Of 1,461 high-risk individuals enrolled into CAPS5, 48.5% had a PV in a PDAC-susceptibility gene.
    - 18.4% had a *BRCA2* PV and 6.4% had an *ATM* PV
    - Around 1/3 of the cohort had a personal history of cancer, with breast cancer being the most commonly reported (15.8% of the entire cohort)
- 10 patients were diagnosed with PDAC, one of whom was diagnosed with metastatic PDAC 4 years after dropping out of surveillance.
    - Of the remaining nine, seven (77.8%) had a stage I PDAC (n = 4 stage IA, n = 3 stage IB) detected by surveillance, one had stage IIB, and one had stage III disease. From the SEER database (national average), in 2016 5.4% of 8,398 PDACs were stage I.
    - 7 of the 9 patients with PDAC are alive, with median OS to date of 3.84 years
    - 8 additional patients underwent surgical resection for worrisome lesions; 3 had high-grade and 5 had low-grade dysplasia in their resected specimens.
- In the entire CAPS cohort (n=1731) over a median follow-up of 2.8 years, 26 PDAC cases have been diagnosed –detection rate of 5.15 PDACs diagnosed per 1,000 person-years of surveillance (1 individual diagnosed with PDAC per year for every 194 screened).
    - 19 of the 26 cases were within surveillance; 57.9% had stage I, 15.8% had stage II, 21.1% had stage III, and 5.2% had stage IV disease. Six of the seven PDACs (85.7%) detected outside surveillance were stage IV, one (14.3%) was stage I.
    - Median age of diagnoses for all PDAC cases was 65.5 years
- 5-year survival to date of patients with a screen-detected PDAC is 73.3%, and median OS is 9.8 years, compared with 1.5 years for patients diagnosed outside of surveillance.
- The authors conclude that most pancreatic cancers diagnosed within the CAPS high-risk cohort in the recent years have had stage I disease with long-term survival, and so recommend that regular pancreatic imaging surveillance *at expert centres by multidisciplinary teams* should be offered to patients who meet recommended pancreatic surveillance criteria.
- They also recommend re-evaluation of the recommendation that a family history of PDAC be required for *BRCA2* and *ATM* PV carriers to be eligible for surveillance, and recommend consideration of more frequent (i.e. biannual) screening for individuals with *CDKN2A* PVs given the more aggressive nature of the disease associated with this gene.

KIT-Associated Familial GIST Syndrome: Response to Tyrosine Kinase Inhibitors and Implications for Risk Management. Brodey *et al*. 2022. *The Oncologist*. https://doi.org/10.1093/oncolo/oyac120

- Sporadic gastrointestinal stromal tumours (GIST) are rare, median age at diagnosis is 60 years, and distribution is equal between males and females.
- Up to 85% of GIST have gain-of-function variants in *KIT* or *PDGFRa* genes. The remaining 15% are associated with other genetic alterations, including in *NF1*, *SDH*, or *BRAF*. Most of these variants are somatic in origin.
    - Treatment with imatinib, a tyrosine kinase inhibitor (TKI), has significantly improved survival for patients with advanced disease from 18 to >70 months

    - Response is dependent on genotype – PVs in *KIT* exon 11 render better response than PVs in *KIT* exon 9. *PDGFRα* p.Asp842Val GIST is known to be resistant to imatinib, but Avapritinib has proven excellent efficacy.
- Familial GISTs are very rare and typically are associated with earlier onset, with an average age at diagnosis of 48 years. Patients often present with multifocal tumours and can be asymptomatic for a long time prior to diagnosis.
    - Families with germline *KIT* variants can also demonstrate variable clinical phenotypes due to mast-cell activation (mastocytosis), which causes urticarial pigmentosa (small tan-red macules on the upper and lower extremities and on the thorax and abdomen).
    - Mast cell disorder can sometimes cause systemic symptoms, such as hepatosplenomegaly, anaemia, dysphagia, as well as anxiety and depression.
- Just over 50 familial cases associated with germline *KIT* or *PDGFRα* variants have been published, which makes managing these patients challenging. Guidelines for type and frequency of surveillance for both affected and unaffected carriers of germline *KIT* PVs are lacking. There is also uncertainty as to whether early diagnosis through asymptomatic screening improves overall survival.
    - Few case reports published to date have documented response to TKIs in familial GIST caused by germline *KIT* variants.
    - The number of known familial GIST cases is likely to increase with the increasing availability of germline testing – GTD criteria for testing (R363): diagnosis <50 or diagnosis any age with ≥1 FDR/SDR/TDR with GIST, phaeochromocytoma, or paraganglioma.
    - Long-term preventative treatment with TKI in patients with a sensitive variant has been proposed, but there is no long-term toxicity data, and studies on murine models have demonstrated potential long-term effects on pregnancy and implantation.
- This case report describes a 53 year-old Caucasian patient diagnosed with multifocal GIST and subsequently found to be heterozygous for a familial PV in *KIT* exon 11.
    - *KIT* alteration initially identified in nephew who presented with unusual progressive hyperpigmentation after his father (patient's brother) developed a GIST in his 40s
    - Patient had baseline abdominal MRI and esophagogastroduodenoscopy which confirmed presence of multifocal GISTs within stomach, oesophagus, pelvis, and small bowel
    - Patient commenced on systemic treatment with imatinib 400 mg once daily
    - 3- and 6-month response assessment scans after commencing therapy with imatinib showed excellent response to therapy, which was maintained at 12 months with minimal side effects
    - Patient underwent surgery to remove residual lesions
- The authors propose a management algorithm based on risk-stratification for familial *KIT*-exon 11 related GISTs. See figure 2 in the article.
- Available here

ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. Pascual *et al*. 2022. *Annals of Oncology – Article in press.* https://doi.org/10.1016/j.annonc.2022.05.520

- Validated and adequately sensitive ctDNA assays have utility in identifying actionable mutations to direct targeted therapy in patients with advanced cancer.
- May be used in routine clinical practice, but important to take into account limitations of the assay.
    - I.e. limitations in detecting fusion events and copy number changes, therefore tissue-based tested still the preferred test for many cancer patients
- However, ctDNA assays may be used when more timely results are needed, or when tissue biopsies are not possible or inappropriate.
- Reflect tumour testing should be considered following a non-informative ctDNA result.
- Other potential uses of ctDNA assays include detection of molecular residual disease (MRD) or molecular relapse (MR) in patients treated for early-stage cancers, identification of patients not responding to therapy, monitoring therapy for development of resistance mutations, and screening asymptomatic people for cancer. Before routine clinical implementation, analytical and clinical validity must be shown, clinical utility demonstrated, and quality requirements must be met with reporting standards clearly defined and supported by evidence.
- The article gives a helpful summary of ctDNA analysis and the challenges around this:
    - Currently no single ctDNA assay that would be fit for all purposes, and different assays have distinct limits of detection and limits of quantitation.
    - In cancer patients, ctDNA fraction varies according to tumour features, including tumour site, disease burden, rates of proliferation and apoptosis, extent of necrosis, inflammation, tumour microenvironment, as well as host-related phenomena.
    - Levels of ctDNA and ctDNA fraction may be affected by treatments including chemotherapy, targeted therapy, immunotherapy and radiation therapy. These can be both acute changes arising from the direct impact of treatment, and longer-term dynamics that relate to tumour shrinkage by treatment. Timing of plasma collection therefore needs to be carefully planned.
    - Several clinical and pathological factors are also associated with ctDNA levels – e.g. in NSCLC higher plasma ctDNA concentrations were found in patients with SCCs versus adenocarcinoma. Need to ensure the plasma sample contains sufficient levels of ctDNA to detect different types of variants and to minimise false-negative results.
    - Somatic alterations and clonal haematopoiesis can risk false-positive ctDNA results, particularly in CHIP-related genes (e.g. *TP53, ATM*), therefore synchronous profiling of plasma DNA and WBC DNA is recommended with certain ctDNA assays.
- The article provides the following recommendations for reporting of results (see table 1), future development of ctDNA assays, and future clinical research:
    - Recommend reporting of pre-analytical parameters, like sample acquisition date and treatment exposure at the time of acquisition.
    - Reporting language should convey the potential for discordance with tumour testing, especially in cases where a variant is not detected in plasma DNA (e.g. language such as ‘non-informative or not detected’ as opposed to ‘negative’).
    - Assays that can measure ctDNA fraction/purity should report this and assist clinicians in estimating whether failure to detect a somatic variant is due to the variant not being

present in the tumour or from insufficient ctDNA. Should also alert clinicians to variants detected in ctDNA assays which may be germline or contributed by non-tumour origins (e.g. CHIP).

- Timing of blood sampling for ctDNA analysis should be carefully selected depending on clinical question.
- Genotyping assays in the future should be adapted to assess tumour purity to allow confident predictions of undetected results, and allow confident true-negative predictions.
- Validated and adequately sensitive ctDNA testing can be used in routine practice for advanced disease genotyping (SNVs and small insertion and deletion variants), provided that limitations are understood and taken into account. Tumour specific recommendations for liquid biopsies are listed in table 2.
- VAF may provide information on subclonal nature of the variant, and theoretically subclonal variants may be less likely to benefit from a targeted therapy, however at the moment VAF should not be used to make decisions in clinical practice because i) it is unclear whether LBs can assess subclonality accurately and ii) there is limited evidence the suggest that true subclonal variants predict for lack of response.
- High ctDNA fractions are required for identification of somatic CNVs, so copy number assessment should only replace tissue assessment when the latter is not possible.
- Currently, evidence suggests patients should not be selected for immunotherapy on the basis of blood tumour mutation burden (bTMB) alone.
- They lay out specific recommendations for advanced cancer genotyping
- Optimal assay for monitoring advanced cancer patients has not been established, and there is currently insufficient evidence to use regular monitoring of ctDNA during therapy. Further studies are needed to define the optimal timing of ctDNA dynamic assessment and the most accurate threshold for response prediction.
- While clinical specificity of ctDNA detection for predicting relapse is high, clinical sensitivity of MRD detection is suboptimal. The clinical utility of ctDNA MRD and MR monitoring remains to be established, and they suggest future clinical trials to do so.
- They suggest large population studies are needed to provide a sufficient level of evidence for the application of ctDNA for screening of asymptomatic populations for cancer.

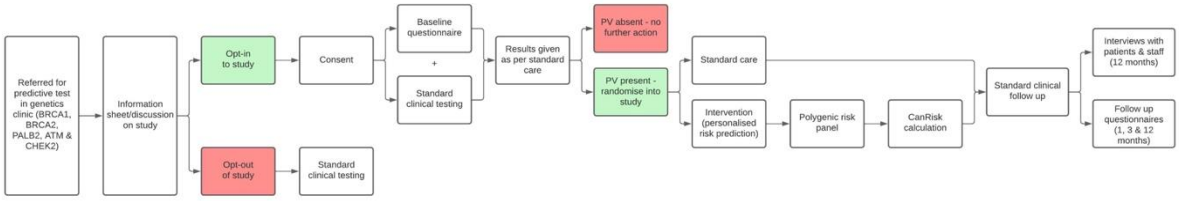
Counselling and ethics

Personalised Risk Prediction in Hereditary Breast and Ovarian Cancer: A Protocol for a Multi-Centre Randomised Controlled Trial. Archer *et al*. 2022. *Cancers*. . https://doi.org/10.3390/cancers14112716

- Most women who are found to have inherited a pathogenic/likely pathogenic gene change in *BRCA1*, *BRCA2*, *PALB2*, *CHEK2* or *ATM* via predictive testing are given broad, non-personalised cancer risk figures (e.g., 44–63% lifetime risk of breast cancer for those with a *PALB2* alteration). These broad risk figures may be problematic for women when considering risk management options (e.g. RRM vs annual screening to manage breast cancer risk). A personalised risk

estimate may aid women's decision-making process and may influence uptake of risk management options.

- This randomised control trial will randomise participants (1:1) to either receive standard, broad cancer risk estimates, or to receive a personalised risk estimate. Personalised risk estimates will be calculated by using CanRisk, combining a patient's genetic result, polygenic risk score (PRS), family history, lifestyle and hormonal factors. Women's decision-making around risk management will be monitored using questionnaires, completed at baseline (pre-appointment) and follow-up (one, three and twelve months after receiving their risk assessment). The primary outcome for this study is the type and timing of risk management options (surveillance, chemoprevention, surgery) taken up over the course of the study (i.e., 12 months). The type of risk-management options planned to be taken up in the future (i.e., beyond the end of the study) and the potential impact of personalised risk estimates on women's psychosocial health will be collected as secondary-outcome measures. This study will also assess the acceptability, feasibility and cost-effectiveness of using personalised risk estimates in clinical care.
- The below diagram is an overview of the study flow:



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