CGG/ERN GENTURIS/ICARE Monthly Journal Round-Up – March 2023

Translational science

Discordance between germline genetic findings and abnormal tumor immunohistochemistry staining of mismatch repair proteins in individuals with suspected Lynch syndrome. Pan *et al.* (2023). *Frontiers in Oncology*; doi: 10.3389/fonc.2023.1069467.

- Assessed 703 individuals with reported abnormal IHC findings and referred for testing using a six-gene (MLH1, MSH2, MSH6, PMS2, EPCAM, and MUTYH) cancer panel for suspected Lynch Syndrome or MUTYH-associated polyposis.
    - Patients most commonly diagnosed with CRC (76%) or endometrial cancer (23.5%).
- Pathogenic variants and VUSs in MMR genes were designated expected or unexpected, relative to IHC results.
    - Expected = variant in MMR gene aligns with protein loss identified by IHC.
- PV positive rate was 23.2% (n=163/703).
    - 8% (n=13/163) of these were in an unexpected MMR gene
- 132 VUSs in MMR genes expected to be mutated based on IHC results were identified in 121 individuals.
    - Independent evidence reclassified 44.7% of these VUSs as likely benign/benign and 12.9% as likely pathogenic/pathogenic.
    - According to the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) MMR variant classification criteria, when the presence of a VUS coincides with the absence of the corresponding MMR protein on IHC in two or more patients, it is deemed supporting evidence for pathogenic/likely pathogenic classification.
    - This study shows that if IHC results had been used as evidence for pathogenicity, there may have been variants classified in error as likely pathogenic, potentially leading to unnecessary overtreatment.
        - In the UK, the ACMG guidelines are recommended for sequence variant classification and interpretation (which do not use IHC results as evidence for or against pathogenicity of a variant).
- Among patients with abnormal IHC results, IHC-guided single gene testing may miss 8% of individuals with Lynch syndrome.

In the clinic

Germline-focused analysis of tumour-detected variants in 49,264 cancer patients: ESMO Precision Medicine Working Group recommendations. Kuzbari et al. 2022. Annals of Oncology. doi.org/10.1016/j.annonc.2022.12.003

- European Society for Medical Oncology Precision Medicine Working Group (ESMO PMWG) update to its 2018/19 recommendations on follow up of putative germline variants detected on tumour-only sequencing.
- Analysed an expanded dataset including 49,264 paired tumour-normal samples.

- Germline conversion rate (GCR; the proportion of variants of true germline origin out of total number of filtered tumour-detected variants) was examined for 58 cancer-susceptibility genes (CSGs).
- Tumour detected variants were filtered based on:
    - Variant minor allele frequency <0.01 in both gnomAD and internal variant frequencies
    - Classification in ClinVar as likely pathogenic or pathogenic and/or truncating variants in known tumour suppressor CSGs
    - Tumour-observed VAF >0.3 (SNVs) or >0.2 (indels)
- Filtering for tumour-observed VAF, 21,351 variants were retained, of which 16.5% were of germline origin.
- Of 3627 true pathogenic germline variants, 3.1% were absent from the final set of filtered tumour-detected variants.
    - However, loss of heterozygosity was present in 86% of these cases and a further 7% had amplification of the wild-type allele.
    - Suggested that modification of the VAF filter would have a modest impact on 'retrieving' these missed true-germline variants. This was demonstrated, for example, in *TP53* where raising the VAF filter from 0.3 (SNVs)/0.2 (indels) to 0.4 only increased the GCR from 0.9 to 1.4% which reflects the frequent somatic loss of the wild-type allele.
- Analysis concluded that strategic filtering improves the GCR with minimal loss of true germline variants present in the tumour.
- In this analysis the in-gene GCR threshold was modified from 10% to 5%.
    - 2018/19 ESMO PMWG recommendations suggested germline-focused follow up when GCR exceeded 10%.
    - 40 genes met criteria for germline based follow up. 34 of which would have met criteria if applying a 10% GCR threshold whereas for 6 genes, recommendations would differ if using a GCR threshold of 10% versus 5%.
    - Because for most genes GCR is either very high or very low, changing the threshold from 10% to 5% yielded only modest uplift in true germline variants detected.
    - Reduction to 5% for per-gene GCR threshold was deemed appropriate by the ESMO PMWG.
- Pan-tumour (across all tumour types) GCR:
    - Very high (≥80%) for 13/56 genes such as *BRCA1* (80%), *BRCA2* (81%), *BRIP1* (83.6%).
    - High (50-80%) for 11/56 genes such as *MLH1* (51.2%), *MSH2* (59.6%) and *ATM* (52.6%).
    - 12/56 genes with GCR between 5% and 50%.
    - Low (0%-5%) for 20/56 genes such as *TP53* (0.9%), *PTEN* (0.6%), *APC* (1.1%) and *BMPR1A, CDK4, HRAS, KIT, MET, NRAS* and *WT1 which all had a GCR of 0%*.
- Generation of clinical recommendations
    - 40 of 58 genes met criteria for inclusion in clinical recommendations for germline follow up (≥5% GCR threshold and three or more true germline variants detected). (Please refer to genes in Box 1 in publication)
- *BRCA1, BRCA2, MLH1, MSH2, MSH6, PALB2* and *RET* considered 'most actionable' (MA-CSGs) in terms of germline follow-up of tumour-only sequencing due to off-tumour GCR being relatively high, as well as better understanding of overall penetrance and efficacy of interventions for prevention/early detection.

- The ESMO PMWG considered four levels of 'clinical conservatism' by which filtered tumour-detected variants may or may not be included for germline follow-up:
    - **Permissive:** germline follow-up for all 40 genes in all tumour types.
    - **Intermediate-permissive:** germline follow-up for all 23 MA-CSGs/high-actionability (HA) CSGs in all tumour types but germline follow-up only in 'associated' tumour types for 17 standard-actionability (SA) CSGs.
    - **Intermediate-conservative:** germline follow-up in all tumour types for the 7 MA-CSGs but germline follow-up only in 'associated' tumour types for the other 33 HA-CSGs/SA-CSGs.
    - **Conservative:** germline follow-up only in 'associated' tumour types for all 40 genes.

Counselling and ethics

Danish heritable retinoblastoma survivors' perspectives on reproductive choices: "It's important for me, not to pass on this condition". Gregersen *et al.* (2021). *Journal of Genetic Counselling*; DOI: 10.1002/jgc4.1618

- Detailed knowledge about the experiences, reflections and decisions of people who have had retinoblastoma (RB) is crucial for tailoring counselling. This study aimed to specifically explore perspectives on reproductive choices
- Patients diagnosed with heritable RB at any time, and aged 18 or over at the time of the study, were invited to participate in the study and interviews were arranged
- 10 semi-structured interviews were carried out, and thematic analysis used to identify important themes.
- All quotes have been translated from Danish to English
- Equal number of male and female participants, mostly under the age of 40 years old. The majority had completed tertiary education and lived with a partner
- Four main themes were identified:
    - A desire to prevent RB: participants expressed a desire to prevent RB being passed on to their children. The lifelong and significant consequences of the condition were noted, and the risk of a more severe primary disease in a child. Participants noted an urge to protect their children and be open and truthful about the condition.
    - No opportunity for prenatal testing: Patients' experiences were dependent on the choices available to them at the time of their decision making. Some described their desire to have children as equally strong as their desire to prevent passing the condition on. There were some feelings of hope and benefits of early screening. Conversely some patients discussed the value of not having to make a deliberate choice if they didn't want to
    - Invasive prenatal testing: The length and complexity of fertility treatment was an important factor to choosing to opt for prenatal testing. The choice to end an affected pregnancy was perceived as very difficult, but a clear decision for some participants. One participant spoke of changing priorities and decisions after having an affected child
    - Preimplantation genetic testing: the ethical elements of this choice appeared to be important. The process was described as "laborious" and the waiting and hoping

was burdensome. Some participants felt guilt about their partners going through the PGT process.

- Reproductive choices were highly context dependent, however all participants expressed a strong wish and responsibility to avoid passing on RB
- The authors recognise that this small group of participants may be different to those who chose not to participate, or those who have not had access to the same genetic counselling. Also, the Danish universal healthcare system provides IVF and PGT free of charge which means this was not an important factor for these participants where it may have been for those in other countries.
- The authors suggest that counselling for RB families is tailored, repeated if needed, and possibly supplemented by peer-to-peer experience sharing

Counselling of path_ *BRCA* carriers who are considering risk-reducing oophorectomy. Manley *et al.* (date). *Post Reproductive Health*; doi.org/10.1177/20533691231156640.

- The most effective method of reducing cancer in path_BRCA carriers is risk-reducing salpingo-oophorectomy (rrSO). It reduces breast and ovarian cancer risk by up to 50% and 95% respectively.
- This paper provides a proforma to aid counselling of women considering rrSO. It prompts discussion of factors such as lifestyle and screening management options, details of rrSO, sequelae of an early menopause, HRT and follow up/support for these patients (Table 1).
- What surgery to offer:
    - Salpingectomy
        - Ongoing studies about the effectiveness of early salpingectomy followed by delayed oophorectomy.
        - Delayed oophorectomy should not be offered outside of a clinical trial.
    - Salpingo-oophorectomy
        - Counselling for rrSO should cover survival outcomes, surgical complication rates and quality of life effects.
        - Occult ovarian and peritoneal cancer rate at the time of surgery is 2.3-17%. Highest risk in BRCA1 carriers (86% of reported cases) and women who have surgery at a later age (1.5% in women <40, 3.8% for women aged 41-50).
        - 1-4% rate of residual peritoneal cancer after rrSO, with the highest risk associated with path_BRCA carriers, which highlights the importance of taking peritoneal washings.
    - Concurrent hysterectomy
        - No evidence that is improves survival outcomes.
        - Decision to offer concurrent hysterectomy should be based on patient preference and individual risk factors.
- In UK path_BRCA carriers, uptake of rrSO is approximately 40%.
    - Promoters of surgery include reducing cancer risk, age >45, family complete, personal or family history of BC, pre-existing gynaecological problems and reducing number of hospital appointments.
    - Barriers to surgery include concerns about sexual function, loss of fertility and surgical complications. BRCA carriers making a decision on rrSO reported concerns

about the impact of menopausal symptoms (achieving symptom control with HRT, potential health risks and impact on body image).

- Uptake of HRT after rrSO is reported to be 40-50%. They key message from this paper is HRT reduces menopause symptoms, thus improving quality of life after a surgical menopause.
- Research presented in this paper suggests referral to a menopause specialist for initial counselling and follow-up should be offered to women considering or undergoing rrSO as it leads to satisfaction and achieving good symptom control compared to reports from women feeling unsupported and struggling with quality-of-life effects without this help.

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