CGG/ERN GENTURIS/ICARE Monthly Journal Round-Up – September 2021

Bigger picture

In a commentary in Nature this month, Professors Mark McCarthy and Ewan Birney give an elegant overview of the need for a more holistic approach to disease risk assessment. They describe how the complex interplay of genetic and non-genetic factors; including environmental, lifestyle and socio-economic factors; contributes to disease risk – and how the risk assessment may be further impacted by changes in other clinical markers over time; or by implementation of risk-reducing strategies. The authors outline the potential use of polygenic risk scores in informing decisions regarding screening and lifestyle choices. (In the UK, studies such as the PIONEER study aim to examine whether knowledge of a woman's polygenic breast cancer risk score will influence her lifestyle choices). The authors outline the limitations of polygenic risk scores as standalone markers of risk and remind us that polymorphisms contributing to such scores and high-risk monogenic variants are not mutually exclusive – application of polygenic risk scores should be done cautiously in individuals where assessment for rare high-risk variants has not been undertaken to avoid inappropriate reassurance if the PRS places an individual in a "low" risk category. Tools such as CanRisk allow us to incorporate reproductive and lifestyle factors alongside family history information and certain monogenic factors and, where available, polygenic risk scores, to generate personalised risk estimates for breast and ovarian cancer; but this risk assessment may be dynamic over time as modifiable factors change and as risk-reducing strategies are implemented. For other cancer types, non-genetic factors may be difficult to quantify – for example the contribution of the gut microbiome to colorectal cancer risk. However, where possible, we should consider non-genetic factors in our risk assessments. The authors also remind us of the potential application of broader sequencing technologies to generate a more comprehensive single genetic risk score. The authors also highlight the issues related to under-representation of non-European cohorts in generating most data related to PRS; and highlight the need for commitment to inclusion of more diverse cohorts in genetic research – flagging, importantly, the difficulties in representation of groups where admixture is common and varied. The authors advocate for adoption of a holistic approach to research – and this approach is equally required in the clinical arena.

Translational science

The IRENA lncRNA converts chemotherapy-polarized tumor-suppressing macrophages to tumor-promoting phenotypes in breast cancer. Liu *et al.* (2021). *Nature Cancer*; 2: 457-473. https://doi.org/10.1038/s43018-021-00196-7

- The authors previously identified a non-coding RNA archetype that functions through post-translational modification of signaling proteins. Thus, as they suggest,

long noncoding RNAs (lncRNAs) may determine the functional status of immune cells by regulating their signaling pathways

- Here, the authors found that Interferon (IFN)-activated proinflammatory macro-phages after neoadjuvant chemotherapy enhanced antitumor immunity but promoted cancer chemoresistance.
- Mechanistically, they showed that IFN induced expression of cytoplasmic long noncoding RNA IFN-responsive nuclear factor-κB activator (IRENA) in macro-phages, which triggered nuclear factor-κB signaling via dimerizing protein kinase R and subsequently increased production of protumor inflammatory cytokines.
- They constructed macrophage-conditional IRENA-knockout mice, we found that targeting IRENA in IFN-activated macrophages abrogated their protumor effects, while retaining their capacity to enhance antitumor immunity.
- These findings indicate that lncRNA can determine the dichotomy of inflammatory cells on cancer progression and antitumor immu-nity and suggest that targeting IRENA is an effective therapeutic strategy to reversing tumor-promoting inflammation

Mutation-specific non-canonical pathway of PTEN as a distinct therapeutic target for glioblastoma. Won Choi *et al.* (2021). *Cell Death & Disease*; 12: 374. https://doi.org/10.1038/s41419-021-03657-0

- This study aimed to understand the functional properties of various PTEN missense mutations and to investigate their clinical relevance.
- The genomic landscape of PTEN alteration was analyzed using the Samsung Medical Center GBM cohort and validated via The Cancer Genome Atlas dataset. Several hotspot mutations were identified, and their subcellular distributions and phenotypes were evaluated.
- The authors in this work established a library of cancer cell lines that overexpress these mutant proteins using the U87MG and patient-derived cell models lacking functional PTEN. They used PTEN-null cells and U87MG cell line which has in-frame deletion within exon 3 of PTEN, and usually used as a negative control for PTEN functional studies
- PTEN mutations were categorized into two major subsets: missense mutations in the phosphatase domain and truncal mutations in the C2 domain. The mutations identified were: D24N, H93Y, C124S, R130Q, G132D, R173C, and K289E. Of these, five missense mutations (D24N, H93Y, R130Q, G132D, and R173C) are known to be maintained throughout the temporal evolution of GBMs while the other two (C124S and K289E) are well-known mutations in previous PTEN studies. The catalytically inactive C124S substitution is a representative loss-of-function substitution, whereas the K289E substitution, which is located within the C2 domain, retains WT phosphatase activity.

- To comprehend the functional implication of PTEN mutations in the present study, the authors examined the subcellular localization of PTEN mutants using U87MG cell lines, infected by lentivirus carrying PTEN mutant DNA (H93Y, D24N, R130Q, G132D, C124S, or R173C).They observed that subcellular compartmentalization varied by PTEN substitutions, even for mutations derived from the same phosphatase domain. The authors then determined the subcellular compartmentalization of four mutant proteins (H93Y, C124S, R130Q, and R173C) from the former group and found that they had distinct localizations; those associated with invasive phenotypes ('edge mutations') localized to the cell periphery, while the R173C mutant localized to the nucleus.
- The authors then used CLUMP (clustering of mutations in protein structure) as a computational method to predict the significance of mutation in a given 3D structure. The crystal structure of the PTEN protein showed that all residues in edge mutations located within the same pocket of the phosphatase domain in the crystal structure, where PI-P3 binds. This finding signified the distinct functions of edge and nuclear mutations, as illustrated by their differential subcellular compartmentalization.
- They also evaluated the limited number of PTEN mutations for their functional characteristics in the current study and found that edge mutations exhibited peculiar subcellular localization and invasive property. Edge mutations exhibited enhanced invasion capacity compared to PTEN-deletion or non-edge mutation, however, the severity of invasion differed by mutation.
- Overall this study emphasizes on PTEN mutations that exhibit distinct functional properties in accordance with their subcellular localization. And highlights the clinical significance of mutation-specific therapeutic options that should be considered in treating GBM patients with PTEN mutations.

In the clinic

Risk of Late-Onset Breast Cancer in Genetically Predisposed Women. Boddicker *et al*. (2021). *Journal of Clinical Oncology*. https://doi.org/10.1200/JCO.21.00531

- The prevalence of germline pathogenic variants in established breast cancer predisposition genes in women in the general population >65y is not well-defined
- Testing guidelines suggest that women diagnosed with breast cancer >65y might have <2.5% likelihood of a PV in a high-penetrance gene
- Study aimed to establish frequency of PVs and remaining risks of breast cancer (risk from age 66-85y) for each gene in women over age 65y
- 26,707 women >65y from population based studies (51.5% with breast cancer, 48.5% unaffected) were tested for PVs in germline predisposition genes
- 25.6% of cases and 17.9% of controls had a FDR with breast cancer

- Frequency of PVs in predisposition genes was 3.18% for women with breast cancer and 1.48% for unaffected women
- *CHEK2* (0.92%), *BRCA2* (0.71%), *ATM* (0.57%), and *PALB2* (0.36%) had the highest frequencies of PVs, whereas *BRCA1* PVs were observed in only 0.28% of women with breast cancer
- PVs in *BRCA1, BRCA2* and *PALB2* were found in 3.42% of women diagnosed with ER negative breast cancer, 1.0% with ER positive BC, and 3.01% with TNBC
- Frequencies of PVs were lower among women with no FDRs with BC
- PVs in *CHEK2, PALB2, BRCA2,* and *BRCA1* were associated with increased risks of breast cancer – equivalent to moderate risk
    - Remaining lifetime risks of breast cancer were approaching 20% for those with PVs in *BRCA1* and *BRCA2*, and were 15% for those with PVs in *PALB2* and *CHEK2*
    - PVs in *PALB2*, *CHEK2*, and *BRCA2* were associated with moderately increased ER-positive breast cancer risk, whereas PVs in *BRCA1*, *BRCA2*, *PALB2*, *RAD51D*, and *BRIP1* were associated with high risks (OR > 4) of ER-negative breast cancer diagnosed over age 65 years
- *ATM* PVs were not statistically significantly associated with risk of breast cancer in women >65y
- Study suggests all women diagnosed with TNBC or ER-negative BC should receive genetic testing and that women >65y with *BRCA1* and *BRCA2* PVs, and perhaps with *PALB2* and *CHEK2* PVs should be considered for MRI screening

The value of clinical breast examination in a breast cancer surveillance program for women with germline *BRCA1* or *BRCA2* mutations. Hettipathirana *et al*. (2021). *Medical Journal of Australia.* https://doi.org/10.5694/mja2.51226

- Retrospective, longitudinal cohort study of women with *BRCA1/2* mutations to assess sensitivity and specificity of clinical breast examination for detecting breast cancer in asymptomatic women
- Participants had not undergone bilateral RRM and had generally undergone breast examination at 6- or 12-month intervals, and annual breast imagine (mammography; and MRI for women aged ≤50y)
- N = 414; 186 women had *BRCA1* mutations and 228 women had *BRCA2* mutations
- 35 of the 414 women were diagnosed with breast cancer during 1761 woman-years of follow-up
    - 27 were screen-detected, 8 (35%) were interval cancers
    - 13 were DCIS, 20 were invasive cancers, 2 unknown (details unavailable)
    - Only 2 were diagnosed based on breast examination alone, neither of whom was undergoing MRI screening
- Sensitivity of breast examination was 6% and specificity was 97%, positive predictive value was 14% and negative predictive value was 92%

- Breast examination alone is not an acceptable test for screening women with *BRCA1/2* mutations.
- Clinical breast examination did not increase the number of breast cancers detected in MRI-screened women with *BRCA1/2* mutations. Removing breast examination from surveillance programs that include MRI may be reasonable for these women.
- If breast examination remains a part of screening for some women, it is important to counsel them about its limited effectiveness, particularly when intensive radiological screening is undertaken, and about the possibility of false positive results.

Counselling and ethics

Impact of personal genomic risk information on melanoma prevention behaviours and psychological outcomes: a randomized controlled trial. Smit *et al.* (2021). *Genet Med.* DOI: https://doi.org/10.1038/s41436-021-01292-w

- The authors hypothesised that providing personalised genomic risk information about melanoma would motivate behaviour change, such as reduced sun exposure, increased sun protection and early detection behaviours
- A randomised, controlled trial design was used. Eligible participants were randomised to one of two study arms
- The intervention group received a DNA testing kit, a personalised booklet about their melanoma risk, a telephone call from a genetic counsellor and a general education booklet. Those in the control arm received an information booklet only.
- Participants were asked to wear electronic UV dosimeters and exposure was logged over a follow-up period. Other follow-up measures included logging sun protection behaviours, sunburn frequency and melanoma-related worry
- No difference in UV exposure between groups, and participants in both arms decreased their time in the sun during the trial. However, there were differences between those who felt genetic information was more or less deterministic
- The most pronounced behavioural effect of the intervention was the reduction of sunburn incidences
- The intervention reduced melanoma-related worry at 12 months
- Those with a higher than average genomic risk reported a greater increased in sun protection behaviours
- The impact of the intervention on behavioural outcomes differed according to population subgroups
- Overall, personalised melanoma genomic risk information did not influence measured patterns of sun exposure, but had beneficial impacts on sun protection, sunburn and skin examinations. The authors note some limitations of the study, and that regular reminders of the relevant information may be required as benefits dropped off at 12-month follow-up.

Breast cancer polygenic risk scores: a 12-month prospective study of patient reported outcomes and risk management behaviour. Yanes *et al*. (2021). *Genetics in Medicine.* https://doi.org/10.1038/s41436-021-01288-6

- The study aimed to assess patient reported outcomes and risk management decisions of women who either accepted ("acceptors") or declined ("decliners") PRS testing
- Women either unaffected or affected by breast cancer and from families with no identified PV in a breast cancer risk gene were invited to receive their PRS
- Participants who accepted PRS testing completed questionnaires at enrolment, 2 weeks post PRS results and 12 months after PRS results. Participants who declined PRS testing completed a questionnaire at enrolment and 12 months later.
- 79% (165/208) of participants opted to receive their PRS
- No increase in anxiety or distress was noted post-test for Acceptors
- Recall of verbal description of PRS category (high or low) was high at 12 months, with ~91% correctly recalling category.
- Acceptors who were found to have a high PRS score reported higher distress, perceived risk and decisional regret compared to their counterparts who received a low risk PRS score
- It was noticed that decliners experienced significantly higher decisional regret compared to acceptors. They also reported fewer perceived benefits and more concerns regards PRS.
- Majority of Acceptors received a moderate risk assessment
- Uptake of risk reducing strategies were low for Acceptors in the first 12 months after receiving PRS

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