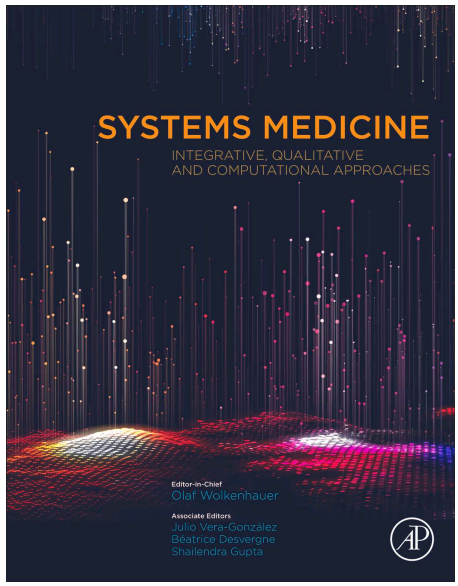

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Germline Genetics in Cancer: The New Frontier

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Introduction

Cancer research is constantly evolving. In order to improve current cancer diagnosis (or prognosis) protocols, new theories and concepts often emerge and are accepted by the scientific community. In some cases, old concepts that were discarded previously due to multiple factors, such as lack of technological or general opinion, can also come back to light. The fundamentals surrounding cancer development have always been that, for a normal cell to be transformed into a cancer cell, a cell needs to acquire a series of mutations that grant selective fitness. Traits obtained by such mutations translate into survival advantages that allow the cell to bypass or increase multiple biological regulatory processes (i.e., cancer hallmarks) (Hanahan and Weinberg, 2000, 2011). These mutations, referred to as somatic mutations, can arise from a variety of external or internal components (i.e., environment, stress, carcinogens, DNA repair, etc.) (Fuchs, 2007; Joshi *et al.*, 2018). Accordingly, building a catalogue characterizing somatic mutations (e.g., COSMIC) was one of the first major step the scientific community took to improve current understanding of cancer (Tate *et al.*, 2019). By increasing our knowledge of somatic mutations, several genes (or driver genes) and mutations have been identified as well as their roles in increasing patients' susceptibility to develop cancer or even other diseases (i.e., psychiatric disorders, cardiovascular diseases, strokes and so on) (Nishioka *et al.*, 2019; Lee *et al.*, 2018; Jaiswal *et al.*, 2017; Chen *et al.*, 2017). Combined with next generation sequencing (NGS) technology, these findings paved the way for many new scientific applications such as gene therapies, genomic testing, bioinformatics, systems and predictive biology. As of today, the somatic mutational landscape of each cancer patient is still used globally in the community. Mutational signatures have allowed a better forecasting of cancer and have improved diagnosis and prognosis to some extent (Kandoth *et al.*, 2013). However, thus far, cancer has been the second leading cause of death in the world. While some cancer types (i.e., breast) now have a 95% of 5-year survival rate, the number of deaths due to cancer in 2018 was estimated to be around 9.6 million (Korsunsky *et al.*, 2014). The lack of success in negating completely cancer associated deaths can be attributed to several issues such as tumoral heterogeneity, carcinogen exposure, age, family history, etc. In hopes of solving this situation, the scientific community has been shifting towards precision medicine underlying that each cancer is genetically different which can lead to diverse biological and clinical outcomes. Dissecting each tumor independently can provide us with a unique genetic and molecular profile allowing for a better monitoring of patients. Additionally, by using the complete mutational landscape, we can perceive the combined effects of mutations on the tumor or the desired phenotype. Other approaches, such a systems biology, also integrates multiple components together attempting to represent cancer dynamically, enabling us to decipher to new pathways, interactions, drug response, cell types and states, and even insights into tumor evolution (Werner *et al.*, 2014; Johnson *et al.*, 2016). In a similar line of thinking, in order to obtain the most representative biological settings, scientists have also started to explore complete transcriptomes, metabolomes, methylomes, etc. of tumors (Chakravarthy *et al.*, 2018; Ma *et al.*, 2018). As such, the concept of germline mutations or germline genomes in cancer has resurfaced over the last decade. Germline mutations (or hereditary mutations) are passed on from parents to offspring and are therefore present in all cells of our body (unlike somatic mutations). Traditionally, germline mutations have largely been ignored because their penetrance is very low; in terms of family history and a handful of cancer risk genes, only 5–10% of cancer population are inherited. While a somatic mutation will arise randomly, its occurrence and its fitness will be based, even driven, upon its germline genomic background. In other words, cancer cells will maximize their survival using their host historical and genetic profile. Thus, germline pathogenic variants not only act as pre-dispositions but also as modulators in shaping tumor evolution. As cancer is a heterogeneous disease and the result of many interactions by multiple genes, unique germline genomic landscapes of patients can therefore have unique phenotypes (similar to unique somatic gene landscapes). Hence, the complete germline genomic landscape of patients can offer valuable insights into cancer development, evolution and clinical outcome. In this article, we will discuss the fundamentals surrounding germline pathogenic variants in cancer, their roles and their current applications. Germline pathogenic variants and germline mutations will be used interchangeably as both phenomena are results from modification in DNA sequence whether caused by polymorphism or single events (functional or not).

Germline variants in cancer were first identified by the geneticist, Albert Knudson in 1971. Knudson investigated a cohort of patients with retinoblastomas from 1944 to 1969, studying specifically mutations on *RB1*. At that time, he already knew that two alleles existed for each gene, therefore both copies would need to be mutated in order to develop a retinoblastoma. Knudson was

first puzzled by the fact that the affected parent could have disease-free children and these unaffected individuals could bore children of their own with retinoblastomas. Furthermore, he noticed that bilateral retinoblastomas were occurring more frequently at a younger age (25–30% of cases), highlighting that germline pathogenic variants were implicated. Because a mutation of both alleles was required to develop cancer, having an inherited mutation in *RB1* was predisposing the children to retinoblastomas. Knudson later proposed his findings as the Knudson hypothesis or what is known today as the "two-hit" hypothesis, explaining that children inheriting from a *RB1* pre-disposition needed to acquire a second mutation in order to develop cancer. Finally, because children without RB1 pre-disposition needed to acquire two mutations, they were less likely to develop a retinoblastoma. Since then, several germline mutations have been reported to be associated with cancer. For example, patients suffering from Li-Fraumeni syndrome have an almost 100% chance of developing a wide range of malignancies before the age of 70. Most patients carry a missing or damaged *p53* gene, a tumor suppressor whose activity is impaired in almost 50% of all cancer patients. *p53* is widely regarded as the most important tumor suppressor in human genome. It acts as one of the major gatekeepers in cancer and has multiple functions such as ensuring genomic integrity and promoting DNA repair, cell cycle regulation and apoptosis, signal transduction and cell adhesion. Other cancer-predisposition genes include *BRCA1* and *BRCA2*, tumor suppressor genes involved mainly in DNA repair, which are associated with breast and ovarian cancer (Maistro *et al.*, 2016; Chan *et al.*, 2017). *BRCA* mutations being autosomal dominant and not gender-specific, each parent possesses a 50% chance to transfer its altered gene copy to its child. For women, germline mutations in *BRCA1* or *BRCA2* have been shown to increase the risk of developing breast cancer by 69–72% and ovarian cancer by 17–44% by the age of 80 (Kuchenbaecker *et al.*, 2017). *BRCA1* and *BRCA2* are now well characterized and used in genetic screening regularly even though the worldwide prevalence is relatively low (5–10%). Other examples include *PTEN* (Liaw *et al.*, 1997), whose mutation results in Cowden syndrome, and *APC*, which is linked to familial adenomatous polyposis (De Queiroz Rossanese *et al.*, 2013). Two distinct types of multiple endocrine neoplasia are associated with the *RET* and *MEN1* genes while *VHL* alterations result in kidney and other types of cancer (Cetani *et al.*, 2017; Moore *et al.*, 2011). Finally, Lynch syndrome, a form of colorectal cancer, is linked to *MSH2*, *MLH1*, *MSH6*, *PMS2*, and *EPCAM* (Gray *et al.*, 2017). Following the same rationale, scientists also conducted many comprehensive analyses of cancer whole exomes and these studies revealed that germline genomic landscape of patients is informative and can be used in genetic testing (Park *et al.*, 2018; Huang *et al.*, 2018). Historically, as mentioned above, germline mutations have been mostly ignored due to their low penetrance. In consequence, massive public genomic databases such as TCGA, GDC or ICGC used to pre-filter germline information providing further analyses quite difficult. In some cases, specific sequencing centers would only sequence tumor samples discarding completely blood (normal DNA) which ultimately became a limiting factor in any germline genomic study. Raw normal and tumor genomic data files, such as BAM and FASTQs, needed to be downloaded and stored locally. Golden standards pre-processing and genomic variant calling pipeline would then be run again to obtain germline genomic information. The complete process often would take several months and sizeable computational resources (CPUs and storage). However, in the past 2–3 years, as germline genomic is becoming a more prominent field, the scientific community and many databases are now starting to offer normal/tumor matched sequencing files as well as germline variants, allowing for an easier accessibility.

Germline Pathogenic Variants in Cancer Diagnosis

As germline mutations are pre-dispositions to cancer, genetic screening of specific mutations (such as *BRCA1* and *BRCA2*) is already being used in cancer diagnosis. However, because these mutations have very low penetrance in cancer population, these genetic tests are often negative and do not provide much improvements at a diagnostic level. Breast cancer is one of the cancer types with the most effective monitoring procedures and it is reflected in its 5 years survival rate, as it is now one of less lethal cancer type. Patients are flagged in clinics following genetic testing or if other risk factors are identified. Following such screenings, patients at risk are required to take regular mammograms. As these mammograms are used mostly for prevention, physicians can diagnose cancer earlier if they find anomalies in images. Other common diagnosis tests used in clinics include colonoscopy and sigmoidoscopy for colorectal cancer, digital rectal exam and the PSA test for prostate cancer, laryngoscopy for larynx and esophageal cancer as well as any biopsy for all cancer types. Yet, because all these tests are considered invasive for patients, they are usually prescribed after detecting anomalies in imaging (X-ray, MRI, PET-CT, ultrasound, etc.). Consequently, doctors are reluctant to submit their patients to a painful procedure if not necessary. Sadly, as imperfection is unavoidable using any method, cancer imaging is not 100% accurate. Breast cancer possess the best detection rate at 90% while other cancer types, such as prostate, only have a 49% detection rate, highlighting that improvements are still required (Sarkar and Das, 2016; Riedl *et al.*, 2015). Furthermore, radiographic imaging is often subject to interpretation which can lead to human error and bias. Estimated imaging human error is around 3–5%, resulting in approximately 40 million radiologist errors every year (Brady, 2017; Bruno *et al.*, 2015). Limiting those errors represent a major challenge in the field. Current clinical protocols are starting to integrate multiple tests results together (i.e., mammography combined with an MRI), but even tests have their own limits, as they can be inconclusive or even conflicting, resulting in a similar interpretation. In order to remain cost-effective, test combination is either not used or reserved to high-risk patients (Nadler *et al.*, 2017). In hopes of diminishing human errors, automated screening methods are currently being explored using computational approaches (Drukker *et al.*, 2014; Giger *et al.*, 2016). Automated image recognition using artificial intelligence could solve any bias or errors related to interpretation. Artificial intelligence (AI) requires a massive amount of information in order to develop an accurate and most representative model. However, as diagnostic images are very abundant, AI could be a perfect approach to improve this issue. For any cancer types, biopsy remain the standard practice

diagnosis test. As mentioned above, not only is it invasive, but depending on the tumor location and the type of biopsy, biopsies can have serious risks (i.e., brain cancer, seizures, strokes, death, etc.). Moreover, biopsies can also fail to detect cancer as extracted sample tissues might not contain cancer cells or too few of them. A few comparative analyses have shown that biopsies can miss 30–45% of prostate cancers, emphasizing that no test is an absolute certainty and that there are still many avenues of improvements (Lecornet *et al.*, 2012; Scott *et al.*, 2015). Nonetheless, in many cases, cancer is diagnosed too late as patients are already exhibiting a variety of symptoms which leads to lower survival chances. As mentioned previously, because cancer is a heterogeneous disease that results from multiple interactions from various genes, it is safe to assume that a different combination of mutations has distinct impacts on cancer (or another specific phenotype). Therefore, unique germline genomes of patients can very well hold crucial genetic information usable to diagnose cancer. In addition, combining multiple germline pathogenic variants together negates the penetrance effect as one individual will most certainly have at least 1 single germline pathogenic variant in its entire genome. If specific biological pathways are already altered in healthy individuals, these same pathways could be targets to cancer cells insinuating that germline variants act as modulators driving the somatic mutational landscape which ultimately, drives tumor development and evolution. Based on this assumption, germline variants could be used not only to predict cancer but even to predict somatic mutations for cancer patients (Zaman *et al.*, unpublished data). Altered germline DNA sequence could act as "hot spots" for future somatic mutations, allowing them to be predictable before occurrence. Germline DNA alterations in a specific gene could increase its susceptibility to develop a somatic mutation (two-hit hypothesis) affecting its function (i.e., DNA repair, antibody affinity, etc.) or even, its implicated biological pathways (Ho and Pastan, 2009; Marty *et al.*, 2017). Based on perceived susceptibility, cancer cells can very well knock down functions in a simple gene or more globally, taking a systems biology approach, maximize a pathway's function in order to improve their survival. As a diagnostic tool, germline DNA can be obtained from a simple non-invasive blood or saliva test which could very well provide an alternative to traditional biopsy. Several genetic tests using tumor samples are already commercially available (see next section). Genetic testing using blood or saliva sample could pave a new way for cancer diagnosis in clinics and allow for a better forecasting of the disease. Liquid biopsies, a new technology, could also improve cancer diagnosis on many fronts. As the tumor proliferates and evolves, cancer cells from the epithelial layer of the infected tissue may eventually enter the bloodstream (epithelial-mesenchymal transition, EMT). These cells are referred to as circulating tumor cells (CTCs). A liquid biopsy is a non-invasive procedure that isolates CTCs in plasma derived from a blood sample. Numerous studies have shown that CTCs can provide many insights on disease progression, treatment effectiveness and key information on somatic events (Mego *et al.*, 2012; Romero, 2018).

Germline Variants in Cancer Prognosis

As highlighted in the previous section, the germline landscape has a profound impact on tumor evolution. As such, germline genetic can be used in cancer diagnosis but also in cancer prognosis. Whether germline pre-dispose, constraint or infer future somatic mutations, a unique set of germline variants will have a unique impact on specific characteristics of cancer enabling us to use this signal to predict clinical outcomes (Wang *et al.*, 2015). Similar to cancer diagnosis, there are several clinical issues with cancer prognosis in the clinical setting. After diagnosis, patients are classified into risk groups or molecular subtypes in order to determine an appropriate treatment course, protocols varying dramatically in terms of dosage and chemotherapeutic agents. Such a classification has been made by a multitude of factors including genomic events, white blood cell count, gene expression, immunophenotypes, karyotypes, etc. (Barry *et al.*, 2007; Alvarnas *et al.*, 2015; Paulsson *et al.*, 2015). For Acute Lymphocytic Leukemia (ALL), aside from the Philadelphia-like subtype, accounting for 4% of ALL cases, pediatric risk misclassification can vary from 20% to 50% of cases and results in a poor treatment response which eventually leads to recurrence (Limvorapitak *et al.*, 2019; Vrooman and Silverman, 2016). Patients stratified as low risk are given a less aggressive protocol and will eventually relapse (undertreatment). Many relapses are located in the bone marrow resulting in more complex and less effective treatment options (stem-cell transplant) (Malempati *et al.*, 2007). In prostate cancer, clinical factors such as the PSA level (ng/mL), Gleason scoring (GS) and tumor stage (T stage) are used to stratify patients into risk groups. Clinical decisions in regards of treatments are then made based on group assignment (low, intermediate and high-risk group) in addition with biopsy results if necessary. In general, based on The Cancer of the Prostate Risk Assessment (CAPRA) score, low-risk group is defined with stage T1 − T2a, GS $<=6$ and PSA $<=10$. Intermediate-risk is attributed to patients with T2b − T2c stage, GS $= 7$ and PSA >10–20. Finally, high-risk group contains patients with $>$T3 stage or PSA >20 or GS 8–10. Furthermore, GS and tumor stage can be interpreted differently by pathologists, which lead to an even more ambiguous stratification of patients. Other studies have also shown that quantitative MRI (qMRI) can improve identification of high-risk cancer patients by providing further images to pathologists in order to assess a better T stage and GS (Mehralivand *et al.*, 2018). However, active surveillance using qMRI remains limited majorly due to its financial burden. Recent reports have shown that 30–50% of stratified low-risk prostate cancer patients are misclassified, having intermediate- or high-risk cancer instead (Patel and Palayapalayam Ganapathi, 2016; Carlsson *et al.*, 2016). As a result, patient anxiety regarding "untreated" diagnosed cancer is increasing, resulting in unnecessary surgeries (Van Den Bergh *et al.*, 2009). In breast cancer, efforts have been to reduce overtreatment, yet 46% of women still do not benefit from chemotherapy emphasizing that risk stratification and current clinical decisions can be further improved (Cardoso *et al.*, 2016). Even with an early diagnosis, many cancer types do not have a powerful risk stratification protocol resulting in poor treatment accuracies, overtreatment and diminished quality of life for patients. As established previously, since the germline genomic landscape possess a constraint over the tumor evolution, susceptibility genes are expected to directly affect, impact and drive cancer-related phenotypes (i.e.,

recurrence, drug response, antigen affinity, etc.). If such phenotypes are derived from germline genomes, then using germline profiles of patients could be helpful in the prognosis field by informing and improving clinical decisions. However, genomic testing remains limited in cancer prognosis. Commercially genomic assays such as Oncotype DX, MammaPrint and PAM50 are available but such tests only focus on small panel of genes and are specific to a few cancer types (Paik *et al.*, 2004; Sparano *et al.*, 2018; van't Veer *et al.*, 2002; Parker *et al.*, 2009). In addition, all these tests rely on gene expression and not genomic alterations, even though the mutational landscape has been linked to cancer prognosis. Very recent studies have shown that germline variants are associated with and even predictive of tumor recurrence in breast cancer (Milanese *et al.*, 2019). This highlights the hypothesis that germline genetics can be used in cancer prognosis. Furthermore, specific germline deletion in the proapoptotic protein BIM, has been shown to increase resistance against kinase inhibitors in cancer (Cheng and Sawyers, 2012). Additionally, germline variants directly affect drug susceptibility, some germline variants having more effect than somatic mutations themselves (Menden *et al.*, 2018). Similarly, germline copy number alterations (CNVs) in breast cancer also have been correlated with risk and prognosis (Kumaran *et al.*, 2017). Integrating both germline and somatic components could very well represent a new frontier and improve decision making in clinical oncology.

As such, germline DNA alterations in immune system related genes could very well have a major impact on either leukocytes recruitment or the tumor immune micro-environment (TIME) itself. A recent study reported that an increase in germline variants in leukocyte genes in breast cancer was associated with a weaker immune response and tumor recurrence (Milanese *et al.*, 2019). Germline variants affecting leukocytes function would have major impact on tumor evolution and clinical outcomes. For example, targeted alterations in T-cells or T-cells receptors α and β chains could reduce T-cell recognition functions allowing cancer cells to avoid detection and escape cell death. According to a recent report, modifications in sequences related to variable, diversity and joining (VDJ) genes are preferentially expressed with a higher adaptive immune response (Findly *et al.*, 2018; Kirik *et al.*, 2017). Consequently, germline variants also shape immune cell abundance, infiltration, overall immune response and TIMEs (Xu *et al.*, 2019; Lim *et al.*, 2018). Based on patient specific germline profiles, more specific variants enriched in natural killer (NK) cells, TIMEs can be classified into three subtypes (TIME-rich, TIME-poor and TIME-desert), each of them has different immunophenotypes and immunotherapy responses, clinical outcomes and survival. The number of inherited variants in NK cells being positively associated with cancer risk, but negatively associated with the fraction of TILs, survival and immunotherapy response, highlighting that germline genomics play an important role in tumorigenesis and metastasis via immune system modifications.

Current immunotherapy protocols involve mainly checkpoint inhibitors (CTLA-4, PD-1, PD-L1, Tim-3, etc.), adoptive cell transfer (ACT) such as chimeric antigen receptor T-cell therapies (CAR-T), monoclonal antibodies (mAbs) and cancer vaccines. One of many cancer hallmarks is the ability to evade the immune response (or destruction), which in turn leads to survival. Cancer vaccine mechanisms are similar to traditional vaccine, where T cells are stimulated to recognize tumor specific antigens, allowing cancer cells to be destroyed by the adaptive immune response (Zhang and Chen, 2018). However, tumor heterogeneity and antigenicity remain the limiting factors in this approach, as cancer cells within the same tumor can often express different antigens allowing them to evade immune response (Ye *et al.*, 2018). mAbs are engineered molecules able to recognize antigens, glycoproteins or glycolipids, carbohydrates and growth factors making them very versatile in cancer immunotherapy treatments (Scott *et al.*, 2012). They act as markers, attaching themselves onto cancer cells, enabling T cells recognition and therefore, boosting the immune response. Depending on the cancer type and/or subtype, mAbs can be designed in order to maximize cancer cells specificity. Yet, mAbs are very expensive to synthetize and produce many side effects experienced by patients (nausea, headache, kidney damage, hypo- and hypertension, etc.) causing their limited usage (Coulson *et al.*, 2014). Checkpoints inhibitors, as for them, are well known and used daily in cancer immunotherapy treatments. Over the past few years, many immune checkpoint inhibitors therapies have been approved by the Food and Drug Administration (FDA), such as PD-1, while several others are still in clinical trials (Darvin *et al.*, 2018). As the tumor grow, cancer cells are subject to selective pressure by the immune system leading to tumor editing (or heterogeneity). Cancer cells often develop the ability to regulate the immune response by inhibiting T cells activity using checkpoint blockade. Checkpoint blockade molecules (i.e., CTLA-4, PD-1, etc.) act as regulators in the immune response. Therefore, checkpoint blockade inhibitors restore anti-tumor response by inhibiting those proteins and increasing T cells stimulation and activity. For example, CTLA-4 act as a competitive ligand in T cell receptor (TCR) signaling. Co-stimulation of CTL-4 with CD28 allows for an inhibition of TCR, leading to inactivation of T cells (Linsley *et al.*, 1994). Another example of checkpoint blockade inhibitor is PD-1 whose binding with PD-L1 and PD-L2 enables recruiting of a tyrosine phosphatase SHP2 directly downregulating TCR (Yokosuka *et al.*, 2012). Finally, the last immunotherapy strategy mentioned above is ACT. Using engineered immune cells or healthy immune cells from another host, these cells are then transferred into the affected individual restoring an immune response or increasing immune system functions altogether. Initially, antigen-specific T cells or TCR genes were simply transduced into affected individuals but later studies shown that TCR therapies were very limited due to major histocompatibility complex (MHC) antigen recognition (or MHC restriction) considering TCRs can only bind to specific MHC molecules (Park *et al.*, 2011). Due to MHC restriction, ACT therapies have shifted towards CAR-Ts, as CAR-Ts have antibodies-like specificity allowing them to be MSH independent. Recent therapies have shown that CAR-Ts, have very good efficacy in leukemia patients accounting for 90% remission in ALL patients with relapse (Maude *et al.*, 2014). However, CAR-Ts toxicity and side effects remain its major limitations. Cytokine release syndrome (CRS), the most common and toxic side effect from CAR-Ts, is an inflammatory response generated by high levels of cytokines. CRS is characterized by a variety of mild symptoms such as diarrhea, headaches, fever and vomiting but can also lead to organ failure and even death. Finally, ACT using CAR-T therapies for solid tumors remains a challenge. Solid tumors exhibit a much higher degree of antigen heterogeneity (or even antigen loss) negating completely CAR-Ts function. In addition, solid tumors also possess an immunosuppressive micro-environment favoring growth

which decreases leukocytes infiltration. Unlike hematological cancers, CAR-Ts delivery in solid tumors represent an additional challenge as they must travel to their destination (Martinez and Moon, 2019). Very recently, ACT therapies have also explored the possibility of transduction NK cells instead T cells. Unlike T cells, NK cells do not require HLA matching avoiding graph-versus-host disease and making them easy to manipulate and transfer from one patient to another. NK cells can recognize cancer cells without any antibodies bypassing completely MHC restriction and antigen heterogeneity. Consequently, CAR-NKs has become a new approach in immunotherapy; using healthy engineered NK-cells in cancer patients (Kloess *et al.*, 2019). NK cells are also involved in immune cells recruitment, interacting with innate and adaptive immune cells (i.e., macrophages, dendritic cells, etc.) and recent studies have shown that many different subsets of NK cells exist, most of them having different functions (Vitale *et al.*, 2019). For example, a new subset of NK cells has been shown to maintain memory against viral infections. In all, CAR-NKs could have several beneficial effects in fighting cancer by eliminating cancer cells directly but also by recruit other immune cells and restoring anti-tumor response in the TIME. As mentioned above, germline NK-deficiency can be detected early, allowing for targeted therapies when patients are diagnosed with cancer. CAR-NKs could be used in patients with TIME-poor or TIME-desert profile, restoring (or boosting) the immune response. Taken together, all these results suggest that germline role in cancer prognosis may very well be expanded not only to risk stratification and chemotherapy responses but also to immunotherapy.

This article discussed a few of many implications of germline in cancer, whether from a diagnostic or a prognostic point of view. Germline mutations are embedded in every single human being, highlighting that our original blueprint automatically influence and guide potential future diseases such as cancer. Surely, as more studies weight in on the matter, germline usage will start increasing daily in clinics. Furthermore, combining the germline and the somatic landscape of patients will lead to better and more informed clinical decisions. Germline usage in personalized medicine and clinical oncology represents a paradigm shift allowing for better prevention, forecasting and treatment of cancer patients.

References

Alvarnas, J.C., Brown, P.A., Aoun, P., *et al.*, 2015. Acute lymphoblastic leukemia, version 2.2015. Journal of the National Comprehensive Cancer Network 13, 1240–1279.

Barry, E., DeAngelo, D.J., Neuberg, D., *et al.*, 2007. Favorable outcome for adolescents with acute lymphoblastic leukemia treated on Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium protocols. Journal of Clinical Oncology 25, 813–819.

Brady, A.P., 2017. Error and discrepancy in radiology: Inevitable or avoidable? Insights Into Imaging 8, 171–182.

Bruno, M.A., Walker, E.A., Abujudeh, H.H., 2015. Understanding and confronting our mistakes: The epidemiology of error in radiology and strategies for error reduction. Radiographics 35, 1668–1676.

Cardoso, F., van't Veer, L.J., Bogaerts, J., *et al.*, 2016. 70-Gene signature as an aid to treatment decisions in early-stage breast cancer. The New England Journal of Medicine 375, 717–729.

Carlsson, S., Jäderling, F., Wallerstedt, A., *et al.*, 2016. Oncological and functional outcomes 1 year after radical prostatectomy for very-low-risk prostate cancer: Results from the prospective LAPPRO trial. BJU International 118, 205–212.

Cetani, F., Pardi, E., Berardi, V., *et al.*, 2017. Incidental occurrence of metastatic medullary thyroid carcinoma in a patient with multiple endocrine neoplasia type 1 carrying germline MEN1 and somatic RET mutations. Journal of Surgical Oncology 116, 1197–1199.

Chakravarthy, A., Furness, A., Joshi, K., *et al.*, 2018. Pan-cancer deconvolution of tumour composition using DNA methylation. Nature Communications 9, 3220.

Chan, S.H., Lim, W.K., Ishak, N.D.B., *et al.*, 2017. Germline mutations in cancer predisposition genes are frequent in sporadic sarcomas. Scientific Reports 7, 10660.

Chen, C.C., Hsu, C.C., Huang, C.E., *et al.*, 2017. Enhanced risk for specific somatic myeloproliferative neoplastic mutations in patients with stroke. Current Neurovascular Research 14, 222–231.

Cheng, E.H., Sawyers, C.L., 2012. In cancer drug resistance, germline matters too. Nature Medicine 18, 494–496.

Coulson, A., Levy, A., Gossell-Williams, M., 2014. Monoclonal antibodies in cancer therapy: Mechanisms, successes and limitations. The West Indian Medical Journal 63, 650–654.

Darvin, D., Toor, S.M., Sasidharan Nair, V., Elkord, E., 2018. Immune checkpoint inhibitors: Recent progress and potential biomarkers. Experimental & Molecular Medicine 50, 165.

De Queiroz Rossanese, L.B., De Lima Marson, F.A., Ribeiro, J.D., Coy, C.S., Bertuzzo, C.S., 2013. APC germline mutations in families with familial adenomatous polyposis. Oncology Reports 30, 2081–2208.

Drukker, K., Sennett, C.A., Giger, M.L., 2014. Computerized detection of breast cancer on automated breast ultrasound imaging of women with dense breasts. Medical Physics 41.012901

Findly, R.C., Niagro, F.D., Sweeney, R.P., Camus, A.C., Dickerson, H.W., 2018. Rearranged T cell receptor sequences in the Germline genome of channel catfish are preferentially expressed in response to infection. Frontiers in Immunology 9, 2117.

Fuchs, E., 2007. Scratching the surface of skin development. Nature 445, 834–842.

Giger, M.L., Inciardi, M.F., Edwards, A., *et al.*, 2016. Automated breast ultrasound in breast cancer screening of women with dense breasts: Reader study of mammography-negative and mammography-positive cancers. AJR. American Journal of Roentgenology 206, 1341–1350.

Gray, P.N., Tsai, P., Chen, D., *et al.*, 2017. TumorNext-lynch-MMR: A comprehensive next generation sequencing assay for the detection of germline and somatic mutations in genes associated with mismatch repair deficiency and lynch syndrome. Oncotarget 9, 20304–20322.

Hanahan, D., Weinberg, R.A., 2000. The hallmarks of cancer. Cell 100, 57–70.

Hanahan, D., Weinberg, R.A., 2011. Hallmarks of cancer: The next generation. Cell 144, 646–674.

Ho, M., Pastan, I., 2009. In vitro antibody affinity maturation targeting germline hotspots. Methods in Molecular Biology 525, 293–308.

Huang, K.L., Mashl, R.J., Wu, Y., *et al.*, 2018. Pathogenic germline variants in 10,389 adult cancers. Cell 173, 355–370.

Jaiswal, S., Natajaran, P., Silver, A.J., *et al.*, 2017. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. The New England Journal of Medicine 377, 111–121.

Johnson, C.H., Ivanisevic, J., Siuzdak, G., 2016. Metabolomics: Beyond biomarkers and towards mechanisms. Nature Reviews. Molecular Cell Biology 17, 451–459.

Joshi, S., Wang, T., Araujo, T.L.S., *et al.*, 2018. Adapting to stress—Chaperome networks in cancer. Nature Reviews Cancer 18, 562–575.

Kandoth, C., McLellan, M.D., Vandin, F., *et al.*, 2013. Mutational landscape and significance across 12 major cancer types. Nature 502, 333–339.

Kirik, U., Persson, H., Levander, F., Greiff, L., Ohlin, M., 2017. Antibody heavy chain variable domains of different germline gene origins diversify through different paths. Frontiers in Immunology 8, 1433.

Kloess, S., Kretschmer, A., Stahl, L., Fricke, S., Koehl, U., 2019. CAR-expressing natural killer cells for cancer retargeting. Transfusion Medicine and Hemotherapy 46, 4–13.

Korsunsky, I., McGovern, K., LaGatta, T., *et al*., 2014. Systems biology of cancer: A challenging expedition for clinical and quantitative biologists. Frontiers in Bioengineering and Biotechnology 2, 27.
Kuchenbaecker, K.B., Hopper, J.L., Barnes, D.R., *et al*., 2017. Risks of breast, ovarian, and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. Journal of the American Medical Association 317, 2402–2416.
Kumaran, M., Cass, C.E., Graham, K., *et al*., 2017. Germline copy number variations are associated with breast cancer risk and prognosis. Scientific Reports 7, 14621.
Lecornet, E., Ahmed, H.U., Hu, Y., *et al*., 2012. The accuracy of different biopsy strategies for the detection of clinically important prostate cancer: A computer simulation. The Journal of Urology 188, 974–980.
Lee, M.H., Siddoway, B., Kaeser, G.E., *et al*., 2018. Somatic APP gene recombination in Alzheimer's disease and normal neurons. Nature 563, 639–645.
Liaw, D., Marsh, D.J., Dahia, P.L., *et al*., 1997. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nature Genetics 16, 64–67.
Lim, Y.W., Chen-Harris, H., Mayba, O., *et al*., 2018. Germline genetic polymorphisms influence tumor gene expression and immune cell infiltration. Proceedings of the National Academy of Sciences of the United States of America 115, E11701–E11710.
Limvorapitak, W., Owattanapanich, W., Utchariyaprasit, E., *et al*., 2019. Better survivals in adolescent and young adults, compared to adults with acute lymphoblastic leukemia—A multicenter prospective registry in Thai population. Leukemia Research 87, 106235.
Linsley, P.S., Greene, J.L., Brady, W., *et al*., 1994. Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity 1, 793–801.
Ma, X., Liu, Y., Alexandrov, L.B., *et al*., 2018. Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. Nature 555, 371–376.
Maistro, S., Teixeira, N., Encinas, G., *et al*., 2016. Germline mutations in BRCA1 and BRCA2 in epithelial ovarian cancer patients in Brazil. BMC Cancer 16, 934.
Malempati, S., Gaynon, P.S., Sather, H., *et al*., 2007. Outcome after relapse among children with standard-risk acute lymphoblastic leukemia: Children's oncology group study CCG-1952. Journal of Clinical Oncology 25, 5800–5807.
Martinez, M., Moon, E.K., 2019. CAR T cells for solid tumors: New strategies for finding, infiltrating, and surviving in the tumor microenvironment. Frontiers in Immunology 10, 128.
Marty, R., Kaabinejadian, S., Rossell, D., *et al*., 2017. MHC-I genotype restricts the oncogenic mutational landscape. Cell 171.1272–1283. e15.
Maude, S., Frey, N., Shaw, P.A., *et al*., 2014. Chimeric antigen receptor T cells for sustained remissions in leukemia. The New England Journal of Medicine 371, 1507–1517.
Mego, M., Gao, H., Lee, B.N., *et al*., 2012. Prognostic value of EMT-circulating tumor cells in metastatic breast cancer patients undergoing high-dose chemotherapy with autologous hematopoietic stem cell transplantation. Journal of Cancer 3, 369–380.
Mehralivand, S., Shih, J.H., Rais-Bahrami, S., *et al*., 2018. A magnetic resonance imaging-based prediction model for prostate biopsy risk stratification. JAMA Oncology 4, 678–685.
Menden, M.P., Casale, F.P., Stephan, J., *et al*., 2018. The germline genetic component of drug sensitivity in cancer cell lines. Nature Communications 9, 3385.
Milanese, J.S., Tibiche, C., Zou, J., *et al*., 2019. Germline variants associated with leukocyte genes predict tumor recurrence in breast cancer patients. NPJ Precision Oncology 3, 28.
Moore, L.E., Nickerson, M.L., Brennan, P., *et al*., 2011. Von Hippel-Lindau (VHL) inactivation in sporadic clear cell renal cancer: Associations with germline VHL polymorphisms and etiologic risk factors. PLoS Genetics 7. e1002312.
Nadler, M., Al-Attar, H., Warner, E., *et al*., 2017. MRI surveillance for women with dense breasts and a previous breast cancer and/or high risk lesion. Breast 34, 77–82.
Nishioka, M., Bundo, M., Iwamoto, K., Kato, T., 2019. Somatic mutations in the human brain: Implications for psychiatric research. Molecular Psychiatry 24, 839–856.
Paik, S., Shak, S., Tang, G., *et al*., 2004. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. The New England Journal of Medicine 351, 2817–2826.
Park, T.S., Rosenberg, S.A., Morgan, R.A., 2011. Treating cancer with genetically engineered T cells. Trends in Biotechnology 29, 550–557.
Park, S., Supek, F., Lehner, B., 2018. Systematic discovery of germline cancer predisposition genes through the identification of somatic second hits. Nature Communications 9, 2601.
Parker, J.S., Mullins, M., Cheang, M.C., *et al*., 2009. Supervised risk predictor of breast cancer based on intrinsic subtypes. Journal of Clinical Oncology 27, 1160–1167.
Patel, V.R., Palayapalayam Ganapathi, H., 2016. Management dilemmas in low-risk prostate cancer. BJU International 118, 180–181.
Paulsson, K., Lilljebjörn, H., Biloglav, A., *et al*., 2015. The genomic landscape of high hyperdiploid childhood acute lymphoblastic leukemia. Nature Genetics 47, 672–676.
Riedl, C.C., Luft, N., Bernhart, C., *et al*., 2015. Triple-modality screening trial for familial breast cancer underlines the importance of magnetic resonance imaging and questions the role of mammography and ultrasound regardless of patient mutation status, age, and breast density. Journal of Clinical Oncology 33, 1128–1135.
Romero, D., 2018. Prostate cancer: CTCs enable early prediction of response. Nature Reviews. Clinical Oncology 15, 134.
Sarkar, S., Das, S., 2016. A review of imaging methods for prostate cancer detection. Biomedical Engineering and Computational Biology 7, 1–15.
Scott, A.M., Allison, J.P., Wolchok, J.D., 2012. Monoclonal antibodies in cancer therapy. Cancer Immunity 12, 14.
Scott, S., Samaratunga, H., Chabert, C., Breckenridge, M., Gianduzzo, T., 2015. Is transperineal prostate biopsy more accurate than transrectal biopsy in determining final Gleason score and clinical risk category? A comparative analysis. BJU International 116, 26–30.
Sparano, J.A., Gray, R.J., Makower, D.F., *et al*., 2018. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. The New England Journal of Medicine 379, 111–121.
Tate, J.G., Bamford, S., Jubb, H.C., *et al*., 2019. COSMIC: The catalogue of somatic mutations in cancer. Nucleic Acids Research 47, D941–D947.
Van Den Bergh, R.C., Essink-Bot, M.L., Roobol, M.J., *et al*., 2009. Anxiety and distress during active surveillance for early prostate cancer. Cancer 115, 3868–3878.
van't Veer, L.J., Dai, H., van de Vijver, M.J., *et al*., 2002. Gene expression profiling predicts clinical outcome of breast cancer. Nature 415, 530–536.
Vitale, M., Cantoni, C., Della Chiesa, M., *et al*., 2019. An historical overview: The discovery of how NK cells can kill enemies, recruit defense troops, and more. Frontiers in Immunology 10, 1415.
Vrooman, L.M., Silverman, L.B., 2016. Treatment of childhood acute lymphoblastic leukemia: Prognostic factors and clinical advances. Current Hematologic Malignancy Reports 11, 385–394.
Wang, E., Zaman, N., Mcgee, S., *et al*., 2015. Predictive genomics: A cancer hallmark network framework for predicting tumor clinical phenotypes using genome sequencing data. Seminars in Cancer Biology 30, 4–12.
Werner, H.M., Mills, G.B., Ram, P.T., 2014. Cancer systems biology: A peek into the future of patient care? Nature Reviews. Clinical Oncology 11, 167–176.
Xu, X., Li, J., Feng, X., *et al*., 2019. Association of germline variants in natural killer cells with tumor immune microenvironment subtypes, tumor-infiltrating lymphocytes, immunotherapy response, clinical outcomes, and cancer risk. JAMA Network Open 2. e199292.
Ye, Z., Qian, Q., Jin, H., Qian, Q., 2018. Cancer vaccine: Learning lessons from immune checkpoint inhibitors. Journal of Cancer 9, 263–268.
Yokosuka, T., Takamatsu, M., Kobayashi-Imanishi, W., *et al*., 2012. Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. The Journal of Experimental Medicine 209, 1201–1217.
Zhang, H., Chen, J., 2018. Current status and future directions of cancer immunotherapy. Journal of Cancer 9, 1773–1781.

Further Reading

Bassan, R., Hoelzer, D., 2011. Modern therapy of acute lymphoblastic leukemia. Journal of Clinical Oncology 29, 532–543.
Choi, S.K., Yoon, S.R., Calabrese, P., Arnheim, N., 2012. Positive selection for new disease mutations in the human germline: Evidence from the heritable cancer syndrome multiple endocrine neoplasia type 2B. PLoS Genetics 8. e1002420.
El Hajj, A., Ploussard, G., de la Taille, A., *et al.*, 2013. Analysis of outcomes after radical prostatectomy in patients eligible for active surveillance (PRIAS). BJU International 111, 53–59.
Knudson, A.G., 1971. Mutation and cancer: Statistical study of retinoblastoma. Proceedings of the National Academy of Sciences of the United States of America 68 (4), 820–823.
Knudson, A.G., 2001. Two genetic hits (more or less) to cancer. Nature Reviews. Cancer 1, 157–162.
Levine, A.J., 1997. p53, the cellular gatekeeper for growth and division. Cell 88, 323–331.
Lim, S.B., Di Lee, W., Vasudevan, J., Lim, W.T., Lim, C.T., 2019. Liquid biopsy: One cell at a time. NPJ precision oncology 3, 23.
Miller, M.C., Doyle, G.V., Terstappen, L.W., 2010. Significance of circulating tumor cells detected by the cell search system in patients with metastatic breast colorectal and prostate cancer. Journal of Oncology 2010, 617421.
Paik, S., Tang, G., Shak, S., *et al.*, 2006. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. Journal of Clinical Oncology 24, 3726–3734.
Palmirotta, R., Lovero, D., Cafforio, P., *et al.*, 2018. Liquid biopsy of cancer: A multimodal diagnostic tool in clinical oncology. Therapeutic Advances in Medical Oncology 10. 1758835918794630.
Richard, V., Conotte, R., Mayne, D., Colet, J.M., 2017. Does the ^{1}H NMR plasma metabolome reflect the host-tumor interactions in human breast cancer? Oncotarget 8, 49915–49930.
Rodrigues, G., Warde, P., Pickles, T., *et al.*, 2012. Pre-treatment risk stratification of prostate cancer patients: A critical review. Canadian Urological Association Journal 6, 121–127.
Stirzaker, C., Zotenko, E., Song, J.Z., *et al.*, 2015. Methylome sequencing in triple-negative breast cancer reveals distinct methylation clusters with prognostic value. Nature Communications 6, 5899.
Zhou, J., Kulasinghe, A., Bogseth, A., *et al.*, 2019. Isolation of circulating tumor cells in non-small-cell-lung-cancer patients using a multi-flow microfluidic channel. Microsystems & Nanoengineering 5, 8.