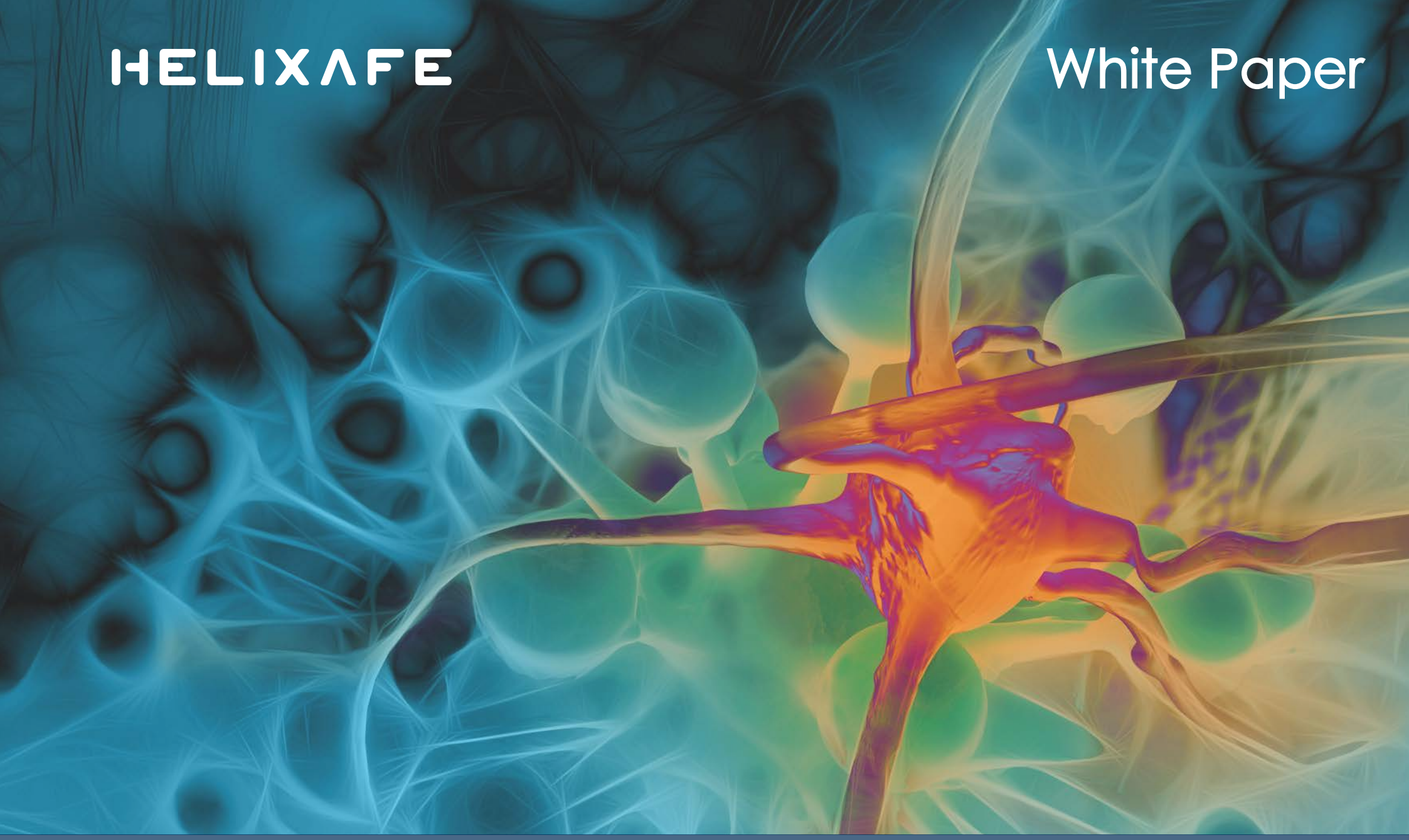
HELIXAFE

White Paper



SOLID CANCER PRODROMAL STAGE DETECTION

INTRODUCTION

Cancer is the second leading cause of death in the world. Estimated death from cancer for 2018 are almost 10 million, with lung, breast an colorectal among the most common form. The disease has is roots into our genes, not only because of inherited alleles predisposing to a higher risk of developing cancer, but also because of mutations and other alterations of the genome occurring lifelong following the exposure to other cancer risk factors. Such mutations and alterations make cells grow abnormally, invading tissues and organs and spreading to other parts of the body.

Fortunately, it is possible to prevent cancer deaths by avoiding or modifying cancer risk factors; current evidence suggests that between 30% and 50% of all cancer deaths could be prevented in such a way. Moreover, early diagnosis and a biomolecular approach to the characterization of cancer are associated to higher success rate in treating cancer. The analysis of genomic instability is an innovative tool helping both diagnosing and treating the disease; what is more, it could help informing decision on risk factor management by the assessment of the prodromal stage of cancer.

GENOMIC INSTABILITY: WHAT IS AND HOW IS IT LINKED TO CANCER

Genomic instability is a phenomenon arising in normal cells through accumulation of genetic and epigenetic changes. It has been shown to occur over a timespan ranging form years to decades, during which the vast majority of cells that acquired it die, probably cleared away by the immune system.

Several studies have been underlying the mechanisms at the basis of such genomic instability – from telomeres integrity to DNA repair capacity of the cells. Resulting abnormalities can be grouped in two distinct form: somatic point mutations (SPMs) and somatic copy number alterations (SCNAs).

In the former case, instability is observed at the nucleotide level; it consists in subtle sequence changes. SCNAs represent instead a form of instability at the chromosome level involving losses and gains of whole chromosomes, chromosome translocations or gene amplification. Chromosome translocation can result in gains or losses of chromosomal material or in the generation of new gene products. Oncogene amplification occur in a subset of many late-stage cancers, clearly associates with tumor progression, has prognostic significance and provides targets for therapeutics; it represents a common way for cultured cells to acquire resistance to chemotherapy too.

Genomic instability is linked to cancer and is an important characteristic of tumor cells. Most cancers develop it at some stage of their progression. It is thought to be involved in cancer genesis too: in fact, a minimal fraction of cells in an organism that have acquired genomic instability are not cleared away, eventually giving rise to a tumor. For example, in a current model for the role of telomeres in carcinogenesis, aging increases the number of cells experiencing telomeres shortening; as a consequence, the number of cells in danger of prooncogenic chromosome instability increases too. Fortunately, in the vast majority of such cells cancer development is prevented by senescence and apoptotic blocks, but in some rare cells – which may be more tolerant to genomic instability because of an alteration of the mechanisms that control telomeres length – this process eventually fails. The consequent instability could reach a lethal level that could prevent cancer development, but it could create a selective pressure for telomeres maintenance too. In fact, in the majority of human cancer telomeres are restabilized by telomere specific reverse transcriptase telomerase, an enzyme stringently repressed in normal somatic cells; otherwise, in a minority of cancers telomeres are maintained by a homologous recombination based mechanisms. In other circumstances, selection pressure can arise from the ability of cancer cells to manipulate or hijack cell's innate quality control mechanisms to have a growth advantage over other cells. In the end, nearly all solid tumors are genetically unstable; or, sometimes, there is a temporary rise of instability which is followed by the return to a relatively stable genome.

Both SPMs and SCNAs are linked to cancer. The mutation in the sequence of K-ras gene that is present in 80% of pancreatic cancers stands as example of cancer-associated SPM; and colon, breast, pancreas or prostate cancers may lose 25% of their alleles. In general, instability observed at the chromosome level is more frequent than instability at the nucleotide level, and evidence supports a greater role for SCNAs than SPMs in developing and maintaining cancer cell population diversity, but whilst uncommon the latter causes dramatic phenotypes.

Genomic instability drives intra-tumor genetic heterogeneity too; it guarantees that no tumors are alike and that no tumor is composed of genetically identical cells. Up to a maximum of four subclones, increasing clone number within the tumor correlates to decreased overall survival. The SCNAs burden per clone limits the viability of the tumor too; in fact, a SCNAs burden greater than 75% is associated

with a favorable outcome, especially when shared among one or two clones. In contrast, a high SNCAs burden spread among many clones is associated with a less favorable outcome.

Studies showed that cancers harboring SCNAs exhibit a true instability; losses or gains of multiple chromosomes are 10-100 times more frequent in their cells than in normal ones. However, the simple presence of genetic alterations, even when frequent, is not a marker of genetic instability. In fact, by definition instability is a matter of rate. That is why the simple analysis of SPMs cannot be used to determine genetic instability. Unfortunately, most studies on cancer genome instability analyze SPMs at a single time point, but to accurately estimate SPMs rate in an individual one needs samples from at least two time points. In fact, a single high frequency of mutation can be a random error causing incorrect evaluations. On the contrary, a somatic mutation frequency growth trend, manifested over a prolonged period, is indicative of genomic instability.

SOMATIC MUTATIONS AND CANCER PRODROMAL STAGE

When discussing about the relationship between DNA mutations and cancer, it is mandatory to differentiate between germline mutations and somatic mutations.

The former are hereditary mutations that have been associated to the risk of cancer development; they occurs in germ cells (cells that will give rise to sperm or eggs) and are passed onto every cell of the offspring. BRCA 1 and 2 mutations stand as examples of germline mutations predisposing to breast cancer, but only a limited percentage of cancers has a clear hereditary component, and even in those cases in which cancer susceptibility is clearly inherited, acquired mutations are needed for cancer to develop.

In contrast, somatic mutations are acquired, non hereditable, changes in DNA sequence in cells other than germ cells; they occurs after conception and can not be passed onto offspring. They occur in specific tissue (e.g. breast or lung) where their progressive accumulation can lead to cancer and is indicative of genomic instability; in particular, trends in somatic mutations are symptomatic of organism capability to repair DNA.

As stated before, genomic instability is a fundamental characteristic of cancer cells. But contrary to germline mutations, genomic instability is not a marker of the risk of cancer; rather, genomic instability is indicative of the cancer prodromal stage, that is the stage, lasting several years, during which cells progressively accumulate somatic mutations in clinically healthy individuals showing no cancer symptoms.

Researchers already gave proof of concept that genomic instability analysis is useful to assess the cancer prodromal stage. And, fortunately, it is possible to prevent and to manage genomic instability. For example, nutrigenetics and nutrigenomics are interesting tools to avoid genomic instability through a simple approach based on lifestyle. Biomarkers of genome integrity such as telomeres length can be utilized to establish recommended daily intakes for nutrients; in turn, optimizing nutrient intake plays a significant role in stabilizing the genome. For example, in smokers carotenoids assumption correlates to lung cancer incidence, and vitamin A or β carotene supplements are associated with a significant increase in mortality; cell cultures studies suggest that whatever concentration of non-vitamin A carotenoids tends to decrease DNA damage, whereas high concentrations of provitamin A carotenoids such as β carotene tend to increase it. Vitamin B3 deficiency impairs the function of enzymes critical to DNA repair, whereas a folate deficiency (especially if combined to suboptimal vitamin B6 and B12 levels) may led to telomeres shortening. Again, vitamin C correlates with various markers of genome stability in the presence of oxidative stress, and vitamin D and selenium concentrations are critical in the maintenance of genome stability too. In general, genome integrity has been shown to be highly sensitive to nutrient status, with optimal nutrients levels differing among individuals.

In a similar way, certain medications are associated with a reduction in genome instability; in particular, when patient with Barrett's esophagus starts taking NSAIDs (non-steroidal anti-inflammatory drugs) their somatic copy number alterations rate drops by an order of magnitude.

GENOMIC INSTABILITY AND CANCER TREATMENT

Upon development of cancer, it is also possible to take advantage of cancer genomic instability to effectively treat it. Some evidences suggest that tumors with more SPMs (e.g. microsatellite instability-positive colorectal cancers and metastatic melanomas with high mutation burden) respond better to immune checkpoint therapies.

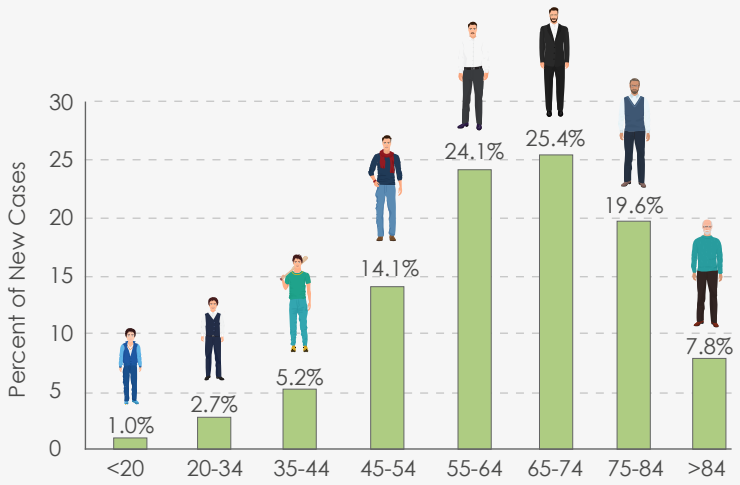
Moreover, it is possible to target the compromised mechanisms that lead to genomic instability to kill cancer cells without affecting healthy cells. For example, chemotherapy dose could be optimized to induce new SCNAs or could be utilized to increase the accumulation of SPMs, thus increasing genomic instability beyond

the tolerable limit and inducing cancer cells clearing.
Genomic instability analysis could also give an explanation for resistance to therapy. In fact, this phenomenon may lie with small clonal lineages more difficult to detect in a tumor. Having evolved most recently, such a small clonal lineages give a more up-to-date picture of the evolutionary dynamics in the tumor too. Moreover, genomic instability analysis allows the identification of resistance-associated genetic alterations to inform treatment decisions and monitoring tumor burden in response to therapy.
Finally, tumor mutational burden can be a biomarker for patient selection. For example, in non-small-cell lung cancer, 1-year progression-free survival rate of patients with a high tumor mutational burden (at least 10 mutations per megabase) is higher with nivolumab plus ipilimumab therapy than with chemotherapy (42.6% vs 13.2%), and the median progression-free survival is 7.2 vs 5.5 months.

ASSESSING GENOMIC INSTABILITY

Measuring genomic instability is difficult because it requires tracing the evolution of multiple subclones coexisting within a tumor. SNCAs rate is even more difficult to measure than SPMs rate: the genomic coordinates of two independently acquired SPMs are highly unlikely to overlap, whereas the same is not true for SNCAs. Hence, unfortunately monitoring genomic instability by means of traditional tissue-based approaches to identify mutational events associated with a higher risk of cancer is largely unfeasible. This limit can be overcome by sampling blood. In fact, compared to healthy people's blood, cancer patients' blood typically presents higher levels of total circulating cell-free DNA (cfDNA).
Discovered for the first time in 1948, cfDNA consists of small DNA fragments circulating in bodily fluids; it contains also circulating-tumor DNA (ctDNA), but in a limited amount (no more than 10%) of the total cfDNA. Both cfDNA and ctDNA are 180-200 nucleotides in size, suggesting their origin from cell death associated to physiological tissue remodelling events. Their analysis offers the possibility of detecting early genomic aberrations and cancer cells evolution; however, the low amount of circulating cfDNA and the very low proportion of mutated cfDNA molecules make such an approach challenging.
Taking advantage of the enhanced sensitivity of sequencing methods allowed by the introduction of molecular barcodes (unique sequences utilized to identify unique DNA fragments for a correct interpretation of DNA sequencing) and of other technological advances such enhanced bioinformatics filtering pipelines, researchers managed to identify clinically relevant genetic alterations in early-stage cancer patients, reaching a sensitivity lower than 1 mutant template molecule per milliliter of plasma. Nevertheless, early detection remains an ambitious clinical application of cfDNA analysis. To establish the sensitivity and specificity required for such an approach a substantial number of cancer patients and healthy controls is needed; furthermore, sources of false positive results exist. In contrast, monitoring overall genomic instability in healthy individuals, rather than the occurrence of a single driving genomic event, could help identifying people that might enter into early access screening prevention programs.
In a proof of principle study, researchers at the University Hospital Basel, the University of Trieste, the Memorial Sloan Kettering Cancer Center in New York, Tor Vergata University in Rome and Bioscience Institute in San Marino demonstrated the technical feasibility of extracting and analyzing healthy individuals' cfDNA to study genomic alterations.

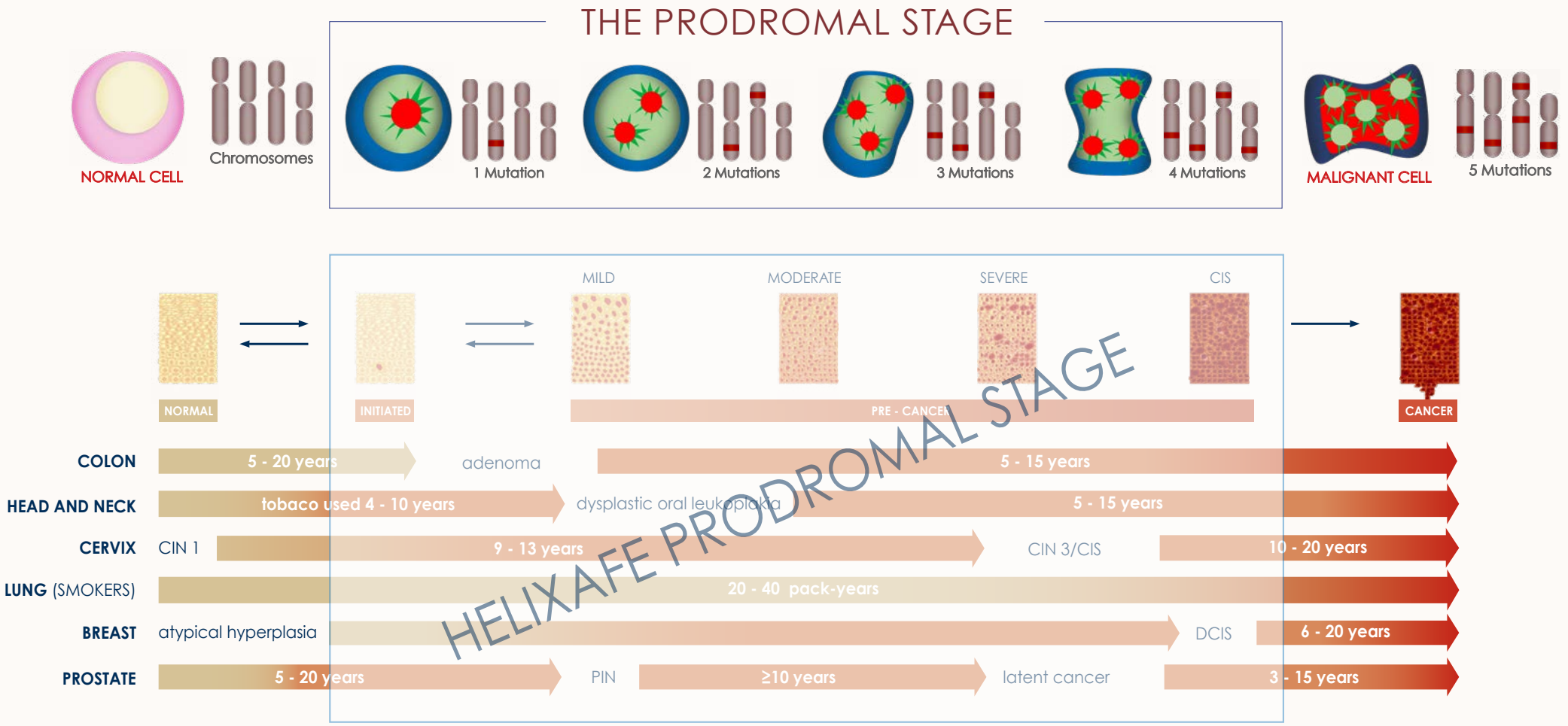
WHEN TO START THE ASSESSMENT PROGRAM



AGE Advancing age is the most important risk factor for cancer development overall, and for many individual cancer types. The median age of a cancer at diagnosis is 66 years but the disease can occur at any age.

ANALYZING cfDNA TO ASSESS GENOMIC INSTABILITY

Alborelli et al studied genomic alterations by means of molecular barcoded ultra-deep sequencing. They first analyzed tissue and blood sample from patients with a histologically confirmed diagnosis of cancer (30 cases of non-small cell lung cancer and 8 cases of breast cancer). Their results highlighted a substantial level of concordance (71%) between blood and tissue mutation profiles, suggesting that cfDNA analysis reliably mimics tissue genomic features. Moreover, certain mutations were detected only by blood cfDNA analysis, confirming the potential clinical value of using this approach in parallel to tissue biopsy, particularly for detecting mutations that are relevant for acquired therapy resistance. Then, they analyzed cfDNA in the blood of 106 women that were healthy at the time of samples collection and that were followed up regularly for the development of a breast cancer or any other malignancy up to 10 years later. A first group of participants did not develop any malignancy during the follow-up; a second group developed fibrocystic breast changes (for example, mastopathy); a third one developed breast cancer, and a fourth one another type of solid tumor. Most healthy individuals (84%) showed no genetic alterations, but 7 out of 55 healthy individuals analyzed showed clinically relevant gene mutations, 4 of which being well known cancer hotspot mutations.



HELIXAFE

In this scenario Bioscience Institute presents HeliXafe, a prevention program allowing the assessment of the prodromal, totally asymptomatic, stage of solid cancer (brain cancer excluded) by means of the analysis and the annual monitoring of mutation rate (hence, genome instability) in cfDNA. Nowadays, cancer screening tools, even when utilized for prevention purposes, manage to identify solid cancer only when already developed and evident. HeliXafe is nor a screening nor a risk assessment, early detection or diagnostic test; it anticipates early detection several years in advance compared to traditional diagnostic technologies.

Monitoring both somatic and germline mutations, HeliXafe allows to obtain a trend of stability for solid cancer associated mutations. Everything that is required is a 10-20 cc blood sample each year, from which cfDNA is obtained. Mutation rate in cfDNA is analyzed by means of Multi Biomarker Next Generation Sequencing (NGS) and a sophisticated management software. NGS is an innovative DNA sequencing technology that allows to sequence a high number of small DNA fragments at the same time, with a very high coverage of a region of interest – especially important for identifying mutation associated to cancer that are present at low fractions.

Based on HeliXafe algorithm, if there are no mutations in cfDNA, or if mutations in cfDNA are detected also in white blood cells DNA (germline control), all that is needed to do is planning the next check-up in one year; in fact, the presence of a mutation in the germline control means that it is an inherited (not acquired) mutation, not indicative of genome instability. But if mutations in cfDNA are not detected in germline control, that means they are somatic, acquired, mutations. In such a case, germline mutations in cancer predisposing genes should be also tested, looking for genes that can promote cancer development in the presence of somatic mutations.

In the end, patients are addressed to a counseling session with a cancer specialist. Genetic instability detected by HeliXafe is analyzed in depth by SCED, a screening assay for non-invasive Solid Cancer Early Detection based on simultaneous NGS analysis of ctDNA, circulating tumoral cells DNA (CTCs) and white blood cells DNA (germline control) obtained from healthy and asymptomatic subjects.

Inside HeliXafe it is possible to choose among 4 specific cancer prevention programs:

. HeliXmoker is specifically designed for smokers and highly pollution-exposed subjects; it analyzes 11 genes and 169 hotspots directly involved in respiratory system cancers with a 100% sensitivity.

. HeliXcolon monitors genes involved in colon cancer with a 99.9% sensitivity, for a total of 14 genes and 245 hotspots.

. HeliXgyn is a lifeline for women exposed to hormone therapies or being at high risk for breast or ovary cancer because of BRCA 1 or 2 mutations, which analyzes 11 genes and 157 hotspots with a 99.9% sensitivity.

. HeliXpan is appropriate for people at low risk for solid cancer; it allows the identification of tissues and organs needing a targeted prevention from somatic mutations linked to genome instability by monitoring 50 genes and 2800 solid cancer associated mutations with a 95% sensitivity. If detected mutations are in genes associated to lung, colon or breast-associated genes, an additional analysis with one of the three other more specific programs (HeliXmoker, HeliXcolon or HeliXgyn) looking for low frequency somatic mutations is recommended.

In the past, cancer prevention was achieved by means of the analysis of the familiar predisposition, and test results needed to be interpreted, potentially resulting non-informative; the analysis and monitoring of mutation rate are instead objective parameters. Aging is the most important cancer risk factor but the disease can hit at every age, and it is never too soon or too late to start prevention by evaluating genomic instability.

REFERENCES

Alborelli 2018 [not yet submitted; cfDNA analysis in healthy individuals]

Alix-Panabières C and Pantel K. Clinical Applications of Circulating Tumor Cells and Circulating Tumor DNA as Liquid Biopsy. Cancer Discov. 2016 May;6(5):479-91. doi: 10.1158/2159-8290.CD-15-1483

Andor N et al. Genomic Instability in Cancer: Teetering on the Limit of Tolerance. Cancer Res. 2017 May 1;77(9):2179-2185. doi: 10.1158/0008-5472.CAN-16-1553

Asatryan AD and Komarova NL. Evolution of genetic instability in heterogeneous tumors. J Theor Biol. 2016 May 7;396:1-12. doi: 10.1016/j.jtbi.2015.11.028
Azad 2015

Bettegowda C et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med. 2014 Feb 19;6(224):224ra24. doi: 10.1126/scitranslmed.3007094.

Bjelakovic G et al. Mortality in randomized trials of antioxidant supplements for primary and sec- ondary prevention: systematic review and meta-analysis. JAMA 2007;297: 842–57

Bracarda S et al. "To Cut or Not to Cut", a Biomolecular Approach to Metastasectomy in Metastatic Clear Cell Renal Cell Carcinoma. Eur Urol. 2018 Oct;74(4):481-482. doi: 10.1016/j.eururo.2018.06.039

Castro-Giner F et al. Cancer Diagnosis Using a Liquid Biopsy: Challenges and Expectations. Diagnostics (Basel). 2018 May 9;8(2). pii: E31. doi: 10.3390/diagnostics8020031.

Cohen JD et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 2018 Feb 23;359(6378):926-930. doi: 10.1126/science.aar3247

Cree IA et al. The evidence base for circulating tumour DNA blood-based biomarkers for the early detection of cancer: a systematic mapping review. BMC Cancer. 2017 Oct 23;17(1):697. doi: 10.1186/s12885-017-3693-7

De Mattos-Arruda L et al. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: a proof-of-principle. Ann Oncol. 2014 Sep;25(9):1729-35. doi: 10.1093/annonc/mdu239

Diehl F et al. Circulating mutant DNA to assess tumor dynamics. Nat Med. 2008 Sep;14(9):985-90. doi: 10.1038/nm.1789

Ferguson LR et al. Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. Semin Cancer Biol. 2015 Dec;35 Suppl:S5-S24. doi: 10.1016/j.semcancer.2015.03.005.

Fribbens C et al. Plasma ESR1 Mutations and the Treatment of Estrogen Receptor-Positive Advanced Breast Cancer. J Clin Oncol. 2016 Sep 1;34(25):2961-8. doi: 10.1200/JCO.2016.67.3061

Gandhi L et al. Pembrolizumab plus Chemotherapy in Metastatic Non-Small-Cell Lung Cancer. N Engl J Med. 2018 May 31;378(22):2078-2092. doi: 10.1056/NEJMoa1801005

Gerlinger M et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012 Mar 8;366(10):883-892. doi: 10.1056/NEJMoa1113205

Hellmann MD et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. N Engl J Med. 2018 May 31;378(22):2093-2104. doi: 10.1056/NEJMoa1801946

Hiom SC. Diagnosing cancer earlier: reviewing the evidence for improving cancer survival
The National Awareness and Early Diagnosis Initiative in England: assessing the evidence 5 years on. Br J Cancer. 2015 Mar 31; 112(Suppl 1): S1–S5. doi: 10.1038/bjc.2015.23

Lengauer C et al. Genetic instabilities in human cancers. Nature. 1998 Dec 17;396(6712):643-9

Misale S et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature. 2012 Jun 28;486(7404):532-6. doi: 10.1038/nature11156

Montagna M et al. Identification of seven new BRCA1 germline mutations in Italian breast and breast/ovarian cancer families. Cancer Res. 1996 Dec 1;56(23):5466-9

Nair-Shalliker V et al. Does vitamin D protect against DNA damage? Mutat Res 2012;733:50–7. doi: 10.1016/j.mrfmmm.2012.02.005

Nakad R and Schumacher B. DNA Damage Response and Immune Defense: Links and Mechanisms. Front Genet. 2016 Aug 9;7:147. doi: 10.3389/fgene.2016.00147

Omenn GS et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta- Carotene and Retinol Efficacy Trial. J Natl Cancer Inst 1996;88:1550–9

Peng Q et al. Reducing amplification artifacts in high multiplex amplicon sequencing by using molecular barcodes. BMC Genomics. 2015 Aug 7;16:589. doi: 10.1186/s12864-015-1806-8

Rahner N and Steinke V. Hereditary Cancer Syndromes. Dtsch Arztebl Int. 2008 Oct; 105(41): 706–714. doi: 10.3238/arztebl.2008.0706

Sethi S et al. Clinical advances in molecular biomarkers for cancer diagnosis and therapy. Int J Mol Sci. 2013 Jul 16;14(7):14771-84. doi: 10.3390/ijms140714771.

Sram RJ et al. Vitamin C for DNA damage prevention. Mutat Res 2012;733:39–49.
doi: 10.1016/j.mrfmmm.2011.12.001

Tomasetti C et al. Stem cell divisions, somatic mutations, cancer etiology, and cancer prevention. Science. 2017 Mar 24;355(6331):1330-1334. doi: 10.1126/science.aaf9011

van Ommen B et al. The Micronutrient Genomics Project: a community-driven knowledge base for micronutrient research. Genes Nutr 2010;5:285–96. doi: 10.1007/s12263-010-0192-8

Volik S et al. Cell-free DNA (cfDNA): Clinical Significance and Utility in Cancer Shaped By Emerging Technologies. Mol Cancer Res. 2016 Oct;14(10):898-908. Doi:
10.1158/1541-7786.MCR-16-0044

Wan JCM et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017 Apr;17(4):223-238. doi: 10.1038/nrc.2017.7. Epub 2017 Feb 24.

Weigelt B et al. Diverse BRCA1 and BRCA2 Reversion Mutations in Circulating Cell-Free DNA of Therapy-Resistant Breast or Ovarian Cancer. Clin Cancer Res. 2017 Nov 1;23(21):6708-6720. doi: 10.1158/1078-0432.CCR-17-0544

World Health Organization. Cancer. http://www.who.int/cancer/en/. 03/10/18

Yates LR et al. Subclonal diversification of primary breast cancer revealed by multiregion sequencing. Nat Med. 2015 Jul;21(7):751-9. doi: 10.1038/nm.3886

Zhang J et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. Science. 2014 Oct 10;346(6206):256-9. doi: 10.1126/science.1256930

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