
RADIATION THERAPY
DRINKING ALCOHOL
REPRODUCTIVE HISTORY
HORMONES
OVERWEIGHT
PHYSICAL INACTIVITY
FAMILY HISTORY
BREAST DENSITY
MAMMOGRAPHIC
BREAST CANCER
UNDERSTAND BREAST CANCER RISK
EXERCISE REGULARLY

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The overarching aim of the Epigenomics and Mechanisms Branch (EGM) is to advance the understanding of the role of (epi)genetic changes and pathways induced by environmental factors and endogenous processes in cancer causation, underpinning studies of etiology, carcinogen evaluation, and prevention. This is achieved by exploiting conceptual and technological advances in laboratory science and molecular epidemiology as well as by capitalizing on IARC's unique role in international cancer research (Figure 1) (Chung et al., 2023; Das et al., 2022; Herceg et al., 2022; Karimi et al., 2023; Talukdar et al., 2022a; Vicente et al., 2022). Key elements of EGM's strategy include developing innovative state-of-the-art molecular and cell biology and functional epigenomics research methodologies and bioinformatics and biostatistics tools, which are applicable to experimental cancer models and human samples from biobanks associated with population-based and case–control studies. EGM's ambition is to place increased emphasis on contributions to translational studies, through the discovery of mechanism-based biomarkers of exposure and risk stratification. Some highlights of EGM's work during the 2022–2023 biennium are described here.

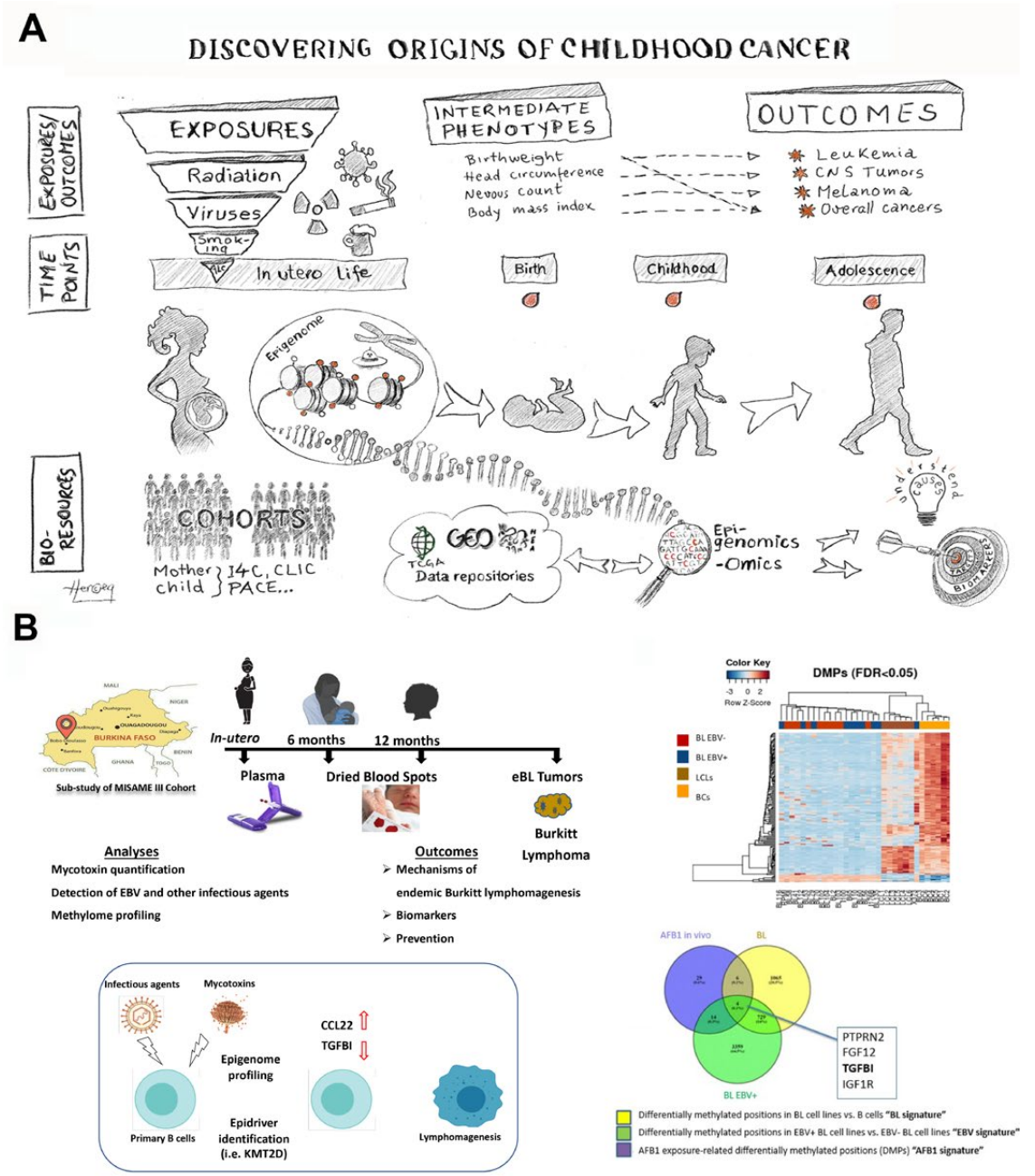
Figure 1. Research aims, collaborators, technological platforms, and resources of the Epigenomics and Mechanisms Branch (EGM). The Branch combines molecular epidemiology and mechanistic studies aimed at investigating the role of (epi)genetic changes and deregulated molecular pathways induced by environmental factors and identifying biomarkers of exposures and cancer risk. EGM also implements (epi)genomic methodologies, profiling strategies, and bioinformatics tools, which are applicable to population-based cohorts (molecular epidemiology studies coordinated by IARC and external collaborators) as well as state-of-the-art in vitro models. EGM's programme is carried out in close collaboration with IARC scientists and epidemiologists as well as external collaborators, many of which are part of international networks established to share technological platforms and biological resources. The emphasis is on enhancing interdisciplinarity and creating synergy within the Branch, thus facilitating the synthesis of scientific evidence from disciplines within the Branch, as well as the development of important transferrable skills between IARC and collaborators in low- and middle-income countries. © IARC.

Identifying epigenetic origins and markers of childhood cancer risk

Epigenome-wide alterations precede diagnosis since birth and affect prognosis of paediatric acute lymphoblastic leukaemia

Paediatric cancer is the leading cause of disease-related mortality in children and adolescents, with increasing incidence worldwide and lifelong sequelae in survivors. The causes of leukaemia, which is the most common form of paediatric cancer, are largely unknown. Growing evidence points to an origin in utero, when global redistribution of the epigenome (DNA methylation) modifications occurs, driving tissue differentiation (Figure 2A). Epigenome-wide DNA methylation was profiled in neonatal blood, with follow-up to paediatric pre-B acute lymphoblastic leukaemia (pre-B ALL), using double-blind analyses between prospective cohorts (from the International Childhood Cancer Cohort Consortium, I4C) extending from birth to diagnosis and retrospective studies backtracking from clinical disease to birth. Validation was done using an independent technology and population (totalling 317 cases and 483 controls) and complemented with pan-tissue methylation-stability (n = 5023 tissues; 30 types) and methylation-expression (n = 2294 tissues; 26 types) analyses. At diagnosis (n = 644 patients with pre-B ALL), methylation analysis was performed in leukaemia tissues from patients with pre-B ALL with at least 10 years of follow-up. Genomic imprinting was found to play a major role among identified loci, and an imprinted immunomodulating tumour suppressor gene was significantly hypermethylated at birth in nested cases relative to controls in all tested populations, including European and Hispanic ancestries. Specific differentially methylated regions (DMRs) were found to be stable over follow-up years after birth and across surrogate blood and target bone marrow tissues. Differential methylation was found to be associated with a change in gene expression and with survival of patients with pre-B ALL, supporting a functional and translational role for epigenetic markers. This study provides a proof of concept to detect epigenetic alterations at birth as potential precursors predisposing to

Figure 2. Identifying origins and causes of childhood cancer. (A) The hypothetical model. Exposure from external sources (general and specific factors) and internal biological processes may induce stable and mitotically heritable changes in the epigenome, which may result in alterations in the gene expression programme of stem and progenitor cells, leading to cancer in childhood and in later life and to cancer-predisposing intermediate phenotypes, which occur during the latency period between the exposure time and disease onset. The intermediate phenotypes, such as birth weight, head circumference, and naevus count, are positively associated with childhood leukaemia or lymphoma, brain tumours, and melanomas (in children, adolescents, and adults), respectively. EGM's studies identify epigenetic (DNA methylation) markers of specific exposures such as tobacco smoking, air pollution, ultraviolet radiation, and infections, as well as general exposures such as socioeconomic status, season of birth, and parental body mass index. (B) In utero and early-life epigenome profiling to decipher the multifactorial origins of endemic Burkitt lymphoma (eBL) in Africa. (left panel) Study design of the cohort-based analyses (top) and in vitro mechanistic analyses aiming to dissect the in utero and early-life epigenome–exposome interplay to decipher the multifactorial origins of eBL in Africa. (right panel) Heat map of differentially methylated positions (DMPs) in the genome of Epstein–Barr virus (EBV)-positive and EBV-negative BL-derived cell lines, primary B cells (BCs), and lymphoblastoid cells (LCLs) (top). Genes commonly affected by methylation changes identified from the comparative analysis of methylome profiles associated with B-cell transformation (BL signature), EBV (EBV signature), and aflatoxin B1 exposure (AFB1 signature) (bottom). The mechanistic analyses confirmed DNA methylation-dependent transcriptional silencing of TGFBI involving the recruitment of DNMT1, which is associated with an activation of the NF-κB pathway. The results revealed a potential common mechanism of B-cell transformation shared by the main risk factors of eBL (EBV and AFB1), suggesting a key determinant of disease that could enable the development of more efficient targeted therapeutic strategies. (A) © IARC/Z. Herceg. (B) (left panel) © IARC, (right panel) Reproduced from Manara et al. (2022). © 2022 by the authors. Licensee MDPI, Basel, Switzerland.



childhood leukaemia, reproducible in three continents and two ethnicities.

Assessment of in utero and early-life epigenome profiles to decipher the multifactorial origins of endemic Burkitt lymphoma in Africa

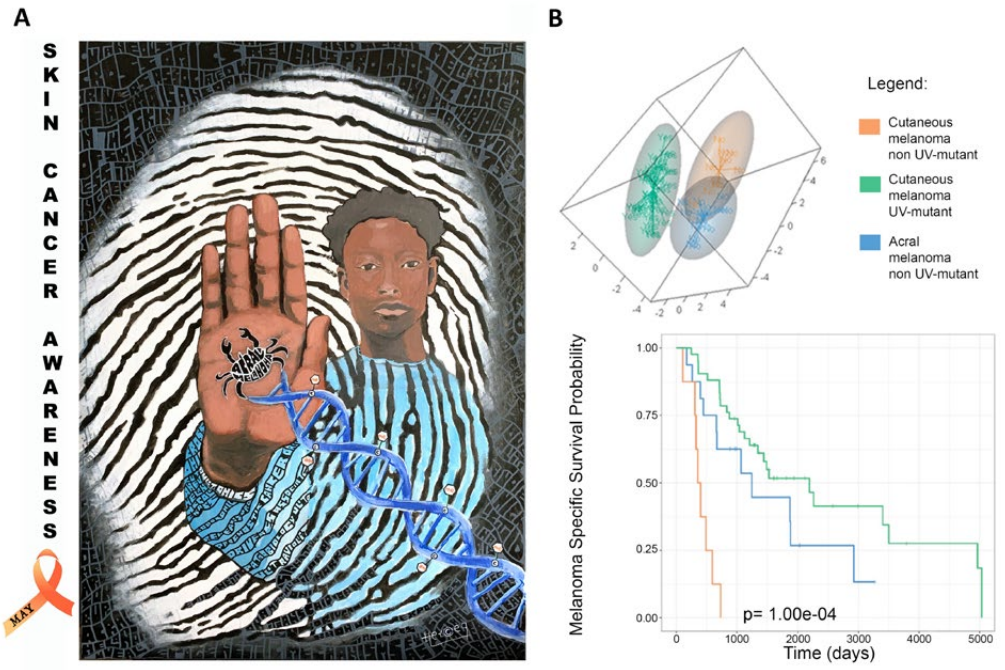
Endemic Burkitt lymphoma (eBL) is the most prevalent childhood cancer in sub-Saharan Africa. Although infection with Epstein–Barr virus (EBV) is necessary and is associated with eBL, it is not sufficient to induce lymphoma; this strongly suggests the multifactorial etiology of eBL. To gain insights into the synergistic impact of co-infections and exposure to mycotoxins on the epigenome, which may underpin eBL development, EGM built on a well-established mother–children cohort from Burkina Faso (the MISAME-III cohort, coordinated by Dr Carl Lachat at Ghent University), an eBL tumours cohort, and state-of-the-art established in vitro approaches (Figure 2B). Using biospecimens collected during pregnancy (mothers) and early in life (children at age 6 months and 12 months) and eBL tumour samples, EGM is performing methylome profiling complemented with in vitro mechanistic analyses to identify biomarkers of exposures, reveal eBL risks, and decipher early mechanisms of eBL development (Figure 2B). The data obtained so far suggest a synergistic impact of the mycotoxin aflatoxin B1 and EBV on immunoregulatory cytokine profiles of B cells and the expression of several cancer-related genes, and revealed putative epigenetic drivers ("epidrivers") of (e)BL among epigenetic regulator genes (Manara et al., 2022). Continuing work should provide a better understanding of environmental factors and mechanisms that underpin eBL carcinogenesis and reveal early biomarkers of the disease, relevant to prevention in low- and middle-income countries.

Cutaneous and acral melanoma cross-omics reveals prognostic cancer drivers associated with pathobiology and ultraviolet exposure

Exposure to ultraviolet (UV) radiation is causally linked to cutaneous melanoma, which occurs mainly in fair-skinned people, but the underlying epigenetic mechanisms, known as molecular sensors of exposure, have not been characterized in clinical biospecimens. EGM integrated clinical, epigenome (DNA methylome), genome, and transcriptome profiling of cutaneous melanoma from two multi-ethnic cohorts (the Barretos Cancer Hospital cohort, in Brazil, and The Cancer Genome Atlas/Skin Cutaneous Melanoma cohort, TCGA/SKCM). The study identified UV-related alterations in immunological pathways, with multi-omics cancer driver potential affecting patient survival. The top hits were validated by targeted sequencing, providing cost-effective opportunities for clinical application.

The study, published in *Nature Communications* (Vicente et al., 2022), also revealed important features of melanomas that are not associated with UV exposure (Figure 3). A subset of cutaneous melanomas did not harbour UV molecular signatures, and their molecular landscape and clinical prognosis not only were different from those of UV-exposed melanomas but also resembled those of the pathologically distinct acral melanoma. Acral melanoma

Figure 3. (A) Molecular fingerprints can infer exposure to ultraviolet (UV) radiation and distinguish between melanoma types, including acral melanoma, which develops in skin areas that are not often exposed to sunlight, such as the palms (as shown), and is the most common type of melanoma in darker-skinned people. (B) (top) Epigenomic maps demonstrating that non-UV-mutant cutaneous melanoma more closely resembles (i.e. overlaps with) acral melanoma rather than UV-exposed cutaneous melanoma. (bottom) Melanoma-specific survival, showing that patients with non-UV-mutant cutaneous melanoma, similarly to those with acral melanoma, have worse survival than patients with UV-exposed cutaneous melanoma. The *P* value was from the log-rank test. (A) © IARC/Z. Herceg. (B) Reproduced from Vicente et al. (2022).



develops in skin areas that are not often exposed to sunlight, such as the palms and soles, and is the most common type of melanoma in darker-skinned people.

By including patients with different skin colours, this study widened the resolution spectrum to various forms of melanoma and gained a better understanding of the origins of this cancer type, which is not necessarily triggered by UV exposure. These gene–environment interactions reveal translationally impactful mechanisms in melanomagenesis (Vicente et al., 2022).

Integrated multi-omics investigations of aristolochic acid-associated urothelial cancers

Aristolochic acids (AAs), natural compounds in Aristolochiaceae plants, pose grave risks of severe nephropathy and urological, hepatobiliary, and other cancers. The tumours arising after exposure to AA-containing herbal medicines or AA-contaminated foods bear a unique mutational signature, a marker of exposure to AA. The ARISTOCANCERS project (https://aristocancers.iarc.who.int/), led by EGM, explored the role of AA in upper tract urothelial carcinoma (UTUC) occurring in southern European regions with prevalent AA nephropathy (Karanović et al., 2022). In this case-series study, EGM and collaborators performed multi-omics analysis of UTUC tumours and patient urine samples, which revealed intricate cancer development processes, including specific DNA adduct formation, multitier gene regulatory network remodelling, and characteristic mutational fingerprints in both the genomic DNA and messenger RNA (mRNA). A microRNA (miRNA)-based urine test for cancer presence and recurrence was devised (Figure 4A). Inadequate regulations have allowed global sales of AA-containing herbal medicines, and environmental exposure routes remain neglected. To raise awareness, EGM published a comprehensive review in *Nature Reviews Cancer*, detailing the mutagenic and carcinogenic effects of AA (Das et al., 2022) and emphasizing the need to eliminate AA exposure sources to reduce cancer rates. The study highlights challenges in assessing AA-induced nephrotoxicity and carcinogenicity worldwide (Figure 4B) and proposes coordinated global actions to limit AA exposure, to prevent far-reaching adverse effects on AA-associated cancers and other pathologies.

Figure 4. The ARISTOCANCERS project on the role of aristolochic acid (AA) in human cancers. (A) The roadmap for multi-omics analysis of AA mutagenicity and carcinogenicity in upper tract urothelial cancers and of biomarkers of tumour presence and recurrence. (B) The global distributions of AA-containing *Aristolochia* plants, AA-associated cancers, reports of AA-associated mutagenesis, and occurrence of AA nephropathy (AAN), as reviewed in Das et al. (2022). mRNA, messenger RNA; miRNA, microRNA. (A) © IARC. (B) Reprinted from Das et al. (2022).

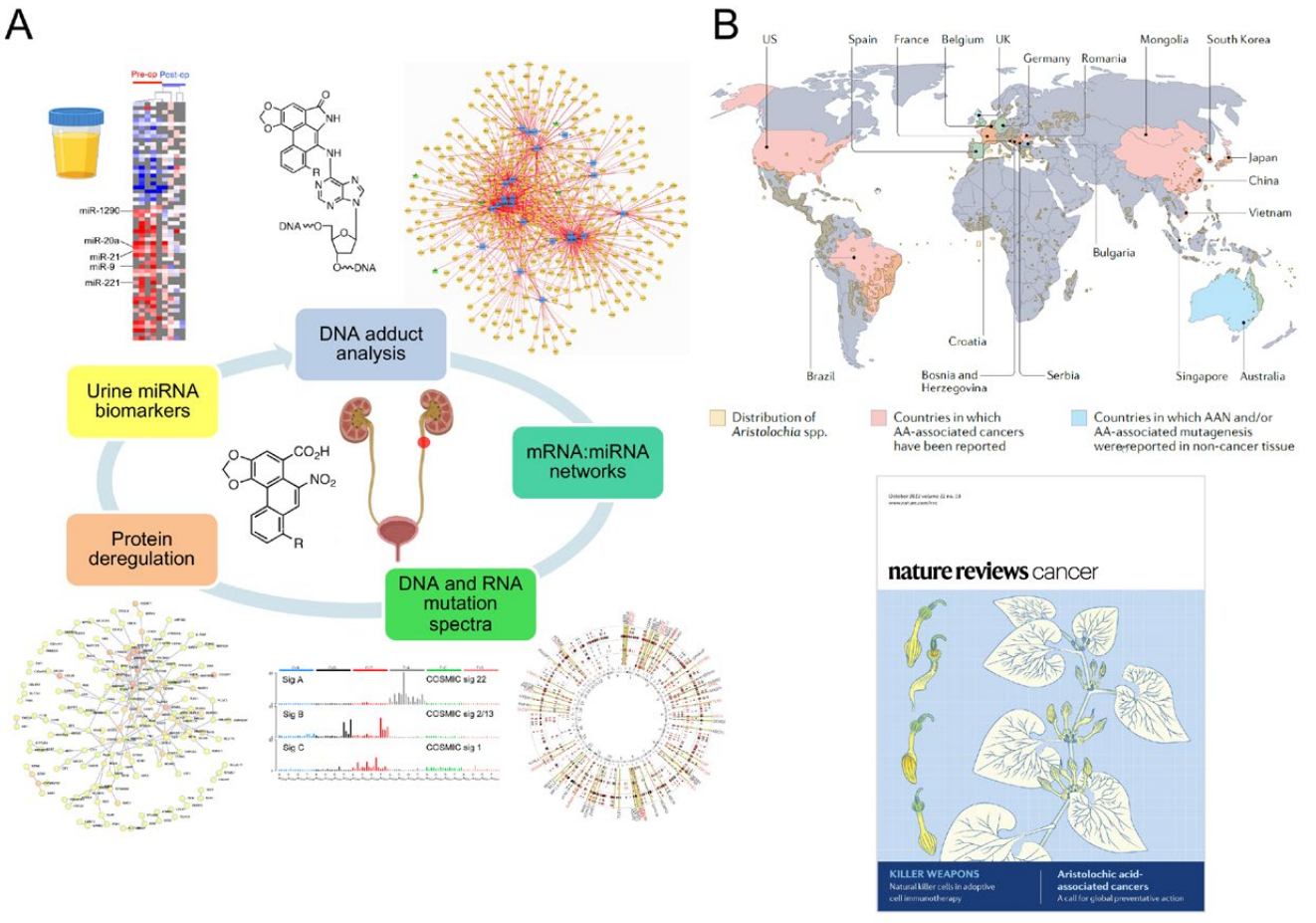
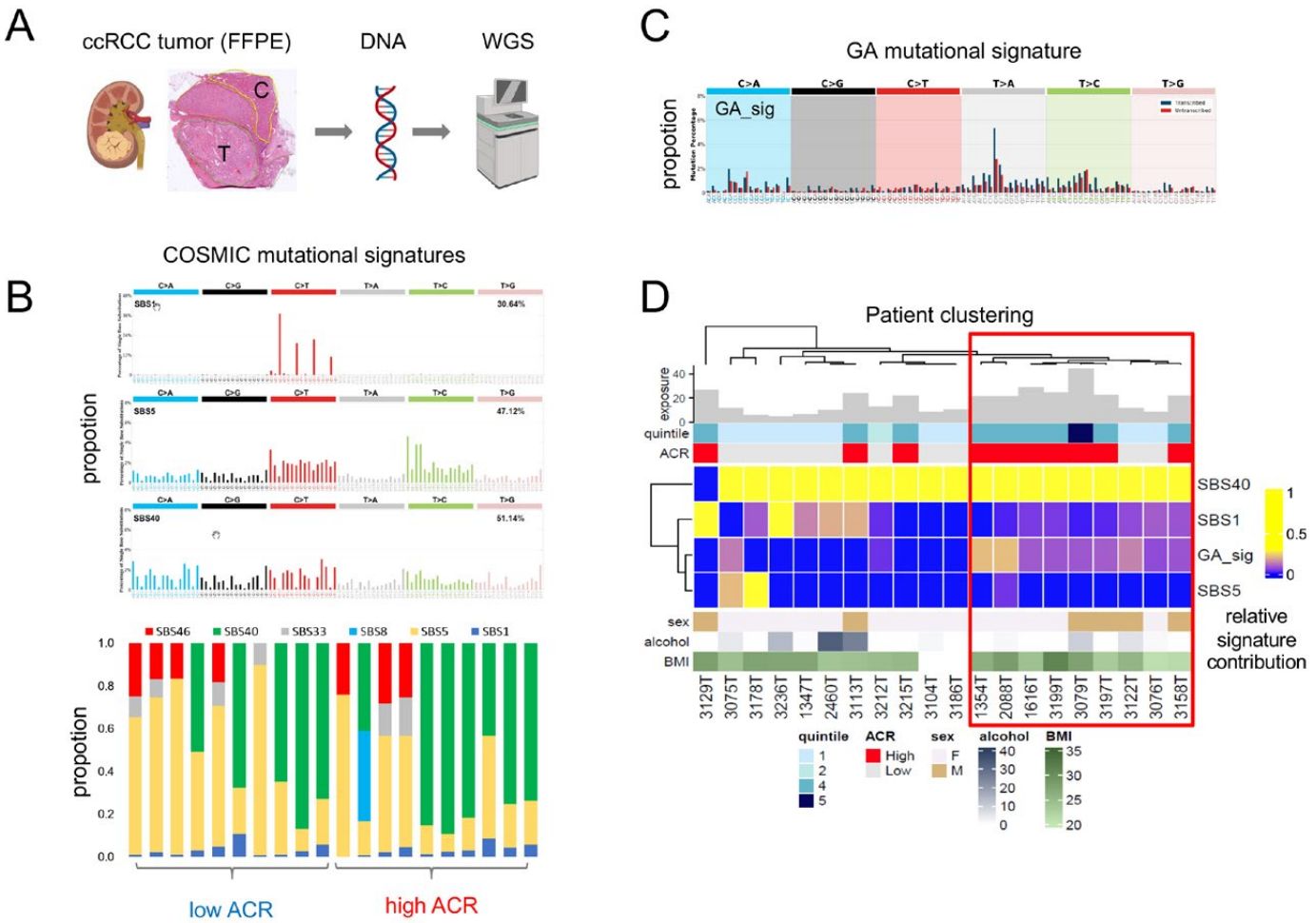


Figure 5. The MODARC project on mutational signatures in renal cancer after dietary exposure to acrylamide (ACR). (A) Schematic of the analysis of genome-scale mutational signatures in tumours of the patients with clear-cell renal cell carcinoma (ccRCC) in the Netherlands Cohort Study on Diet and Cancer (NLCS). T, tumour tissue area; C, non-tumour control tissue. (B) COSMIC mutational signatures identified in NLCS ccRCCs, and their distribution in samples of each ACR exposure group. (C) The mutational signature of glycidamide (GA), the reactive metabolite of ACR. (D) Hierarchical clustering of NLCS ccRCC samples based on mutational signatures reveals a relative enrichment of the presence of the GA signature (GA_sig) in the group with high dietary ACR exposure (red rectangle). BMI, body mass index; FFPE, formalin-fixed, paraffin-embedded; SBS, single-base substitution; WGS, whole-genome sequencing. © IARC.



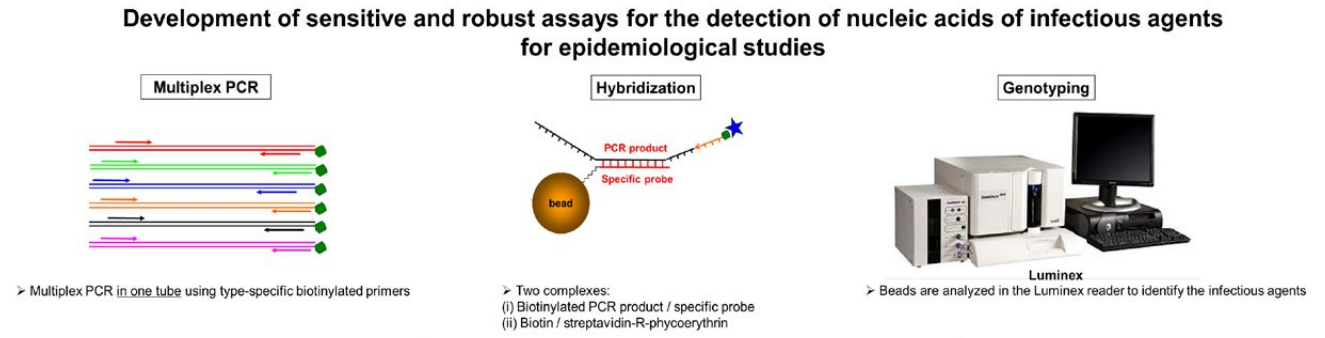
Mutational signatures of dietary acrylamide in renal cancer

Acrylamide, which was classified by the *IARC Monographs* programme as probably carcinogenic to humans (in 1994), is found in heated starchy foods and tobacco smoke. Previous studies on dietary acrylamide exposure and cancer yielded inconclusive results, although a potential elevated acrylamide-associated risk of clear-cell renal cell carcinoma (ccRCC) in non-smokers was proposed. EGM's MODARC project, a World Cancer Research Fund International-funded collaboration between EGM, Maastricht University, and the United States Food and Drug Administration National Center for Toxicological Research, investigates a molecular link between dietary acrylamide intake and ccRCC in the Netherlands Cohort Study on Diet and Cancer (NLCS), which involved 120 852 participants, including 480 with renal cancer. EGM's genomic investigations revealed the presence of endogenous COSMIC mutational signatures in all tumour samples, regardless of the dietary acrylamide exposure history (Figure 5A, B). However, an optimized in silico signature attribution approach showed a 2-fold enrichment of the mutational signature of glycidamide, a reactive metabolite of acrylamide, previously described by EGM (Figure 5C), in the cases with high acrylamide exposure (Figure 5D). This indicates a possible link between glycidamide-induced mutagenesis and ccRCC development, warranting further investigations on a larger scale. Furthermore, the findings can inform measures aiming to reduce acrylamide exposure and prevent related cancer formation.

Laboratory tools for epidemiological studies on virus-induced cancers

The IARC platform has established highly sensitive Luminex-based assays that enable the identification of viral biomarkers in body fluids, including circulating human papillomavirus DNA (HPV ctDNA) (Galati et al., 2022a)

Figure 6. Development of sensitive and robust assays for the detection of nucleic acids of about 250 infectious agents, including viruses, parasites, and bacteria. The assays combine two different steps: (i) multiplex polymerase chain reaction (PCR) using type-specific primers for the amplification of DNA, and (ii) bead-based hybridization for the identification of the infectious agents (Luminex technology). © IARC.



Infectious agents detected by the Luminex platform

Infectious agents	No. of infectious agents
High-risk and two Low-risk (HPV6 and 11) alpha HPV types	21
Low-risk alpha HPV types	29
gamma HPV types	52
beta HPV types	46
Polyomaviruses	12
Herpesviruses	8
Adenoviruses	17
Other infectious agents (Chlamydia T., HBV, MMTV, Schistosoma (haematobium, mansoni, japonicum), HPV1, Bocavirus)	8
Microbiome (bacteria suspected to be involved in human cancer and other diseases)	50
	Total = 241

(Figure 6). Potential advantages of the use of body fluid-based assays include reduced time and easier management of patients, especially for early diagnosis and disease monitoring (Karimi et al., 2023). In collaboration with the European Institute of Oncology (Italy), a proof-of concept biomarker study was designed to compare several non-invasive diagnostic approaches to identify HPV-associated head and neck squamous cell carcinomas (HNSCC) using a combination of HPV ctDNA in plasma and HPV DNA in oral samples from patients with HNSCC (n = 132) and non-SCC HNC (n = 10). EGM found that oral HPV DNA and, slightly more, HPV16 ctDNA in plasma represent highly sensitive and reliable biomarkers for the identification of HPV-positive oropharyngeal squamous cell carcinoma (OPSCC). The use of combined biomarkers, such as HPV16 ctDNA and oral HPV16 DNA in gargle, resulted in the identification of 100% of HPV16-related OPSCCs (15 of 15; 95% confidence interval, 76.14–100.00), even at earlier cT stages. This proof-of-concept study, which complements and extends the work described by Robbins et al. (2022), indicates that non-invasive body-fluid biomarkers could be adjunctive tools, which can be easily applied together with the available methods, in a diagnostic algorithm of HPV-driven OPSCCs.

Bariatric surgery-induced weight loss and associated genome-wide DNA methylation alterations in obese individuals

Obesity is a multifactorial and chronic disease that adversely affects human health, including cancer risk. EGM took advantage of intervention studies (including the ISS-Rome bariatric surgery and caloric restriction cohort) to investigate the effects of bariatric surgery-induced weight loss on clinical parameters and epigenome alterations in individuals with severe obesity (Figure 7). The study collected blood samples and follow-up data, based on which EGM performed DNA methylome analysis to identify differentially methylated genes and pathways linked to weight loss. To substantiate the results, a replication set of samples from body mass index (BMI)-discordant monozygotic twins was included. The obese twins in the replication set lost weight due to caloric restriction, thus serving as a control group that did not undergo bariatric surgery. The analysis revealed 41 significant (Bonferroni $P < 0.05$) and 1169 suggestive differentially methylated positions (DMPs) associated with weight loss due to bariatric surgery. Among the significant DMPs, the top hits were replicated in an independent cohort of BMI-discordant monozygotic twins (where the heavier twin underwent diet-induced weight loss). Pathway enrichment analysis of the DMR-associated genes showed that functional pathways related to immune function and type 1 diabetes were significant. Weight loss due to bariatric surgery also significantly decelerated epigenetic age 12 months after the intervention (Figure 7) (Talukdar et al., 2022a). EGM's findings provide evidence that weight loss brings about an improvement in biological (epigenetic) age and in the clinical/metabolic profile of obese individuals. Continuing studies are aimed at addressing whether specific epigenetic changes that occur as early events in response to weight loss may contribute to the reduction of obesity-associated cancer risk.

Figure 7. Weight loss and associated genome-wide DNA methylation alterations in obese individuals. (A) Study design with participant details and collection time points. (B) BMI-change trajectory at 6 months and 12 months after bariatric surgery. (C) Manhattan plot showing all differentially methylated positions (DMPs) across autosomes after weight loss. (D) Volcano plot showing hypermethylated and hypomethylated DMPs. (E) Heat map showing DNA methylation patterns of DMPs with weight loss. (F) Epigenetic age acceleration (EAA) at different time points during the course of weight loss. EAA analysis was performed using the Hannum method by taking the residual from the regression of epigenetic age (based on β values of 71 CpG probes) on chronological age. Positive EAA values suggest that the epigenetic age is greater than expected based on chronological age. Reproduced with permission from Talukdar et al. (2022a).