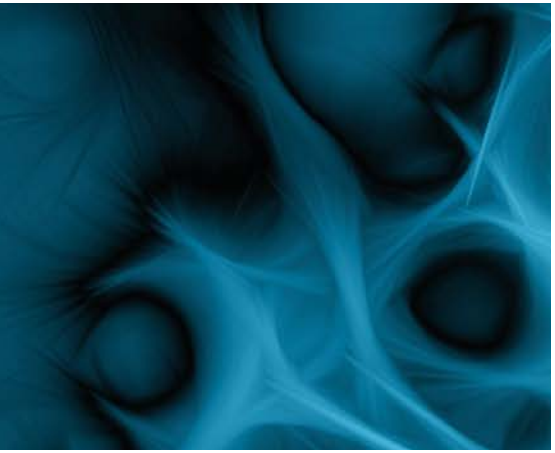
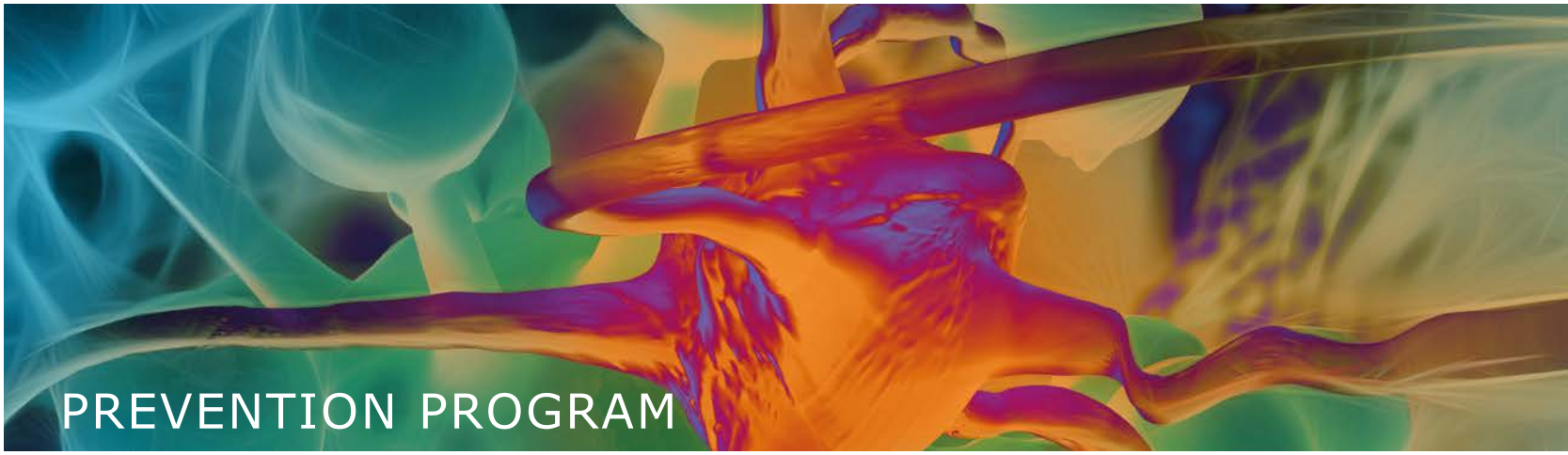
HELIXAFE

CANCER PRODROMAL STAGE ASSESSMENT



SOMATIC MUTATION FREQUENCIES MONITORING



BIOSCIENCE GENOMICS

Università di Roma Tor Vergata spin off

SOLID CANCER PRODROMAL STAGE ASSESSMENT

By yearly monitoring the occurrence of cancer-associated mutations in asymptomatic patients, we can potentially detect the solid cancer prodromal stage, before a clear clinical manifestation. It now acquired that it might take several years between the appearance of a first mutation and the actual development of cancer. Thus, the detection of certain mutations driving the early identification of neoplastic lesions can significantly improve survival rates.

Current diagnostic methods (such as mammography, X-rays, colonoscopy and dermoscopy) detect cancers when they already exist as established tumor. Identifying the cancer-associated mutations as soon as they occur and before tumor is detectable with current diagnostic methods may enable the full eradication of the disease and significantly better outcome for the patient.

SOMATIC MUTATIONS

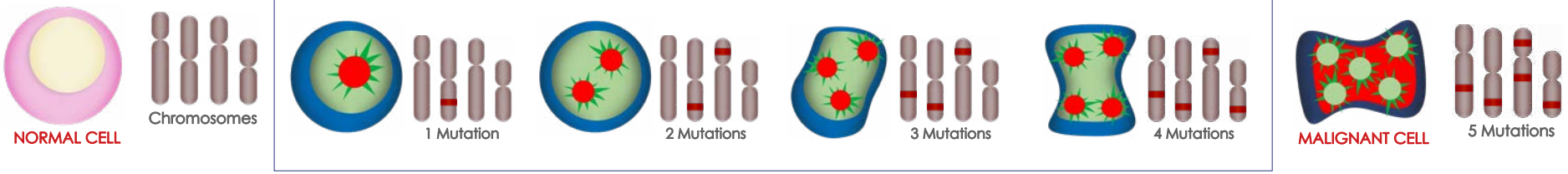
A SOMATIC mutation is not present in the zygote, but is acquired during a patient's life as a consequence to various factors, such as dangerous environment agents or life-style behavior (e.g. cigarette smoke).

SOMATIC mutations may occur in a single cell that by sub sequentially acquiring a proliferative advantage leading to multiple and sustained rounds of cell divisions will carrying on these mutations along in all 'offspring' cells.

Nonetheless since SOMATIC mutations are not hereditary, they are not passed onto future generations.

Most cancers result from the accumulation of acquired (SOMATIC) mutations because they are more common than their germline (inherited) counterpart.

THE PRODROMAL STAGE



MUTATIONS AND GENETIC INSTABILITY ARE INDICATORS IN CANCER PRODROMAL STAGE

Environmental and lifestyle factors such as smoking, poor and fat-rich diet, hormone imbalance and radiation/sun exposure can lead to the acquisition of SOMATIC mutations in cancer-relevant genes. Occasionally, random mutations occur during cell division, seemingly without cause. During normal cell division, the original cell must replicate its DNA; such process leads to occasional errors and the introduction of mutations within the DNA sequence.

Although our cells have developed a highly sophisticated and efficient DNA replication proofreading system, every cell division represents an new opportunity for a mutation to occur. Usually, healthy cells detect mutations in their DNA sequence and promptly repair them. If particular mutation cannot be repaired, the cell turns up a cell death guided process called apoptosis.

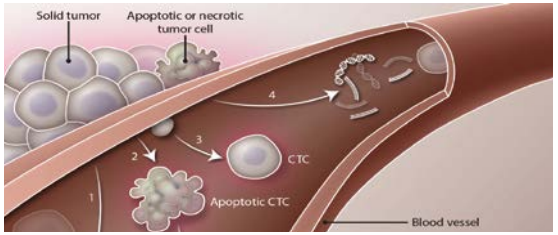
However, if cell death does not occur and the mutation is not repaired, the cell might start dividing and eventually leading to cancer. As we get older, the number of somatic mutations accumulates and our DNA replication proofreading system reduces its efficiency, that is why age poses a greater risk of developing cancer.

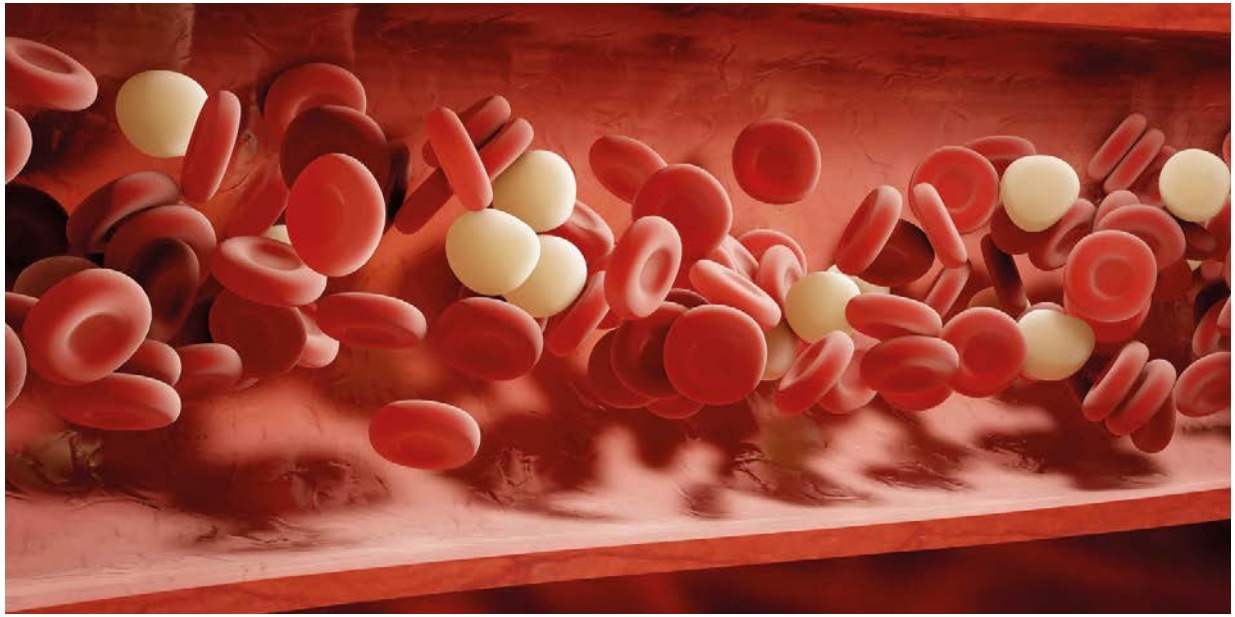
WHAT IS LIQUID BIOPSY

Upon tumor cell death (via necrosis or apoptosis) occurring as a consequence of rapid proliferation and cellular turnover, small fragments of DNA, known as circulating tumor DNA (ctDNA) are released into the bloodstream. In parallel, cancer cells are shed from a solid tumor mass into the bloodstream, and they are referred to as circulating tumor cells (CTCs). The term Liquid Biopsy refers to body fluid sampling (blood, saliva, urine, etc.) and analysis of ctDNA and/or CTCs. Interrogation of ctDNA and CTCs extracted from a peripheral blood sample enables the detection and characterization of a patient's cancer.

cfDNA

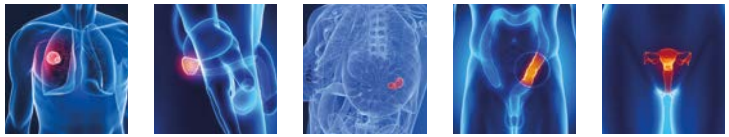
Circulating cell-free DNA (cfDNA) are small DNA fragments found circulating in plasma or serum, as well as other body fluids such as saliva and urine. In healthy individuals, the levels of cfDNA in plasma/serum are generally low, however during pregnancy, illness, and exacerbation of tissue (intensive exercise or injury) the levels of cfDNA generally increase.





TISSUE BIOPSY	VS	SCED - HELIXAFE
Invasive		Non-invasive
Specific to primary tumor site		Independent from primary tumor
Poor evaluation of tumor heterogeneity		Informative on tumor heterogeneity
Often difficult to obtain		Blood withdrawal easily obtainable
Not relevant in cases where primary tumor has been surgically removed		Informative even prior to development of measurable primary tumors or metastasis
Difficult to repeat		Repeatable
Risk of inaccurate localization of the area to be analyzed		Risk of low amount of blood withdrawal

HELIXAFE



SOLID CANCERS

Cancer is a genetic disease. The accumulation of somatic mutations in the DNA of a cell leads to deregulation of its physiological functions resulting in uncontrolled growth eventually leading to cancer development. Once established, tumors shed DNA that can reach the circulatory system (circulating-free DNA or cfDNA). Thus, monitoring the emergence of somatic mutations present in cfDNA may allow the detection of cancer at very early stages.

Early stage tumors with no metastatic spread have a significant better chance to be successfully treated. Based on the analysis of cfDNA using Next Generation Sequencing technology (NGS), **HELIXAFE**, performed on a regular base, provides a mutation frequency monitoring system and a risk assessment for tumor incidence. In the last decade, the NGS technology has revolutionized medical genetics and pathology allowing to inexpensively sequence the human genome at high speed and accuracy. Using a proprietary algorithm developed at the Bioscience Genomics, **HELIXAFE** annual prevention program allows the monitoring of mutations frequency via the interrogation of 2800 hotspots of mutations over 50 solid tumors-associated genes.

NON-INVASIVE

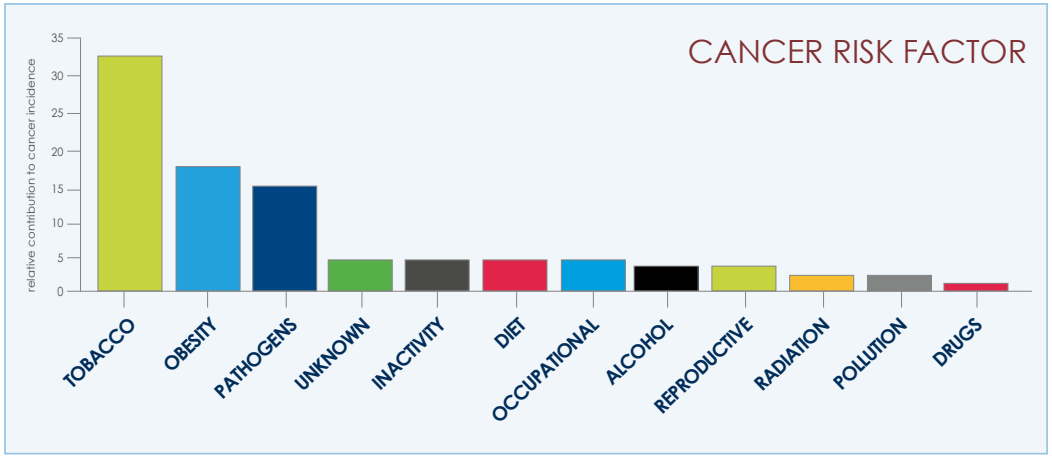
cfDNA can be easily obtained from just a 10 ml sample of full blood. **HELIXAFE** annual prevention program enables the detection of mutations associated with cancer risk development in real-time, or to follow-up mutations already detected in previous analyses, thus informing on the evolution of genetic aberrations. For patients at high-risk as defined using **HELIXAFE**, cancer-associated genetic alterations will be measured using SCED (Solid Cancer Early Detection). SCED ensures an accurate mutational analysis of cfDNA (at allelic frequency as low as 0.05%) with the aim of early cancer onset detection at stage were chances to be cured are high.

50 genes and 2800 mutations									
ABL1	AKT1	ALK	APC	ATM	BRAF	CDH1	CDKN2A	CSF1R	CTNNB1
EGFR	ERBB2	ERBB4	EZH2	FBXW7	FGFR1	FGFR2	FGFR3	FLT3	GNA11
GNAS	GNAQ	HNF1A	HRAS	IDH1	JAK2	JAK3	IDH2	KDR	KIT
KRAS	MET	MLH1	MPL	NOTCH1	NPM1	NRAS	PDGFRA	PIK3CA	PTEN
PTPN11	RB1	RET	SMAD4	SMARCB1	SMO	SRC	STK11	TP53	VHL

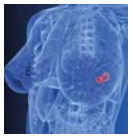
OBJECTIVE ASSESSMENT RISK

Currently the risk assessment of solid cancers is based on the evaluation of the clinical medical history and familiarity of a patient, along with the analysis of certain biomarkers (e.g. PSA for prostate tumor) at a specific time point. In many cases however, this approach fails to detect cancer at its early onset.

Using a monitoring approach, **HELIXAFE** analyzes in real-time the emergence of cancer-associated mutations through NGS technology generating a risk score via customized proprietary software.



HELIXGYN



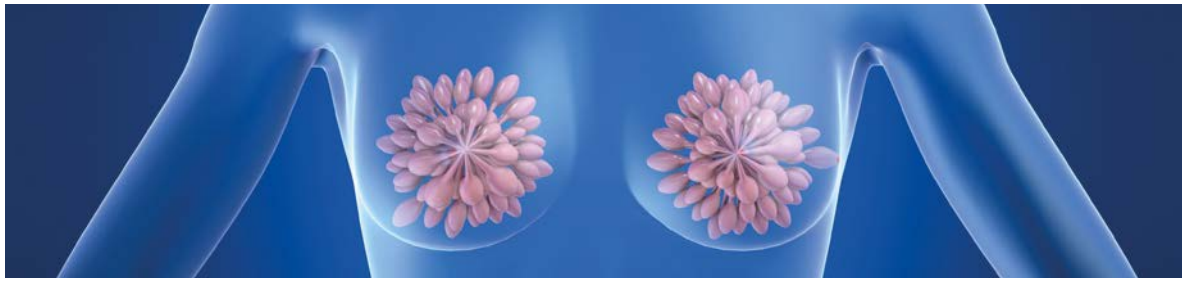
BREAST - OVARIAN CANCER

Breast and ovarian cancer are diagnosed to almost two million women each year worldwide. Risk factors include genetic predisposition, late pregnancy, a history of hormone replacement therapy, contraception or ovarian stimulation.

HELIXGYN is a yearly prevention program for women at risk of developing breast or ovarian cancer.
HELIXGYN monitors the occurrence of specific mutations that arise in the context of breast and ovarian cancer.

10 Genes	
AKT1	FBXW7
EGFR	KRAS
ERBB2	PIK3CA
ERBB3	SF3B1
ESR1	TP53

157 HOTSPOT	
PIK3CA:	E545K and H1047R
AKT1:	E17K
ESR1:	Mutations associated with anti-estrogen resistance
TP53:	Mutations associated with loss of function
ERBB2:	Mutations associated with sensitivity to anti-ERBB2 therapies

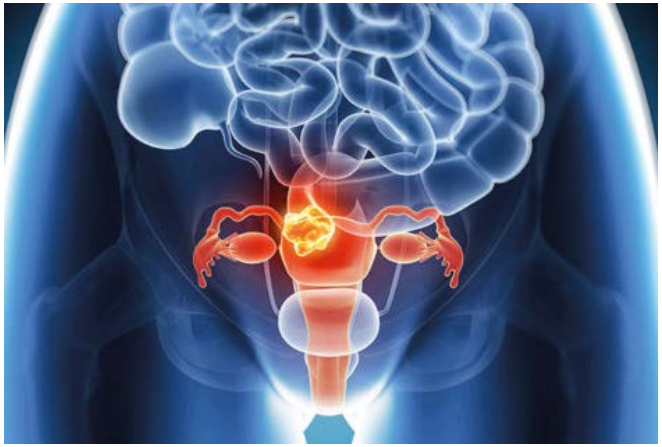


CONTRACEPTION Hormonal therapy used as contraceptive contains endocrine hormones, which may stimulate the growth of tumors cells.

HELIXGYN can help to assess the safety of contraceptive hormone treatment or hormone replacement therapy by monitoring the emergence of mutations that are associated to breast and ovarian cancer development.

FIVET growing scientific evidence suggest that some sensitive tissues such as those of breast, uterus, cervix and ovary, may be subject to tumor formation after prolonged hormone stimulation.
Studies have underlined a link between cancer development and treatment against infertility. Also in this case, hormones can accelerate the growth of tumor cells that may be already present in some tissues. HELIXGYN can monitor the occurence of cancer-associated mutations that may arise as a consequence to prolonged hormone stimulation.

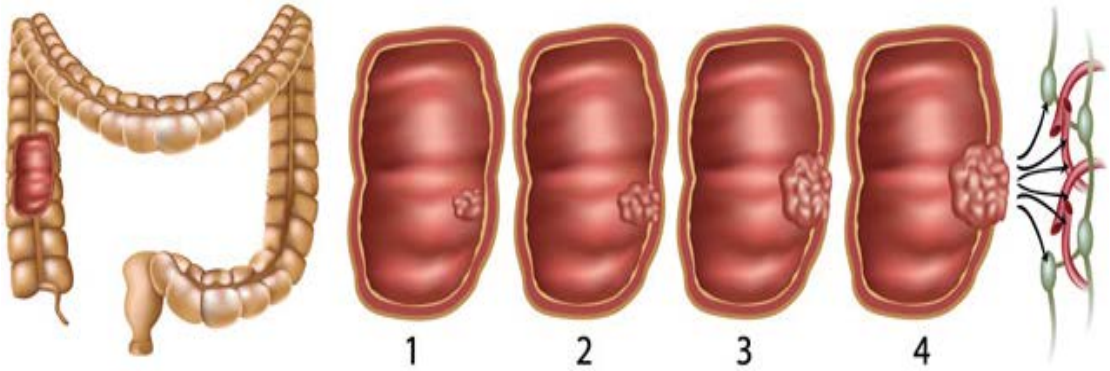
TOS the relationship between the hormone replacement therapy during menopause and the risk to develop several tumors has been a debated subject for decades.
Hormone replacement therapy taken after menopause increases the risk to develop breast cancer as a function of treatment duration.
The risk of endometrial hyperplasia, which could be a precursor for endometrial cancer, has been shown to increase in cases where only estrogens are administered.
HELIXGYN is also designed to assess the safety of hormone replacement therapy.



HELIXCOLON

COLORECTAL CANCER

HELIXCOLON is a prevention program that provides yearly assessment of 14 genes and 246 hotspots associated to the development of colorectal cancer.

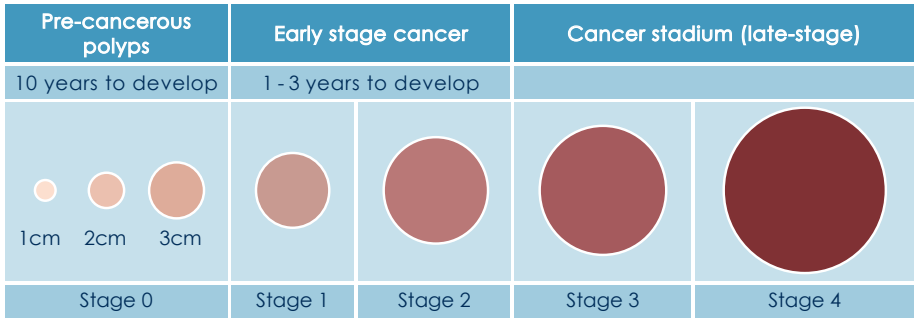


Currently, screening tests used to detect colorectal cancer are based on blood detection in fecal samples, or in some cases the rectum sigmoidoscopy.
In western countries, colon-rectum cancer represents the second most common malignant tumor and it has a mortality rate of 13% at 5-years after diagnosis.
The occurrence of colorectal cancer is quite rare before the age of 40y and it is increasingly frequent from the age of 60y reaching a peak at age 80y, affecting men and women equally.

Risk factors concerning colorectal cancer development obesity, genetic predisposition, age, inflammatory chronic bowel diseases and clinical history of colon polyps play a major role.

14 Genes	
AKT1	KRAS
BRAF	MAP2K1
CTNNB1	NRAS
EGFR	PIK3CA
ERBB2	SMAD4
FBXW7	TP53
GNAS	APC

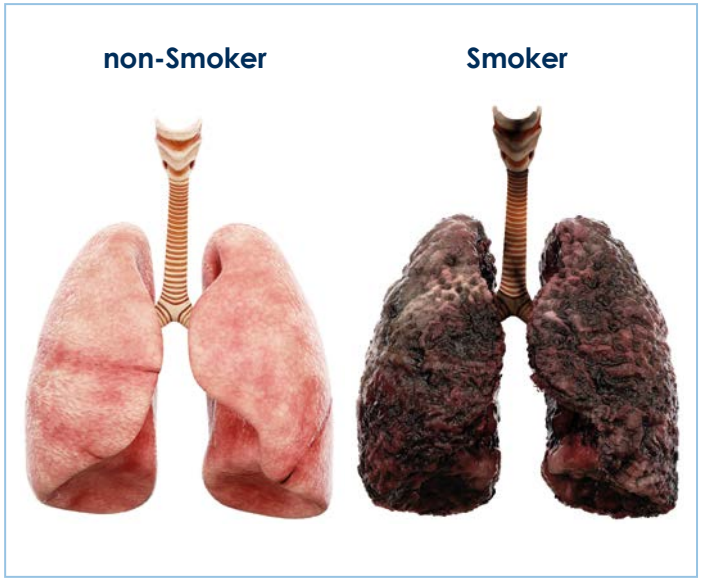
245 HOTSPOT	
KRAS/NRAS:	G12/G13/Q61
BRAF:	V600E
PIK3CA:	E545K, H1047R
TP53:	R175H R273H/C/L
Recurent deleterious APC mutations (including p.R876*, p.Q1378*, and p.R1450*)	
SMAD:	R361C/H
CTNNB1:	S45F, T41A



Colorectal cancer is often silent, having mild clinical symptoms till late stages and is often resulting from the evolution of a benign lesion (as the adenomatous polyps) acquiring genetic abnormalities over relative long period of time (usually several years to decades).
Thus, **HELIXCOLON** prevention program would ensure a timely identification of degenerating lesions allowing a prompt intervention strategy.

HELIXMOKER

LUNG CANCER



HELIXMOKER is a cancer prevention program specifically designed for smokers. Upon the mutational status analysis of genes directly involved in lung cancer onset, a patient has the possibility to discover at a very early stage if he/she is affected by lung cancer.

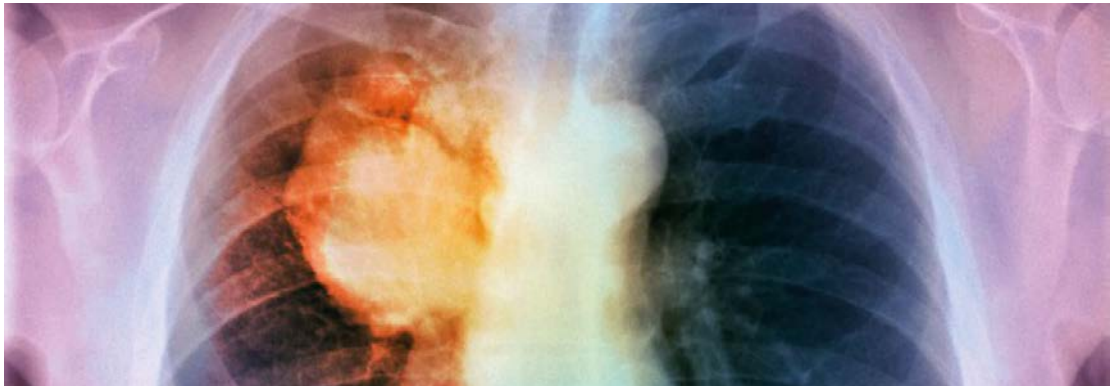
11 Genes	
ALK	MET
BRAF	NRAS
EGFR	PIK3CA
ERBB2	ROS1
KRAS	TP53
MAP2K1	

169 HOTSPOT	
EGFR:	T790M, L858R, Exon19 del, C797S
KRAS:	G12X, G13X, Q61X
ALK:	1151 Tins, L1152R, C1156Y
BRAF:	V600E

The main cause of lung cancer is cigarette smoke. Several studies indicate that there is a clear dose-effect relationship between inhaling tobacco and development of lung cancer.
Studies report that the risk of having lung cancer is 14 times higher among smokers than non-smokers (up to 20 times for heavy smokers, e.g. 20 cigarettes per day).
Cigarette smoke is responsible for the vest majority of lung tumors.
Air pollution, family history of lung cancer, as well as various pathological conditions affecting the normal lung physiology may also contribute to increase the possibility of being affected by this disease.

THE CONTINUOUS MONITORING OF A GENETIC MUTATIONAL PROFILE

Smokers, or people at risk of developing lung cancer, can undergo a yearly checkup of their mutational profile to monitor the potential onset of lung cancer.



EARLY DETECTION

SCED (Solid Cancer Early Detection) is used when HELIXAFE reveals events underlying possible genetic instability and an in-depth analysis is required. SCED enables a high-resolution analysis of cancer-associated genes through cfDNA analysis. Given that ctDNA can be isolated from the peripheral blood, the test is non-invasive and can be easily repeated several times at no risk.

52 Hotspot genes				
AKT1	EGFR	FLT3	KRAS	PDGFRA
ALK	ERBB2	GNA11	MAP2K1	PIK3CA
AR	ERBB3	GNAQ	MAP2K2	RAF1
ARAF	ESR1	GNAS	MET	RET
BRAF	FGFR1	HRAS	MTOR	ROS1
CHEK2	FGFR2	IDH1	NRAS	SF3B1
CTNNB1	FGFR3	IDH2	NTRK1	SMAD4
DDR2	FGFR4	KIT	NTRK3	SMO

Tumor suppressor genes
APC
FBXW7
PTEN
TP53

12 CNVs genes	
CCND1	ERBB2
CCND2	FGFR1
CCND3	FGFR2
CDK4	FGFR3
CDK6	MET
EGFR	MYC

Genes fusions	
ALK	FGFR3
BRAF	MET
ERG	NTRK1
ETV1	NTRK3
FGFR1	RET
FGFR2	ROS1

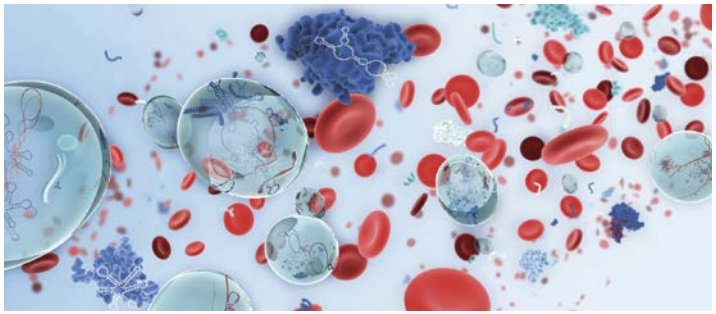
52 genes, Single library from DNA and RNA, 272 amplicons, >900 hotspots and indels, Extended coverage of TP53, 96 fusions, 12 CNVs, MET exon 14 skipping

THERAPY MONITORING

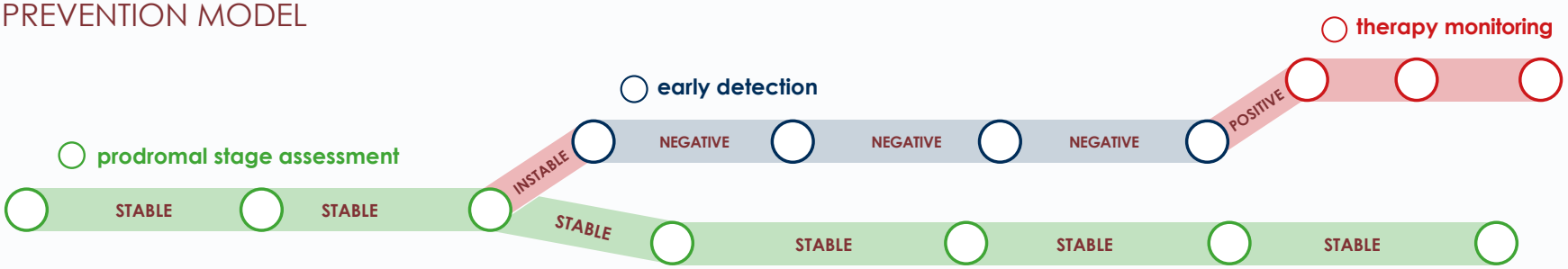
SCED 3D allows the highest possible accuracy as it cross-checks data obtained from circulating tumor cells (CTC), circulating tumor DNA (ctDNA) and germline DNA. This test represents the ideal approach for the treatment monitoring because it uses a peripheral blood sample and it is not associated to any risk for the patient.

CTCs and ctDNA

Solid cancers are often asymptomatic and clinically undetectable until they vascularize and reach a considerable mass (normally 1-2 cm in diameter). However, smaller lesions (less than 1cm) might be capable of releasing ctDNA (circulating tumor DNA) and CTCs (circulating tumor cells) into the bloodstream, giving insight into the genetic portrait of the primary tumor.
CTCs represent cancer cells detached from a solid tumor mass that are in the process of colonizing distant organs and potentially giving rise to metastasis. CTCs and ctDNA released in the bloodstream provide genetic information that is indicative of the tumor of origin.

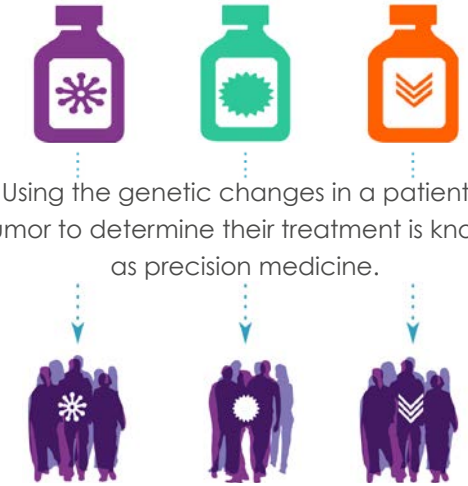


PREVENTION MODEL



	Prodromal Stage Assessment				Early Detection	Therapy Monitoring
PERFORMANCE	HELIXAFE	HELIXMOKER	HELIXGYN	HELIXCOLON	SCED	SCED 3D
Genes	50	ALK,BRAF,EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, TP53	AKT1, EGFR, ERBB2, ERBB3, ESR1, FBXW7, KRAS, PIK3CA, SF3B1, TP53	AKT1, BRAF, CTNNB1, EGFR, ERBB2, FBXW7, GNAS, KRAS, MAP2K1, NRAS, PIK3CA, SMAD4, TP53, and APC	52 genes, Single library from DNA and RNA, 272 amplicons, >900 hotspots and indels, Extended coverage of TP53, 96 fusions, 12 CNVs, MET exon 14 skipping	50
Mutations	2800	169 Hotspot	157 Hotspot	245 Hotspot	Selected mutations	2800
Sensitivity	95%*	100%	>99,9%	>99,9%	>99,9%	95%*
Specificity	98%*	98%	>99,9%	>99,9%	>99,9%	98%*
Allele Frequencies %	>1%	>0,50%	>0,50%	>0,50%	>0,50%	>1%
cfDNA	YES	YES	YES	YES	YES	YES
Germline DNA	YES	YES	YES	YES	-	YES
CTCs	-	-	-	-	-	YES
ctDNA	-	-	-	-	YES	-

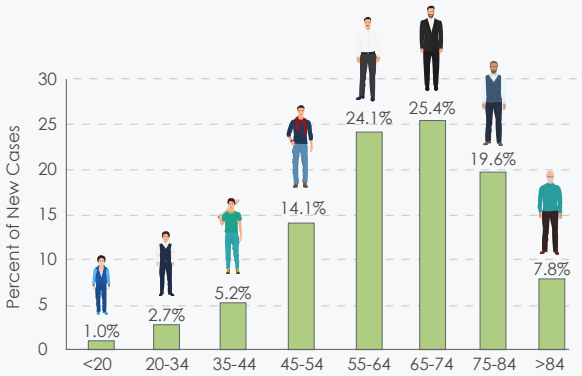
PRECISION MEDICINE IN CANCER TREATMENT



Using the genetic changes in a patient's tumor to determine their treatment is known as precision medicine.

Precision medicine is an approach that allows physicians to select treatments based on the genetic abnormalities of each individual patient.
Currently, patients diagnosed with cancer usually receive the same standard of care treatment as others who have the same cancer type or subtype. As a consequence, not all patients respond equally to a given treatment, and many patients are given sub-optimal therapies.
In contrast, with a precision medicine approach, the mutational profile of each patient serves as a reference for treatment decisions, and allows clinicians to identify the best treatment for each patient.

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.

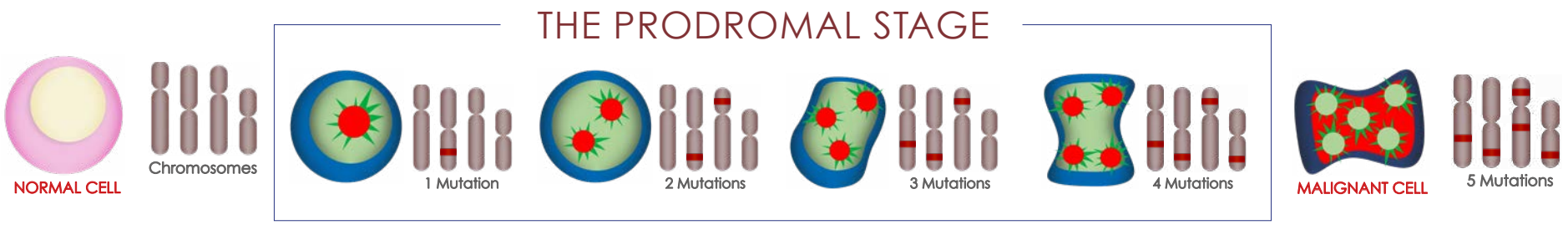
FDA APPROVED DRUGS CANCER TARGETED THERAPY AFTER A DIAGNOSIS BASED ON THE MUTATION

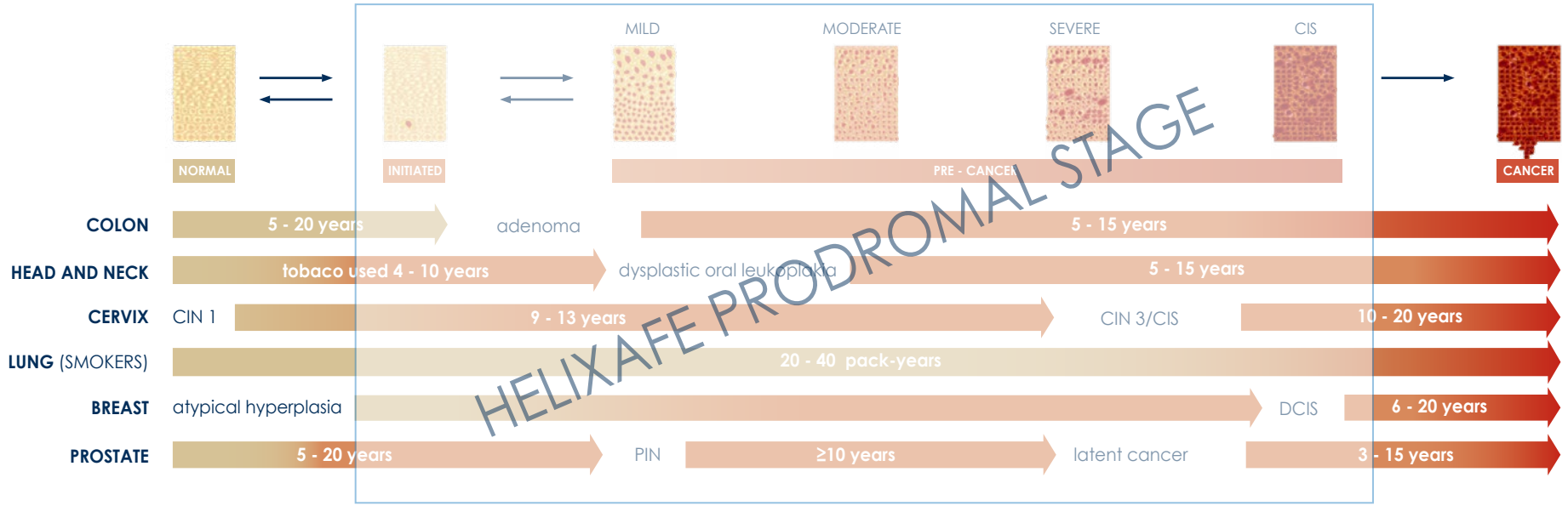
The target of the therapy is the mutation (which causes the cancer) and not the cancer tissue that is a consequence of the mutations.

Ado-Trastuzumab Emtansine	ERBB2	HER2 protein overexpression
Afatinib	EGFR	EGFR exon 19 deletion
Alectinib	ALK	ALK gene rearrangement positive
Anastrozole	ESR1, PGR	Hormone receptor positive
Arsenic Trioxide	PML-RARA	PML-RARα translocation positive
Belinostat	UGT1A1	UGT1A1*28 allele homozygotes
Blinatumomab	BCR-ABL1	Philadelphia chromosome negative
Bosutinib	BCR-ABL1	Philadelphia chromosome positive
Busulfan	BCR-ABL1	Philadelphia chromosome negative
Cabozantinib	RET	RET mutation positive
Capecitabine	DPYD	DPD deficient
Ceritinib	ALK	ALK gene rearrangement positive
Cetuximab (1)	EGFR	EGFR protein expression positive
Cetuximab (2)	KRAS	KRAS codon 12 and 13 mutation negative
Cisplatin	TPMT	TPMT intermediate or poor metabolizers
Cobimetinib	BRAF	BRAF V600E/K mutation positive
Crizotinib	ALK	ALK gene rearrangement positive
Dabrafenib (1)	BRAF	BRAF V600E/K mutation positive
Dabrafenib (2)	G6PD	G6PD deficient
Dasatinib	BCR-ABL1	Philadelphia chromosome positive
Denileukin Diftitox	IL2RA	CD25 antigen positive
Dinutuximab	MYCN	MYCN amplification positive
Erlotinib (1)	EGFR	EGFR protein expression positive
Erlotinib (2)	EGFR	EGFR exon 19 deletion
Everolimus (1)	ERBB2	HER2 protein overexpression negative
Everolimus (2)	ESR1	Estrogen receptor positive
Exemestane (1)	ESR1	Estrogen receptor positive
Exemestane (2)	PGR	Progesterone receptor positive
Fluorouracil (2)	DPYD	DPD deficient
Fulvestrant	ESR1, PGR	Hormone receptor positive
Gefitinib	EGFR	EGFR exon 19 deletions
Ibrutinib	del (17p)	Chromosome 17p deletion positive
Imatinib (1)	KIT	KIT protein expression positive
Imatinib (2)	BCR-ABL1	Philadelphia chromosome positive
Imatinib (3)	PDGFRB	PDGFR gene rearrangement positive
Imatinib (4)	FIP1L1-PDGFRA	FIP1L1-PDGFRα fusion kinase
Irinotecan	UGT1A1	UGT1A1*28 allele carriers
Lapatinib (1)	ERBB2	HER2 protein overexpression positive
Lapatinib (2)	HLA-DQA1,HLA-DRB1	HLA-DQA1*0201 or HLA-DRB1*0701
Letrozole	ESR1, PGR	Hormone receptor positive
Mercaptopurine	TPMT	TPMT intermediate or poor metabolizers
Nilotinib (1)	BCR-ABL1	Philadelphia chromosome positive
Nilotinib (2)	UGT1A1	UGT1A1*28 allele homozygotes
Nivolumab (1)	BRAF	BRAF V600 mutation positive
Nivolumab (2)	CD274	PD-L1 protein expression positive
Obinutuzumab	MS4A1	CD20 antigen positive
Olaparib	BRCA1-2	BRCA1-2 mutation positive
Omacetaxine	BCR-ABL1	Philadelphia chromosome positive
Osimertinib	EGFR	EGFR T790M mutation positive
Palbociclib (1)	ESR1	Estrogen receptor positive
Palbociclib (2)	ERBB2	HER2 protein overexpression negative
Panitumumab (1)	EGFR	EGFR protein expression positive
Panitumumab (2)	KRAS	KRAS codon 12 and 13 mutation negative
Pazopanib	UGT1A1	UGT1A1*28 allele homozygotes
Pembrolizumab (1)	BRAF	BRAF V600 mutation positive
Pembrolizumab (2)	CD274	PD-L1 protein expression positive
Pertuzumab	ERBB2	HER2 protein overexpression positive
Ponatinib	BCR-ABL1	Philadelphia chromosome positive
Rasburicase (1)	G6PD	G6PD deficient
Rasburicase (2)	CYB5R1-4	NADH cytochrome b5 reductase deficient
Rituximab	MS4A1	CD20 antigen positive
Tamoxifen (1)	ESR1, PGR	Hormone receptor positive
Tamoxifen (2)	F5	Factor V Leiden carriers
Tamoxifen (3)	F2	Prothrombin G20210A allele positive
Thioguanine	TPMT	TPMT intermediate or poor metabolizers
Tositumomab	MS4A1	CD20 antigen positive
Trametinib	BRAF	BRAF V600E/K mutation positive
Trastuzumab	ERBB2	HER2 protein overexpression
Tretinoin	PML-RARA	PML-RARA translocation positive
Vemurafenib (1)	BRAF	BRAF V600E mutation positive
Vemurafenib (2)	NRAS	NRAS mutation positive

CANCER PROGRESSION

Many tumor types initially develop with no symptoms, and most patients are diagnosed only at late stages, when surgical resection of the tumor and cancer eradication is no longer applicable. It is demonstrated that for several tumor types, it could take decades since the initiating driver mutation has occurred until a patient's death. Hence, early cancer detection could enable a prompt medical intervention (e.g. tumor resection) resulting in substantially higher chances of surviving tumor occurrence.





BIOSCIENCE GENOMICS

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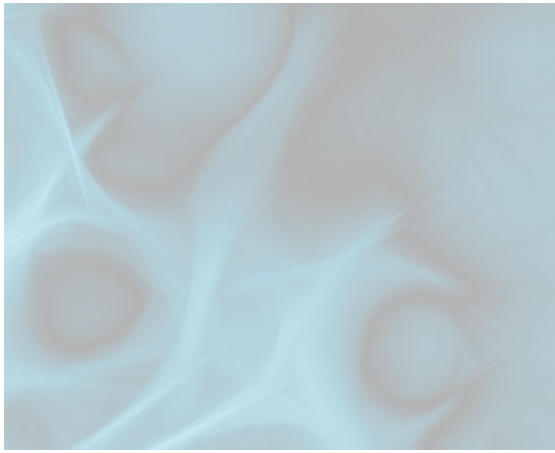

info@bioinst.com

ROME

Tor Vergata - University of Rome

Via della Ricerca Scientifica 1

00133 Rome - Italy

MILAN

San Raffaele Hospital

DIBIT 1 - via Olgettina 58

20132 Milan - Italy

SAN MARINO

Strada Rovereta, 42

47891 Falciano

San Marino

www.bioinst.com