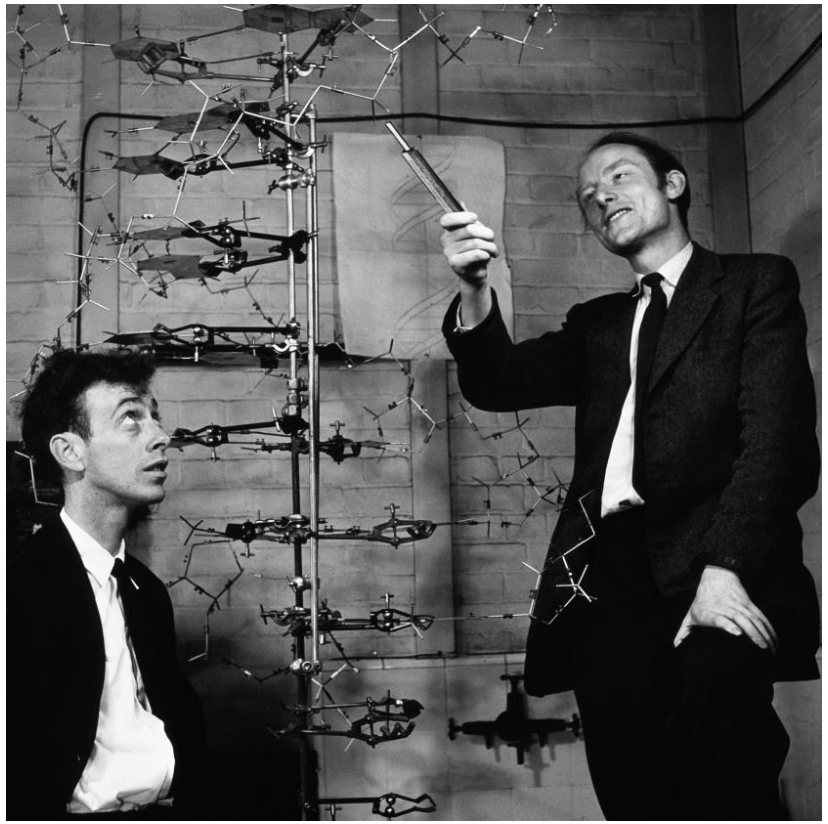
Prezentare generală CEMT: de la biobanca de tumori la biologia moleculară

Prof. Irinel Popescu

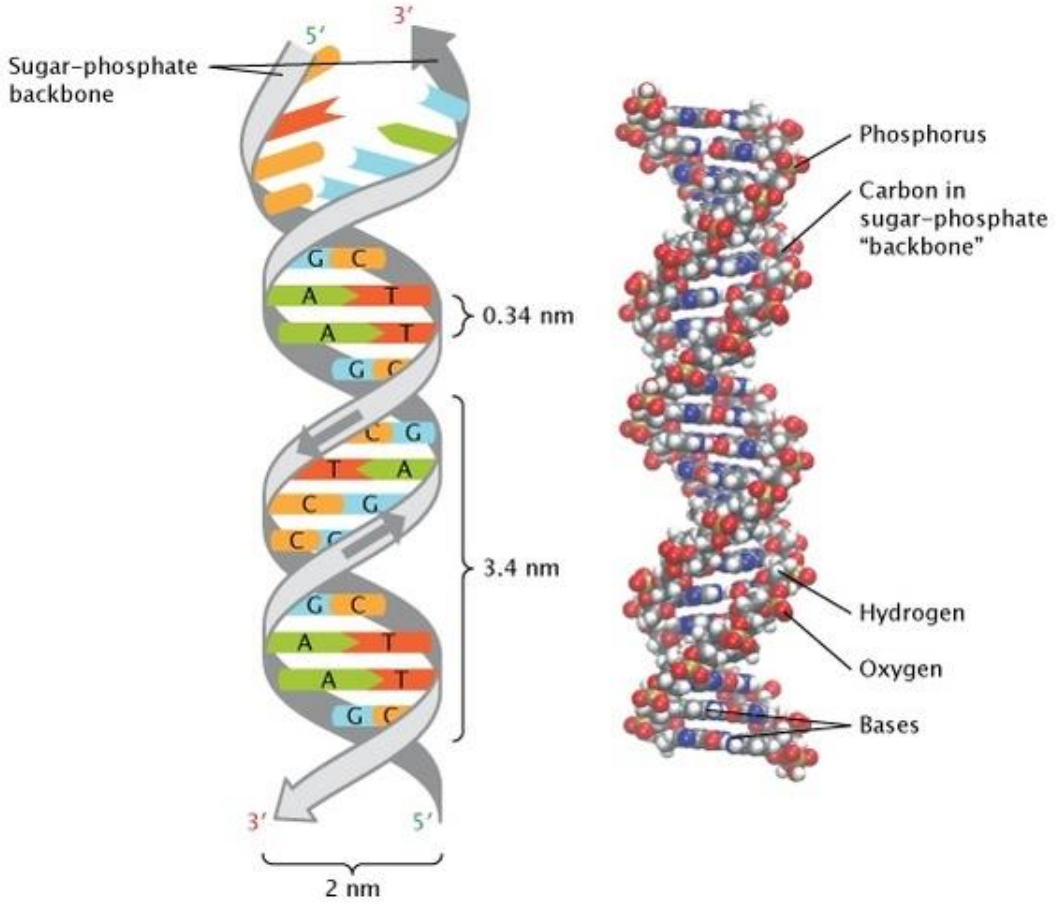
Institutul Clinic Fundeni

Centrul de Excelenta in Medicina Translationala

1953- Model Watson si Crick



Science Photo Library website

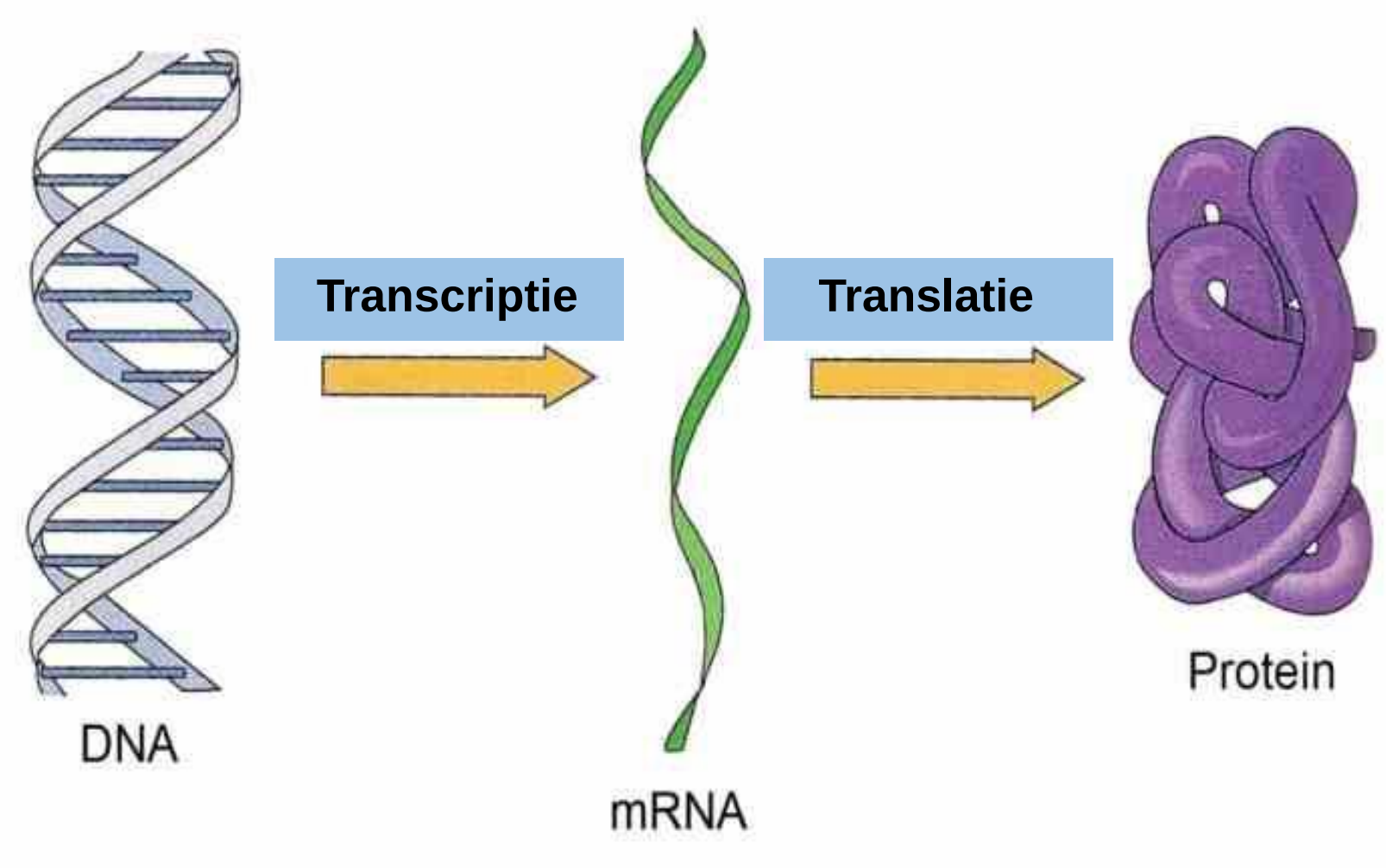


Structura dublu-helix a ADN.

Structura tridimensionala de dublu-helix a ADN a fost descrisa pentru prima data de James Watson si Francis Crick. Bazele azotate sunt unite prin legaturi de hidrogen pe baza complementaritatii.

GENOMICA

PROTEOMICA



Proiectul Genomului Uman (HGP)

International Human Genome Sequencing Consortium

(‘proiect public‘)

- **20 centers from 6 countries: US, UK, China, France, Germany, Japan**

Celera Genomics –

(‘proiect privat’)

*“There appear to be about **30,000–40,000 protein-coding genes** in the human genome”*

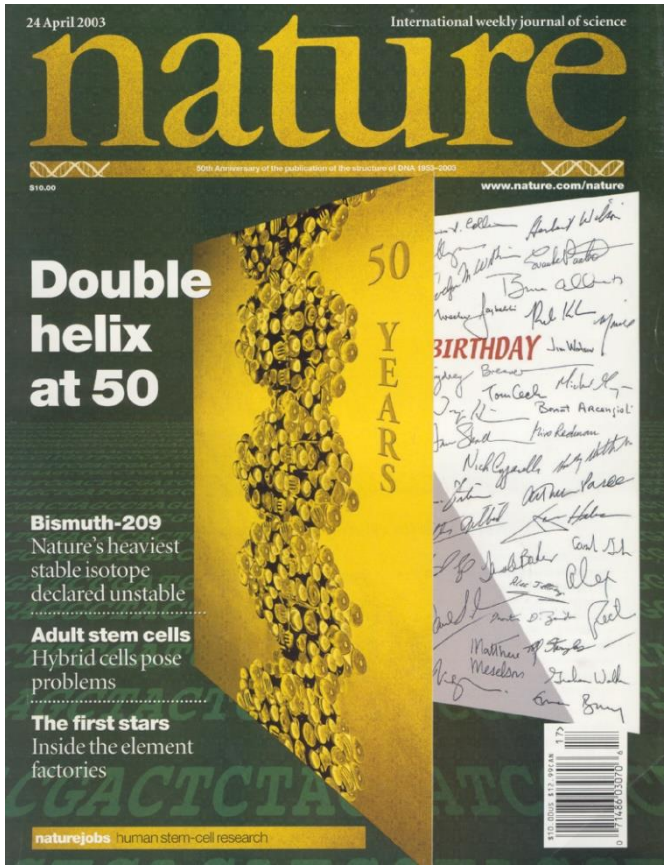
“Analysis of the genome sequence revealed **26,588 protein-encoding transcripts**”

Aprilie 2003 - “Human Genome Month”

- **International Human Genome Sequencing Consortium –complete human genome sequence in baze de date publice**
- **Baza de date Celera – disponibila comercial**

25 Aprilie 2003 - “DNA Day”

- **Marcheaza a 50 –a aniversare de la descriereea structurii doublu helix de catre Watson si Crick**



Metode de investigatii

- **Microarray**
  - Analiza expresiei genice
  - Analiza mutatiilor SNP
- **"*High-throughput next generation sequencing*" (HT-NGS)**
  - Secventierea intregului genom
  - Secventierea intregului exom
  - Secventierea tintita (panel de gene tinta)
- **DNA sequencing - Sanger**
  - Determinarea secventei nucleotidice

Metode de investigatii

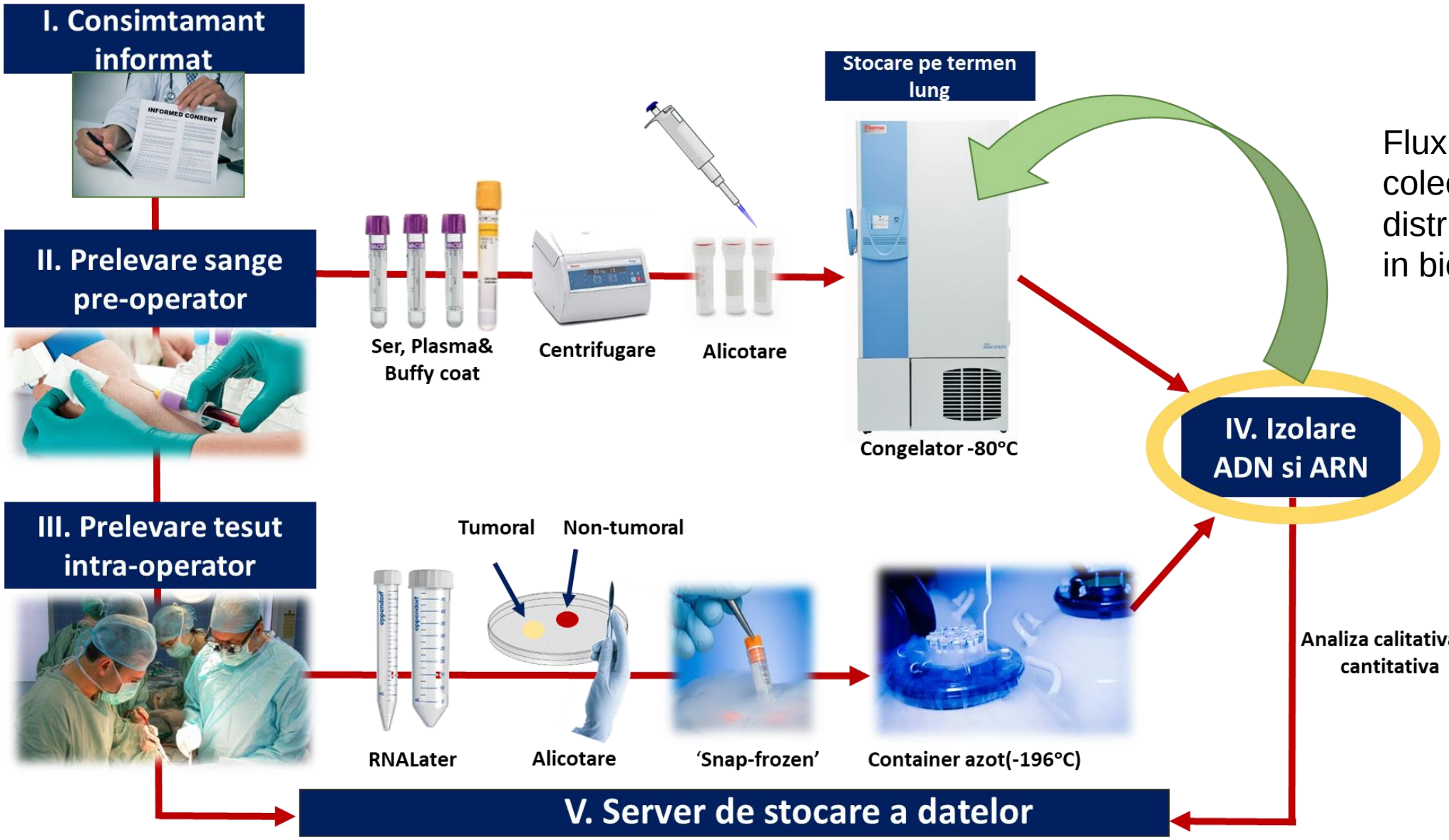
- dd PCR (Digital Droplet PCR)

- Necesitatea de a banca obligatoriu probe de singe o data cu tumorile

Specimene biologice – Procesul de biobancare

Din sala de operatie in Biobanca :

INSTITUTUL CLINIC FUNDENI



Flux de lucru pentru colectarea, stocarea si distribuirea esantioanelor in biobanca

Procesul de biobancare- De la esantion la Big Data

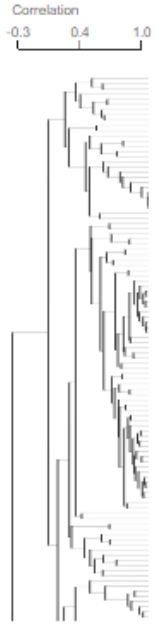
DATE GENOMICE

FEATURES	Feature Nu	Row	Col	SubTypeMa	Control Typ	ProbeName	SystematicName	PositionX	PositionY	gProcessedSignal	gProcessedSigError	gMedianSignal	gBGMedianSignal	gBGPixSDev	gIsSaturated	gIsFeatNonUnif	gIsBGNonUnif	gIsFeatPOnOL	gIsBGPopnOL	gIsManualFlag	gBGSubSignal	gIsPosAndSig	gIsWellAbove	SpotExtentX	gBGMeanSignal
DATA	1	1	1	260	1	GE_Bright	GE_Bright	686.965	340.224	5.83E+04	5.83E+03	50809	42	9.37E+00	0	0	0	0	1	0	51327.5	1	1	25.7804	42.8881
DATA	2	1	2	66	1	DarkCorne	DarkCorne	708.152	340.5	3.37E+00	3.38E+00	51	42	9.31E+00	0	0	0	0	1	0	-2.69591	0	0	26.6546	43.249
DATA	3	1	3	66	1	DarkCorne	DarkCorne	729.4	340.586	3.36E+00	3.37E+00	52	43	9.64E+00	0	0	0	0	1	0	-2.97775	0	0	30	43.7134
DATA	4	1	4	0	0	A_23_P11	NM_0159	750.683	340.349	6.04E+03	6.04E+02	5259	43	9.40E+00	0	0	0	0	1	0	5370.19	1	1	26.2212	43.4311
DATA	5	1	5	0	0	A_33_P32	NM_0806	772	340.7	3.28E+02	3.30E+01	344	43	9.33E+00	0	0	0	0	1	0	292.463	1	1	26.2212	43.3121
DATA	6	1	6	0	0	A_33_P33	NM_1784	793.358	340.566	3.62E+00	3.36E+00	60	42	9.28E+00	0	0	0	0	1	0	3.23926	1	0	24.4106	43.2797
DATA	7	1	7	0	0	A_33_P32	ENST0000	813.929	340.804	3.31E+01	4.69E+00	82.5	43	9.16E+00	0	0	0	0	1	0	29.6969	1	1	25.1049	43.3526
DATA	8	1	8	0	0	A_33_P33	NM_0012	835.478	340.739	1.25E+01	3.54E+00	66.5	43	9.25E+00	0	0	0	0	1	0	11.2754	1	0	22.9591	43.381
DATA	9	1	9	0	0	A_21_P00	NR_02424	856.491	340.947	4.36E+03	4.36E+02	3983	42	9.20E+00	0	0	0	0	1	0	3935.63	1	1	24.8756	43.2913
DATA	10	1	10	0	0	A_21_P00	NR_03826	877.5	341.324	1.49E+02	1.52E+01	184.5	43	9.23E+00	0	0	0	0	1	0	134.709	1	1	30	43.3021
DATA	11	1	11	0	0	A_24_P21	NM_0169	898.875	341.425	2.74E+01	4.28E+00	81	43	9.60E+00	0	0	0	0	1	0	24.8674	1	1	21.4095	43.5937
DATA	12	1	12	0	0	A_23_P11	NM_0024	919.897	341.397	1.46E+04	1.46E+03	13485	43	9.56E+00	0	0	0	0	1	0	13330.7	1	1	25.7804	43.6295
DATA	13	1	13	0	0	A_33_P32	NM_0011	941	341.5	5.49E+02	5.50E+01	567	43	9.65E+00	0	0	0	0	1	0	501.676	1	1	26.2212	43.6221
DATA	14	1	14	0	0	A_23_P10	NM_0223	962	341.5	7.87E+00	3.35E+00	62	43	9.58E+00	0	0	0	0	1	0	7.21002	1	0	26.2212	43.6216
DATA	15	1	15	0	0	A_32_P61	NR_02694	983.333	341.609	3.24E+00	3.25E+00	56	42	9.45E+00	0	0	0	0	1	0	0.746758	0	0	30	43.3017
DATA	16	1	16	0	0	A_33_P37	NR_02664	1004.5	341.676	6.86E+01	7.59E+00	115	42	9.39E+00	0	0	0	0	1	0	63.1997	1	1	27.0811	43.3905
DATA	17	1	17	0	0	A_33_P34	NM_0011	1025.9	341.597	1.51E+03	1.51E+02	1445	43	9.65E+00	0	0	0	0	1	0	1392.05	1	1	26.2212	43.6858
DATA	18	1	18	0	0	A_33_P33	NM_0162	1046.9	341.597	4.06E+02	4.07E+01	429.5	43	9.21E+00	0	0	0	0	1	0	375.812	1	1	26.2212	43.4613
DATA	19	1	19	0	0	A_33_P33	NM_0146	1067.78	342.312	1.28E+02	1.32E+01	176	43	9.16E+00	0	0	0	0	1	0	118.944	1	1	26.4388	43.5517
DATA	20	1	20	0	0	A_33_P32	NM_0162	1089.21	341.947	7.91E+03	7.91E+02	7239	43	9.15E+00	0	0	0	0	1	0	7366.85	1	1	24.8756	43.6363
DATA	21	1	21	0	0	A_33_P32	ENST0000	1110.33	342.391	4.61E+00	3.23E+00	59.5	43	9.12E+00	0	0	0	0	1	0	4.30179	1	0	27.2919	43.4184
DATA	22	1	22	0	0	A_24_P27	NM_0322	1131.57	342.328	9.25E+01	9.79E+00	138	43	9.31E+00	0	0	0	0	1	0	86.5595	1	1	25.7804	43.4664
DATA	23	1	23	0	0	A_21_P00	TCONS_I2	1152.46	342.246	3.17E+00	3.18E+00	54	43	9.46E+00	0	0	0	0	1	0	-0.11828	0	0	25.3321	43.4048
DATA	24	1	24	0	0	A_21_P00	NR_11029	1174.26	342.5	2.78E+01	4.22E+00	82	43	9.56E+00	0	0	0	0	1	0	26.1236	1	1	21.9382	43.4841
DATA	25	1	25	0	0	A_24_P28	NM_0012	1194.85	342.5	5.86E+01	6.66E+00	107	43	9.50E+00	0	0	0	0	1	0	55.182	1	1	26.6546	43.5617
DATA	26	1	26	0	0	A_23_P34	NM_0011	1216.18	342.421	7.32E+02	7.32E+01	748	43	9.67E+00	0	0	0	0	1	0	691.07	1	1	25.7804	43.7528
DATA	27	1	27	0	0	A_21_P00	XR_42806	1237.22	342.522	1.14E+01	3.35E+00	65	43	9.67E+00	0	0	0	0	1	0	10.7901	1	0	27.0811	43.7298
DATA	28	1	28	0	0	A_21_P00	NM_0309	1258.2	342.682	1.28E+03	1.28E+02	1281	43	9.54E+00	0	0	0	0	1	0	1212	1	1	26.6546	43.7219
DATA	29	1	29	0	0	A_21_P00	ENST0000	1279.5	342.676	3.13E+00	3.14E+00	50	43	9.24E+00	0	0	0	0	1	0	-2.45748	0	0	30	43.5413
DATA	30	1	30	0	0	A_23_P89	NM_1944	1300.37	343.309	4.64E+01	5.60E+00	99	43	9.31E+00	0	0	0	0	1	0	44.2631	1	1	27.0811	43.5992
DATA	31	1	31	0	0	A_33_P33	NM_0061	1321.57	343.298	7.66E+00	3.22E+00	64	42	9.25E+00	0	0	0	0	1	0	7.31717	1	0	30	43.426
DATA	32	1	32	0	0	A_23_P10	NM_0029	1343.16	343.377	7.52E+03	7.52E+02	7189.5	42	9.23E+00	0	0	0	0	1	0	7195.01	1	1	26.0017	43.2628
DATA	33	1	33	0	0	A_21_P00	Inc-METTL	1364.77	343.2	3.10E+00	3.11E+00	54	43	9.51E+00	0	0	0	0	1	0	-0.43473	0	0	30	43.3963
DATA	34	1	34	0	0	A_33_P32	ENST0000	1385.29	343.5	7.25E+01	7.89E+00	125	42	9.46E+00	0	0	0	0	1	0	69.648	1	1	27.0811	43.3095
DATA	35	1	35	0	0	A_33_P33	ENST0000	1406.5	343.5	3.09E+00	3.10E+00	58.5	42	9.26E+00	0	0	0	0	1	0	1.50109	0	0	27.9146	43.2458
DATA	36	1	36	0	0	A_33_P37	XR_11089	1427.9	343.597	5.64E+02	5.64E+01	594	42	9.47E+00	0	0	0	0	1	0	543.642	1	1	26.2212	43.4221
DATA	37	1	37	0	0	A_33_P34	A_33_P34	1449	343.5	6.83E+02	6.84E+01	705	42	9.43E+00	0	0	0	0	1	0	659.963	1	1	26.2212	43.2915
DATA	38	1	38	0	0	A_33_P36	AK091675	1470	343.5	1.94E+02	1.96E+01	243	43	9.56E+00	0	0	0	0	1	0	187.835	1	1	26.2212	43.5536

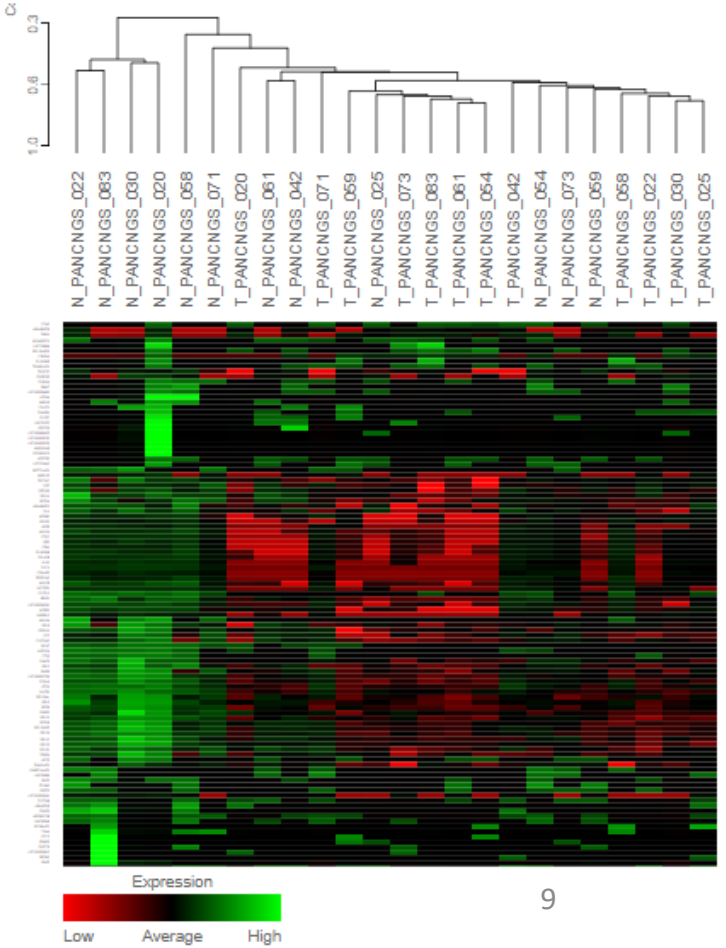
BIOINFORMATICA

PRELUCRAREA DATELOR

Aliniere, asamblare, identificare, comprimarea datelor, confidentialitate, arhivare, variatii structurale…



HEATMAP EXPRESIE DIFERENTIALA



Aplicatii clinice

- **Risc oncologic**
  - BRCA : ex: mutația BRCA1 or BRCA2 (cancer de sân, ovar)
  - adenomatous polyposis coli (APC) – Familial adenomatous polyposis (FAP)
  - CDH1- gena care codifica caderina E: cancer gastric
  - Sindromul Lynch

- **Medicina personalizată**

- **Her-2** - Selectează pacienţii pentru tratament cu Herceptin (Trastuzumab) în cancerul de sân (aprobat FDA în 1998 în SUA şi în anul 2000 în UE); supraexprimare Her2 în tumorile de sân - în 1986 (NIH)

- **KRAS** - pacienţii cu cancer de colon care au mutaţiile ale genei KRAS cel mai probabil nu vor răspunde la terapia cu anticorpi anti- receptor pentru factorul de creștere epidermală (EGFR)- Cetuximab, Panitumumab
- Instabilitatea microsatelitara: raspunsul la imunoterapie in cancerul de colon

Aplicatii clinice

- Proteomica:
  - Diagnostic
  - Monitorizare postterapeutica (depistare precoce a recidivei)

Context istoric

Termenul **“biobanca”** a fost utilizat pentru prima data in vederea descrierii materialului biologic uman, in anul **1996**, intr-un articol publicat in *Journal of Molecular Medicine.*

Autorii, Loft si Poulsen au investigat rolul degradarii oxidative a ADN ca factor de risc independent in dezvoltarea cancerului.

Loft S, Poulsen HE, J Mol Med (Berl). 1996 Jun;74(6):297-312

2009 Time Magazine's Top 10 Ideas Changing the World

#8 este BIOBANCA

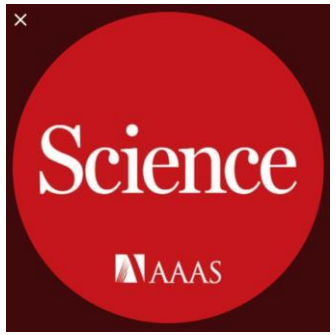
"***Biobanks will transform the way we see disease developing***" (Dr. Carolyn Compton, director, NCI's Office of Biorepositories and Biospecimen Research)

Time Magazine, March 12, 2009

Presedintele Obama sprijina medicina personalizata

Biobancarea este un element cheie al medicinei personalizate

July 2015- Science-”The precision medicine initiative **proposed by President Barack Obama last week** would center on a huge new biobank containing medical records and genetic information for perhaps a million Americans.”



doi:10.1126/science.aaa6418

Care sunt elementele necesare in biobancare?

- ✓ Regulamente
- ✓ Proceduri standardizate
- ✓ Acces la probe biologice provenind de la pacienti
- ✓ Personal si echipamente pentru procesele de colectare, procesare, prezervare si distribuire a probelor
- ✓ Sisteme specializate de management al informatiei in laborator (LIMS)
- ✓ Colaborari in cadrul retelei institutiilor implicate

Adaptate dupa Dr Yehudit Cohen. Sheba Medical Center, Tel Hashomer, Israel. “Biobanking on shoestring budgets”, ESSB, Verona, October 2013 (http://www.esbb.org/verona/programme_thur.html)

ISTORIA BIOBANCII Institutului Clinic Fundeni

- Din 2002, un parteneriat public - privat a reunit laboratorul **RNTECH (Franta)** si **Institutul Clinic Fundeni.**
- **Vladimir Lazar, Liliana Paslaru, Liviu Badea, Simona Dima**

”Centrul de Excelenta in Medicina Translationala”

CERCETAREA TRANSLATIONALA: From ”Bed To Bench” And From ”Bench To Bed”

Obiectivul strategic CEMT- de a utiliza platforma nou creata (foto dreapta) pentru a efectua cercetari pe mai mult de 60.000 de bolnavi care se interneaza anual in Insitutul Clinic Fundeni (foto stinga)

Structura CEMT

- www.cemt.ro
- Centrul de gastroenterologie
- Laboratoa de anatomie patologica
- Biobanca
- Laborator de genomica
- Laborator de proteomica
- Laborator de culturi celulare si organoizi
- Centru de bioinformatica: inteligenta artificiala
- Biobaza
- Laborator de chirurgie experimentala
- Laborator de microchirurgie
- Centru de simulare

BIOBANCA

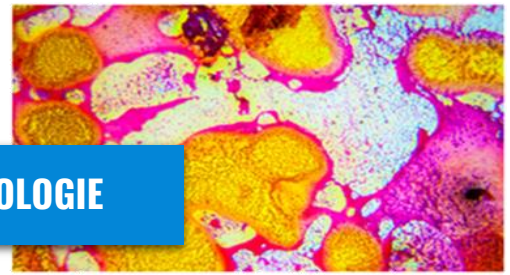
CHIRURGIE EXPERIMENTALA

MODUL DE STOCARE DE DATE

GASTROENTEROLOGIE

BIOLOGIE MOLECULARA

CENTRU DE SIMULARE



HISTOPATOLOGIE


Thermo
SCIENTIFIC
SANYO
VIP PLUS
VIP SERIES
TSU SERIES


EURO-CYL

Rolul Procedurilor Standard Operationale (SOP)

SOP mentioneaza modul de obtinere, prelucrare si stocare a specimenelor biologice pentru aplicatii ulterioare

SOP permite armonizarea protocoalelor studiilor realizate in centre diferite

- ghiduri pentru SOP:

- Medical Research Council, UK
- Council of Europe, Committee of Ministers
- National Cancer Institute, US
- National Cancer Center, Singapore
- Australasian Biospecimen Network
- International Society for Biological and Environmental Biorepositories
- Organization for Economic Cooperation & Development
- TUBAFROST, European Union
- BBMRI Technical Standards& Protocols for Biological Resource Centers
- CryoBiosystems, Inc.

Regulamente & Proceduri (SOP) – Institutul Clinic Fundeni

Clinical Institute Fundeni – Center for Digestive Diseases and Hepatic Transplant

INFORMED CONSENT DECLARATION
Project title: Role of S100A4 and MAP4K4 in pancreatic ductal adenocarcinoma progression

Patient Numerical Code:

I, the undersigned, certify that MD .....
has asked me to participate in a research study **Role of S100A4 and MAP4K4 in pancreatic ductal adenocarcinoma progression** that intents to identify the biological mechanisms of my disease.

I have been fully informed of the aims of this study and how will be implementated, as well as any risks it may involve.I have received answers to all my questions about the study.

I freely give my consent to participate in this study and I understand that I have the right to withraw from it at any time without incurring any preiudice.

I agree that for the purpose of the study the promoter and/or the health authorities may have direct access to original medical documents in order to check all the clinical procedures and/or data, but without violating their confidentiality.

I agree that the medical data recorded during this research study may undergo computer processing by the promoter or by others acting on its behalf. I have noted that my right of access, provided by the law ," Law Nr. 677/2001- Romania regarding the people protection concerning the personal data processing and their free spreading (article 13 and follows) may by exercised at any time through the study physician. I can also exercise my rights to correct data through the same physician.

I have received a copy of this document and I have been informed that another copy will be kept by the MD that asked me to participate of this study under conditions which guarantee confidentiality, to which I consent.

My consent does not discharge the organiser of the research study of their responsabilities. I retain all my rights as guarateed by the law. I hereby declar that I have received answers to all the questions I have asked about the study and I have answered all the questions concerning my medical history.

I state that I had response to all my questions that I asked about these study; having answered to all questions that were asked to me in particular on my medical antecedents and I undertake to follow all the advices and instructions I will be given by the medical team and which are described in detail in the information sheet.

First and last name of the patient:
.....
.....
Date: ..... Signature:

First and last name of the MD encharge with filling in the forms:
.....
Date: ..... Signature:

INFORMATION SHEET

Before you participate in this biomedical research study it is important to read this document, which will informe you about the implementation of the study.

Please take all the time that you need before giving your consent.
By participating in this study you will contribute to research which could help another people.
The treatment of your disease will impose a surgical intervention.
We are asking you to participate in a research project Role of S100A4 and MAP4K4 in pancreatic ductal adenocarcinoma progression being carried out by Prof Lionel Popescu at Fundeni Clinical Institute, concerning the biological mechanisms which led to the develope- ment of your disease. A clear understanding of these mechanisms would allow the identification of new diagnostic/prognostic markers and of new therapeutic targets. This will provide an improvement in the management of patients suffering of this disease in the future.

You were informed by your treatant MD that the surgical sample (extracted tissue) will be send in the Anatomypathology Department of the Fundeni Clinical Institute. A part of this tissue will be used for establishing the histopathological diagnosis and the other one will normally be distroied by incineration as operatory waste. We are asking your consent for taking fragments from this operatory waste with the aim to collect and use them in different research projects of the Fundeni Clinical Institute.
For the surgery, a blood sample must be prelevated. We ask your consent to prelevate an additional blood sample and also an urine sample in order to use them in the research projects of the Fundeni Clinical Institute.
For your disease follow-up other blood samples will have to be prelevated periodical. We ask your consent to take additional blood samples which will be used in the research programs of the Institute.

The MD asking you to participate in this study will allow you plenty of time to think about it. You are free to take part or not of this study. You have the right at any time to withdraw your participation without this affecting the care you receive and without having to give any reasons.

The medical information collected in the context of this study will be processed under anonymous and confidential conditions; it may be consulted by the representatives of the promoter or by relevant representatives from the Health Authorities in Romania or another countries. Your identity will never be mentioned and all biological samples will remain anonymous throughout the imple- mentation of any research project.

The Institute will be permitted to use these samples for any research project having the following objectives described (a better comprehension of the mechanisms leading to my disease which could make it possible to identify new diagnostic/prognostic markers and to identify new therapeutic targets) without me being able to place any restrictions on their use, except concerning preservation of my anonymity.
Any research will be carried out in compliance with the recommandations of the Declaration of Helsinki (1964) and its amendments. Medical data concerning you and necessary for this research will be the subject of computer processing and will only be transmitted to the promoter and if necessary to the appropriate health authorities under conditions which guarantee their confidentiality. You can at any time exercise your right of acces to and correction of these data, through the MD encharge with the study. You are free to withdraw at any time from this protocol simply by a written request. Such a withdrawal will mean that the Institute will remove your samples from the collection and ensure their destruction by incineration.
As required by law if you aree to participate in this study we will ask you to sign the consent form above.

STANDARD OPERATING PROCEDURE FOR BIOLOGICAL SAMPLE HARVESTING AND COLLECTING

Patient

↓

Signed informed consent (patient, physician)

↓

Blood collection:

3x EDTA (purple cap)

1x Clot Activator (yellow cap)

↓

Tissue collection

Normal/ Non-tumoral tissue	Tumoral tissue
➢ 1 fragment of 0.5-1 cm^3- in 15 ml tubes with RNA Later solution	➢ 1 fragment of 0.5-1 cm^3- in 15 ml tubes with RNA Later solution

! Samples must be stored at + 4 ° C in the refrigerator, until processing

Personal calificat cu expertiza in domeniu

- Recrutarea pacientilor si obtinerea consimtamantului informat
- Colectarea datelor clinice si biologice
- Inregistrarea si procesarea datelor, analiza statistica, stocarea si actualizarea datelor
- Proceduri standard operationale (SOP) si suport tehnic specific
- Procesarea si stocarea specimenelor biologice
- Educatie si training
- Dezvoltarea de contracte, parteneriate in mediul de afaceri

Biobanca- faciliteaza networking national si international

INSTITUTUL CLINIC FUNDENI

Institutul Clinic Fundeni:

Peste 5000 de pacienți internați și peste 3500 de pacienți operați anual.

Experiență în studii clinice.

Biobancă, în care sunt stocate probele biologice de la pacienții operați și apoi urmăriți.

Laboratorul de genomica.

Laboratorul de terapii celulare.

Centrul de chirurgie experimentală.

Rețele naționale:

Institutul Național de Virusologie " Ștefan S. Nicolau;

Institutul de Biologie și Patologie Celulară "N. Simionescu"

Institutul Oncologic "Prof. Dr. Al. Trestioreanu"

Rețele internaționale

Prof. Bin Teh, **National Cancer Center Singapore**, Translational Research Laboratory

Prof. Dan Duda, **Harvard School of Medicine**, Boston, USA

Prof. Victor Velculescu, **Johns Hopkins University**, Baltimore, USA

Prof. Florin Selaru, **Gastroenterology and Hepatology, Johns Hopkins University**, Baltimore, USA

Publicarea de articole;

Noi colaborări;

Sprijin pentru proiecte transnaționale

Procesul de biobancare

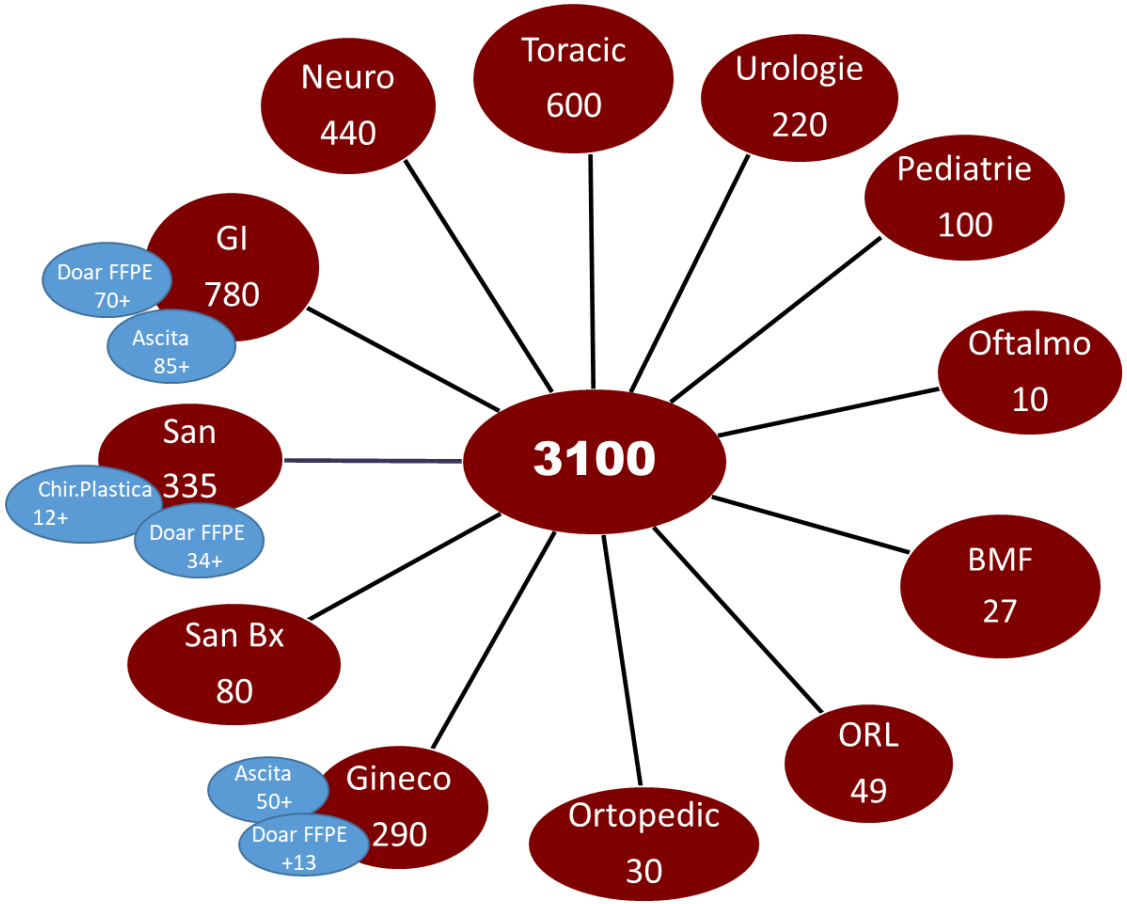
INSTITUTUL CLINIC FUNDENI

BIOBANCA Institutului Clinic Fundeni

Tip de tesut (tumoral sau non-tumoral)	Numar de patienti
Colon	598
Rect	230
Stomac	498
Pancreas	764
Ficat	864
Joctiune recto-sigmoidiana	91
Metastaza	52
Ovar	82
San	19
Altele	168
TOTAL	**3366**

Intre **2003-2019** au fost stocate probe biologice de la peste **3300** de pacienti cu afectiuni oncologice

Banca de tesut- **SHEBA MEDICAL CENTER**



Adaptata dupa Dr Yehudit Cohen. Sheba Medical Center, *2013*

SUSTENABILITATE

- Biospecimenele si produsii derivati din acestea pot fi oferite de catre biobanca pentru sistemul public sau privat.
- Proiecte de cercetare prospective
- Servicii specifice legate de materialul biologic, precum accesul, stocarea, colectarea, transportul, procesarea, testarea probelor biologice și analiza datelor
- c**omercializarea rezultatelor de cercetare și a produșilor derivați (Brevete)**
- O oportunitate specifică de dezvoltare poate implica comercializarea de **linii celulare tumorale** primare obținute din materialul biologic tumoral colectat și stocat în biobancă.

Proiecte Finalizate in care s-a folosit biobanca

Acronim	Titlul proiectului	Perioada	Buget
HEPMARK	**Stratificarea pacientilor cu carcinom hepatocelular prin identificarea unor markeri neinvazivi**, "Research in Priority Areas" Program, Financial Mechanism of the European Economic Area (EEA). 4SEE/30.06.2014 Director Proiect: Prof. Dr. Irinel Popescu	2014-2017	1.125.000
S100MAP	**Rolul markerior S100A4 si MAP4K4 in progresia adenocarcinomului pancreatic ductal**; Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI). Director Proiect: Prof. Dr. Irinel Popescu	2012-2015	3.000.000
TRACER	**Subtipuri transcriptomice de cancer hepatocelular si raspunsul acestora la terapie**; 10.10.2005- 01.04.2008. Director Proiect: Prof. Dr. Paslaru Liliana	2017-2018	475,000
GENOPACT	**Profilul expresiei genice si studiul biomarkerilor corelati cu parametrii clinici si patologici in cancerul de pancreas**; CEEX - Module I-56/2005. Director Proiect: Prof. Dr. Irinel Popescu	2005-2008	15,000,000
PACAGENTER	**Profilul genic indus de supresia transcriptionala Ets1 in cancerul de pancreas**; CEEX Grant, Module I -62/2006- Director Proiect: Prof. Dr. Irinel Popescu	2006-2008	15,000,000
MOLPANC	**Studiu comparativ al mecanismelor moleculare in pancreatita cronica si adenocarcinomul pancreatic ductal**; PNCD II P024. Director Proiect: CSI Dr. Simona Dima	2007-2010	2,000,000

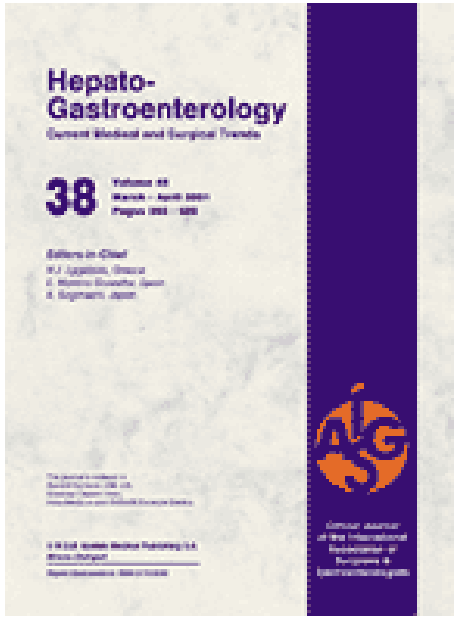
Proiecte desfasurate in CEMT- in care s-a folosit biobanca

INSTITUTUL CLINIC FUNDENI

Acronim	Titlu proiect	Nr. Pacienti	Buget ICF (RON)	Durata Proiect
PANCNGS	Mecanisme patogenice si tratamentul personalizat in cancerul de pancreas utilizand tehnologii multi-omice; UEFISCDI; **Director Proiect: Prof. Dr. Irinel Popescu**	70	**2.573.670**	2018-2020
THERRES	Mecanisme si biomarkeri de raspuns si rezistenta la terapiile curente tintite ale cancerului gastric; UEFISCDI; **Director Proiect: Prof. Dr. Irinel Popescu**	60	**3.400.000**	2018-2022
ONCOGIN	Consorțiu multidisciplinar pentru susținerea competențelor de cercetare în diagnosticarea, tratarea și identificarea de factori predictivi ai afecțiunilor maligne ginecologice; UEFISCDI; **Director Proiect: CSII Dr.Bacalbasa Nicolae**	60	**1.035.300**	2018-2020
ONCORAD	Dezvoltarea de radiofarmaceutice si tehnici nucleare in oncologie pentru imagistica si tratament personalizat la nivel molecular; UEFISCDI; **Director Proiect: CSI Dr. Simona Olimpia Dima**	0	**381.836**	2018-2020
MIMOSA	Metode noi de monitorizare a sarcinii si diagnostic prenatal; UEFISCDI; **Director Proiect: Prof. Dr. Lorand Savu**	0	**855.000**	2018-2020

Impactul datelor publicate de Institutul Clinic Fundeni in cancerul pancreatic:

de la biobanca la subtipuri moleculare de cancer de pancreas



"Badea L, Herlea V, Dima SO, Dumitrascu T et al. Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. Hepatogastroenterology 2008 Nov-Dec;55(88):2016-27"

Citation : 388

Am realizat analiza expresiei genice a 36 adenocarcinoame de pancreas si tesut pancreatic normal (peritumoral) provenite de la pacienti operati in centrul de Chirurgie si Transplant Hepatic din Institutul Clinic Fundeni

Metodologie: microarray Affymetrix U133 Plus 2.0 65 de gene au avut un log-fold change de 1 (corespunde cu supraexpresie de 2-fold)

Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy

Nat Med. 2011 Apr; 17(4): 500–503.

Eric A Collisson[1,2,10], Anguraj Sadanandam[1,3,10], Peter Olson[4,9], William J Gibb[1,9], Morgan Truitt[4], Shenda Gu[1], Janine Cooc[5], Jennifer Weinkle[1], Grace E Kim[6], Lakshmi Jakkula[1], Heidi S Feiler[1], Andrew H Ko[2], Adam B Olshen[7], Kathleen L Danenberg[5], Margaret A Tempero[2], Paul T Spellman[1], Douglas Hanahan[3,4] & Joe W Gray[1,8]

Au analizat expresia genica CP prin microarray (UCSF tumors, n=27) si setul de date publicat de **Badea, et al. 2008.**

Probe biologice UCSF: ARN extras din parafina provenita de la pacienti cu adenocarcinom de pancreas rezecat si linii de celule de cancer de pancreas

Subtipurile de cancer de pancreas au fost obtinute folosind NMF (nonnegative matrix factorization) si clustering.

62 semnaturi genice
(semnaturi genice specifice unui subtip)

Subtypes of pancreatic ductal adenocarcinoma and their differing responses to therapy

Nat Med. 2011 Apr; 17(4): 500–503.

Eric A Collisson[1,2,10], Anguraj Sadanandam[1,3,10], Peter Olson[4,9], William J Gibb[1,9], Morgan Truitt[4], Shenda Gu[1], Janine Cooc[5], Jennifer Weinkle[1], Grace E Kim[6], Lakshmi Jakkula[1], Heidi S Feiler[1], Andrew H Ko[2], Adam B Olshen[7], Kathleen L Danenberg[5], Margaret A Tempero[2], Paul T Spellman[1], Douglas Hanahan[3,4] & Joe W Gray[1,8]

Global gene expression analysis has proved useful for subtype identification in many human tumor types[4]. We approached PDA subtype identification by first identifying intrinsically variable (standard deviation > 0.8) genes in two gene expression microarray datasets from resected PDA. We generated one dataset using microdissected PDA material (UCSF tumors, n=27) from clinical samples for which information on survival duration was available and the second was previously published (Badea, et al.)[5]. To increase power, we merged these two datasets using the distance weighted discrimination (DWD) method[6,7] and included intrinsically variable genes common to both studies. We then performed nonnegative matrix factorization (NMF) analysis with consensus clustering[8] to identify subtypes of the disease.

Rezultatele au fost validate in trei seturi de date publice (n = 102 esantioane)

Table 2. Molecular Subgrouping of Pancreatic Cancers in Selected Seminal Studies From 2008 to 2016

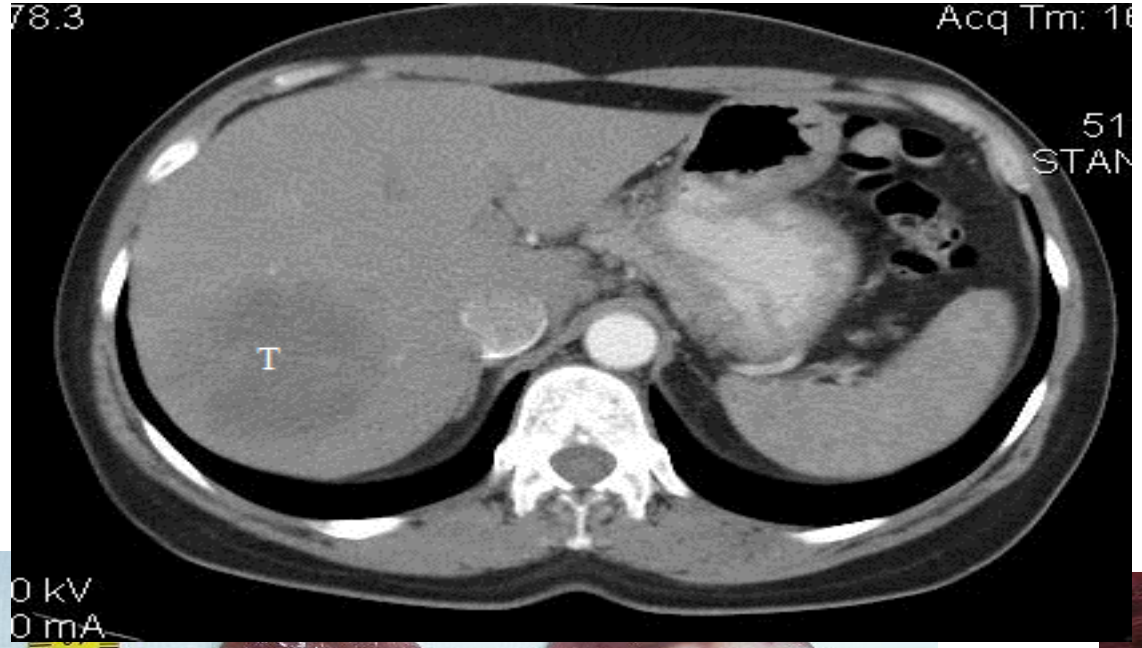
Study	Year	Sample Number	Patient Subtypes	Technique(s) Used	Molecular Subgroups	Therapeutic Implications	Comments
Jones et al[17]	2008	24 + 90	Cell lines/ PDX + resected tumors	Sequencing of protein coding exons with PCR	12 core signaling pathways	None directly	Sequenced 23,219 transcripts; assessed deletions and amplifications by DNA microarray
Collisson et al[18]	2011	27 + 34	Resected, microdissected + cell lines	Gene expression microarrays	3 subtypes	Yes	Assessed 27 UCSF samples; validated the 3 subtypes in 19 human PDX and 15 mouse cell lines; cross validated across 4 published data sets[11-14]; drug sensitivity analyses done in vitro
Biankin et al[23]	2012	142	Primary resected tumors	Exon sequencing and copy number analysis	Multiple core signaling pathways	None directly	Subgroups: G1/S checkpoint, apoptosis, angiogenesis, TGF-β signaling, and axon guidance
Waddell et al[24]	2015	142	Primary resected tumors	Whole-genome sequencing and copy number variation	4 subtypes	Yes	Subtype analysis and correlation with outcomes
Witkiewicz et al[26]	2015	109	Primary resected tumors	Virtual microdissection with copy number analysis and whole-exome sequencing	7 key pathways	Yes	Identification of multiple signaling pathways, association of mutations with prognosis, and identification of potentially targetable molecular abnormalities
Moffitt et al[25]	2015	357	Primary, metastatic, cell lines, and normal tissue	Virtual microdissection with microarrays and RNA sequencing	2 subtypes	Yes	Multiple specimens used, and digital separation of tumor, stroma, and "normal" tissue
Bailey et al[27]	2016	456	Primary resected tumors	Integrated genomic analysis	10 subgroups	Yes: 4 subtypes	Subtype analysis *and* signaling pathway analysis

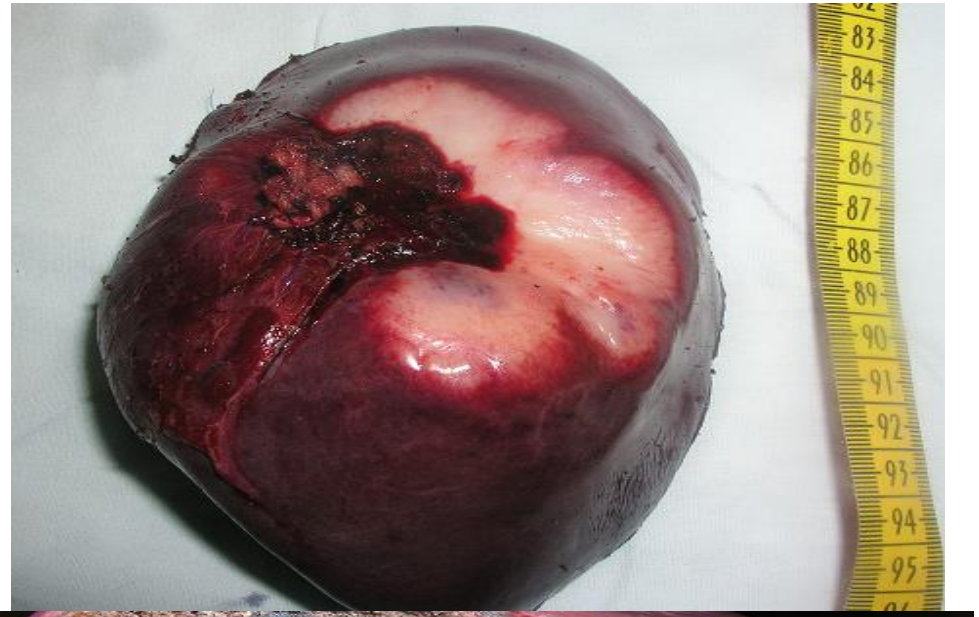
Techniques and sample types used for each of the key studies discussed in the article are summarized. A subjective assessment of the therapeutic implications of the study findings has been made based on the direct assessment of patient outcomes, and/or results of preclinical testing of the subgroups/pathways identified. PCR = polymerase chain reaction; PDX = patient-derived xenograft; TGF = transforming growth factor; USCF = University of California, San Francisco.

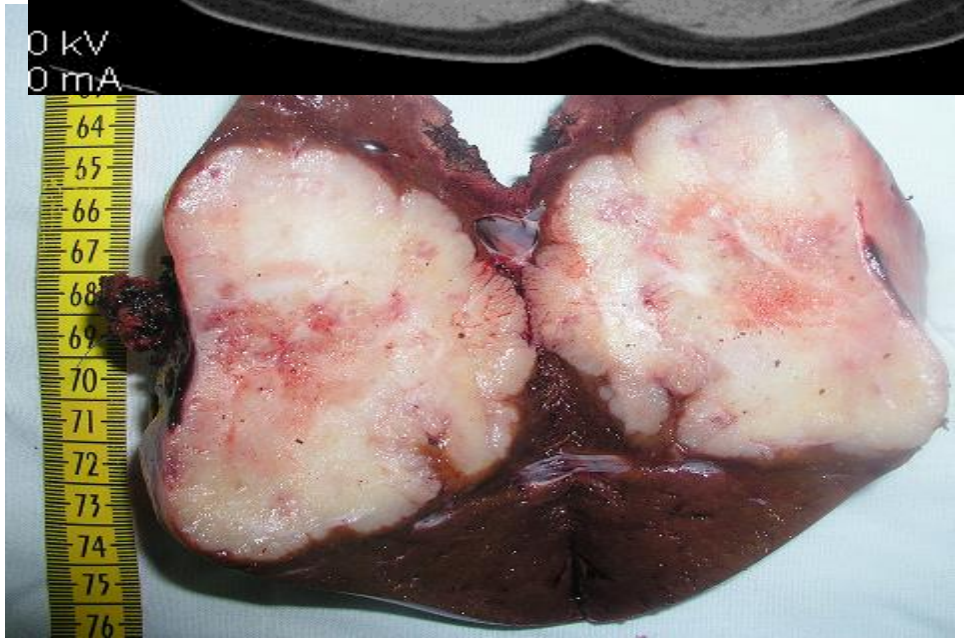
Impactul datelor publicate de Institutul Clinic Fundeni in cancerul pancreatic:

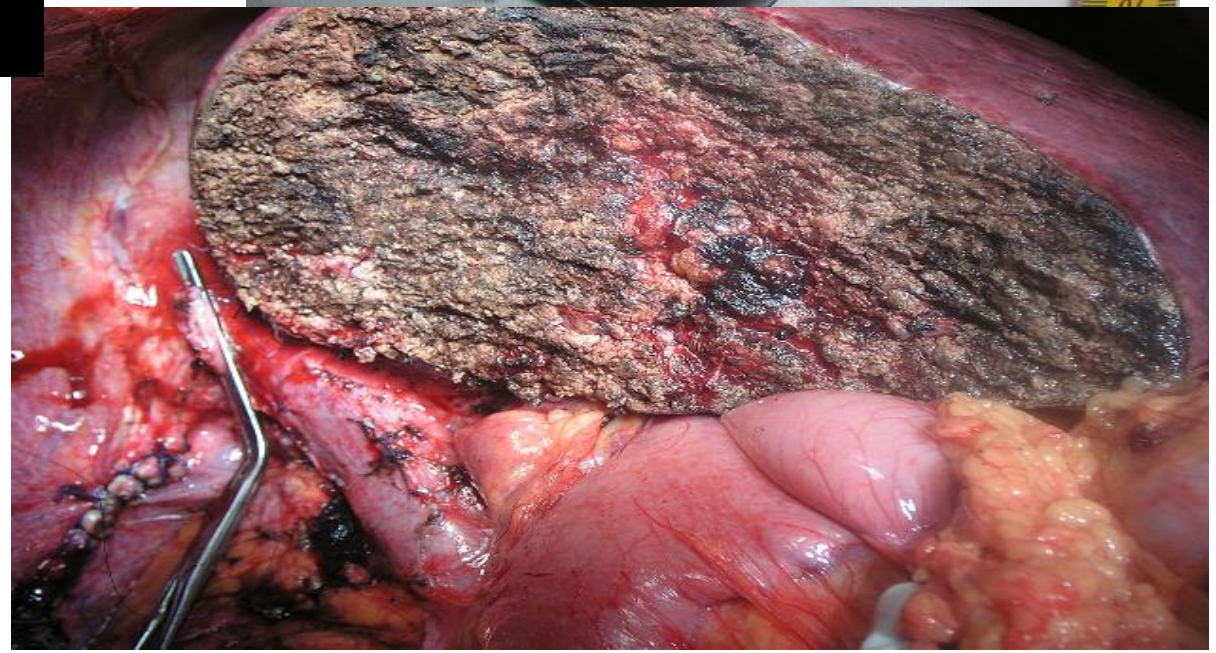
de la biobanca la caracterizare moleculara in colangiocarcinom

Colangiocarcinom









Colangiocarcinom

Obiectiv principal: identificarea de mutatii genetice implicate in dezvoltarea colangiocarcinomului

1. Studiul 1 : 209 cazuri de etiologie infectioasa si non infectioasa (Singapore si **Romania**)- (exome sequencing)

2. Studiul 2: 489 cazuri de etiologie infectioasa si non infectioasa (10 tari, incluzand si **Romania**)- (whole genome sequencing - 71 cases)

3. Studiul 3: 64 cazuri de clangiocarcinom (USA si **Romania**)- exome sequencing

Chan-on W et al., NG 2013; Jusakul A et al. Cancer Discov. 2017; Jiao Y et al. NG 2013

Studiul 1

nature genetics

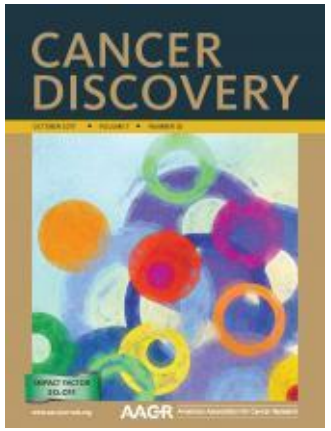
Alterari genomice in colangiocarcinom in functie de etiologie

Citations : 339

Exome sequencing identifies distinct mutational patterns in liver fluke–related and non-infection-related bile duct cancers

Waraporn Chan-on[1–3,13], Maarja-Liisa Nairismägi[1,2,13], Choon Kiat Ong[1,2,13], Weng Khong Lim[1,2,13], Simona Dima[4], Chawalit Pairojkul[3], Kiat Hon Lim[5], John R McPherson[6], Ioana Cutcutache[6], Hong Lee Heng[1,2], London Ooi[7], Alexander Chung[7], Pierce Chow[7], Peng Chung Cheow[7], Ser Yee Lee[7], Su Pin Choo[8], Iain Bee Huat Tan[8], Dan Duda[9], Anca Nastase[4], Swe Swe Myint[1,2], Bernice Huimin Wong[1,2], Anna Gan[1,2], Vikneswari Rajasegaran[1,2], Cedric Chuan Young Ng[1,2], Sanjanaa Nagarajan[1,2], Apinya Jusakul[1,2], Shenli Zhang[2], Priya Vohra[2], Willie Yu[1,2,10], DaChuan Huang[1,2], Paiboon Sithithaworn[3], Puangrat Yongvanit[3], Sopit Wongkham[3], Narong Khuntikeo[3], Vajaraphongsa Bhudhisawasdi[3], Irinel Popescu[4], Steven G Rozen[6], Patrick Tan[2,11,12] & Bin Tean Teh[1,2,12]

[1]NCCS-VARI Translational Research Laboratory, Division of Medical Sciences, National Cancer Centre Singapore, Singapore. [2]Division of Cancer and Stem Cell Biology, Duke–National University of Singapore Graduate Medical School, Singapore. [3]Liver Fluke and Cholangiocarcinoma Research Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. [4]Center of Digestive Diseases and Liver Transplantation, Fundeni Clinical Institute, Bucharest, Romania. [5]Department of Pathology, Singapore General Hospital, Singapore. [6]Division of Neuroscience and Behavioral Disorders, Duke–National University of Singapore Graduate Medical School, Singapore. [7]Department of General Surgery, Singapore General Hospital, Singapore. [8]Department of Medical Oncology, National Cancer Centre Singapore, Singapore. [9]Edwin L. Steele Laboratory for Tumor Biology, Department of Radiation Oncology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA. [10]National University of Singapore Graduate School for Integrative Sciences and Engineering, Singapore. [11]Genome Institute of Singapore, Singapore. [12]Cancer Science Institute of Singapore, National University of Singapore, Singapore. [13]These authors contributed equally to this work. Correspondence should be addressed to B.T.T. (teh.bin.tean@singhealth.com.sg), P.T. (gmstanp@duke-nus.edu.sg), S.G.R. (steve.rozen@duke-nus.edu.sg) or I.P. (irinel.popescu@icfundeni.ro).



October 2017
Volume 7,
Issue 10

Whole-Genome and Epigenomic Landscapes of Etiologically Distinct Subtypes of Cholangiocarcinoma

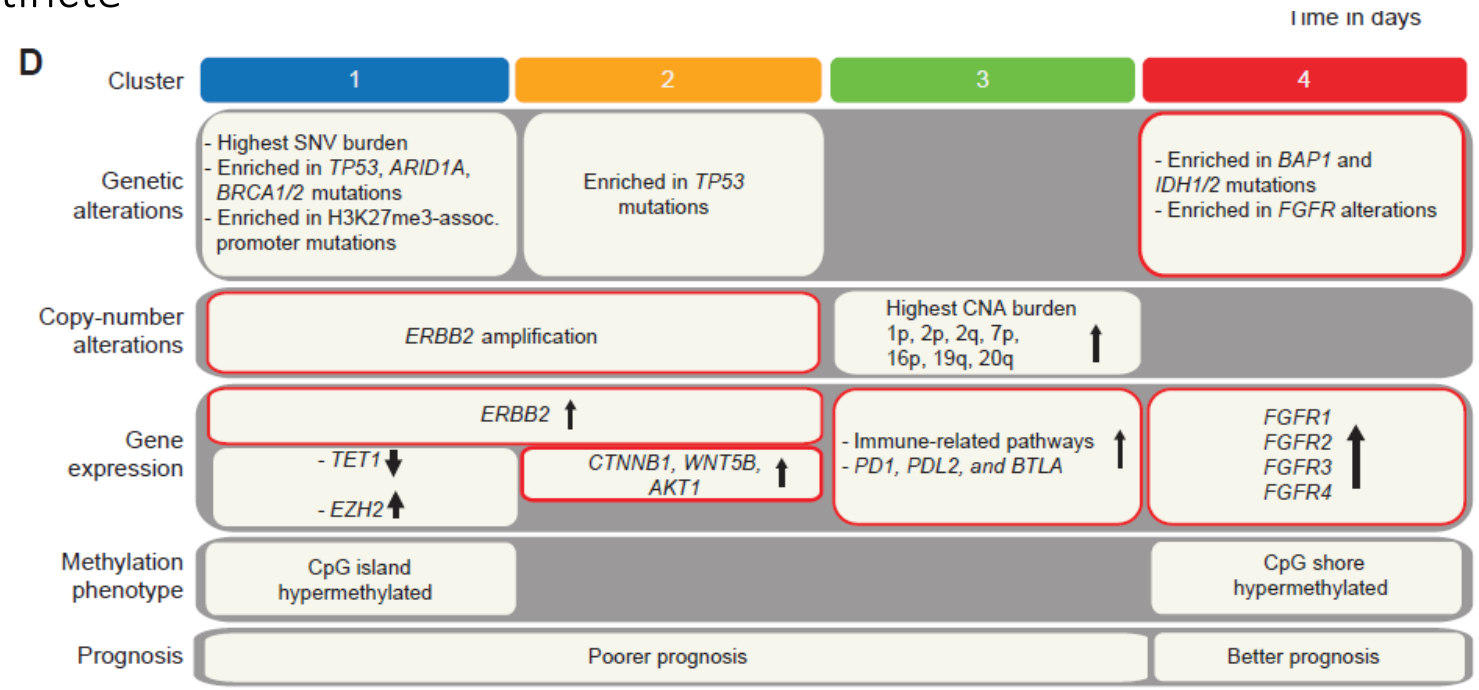
Apinya Jusakul[1,2,3], Ioana Cutcutache[4], Chern Han Yong[1,4], Jing Quan Lim[2,5], Mi Ni Huang[4], Nisha Padmanabhan[1], Vishwa Nellore[6], Sarinya Kongpetch[2,7,8], Alvin Wei Tian Ng[9], Ley Moy Ng[10], Su Pin Choo[11], Swe Swe Myint[2], Raynoo Thanan[12], Sanjanaa Nagarajan[2], Weng Khong Lim[1,2], Cedric Chuan Young Ng[2], Arnoud Boot[1,4], Mo Liu[1,4], Choon Kiat Ong[5], Vikneswari Rajasegaran[2], Stefanus Lie[2,13], Alvin Soon Tiong Lim[14], Tse Hui Lim[14], Jing Tan[2], Jia Liang Loh[2], John R. McPherson[4], Narong Khuntikeo[7,15], Vajaraphongsa Bhudhisawasdi[15], Puangrat Yongvanit[7], Sopit Wongkham[12], Yasushi Totoki[16], Hiromi Nakamura[16], Yasuhito Arai[16], Satoshi Yamasaki[17], Pierce Kah-Hoe Chow[18], Alexander Yaw Fui Chung[19], London Lucien Peng Jin Ooi[19], Kiat Hon Lim[20], Simona Dima[21], Dan G. Duda[22], Irinel Popescu[21], Philippe Broet[23], Sen-Yung Hsieh[24], Ming-Chin Yu[25], Aldo Scarpa[26], Jiaming Lai[27], Di-Xian Luo[28], André Lopes Carvalho[29], André Luiz Vettore[30], Hyungjin Rhee[31], Young Nyun Park[31], Ludmil B. Alexandrov[32], Raluca Gordân[6,33], Steven G. Rozen[1,4,34], Tatsuhiro Shibata[16,17], Chawalit Pairojkul[35], Bin Tean Teh[1,2,10,34,36], and Patrick Tan[1,10,34,37]

Studiul 2

Citations : 414

Whole-genome sequencing (WGS) la 71 cazuri de colangiocarcinom si tesut non-tumoral

Datele epigenetice au dus la definirea a **4 subtipuri de colangiocacinom** (4 clustere), fiecare prezentand caracteristici moleculare si clinic distincte



Studiul 3

nature genetics

Citations : 477

Exome sequencing identifies frequent inactivating mutations in *BAP1*, *ARID1A* and *PBRM1* in intrahepatic cholangiocarcinomas

Yuchen Jiao[1,2,19], Timothy M Pawlik[3,19], Robert A Anders[4,19], Florin M Selaru[5], Mirte M Streppel[4], Donald J Lucas[6], Noushin Niknafs[7], Violeta Beleva Guthrie[7], Anirban Maitra[4], Pedram Argani[4], G Johan A Offerhaus[8], Juan Carlos Roa[9], Lewis R Roberts[10], Gregory J Gores[10], Irinel Popescu[11], Sorin T Alexandrescu[11], Simona Dima[11], Matteo Fassan[12,13], Michele Simbolo[12,13], Andrea Mafficini[12], Paola Capelli[13], Rita T Lawlor[12,13], Andrea Ruzzenente[14], Alfredo Guglielmi[14], Giampaolo Tortora[15], Filippo de Braud[16], Aldo Scarpa[12,13], William Jarnagin[17], David Klimstra[18], Rachel Karchin[7], Victor E Velculescu[1,2], Ralph H Hruban[4], Bert Vogelstein[1,2], Kenneth W Kinzler[1,2], Nickolas Papadopoulos[1,2] & Laura D Wood[4]

[1]The Ludwig Center, Johns Hopkins University and Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA. [2]Howard Hughes Medical Institute, Johns Hopkins University and Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA. [3]Department of Surgery, Johns Hopkins University and Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA. [4]Department of Pathology, Johns Hopkins University and Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA. [5]Department of Gastroenterology and Hepatology, Johns Hopkins University and Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA. [6]Department of Surgery, Walter Reed National Military Medical Center, Bethesda, Maryland, USA. [7]Department of Biomedical Engineering, Institute for Computational Medicine, Johns Hopkins University and Sidney Kimmel Comprehensive Cancer Center, Baltimore, Maryland, USA. [8]Department of Pathology, University Medical Center, Utrecht, The Netherlands. [9]Department of Pathology, BIOREN-CEGIN, Universidad de La Frontera, Temuco, Chile. [10]Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, USA. [11]Dan Setlacec Center of General Surgery and Liver Transplantation, Fundeni Clinical Institute, Bucharest, Romania. [12]ARC-NET Miriam Cherubini Research Centre, University of Verona, Verona, Italy. [13]Department of Pathology and Diagnostics, University of Verona, Verona, Italy. [14]Department of Surgery, University of Verona, Verona, Italy. [15]Department of Medicine, Medical Oncology Unit, University of Verona, Verona, Italy. [16]Medical Oncology Unit 1, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. [17]Department of Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York, USA. [18]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, New York, USA. [19]These authors contributed equally to this work. Correspondence should be addressed to A.S. (aldo.scarpa@univr.it), K.W.K. (kinzlke@jhmi.edu), N.P. (npapado1@jhmi.edu) or L.D.W. (ldwood@jhmi.edu).

Jiao Y *et al*, Nature Genetics 45 (2013)

Studiul 3

Citations : 477

Table 1 Genes with frequent somatic alterations in intrahepatic cholangiocarcinoma

Gene	Number of mutations (DS) (%)	Types of mutations (DS)[a]	*P* value (DS)[b]	Number of mutations (PS) (%)	Types of mutations (PS)[a]	Mutant 3-year survival (%)[c]	Wild-type 3-year survival (%)[c]	*P* value for 3-year survival[c]
BAP1	8 (25)	3 FS, 1 IF, 2 SS, 2 MS	7.4×10^{-12}	5 (16)	1 SS, 4 MS	27	81	0.102
ARID1A	6 (19)	3 NS, 1 FS, 2 MS	4.3×10^{-3}	3 (9)	1 NS, 1 FS, 1 MS	40	79	0.51
PBRM1	5 (17)	1 NS, 4 FS	1.8×10^{-5}	3 (9)	1 NS, 1 FS, 1 MS	50	76	0.75
IDH1 or *IDH2*	6 (19)	6 MS	5.4×10^{-3}	7 (22)	7 MS	33	81	0.0034

DS, discovery screen; PS, prevalence screen.

[a]FS, frameshift insertion-deletion; IF, in-frame insertion-deletion; SS, splice-site mutation; MS, missense mutation; NS, nonsense mutation. [b]Calculated using Bonferroni correction for multiple comparisons (see **Supplementary Table 6a** for additional data). [c]Calculated using clinical data from discovery screen samples.

BAP 1 - BRCA1 associated protein-1 (ubiquitin carboxy-terminal hydrolase), cromozom 3

ARID1A - AT-rich interactive domain-containing protein 1A (SWI-like), cromozom 1

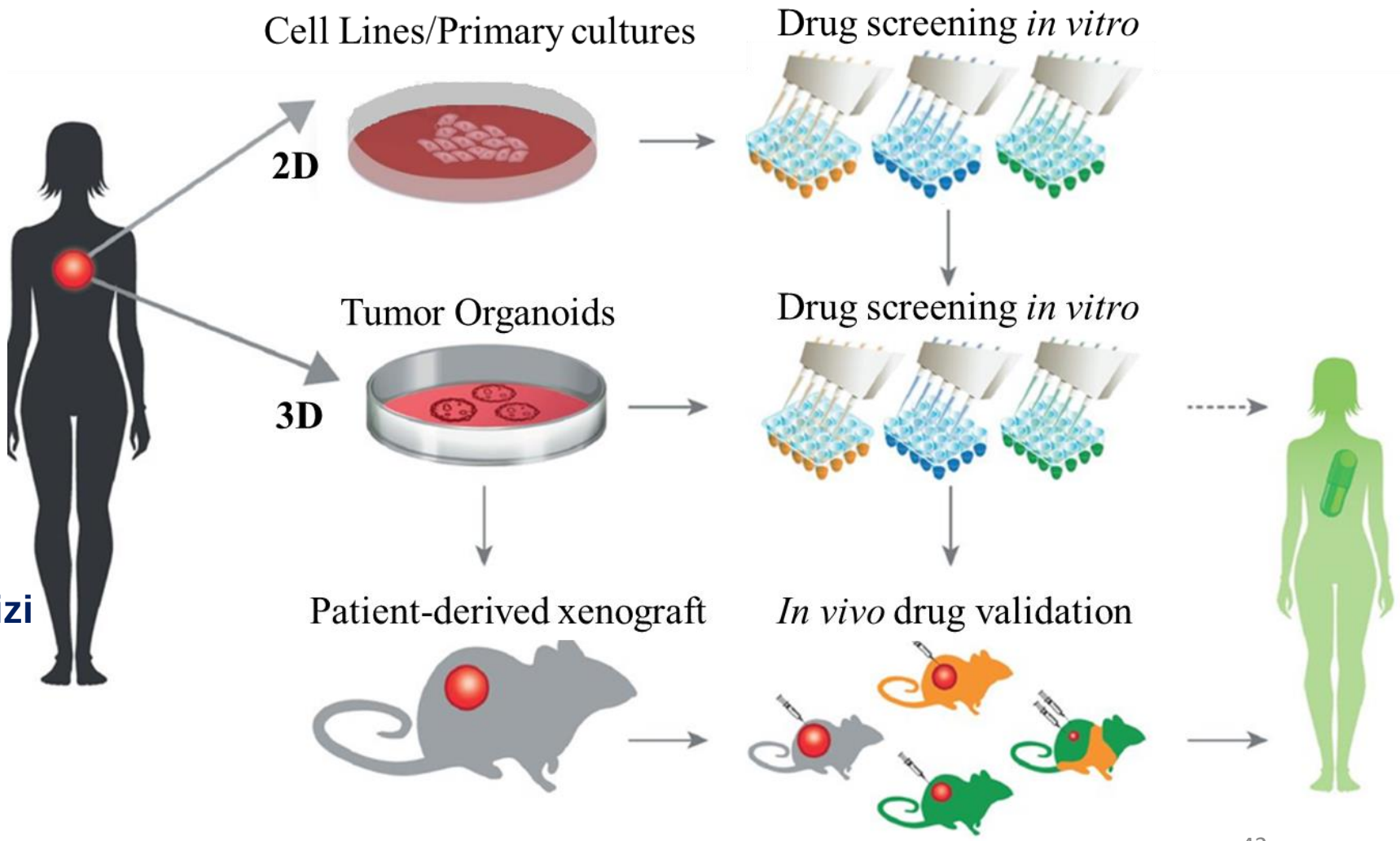
PBRM1 - polybromo 1, cromozom 3,

IDH1, IDH2 - isocitrate dehydrogenase 1 (cromozom 2), isocitrate dehydrogenase 2 (cromozom 15)

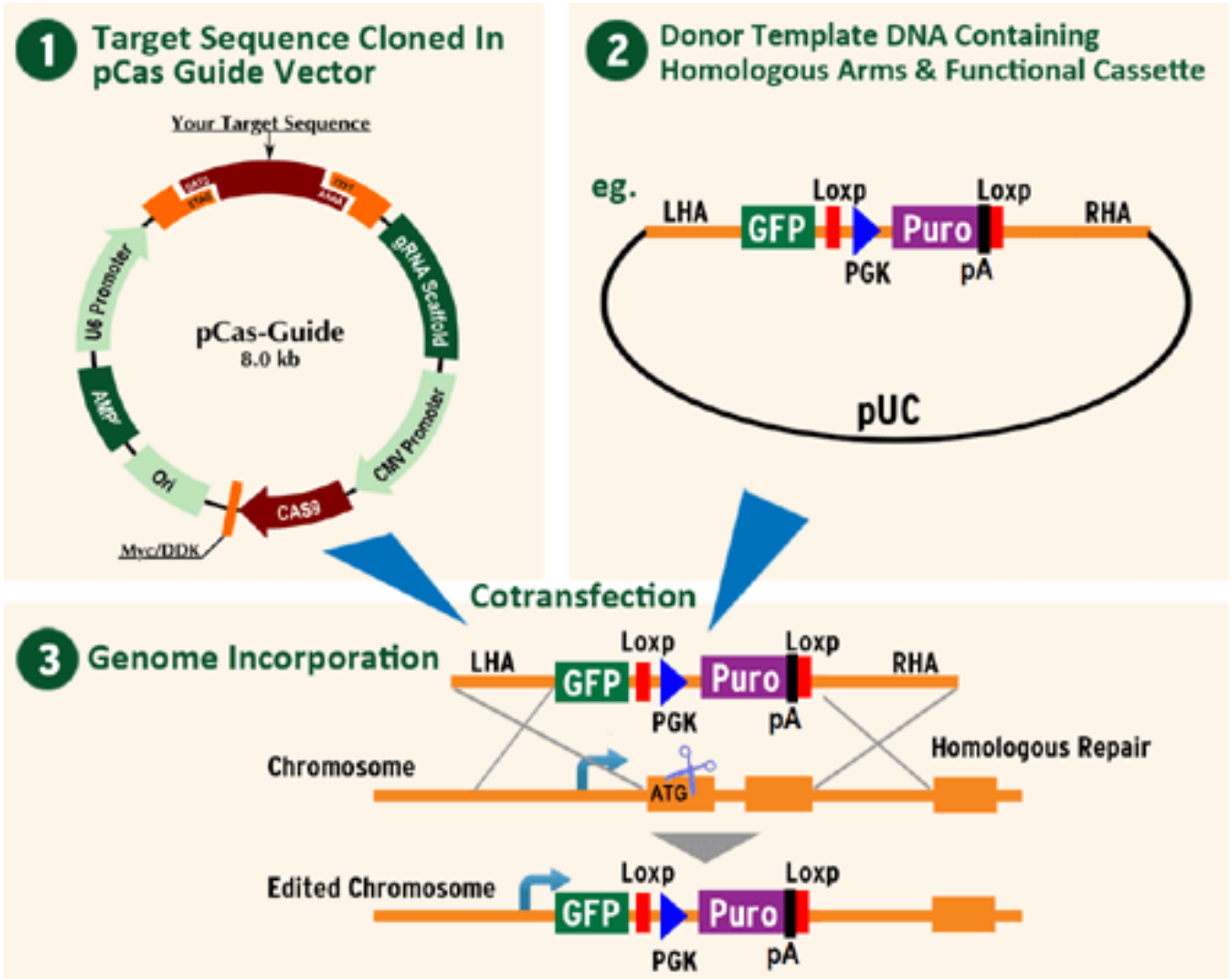
Despre viitor…

INSTITUTUL CLINIC FUNDENI

- **Linii celulare/Culturi primare**
- **Culturi de organoizi tumorali (modele personalizate *in vitro*)**
- **Xenografe derivate din organoizi**



Despre viitor…



Target gene knocked out, GFP under native gene promoter, Puro under PGK promoter

Sursa Origene

- CRISPR-CAS9

Reprezinta un instrument de editare genica tintita utilizat recent pentru tratamentul malignitatilor hematologice. (Jensen T., et al, 2019)

Potential terapeutic in cancerele solide

Ce urmeaza?

Avantajele constituirii unei biobanci:

- ✓ Crearea unei retele nationale care sa permita profesionistilor din domeniu sa contribuie cu specimene biologice pentru cercetarea fundamentala, translationala si clinica.

- ✓ contributii considerabile aduse sistemului de cercetare national.