LA CARATTERIZZAZIONE MOLECOLARE DEI TUMORI DEL PANCREAS





"LA TERAPIA PERSONALIZZATA IN ONCOLOGIA"

Dpt. Medicina Translazionale

Università "La Sapienza"

Roma

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gdf 2011

Pancreatic Cancer

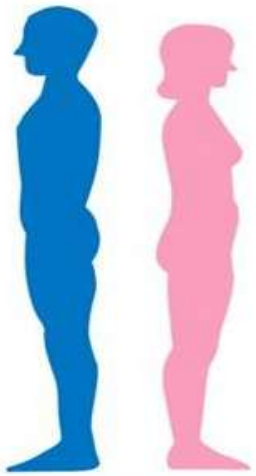
- ✓Epidemiology

Sopravvivenza e mortalità

10° neoplasia per frequenza, 4° causa di morte per tumore l'anno nei paesi occidentali (10/100000, uguale ad incidenza).

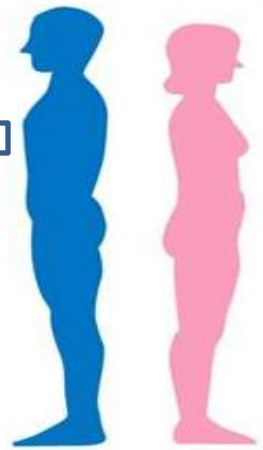
Estimated new cases

Males			Females		
Prostate	218,890	29%	Breast	178,480	26%
Lung & bronchus	114,760	15%	Lung & bronchus	98,620	15%
Colon & rectum	79,130	10%	Colon & rectum	74,630	11%
Urinary bladder	50,040	7%	Uterine corpus	39,080	6%
Non-Hodgkin lymphoma	34,200	4%	Non-Hodgkin lymphoma	28,990	4%
Melanoma of the skin	33,910	4%	Melanoma of the skin	26,030	4%
Kidney & renal pelvis	31,590	4%	Thyroid	25,480	4%
Leukemia	24,800	3%	Ovary	22,430	3%
Oral cavity & pharynx	24,180	3%	Kidney & renal pelvis	19,600	3%
Pancreas	18,830	2%	Leukemia	19,440	3%
All Sites	**766,860**	**100%**	**All Sites**	**678,060**	**100%**

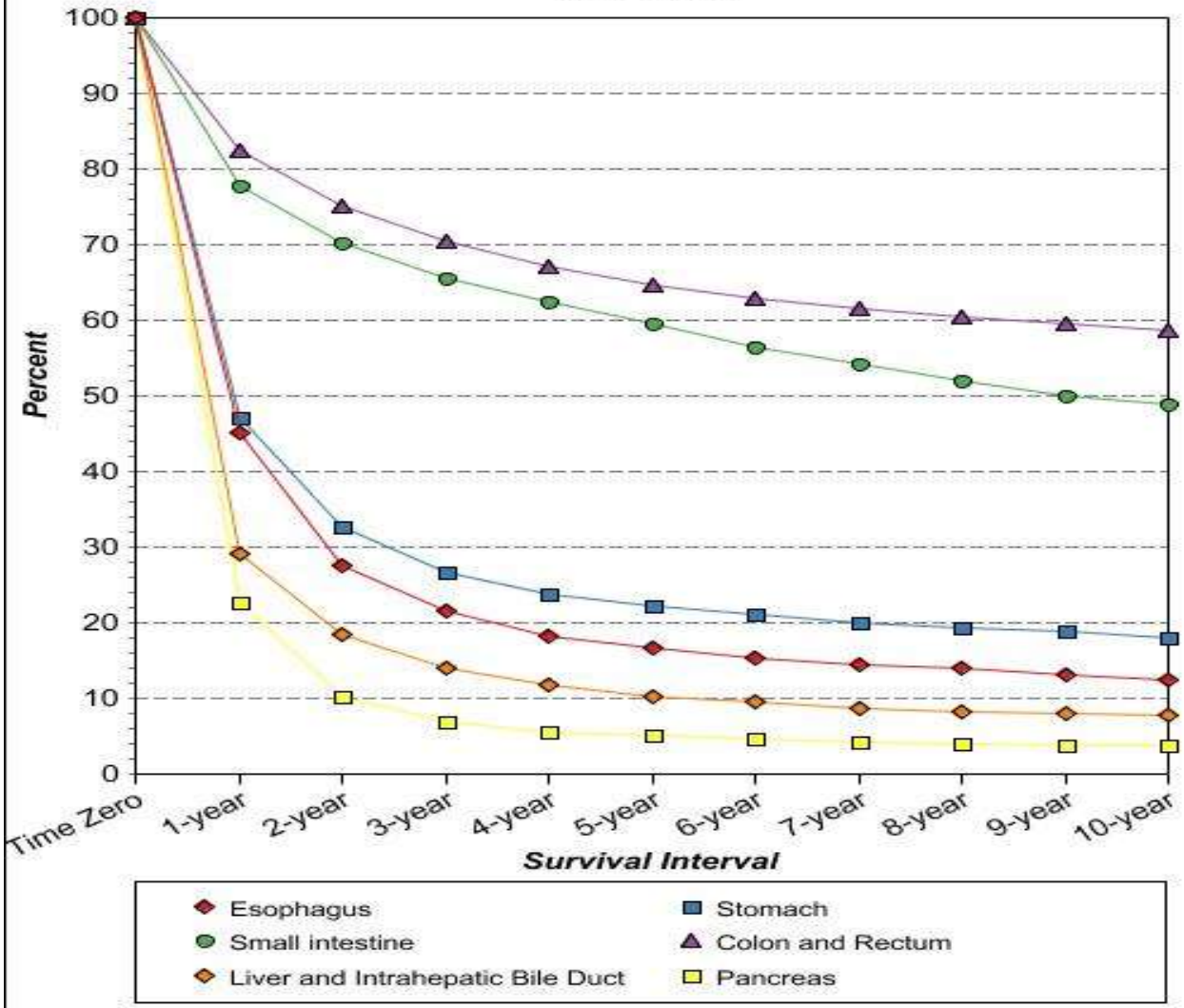


Estimated deaths

Males			Females		
ung & bronchus	89,510	31%	Lung & bronchus	70,880	26%
Prostate	27,050	9%	Breast	40,460	15%
Colon & rectum	26,000	9%	Colon & rectum	26,180	10%
Pancreas	16,840	6%	Pancreas	16,530	6%
Leukemia	12,320	4%	Ovary	15,280	6%
Liver & intrahepatic bile duct	11,280	4%	Leukemia	9,470	4%
Esophagus	10,900	4%	Non-Hodgkin lymphoma	9,060	3%
Urinary bladder	9,630	3%	Uterine corpus	7,400	3%
Non-Hodgkin lymphoma	9,600	3%	Brain & other nervous system	5,590	2%
Kidney & renal pelvis	8,080	3%	Liver & intrahepatic bile duct	5,500	2%
All Sites	**289,550**	**100%**	**All Sites**	**270,100**	**100%**

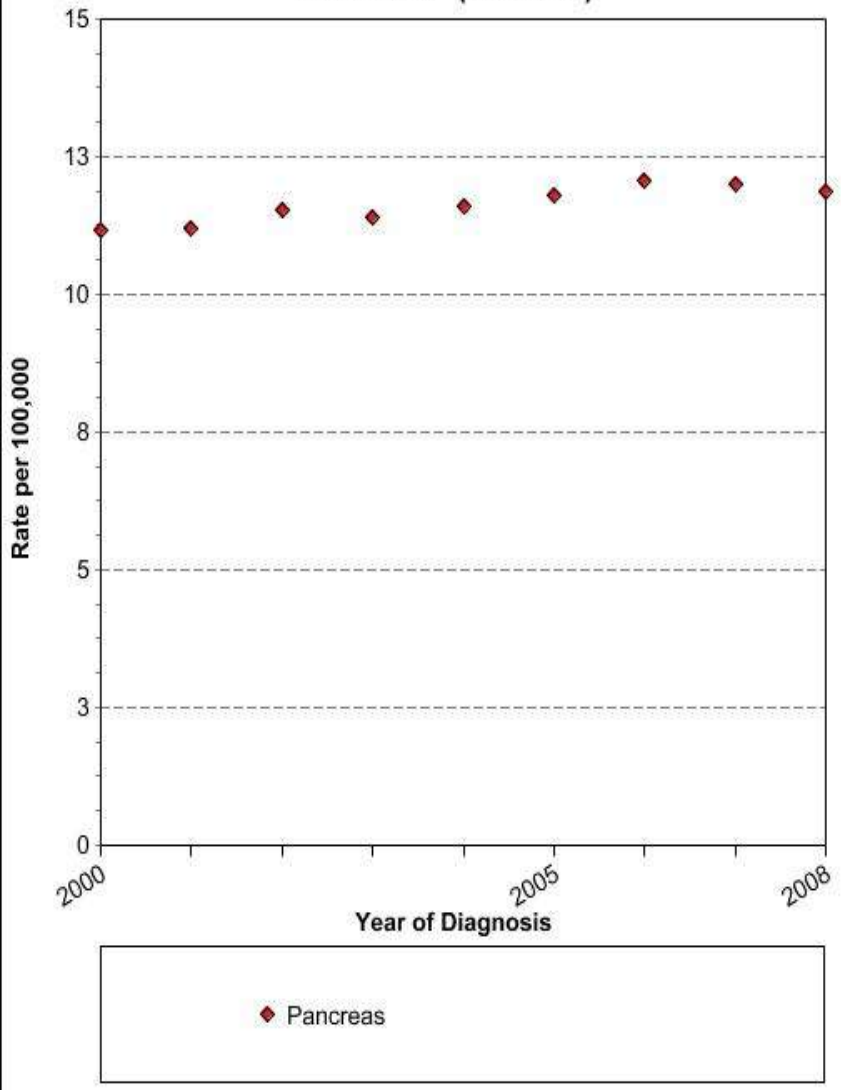


Relative Survival By Survival Time
By Cancer Site
All Ages, White, Both Sexes
1988-2006



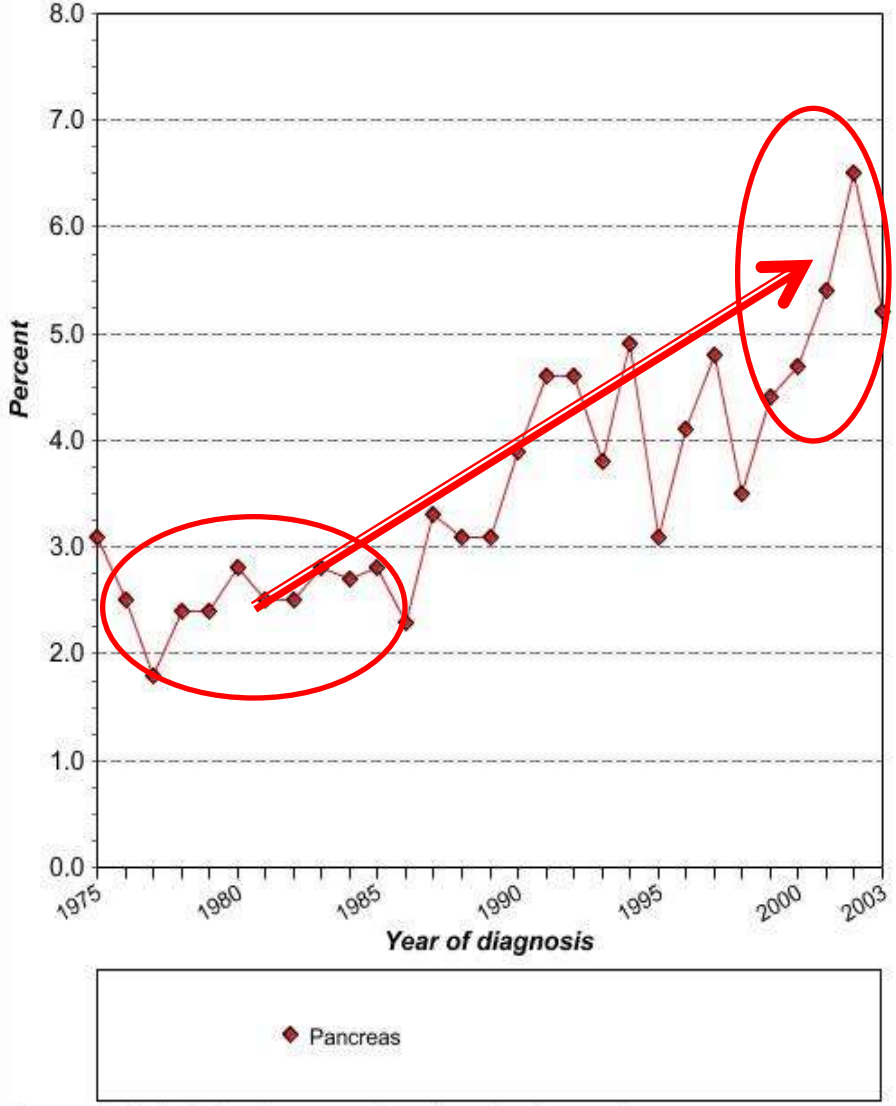
Cancer sites include invasive cases only unless otherwise noted.
Survival source: SEER 9 areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, and Atlanta).
The annual survival estimates are calculated using monthly intervals.

Age-Adjusted SEER Incidence Rates By Cancer Site All Ages, White, Both Sexes 2000-2008 (SEER 17)



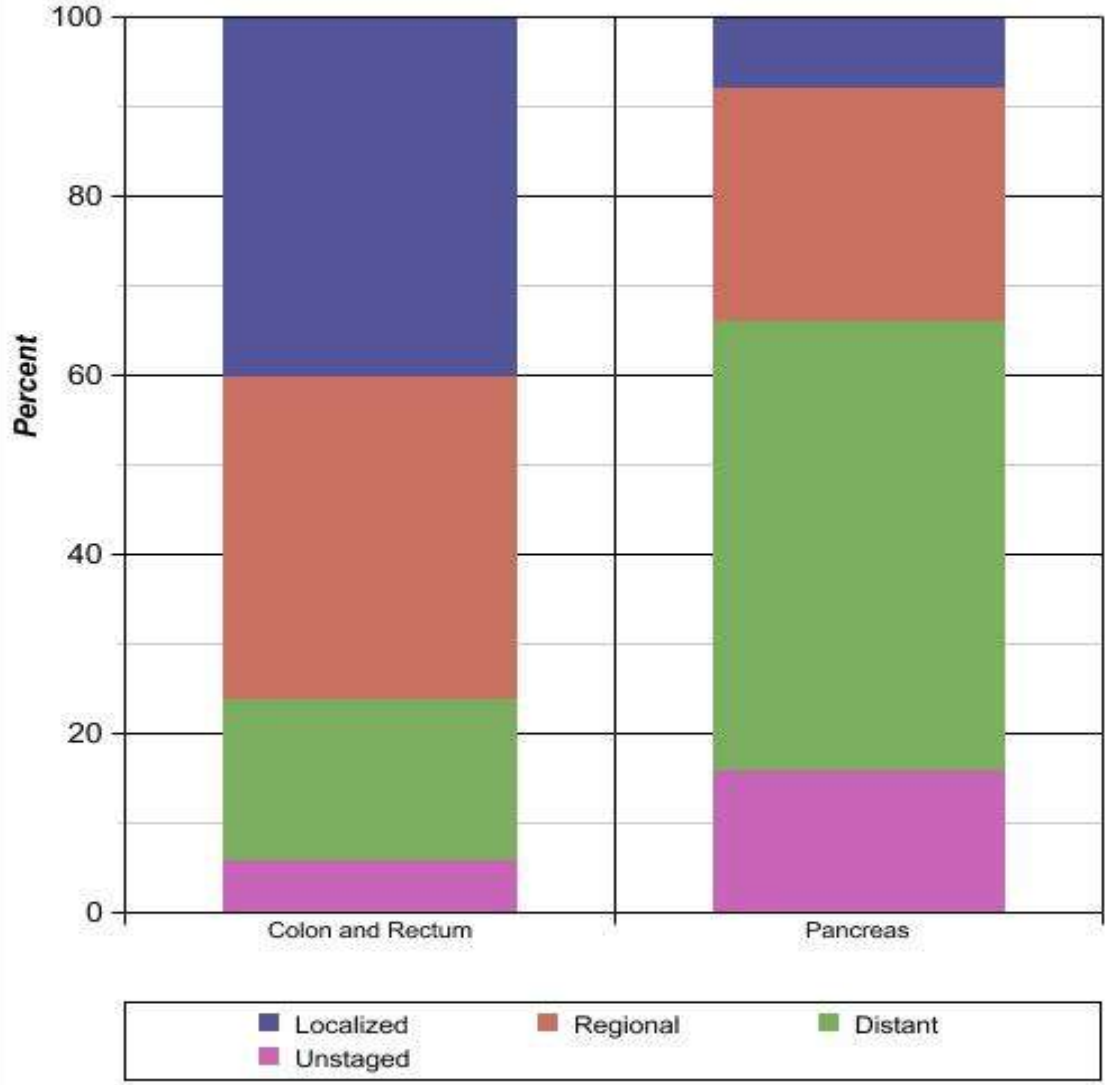
Cancer sites include invasive cases only unless otherwise noted.
Incidence source: SEER 17 areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, Atlanta, San Jose-Monterey, Los Angeles, Alaska Native Registry, Rural Georgia, California excluding SF/SJM/LA, Kentucky, Louisiana and New Jersey).
Rates are per 100,000 and are age-adjusted to the 2000 US Std Population (19 age groups - Census P25-1130). Regression lines are calculated using the Joinpoint Regression Program Version 3.5, April 2011, National Cancer Institute.

5-Year Relative Survival By Year Dx By Cancer Site All Ages, White, Both Sexes 1975-2003



Cancer sites include invasive cases only unless otherwise noted.
Survival source: SEER 9 areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, and Atlanta).
The 5-year survival estimates are calculated using monthly intervals.

Stage Distribution (SEER Summary Stage 2000)
By Cancer Site
All Ages, White, Both Sexes
2000-2007



Cancer sites include invasive cases only unless otherwise noted.
Incidence source: SEER 17 areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, Atlanta, San Jose-Monterey, Los Angeles, Alaska Native Registry, Rural Georgia, California excluding SF/SJM/LA, Kentucky, Louisiana and New Jersey).

Pancreatic Cancer

- ✓Epidemiology
- ✓Risk Factors

Major Risk Factors for Pancreatic Cancer

	Increased Risk	Proportion of total Pancreatic Cancer (%)
Smoking	**X 2**	30
Genetic Factors (familiarity)	**X 5-10**	10
Chronic Pancreatitis	**X 10-20**	1
Hereditary Pancreatitis	**X 35 -70**	1
Age > 70	**X 5**	-
Type 2 Diabetes	**X 2**	-
Obesity	**X 2**	-
Higth-fat diet	**X 2**	-

Pancreatic Cancer

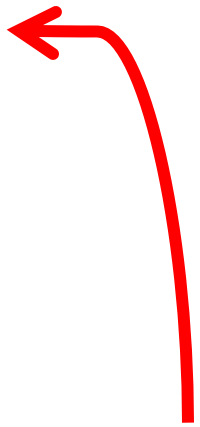
- ✓Epidemiology
- ✓Risk Factors
- ✓Precancerous Lesions

Pancreatic Tumors

	Frequency	Prognosis
Solid tumors		
– Ductal Adenocarcinomas	90 %	unfavorable
– Acinar cell Carcinoma	1 %	unfavorable
– Endocrine Tumors	9 %	intermediate
– …….		
Cystic Tumors		
– **I**ntraductal **P**apillary **M**ucinous **N**eoplasm	2 %	good
– **M**ucinous **C**ystic **N**eoplasm	1 %	good
– Serous cystic neoplasm	1 %	good
– ……		

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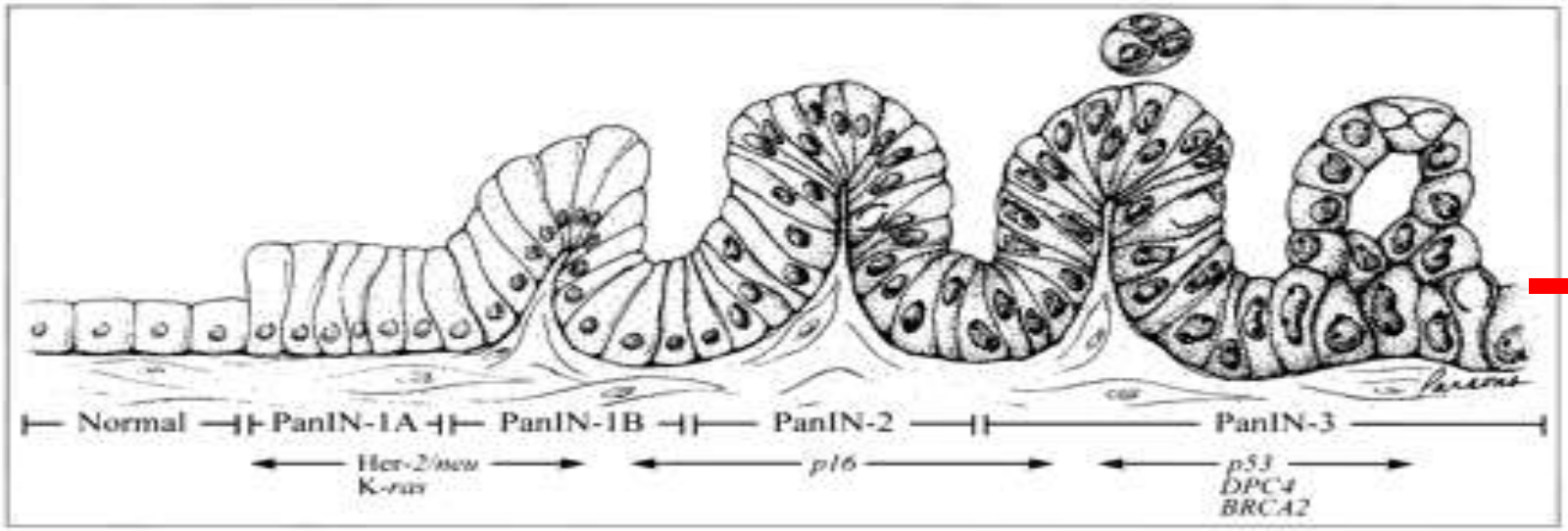


Pancreatic Intraepithelial Neoplasia

Pancreatic Intraepithelial Neoplasia

- Histological criteria (Achitectural and Cytonuclear Atypia)
  - PanIN 1
    - A ductal cell elongation
    - B papillary instead of flat architecture
  - PanIN 2
    - Nuclear abnormalities, abnormal mitoses
  - PanIN 3
    - Budding cells into the lumen= **Carcinoma *in situ***

Biologia Molecolare della progessione neoplastica

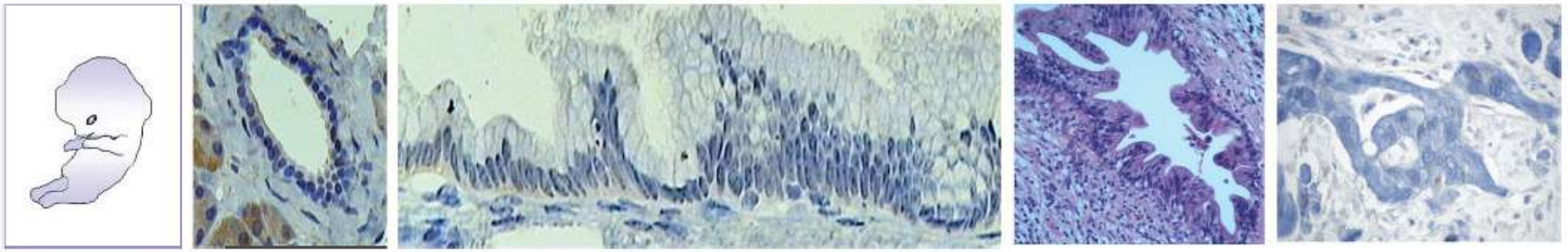


PDA

(Wilentz et al., 2000)

Gene	Mutation Frequency	Method	
KRAS	> 90%	Point mutation	Hingorani. 2003
CDKN2A/INK4A	> 95%	Deletion; Methylation	Aguirre, 2003
TP53	50 – 75%	Point mutation	Hingorani, 2005
SMAD4/DPC4	55%	Deletion; Point mutation	Bardeesy, 2006; Izeradjene, 2007
BRCA2	< 10%	Point mutation	

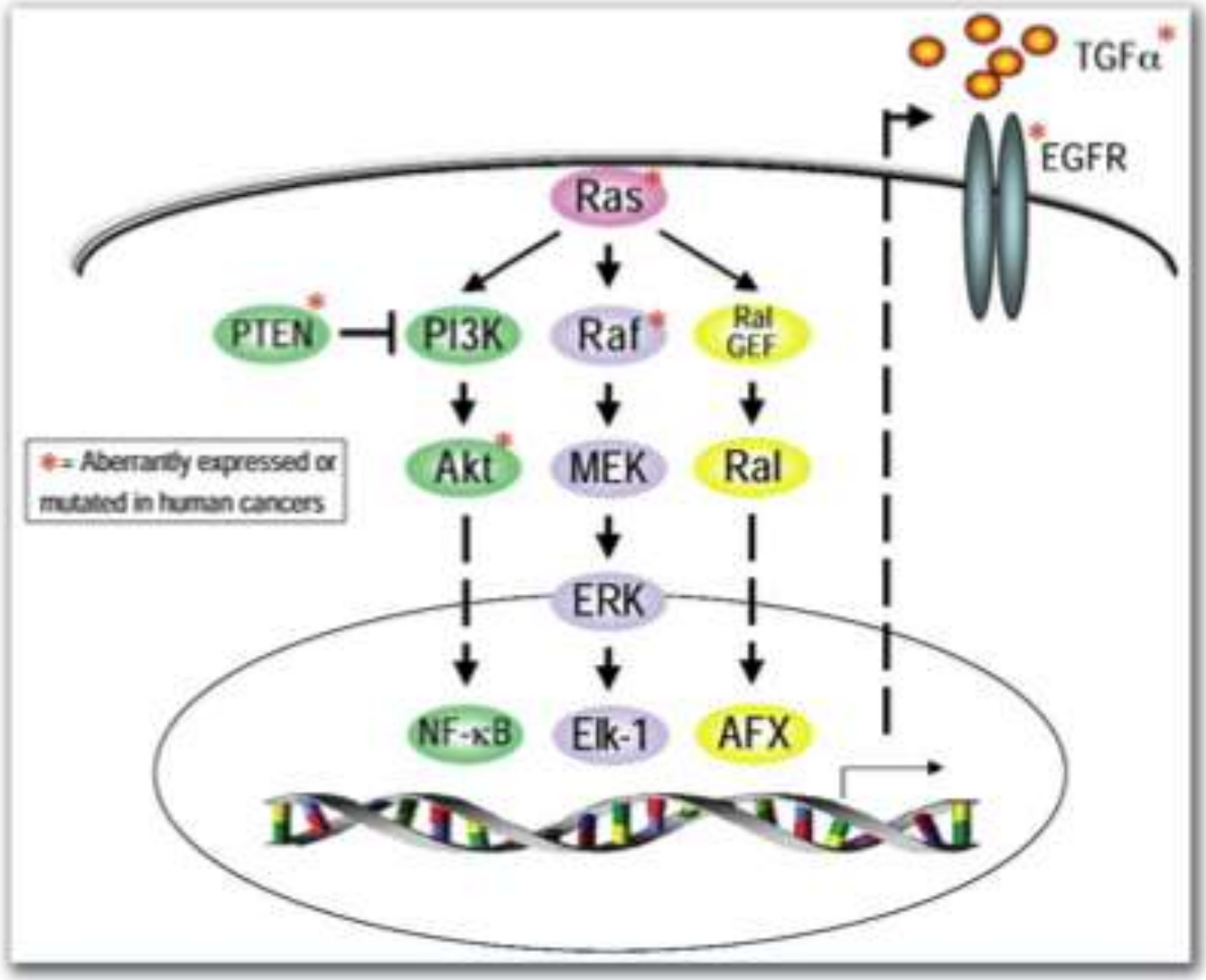
Biologia Molecolare della progessione neoplastica



Embyro	Benign ductal cells	PanIN-1	PanIN-2	PanIN-3	Invasive carcinoma
Pdx-1 Sonic hedge-hog (SHH) Serine/threonine kinase 11 (STK11/LKB1)		K-ras Telomere shortening p21 (WAF1/CIP1) Human epidermal growth factor receptor 2 (HER2/neu) Mucin 1 (MUC1) MUC 6 Trefoil factor 1 (TFF 1) p16 (INK4a) S100A11 MUC5AC S100A6	Cyclin D1 Cyclo-oxygenase-2 (COX-2) Hes1 (Hair and enchancer-of split 1) Notch 1 Pepsinogen C Kruppel-like factor 4 (KLF4) HOXA5 GATA5 Gastrin Villin-1 Villin-2 Cellular retinoic acid binding protein 1 (CRABP1)	p53 SMAD4 BRCA2 S100P SHH SialyT (Mucin-associated-carbohydrate antigen) Maspin MUC4 Tumour suppressor in-lung cancer-1 (TSLC-1) Familial adenamatous-polyposis (FAP)	

Figure 1 Histological images of benign pancreatic ductal epithelial cells, progressive PanIN lesions and invasive carcinoma, with associated genetic alterations.

Ras Pathway


TGFα*
*EGFR
Ras*
PTEN*
PI3K
Raf*
Ral GEF
Akt*
MEK
Ral
ERK
NF-κB
Elk-1
AFX
*= Aberrantly expressed or mutated in human cancers

K ras

- **Oncogene** codificante proteina GTP accoppiata coinvolta nelle pathways di trasduzione del segnale mediato dai Fattori di crescita

Mutazioni di K Ras (codone 12, 13 o 61)

forma costitutivamente attivata (bloccata nello stato GTP-blind)



cascata del segnale: raf-MAPKK-ERK

- **Mutazioni attivanti di K Ras sono presenti fino al 75-90% delle neoplasie pancreatiche, ed in paricolare sono state trovate anche in precursori (PAN-IN) – mutazione iniziante**

K Ras

Potenziali target terapeutici

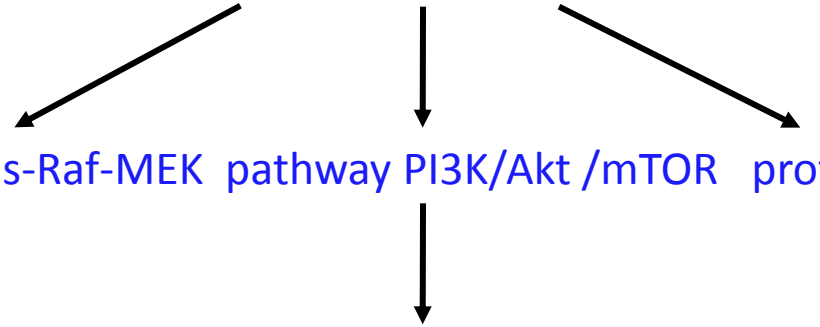
- Inibizione delle **modificazioni post trasduzionali** (inibitore della franesil transferas): non efficacia negli studi di Fase 3

- **RNA *interference*** promettente sia da solo che in combinazine alla terapia radiante

- **Inibizione della cascata del segnale**: Sorafenib (inibitore della Raf-1 chinasi) inattivo sul cancro pacreatico

Fattori di Crescita implicazioni sul segnale intracellulare

- **EGFR-1** proteina transmembrana ad attività tirosinkinasico: ligandi EGF e TGFa



- **Akt, e EGFR-1** (così come le altre proteine della famiglia ErbB) sono overespresse nel cancro pancreatico

Fattori di Crescita implicazioni sul segnale intracellulare

- Cetuximab, anticorpo monoclonale anti **EGFR**: in combinazione con Gemcitabina incremento della sopravvivenza a 1 aa (32%)

- Inibitori di **MTOR** attività antitumorale in vitro, fase di sperimentazione in vivo

- Inibitori di **NFkB** (attivato dalla cascata Akt): studi di fase I su (Bortezomib)

- Inibitore del'attività tirosin kinasica intracellulare associata ai Recettori: **Erlotinib** (Tarceva) efficace con Gemcitabina approvato nella malattia metastatica

- **VEGF** overesppresso nel k pancreas: Bevacizumab (anticorpo monoclonale contro VEGF) inefficace da solo e/o con Gemcitabina, Sunitinib (inibitore VEGFR 1,2,3) potenziale ruolo terapeutico.

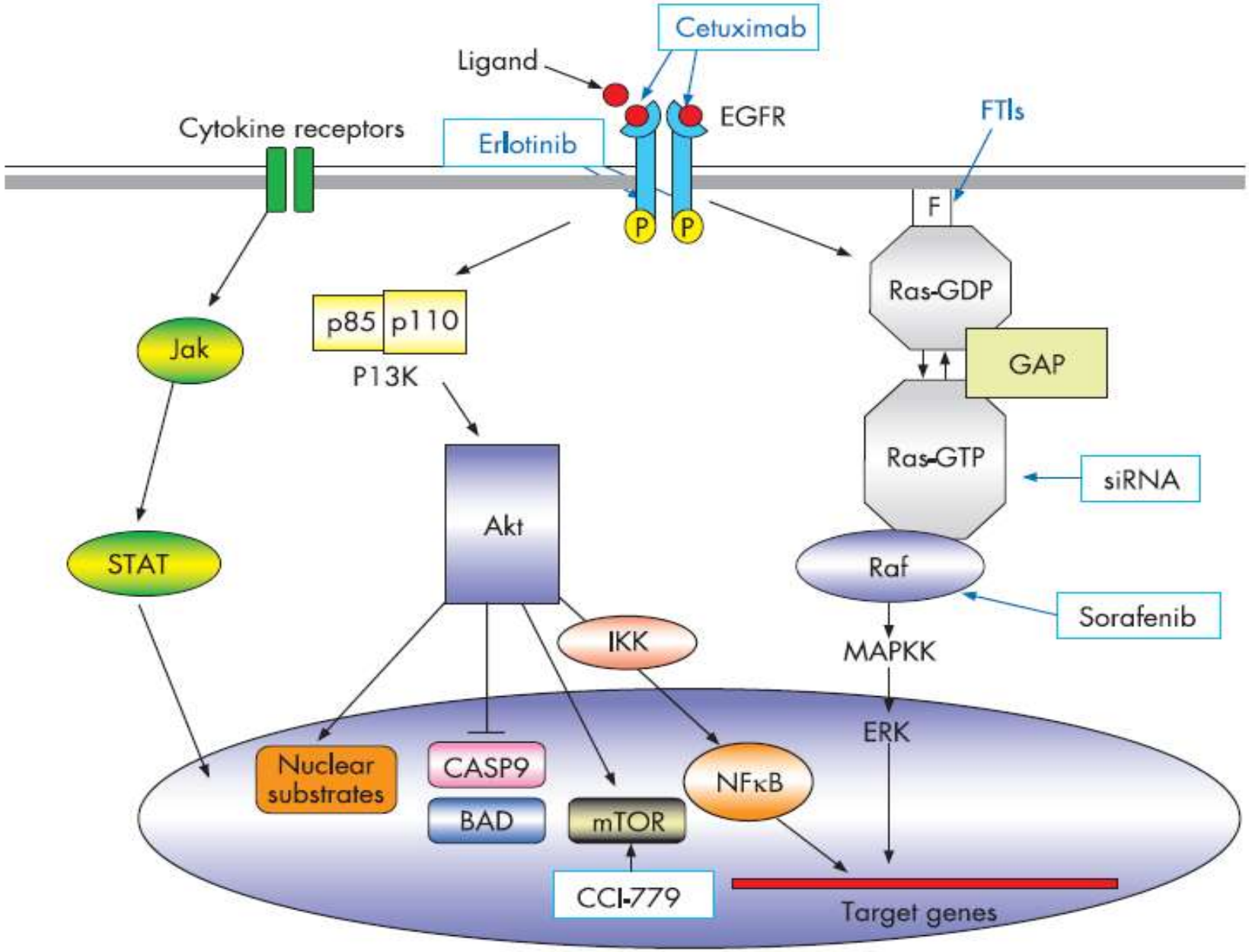


Figure 2 Schematic representation of molecular oncogenic signalling pathways in pancreatic cancer. Agents targeting specific aspects of these pathways are indicated in blue boxes.

Nuovi Potenziali *target:* Oncosoppressori

- Mutazioni della **p53** sono prsenti in oltre **il 50% dei tumori pancreatici**. A differenza del cancro del colon mutazione non presente nelle fasi preneoplastiche ma nei tumori avanzati.

- **SMAD4** media la trasmissione del segnale di TGFB e delle citochine. Nel **90% dei cancri pancreatici** perdita di eterozigosi a carico della regione codificante SMAD4, perdita delle sue funzioni oncosoppressive mediate da TGFB

- **p16INK4A**, lega le CDK4/CDK6, prevenendo la formazione del complesso mitogenico ciclina D-CDK4/CDK6 e inibendo quindi la fosorilazione di Rb e l'ingresso della cellula in fase S. **Nel cancro pancreatico** è presente una perdita della funzione oncosoppressiva di P16ink4a , per inattivazione del gene INK4A, fino al **95% dei casi**

Nuovi Potenziali *target*: embriogenesi

- *Il* ***pathway*** **Notch**, una famiglia di recettori di superfice cellulare, attivo nella fase embriogenica, ha un importante ruolo nei prcessi di apoptosi, differenziazione e sviluppo pancreatico e nell'insorgenza del cancro.

- ***Upregulation*** del Pathway Notch , normalmente inattivo dopo lo sviluppo, è stata osservata nelle lesioni neoplastiche e preneoplastiche pancreatiche. Potenziale target terapeutico.

- **Il segnale Hedgehog**, attraverso I suoi ligandi Sonic, Indian e Desert, è cruciale nello sviluppo del tratto Gastrointestinale, e risulta implicato nelle fasi iniziali dell'insorgenza e della progressione del K pancreas.
- Sono in fase di studio trial su un inibitore del segnale Notch (Cyclopamina)

Microenvironnement

- Il Cancro del Pancreas ha una intensa e peculiare reazione stromale desmoplastica che coinvolge larga parte del tumore. Tale reazione è costituita da matrice extracellulare, fibroblasti, cellule endoteliali ed infiammatorie.

- Il microenvironnement ha un ruolo fondamentale nella resistenza della neoplasia alla terapia.

- Farmaci inibitori della matrice sono potenzialmente degli adiuvanti per altre molecole terapeutiche.

- Inibitori delle Metalloproteinasi di matrice inefficaci da soli e/o con Gemcitabina.

Pancreatic Cancer

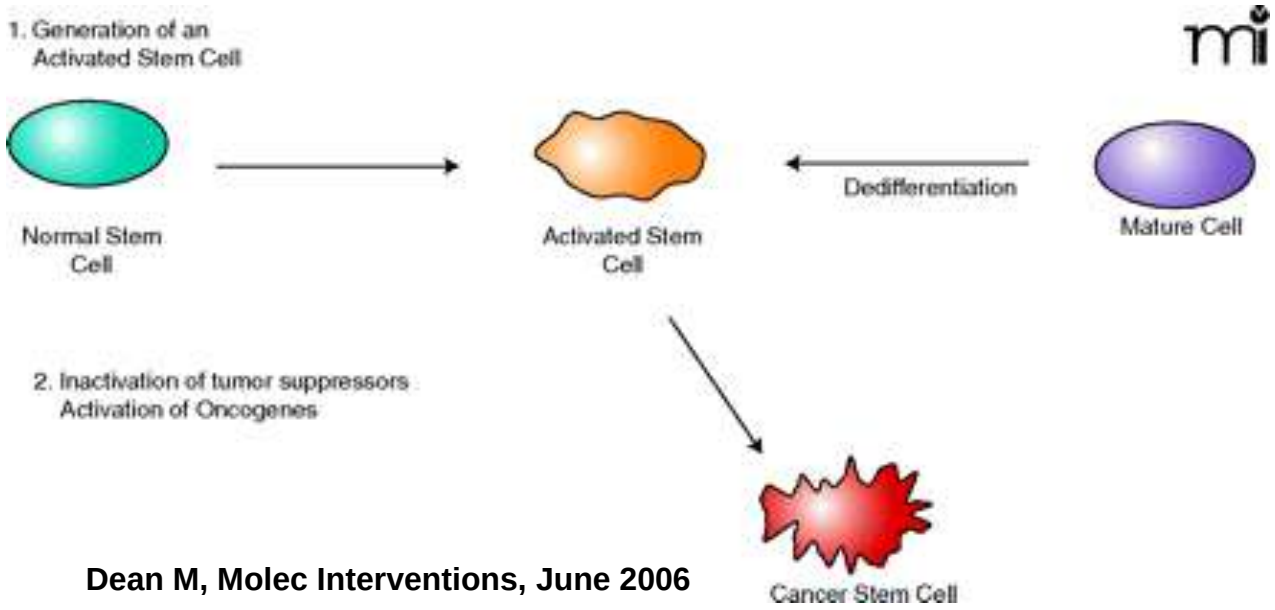
- ✓Epidemiology
- ✓Risk Factors
- ✓Precancerous Lesions
- ✓Diagnosis
- ✓Therapy

Table 3 Phase III trials of new agents in pancreatic cancer[196–201]

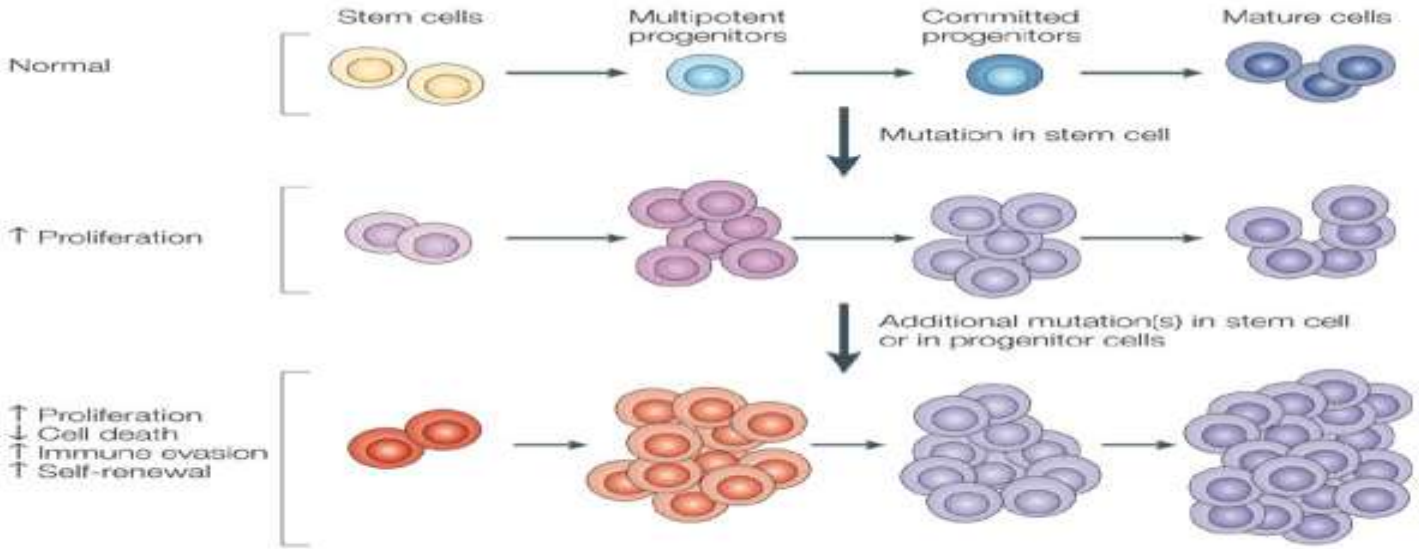
Trial	Patients (n)	Regimen	Comments
PA3 (Canada, USA)[196]	569	Gemcitabine vs	Median survival = 5.91 months; 1 year survival = 17%
		Gemcitabine + erlotinib	Median survival = 6.37 months; 1 year survival = 24% (NS) Erlotinib = EGFR tyrosine kinase inhibitor (oral)
SWOG S0205 (USA)[197]	704	Gemcitabine vs Gemcitabine + cetuximab	Active cetuximab = monoclonal antibody to EGFR
CALGB 80303 (USA)[198]	590	Gemcitabine vs Gemcitabine + bevacizumab (Avastin)	Closed (NS) bevacizumab = anti-VEGFR antibody
Avita (Europe)[199]	600	Gemcitabine + erlotinib vs Gemcitabine + bevacizumab + erlotinib	Closed prematurely
GV1001 (Europe, Australia)[200]	520	Gemcitabine vs GV1001 [+ GMCSF] + gemcitabine	Active GV1001 = peptide vaccine targeting telomerase
TeloVac (UK)[201]	1100	Gemcitabine + capecitabine vs Gemcitabine + capecitabine then GV1001 [+ GMCSF] vs Gemcitabine + capecitabine + GV1001[+ GMCSF]	Active

EGFR, epidermal growth factor receptor; GMCSF, granulocyte monocyte colony-stimulating factor.

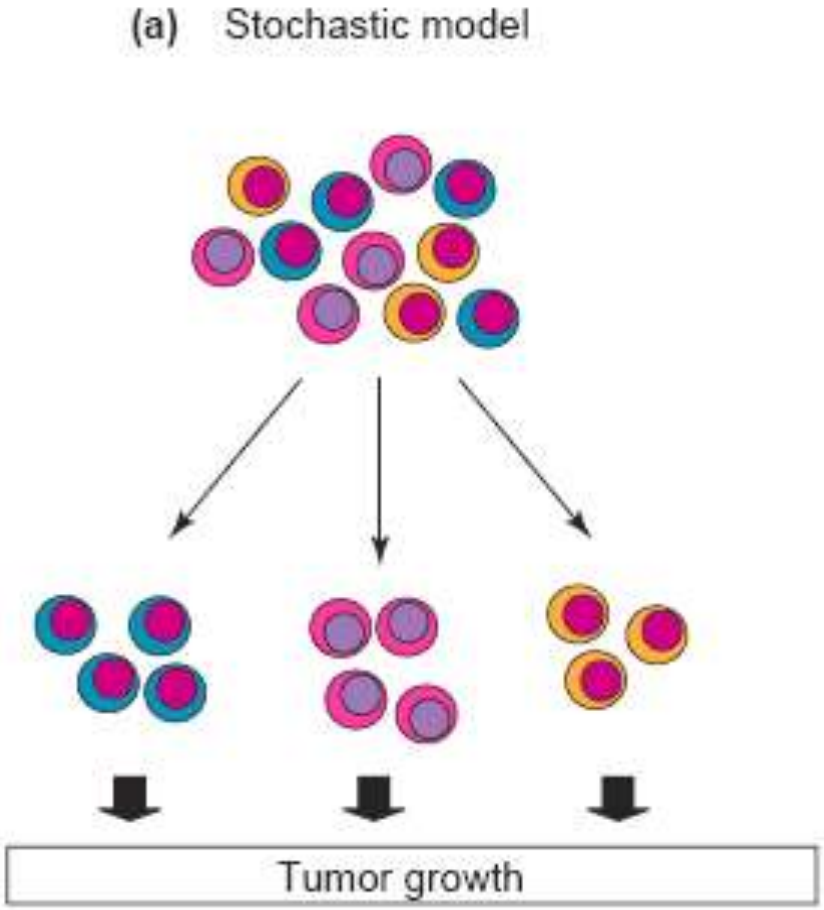
Stem Cell Hypothesis

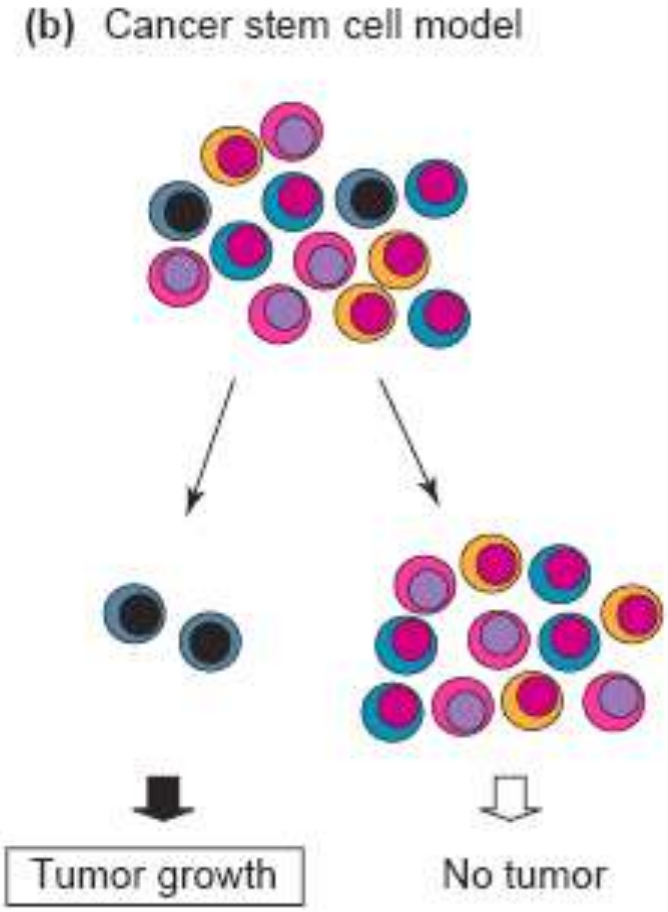


Dean M, Molec Interventions, June 2006



Dean M, et al, Nature Rev Cancer, April 2005



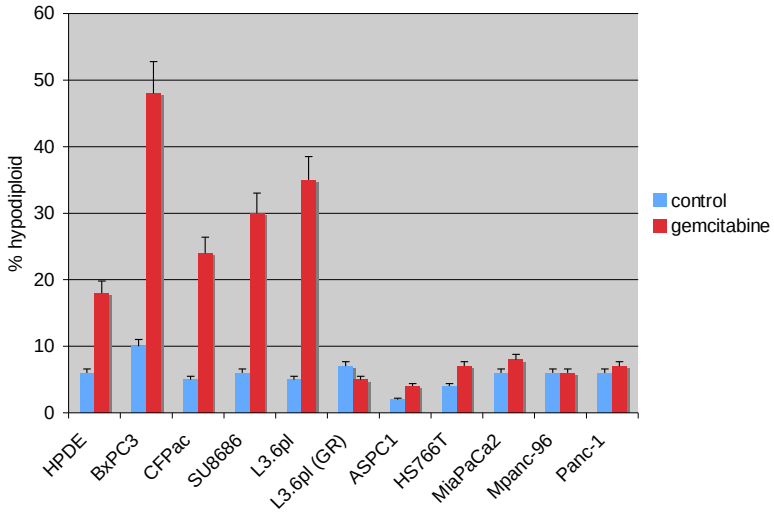


Unregulated growth is due to serial acquisition of genetic events

Unregulated cell growth is due to a disruption in the regulatory mechanism in stem cell renewal

Gemcitabine resistance in a panel of cell lines

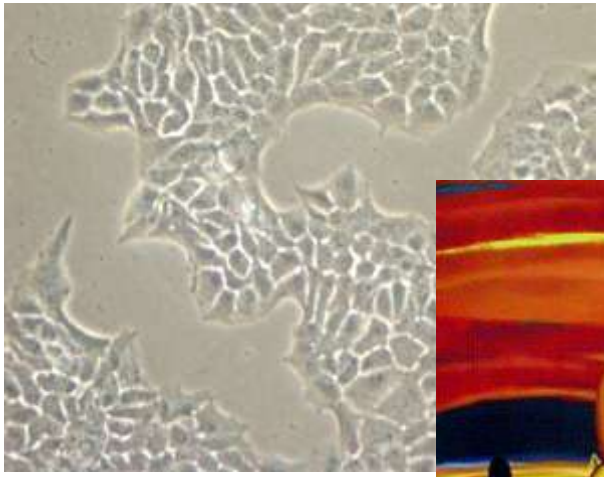
- **Selected a panel of 10 cancer lines and one “normal” line (HPDE)**
- **Included in the panel was a gem-resistant variant of L3.6pl (GR)**
- **Good consensus among 3 labs and reproducible over time**



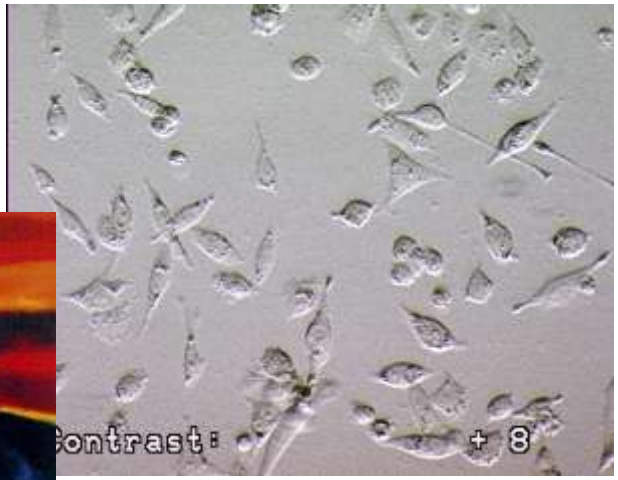
Thiru Arumugam

Wrong therapies select stem cells (gemcitabine resistant pancreatic adenocarcinoma cells)

L3.6pl Parental

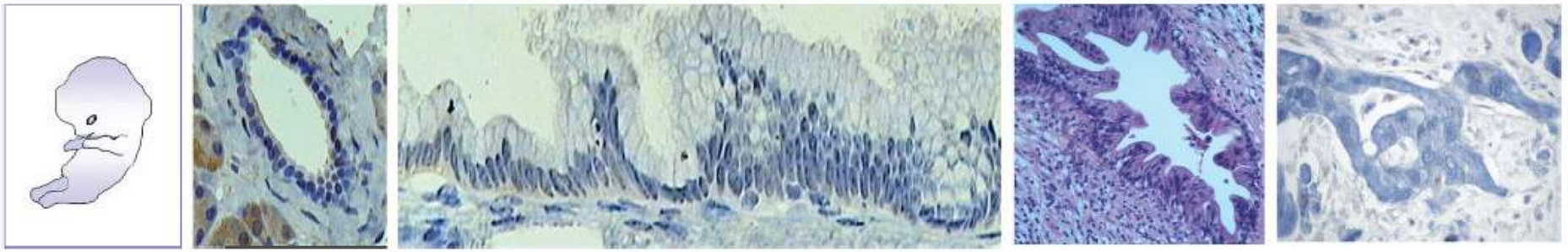


Gemcitabine resistant Cells



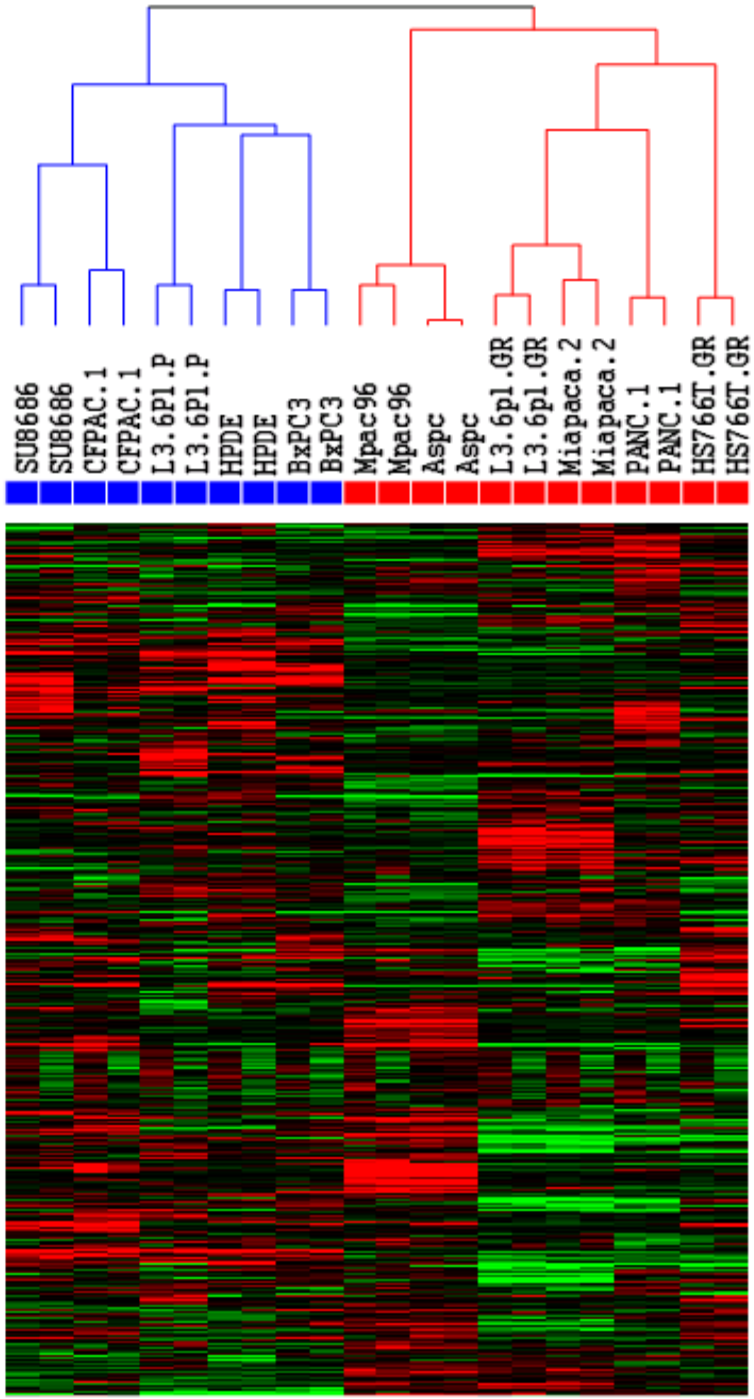


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Figure 1 Histological images of benign pancreatic ductal epithelial cells, progressive PanIN lesions and invasive carcinoma, with associated genetic alterations.



Global gene expression patterns of pancreatic cancer cell lines

(Complete linkage clustering)

Sensitive cell lines

Resistant cell lines

Woonyoung Choi

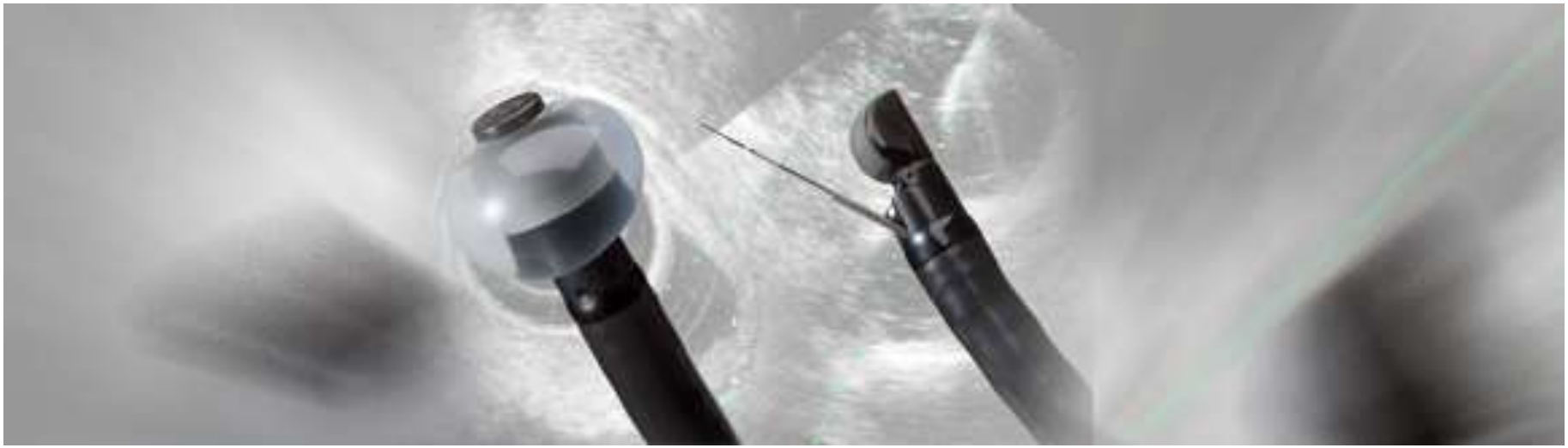
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- ✓Precancerous Lesions
- ✓Diagnosis

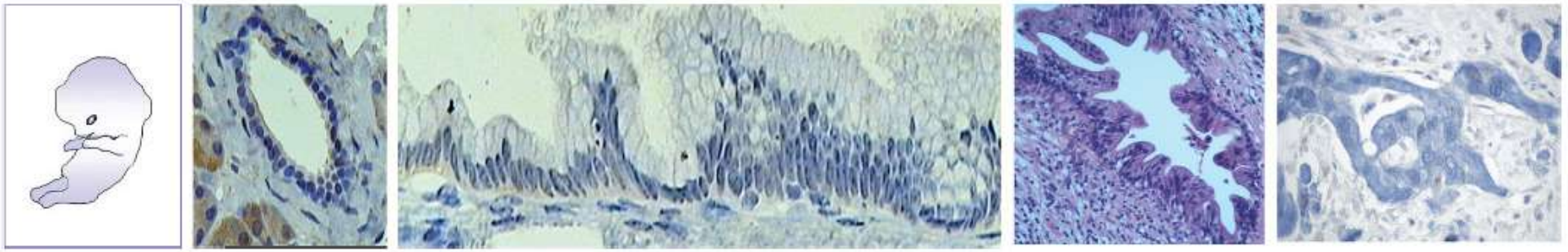
Diagnosis: Imaging

Ultrasound	Average
Endoscopic Ultrasound	**Platinum (?) Standard**
MR +	**Gold Standard**
Colangio MR	
+ secretin	
CT scan	Staging
PET (FDG)	Staging

Eco-Endoscopia

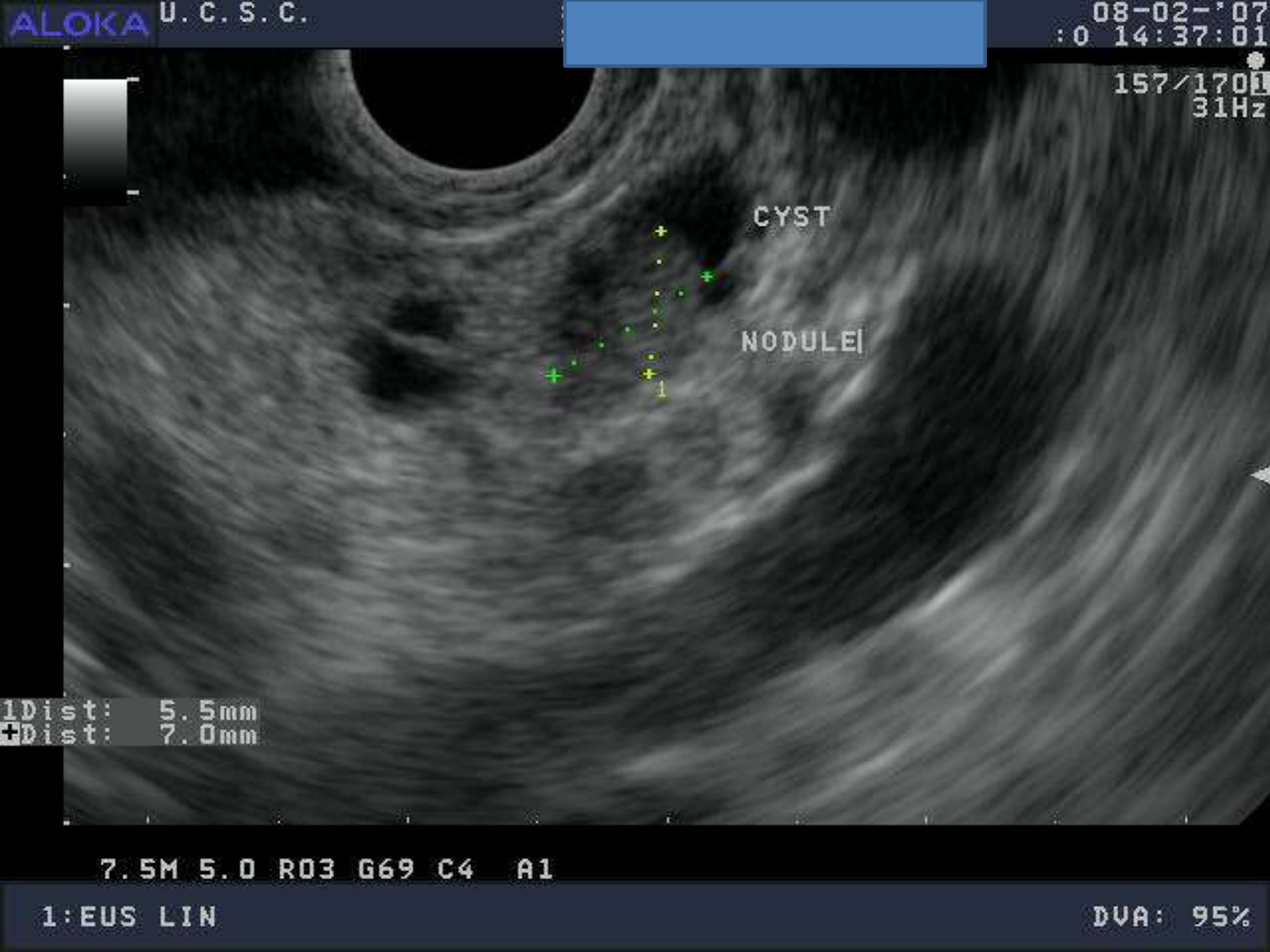


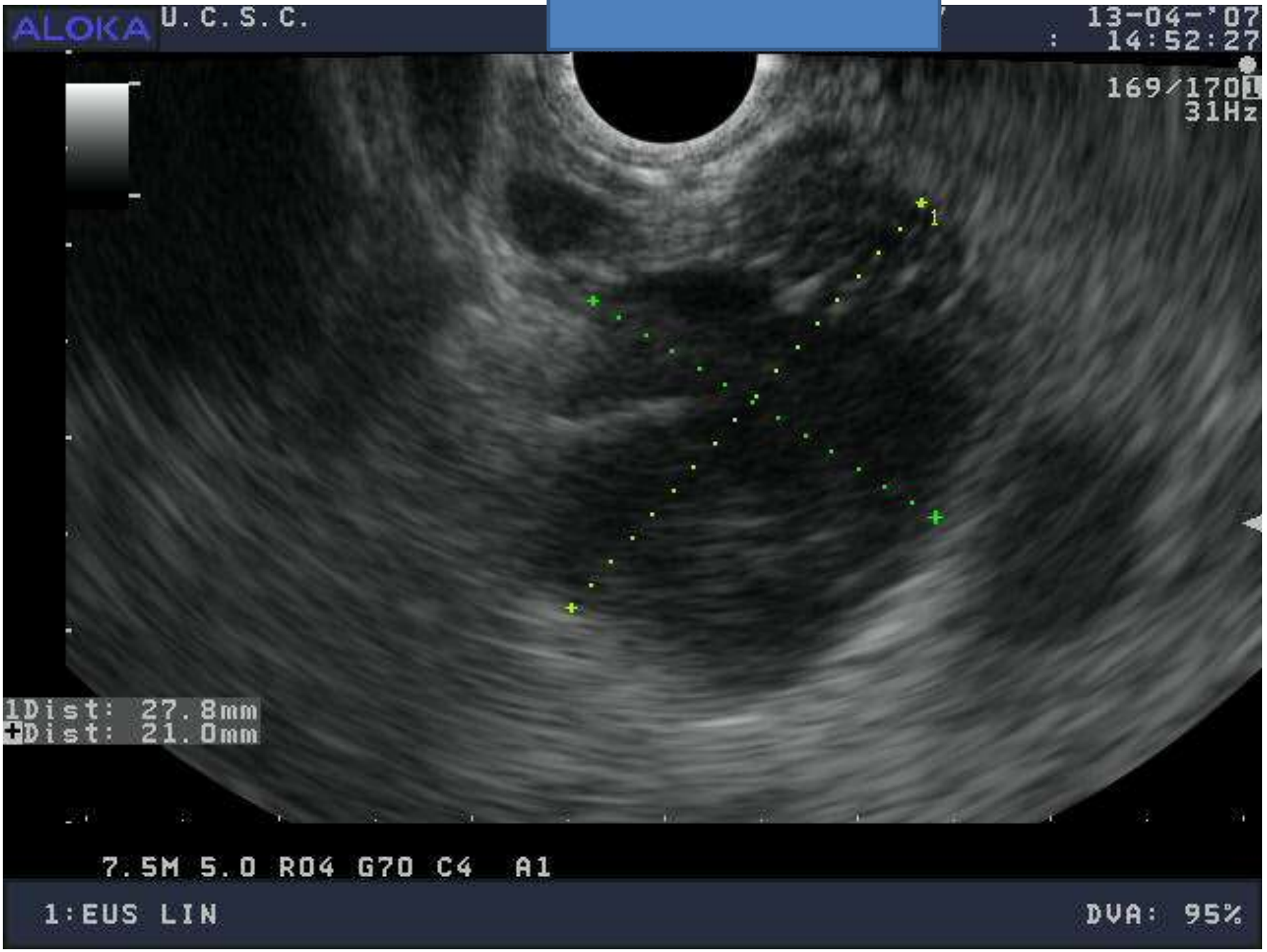
Biologia Molecolare della progessione neoplastica



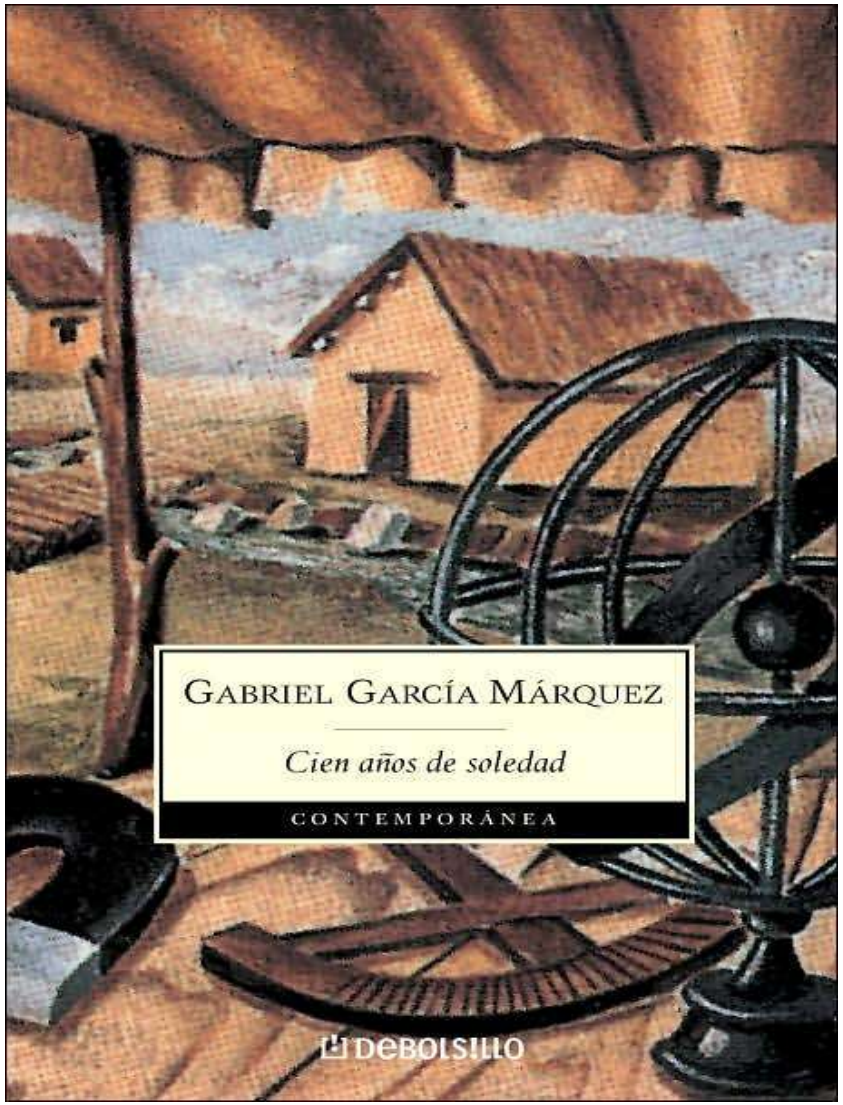
Embyro	Benign ductal cells	PanIN-1	PanIN-2	PanIN-3	Invasive carcinoma
Pdx-1 Sonic hedge-hog (SHH) Serine/threonine kinase 11 (STK11/LKB1)		K-ras Telomere shortening p21 (WAF1/CIP1) Human epidermal growth factor receptor 2 (HER2/neu) Mucin 1 (MUC1) MUC 6 Trefoil factor 1 (TFF 1) p16 (INK4a) S100A11 MUC5AC S100A6	Cyclin D1 Cyclo-oxygenase-2 (COX-2) Hes1 (Hair and enchancer-of split 1) Notch 1 Pepsinogen C Kruppel-like factor 4 (KLF4) HOXA5 GATA5 Gastrin Villin-1 Villin-2 Cellular retinoic acid binding protein 1 (CRABP1)	p53 SMAD4 BRCA2 S100P SHH SialyT (Mucin-associated-carbohydrate antigen) Maspin MUC4 Tumour suppressor in-lung cancer-1 (TSLC-1) Familial adenamatous-polyposis (FAP)	

Figure 1 Histological images of benign pancreatic ductal epithelial cells, progressive PanIN lesions and invasive carcinoma, with associated genetic alterations.


ALOKA
U.C.S.C.
08-02-'07
:0 14:37:01
157/170
31Hz
CYST
NODULE
1
1Dist: 5.5mm
+Dist: 7.0mm
7.5M 5.0 R03 G69 C4 A1
1:EUS LIN
DVA: 95%


ALOKA
U.C.S.C.
13-04-'07
14:52:27
169/170
31Hz
1
1Dist: 27.8mm
+Dist: 21.0mm
7.5M 5.0 R04 G70 C4 A1
1:EUS LIN
DVA: 95%

Tumori Neuro-Endocrini

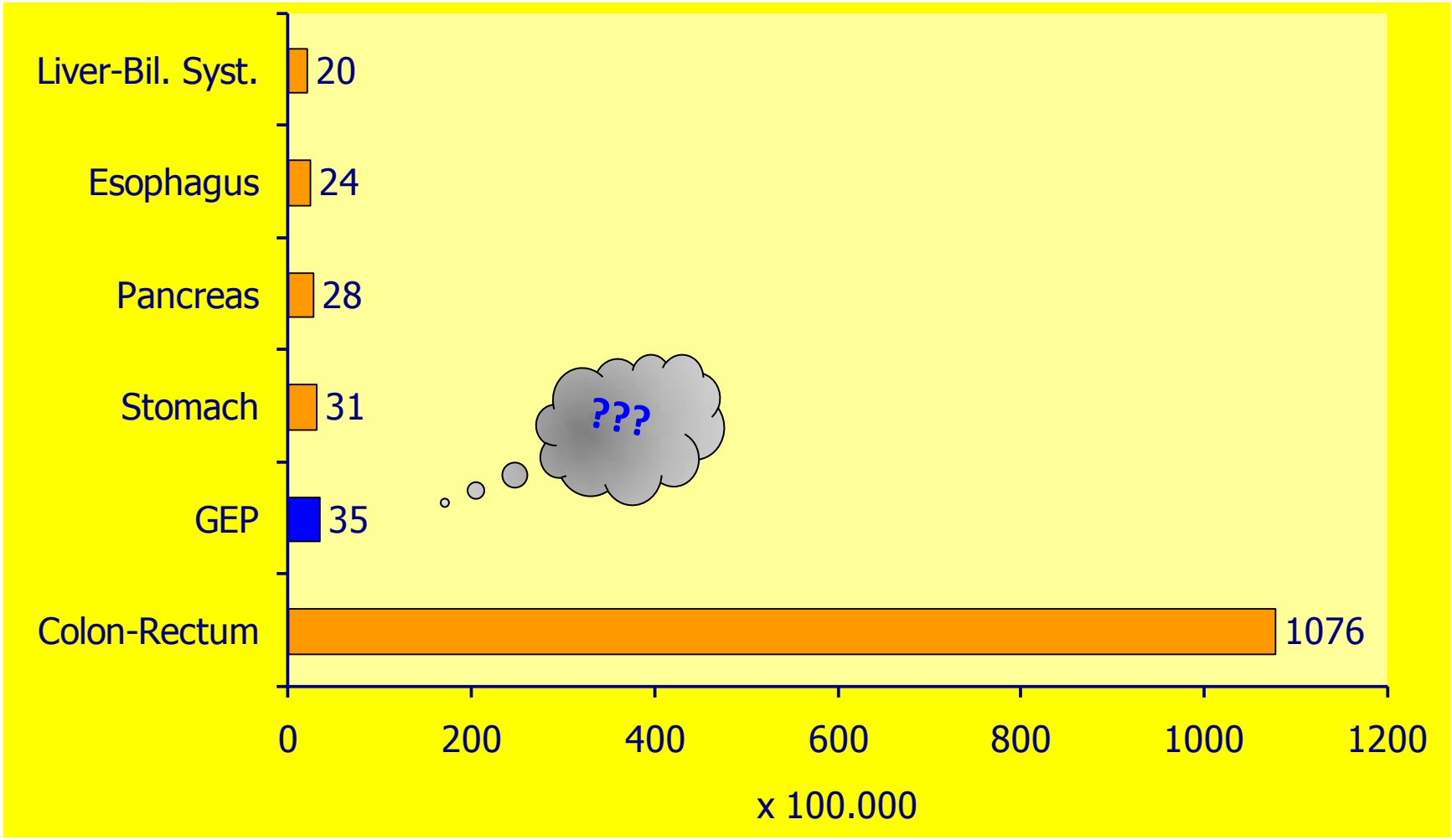


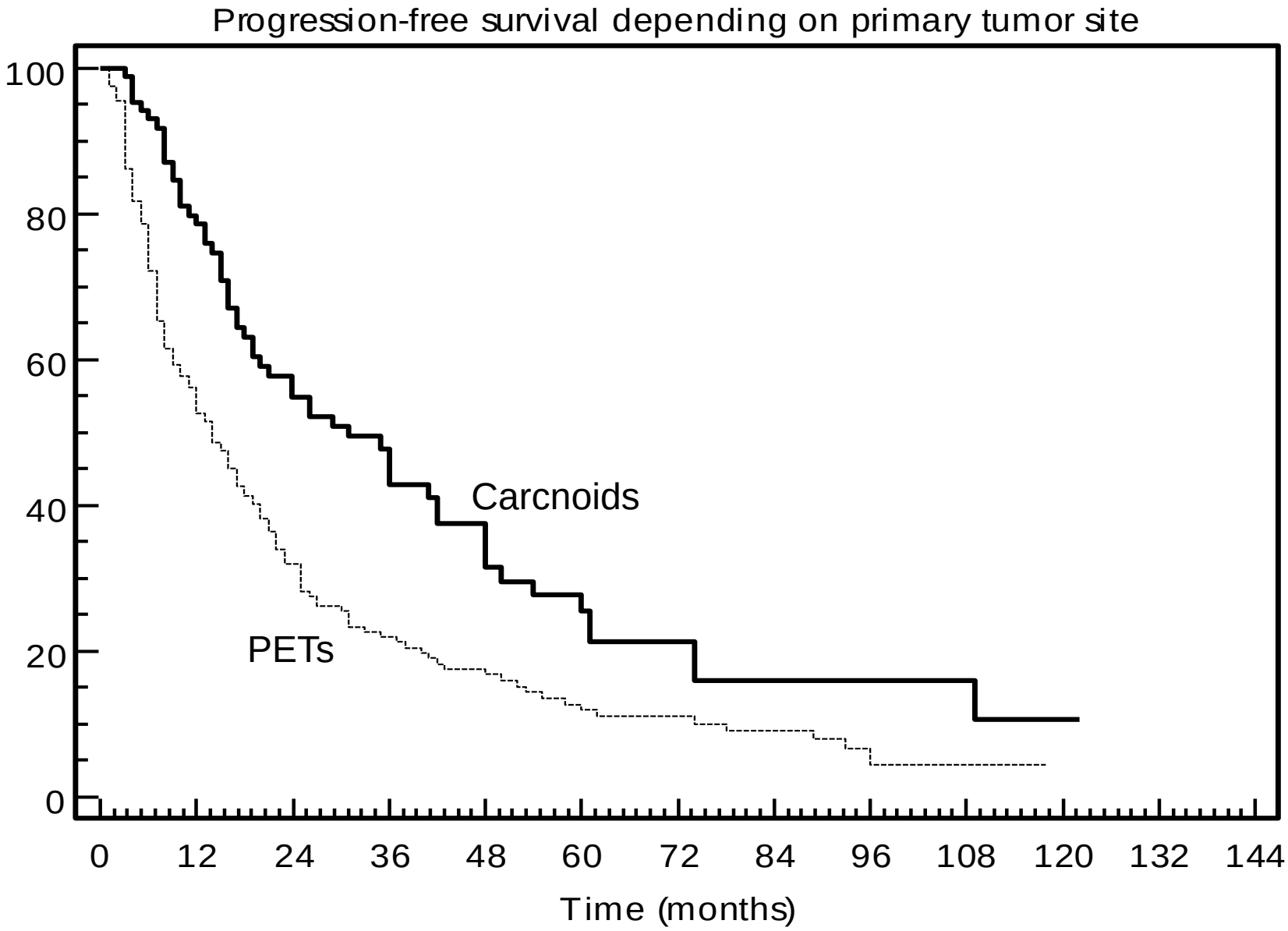
NET Tumors: 2011

“!” or/and “?”

Digestive Tumors: Prevalence (M+F)

SEER: 2004 (modified from: *JC Yao, 2008, JCO*)

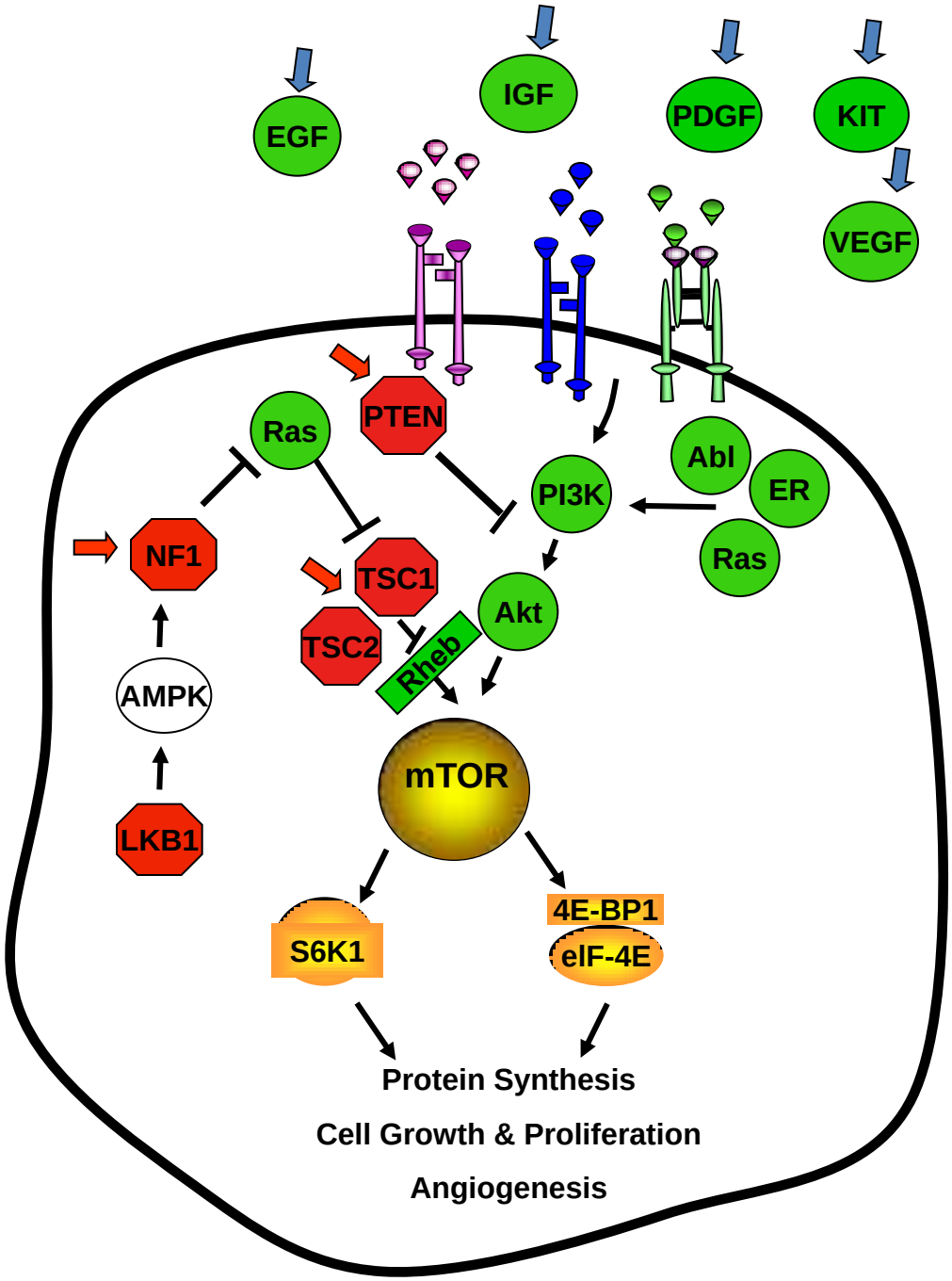



Progression-free survival depending on primary tumor site
100
80
60
40
20
0
Carcnoids
PETs
0
12
24
36
48
60
72
84
96
108
120
132
144
Time (months)

Problem

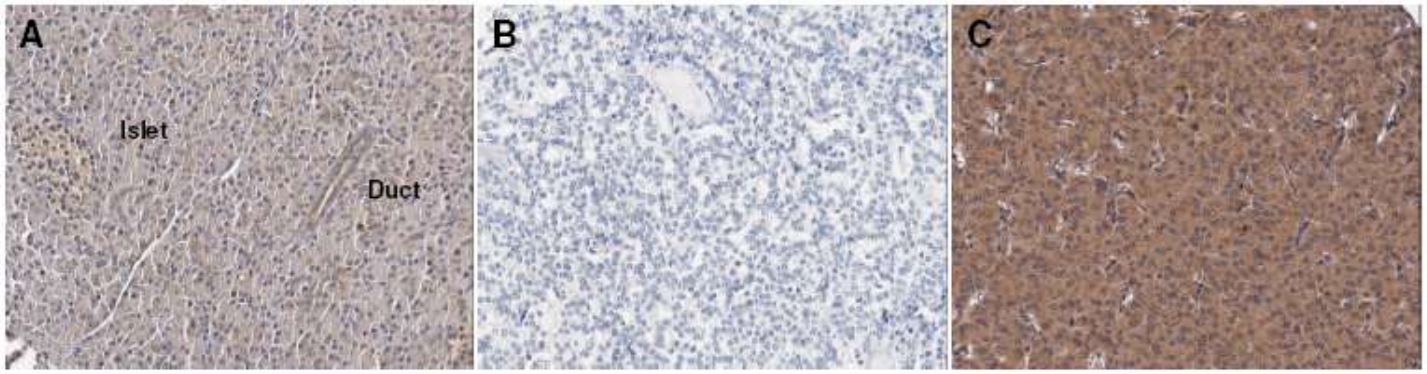
- Incidence (W+M)= 2.43 x 100.000

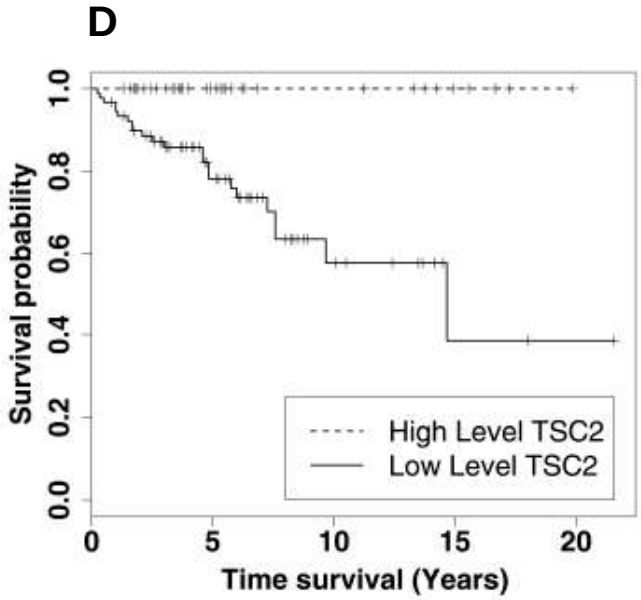
- In Italy:
  - 60.000.000 inhabitants
  - 1458 new cases x one year **“!?!?!?”**

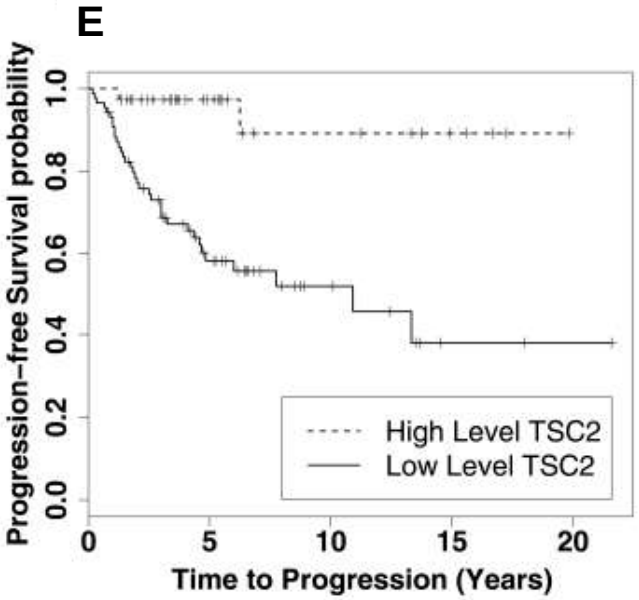


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TSC 2 is downregulated in PETs, and its levels are related with disease outcome

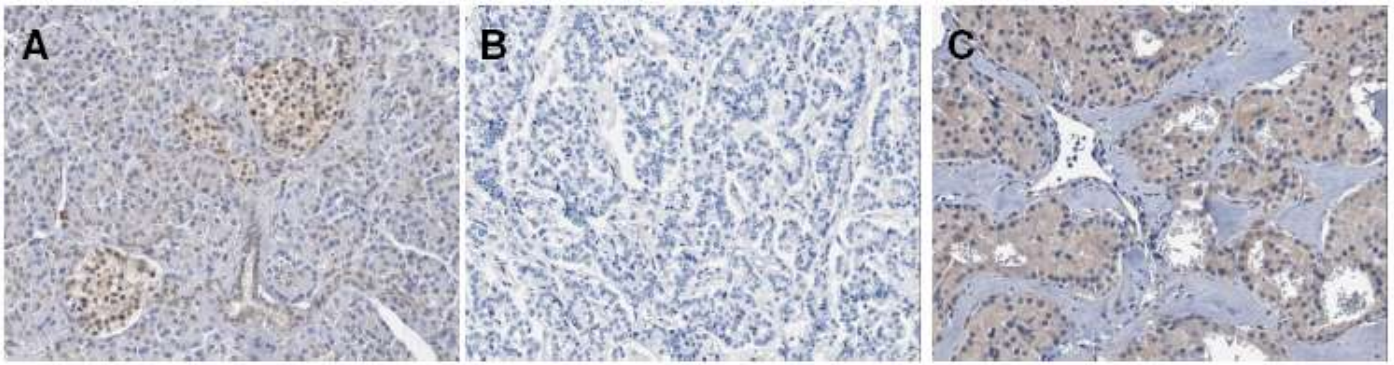






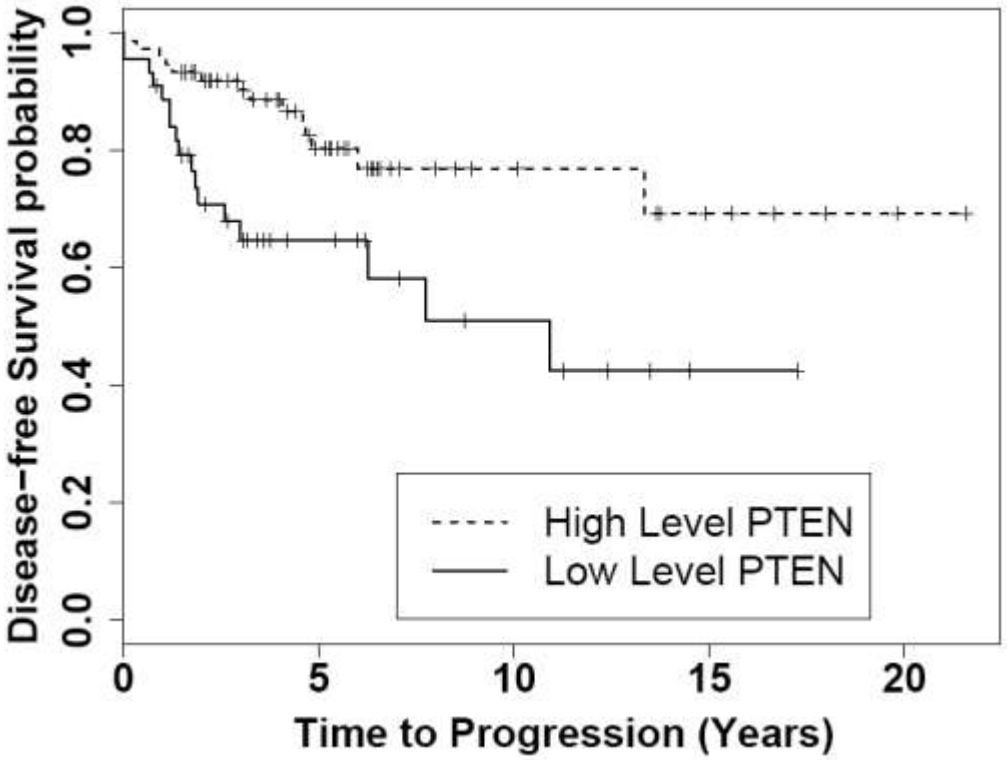
Missiaglia et al. JCO 2010

PTEN is downregulated in PETs, and its levels are related with disease outcome



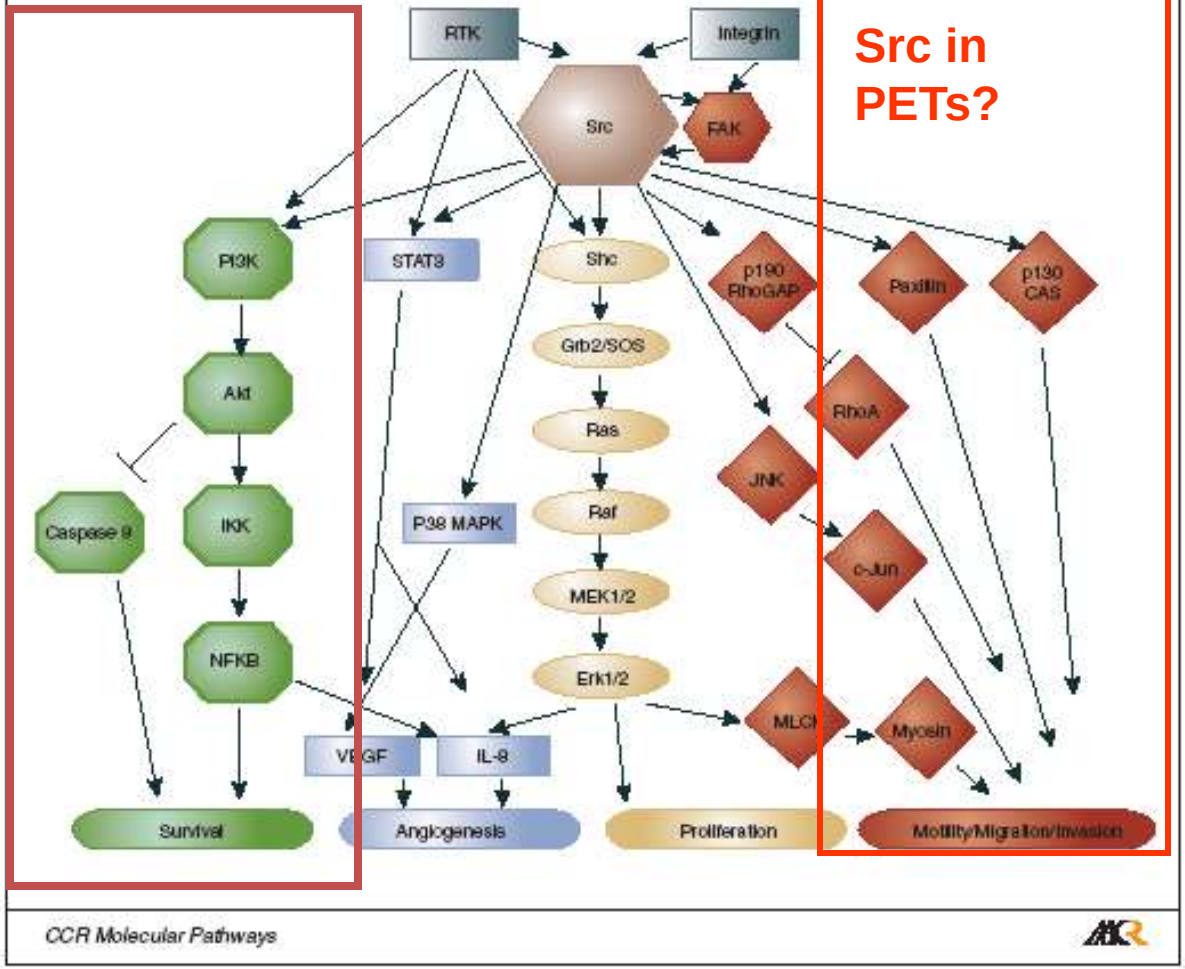
E

D

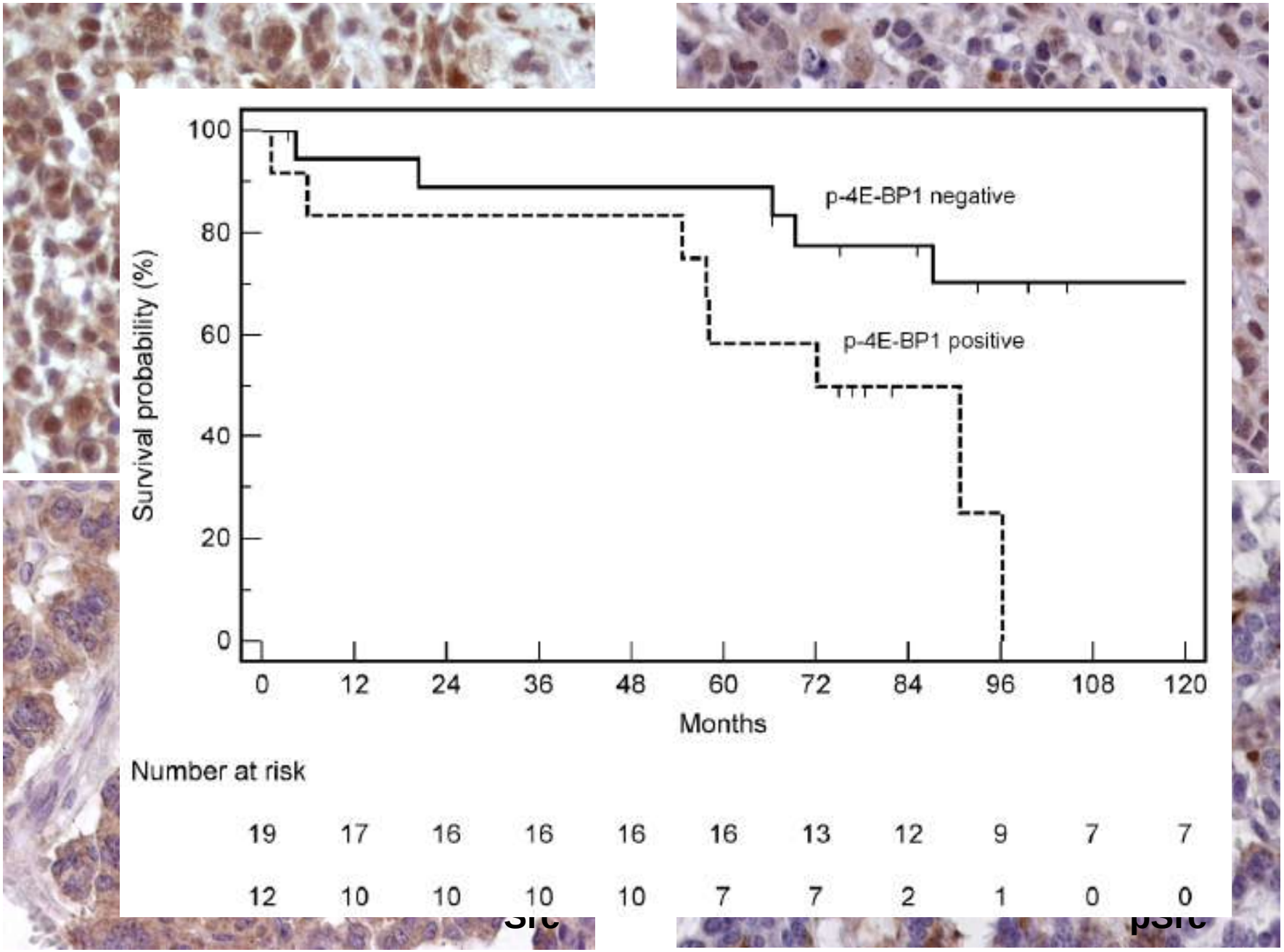


Missiaglia et al. JCO 2010

Src-mediated Signaling Pathways

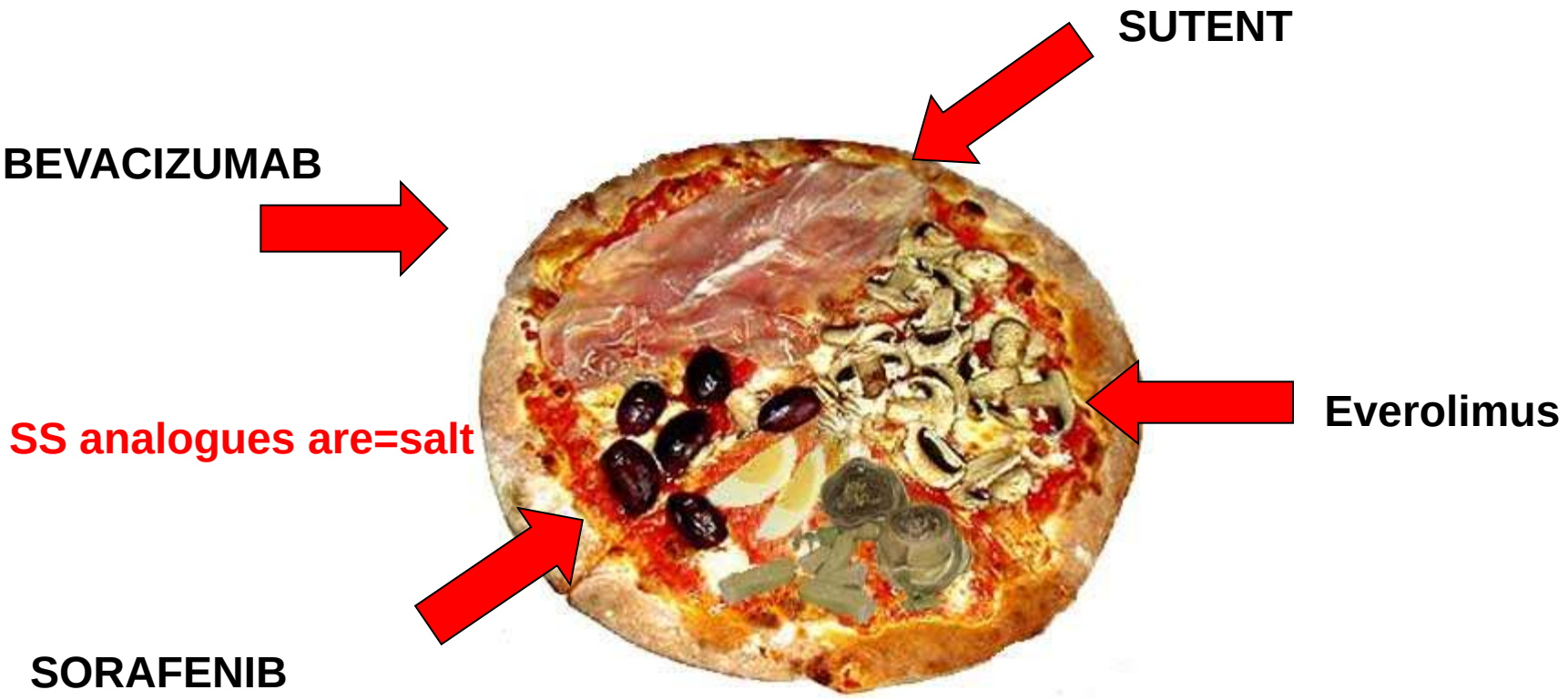


Summy and Gallick, CCR 2006



Di Florio A et al. submitted

A patient is not a pizza!



Enjoy bit of XELODA or ERLOTINIB maybe?

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"Alle migliaia di Ricercatori Precari... che fan Ricerca per pochi Denari"







It.A.NET

Italian Association for Endocrine Tumours

Gianfranco Delle Fave

Dpt. Digestive and Liver Diseases

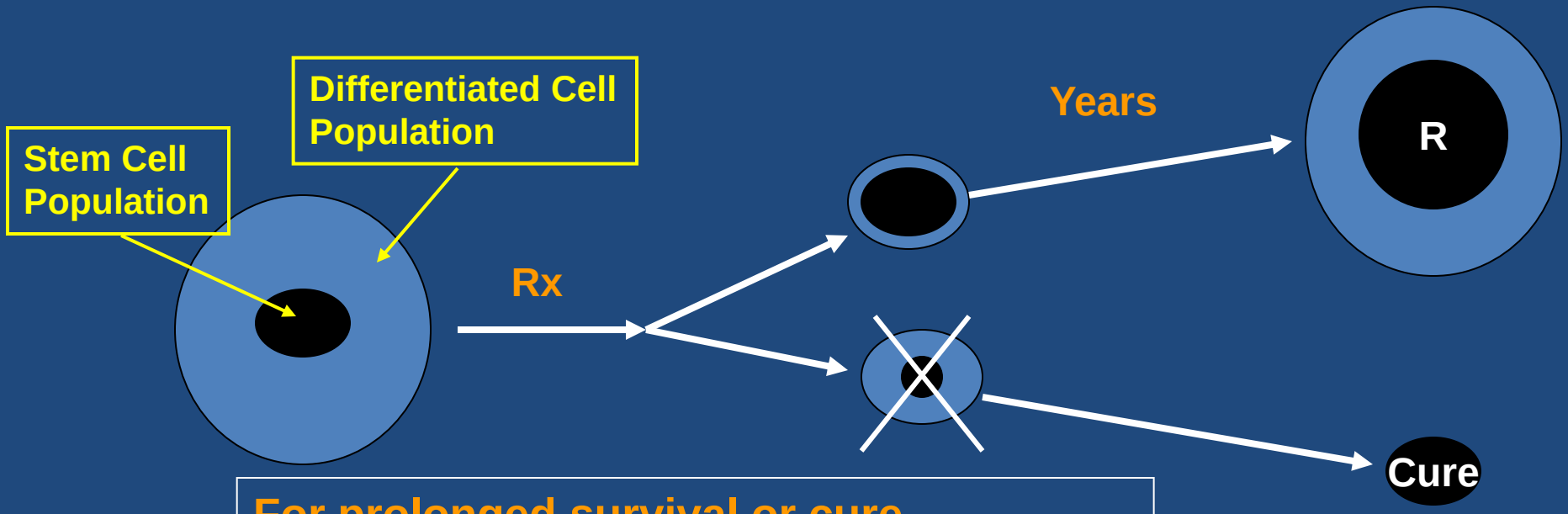
2nd School of Medicine

gianfranco.dellefave@uniroma1.it

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Models of Stem Cell Therapeutics

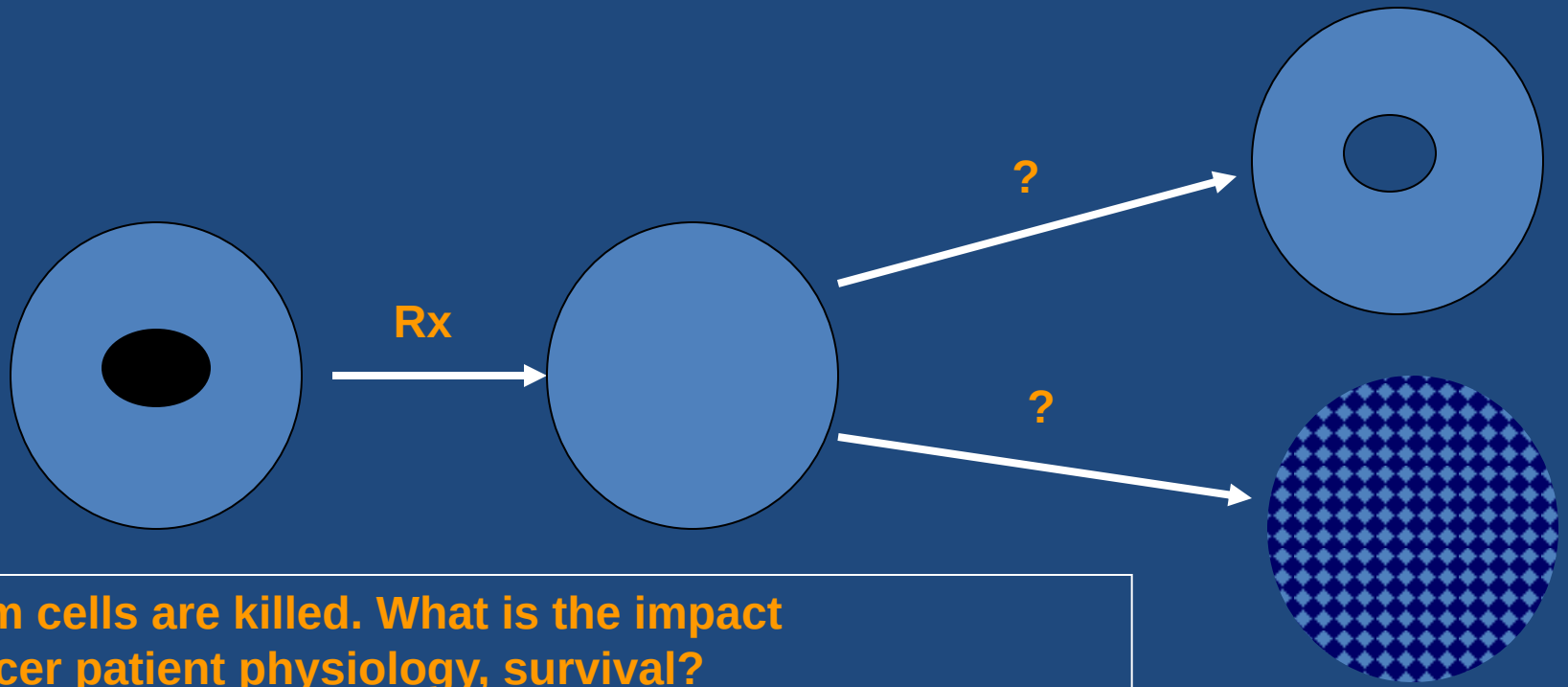
- **Model 1A: Highly effective therapy; only microscopic tumor cells remain; minimal residual disease**



For prolonged survival or cure the vast majority or <u>all</u> stem cells need to be killed.

Models of Stem Cell Therapeutics

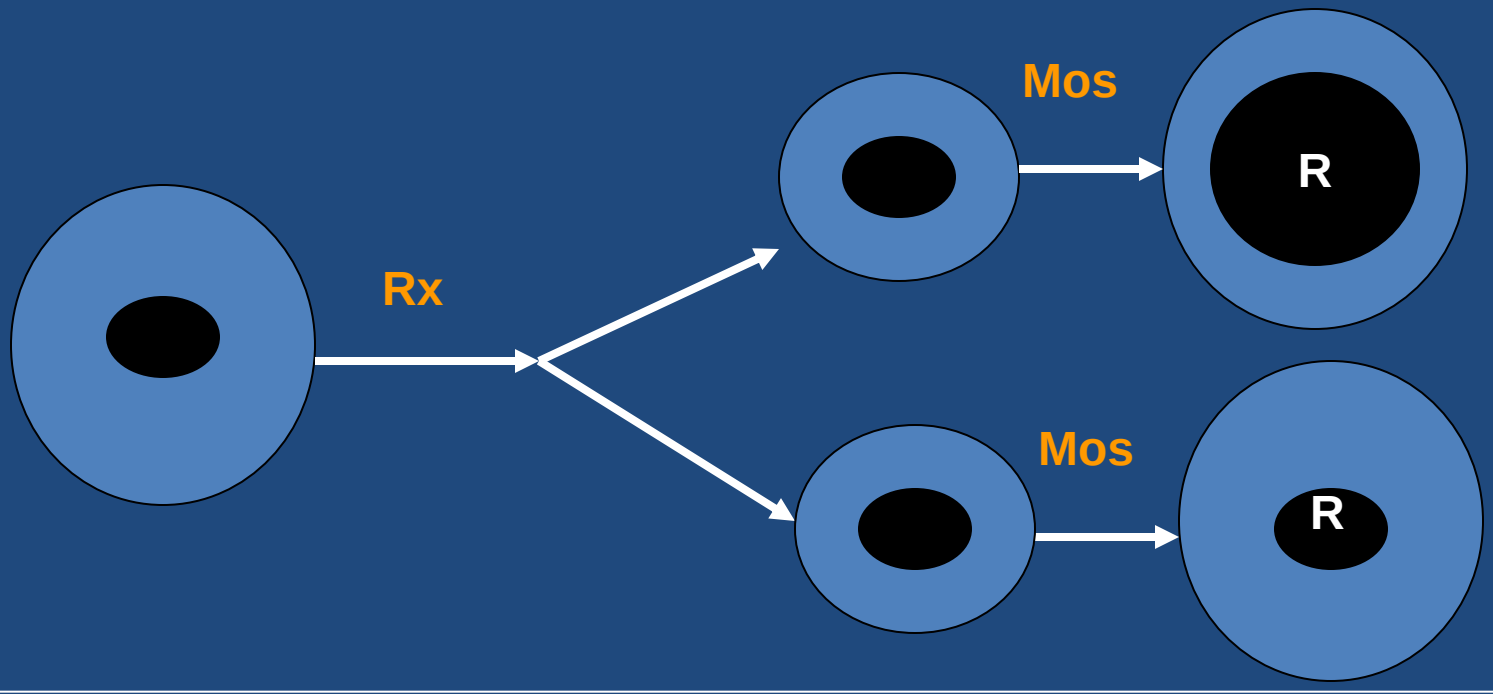
- **Model 1B: Highly effective therapy specifically targeting stem cells; no effect on non-stem cells**



All stem cells are killed. What is the impact on cancer patient physiology, survival?

Models of Stem Cell Therapeutics

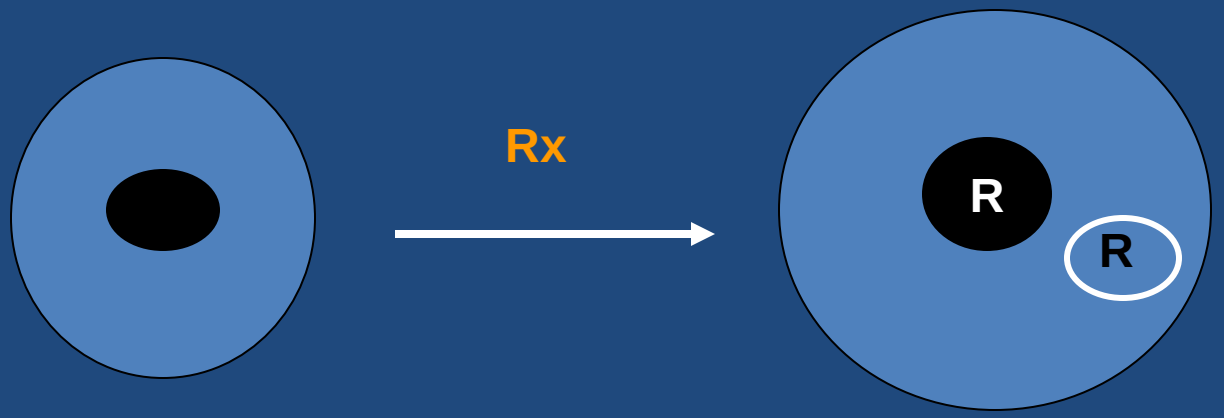
- **Model 2: Partially effective therapy; partial response**



Not necessary to postulate that a minor population of stem cells are responsible for treatment failure; Stem & differentiated cells could reconstitute the original tumor mass.

Models of Stem Cell Therapeutics

- **Model 3: Totally ineffective therapy; no response or progressive disease**

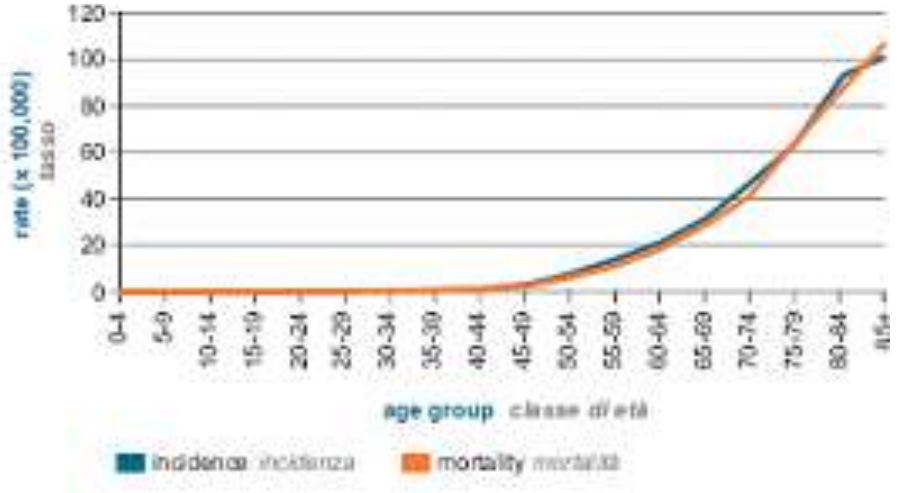


Both stem cells and non-stem cells resistant to treatment. Failure of pre-clinical studies to identify agents with activity against tumor stem cells & differentiated cells.

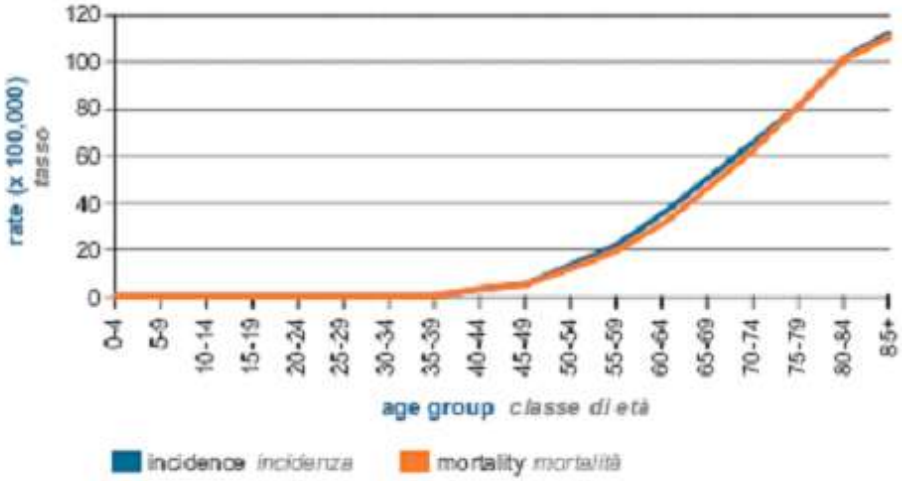
Incidenza

- **L' Adenocarcinoma Pancreatico Duttale (PDAC) è la neoplasia più frequente a carico del pancreas.**
- **Incidenza in aumento (10-12/100000):** 30000 nuovi casi l'anno in USA, 60000 in Europa (*lifestyle*?).
- Età media 66 aa.

♀ Femmine Females



♂ Maschi Males



Maisonneuve et al. Nat Rev Gastroenterol Hepatol. 2009 Oct 6.