MASTER DI II LIVELLO

BIOTECNOLOGIE DI LABORATORIO E MEDICINA TRASFUSIONALE

COLTURE CELLULARI

Colture primarie

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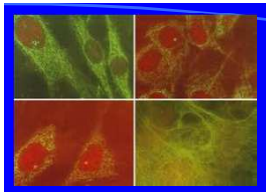
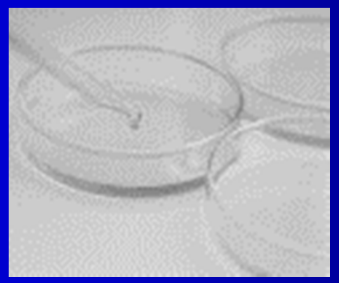


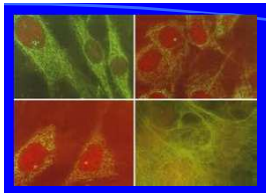
Colture cellulari

DEFINIZIONE

Procedimento complesso mediante il quale le cellule sono coltivate in condizioni controllate, al di fuori del loro ambiente naturale.

organismi multicellulari	organismi unicellulari
cellule animali	virus
piante	batteri
miceti	protozoi





Colture primarie

Isolamento delle cellule

Purificazione da sangue

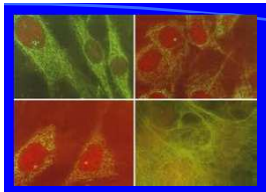
- Leucociti
  - proliferazione in vitro
- ~~Eritrociti~~

Digestione enzimatica di tessuti molli

- Cellule nucleate

Coltura di espianto

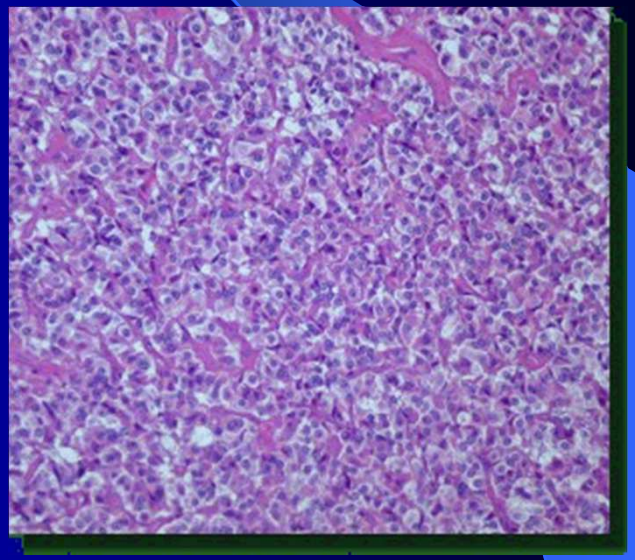
- Frammenti di tessuto in terreno di coltura

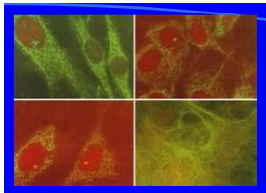


Colture primarie

Colture cellulari

- Colture primarie
- Linee stabilizzate
- Linee trasformate
- Linee ingegnerizzate





Colture primarie

Colture primarie

Colture cellulari derivanti da un singolo individuo

➔ durata limitata

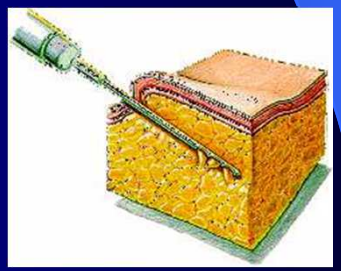
➔ sviluppo di senescenza – interruzione della proliferazione

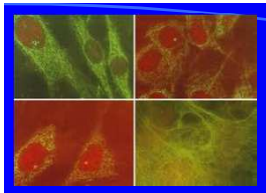
Linee stabilizzate

Le linee cellulari immortalizzate hanno acquisito la capacità di proliferare in modo indefinito grazie a mutazioni casuali o modifiche apposite

Colture primarie

- Organo e/o tessuto disgregato meccanicamente o enzimaticamente in cellule singole o clumps
- Coltura in sospensione e/o su substrato solido
- Capacita' proliferativa limitata

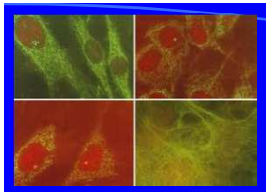




Colture primarie

Perché si coltivano?

- Per espandere i cloni cellulari da indagare
- Per investigare le caratteristiche biochimiche e fisiologiche delle cellule (endocrine)
- Per capire le basi molecolari e cellulari delle patologie d'organo
- Per valutare l'efficacia di agenti terapeutici
- Per testare e sviluppare nuovi materiali



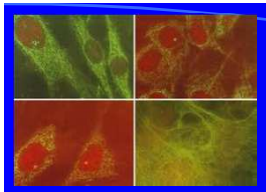
Colture primarie

Cosa serve?

Incubatore per colture cellulari

Temperatura = 37°C

Atmosfera controllata = 5% CO_2 e 95% aria per le cellule di mammifero



Colture primarie

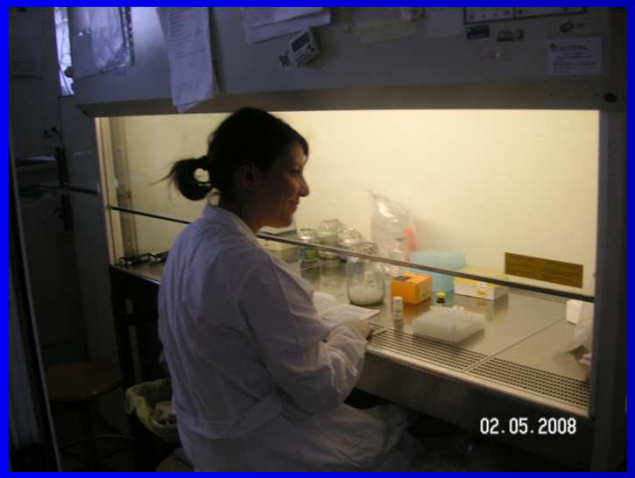
Cosa serve?

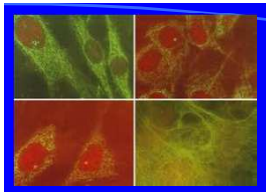
Cappa a flusso laminare "biohazard"

flusso continuo

garanzia di sterilità all'interno della cappa

sterilizzazione mediante UV



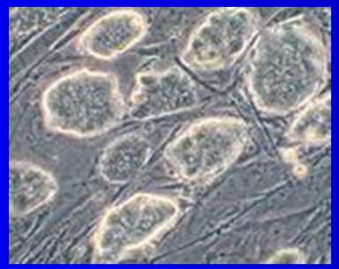


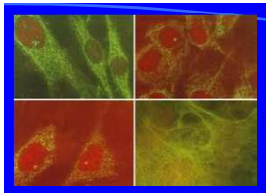
Colture primarie

Cosa serve?

Microscopio ottico

visione invertita
contrasto di fase
possibilità di fotografare





Colture primarie

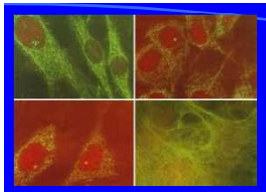
Cosa serve?

Terreno di coltura

A seconda delle caratteristiche del tessuto è possibile preparare (o acquistare) uno specifico terreno di coltura,
Le variabili più importanti sono

pH
concentrazione di glucosio
presenza di fattori di crescita
concentrazione del siero
(solitamente siero fetale bovino)
altri nutrienti (aminoacidi, vitamine)

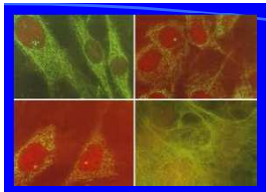
Potenziale contaminazione con virus o prioni



Colture primarie

Come si fa?

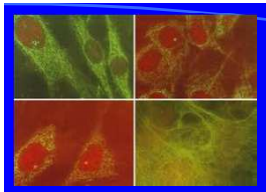
- Prelevare il tessuto fresco in condizioni di sterilità
- Trasferire il tessuto fresco in una petri
- Lavare con PBS
- Dissezionare eliminando grasso e tessuto necrotico



Colture primarie

Come si fa?

- Lavare ripetutamente
- Rimuovere il terreno in eccesso
  e ricoprire con terreno di coltura

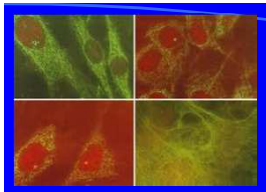


Colture primarie

Come si fa?

- Trasferire i pezzi in una fiasca da 25 cm^2

- Aggiungere <u>enzimi</u> e porre in orbital incubator a 37° per 1-2 ore



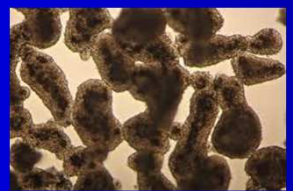
Colture primarie

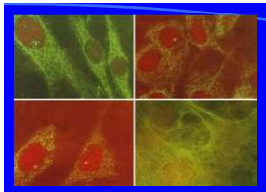
Enzimi

- Tripsina : (idrolasi) massima attività a 37°, induce una maggiore disgregazione ma può danneggiare le cellule. Per minimizzare il danno è possibile usare tripsina a 4°C per permettere all'enzima di penetrare nel tessuto con poca attività

- Collagenasi : (metallo-proteinasi) determina una minore disgregazione ma è meno aggressiva

- Ogni tessuto richiede condizioni diverse



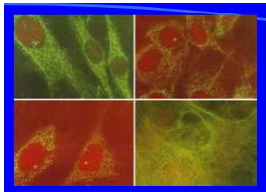


Colture primarie

Come si fa?

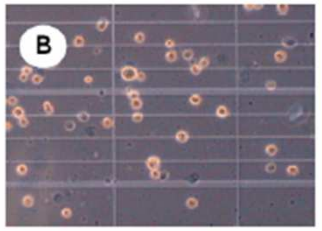
- Passaggi in aghi di diametro sempre minore (18 – 22 g)

Si ottiene una sospensione cellulare mista

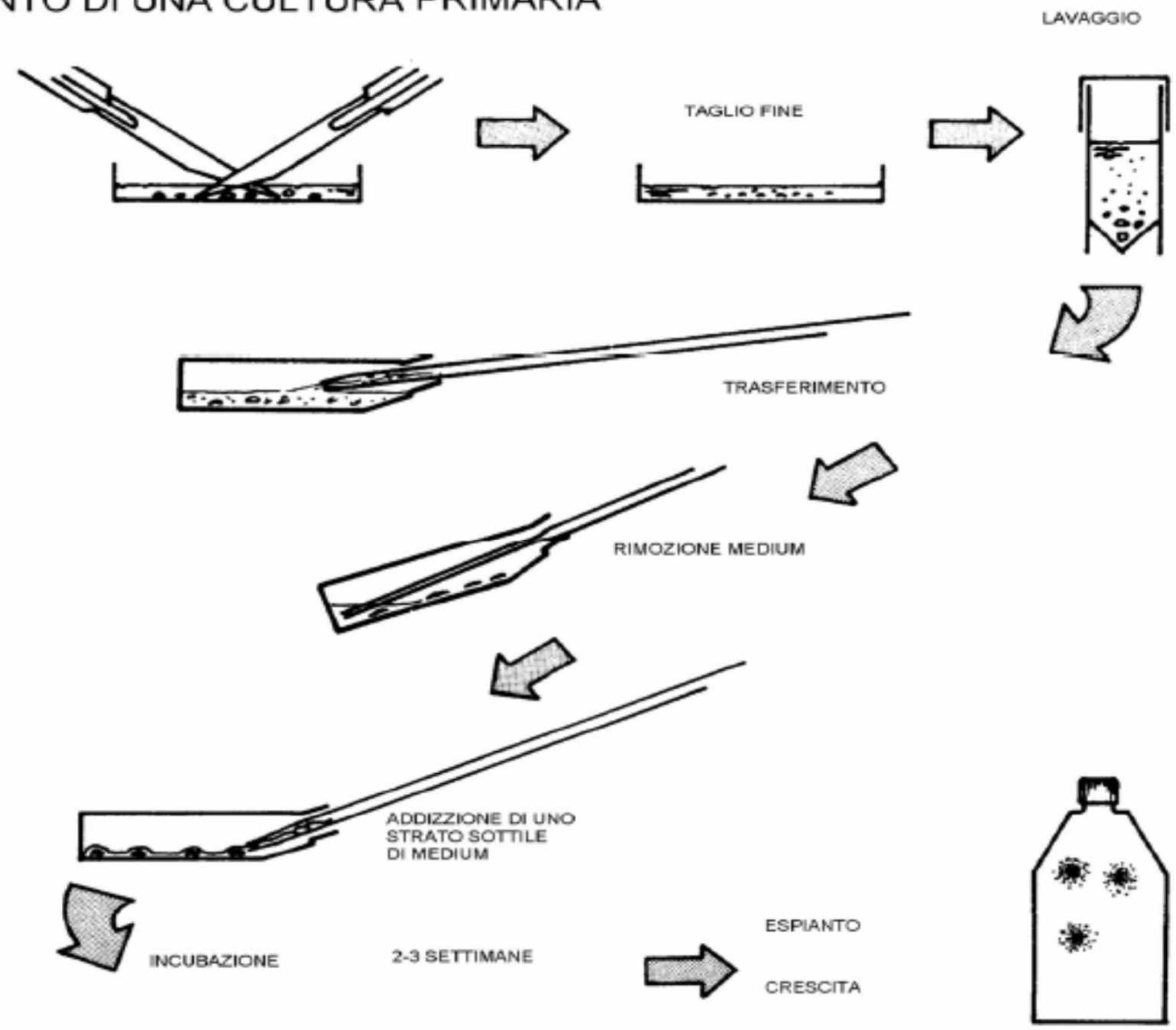


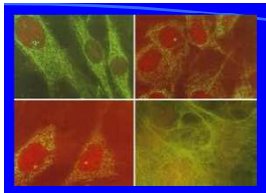
Colture primarie

- Centrifugare ed eliminare il surnatante
- Risospendere il pellet in 1-5 ml di RPMI
- Pelevare 10 ul della sospensione cellulare e contare in camera di Burker



ESPIANTO DI UNA CULTURA PRIMARIA

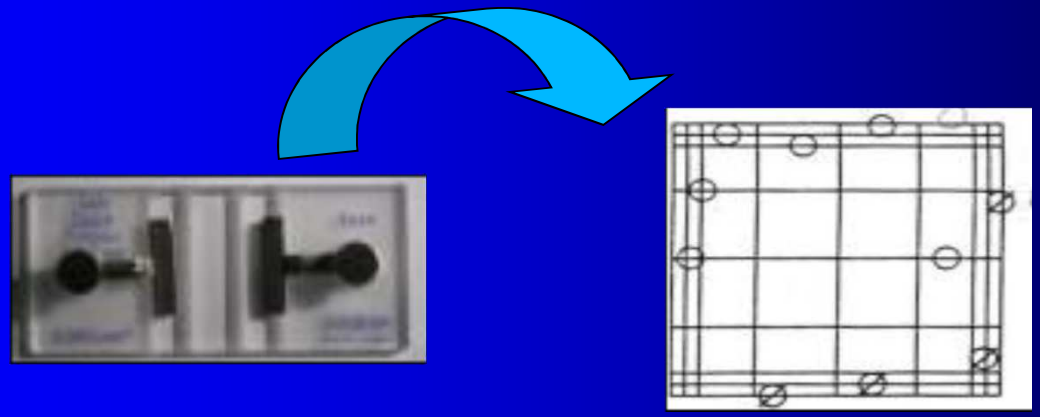


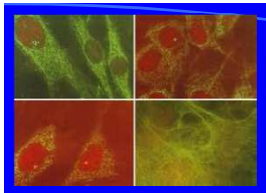


Colture primarie

Camera di burker

- E' caratterizzata da una griglia standardizzata costituita da 9 quadrati.
- La media del numero di cellule che si contano nei 4 quadrati viene moltiplicata per un valore fisso 10^4 (poichè ogni quadrato rappresenta un volume totale di 0,1 mm^3) e si ottiene quindi il numero di cellule in 1 ml di soluzione

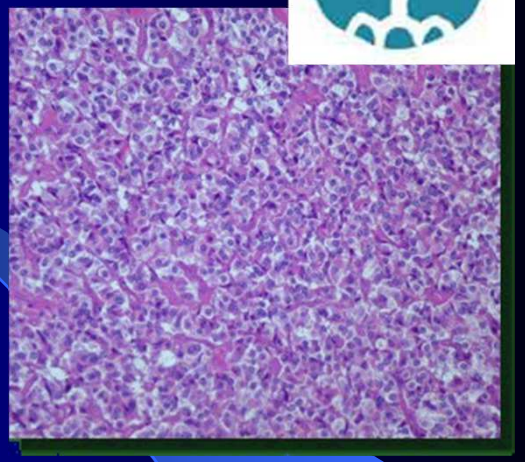


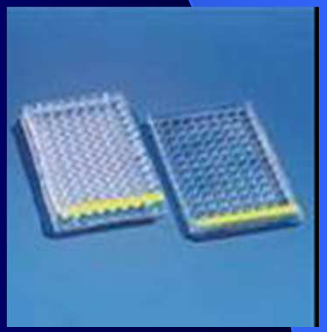


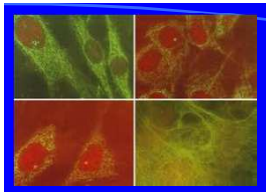
Colture primarie

Come si fa?

- Osservare al microscopio ottico le cellule galleggianti nel mezzo di coltura, in modo da valutare la congruità delle cellule piastrate
- Seminare nelle piastre da 96 well 20.000 cells/wells
- Porre nell'incubatore termostatato a 37°C e 5% CO_2, dopo circa 2-3 ore è possibile osservare le cellule che iniziano ad attaccarsi alla piastra ed acquisire la propria forma







Colture primarie

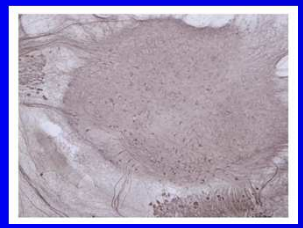
Come si fa?

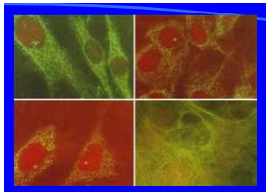
Densità di semina (numero di cellule/ml di terreno di coltura)
➔ Influenza le caratteristiche e la differenziazione cellulare

Coltura in sospensione vs. in adesione

- alta densità
- colture organitipiche
- Superficie di crescita:
  - plastica
  - microcarriers
  - coating

Crescita cellulare in 3-D
Più simile al tessuto in vivo
Tecnicamente molto complessa

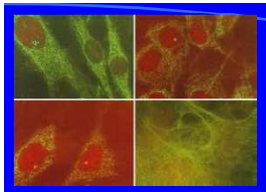




Colture primarie

Come si fa?

Attenzione alla cross-contaminazione!!!



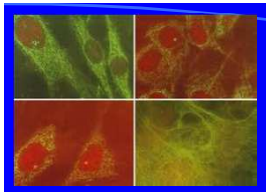
Colture primarie

Come si fa?

sospensione/monostrato

Le cellule continuano a crescere fino a riempire tutto lo spazio disponibile

Deplezione di nutrienti
Accumulo di cellule apoptotiche/mecrotiche
Inibizione da contatto
Induzione della differenziazione

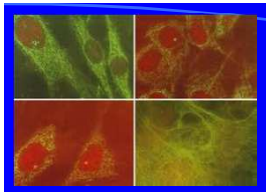


Colture primarie

Come si fa?

sospensione/monostrato

Tutte le operazioni
(cambio del mezzo do cuùoltura, passaggio delle cellule, trasfezioni)
devono essere svolte in
CONDIZIONI DI STERILITA'



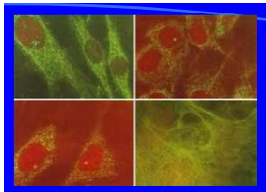
Colture primarie

Come si fa?

- Cappa a flusso laminare

Antibiotici
Antimicotici

Indicatori di variazione del pH



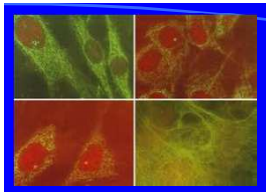
Colture primarie

Sostituzione del mezzo di coltura

Passaggio

Trasferimento di un piccolo numero di cellule in una nuova fiasca

Se fatto regolarmente evita la senescenza



Colture primarie

E ora ?

Semina in piastra

↓

Incubazione

↓

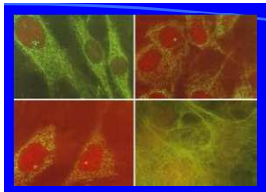
Raccolta del mezzo di coltura

↓

Dosaggi (ormonali)

↘

Saggi di Vitalità Apoptosi Attivazione vie del segnale

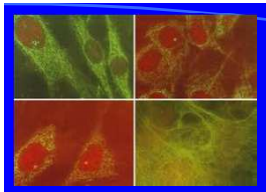


Colture primarie

Lettura allo spettrofotometro/fluorimetro

⬇

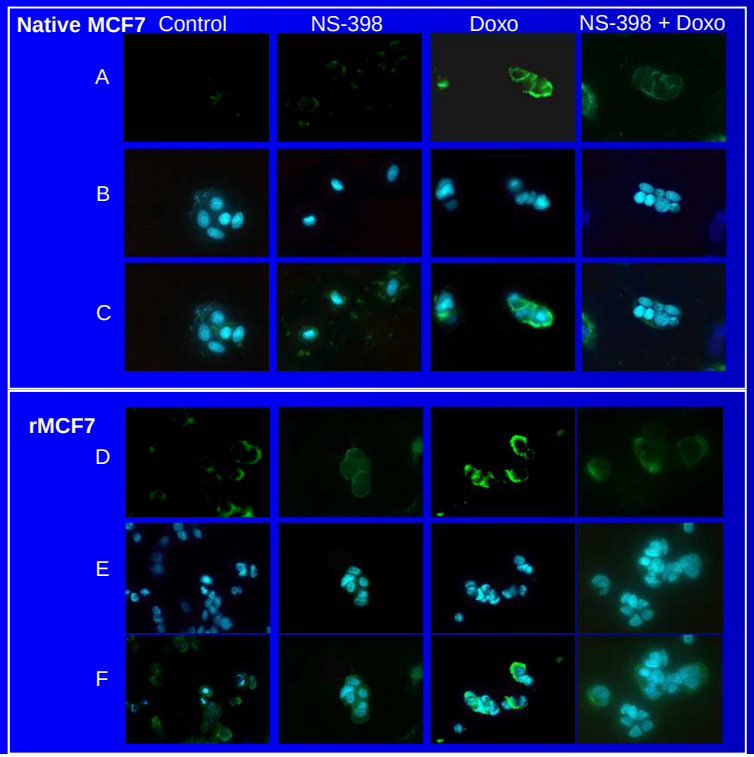
RISULTATI!!

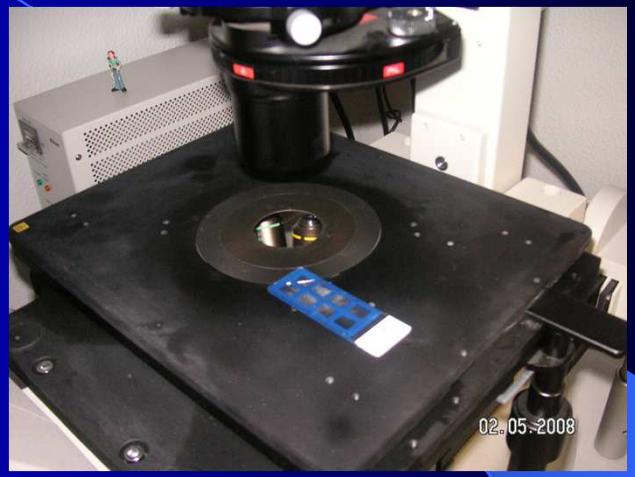


Colture primarie

Fissazione su vetrino

Immunofluorescenza





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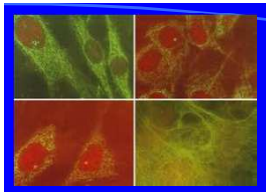


Colture primarie

Applicazioni

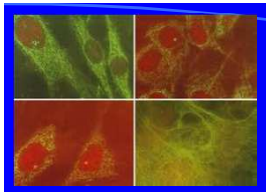
Produzione di vaccini
Produzione biotecnologica con tecnica del DNA ricombinante di

- enzimi
- ormoni
- anticorpi monoclonali
- interlauchine
- linfochine
- agenti antineoplastici

➔Soprattutto per proteine glicosilate e/o dotate di modifiche post-traduzionali che non possono essere riprodotte in ambiente batterico

ERITROPOIETINA

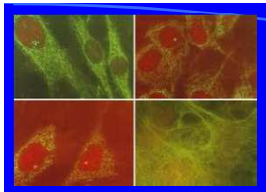
L'alternativa alle colture cellulari di mammifero sono costituite da cellule di insetto, cellule vegetali, cellule embrionali



Colture primarie

New biotechnology approaches

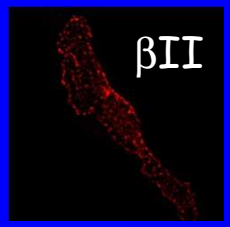
NEW MOLECULAR TARGETS

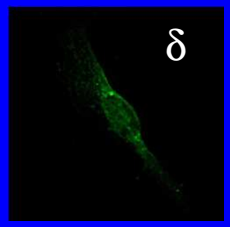


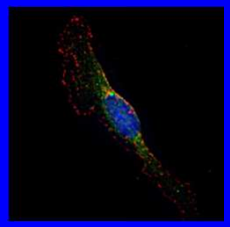
Colture primarie

Pancreatic endocrine tumors

PKC

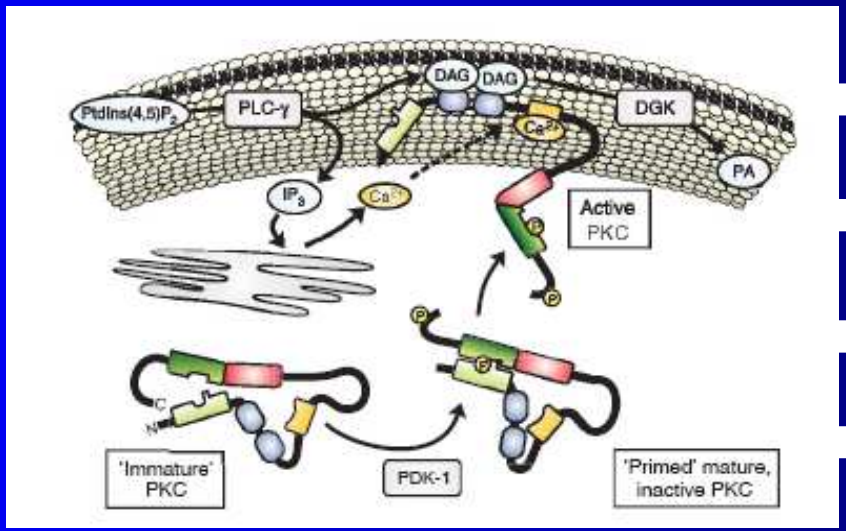




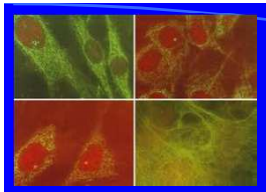


Does targeting Protein Kinase restrain proliferation in human pancreatic endocrine tumors?

PKC: key enzymes of cellular dynamics



- SURVIVAL
- APOPTOSIS
- ANGIOGENESIS
- PROLIFERATION



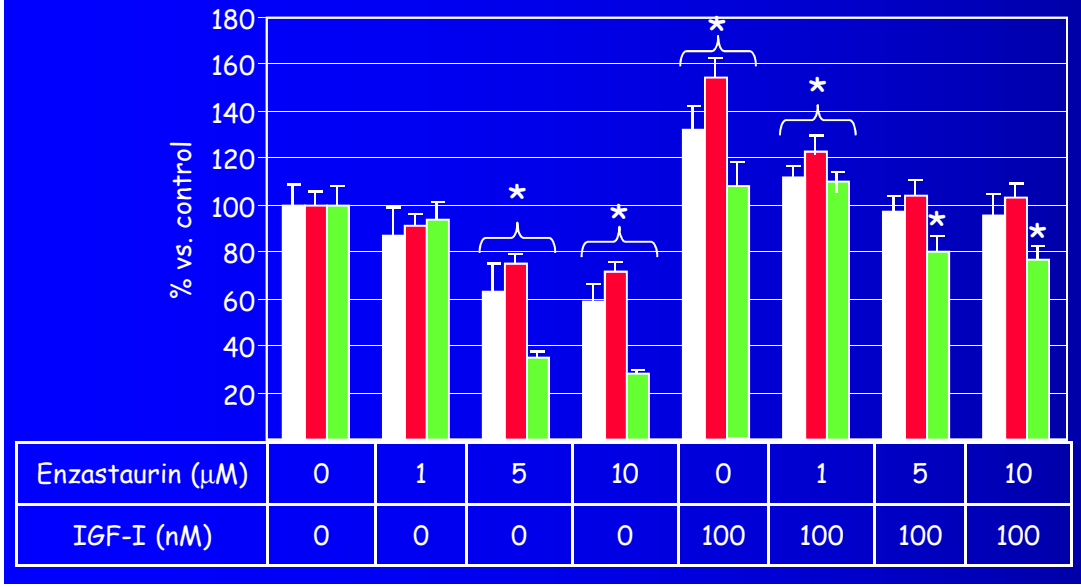
Colture primarie

Pancreatic endocrine tumors

ENZASTAURIN

PKCβII inhibitor

- Riduces neoplastic cell division
- Enhances apoptosis
- Inhibits tumor vascularization

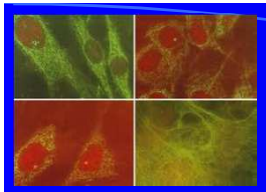


Enzastaurin (μM)	0	1	5	10	0	1	5	10
IGF-I (nM)	0	0	0	0	100	100	100	100

Reduces PNN primary culture cell viability and CgA secretion

Molè et al. submitted to Endocr-Relat Can

EFE 2012



Colture primarie

Pancreatic endocrine tumors

ENZASTAURIN

BON-1 cell line

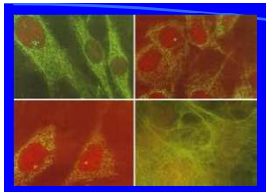
- ↓ cell viability
- ↓ DNA synthesis
- ↑ apoptosis
- ↓ GSK3β phosphorylation

→ antiproliferative effects

- ↓ CgA secretion

→ antisecretory effects

Molè et al. submitted to Endocr-Relat Can



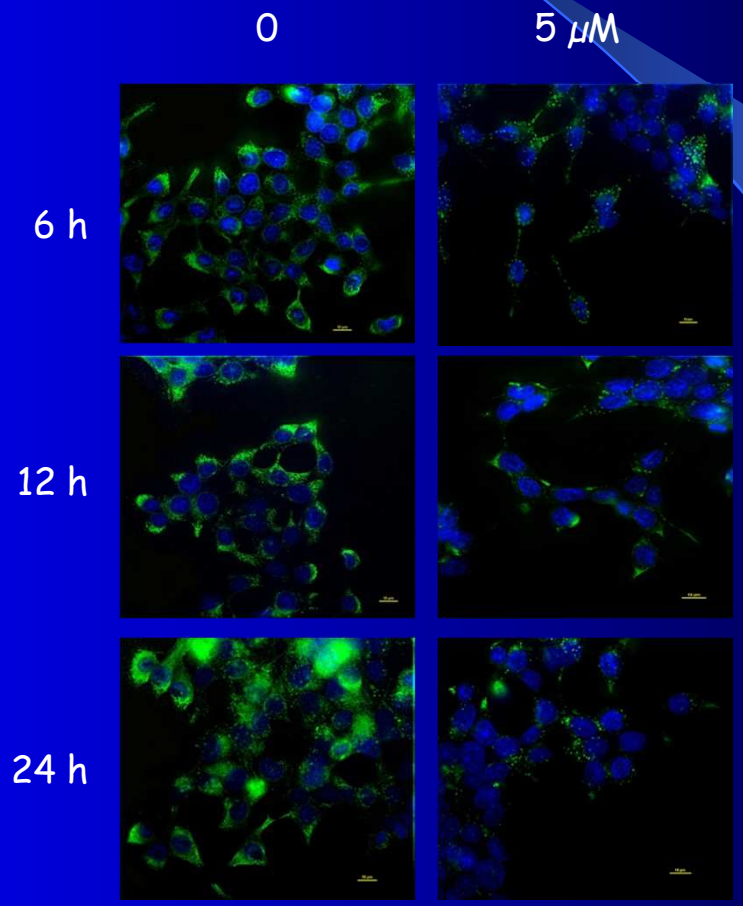
Colture primarie

Pancreatic endocrine tumors

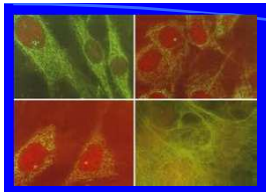
ENZASTAURIN

BON-1 cell line

↓ CgA expression



Molè et al. submitted to Endocr-Relat Can



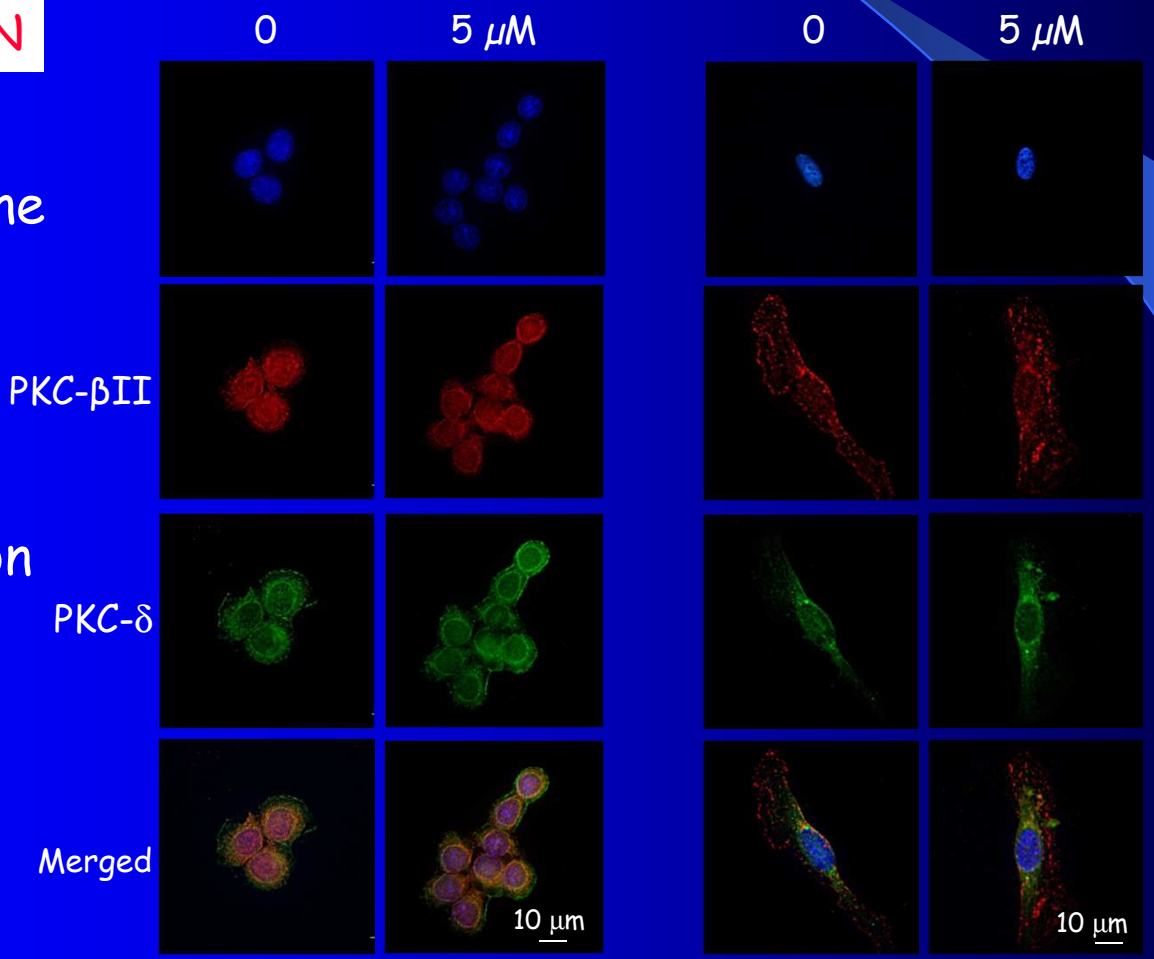
Colture primarie

Pancreatic endocrine tumors

ENZASTAURIN

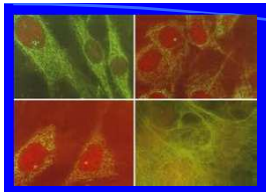
BON-1 cell line

isoform delocalization



Molè et al. submitted to Endocr-Relat Can

EFE 2012



Colture primarie

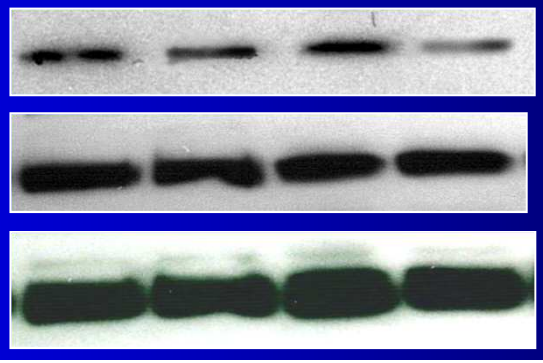
Pancreatic endocrine tumors

ENZASTAURIN

BON-1 cell line

↓

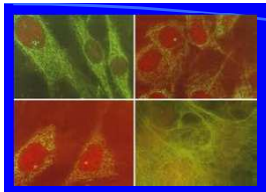
no protein changes



Enzastaurin (μM)	0	5	0	5
IGF-I (nM)	0	0	100	100

Molè et al. submitted to Endocr-Relat Can

EFE 2012

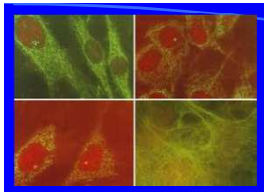


Colture primarie

Pancreatic endocrine tumors

ENZASTAURIN

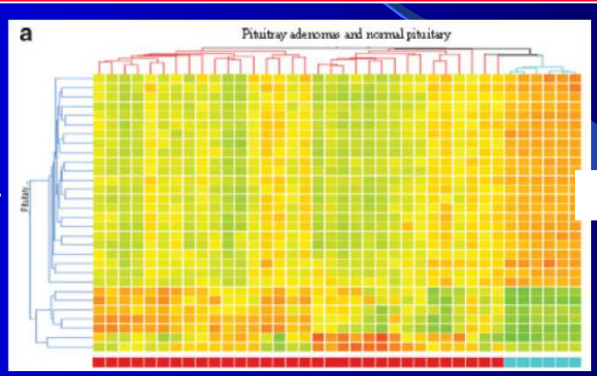
PKC may represent
a new pharmacological target
for pancreatic endocrine tumors
medical therapy



Colture primarie

Pancreatic endocrine tumors

72 primary PETs
7 matched metastases
10 normal pancreatic samples



validation by
QPCR or IHC

Absent or low SSTR2
in insulinomas
vs. nonfunctioning tumors

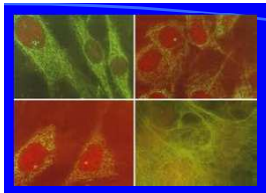
FGF13 expression
↑ liver metastases
↓ disease-free survival

↓ TSC2 and PTEN
↳ ↓ disease-free
and overall survival

possible molecular signatures?

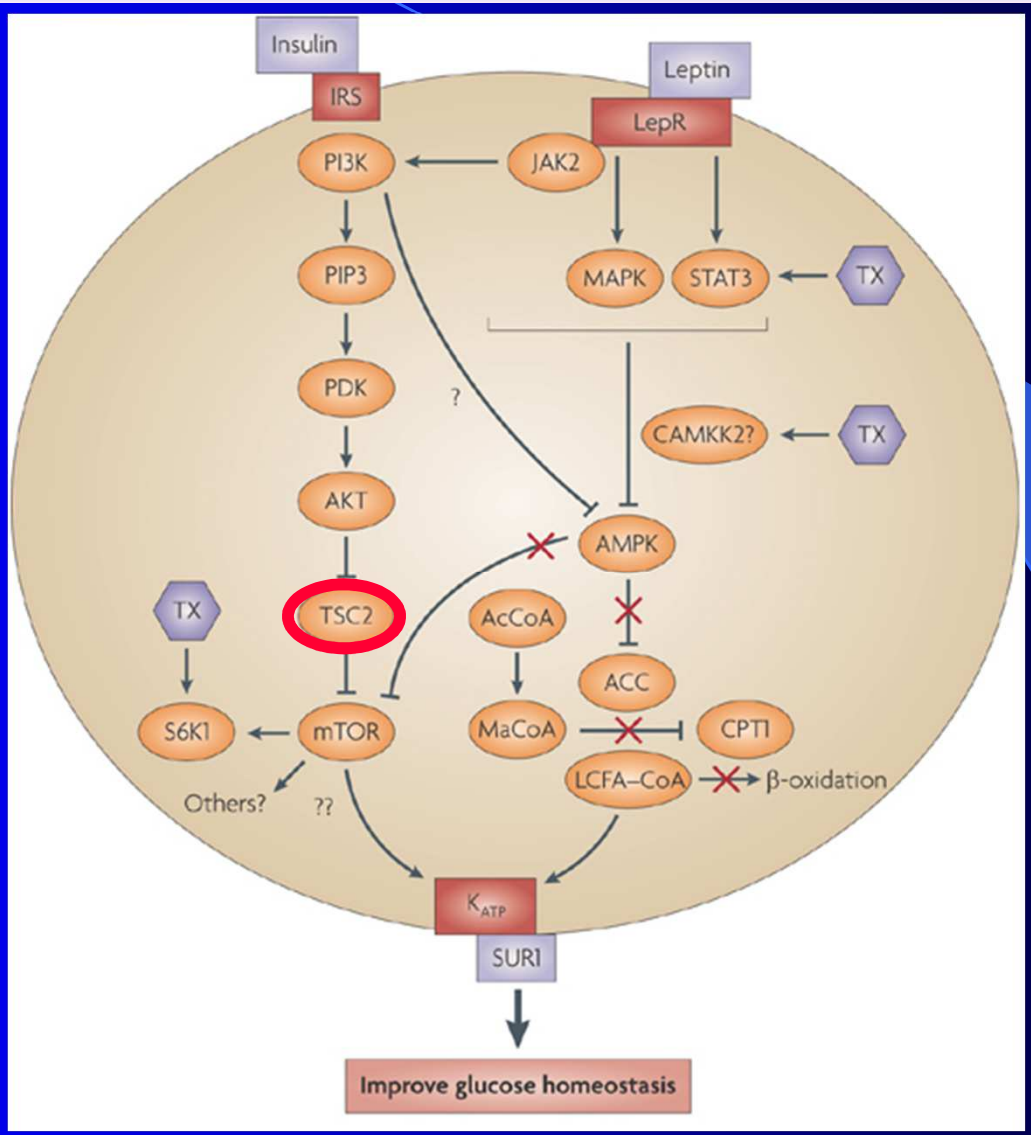
Missiaglia et al 2010 J Clin Oncol 28:245-55

EFE 2012

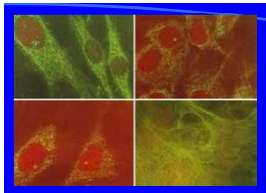


Colture primarie

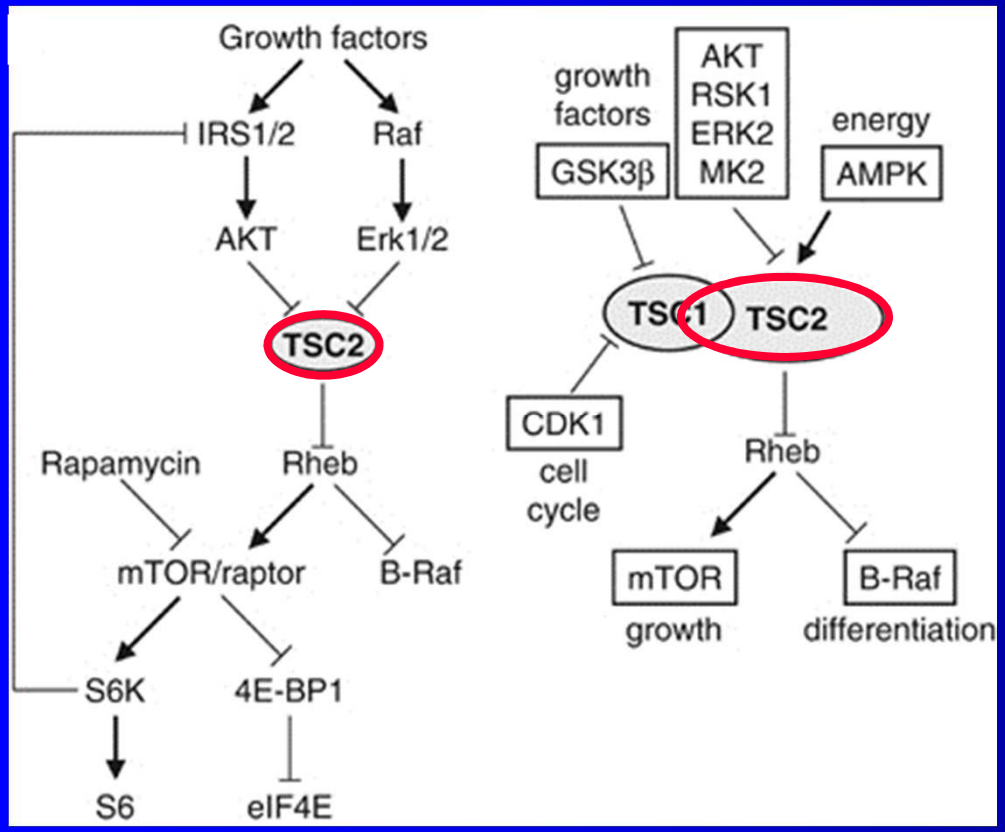
TSC2
mTOR up-stream signalling pathway



Sandoval et al. 2009 Nat Rev Drug Disc 8: 386-98

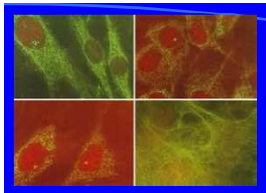


Colture primarie



Astrinidis et al. 2005 Oncogene 24: 7475–7481

TSC2 physiologically inhibits mTOR activation



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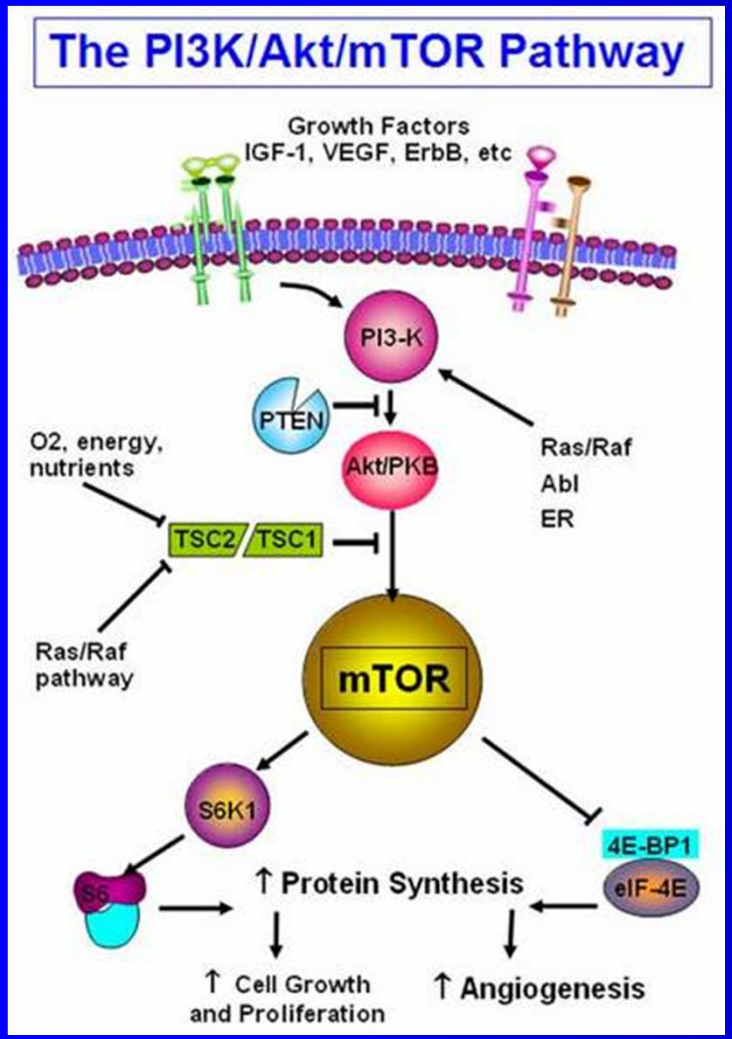
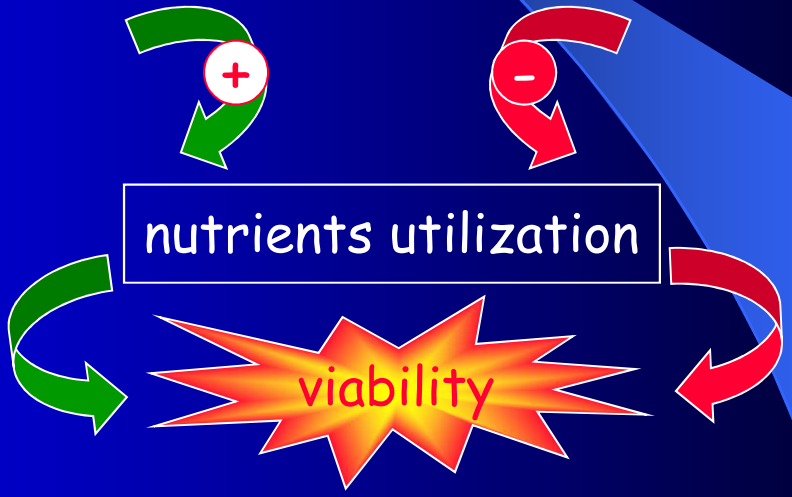
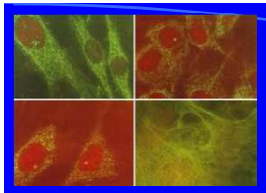


mTOR

large serine-threonine kinase



biological switch sensing changes in the cellular environment and helping cells respond



Colture primarie

Normal neuroendocrine cell

growth signals

+

AKT

-

TSC2

-

mTOR

+

growth

TSC2 mutated neuroendocrine cell

growth signals

+

AKT

-

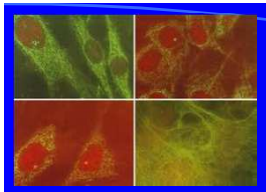
TSC2

mTOR

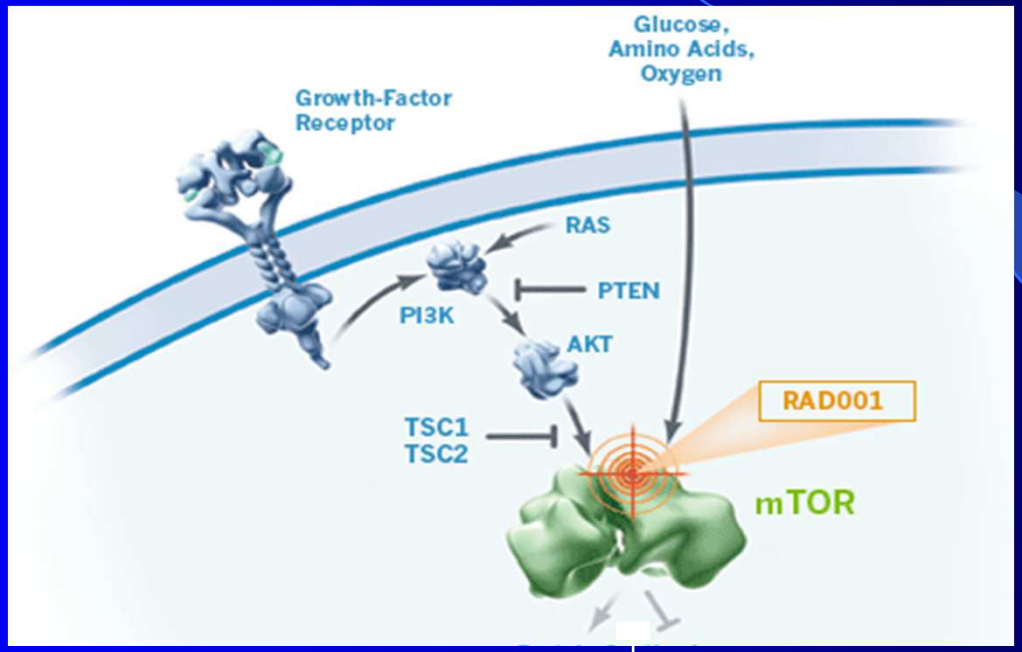
+

growth

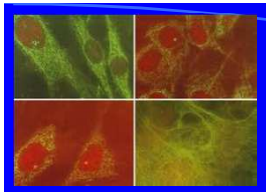
EFE 2012



Colture primarie



- ↓ nutrient uptake
- ↓ cell growth and proliferation rate
- ↓ angiogenesis



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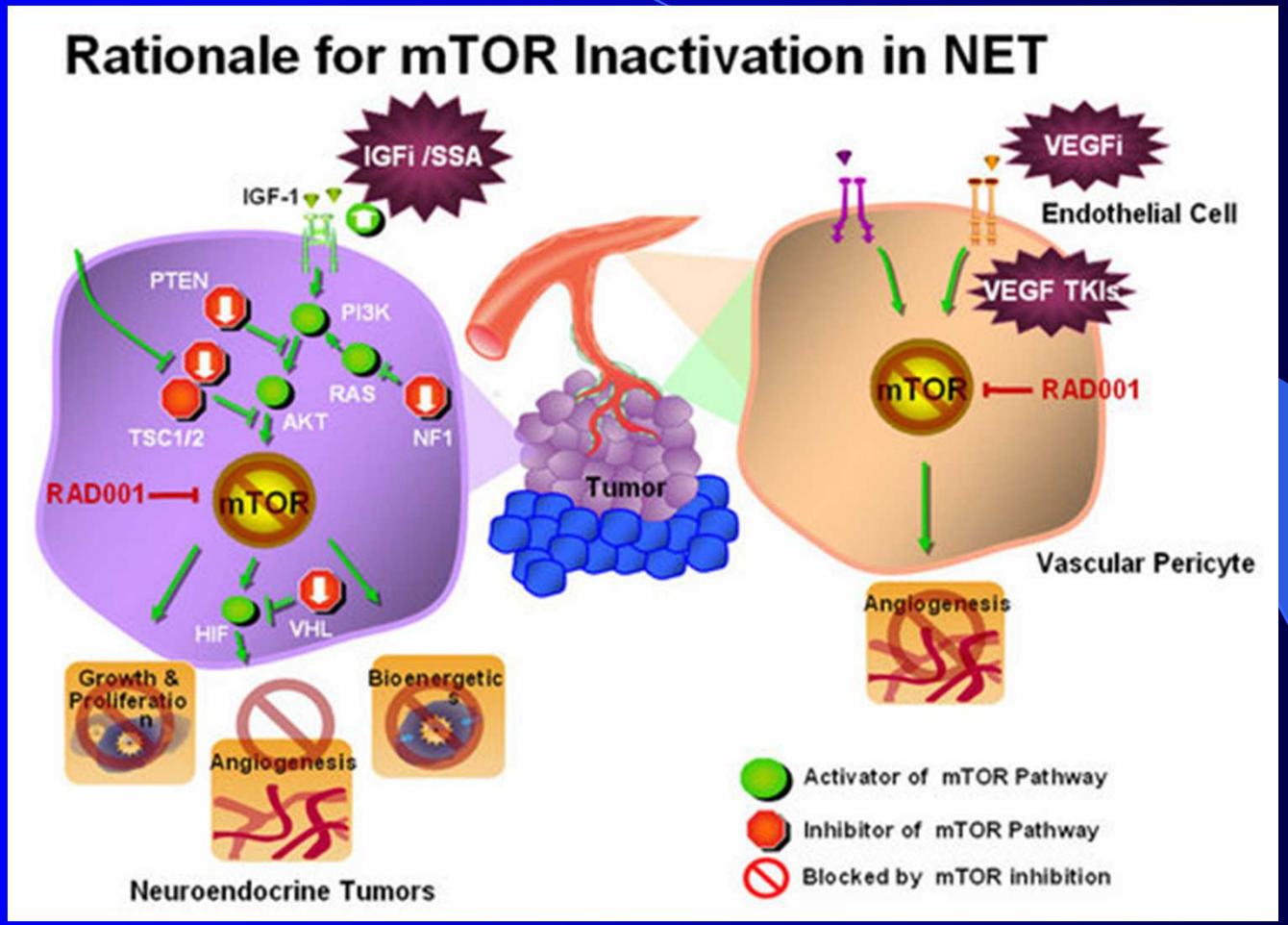
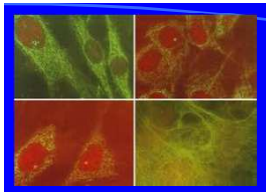




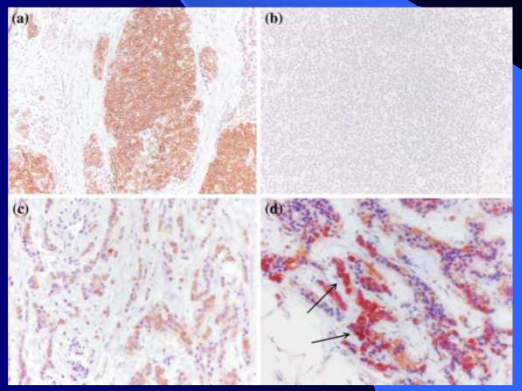
Colture primarie

High p-mTOR expression in poorly differentiated neuroendocrine carcinomas

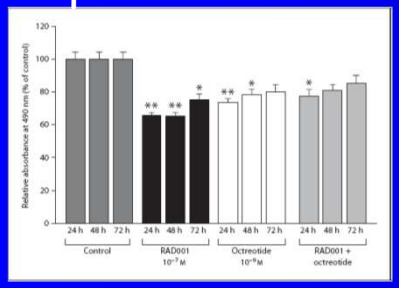
WHO classification	p-mTOR (high)	p-mT (low)	
Well-differentiated neuroendocrine tumor and well-differentiated neuroendocrine carcinoma	3	8	11
Poorly differentiated neuroendocrine carcinoma	6	3	9
Total	9	11	20

Shimizu et al 2010 Cancer Chemother Pharmacol 65:889-93

potential therapeutic role for mTOR inhibitors in neuroendocrine tumors

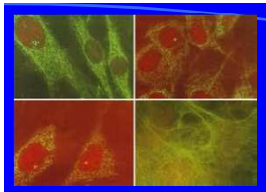


RAD-001 inhibits neuroendocrine cell proliferation



Clinical trials

Grozinsky-Glasberg et al 2008 Neuroendocrinology 87:168
Zitzmann et al. 2007 Neuroendocrinology 85:54-60



Colture primarie

RAD-001 In Advanced Neuroendocrine Tumors (RADIANT)

RADIANT-1: Phase 2 open label study of RAD-001 in advanced pancreatic neuroendocrine tumors after failure of chemotherapy

Stratum 1
115 patients

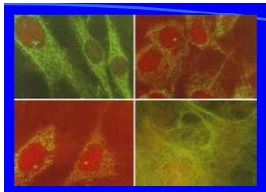
RAD001 10 mg/d

RAD001 10 mg/d + octreotide LAR 30 mg/4w

Stratum 2
45 patients

clinical benefit

Yao et al. 2010 J Clin Oncol 28: 69-76



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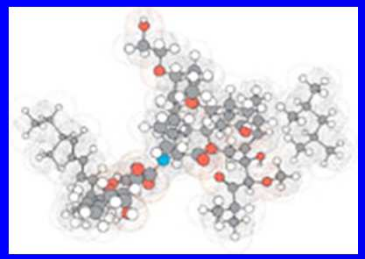
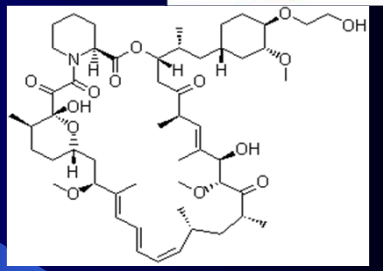


Everolimus (RAD001)

derivative of Rapamycin



immunosuppressant agent

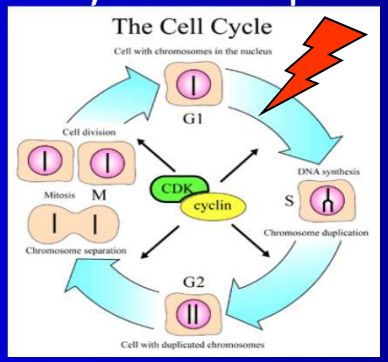
antineoplastic activity

⬇ organ transplants rejection

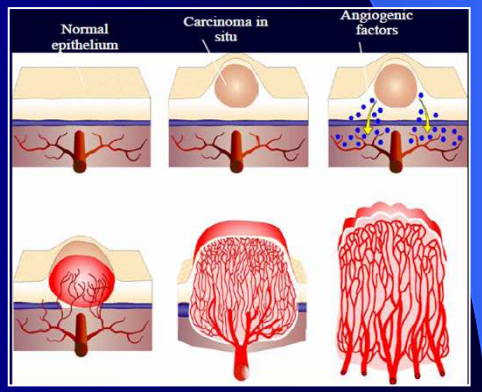
mTOR inhibitor

mTORC1 protein

cell cycle G1-S phase



⬇ VEGF secretion



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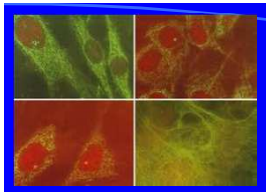
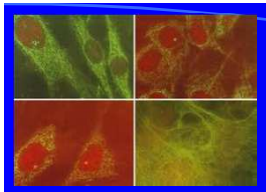


Colture primarie

What about other endocrine tumors?

BRONCHIAL CARCINOIDS

Inoperable bronchial carcinoids are still orphan of medical therapy



Colture primarie

BC

Multidisciplinary group

Section of Endocrinology, Department of Biomedical Sciences and Advanced Therapies - University of Ferrara
Department of Medical and Surgical Sciences, University of Padova
Department of Thoracic Surgery, University of Padova
Department of Diagnostic Medical Sciences and Special Therapies, University of Padova
Institute of Clinical Surgery, University of Ferrara

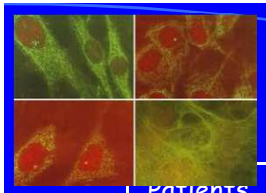
24 bronchial carcinoids

surgery

Biomolecular analysis

Pathology

Zatelli et al. Endocr Relat Cancer. 2010;17:719-29



Colture primarie

Patients characteristics

Patients	Age	Sex	Diagnosis	TNM
1	49	F	Typical	T2N2
2	36	F	Atypical	T2N1
3	49	F	Typical	T3N0
4	29	M	Typical	T2N0
5	45	M	Typical	T2N0
6	52	M	Typical	T1N0
7	36	F	Typical	T2N1
8	74	F	Typical	T1N0
9	68	M	Atypical	T2N0
10	39	M	Atypical	T3N0
11	47	F	Typical	T2N0
12	76	M	Typical	T1N1
13	42	M	Typical	T2N0
14	30	M	Typical	T2N0
15	72	F	Typical	T1N0
16	72	M	Typical	T1N1
17	39	F	Typical	T2N0
18	72	M	Typical	T1N1
19	68	M	Typical	T2N0
20	41	M	Atypical	T3N0
21	50	F	Typical	T1N0
22	57	M	Typical	T2N1
23	45	M	Typical	T1N0
24	49	F	Atypical	T2N0

14 ♂, 10 ♀

51.5 ± 3.1 y

19 typical carcinoids

5 atypical carcinoids

primary cultures

Cell viability response to RAD001

responders

non responders

BC

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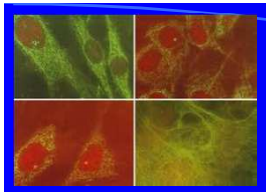
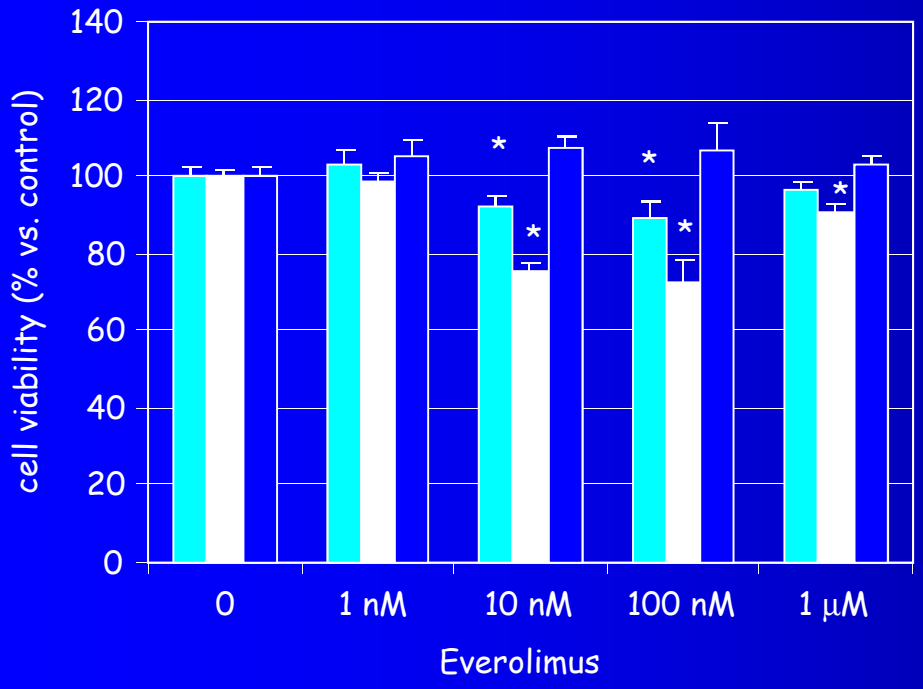


Colture primarie

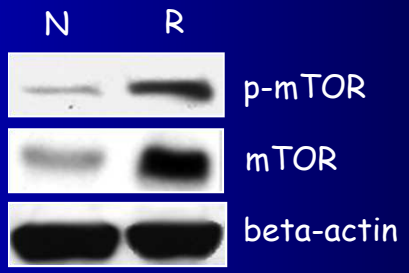
BC



All

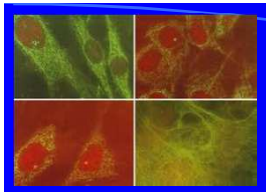
Responder (15)

Non responder (9)



RAD001 reduces cell viability in BC with higher total and phosphorylated mTOR

Zatelli et al. Endocr Relat Cancer. 2010;17:719-29



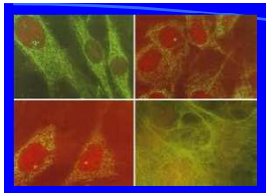
Colture primarie

BC characteristics according to response to everolimus

	Responders	Non responders	p (responders vs. non responders)
Age	53 ± 5.4	48 ± 4.7	n.s.
Gender (M/F)	8/7	5/4	n.s.
Smoking history (yes/no)	6/9	4/5	n.s.
Diameter (cm)	3.6 ± 0.4	2.16 ± 0.3	< 0.05
Lymphnode metastases (%)	10.4	3.1	< 0.02
Typical /atypical	10/5	9/0	< 0.05
Mitotic figures/mm^2	1.7 ± 0.2	0.8 ± 0.1	< 0.01
CD105 (counts/mm^2)	43.3 ± 9	25.3 ± 4.3	< 0.05
Plasma CgA levels (ng/ml)	496.8 ± 144	57.6 ± 2.1	< 0.05
Plasma PP levels (ng/L)	116.6 ± 21	51.2 ± 7.6	< 0.05
mTOR mRNA expression (fold vs. non responders)	900	1	< 0.01

BC

Zatelli et al. Endocr Relat Cancer. 2010;17:719-29



Colture primarie

BC

Cell viability

24 BC

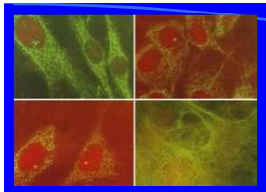
Everolimus

15 RAD-responders

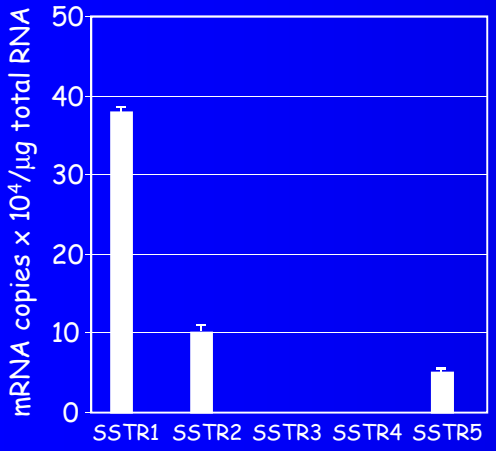
tumor diameter
% local metastases
mitoses
angiogenetic marker
CgA and PP plasma levels
mTOR expression

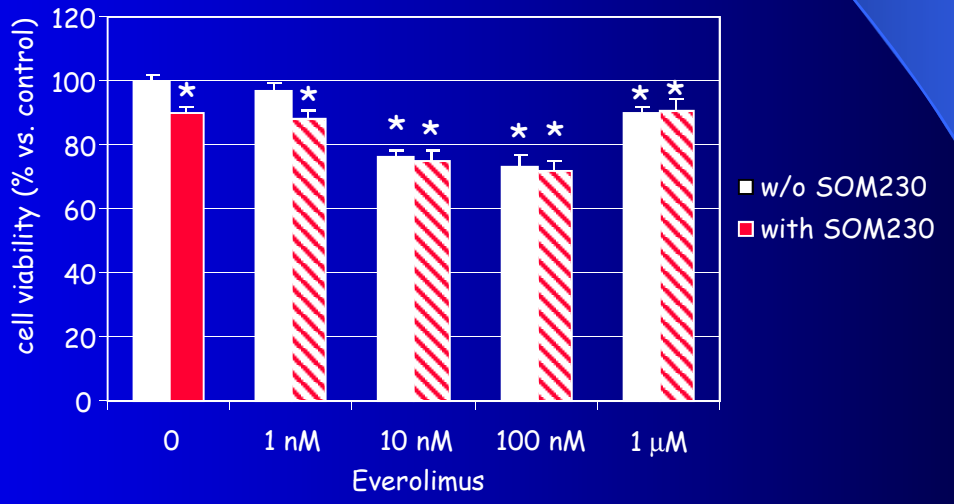
BC responding to everolimus are more aggressive

Zatelli et al. Endocr Relat Cancer. 2010;17:719-29



Colture primarie





SOM230 reduces cell viability
but
has no cooperative effect

Zatelli et al. Endocr Relat Cancer. 2010;17:719-29

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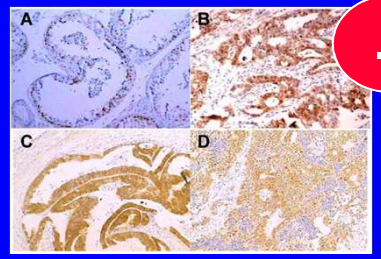
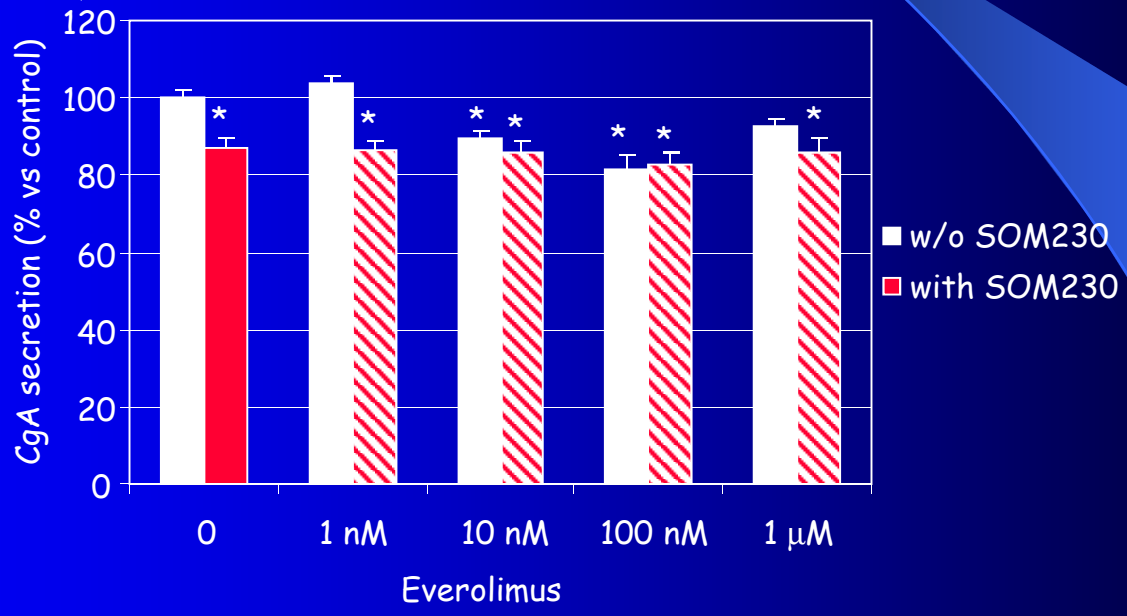
Colture primarie



differentiation



Everolimus and SOM230 reduce CgA secretion
without
cooperative effects

Zatelli et al. Endocr Relat Cancer. 2010;17:719-29

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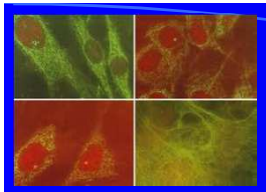
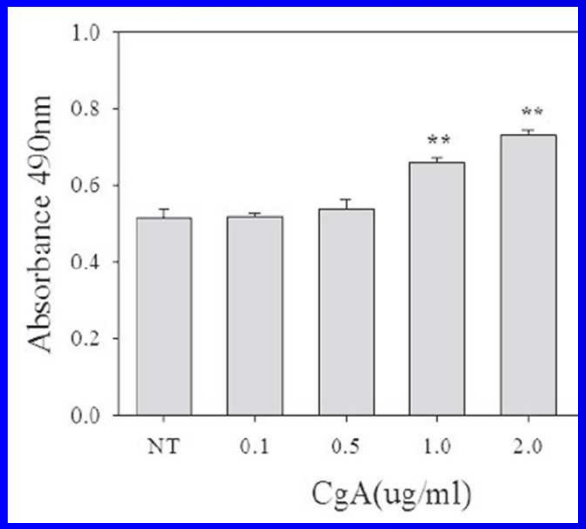
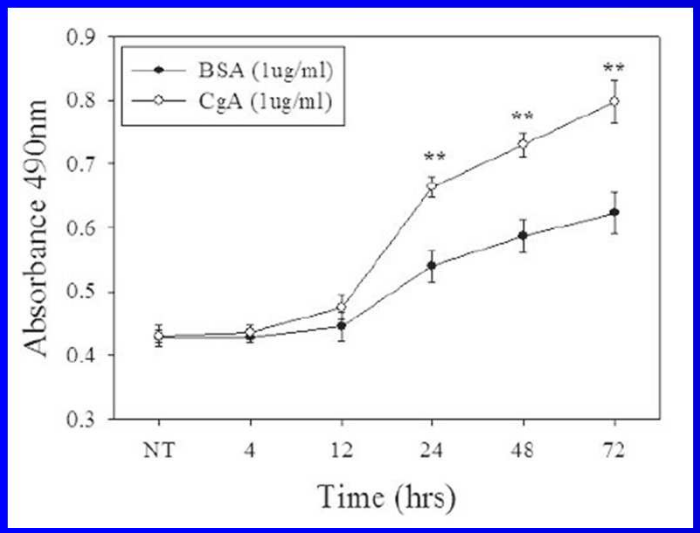
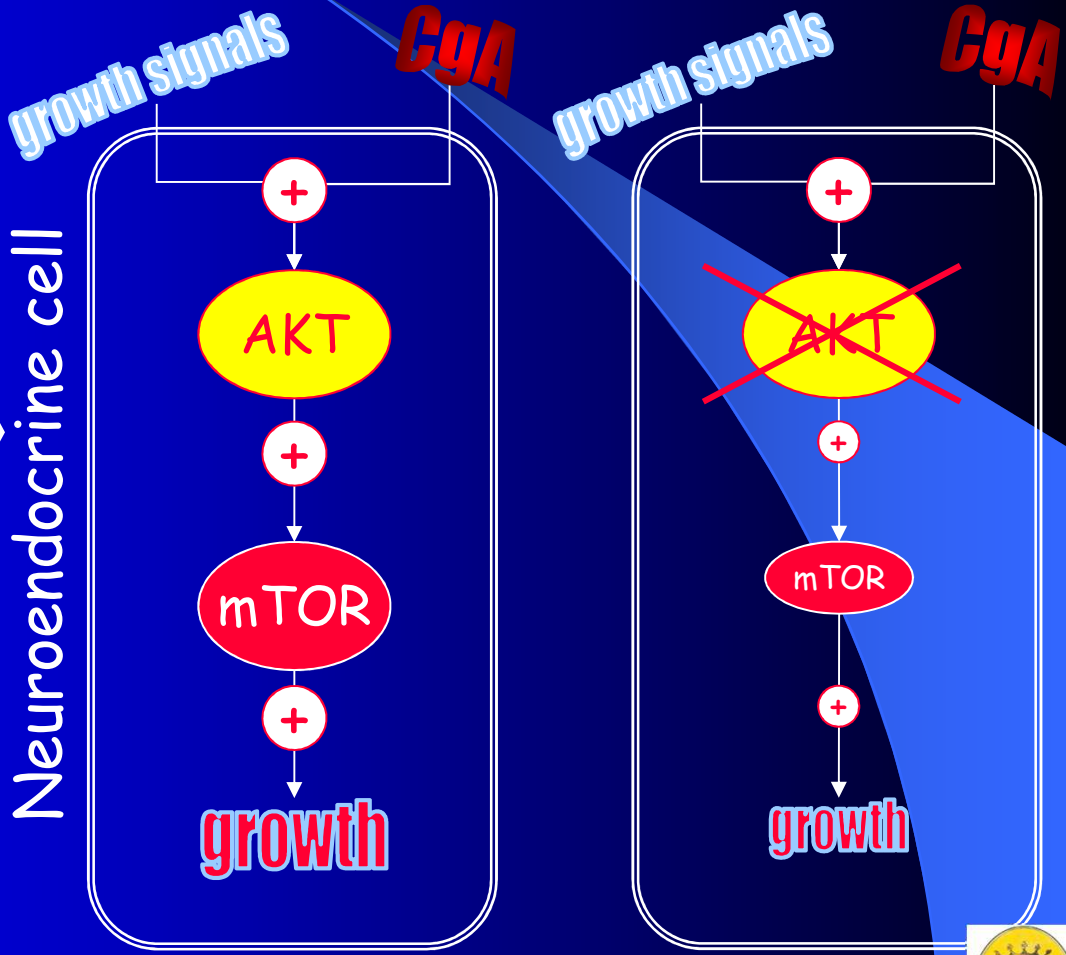


Colture primarie

but not only differentiation.. !!

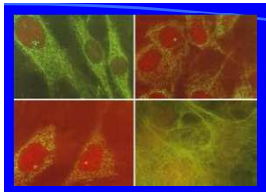




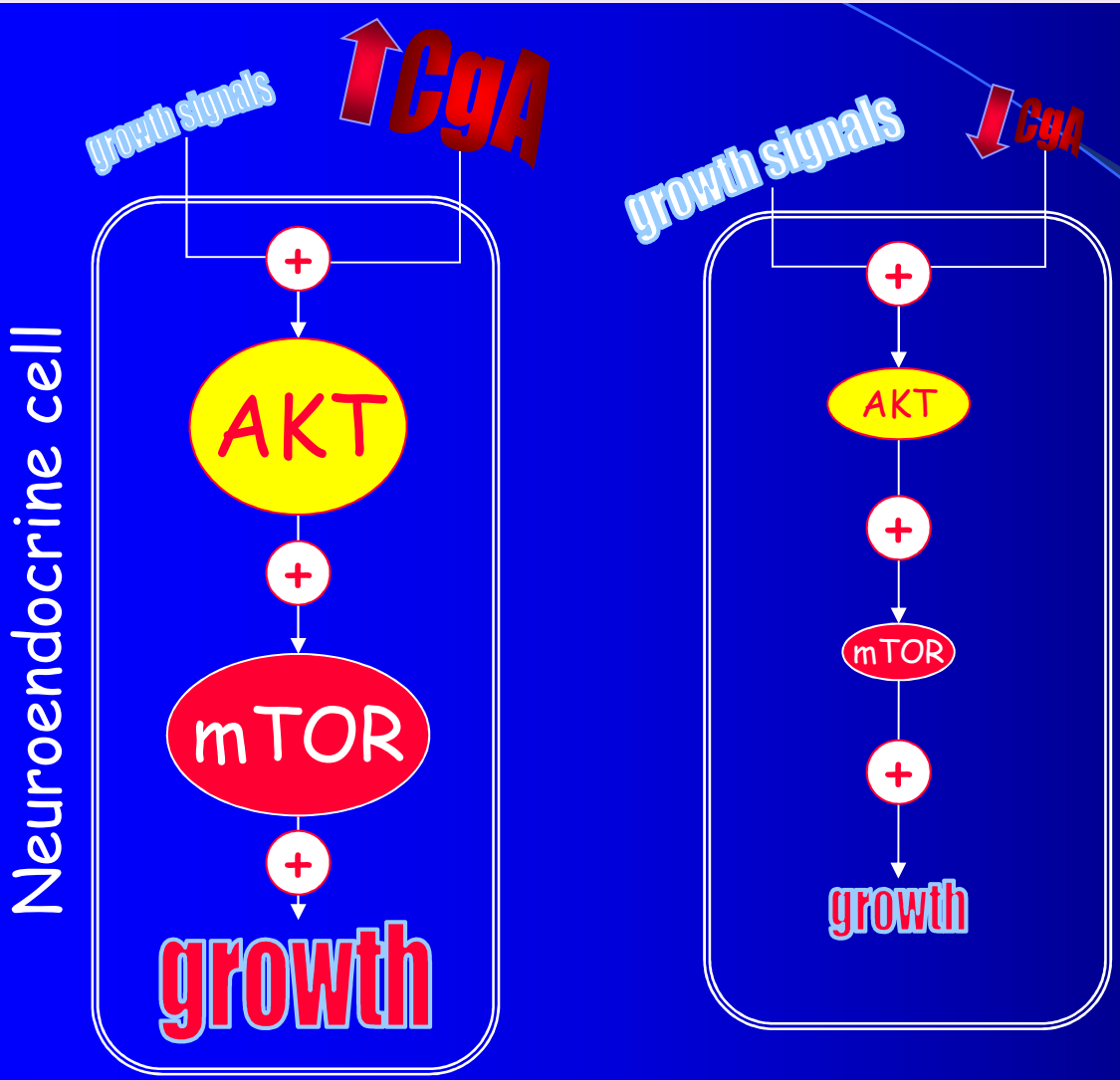


Gong et al. 2007 Endocrinology 148:4489

EFE 2012



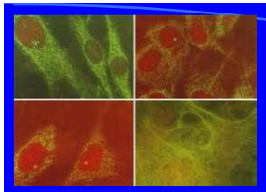
Colture primarie



lower CgA secretion might result in reduced cell growth

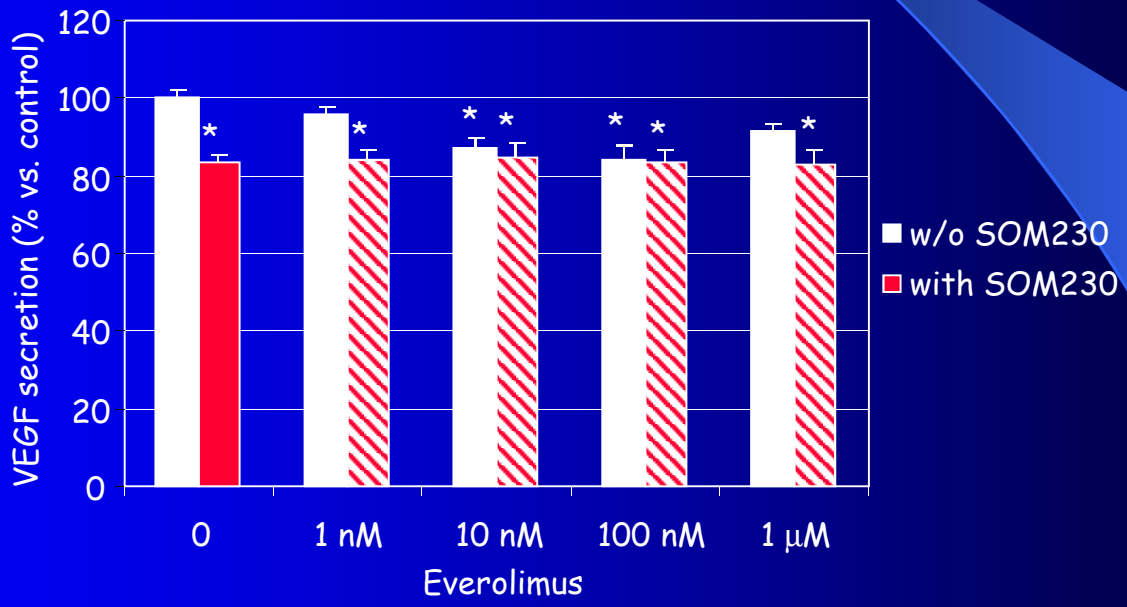
Gong et al. 2007 Endocrinology 148:4489

EFE 2012



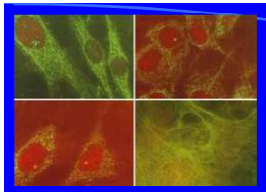
Colture primarie

angiogenesis



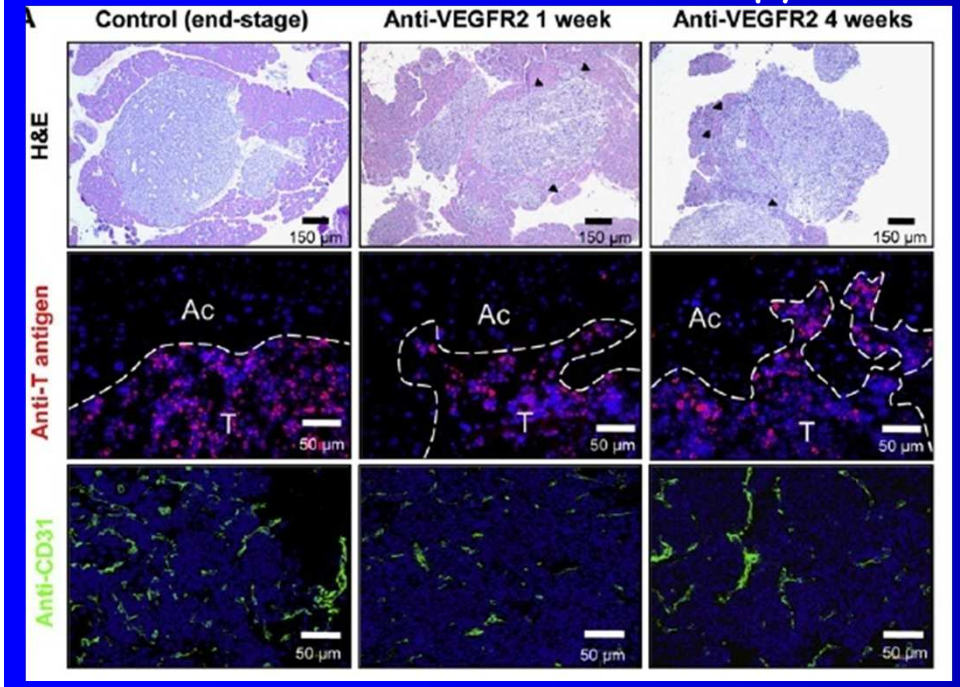
Everolimus and SOM230 reduce VEGF secretion
without
cooperative effects

Zatelli et al. Endocr Relat Cancer. 2010;17:719-29

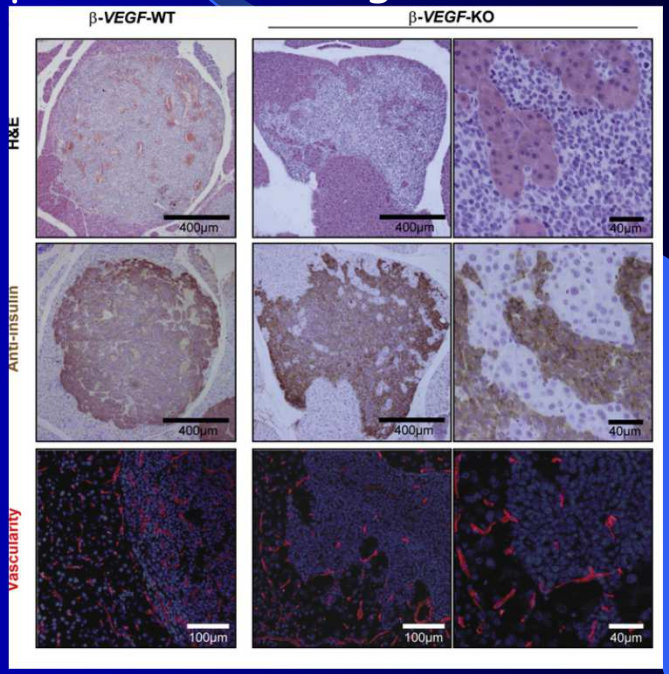


Colture primarie

Increased invasive phenotype after anti-VEGFR2 therapy

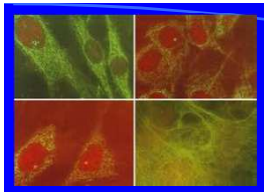


Increased tumor invasion after tumor-specific VEGF-A gene deletion



Paez-Ribes et al. 2009 Cancer Cell 15: 220–231

"…potent angiogenesis inhibition can alter the natural history of tumors by increasing invasion and metastasis …"



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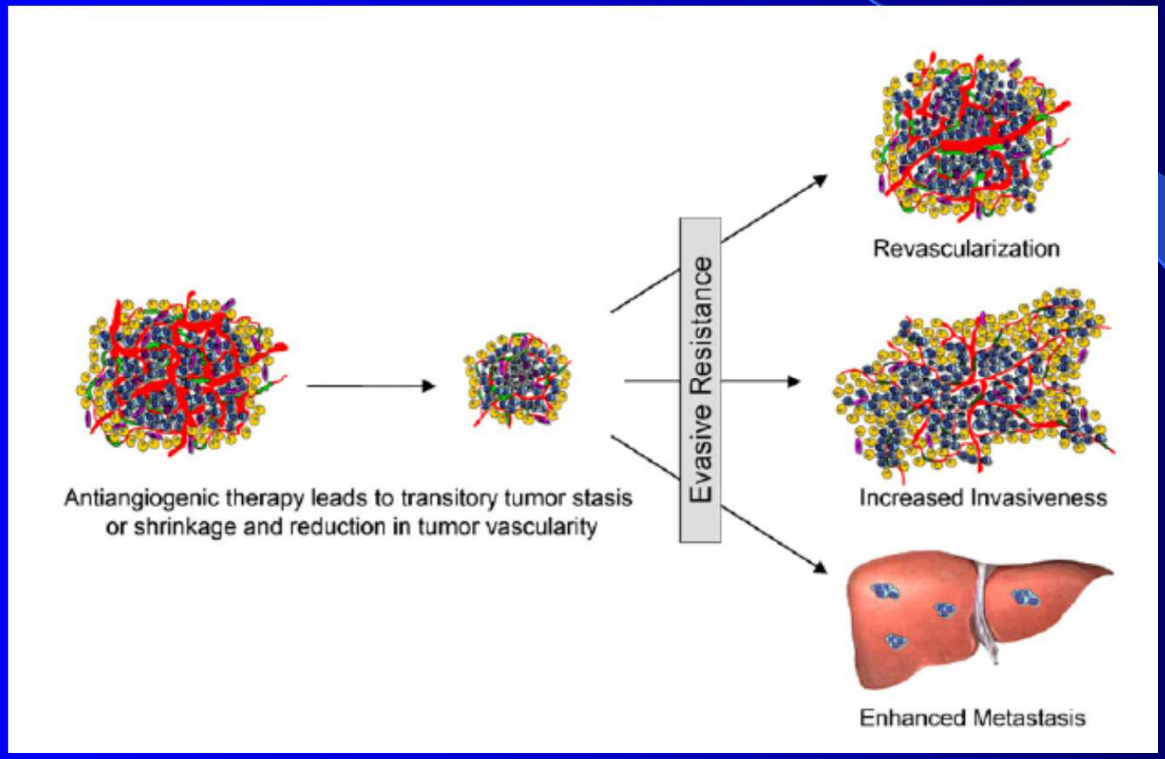
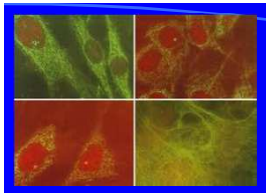


Paez-Ribes et al. 2009 Cancer Cell 15: 220–231

combined therapy!



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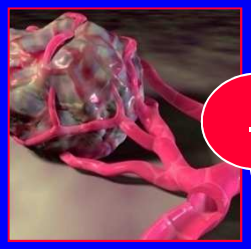
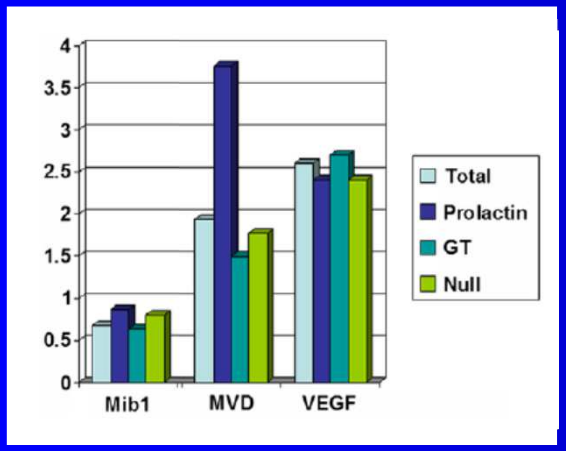


Everolimus

-

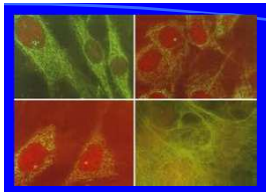
angiogenesis

Angiogenesis is enhanced in aggressive pituitary adenomas



Chacko et al. 2009 J Clin Neurosci 16: 660–665

mTOR inhibitors in invasive pituitary adenomas??

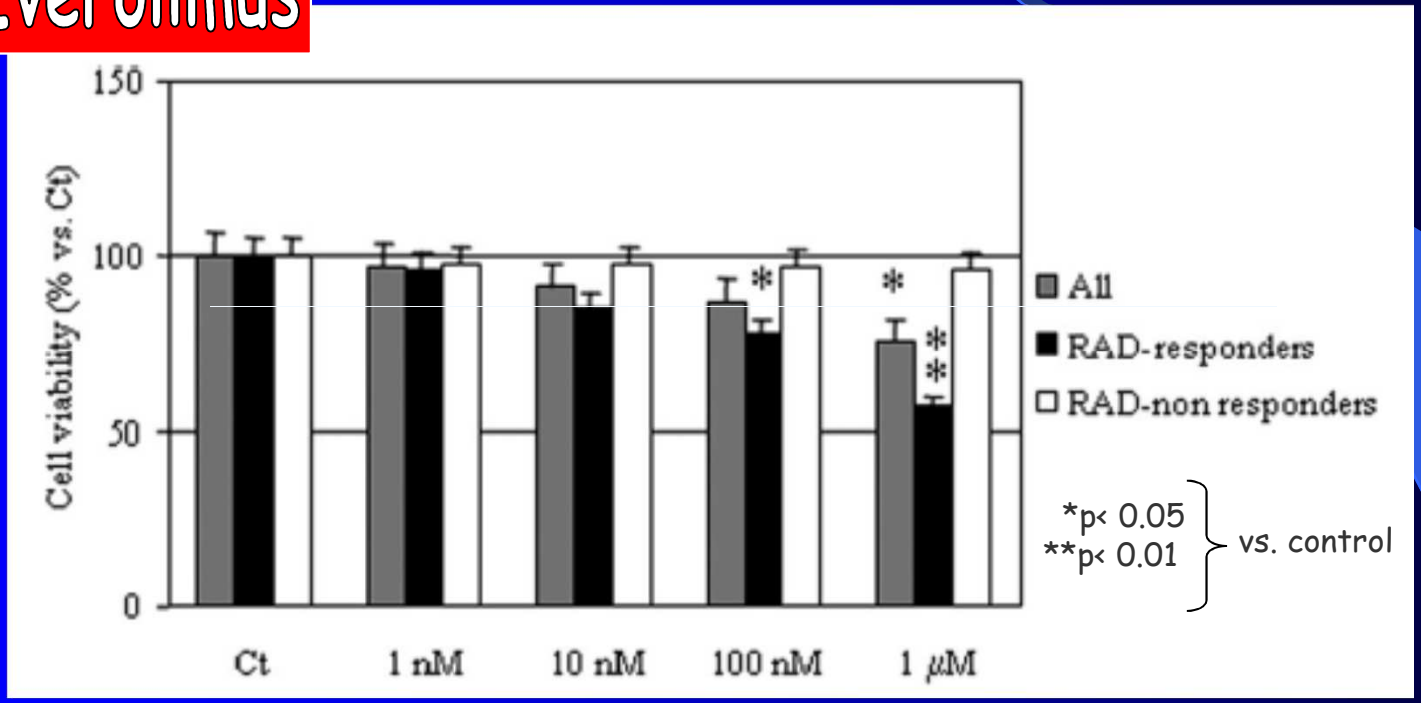


Colture primarie

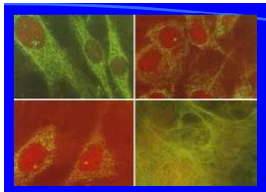
NFA

Cell viability

Everolimus



Everolimus dose-dependently reduces NFA cell viability in vitro



Colture primarie

Cell viability

40 NFA

Everolimus

28 RAD-responders

12 RAD-non responders

median age (yr)	57	69
M/F	0.65	1.4
invasive	20 (71%)	7 (58%)

Zatelli et al. 2010 J Clin Endocrinol Metab 95(2):968-76

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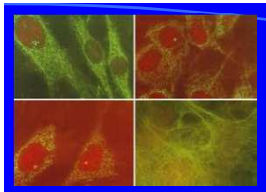


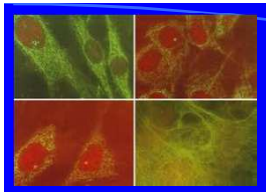
Colture primarie

RAD-responders

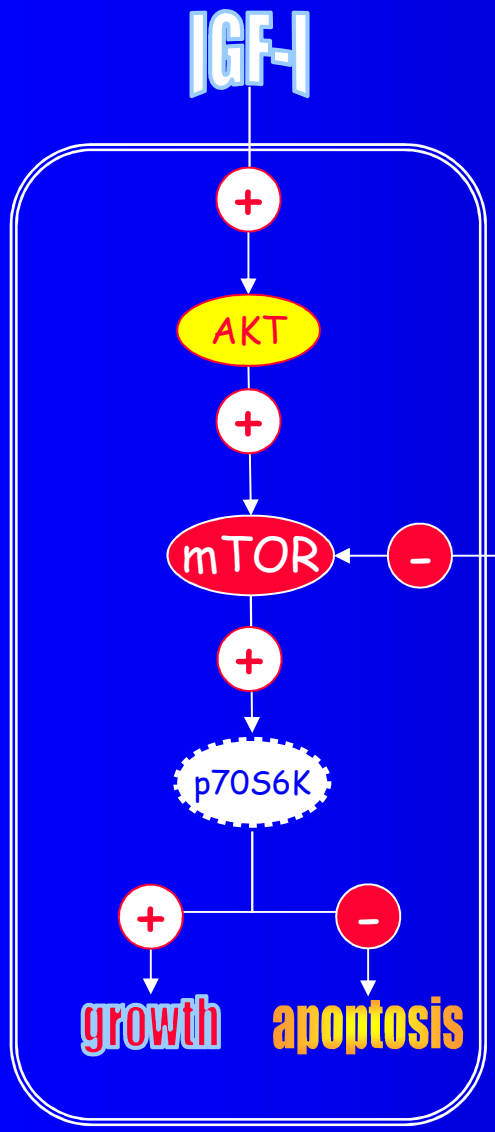
70% of the examined tumors deriving mostly from younger female patients with invasive macroadenomas

patients likely not cured by surgery could be eligible for medical therapy with mTOR inhibitors

Zatelli et al. 2010 J Clin Endocrinol Metab 95(2):968-76



Colture primarie



- reduces NFA cell viability
- promotes apoptosis
- reduces p70S6k activity

blocking IGF-I stimulatory effects

Zatelli et al. 2010 J Clin Endocrinol Metab 95:968

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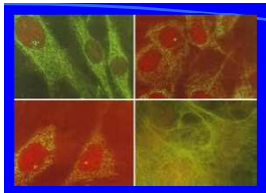
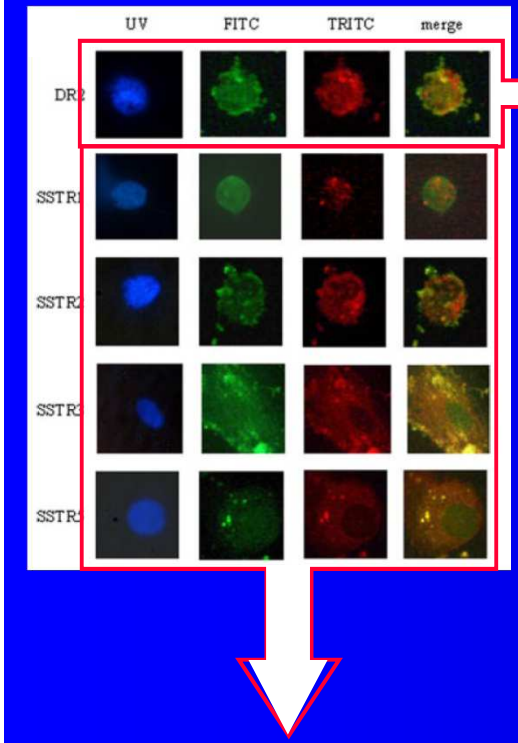


Colture primarie

NFA

Any synergism with DA and SSTR agonists?



Cabergoline significantly reduces NFA cell viability

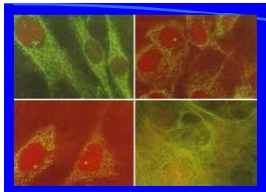
but

no additive effects with RAD001

SOM230 significantly reduces cell viability in SSTR5- NFA

with additive effects with RAD001

Zatelli et al. 2010 J Clin Endocrinol Metab 95(2):968-76



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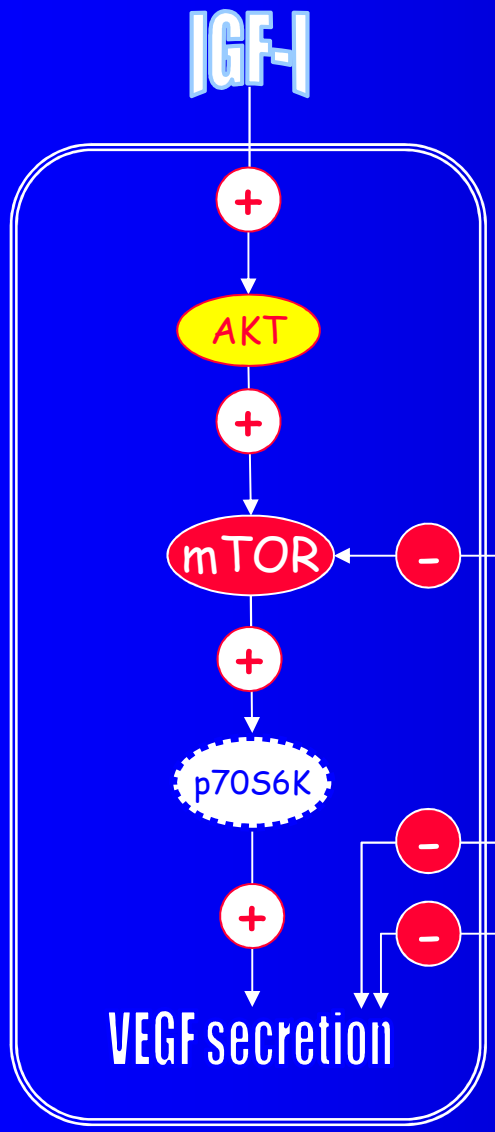
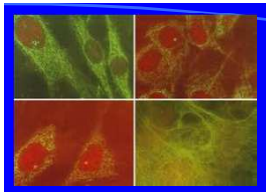


- blocks IGF-I induced VEGF secretion
- does not enhance the antisecretory effects of DR and SSTR agonists

Zatelli et al. 2010 J Clin Endocrinol Metab 95:968

EFE 2012

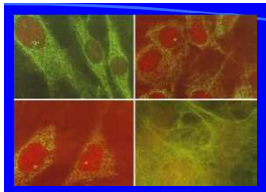


Colture primarie

NFA BC

everolimus reduces cell viability of
invasive neuroendocrine tumors in vitro
with possible cooperative effects with SOM230

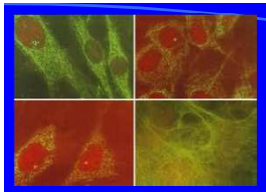
mTOR inhibitors may represent a possible medical therapy for aggressive neuroendocrine tumors



Colture primarie

Everolimus

clinical trials are needed to confirm these promising pre-clinical findings



Colture primarie

RADIANT-1
RAD001 In Advanced Neuroendocrine Tumors

Phase 2 Open Label Study of Everolimus in Advanced Pancreatic Neuroendocrine tumors after Failure of Chemotherapy

Primary end-point: Response Rate; Recruitment closed: 160 patients

(PUBLISHED *JCO*)

RADIANT-2
RAD001 In Advanced Neuroendocrine Tumors

Phase 3 Placebo-Controlled Study of Everolimus in Patients Receiving Sandostatin LAR for Advanced Carcinoid Tumors

Primary end-point: PFS; Recruitment closed: 415 patients

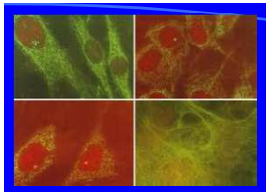
(PRESENTED AT THE *ASCO GI* 2011)

RADIANT-3
RAD001 In Advanced Neuroendocrine Tumors

Phase 3 Placebo-Controlled Study of Everolimus in Advanced Pancreatic Neuroendocrine Tumors

Primary end-point: PFS; Recruitment closed: 410 patients

(PUBLISHED NEJM)



Colture primarie

Prolongation of progression-free survival in several NETS

everolimus slows tumor growth and improves progression free survival

RAMSETE efficacy and safety of daily everolimus for the treatment of advanced patients with nonfunctioning NETs, including bronchial carcinoid

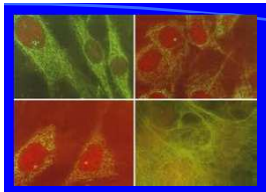
APPLICATIONS OF RAD001 IN ENDOCRINE-RELATED TUMORS

Sezione di Endocrinologia

Dipartimento di Scienze Biomediche e Terapie Avanzate

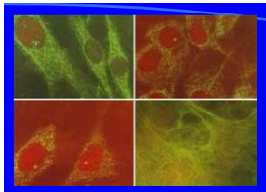
Università degli Studi di Ferrara

Direttore Prof. Ettore degli Uberti



Colture primarie

MTC



Colture primarie

MTC → Surgery

20 patients: 6 ♂ and 14 ♀

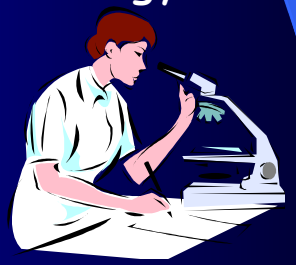
age = 50 ± 3.8 yr

19 primary tumors

1 metastatic lymph node

Biomolecular analysis

Pathology



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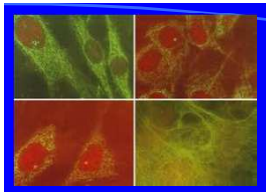
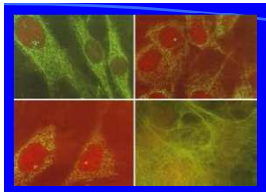


Colture primarie

Patients

N°	Sex	Age	Plasma CT (pg/ml)	TNM	Stage	Inheritance
# 1	M	46	157	T2N0Mx	II	SP
# 2	M	40	1500	T2N0Mx	II	FMTC
# 3	F	35	97	T2N0Mx	II	SP
# 4	F	31	306	T1N0Mx	II	SP
# 5	M	47	940	T2N0Mx	II	MEN2A
# 6	M	39	1500	T2NxMx	II	SP
# 7	F	35	700	T2N0Mx	II	SP
# 8	F	32	28	T1N+M0	II	MEN2A
# 9	F	35	74	T1N0Mx	I	SP
# 10	F	33	19	T1N0M0	I	FMTC
# 11	F	42	153	T2N0Mx	II	SP
# 12	M	52	207	T2N0Mx	II	SP
# 13	F	44	2350	T2N0Mx	II	SP
# 14	M	56	1258	T2N0Mx	II	SP
# 15	F	79	1500	T2N+Mx	III	SP
# 16	F	71	2578	T4N+M+	III	SP
# 17	F	73	3848	T1N+M1	IVc	SP
# 18	F	75	1500	T3N0Mx	III	SP
# 19	F	69	3405	T2N+Mx	III	SP
# 20	F	70	9227	T4N1bM+	IVb	SP



Colture primarie

20 MTC

Tissue bank

Freezer -80°C

Tissue

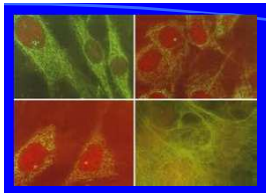
Primary coltures

In vitro experiments:
cell viavility
(colorimetric method)

1 nM to 1 µM RAD001

10 nM SOM230

50 nM IGF-1



Colture primarie

Human MTC primary cultures

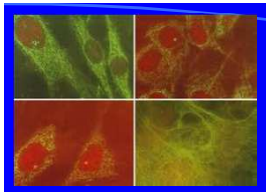
20 samples

primary cultures

Cell viability response to RAD001

14 responders

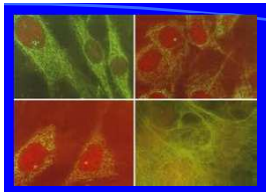
6 non responders



Colture primarie

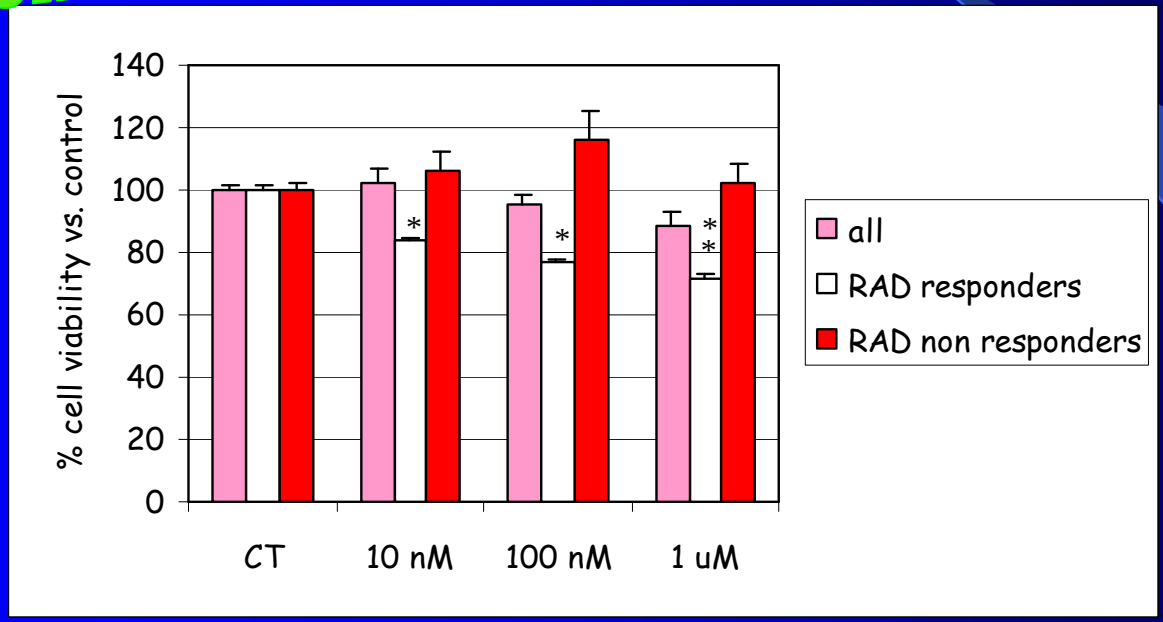
Patients

	Responders	Non responders	p
Age (yr)	40,5 ± 2,1	72,8 ± 1,7	<0,01
F/M	8:6	6:0	-
Plasma calcitonin (pg/ml)	663,5 ± 203,8	3676,3 ± 1289,8	<0,01
Stage	I or II	III or higher	-

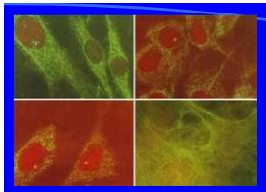


Colture primarie

cell viability

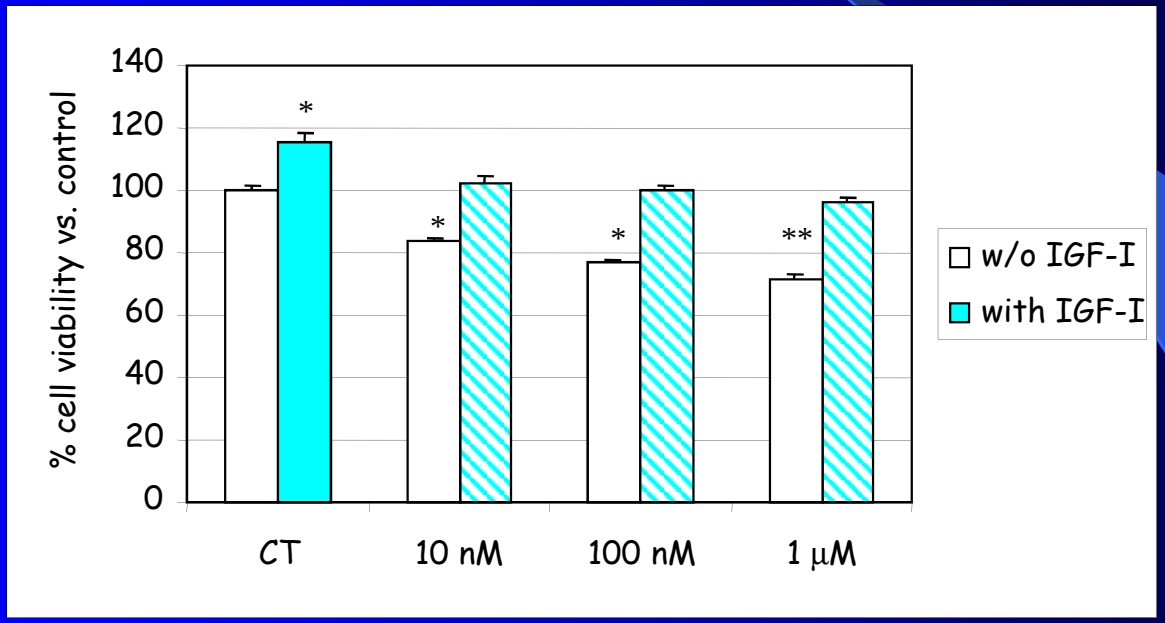


RAD001 significantly reduces cell viability in responder MTC

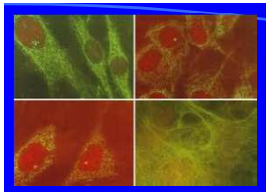


Colture primarie

cell viability

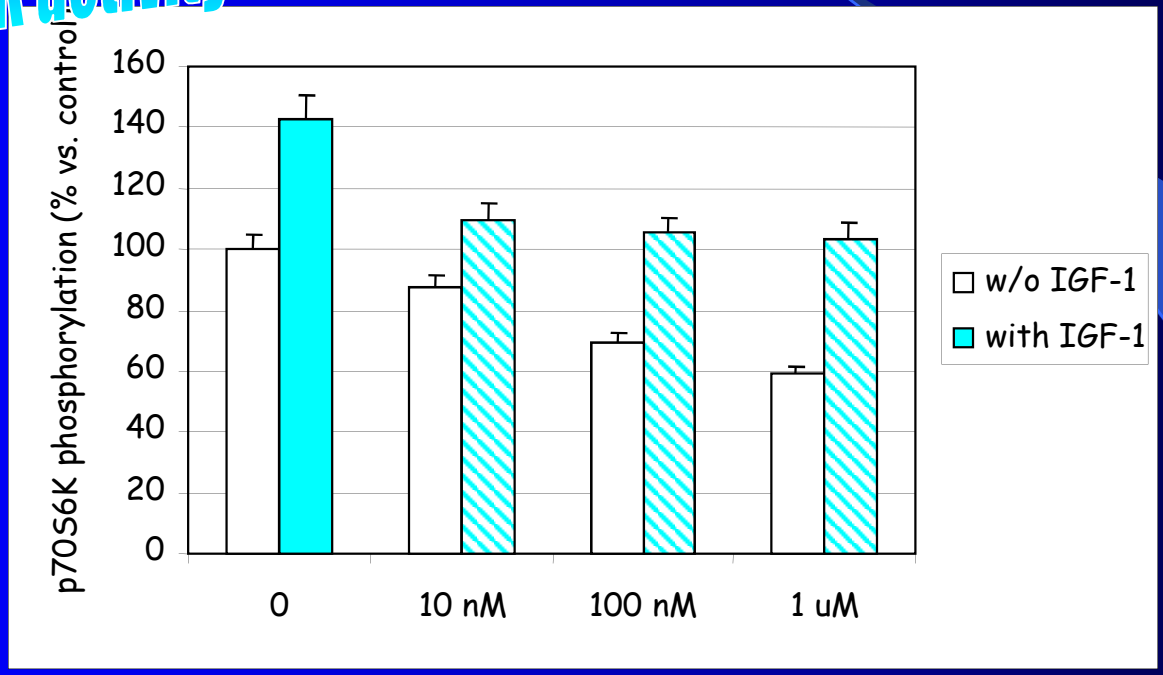


RAD001 abrogates the proliferative effect of IGF-I

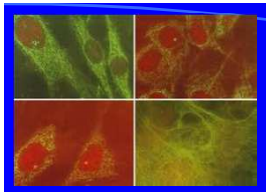


Colture primarie

p70S6K activity



RAD001 significantly reduces p70S6K phosphorylation and abrogates the stimulatory effects of IGF-I



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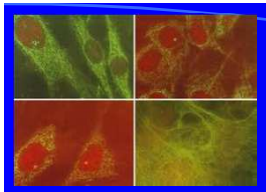
Any synergistic effect with somatostatin analogs?



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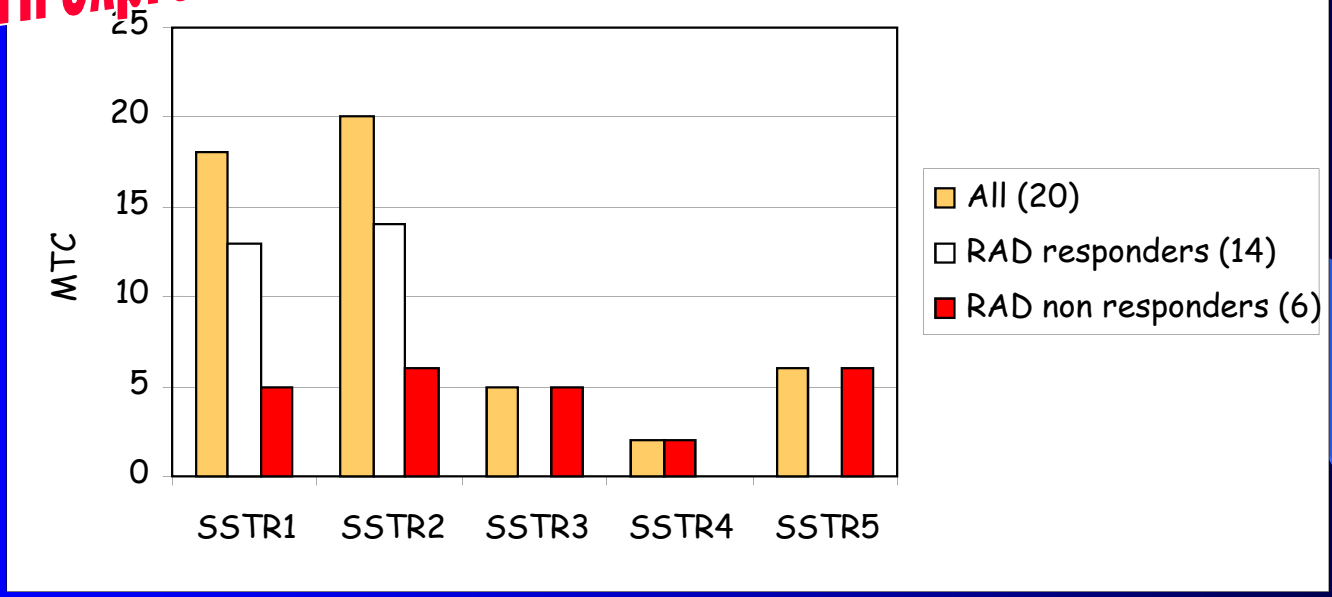
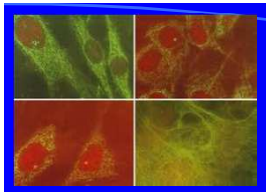
SSTR expression



MTC predominantly express SSTR1 and SSTR2

Responder MTC lack SSTR5 expression



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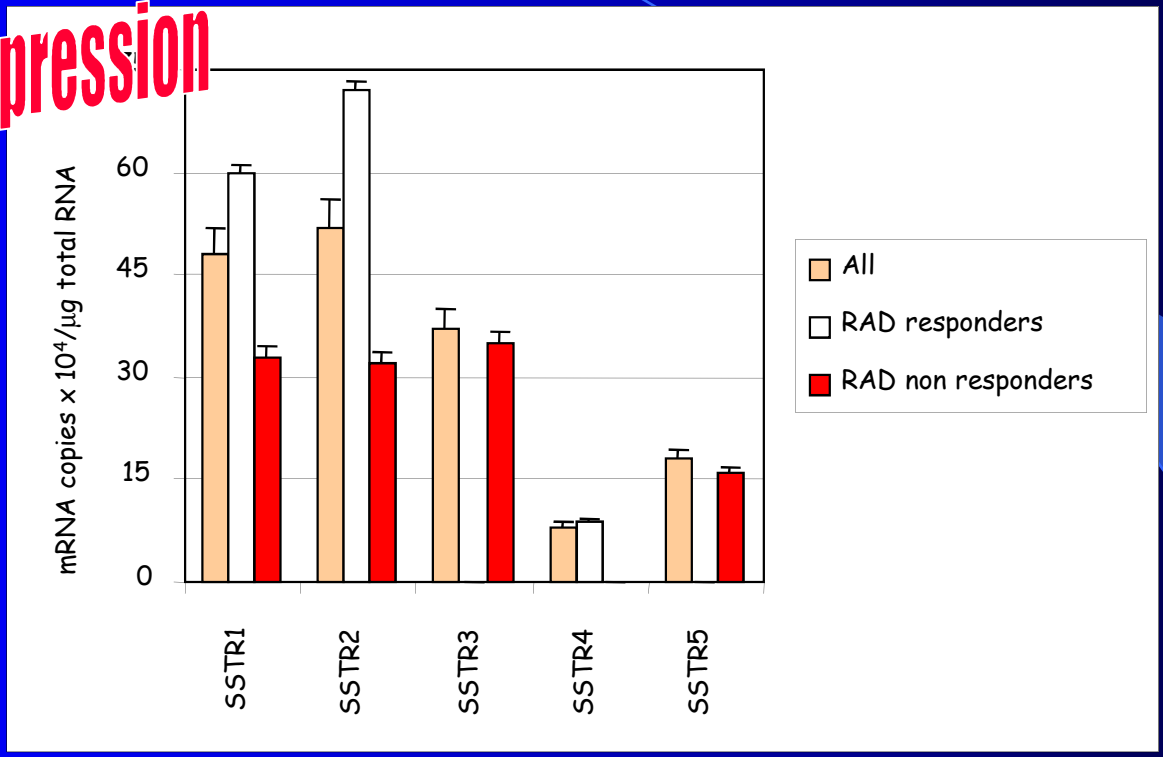
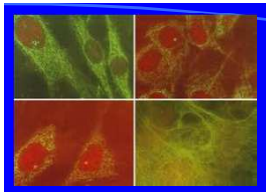
SSTR expression



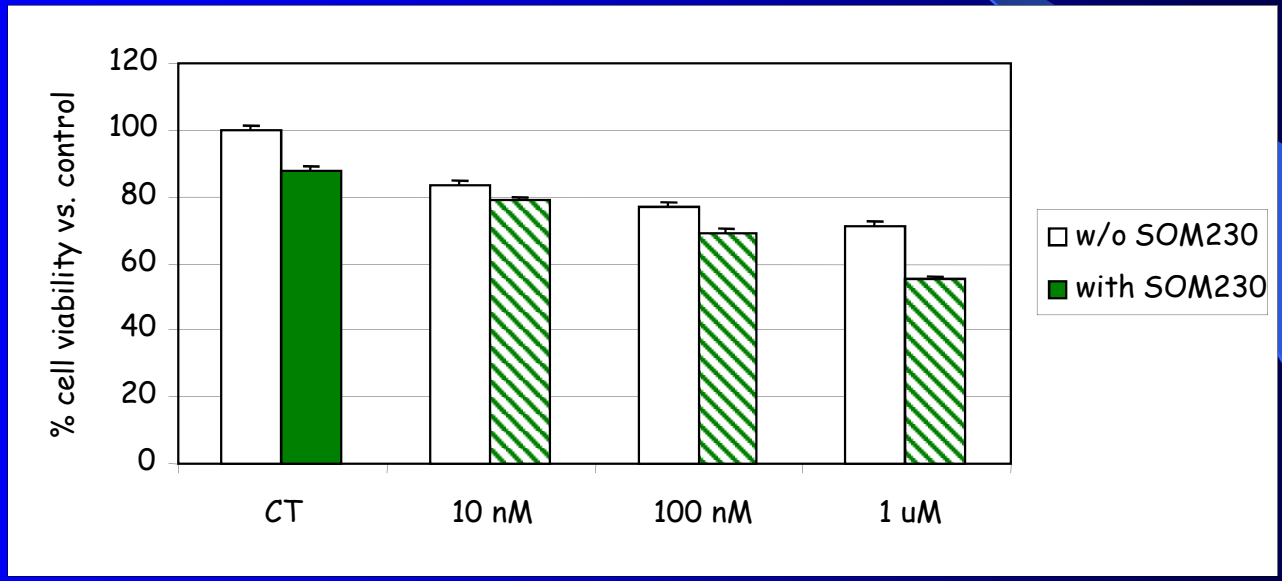
MTC predominantly express SSTR1 and SSTR2

Responder MTC lack SSTR5 expression

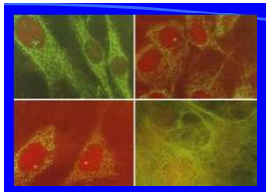


Colture primarie

cell viability



SOM230 potentiates
RAD001 antiproliferative effects

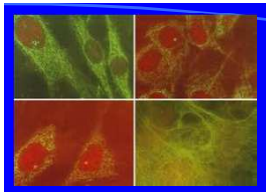


Colture primarie

In conclusion

- RAD001 reduces cell viability in 70% of MTC primary cultures
- MTC responder coltures do not express SSTR5
- the antiproliferative effect of RAD001 is blocked by IGF-1 and enhanced by co-treatment with SOM230

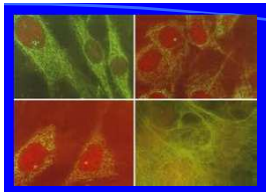
Everolimus might represent a possible therapeutic tool in MTC, also in association with SRIF analogs



Colture primarie

GH-omas

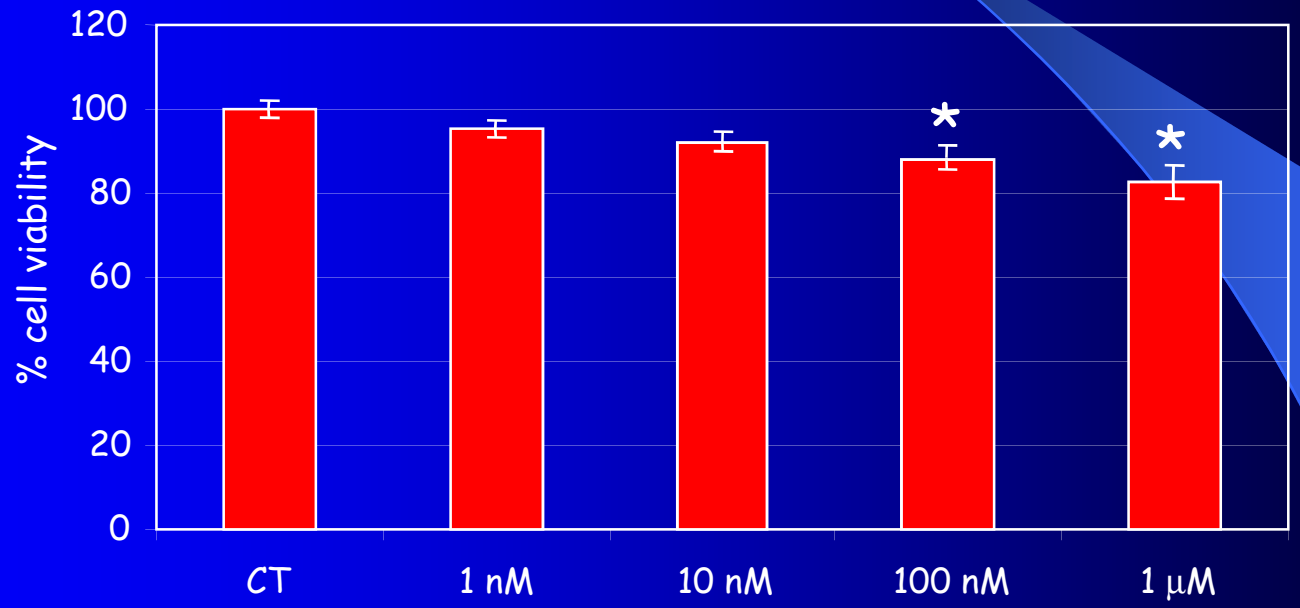
EFE 2012



Colture primarie

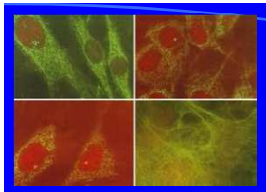
pituitary adenoma

RAD001 in 7 GH-omas



*P<0.05

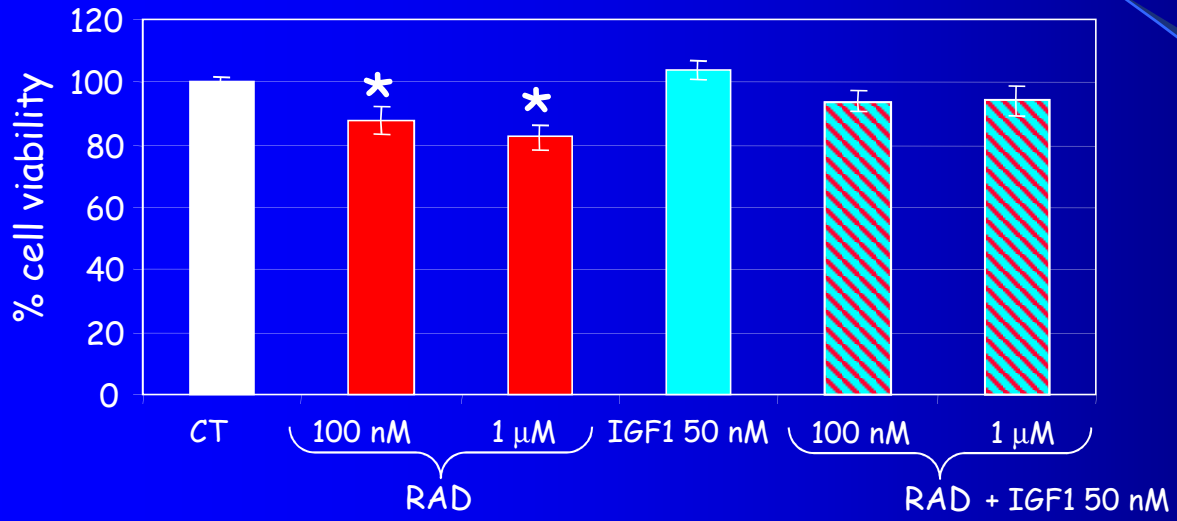
RAD001 dose-dependently
reduced somatotropinoma cell viability in vitro



Colture primarie

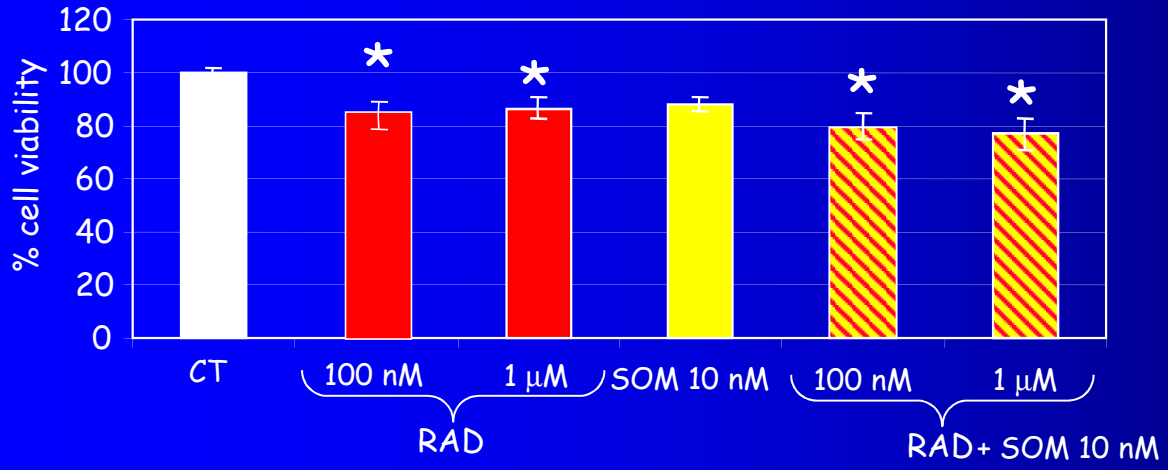
pituitary adenoma

RAD001 in GH-omas



RAD001 is blocked by IGF-1

*P<0.05; **P<0.01



SOM230 enhances RAD001 effects

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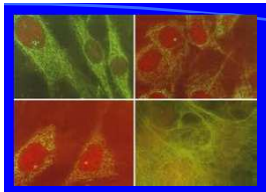
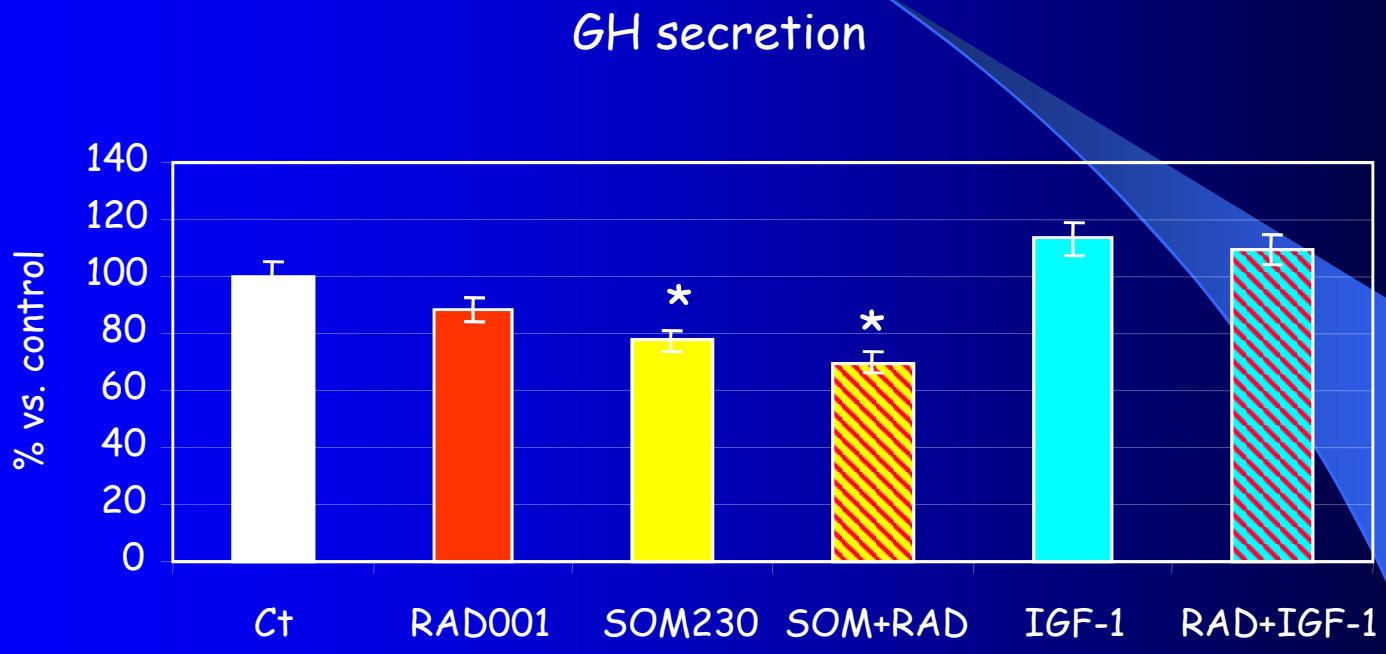


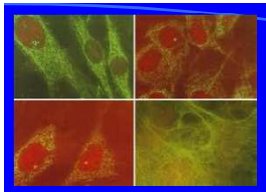
Colture primarie

pituitary adenoma

RAD001 in GH-omas



RAD001 reduces GH secretion
IGF-1 co-treatment blocks this effect



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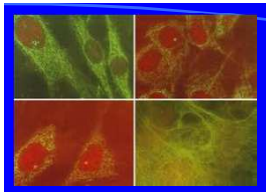
ACTH-omas



Colture primarie

pituitary adenoma

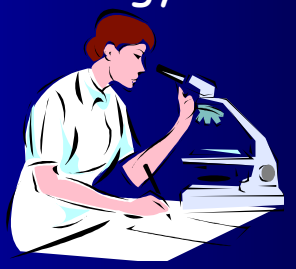
Pituitary Unit

TNS surgery

10 ACTH-secreting pituitary adenomas

Biomolecular analysis

Pathology



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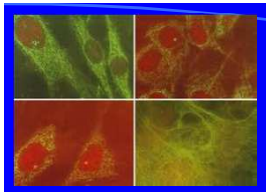


Colture primarie

pituitary adenoma

Tissue bank

Freezer -80°C

Tissue

Primary coltures

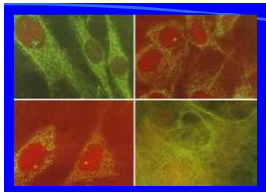
In vitro experiments:
cell viavility (colorimetric method)
apoptosis with caspase 3/7 assays
mTOR phosphorylation by ELISA

50 nM IGF-1

10 nM -1 μM RAD001

10 nM SOM230

EFE 2012

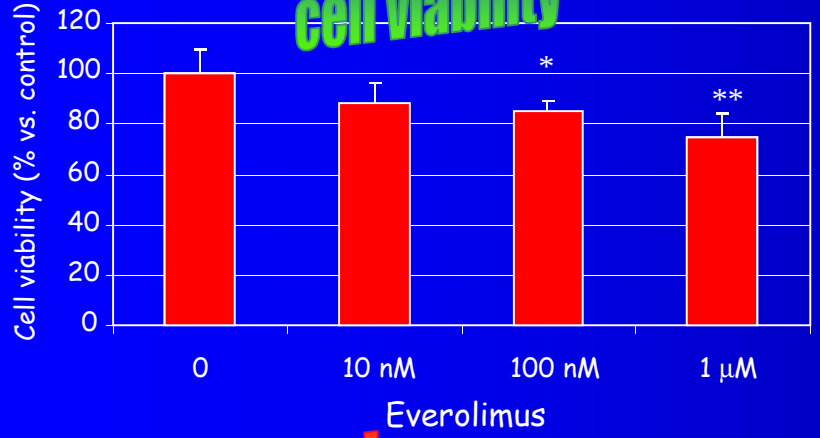


Colture primarie

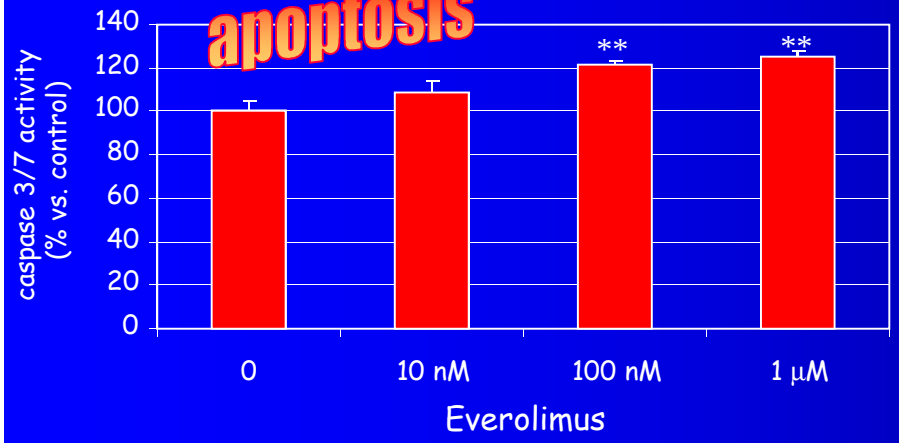
pituitary adenoma

RAD001

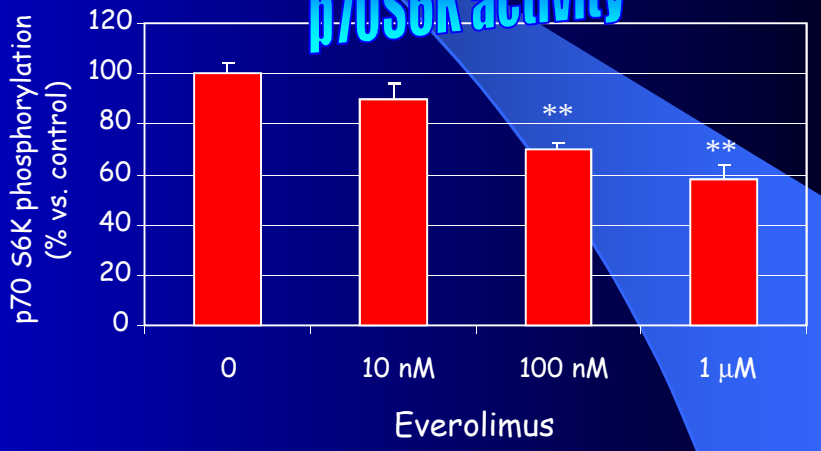
cell viability



apoptosis



p70S6K activity



*P<0.05; **P<0.01

RAD001 dose-dependently reduces cell viability, induces apoptosis and inhibits p70S6K phosphorylation

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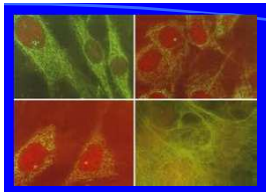
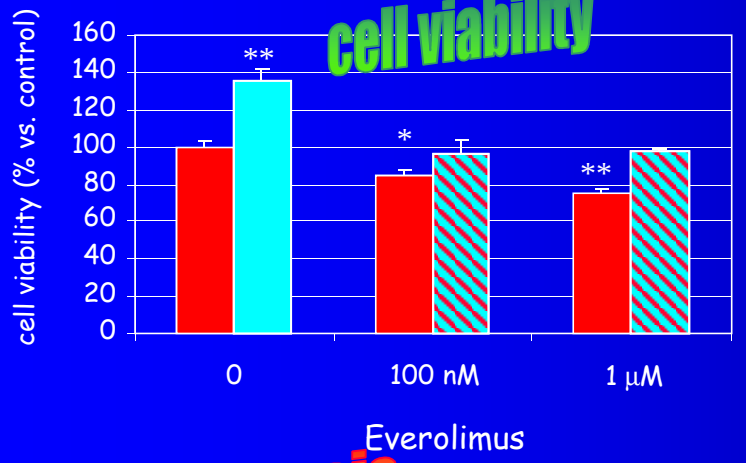


Colture primarie

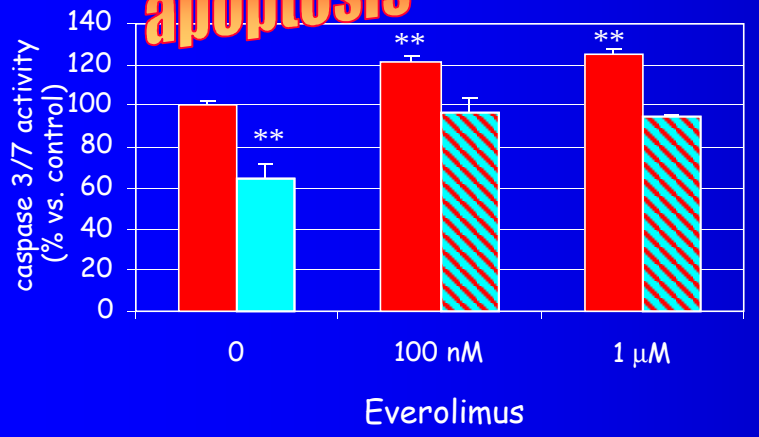
pituitary adenoma

IGF-I

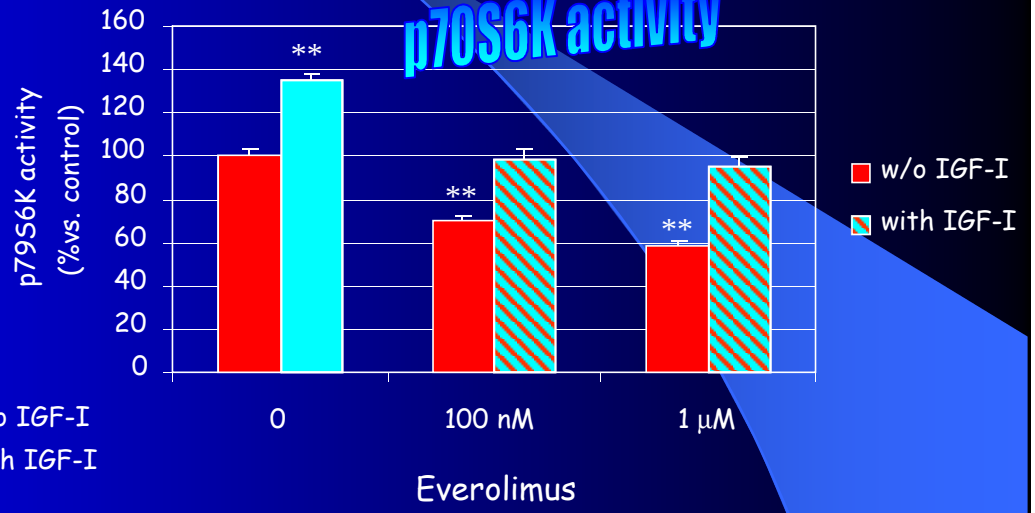
cell viability



apoptosis



p70S6K activity



*P<0.05; **P<0.01

RAD001 blocks the effects of IGF-I on cell viability, apoptosis and p70S6K phosphorylation

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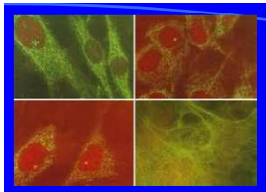
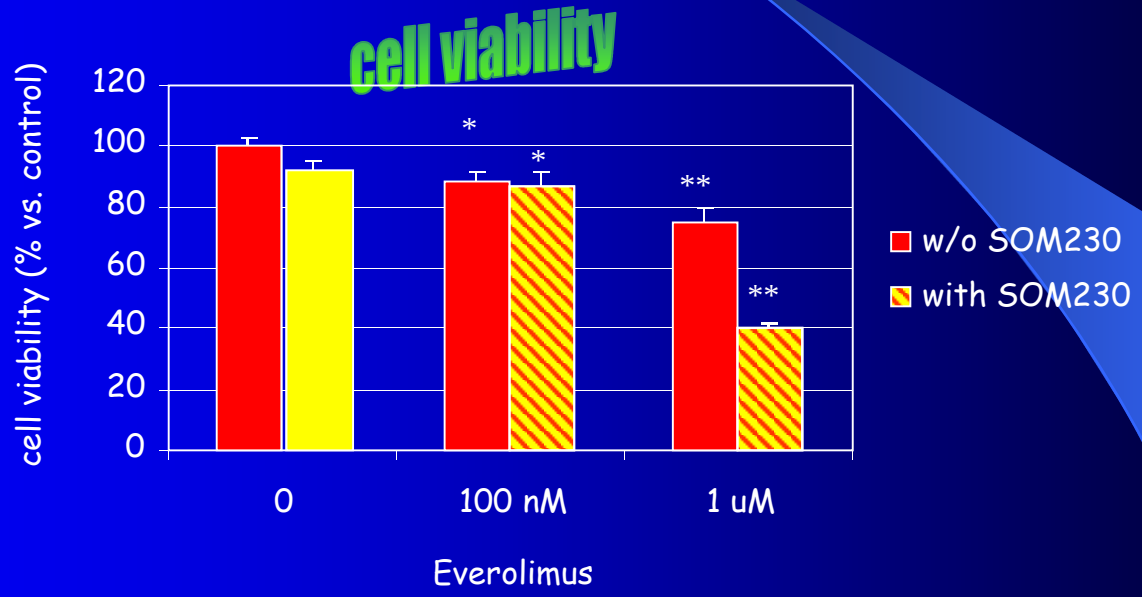


Colture primarie

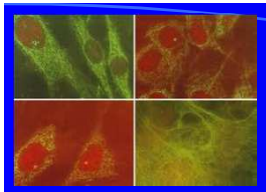
pituitary adenoma

SOM230



*P<0.05; **P<0.01

SOM230 enhances the antiproliferative effects of RAD001



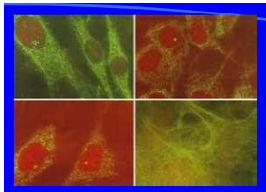
Colture primarie

pituitary adenoma

CONCLUSION

- RAD001 dose-dependently inhibits ACTH-secreting pituitary adenoma cell viability in primary culture by inducing apoptosis and inhibiting p70S6K activity
- These effects are blocked by IGF-I
- SOM230 enhances RAD001 effects

everolimus might represent a possible medical treatment aiming at controlling ACTH-secreting adenomas

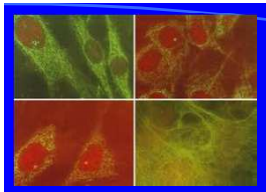


Colture primarie

pituitary adenoma

CONCLUSION

These preliminary results indicate that everolimus might represent a possible medical treatment aiming at controlling pituitary adenoma growth



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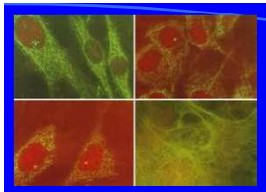
FUTURE PERSPECTIVES

- Evaluate antisecretory activity on GEP NETs
- Evaluate the possible influence of RAD001 on chemoresistance
- Evaluate the possible influence of mTOR inhibition of GH peripheral effects



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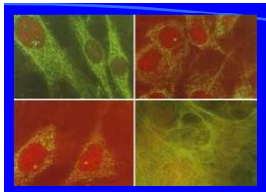

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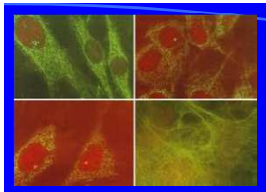

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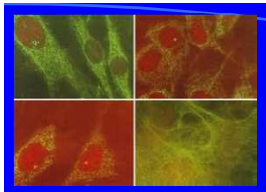

Colture primarie

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