Hedgehog embrioģenēzi regulējošais signālceļš un sīkšūnu plaušu vēža rezistence.

Viktors Kozirovskis (PSKUS)

15.06.2011

Latvijas dati: 2009. gads

Jaunatklātas onkoloģiskās saslimšanas		10020	
	♂		4924
	♀		5096
Plaušu vēzis		1119	
	♂		896
	♀		223

Uzskaitē 2320 plaušu vēža slimnieki

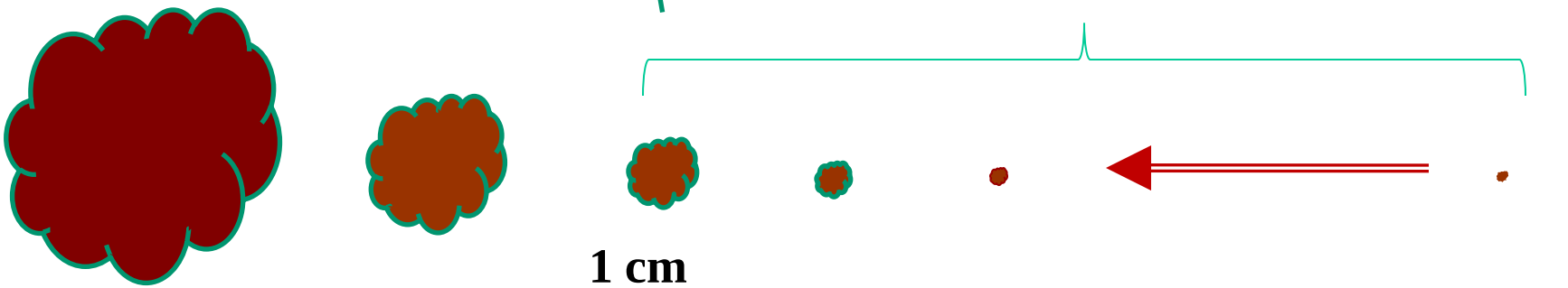
	Sīkšūnu	Ne-sīkšūnu
Īpatsvars	15 %	85 %
Šūnu dubultošanās laiks	30 dienas	100 dienas
Primāra terapija	Ķīmijterapija	Ķirurģija Staru terapija Ķīmijterapija

10 X 100 = 1000 dienas
līdz 1 kg audzēja !

8 - 10 gadi !

100 dienas

audzēja masas
dubultošanās 30 reizes



4 cm **2 cm** **1 cm (1 g) (10 ^{9})** **0,5 cm** **0,25 cm** **1 šūna**

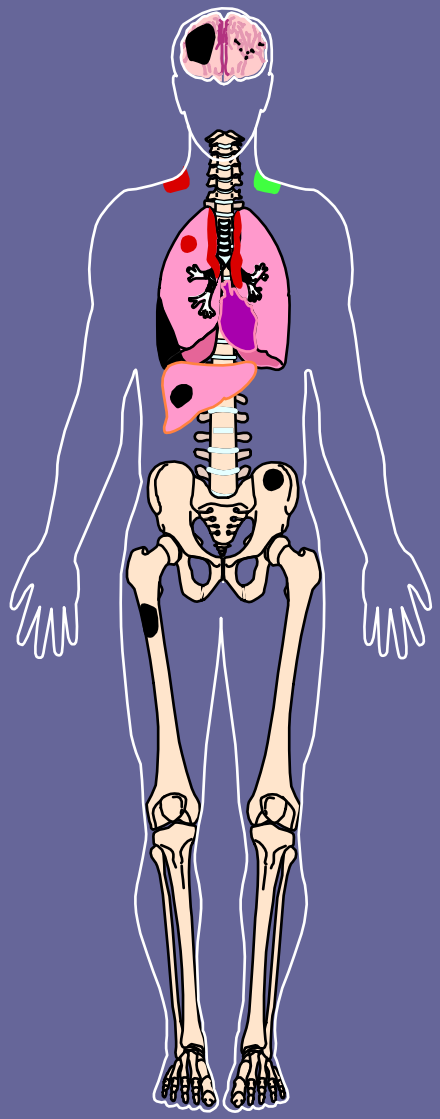
30 dienas

10 X 30 = 300 dienas
līdz 1 kg audzēja !

2,5 gadi !

Sīkšūnu plaušu vēža stadijas

- **Lokalizēts**
- ?
- **Izplatīts**



1/3 Lokalizēts

2/3 Izplatīts

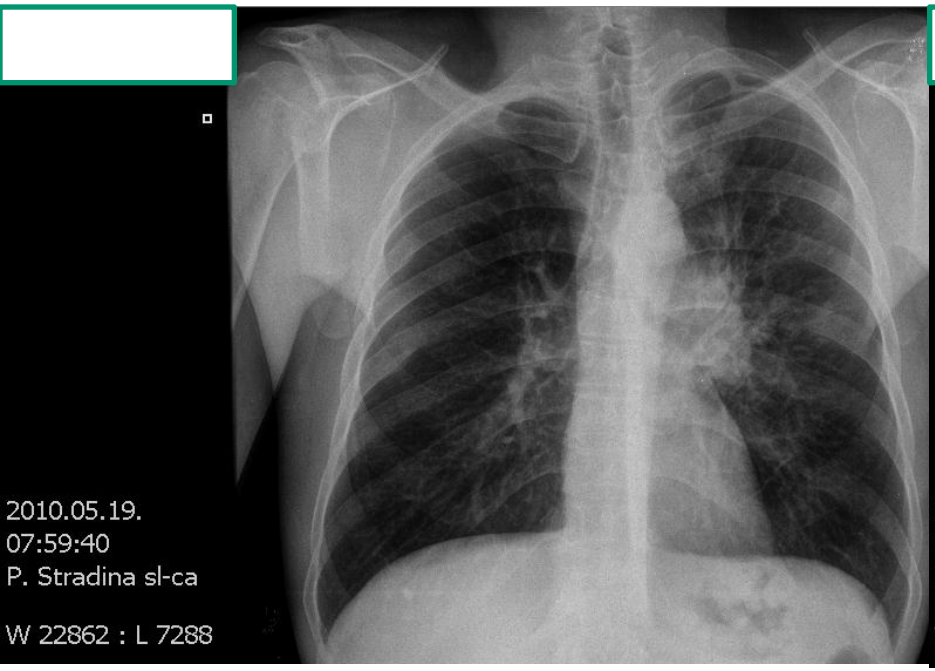
Sīkšūnu plaušu vēzis līdz ķīmijterapijas ērai (1960-ie gadi)

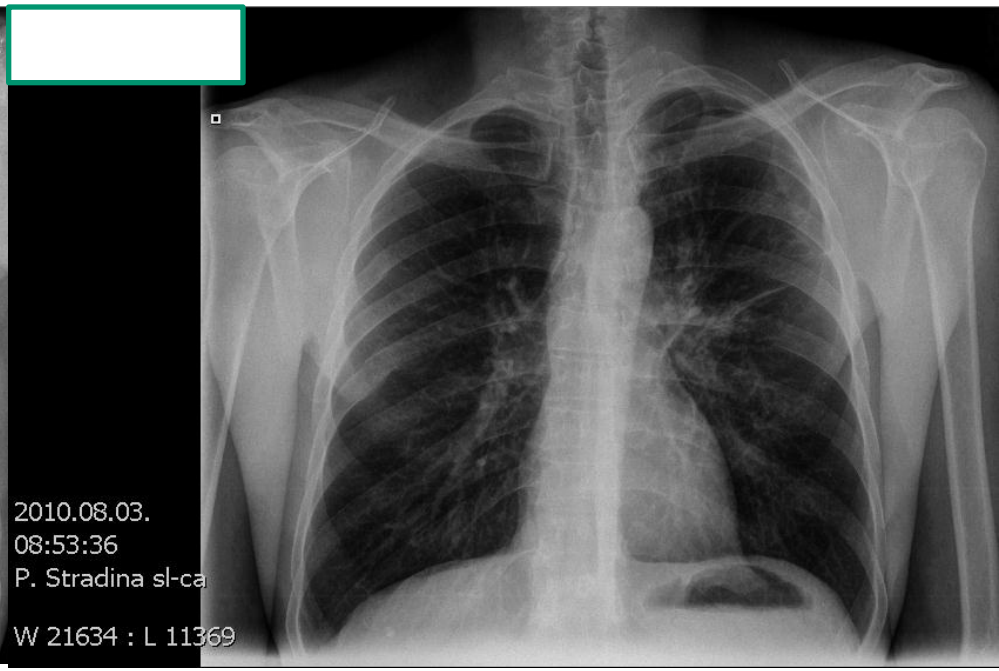
	Vidējā dzīvildze
• Lokalizēts (LD)	3 mēneši
• Izplatīts (ED)	1 mēnesis

Zelen, M. Keynote address on biostatistics and data retrieval. Cancer Chemother Rep 3 1973; 4:31.

Sīkšūnu plaušu vēzis ķīmijterapijas ērā (no 1970-iem gadiem)

Stage	Response rate		OS	2 yr	5 yr
	CR	CR+PR	Median		
	survival		survival		
LD	40-60%	>80%	14-20 Mo	20-40%	10%
ED	15-30%	70-80%	7-10 Mo	<5%	<1%
relapsed	variable		~ 4 Mo	<1%	


2010.05.19.
07:59:40
P. Stradina sl-ca
W 22862 : L 7288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08:53:36
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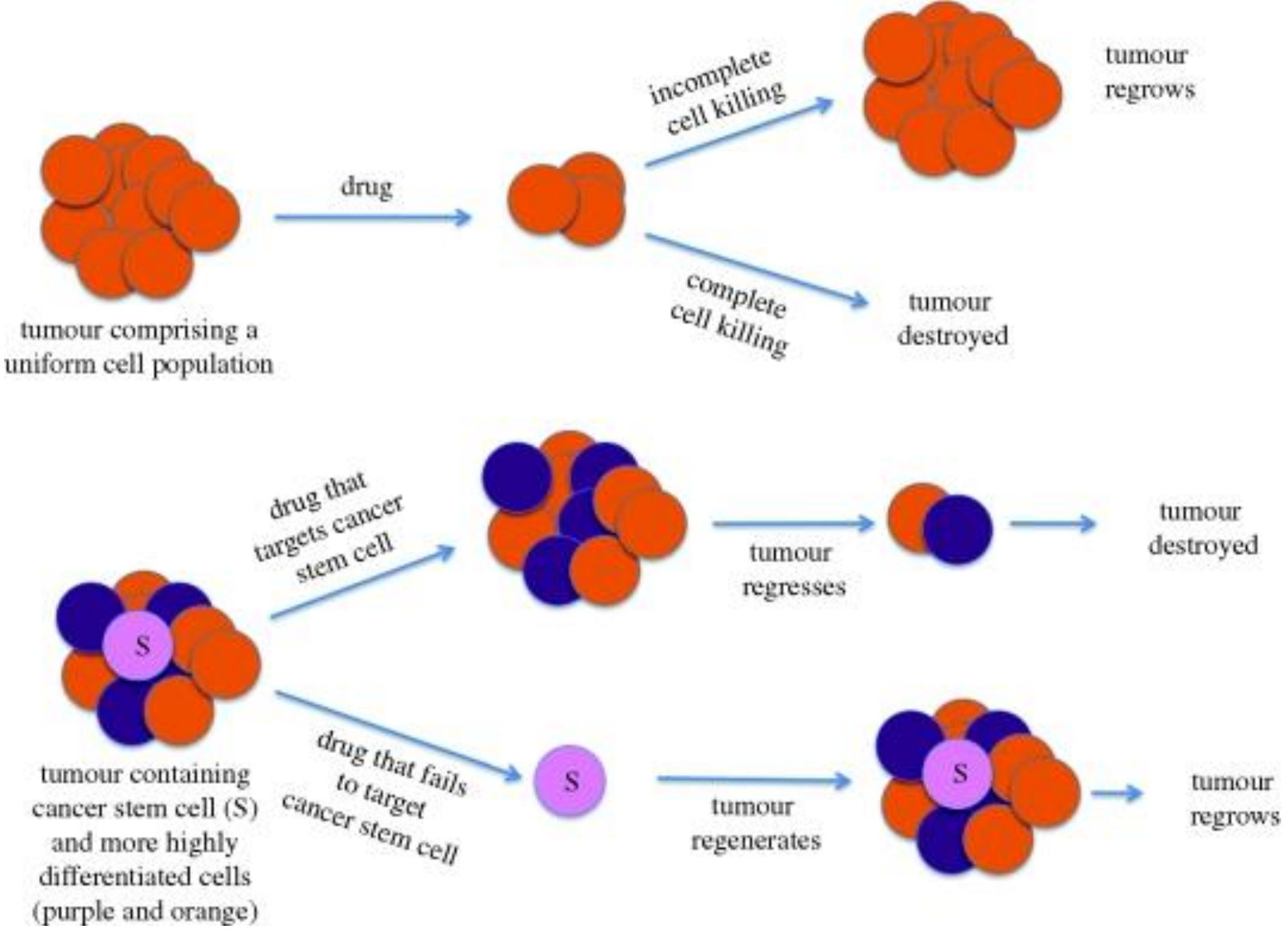
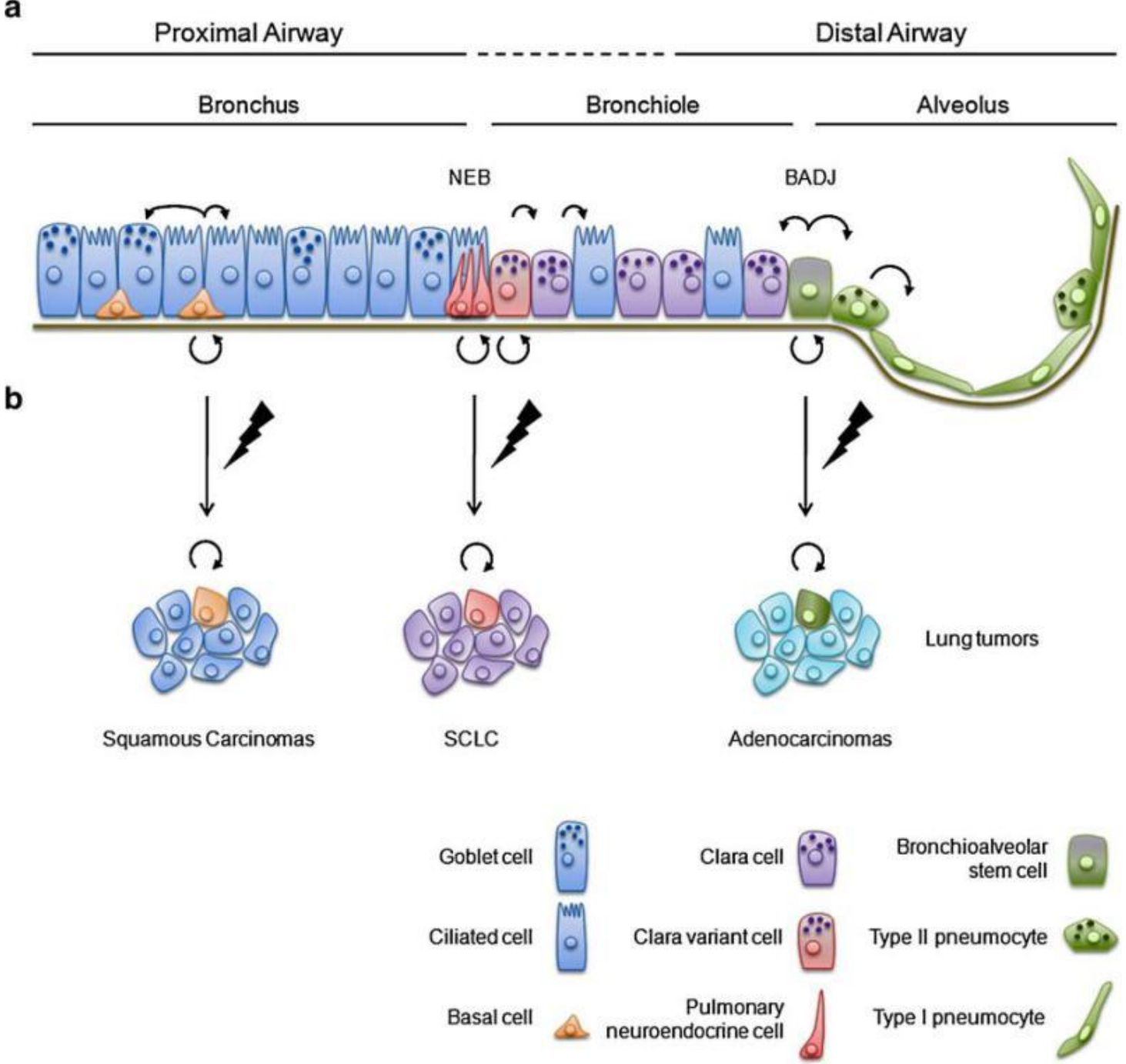
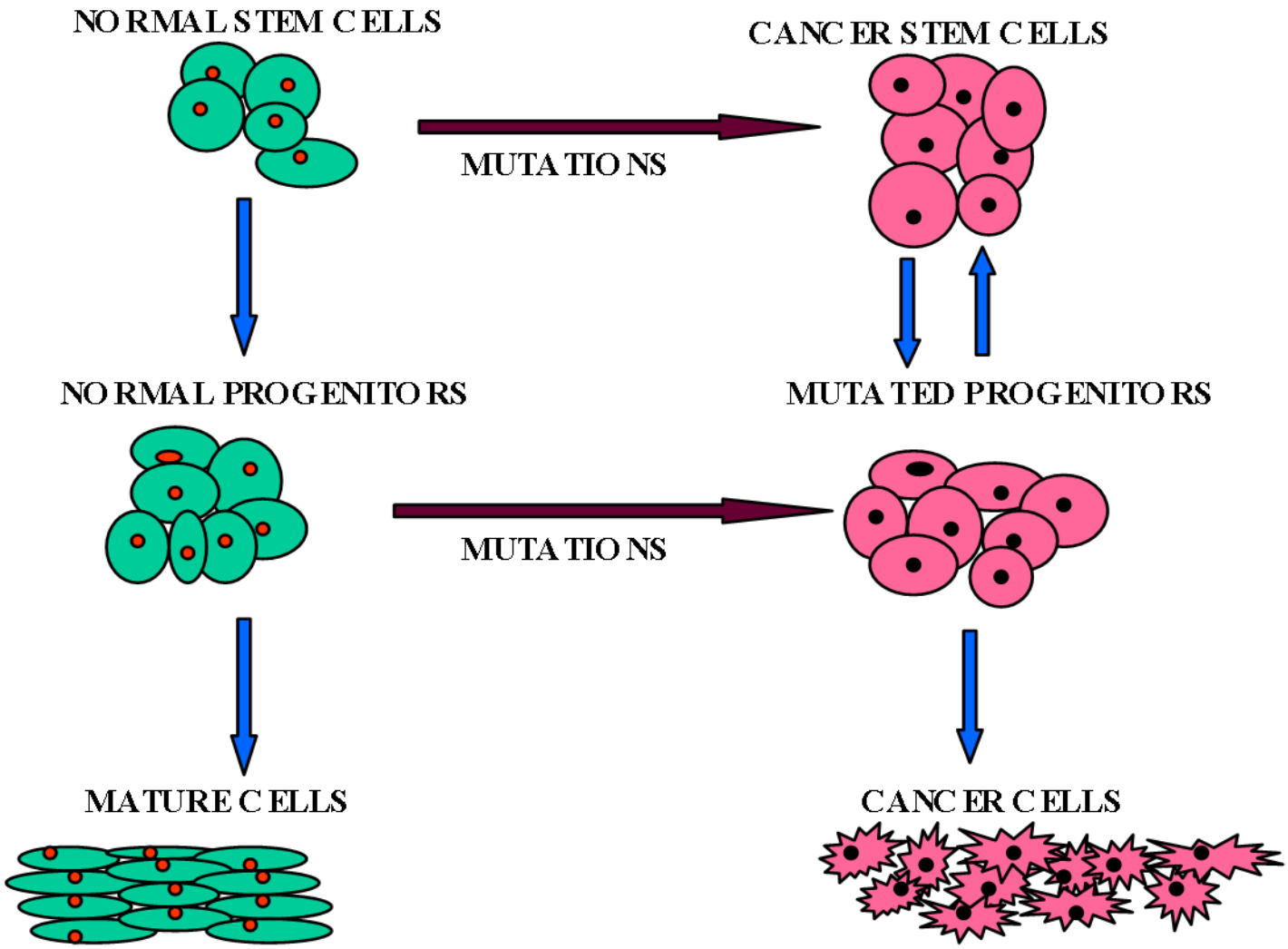


Philos Trans R Soc Lond B Biol Sci. 2010 January 12; 365(1537): 155–163.



Cancer Metastasis Rev. 2010 March; 29(1): 61–72.



Sagar et al. Cancer Cell International 2007 7:9

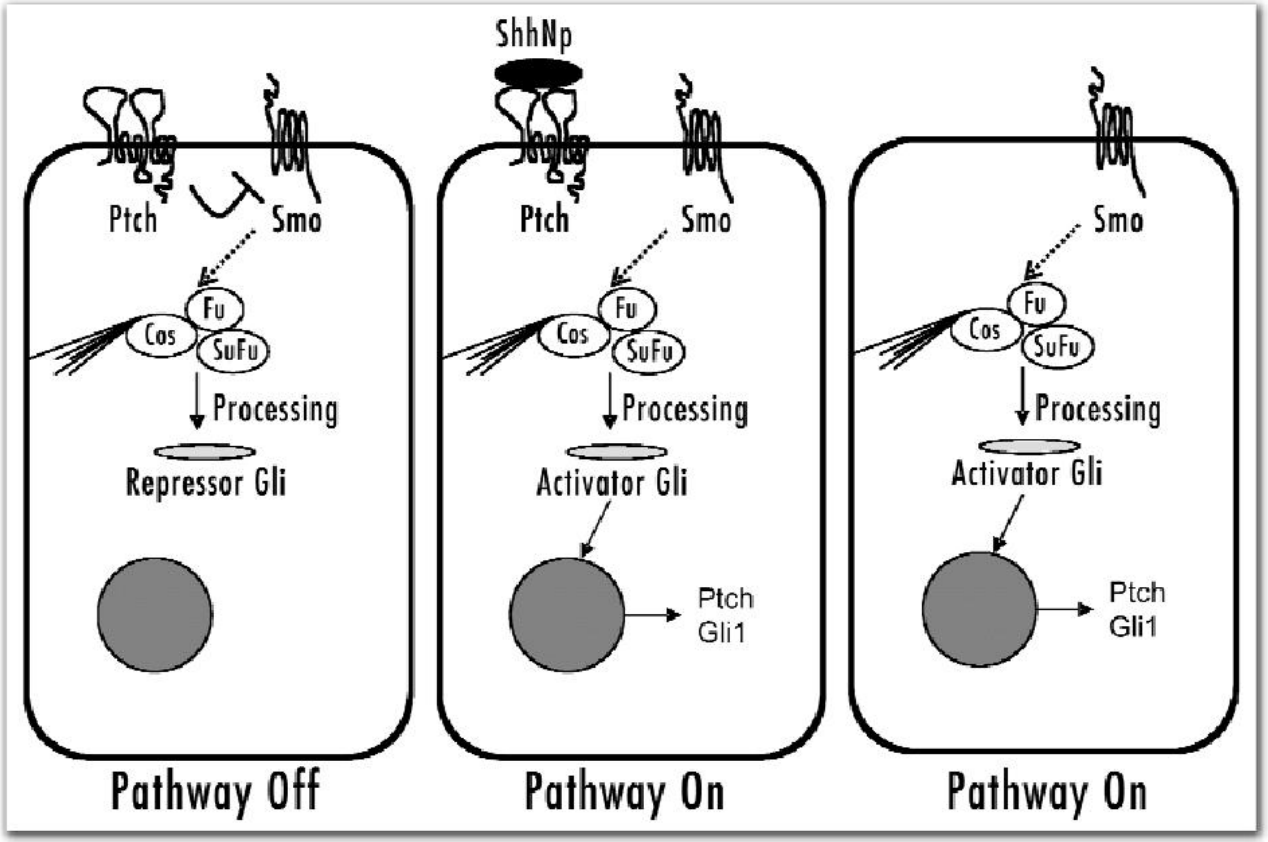


Figure 1. A Simplified model of hedgehog (Hh) signaling. In the absence of the ligand Sonic Hedgehog (Shh), the receptor Patched (Ptch) constitutively inhibits Smoothened (Smo). This promotes cytoplasmic tethering and processing of the Gli transcription factors by a multi-protein, microtubule associated complex consisting of the kinase Fused (Fu), the novel protein Suppressor of Fused (SuFu) and Costal2 (Cos). Processing favors repressor forms of Gli proteins, which silence the Hh transcriptional program. Binding of Shh inhibits Ptch. This in turn de-represses Smo, releasing unprocessed Gli activators from the complex to promote transcription of Hh targets in the nucleus, including Gli1 and Ptch. Similar pathway activation is achieved in the absence of Ptch function through mutation or epigenetic silencing.

[Cell Cycle 2:3, 196-198, May/June 2003];

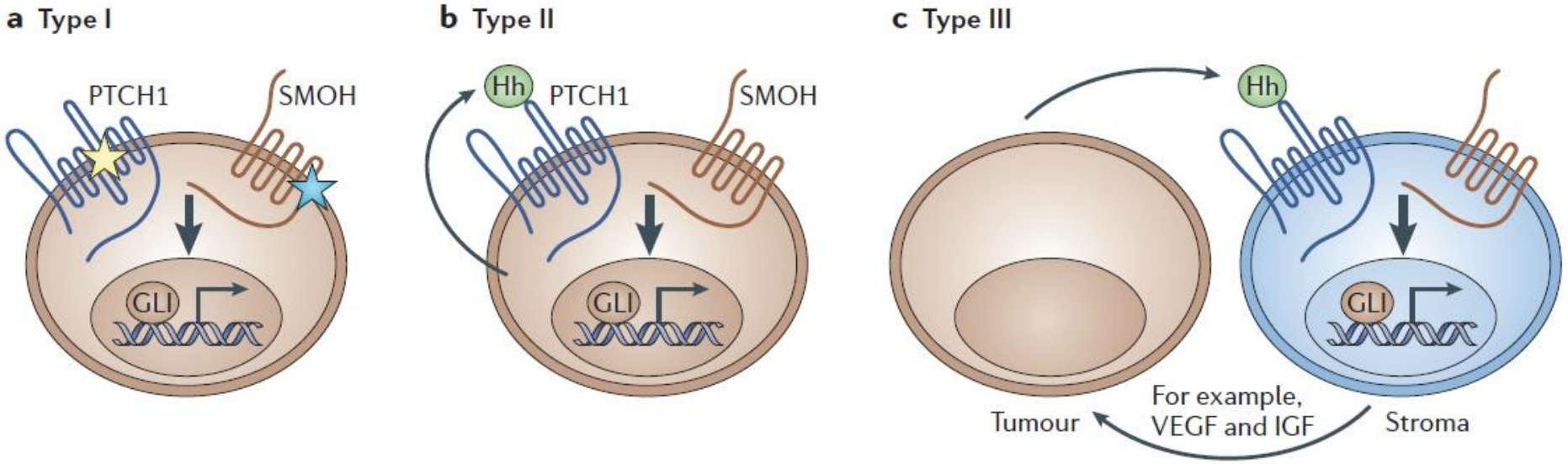


Figure 2 | **Models of Hh pathway activation in cancer. a** | Type 1: loss-of-function mutations in Patched 1 (PTCH1; yellow star) or gain-of-function mutations in Smoothened (SMOH; blue star) lead to constitutive Hedgehog (Hh) pathway activation. **b** | Type 2: autocrine model in which tumour cells produce and respond to Hh ligand. Pathway activation may occur in all tumour cells or in a small number of tumour stem cells. **c** | Type 3: paracrine model in which tumour cells produce Hh ligand and surrounding stromal cells respond by producing additional growth factors to support tumour growth or survival, for example. IGF, insulin-like growth factor; VEGF, vascular endothelial growth factor.

Nature Reviews Drug Discovery 5, 1026-1033 (December 2006)

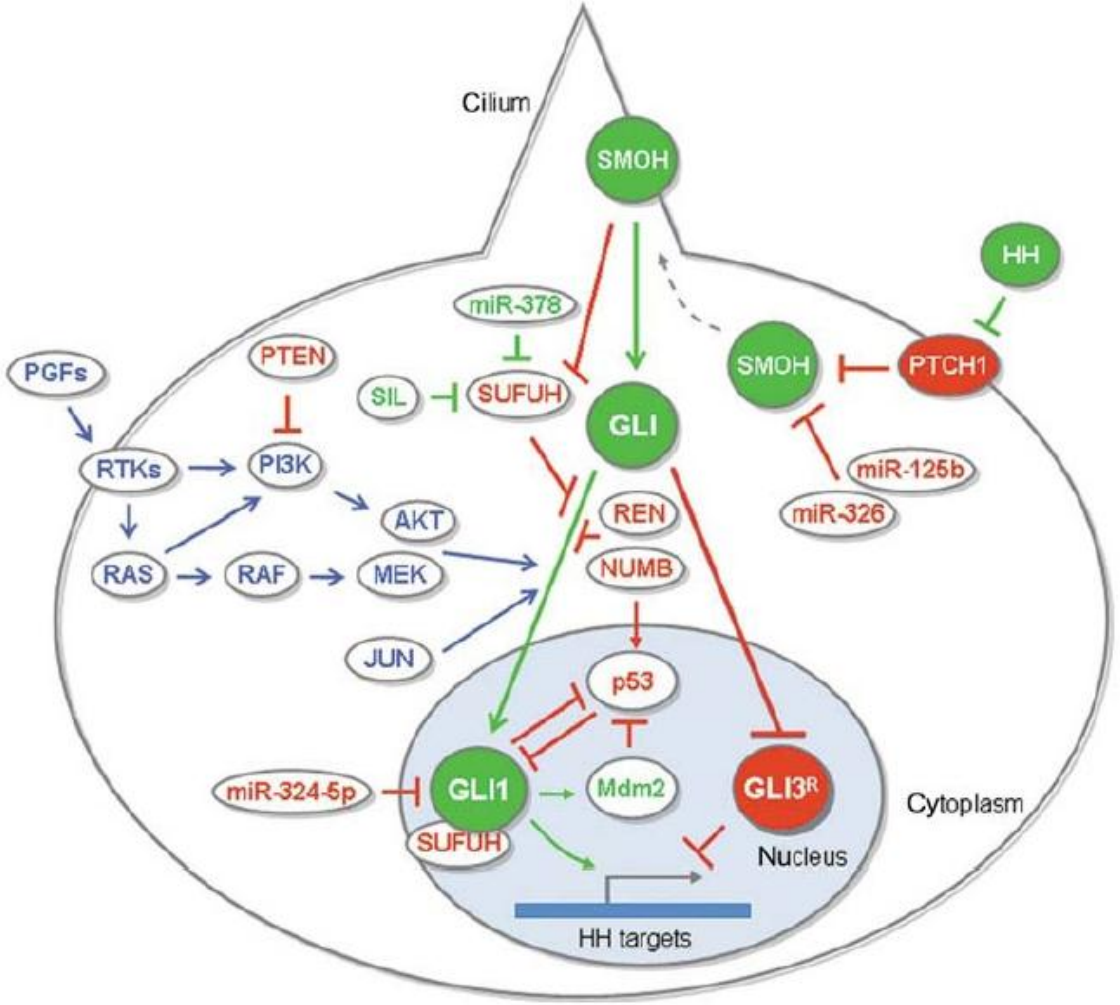


Figure 2 Integration of oncogenic and tumor suppressor inputs by the GLI code in cancer. Upon inhibition of PTCH1 function by HH ligands, the repression on SMOH is released, SMOH moves into the primary cilium and activates downstream signaling by stabilizing activating full-length GLI proteins (GLI1) and blocking the production of GLI repressors (GLI3R). The mammalian GLI code includes three proteins. Generally, GLI1 is an activator although it exists in N′ and C′ Δ deleted activator and repressor forms, respectively, GLI2 has activator and C′ Δ repressor functions and GLI3 is a weak activator and its C′ Δ form is a strong repressor. Components of the classical HH pathway are in filled circles, in red for inhibitors and in green for activators. Positive and negative regulators of HH-GLI signaling are in unfilled circles, in blue for the PGF-RTK-RAS-RAF-MEK, PI3K-AKT and JUN pathways, in green for activators and in red for repressors. The color of the arrow is dictated by the final effect on the GLI code: red arrow for a final repressive effect, green arrow for a final activating effect on the GLI code. See text for further details.

Table 1. List of Representative Hedgehog Target Genes

Function	Gene	Direct or Indirect	References
Positive feedback	*GLI1*	Direct target	Yoon *et al.* 2002
Negative feedback	*PTCH1*	Direct target	Yoon *et al.* 2002
	PTCH2	Direct target	Vokes *et al.* 2007
	HHIP1	Direct target	Chuang & McMahon, 1999 Vokes *et al.* 2007
Proliferation	*MYCN*	Direct target	Kenney *et al.* 2003 Hallikas *et al.* 2006
	CCND1	Direct target	Kasper *et al.* 2006
	CCND2	Direct target	Yoon *et al.* 2002
	CCNE		Kenney & Rowitch, 2000
	FOXM1		Teh *et al.* 2002
	CCNB1	Indirect target	Schüller *et al.* 2007
	CDC25B	Indirect target	Schüller *et al.* 2007
Stem-cell signaling network	*JAG2*	Direct target	Kasper *et al.* 2006
	FST	Direct target	Eichberger *et al.* 2008
	GREM1	Direct target	Vokes *et al.* 2008
	BMP4	Indirect target	van den Brink *et al.* 2001 Katoh & Katoh, 2006
	WNT2B		Bonifas *et al.* 2001
	WNT5A		Bonifas *et al.* 2001
	PDGFRA		Xie *et al.* 2001

Stem-cell marker	*BMI1*		Leung *et al.* 2004 Liu *et al.* 2006 Sangiorgi & Capecchi, 2008
	LGR5		Barker *et al.* 2008 Tanese *et al.* 2008
	CD44		Chen *et al.* 2007
	CD133		Clement *et al.* 2007
Survival	*BCL2*	Direct target	Regl *et al.* 2004
	CFLAR	Direct target	Kump *et al.* 2008
Epithelial-to-mesenchymal transition	*FOXC2*		Yamanashi *et al.* 2003 Hallikas *et al.* 2006
	SNAI1		Li *et al.* 2007
	TWIST2		Li *et al.* 2007
	ZEB1		Katoh & Katoh, 2008
	ZEB2		Katoh & Katoh, 2008
Others	*FOXF1*	Direct target	Mahlapuu *et al.* 2001 Madison *et al.* 2009
	FOXL1	Direct target	Hallikas *et al.* 2006 Madison *et al.* 2009
	PRDM1	Direct target	Vokes *et al.* 2008
	PTHLH		Sterling *et al.* 2006

Oncogene (2007) 26, 5674–5679

www.nature.com/onc

SHORT COMMUNICATION

Sonic Hedgehog promotes multiple drug resistance by regulation of drug transport

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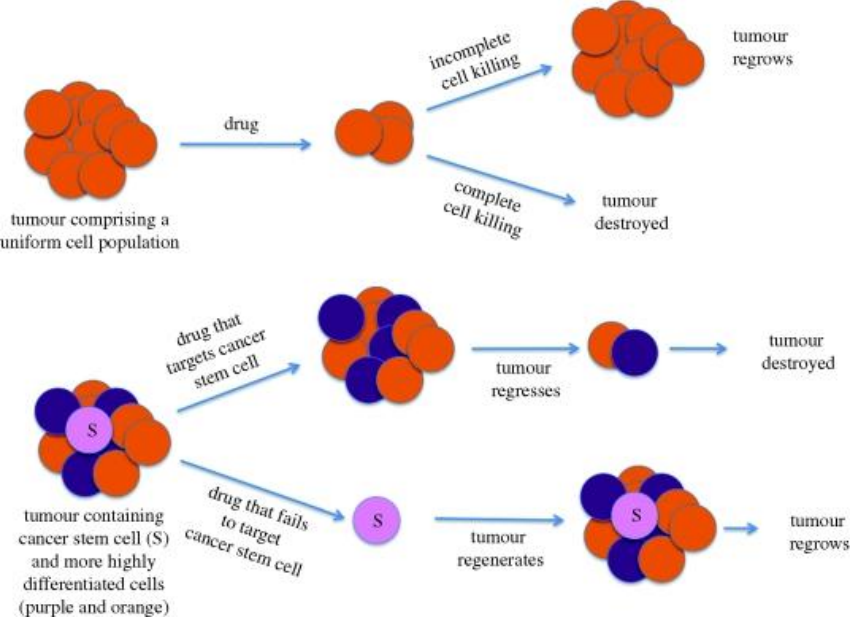
A major obstacle to successful chemotherapy is intrinsic or acquired multi-drug resistance (MDR). The most common cause of MDR involves increased drug efflux from cancer cells mediated by members of the ATP-binding cassette (ABC) transporter family. The regulation of ABC transporters in the context of cancer is poorly understood, and clinical efforts to inhibit their function have not been fruitful. Constitutive activation of the Hedgehog (Hh) pathway has been shown to contribute to the growth and maintenance of various cancers. Here, we show that inhibition of Hh signaling increases the response of cancer cells to multiple structurally unrelated chemotherapies. We further show that Hh pathway activation induces chemoresistance in part by increasing drug efflux in an ABC transporter-dependent manner. We found that Hh signaling regulates the expression of the ABC transporter proteins multi-drug resistance protein-1 (MDR1, ABCB1, P-glycoprotein) and (BCRP, ABCG2), and that targeted knockdown of MDR1 and BCRP expression by small interfering RNA partially reverses Hh-induced chemoresistance. These results suggest that the Hh pathway may be a target to overcome MDR and increase chemotherapeutic response.
Oncogene (2007) **26**, 5674–5679; doi:10.1038/sj.onc.1210356; published online 12 March 2007

certain classes of chemotherapeutic agents and toxins from cells, causing a decrease in intracellular drug accumulation and rendering the agents ineffective. To date, 48 members of the ABC transporter family have been identified in humans (Szakacs *et al*., 2006). These genes are classified into seven structural subfamilies (ABCA through ABCG) which have a broad range of expression in normal and malignant tissues. Although ABC transporters are highly expressed in stem cells and during tissue regeneration, their regulation in the context of cancer is poorly understood (Szakacs *et al*., 2006).

The hedgehog (Hh) signaling pathway is critical for growth and differentiation during embryonic development and is required for maintenance of somatic stem cells (Ingham and McMahon, 2001). Hh ligands (Sonic, Desert and Indian) bind to and antagonize the cell surface receptor patched (PTCH), relieving the PTCH-mediated suppression of the transmembrane protein smoothened (SMO). SMO then initiates an intracellular signaling cascade that leads to the activation and nuclear translocation of the Gli family of transcription factors (Gli-1, 2 and 3). Gli family members mediate transcription of genes controlling proliferation, differentiation and survival (Ingham and McMahon, 2001;



Hipotēze:

Embrioģenēzi regulējošā signālceļa Hedgehog aktivitātei ir nozīme sīkšūnu plaušu vēža rezistences attīstībā

Hedgehog signālceļa paaugstināta aktivitāte:

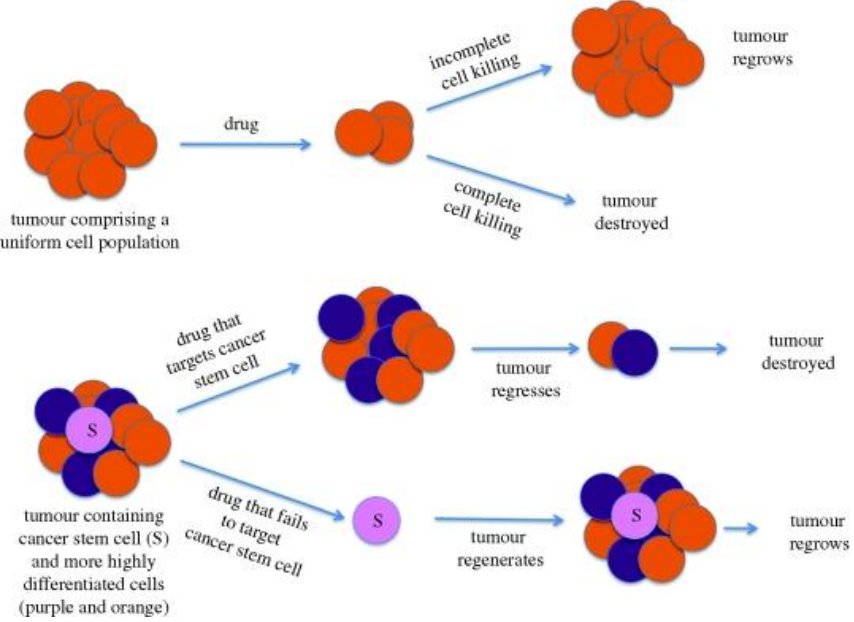
1. raksturīga vēža cilmšūnām

pie sīkšūnu plaušu vēža:

2. aprakstīta sīkšūnu plaušu vēža šūnu līnijās un audzēju paraugos
3. "In vitro" pierādījumi par to saistību ar audzēja šūnu proliferāciju

pie citiem audzējiem:

4. saistīta ar rezistenci pret terapiju
5. uztur audzēja šūnu proliferāciju, nomāc apoptozi
6. saistīta ar klīniski nelabvēlīgu slimības gaitu

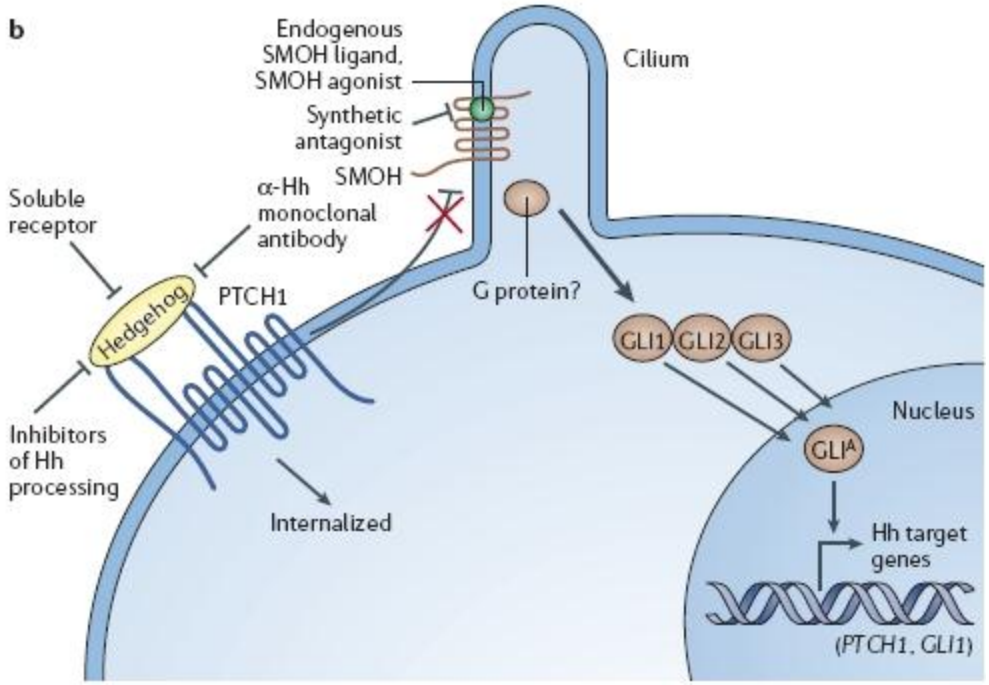


Mērķis:

Izvērtēt, vai ir atšķirība starp Hedgehog signālceļa aktivitāti sīkšūnu plaušu vēža šūnās recidīva laikā, salīdzinot ar stāvokli pirms terapijas

Pētījumu skaits, kas novērtē izmaiņas sīkšūnu plaušu vēža šūnās slimības gaitā līdz šim ir stipri ierobežots.

Literatūrā pagaidām nav datu par pētījumiem, kas analizē Hedgehog signālceļa aktivitāti un slimības klīnisku gaitu sīkšūnu plaušu vēža slimniekiem.

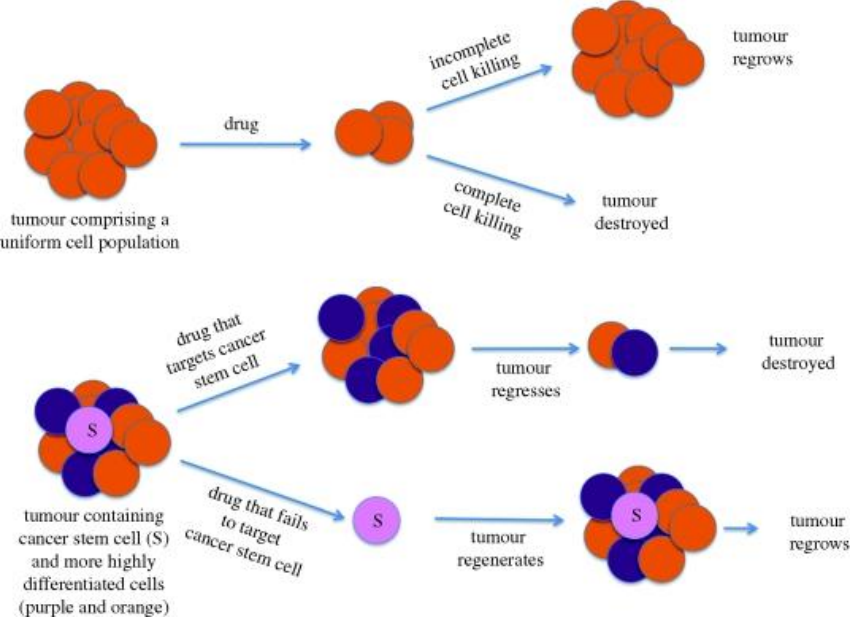


Materiāli un Metodes:

1. Sīkšūnu plaušu vēža pacientu audzēju biopsiju paraugu iegūšana pirms terapijas un recidīva laikā

2. Hedgehog signālceļa mērķa gēnu (Gli1, PTCH1, citi?) ekspresijas analīze pētījuma sīkšūnu plaušu vēža pacientu audzēju biopsiju paraugos, kas ir ņemti pirms terapijas un recidīva laikā, izmantojot reālā laika kvantitatīvo RT-PCR

3. Laboratoriski iegūto datu un pētījuma pacientu klīniskas informācijas analīze.



Paredzamais darba apjoms
25-30 pacienti

(5-10 pacienti gadā).

Sagaidāmie rezultāti:

1. Zinātnisks pamats pielietot Hedgehog signālceļu inhibējošos medikamentus sīkšūnu plaušu vēža ārstēšanā.

2. Jauni prognostiski biomarķieri terapijas efektivitātes izvērtēšanai.