X Congresso Internacional de Uro-Oncologia

V SIMPÓSIO MULTIPROFISSIONAL DE URO-ONCOLOGIA

4 a 6 de Abril de 2019

Biomarcadores em câncer de testículo

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Titular do Deptº de Oncologia Clínica do A C Camargo Cancer Center ,

Co coordenador do Centro de Referência em Urologia do A C Camargo Cancer Center

14:30 às 14:50 – Sala Matisse

Declaração de Conflitos de Interesse

De acordo com a Resolução 1931/2009 do Conselho Federal de Medicina e com a RDC 96 / 2008 da ANVISA, declaro que:

- Honorários por palestra ministradas:
  - Bayer HealthCare Pharmaceuticals
  - Produtos Roche Químicos e Farmacêuticos S.A
  - Eli Lilly do Brasil Ltda
  - Janssen Biotech, Inc
  - Laboratórios Pfizer Ltda
  - Sanofi-aventis
  - GlaxoSmithKline (GSK) Brasil LTDA
  - Bristol-Myers Squibb
  - Merck Sharp & Dohme Corp
  - Novartis Biociências S.A

- Investigador Principal ou Sub[illegible]linicos das seguintes industrias farmacêuticas:
  - AMGEN
  - Bristol-Myers Squibb
  - Laboratórios Pfizer Ltda
  - Produtos Roche Químicos e Farmacêuticos S.A
  - Novartis Biociências S.A.
  - Merck Sharp & Dohme Corp

- Participação em Congressos Nacionais e Internaci[illegible]com despesas pagas por :
  - [illegible]e Químicos e Farmacêuticos S.A
  - [illegible]sil Ltda
  - [illegible]ios Pfizer Ltda
  - [illegible]ringer Ingelheim do Brasil Química e Farmacêutica
  - Bayer HealthCare Pharmaceuticals
  - Janssen Biotech, Inc
  - Sanofi-aventis
  - Bristol-Myers Squibb

- *Advisory Board*
  - Produtos Roche Químicos e Farmacêuticos S.A
  - Novartis Biociências S.A
  - Laboratórios Pfizer Ltda
  - Sanofi-aventis
  - Boehringer Ingelheim do Brasil Química e Farmacêutica
  - Bayer HealthCare Pharmaceuticals

Nenhum para esta aula

Declaro não ter ações em bolsa de valores das empresas supracitadas.

Meus pré-requisitos para participar destas atividades são o intercâmbio científico, a autonomia do pensamento científico, a independência de opinião e a liberdade de expressão, aspectos estes respeitados pela Novartis.

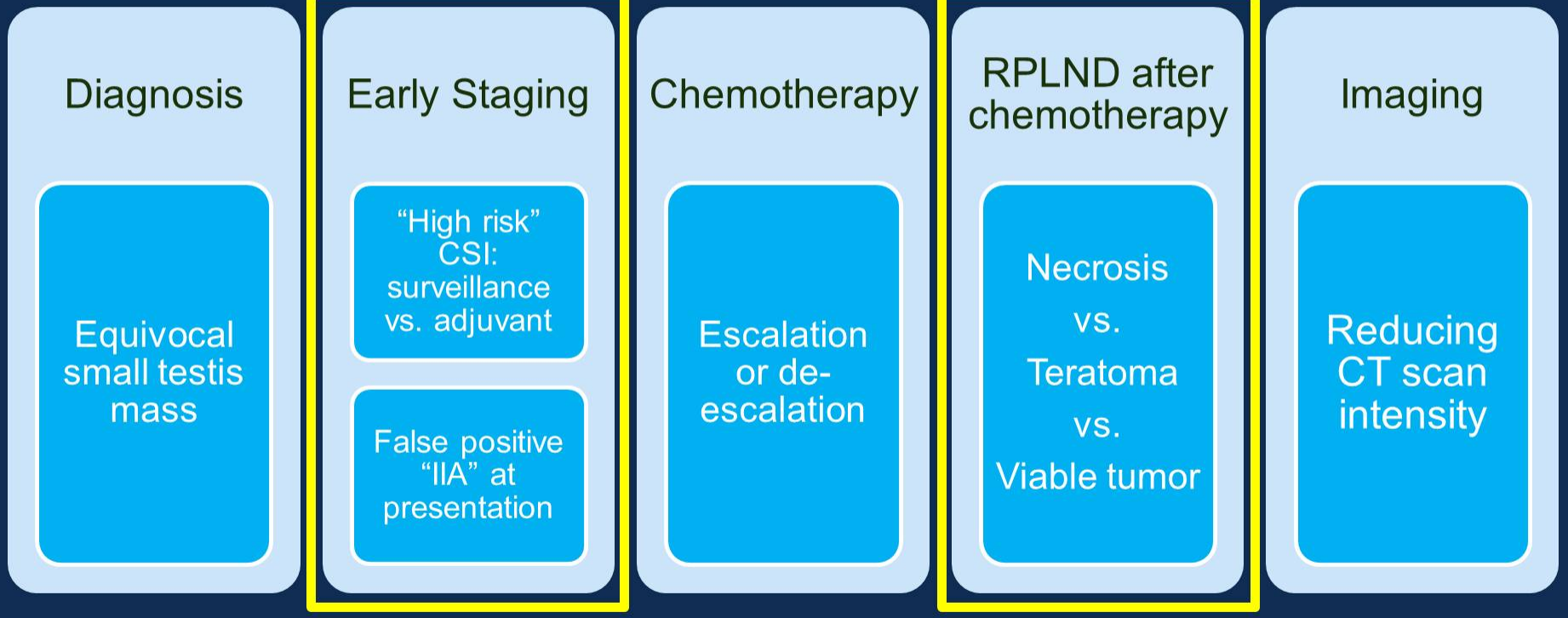
Biomarcador: Definição

- **FDA**: a characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions.

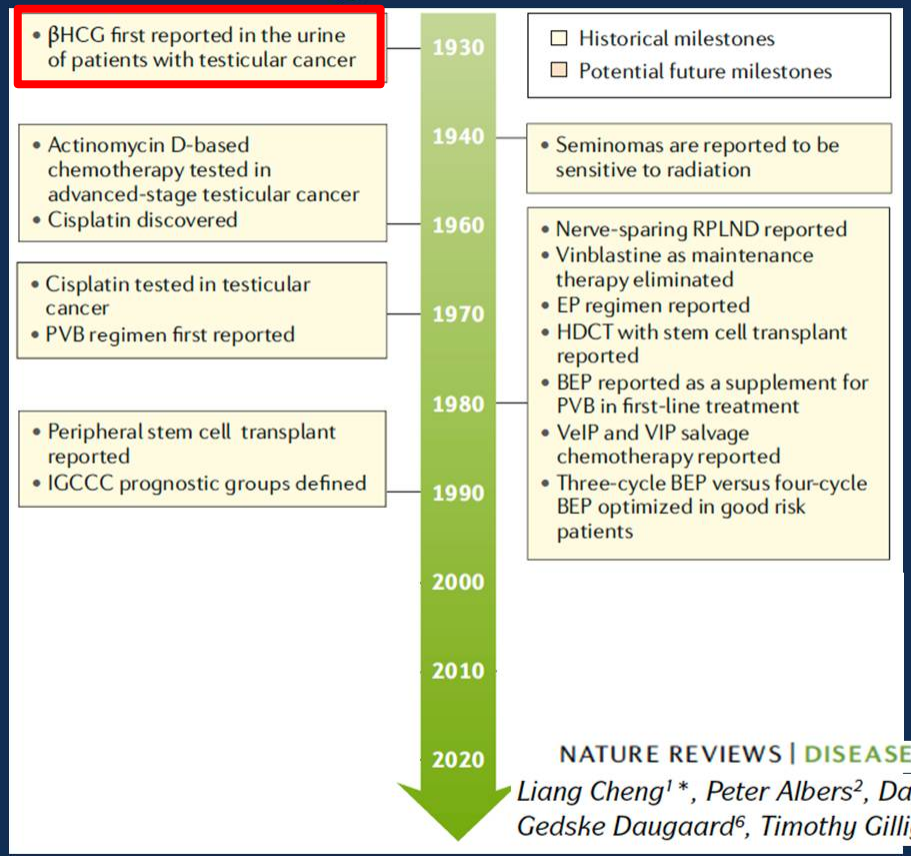
- **Types:** Molecular, histologic, radiographic, and physiologic characteristics.

http://www.ncbi.nlm.nih.gov/books/NBK326791/

Clinical States in testis cancer in need of better biomarkers



History of breakthroughs in testicular cancer



NATURE REVIEWS | DISEASE PRIMERS | Article citation ID: (2018) 4:2

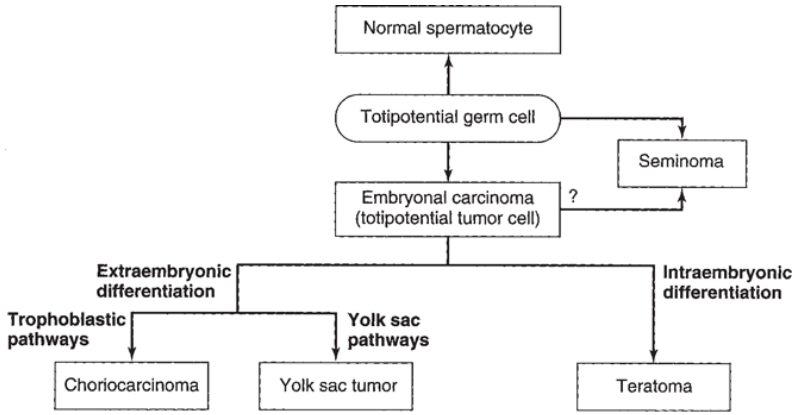
Liang Cheng[1], Peter Albers[2], Daniel M. Berney[3], Darren R. Feldman[4,5], Gedske Daugaard[6], Timothy Gilligan[7] and Leendert H. J. Looijenga[8]*

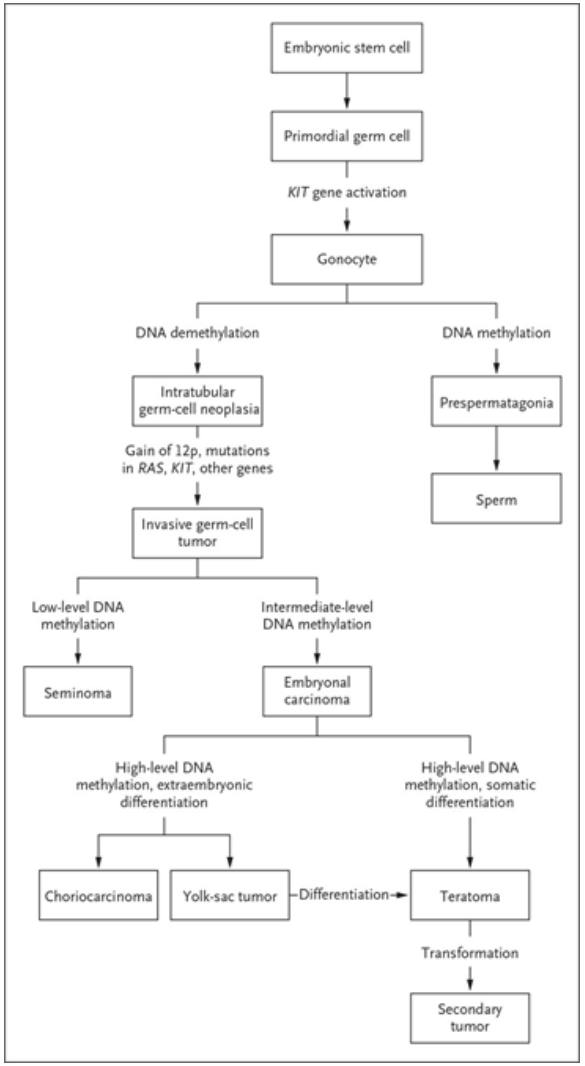
Presented by: Robert Hamilton

Marcadores Tumorais em Tumor de Testículo

- Beta- *human chorionic gonadotropin* (hCG)
- Alfa Feto Proteína (AFP)
- Lactate dehydrogenase (LDH)

- Não seminomatosos 80-85%
- Seminomas 15-25%
  - somente B-hCG ou LDH,
  - jamais AFP (em seminomas puros)





Hanna NH and Einhorn LH. N Engl J Med Nov 20;371(21):2005-16, 2014.

human Chorionic Gonadotropin (hCG)

- Maioria dos exames mensuram a subunidade beta, pois a alpha é comum a vários hormonios pituitários
- ASCO/NACB: uso de um ensaio imunométrico de duplo anticorpo que meça o beta-hCG total (tanto o dímero alfa / beta intacto como o monômero beta livre)
- Normalidade 5-10 UI/L
- Meia vida 1- 3 dias
- Varia de acordo com tipo histológico e estágio tumoral
  - 10-20% EC I NS a > 40% EC III
  - Carcinomas Embrionários, Coriocarcinoma e Mistos
- beta-hCG >50.000 UI/L => 3.5% Hipertireoidismo (ligação cruzada no receptor com TSH)
- Falso positivos:
  - Hipoganadismo => ↑ LH = reação cruzada na mensuração laboratorial com beta hCG
  - Lise tumoral pós tratamento (especialmente após C1)
  - Anticorpos heterófilos que interferem na mensuração laboratorial
  - Maconha (único relato)
  - Produção por outros tumores como neuroendocrino, bexiga, rim, próstata, pulmão, cabeça e pescoço, gastrointestinal, colo de útero, útero, vulva, linfoma e leucemias

Kricka LJ. Clin Chem; 45:942, 1999.
http://www.fda.gov
Garnick MB. N Engl J Med; 303:1177, 1980.
Braunstein GD. Urology ; 25:605, 1985.
Stenman UH. Clin Biochem ; 37:549, 2004.
Oosting SF. Ann Oncol. ;21(1):104, 2010.

Alfa Feto Proteína (AFP)

- normal < 10-15 micrograms/L
- Meia vida 5-7 dias
- Seminona não produz por definição
- Tipos Não Seminomatosos: tumores do saco vitelínico/seio endodérmico e carcinoma embrionário.
  - 10-20% EC I e até 40-60% EC III
- Falso positivo:
  - Outros tumores como Hepatocarcinoma, metástases hepáticas de quaisquer tumores e tumores gastrointestinais
  - Outras doenças que acometem o fígado como cirrose, hepatite, abuso de álcool
  - Lise tumoral após C1 de tratamento

Gilligan TD. J Clin Oncol;28(20):3388, 2010.
Yuasa T. J Androl;20(3):336, 1999.

Lactate dehydrogenase (LDH)

- ↑ em 40 -60% dos tumores de testículo
- < sensibilidade e especificidade que HCG e AFP em Não Seminomas
- Pode ser único marcador de Seminomas
- Relacionado a volume de doença e taxa proliferativa
- FATOR PROGNÓSTICO independente
- Sensível, mas não específico na recorrência
- Falso positivos:
  - Inúmeros processos inflamatórios, infecciosos ou de quaisquer danos teciduais agudo ou crônico

Gilligan TD. J Clin Oncol;28(20):3388, 2010
Mencel PJ. J Clin Oncol;12(1):120, 1994

International Germ Cell Collaborative Group (IGCCCG)

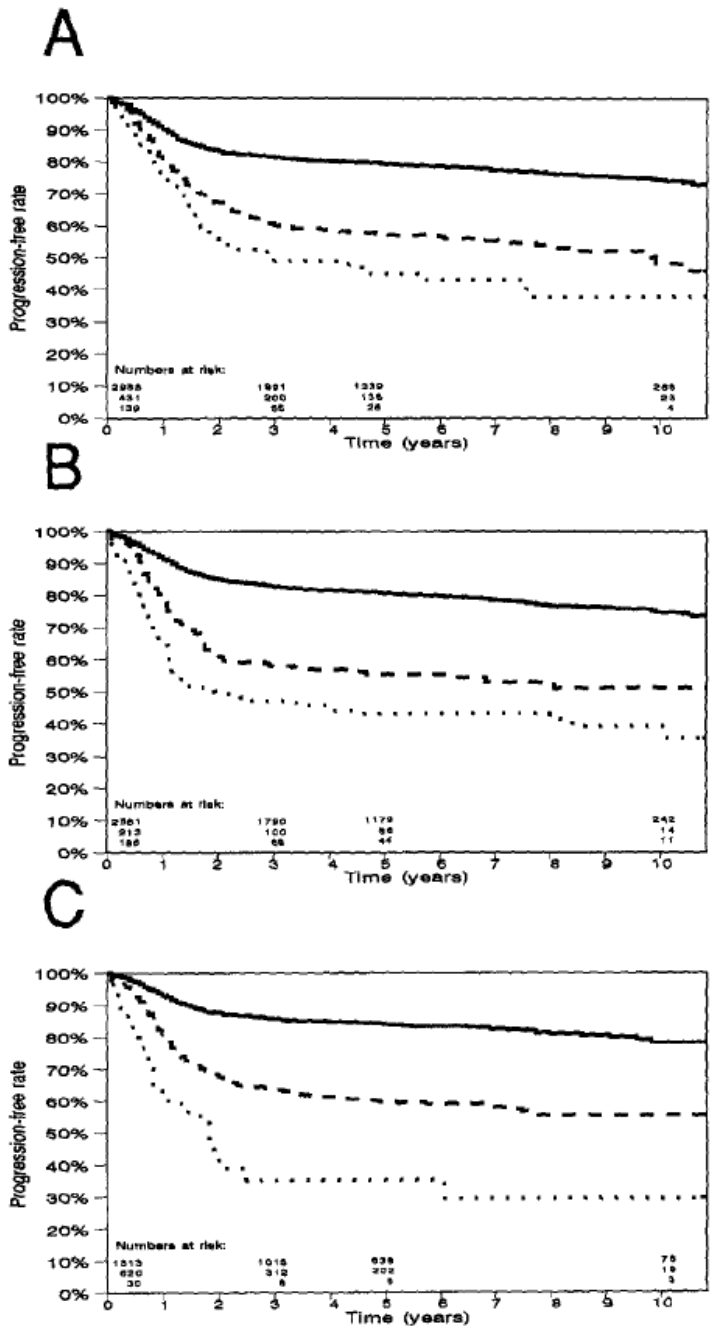
- 1991
- British Medical Research Council(MRC)
- European Organisation for Research and Treatment of Cancer (EORTC)
- EUA:
  - Memorial Sloan-Kettering Cancer Center
  - Indiana University Hospital
  - University of Texas MD Anderson Cancer Center
- National Germ Cell Groups:
  - Canada, Austrália, Nova Zelândia, Espanha, França, Dinamarca, e Itália

IGCCCG, J Clin Oncol 15:594-603. 1997

International Germ Cell Cancer Collaborative Group 1997 - (IGCCCG)

- 1975-1990: 5.202 (CDDP ou Carboplatina)
  - 85% Não Seminomatoso (NS): 4.454
  - 15% Seminoma: 660
- Seguimento: 5 anos
- Validação: 30% Não Seminomatoso
- **Análise Multivariada:**
  - **Sítio da Doença**
    - **Extragonadal - Mediastinal (Não Seminiomas)**
  - **Metastase ExtraPulmonar**
    - **Único fator em Seminomas**
  - **Marcadores: AFP, HCG e LDH**

IGCCCG, J Clin Oncol 15:594-603. 1997



Sobrevida Livre de Progressão

- (A) AFP
  - < 1.000 ng/mL
  - 1.000 – 10.000 ng/mL
  - >10.000 ng/mL
- (B) HCG
  - < 5.000 UI/L (1.000ng/mL)
  - 5.000 – 50.000 UI/L (1.000–10.000ng/mL)
  - > 50.000 UI/L (10.000 ng/mL)
- (C) LDH
  - < 1.5xLSN
  - 1.5-10xLSN
  - >10xLSN

IGCCCG, J Clin Oncol 15:594-603. 1997

GOOD PROGNOSIS	
NON-SEMINOMA	**SEMINOMA**
Testis/retroperitoneal primary ***and*** No non-pulmonary visceral metastases ***and*** Good markers - all of *AFP < 1000 ng/ml and* *hCG < 5000 iu/l (1000 ng/ml) and* *LDH < 1.5 x upper limit of normal* **56% of non-seminomas** **5 year PFS 89%** **5 year Survival 92%**	Any primary site **and** No non-pulmonary visceral metastases **and** Normal AFP, any hCG, any LDH **90% of seminomas** **5 year PFS 82%** **5 year Survival 86%**
INTERMEDIATE PROGNOSIS	
NON-SEMINOMA	**SEMINOMA**
Testis/retroperitoneal primary ***and*** No non-pulmonary visceral metastases ***and*** **Intermediate markers - any of:** *AFP ≥ 1000 and ≤ 10,000 ng/mL or* *hCG ≥ 5000 iu/l and ≤ 50,000 iu/l or* *LDH ≥ 1.5 x N and ≤ 10 x N* **28% of non-seminomas** **5 year PFS 75%** **5 year Survival 80%**	Any primary site ***and*** **Non-pulmonary visceral metastases** ***and*** Normal AFP, any hCG, any LDH **10% of seminomas** **5 year PFS 67%** **5 year Survival 72%**
POOR PROGNOSIS	
NON-SEMINOMA	**SEMINOMA**
Mediastinal primary ***or*** **Non-pulmonary visceral metastases** ***or*** **Poor markers - any of:** *AFP > 10,000 ng/ml or* *hCG > 50,000 iu/l (10000 ng/ml) or* *LDH > 10 x upper limit of normal* **16% of non-seminomas** **5 year PFS 41%** **5 year Survival 48%**	**No patients classified as poor prognosis**

Classificação Prognóstica IGCCCG

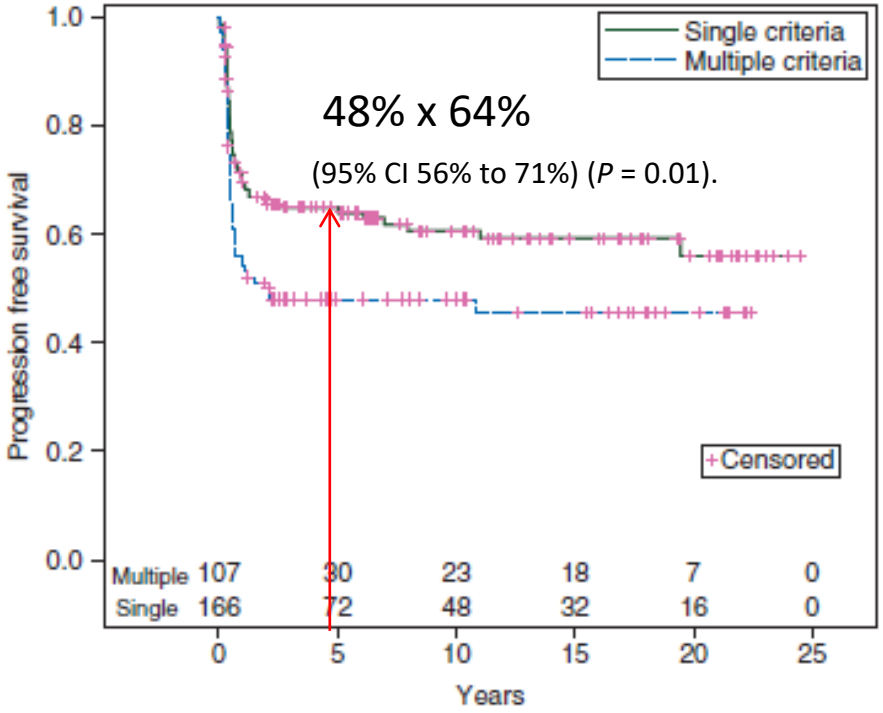
1975-1990: 5.202 (CDDP ou Carboplatina)

IGCCCG, J Clin Oncol 15:594-603. 1997

Subgrupos de Pobre Prognóstico

- Indiana University (N = 273, 1990 to 2014)
- SLP (5a) 58%
- SG (5a) 73%

	Hazard ratio (95% CI)	*P* value
Multivariate 5-year PFS analysis		
Liver metastasis	1.66 (1.05–2.64)	0.03
Brain metastasis	2.30 (1.80–4.91)	<0.001
Bone metastasis	2.09 (0.97–4.50)	0.06
Primary mediastinal nonseminoma	3.14 (1.72–5.71)	<0.001
Elevation in logarithmic AFP (ng/ml)	1.00 (0.92–1.05)	0.67
Elevation in logarithmic β-hCG (mIU/ml)	1.07 (1.01–1.14)	0.04
Age	1.02 (1.00–1.04)	0.06
Multivariate 5-year OS analysis		
Liver metastasis	1.44 (0.78–2.67)	0.24
Brain metastasis	3.30 (1.74–6.23)	<0.001
Bone metastasis	2.43 (0.98–6.00)	0.06
Primary mediastinal nonseminoma	4.63 (2.25–9.56)	<0.001
Elevation in logarithmic AFP (ng/ml)	1.03 (0.95–1.12)	0.45
Elevation in logarithmic β-hCG (mIU/ml)	1.04 (0.97–1.13)	0.28
Age	1.03 (1.01–1.06)	0.02



SG (5ª) 69% x 76%
(95% CI 68% to 82%) (*P* = 0.17)

Adra N. Ann Oncol. 2016 May;27(5):875-9, 2016.

Subgrupos de Intermediário e Pobre Prognóstico

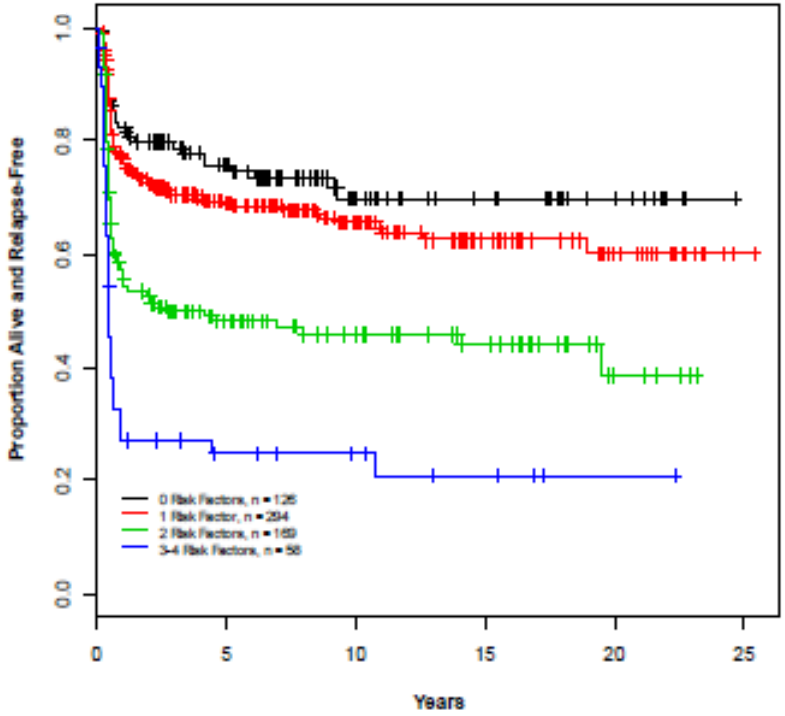
- Indiana University e Fondazione IRCCS Istituto Nazionale dei Tumori
- N = 647

Factor	Univariable Analysis			Multivariable Analysis		
	HR	95% CI	P[a]	HR	95% CI	P[a]
Age at the Time of Diagnosis, Continuous/Year	1.04	1.02-1.06	<.001	1.03	1.01-1.05	.003
IGCCCG Risk Category, Poor vs. Intermediate	2.21	1.52-3.23	<.001			
AFP, IU/mL			.98			
<1000	1.03	0.65-1.64				
1000-10,000	1.05	0.64-1.71				
>10,000	Ref	Ref				
HCG, IU/L			.26			
<5000	1.11	0.76-1.63				
5000-50,000	0.76	0.45-1.28				
>50,000	Ref	Ref				
Site of the Primary Tumor, Mediastinum vs. Other	2.71	1.94-3.79	<.001	3.09	2.14-4.44	<.001
Brain Metastases, Yes vs. No	2.53	1.67-3.83	<.001	2.21	1.40-3.48	<.001
Liver Metastases, Yes vs. No	1.11	0.76-1.61	.59			
Bone Metastases, Yes vs. No	1.78	1.01-3.14	.047			
Nonpulmonary Visceral Metastases, Yes vs. No	1.43	1.04-1.97	.029			
Retroperitoneal Metastases			<.001			
None	1.81	1.09-3.02				
<3 cm	0.61	0.27-1.39				
3-5 cm	0.45	0.22-0.91				
>5 cm	0.71	0.42-1.19				
Unknown	Ref	Ref				
Lung Metastases			.005			.016
None	Ref	Ref		Ref	Ref	
<3 cm	1.31	0.88-1.95		1.43	0.93-2.19	
3-5 cm	1.56	0.93-2.61		1.79	1.03-3.12	
>5 cm	2.29	1.35-3.88		2.09	1.18-3.70	
Unknown	2.13	1.30-3.48		2.27	1.33-3.87	

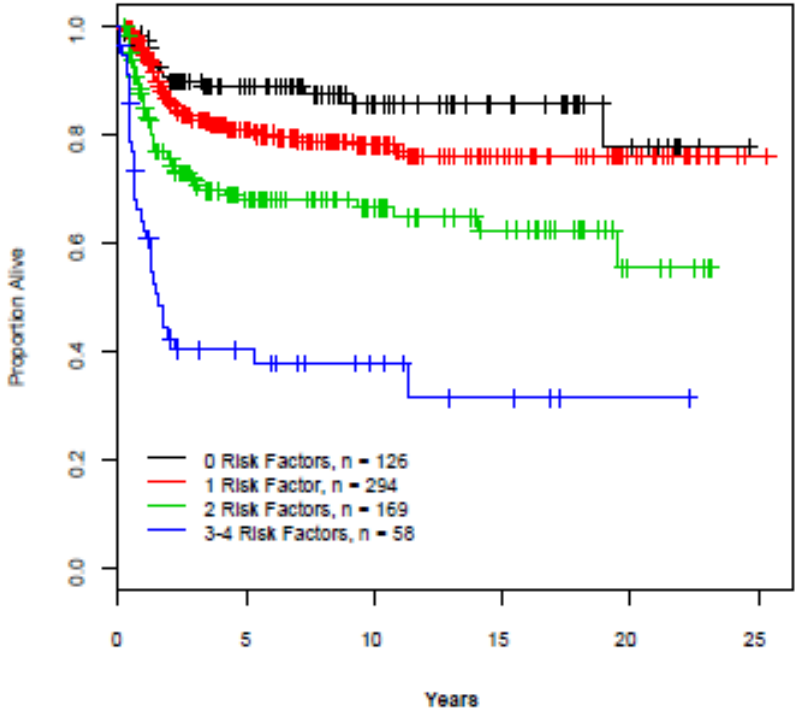
Necchi A. Clin Genitourin Cancer. Apr;15(2):306-312.e3, 2017.

Subgrupos de Intermediário e Pobre Prognóstico

SLP



SG



Necchi A. Clin Genitourin Cancer. Apr;15(2):306-312.e3, 2017.

Biomarcadores & Tratamento

- Fator Prognóstico

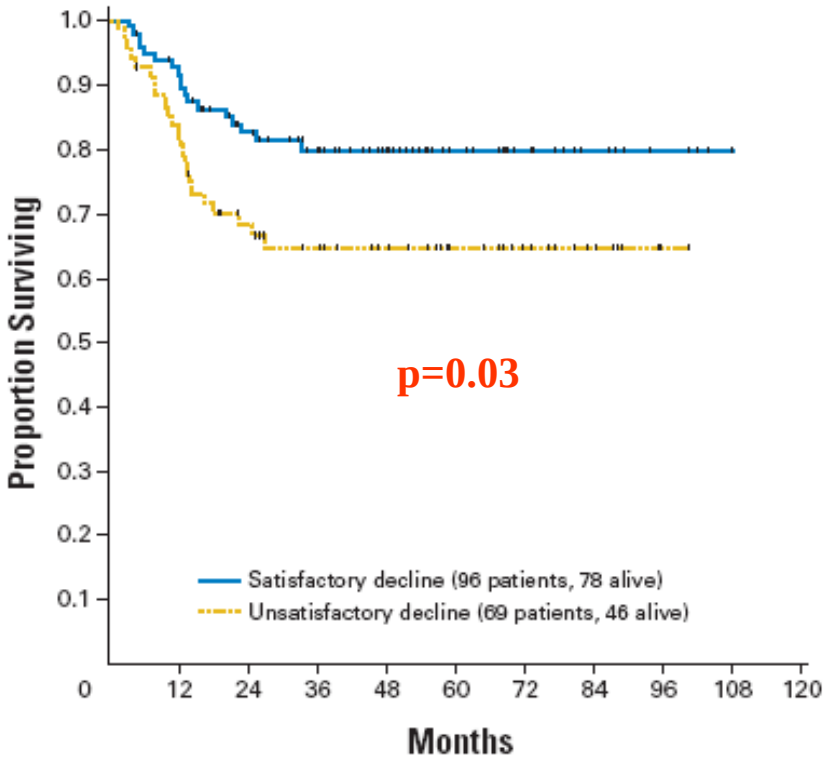
X

- Fator Preditivo

Mazumdar M. J Clin Oncol. May 1;19(9):2534-41, 2001

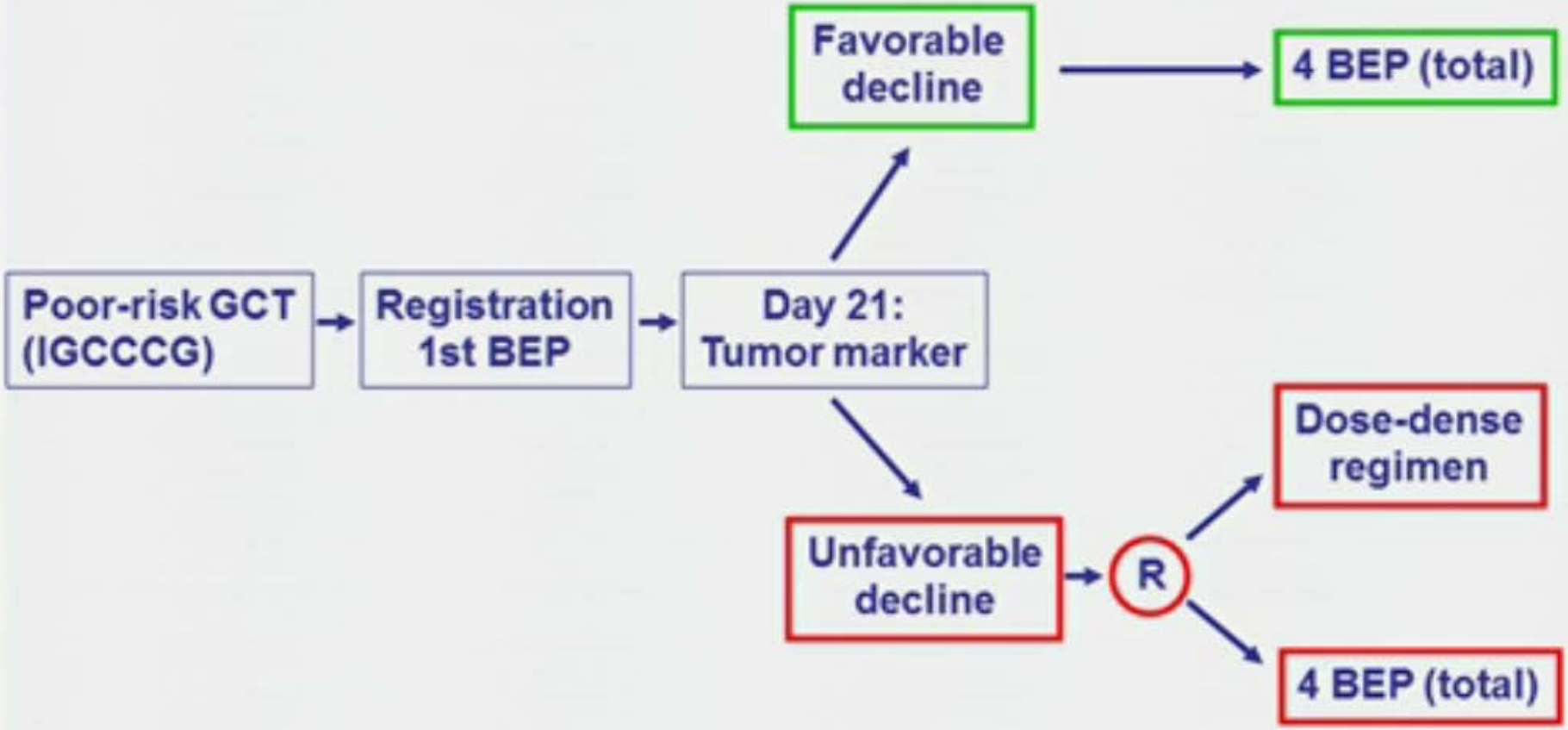
Alta dose 1ª Linha
MSKCC, ECOG, SWOG e CALGB

- Declínio dos Marcadores:
- Satisfatório: 58%
  - Normal após C3
  - Meia Vida 7 dias AFP e 3.5 dias HCG
- Insatisfatório: 42%
  - 1 ou mais marcadores com queda > 7 dias AFP ou > 3.5 dias HCG



Motzer RJ, *J Clin Oncol 25:247-256, 2007*

GETUG 13 Phase III design

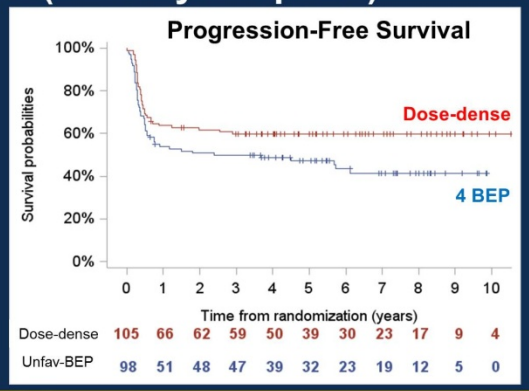


Fizazi K. Lancet Oncol. 2014 Dec;15(13):1442-50.

GETUG 13
SLR – Queda Desfavorável

Results (2016): PFS in patients with an unfavorable tumor marker decline (Primary endpoint)

5-year PFS: 60% vs 47%
HR: 0.65 [0.43-0.97]
p=0.037



PRESENTED AT: ASCO ANNUAL MEETING '16
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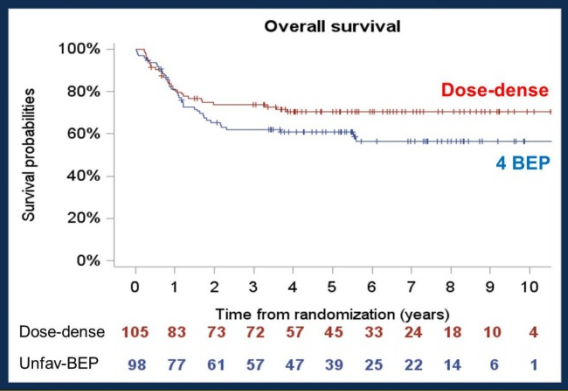
Presented by: Karim Fizazi

Results (2016): OS in patients with an unfavorable tumor marker decline (Secondary endpoint)

5-year OS: 70.4% vs 60.8%
HR: 0.69 [0.43-1.11]
p=0.12

75 vs 59 pts alive at last follow-up



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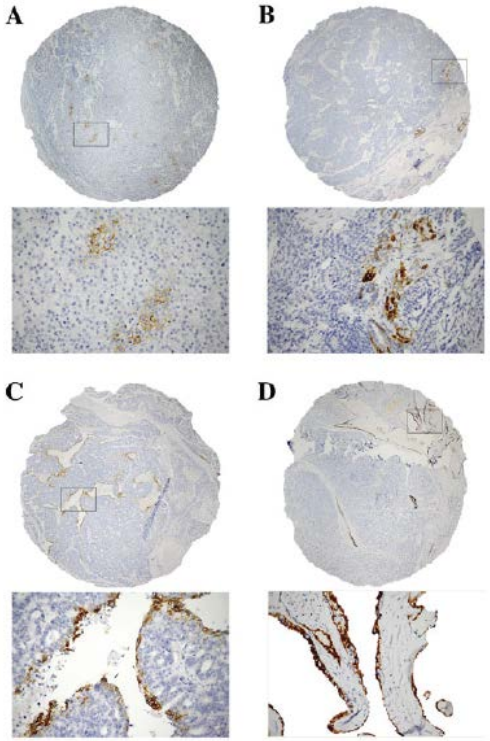
Presented by: Karim Fizazi

Fizazi K. J Clin Oncol, 34:4504, (suppl),2016

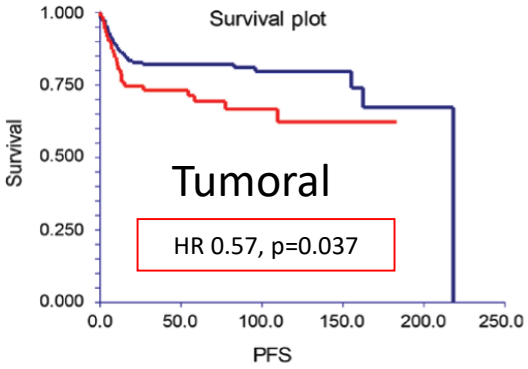
Biomarcadores através da Imunohistoquímica

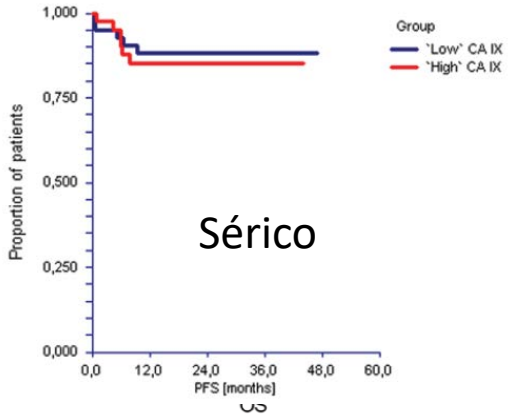
- Anidrase Carbônica IX (N = 228)



		CA IX expression				
		Absent		Present		
Histological subtype	No.	No.	%	No.	%	P-value
Healthy testis	107	107	100.0	0	0.0	N/A
Testicular germ cell tumors	205	143	69.8	62	30.2	<0.001
Seminoma	75	58	77.3	17	22.7	<0.001
Embryonal carcinoma	118	104	88.1	14	11.9	<0.001
Yolc sac tumor	36	28	77.8	8	22.2	<0.001
Choriocarcinoma	13	12	92.3	1	7.7	0.11
Teratoma	59	36	61.0	23	39.0	<0.001
GCNIS	76	76	100.0	0	0.0	N/A

GCNIS, germ cell neoplasia *in situ*; CA IX, carbonic anhydrase IX.

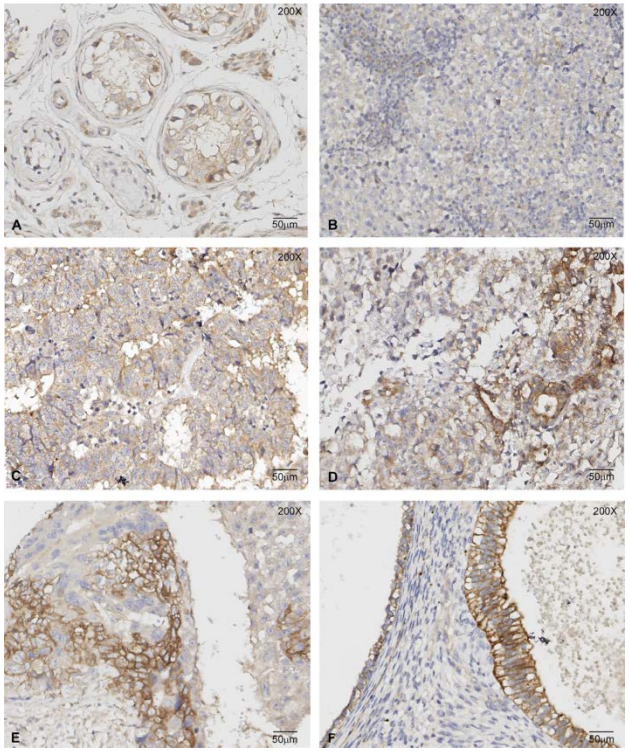




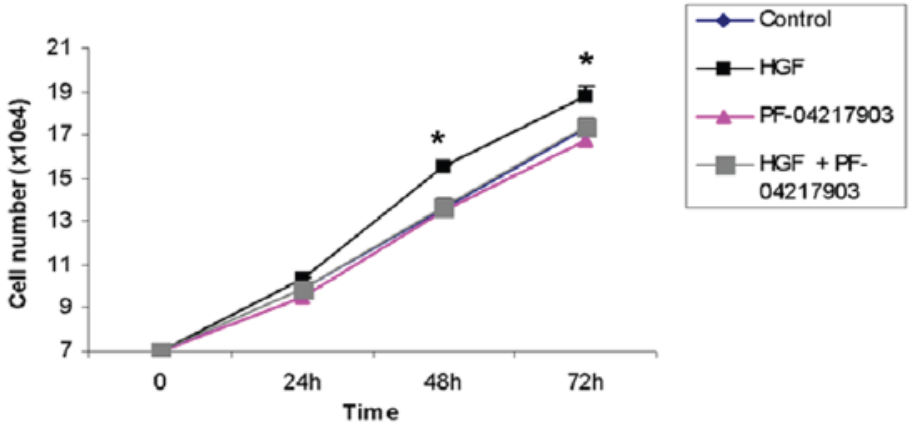
Kalavska K. Oncol Lett. Oct;12(4):2590-2598, 2016.
Kalavska K. Oncol Lett. Apr;13(4):2177-2185, 2017.

Biomarcadores através da Imunohistoquímica

- Receptor c-MET do Fator de Crescimento de Hepatócito



Tumor component	M	C	N
GCNIS	++	–	+
SE	+	+	–
EC	++	++	–
YST (E)	+++	+++	–
(NE)	++	++	–
CHC (C)	+++	++	–
(S)	–	+	–
TE	+++	++	–



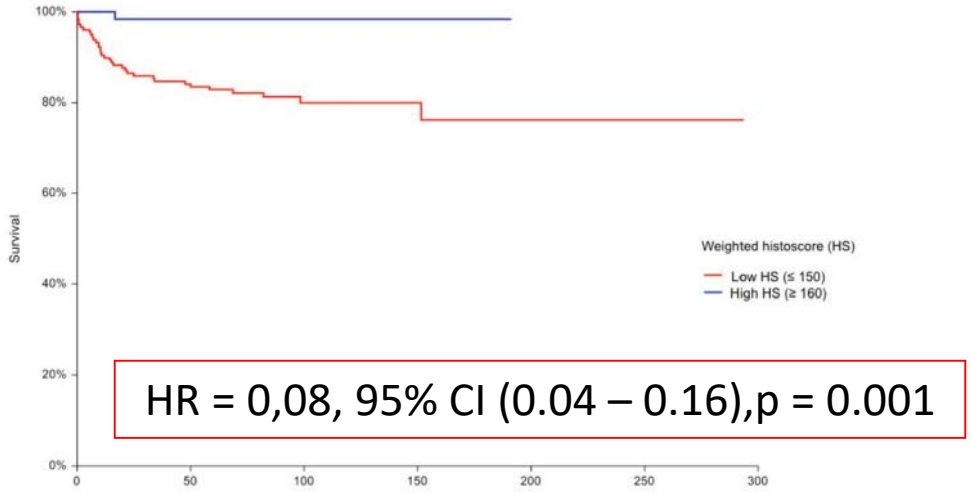
Scheri KC. Oncotarget. Aug 7;9(61):31842-31860, 2018

Biomarcadores Imunorelacionados

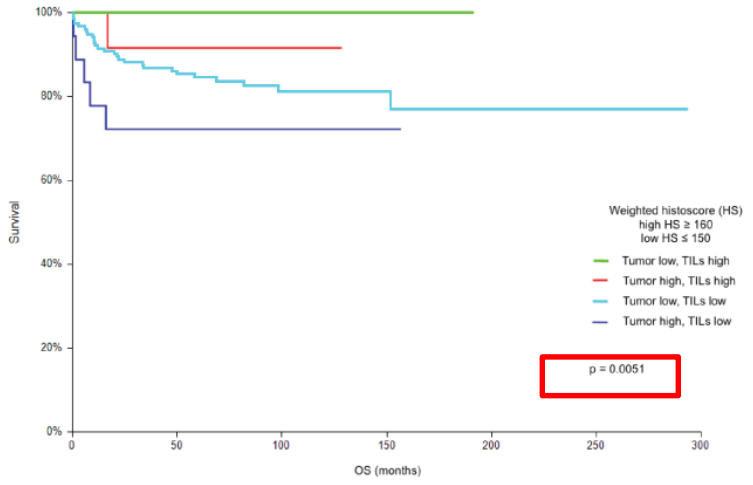
- PD-L1 Tumoral

Tissue types	Negative	Weak	Strong	Positive cases (%)
Seminoma ($n = 208$)	57	67	84	73%
Non-seminoma ($n = 121$)	43	35	43	64%
Intratubular germ cell neoplasia	20	0	0	0%
Normal testis	20	0	0	0%

- Infiltrado Linfocitário Tumoral

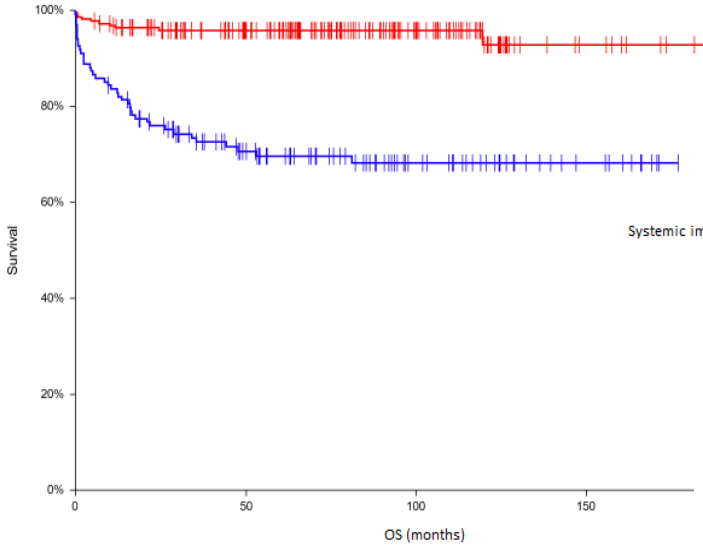


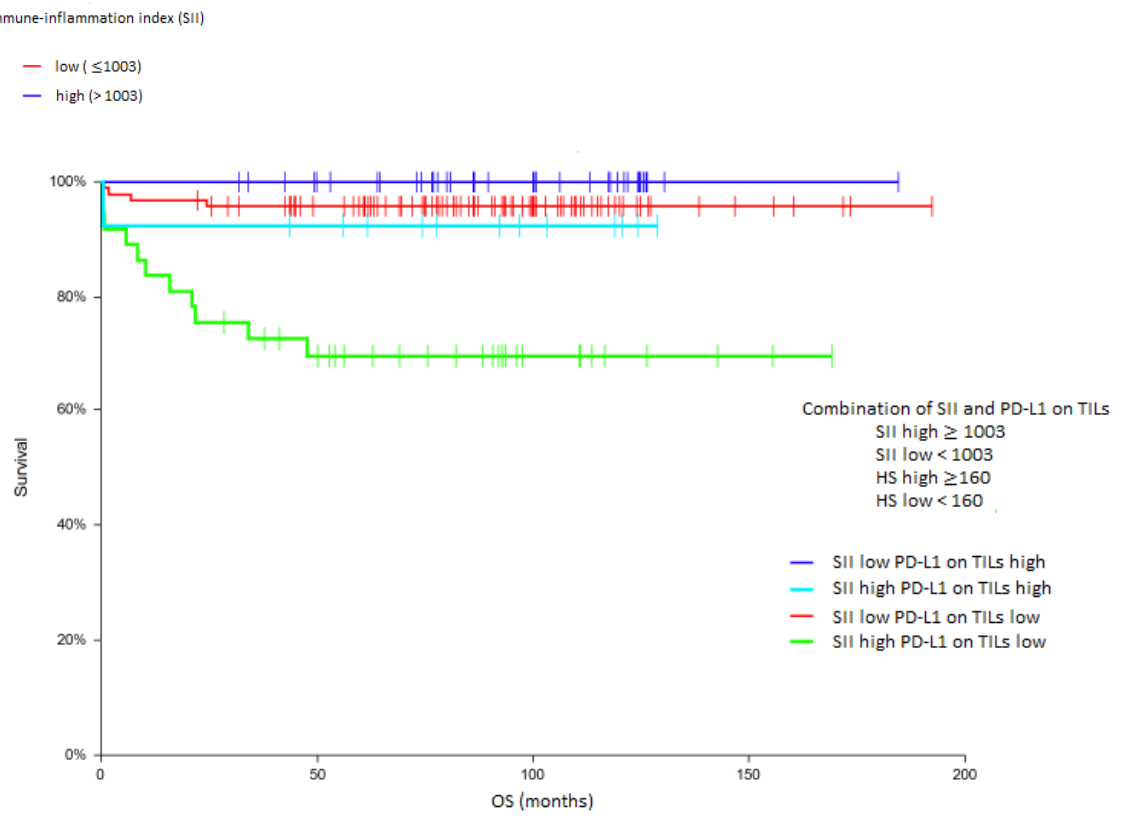
HR = 0,08, 95% CI (0.04 – 0.16),p = 0.001



Fankhauser CD. Br J Cancer. Jul 28;113(3):411-3, 2015
Chovanec M. Oncotarget. Mar 28;8(13):21794-21805, 2017

Systemic immune-inflammation index (SII)





- SII = P x N/L

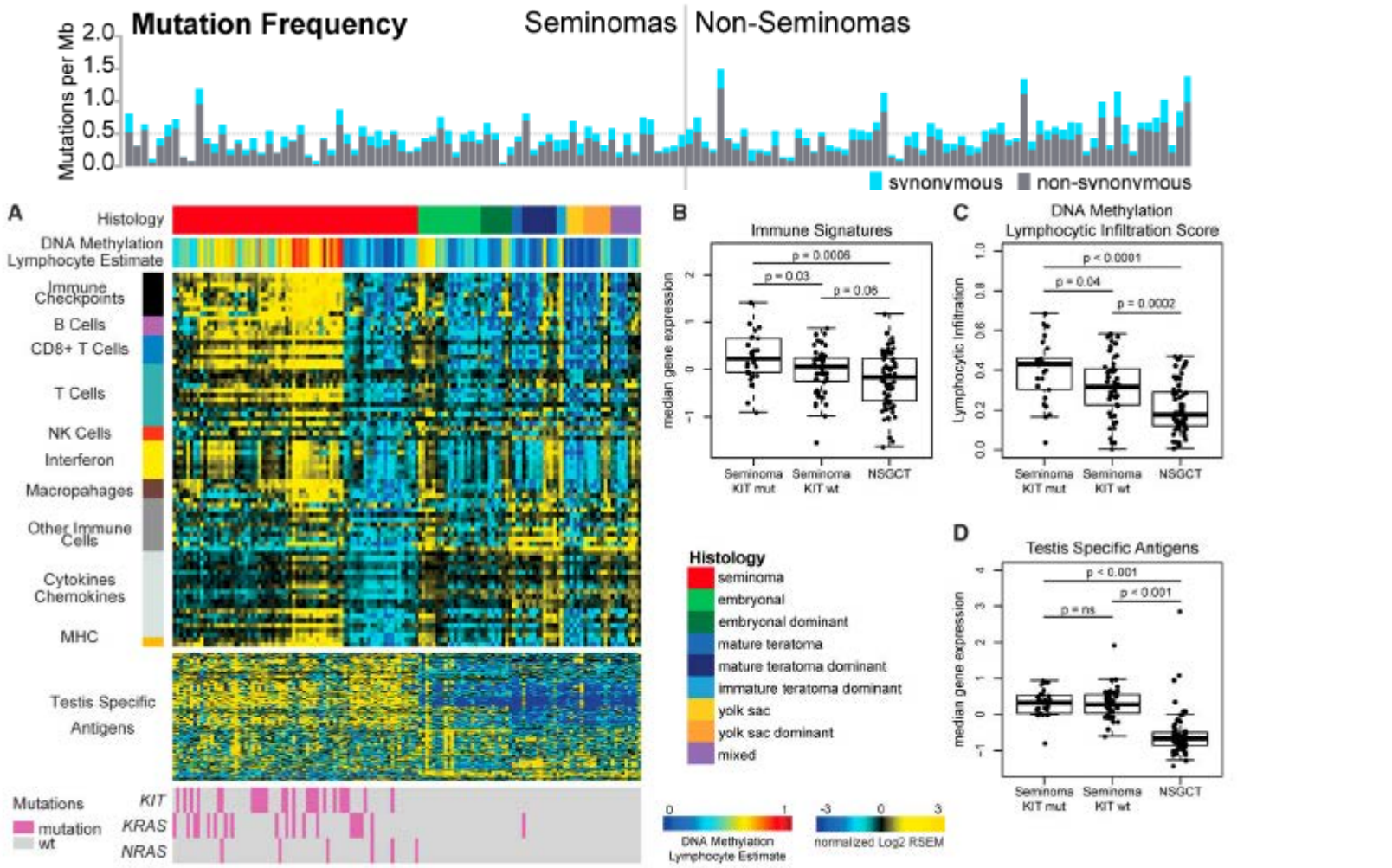
Chovanec M. Br J Cancer. Mar 20;118(6):831-838, 2018.

Biomarcadores Imunorelacionados

- Fase II Pembrolizumab (anti-PD1)
- N = 12
- PD-L1 (+) = 2 (PD ambos)
- TR (RC+RP) = 0
- DE = 2

Adra N. Ann Oncol. Jan 1;29(1):209-214, 2018.

Caracterização Molecular do Tu de Testículo

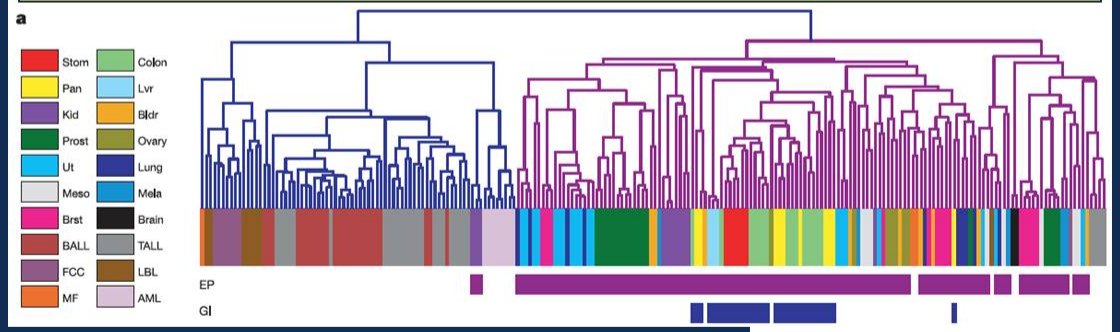


Shen H. Cell Rep. Jun 12;23(11):3392-3406, 2018.

miRNA: biology and biomarker potential

- miRNA expression dysregulated in cancer:
  - Amplification/deletion of miRNA genes
  - Aberrant transcriptional control
  - Epigenetic changes
  - Altered biogenesis
- Can function as:
  - Oncogenes
  - Tumor suppressor

Expression retains characteristics of cell of origin



Lu *et al.*, Nature 2005

Presented by: Robert Hamilton

miRNA: biology and biomarker potential

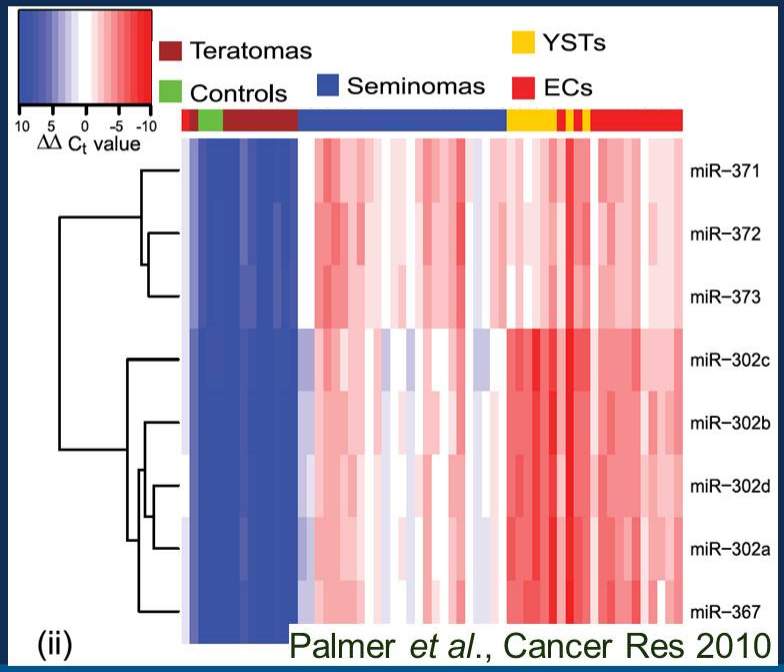
- First report of miR-371-373 highly expressed in testis cancer

A Genetic Screen Implicates miRNA-372 and miRNA-373 As Oncogenes in Testicular Germ Cell Tumors

Cell

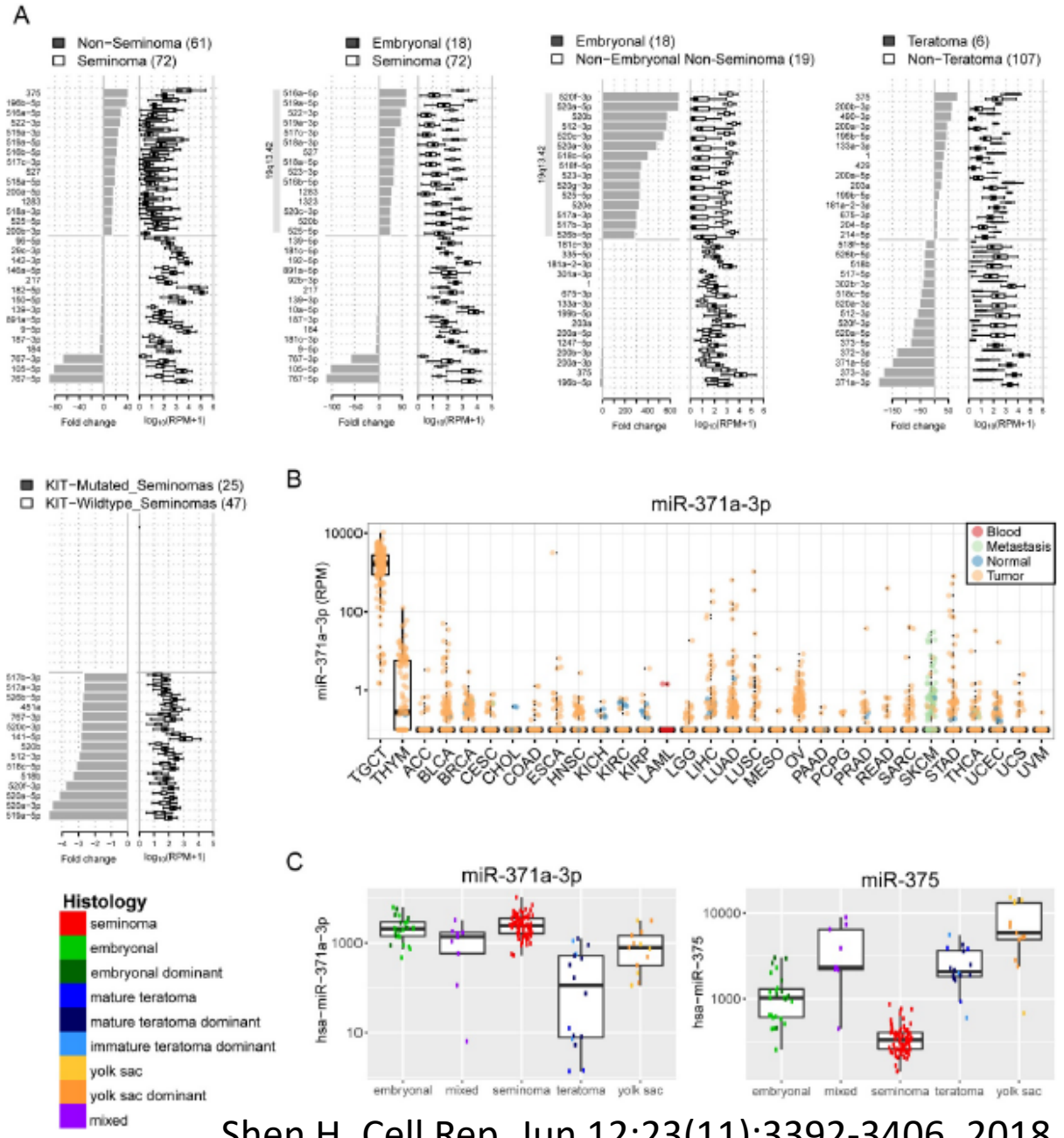
Voorhoeve *et al.*, Cell *124*, 1169–1181, March 24, 2006

- 2010: miR-371 & miR-302 cluster
  - Overexpressed regardless of:
    - Tumor type (Sem vs. NSGCT)
    - NSGCT subtype
    - Pre- vs. post-pubertal
    - Anatomical site (extra- vs. gonadal)



Presented by: Robert Hamilton

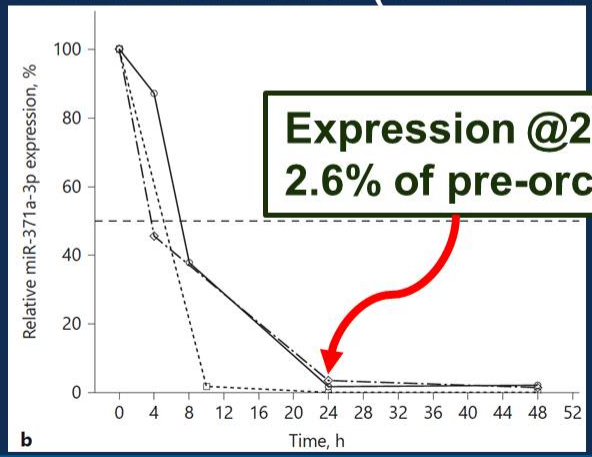
- miRNA



Shen H. Cell Rep. Jun 12;23(11):3392-3406, 2018.

miRNA: biology and biomarker potential

- Detectable in the serum
  - Released to blood stream via exosomes
  - Resistant to degradation (complexed to Ago2)
- Short half-life (t-½ 3-7hrs)



Compare to:
- **HCG: 36 hrs**
- **AFP: 5-7 days**

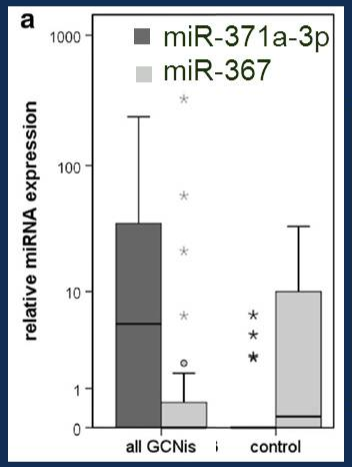
Murray *et al*., Am J Clin Pathol 2011
Hunter *et al*., PLoS One 2008
Arroyo *et al*., PNAS 2011
Radtke *et al*., Urol Int 2017

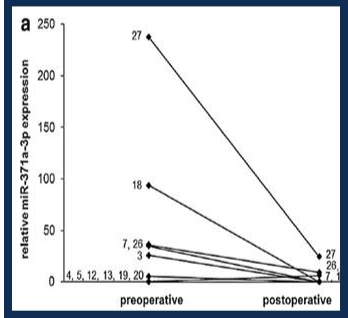
Can germ cell neoplasia in situ be diagnosed by measuring serum levels of microRNA371a-3p?

A. Radtke[1] · J.-F. Cremers[2] · S. Kliesch[2] · S. Riek[1] · K. Junker[3] · S. A. Mohamed[4] · P. Anheuser[5] · G. Belge[1] · K.-P. Dieckmann[5]

J Cancer Res Clin Oncol (2017) 143:2383–2392

- 27 patients with GCNIS only (no invasive GCT) vs. controls
- 52% had elevated miR-371a-3p
- Highest in bilateral cases
- Normalized after RT or orch in all





Suggests miR-371a-3p overexpression is an early molecular change in GCT pathogenesis

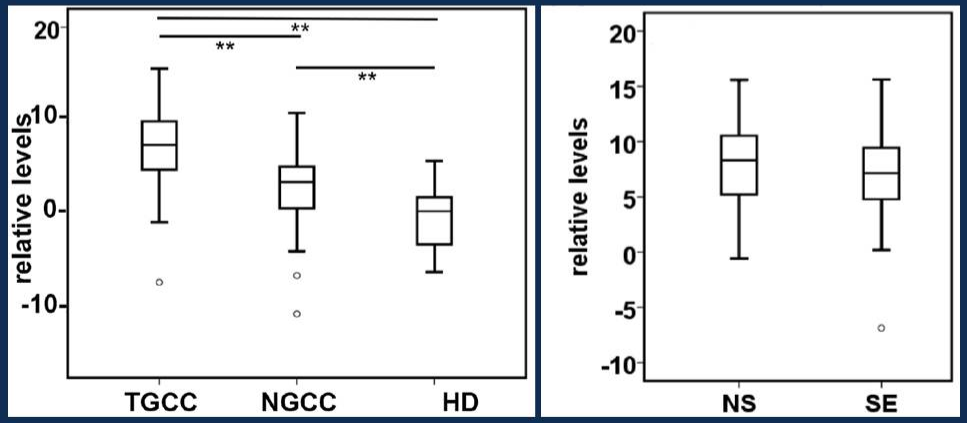
Presented by: Robert Hamilton

Accurate primary germ cell cancer diagnosis using serum based microRNA detection (ampTSmiR test)

Oncotarget, 2017, Vol. 8, (No. 35), pp: 58037-58049

Ton van Agthoven[1] and Leendert H.J. Looijenga[1]

- Compared miRNA 371a-3p at diagnosis across
  - 250 germ cell cancers
  - 60 non-germ cell cases (e.g. torsion, Leydig etc.)
  - 104 healthy controls
- Characteristics:
  - Sensitivity: 90%
  - Specificity: 86%
  - AUC 0.95



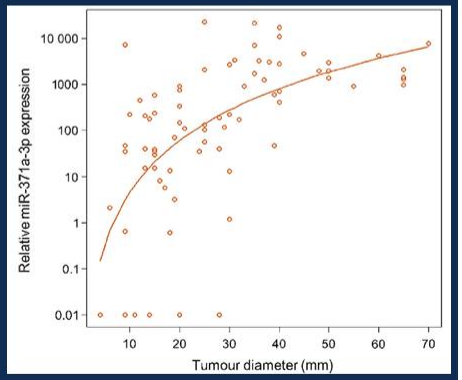
Presented by: Robert Hamilton

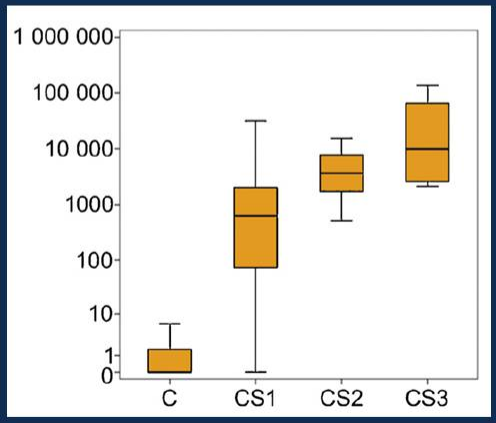
Serum Levels of MicroRNA miR-371a-3p: A Sensitive and Specific New Biomarker for Germ Cell Tumours

EUROPEAN UROLOGY 71 (2017) 213–220

Klaus-Peter Dieckmann [a,†,*], *Arlo Radtke* [b,†], *Meike Spiekermann* [b,†], *Thomas Balks* [a], *Cord Matthies* [c], *Pascal Becker* [c], *Christian Ruf* [c], *Christoph Oing* [d], *Karin Oechsle* [d], *Carsten Bokemeyer* [d], *Johannes Hammel* [e], *Sebastian Melchior* [e], *Werner Wosniok* [f], *Gazanfer Belge* [b]

- miR-371a-3p measured in 166 consecutive germ cell tumor patients and 106 controls
- Expression correlated to:
  - Tumor size in CSI
  - Clinical stage





Presented by: Robert Hamilton

Clinical utility of plasma miR-371a-3p in germ cell tumors

J Cell Mol Med. 2019;23:1128–1136.

Michal Mego[1,2] | Ton van Agthoven[3] | Paulina Gronesova[4] | Michal Chovanec[2] | Vera Miskovska[5] | Jozef Mardiak[2] | Leendert H. J. Looijenga[3,6]

- 180 patients starting first line chemotherapy
- miR371a-3p associated with PFS and OS
- Held for seminoma and NSGCT

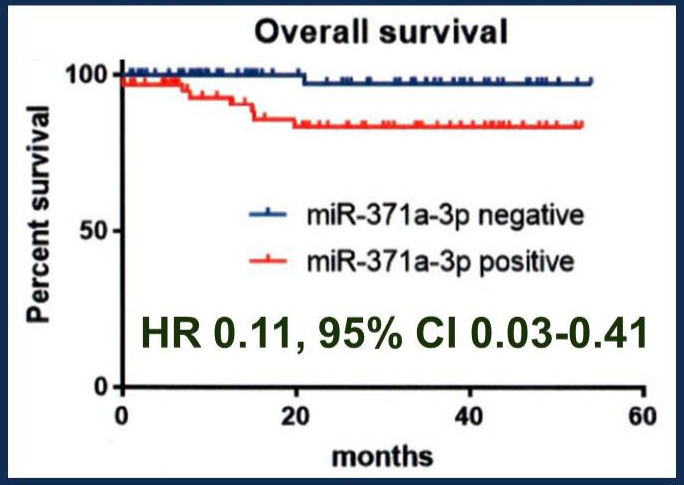


TABLE 4 Prognostic value of plasma miR-371a-3p before the first cycle of chemotherapy

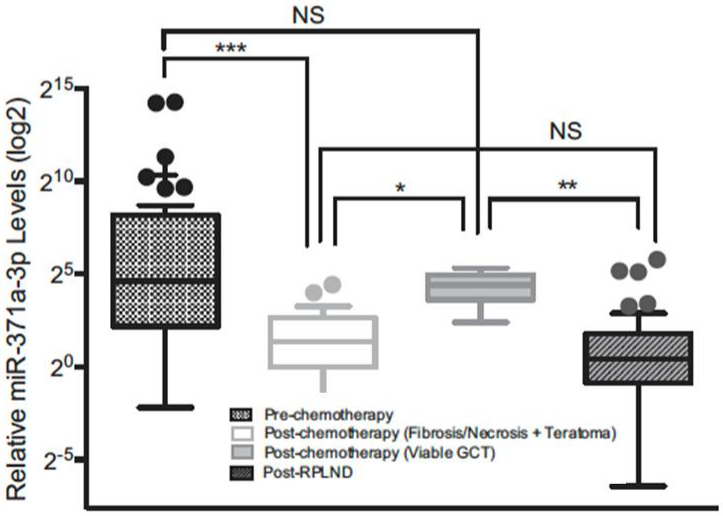
	HR (95% CI), *P*-value	
	Overall survival	
Variable	Univariate analysis	Multivariate analysis
Plasma miR-371a-3p		
Negative vs. positive	0.21 (0.07-0.67), **0.03**	0.42 (0.09-1.98), 0.33
IGCCCG risk group		
Good risk vs. intermediate/poor risk	0.07 (0.02-0.25), **<0.00001**	0.08 (0.020.39), **0.002**

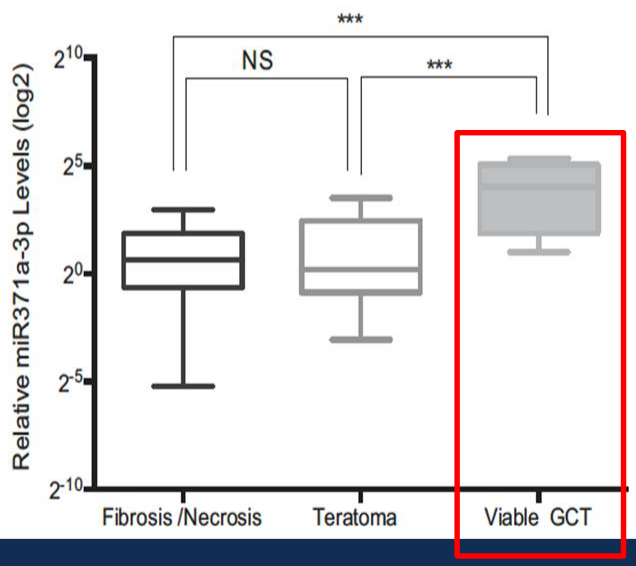
Presented by: Robert Hamilton

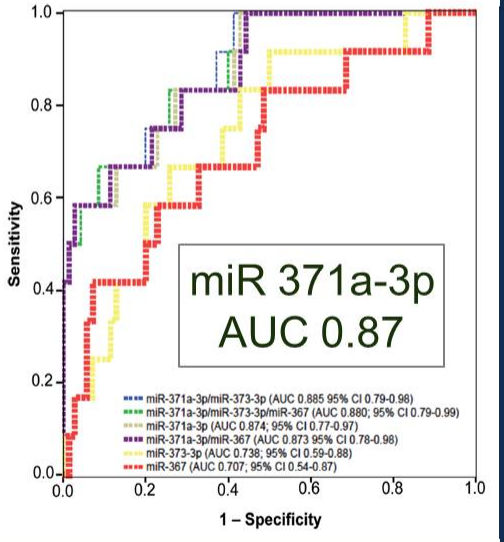
THE JOURNAL OF UROLOGY® Vol. 200, 126-135, July 2018

Serum miRNA Predicts Viable Disease after Chemotherapy in Patients with Testicular Nonseminoma Germ Cell Tumor

Ricardo Leão,* Ton van Agthoven,* Arnaldo Figueiredo, Michael A. S. Jewett, Kamel Fadaak, Joan Sweet, Ardalan E. Ahmad, Lynn Anson-Cartwright, Peter Chung, Aaron Hansen, Padraig Warde, Pedro Castelo-Branco, Martin O'Malley, Philippe L. Bedard, Leendert H. J. Looijenga*,† and Robert J. Hamilton*,†







THE JOURNAL OF UROLOGY® Vol. 200, 126-135, July 2018

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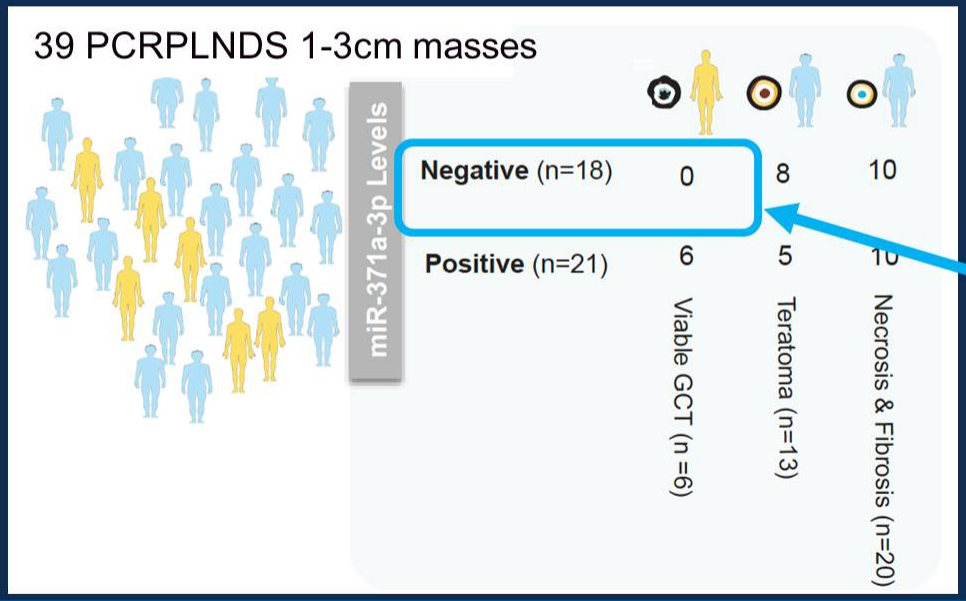
Consider watching masses at 3cm cut-off?



No Viable Disease

Presented by: Robert Hamilton

Varied methods: table courtesy of Lucia Nappi, BC Cancer Agency

	TSmiR (Looijenga)	Serum miR extraction (Dieckmann)	Serum miR extraction (Murray)	Plasma miR extraction (Nappi)
Blood collection	Serum Separator Tubes	Serum Separator Tubes	Serum Separator Tubes	Streck
Extraction method	Magnetic beads	Serum with extraction kit	Serum with extraction kit	Plasma with extraction kit
Preamplifications	none	1	1	1
Normalization	miR-20a miR-93	miR-93	miR-30b-5p cel–miR–39–3p	miR-30b-5p cel–miR–39–3p miR451
Spike in miRNAs	Cel–miR–39–3p Ath-miR159a	Cel-miR-39-3p	Cel-miR-39-3p	Cel-miR-39-3p
Quality control	Intra and inter plates variability	NA	Ct vaues of miR-30b-5p cel–miR–39–3p miR451 miR23a hemolysis	Ct vaues of miR-30b-5p cel–miR–39–3p miR451 miR23a hemolysis
Serum Volume	50 uL	200 uL	200 uL	200 uL
Data analysis	No pre-established cutoffs. Cutoffs of RQ adjusted to reach a certain sensitivity	Cutoff: RQ ≥ 5 to evaluate sensitivity and specificity	Cutoff: RQ≥ 2 to define miRNa overexpression	Quantitative and qualitative analysis. +ve or -ve miR371 based on Ct value ≤40

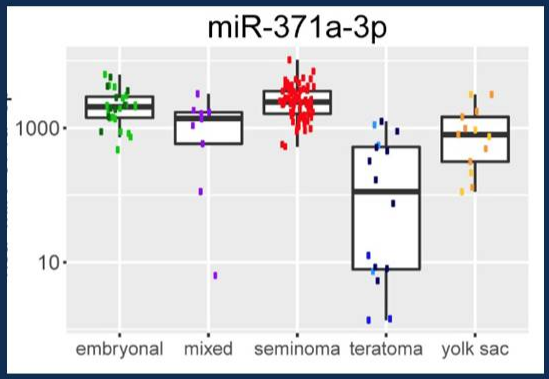
Overcoming the teratoma issue

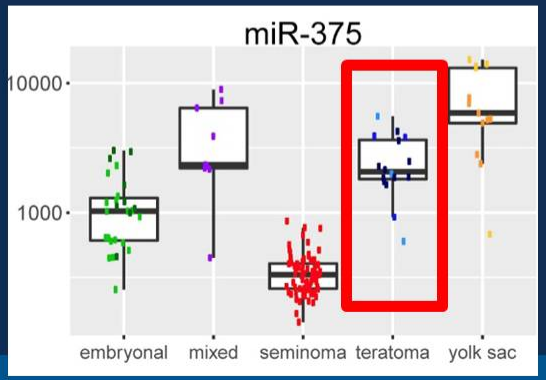
Integrated Molecular Characterization of Testicular Germ Cell Tumors

Cell Reports *23*, 3392–3406, June 12, 2018

Hui Shen,[1,32] Juliann Shih,[2,3,4,32] Daniel P. Hollern,[5,32] Linghua Wang,[6,7,32] Reanne Bowlby,[8,32] Satish K. Tickoo,[9,32] Vésteinn Thorsson,[10] Andrew J. Mungall,[8] Yulia Newton,[11] Apurva M. Hegde,[12] Joshua Armenia,[13] Francisco Sánchez-Vega,[13] John Pluta,[14] Louise C. Pyle,[14,15] Rohit Mehra,[16] Victor E. Reuter,[9] Guilherme Godoy,[17] Jeffrey Jones,[17] Carl S. Shelley,[18] Darren R. Feldman,[19] Daniel O. Vidal,[20] Davor Lessel,[21,22] Tomislav Kulis,[23] Flavio M. Cárcano,[24] Kristen M. Leraas,[25] Tara M. Lichtenberg,[25] Denise Brooks,[8] Andrew D. Cherniack,[2,3] Juok Cho,[2]

- 137 primary testicular tumors through TCGA
- Tissue-based (not serum) miRNA sequencing
- Found miR371a-3p elevated in all subtypes
  - *Except teratoma*
- Found miR375 elevated in teratoma
- Requires validation and analysis in serum





Presented by: Robert Hamilton

Estudos Prospectivos de Validação

SWOG CANCER RESEARCH NETWORK

S1823: Prospective cohort study of miR-371a-3p for Tumor Surveillance in Stage I Testicular Cancer

- **PIs:** Christian Kollmannsberger & Craig Nichols
- **Primary Objective:** Correlate miR-371 expression with relapse in CSI

Pre-orch → Post-orch → q3 months for 2 years

- Blood sent to Vancouver for analysis (Lucia Nappi)
- Pragmatic design for "real world" performance characteristics
- Aim: 1200 patients accrued over 24 months
- Estimated start date: end of 2019

CHILDREN'S ONCOLOGY GROUP

AGCT1531: Minimizing Toxicity for Low and Standard Risk Pediatric, Adolescent and Young Adult Germ Cell Tumor Patients

- **PI:** Lindsay Frazier; **Co-PI**: Furqan Shaikh & Farzana Pashankar
- Age 0-50 years; CSI A/B Seminoma or NSGCT
- **Secondary Objective:** To assess the utility four circulating microRNAs (miRNA 371-373 and miR302)

Pre-orch → q1 mos x 3 → q3 mos for 1yr → q6 mos for 1 yr

- Blood sent to Jim Amatruda (UT Southwestern) & Matt Murray (Cambridge)
- Aim: 946 CSI testicular, ovarian and EGCT
- Opened May 2017

Presented By Robert Hamilton at 2019 Genitourinary Cancers Symposium

Biomarcadores em Câncer de Testículo Conclusões

- HCG, AFP e LDH ainda são os principais
  - Diagnóstico, estagiamento (prognóstico), direcionamento e avaliação de tratamento, além de seguimento
- miRNA forte promessa
  - Falta padronização e validação
- Caracterização molecular
  - Oportunidades de biomarcadores e novas terapias

OBRIGADO