Human germ cell tumors: views and news.

12:00–13:40 **SESSION 1. BIOMARKERS**
Chair: Theresa WHITESIDE, University of Pittsburgh Cancer Institute, PA, USA
Magdalena CHECHLIŃSKA, Warsaw, PL

12:00–12:25 **Makers of progression risk in patients with squamous cell vulvar carcinoma**
Magdalena KOWALEWSKA, Warsaw, PL

12:25–12:50 **LnRNA in ovarian cancer**
Magdalena CHECHLIŃSKA, Warsaw, PL

12:50–13:15 **Melanoma-derived exosomes in plasma of melanoma patients: biomarkers of tumor progression?**
Theresa WHITESIDE, University of Pittsburgh Cancer Institute, PA, USA

13:15–13:40 **Human Germ Cell Tumors - News and Views**
Lendert H. LOOIJENGA, Rotterdam, Utrecht, The Netherlands

Diagnosis -- Treatment -- Follow-up

BIOMARKERS (tissue/DNA and body fluids)

(p)GCTs & fertility (gonadal development)

Historical (over)view: classification(s).

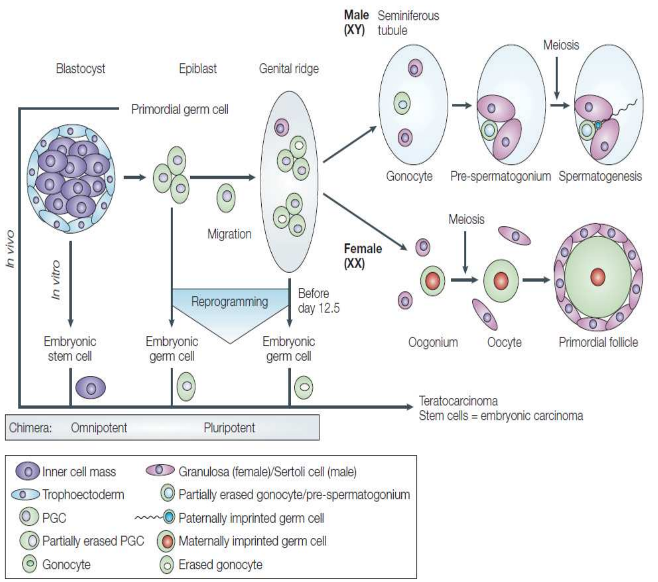
Diagnosis -- Treatment -- Follow-up

Princess máxima center
pediatric oncology

Table 18.2 Comparison of classifications of testicular germ cell tumors

Dixon and Moore[102]	Collins and Pugh[100]	British Testicular Tumour Panel[109]	Mostofi and Price[107]	WHO[99]
Seminoma	Seminoma Classic Spermatocytic	Seminoma Classic Spermatocytic	Seminoma Typical Spermatocytic Anaplastic	Seminoma Spermatocytic seminoma
Embryonal carcinoma	Malignant teratoma, anaplastic (MTA) Malignant teratoma, intermediate, with no differentiated or organoid elements (MTIB)	Malignant teratoma, undifferentiated (MTU)	Embryonal carcinoma Adult Polyembryoma	Embryonal carcinoma Polyembryoma
Teratoma with embryonal carcinoma ("teratocarcinoma")	Malignant teratoma, intermediate, with differentiated or organoid elements (MTIA)	Malignant teratoma, intermediate	Embryonal carcinoma and teratoma ("teratocarcinoma")	Embryonal carcinoma and teratoma ("teratocarcinoma")
Teratoma, adult	Teratoma, differentiated (TD)	Teratoma, differentiated	Teratoma Mature Immature	Teratoma Mature Immature With malignant transformation
Choriocarcinoma	Malignant teratoma, trophoblastic (MTT)	Malignant teratoma, trophoblastic	Choriocarcinoma	Choriocarcinoma
	Orchioblastoma	Yolk sac tumor	Embryonal carcinoma, infantile (juvenile)	Yolk sac tumor

From (with last two columns updated) Nochomovitz LE, De La Torre FE, Rosai J. Pathology of germ cell tumors of the testis. Urol Clin North Am 1977, **4**: 359–378.

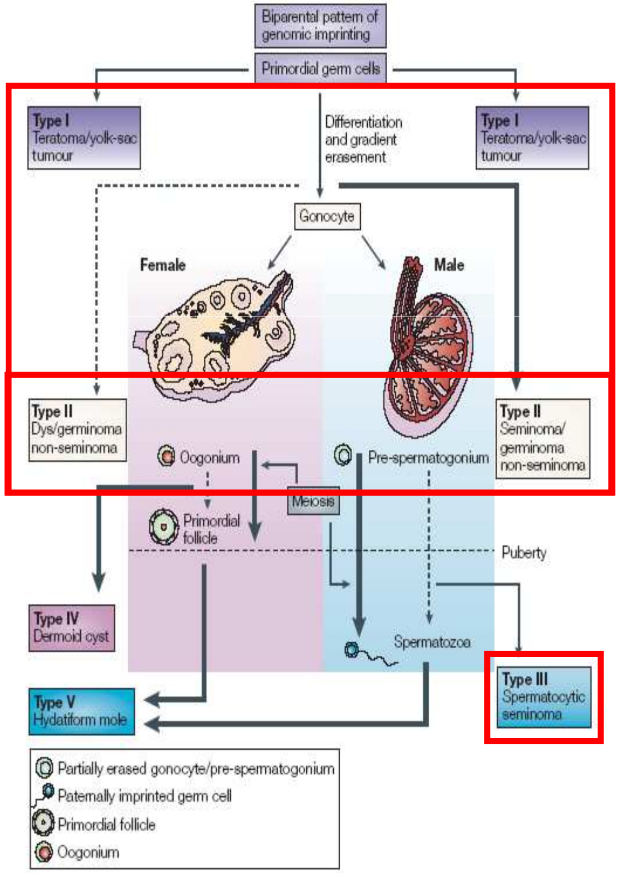


TESTICULAR GERM-CELL TUMOURS IN A BROADER PERSPECTIVE

J. Wolter Oosterhuis and Leendert H. J. Looijenga

MARCH 2005 | VOLUME 5

World Health Organization



PRECURSOR TERMINOLOGY TYI

CIS - (TIN) - IGCNU

CIS IGCNU

IGCNU
IGCN
GCNI
GCNIS
GCNIS

Germ Cell Neoplasia In Situ

Germ cell neoplasia *in situ* (GCNIS): evolution of the current nomenclature for testicular pre-invasive germ cell malignancy

Histopathology 2016 DOI: 10.1111/his.12958

Daniel M Berney,[1] Leendert H J Looijenga,[2] Muhammad Idrees,[3] J Wolter Oosterhuis,[2] Ewa Rajpert-De Meyts,[4] Thomas M Ulbright[3] & Niels E Skakkebaek[4]

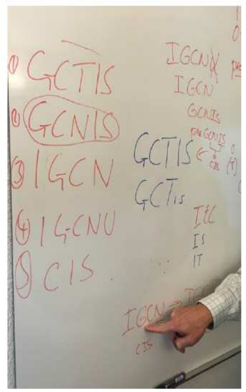


Figure 2. Workshopping the new name in Zurich, March 2015. The final proposal is indicated by the circle.

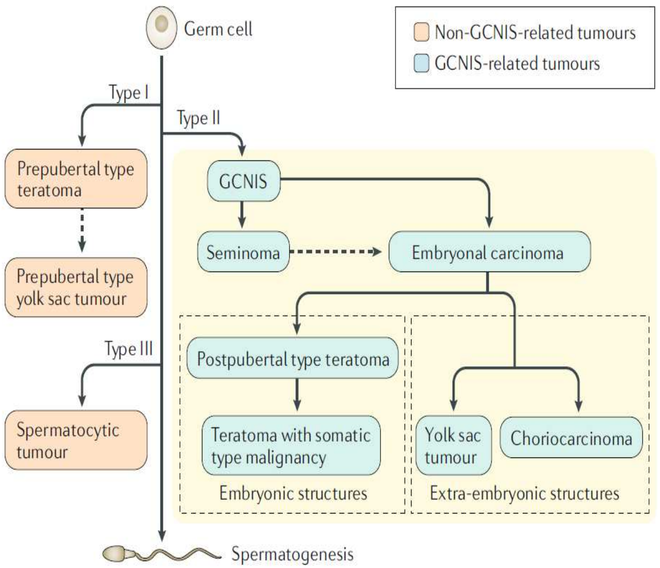
Focus Testicular (Type II) GCTs.

Diagnosis -- Treatment -- Follow-up

Testicular cancer

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]

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



Histopathology 2011, 58, 440–446. DOI: 10.1111/j.1365-2559.2011.03767.x

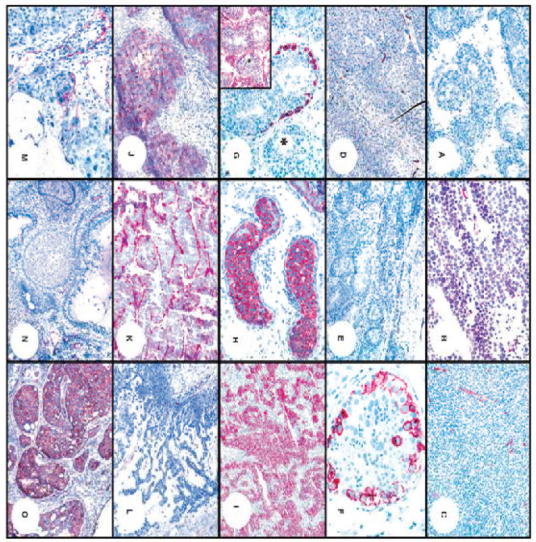
Diagnosis of testicular carcinoma *in situ* '(intratubular and microinvasive)' seminoma and embryonal carcinoma using direct enzymatic alkaline phosphatase reactivity on frozen histological sections

Hans Stoop,[1] Wim Kirkels,[2] Gert R Dohle,[2] Ad J M Gillis,[1] Michael A den Bakker,[1] Katharina Biermann,[1] Wolter Oosterhuis[1] & Leendert H J Looijenga[1]

Table 4.04 Usual patterns of immunoreactivity in testicular germ cell tumours and metastatic carcinoma (markers are listed alphabetically and proportions of reported positive reactivities are shown in parentheses)

Marker	Germ cell neoplasia in situ	Seminoma	Embryonal carcinoma	Yolk sac tumour	Choriocarcinoma	Teratoma	Spermatocytic tumour	Metastatic carcinoma	Other positive tumours
NANOG	+ (100%)	+ (100%)	+ (100%)	–	–	–	–	–	Gliomas, some carcinomas
OCT3/4 (POU5F1, OCT3, OCT4)	+ (100%)	+ (100%)	+ (100%)	–	–	–	–	–	Rare non-small cell lung cancer, clear cell renal carcinoma, and large cell lymphomas
SOX17	+	+ (95%)	–	± (50%)	–	±	ND	ND	ND
SOX2	–	– (< 1%)	+ (96%)	–	–	±	ND	ND	Immature elements in teratoma, melanoma, rhabdoid tumour
AFP	–	–	± (8–33%)	+ (74–100%)	–	±	–	±	Hepatocellular neoplasms, hepatoid carcinomas, occasional other non-germ cell tumours
βhCG	–	–	–	–	+ (100%)[a]	–	–	±	Other trophoblastic tumours, syncytiotrophoblasts in germ cell tumours, some non-germ cell tumours

World Health Organization



Impact all ages/anatomical localizations

Earliest pathogenetic changes.

Diagnosis -- Treatment -- Follow-up

Stem cell factor as a novel diagnostic marker for early malignant germ cells‡

J Pathol 2008; 216: 43–54

H Stoop,[1†] F Honecker,[1,2†] GJM van de Geijn,[1†] AJM Gillis,[1] MC Cools,[1,3] M de Boer,[1] C Bokemeyer,[2] KP Wolffenbuttel,[4] SLS Drop,[5] RR de Krijger,[1] N Dennis,[6] B Summersgill,[6] A McIntyre,[6] J Shipley,[6] JW Oosterhuis[1] and LHJ Looijenga[1]*

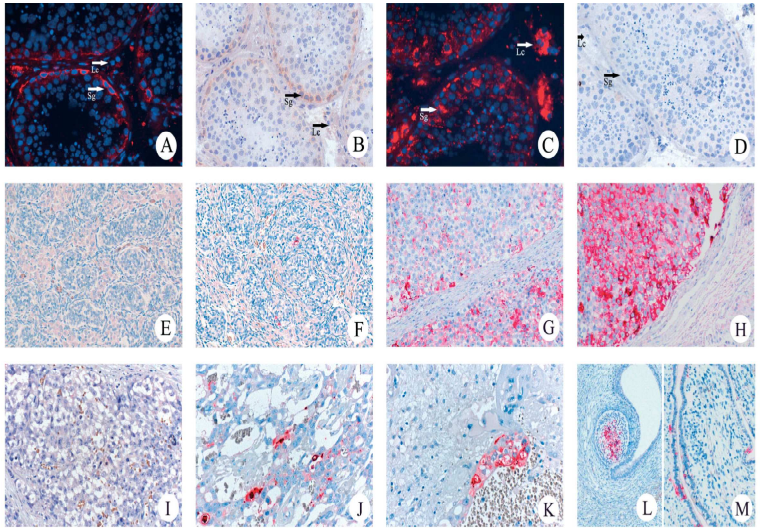
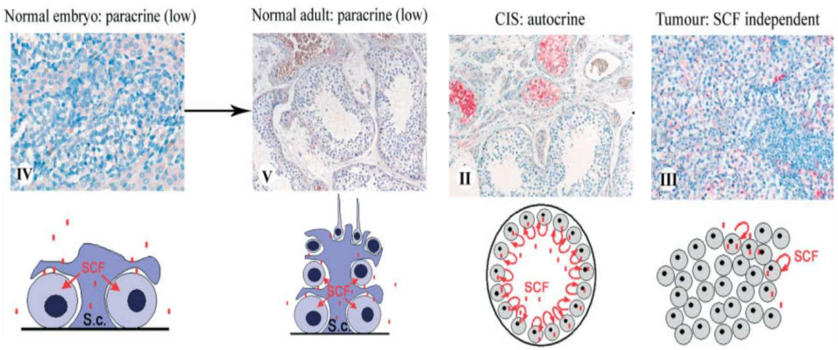
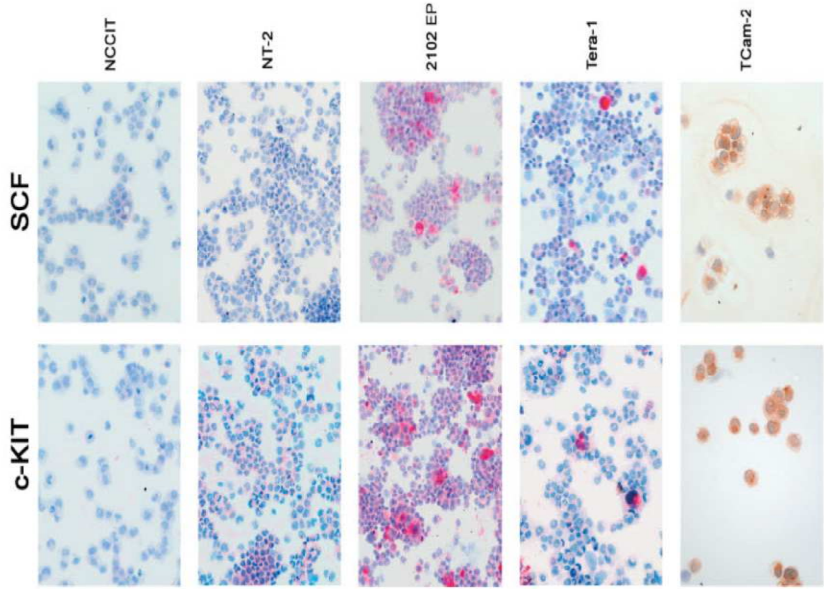
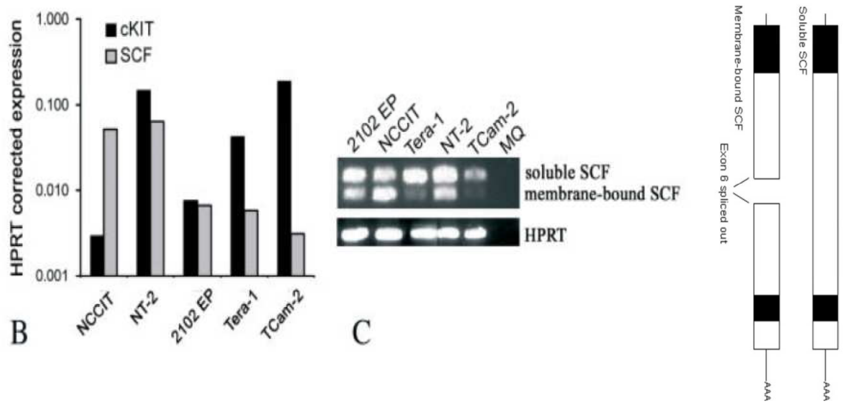


Figure 1. Immunohistochemistry for c-KIT (A, B) and SCF (C, D) on frozen normal adult testicular tissue: (A) fluorescent detection and (B) bright field detection of c-KIT. Arrows indicate positive spermatogonia (Sg) and Leydig cells (Lc). SCF immunostaining with (C) fluorescent detection (showing diffusely positive staining in the tubules and Leydig cells) and (D) non-fluorescent detection of SCF, being negative in the latter. SCF staining is (E) negative in a fetal male gonad of 15 weeks' gestational age and (F) reveals a few SCF-positive cells in a gonad of an 18-week-old male fetus with trisomy 21. Immunohistochemistry for SCF of invasive TGCTs (G–M) demonstrates heterogeneity in the different histological subtypes: (G, H) seminoma; (I) embryonal carcinoma; (J) yolk sac tumour; (K) choriocarcinoma; (L, M) two elements of mature teratoma







international journal of andrology ISSN 0105-6263

ORIGINAL ARTICLE

KIT and RAS signalling pathways in testicular germ cell tumours: new data and a review of the literature

N. C. Goddard,*[1] A. McIntyre,*[1] B. Summersgill,* D. Gilbert,* S. Kitazawa† and J. Shipley*

Earliest pathogenetic changes: screening (DSD).

Diagnosis -- Treatment -- Follow-up

Histological Assessment of Gonads in DSD: Relevance for Clinical Management

Johannes A. Spoor[a] J. Wolter Oosterhuis[b] Remko Hersmus[b] Katharina Biermann[b] Katja P. Wolffenbuttel[c] Martine Cools[d] Zainab Kazmi[a] Syed F. Ahmed[a] Leendert H.J. Looijenga[b]

Sex Dev 2018;12:106–122

Table 1. Diagnostic criteria of GCC precursor lesi...

Diagnosis	Type of gonad	Age	Germ cells: morphology	Germ cells: location in gonad	Germ cells: location in seminiferous tubule	Immunohistochemistry: germ cells	Immunohistochemistry: supportive cells
Delayed maturation	undervirilized testis or dysgenetic gonad	>6 months	typical gonocytes	wide spread	central	OCT3/4 + TSPY +/– KITLG –	SOX9+
Pre-GCNIS	undervirilized testis or dysgenetic gonad	any	typical gonocytes	focal	basement membrane	OCT3/4 + TSPY +[a] KITLG + (focal)	SOX9+
GCNIS	undervirilized testis	any	atypical gonocytes	focal	basement membrane	OCT3/4 + TSPY +[b] KITLG +[c]	SOX9+
Gonado-blastoma	dysgenetic gonad	any	atypical gonocytes	focal, in nests	in cord like structures or stroma	OCT3/4 + TSPY +[b] KITLG +	SOX9+ FOXL2+

Supportive cells can be Sertoli cells (SOX9+) or granulosa cells (FOXL2+).
[a] Co-expression of OCT3/4 and TSPY in a heterogeneous pattern. [b] Co-expression of OCT3/4 and TSPY in a homogenous pattern.
[c] Diffuse expression in Sertoli cells and in GCNIS cells.

"INTERSEX"

Gonadal Maldevelopment as Risk Factor for Germ Cell Cancer: Towards a Clinical Decision Model

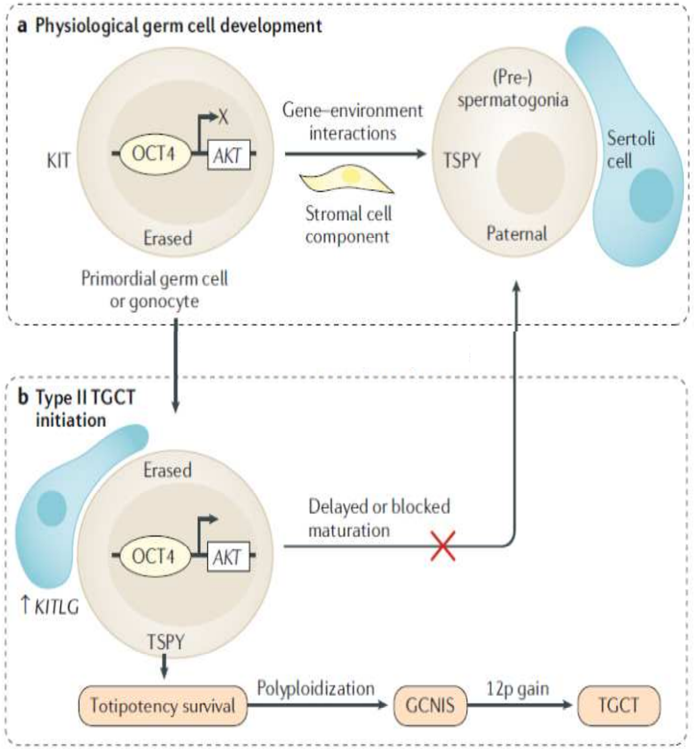
Yvonne G. van der Zwan[a], Katharina Biermann[a], Katja P. Wolffenbuttel[b], Martine Cools[c], Leendert HJ. Looijenga[a,*]

EUROPEAN UROLOGY 67 (2015) 692–701

NATURE REVIEWS | DISEASE PRIMERS | Article citation ID: (2018) 4:29

Testicular cancer

Liang Cheng, Peter Albers, Daniel M. Berney, Darren R. Feldman, Gedske Daugaard, Timothy Gilligan and Leendert H. J. Looijenga



Erasmus MC
NFU Nederlandse Federatie van Universitair Medische Centra
Ministerie van Volksgezondheid, Welzijn en Sport

ERASMUS MC - DSD

DSDNederland
VERENIGING VOOR MENSEN MET EEN XY-DSD (XY-DIFFERENCES OF SEX DEVELOPMENT) EN HUN OUDERS

KITLG & GWAS - TGCTs.

Diagnosis -- Treatment -- Follow-up

Princess máxima center
pediatric oncology

Rapley et al., 2009; Kanetsky et al. 2009; Turnbull et al. 2010; Kratz et al., 2011; Ruark et al., 2013; Chung et al., 2013.

+ KITLG [OR = 2.69 highest to date]; + SPRY4 [inh. MAPK, downstream KITLG]; + BAK1 [downstream KITLG]
+ DMRT1 [sex determination]
+ TERT, ATFIP [telomere maintenance]
+ UCK2, HPGDS, CENPE, CLPTM1L, MAD1L1, RFWD3, TEX14, PPM1E

Risk alleles are the major alleles (lower in Asian & African = low risk (T)GCC)

Independent:
Cryptorchidism; fam. predisposition, spermatogenic function

TECAC
TEstis CAncer Consortium

SUSCEPTIBILITY ALLELE(S)

< 1% of patients carry low risk *KITLG* allele
< 3% of patients carry low risk

double homozygous high risk alleles *KITLG* + *DMRT1* (28X TGCC)

OPEN ACCESS Freely available online   PLOS | GENETICS

Mutations in *LRRC50* Predispose Zebrafish and Humans to Seminomas

Sander G. Basten[1], Erica E. Davis[2,9], Ad J. M. Gillis[3,9], Ellen van Rooijen[1,4], Hans Stoop[3], Nikolina Babala[1], Ive Logister[1,4], Zachary G. Heath[2], Trudy N. Jonges[5], Nicholas Katsanis[2], Emile E. Voest[1], Freek J. van Eeden[4], Rene H. Medema[1], René F. Ketting[4], Stefan Schulte-Merker[4], Leendert H. J. Looijenga[4], Rachel H. Giles[1,6*]

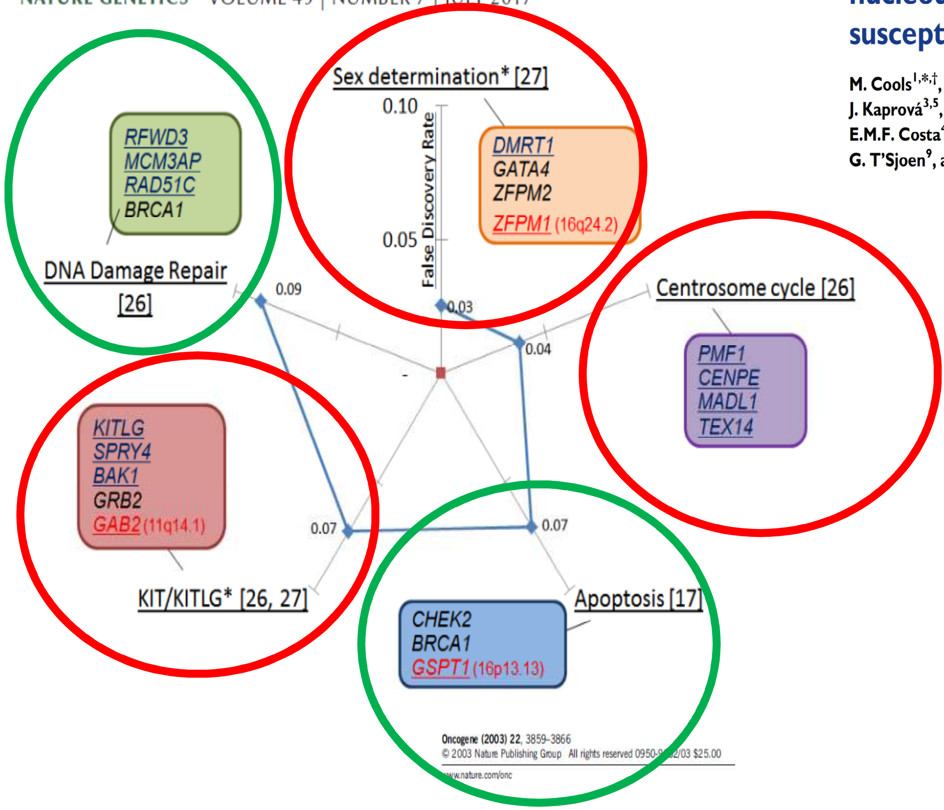
SNP susceptibility TGCTs (GWAS):

Diagnosis -- Treatment -- Follow-up

Meta-analysis of five genome-wide association studies identifies multiple new loci associated with testicular germ cell tumor

Identification of 19 new risk loci and potential regulatory mechanisms influencing susceptibility to testicular germ cell tumor

NATURE GENETICS VOLUME 49 | NUMBER 7 | JULY 2017



Aneuploidy of human testicular germ cell tumors is associated with amplification of centrosomes

Frank Mayer[1,2], Hans Stoop[1], Subrata Sen[3], Carsten Bokemeyer[2], J Wolter Oosterhuis[1] and Leendert HJ Looijenga*[1]

Human Reproduction, Vol.32, No.12 pp. 2561–2573, 2017
Advanced Access publication on November 7, 2017 doi:10.1093/humrep/dex300

human reproduction ORIGINAL ARTICLE *Reproductive genetics*

Malignant testicular germ cell tumors in postpubertal individuals with androgen insensitivity: prevalence, pathology and relevance of single nucleotide polymorphism-based susceptibility profiling

M. Cools[1,*,†], K.P. Wolffenbuttel[2,†], R. Hersmus[3], B.B. Mendonca[4], J. Kaprová[3,5], S.L.S. Drop[6], H. Stoop[3], A.J.M. Gillis[3], J.W. Oosterhuis[3], E.M.F. Costa[4], S. Domenice[4], M.Y. Nishi[4], L. Wunsch[7], C.A. Quigley[8], G. T'Sjoen[9], and L.H.J. Looijenga[3,*]

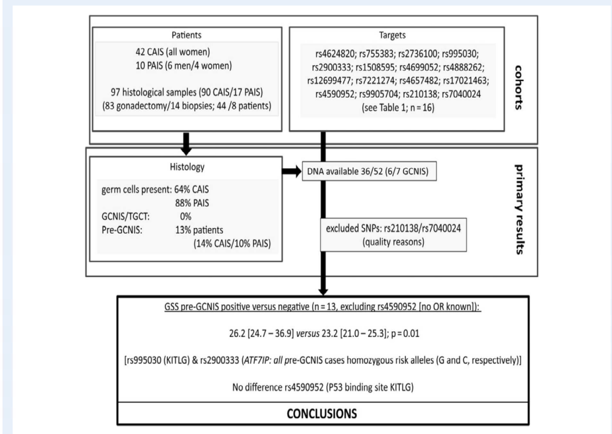
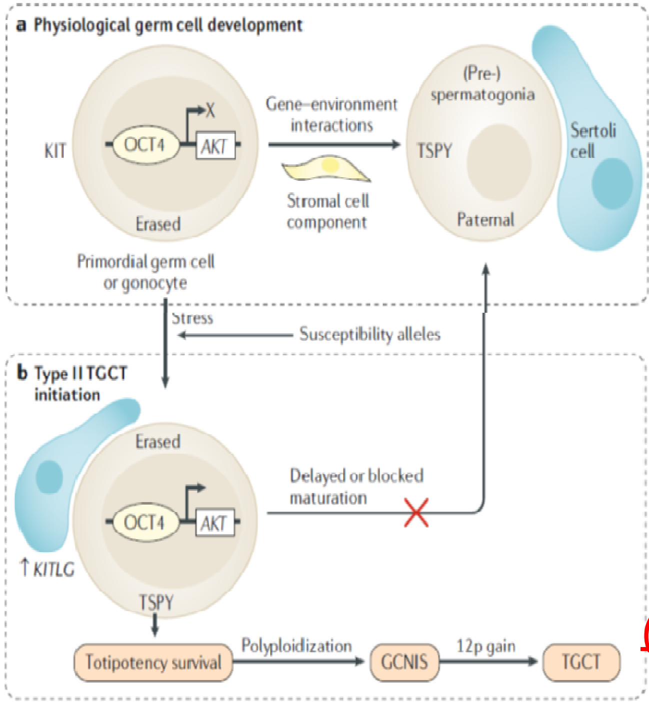
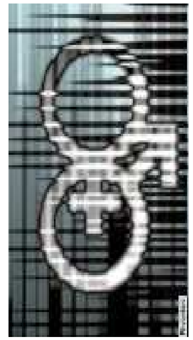


Figure 1 Schematic overview of the design of the study, both related to patients included as well as to SNPs investigated. Note that two of the original series of targets were excluded due to technical reasons (see text), resulting in a final list of 14 SNPs (see Table I). One of these SNPs lacks an odds ratio (OR), and was therefore not included in the final genetic susceptibility score (GSS). CAIS/PAIS: complete/partial androgen insensitivity syndrome: GCNIS: germ cell neoplasia in situ; TGCT: testicular germ cell tumor.





INTEGRATED RISK ASSESSMENT (INDIVIDUAL BASIS)

From constitutional DNA to tumor specific changes (epigenetics): primary tumors

Princess máxima center pediatric oncology

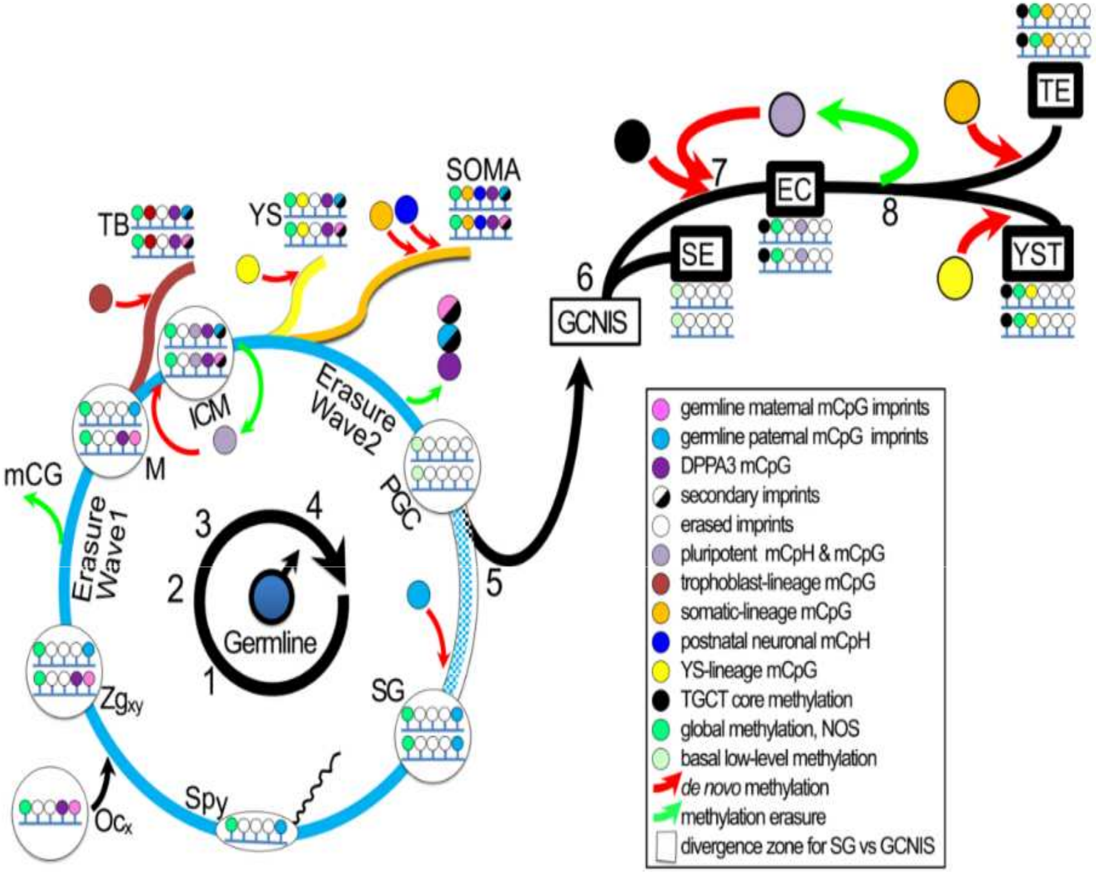
Imprints and *DPPA3* are bypassed during pluripotency- and differentiation-coupled methylation reprogramming in testicular germ cell tumors

J. Keith Killian,[1] Lambert C.J. Dorssers,[2] Britton Trabert,[3] Ad J.M. Gillis,[2] Michael B. Cook,[3] Yonghong Wang,[1] Joshua J. Waterfall,[1] Holly Stevenson,[1] William I. Smith Jr.,[4] Natalia Noyes,[1] Parvathy Retnakumar,[1] J. Hans Stoop,[2] J. Wolter Oosterhuis,[2] Paul S. Meltzer,[1] Katherine A. McGlynn,[3] and Leendert H.J. Looijenga[2]

Genome Research 2016 26:1490–1504
www.genome.org

Supplemental Table S1: Summary of Original and Reference samples in this study

	Sample type	GEO Accession#	n
Original			
	SE	GSE74104	61
	EC	GSE74104	43
	YST	GSE74104	6
	TE	GSE74104	20
	BNT	GSE74104	128
	OVT	GSE74104	9
	Total Original		**267**
Public database			
	PGC	GSE63818	13
	Soma	GSE52576,GSE30654,GSE63818	95
	ESC	GSE52576,GSE30654	29
	iPSC	GSE30654	35
	sperm	GSE47627	8
	lymphoid	GSE35069	18
	hydatidiform mole	GSE52576,GSE30654	9
	phESC	GSE52576,GSE30654,GSE57992	12
	placenta/villi	GSE41336,GSE57767	23
	triploid placenta	GSE74738	10
	TGCT cell lines	GSE60787	12
	chimp iPSC	GSE61343	28
	leukocyte	GSE67393	117
	brain cortex- prenatal	GSE74193	65
	brain cortex- postnatal	GSE64509	35
	cerebellum- postnatal	GSE64509	32
	brain neurons- adult (FACS)	GSE50798	12
	brain glia- adult (FACS)	GSE50798	12
	neuroblastoma tissue	GSE54719	35
	cancer compendium cell lines	GSE68379	1028
	benign prostate	TCGA	50
	TGCT	TCGA	94
	Total Reference		**1772**



All pure histology (none mixed)

GCNIS & SE = PGC; EC = ES/IPS; Diff. NS = embryonic -; TE = somatic adult tissue.

From constitutional DNA to tumor specific changes (epigenetics): pathogenesis.

Int. J. Mol. Sci. **2019**, *20*, 258; doi:10.3390/ijms20020258

Review

Human Germ Cell Tumors are Developmental Cancers: Impact of Epigenetics on Pathobiology and Clinic

João Lobo [1,2,3], Ad J. M. Gillis [4,5], Carmen Jerónimo [1,3], Rui Henrique [1,2,3] and Leendert H. J. Looijenga [4,5,*]

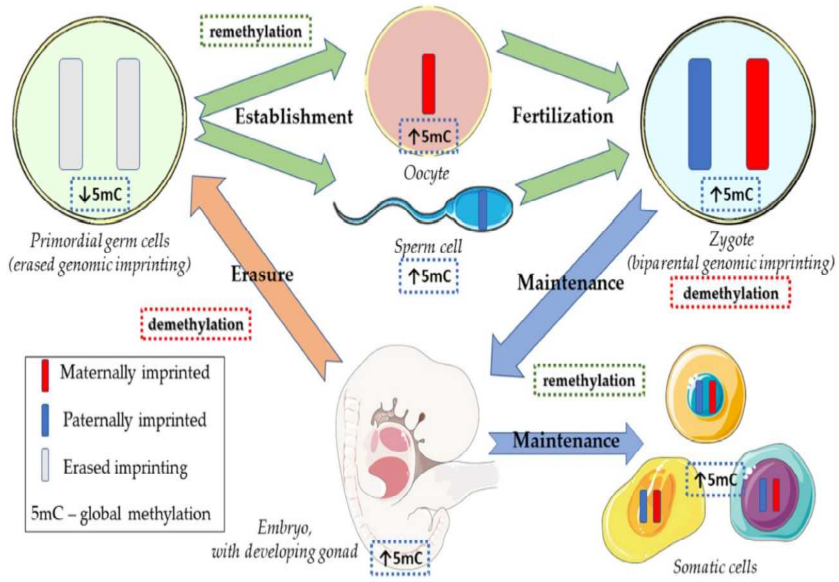
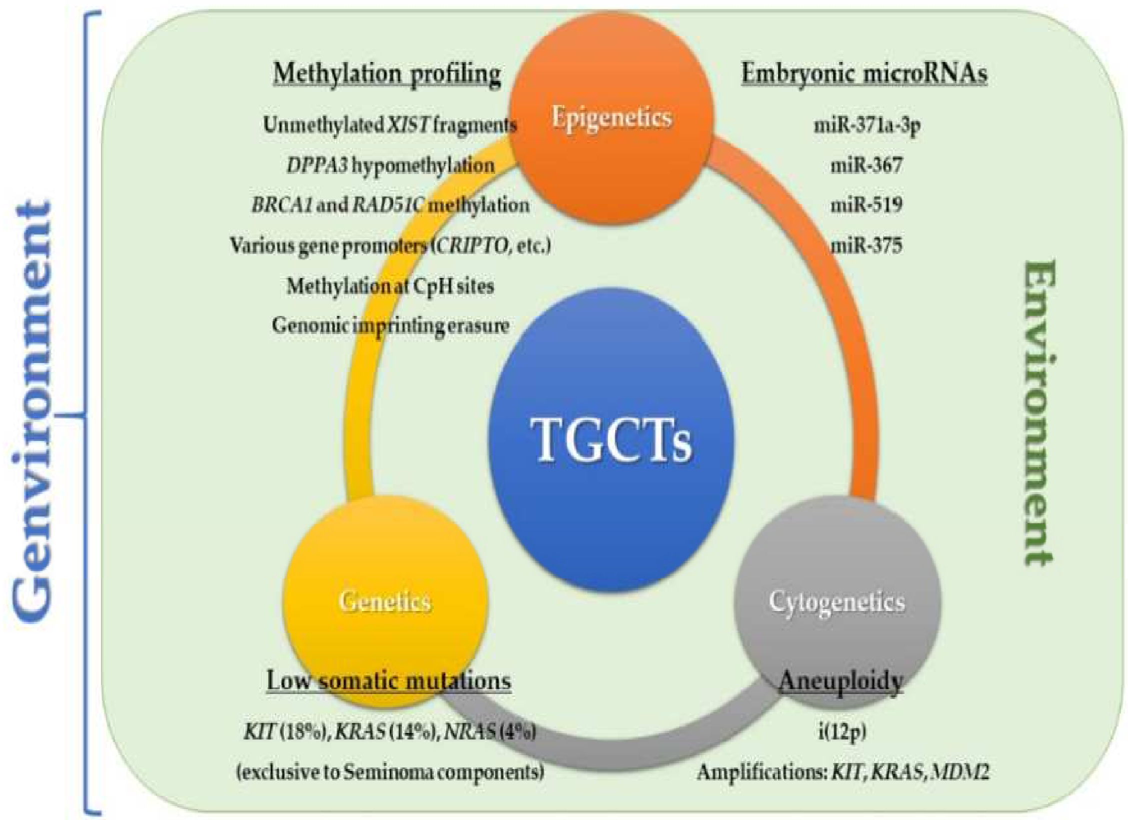


Figure 1. Cycle of genomic imprinting and global methylation.



QUESTIONS OF RELEVANCE:
Does GCNIS always progress to SE/NS?; What is risk of SE to progress into NS?
What is risk for metastases in (occult) stage I SE and NS?; How to predict RMT?
How to predict (standard) treatment resistance?

From constitutional DNA to tumor specific changes (genetics): primary tumors.

Whole-exome sequencing reveals the mutational spectrum of testicular germ cell tumours

Kevin Litchfield[1], Brenda Summersgill[2], Shawn Yost[1], Razvan Sultana[1], Karim Labreche[1,3], Darshna Dudakia[1], Anthony Renwick[1], Sheila Seal[1], Reem Al-Saadi[2], Peter Broderick[1], Nicholas C. Turner[4], Richard S. Houlston[1], Robert Huddart[5], Janet Shipley[2] & Clare Turnbull[1,6]

Received 26 Sep 2014 | Accepted 25 Nov 2014 | Published 22 Jan 2015

Exome-wide Sequencing Shows Low Mutation Rates and Identifies Novel Mutated Genes in Seminomas

EUROPEAN UROLOGY 68 (2015) 77–83

Ioana Cutcutache[a,b], Yuka Suzuki[a,b], Iain Beehuat Tan[c,d], Subhashini Ramgopal[a,b], Shenli Zhang[b], Kalpana Ramnarayanan[b], Anna Gan[b,e], Heng Hong Lee[b,e], Su Ting Tay[b], Aikseng Ooi[f], Choon Kiat Ong[e], Jonathan T. Bolthouse[g], Brian R. Lane[g], John G. Anema[g], Richard J. Kahnoski[g], Patrick Tan[b,d,h,], Bin Tean Teh[b,e,h,*], Steven G. Rozen[a,b,*]*

Exome Sequencing of Bilateral Testicular Germ Cell Tumors Suggests Independent Development Lineages[1,2]

Sigmund Brabrand[*,†,‡,3], Bjarne Johannessen[*,†,3], Ulrika Axcrona[§], Sigrid M. Kraggerud[*,†], Kaja G. Berg[*,†], Anne C. Bakken[*,†], Jarle Bruun[*,†], Sophie D. Fosså[¶], Ragnhild A. Lothe[*,†], Gustav Lehne[¶] and Rolf I. Skotheim[*,†]

*Department of Molecular Oncology, Institute for Cancer

Neoplasia (2015) 17, 167–174

Genomic evolution and chemoresistance in germ-cell tumours

NATURE | VOL 540 | 1 DECEMBER 2016

Amaro Taylor-Weiner[1,2*], Travis Zack[1,3*], Elizabeth O'Donnell[4,5], Jennifer L. Guerriero[4], Brandon Bernard[4], Anita Reddy[6], G. Celine Han[2,4], Saud AlDubayan[7,8], Ali Amin-Mansour[2], Steven E. Schumacher[2,9], Kevin Litchfield[10,11], Clare Turnbull[10,11], Stacey Gabriel[2], Rameen Beroukhim[2,4], Gad Getz[2,12], Scott L. Carter[2,13,14,15], Michelle S. Hirsch[16], Anthony Letai[4], Christopher Sweeney[4]§ & Eliezer M. Van Allen[2,4,13]§

Rare disruptive mutations in ciliary function genes contribute to testicular cancer susceptibility

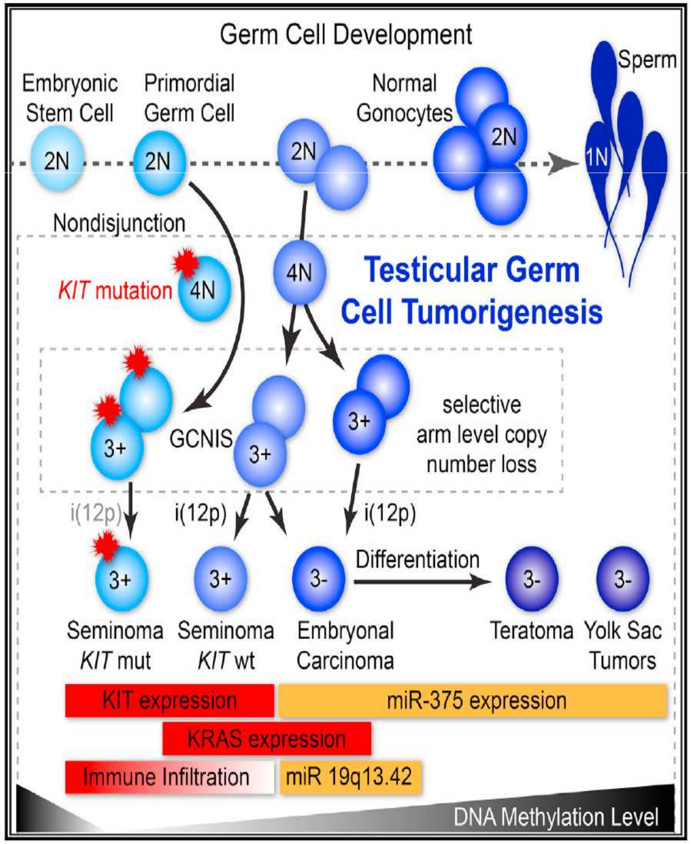
Kevin Litchfield[1], Max Levy[1], Darshna Dudakia[1], Paula Proszek[2], Claire Shipley[2], Sander Basten[3], Elizabeth Rapley[1], D. Timothy Bishop[4], Alison Reid[5], Robert Huddart[5], Peter Broderick[1], David Gonzalez de Castro[2,6], Simon O'Connor[2], Rachel H. Giles[3], Richard S. Houlston[1,7] & Clare Turnbull[1,8,9]

Received 5 May 2016 | Accepted 4 Nov 2016 | Published 20 Dec 2016

Integrated Molecular Characterization of Testicular Germ Cell Tumors

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] David I. Heiman,[2] Katayoon Kasaian,[8] Minwei Liu,[26] Michael S. Noble,[2] Liu Xi,[6] Hailei Zhang,[2] Wanding Zhou,[1] Jean C. ZenKlusen,[27] Carolyn M. Hutter,[28] Ina Felau,[27] Jiashan Zhang,[27] Nikolaus Schultz,[13] Gad Getz,[2,29] Matthew Meyerson,[2,3] Joshua M. Stuart,[11] The Cancer Genome Atlas Research Network, Rehan Akbani,[12] David A. Wheeler,[6] Peter W. Laird,[1] Katherine L. Nathanson,[14,30] Victoria K. Cortessis,[31,*] and Katherine A. Hoadley[5,33,*]

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



From constitutional DNA to tumor specific changes (epi/genetics): primary

Princess máxima center pediatric oncology

Molecular heterogeneity and early metastatic clone selection in testicular germ cell cancer development

Lambert C. J. Dorssers[1], Ad J. M. Gillis[1], Hans Stoop[1], Ronald van Marion[1], Marleen M. Nieboer[2], Job van Riet[3,4], Harmen J. G. van de Werken[3,4], J. Wolter Oosterhuis[1], Jeroen de Ridder[2] and Leendert H. J. Looijenga[1,5]

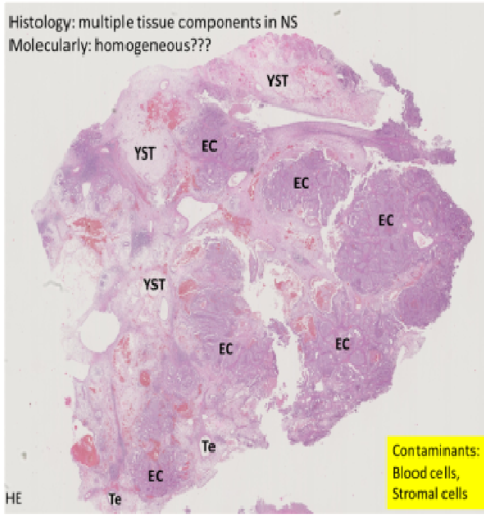
BJC British Journal of Cancer

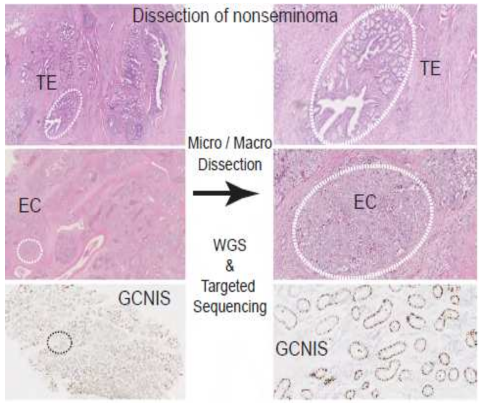
Whole genome & targeted seq. RNA Seq. & Methyl. Profiling

42 samples of 4 resistant NS (chemo-naive)

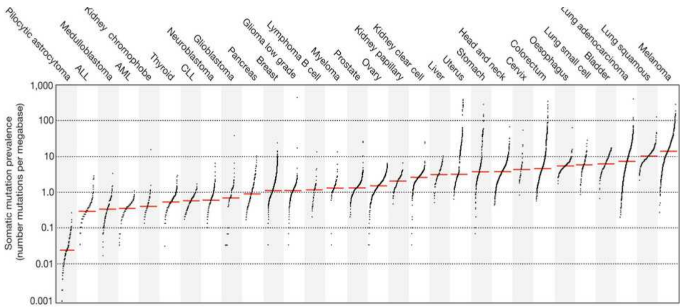
Case	T3209	T6107	T618	T1382
	NS / PBL	NS / PBL	NS / NAP	NS / PBL
Whole Genome Sequencing				
CG sSNV	558	635	455	494
Putative sSNV	374	197	365	303
Validated sSNV / tested	55 / 56	24 / 25	40 / 45	31 / 32
CG SV	33	9	26	24
Validated SV / tested	2 / 2	1 / 1	4 / 8	2 / 4
Splicing mutation	0 / 4	1 / 2	3 / 4	0 / 1
Protein change*	12	3	14	16
Micro- or macro dissection of specific components				
GCNIS	2	2	3	
EC	3	4		1
EB	7			
TE	4	1		5
YST	3	3		4
Normal			1	

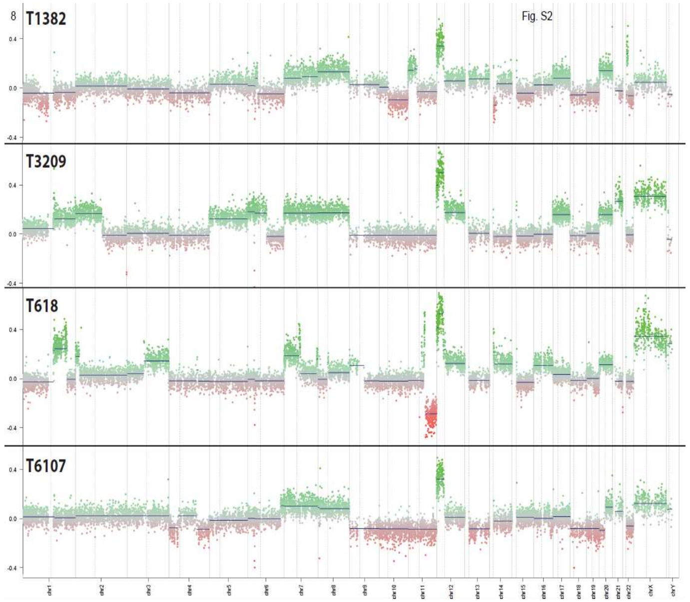
BRCA signature





Nature. 2013 August 22; 500(7463): 415–421. doi:10.1038/nature12477.





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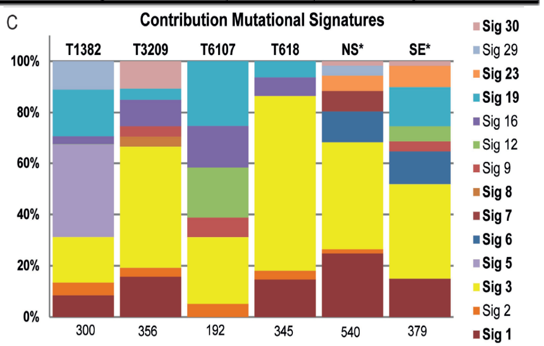
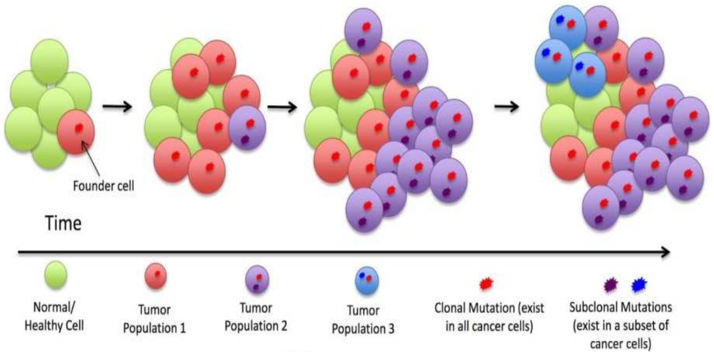
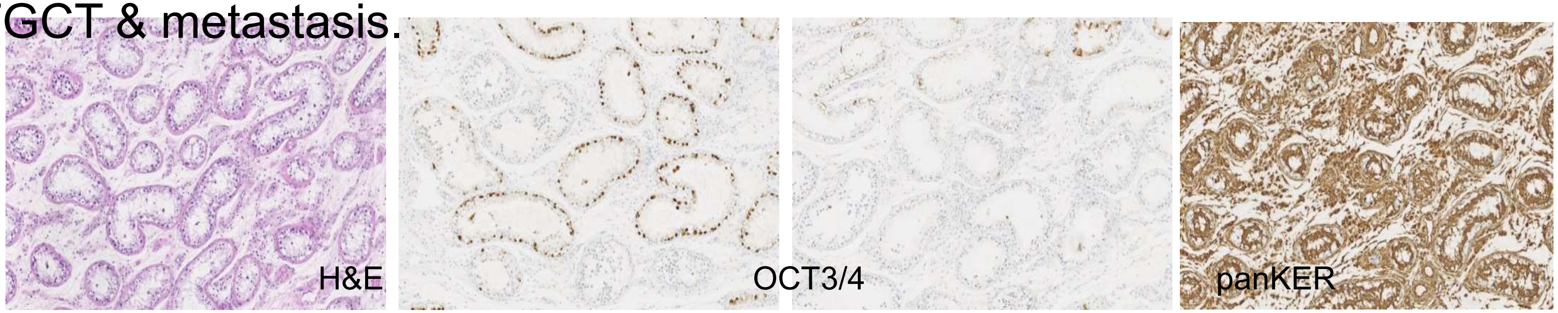
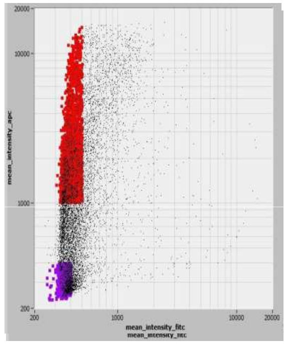


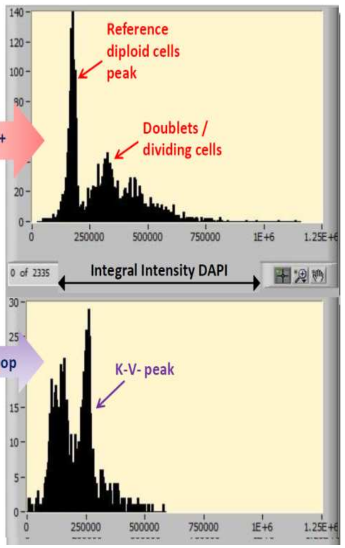


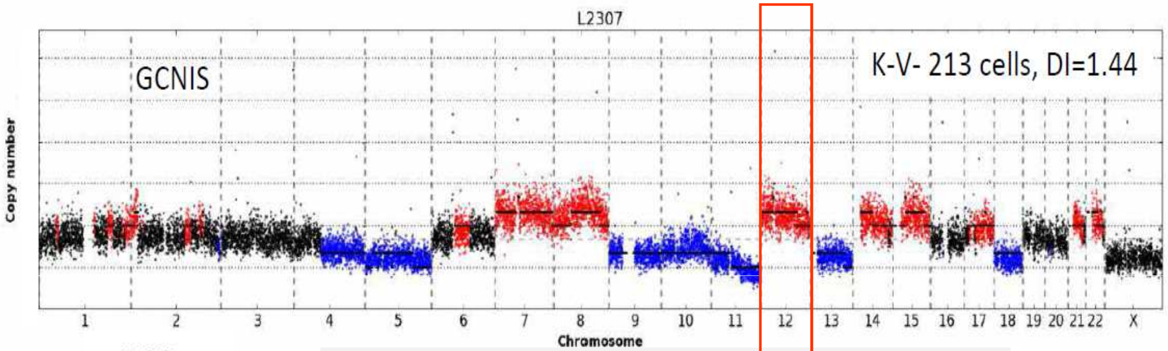
From constitutional DNA to tumor specific changes (epi/genetics): primary TGCT & metastasis.

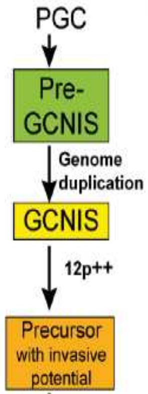


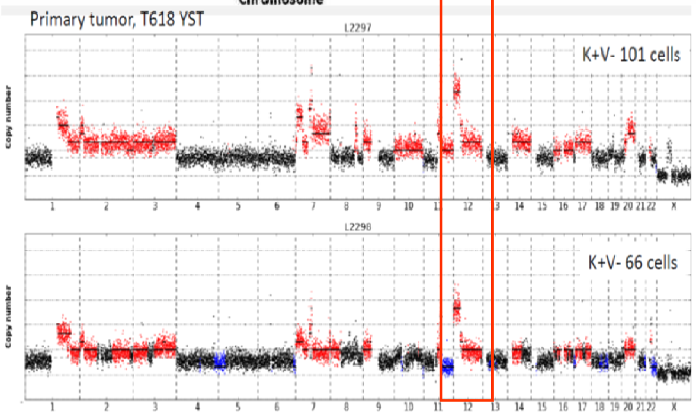
Vimentin

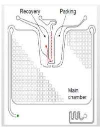












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Menarini team:
Rossana Lanzelotti, Castel Maggiore, IT
Alberto Ferrarini
Béchir Boughaba
Raimo Tanzi
Francesca Fontana

Oncogene. 2000 Nov 30;19(51):5858-62.

Overrepresentation of the short arm of chromosome 12 is related to invasive growth of human testicular seminomas and nonseminomas.

Rosenberg C[1], Van Gurp RJ, Geelen E, Oosterhuis JW, Looijenga LH.

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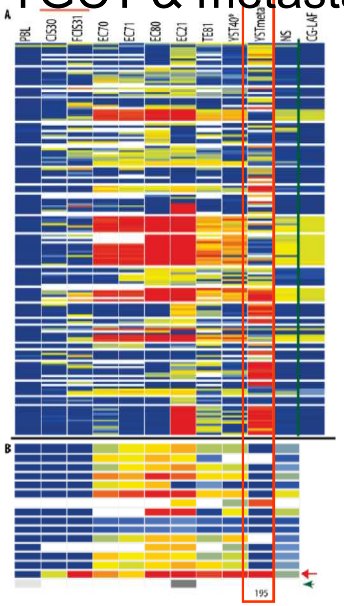
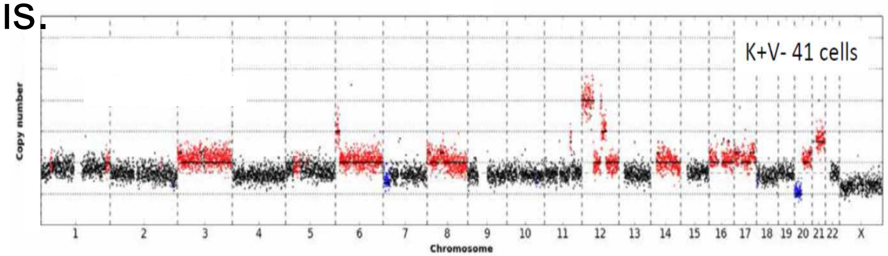
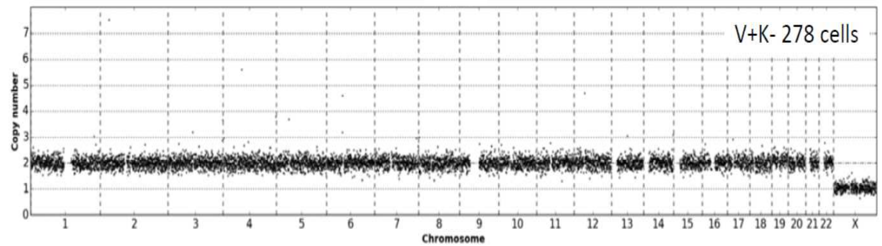
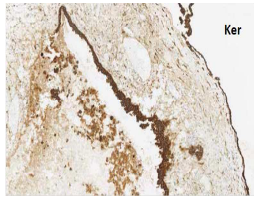
From constitutional DNA to tumor specific changes (epi/genetics): primary TGCT & metastasis.

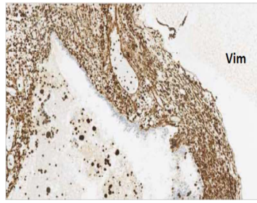






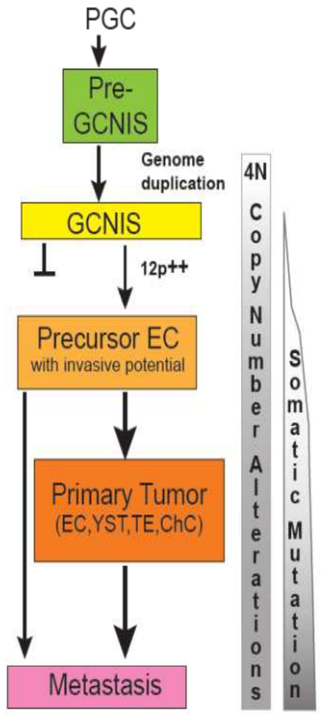


RMT (15 months after orchiectomy)

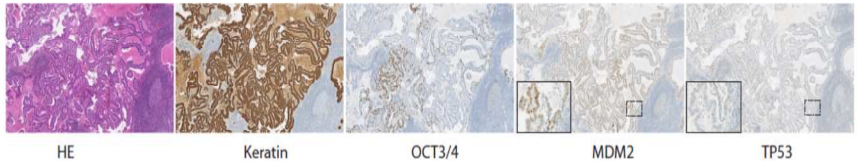


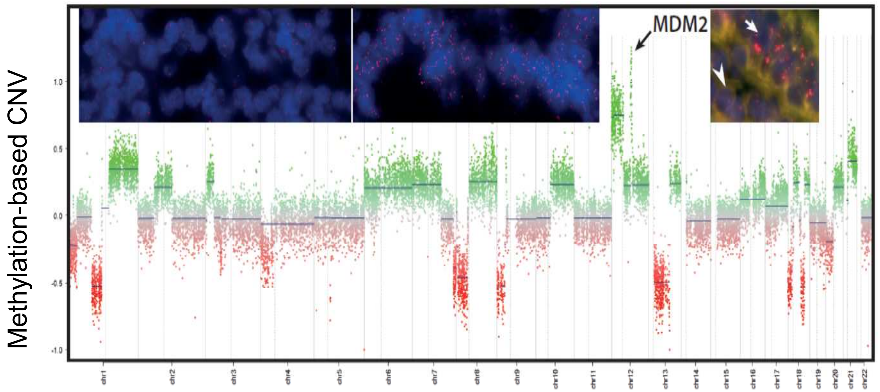
Therapeutic Potential of Mdm2 Inhibition in Malignant Germ Cell Tumours EUROPEAN UROLOGY 57 (2010) 679–687

Sebastian Bauer[a,b,], Thomas Mühlenberg[a,b], Michael Leahy[c], Mathias Hoiczyk[a,b], Thomas Gauler[b], Martin Schuler[a,b], Leendert Looijenga[d]*



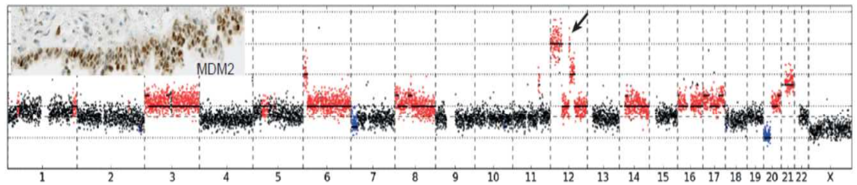
Nonseminoma Model





Genetic Determinants of Cisplatin Resistance in Patients With Advanced Germ Cell Tumors *J Clin Oncol 34:4000-4007. © 2016*

Aditya Bagrodia, Byron H. Lee, William Lee, Eugene K. Cha, John P. Sfakianos, Gopa Iyer, Eugene J. Pietzak, Sizhi Paul Gao, Emily C. Zabor, Irina Ostrovnaya, Samuel D. Kaffenberger, Aijazuddin Syed, Maria E. Arcila, Raju S. Chaganti, Ritika Kundra, Jana Eng, Joseph Hreiki, Vladimir Vacic, Kanika Arora, Dayna M. Oschwald, Michael F. Berger, Dean F. Bajorin, Manjit S. Bains, Nikolaus Schultz, Victor E. Reuter, Joel Sheinfeld, George J. Bosl, Hikmat A. Al-Ahmadie, David B. Solit, and Darren R. Feldman



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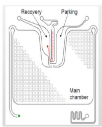
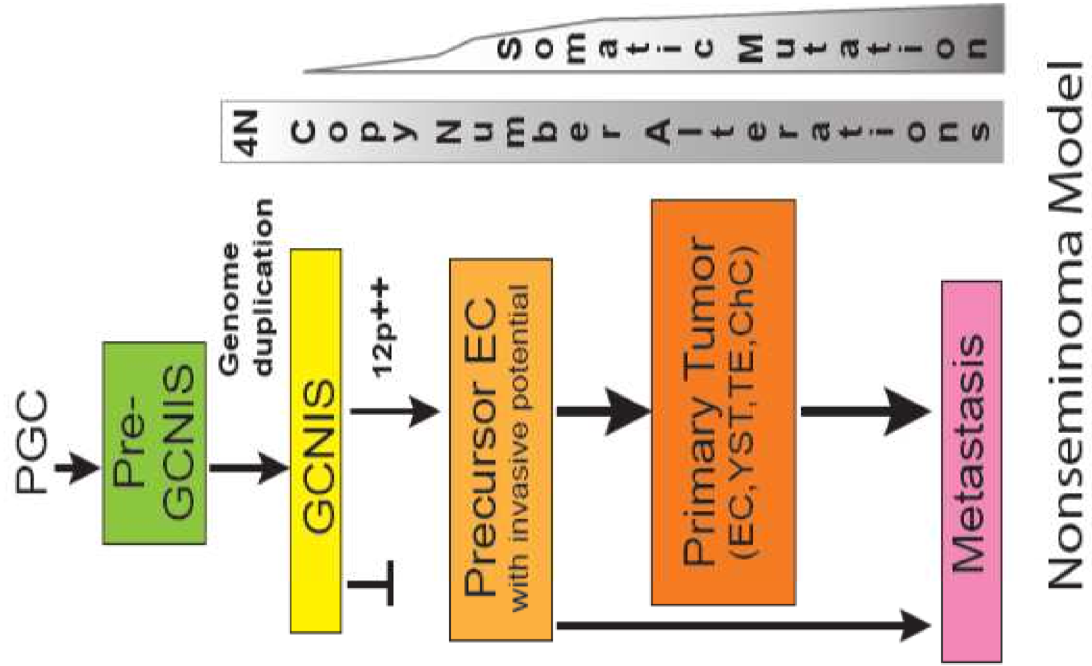


From constitutional DNA to tumor specific changes (epi/genetics): primary TGCT & metastasis.



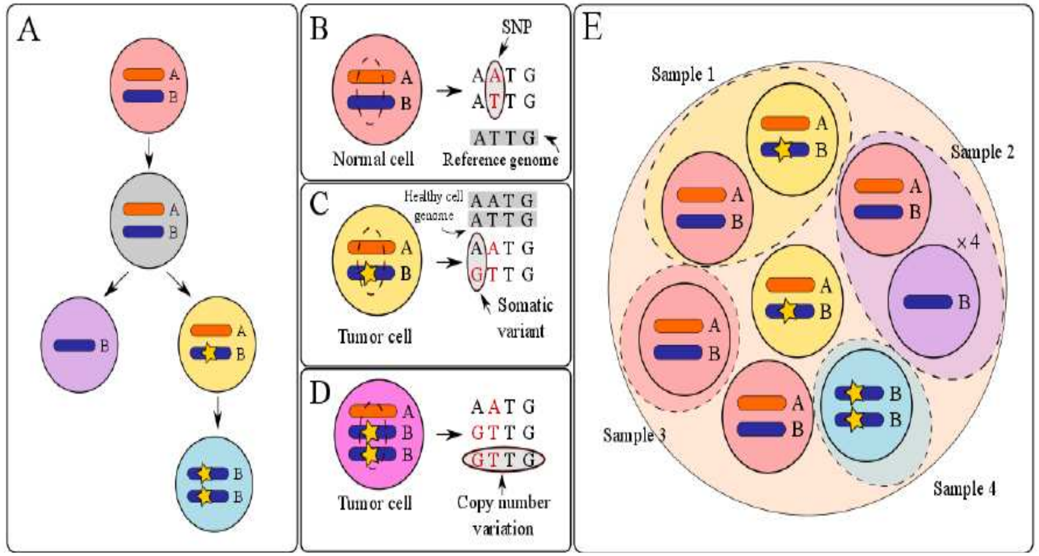
Epigenetic initiation

Main question(s): (Epi)genetics
- Early pathogenetic hit
- Tumor heterogeneity
- Treatment sensitivity/resista

TargetClone: A multi-sample approach for reconstructing subclonal evolution of tumors

Marleen M. Nieboer[1], Lambert C. J. Dorssers[2], Roy Straver[1], Leendert H. J. Looijenga[2,3], Jeroen de Ridder[1]*

PLOS ONE | https://doi.org/10.1371/journal.pone.0208002 November 29, 2018



Liquid biopsy analyses (T)GCTs.

Diagnosis -- Treatment -- Follow-up

?? - UNIVERSAL MARKER - ??

Literature: sensitivity (%)

	SE	TGCT NS
AFP	36	3
	45	

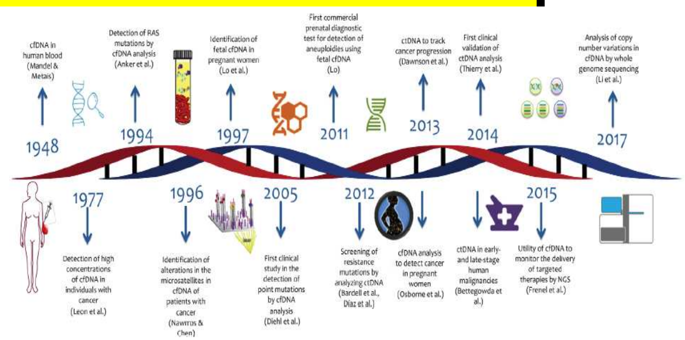


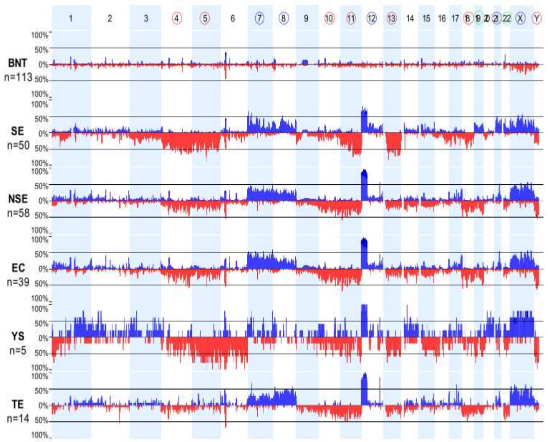
Figure 1: Timeline of liquid biopsy development.

www.impactjournals.com/oncotarget/ Oncotarget, 2018, Vol. 9, (No. 2), pp: 2912-2922

Review

The dawn of the liquid biopsy in the fight against cancer

Irma G. Domínguez-Vigil[1], Ana K. Moreno-Martínez[1,2], Julia Y. Wang[3], Michael H. A. Roehrl[4] and Hugo A. Barrera-Saldaña[1,5]



XIST unmethylated DNA fragments in male-derived plasma as a tumour marker for testicular cancer

Takahiro Kawakami, Keisei Okamoto, Osamu Ogawa, Yusaku Okada

Testicular germ-cell tumours (TGCTs) are the most common malignant diseases among men aged 20–40 years. We developed a DNA tumour marker for TGCTs based on the unmethylated DNA profile of a neoplasm. The 5′ end of the *XIST* gene is mainly hypomethylated in TGCTs irrespective of *XIST* expression. Male somatic cells, however, show complete methylation through the CpG sites, including the minimum promoter and *XIST*-conserved repeats. Identification of a *XIST* unmethylated fragment in male plasma might be diagnostic for TGCTs.

Lancet 2004; **363:** 40–42

See Commentary page 6

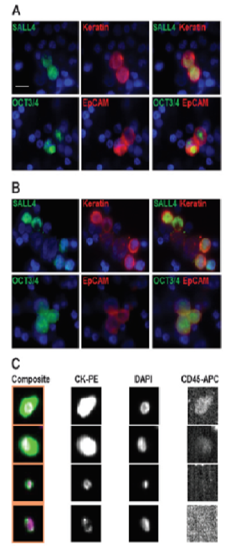
J Clin Oncol. 2001 Jun 15;19(12):3029-36. Clinical impact of germ cell tumor cells in apheresis products of patients receiving high-dose chemotherapy. Bokemeyer C, Gillis AJ, Pompe K, Mayer F, Metzner B, Schleucher N, Schleicher J, Pflugrad-Jauch G, Oosterhuis JW, Kanz L, Looijenga LH.
PBSC preparations from 57 patients were investigated for the presence of contaminating tumor cells using this set of targets, including beta human chorionic gonadotropin (beta-hCG), fibronectin (EDB variant), epidermal growth factor receptor (EGFR), CD44 (v8 to 10 variant), germ cell and placental alkaline phosphatase (AP), human endogenous retrovirus type K (ENV and GAG), and XIST. Despite the presence of tumor cells, retransplantation of the PBSC products did not effect long-term outcome.

Circulating Tumor Cells in Patients with Testicular Germ Cell Tumors

Clin Cancer Res; 20(14) July 15, 2014

Paulina Nastały[1], Christian Ruf[2,4], Pascal Becker[4], Natalia Bednarz-Knoll[1], Małgorzata Stoupiec[1], Refik Kavsur[1], Hendrik Isbarn[2], Cord Matthies[4], Walter Wagner[4], Dirk Höppner[5], Margit Fisch[2], Carsten Bokemeyer[3], Sascha Ahyai[2], Friedemann Honecker[3], Sabine Riethdorf[1], and Klaus Pantel[1]

Results: In total, CTCs were detected in 25 of 143 (17.5%) peripheral blood samples, whereas only 11.5% of patients were CTC-positive when considering exclusively the CellSearch assay. The presence of CTCs in peripheral blood correlated with clinical stage ($P < 0.001$) with 41% of CTC positivity in patients with metastasized tumors and 100% in patients with relapsed and chemotherapy-refractory disease. Histologically, CTC-positive patients suffered more frequently from nonseminomatous primary tumors ($P < 0.001$), with higher percentage of yolk sac ($P < 0.001$) and teratoma ($P = 0.004$) components. Furthermore, CTC detection was associated with elevated serum levels of α-fetoprotein (AFP; $P = 0.025$), β-human chorionic gonadotropin (βHCG; $P = 0.002$), and lactate dehydrogenase (LDH; $P = 0.002$). Incidence and numbers of CTCs in TVB were much higher than in peripheral blood.

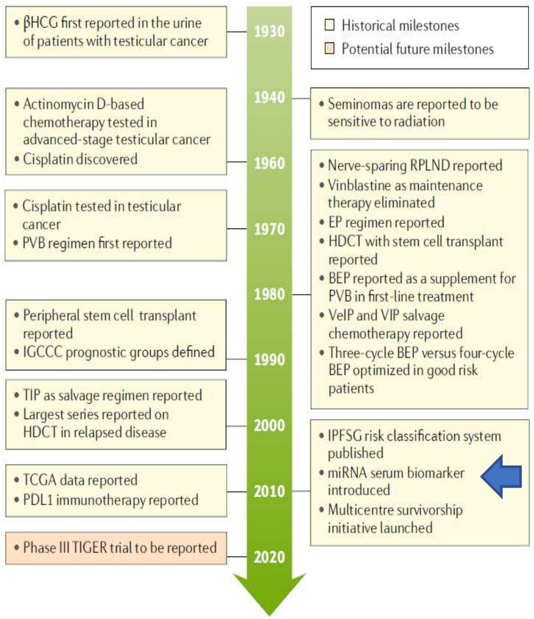


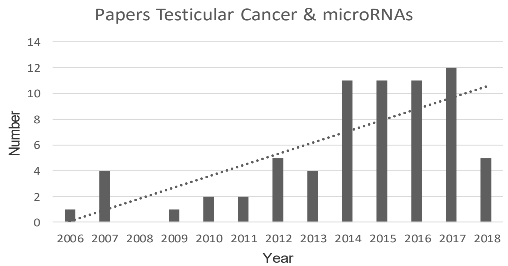
Liquid biopsy analyses (T)GCTs: microRI Diagnosis -- Treatment -- Follow-up

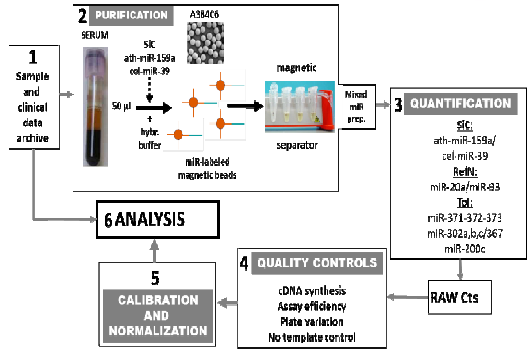
Testicular cancer

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]

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







miR-371a-3p: positive in SE, EC, YST, CH (not TE)

Sens./Spec. 90/86%

AUC: 95.1%

PPV/NPV:

“False negatives”:
- Small tumors
- Pure teratoma

(T)GCT liquid biopsies: 174 + 89 + 34 + 603 + 10 = 910; TE: 6 = 6; GCNIS only: 33 + 6 = 39; total: 955

Controls: 94 + 119 + 276 = 489

Involved LEPO:
Ton van Agthoven, Lambert Dorssers, Ad Gillis, Wolter Oosterhuis, Hans Stoop (and collaborators)

Students:
Annemarie Bakkum, Anna Daamen, Mark van der Lee, Jeffrey Oliviera, Arina Puchina, Kabir Razack, Kasper Smits

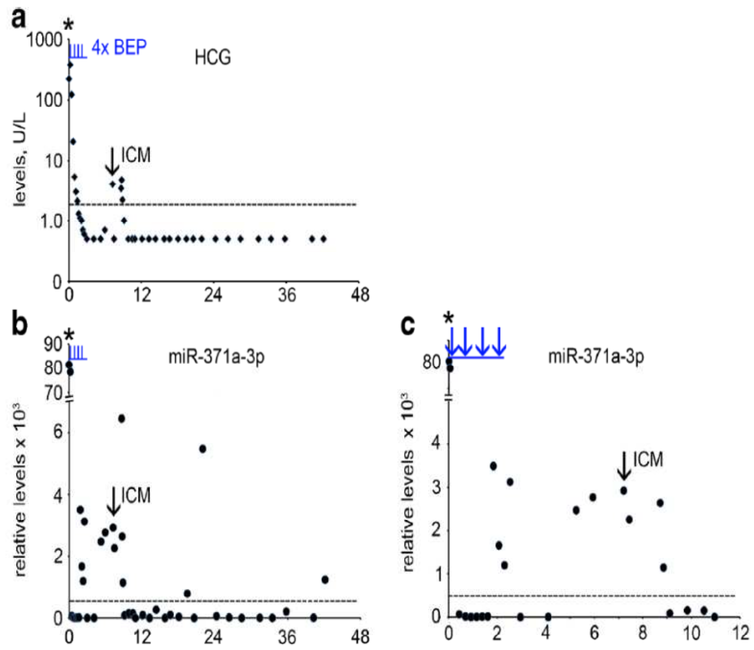
Liquid biopsy analyses (T)GCTs: miR-371a-3p (longitudinal series I)

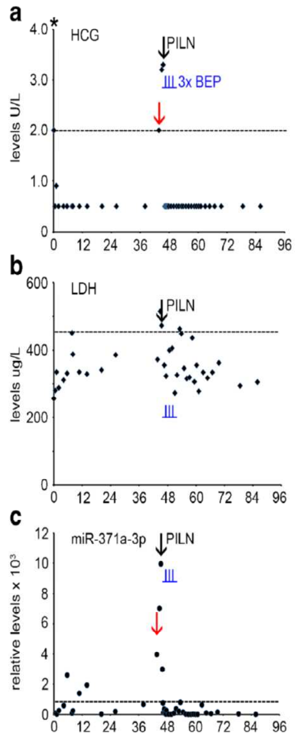
Cell Oncol. (2017) 40:379–388
DOI 10.1007/s13402-017-0333-9

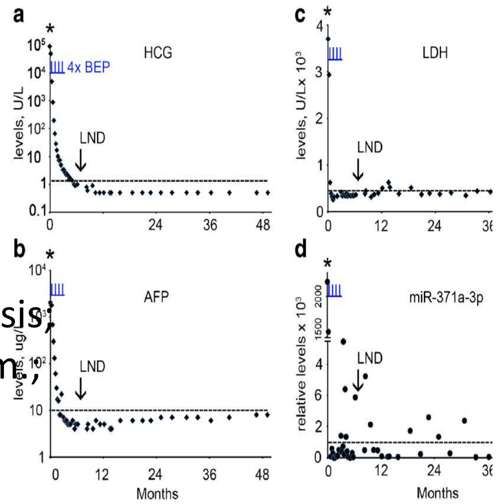
ORIGINAL PAPER

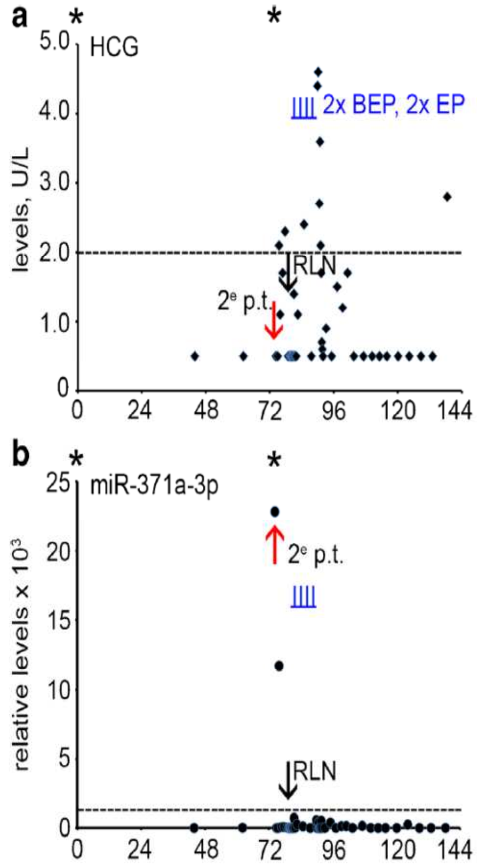
microRNA-371a-3p as informative biomarker for the follow-up of testicular germ cell cancer patients

Ton van Agthoven[1] · Wil M. H. Eijkenboom[1] · Leendert H. J. Looijenga[1]



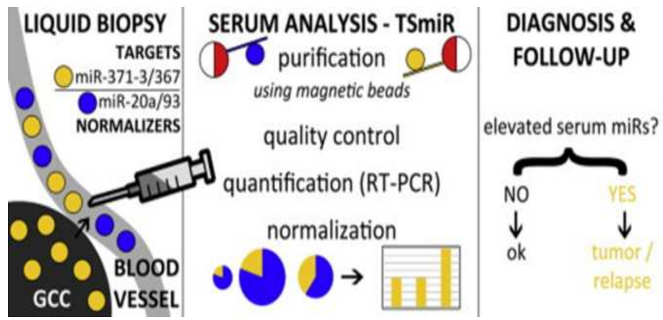






* = orchiectomy; ICM = intracranial metastasis;
PILN = para-iliac lymph node; p.t = prim. tum.;
LND = lymph nodfe dissection

6 TGCT patients



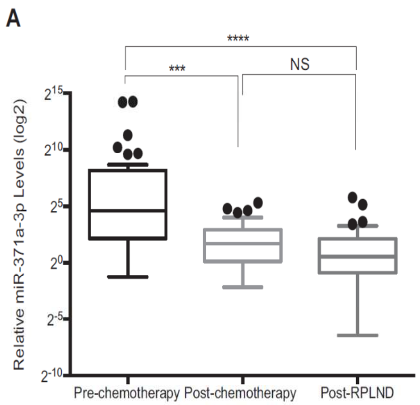
Liquid biopsy analyses (T)GCTs: miR-371a-3p (longitudinal series II).

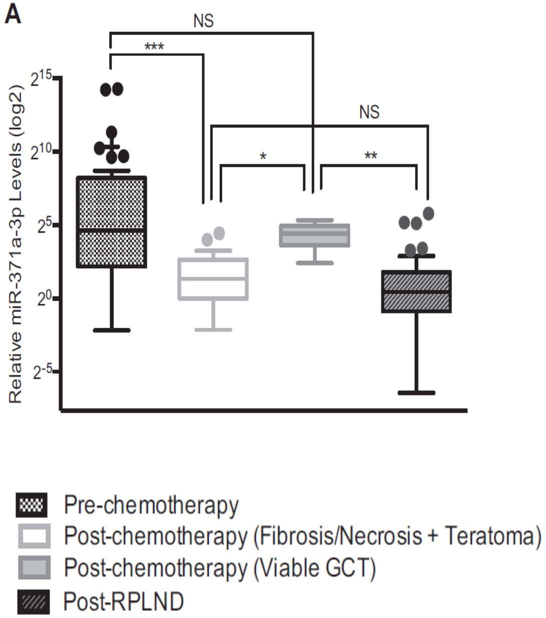
Serum miRNA Predicts Viable Disease after Chemotherapy in Patients with Testicular Nonseminoma Germ Cell Tumor

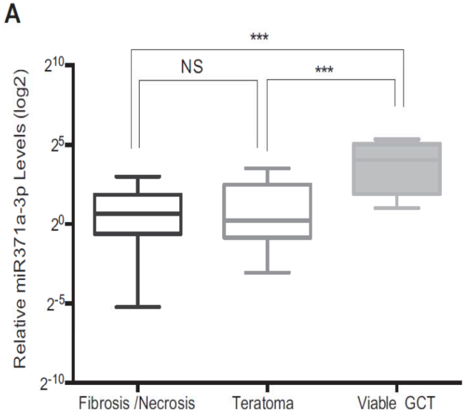
Ricardo Leão,* Ton van Agthoven,* Arnaldo Figueiredo, Michael A. S. Jewett, Kamel Fadaak, Joan Sweet, Ardalan E. Ahmad, Lynn Anson-Cartwright, Peter Chung, Aaron Hansen, Padraig Warde, Pedro Castelo-Branco, Martin O'Malley, Philippe L. Bedard, Leendert H. J. Looijenga*,† and Robert J. Hamilton*,†

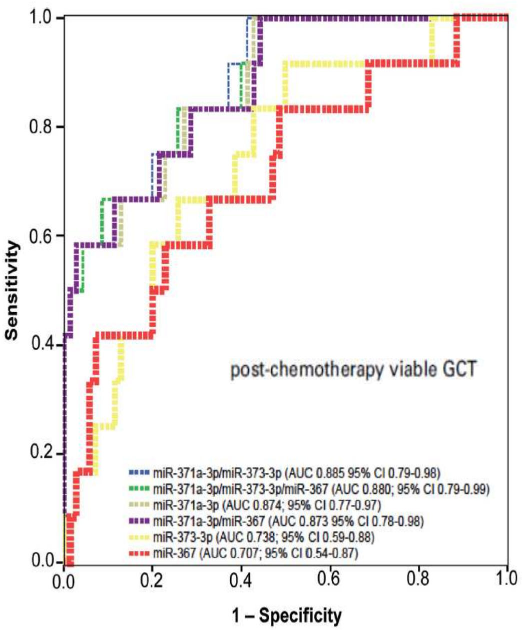
THE JOURNAL OF UROLOGY® Vol. 200, 126-135, July 2018

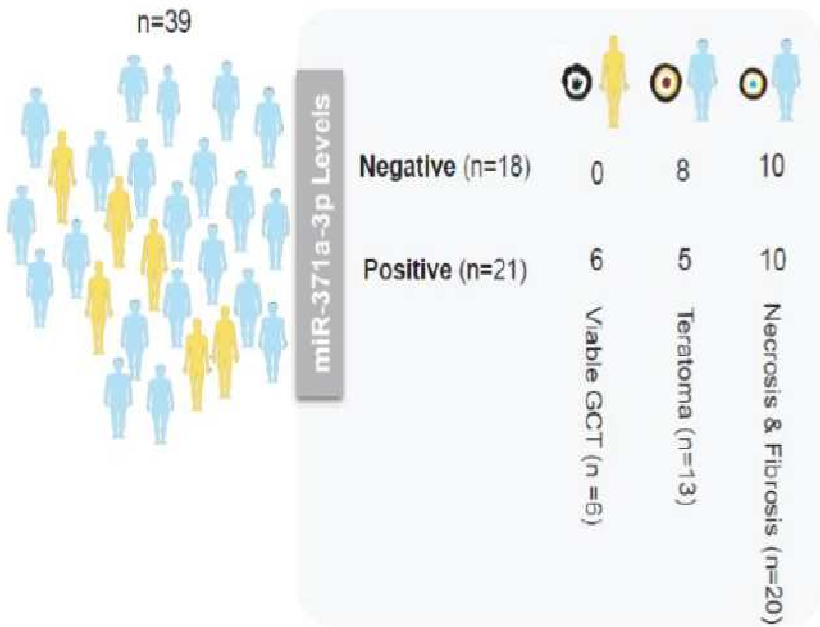
82 patients: cohort 1 (39)/cohort 2 (43)

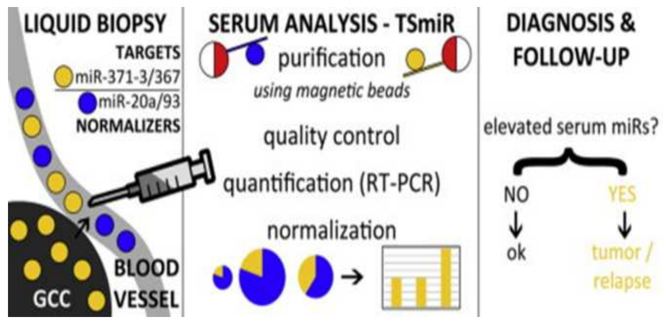












Liquid biopsy analyses (T)GCTs: miR-371a-3p (longitudinal series III).

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: start of chemotherapy & 101 second cycle)

TABLE 3 Correlation between pretreatment plasma miR-371a-3p and serum tumour markers

	miR-371a-3p continuous		miR-371a-3p dichotomized	
Variable	Pearson correlation	P-value	Pearson correlation	P-value
AFP	0.26	**0.0025**	0.13	0.14
HCG	−0.02	0.78	0.15	0.08
LDH	0.61	**<0.00001**	0.33	**0.0001**
S-stage	0.41	**<0.00001**	0.42	**<0.00001**

Statistically significant indicated bold.
HCG, human chorionic gonadotropin; AFP, alpha-fetoprotein; LDH, lactate dehydrogenase.

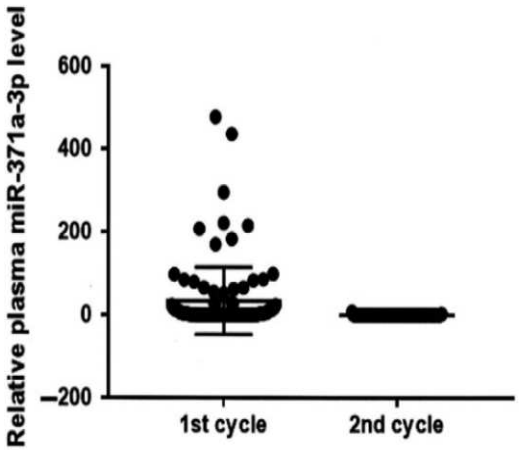
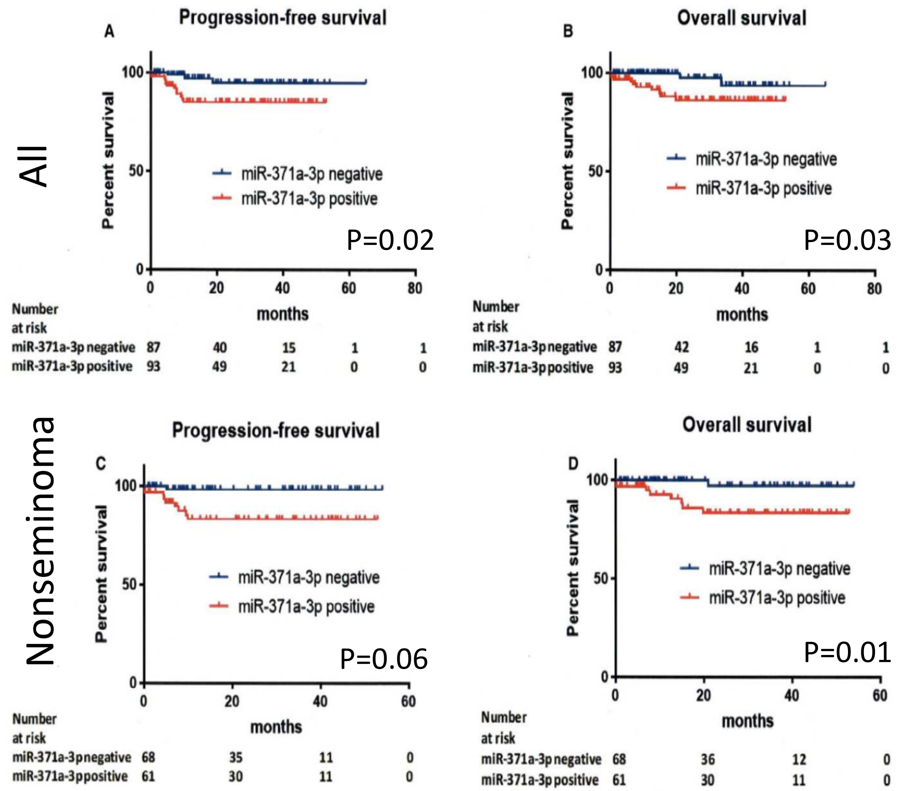


TABLE 4 Prognostic value of plasma miR-371a-3p before the first cycle of chemotherapy

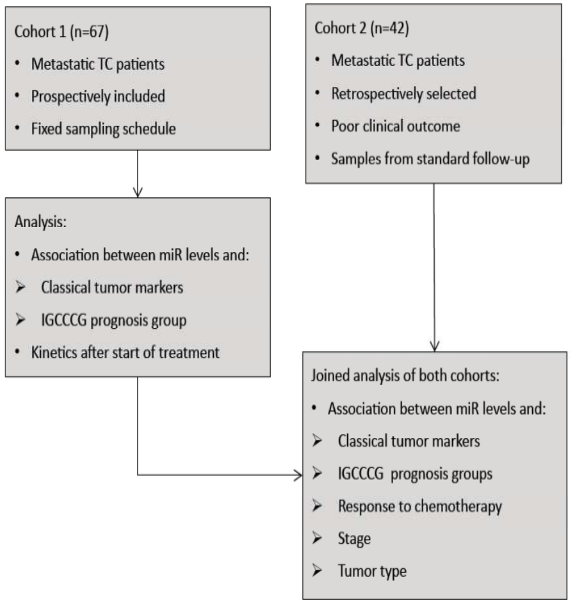
	HR (95% CI), P-value			
	Progression-free survival		Overall survival	
Variable	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis
Plasma miR-371a-3p				
Negative vs. positive	0.26 (0.09-0.71), **0.02**	0.40 (0.11-1.47), 0.20	0.21 (0.07-0.67), **0.03**	0.42 (0.09-1.98), 0.33
IGCCCG risk group				
Good risk vs. intermediate/poor risk	0.15 (0.05-0.51), **0.0001**	0.19 (0.06-0.58), **0.003**	0.07 (0.02-0.25), **<0.00001**	0.08 (0.020.39), **0.002**

Statistically significant indicated bold.

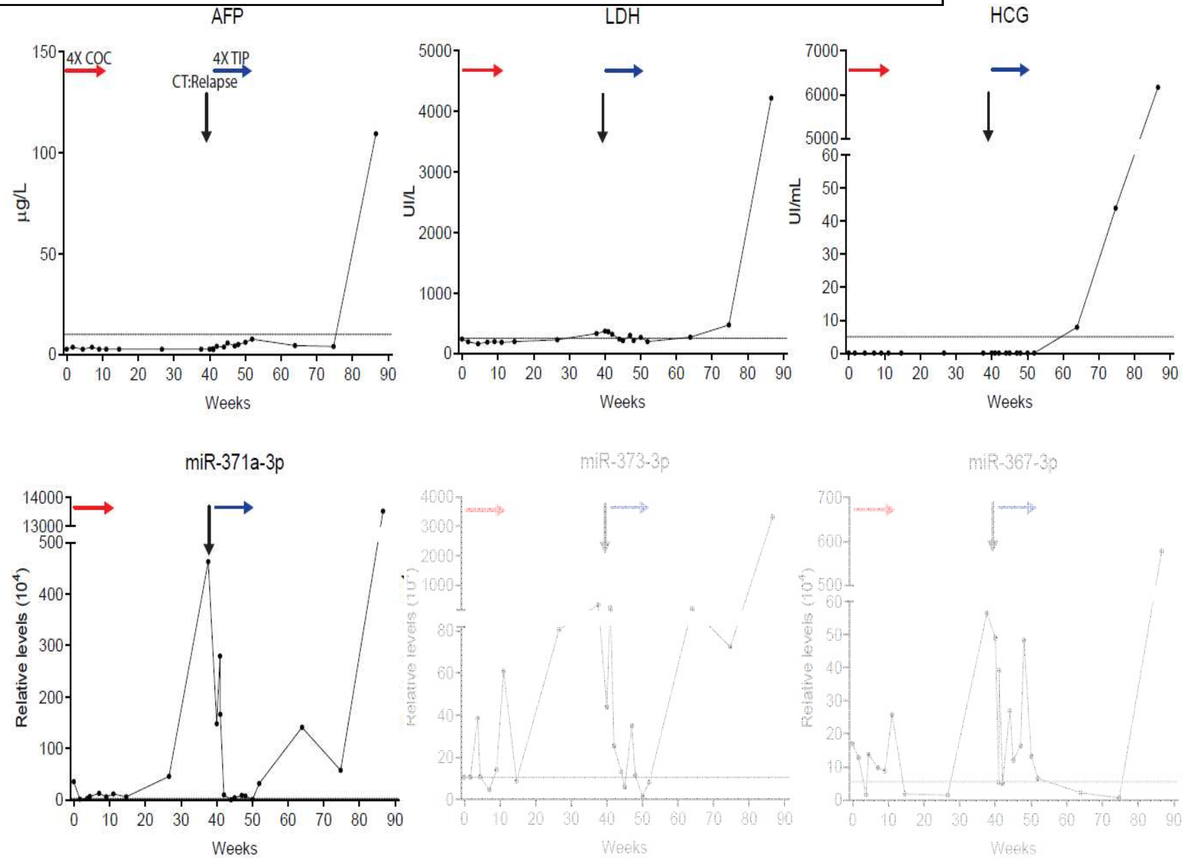


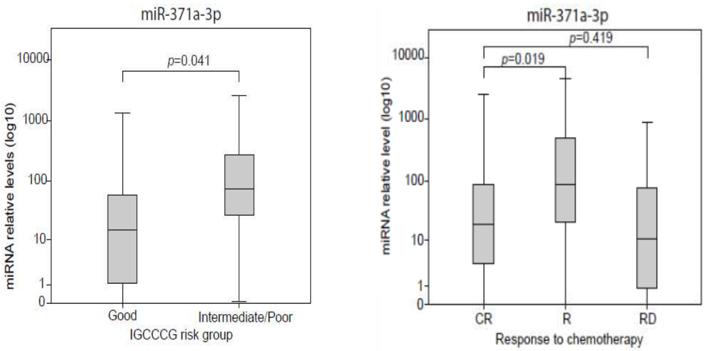
Liquid biopsy analyses (T)GCTs: miR-371a-3p (longitudinal series IV).

	Good	Intermediate	Poor	Relapse	Refractory
I:	83.6%	14.9%	1.5%	n= 6	n= 1
II:	26.2%	35.7%	38.1%	n= 28	n= 14



88 patients protein marker neg. (10.1%): 9 positive at least one miR.





patients + relapse (34/21 samples):
12x elevated miR before protein marker (57.1%; 2 only miR)
patients refractory (15/13 samples):
12x elevated miR (86.6%; 6x during protein markers

Kinetics: 12 patients first week chemo
T1/2 35 hours (SD+/- 25, 6-86 hours)

?Relapse versus Refractory?

Rosas Plaza & Van Agthoven *et al.* To be sub

Preclinical model.

Diagnosis -- Treatment -- Follow-up

The MicroRNA-371 Family as Plasma Biomarkers for Monitoring Undifferentiated and Potentially Malignant Human Pluripotent Stem Cells in Teratoma Assays

Daniela C.F. Salvatori,[1,5,*] Lambert C.J. Dorssers,[2,5] Ad J.M. Gillis,[2] Gemma Perretta,[3] Ton van Agthoven,[2] Maria Gomes Fernandes,[1] Hans Stoop,[2] Jan-Bas Prins,[1] J. Wolter Oosterhuis,[2] Christine Mummery,[4] and Leendert H.J. Looijenga[2,*]

Stem Cell Reports | Vol. 11 | 1493–1505 | December 11, 2018

Lessons from human teratomas to guide development of safe stem cell therapies

NATURE BIOTECHNOLOGY VOLUME 30 NUMBER 9 SEPTEMBER 2012

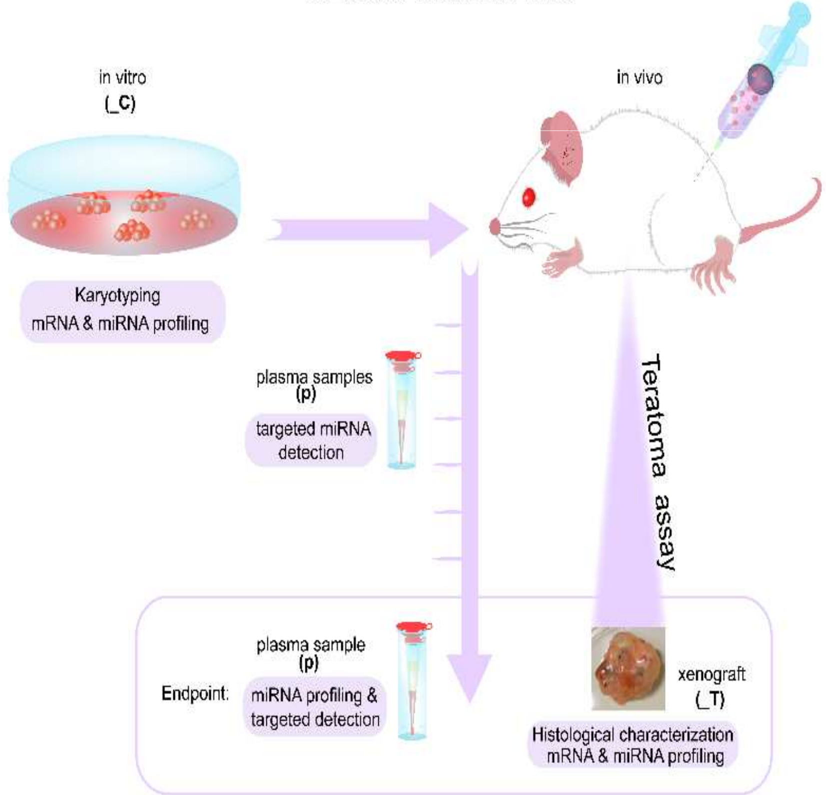
Justine J Cunningham[1], Thomas M Ulbright[2], Martin F Pera[3] & Leendert H J Looijenga[4]

Table 4 Methods for assaying residual pluripotent stem cells in clinical product or patient monitoring

Method of detection	Molecular compartment	Sensitivity	Limitations	Potential stage of application: Preclinical	CMC	Clinical
Methods with accepted clinical utility						
ASO-qRT-PCR	Tumor DNA	0.001%, that is, 1 CTC in 100,000 normal[83]	Requires large number of samples for repeated testing to assure statistical certainty	Yes	Yes	Yes
Flow cytometry	Tumor cell	0.01%, that is, 1 CTC in 10,000 normal[84]	Requires four- to six-color flow, necessitating multiple cell surface markers	Yes	Yes	Yes
ELISAs	Tumor protein	Ultrasensitive assays detect in sub-pg/ml range[85]	Requires unique protein expression & correlation of protein signal with cell number	Yes	Yes	Yes
MRI	Tumor cell	Masses >0.3 cm	Unknown effect of imaging labels on stem cell phenotype or genotoxic potential	Yes	No	Yes
FDG-PET	Tumor size	Masses >1 cm (ref. 86)	Poor spatial resolution	No	No	Yes
Methods with emerging evidence						
qRT-PCR	Tumor miRNA	Limit of detection down to 10 copies of miRNA[87]	Requires identification of miRNAs with known association with pluripotent cells	Yes	Yes	Yes
Immuno-PCR (TPA)	Tumor protein	Limit of detection in femtogram range[88]	Requires unique protein expression & correlation of protein signal with cell number	Yes	Yes	Yes
Fluorescent nanocrystals & cation exchange	Tumor miRNA	Limit of detection in femtomole range[89]	Requires identification of miRNAs with known association with pluripotent cells	Yes	Yes	Yes
Nanoparticle surface plasmon resonance	Tumor miRNA	Limit of detection in attomole range[90]	Requires identification of miRNAs with known association with pluripotent cells	Yes	Yes	Yes
Bioluminescence (BLI)	Tumor cell	Limit of detection to be determined[91,92]	Requires demonstration that vectors used to label cells have no effect on cell product profile	Yes	No	No

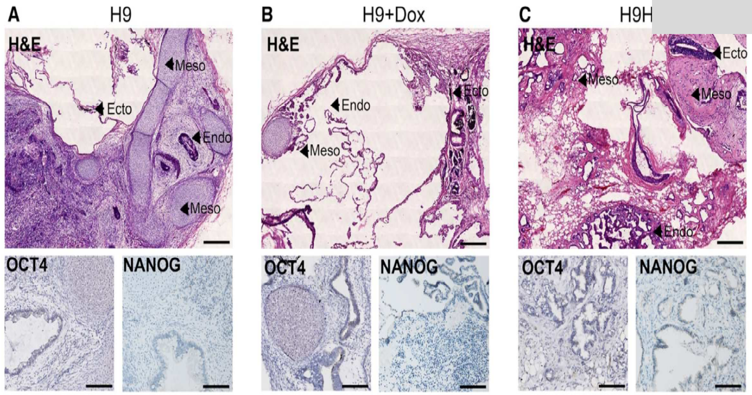
CMC, product chemistry, manufacturing and controls; CTC, circulating tumor cell; ELISA, enzyme-linked immunosorbent assay; miRNA, microRNA; MRI, magnetic resonance imaging; PET, positron emission tomography; TPA, TaqMan protein assay.

hESC/hiPSC/hGCT lines



Preclinical model.

Diagnosis -- Treatment -- Follow-up



H Histology Xenografts

Cell line	Meso	Ecto	Endo	EC	YS	SE
H9	+	+	+	-	-	-
H9+Dox	+	+	+	-	-	-
H9Hyb	+	+	+	-	-	-
Lu07	+	+	+	-	+	-
Lu07+Dox	-	-	-	+	+	-
2102EpL	-	-	-	+	-	-
TCam-2	-	-	-	+	+	+

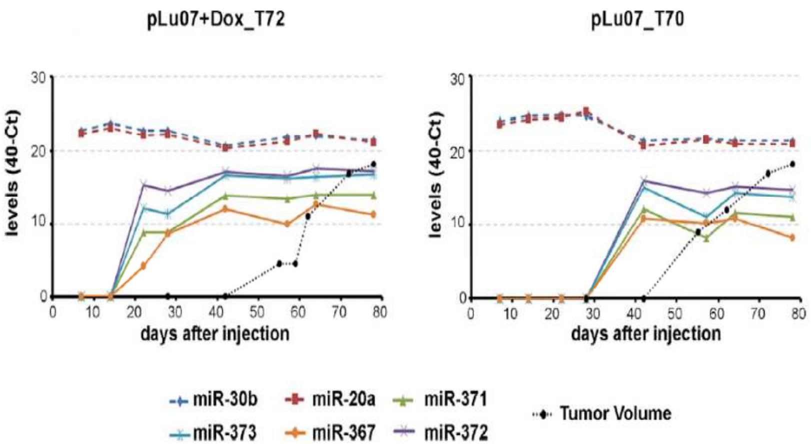


Figure 6. Time Course of miR-371, -372, -373, and -367 Accumulation in the Mouse Plasma Samples of Two Representative Mice Injected with Lu07 (Right Panel) and Lu07 + Dox Cells (Left Panel)

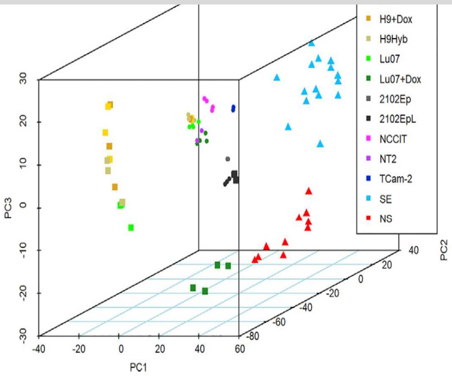


Figure 3. Principal-Component Analysis of mRNA Expression of hPSCs, hPSC-Derived Xenografts, hGCT-Derived Cell Lines and Xenografts and Primary Testicular hGCTs

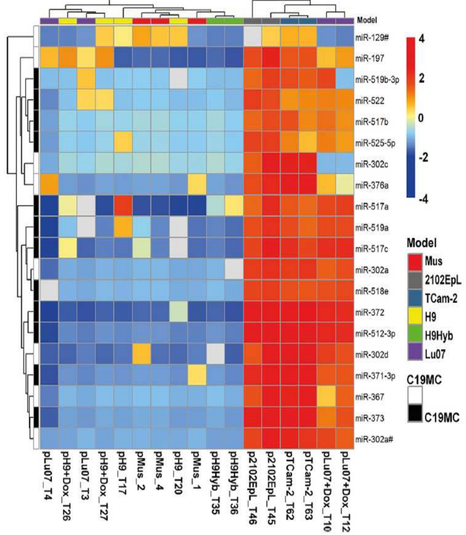


Figure 5. MicroRNA Expression Profile of the Xenograft Endpoint Plasma

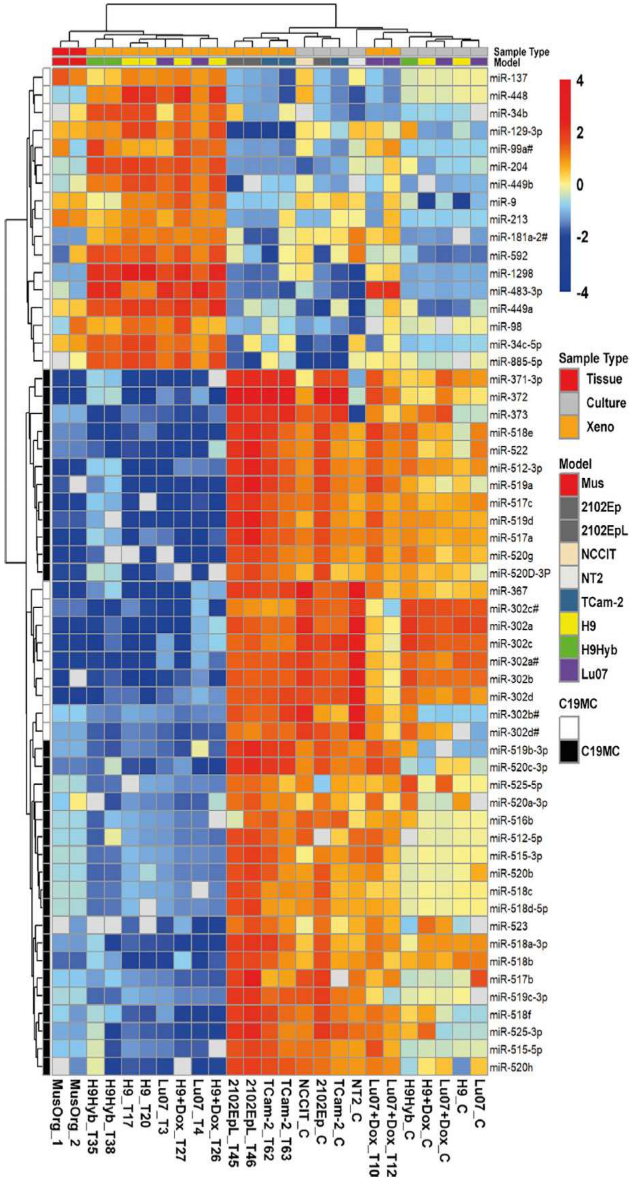


Figure 4. miRNA Expression Profile Comparison between hPSCs and hGCT-Derived Cell Lines and Respective Xenografts

Conclusions and take home messages.

- Clinically relevant subtypes of (T)GCTs
- Informative histology based diagnostic (protein) biomarkers
- GWAS SNPS & biology matches (KITLG, gonadal development, ….)
- Similarity embryogenesis and Type II TGCTs (epigenetics)
- High level of genetic heterogeneity within Type II TGCTs (no driver mutat
- Treatment resistance “markers” not identified in primary tumor
- miR-371a-3p (almost absolute) liquid biopsy markers for malignant GCT c

Princess máxima center pediatric oncology

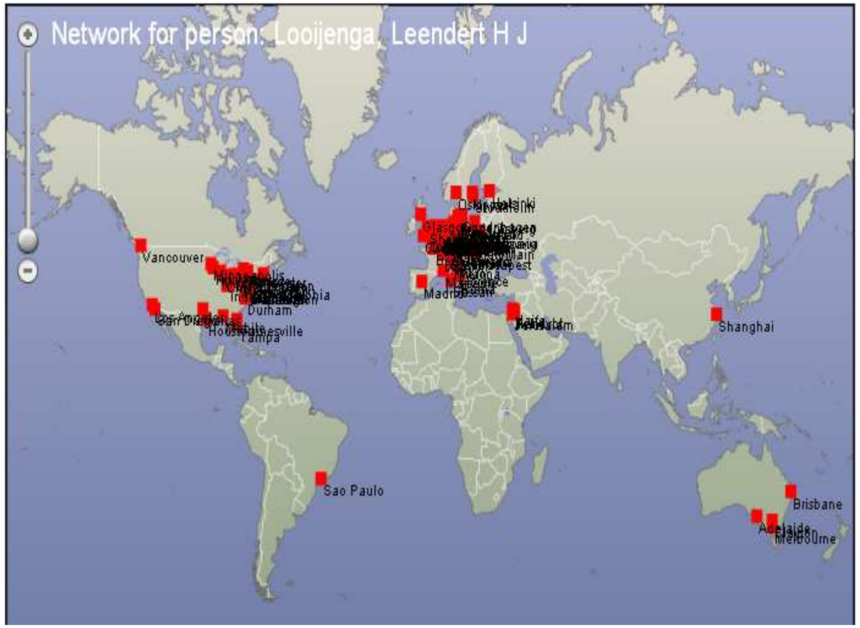
Our ambition: curing every (T)GCT patient,
while providing an optimal quality of life

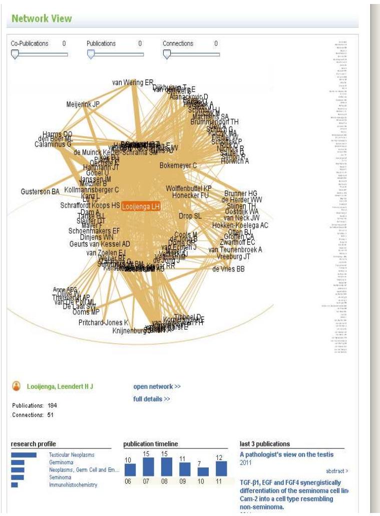
About us

Fertility-preservation

Acknowledgements.

(missing KW, KB, JWO, HS, YvB)





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Urologists and Pathologists, Patients