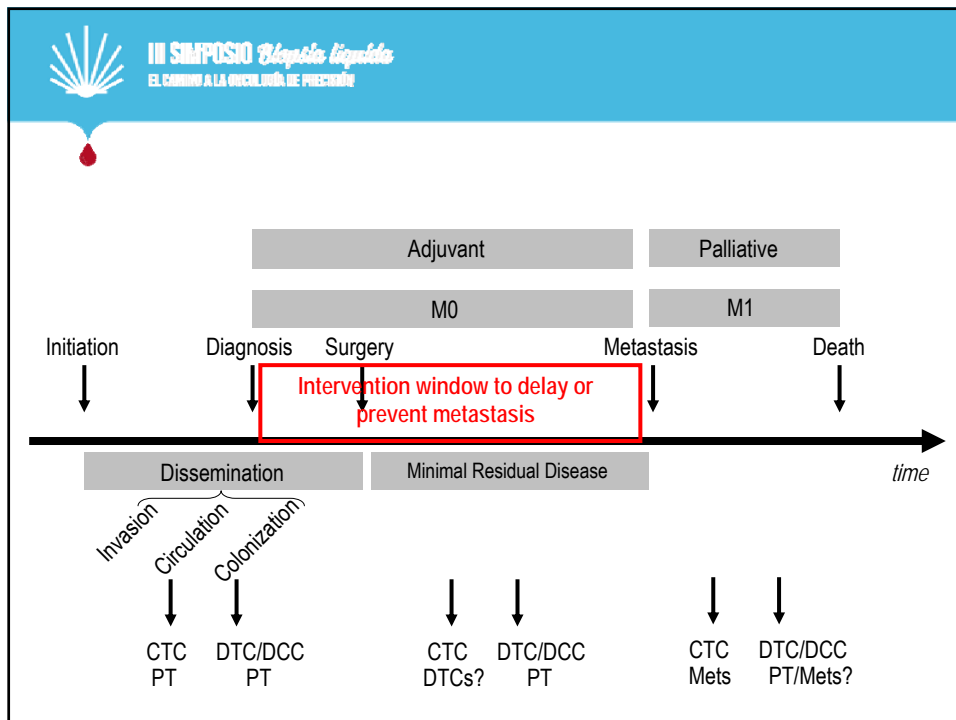


III SIMPOSIO
Biopsia líquida
 EL CAMINO A LA ONCOLOGÍA DE PRECISIÓN

26 - 27 DE ENERO 2018 - JANUARY 25th - 27th - SANTIAGO DE COMPOSTELA

Early dissemination in prostate cancer

Miodrag Guzvic, University of Regensburg, Germany





Background

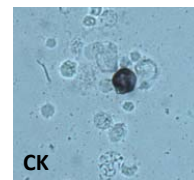
- 90% of cancer-related deaths are due to metastasis
- Minimal Residual Disease comprises of Disseminated Cancer Cells (DCC) remaining in the body after surgery
- DCCs are founder cells of later arising lethal metastasis metastasis

Klein, Nat Rev Cancer, 2009
Stoecklein et al., Cancer Cell, 2008



Disseminated Cancer Cells

- Can be detected in bone marrow (BM) or lymph nodes of cancer patients
- Detected using staining for cytokeratins (CK) or EpCAM
- Frequency: 10^{-6}
- Usually detected as individual cells



CK



EpCAM



Hypothesis

Targeting founder cells of metastasis
may lead to prevention or delay of
death due to metastasis

Klein, Nat Rev Cancer, 2009
Stoecklein et al., Cancer Cell, 2008



Aim

Molecular characterization of
genome and transcriptome of early
disseminated cancer cells



DCCs in prostate cancer (PC)

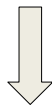
- Bone is the major metastatic site in PC
- PC is slow progressing – currently impossible to identify patients that may benefit from therapy
- → Considerable overtreatment

Guzvic & Klein, Transl Cancer Res, 2016

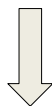


Clinical Progression of PC

stage M0 no sign of metastasis



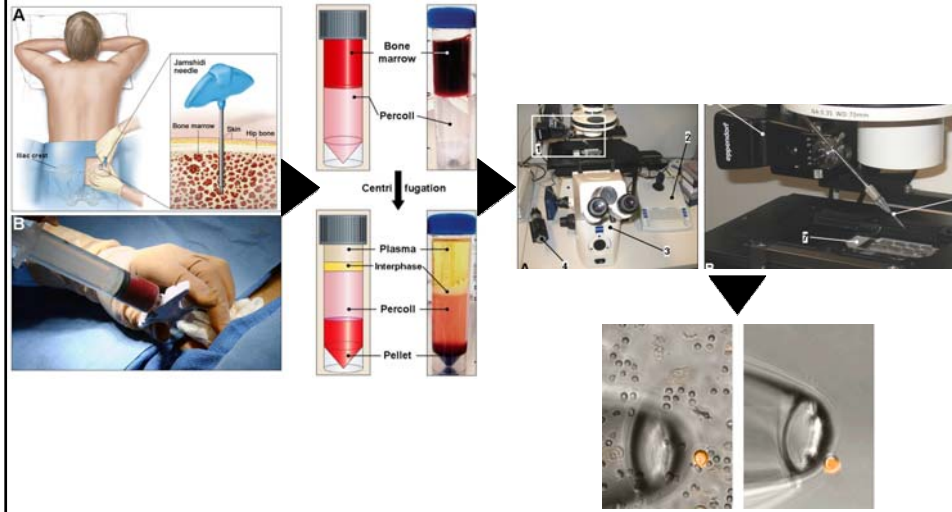
stage BR PSA-increase after surgery (biochemical relapse, BR)



stage M1 distant metastasis (> 85% bone metastasis)

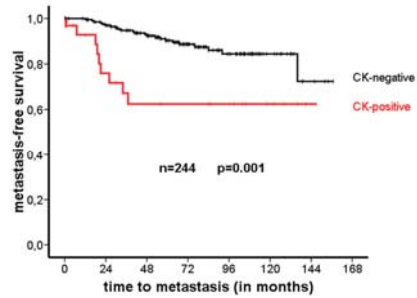
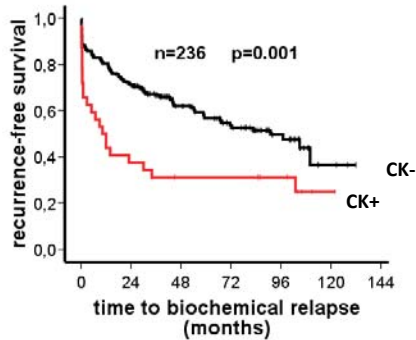


III SIMPOSIO *Biopsia líquida* EL CAMINO A LA QUIMIOTERAPIA DE PREVENCIÓN



III SIMPOSIO *Biopsia líquida* EL CAMINO A LA QUIMIOTERAPIA DE PREVENCIÓN

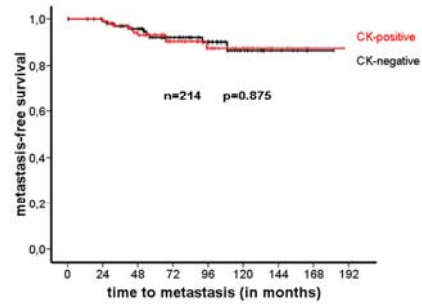
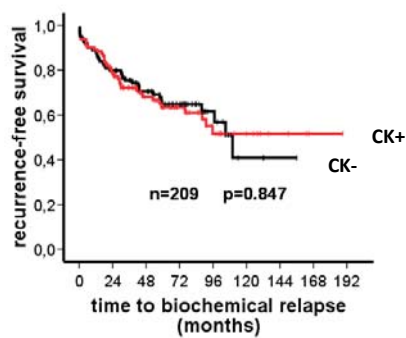
Strong prognostic impact of CK⁺ DCCs in bone marrow of prostate cancer patients



Weckermann, Polzer et al., JCO, 2008



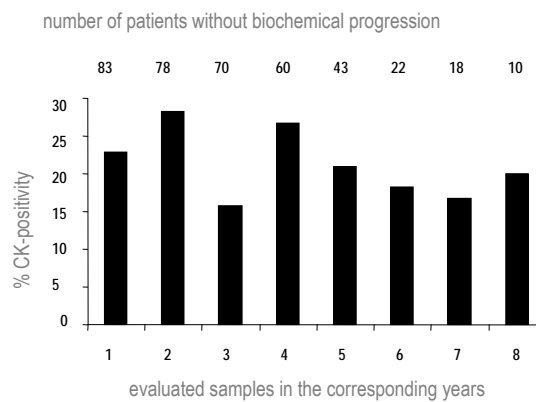
No prognostic impact of CK⁺ DCCs during follow-up BM sampling in PC



Weckermann, Polzer et al., JCO, 2008



Dormancy of CK-positive DCCs?



Frequency of CK⁺ cells in the BM of patients persists over time



Single cell analysis

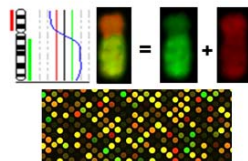


Genomic
DNA

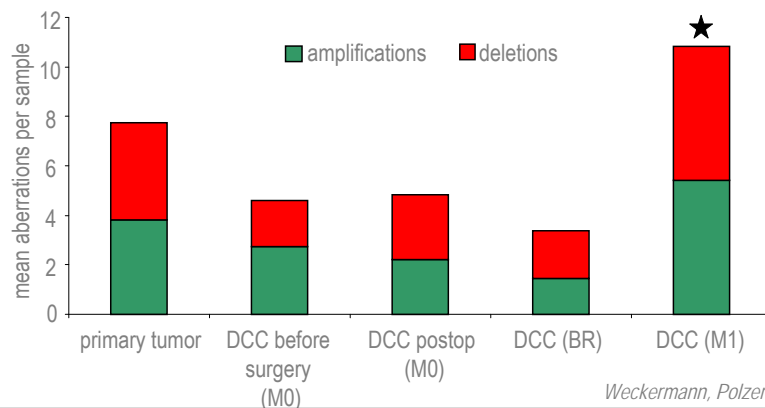


Whole genome amplification

Klein et al., PNAS, 1999
Czyz et al., PloS 1, 2014



Number of genomic aberrations before M1 stage stays relatively constant

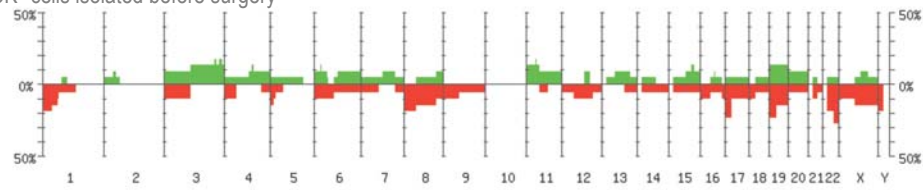


Weckermann, Polzer et al., JCO, 2008

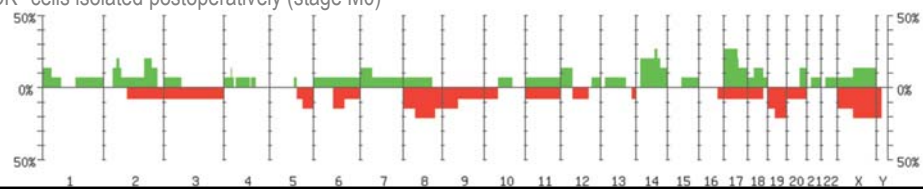


No characteristic genetic differences between DCCs isolated before and after surgery

CK⁺ cells isolated before surgery



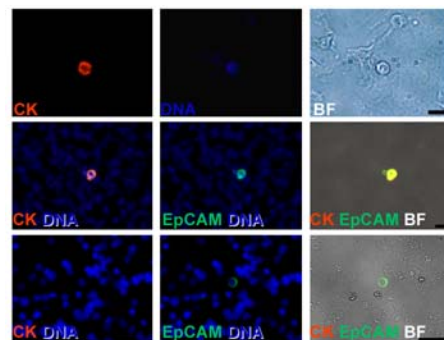
CK⁺ cells isolated postoperatively (stage M0)



Weckermann, Polzer et al., JCO, 2008



EpCAM⁺ DCCs in prostate cancer



Guzvic et al., in preparation



III SIMPOSIO *Biopsia líquida*
EL CAMINO A LA OMOLOGÍA DE PREVENCIÓN



EpCAM⁺ DCCs identify high-risk patients

Guzvic et al., in preparation



III SIMPOSIO *Biopsia líquida*
EL CAMINO A LA OMOLOGÍA DE PREVENCIÓN



Genomic progression of CK⁺ and EpCAM⁺ DCCs

Guzvic et al., in preparation



III SIMPOSIO *Hematología*
EL CAMINO A LA OROLOGÍA DE PREVENCIÓN



CK⁺ and EpCAM⁺ DCCs display different genomic changes

Guzvic et al., in preparation



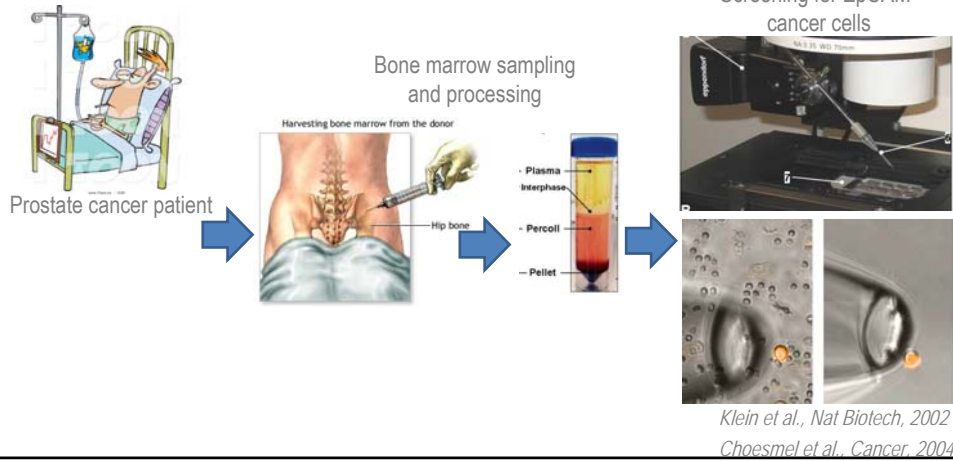
III SIMPOSIO *Hematología*
EL CAMINO A LA OROLOGÍA DE PREVENCIÓN



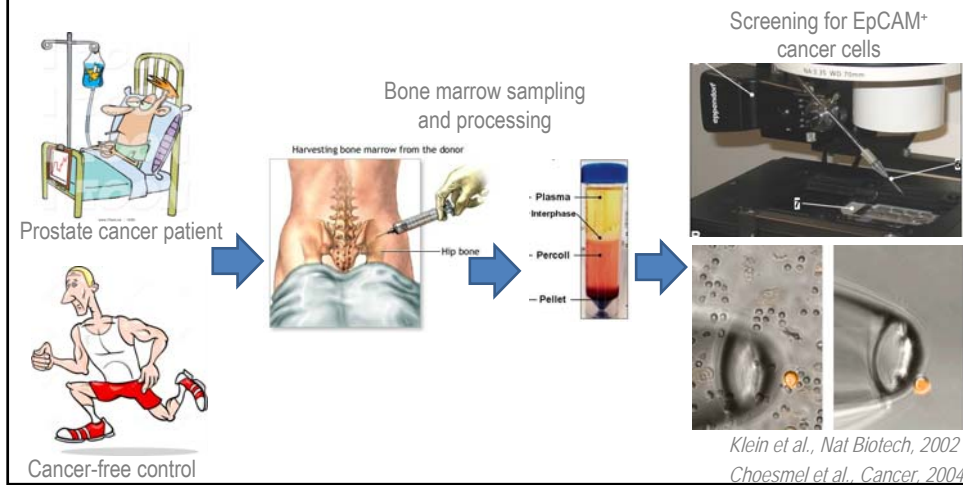
Expression profiling and functional characterization of EpCAM⁺ DCCs



Workflow

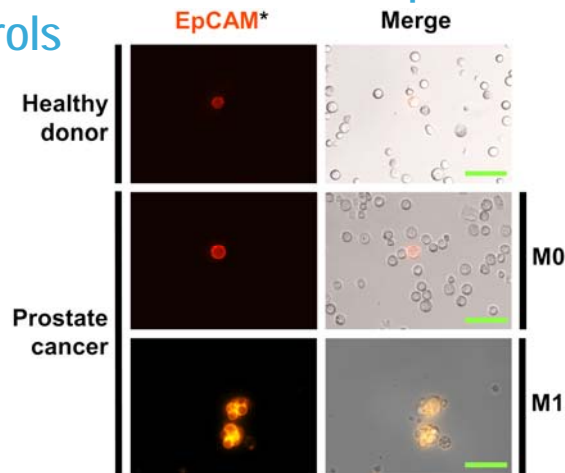


Workflow





EpCAM⁺ cells are found in patients and controls



Guzvic et al., *Cancer Res*, 2014



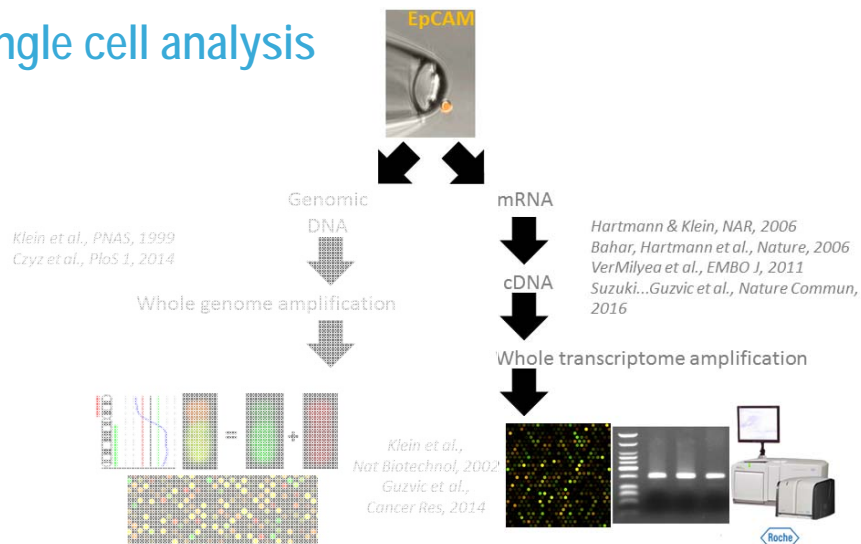
EpCAM⁺ cells are detected at similar frequency in patients and controls

	M0	N	M1
Screened BM samples	105	18	2
EpCAM ⁺ cells detected in	65 (62%)	10 (56%)	2 (100%)

Guzvic et al., *Cancer Res*, 2014



Single cell analysis



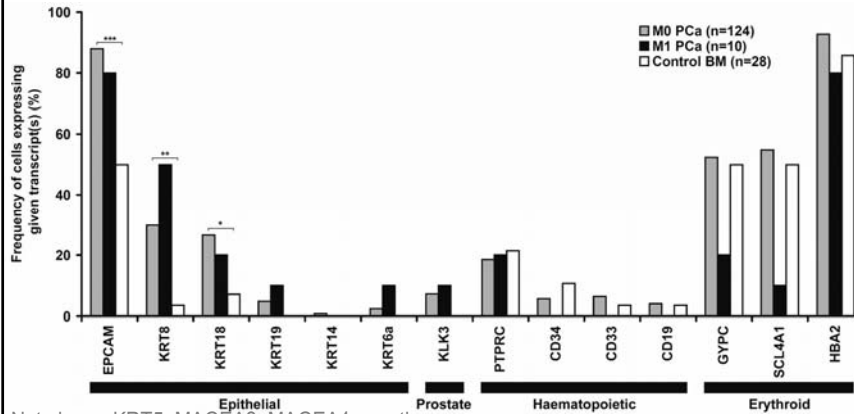
What is a DCC?

Limited transcriptome analysis for a panel of relevant markers

- Epithelial-specific: EPCAM, KRT5, KRT6A, KRT8, KRT14, KRT18, KRT19
- Prostate/cancer specific: KLK3 (PSA), MAGEA2, MAGEA4
- Hematopoietic: CD19, CD33, CD34, PTPRC (CD45)
- Erythroid: HBA2, GYPC, SCL4A1 (band 3)



Erythroid transcripts are expressed in majority of EpCAM⁺ cells from patients and controls



Nota bene: KRT5, MAGEA2, MAGEA4 negative

Guzvic et al., *Cancer Res*, 2014

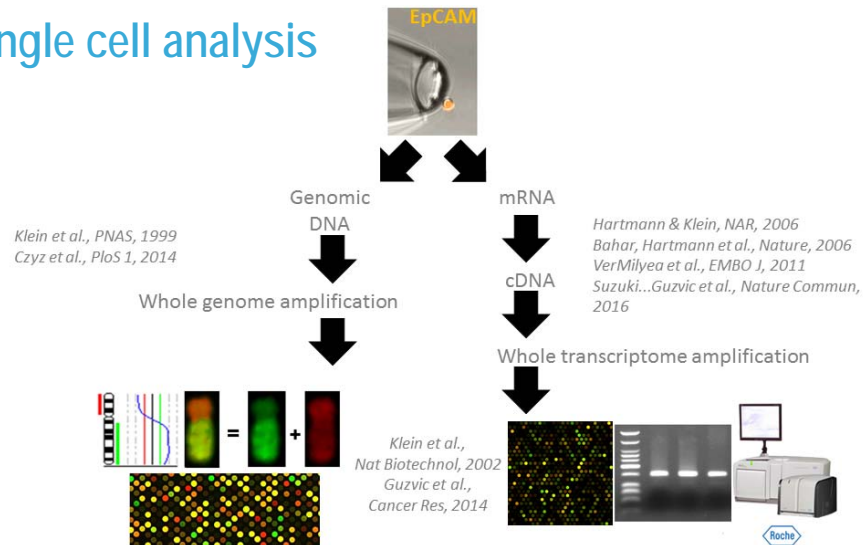


What is a DCC?

How to reliably identify true DCCs?



Single cell analysis



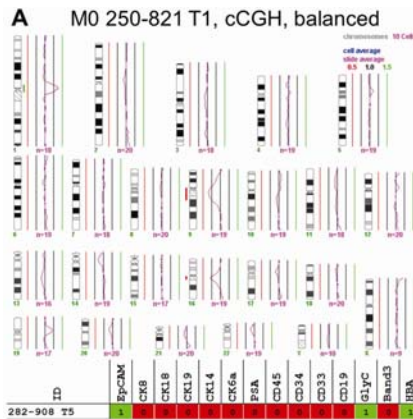
Combined analysis of genome and transcriptome of putative DCCs

35% of EpCAM⁺ cells from patients have aberrant genomes
→ true DCCs

Absence of genomic aberrations does not mean that the cell is not a cancer cell



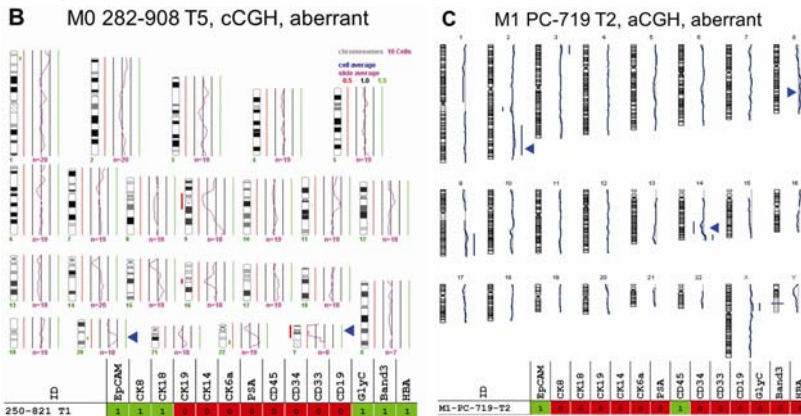
Some EpCAM⁺ cells from patients have no aberrations



Guzvic et al., *Cancer Res*, 2014



True DCCs express hematopoietic and erythroid transcripts



Guzvic et al., *Cancer Res*, 2014



M	EpCAM ⁺ cell	CGH	Transcriptome analysis														
			Genome	EPKAM	KRT8	KRT18	KRT19	KRT14	KRT6a	KRT5	KLK3	PTPRC	CD34	CD33	CD19	GYPC	SCL4A1
	284-910 T2	B	1	0	0	0	0	0	0	0	1	0	0	0	1	1	1
	338-1009 T1	B	1	1	1	0	0	0	0	0	1	1	0	0	1	1	1
	338-1009 T3	B	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1
	348-1035 T1	B	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	362-1085 T1	B	1	1	1	0	0	0	0	0	1	0	1	1	1	1	1
	362-1085 T2	B	1	1	1	0	0	0	0	0	1	0	0	1	1	1	1
	362-1085 T3	B	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1
	363-1086 T1	B	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1
	363-1086 T2	B	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1
	363-1086 T3	B	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1
MO	252-825 T2	A	1	1	0	0	1	1	0	0	0	0	0	0	0	1	1
	256-830 T1	A	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1
	256-830 T2	A	1	0	1	0	0	0	0	0	1	0	0	0	1	1	1
	256-830 T3	A	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	256-830 T4	A	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1
	282-908 T5	A	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	286-914 T3	A	1	0	1	0	0	0	0	0	0	0	0	0	1	1	0
	333-1003 T2	A	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	336-1006 T3	A	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	452-2244-T1	A	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
	452-2244-T3	A	1	1	1	1	0	0	0	1	0	0	0	0	0	0	1
	452-2244-T4	A	1	1	1	0	0	0	0	1	0	0	0	0	0	0	1
	452-2244-T5	A	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
	452-2244-T8	A	1	1	1	1	0	0	0	1	0	0	0	0	0	0	1
M1	M1-PC-719-T1	A	1	1	0	0	0	1	0	0	1	0	0	0	0	0	1
	M1-PC-719-T2	A	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	M1-PC-719-T8	A	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0

B Balanced
A Aberrant
1 Positive
0 Negative

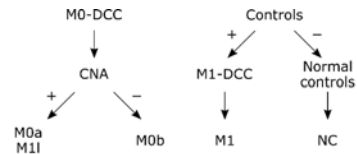


Deeper analysis of DCC heterogeneity

- Transcriptome sequencing of DCCs



Three distinct groups of EpCAM⁺ cells based on gene expression



Guzvic et al., in preparation



Subgroups of EpCAM⁺ cells differ in the number of active genes

Guzvic et al., in preparation



III SIMPOSIO *Hepática líquida*
EL CAMINO A LA QUIMIOTERAPIA DE PREVENCIÓN



Subpopulations of EpCAM⁺ cells display distinct mutation signatures

Guzvic et al., in preparation



III SIMPOSIO *Hepática líquida*
EL CAMINO A LA QUIMIOTERAPIA DE PREVENCIÓN

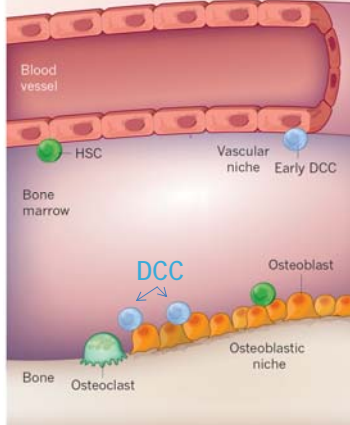


Panel-sequencing reveals similar groups



The fate of DCCs at distant site

Current thinking ("dogma")

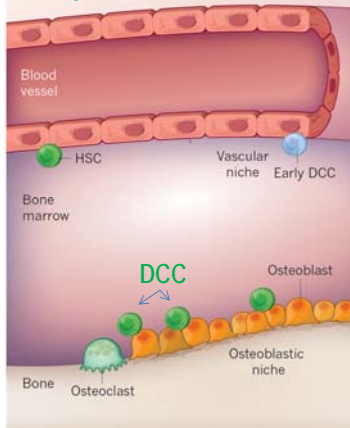


Prostate epithelial cancer DCCs "look" like prostate epithelial cancer cells



The fate of DCCs at distant site

Reality



Prostate epithelial cancer DCCs have "mixed" haematopoietic/epithelial phenotype





The fate of DCCs at distant site

- Unexpected DCC transcriptome plasticity
- Adaptation of DCCs to the BM microenvironment



Expression of β -globin by cancer cells promotes cell survival during blood-borne dissemination

Yu Zheng^{1,2,*}, David T. Miyamoto^{3,4,*}, Ben S. Wittner^{1,4}, James P. Sullivan^{1,5}, Nicola Aceto^{1,7}, Nicole Vincent Jordan^{1,2}, Min Yu^{1,1}, Nezhil Murat Karabacak⁵, Valentine Comolli⁵, Robert Morris¹, Rushil Desai¹, Niyati Desai^{1,6}, Erin Emmons¹, John D. Milner¹, Richard J. Lee^{1,4}, Chin-Lee Wu^{1,6}, Lecia V. Sequist^{1,4}, Wilhelm Haas^{1,4}, David T. Ting^{1,4}, Mehmet Toner⁵, Sridhar Ramaswamy^{1,4}, Shyamala Maheswaran^{1,7} & Daniel A. Haber^{1,2,4}

Human breast cancer metastases to the brain display GABAergic properties in the neural niche

Jooh Neman¹, John Termini², Sharon Wilczynski², Nagarajan Valdeh², Cecilia Choy^{2*}, Claudia M. Kowolik², Hubert Li^{2*}, Amanda C. Hambrecht^{2*}, Eugene Roberts^{2*}, and Rahul Jindal^{2,11}

Human prostate cancer metastases target the hematopoietic stem cell niche to establish footholds in mouse bone marrow

Yusuke Shiozawa¹, Elisabeth A. Pedersen¹, Aaron M. Havens^{1,4}, Younghun Jung¹, Anjali Mishra¹, Jeena Joseph¹, Jin Koo Kim¹, Lait R. Patel¹, Chi Ying¹, Anne M. Ziegler¹, Michael J. Pienta¹, Junhui Song⁴, Jingsheng Wang¹, Robert D. Loberg¹, Paul H. Krebsbach⁴, Kenneth J. Pienta¹ and Russell S. Taichman¹

984-989 | PNAS | January 21, 2014 | vol. 111 | no. 3 | *J Clin Invest.* 2011;121(4):1298-1312. doi:10.1172/JCI43414.



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BayGene
Bayerisches Genomforschungsnetzwerk



Sixth Framework Programme



Federal Ministry of Education and Research
Deutsche Forschungsgemeinschaft
DFG



Helping Researching Informing

Dr. Josef Steiner
Cancer Research
Foundation
Award

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