

Fronteras de la Ciencias
Salamanca, Spain
December 2012

Using Genomics to Improve Response to Neoadjuvant Therapy in Rectal Cancer Patients



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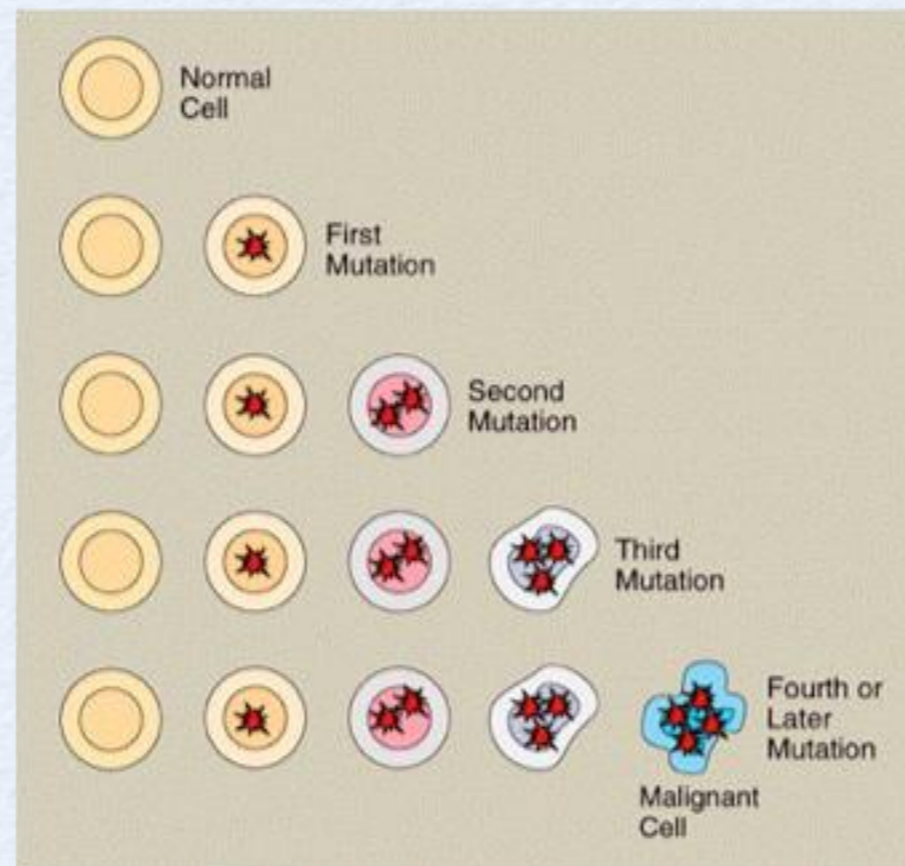


Cancer is a Genetic Disease

Caused by the accumulation of genetic and epigenetic alterations in DNA of normal somatic cells

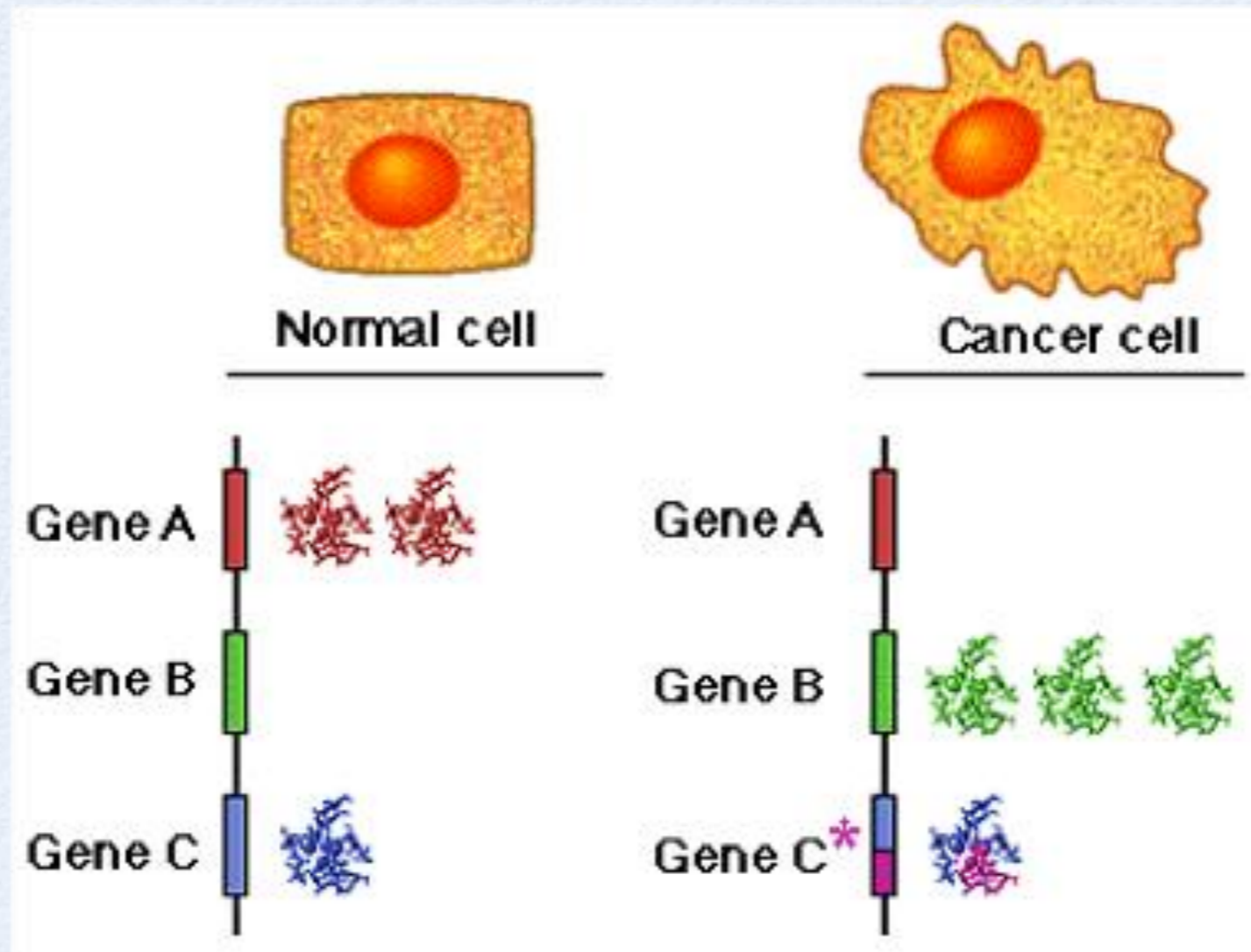
DNA replication errors

Endogenous and exogenous mutagens

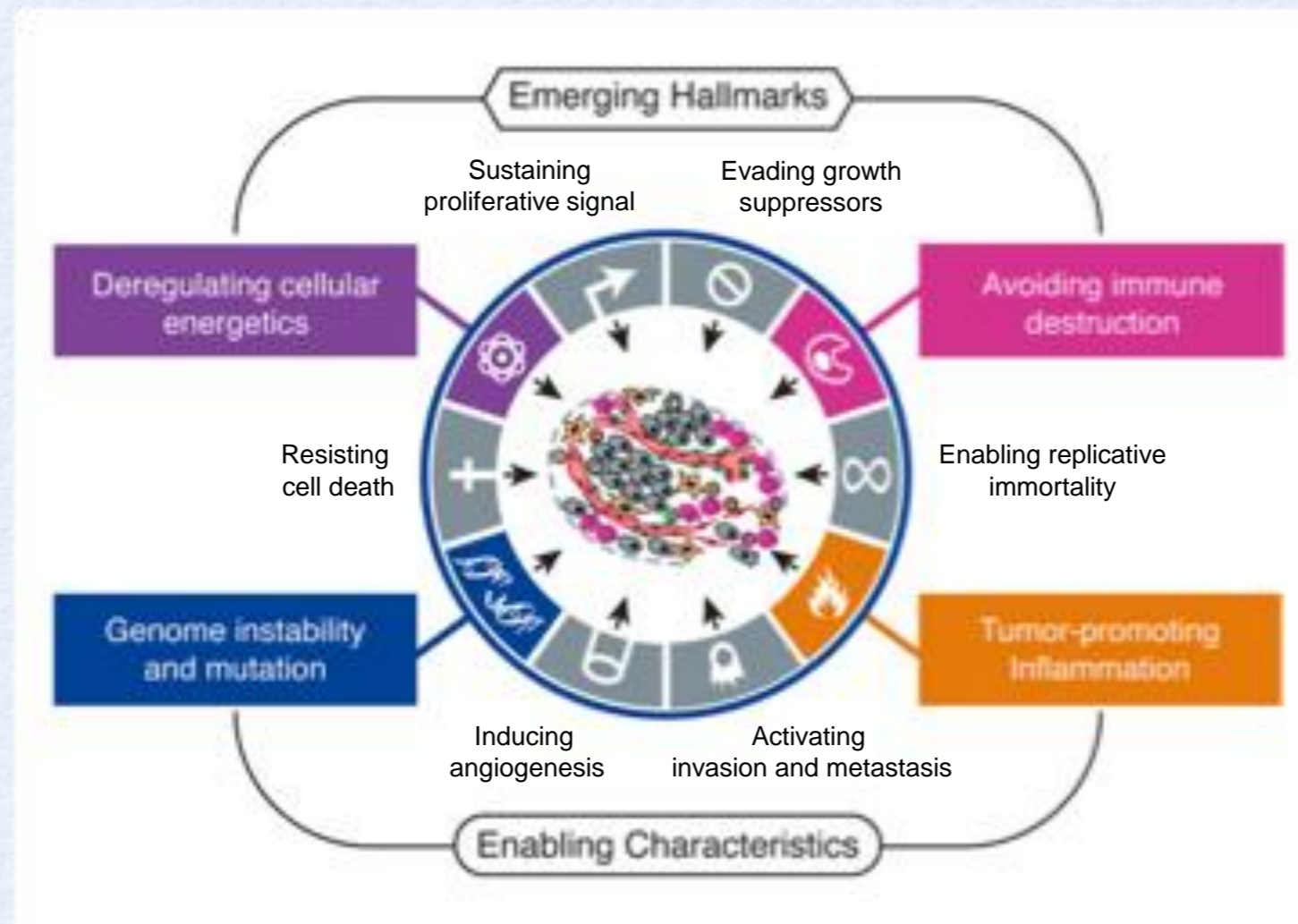


3,000 point mutations
hundreds chromosomal aberrations

Alterations in gene expression and cell reprogramming

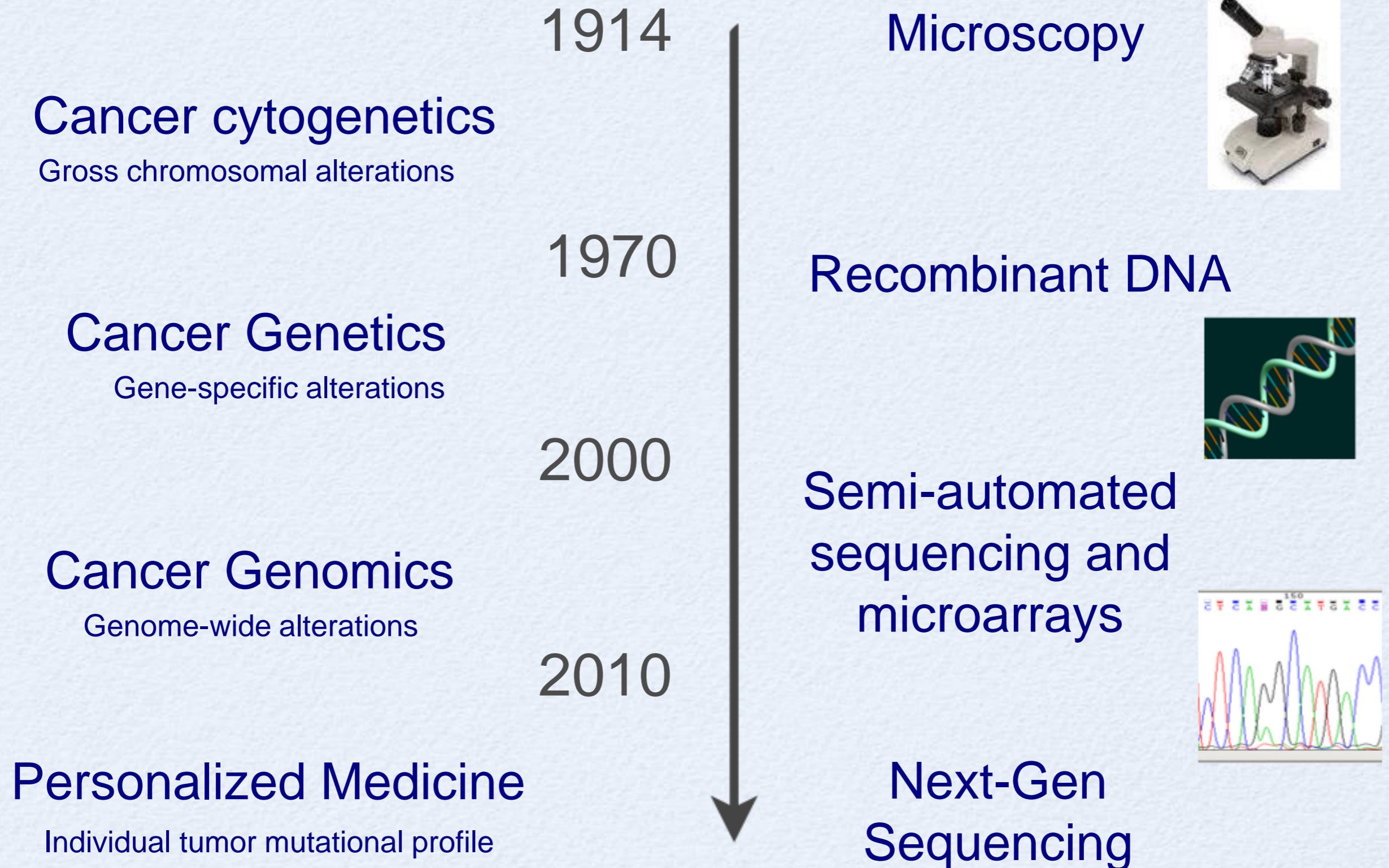


The hallmarks of cancer



Hanahan & Weinberg Cell, 2011

From Cancer Genetics to Cancer Genomics



Why search for genetic alterations in tumor genomes?

Development of alternative therapies

Indirect detection of tumor cells

Specific for tumor cells

Genetic Alterations

Determine tumor characteristics

Predict disease outcome

Predict treatment response

Laboratory of Cancer Genetics and Genomics at the Ludwig Institute



Major Interest: Cancer Genetics and Genomics

Expertise: Genome-wide methodologies for:

- gene expression analysis (quantitative, qualitative)
- germline polymorphisms (CNV, SNPs)
- somatic alterations (genetic, epigenetic)
- bioinformatics

Early Genome Initiatives in Brazil



NATURE | VOL 406 | 13 JULY 2000 | www.nature.com

articles

The genome sequence of the plant pathogen *Xylella fastidiosa*

The *Xylella fastidiosa* Consortium of the Organization for Nucleotide Sequencing and Analysis*, São Paulo, Brazil

The complete genome sequence of *Chromobacterium violaceum* reveals remarkable and exploitable bacterial adaptability

11660-11665 | PNAS | September 30, 2003 | vol. 100 | no. 20

Brazilian National Genome Project Consortium*

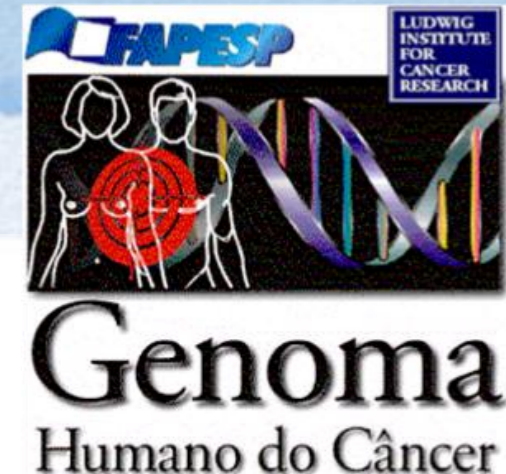
JOURNAL OF BACTERIOLOGY, Aug. 2005, p. 5568-5577
0021-9193/05/\$08.00+0 doi:10.1128/JB.187.16.5568-5577.2005
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Vol. 187, No. 16

Swine and Poultry Pathogens: the Complete Genome Sequences of Two Strains of *Mycoplasma hyopneumoniae* and a Strain of *Mycoplasma synoviae*†

Ana Tereza R. Vasconcelos,¹ Henrique B. Ferreira,² Cristiano V. Bizarro,² Sandro L. Bonatto,³ Marcos O. Carvalho,² Paulo M. Pinto,² Darcy F. Almeida,⁴ Luiz G. P. Almeida,¹ Rosana Almeida,⁵ Leonardo Alves-Filho,² Enequina N. Assunção,⁶ Vasco A. C. Azevedo,⁷ Mauricio R. Bogo,³ Marcelo M. Brigido,⁸ Marcelo Brocchi,^{2,9} Helio A. Burity,¹⁰ Anamaria A. Camargo,¹¹

Cancer Genome Initiative in Brazil



PNAS | October 9, 2001 | vol. 98 | no. 21 | 12103-12108

The contribution of 700,000 ORF sequence tags to the definition of the human transcriptome

Anamaria A. Camargo^a, Helena P. B. Samaia^a, Emmanuel Dias-Neto^a, Daniel F. Simão^a, Italo A. Migotto^a, Marcelo R. S. Briones^b, Fernando F. Costa^a, Maria Aparecida Nagai^a, Sergio Verjovski-Almeida^a, Marco A. Zago^c, Luis Eduardo C. Andrade^d, Helaine Camer^e, Hamza F. A. El-Dorry^a, Enilza M. Espreafico^f, Angelita Habr-Gama^g, Daniel Giannella-Neto^h, Gustavo H. Goldmanⁱ, Arthur Gruber^j, Christine Hackel^k, Edna T. Kimura^l, Rui M. B. Maciel^m, Suelly K. N. Marieⁿ, Elizabeth A. L. Martins^o, Marina P. Nóbrega^p, Maria Luisa Paço-Larson^q, Maria Inês M. C. Pardini^r, Gonçalo G. Pereira^s, João Bosco Pesquero^t, Vanderlei Rodrigues^u, Sílvia R. Rogatto^v, Ismael D. C. G. da Silva^w,

13418-13423 | PNAS | November 11, 2003 | vol. 100 | no. 23

The generation and utilization of a cancer-oriented representation of the human transcriptome by using expressed sequence tags

Helena Brentani^a, Otávia L. Caballero^a, Anamaria A. Camargo^a, Aline M. da Silva^b, Wilson Araújo da Silva, Jr.^c, Emmanuel Dias Neto^d, Marco Grivet^e, Arthur Gruber^f, Pedro Edson Moreira Guimaraes^g, Winston Hide^h, Christian Iseliⁱ, C. Victor Jongeneel^j, Janet Kelso^k, Maria Aparecida Nagai^l, Elida Paula Benquique Ojopi^m, Elisson C. Osorioⁿ, Eduardo M. R. Reis^o, Gregory J. Riggins^p, Andrew John George Simpson^{q-k}, Sandro de Souza^a,

Understand Tumor Biology

Oncogene (2003) 22, 1–8

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www.nature.com/ncr

ORIGINAL PAPER

Epigenetic silencing of the adhesion molecule ADAM23 is highly frequent in breast tumors

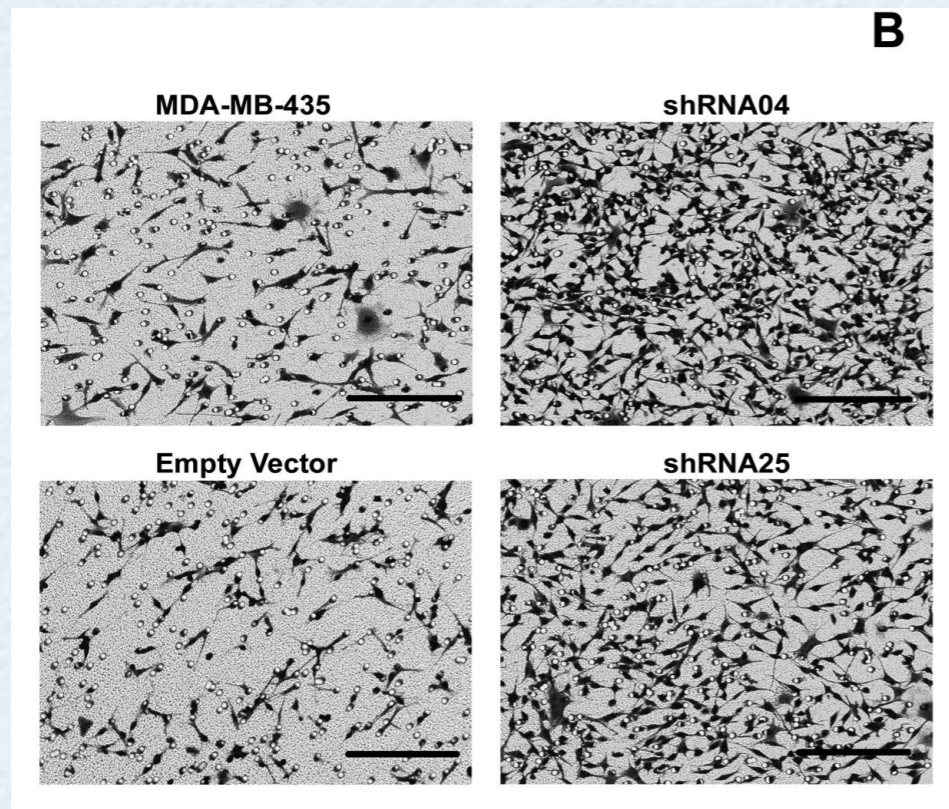
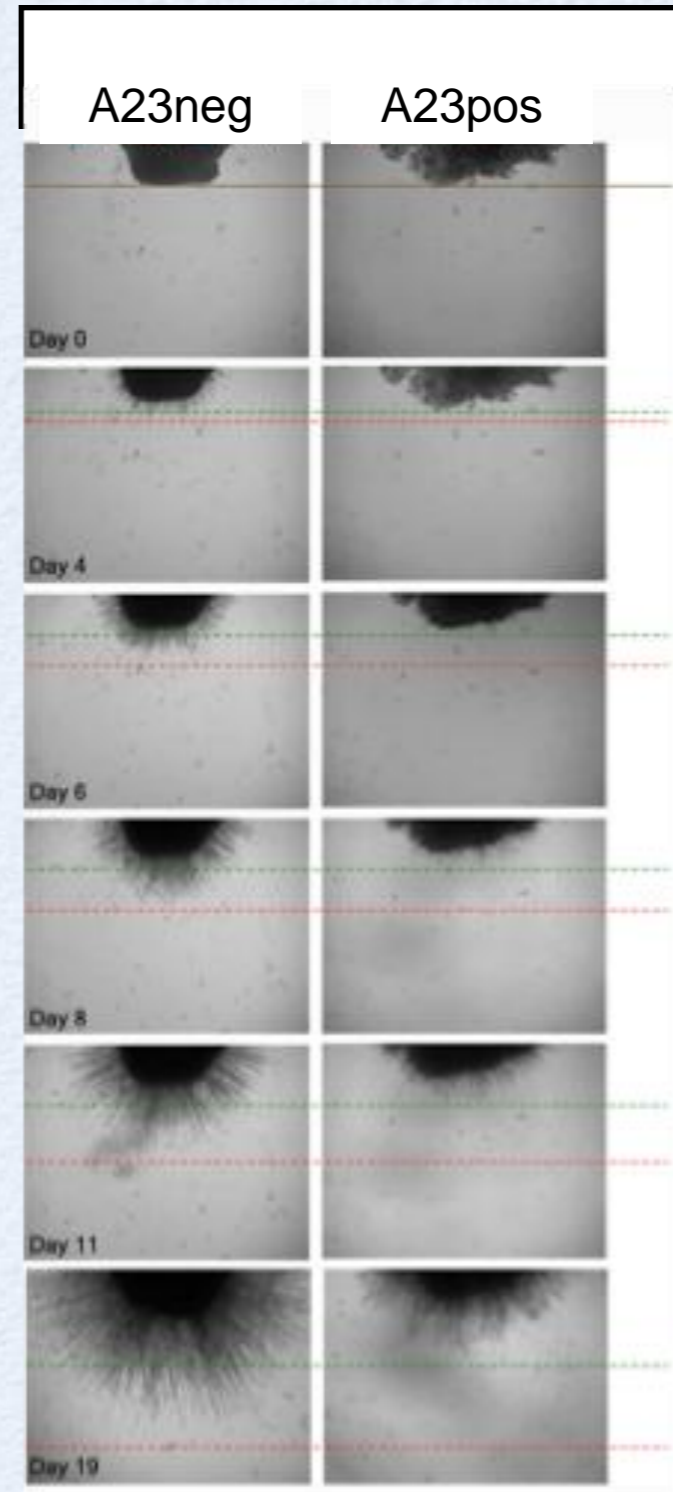
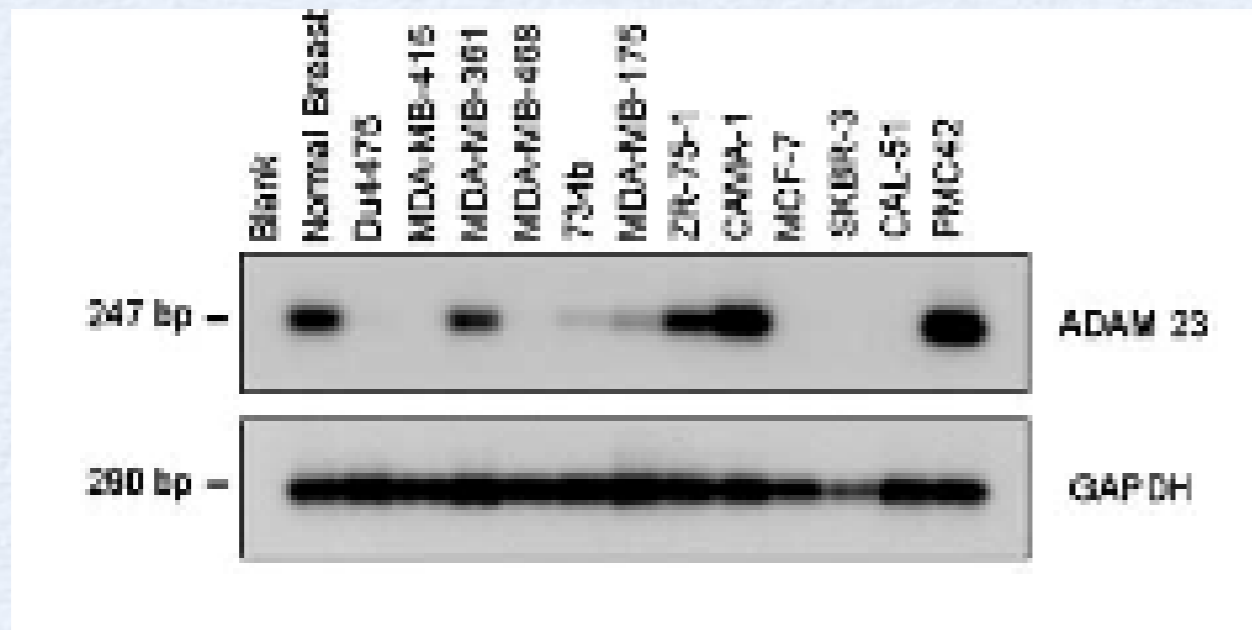
Fabricio F Costa¹, Newton V Verbisck¹, Anna Christina M Salim¹, Daniela F Ierardi¹, Lilian C Pires¹, Regina M Sasahara², Mari C Sogayar², Silvio M Zanata³, Alan Mackay⁴, Michael O'Hare⁴, Fernando Soares⁵, Andrew JG Simpson⁵, Anamaria A Camargo^{6,1}

ADAM23 Negatively Modulates $\alpha_v\beta_3$ Integrin Activation during Metastasis

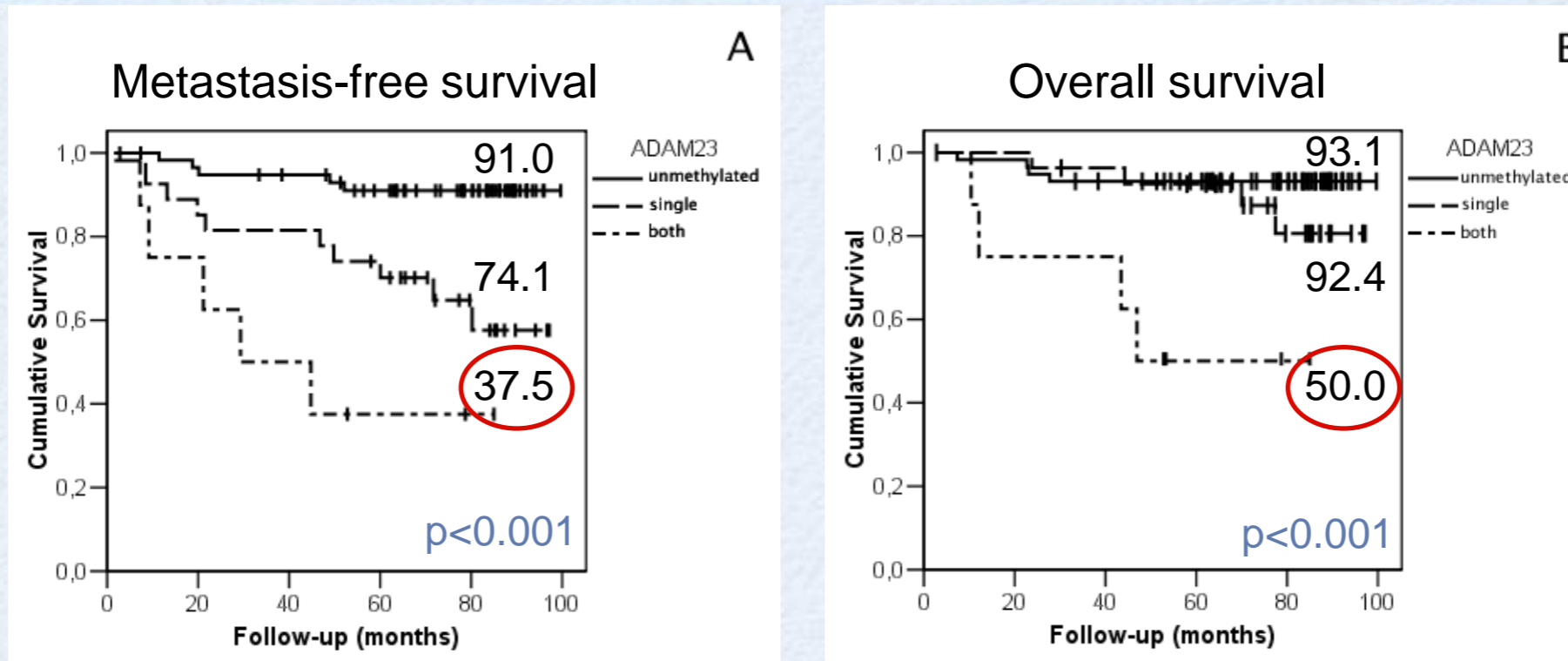
Cancer Res 2009; 69: (13). July 1, 2009

Newton V. Verbisck,¹ Érico T. Costa,¹ Fabricio F. Costa,¹ Felícia P. Cavalher,¹ Michele D.M. Costa,¹ Angelita Muras,¹ Valéria A. Paixão,¹ Ricardo Moura,¹ Mariana F. Granato,¹ Daniela F Ierardi,¹ Tamara Machado,¹ Fabiana Melo,¹ Karina B. Ribeiro,¹ Isabela W. Cunha,¹ Vladimir C.C. Lima,¹ Maria do Socorro Maciel,¹ André L. Carvalho,¹ Fernando F. Soares,¹ Silvio Zanata,¹ Mari C. Sogayar,² Roger Chammas,² and Anamaria A. Camargo¹

Understand Tumor Biology



Identify Tumor Biomarkers



Variables	Distant metastasis free survival			Disease specific survival		
	HR	95% CI	p	HR	95% CI	p
Positive Lymph Nodes						
0	1.0	ref.		1.0	ref.	
1-3	12.65	2.84 - 56.28	0.001	26.6 6	2.80 - 254.08	0.004
≥4	14.37	3.75 - 55.08	<0.001	16.5 6	1.91 - 143.74	0.011
Tamoxifen						
Absent	1.0	ref.		1.0	ref.	
Present	0.14	0.05 - 0.39	<0.001	0.05	0.01 - 0.28	0.001
ADAM23						
<u>Unmethylated</u>	1.0	ref.		1.0	ref.	
Single methylated region	6.53	2.07 - 20.57	0.001	2.30	0.52 - 10.07	0.270
Both methylated regions	8.84	2.31 - 33.76	0.001	9.97	1.96 - 50.61	0.006

Identify New Therapeutic Targets

18066-18071 | PNAS | November 28, 2006 | vol. 103 | no. 48

Characterization of a cancer/testis (CT) antigen gene family capable of eliciting humoral response in cancer patients

Raphael B. Parmigiani^{*}, Fabiana Bettoni^{*}, Maria D. Vibranovski^{*1}, Marilene H. Lopes^{*}, Waleska K. Martins^{*}, Isabela W. Cunha¹, Fernando A. Soares¹, Andrew J. G. Simpson¹, Sandro J. de Souza^{*}, and Anamaria A. Camargo^{*2}

^{*}Ludwig Institute for Cancer Research, SP 05508-010, São Paulo, Brazil; ¹Biochemistry Department, University of São Paulo, SP 05508-900, São Paulo, Brazil; ²Pathology Department, Hospital A. C. Camargo, São Paulo, SP 01528-010, Brazil; and ³Ludwig Institute for Cancer Research, Memorial Sloan-Kettering Cancer Center, New York, NY 10058

PNAS

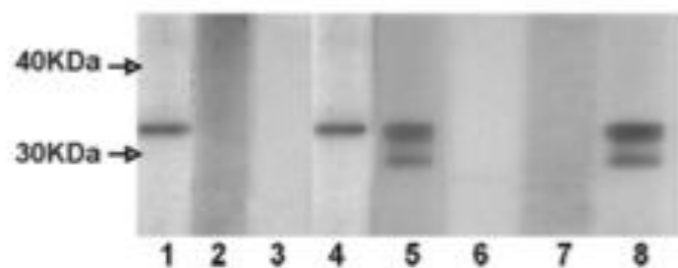
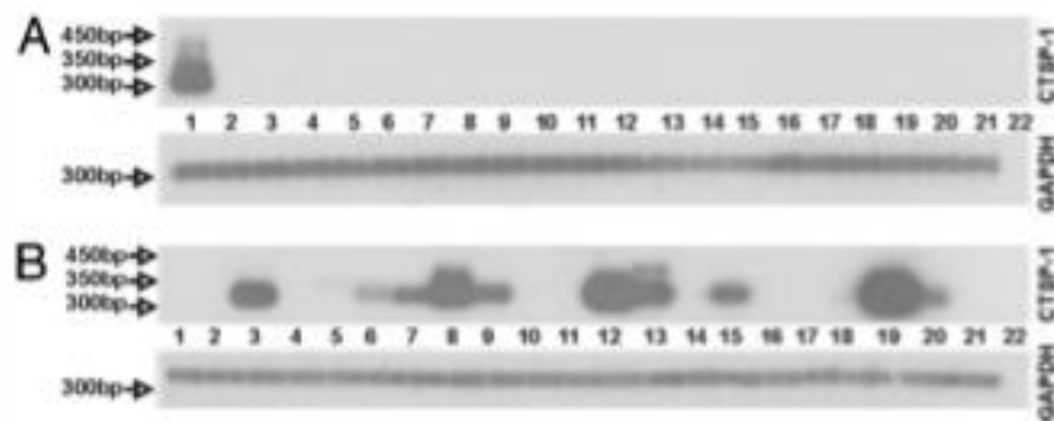
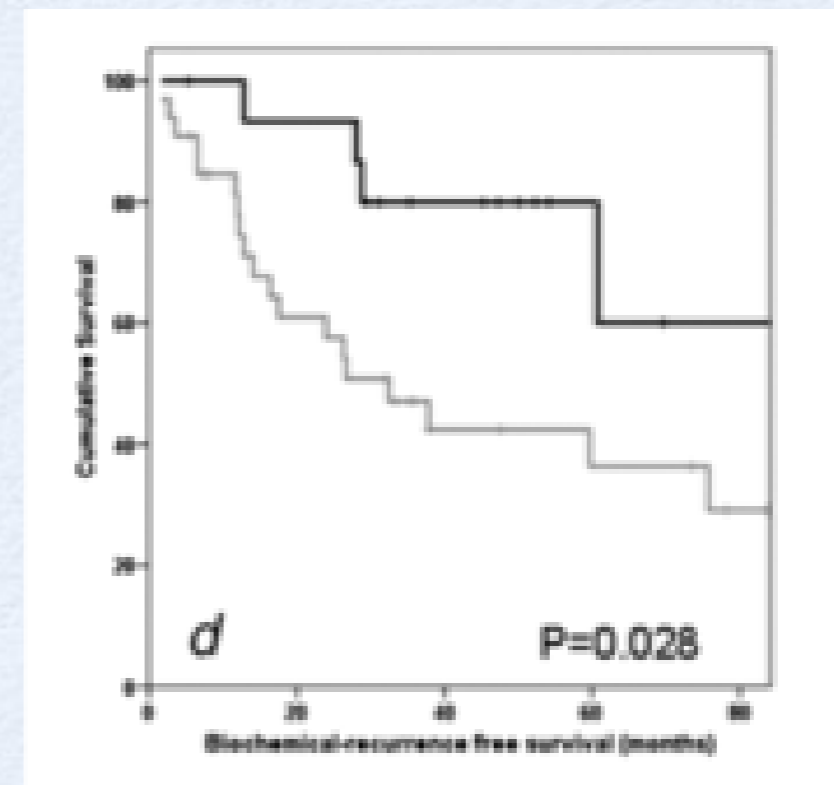


Fig. 6. Detection of antibodies against CTSP-1 recombinant protein in plasma samples from cancer patients. A Western blot using CTSP-1 recombinant protein and plasma samples from prostate cancer patients (lanes 1-7) is shown. An anti-HisTag antibody was used as positive control (lane 8). Molecular weight markers are indicated.



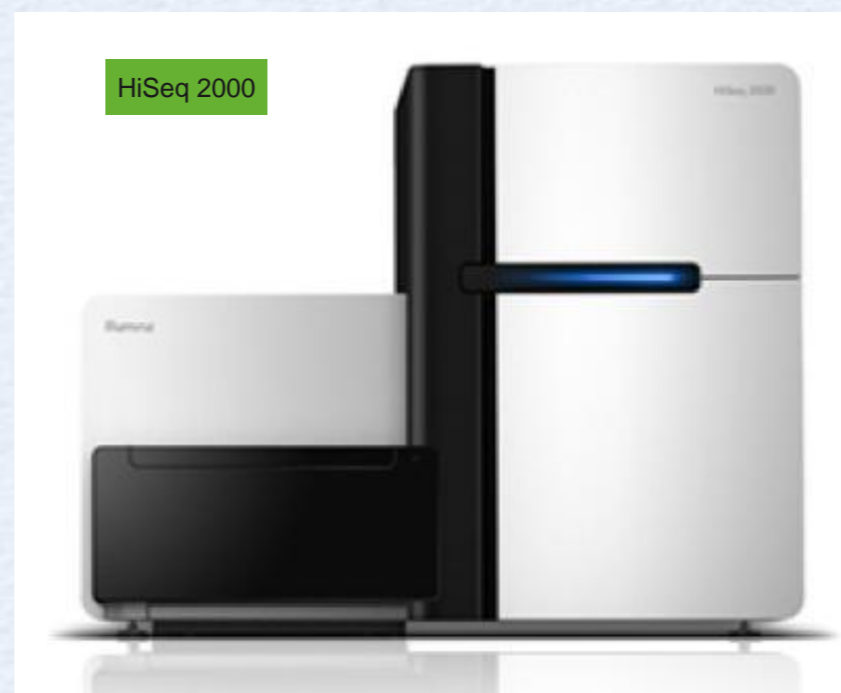
Next-Generation Sequencing Platforms

Roche-454



Reads: 400nt
Run: 1.2Mi reads
Run: 480M bases
Cost: 48k bases/\$
Time: 480k bases/h

Illumina-Solexa



Reads: 75 a 100nt
Run: 2Bi reads
Run: 200G bases
Cost: 10M bases/\$
Time: 1G base/h

AB-SOLiD



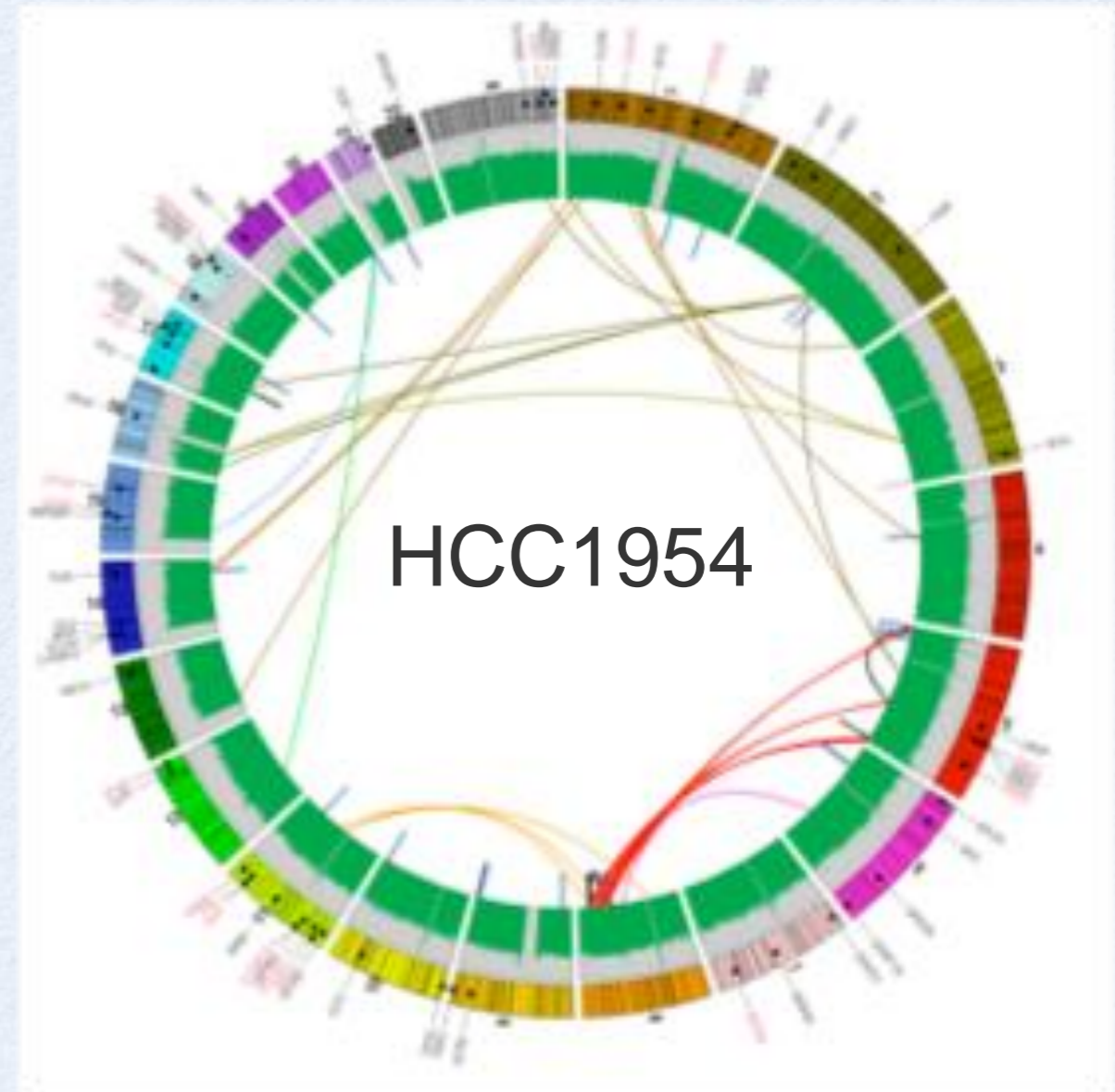
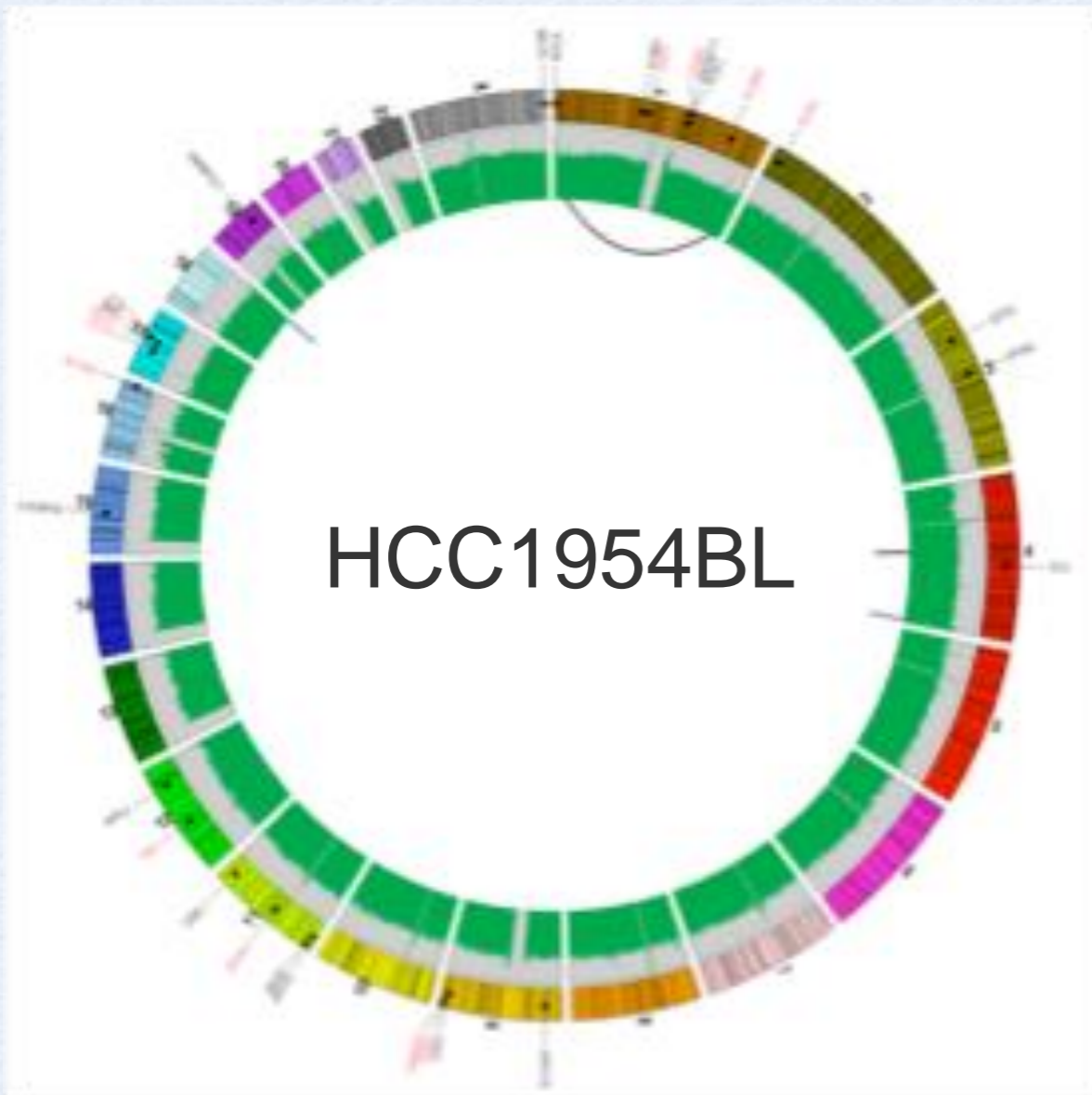
Reads: 50nt
Run: 2Bi reads
Run: 100G bases
Cost: 10M bases/\$
Time: 0.7G bases/h

Sequencing Tumor Genomes

Distinct patterns of somatic alterations in a lymphoblastoid and a tumor genome derived from the same individual

6056–6068 *Nucleic Acids Research*, 2011, Vol. 39, No. 14
doi:10.1093/nar/gkr221

Pedro A. F. Galante¹, Raphael B. Parmigiani¹, Qi Zhao^{2,3}, Otávia L. Caballero³,
Jorge E. de Souza¹, Fábio C. P. Navarro¹, Alexandra L. Gerber⁴, Marisa F. Nicolás⁴,
Anna Christina M. Salim¹, Ana Paula M. Silva¹, Lee Edsall⁵, Sylvie Devalle³,



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Anna Christina M. Salim¹, Ana Paula M. Silva¹, Lee Edsall⁵, Sylvie Devalle³,

Somatic Variations	HCC1954		HCC1954BL	
	Number	Percentage	Number	Percentage
Point mutations	274	100	173	100.00
Coding	64	23.36	30	17.3
Nonsense	2	0.73	3	1.7
Missense	45	16.42	15	8.7
Synonymous	17	6.20	12	6.9
Non-coding	14	5.11	15	8.7
UTR	13	4.74	13	7.5
ncRNA	1	0.36	2	1.2
miRNA	0	0	0	0
Intronic	179	65.33	114	65.9
Splice site	0	0	0	0
Other intronic	179	65.33	114	65.9
Intergenic	17	6.20	14	8.1

Sequencing Tumor Genomes

Systematic detection of putative tumor suppressor genes through the combined use of exome and transcriptome sequencing

Zhao et al. *Genome Biology* 2010, 11:R114
<http://genomebiology.com/2010/11/11/R114>

Qi Zhao^{1†}, Ewen F Kirkness^{2†}, Otavia L Caballero^{1†}, Pedro A Galante³, Raphael B Parmigiani³, Lee Edsall⁴, Samantha Kuan⁴, Zhen Ye⁴, Samuel Levy⁵, Ana Tereza R Vasconcelos⁶, Bing Ren⁴, Sandro J de Souza³, Anamaria A Camargo³, Andrew JG Simpson^{1*}, Robert L Strausberg^{1*}

Global DNA hypomethylation coupled to repressive chromatin domain formation and gene silencing in breast cancer

246 *Genome Research*
www.genome.org

22:246–258 © 2012 by Cold Spring Harbor Laboratory Press; ISSN 1088-9051/12; www.genome.org

Gary C. Hon,¹ R. David Hawkins,¹ Otavia L. Caballero,² Christine Lo,³ Ryan Lister,⁴ Mattia Pelizzola,⁴ Armand Valsesia,⁵ Zhen Ye,¹ Samantha Kuan,¹ Lee E. Edsall,¹ Anamaria Aranha Camargo,⁶ Brian J. Stevenson,⁵ Joseph R. Ecker,⁴ Vineet Bafna,³ Robert L. Strausberg,^{2,7} Andrew J. Simpson,^{2,7} and Bing Ren^{1,8,9}

Molecular Oncology Center at Hospital Sírio-Libanês



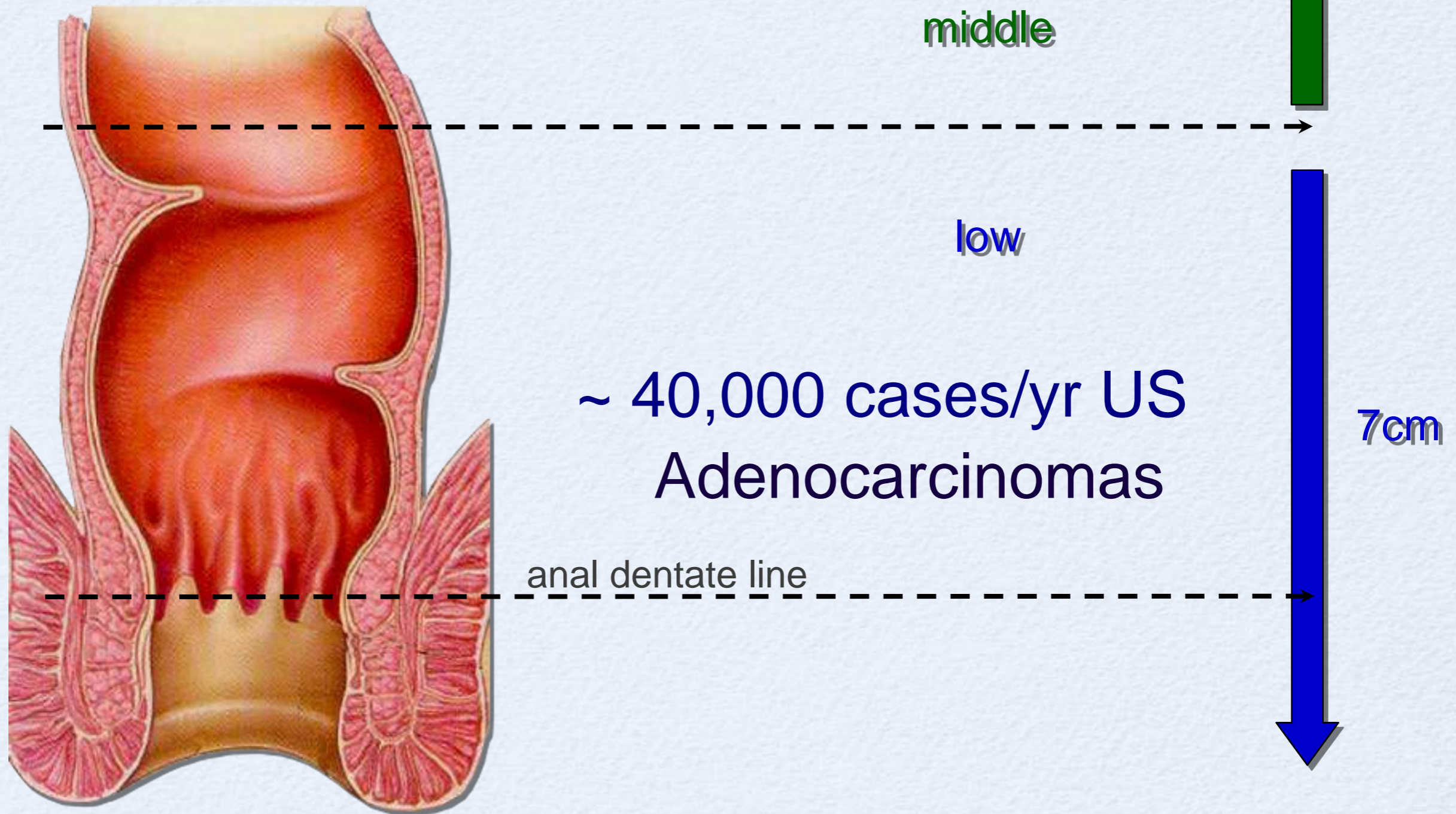
Major Interest: Translational Research in Oncology

Available Platforms:

- NextGen sequencing (5500XL and Illumina)
- Dedicated Bioinformatics Group

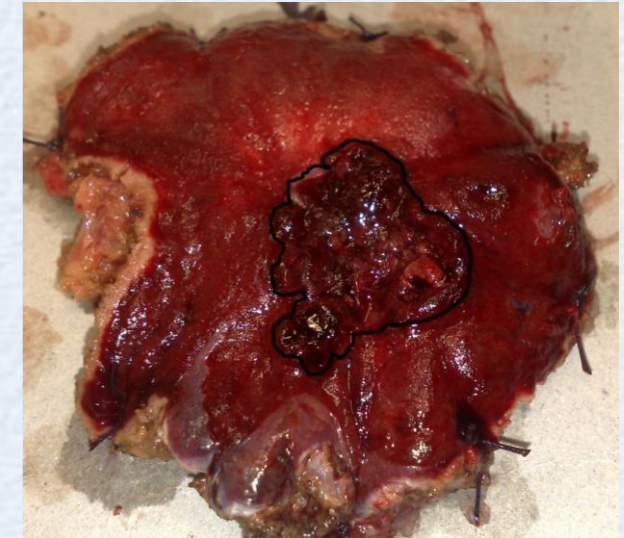
Ongoing Projects at MOC-HSL

Rectal Tumors

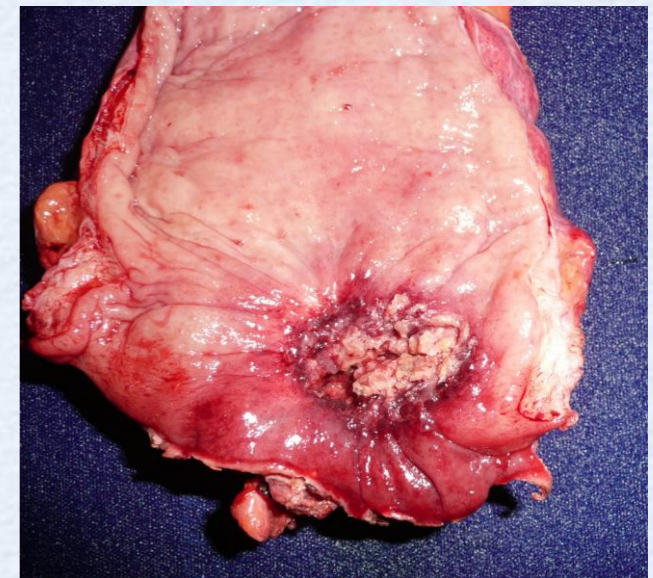


Management of Rectal Cancer Surgical Intervention

Local Excision
T1/T2 initial tumors



Radical Surgery
T3/T4 locally advanced tumors



Management of Rectal Cancer

Why search for Alternatives to Radical Surgery?

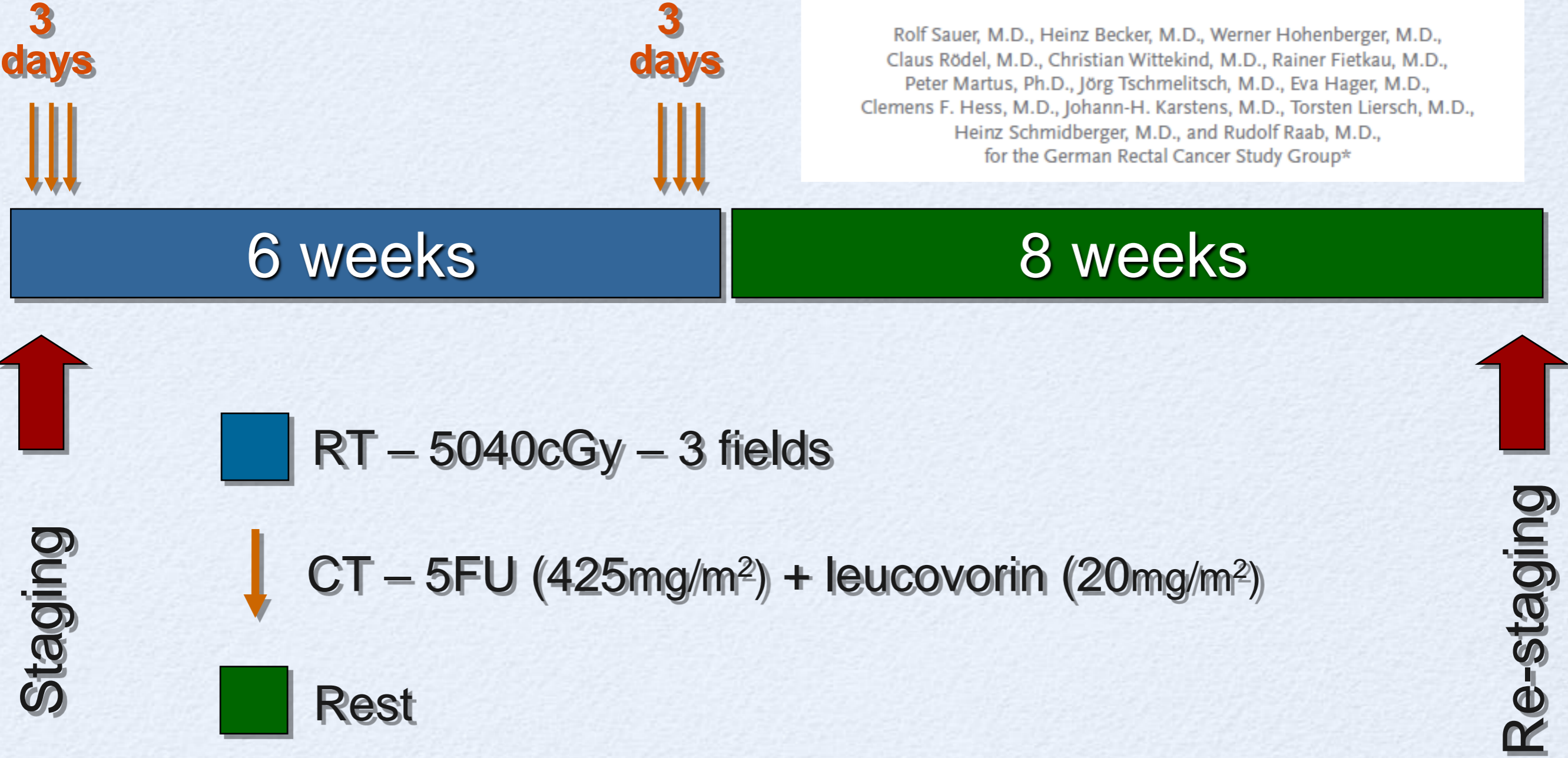
Overall Morbidity	38%
Mortality	2-3%
Urinary Dysfunction	20%
Sexual Dysfunction	15%
Anorectal Dysfunction	20%
Recurrence Rates	8-40%

Management of Rectal Cancer

Neoadjuvant Therapy - for locally advanced tumors

Preoperative versus Postoperative Chemoradiotherapy for Rectal Cancer

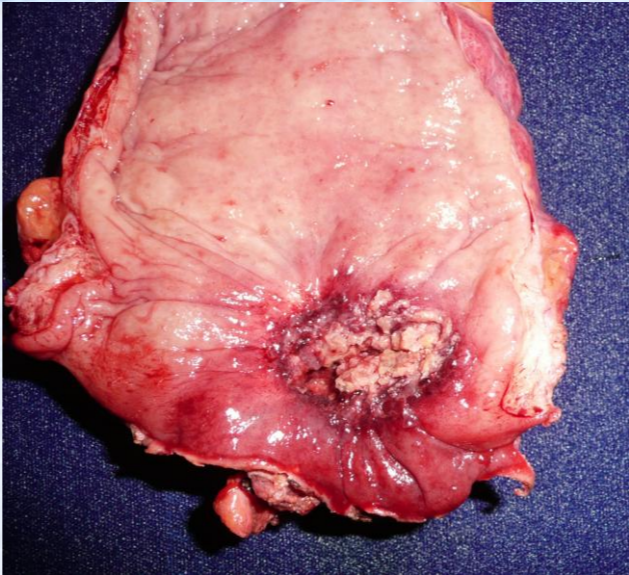
Rolf Sauer, M.D., Heinz Becker, M.D., Werner Hohenberger, M.D.,
Claus Rödel, M.D., Christian Wittekind, M.D., Rainer Fietkau, M.D.,
Peter Martus, Ph.D., Jörg Tschmelitsch, M.D., Eva Hager, M.D.,
Clemens F. Hess, M.D., Johann-H. Karstens, M.D., Torsten Liersch, M.D.,
Heinz Schmidberger, M.D., and Rudolf Raab, M.D.,
for the German Rectal Cancer Study Group*



Management of Rectal Cancer

Variable clinical response

No Response



Radical Surgery

Near Complete



Conservative Surgery

Complete Response

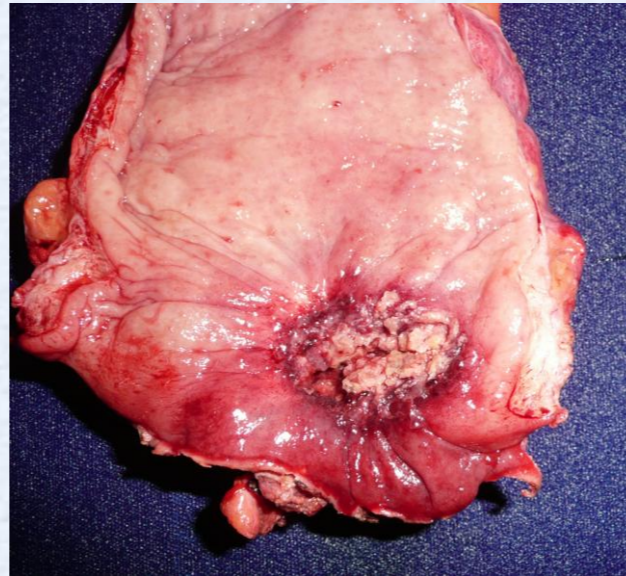


???????

Management of Rectal Cancer Major Challenges

#1 Can we avoid the unnecessary toxic effects of QRT in patients with no clinical evidence of response to therapy?

No Response



Radical Surgery

#2 Can we avoid the unnecessary surgery and comorbidities in patients with complete clinical response to therapy?

Complete Response



Conservative Surgery

Management of Rectal Cancer



#1 Avoid unnecessary CRT
toxic effects

#2 Avoid unnecessary
surgery and morbidity

Next Generation Sequencing
SOLiD platform

RNA-seq
Gene Expression analysis

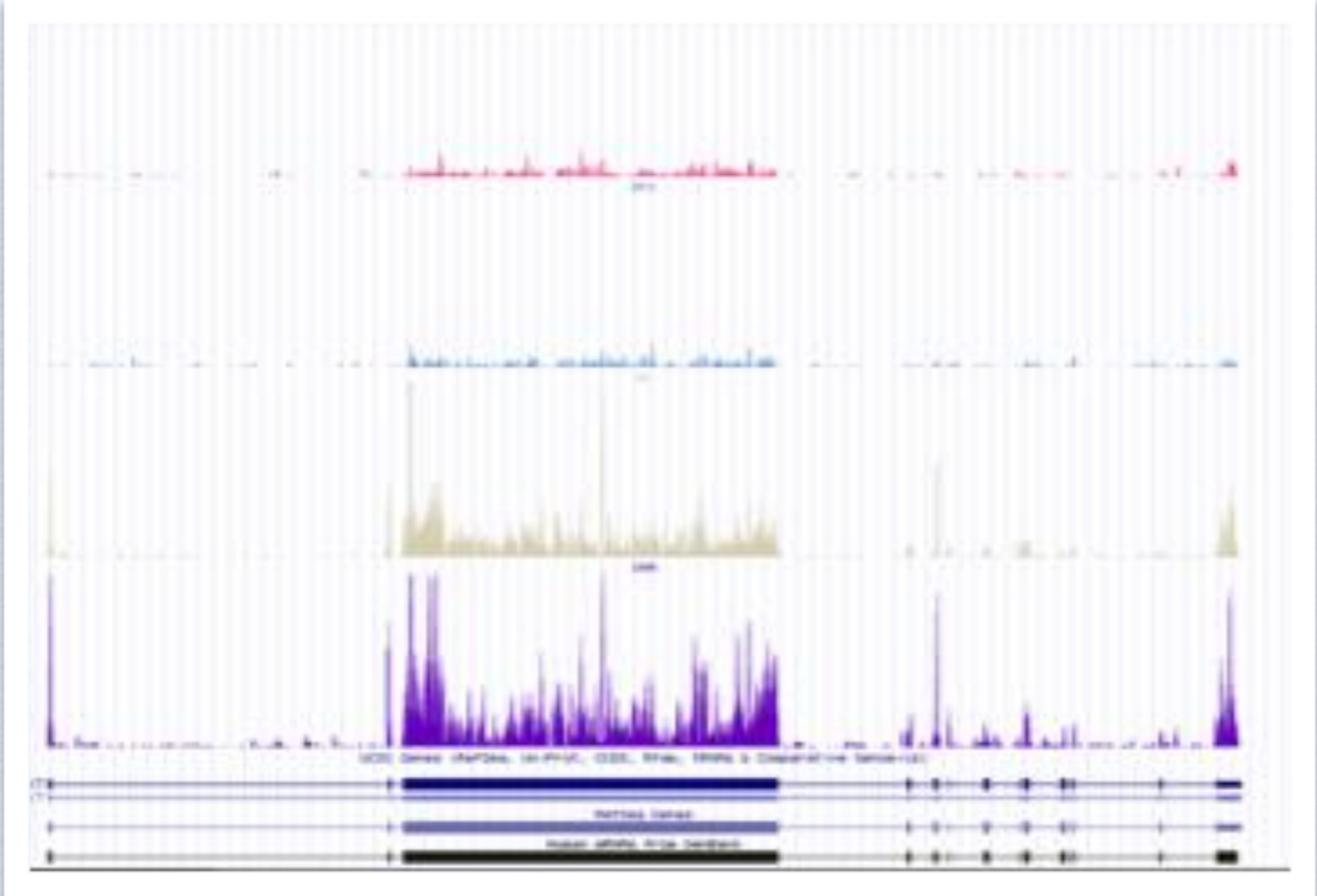
Paired-end gDNA-seq
Chromosomal Rearrangements

Develop a predictive
marker for therapeutic
response

Develop a biomarker
for detection of residual
disease

Predicting Response to Neoadjuvant Therapy

A Total of 47 Differentially Expressed Genes



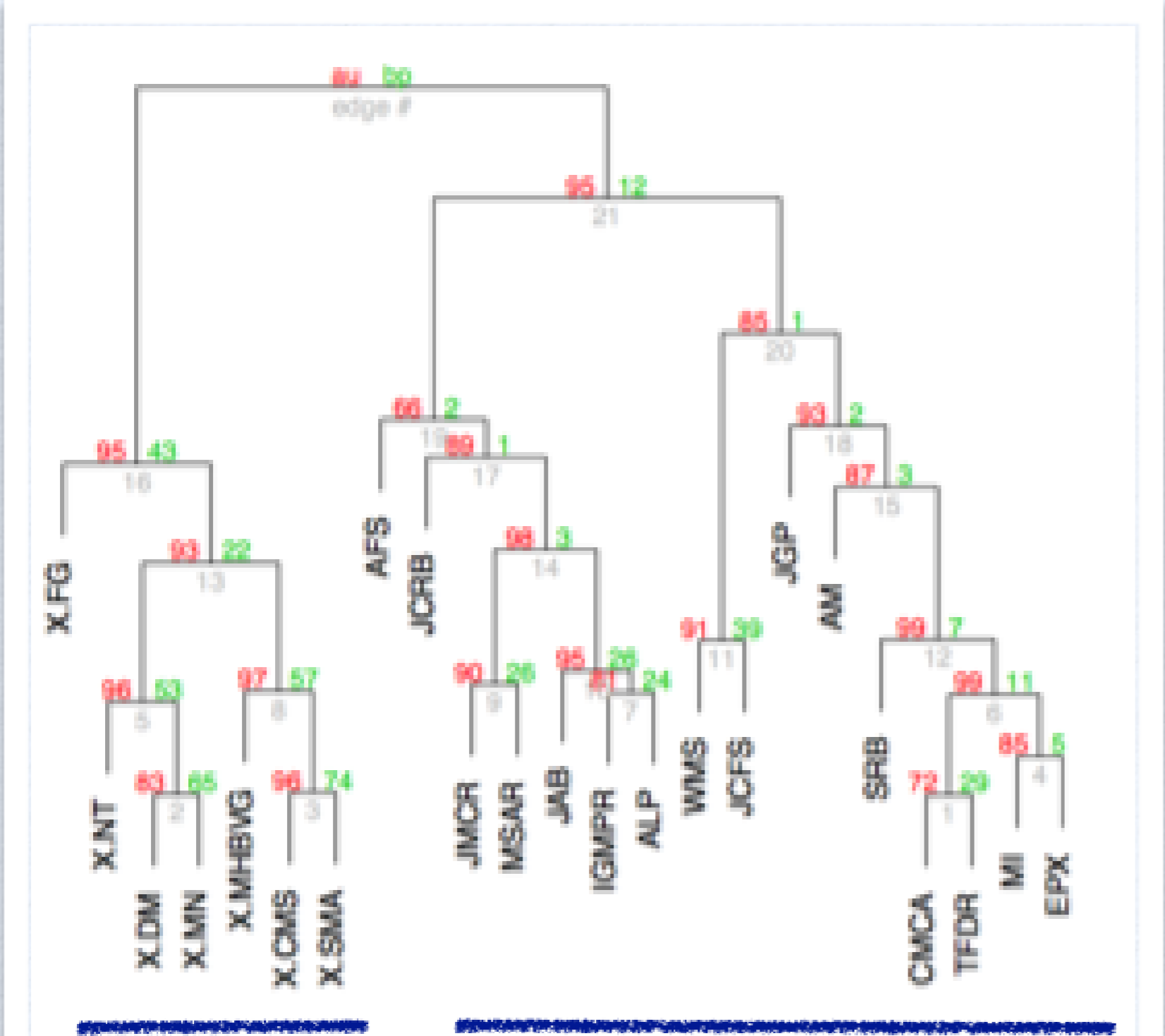
Incomplete Response

Complete Response

MUC17

Predicting Response to Neoadjuvant Therapy

Gene Signature - Training Set



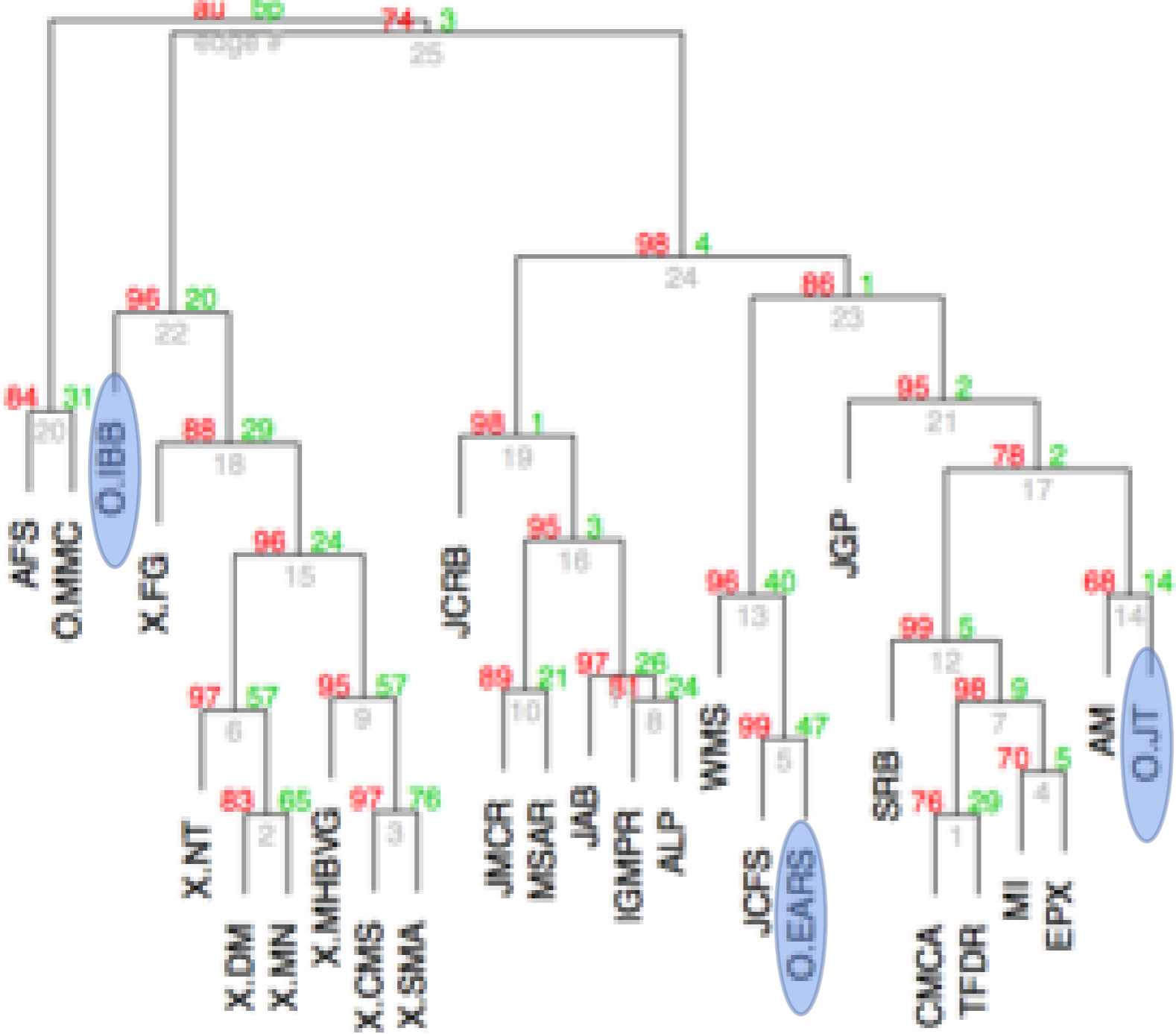
Complete

Incomplete

47 Differentially Expressed Genes

Predicting Response to Neoadjuvant Therapy

Gene Signature -Validation Set



47 Differentially Expressed Genes

Management of Rectal Cancer Major Challenges

#1 Avoid unnecessary CRT
toxic effects

#2 Avoid unnecessary
surgery and morbidity

Next Generation Sequencing
SOLiD platform

RNA-seq
Gene Expression analysis

Paired-end gDNA-seq
Chromosomal Rearrangements

Develop a predictive
marker for therapeutic
response

Develop a biomarker
for detection of residual
disease

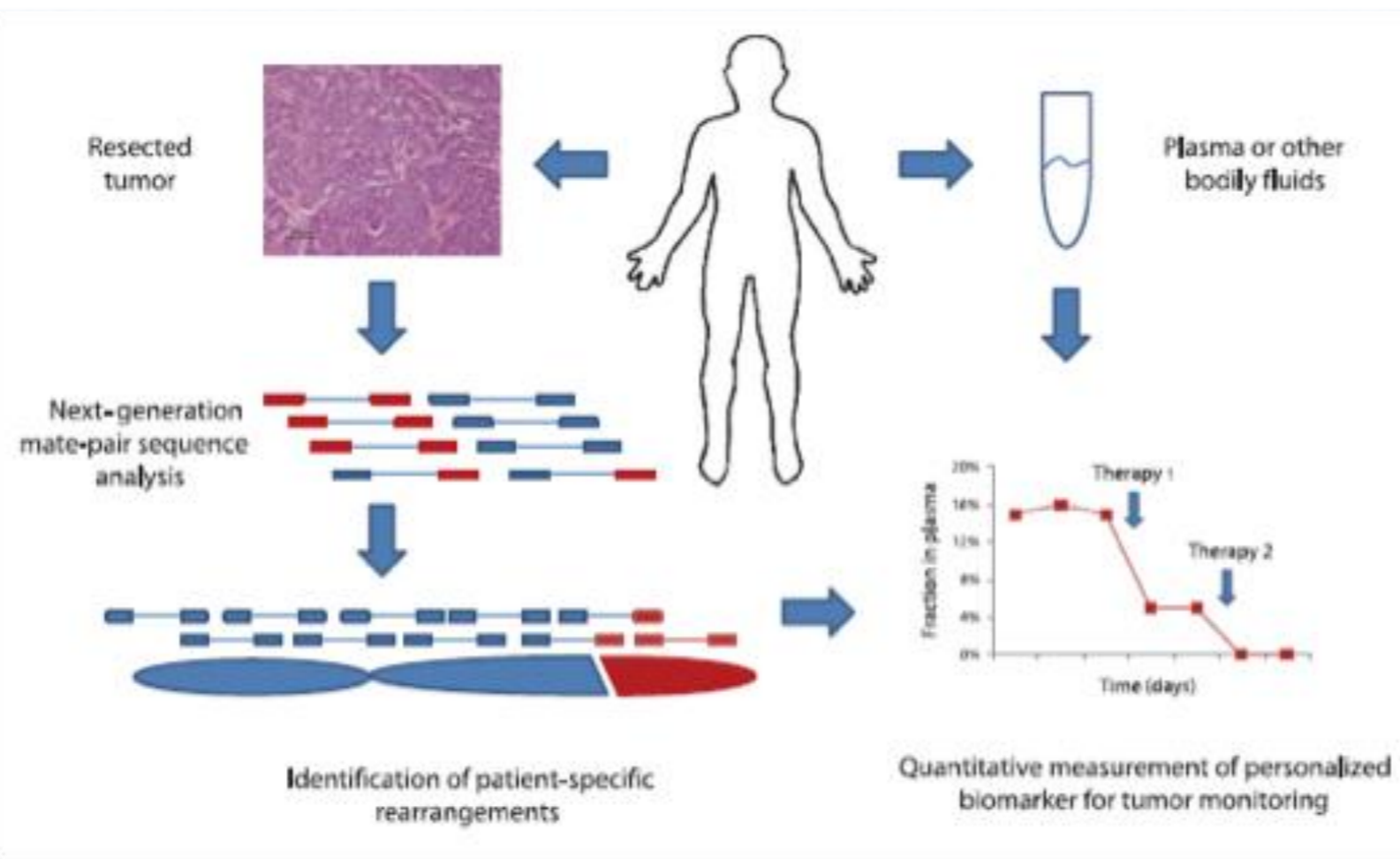
Assessing Response to Neoadjuvant Therapy

Personalized Biomarkers

Development of Personalized Tumor Biomarkers Using Massively Parallel Sequencing

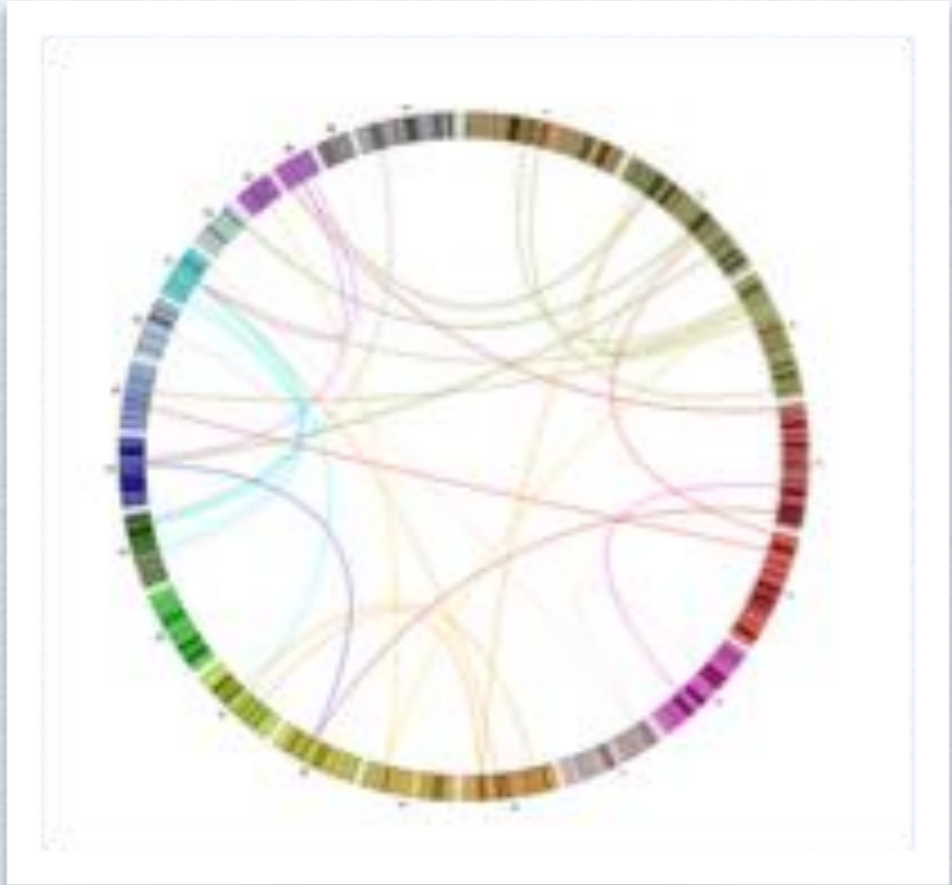
Rebecca J. Leary¹, Isaac Kinde¹, Frank Diehl¹, Kerstin Schmidt¹, Chris Clouser², Cisilya Duncan², Alena Antipova², Clarence Lee², Kevin McKernan², Francisco M. De La Vega³, Kenneth W. Kinzler¹, Bert Vogelstein¹, Luis A. Diaz Jr.¹, and Victor E. Velculescu^{1,*}

¹Ludwig Center for Cancer Genetics and Therapeutics and Howard Hughes Medical Institute, Johns Hopkins Kimmel Cancer Center, Baltimore, MD 21231, USA.

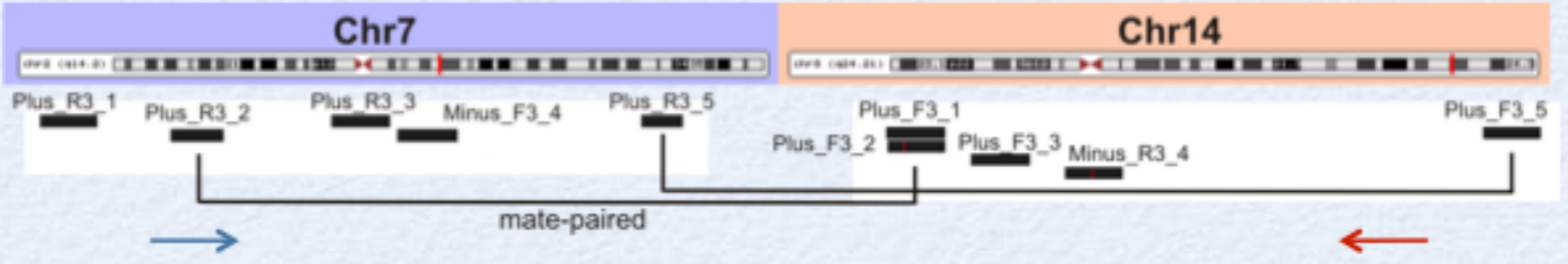
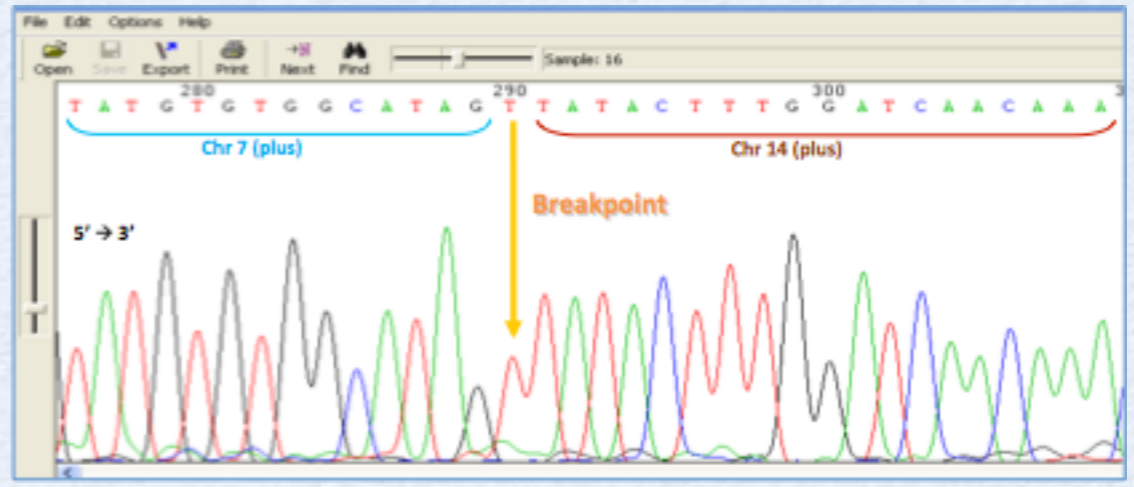
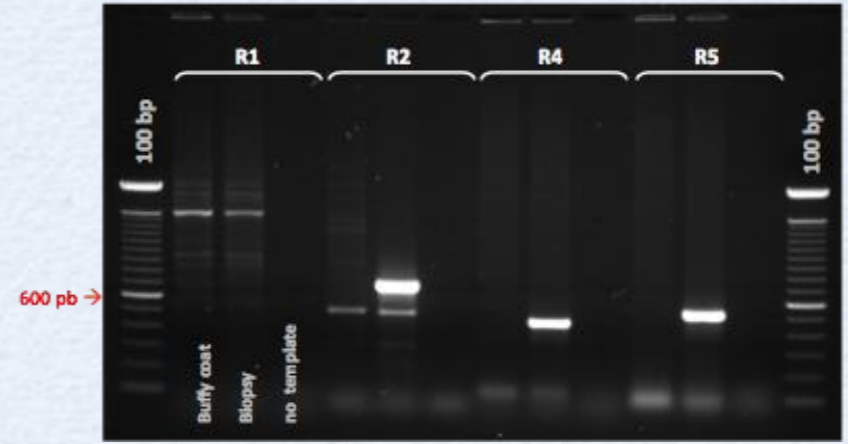


Assessing Response to Neoadjuvant Therapy

Intrachromosomal rearrangements



Patient # 1

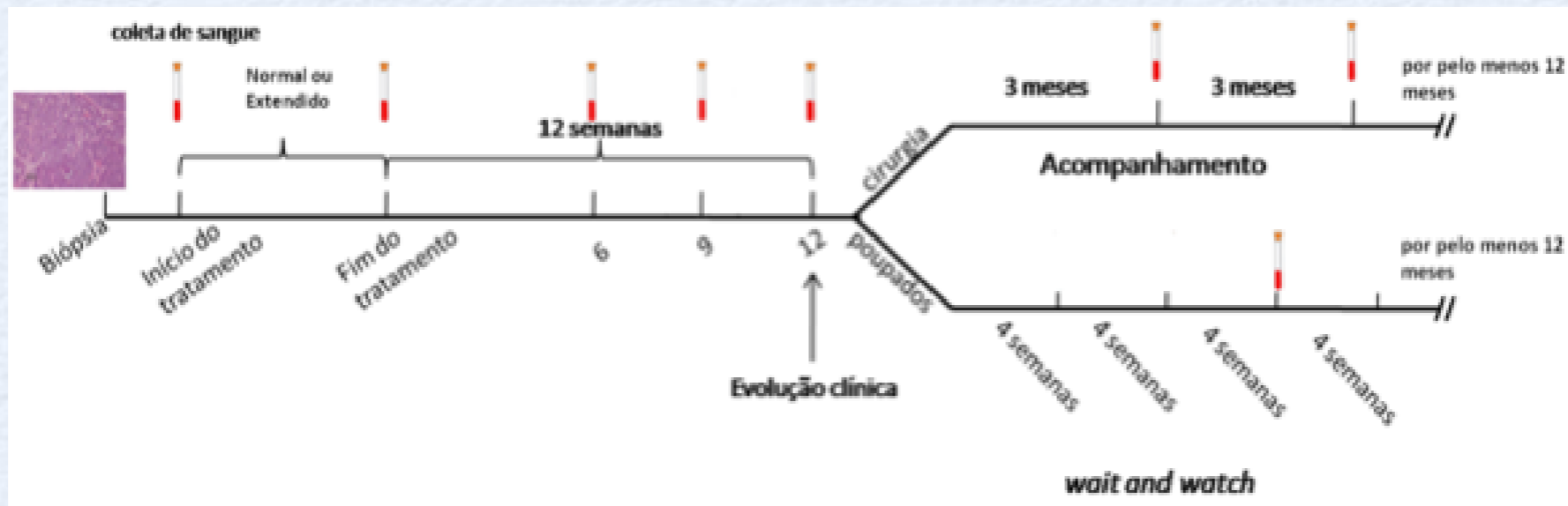
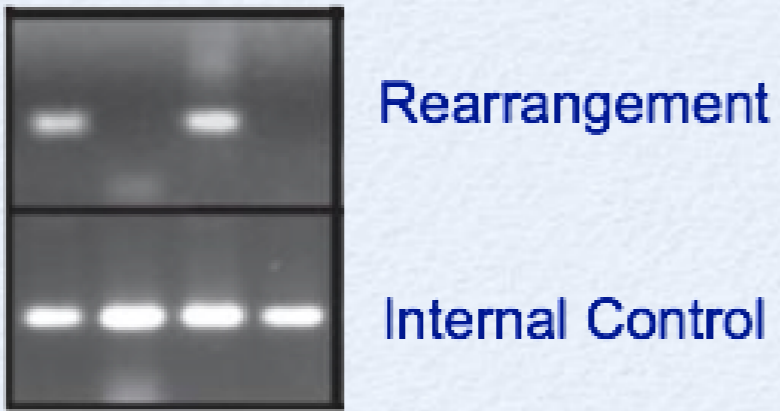


Assessing Response to Neoadjuvant Therapy

Detecting Circulating DNA in the plasma samples

Patient 1
Incomplete Response

Patient 2
Complete Response



Rectal Cancer Multidisciplinary Approach



Surgeons

Angelita Habr-Gama
Rodrigo Oliva Perez
Joaquim Gama-Rodrigues

Oncologist

Jorge Sabbaga

Radiotherapist

Wladimir Nadalin
Patricia Bailão Aguilar



The Next-Generation



Centro de Oncologia
Molecular

Pedro AF Galante
Raphael Parmigiani
Fernanda Koyama
Paula Asprino
Fabiana Bettoni
Paola Carpinetti
Elisa Donnard
Fábio Casarotti
Bruna Quevedo
Natalia Felício

