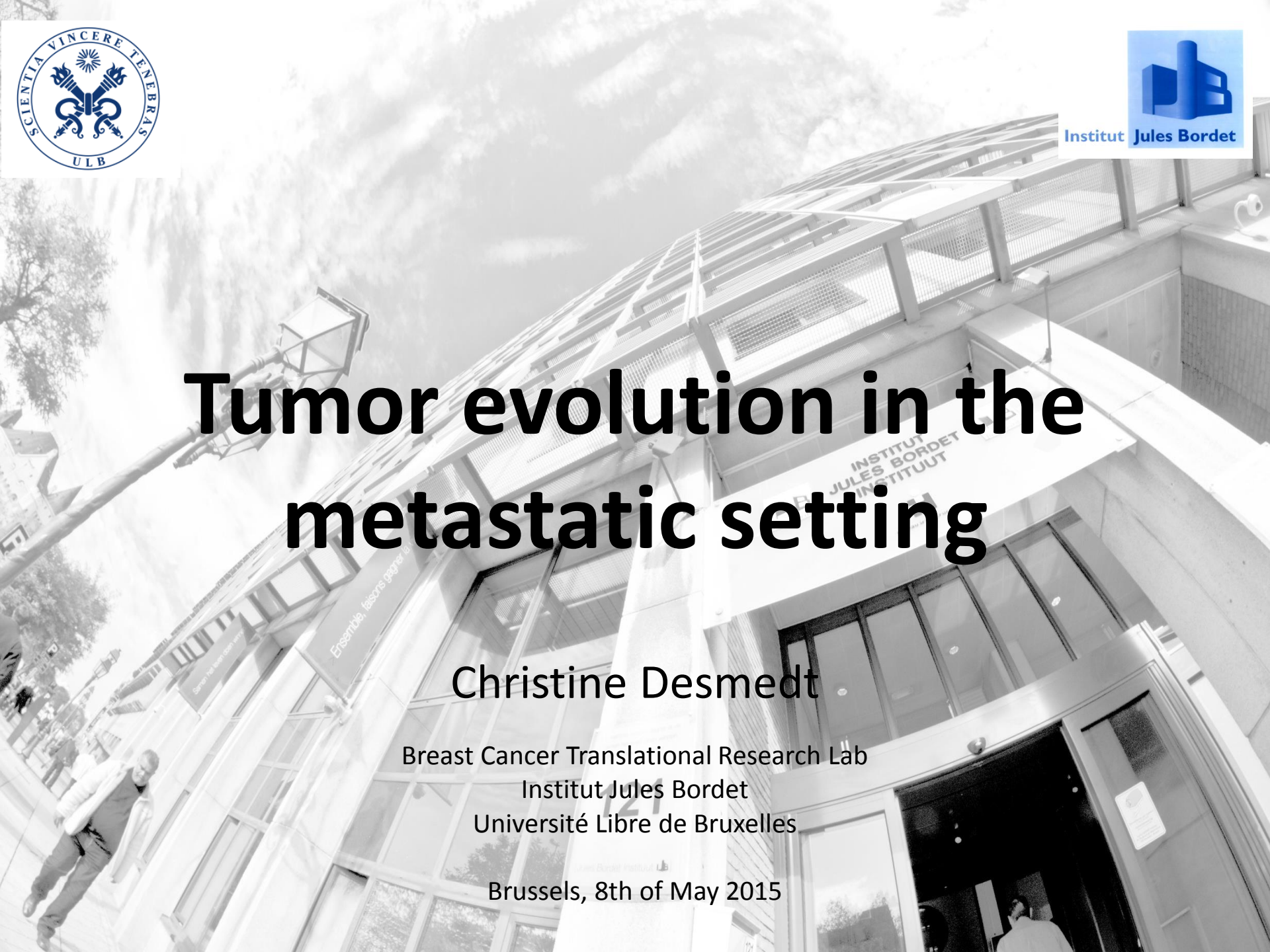


Tumor evolution in the metastatic setting

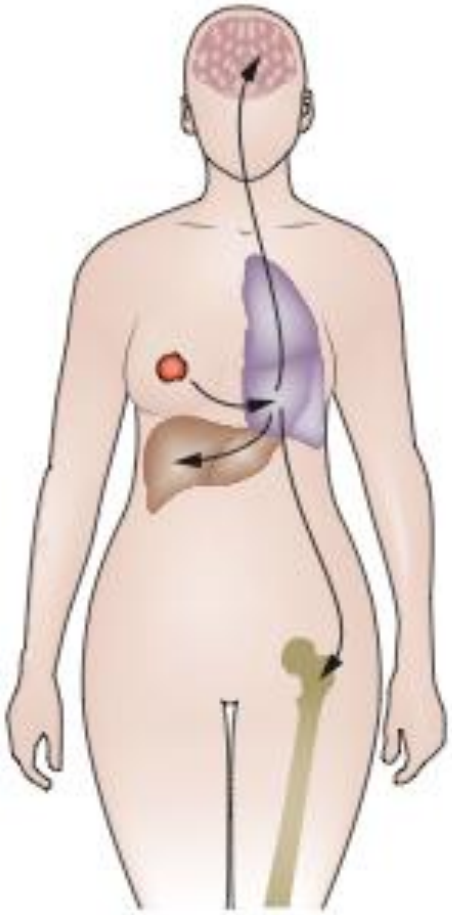
Christine Desmedt

Breast Cancer Translational Research Lab
Institut Jules Bordet
Université Libre de Bruxelles

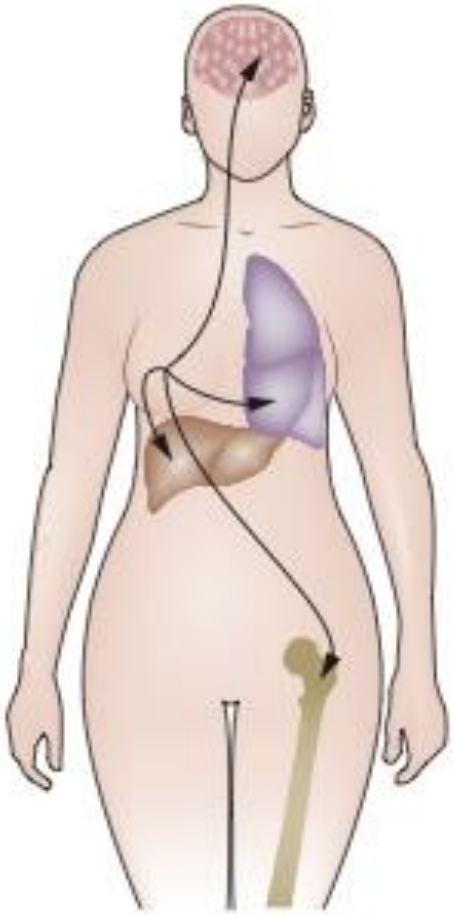
Brussels, 8th of May 2015

Introduction

What are the origins of metastases?



AND/OR?

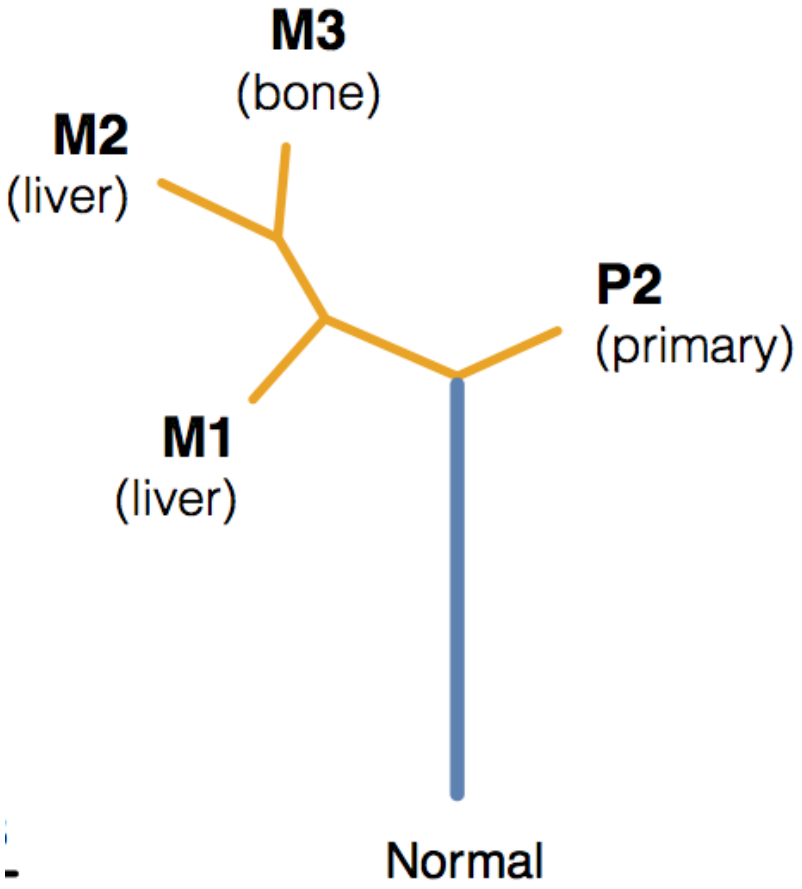


Metastatic cascade

Parallel progression

Naxerova & Jain, Nat Rev2015

Phylogenetics



Tree of life=
Evolutionary trajectory
of the disease

Whole exome/genome studies

Table 1 | Genome-wide comparisons of solid primary tumours and their metastases

Study	Primary cancer	Number of patients	Time between resection of primary tumour and metastasis	Genetic relationship between primary tumour and metastases	Evidence of possible metastatic cascade*
Jones *et al.* (2008)[32]	Colon	10	Ranged from synchronous to 20 months	High similarity	NA
Liu *et al.* (2009)[52]	Prostate	24	Synchronous	High similarity; primary only available in 5 cases	Yes
Shah *et al.* (2009)[57]	Breast	1	9 years	Divergent	NA
Campbell *et al.* (2010)[51]	Pancreas	13	Synchronous	High similarity in most patients; primary tumour not available in some cases	Yes, in some patients
Ding *et al.* (2010)[55]	Breast	1	8 months	High similarity	NA
Yachida *et al.* (2010)[33]	Pancreas	7	Synchronous	Similarity between metastases and localized area of primary	No
Navin *et al.* (2011)[49]	Breast	1	Not specified	High similarity	NA
Gerlinger *et al.* (2012)[5]	Kidney	2	Synchronous	Divergent	Yes
Wu *et al.* (2012)[58]	Medulloblastoma	7	Not specified	Divergent	Yes
Haffner *et al.* (2013)[54]	Prostate	1	17 years	Similarity between metastases and localized area of primary	Yes

*That is, metastasis giving rise to metastasis; NA for studies that did not assess multiple metastases. Abbreviation: NA, not applicable.

N= 1 pt with only one metastatic site

Naxerova & Jain, Nat Rev2015

Autopsy-based BC studies

Study	Nr of pts	Main findings
Viadana (1973)	647 (<1970)	Comparison of metastases in young and older pts: 1. More extensive disease in younger pts 2. More liver, thyroid and bone mets in younger pts
Harris (1984)	92 (1972-83)	Comparison of metastatic pattern IDC vs ILC: 1. More lung mets in IDC 2. More bone peritoneal, car meningitis mets in ILC
Parham (1989)	85 (1973-86)	Confirmation of cancer-related death in 75% of the cases with BC history : tendency to over-estimate BC as cause of death.
Cummings (2014)	197 (1960-79)	1. Pts with CNS mets more likely to present with bone mets 2. More liver and gynecological mets in young pts 3. (n=55): ER and PgR downregulation in mets compared to prim. 4. (n=6): CGH analysis: Prim differs from mets, but mets are similar
Juric (2015)	1	1. Comparison of primary and metastatic lesions. 2. Heterogeneity between lesions regarding PTEN alterations, which correlated to response to PI3K inhibition

Aim of the current study=
Reconstructing evolutionary trajectories

Autopsy patients

Eligibility criteria:

(1) Patients died from breast cancer;

(2) Availability of FFPE tissue blocks from the primary breast tumor, a non-cancerous tissue as germline reference and at least one metastatic sample;

(3) Minimum 30% tumor cellularity at central pathological review;

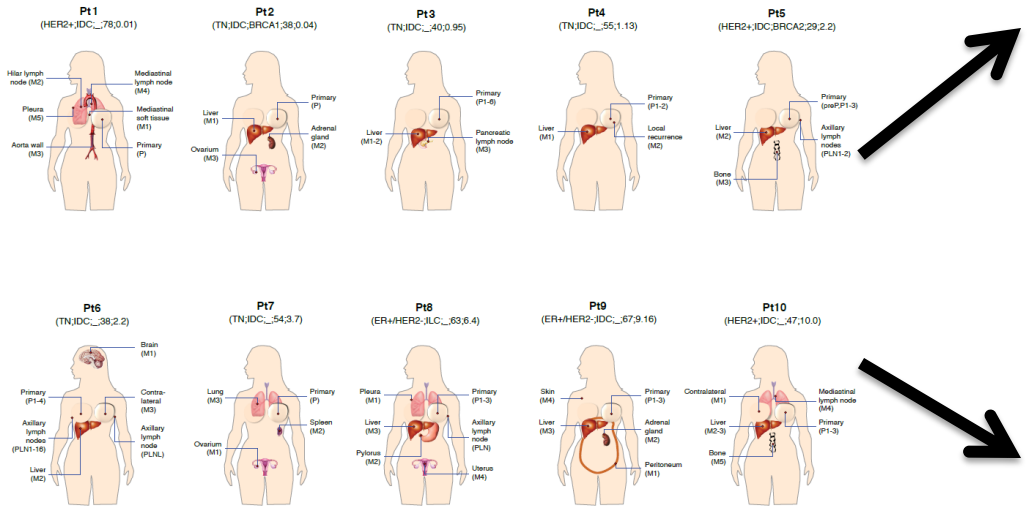
(4) >1µg of dsDNA for from at least the primary breast tumor, a non-cancerous tissue as germline reference and at least one metastatic sample

N=10 patients

Patients and samples

Time between death and autopsy	Average=2.8 days (range= 1.5 - 4.2)
Nr of distant metastatic samples/pt	Average=3 (range= 1-4)
Nr of patients with multiple primary samples	7 (range= 2-8)
Molecular subtype	5 ER-/HER2-, 2 HER2+, 3 ER+/HER2-
Age at diagnosis	4 young patients (≤40), 3 between 40-60, 3 older patients >60
Histologic subtype	9 IDC and 1 ILC
Treatment	2 treatment naive, 8 with systemic treatment (3/8 with neo-adjuvant treatment)

Strategy



Substitutions/indels (mutations):

1/ Whole-exome sequencing

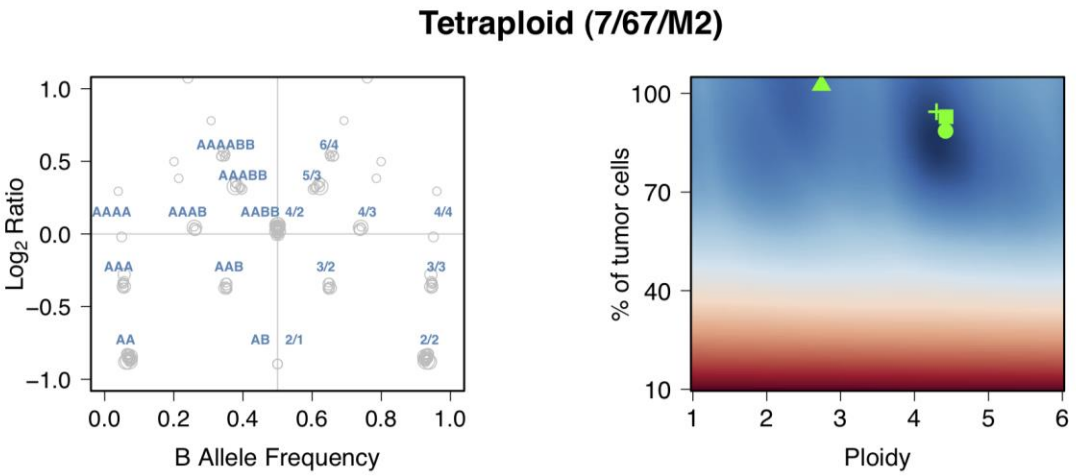
2/ Deep re-sequencing

Copy number alterations (CNAs):

Affymetrix Oncoscan FFPE Express 2.0 assay

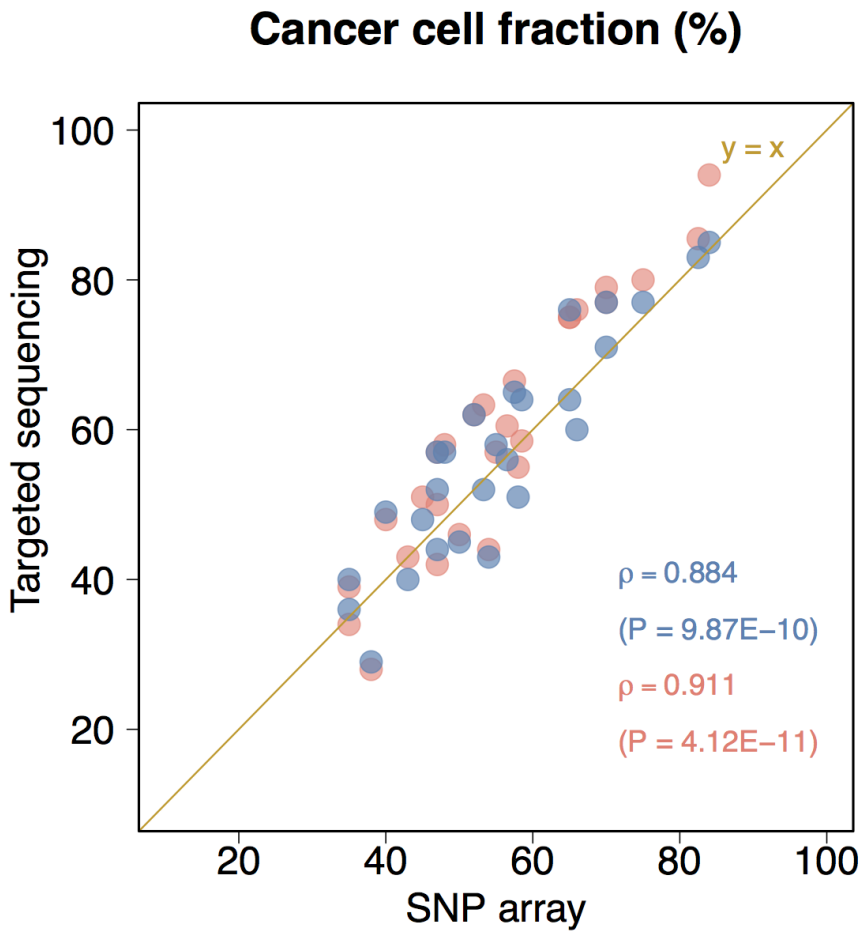
Results

1. Estimation of ploidy and CCF



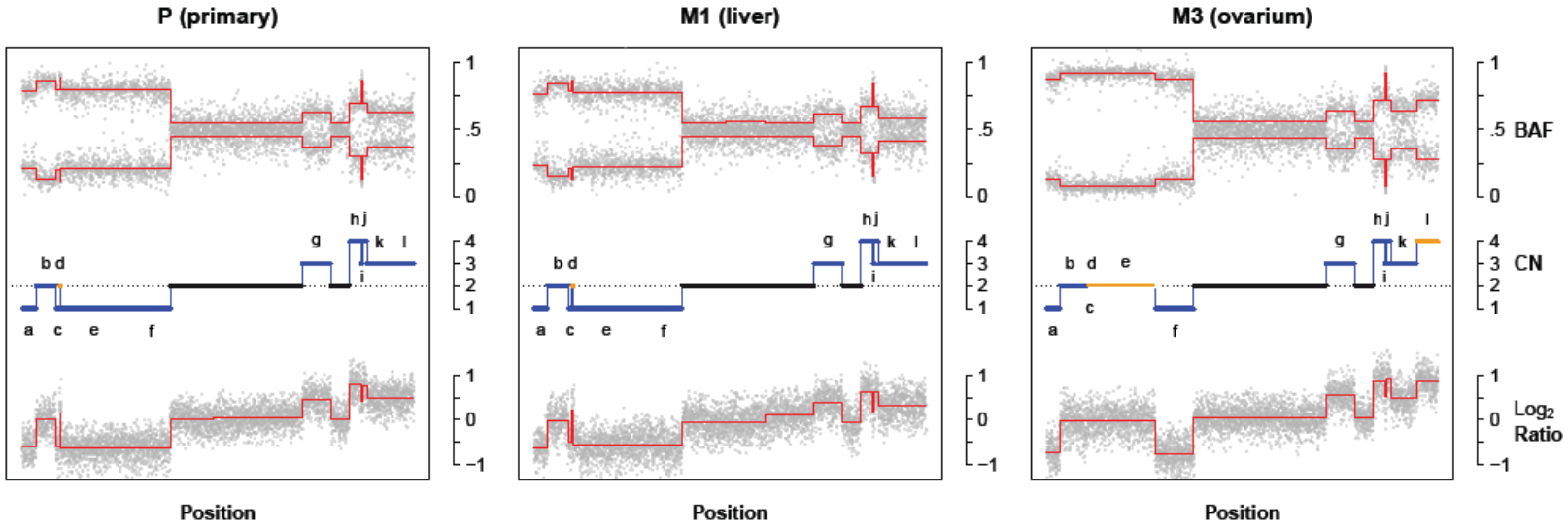
- High ploidy samples: potential different estimation according to the algorithm used;
- Comparison with FACS analysis
- Only in 4/10: diploid tumors
- Change in ploidy occurred always in primary tumor

2. Estimation of cancer cell fractions (from SNPs and SNVs)

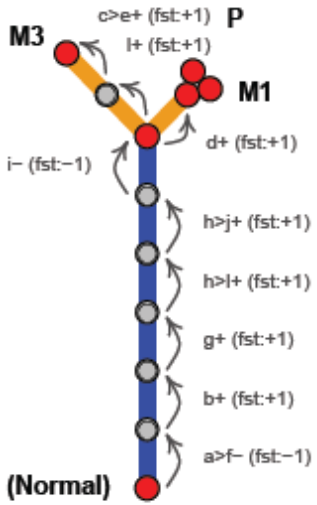


- CCF estimated from SNP arrays with GAP;
- CCF estimated from SNVs with PyClone
- Excellent correlation between both estimates

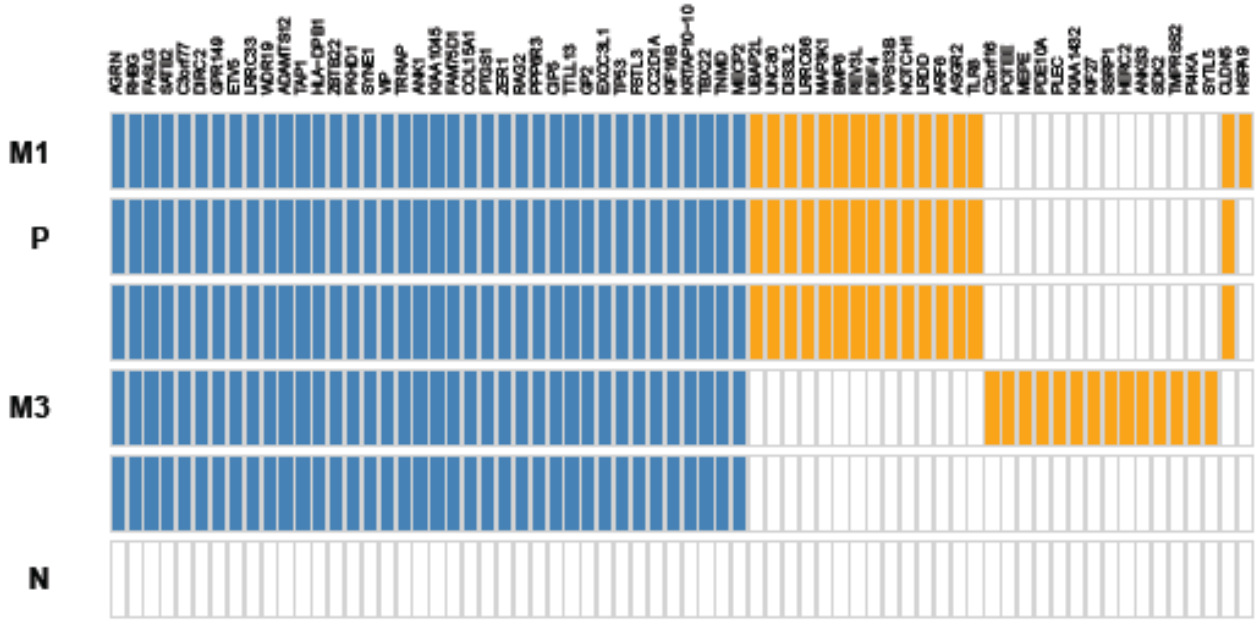
3. Phylogenetic reconstruction (2n)

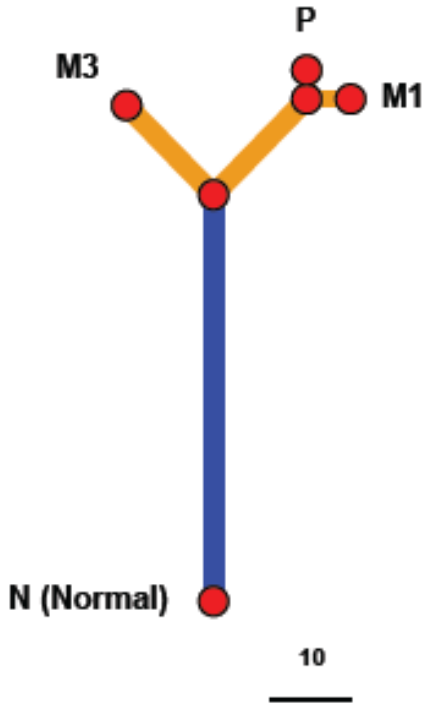


1st step: CNAs



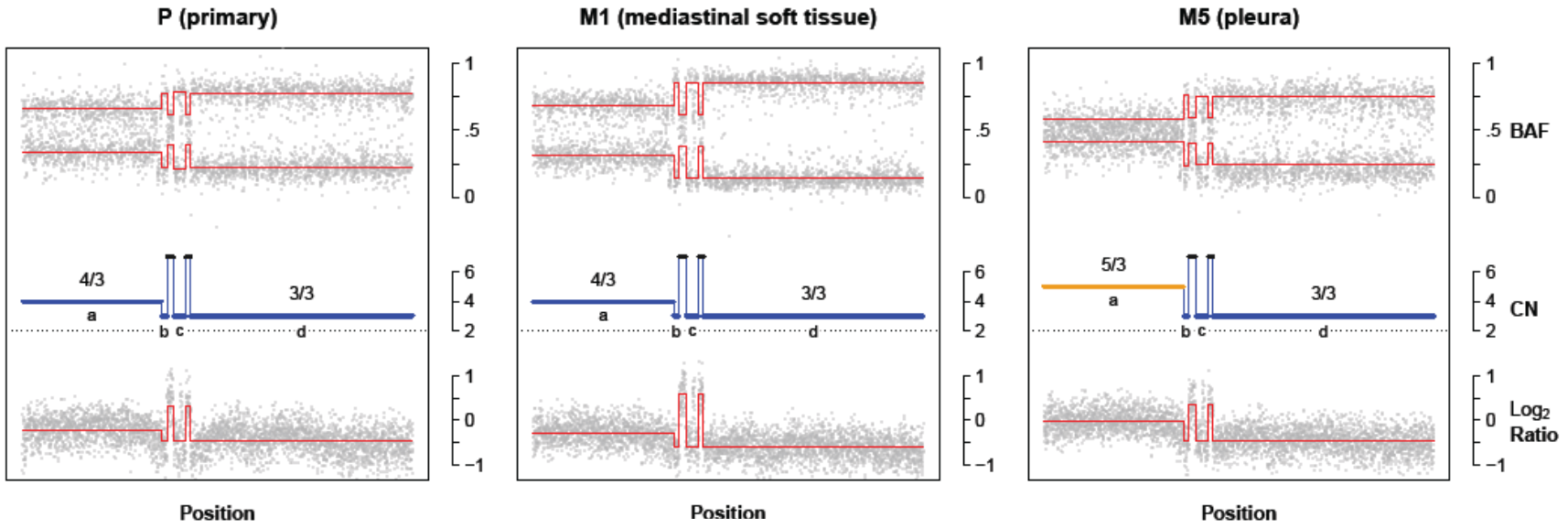
3. Phylogenetic reconstruction (2n)



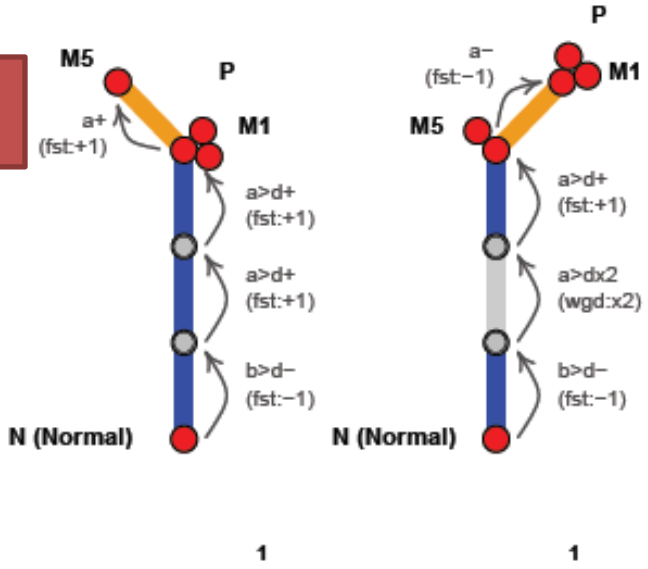


2nd step: SNVs

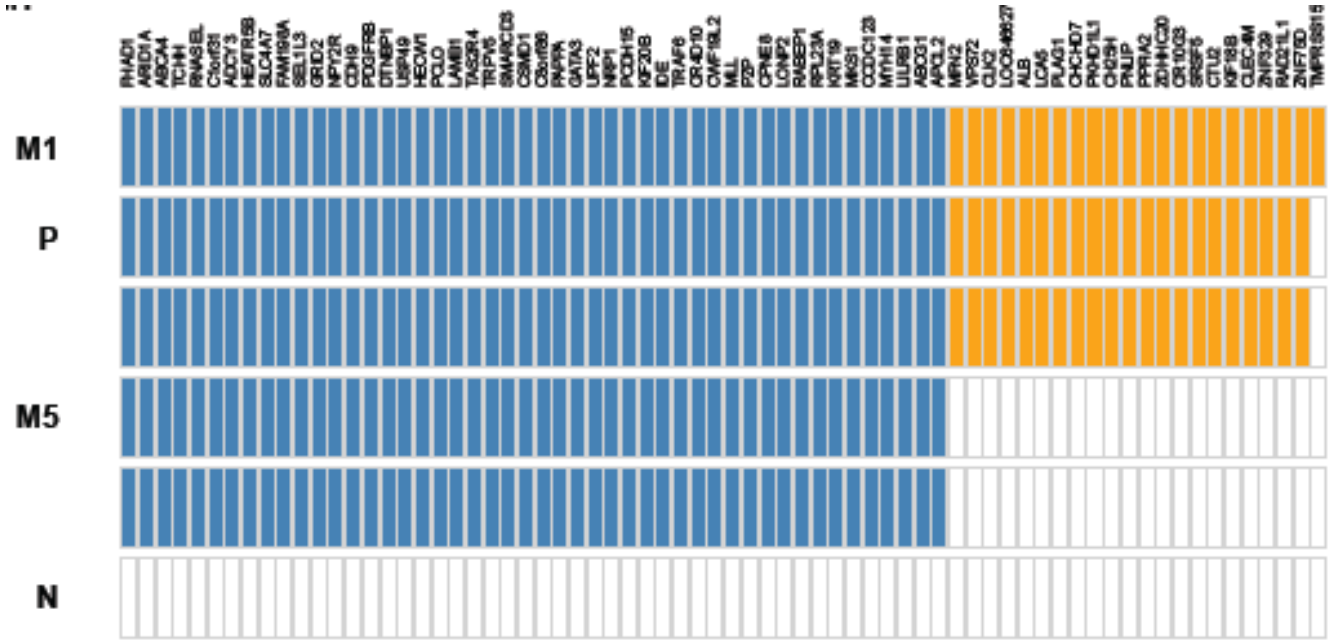
3. Phylogenetic reconstruction (4n)

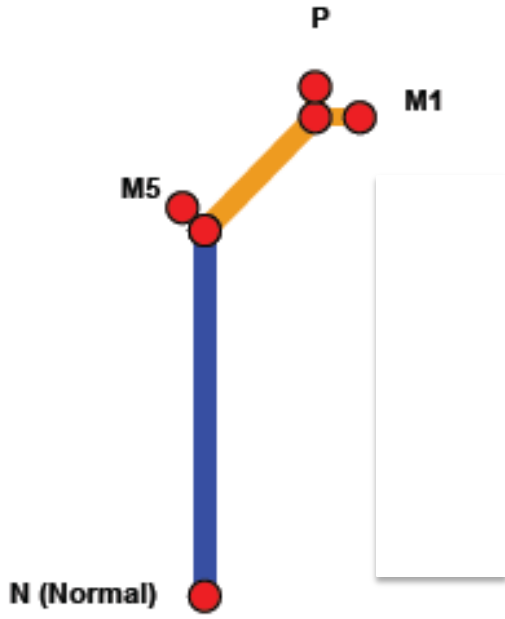


1st step: CNAs



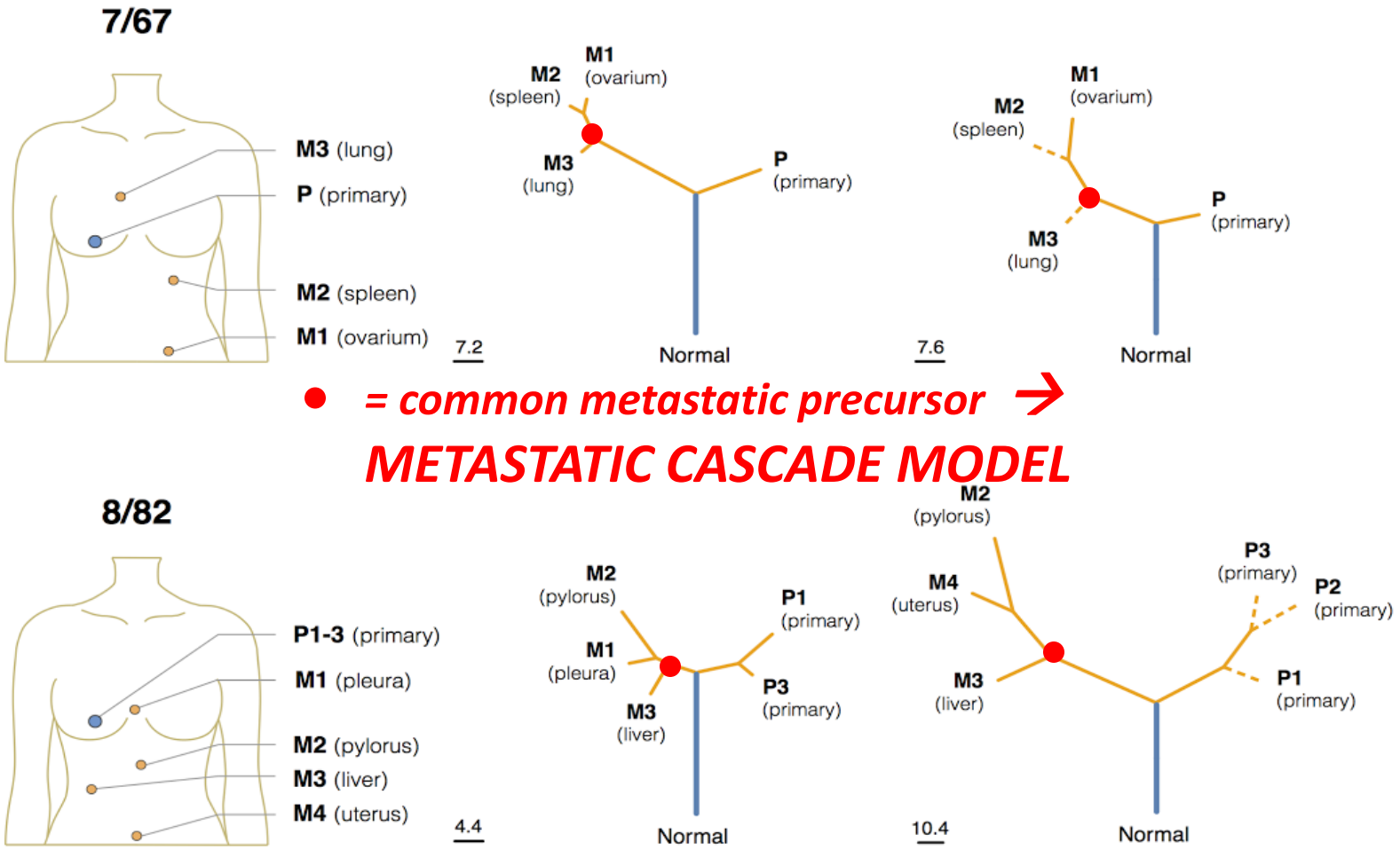
3. Phylogenetic reconstruction (4n)





2nd step: SNVs

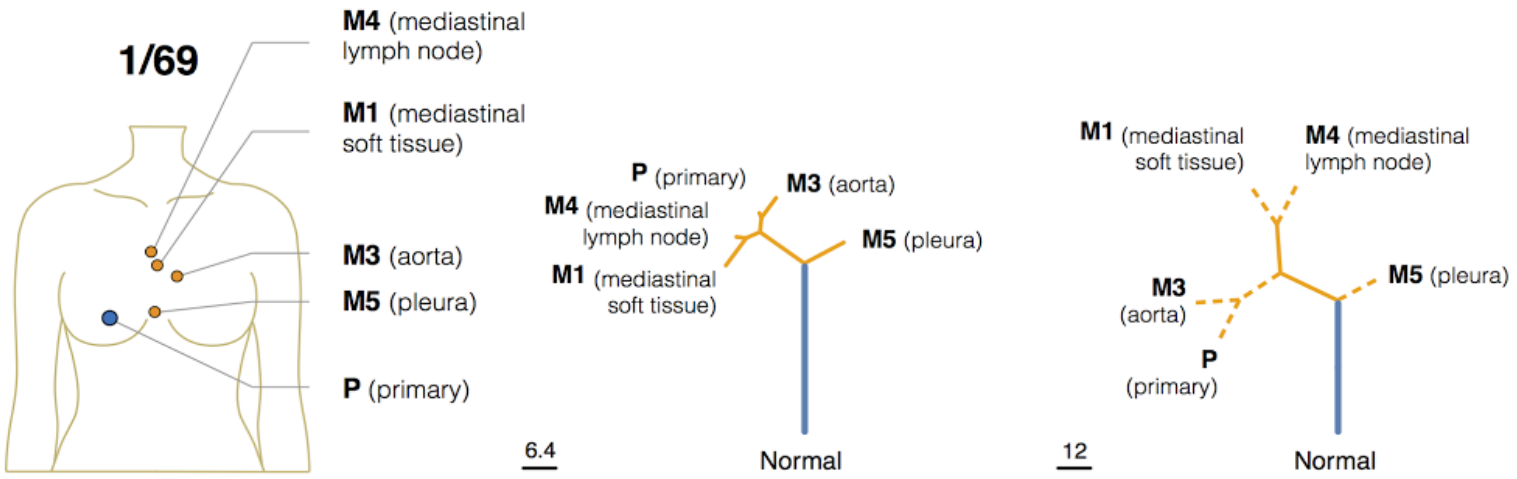
3. Progression trajectories-I



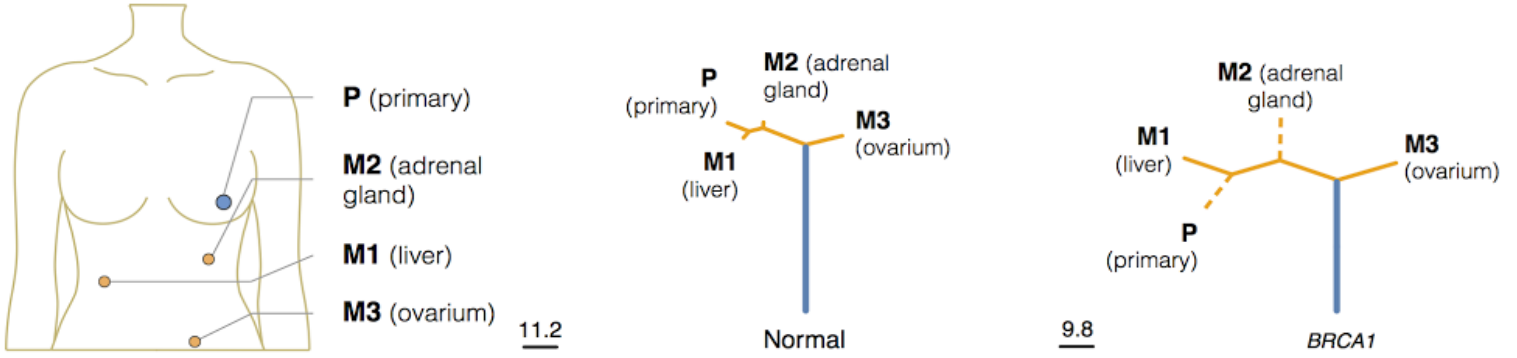
- ***= common metastatic precursor →***

METASTATIC CASCADE MODEL

3. Progression trajectories -II

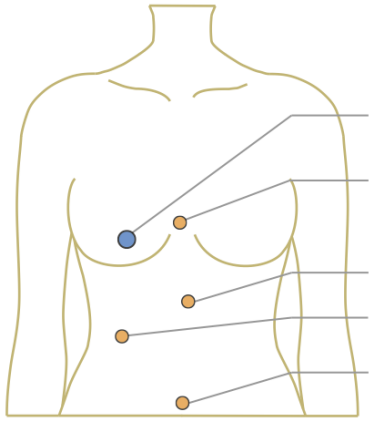


PARALLEL PROGRESSION MODEL

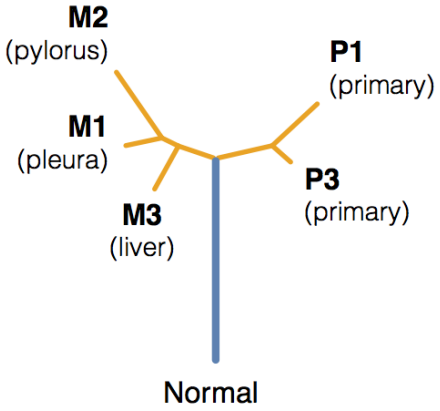


4. Multiple primary samples

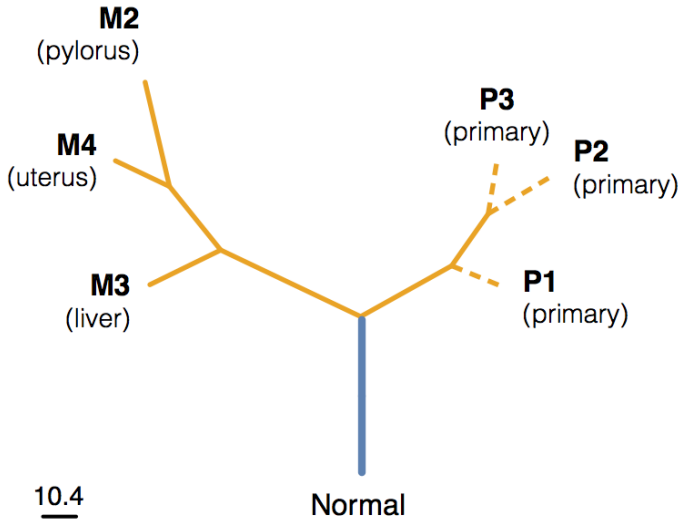
8/82



P1-3 (primary)
M1 (pleura)
M2 (pylorus)
M3 (liver)
M4 (uterus)

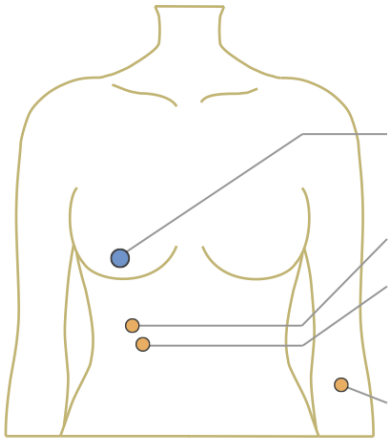


4.4

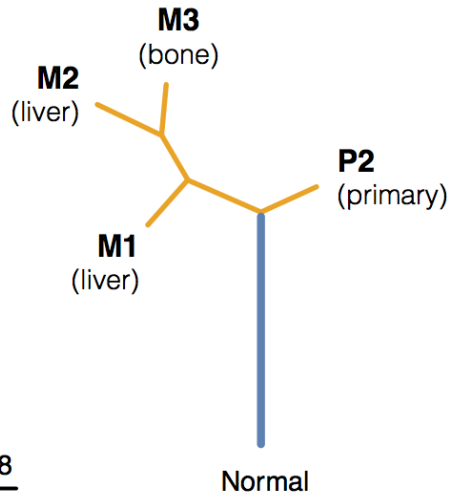


10.4

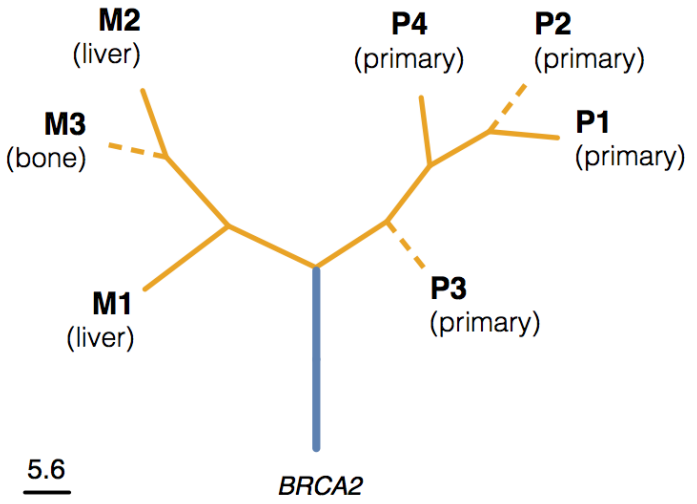
5/87



P1-4 (primary)
M1 (liver)
M2 (liver)
M3 (bone)

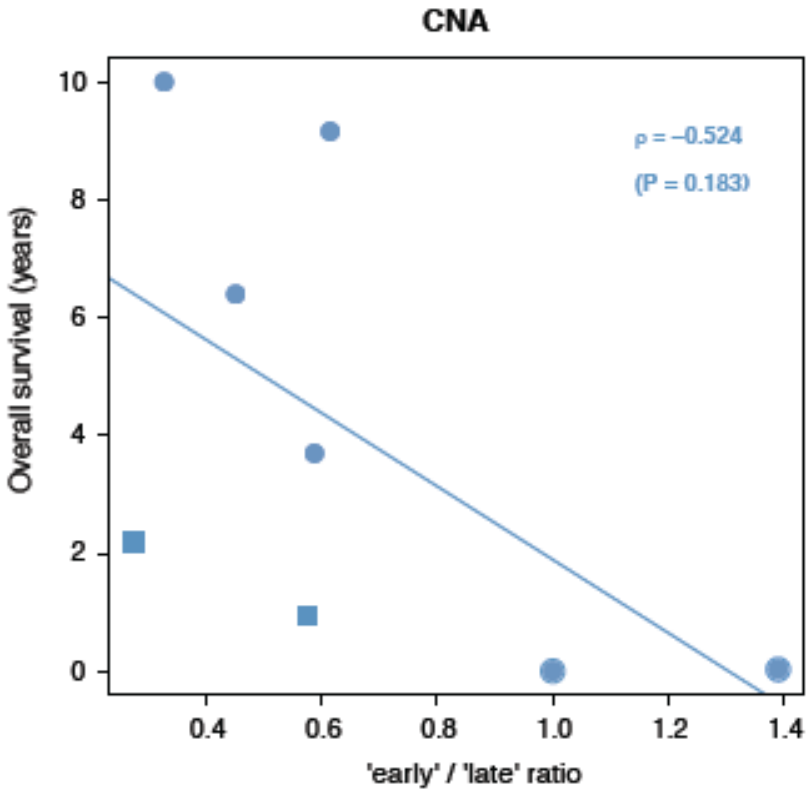


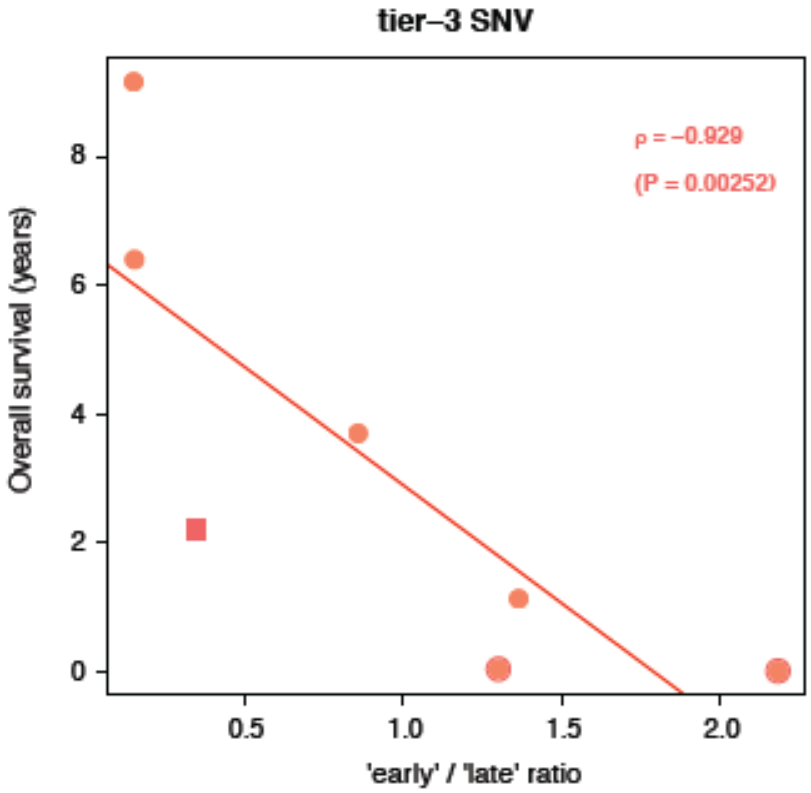
7.8



5.6

5. ‘Early’ to ‘late’ ratio





ARTICLES

Genome remodelling in a basal-like breast cancer metastasis and xenograft

Li Ding[1,2]*, Matthew J. Ellis[3,4]*, Shunqiang Li[3], David E. Larson[1], Ken Chen[1], John W. Wallis[1,2], Christopher C. Harris[1], Michael D. McLellan[1], Robert S. Fulton[1,2], Lucinda L. Fulton[1,2], Rachel M. Abbott[1], Jeremy Hoog[3], David J. Dooling[1,2], Daniel C. Koboldt[1], Heather Schmidt[1], Joelle Kalicki[1], Qunyuan Zhang[2,5], Lei Chen[1], Ling Lin[1], Michael C. Wendl[1,2], Joshua F. McMichael[1], Vincent J. Magrini[1,2], Lisa Cook[1], Sean D. McGrath[1], Tammi L. Vickery[1], Elizabeth Appelbaum[1], Katherine DeSchryver[3], Sherri Davies[3], Therese Guintoli[3], Li Lin[3], Robert Crowder[3], Yu Tao[6], Jacqueline E. Snider[3], Scott M. Smith[1], Adam F. Dukes[1], Gabriel E. Sanderson[1], Craig S. Pohl[1], Kim D. Delehaunty[1], Catrina C. Fronick[1], Kimberley A. Pape[1], Jerry S. Reed[1], Jody S. Robinson[1], Jennifer S. Hodges[1], William Schierding[1], Nathan D. Dees[1], Dong Shen[1], Devin P. Locke[1], Madeline E. Wiechert[1], James M. Eldred[1], Josh B. Peck[1], Benjamin J. Oberkfell[1], Justin T. Lolofie[1], Feiyu Du[1], Amy E. Hawkins[1], Michelle D. O'Laughlin[1], Kelly E. Bernard[1], Mark Cunningham[1], Glendoria Elliott[1], Mark S. Morton[1], Donna M. Thompson Jr[7], Jennifer L. Ivanovich[7], Paul J. Goodfellow[7], Charles M. Perou[8], George M. Weinstock[1], Rebecca Aft[7], Mark Watson[9], Timothy J. Ley[1,2,3,4], Richard K. Wilson[1,2,4] & Elaine R. Mardis[1,2,4]

Massively parallel DNA sequencing technologies provide an unprecedented ability to screen entire genomes for genetic changes associated with tumour progression. Here we describe the genomic analyses of four DNA samples from an African-American patient with basal-like breast cancer: peripheral blood, the primary tumour, a brain metastasis and a xenograft derived from the primary tumour. The metastasis contained two single-nucleotide mutations and a focal deletion not present in the primary tumour, and was significantly enriched for 20 shared mutations. The xenograft retained all primary tumour mutations and displayed a mutation enrichment pattern that resembled the metastasis. Two overlapping large deletions, encompassing *CTNNA1*, were present in all three tumour samples. The differential mutation frequencies and structural variation patterns in metastasis and xenograft compared with the primary tumour indicate that secondary tumours may arise from a minority of cells within the primary tumour.

Basal-like breast cancer is characterized by the absence of oestrogen receptor (ER) expression, the lack of *ERBB2* gene amplification, and a high mitotic index[1]. The consequent absence of approved targeted therapy options and poor response to standard chemotherapy often results in rapid metastatic disease. This subtype accounts for an elevated percentage of breast cancers in patients with African ancestry[1]. Clinical progress has been limited by a poor understanding of the genetic events responsible for this tumour subtype and by limited preclinical models to study the disease. Because basal-like breast cancer has a highly unstable genome[2], the question is whether the fatal metastatic process is driven by a few critical mutations after the tumour cells arrive at the distant site, or whether the primary tumour generates cells with a complete repertoire of somatic mutations required for metastatic growth. The rapid advancement of next-generation sequencing technologies allows comprehensive characterization of genomic changes, facilitating the comparison of multiple samples taken from the same patient to address the genetic basis for tumour progression and metastasis.

Case presentation and previous characterization of samples

A 44-year-old African-American woman was diagnosed with an ERBB2-negative and ER-negative inflammatory breast cancer. She was treated with neoadjuvant dose-dense chemotherapy[3], but significant residual tumour was present in the breast and axillary lymph nodes at mastectomy. This indicated chemotherapy resistance and she subsequently underwent radiation therapy. Eight months later she developed cerebellar metastasis and, despite resection, rapidly succumbed to widely disseminated disease. A transplantable human-in-mouse (HIM) xenograft tumour line was generated from a sample of her primary tumour biopsied before treatment[3]. The xenograft in the mammary fat pad was locally invasive and produced metastatic deposits in the lungs and ovaries. Informed consent for full genome sequencing was obtained and DNA samples were prepared from her peripheral blood, primary tumour, brain metastasis and an early passage xenograft (harvested 101 days after initial engrafting into the mouse host). Application of the PAM50 intrinsic subtype algorithm identified the primary tumour, brain metastasis and xenograft line as basal-like subtype, with high risk of relapse (ROR) scores[4].

Sequence coverage and mutation analysis

Using a paired-end sequencing strategy, we generated 130.7, 124.9, 111.8 and 149.2 billion base pairs of sequence data from genomic DNA derived from blood, primary tumour, brain metastasis and

[1]The Genome Center at Washington University, [2]Department of Genetics, [3]Department of Medicine, [4]Siteman Cancer Center, [5]Division of Statistical Genomics, [6]Division of Biostatistics, [7]Department of Surgery and the Young Women's Breast Cancer Program, [8]Department of Pathology and Immunology, Washington University School of Medicine, St Louis, Missouri 63108, USA. [9]Department of Genetics, Lineberger Cancer Center, University of North Carolina, Chapel Hill, North Carolina 27599, USA.
*These authors contributed equally to this work.

LETTERS

Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution

Sohrab P. Shah[1,2]*, Ryan D. Morin[3]*, Jaswinder Khattra[1], Leah Prentice[1], Trevor Pugh[3], Angela Burleigh[1], Allen Delaney[3], Karen Gelmon[4], Ryan Guliany[1], Janine Senz[2], Christian Steidl[2,5], Robert A. Holt[3], Steven Jones[3], Mark Sun[1], Gillian Leung[1], Richard Moore[3], Tesa Severson[3], Greg A. Taylor[3], Andrew E. Teschendorff[6], Kane Tse[1], Gulisa Turashvili[1], Richard Varhol[3], René L. Warren[3], Peter Watson[7], Yongjun Zhao[3], Carlos Caldas[6], David Huntsman[2,5], Martin Hirst[3], Marco A. Marra[3] & Samuel Aparicio[1,2,5]

Recent advances in next generation sequencing[1–4] have made it possible to precisely characterize all somatic coding mutations that occur during the development and progression of individual cancers. Here we used these approaches to sequence the genome (43-fold coverage) and transcriptomes of an oestrogen receptor-positive metastatic lobular breast cancer at depth. We found 32 somatic non-synonymous coding mutations present in the metastasis, and measured the frequency of these somatic mutations in DNA from the primary tumour of the same patient, which arose 9 years earlier. Five of the 32 mutations (in *ABI1*, *HAS3*, *SLC24A4*, *SNX13* and *ZNF521*) were present in the DNA of the primary tumour even at deep coverage. Six mutations (in *KIF1C*, *USP28*, *MYH8*, *MORC1*, *KIAA1468* and *RNASEH2A*) were present at lower frequencies (1–13%), 19 were not detected in the primary tumour, and two were undetermined. The combined analysis of genome and transcriptome data revealed two new RNA-editing events that recode the amino acid sequence of *SRP9* and *COG3*. Taken together, our data show that single nucleotide mutational heterogeneity can be a property of low or intermediate grade primary breast cancers and that significant evolution can occur with disease progression.

Lobular breast cancer is an oestrogen receptor-positive (ER+) cancer, also known as ESR1[+] subtype of breast cancer (approximately 10% of breast cancers). It is usually low- or intermediate-histological grade and can recur many years after initial diagnosis. To interrogate the genomic landscape of this class of tumour, we re-sequenced[1–4] the DNA from a metastatic lobular breast cancer specimen (89% tumour cellularity; Supplementary Fig. 1) at approximately 43.1-fold aligned, haploid reference genome coverage (12[illegible] paired-end sequence; Supplementary Fig. 1[illegible] tary Methods). Deep high-throughput transcriptome sequencing (RNA-seq)[5] performed on the same sample generated 160.9-million reads that could be aligned (Supplementary Table 1, see also Supplementary Fig. 2 and Supplementary Methods). The saturation of the genome (Table 1) and RNA-seq (Supplementary Table 1) libraries for single nucleotide variant (SNV) detection is discussed in Supplementary Information. The aligned (hg18) reads were used to identify (Supplementary Fig. 2) the presence of genomic aberrations, including SNVs (Supplementary Table 2), insertions/deletions (indels), gene fusions, translocations, inversions and copy number alterations (Supplementary Methods). We examined predicted coding indels and predicted inversions (coding or non-coding; Supplementary Methods); however, all of the events that were validated by Sanger re-sequencing were also present in the germ line (Supplementary Tables 3 and 4). None of the 12 predicted gene fusions [illegible] validated. We also computed the segmental copy number (Supplementary Methods and Supplementary Table 5a) from aligned reads, and revalidated high level amplicons by fluorescence *in situ* hybridization (FISH) (Supplementary Table 5b), revealing the presence of a new low-level amplicon in the *INSR* locus (Supplementary Fig. 3).

We identified [illegible] SNVs [illegible] using a Binomial [illegible] model, SNVMix (Supplementary Table 2, Methods and Supplementary Appendix 1). From the RNA-seq (WTSS-PE) and genome (WGSS-PE) libraries we predicted 1,456 new coding non-synonymous SNVMix variants (Supplementary Table 2). After the removal of pseudogene and HLA sequences (1,178 positions remaining) and after primer design, we re-sequenced (Sanger amplicons) 1,120 non-synonymous coding SNV positions in the tumour DNA and normal lymphocyte DNA. Some 437 positions (268 unique to WGSS-PE, 15 unique to WTSS-PE, and 154 in common) were confirmed as non-synonymous coding variants. Of these, 405 were new

[illegible] | Summary of sequence [illegible] coverage

	WGSS-PE	WTSS-PE
Total number of reads	2,922,713,774	182,532,650
Total nucleotides (Gb)	140.991	7.108
Number of aligned reads	2,502,465,226	160,819,484
[illegible]	85.718	6.266
[illegible]	0.21	0.013
[illegible]	2.14	NA
regions)		
Canonically aligned reads	2,294,067,534	109,093,616
Exons covered	93.5 at >10 reads; 95.7 at >5 reads	82,200 at 10 reads (see also Supplementary Table 1)
Reads aligned canonically (%)	78.49	67.79
Unaligned reads	420,248,548	21,613,166
Mean read length (bp)	48.24	38.94

The WGSS-PE column shows the genome paired-end read coverage for DNA from the metastatic pleural effusion sample. The WTSS-PE column shows coverage for the complementary DNA reads from the matched transcriptome libraries of the metastatic pleural effusion. Coverage of exon bases in the reference genome (hg18) is shown at 5or more reads per position, and 10 or more reads per position for the metastatic genome. bp, base pairs; NA, not applicable.

[1]Molecular Oncology, [2]Centre for Translational and Applied Genomics, [3]Michael Smith Genome Sciences Centre, BC Cancer Agency, 675 West 10th Avenue, Vancouver V5Z 1L3, Canada. [4]Medical Oncology, BC Cancer Agency, 600 West 10th Avenue, Vancouver V5Z 1L3, Canada. [5]Department of Pathology, University of British Columbia, G227-2211 Wesbrook Mall, British Columbia, Vancouver V6T 2B5, Canada. [6]Cancer Research UK, Cambridge Research Institute, Li Ka Shing Centre, Robinson Way, Cambridge CB2 0RE, UK. [7]Deeley Research Centre, BC Cancer Agency, Victoria V8R 6V5, Canada.
*These authors contributed equally to this work.

TNBC

Metastatic <1 year

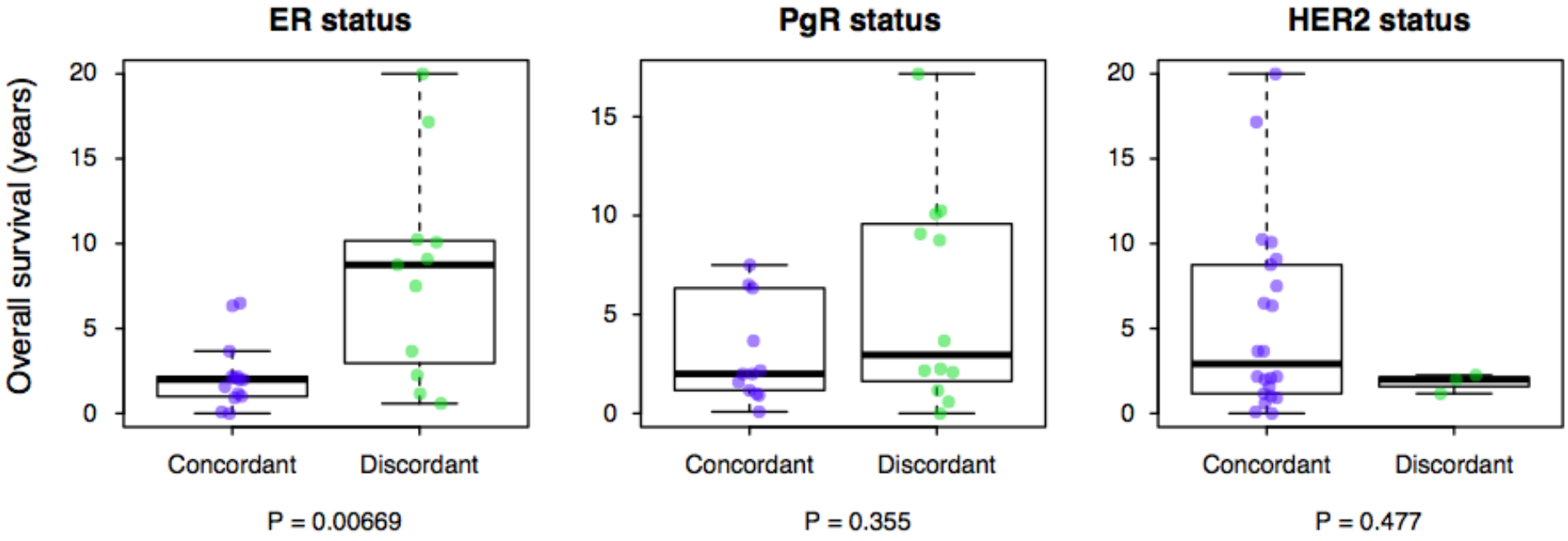
96% of common mutations

ER+ LC

Metastatic >9 years

34% of common mutations

6. Discordances ER, PR & HER2



Discordances ER, PgR and HER2

ELSEVIER

Critical Reviews in Oncology/Hematology 84 (2012) 301–313

CRITICAL REVIEWS IN Oncology Hematology Incorporating Geriatric Oncology

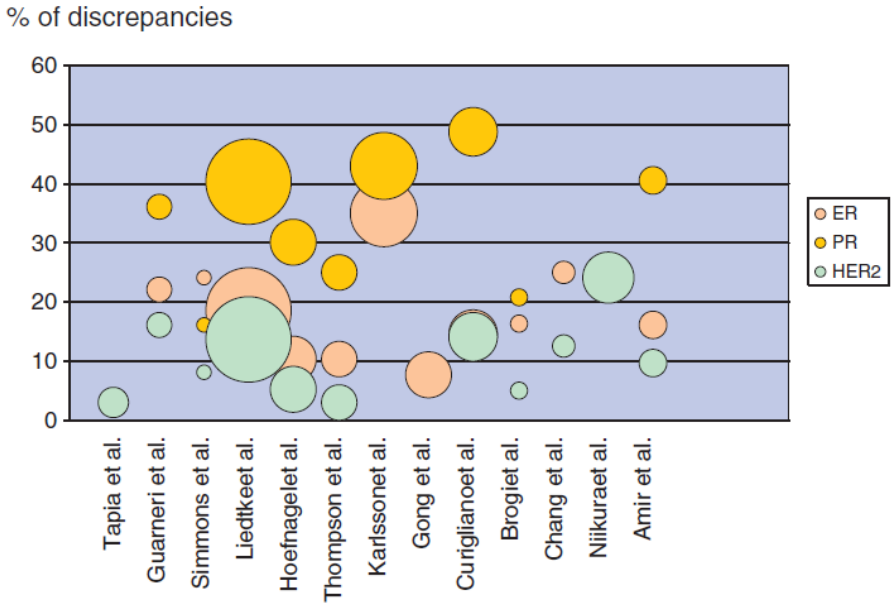
www.elsevier.com/locate/critrevonc

Discrepancies between primary tumor and metastasis: A literature review on clinically established biomarkers

Stéphane Vignot [a,*], Benjamin Besse [b], Fabrice André [b], Jean-Philippe Spano [a], Jean-Charles Soria [b]

[a] Service d'Oncologie Médicale, Groupe Hospitalier Pitié Salpêtrière, Paris, France
[b] Unité INSERM U 981, Université Paris Sud, Département de Médecine, Institut Gustave Roussy, Villejuif, France

Received 6 February 2012; received in revised form 1 May 2012; accepted 16 May 2012



Metastatic progression of breast cancer: insights from 50 years of autopsies

Margaret C. Cummings,[1,2,3#*] Peter T. Simpson,[1#] Lynne E. Reid,[1] Janani Jayanthan,[1] Joanna Skerman,[2] Sarah Song,[4] Amy E. McCart Reed,[1] Jamie R. Kutasovic,[1] Adrienne L. Morey,[5] Louise Marquart,[6] Peter O'Rourke,[6] Sunil R. Lakhani[1,2,3]

Biomarker	Proportion of patients (%) with concordant staining of all metastases
ER	31/55 (56·4%)
PgR	37/55 (67·3%)
HER2	53/55 (96·4%)
Ki67	39/55 (70·9 %)
p53	40/55 (72·7%)
CK AE1/AE3	53/55 (96·4%)
EGFR	49/55 (89·1%)
c-kit	54/55 (98·2%)

Messages regarding breast cancer progression

- Autopsies → reconstruction of breast cancer progression;
- Accurate reconstruction needs combination of mutation and copy number data;
- Different progression trajectories are possible in breast cancer (parallel and in cascade);
- Metastases can differ from their primary tumor, especially if the patients developed their metastases many years after initial diagnosis.

Limitations of the study

- Only two time points (diagnosis and death) were investigated;
- Heterogeneity of primary tumor not formally investigated;
- Heterogeneity of treatment received over the course of the disease;
- All agressive cancers (what about more indolent ones?);
- Only genomic changes were investigated;
- Relatively small nr of patients.

Bordet Institute - ULB

David Brown
Françoise Rothé
Pierre-Yves Adnet
Samira Majjaj
Gabriele Zoppoli
Christos Sotiriou

Hughes Duvillier
Martine Piccart
Denis Larsimont

Semmelweis University

Borbala Szekely
Marcell Szaz
Magdolna Dank
Gyöngyvér Szentmártoni
Nora Udvarhelyi
Zsofia I. Nagy
Zsofia Farago
Anna-Maria Tokes
Janina Kulka

Yale University

Lajos Puzstai

VIB- KUL

Dominiek Smeets
Diether Lambrechts

and…the patients & their family!

fnrs FREEDOM TO RESEARCH

FONDATION MEDIC

Les Amis de l'Institut Bordet

IRSIB