Clonal Evolution of Childhood Cancers

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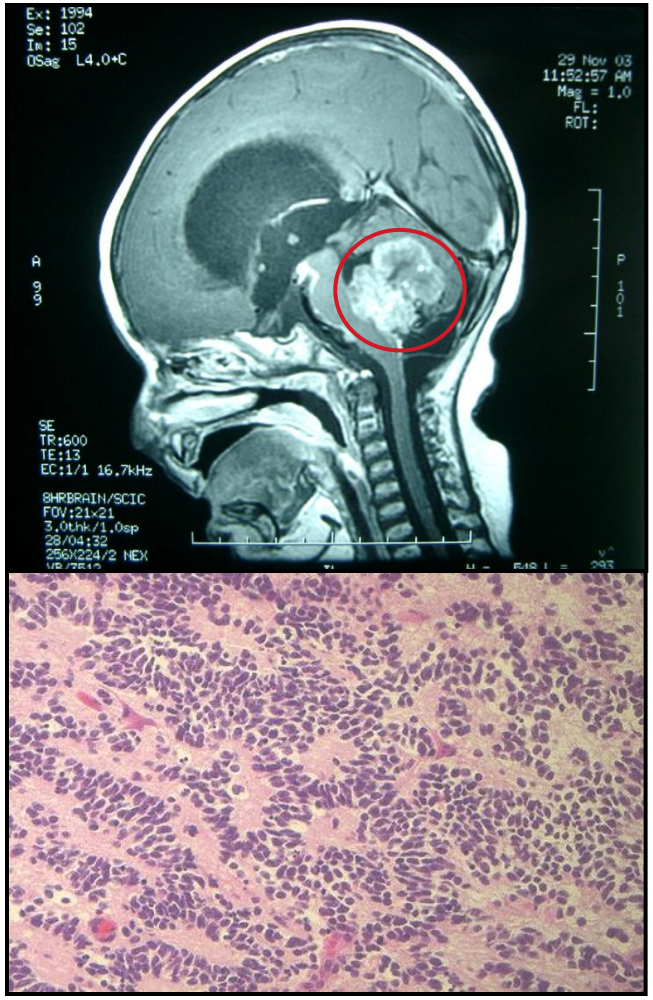
SickKids

MAGIC

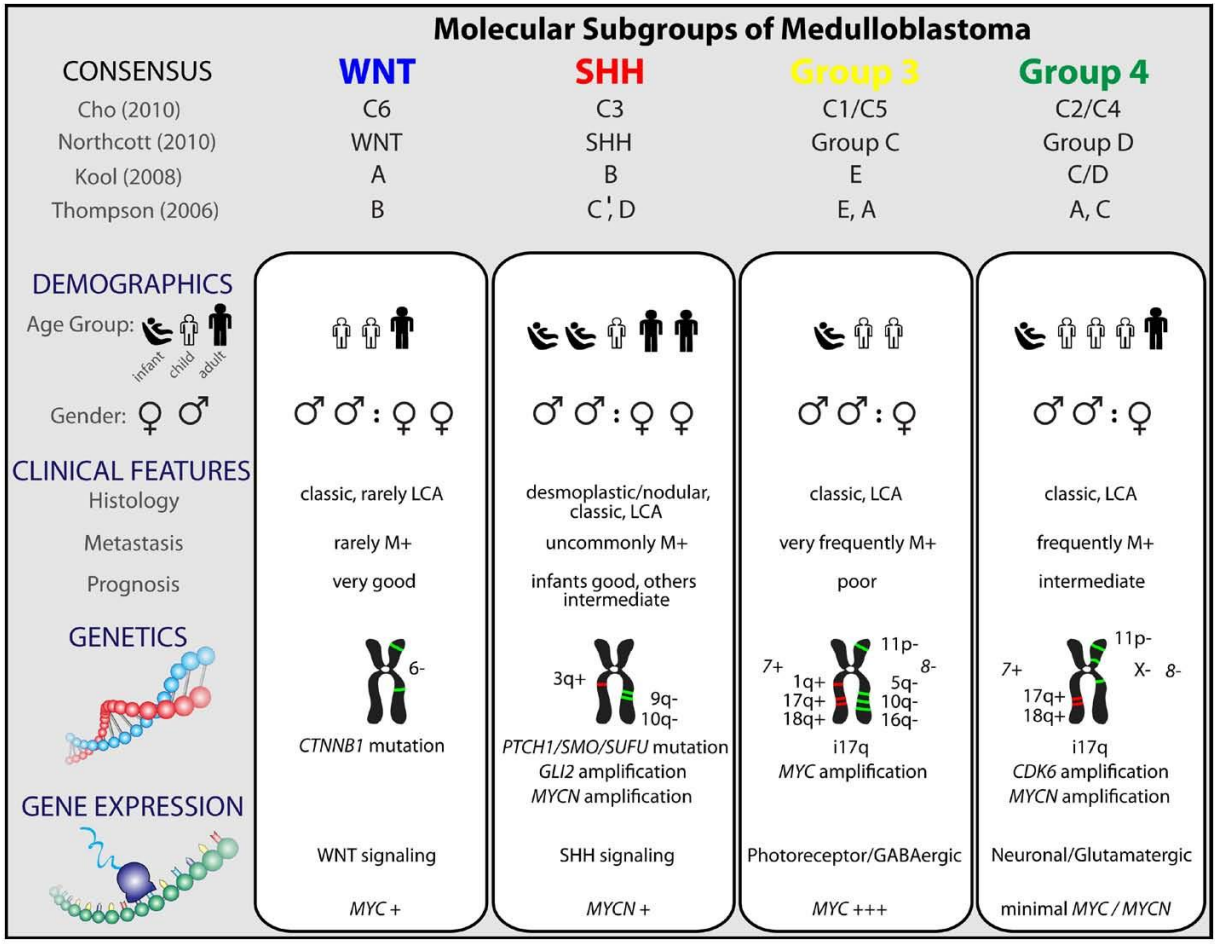
Medulloblastoma

- As a group, most common malignant childhood brain tumor
- Current therapy is maximal safe surgery, followed by radiation and non-specific cytotoxic chemotherapy, possibly with BMT
- Patients with high risk disease get more therapy and more toxicity
- No targeted agents as part of routine clinical care anywhere in the world

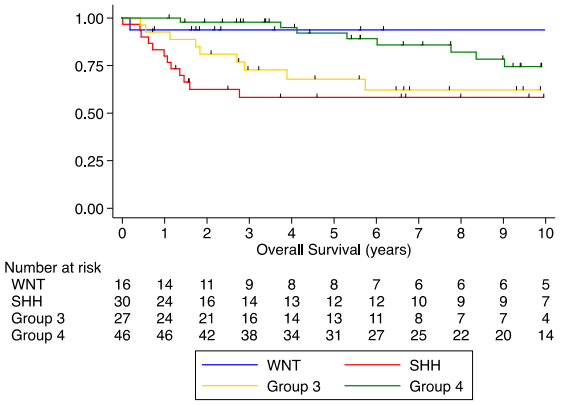


Molecular subgroups of medulloblastoma: the current consensus

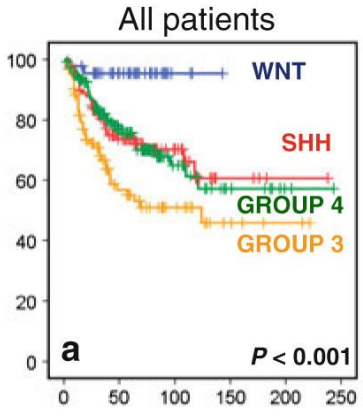
Michael D. Taylor · Paul A. Northcott · Andrey Korshunov · Marc Remke · Yoon-Jae Cho · Steven C. Clifford · Charles G. Eberhart · D. Williams Parsons · Stefan Rutkowski · Amar Gajjar · David W. Ellison · Peter Lichter · Richard J. Gilbertson · Scott L. Pomeroy · Marcel Kool · Stefan M. Pfister



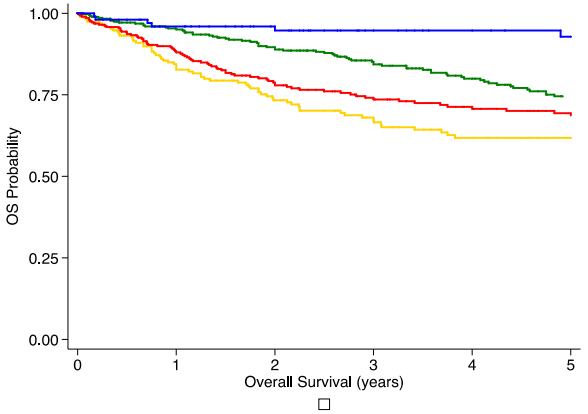
Subgroup is prognostic everywhere



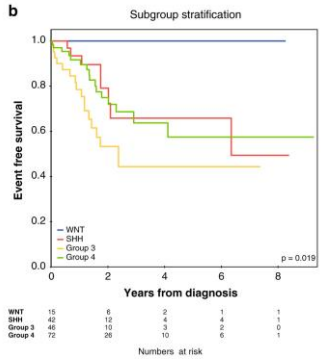
Sick Kids, Toronto



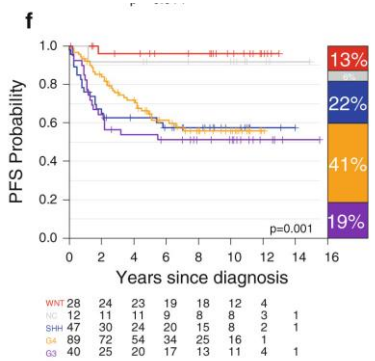
Kool, Meta-analysis



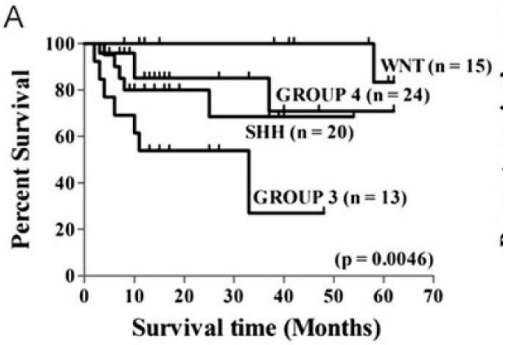
MAGIC cohort



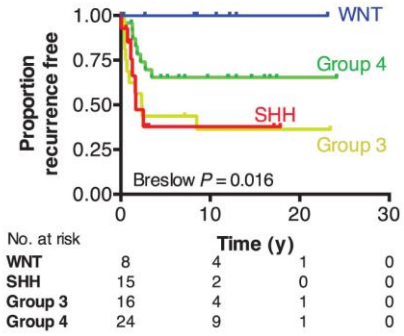
HIT 2000, Germany



United Kingdom



India

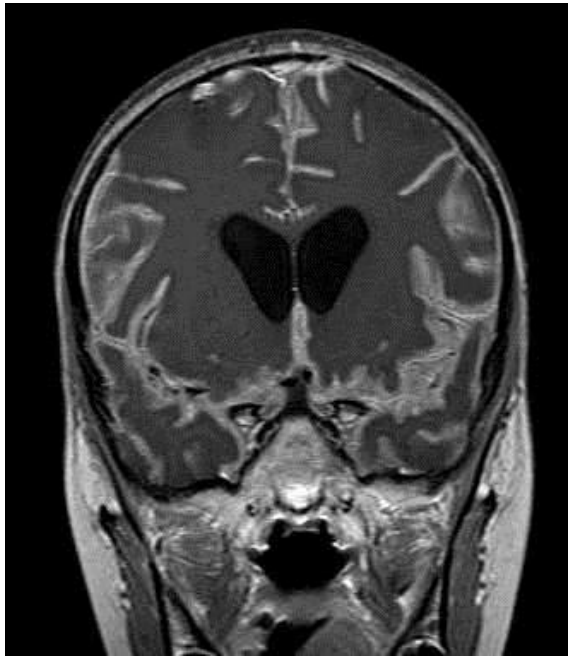


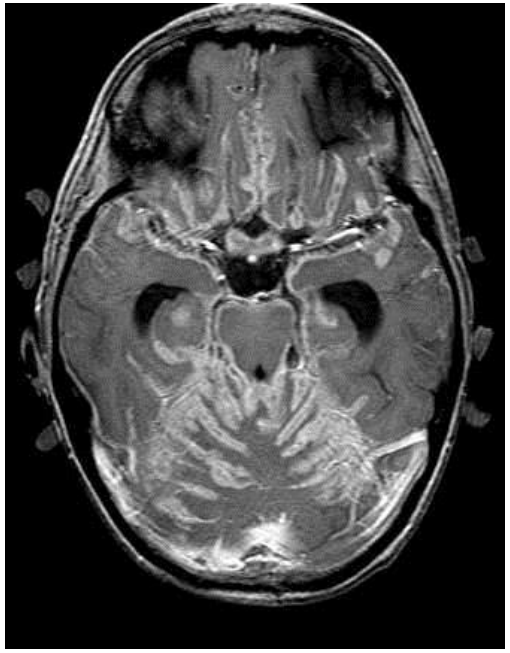
Vancouver, British Columbia

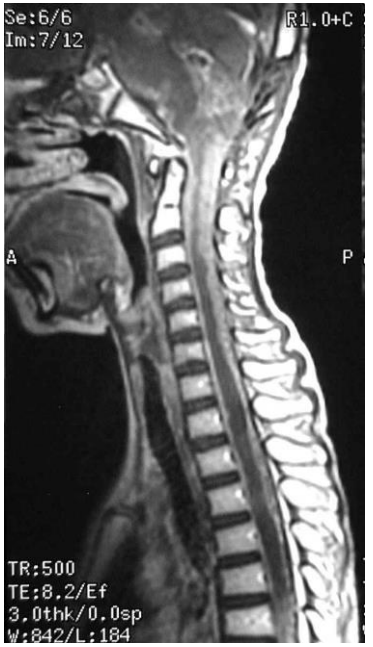
LETTER

doi:10.1038/nature10825

Clonal selection drives genetic divergence of metastatic medulloblastoma

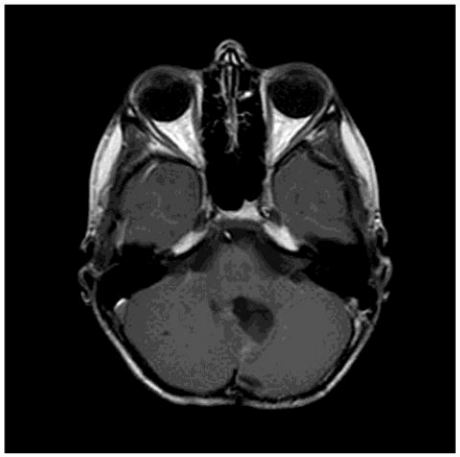
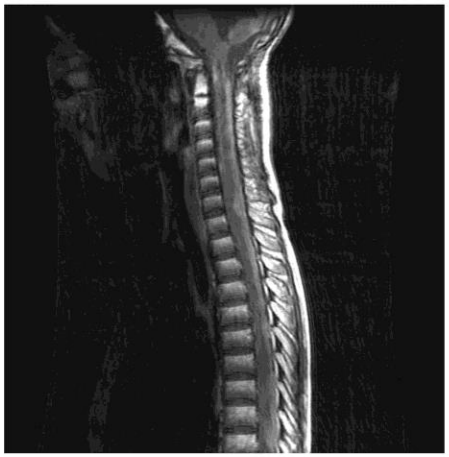
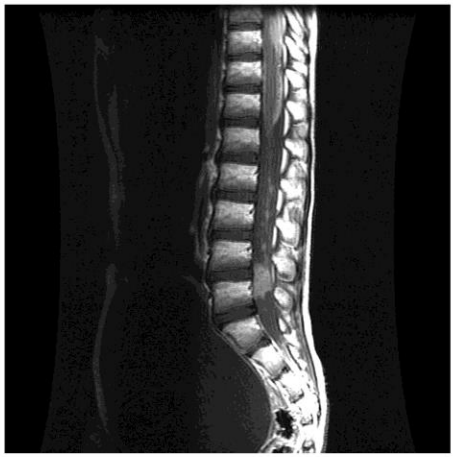




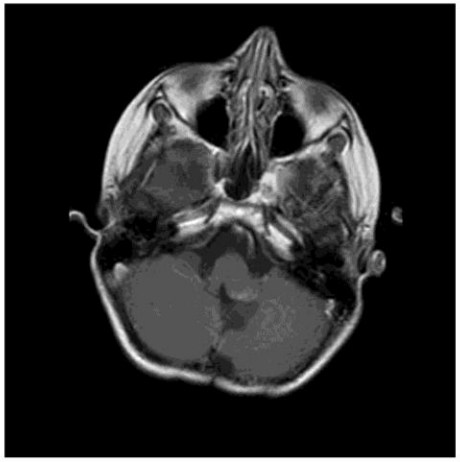
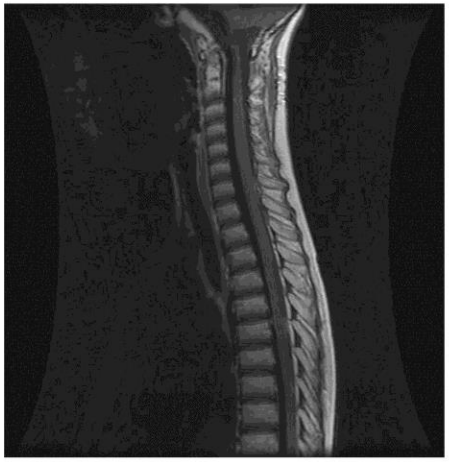
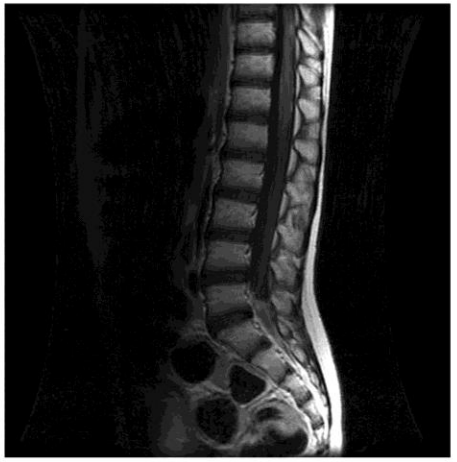


- Metastases present in 30% of patients at presentation
- Very poor prognostic marker
- Largest cause of morbidity in children with medulloblastoma (craniospinal rads)
- Present in most children at time of relapse
- Major cause of death

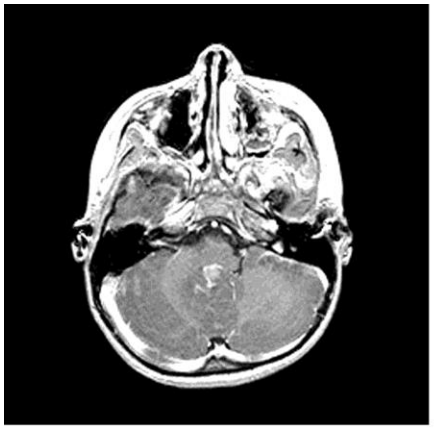
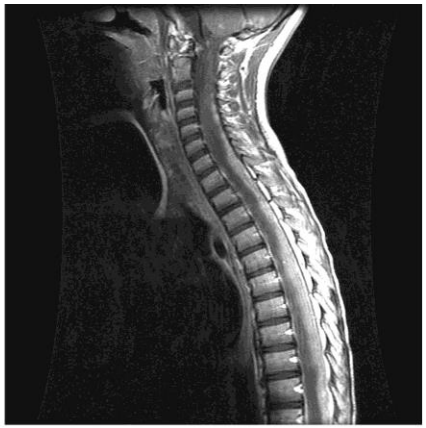
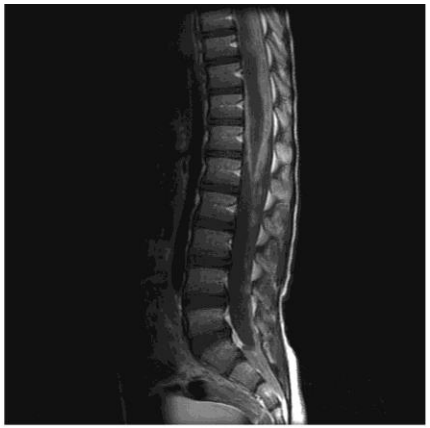
Metastases Respond, Primary Does Not

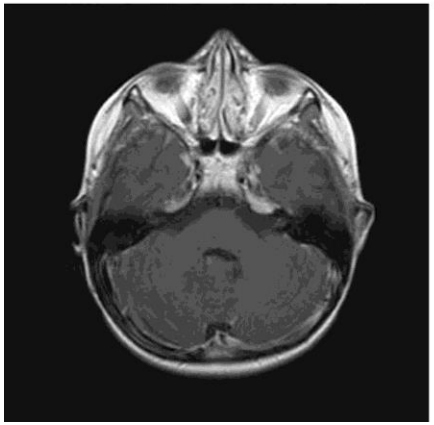
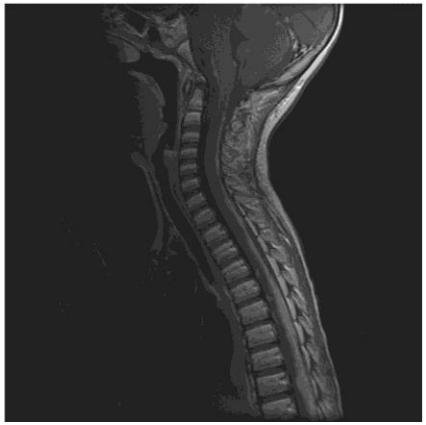
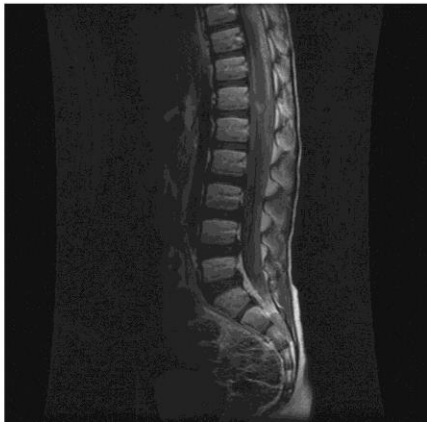
Chemotherapy

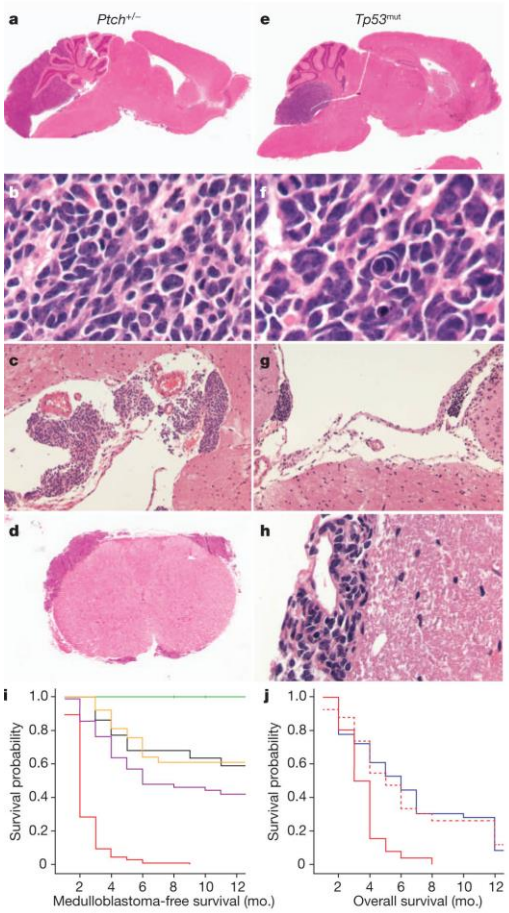
Primary Responds, Metastases Do Not

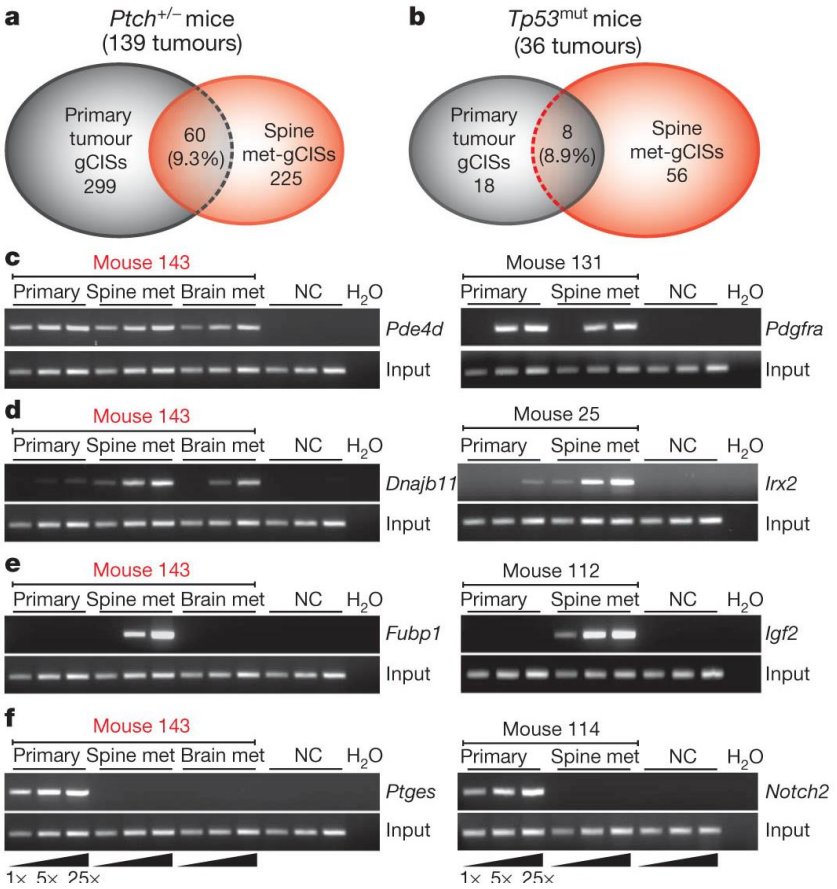




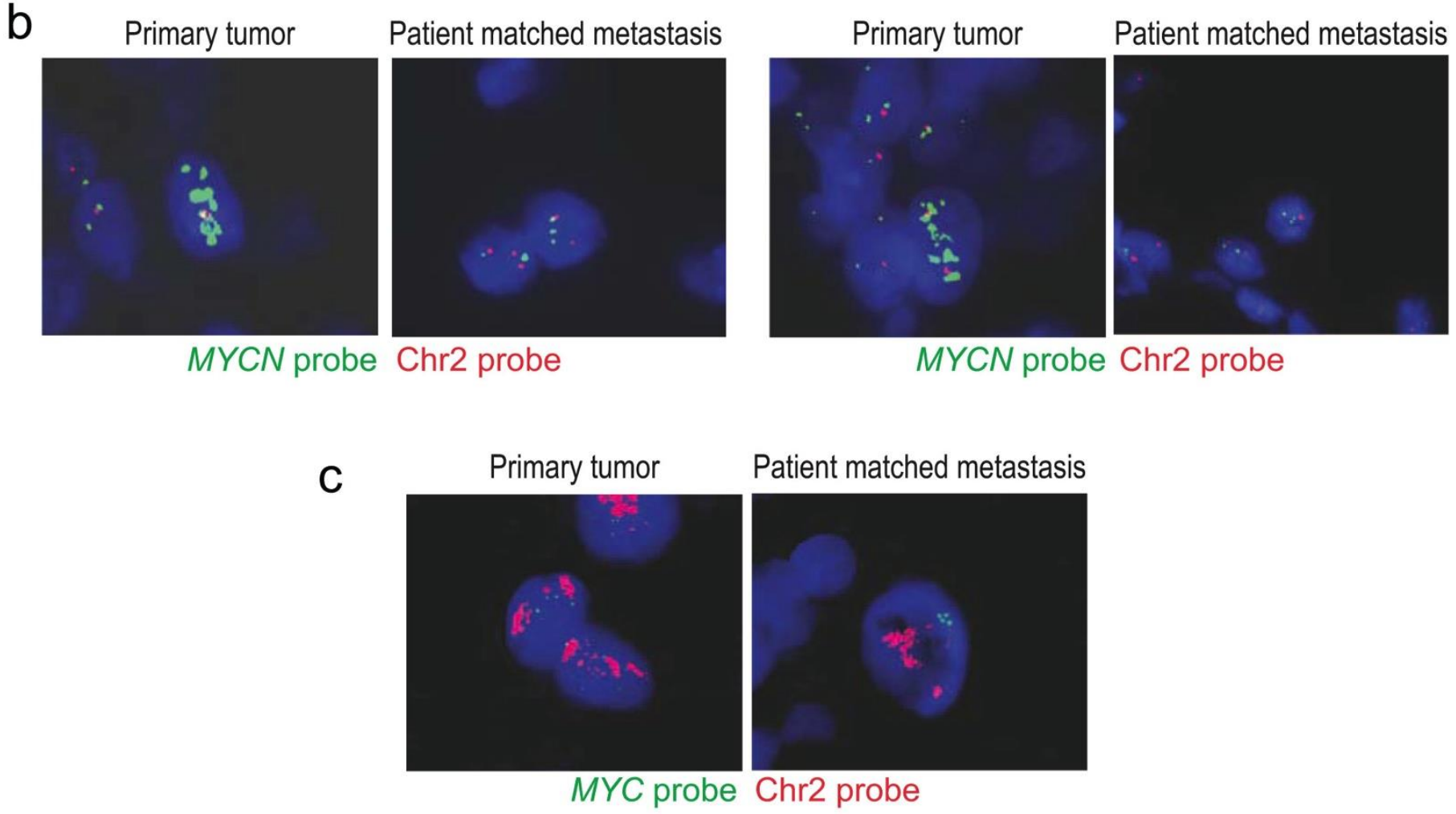
Chemotherapy

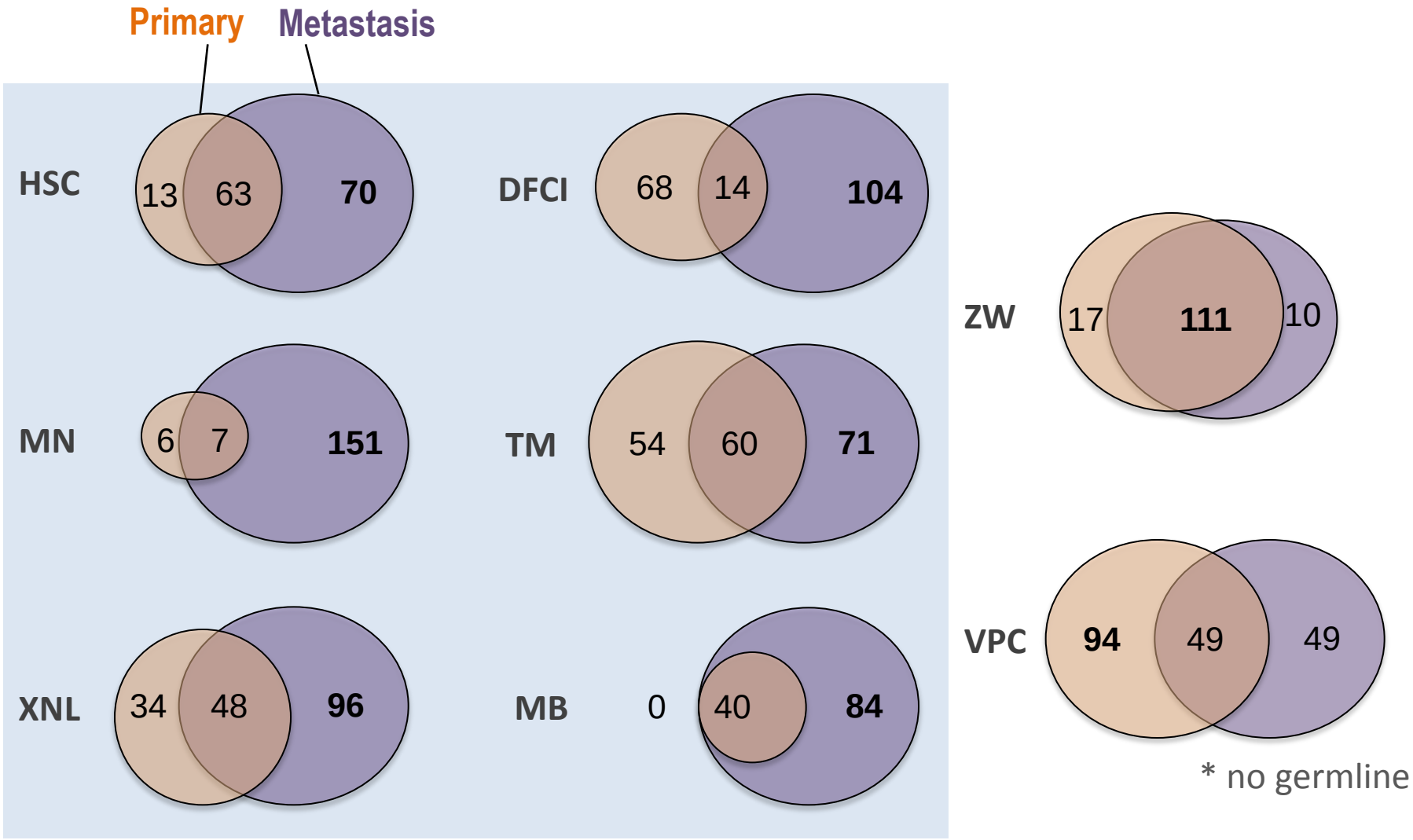
Leptomeningeal Metastases Arise Through Clonal Selection in a Transposon Driven Mouse Model of Medulloblastoma





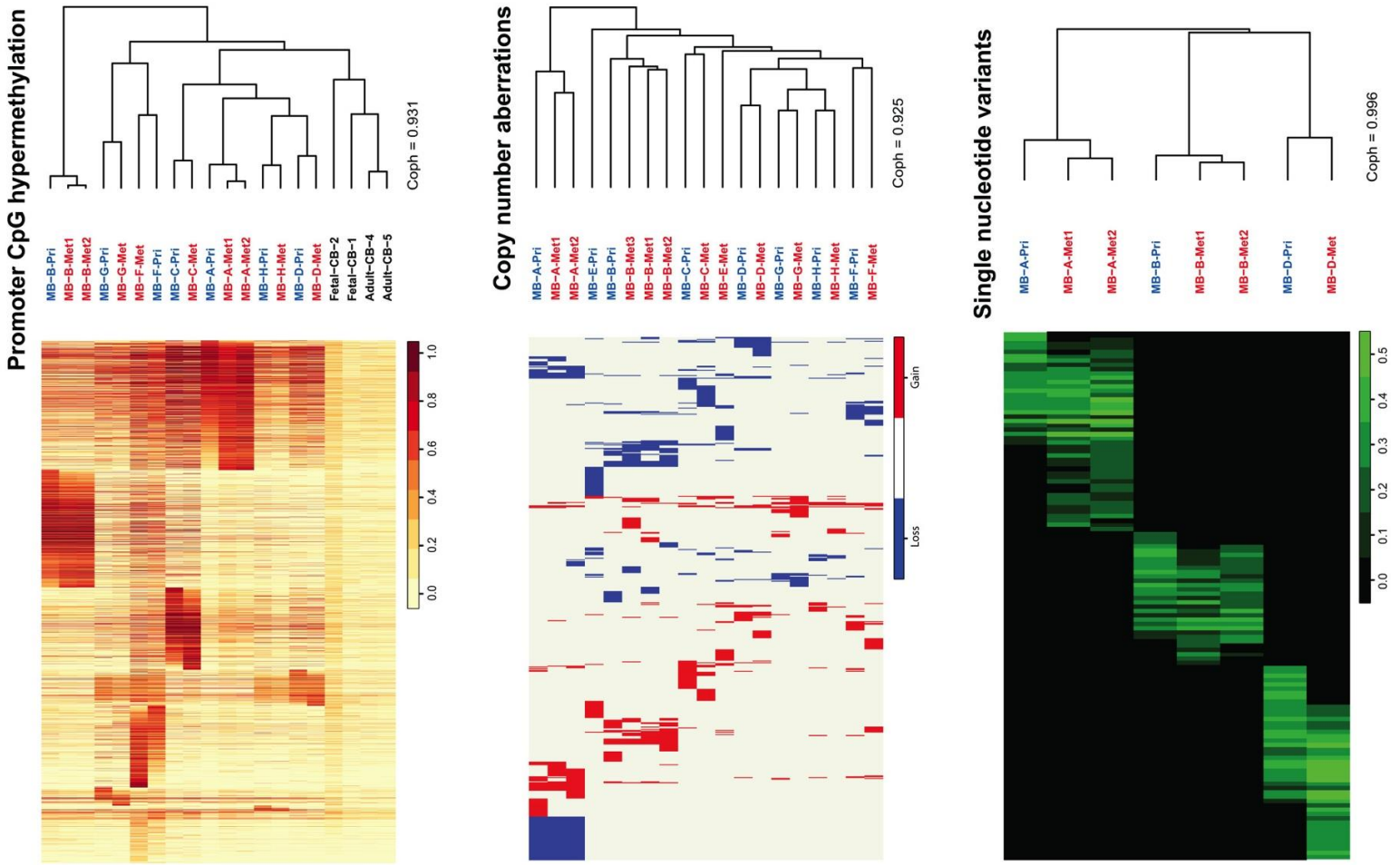

b
Primary tumor
Patient matched metastasis
Primary tumor
Patient matched metastasis
MYCN probe
Chr2 probe
MYCN probe
Chr2 probe
c
Primary tumor
Patient matched metastasis
MYC probe
Chr2 probe

SNVs are typically Metastasis-specific



* no germline

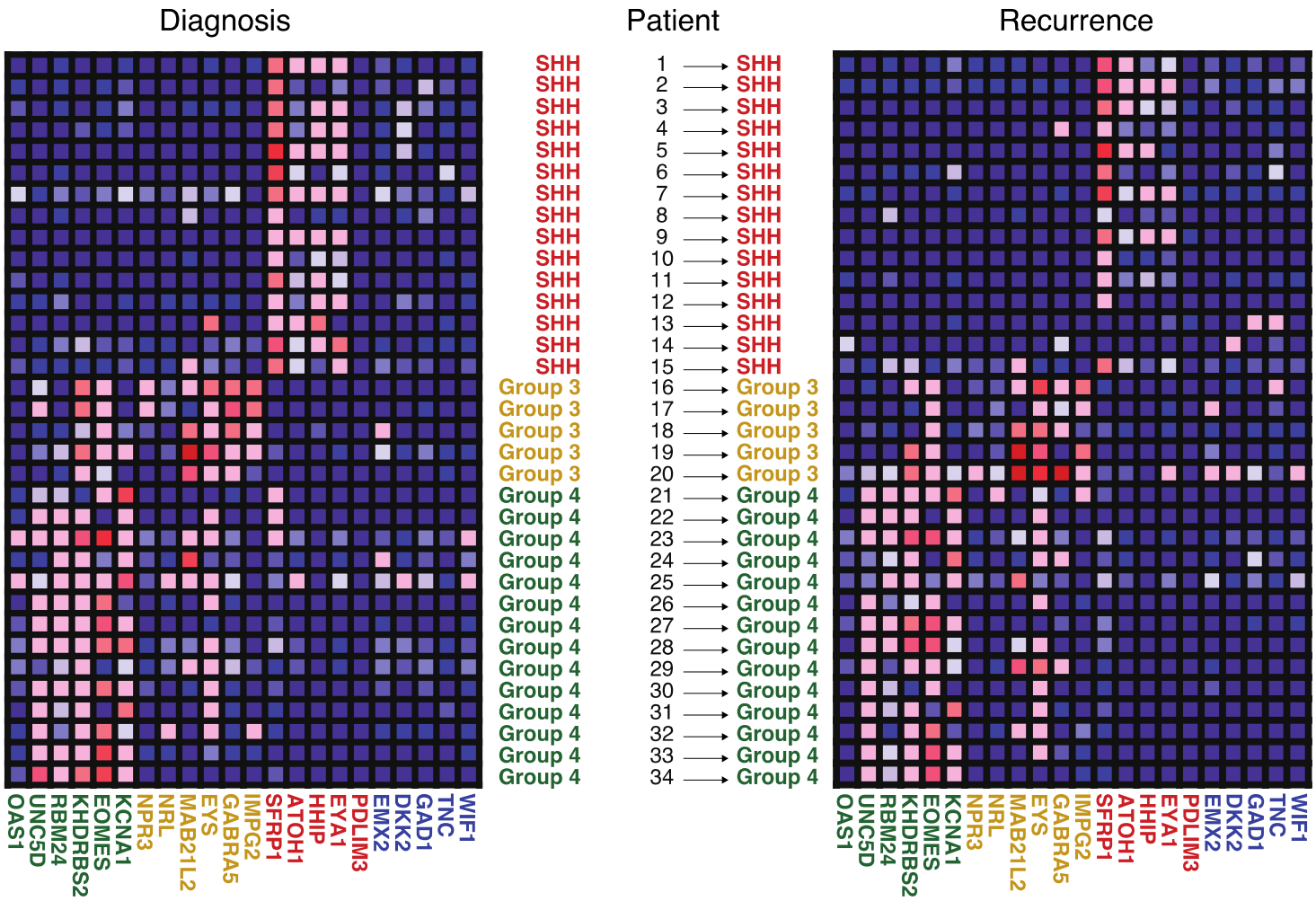
Every child with medulloblastoma really has two diseases – the primary compartment and the metastatic compartment



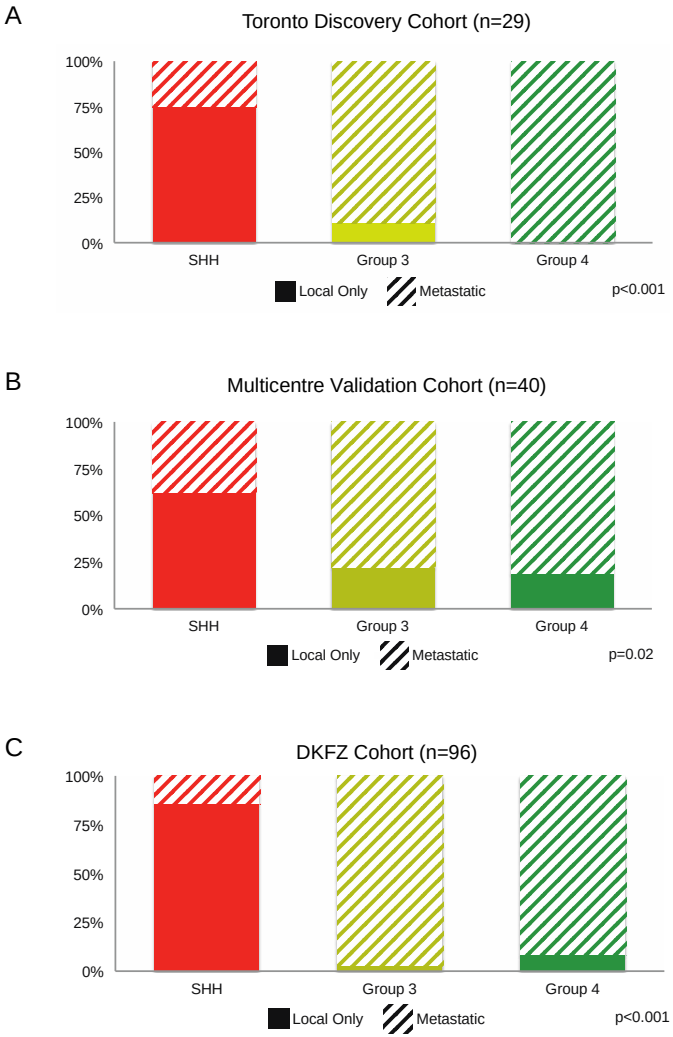
Conclusions: Metastases

- Metastases are very genetically divergent from their matched primary tumor
- Metastases likely arise through clonal selection of a pre-existing minor clone of the primary tumor
- Targeted therapies developed against primary medulloblastoma are unlikely to be effective against metastases as their genetic repertoire is so very different.

Subgroup affiliation is stable at recurrence



Pattern of Recurrence is Subgroup Specific



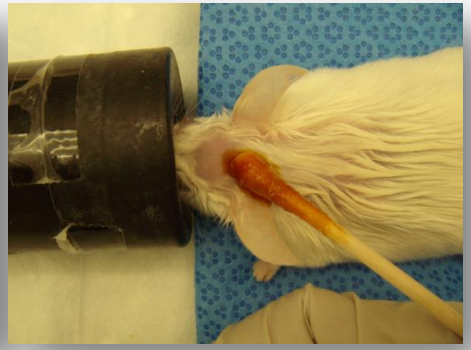
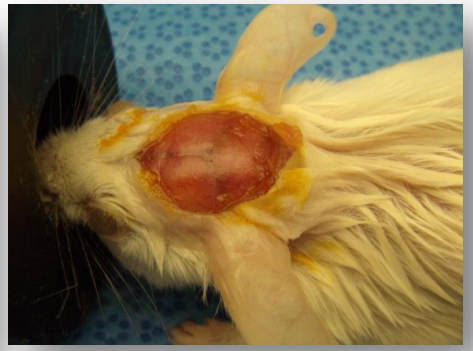
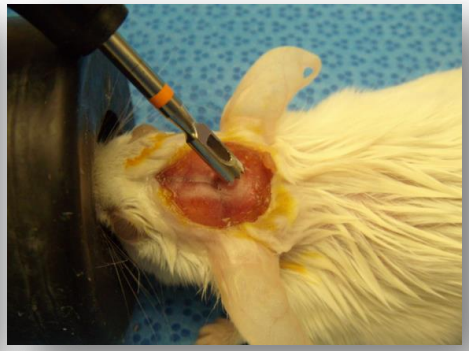
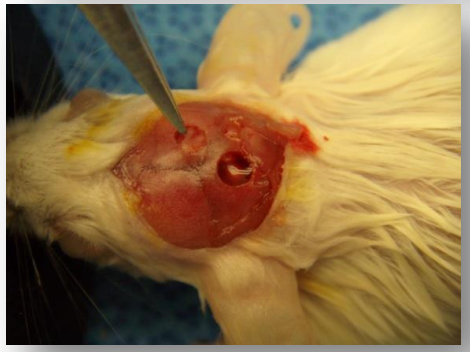
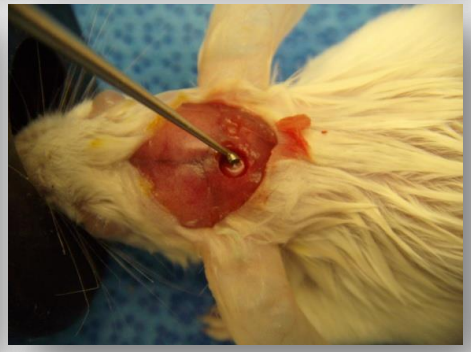
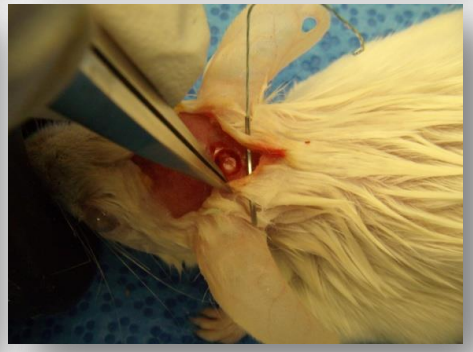
A very big assumption……

- The vast majority of molecular biology research on medulloblastoma is based on studies of primary, untreated tumors

- Molecular targets discovered in the untreated primary are assumed to still be present in the highly treated recurrence.

- IF the target is no longer present, therapy will undoubtedly fail.

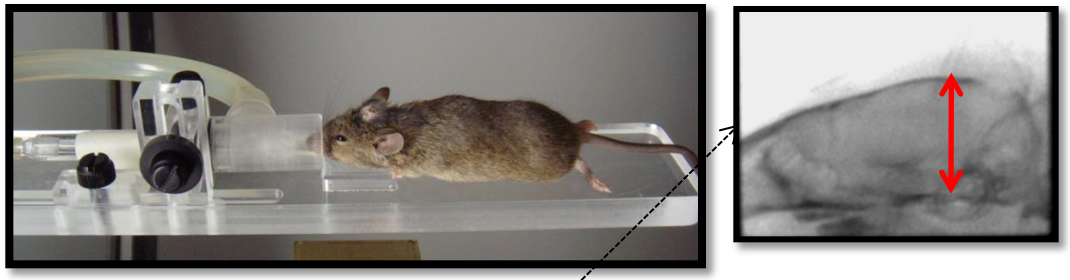
A survival surgery procedure to cure MB affected mice.

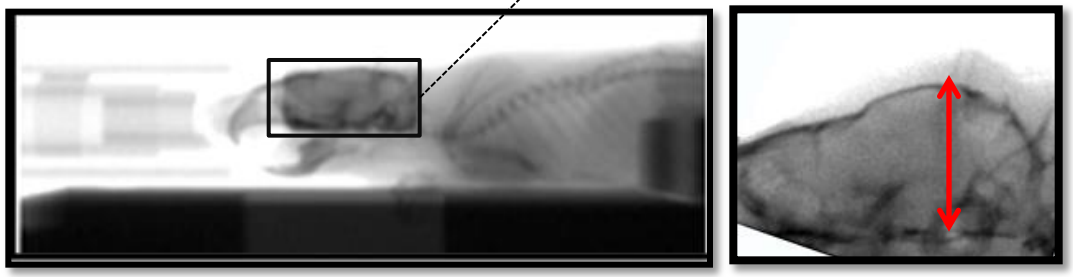
Post surgical CT guided craniospinal irradiation of tumor bearing mice.

CT guided irradiator

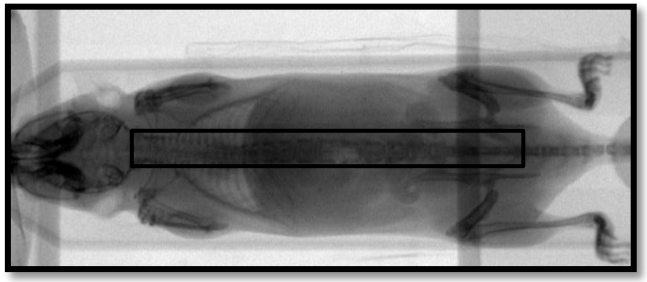
Brain irradiation setup



Brain collimator field



Spinal cord irradiation setup

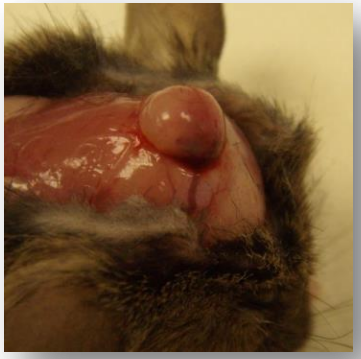


Mon	Tue	Wed	Thur	Friday
2Gy		2Gy		2Gy

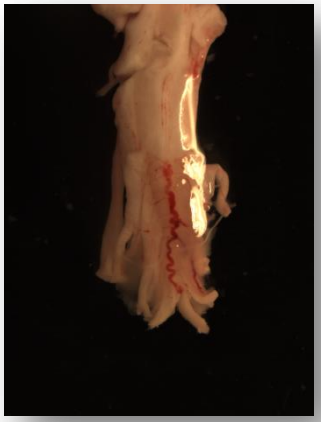
Mon	Tue	Wed	Thur	Friday
2Gy	2Gy	2Gy	2Gy	2Gy

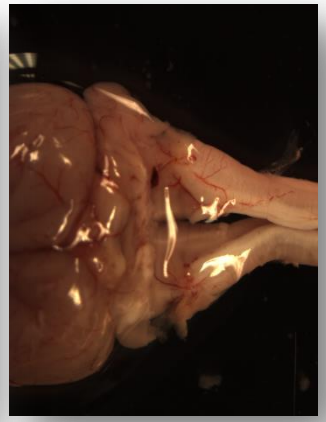
Mon	Tue	Wed	Thur	Friday
2Gy	2Gy	2Gy	2Gy	2Gy

Mon	Tue	Wed	Thur	Friday
2Gy	2Gy	2Gy	2Gy	2Gy



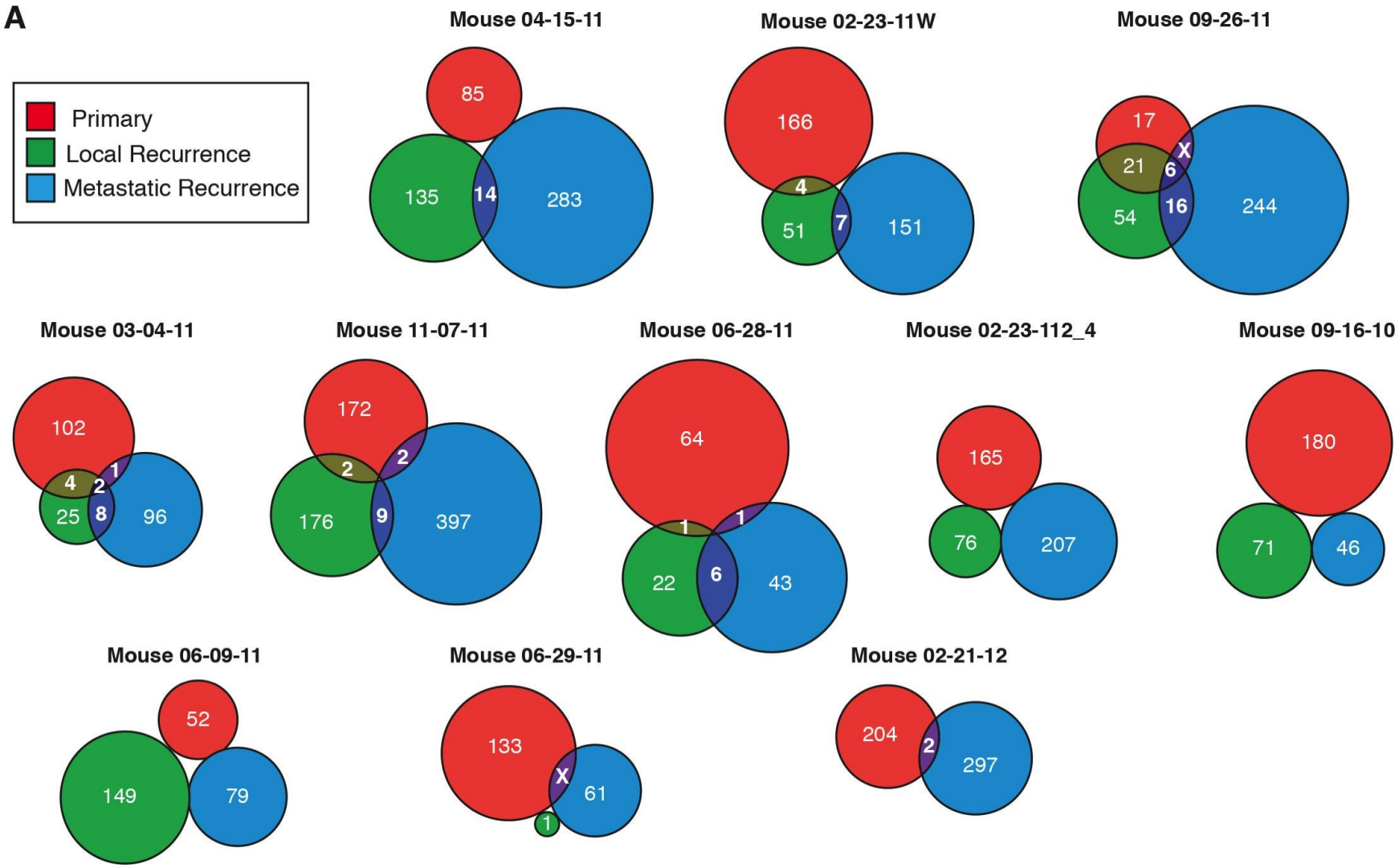
Local recurrence





Recurrence in the spine

Cured mouse


A
Primary
Local Recurrence
Metastatic Recurrence
Mouse 04-15-11
85
135
14
283
Mouse 02-23-11W
166
4
51
7
151
Mouse 09-26-11
17
X
21
6
54
16
244
Mouse 03-04-11
102
4
2
1
25
8
96
Mouse 11-07-11
172
2
2
176
9
397
Mouse 06-28-11
64
1
1
22
6
43
Mouse 02-23-112_4
165
76
207
Mouse 09-16-10
180
71
46
Mouse 06-09-11
52
149
79
Mouse 06-29-11
133
X
61
1
Mouse 02-21-12
204
2
297

A

Primary Tumors (N=11)

Gene	q-value	Mice #
Atxn7l1	6.24E-14	2
Baz1a	3.38E-11	2
Bdp1	2.08E-11	2
C80913	7.85E-17	2
Col4a3bp	3.87E-09	2
Crebbp	9.57E-20	3
Efcab6	2.39E-08	2
F730043M19Rik	1.36E-32	2
Fgfr3	5.44E-72	2
Gramd1b	3.75E-09	2
Mrpl3	1.92E-35	2
Nup98	6.26E-12	2
Pcx	2.69E-12	2
Pde8b	1.58E-05	2
Prmt7	4.85E-27	2
Setbp1	1.27E-07	3
Sntg2	1.23E-05	2
Snx27	1.29E-12	2
Sprr2h	2.09E-69	2
Strn3	1.41E-10	2
Tead1	2.01E-14	3
Zfp423	1.13E-05	2
Zfp644	3.10E-12	2

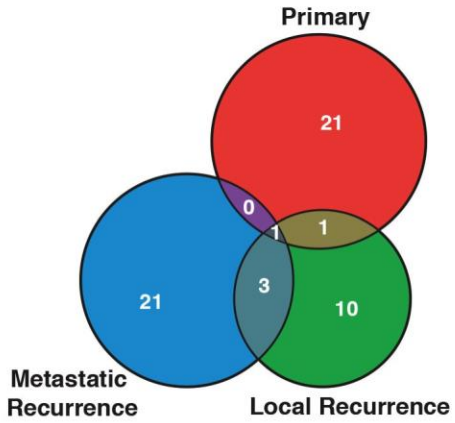
Metastatic Recurrence (N=11)

Gene	q-value	Mice #
1700069L16Rik	5.81E-29	2
Adamts20	4.21E-05	2
Arid1b	1.20E-05	3
Atp2b2	1.10E-08	3
Chchd3	6.61E-06	3
Chst10	8.49E-23	2
Crebbp	4.94E-13	3
Cul2	1.22E-11	2
Dkk3	2.23E-21	2
Exoc6	3.10E-05	2
Fnbp1	9.83E-09	2
Gak	6.67E-12	2
Gpatch1	1.42E-18	2
Megf10	8.06E-11	3
Mllt1	2.85E-21	2
Myod1	9.81E-81	2
Rftn1	7.00E-05	2
Slc16a7	1.76E-06	2
Sympk	1.46E-29	2
Tcf4	8.14E-05	3
Tmem154	1.53E-21	2
Trp53	>2E-308	4
Usp33	1.98E-14	2
Vac14	2.73E-10	2
Wwp2	5.51E-08	2

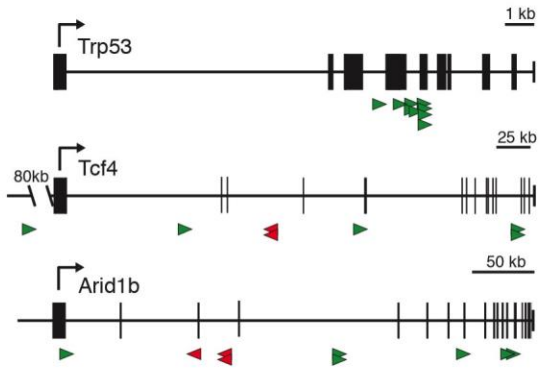
Local Recurrence (N=10)

Gene	q-value	Mice #
Arid1b	1.47E-07	2
Cbfa2t2	4.77E-21	2
Crebbp	6.23E-17	2
Dgkg	8.18E-15	2
Eras	>2E-308	2
Foxr2	2.15E-72	2
Jazf1	5.18E-08	2
Lnpep	1.85E-18	2
Ptch1	9.41E-49	2
Pten	1.61E-26	2
Strn3	7.24E-21	2
Tcf4	1.17E-15	3
Trim33	6.44E-22	2
Trp53	>2E-308	3
Xpo1	4.71E-43	2

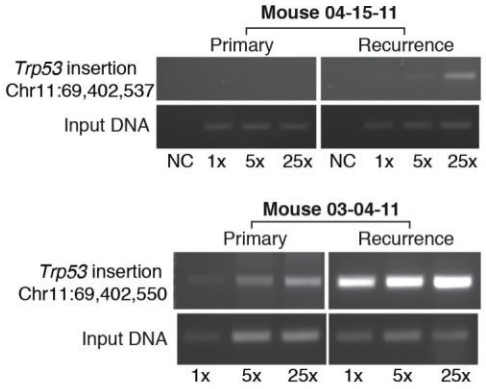
B



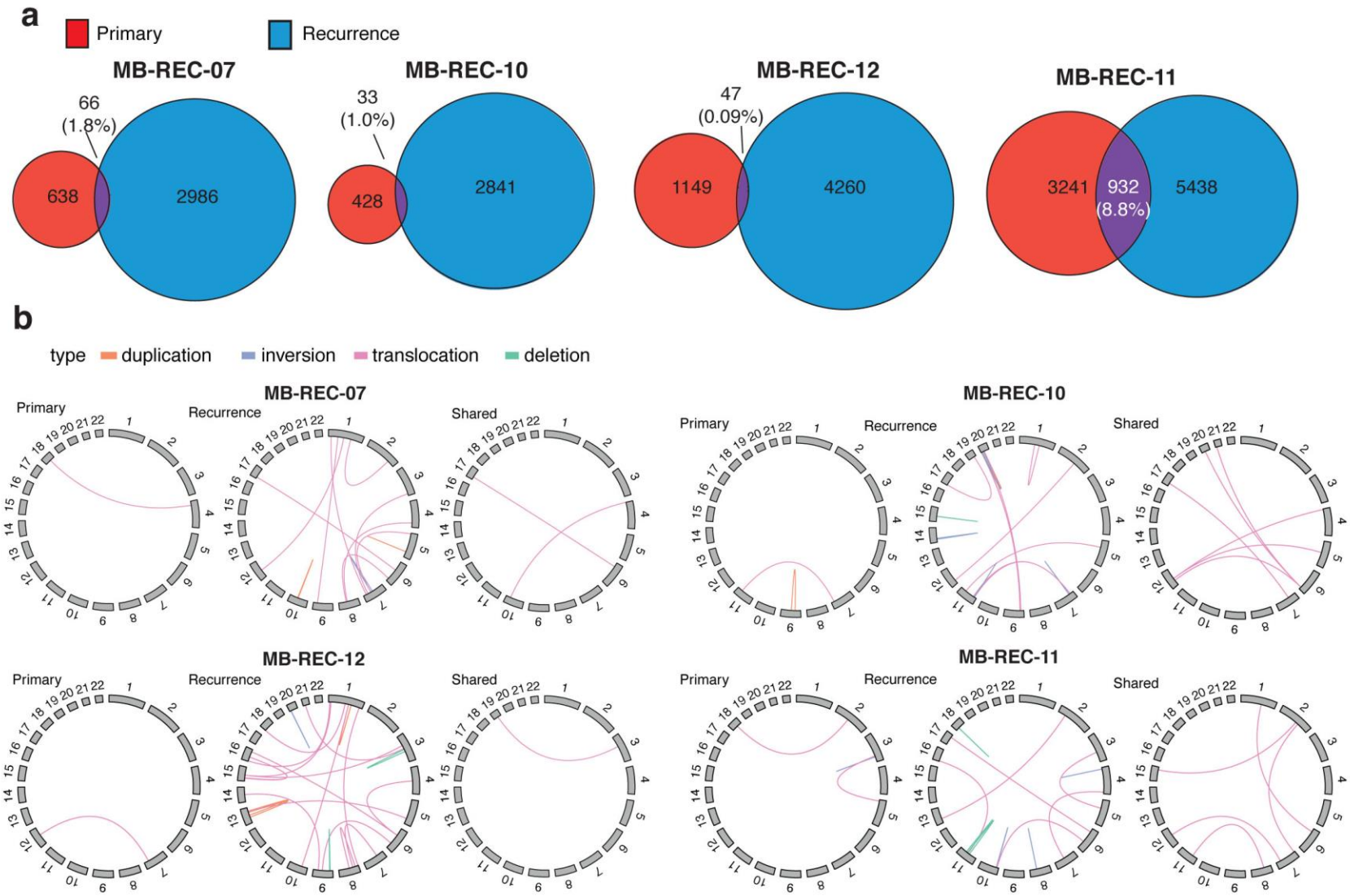
C



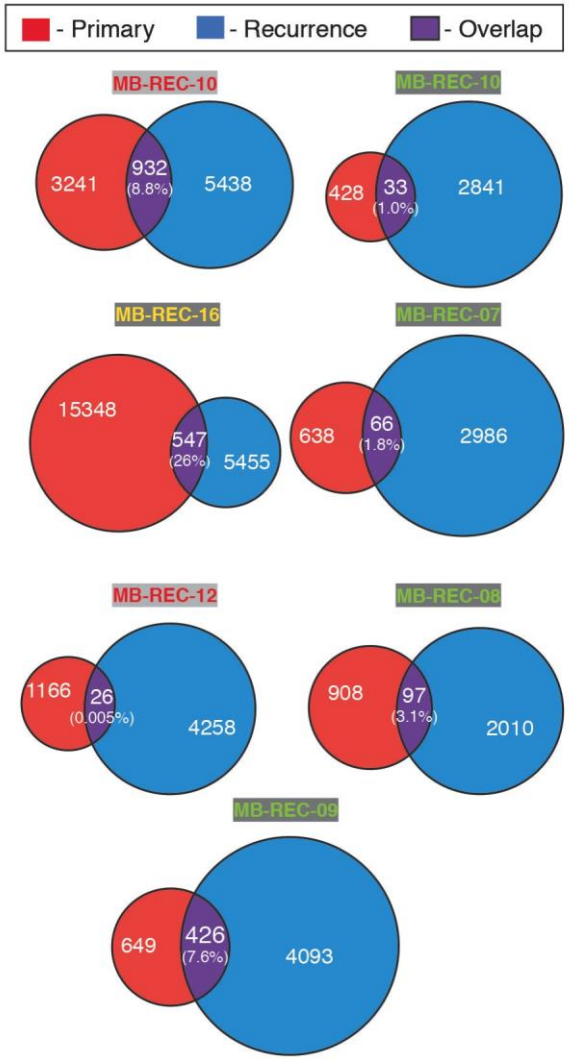
D



WGS of 38 diagnostic/recurrent MBs



Human Primary/Recurrent WGS SNVs



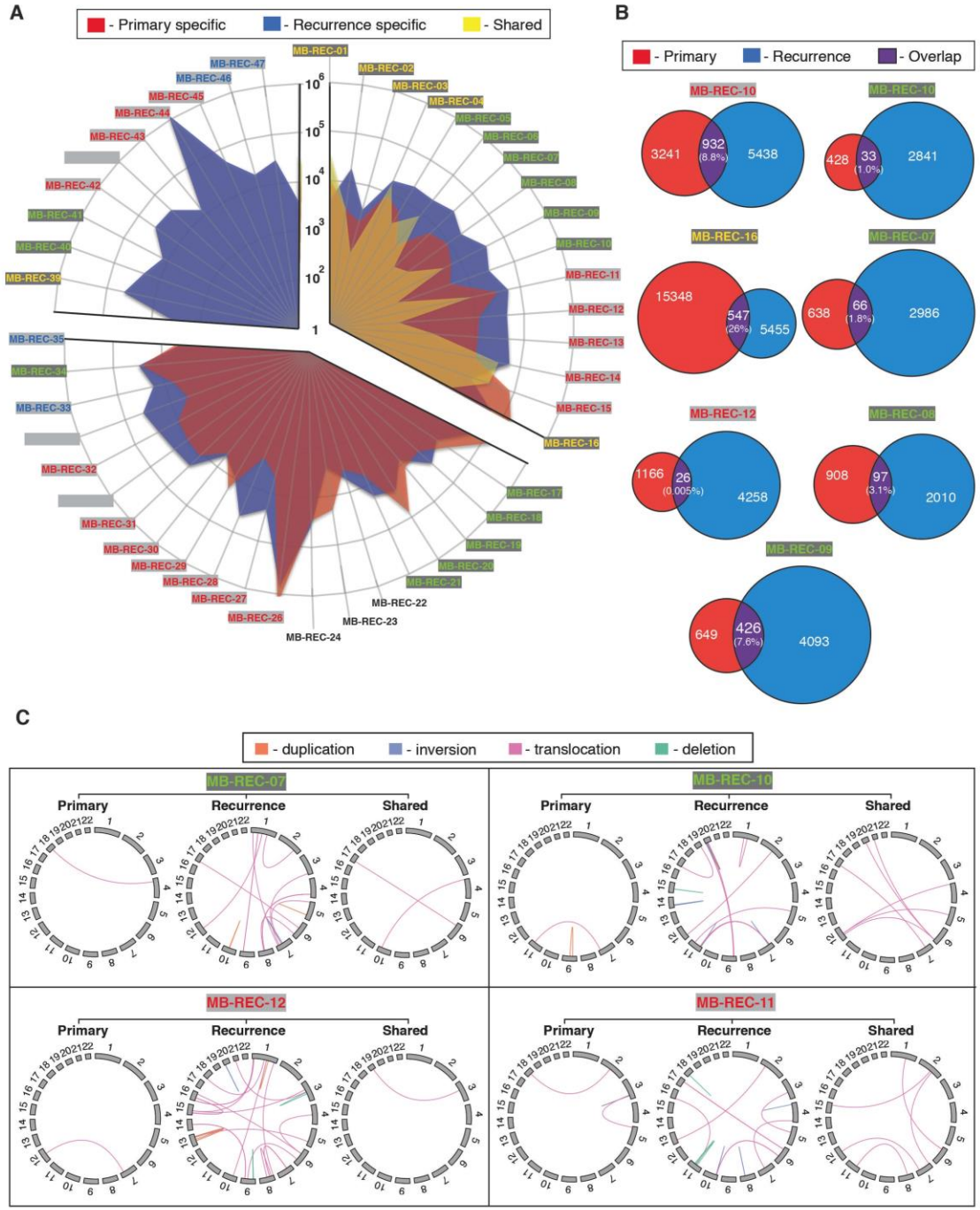
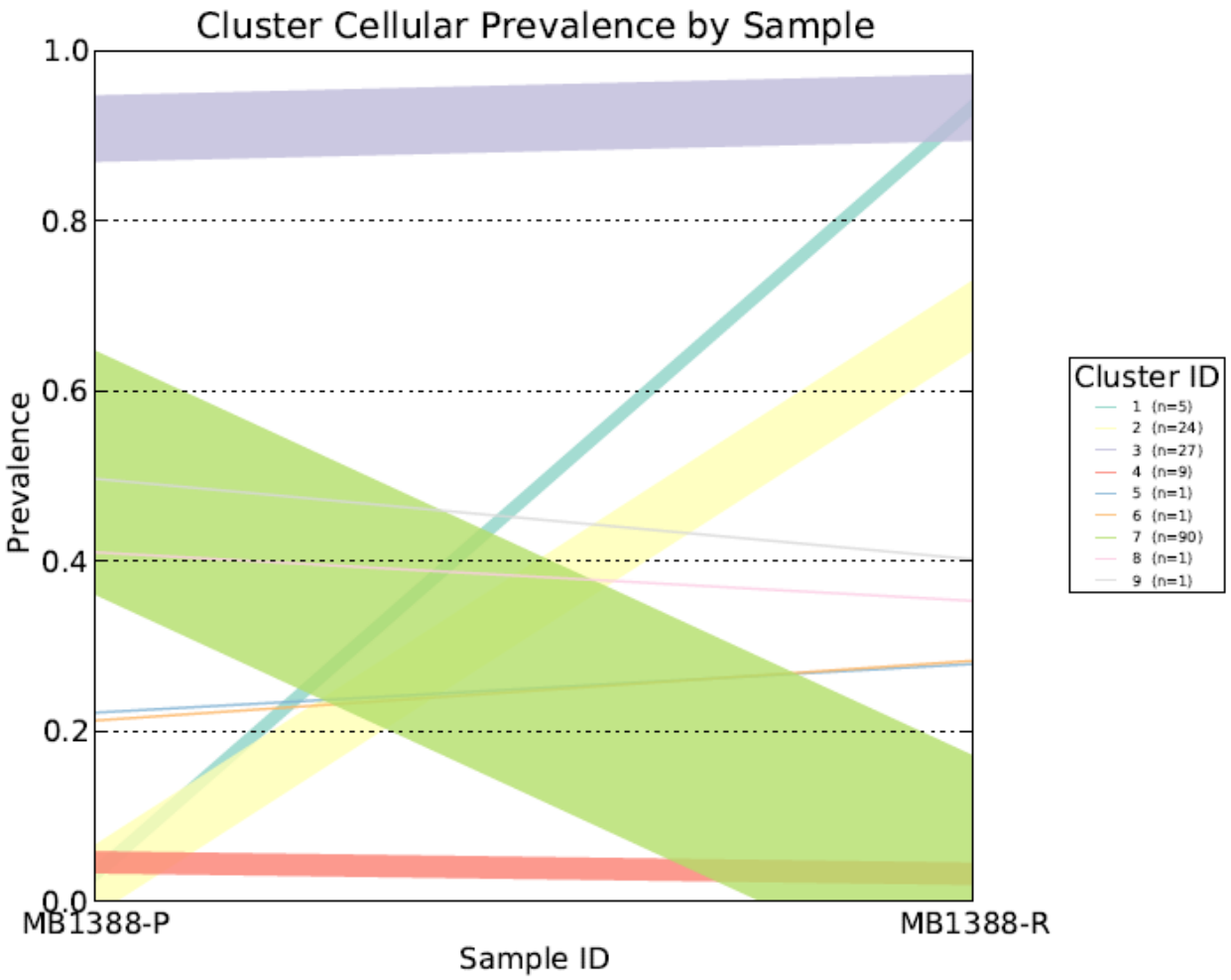
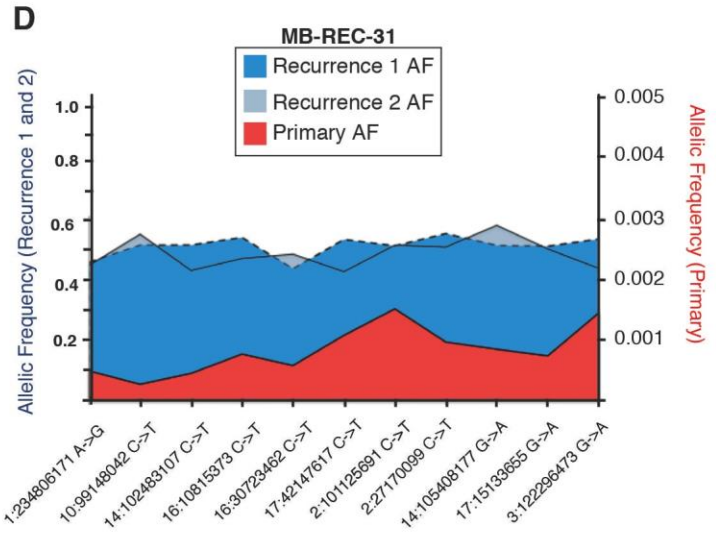
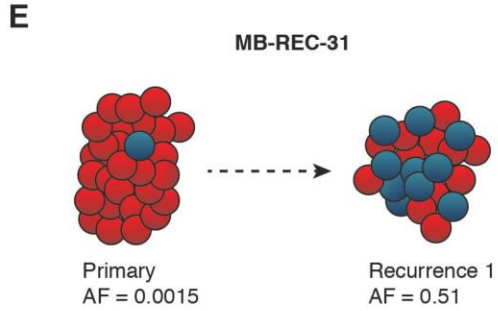


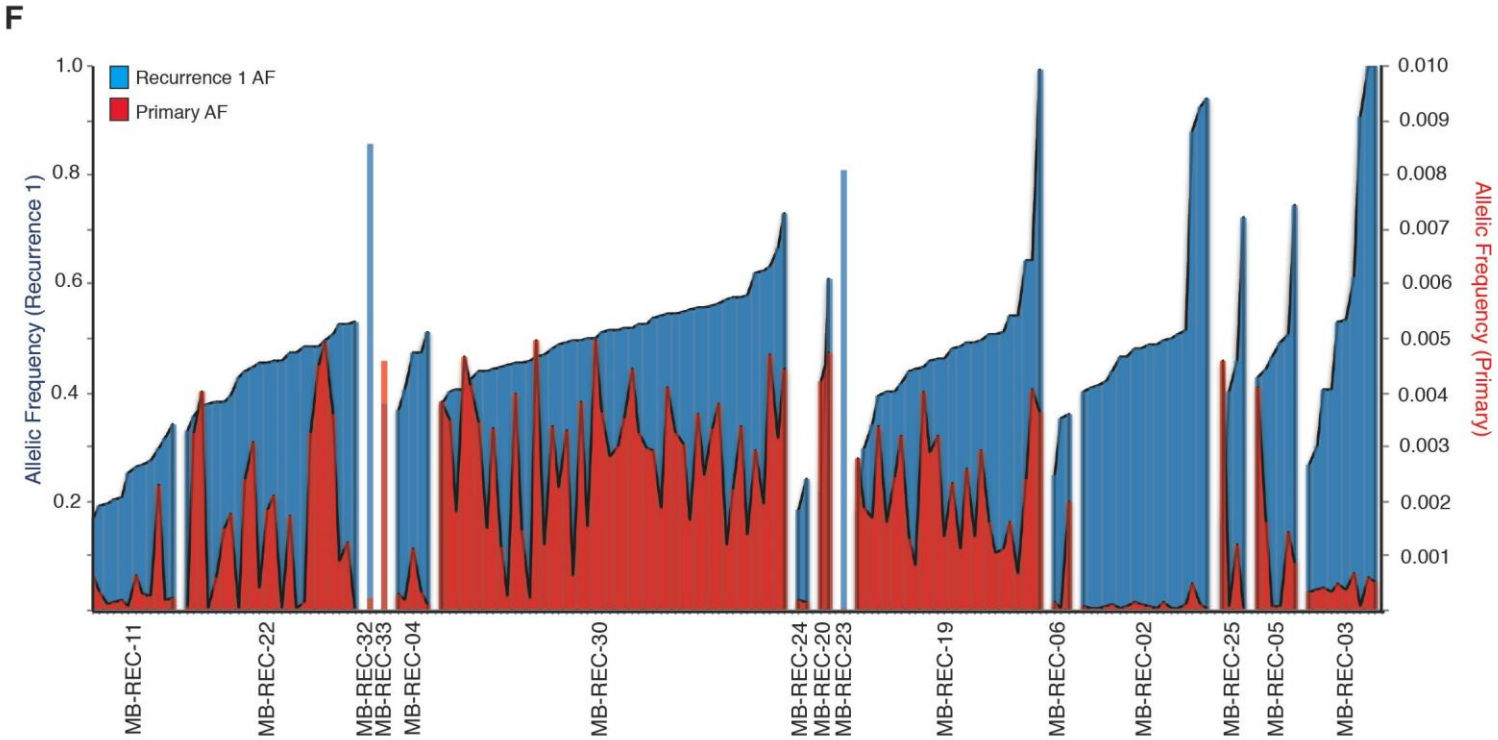
Fig. 5 MD Taylor, 2014

Pyclone – comparing mutation clusters, prim vs. recur

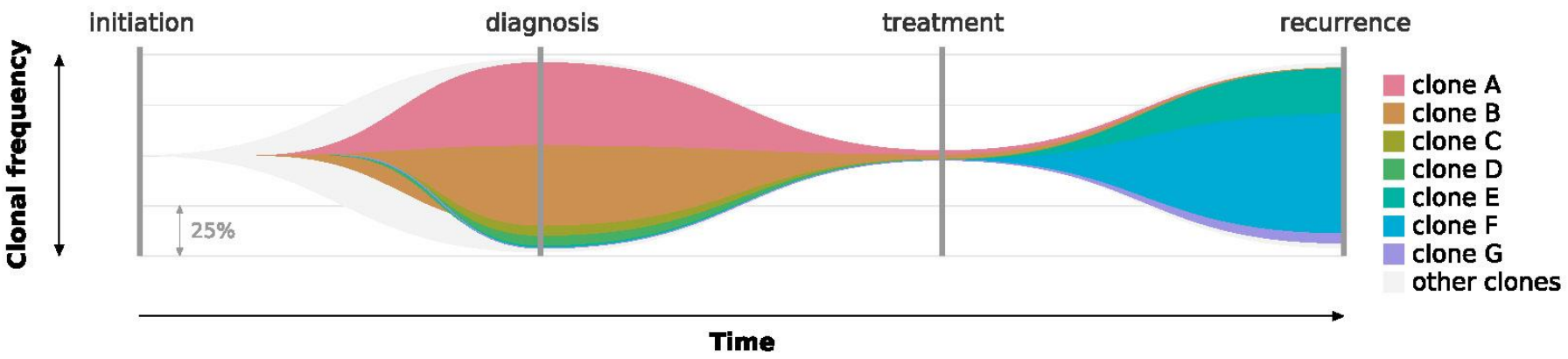



D
MB-REC-31
Recurrence 1 AF
Recurrence 2 AF
Primary AF
Allelic Frequency (Recurrence 1 and 2)
Allelic Frequency (Primary)
1.0
0.8
0.6
0.4
0.2
0.005
0.004
0.003
0.002
0.001
1:234806171 A->G
10:99148042 C->T
14:102483107 C->T
16:10815373 C->T
16:30723462 C->T
17:42147617 C->T
2:101125691 C->T
2:27170099 C->T
14:105408177 G->A
17:15133655 G->A
3:122296473 G->A


E
MB-REC-31
Primary
AF = 0.0015
Recurrence 1
AF = 0.51


F
Recurrence 1 AF
Primary AF
Allelic Frequency (Recurrence 1)
Allelic Frequency (Primary)
1.0
0.8
0.6
0.4
0.2
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
MB-REC-11
MB-REC-22
MB-REC-32
MB-REC-33
MB-REC-04
MB-REC-30
MB-REC-24
MB-REC-20
MB-REC-23
MB-REC-19
MB-REC-06
MB-REC-02
MB-REC-25
MB-REC-05
MB-REC-03

Clonal evolution streamgraph



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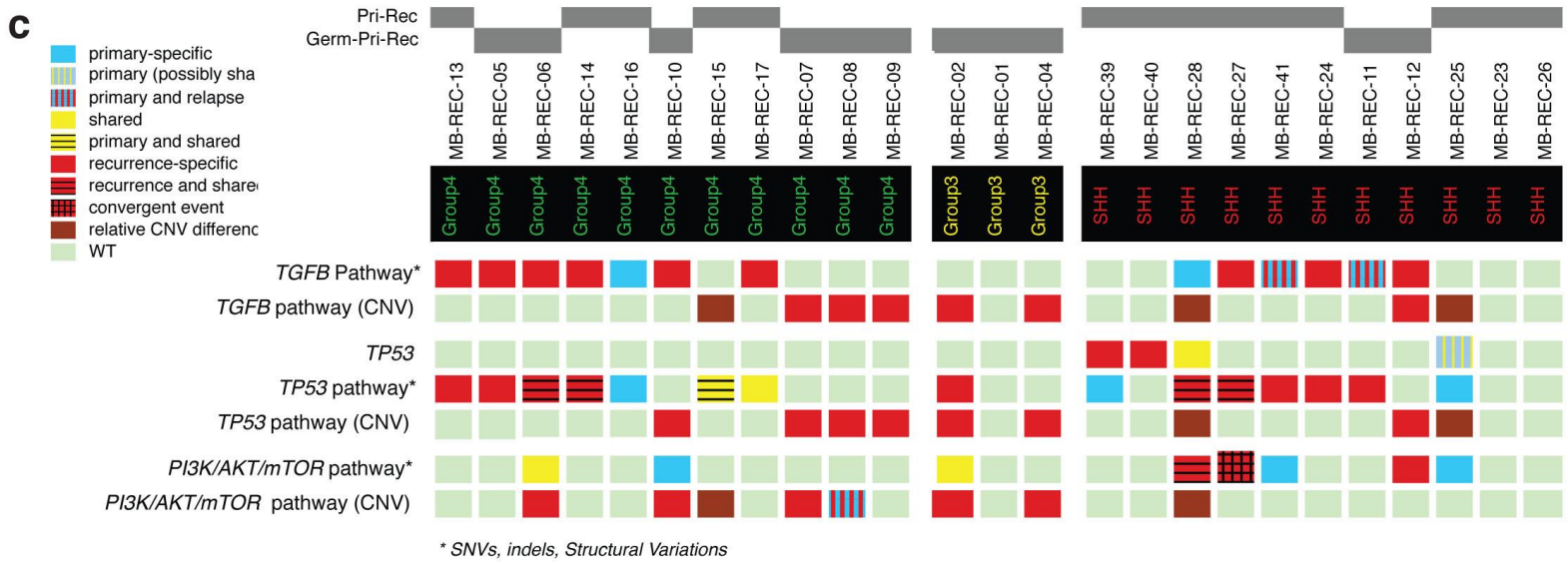
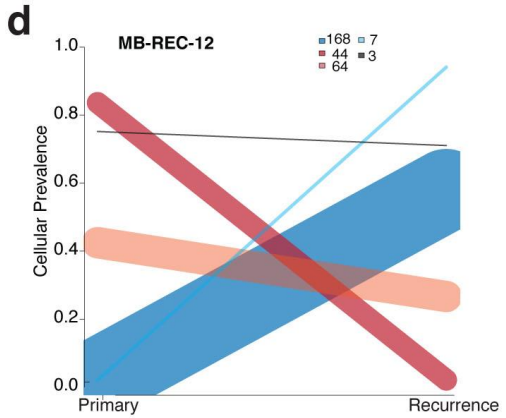
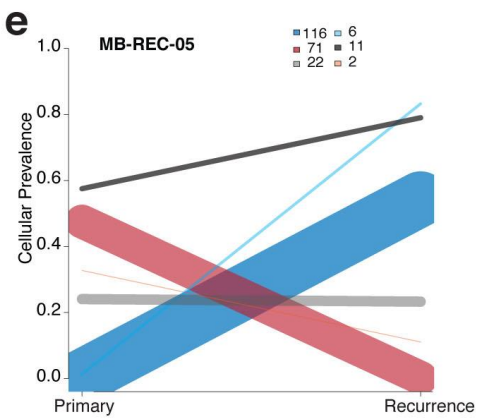
Clonal divergence, but Pathway convergence





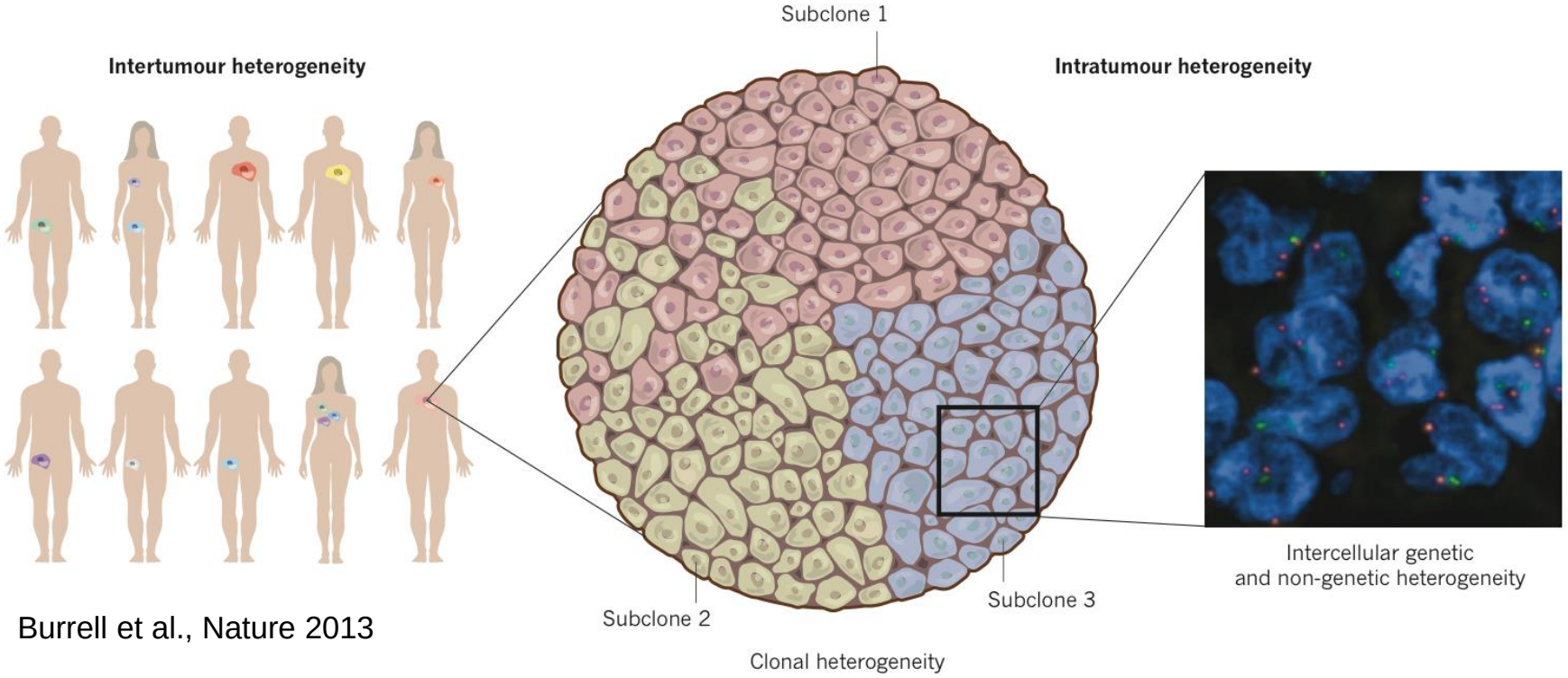


Recurrence: Conclusions

- Large genetic divergence of somatic events between the untreated primary tumor and its recurrence.
- Targets for rational therapy discovered and documented at presentation may not be present at recurrence.
- Our current design for Phase II trials is critically flawed, and doomed to failure.

A million oncologists doing a million clinical trials for a million years…….

Concept of tumor heterogeneity



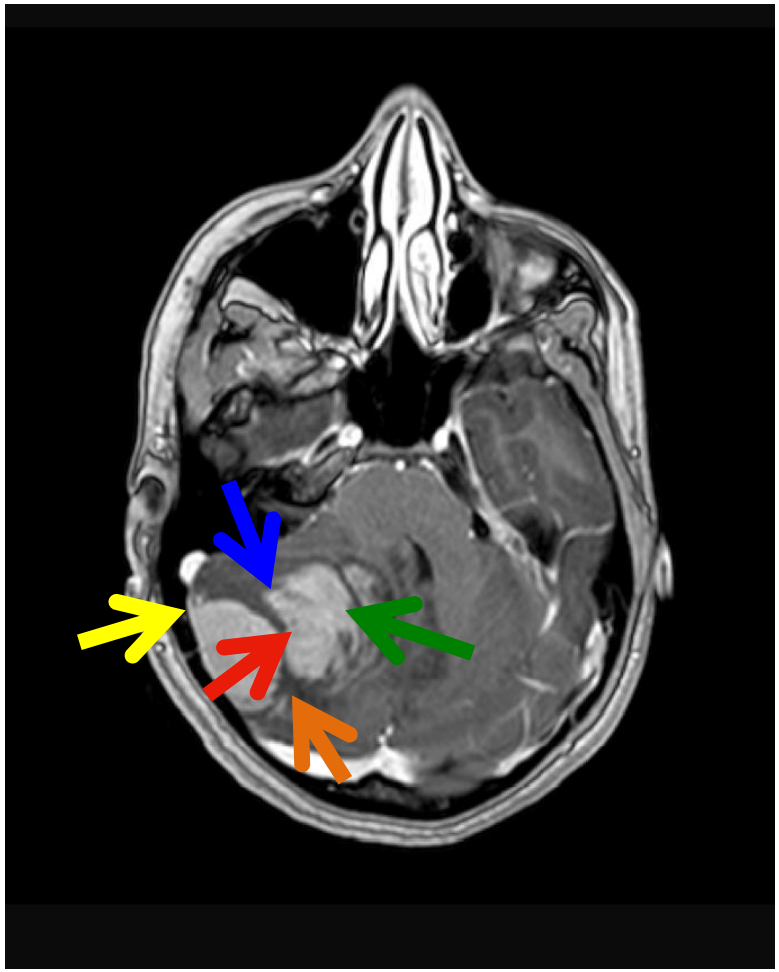
Burrell et al., Nature 2013

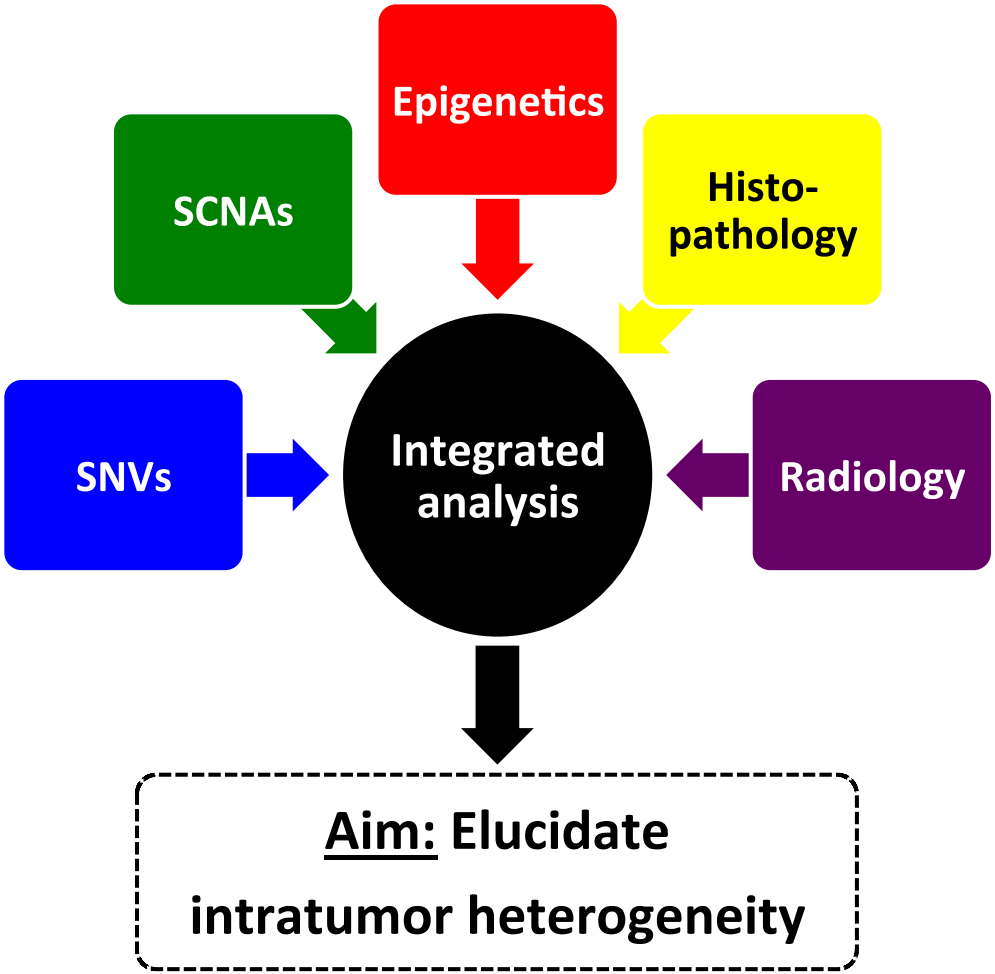
Examples of spatial heterogeneity in other cancer types:
Renal cell carcinoma – Gerlinger et al., NEJM 2012; Martinez et al., AMJP 2013; Gerlinger et al., Nat Med 2014
Glioblastoma – Nickel et al., PlosOne 2012; Sottoriva et al., PNAS 2013; Johnson et al., Nat Med 2014

Important implication:

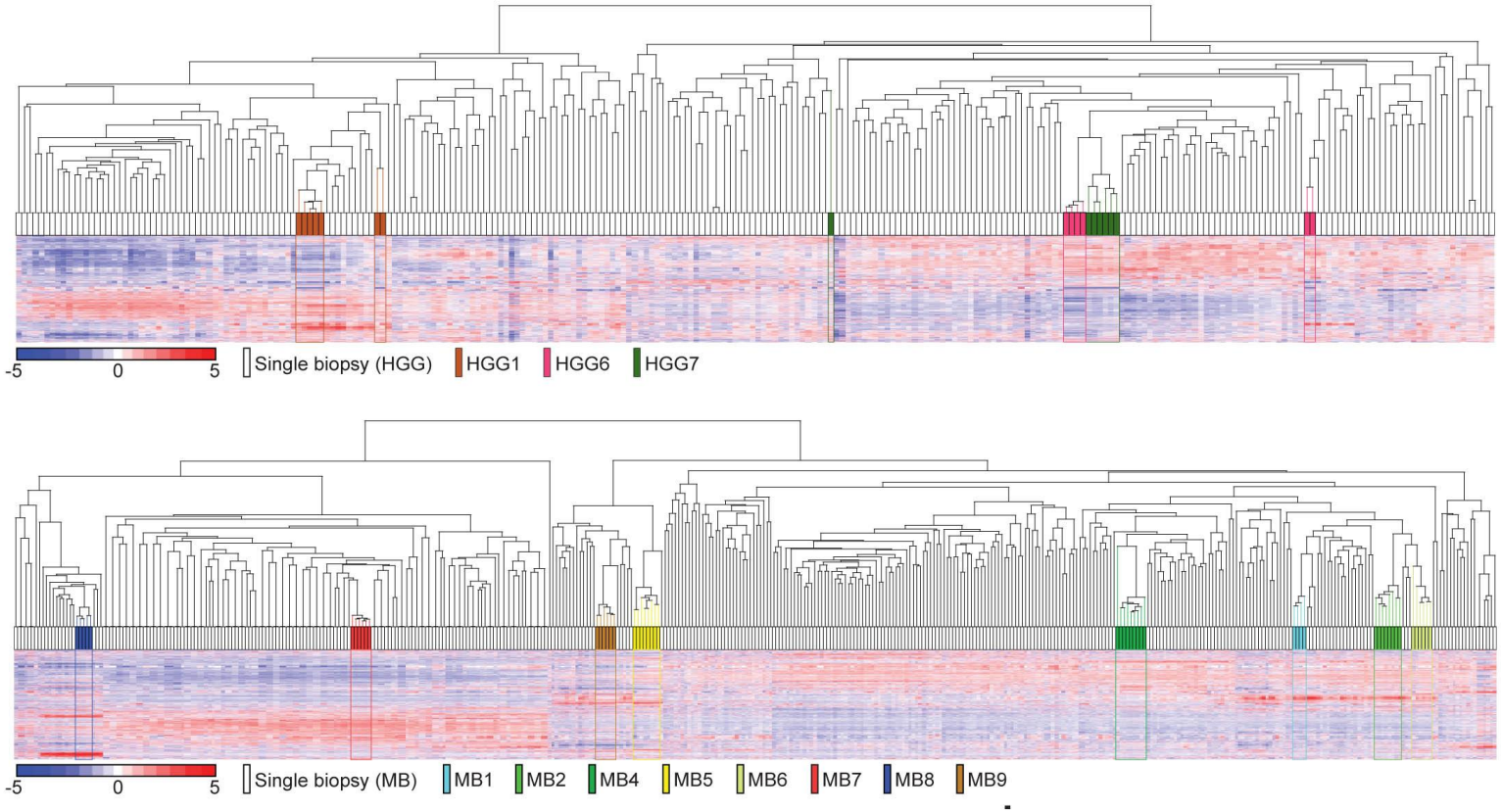
Spatial heterogeneity may preclude development of molecular based targeted therapy in pediatric brain tumors.

Experimental strategy





Malignant Gliomas



Medulloblastomas

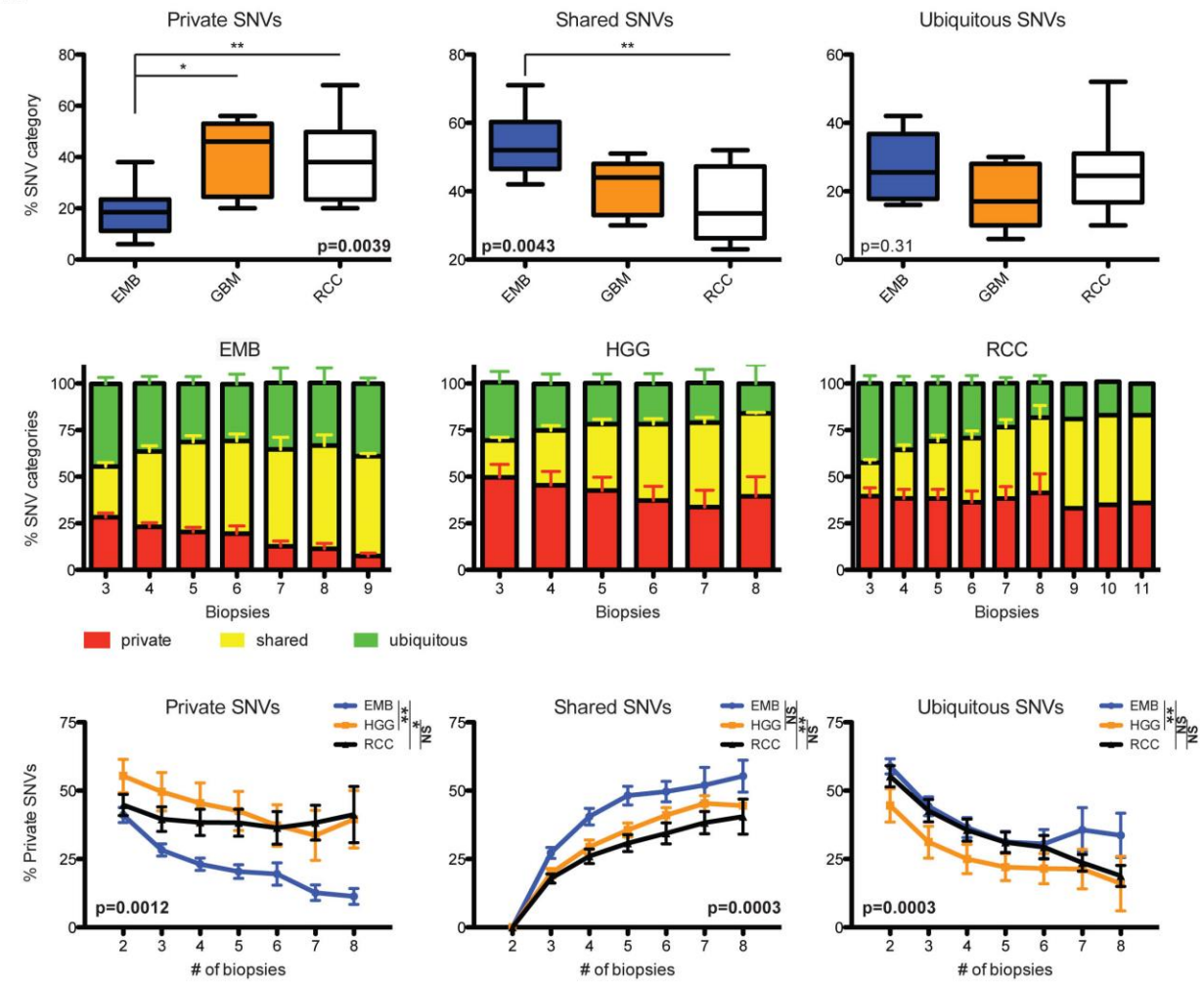
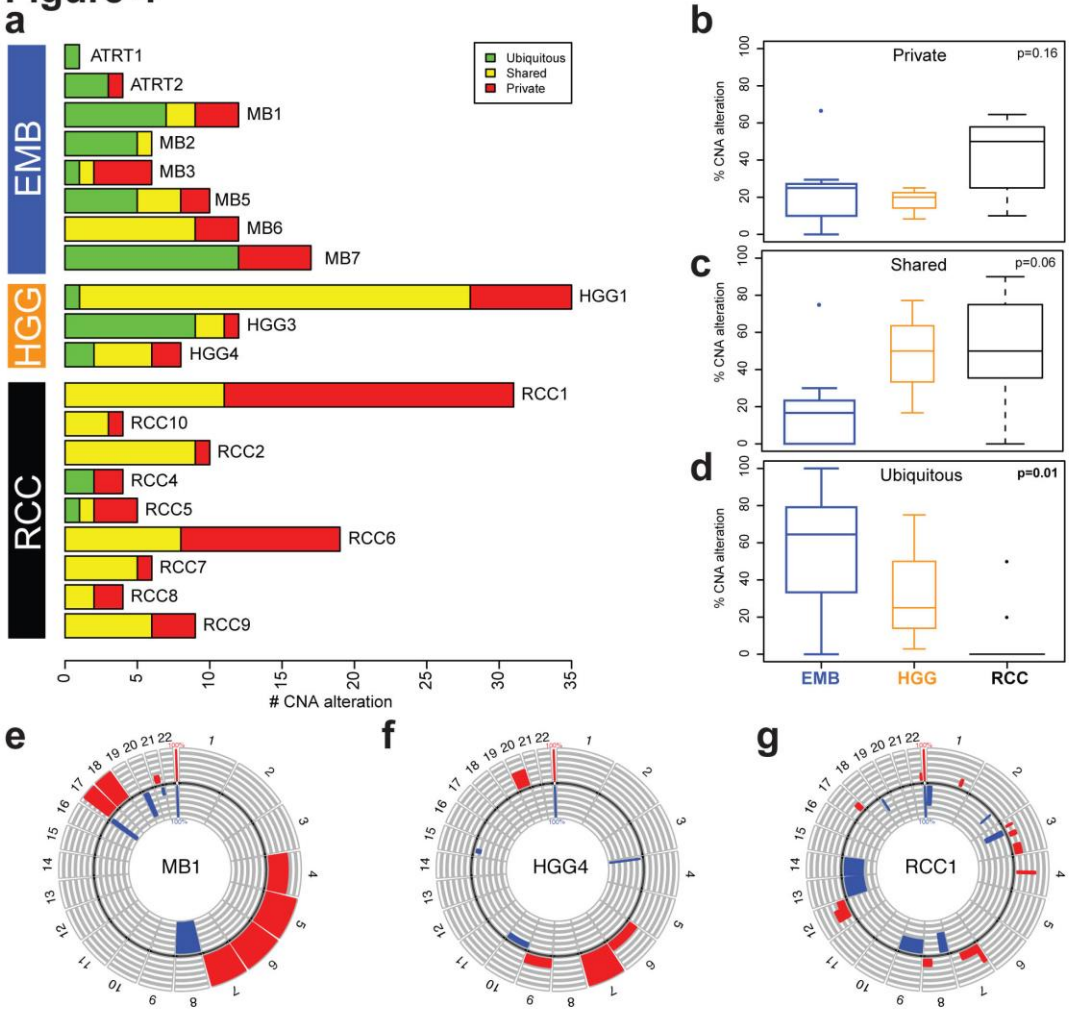
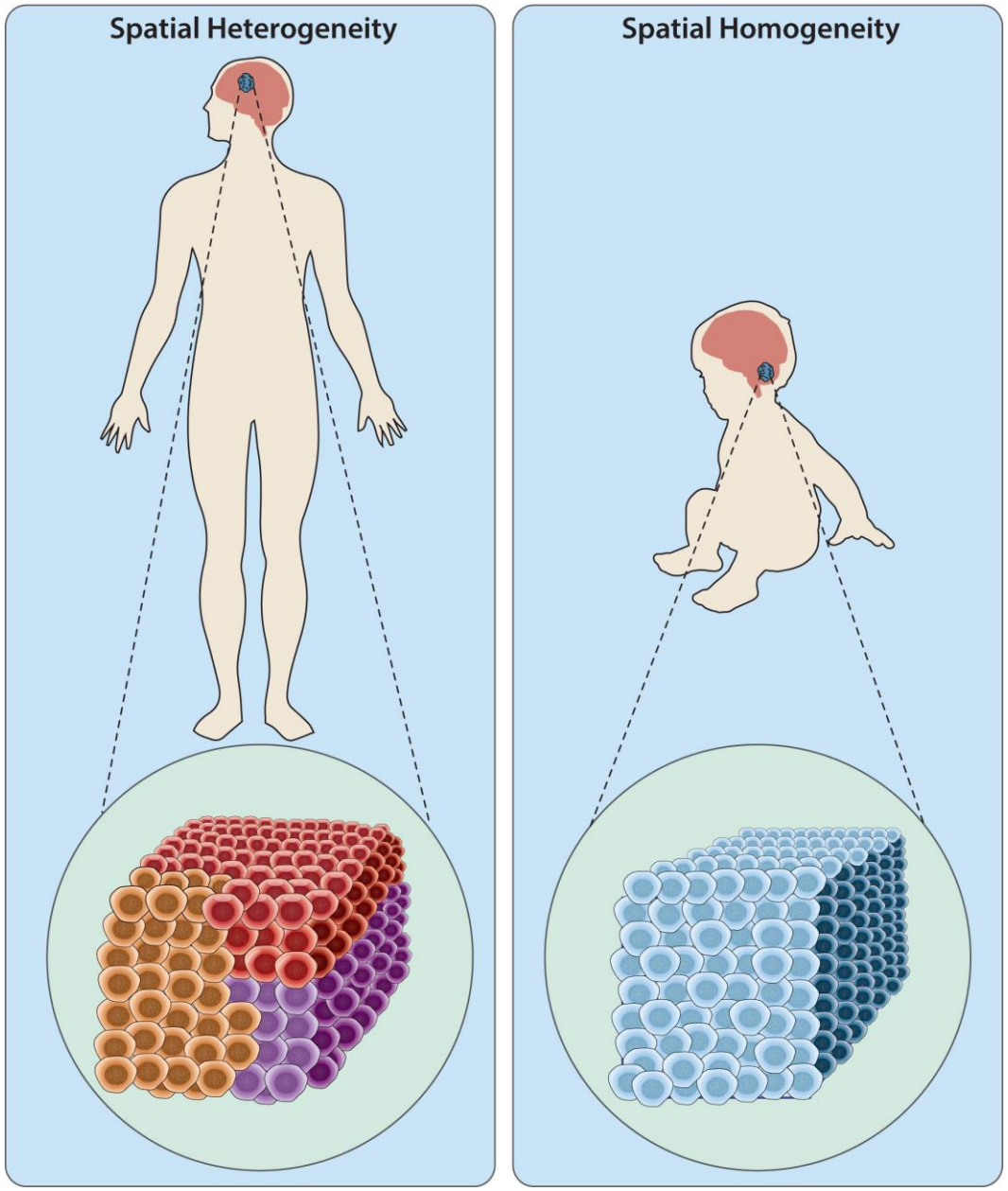

Private SNVs
Shared SNVs
Ubiquitous SNVs
% SNV category
p=0.0039
p=0.0043
p=0.31
EMB
GBM
RCC
EMB
HGG
RCC
% SNV categories
Biopsies
private
shared
ubiquitous
Private SNVs
Shared SNVs
Ubiquitous SNVs
% Private SNVs
p=0.0012
p=0.0003
p=0.0003
# of biopsies
NS

Figure 4


a
EMB
HGG
RCC
ATRT1
ATRT2
MB1
MB2
MB3
MB5
MB6
MB7
HGG1
HGG3
HGG4
RCC1
RCC10
RCC2
RCC4
RCC5
RCC6
RCC7
RCC8
RCC9
Ubiquitous
Shared
Private
0 5 10 15 20 25 30 35
CNA alteration
b
Private
p=0.16
c
Shared
p=0.06
d
Ubiquitous
p=0.01
% CNA alteration
EMB
HGG
RCC
e
MB1
f
HGG4
g
RCC1


Spatial Heterogeneity
Spatial Homogeneity

Acknowledgements