

Supplemental Methods:

DNA extraction from FFPE:

One representative block per case containing >90% cellularity and minimal necrosis was selected. Twenty five mg of tissue was obtained in 40 um histological sections. Where possible, cases that had a repeat resection at the time of recurrence were obtained and profiled. These are included as individual tumors in the analysis, although the annotation afforded the opportunity to examine several loci in detail for changes from initial to recurrent. However, frequency of chromosomal changes is reported as 1 sample per patient (see analysis below).

High quality DNA (>10Kb fragments) was extracted from FFPE sections by modifications of Qiagen tissue extraction protocol (cat #69504). Samples were deparaffinized by treatment with mixed xylenes (1.2 ml, vortex, centrifuge 3' at 25°C, decant, repeat 1-3x until clear). Xylene was removed by addition of 100% ETOH (1.2 ml, vortex, centrifuge 3' at 25°C, decant, repeat 1-2x until clear) and then vaporized for 10 min at 37°C and rehydrated in PBS (1.2 ml, vortex, centrifuge 3' at 25°C, decant). Samples were lysed in 360 ul of Qiagen lysis buffer and 40 ul proteinase K (20 mg/ml) and inverted at 55°C for 24-72 hours as needed for full digestion. At 24 and 48 hrs, an additional 40 ul of proteinase K (20 mg/ml) was added if further digestion time was required. The preparation of DNA from tissue lysates was according to protocol (Qiagen) using buffer volumes at equi-volume with digested sample. DNA was eluted in 2-3 elutions of 50 ul H₂O per elution after letting the solution sit on the column for 10 minutes before centrifugation, mixed and quality examined by gel electrophoresis of 1 ug in a 1% agarose gel. Samples with at least 50% of total DNA running above the 10 Kb marker (NEB) were of sufficient quality to proceed with array CGH (Supplemental Figure 1A) as determined by a series of samples with variable quality (data not shown).

MCR definition

Loci of amplification and deletion are evaluated across samples with an effort to define MCRs targeted by overlapping events in two or more samples. An algorithmic approach has been previously described (Aguirre et al., 2004, Tonon et al., 2005 and Carrasco et al., 2006). It is applied to the segmented data as follows:

1. Segments with values >0.4 or <-0.4 are identified as altered.
2. If two or more similarly altered segments are adjacent in a single profile or separated by <500 kb, the entire region spanned by the segments is considered to be an altered span.
3. Altered segments or spans <20 Mb are retained as "informative spans" for defining discrete locus boundaries. Longer regions are not discarded, but are not included in defining locus boundaries.
4. Informative spans are compared across samples to identify overlapping amplified or deleted regions (informative spans only); each is called an "overlap group."
5. Overlap groups are divided into separate groups wherever the recurrence rate falls <25% of the peak recurrence for the whole group. Recurrence is calculated by counting the number of samples with alteration at high threshold (± 0.4).
6. MCRs are defined as contiguous spans within an overlap group, having at least 75% of the peak recurrence. If there are more than three MCRs in a locus, the whole

region is reported as a single complex MCR. In cases where MCRs were defined by two overlapping CNAs, MCR inclusion in the final list and boundary definition was subjected to individual review.