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# Finding the way to Wilms tumor by comparing the primary and relapse tumor samples

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**In this issue of *Cell Reports Medicine*, Gadd and colleagues presented on behalf of the Children's Oncology Group their comprehensive analysis of genetic changes associated with relapse in children with favorable histology Wilms tumor.**

Whereas the genetic landscape of Wilms tumor has been widely investigated at diagnosis, the study by Gadd et al. represents a relevant action in order to dissect Wilms tumor relapse.[1] We have read this paper with much interest, given that it implements a modern paradigm of research in Wilms tumor that centralizes gaining knowledge when investigating relapsing tumors, their environment, and the host, knowledge to which others and we have recently contributed.[2–4]

Mutations in many of the genes involved in renal development also play a key role in the growth of Wilms tumor, including *SIX1*, *WT1*, *MYCN*, *WTX*, *MLLT1*, and *CHD4*.[5] How do these genes underline evolution of cell clusters in recurrent or metastatic tumors?

By focusing on the comparison between matched germline-primary-relapse trios, germline-relapse pairs, and relapsed Wilms tumors, Gadd and colleagues identified mutations in *SIX1* gene or in genes of the MYCN network in more than 40% of relapse samples.[1] They showed that in several patients, mutations in *SIX1*, *MYCN* (genes involved in maintaining the progenitor state in early nephrogenesis), and *WTX* were present in the relapse but not in the corresponding primary tumor. In particular, they found *SIX1* mutations in 11 of 82 (13.4%) relapse samples, similar to our previous findings (we found four mutations in 27 analyzed relapse samples, corresponding to 14.8%). Interestingly, they identified *SIX1* mutations in the relapse sample but not in the primary sample in three out of six evaluable patients with available matched pairs. In our original experience, we could find *SIX1* mutations in four relapses but only in two paired primary samples, although we examined multiple tissue blocks taken from different topographical areas of the primary tumor.[3,4] These findings by Gadd et al. and our group could suggest that *SIX1* mutation is not required for tumor origin in many patients, but it may underline tumor progression or resistance to chemotherapy.

In the present paper, mutations in microRNA-processing genes were concordant in pairs of primary tumor and relapse samples. In our previous paired analysis, by multiple sampling we found that in some cases mutations in microprocessing genes, although present in the relapse samples, showed to be clonal events in the corresponding primary tumor.[3,4] Altogether, these observations are consistent with a Darwinian clonal evolution in which Wilms tumor becomes aggressive when it grows up, because of late developments of genetic events most influencing relapse (like mutations involving *TP53* gene, microRNA biogenesis, or SIX1/2 pathway—all likely underling high-risk Wilms tumors, as reviewed in Spreafico et al.[5]), differently from other childhood embryonal tumors.[6]

Additional patients in this report had variants in other members of the MYC transcription factor network that are expected to result in cellular impacts similar to MYCN over-expression, including *MAX* (two patients), *MGA* (five patients), and *NONO* (one patient). The MYCN network is also involved with preservation of the progenitor state in the kidney. Other genes recurrently mutated in the relapse tumors, and not previously identified in primary Wilms tumors were *DIS3* and *TERT*.

When investigating copy number changes, Gadd and colleagues reported a high proportion of relapses (75%) with gain of chromosome 1q, whereas this anomaly was present in 47% of paired primary tumor samples, further supporting its adverse prognostic role.[1] Similarly, yet in a smaller series of cases, we found chromosome 1q gain and/or allelic imbalance in three of eight (37.5%) primary tumor samples and in six of eight (75%) paired relapse samples.[4] Altogether these data seem to indicate that this chromosomal anomaly arises during evolution of cells in relapse or metastatic disease.

How intratumoral heterogeneity is going to influence sampling bias to detect this and other biomarkers is challenging and deserves much attention for diagnostic and prognostic purposes. Cresswell et al. estimated that multiregional sampling $\geq$three samples per tumor could guarantee that >95% of tumors with chromosome 1q gain would be detected.[7] Other colleagues, acknowledging that heterogeneity in tumors might induce a sampling bias, recently suggested studying circulating tumor DNA at the time of diagnosis to enable the detection of clonal 1q gain and, afterwards, to monitor response or to detect recurrent disease.[8]

The opportunity to investigate germline-primary-relapse trios enabled Gadd and colleagues to gain insights in the temporal acquisition of the mutations in Wilms tumor, although there is no clear evidence to support a specific sequence of genetic events. It is, in fact, possible that the

combination of mutations or structural anomalies is critical, rather than the temporal order of their acquisition. For example, the co-occurrence of mutations in genes supporting continued progenitor proliferation (like the highly homologous *SIX1* and *SIX2* genes) with those preventing differentiation might be most important rather than the temporal order of their accumulation.

Deeply studying relapsed Wilms tumors could be helpful to patients themselves, on different levels. First, the value of biological characterization of relapse is instrumental to elucidate the genomic mechanisms underlying metastasis and progression, as we and other colleagues have pointed out.[1–4] The availability of samples to compare between primary, relapsed Wilms tumors and the corresponding normal tissue from many patients enables us to gain insights into their spatial and temporal phylogenetic relation. Effective treatment options for many relapsing patients are still limited, and we could facilitate molecular-targeted therapies by screening relapsed tumor for actionable alterations, which could be different from primary tumor. Lastly, the relapse setting gives us opportunities to test new drugs (that can be moved upfront in the future), and also to develop tumor models, like organoids or patient-derived xenografts.[9]

Studies like this one are possible because of the work done by cooperative groups, like Children's Oncology Group Renal Tumor Committee, SIOP Renal Tumor Study Group,[10] Associazione Italiana Ematologia Oncologia Pediatrica,[3,4] and the U.K. Children's Cancer and Leukemia Group,[2] which included biology and classification registries that served as the entry portal to therapeutic protocols.

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## DECLARATION OF INTERESTS

The authors declare no competing interests.

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