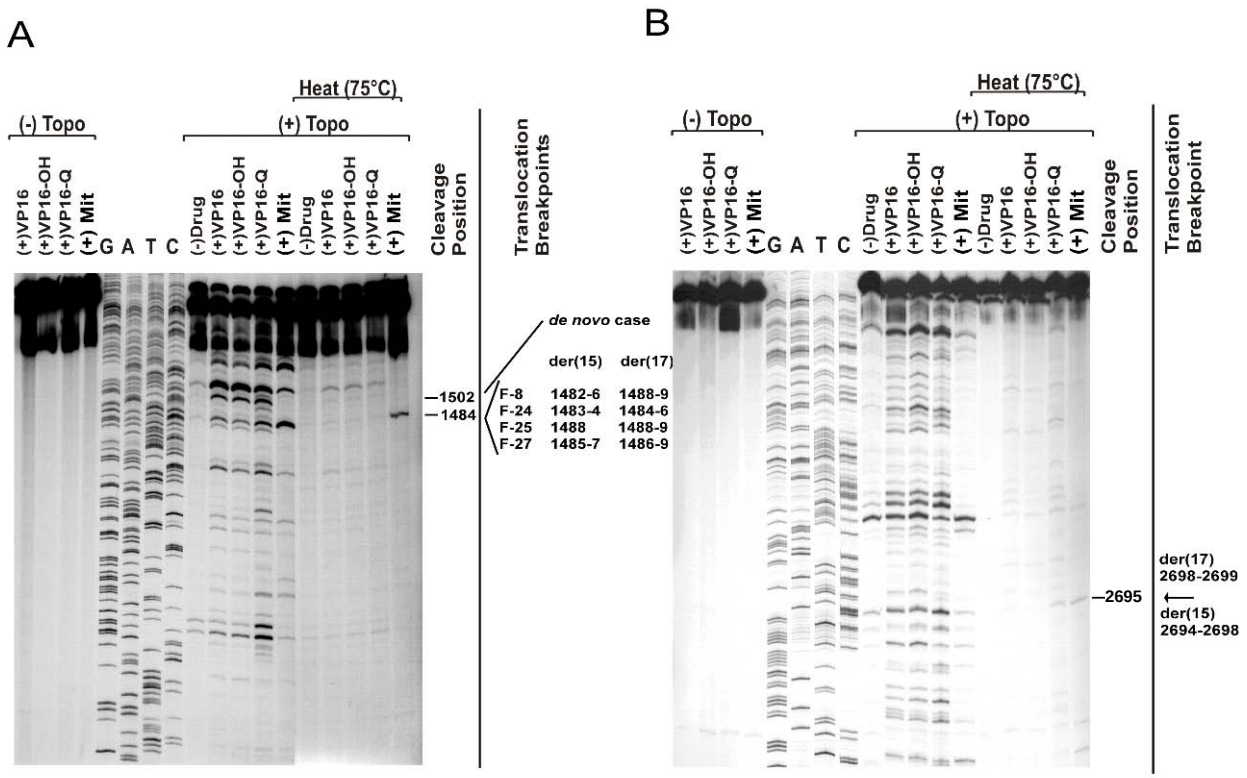

Figure 2. **Demonstration of mitoxantrone-induced topoisomerase II dependent DNA cleavage at translocation breakpoints in therapy-related APL**

A) *In vitro* DNA topoisomerase II cleavage assay carried out for a *PML* substrate containing the breakpoints of 4 treatment-related APL (t-APL) cases (F-8,-24,-25,-27) within a 8bp breakpoint hotspot (positions 1482-1489). Patients received combination chemotherapy including the topoisomerase II poison mitoxantrone for breast carcinoma. Control reactions were carried out in the absence of DNA topoisomerase II (lanes 1-4), and in the presence of etoposide (VP16), etoposide catechol (VP16-OH), etoposide quinone (VP16-Q) and mitoxantrone (Mit). Dideoxy sequencing reactions of the substrate are shown in lanes 5-8. Cleavage reactions were carried out by exposure to human DNA topoisomerase IIα in the absence of drug (lane 9), and in the presence of etoposide (lane 10), etoposide catechol (lane 11), etoposide quinone (lane 12) and mitoxantrone (lane 13). Additional cleavage reactions were carried out to evaluate the heat-stability of cleavage complexes formed by incubation at 75°C for 10 min (lanes 14-18). The nucleotide shown by the dash is the 5'side of the cleavage site (-1 position), which corresponds to the der(15) and der(17) translocation breakpoints in 4 cases of mitoxantrone-related APL (far right). The cleavage site at position 1484 was observed in the absence of drug, and in the presence of etoposide, both etoposide metabolites and mitoxantrone (lanes 9-13). Cleavage at this position was the strongest site observed in the presence of mitoxantrone (lane 13). Furthermore, the complexes formed at this site were shown to be heat-stable in the presence of mitoxantrone (lane 18). Interestingly, a cleavage site at position 1502 is also observed, which corresponds to a breakpoint detected in a case of *de novo* APL.

B) DNA topoisomerase II cleavage assay of normal homologue of der(15) and der(17) *RARA* translocation breakpoints in APL of one of the mitoxantrone-related cases (F-8). The substrate spanning positions 2603 to 2871 of *RARA* intron 2 contained the translocation breakpoints. Dash at right shows (-1) position of cleavage site corresponding to der(15) and der(17) translocation breakpoints (arrow far right). (Mistry *et al, New Engl J Med* 2005)