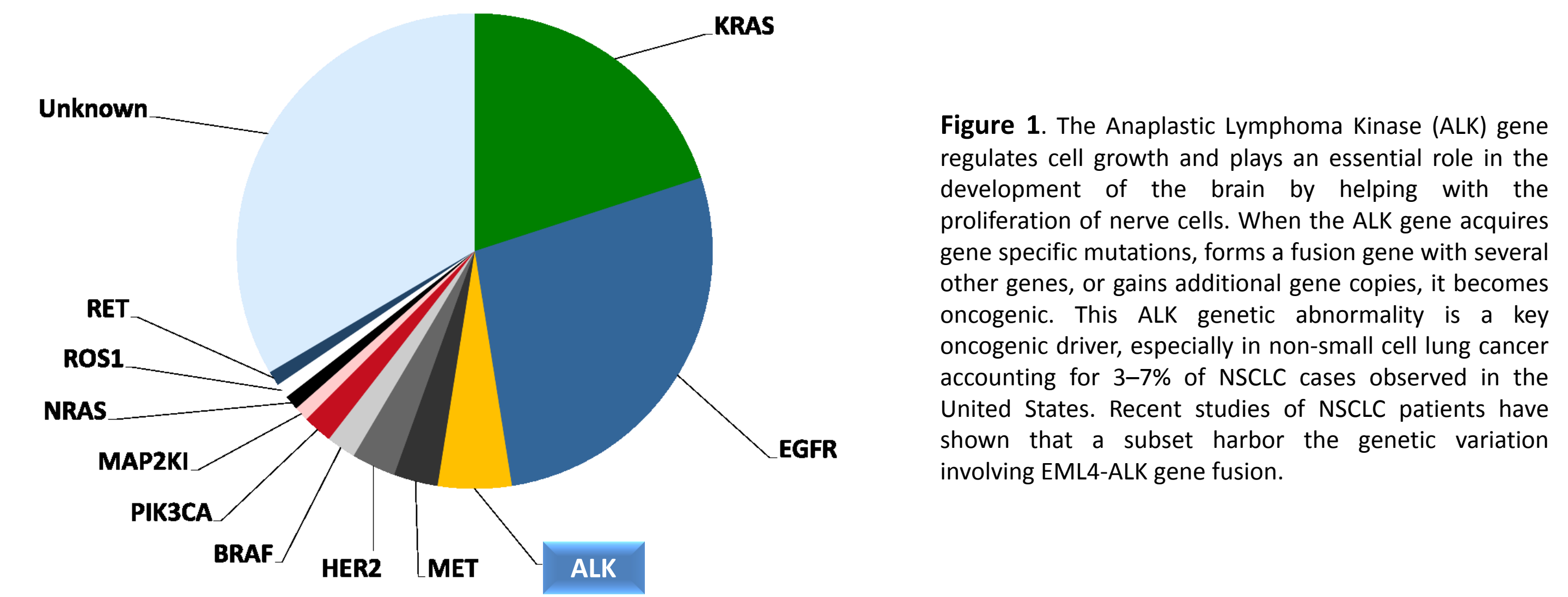


Abstract

Recent studies show that tumor cells derived from a subset of patients with non-small-cell lung cancer (NSCLC) harbor the echinoderm microtubule-associated protein-like 4 (EML4)-anaplastic lymphoma kinase (ALK) fusion oncogene; the result of a Paracentric chromosomal inversion on the short arm of chromosome 2. The EML4-ALK oncogene, like other ALK fusion oncogenes, is a druggable target that is responsive to ALK inhibitors. However, there is a lack of EML4-ALK *in vitro* models for drug screening. Here we set out to generate an isogenic EML4-ALK fusion non-small cell lung cancer model in the A549 lung cancer cell line, which harbors other naturally occurring genomic aberrations inherent in non-small cell lung cancer. This model could serve as a clinically relevant drug screening cell model. In this study, we utilized the CRISPR/Cas9 genome editing platform to target endogenous loci in human cells and create the intended genomic translocation event. By employing sgRNAs-Cas9 constructs designed to cut precisely at relevant translocation breakpoints, we induced cancer-relevant genomic rearrangements that resulted in the expression of EML4–ALK fusions. Breakpoint junction analysis tested after sgRNA-CRISPR/Cas9 mediated genomic DNA cleavage in A549 cells showed the successful creation of the EML4-ALK fusion found in tumor cells from a subpopulation of NSCLC patients. Furthermore, single clonal isolation and functional screening demonstrated that the EML4-ALK isogenic cell line was sensitive to ALK inhibitors relative to the parental A549 cell line. This newly developed EML4-ALK isogenic lung cancer cell line could provide a very useful tool for oncology drug discovery and development.

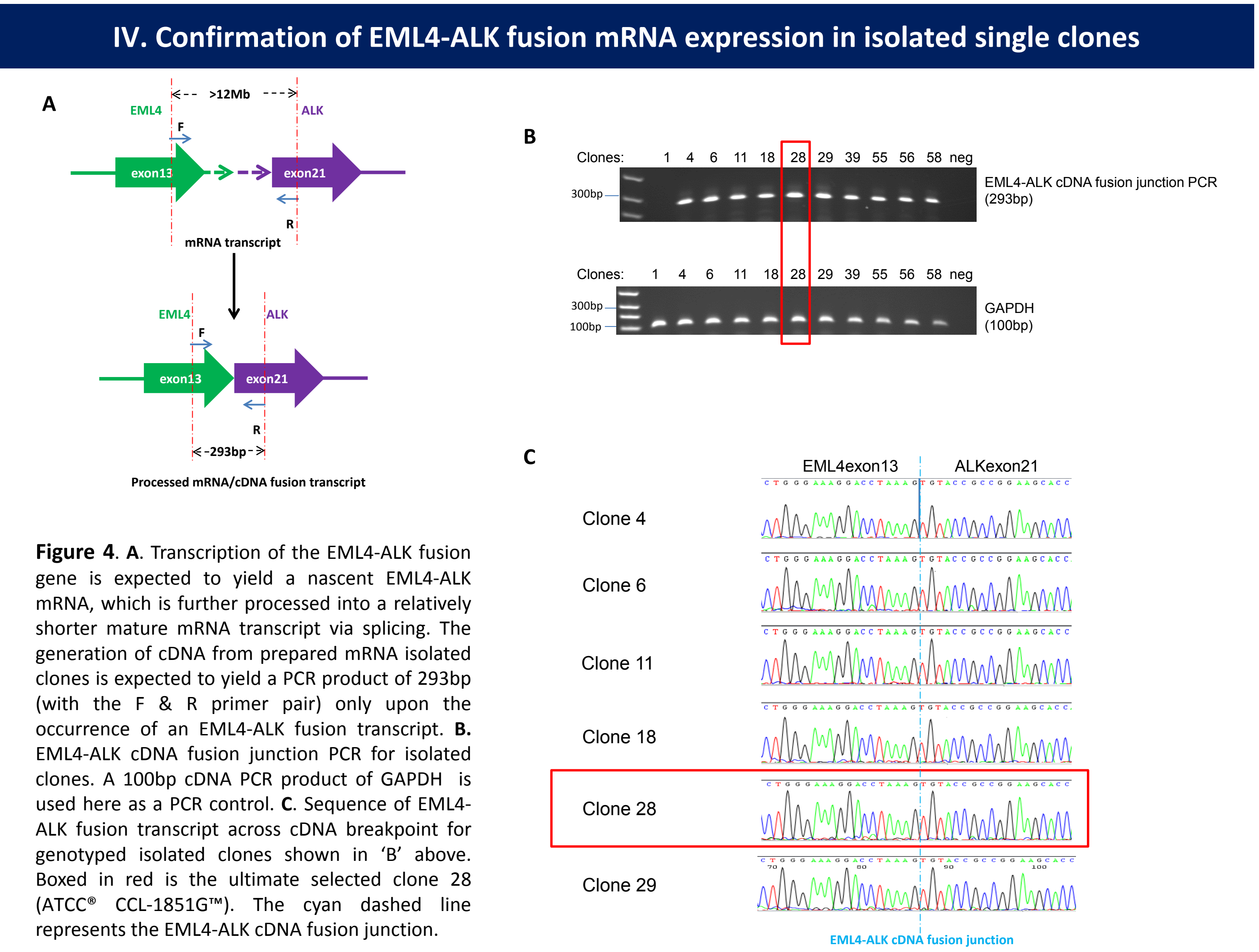
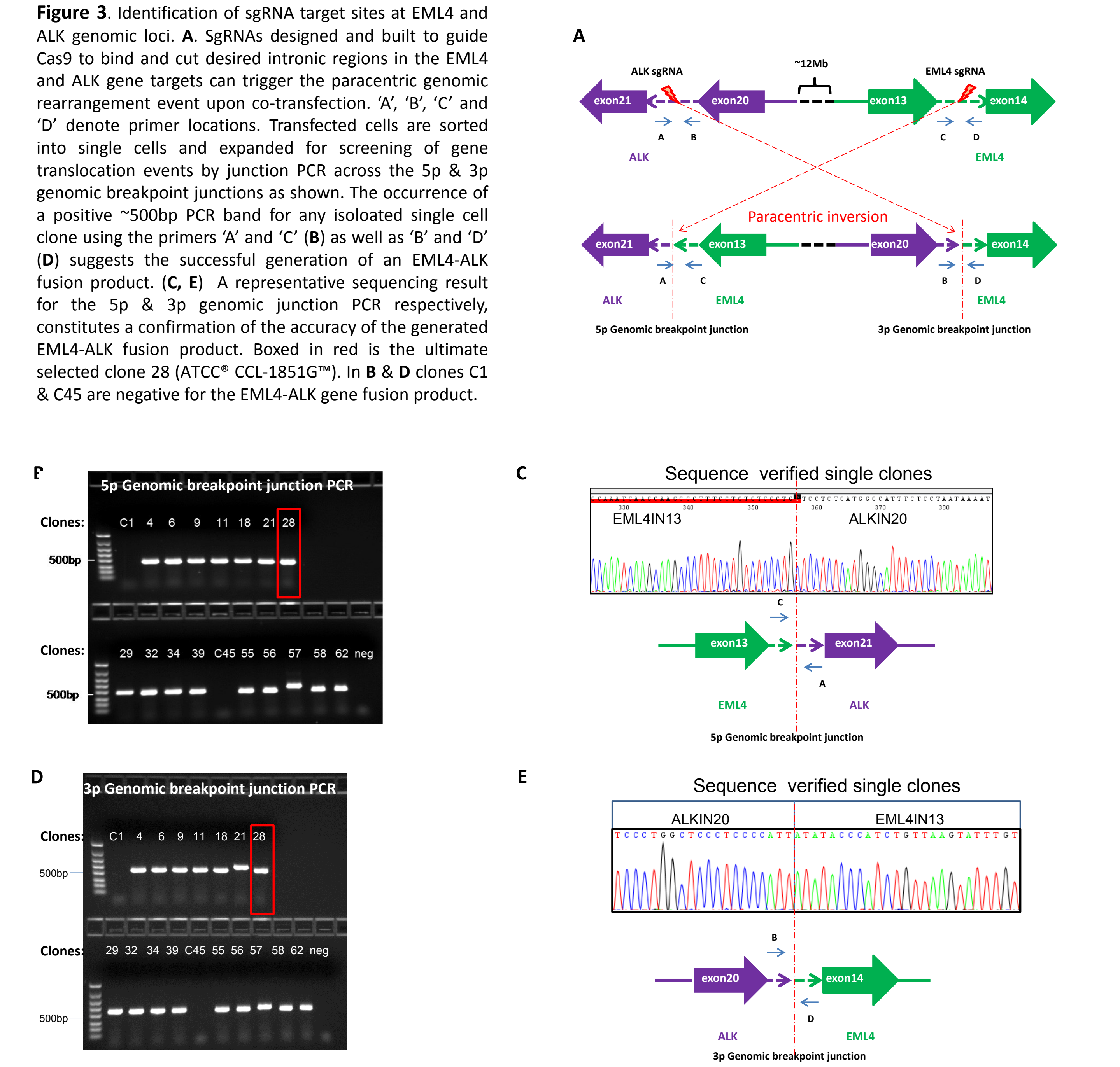
I. Background information: ALK is a drug target and a diagnostic marker



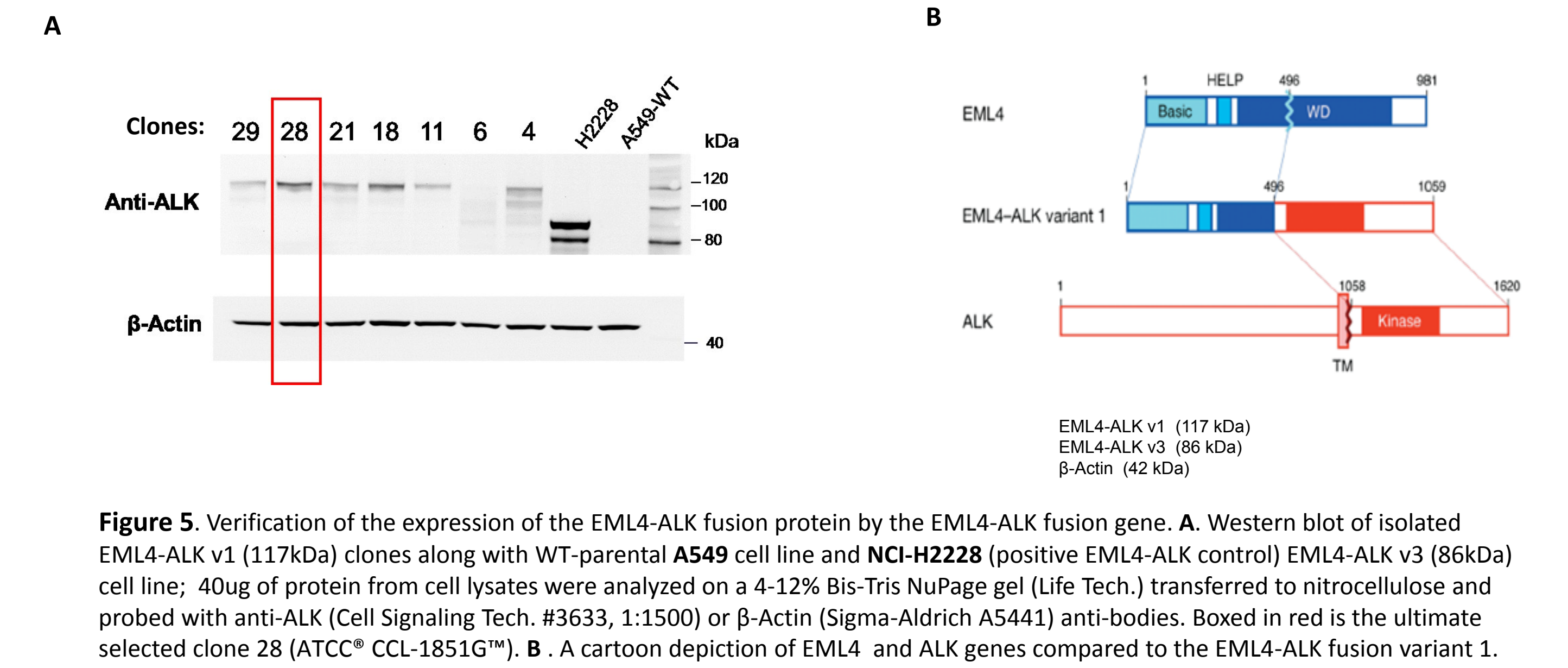
II. Introduction to the EML4-ALK gene fusion: The EML4-ALK gene fusion is a gain of function mutation



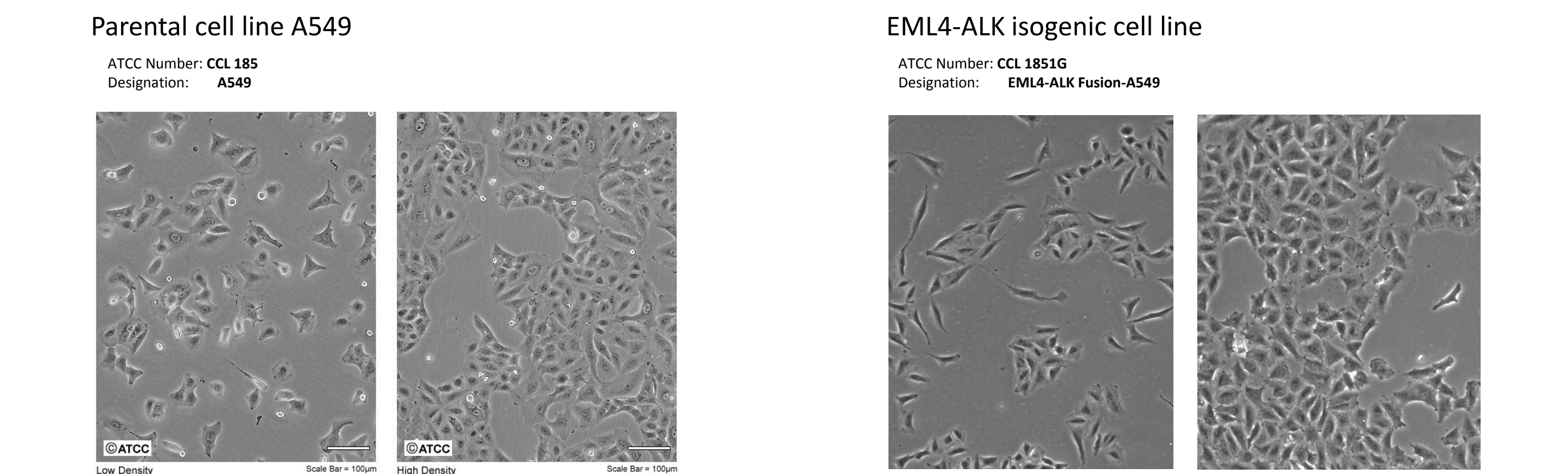
III. Project design and execution: Design and constructed CRISPR/Cas9 reagents to generate EML4-ALK gene fusion in A549 cells (ATCC - CCL-185)



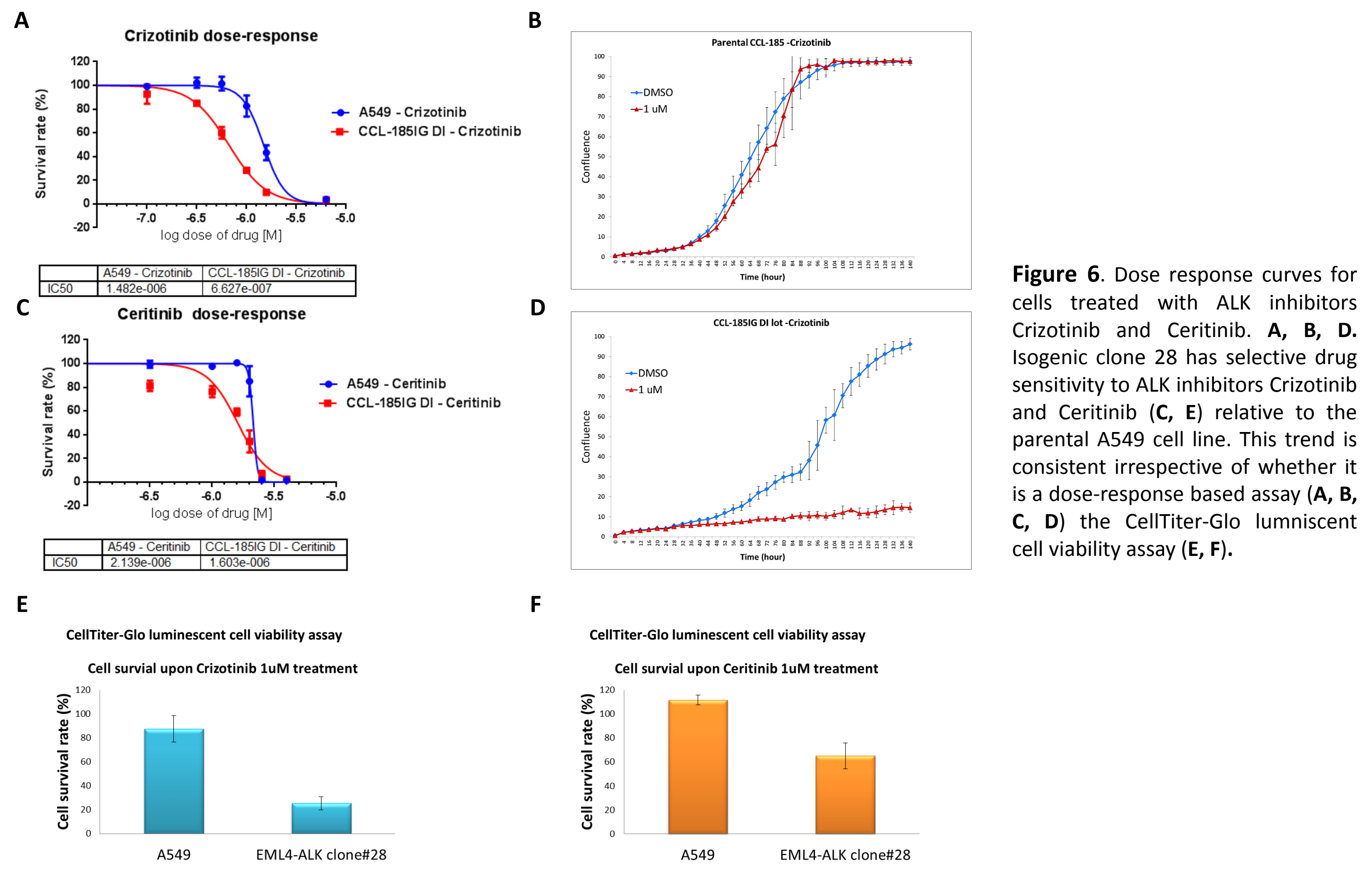
V. Confirmation of EML4-ALK fusion protein expression in isolated single clones



VI. Comparison of the morphology of isogenic clone and parental cell line: The morphology of isogenic clone 28 is similar to the parental line A549



VII. Relative drug response of isogenic clone: EML4-ALK A549 isogenic clone 28 is sensitive to ALK inhibitor drugs



VIII. CONCLUSION

- The ATCC isogenic cell line CCL-1851G derived from the A549 NSCLC cell line (CRL-185), contains the EML4-ALK fusion variant 1 (E13; A20). It has been validated at the genomic, transcript and protein levels.
- Furthermore, CCL-1851G is more sensitive to ALK inhibitors crizotinib and ceritinib when compared to the A549 parental cell line. CCL-1851G can be a useful model to study the tyrosine kinase signaling pathway, and to screen for novel ALK inhibitors in anti-cancer drug discovery and development.