Somatic mutations found within the tyrosine kinase domain of the human epidermal growth factor (HER) or the human erythroblastoma virus B (ErbB) family of transmembrane receptors have been implicated in the development and progression of non-small cell lung cancer (NSCLC). These mutations have been identified in the EGFR (epidermal growth factor receptor [HER1; ErbB1]), HER2 (ErbB2) and HER4 (ErbB4) genes, but no functional mutations have been described to date in the kinase inactive HER3 (ErbB3) gene. Here, we report the case of an adolescent patient with advanced NSCLC in whom we identified a novel V855A (Valine → Alanine) somatic mutation situated in exon 21 of the HER3 tyrosine kinase domain. Remarkably, the mutation maps at a position homologous to the frequently described EGFR tyrosine kinase inhibitor (TKI)-sensitive L858R (Leucine → Arginine) activating mutation situated in exon 21 of the EGFR tyrosine kinase domain. In vitro functional analysis in a null Ba/F3 background reveals that HER3-V855A when combined with its HER2 dimerization partner leads to neuregulin 1\(\beta\)-induced HER3 and HER2 receptor activation and transforms interleukin-3 (IL-3) dependent Ba/F3 cells to neuregulin 1\(\beta\)-dependent growth. Afatinib, an ErbB family inhibitor, has anti-proliferative and pro-apoptotic effects on the mutant HER3: wild-type HER2 Ba/F3 derivative that is logarithmically higher than the effect obtained in the wild-type HER3: wild-type HER2 combination. Together, our findings demonstrate that kinase impaired HER3 can be activated and that the HER3-V855A mutation confers a gain-of-function phenotype that is associated with sensitivity to afatinib. This finding could be relevant for the treatment of NSCLC or other cancers carrying such mutations.