Interleukin 7 (IL-7), a product of stromal cells, and its receptor, formed by IL-7Rα (encoded by IL7R) and the common gamma chain (γc), are essential for normal T-cell development and homeostasis of mature peripheral T cells. IL-7Rα deficiency mice showed a diminished T cell number and impaired lymphocyte development. Further, the IL-7/IL-7Rα pathway is important for T-acute lymphoblastic leukemia (T-ALL) proliferation and survival. Hypothesizing that IL-7Rα gain-of-function mutation could be occurring in T-ALL, 50 T-ALL samples were analyzed for mutations. About 9% of childhood T-ALL presented mutations at the transmembrane domain, and all of them were in-frame insertions and deletions. In all but three cases there was an insertion of cysteine that is essential for disulfide bond formation and constitutive activation of the receptor independent of IL-7. The constitutive signaling was confirmed by phosphorylation of Jak1, STAT5, AKT and Bad, and analysis of IL-7Rα artificial mutants with or without cysteine. The position of cysteine insertion is very important to disulfide bond formation, to activate the Jak/STAT pathway and to support the proliferation of Ba/F3 and D1 cell line in the absence of cytokine. Moreover, IL-7Rα mutant transduced D1 cells injected into IL-7−/− mice caused splenomegaly, metastasis and tumor at the injection site. Similar results were obtained with the ectopic expression of the IL7Rα mutant in hematopoietic progenitor cells of IL7R−/−, JAK3−/− and/or IL2Rγ−/− mice, demonstrating that the mutant homodimer IL-7Rα operates independently of these molecules. Overall, our findings indicate that IL7R mutational activation is involved in human T-cell leukemogenesis, paving the way for therapeutic targeting of IL-7R–mediated signaling in T-ALL.