Identification of translocation products but not K-RAS mutations in memory B cells from patients with multiple myeloma

Thomas Rasmussen,1 Jacob Haaber,2 Inger Marie Dahl,3 Lene M. Knudsen,1,2 Gitte B. Kerndrup,2 Marianne Lodahl,1 Hans E. Johnsen,1,4 and Michael Kuehl5

1Department of Hematology, Herlev Hospital, University of Copenhagen, Herlev, Denmark 2Department of Hematology, Odense University Hospital, Odense Denmark, 3Section of Hematology, University Hospital, Tromsoe, Norway; 4Department of Hematology, Aalborg Hospital, Aarhus University Hospitals; 5Genetics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda Naval Hospital, Building 8, Room 5105, Bethesda, MD, USA


Online Supplementary Figure S1. Identifying MM tumors that are CCND1 high and low expressors. The actual CCND1/β-actin ratio was determined in FACS-purified MM plasma cells using real-time PCR as described in the Design and Methods section. Plasma cells with high CCND1 expression (CCND1/β-actin >0.1) and low to medium expression (CCND1/β-actin >0.001 and <0.1) were identified.

Online Supplementary Figure S2. Variations in gene expression at the single-cell level. (A) Variation introduced by global RT-PCR. Plasma cells from a MM patient were FACS-sorted (100 cells/PCR tube) and global RT-PCR were performed in five separate runs and the CCND1/β-actin ratios were determined, with the largest difference being 1.7-fold. (B) Variation due to real-time measurements in the same run (100 plasma cells/PCR tube), with the maximum difference in the CCND1/β-actin ratio being 1.15-fold. (C) Variation due to real-time measurements on separate days (100 plasma cells/PCR tube), with the maximum difference in the CCND1/β-actin ratio being 1.42-fold. (D) From a patient with CCND1-positive plasma cells, the plasma cells were FACS-sorted to one cell/PCR tube, with the maximum difference in the CCND1/β-actin ratio being 5.4-fold. (E) From a patient with FGFR3-positive plasma cells, the plasma cells were FACS-sorted to one cell/PCR tube, with the maximum difference in the FGFR3/β-actin ratio being 4.3-fold. (F) From a patient with CCND1-positive plasma cells, the plasma cells were FACS-sorted to 100 cells/PCR tube (MM-2, Table 1), with the maximum difference in the CCND1/β-actin ratio being 20.5-fold.