The supportive effect requires communication between CLL cells and viable stromal cells
Since several cytokines such as IL-4 are known to support survival of CLL cells in vitro, and since TGF-β is produced by the leukemic cells and stromal cells, we investigated in samples of 3 patients whether these cytokines are involved in the supportive functions of BMSC. As shown in Fig. S1 A, recombinant human IL-4 (10 ng/ml) and anti–IL-4 antibody (20 µg/ml) had a minor influence on the supportive effect of BMSC after 4 days in co-cultures. The same was true for TGF-β1 (1 ng/mL). However, anti–TGF-β antibody (20 µg/ml) moderately reduced the supportive effect of BMSC (Fig. S1B).

We further explored whether the supportive effect could be due to other soluble mediators produced in the co-culture or through the interaction between CLL cells and receptors expressed on BMSC or extracellular matrix proteins produced by these cells. CLL cells from 6 patients were incubated for 4 days in suspension culture supplemented with 10–50% culture supernatant obtained from 3 days old confluent BMSC in serum free medium. CLL cells were also incubated with methanol fixed BMSC which retained surface structures and matrix proteins. As shown in Fig. S1 C, a moderate supportive effect was obtained by the supernatant of BMSC and by methanol fixed stromal cells. However, the percentage of apoptotic CLL cells increased when BMSC either directly or indirectly using transwell culture plates (membrane pores 0,4 µm) was significantly lower (11 ± 4% and 14 ± 5% respectively) compared to 54 ± 12% in suspension control culture (p<0,01). Collectively, these data indicate that the supportive effect of stromal cells is a complex process which requires several soluble mediators, direct contact and bidirectional communication between CLL cells and viable stromal cells.

Inhibition of PI3-K/Akt selectively induces apoptosis in CLL cells
Since the effect of PI3-K or Akt inhibition might be due to a general toxicity, we analyzed the flow cytometry data from 30 patients by gating on the viable population after exposure to the inhibitors in co-cultures for 4 days. As demonstrated in Fig. S2 A, there was a significant decrease (p<0,01) in the percentage of the leukemic CD19+/CD5+/CD23 cells within the living cell population after exposure to 1 µM of LY294002 or 0,5 µM of wortmannin. In contrast, there was a relative increase in the percentages of CD3+ T cells and CD14+ monocytes (S2 B). The monocytes also retained their phagocytotic capacity in spite of the exposure to LY294002 or wortmannin (S2 C: a–c). Also the stromal cells in treated co-cultures remained intact, adherent and viable (S2 C: d–f)) but the adhesion of CLL cells to BMSC was significantly reduced (S2 e–f). These results indicate that the malignant cells are more sensitive to PI3-K inhibitors than T cells, monocytes and BMSC. This could be confirmed by exposing purified B cells from CLL patients and healthy individuals to the inhibitors which demonstrates that the CLL cells were far more sensitive than normal B cells (S2 D). The molecular mechanisms for the selective effect on the leukemic CLL cells remain to be investigated. However, it has been demonstrated that the inactivation of PI3-K (p85α subunit) in mice leads to an impaired development and maturation of B cells while T cell development and proliferation was normal.\(^1\,^2\) It has also been demonstrated that PI3-K is tightly linked to the antigen receptor-mediated signaling and is pivotal for B cell development and function.\(^3\) In addition, CD19 which is essentially expressed on B cells and CLL cells binds to PI3-K and plays a key regulatory role in the activation of PI3-K/Akt pathway.\(^4\) Furthermore, it has been recently shown that the inhibition of PI3-K leads to apoptosis in T-ALL cells without affecting the normal T cell precursors.\(^5\) Therefore, it seems that CLL cells are addicted to or dependent on PI3-K signaling than normal cells.
Effect of pre-exposure of BMSC to PI3-K inhibitors on their survival supporting capacity
Since the effect of the PI3-K inhibitors on the decreased viability of CLL cells in co-culture might be due to an indirect effect on the BMSC, we pre-exposed BMSC to LY294002 or wortmannin for 6 hours followed by removal of the inhibitors. Then co-cultures were initiated for 3 days and the capacity of BMSC to support the viability of CLL cells was assessed by FACS analysis. As shown in figure S3, both inhibitors at the applied concentrations had no significant effect on the viability of BMSCs. Furthermore, pre-exposure to the inhibitors did not affect the capacity of BMSC to support survival of CLL cells. The figure demonstrates a representative example of 3 independent experiments. These data suggest that pre-exposure of the BMSC to the PI3-K inhibitors might not significantly influence their survival supporting capacity of CLL cells.

Effect of pan–PI3-K and isotype-specific inhibitors on the viability of CLL cells in suspension and in co-culture
Since the pan–PI3-K inhibitors LY294002 and wortmannin might have a wider range of targets, we tested the effect of a more selective PI3-K inhibitor (PI-103) and selective inhibitors of p110α, p110β, p110γ isotypes. Cell viability was measured by MTT assays. As shown in Fig. S4, a minimal effect on the viability of BMSC was observed although a moderate effect could be induced by PI-103. CLL cells in suspension cultures appeared sensitive to all inhibitors although this effect was relatively reduced in the co-culture. These data suggest that CLL cells are sensitive to a variable degree to the pan- and isotype-specific inhibitors while BMSC appeared to be less sensitive. This confirms our data on the selective effect of PI3-K inhibition in inducing apoptosis in CLL cells as presented in this work.
Figure S1. Effect of selected soluble mediators
To explore the potential effect of soluble mediators which could be produced by CLL cells and BMSC, cell cultures were supplemented with IL-4 (10 ng/ml) or anti–IL-4 antibodies (20µg/ml) (A), or with TGF-β1 (1ng/ml) and anti–TGF-β1 antibodies (20 µg/ml) (B). The effect of soluble mediators in the supernatants of BMSC and direct cell-cell, cell-matrix contact is demonstrated in (C). Cell viability was assessed by Annexin V/PI staining after 4 days in culture. A significant decrease in the apoptosis rate is mainly observed in co-cultures which allow direct and indirect communication between CLL cells and viable BMSC (**p<0,01).

Figure S2. PI3-K inhibition selectively induces apoptosis in CLL cells
PBMC from 30 CLL patients were exposed to LY294002 in co-cultures for 4 days and cell viability was assessed by flow cytometry. A significant decrease in the percentage of viable leukemic cells (CD19/CD5/CD23) is observed after treatment with LY294002 compared to untreated cultures (A). This was associated with a relative increase in T cells and monocytes (B). The monocytes (large arrows) retained the capacity to phagocytose apoptotic cells and cell fragments (small arrows) (C & D). As demonstrated in (E) CLL cells adhere to BMSC under control conditions. The addition of LY290042 prevented this adhesion and induced death in the leukemic cells while BMSC remained adherent and viable (F).

Figure S3. Effect of pre-exposure of BMSC to PI3-K inhibitors on their survival supporting capacity
Short exposure of BMSC to PI3-K inhibitors (LY294002 and wortmannin) for 6 hours followed by removal of the inhibitors and co-culture initiation with CLL cells did not significantly influence the endogenous capacity of BMSC to support survival of CLL cells.

Figure S4. Effect of pan–PI3-K and isotype-specific inhibitors on the viability of CLL cells in suspension and in co-culture
Exposure of BMSC to pan- and isotype-specific PI3-K inhibitors did not significantly affect their viability. However, they were more effective in reducing the viability of CLL cells in suspension culture than in co-culture.

REFERENCES
Figure S1
Figure S2
Figure S3
Figure S4